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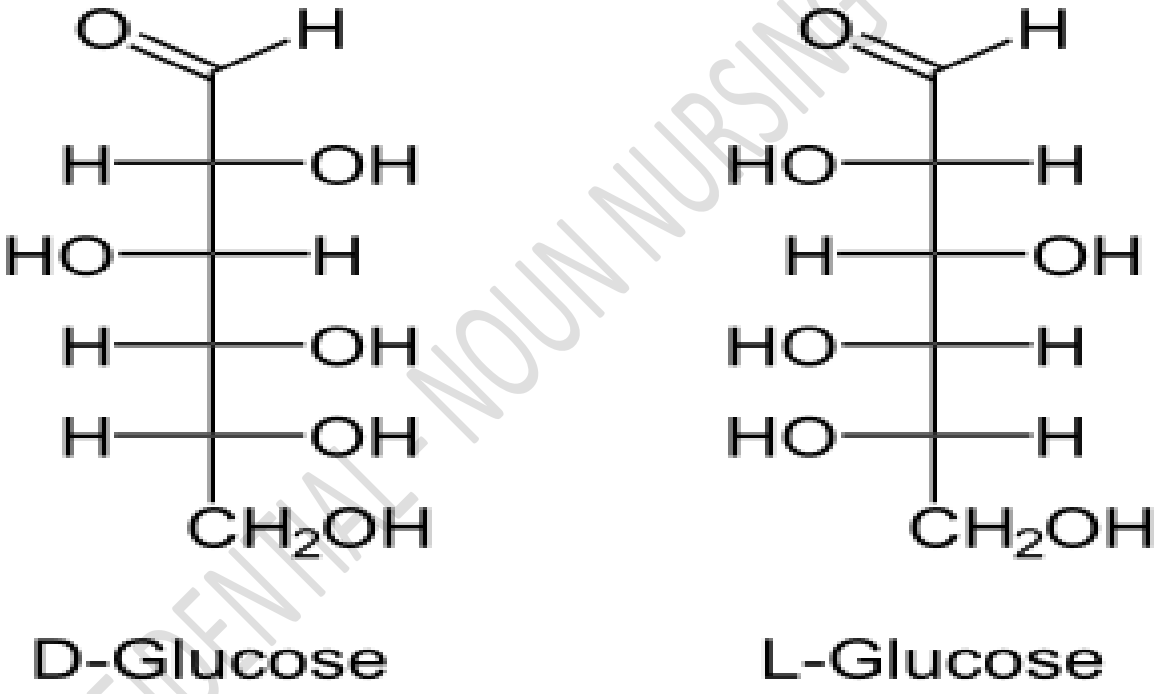
NATIONAL OPEN UNIVERSITY OF NIGERIA
SCHOOL OF HEALTH SCIENCES



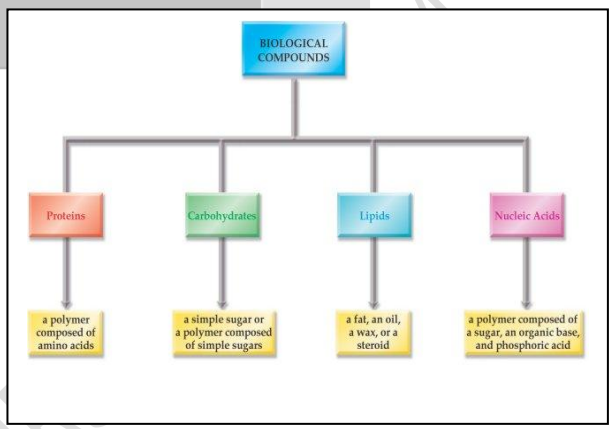
COURSE CODE: NSC 108

COURSE TITLE: MEDICAL BIOCHEMISTRY I

COURSE UNITS: 3 (2-1-2)

NATIONAL OPEN UNIVERSITY OF NIGERIA

COURSE GUIDE
NSC 108 MEDICAL BIOCHEMISTRY I



COURSE CODE: NSC 108
COURSE TITLE: Medical Biochemistry I
COURSE UNITS: 3 Credit units (24 hours of instruction online; 12 hours of Discussion forum online/tutorial; 48 hours of laboratory practical).
YEAR: 1
SEMESTER: 2nd Semester
PRE-REQUISITE COURSES: All courses in the BNSC degree programme in the first semester of the first year.
CON-CURRENT COURSES: NSc 102, 104, 106
SESSION: 2015/2016 **COURSE WEBSITE:** *www.noun.edu.ng/*

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COURSE COORDINATOR: To be appointed by the School
COURSE FACILITATORS: To be appointed by the School

COURSE GUIDE

GENERAL INTRODUCTION

This course is thought as Medical Biochemistry; medical biochemistry is a subset of general biochemistry. In this course, you will learn the biochemical activities that occur inside the cell and how these activities determine our state of health and what happens in disease. The reason why we fall sick and the root causes of disease will be clearer to you at the end of the course. Take a second look at yourself in the mirror; note the shape of your face, the shape of your nose, ear and lips. Examine your complexion, your height and the colour of your hair. Now look at your mother carefully, how many of these features do you share with her? Do the same to your father. Do you observe any features not shared with either of your parents? If there is any, it may be the feature you inherited from your grandparents. Scientific basis for this simple experiment will be explained to you in this course. Diseases can be caused by microorganisms, what we eat, drink and by our lifestyle; it can also be inherited from our parents. You will be in a position to explain the source or cause of a particular disease and the mechanism of its treatment if you understand the biochemistry of human body. The knowledge that will be acquired in this course will assist you in understanding the effects of drugs on the body and the effects of our body on the drugs we use for therapeutic purposes.

COURSE AIM

The aim of this course is to build your foundation for application of the understanding of the chemical make up of the body in development and implementation of care of patients

COURSE OBJECTIVES

At the completion of this course, you should be able to:

Explain the context of medical biochemistry in health and health care

WORKING THROUGH THIS COURSE

The course will be delivered adopting the blended learning mode, 70% of online but interactive sessions and 30% of face-to-face during laboratory sessions. You are expected to register for this course online before you can have access to all the materials and have access to the class sessions online. You will have hard and soft copies of course materials, you will also have online interactive sessions, face-to-face sessions with instructors during practical sessions in the laboratory. The interactive online activities will be available to you on the course link on the Website of NOUN. There are activities and assignments online for every unit every week. It is important that you visit the course sites weekly and do all assignments to meet deadlines and to contribute to the topical issues that would be raised for everyone's contribution.

You will be expected to read every module along with all assigned readings to prepare you to have meaningful contributions to all sessions and to complete all activities. It is important that you attempt all the Tutor Marked Assignments (TMA) and other Self Assessment Questions (SAQ) at the end of the Module or Units to help your understanding of the contents and to help you prepare for the in-course tests and the final examination. You will also be expected to keep a portfolio where you keep all your completed assignments.

STUDY UNITS

This course, the first of 2 courses that you will take is divided into 3 Modules of 14 study units.

Module 1- General Introduction to Medical Biochemistry

Unit 1: Introduction to Physiological and Pathological Chemistry
Unit 2: Cell Structure and Functions 1
Unit 3: Functions of the Cell Membrane and the Organelles

Module 2- Chemistry of the Nutrients
Unit 1: Chemistry of Sugars
Unit 2: Chemistry of Sugars (II)
Unit 3: Water, Acids, Bases and Buffer
Unit 4: Chemistry of Amino Acids and Proteins (I)
Unit 5: Chemistry of Amino Acids and Proteins (II)
Unit 6: Classification of Lipids
Unit 7: Introduction to Nucleic Acid Biochemistry-I
Unit 8: The Structures of DNA and RNA

Module 3 - Enzymology
Unit 1: Enzymology 1
Unit 2: Enzyme Kinetics
Unit 3: Enzymology 3

REFERENCE TEXTBOOKS

1. Murray, R.K., Bender, D.A., Botham, K., M., Kennelly, P.J., Rodwell V.W. and Well, P.A., (2012). Harper's Illustrated Biochemistry (29th Edition) McGraw-Hill Medical
2. Devlin T.M. (2010) Textbook of Biochemistry with Clinical Correlation 7th Edition. JohnWiley & Sons Inc.
3. Nelson, D.L. and Cox M.M., (2009) Lehninger Principles of Biochemistry 4th edition

COURSE REQUIREMENTS AND EXPECTATIONS OF YOU

Attendance of 95% of all interactive sessions, submission of all assignments to meet deadlines; participation in all CMA, attendance of all laboratory sessions with evidence as provided in the log book, submission of reports from all laboratory practical sessions and attendance of the final course examination. You are also expected to:

1. Be versatile in basic computer skills
2. Participate in all laboratory practical up to 90% of the time
3. Submit personal reports from laboratory practical sessions on schedule
4. Log in to the class online discussion board at least once a week and contribute to ongoing discussions.
5. Contribute actively to group seminar presentations.

EQUIPMENT AND SOFTWARE NEEDED TO ACCESS COURSE

You will be expected to have the following tools:

1. A computer (laptop or desktop or a tablet)
2. Internet access, preferably broadband rather than dial-up access
3. MS Office software – Word PROCESSOR, Powerpoint, Spreadsheet
4. Browser – Preferably Internet Explorer, Moxilla Firefox
5. Adobe Acrobat Reader

NUMBER AND PLACES OF MEETING (ONLINE, FACE-TO-FACE, LABORATORY PRACTICALS)

The details of these will be provided to you at the time of commencement of this course

DISCUSSION FORUM

There will be an online discussion forum and topics for discussion will be available for your contributions. It is mandatory that you participate in every discussion every week. You

participation link you, your face, your ideas and views to that of every member of the class and earns you some mark.

COURSE EVALUATION

There are two forms of evaluation of the progress you are making in this course. The first are the series of activities, assignments and end of unit, computer or tutor marked assignments, and laboratory practical sessions and report that constitute the continuous assessment that all carry 30% of the total mark. The second is a written examination with multiple choice, short answers and essay questions that take 70% of the total mark that you will do on completion of the course.

Students evaluation: The students will be assessed and evaluated based on the following criteria

- **In-Course Examination:**
  In-course examination will come up in the middle of the semester. This would come in form of Computer Marked Assignment. This will be in addition to one compulsory Tutor Marked Assignment (TMA's) and three Computer Marked Assignment that comes as specified after the modules.
- **Laboratory practical:** Attendance, record of participation and other assignments will be graded and added to the other scores form other forms of examinations.
- **Final Examination:** The final written examination will come up at the end of the semester comprising essay and objective questions covering all the contents covered in the course. The final examination will amount to 60% of the total grade for the course.

Learner-Facilitator evaluation of the course

This will be done through group review, written assessment of learning (theory and laboratory practical) by you and the facilitators.

GRADING CRITERIA

Grades will be based on the following Percentages

Tutor Marked Individual Assignments	10%	
Computer marked Assignment	10%	
Group assignment	5%	40%
Discussion Topic participation	5%	
Laboratory practical	10%	
End of Course examination	60%	

GRADING SCALE

A = 70-100

B = 60 - 69

C= 50 - 59

F = $\leq$ 49

SCHEDULE OF ASSIGNMENTS WITH DATES

To be provided for each module by the facilitator in addition to the ones already spelt out in the course materials.

SPECIFIC READING ASSIGNMENTS

To be provided by each module

COURSE OVERVIEW

Medical Biochemistry (I)

Medical Biochemistry (I) is the first of two courses in that runs in the second year second of two courses that covers the .....

HOW TO GET THE MOST FROM THIS COURSE

1. Read and understand the context of this course by reading through this course guide paying attention to details. You must know the requirements before you will do well.
2. Develop a study plan for yourself.
3. Follow instructions about registration and master expectations in terms of reading, participation in discussion forum, end of unit and module assignments, laboratory practical and other directives given by the course coordinator, facilitators and tutors.
4. Read your course texts and other reference textbooks.
5. Listen to audio files, watch the video clips and consult websites when given.
6. Participate actively in online discussion forum and make sure you are in touch with your study group and your course coordinator.
7. Submit your assignments as at when due.
8. Work ahead of the interactive sessions.
9. Work through your assignments when returned to you and do not wait until when examination is approaching before resolving any challenge you have with any unit or any topic.
10. Keep in touch with your study centre, the NOUN, School of Health Sciences websites as information will be provided continuously on these sites.
11. Be optimistic about doing well.

COURSE MATERIAL

Table of Contents Page

Module 1 - General Introduction to Medical Biochemistry

Introduction

This module introduces you to the chemical context of the functioning of the cell as the basic unit of the body. The structure and functioning of the organelles of the cell are also presented.

Modular Objectives:

At the end of this module, you should be able to:

i. Discuss in details Physiological and Pathological chemistry
ii. Explain the cell structure with the aid of a diagram
iii. Enumerate and discuss the functions of Membrane and the Organelles

Contents

Unit 1: Introduction to Physiological and Pathological Chemistry
Unit 2: Cell Structure and Functions 1
Unit 3: Functions of the Cell Membrane and the Organelles

UNIT ONE - INTRODUCTION TO PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY

CONTENT

1.0 Introduction

This unit introduces you to medical biochemistry with some explanations of the relevance of the course to your practice. You will also be exposed to different branches of biochemistry.

2.0 Objectives

At the end of this unit, you should be able to:

i. Define medical biochemistry.
ii. Mention one major breakthrough in the field of biochemistry
iii. Explain the relevance of biochemistry to Nursing, Medicine and other biological sciences.
iv. Describe different branches of biochemistry.

3.0 Main Content

3.1 Definition of Biochemistry

Biochemistry is the study of chemical processes in living organisms. It can also be defined as the application of chemistry to the study of biological processes in living organisms. Biochemistry is both a life science and a chemical science; it explores the chemistry of living organisms and the molecular basis for the changes occurring in living cells.

Medical biochemistry can be described as a branch of General biochemistry; its scope is not as broad as general biochemistry. Medical biochemistry is defined as the study of biochemical processes that occur within the human body in relation with their application in the field of medicine. Millions of complex chemical reactions are going on in the human body at any given time, ranging from the balance of the endocrine system to the storage and utilization of fuel molecules such as glucose. By studying and understanding these highly complex reactions, medical biochemists have found better ways to fight infections and diseases at the molecular level. Since an Engineer cannot repair a vehicle if he does not understand how it works, so a Nurse must understand how human body works before she can treat her patient effectively.

Much of biochemistry deals with the structures and functions of cellular components such as proteins, carbohydrates, lipids and nucleic acids collectively known as biomolecules. The main focus of medical biochemistry is in understanding how biological molecules give rise to the processes that occur within living cells, which in turn relates greatly to the study and understanding of the whole organism (human being).

3.2 Breakthrough in Biochemistry

Our present knowledge of human body came from series of experiments and research conducted several years ago. Transfer of genetic information from one generation to the next was not thoroughly understood until the major breakthrough of 1953/54.

One of the major breakthroughs in medical biochemistry was the discovery of an accurate model of DEOXYRIBONUCLEIC ACID (DNA) by James Watson and Francis Crick in 1953. This discovery opened up possibilities in the realm of medical biochemistry that had been inaccessible until that time.

The human genome was mapped completely in 2003 as a result of the 13 year Human Genome project (HGP). Since then, medical biochemists have had access to vital genetic information which has allowed for manipulation within the cell nucleus. They are also finding ways to isolate harmful traits within human DNA, and have found methods of sometimes causing them to completely shut down prior to manifestation.

Intense effort on the parts of the scientific and medical communities applied to biochemical research has led to the discovery of many vaccines, anti-depressants and other useful medicinal drugs. These drugs often work hand-in-hand with the chemical makeup of the human anatomy. Without medical biochemistry, much of modern medicine would not be practiced as it is known today.

3.3 Relevance of Medical Biochemistry to other life Sciences

Medical Biochemistry provides foundation for other life sciences such as medicine, Nursing, pharmacy, zoology, microbiology etc. Various methods are used by Biochemists to isolate, purify, characterize and study the reactions of all cellular components. Biochemists have contributed greatly to the discovery of new drugs to treat chronic diseases such as cancer, viral infections and metabolic disorders. They are able to do this because they have thorough understanding of what happens at the molecular level i.e inside the cell.

3.4 Branches of Biochemistry

i. Toxicology: This field studies the adverse effects of toxic or foreign chemical substances on the organisms. Environmental and food toxicology also fall under this branch of biochemistry.
ii. Enzymology: The study of enzymes, their functions, deficiency and the consequence of such deficiency in diseases.
iii. Molecular biology and Biotechnology: This field evolved directly from Nucleic acid biochemistry and it involves manipulation of DNA to improve drug research and solve health problems. It has wide applications in other fields of science which includes cancer research.
iv. Lipid and Carbohydrate biochemistry: These fields study the biochemical basis of metabolic disorders such as diabetes, obesity and Cardiovascular diseases.
v. Natural products biochemistry: This is a new area of research in biochemistry; it evolved as a result of interest of scientists across the world in searching for new drugs from plants. Quinine and Artesunate (antimalaria drugs) were isolated from plants.

4.0 Conclusion

This introductory unit has shown that biochemistry as a study of the chemical processes in the body has made many breakthroughs that is helping in better understanding and planning of care for people. The five main branches of biochemistry presented have different dimensions to the discourse of health and health care.

5.0 Summary

In this unit, you have learnt about the following:

i. Definition of Biochemistry
ii. Breakthrough in Biochemistry
iii. Relevance of Medical Biochemistry to other life Sciences
iv. Branches of Biochemistry

6.0 Tutor Marked Assignments

6.1 Activity – As provided by the facilitator

6.2 Answer the following questions:

i. What is biochemistry?
ii. Why is it important for nursing students to study biochemistry?
iii. Assuming there were no biochemists in the world up till year 2010, do you think medicine and nursing sciences will be practiced the way they are practiced today? Explain your answer.
iv. Mention 3 branches of biochemistry and explain the area of biochemistry they study.

7.0 References and other resources

Specific reading text to be provided by the Facilitator

UNIT TWO - CELL STRUCTURE AND FUNCTIONS 1

CONTENT

1.0 Introduction

The living cells we are discussing here is not different from the cell you learnt in Biology when you were in secondary school. Cells are the monomeric unit through which the complex human body was constructed; my body and your body contain several billion cells! Biochemical arrangement of cells and how these cells interact to perform various functions in man are not only fascinating but also very interesting. Imagine the sensitivity of cells responsible for taste; different region of your tongue detects different taste.

Some cells are replaced every 72 hours in our body while some spend up to ten years before they die. Also some cells remained in our body throughout our lifetime. It is important to understand the importance of compartmentalization in cells and the functions of various organelles present in the cells. This knowledge will help you in subsequent modules; most biochemical reactions take place inside the cell but in different organelles; for example, energy generation takes place inside the mitochondria. Thorough understanding of cell structure will help you to understand the root causes of many diseases and the biochemical mechanisms of their treatment. These mechanisms will also be relevant in other physiological courses you are going to offer.

2.0 Objectives

At the end of this unit, you should be able to:

i. Define a cell and draw the structure of a typical animal cell
ii. Differentiate between prokaryotic and eukaryotic cells
iii. Describe the types, classification and life span of animal cells
iv. Describe the chemical components of plasma membranes

3.0 Main Content

3.1 The definition and structure of Animal cell

A living cell is defined as the fundamental unit of life and it is the smallest unit capable of exhibiting the characteristics of life. Cell was accidentally discovered in 1665 by Robert Hooke while examining a thin slice of cork under his new crude microscope. He observed numerous porous structures (dead cells made of cellulose found in plants) and named them

CELLS (Latin, cellula means little room or small chamber). Further research later confirmed that all living things are composed of cells.

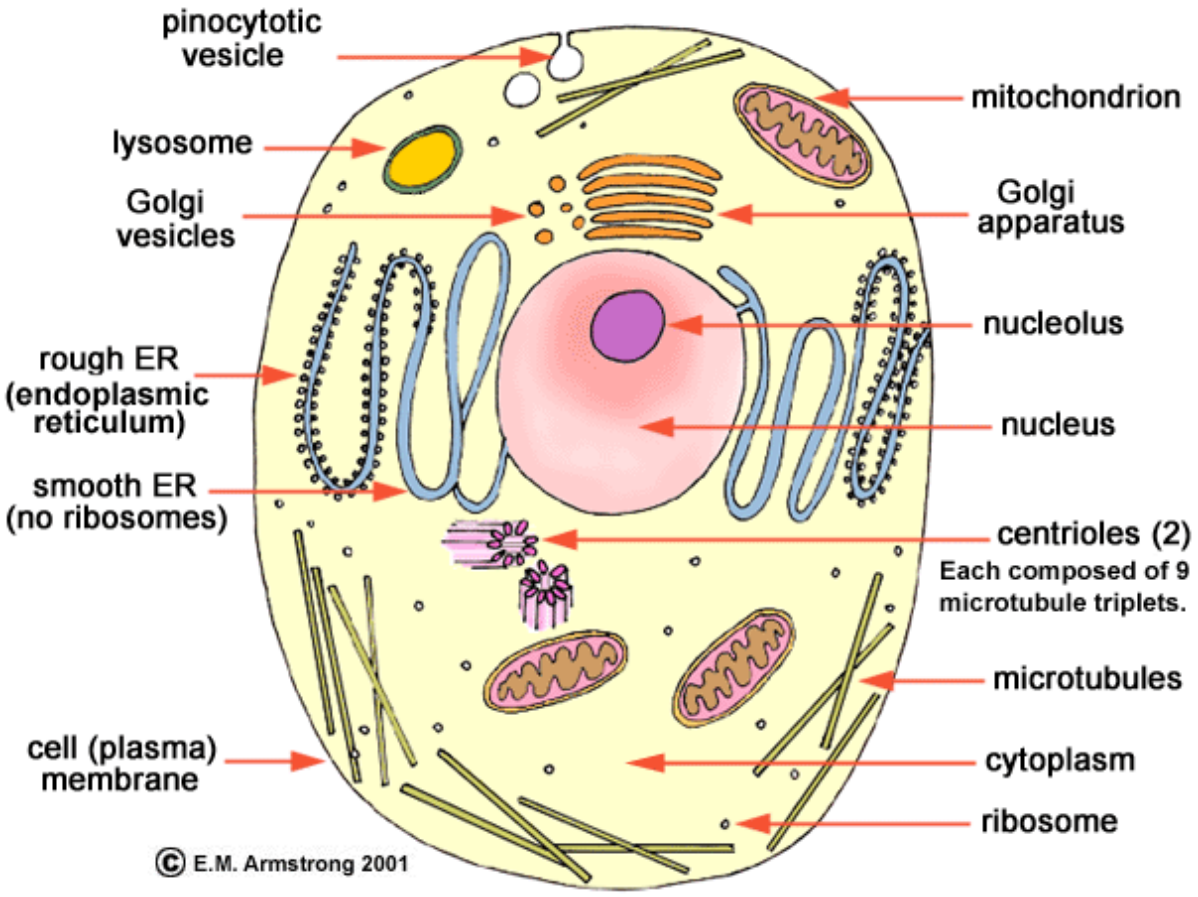


Fig. 2.1: **Structure of Animal Cell (source: google images)**

Animal cells have different shapes and sizes; some are circular, spherical, cylindrical, Fibrous etc. Red blood cells called erythrocytes are one of the smallest animal cells while ova are among the largest. In terms of length, nerve cells are the longest. For ease of representation, circular structure is commonly used to illustrate the structure of animal cells. Study the structure of animal cell (Figure 2.1), take note of the organelles shown in the structure (mitochondria, ribosome, Golgi, endoplasmic reticulum, and lysosome). These organelles have different functions they perform in the cell; the functions will be discussed in subsequent section. A cell can be subdivided into 3 parts namely:

i. The plasma membrane- This is the thin cover that separates a cell from its environment, it also protect the components of the cell from leaking. It prevents the fluid outside the cell called extracellular fluid (ECF) from mixing with the fluid inside the cell called intracellular fluid (ICF). Plasma membranes regulate the materials that enters or leaves the cell, for this reason, it is said to be semi-permeable. In addition, the plasma membrane has some glycoproteins and glycolypids on its surface; these molecules serve as signal molecule between cells.

ii. The cytoplasm: This is the fluid-like space between the plasma and nuclear membrane. Cytoplasm is the cavity where the organelles are found. It provides space

for the movement of synthesized products from one compartment to another for further processing. The organelles are suspended in the cytoplasm by cytoskeleton network that resemble nets.

iii. Nucleus: This is the most important part of the cell, the nucleus is always centrally located. It has its own membrane called nuclear membranes which protects the content of the nucleus. Nucleus is very important to the cell because it contains the genetic materials (DNA and RNA) that control all the activities of the cell. Nucleus regulates the rate and time of cell division. It also determines the materials that enter or exit the cell.

3.2 Differences between Prokaryotes and Eukaryotes cells

The electron microscope allowed classification of cells into two major groups, **prokaryotes** and **eukaryotes** based on the presence and absence of the true nucleus. Eukaryotes have nucleus which is covered by nuclear membrane; Animals, plants and fungi belong to the eukaryotes. Prokaryotes have no typical nucleus (no true nucleus), bacteria and blue green algae belong to the prokaryotes. Eukaryotic cells are much larger than prokaryotes, they also have a variety of other membrane bound organelles in their cytoplasm, and example includes mitochondria, lysosomes, endoplasmic reticulum (ER) and Golgi complexes.

3.3 Types, Classification and life -span of animal cells

There are about 210 distinct human cell types and there are between 50 and 100 trillion cells in adult human body. Animals grow as a result of cell division and cell enlargement. All animals begin their existence as a simple cell i.e. fertilized egg. This cell divides into 2, 4, 8, 16, 32 etc. to produce a body consisting of numerous cells. Ovum is the largest cell cell in man while red blood cell is the smallest.

Multicellular organisms are able to specialise cells to perform specific functions. A group of such cells is a tissue and in animals these occurs as four basic types namely epithelium tissues, nervous tissues, muscle tissues and connective tissues. Several types of tissues work together as an organ to produce a particular function such as the pumping of blood by the heart. This pattern continues to a higher level with several organs functioning as an organ system to allow for reproduction, digestion etc. Multicellular organisms consist of several organ systems.

Cells within the human body have different lifespan based on the type and function of that cell. Although some types of cells are short lived, others remain in person's body for months, years or throughout life. Taste receptor cells in the mouth live for 10 days, one month for the skin cells, 15 years for muscle cells and a lifetime for nerve cells.

Normal red blood cell lives for about 3-4months while sicle shaped red blood cells live for only 10-20 days. White blood cells live for about a year and sperm cells have a lifespan of about 3 days.

3.4 The chemical components of plasma membranes

Plasma membrane mainly consists of lipids and proteins. There is a wide variation in lipid-protein ratio between different cell membranes. The functions performed by cell and the location determine the quantity of proteins and lipids present in their membranes. Here are some examples of cell membranes and their percentage protein-lipid ratio:

Table 2.1: Protein-Lipid ratio of some plasma membranes

Membrane	Protein (%)	Lipid (%)
Erythrocyte	49	41
Liver	60	40
CNS myelin	20	79
Outer mitochondria	50	46
Inner mitochondria	75	23

Membrane Lipids

There are several types of membrane lipids. The fundamental building blocks of cell membranes are the **phospholipids**. Other lipids present in the cell membranes are cholesterol and glycolipids. Membrane lipids are ***amphipathic molecules*** (they have both hydrophilic and hydrophobic ends, hydrophilic means "water loving"; this part readily associates with water while hydrophobic ends means "water hating"; they tend to move away from water). Formation of bilayers is another common property shared by all membrane lipids (Figure 1.2).

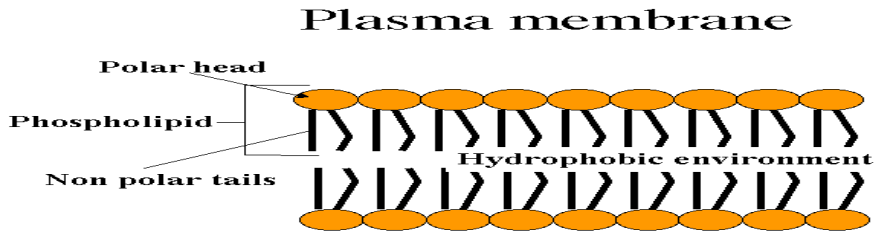


Fig. 2.2: **Simplified structure of Plasma membrane bilayer (source- google images)**

(a) Phospholipids are the most abundant membrane lipids. They have a polar head group and two hydrophilic hydrocarbon tails. The tails are usually fatty acids and they can differ in length (20-24 carbon atoms. One tail usually has one or more double bonds (unsaturated) while the other tail may not contain double bond (saturated). Each double bond creates a bend or kink in the tail. Lipid molecules spontaneously aggregate to bury their hydrophobic tails in the interior and expose their hydrophilic heads to water. Depending on their shape, they can do this in either of two ways; they can form spherical micelles, with the tails inward or they can form bilayers with the hydrophobic tails sandwiched between the hydrophobic head groups. The same forces that drive phospholipids to form bilayers also provide a self-healing property for the cell membrane. A lipid bilayer has other characteristics beside its self-healing properties that make it an ideal structure for cell membranes. One of the most important of these is its fluidity which is crucial to many membrane functions.

A shorter chain length and the presence of double bonds (unsaturated fatty acids) in fatty acid components of phospholipids promote fluidity at low temperature and vice versa. In animals, cholesterol is the key regulator of membrane fluidity

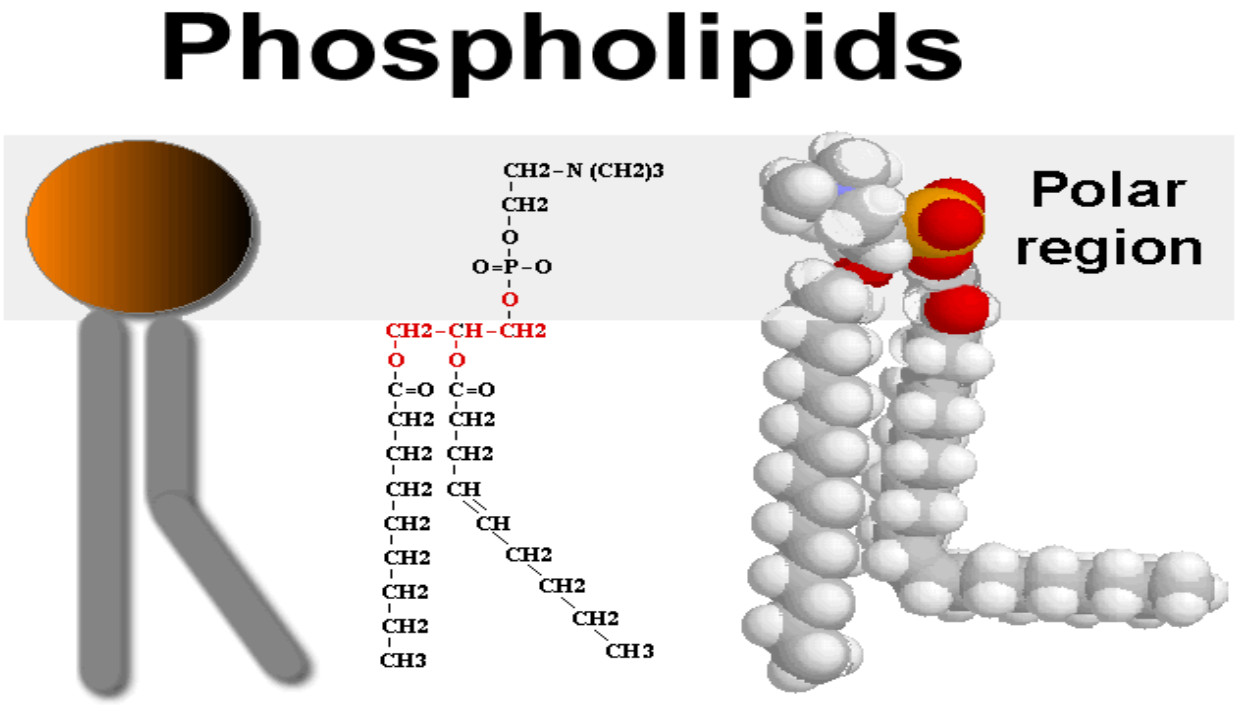


Fig. 2.3: **Three different ways of representing phospholipid orientation in the plasma membranes (source: google images)**

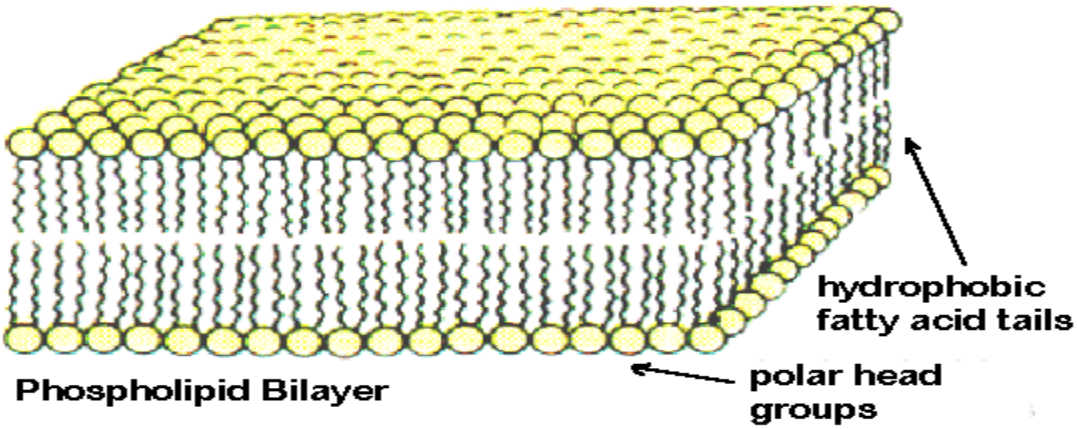


Fig. 2.4: **Three dimensional structure of plasma membrane showing the two bilayers (source: google images).**

The major phospholipids predominate in the plasma membrane of many mammalian cells are; phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine. Only phosphatidyl serine carries a negative charge, the other two are electrically neutral at physiological pH. Some phospholipids, such as the inositol phospholipids are present in smaller quantities. They are usually referred to as phosphoglycerides.

(b) Sphingomyelin is another group of phospholipids; it contains a sphingosine back bone rather than glycerol to which fatty acids and phosphoryl choline are attached. It is electrically neutral at physiological pH; they are prominent in myelin sheaths.

The Asymmetry of the Lipid Bilayer

The lipid compositions of the two monolayer of the lipid bilayer in all membranes are quite different. In the human red blood cell membrane, almost all phospholipids that have choline (phoshphotidyl choline and sphingomyelin) are in the outer monolayer, whereas phospholipids molecules that contain primary amino group (phosphatidyl ethanolamine and phosphatidyl serine) are in the inner monolayer. Glycolipids and glycoprotein are found on the outer monolayer. Animals exploit the phospholipids asymmetry of their plasma membrane to distinguish between live and dead cells. When animal cells undergo apoptosis, phosphatidyl serine which is normally confined to the cytosolic monolayer of the plasma membrane rapidly translocates to the extracellular monolayer. The phosphatidyl serine exposed on the cell surface serves as a signal to induce the macrophages to ingest and digest the dead cell.

Cholesterol

Cholesterol is another important membrane lipid found almost exclusively in the plasma membrane of mammalian cells. It provides stability to the membrane, and it is neutral at physiological pH. In the erythrocyte membrane, the outer membrane leaflet is a rigid four-ringed molecule with a tiny hydrophilic end (hydroxyl group).

Glycolipids

Glycolipids are sugar-containing lipids, like sphingomyelin, the glycolipids in animal cells are derived from sphingosine. In glycolipids, one or more sugars rather than phosphoryl choline are attached to this group. The simplest glycolipid is called cerebroside and it contains a simple sugar residue, either glucose or galactose. More complex glycolipids such as gangliosides contain a branched chain of as many as seven sugar residues. Glycolipids are oriented in such a way that the sugar residues are always on the extracellular side of the membrane.

Lipid vesicle or Liposome

The ability of phospholipids to form lipid bilayer has been used to create an important clinical tool called liposome or lipid vesicle. Liposome is an aqueous compartment enclosed by a lipid bilayer (Figure 2.5). The liposome can be used to deliver drugs to target cells or DNA to specific cells for gene therapy. This liposome fuse with the plasma membrane of target cell, introducing the drugs or chemicals it contains into the cell. The selective fusion of lipid vesicle with particular kinds of cells is a promising means of controlling the delivery of drugs to target cells. Research is ongoing on the use of liposome for cancer chemotherapy.

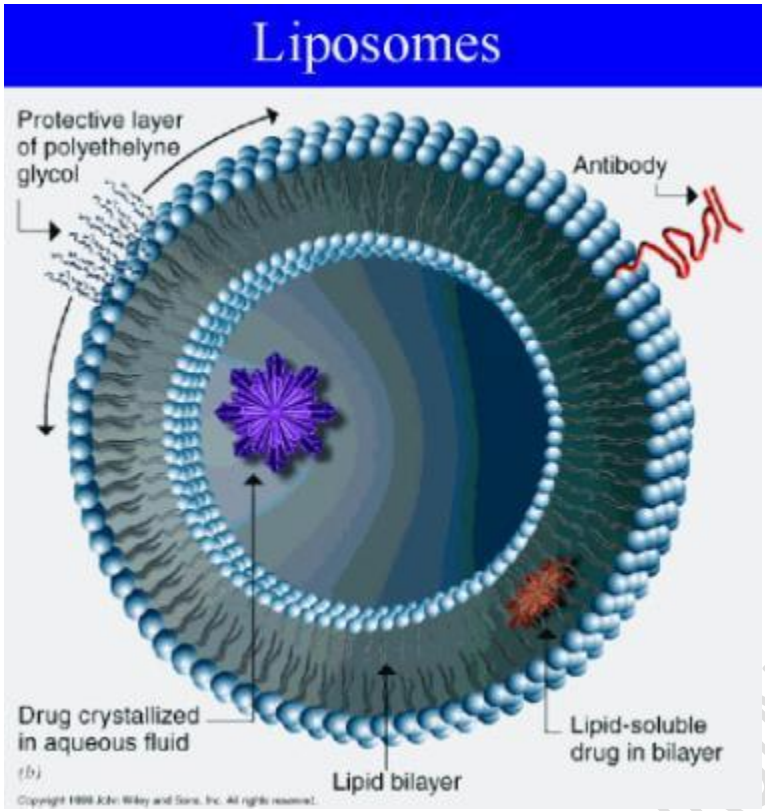


Fig. 2. 5: **The structure of a cross section of liposome (source: google images)**

Membrane Proteins

Membrane proteins perform most of the specific functions of the membranes. They give each stype of membrane in the cell its characteristic functional properties. The amount and types of proteins in a membrane also varies, for example, in the myelin membrane, which serves mainly as electrical insulator for nerve cells, less than 25% of the membrane mass is protein. In the mitochondria, 75% of the membrane component is protein due to the presence of many enzymes and electron pumps.

Membrane proteins can be classified as being either peripheral or integral on the basis of their association with the membrane lipids. Integral membrane proteins interact extensively with the hydrocarbon chains of membrane lipids; in fact, most of them span the lipid bilayer, protruding at both ends. They have high percentage of non-polar amino acids and represent about 70% of total membrane proteins. Examples are membrane enzymes, hormone receptors, pumps and channels. In contrast, peripheral proteins are bound to the surface of lipid bilayer primarily by electrostatic and hydrogen bonds. Many peripheral membrane proteins are bound to the surfaces of integral proteins, on either the cytosolic or extra cellular side of the membrane. Examples include cytochrome c and acetyl choline esterase.

Glycoproteins in blood typing

Carbohydrate groups are covalently attached to many different proteins to form glycoprotein. Many glycoproteins are components of cell membranes, where they play a variety of roles in processes such as cell adhesion, receptors for hormones, responsible for negative charges on many cell surface and binding of sperm to eggs. The carbohydrates of glycoprotein determine the blood group antigens that have been used in blood typing. Carbohydrates are attached to glycoprotein and glycolipids on the surface of red blood cells. For one type of blood group one of the three different structures termed A, B and O may be present. These structures have

in common oligosaccharide foundation called the "O" antigen. Those people that belong to blood group A have one extra monosaccharide called N-acetylgalactosamine in addition to the common oligosaccharide present in all humans. People in blood group B contain an extra monosaccharide called galactose, through an α-1,3 linkage to a glactose moiety of the common oligosaccharide present in all humans.

4.0 Conclusion

The organelles of the basic cell, through the chemical structure perform different functions.

5.0 Summary

In this unit, you have learnt about the following:

i. The definition and structure of Animal cell
ii. Differences between Prokaryotes and Eukaryotes cells
iii. Types, Classification and life -span of animal cells
iv. The chemical components of plasma membranes

6.0 Tutor Marked Assignments

6.1 **Activity:** To be provided by the facilitator.

6.2 Answer the following questions:

i. Draw a well labeled structure of a circular animal cell
ii. List three differences between prokaryotic and eukaryotic cells, give two examples of each.
iii. Give an example of a cell that live for (a) Less than 5 days (b) 10 days (c) 1 month (d) 1 year (e) 15 years (f) Life time
iv. What are the major components of the plasma membranes? List all other components of plasma membranes.
v. List 2 functions for each of the following organelles: (a) Nucleus (b) Mitochondria (c) Rough endoplasmic reticulum (d) Peroxisomes

7.0 References and other resources

Devlin T.M. (2010) Textbook of Biochemistry with Clinical Correlation 7th Edition. JohnWiley & Sons Inc.

UNIT THREE - FUNCTIONS OF THE CELL MEMBRANE AND THE ORGANELLES

CONTENT

1.0 Introduction

The cell membrane and the organelles carry out all the functions performed by living cells. The cell or plasma membrane can be referred to as 'the wall of a city' it protects the components of the cell and also regulates what enters or leaves the cell. The plasma membrane is very important to all cells; the cell owes its survival to intact and functional cell membrane. If there is injury to the cell membrane, the whole cell may be destroyed. The organelles are the various mini-cells found inside the cell, they help the cell to perform its diverse functions such as synthesis, storage, energy generation and excretion of waste materials. Most of them contain a separate membrane that encloses their individual contents. The presence of separate membrane prevents the destructive activities of degrading enzymes present in the lysosome. The pH of fluid in the lysosome is also different from the pH of the cytoplasmic fluid. The collective functions of the plasma membrane and the organelles are referred to as the cell functions.

2.0 Objectives

At the end of this unit, you should be able to:

i. Describe the organelles and give examples and explain the functions of each organelle
ii. Describe plasma membrane and list its functions
iii. List the factors that regulate the movement of materials across the membranes

3.0 Main Content

3.1 Types and Functions of Organelles

Organelles and their functions

i. Rough Endoplasmic Reticulum: Synthesis of protein (due to the presence of ribosomes) and degradation of worn out organelles.
ii. Smooth Endoplasmic Reticulum: Synthesis of lipids and steroids, storage and metabolism of calcium and detoxification of toxic substances.
iii. Golgi apparatus: Processing, packaging, labeling and delivery of proteins and lipids to thir destination within cell.

iv. Lysosome: Degradation of macromolecules, worn out organelles and the removal of excess secretory products. It has the thickest membrane to prevent the leakage of hydrolytic enzymes. It contains more than 40 different hydrolytic enzymes and they are collectively known as LYSOZYMES.

v. Peroxisomes: Detoxification of hydrogen peroxide and other radicals because it contains antioxidant enzymes such as peroxidases and catalases. Break down of excess fatty acids, degradation of purine to uric acid and formation of bile acids.

vi. Mitochondria: Production of energy in form of ATP (citric acid cycle), it is maternally inherited but absent in red blood cells and initiation of apoptosis.

vii. Ribosomes: Synthesis of proteins.

viii. Nucleus: It controls all the cellular activities, absent in matured red blood cells. DNA and RNA synthesis and storage of genetic information.

3.2 The functions of plasma membranes

i. Protection: The plasma membrane protects the cytoplasm and the organelles present in the cytoplasm. It is responsible for the maintenance of shape and size of cells. The most important function of cell membrane is transportation and regulation of materials across the membrane.

ii. Transportation of materials across the cell membrane: The cell membrane act as semi permeable membrane which allows only some substances to pass through it and act as a barrier for other substances. For example, small hydrophobic molecules such as CO_2, O_2 and small lipids dissolve in the membrane and pass through readily. Tiny polar molecules such as H_2O and alcohol can also slip between the phospholipids molecules. Ions and most nutrient molecules do not move freely through the membrane, but are often carried by the transport protein channels, either with or without the use of energy.

Gradients are important in moving materials through membranes both passively (without the use of energy by the cell) and actively (transport requiring cell energy).

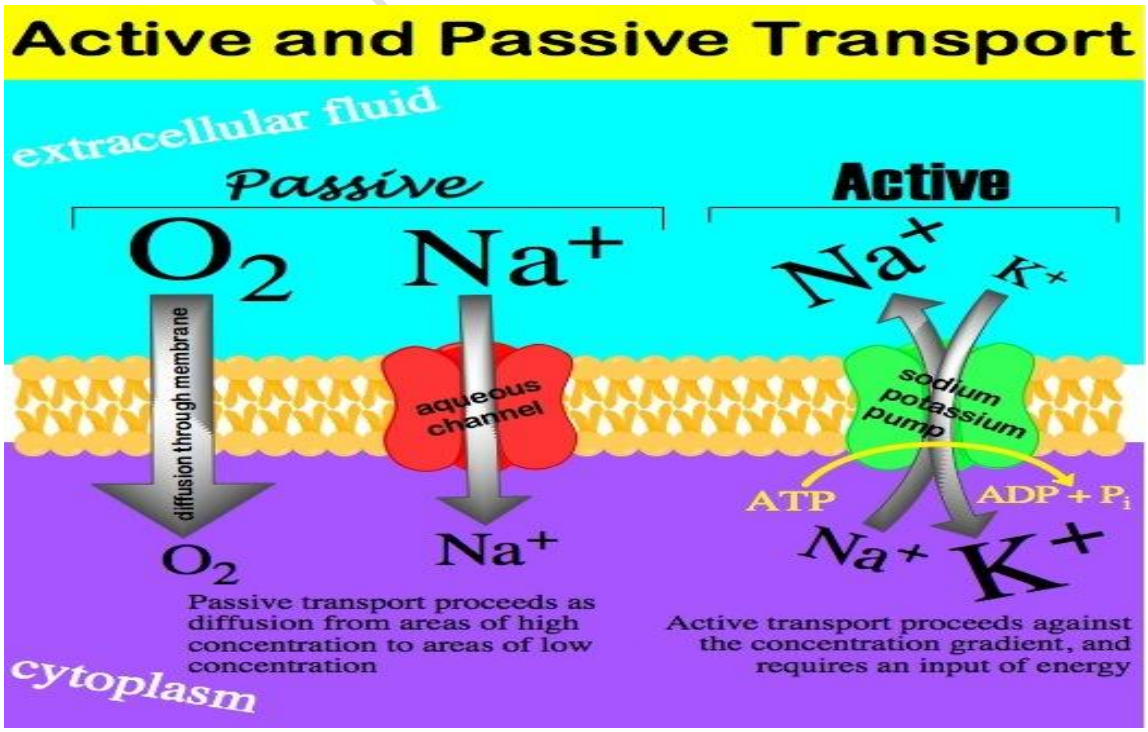


Fig 3.1:

Passive Transport - Passive transport in cells involves the process of diffusion, the diffusion can be simple or facilitated.

Simple diffusion – In terms of cellular activity, the rate of simple diffusion can be affected by temperature, molecular size, concentration of the gradient. Materials that are moved through membranes by simple diffusion include: water, carbon dioxide, oxygen, some lipid soluble molecules such as alcohol.

Facilitated diffusion – Most molecules cannot move freely through the membrane, but do cross membranes with the help of membrane transport proteins, which temporarily bind to the substance to be moved through the membrane, a process called facilitated diffusion or passive transport. No energy is involved in the process; both carrier proteins and channel proteins are involved in facilitated diffusion. Materials that pass through membranes by facilitated diffusion include glucose, amino acids and many small ions. The movement of water through membranes also involves facilitated diffusion, the special protin channel used for this is called *aquaporins*, and it facilitates the movement of water at a rate needed for cell activities.

Facilitated diffusion process may be coupled to the movement of other molecules in the same direction or opposite direction. In co-transport; the transport of one molecule depends on sequential transfer of another molecule. Co-transport may be symport or antiport. A symport moves two molecules in the same direction e.g. sodium-glucose transporter. Antiport system moves two molecules in opposite direction, it is also known as counter transport e.g. sodium-potassium transporter.

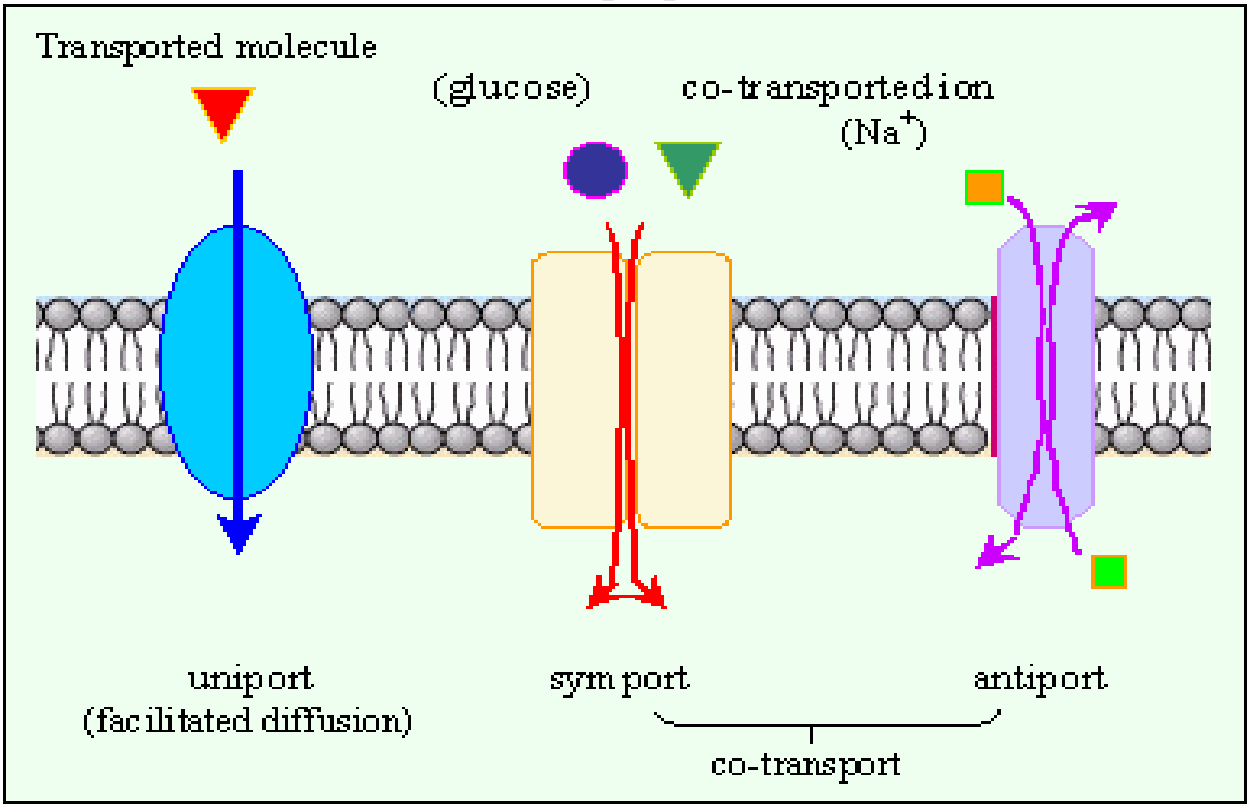


Fig 3.2:

Active transport - Energy requiring transport across membranes

All cells need to move some substances through membrane in a direction counter to the gradient or move substances that are too large or bulky with the use of cell energy. Cells have a number of ways to transport materials across the cell membrane with the use of energy. Some transport proteins (carrier proteins) can move substances through the membrane against the concentration gradient. Active transport typically requires two carrier protein active sites. One recognizes the substances to be carried while the other releases ATP to provide energy for the protein carrier. In some cases, concentration gradients of ions typically (H^+) protons or (Na^+) sodium ions can be used to provide the energy needed to move molecules through the membranes.

Active transport is classified into two types according to the source of energy used. Primary active transport derives its energy directly from the hydrolysis of ATP while the secondary active transport uses an indirect energy of an electrochemical gradient or membrane potential produced originally by primary active transport. An example of primary active transport is sodium-potassium pump (Na^+-K^+ ATPase). It is the protein or enzyme responsible for the transportation of Na^+ and K^+ across the cell membrane. The enzyme is known as sodium-potassium Adenosine triphosphatase.

The energy required for the transportation of sodium and potassium ions are derived from the hydrolysis of ATP. For every three Na^+ pumped out of the cell, two K^+ are released into the cytosol. Physiological importance of Na^+- K^+ gradient in animal cells are: the control of cell volume, it renders neurons and muscle cells electrically excitable and It drives the active transport of sugars and amino acids.

3.3 Endocytosis and Exocytosis

Vesicle mediated transport- Transportation of large substances may require changes in membrane shape and the fusion of the plasma membrane with vesicles containing the substances to be moved. Such changes in membranes occur throughout the life time of the cell. Movement of materials of the cell involving changes of the membrane and formation of vesicles is called exocytosis while the movement of materials into the cell is called endocytosis.

Exocytosis- Materials can be exported from the cell by fusing vesicles with the plasma membrane. For example, insulin made inside the cells of the pancreas is released to the blood stream by exocytosis.

Endocytosis- There is a variety of endocytosis processes in the cell, examples includes pinocytosis, receptor mediated endocytosis and phagocytosis.

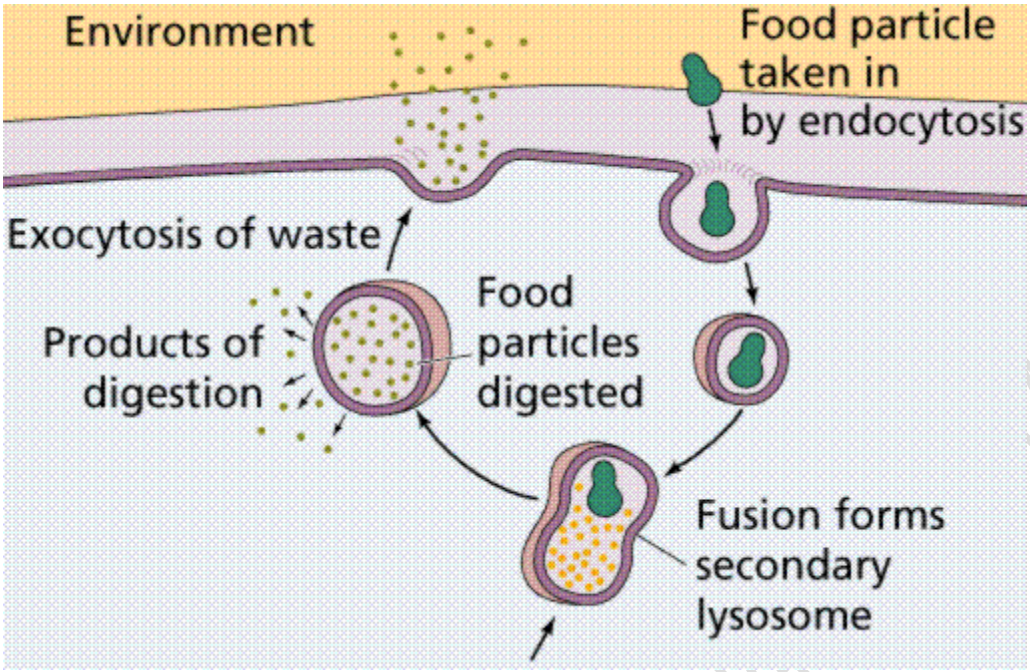


Fig 3.3:

4.0 Conclusion

Beyond the functions perform by the different organelles of the cell, the chemical structure of the plasma membrane explains the process of transportation across different gradients of the cell.

5.0 Summary

In this unit, you have learnt about the eight organelles in a cell with different functions perform. We have also related the structure of the cell membrane to the different forms of mechanism of transportation of material across different media with explanation of factors that drive such exchange.

6.0 Tutor Marked Assignments

6.1 **Activity** – See Laboratory manual

6.2 Answer the following questions**:**

i. List 4 organelles and explain their functions
ii. Explain the passive mechanism of transportation across the cell membrane, draw diagrams to illustrate your answers.
iii. Differentiate between the active and passive methods of transportation.
iv. Explain with a well labelled diagram the mechanism of endocytosis and exocytosis

7.0 References and other resources

Specific reading text in addition to the reference textbooks to be provided by the Facilitator

Module 2 - Chemistry of the Nutrients

Introduction

The nutrients that we take are made of chemicals combined in diverse proportions. The food that we eat must be broken down into simple forms that are absorbable by the body through the cell membranes. In this module, you are going to learn about the chemistry of sugar, the smaller form of carbohydrate, amino acids from proteins, water and other liquids. You are also going to learn about the chemical composition of DNA and RNA, the hereditary maps of the individual.

Module Objectives

At the end of this module, you should be able to discuss the following in details:

i. Chemistry of sugar
ii. Water, Acids, Bases and Buffer
iii. Chemistry of Amino Acids and Proteins
iv. Classification of Lipids
v. The structures of DNA and RNA

CONTENTS

UNIT ONE - CHEMISTRY OF SUGARS

CONTENT

1.0 Introduction

Biochemistry is concerned with the chemical components of the human body- their properties, functions, synthesis, transport and degradation. These are called Biomolecules Major classes of these molecules include carbohydrates, lipids, proteins and Nucleic acids. The building blocks of these compounds are the simple sugars, fatty acids, amino acids and mononucleotides. Carbohydrates (CHOs) are compounds containing C, H and O and having the general formula $C_nH_{2n}O_n$ Carbohydrates are widely distributed in plants and animals where they play important structural and metabolic roles. Glucose is the most important carbohydrate.

Most dietary CHO is absorbed into the bloodstream as glucose formed by the hydrolysis (breakdown) of dietary starch and disaccharides. Other sugars are also converted to glucose in the liver. Glucose is the major metabolic fuel of mammals and a universal fuel of fetus. It is the precursor for the synthesis of all other CHOs in the body, including glycogen for storage , ribose and deoxyribose in nucleic acids , galactose for synthesis of lactose in milk, in glycolipids and in combination with proteins in glycoproteins and proteoglycans. Diseases associated with CHO metabolism include Diabetes Mellitus, Galactosemia, Glycogen storage diseases and Lactose intolerance.

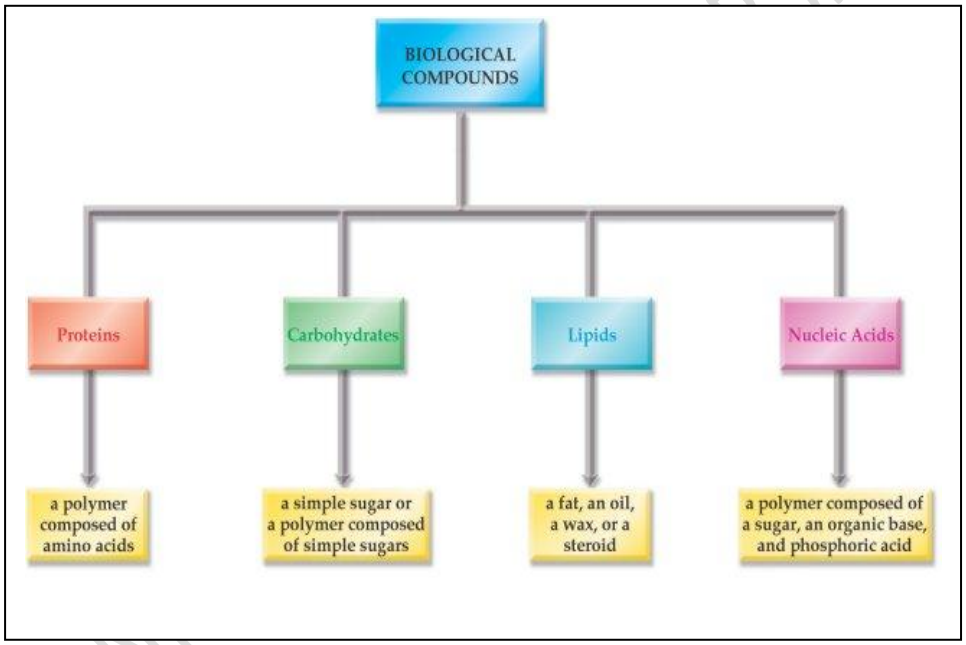


*Fig 1.1:*Biomolecules

2.0 Objectives

At the end of this unit, you should be able to:

i. Define the terms monosaccharide, disaccharide, oligosaccharide and polysaccharide.
ii. Explain the different ways in which the structures of glucose and other monosaccharides can be represented,

iii. Describe the occurrence of isomerism in sugars.

3.0 Main Content

3.1. Definition by Classification of Carbohydrates

Carbohydrates are classified according to the number of sugar units in the molecule as follows:

Monosaccharides: These are sugars that contain one sugar unit and thus cannot be further hydrolyzed. They represent the end product of CHO digestion in the human body. They may be classified as trioses, tetroses, pentoses, hexoses or heptoses, depending upon the no of C atoms, and as aldoses and ketoses depending on whether they have an aldehyde or ketone group.

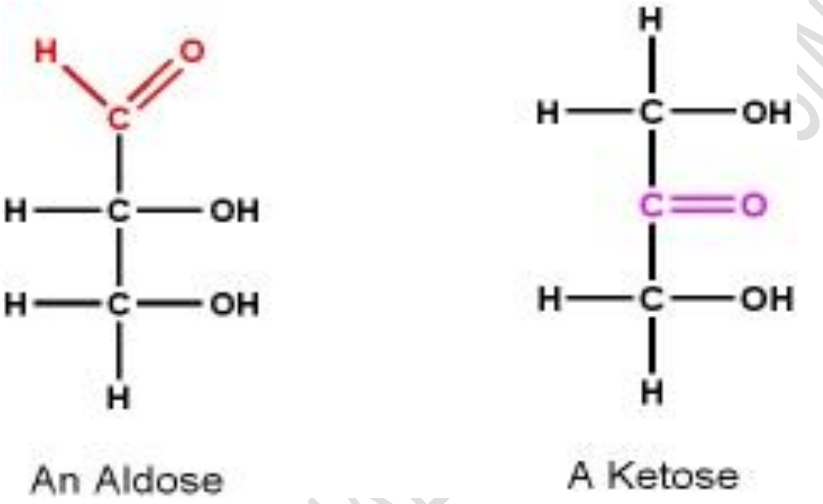


Fig 2.2 Aldose vs Ketose

Disaccharides : These are condensation products of 2 monosaccharide units e.g lactose (galactose + glucose) ,maltose (2 glucose) and sucrose (glucose + fructose)

Oligosaccharides: Condensation products of 3-10 monosaccharides. Most are not digested by

human enzymes. Rather, they play structural roles.

Polysaccharides: Condensation products of > 10 monosaccharide units. E.g starch and Dextrin which may be linear or branched polymers. Food also contains a wide variety of other polysaccharides, collectively known as non starch polysacchs, they are not digested by human enzymes and are the major components of dietary fibre Examples include Cellulose (a glucose polymer from plant cell walls) and Inulin (a fructose polymer which the storage CHO in some plants.

Table 2.1: Classification of Sugars

	Aldoses	**Ketoses**
Trioses ($C_3H_6O_3$)	Glyceraldehyde	Dihydroxyacetone
Tetroses ($C_4H_8O_4$)	Erythrose	Erythrulose
Pentoses ($C_5H_{10}O_5$)	Ribose	Ribulose
Hexoses ($C_6H_{12}O_6$)	Glucose	Fructose
Heptoses ($C_7H_{14}O_7$)	-	Sedoheptulose

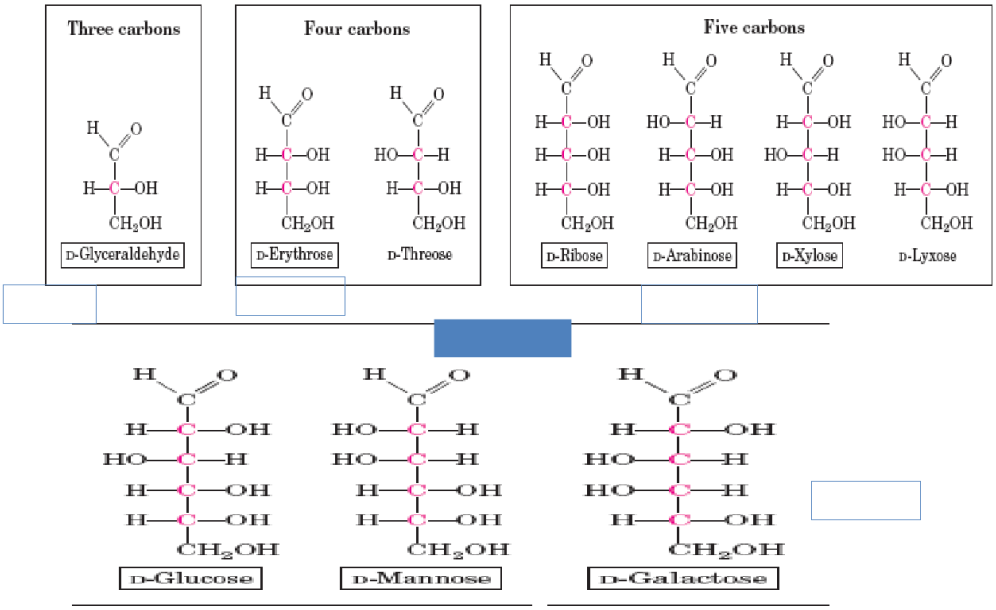
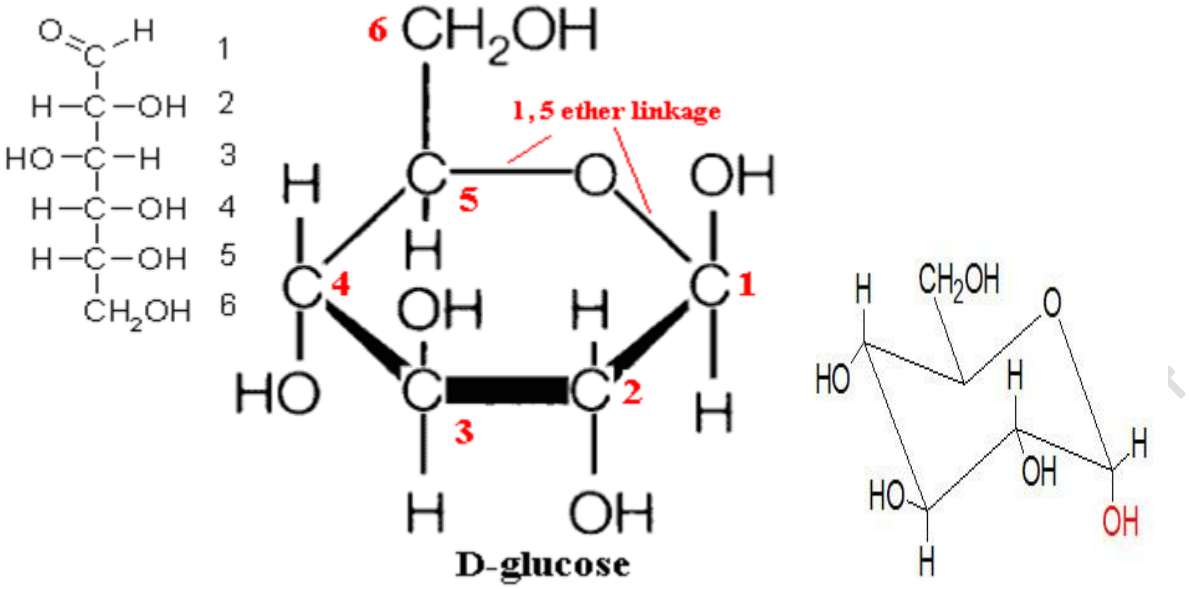


Fig 2.3: Sugars with different no of carbon atoms

Structure of Glucose

The straight chain structural formula of glucose can account for some of its properties, but a cyclic structure, formed by a reaction between the aldehyde group and an OH group is thermodynamically more favoured and accounts for other properties. The cyclic structure results from the reaction between the aldehyde group in C1 and the OH group in C5, forming a hemiacetal linkage and producing either of 2 stereoisomers. The structure can also be represented in form of a chair, with the 6 membered ring containing one oxygen atom.



Straight chain Haworth projection Chair form

Fig 2.4: Structures of Glucose

3.2 Isomers of Glucose

Isomerism is the occurrence of compounds with the same chemical formula but different structural formula. The important types of isomerism found in glucose are

D and L isomerism. The designation of a sugar isomer as the D form or of its mirror image as the L form is determined by its spatial relationship to the parent compound of the carbohydrates, glyceraldehydes. The orientation of the –H and –OH groups around the c atom adjacent to the terminal primary alcohol carbon determines whether the sugar belongs to the D or L series (when the –OH grp on this C is on the right, the sugar is the D isomer, when it is on the left, it is the L-isomer) Most of the naturally occurring monosaccharide are D sugars. The presence of asymmetric c atom also confers optical activity on the compound. When a beam of plane-polarized light is passed through a solution of an optical isomer, it rotates either to the right (dextrorotatory, +) or to the left ,(levorotatory -)

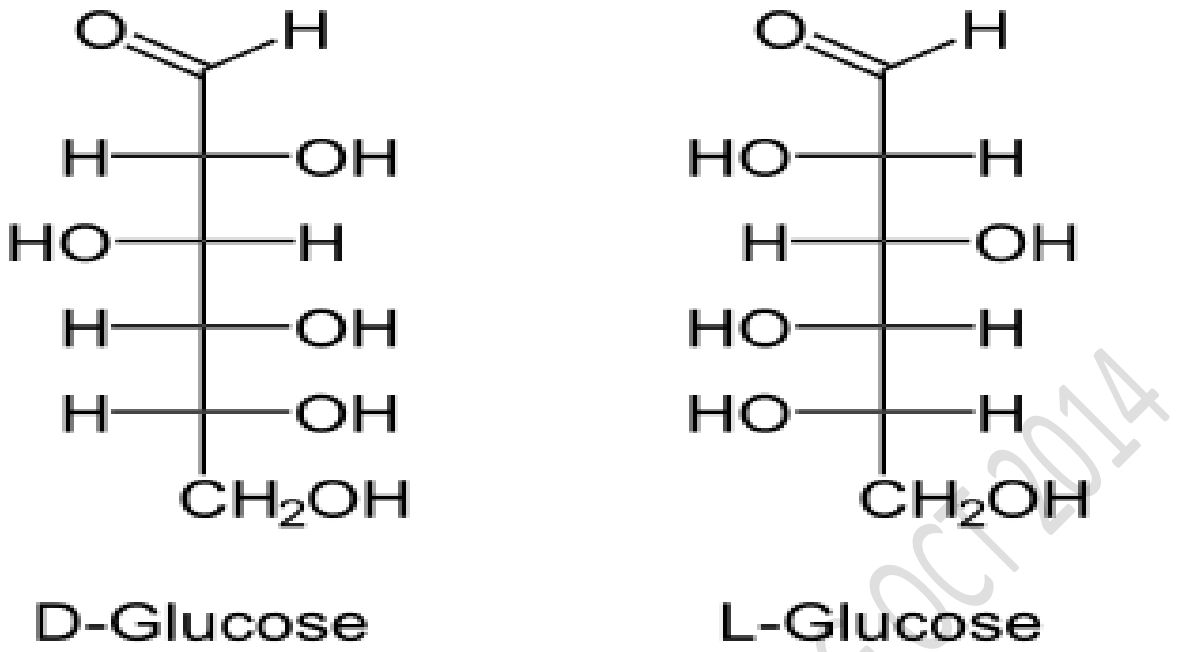


Fig 2.5: D & L forms of Glucose

Pyranose and Furanose ring structures: Most monosaccharide spontaneously form ring structures in which the aldehyde or keto group forms a bond with one of its OH groups. If the ring contains 5 atoms, it is called a furanose ring; if it contains 6 atoms, it is called a pyranose ring. For glucose in solution > 99% is in the pyranose form.

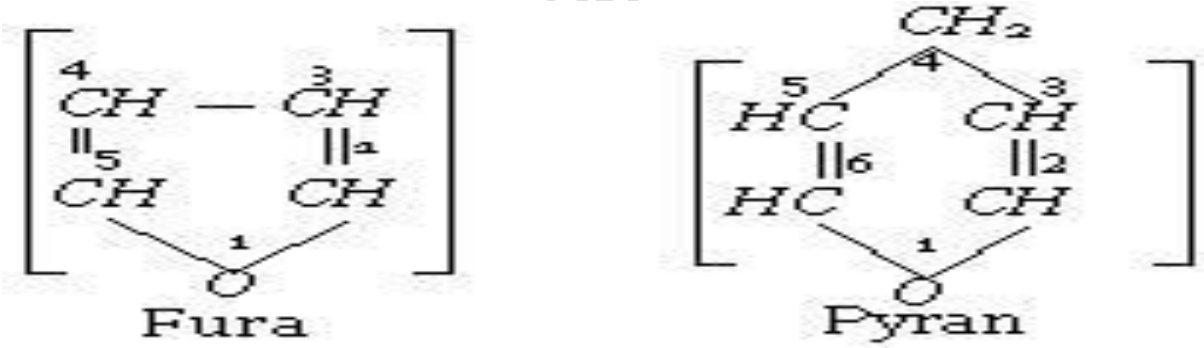


Fig 2.6: Furanose vs Pyranose rings

Alpha and Beta Anomers: Anomerism is the formation of rings that result in the creation of an assymetric carbon, called the anomeric carbon . If the –OH group on the anomeric carbon lies to the right of the carbon chain, it is considered to be in the α- configuration, and in β configuration if it lies to the left of the chain.(One of 2 stereoisomers of a cyclic saccharide that differs only in its configuration at the hemiacetal carbon , a hemiacetal is formed by combination of an aldehyde and an alcohol group).

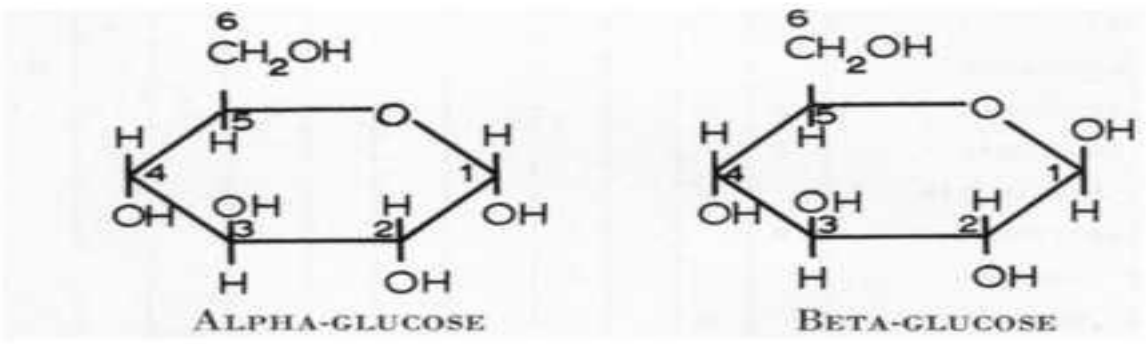


Fig 2.7: Anomeric forms of Glucose

Epimers: These are isomers differing as a result of variations in configurations of the –OH and –H on C atoms 2,3 and 4 of glucose. The most important biological isomers of glucose are mannose (C2) and galactose C4).

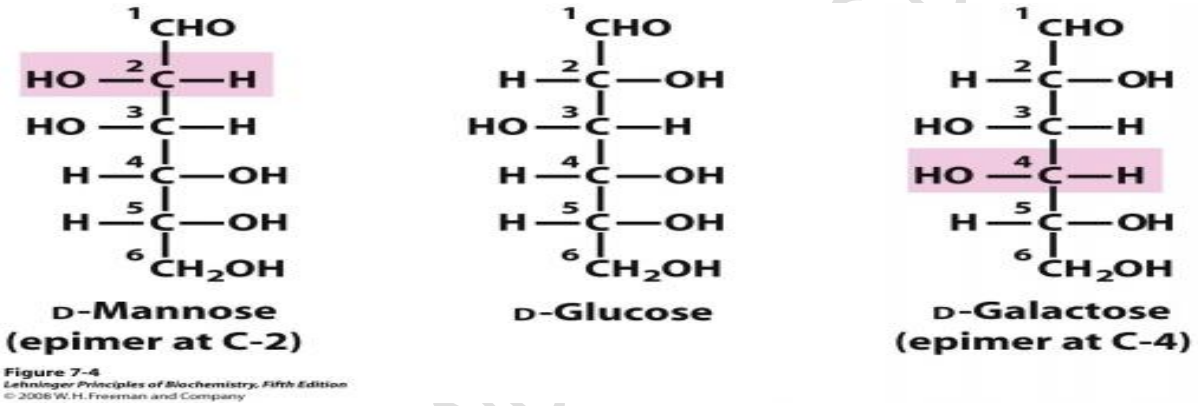


Fig 2.8: Epimers of Glucose

Aldose-Ketose Isomerism: Fructose has the same molecular formula as glucose but differs in its structure. Since it has a keto grp in position 2 whereas there is an aldehyde group in position 1 of glucose.

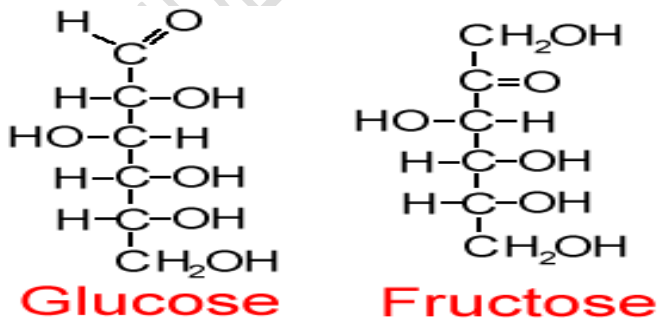


Fig 2.9:

4.0 Summary

In this unit, you have learnt about the following:

i. The meaning of the terms monosaccharide, disaccharide, oligosaccharide and polysaccharide.
ii. The different ways in which the structures of glucose (the most important carbohydrate) and other monosaccharides can be represented.
iii. The types of isomerism found in sugars.

5.0 Tutor Marked Assignments

6.1 Activity – draw the chemical structures of the different forms of glucose over and over again until you become conversant with the chemical composition

6.2 Answer the following questions:

i. Explain the types of isomerism found in glucose
ii. Give 2 examples each of Monosaccharides, Oligosaccharides and Polysaccharides found in nature.

7.0 References and other resources

1. Murray, R.K., Bender, D.A., Botham, K., M., Kennelly, P.J., Rodwell V.W. and Well, P.A., (2012). Harper's Illustrated Biochemistry (29th Edition) McGraw-Hill Medical
2. Devlin T.M. (2010) Textbook of Biochemistry with Clinical Correlation 7th Edition. JohnWiley & Sons Inc.
3. Nelson, D.L. and Cox M.M., (2009) Lehninger Principles of Biochemistry 4th edition

UNIT TWO - CHEMISTRY OF SUGARS (II)

CONTENT

1.0 Introduction

The previous study session has provided information on the basic chemistry of different types of Sugars. In this session, you will learn more about these sugars and their physiological relevance. You will also get to know about some derivatives of these sugars and their functions.

2.0 Objectives

At the end of this unit, you will be able to:

i. State the composition and give examples of disaccharides, oligosaccharides and polysaccharides found in Biological systems.
ii. Describe the roles of carbohydrates in cell membranes and lipoproteins.
iii. Carbohydrates in Biological systems

3.0 Main Content

3.1 Glucose, Galactose, Fructose and Mannose

Glucose, galactose, fructose and mannose are physiologically the most important hexoses. Pentoses are important in nucleotides, nucleic acids and several coenzymes. Derivatives of trioses, tetroses, and pentoses are also formed as metabolic intermediates in some pathways of CHO metabolism (glycolysis and pentose phosphate pathway).Carboxylic acid derivatives of glucose are also important. They include D-glucuronate, L-iduronate and L-gulonate. Deoxy sugars are those in which one OH group has been replaced by H e.g deoxyribose in DNA. Amino sugars include D-glucosamine, D-galactosamine and D-mannosamine.

Physiologically important disaccharides include maltose, sucrose and Lactose. Starch and glycogen are storage polymers of glucose in plants and animals respectively. Starch is the major source of energy in the diet.It forms an α-glucosidic chain, called a glucosan or glucan. The 2 main constituents are amylose (13-20%), which has a non-branching helical structure , and amylopectin (80-87%) which consists of branched chains composed of 24-30 glucose residues with α1→4 linkages in the chains and by α1→6 linkages at the branch points. The glycemic index of a starchy food is a measure of its digestibility, based on the extent to which it raises the blood concentration of glucose compared with an equivalent amount of glucose.

Table 2.1:1 Disaccharides

Sugar	**Composition**	**Source**
Sucrose	Glucose , Fructose	Cane sugar, sorghum, some fruits and vegetables
Lactose	Galactose, Glucose	Milk
Maltose	2 Glucose units(in α-1→4 linkage)	Enzymatic hydrolysis of starch
Lactulose	Galactose, Fructose	Heated Milk
Trehalose	2 Glucose units (in α-1→1 linkage)	

Table 2.1: 2 Pentoses

Sugar	**Source**
D-Ribose	Nucleic acids and metabolic intermediate
D- Ribulose	Metabolic intermediate
D- Arabinose	Plant gums
D- Xylose	Plant gums, Proteoglycans
L-Xylulose	Metabolic intermediate

Table 2.1:3 Hexoses

Sugar	Source	Biochemical importance
D-Glucose	Cane sugar, Fruit juices, starch, maltose and Lactose	Main metabolic fuel
D-Fructose	Fruit juices, honey, Inulin	Readily metabolized via glucose
D-Galactose	Lactose	Readily metabolized to glucose. Synthesized in mammary gland for synthesis of lactose
D-Mannose	Plant gums	Constituent of glycoproteins

3.2 Roles of Carbohydrates in cell membranes

Glycogen is the storage polysaccharide in animals. It is a more highly branched structure than amylopectin. Muscle glycogen granules are made of up to 60,000 glucose residues. Inulin is a polysaccharide of fructose while dextrins are intermediates in the hydrolysis of starch. **Cellulose** is the chief constituent of plant cell walls. It is insoluble and consists of of glucopyranose units linked by β 1→4 bonds to form long, straight chains strengthened by cross-linking H bonds. Mammals lack any enzyme that hydrolyzes the β 1→4 bonds, and so cannot digest cellulose. It is an important source of bulk in the diet, and the major component of dietary fibre. **Chitin** is a structural polysaccharide in the exoskeleton of crustaceans and insects and also in mushrooms. **Glycosaminoglycans** are complex CHOs containing amino sugars and uronic acids. They may be attached to a protein molecule to form a proteoglycan. **Proteoglycans** provide the ground or packing substance of connective tissue Examples include hyaluronic acid, chondroitin sulphate and heparin. **Glycoproteins** (mucoproteins) are ptoteins containing branched or unbranched oligosacc chains. They occur in cell membranes and include serum albumin. Approximately 5% of the weight of cell membranes is CHO in glycoproteins and glycolipids.

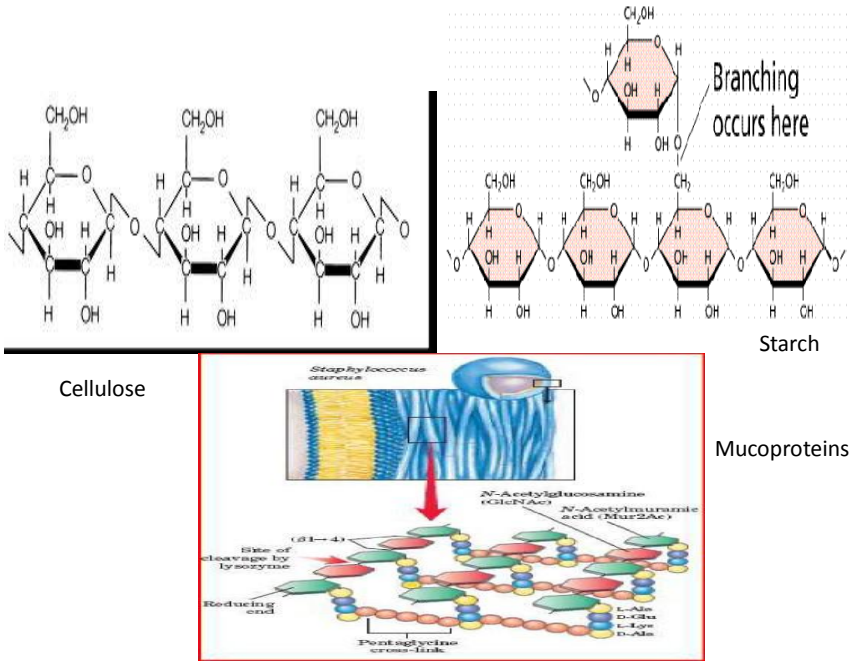


Fig 2.1: Structures of cellulose, starch and Mucoproteins

5.0 Summary

In this unit, you have learnt about distinguishing between Glucose, Galactose, Fructose and Mannose. You have also learnt about the roles of Carbohydrates in cell membranes.

6.0 Tutor Marked Assignments

6.1 Activity

6.2 Answer the following questions:

i. Using structures only, distinguish the 3 epimers of glucose.
ii. State the components of the following sugars and the types of linkage: Sucrose,Lactulose, Trehalose and Maltose.
iii. Draw a table showing the generic name, ketose and aldose forms of sugars with 3,4, 5 and 6 carbon atoms.

7.0 References and other resources

1. Murray, R.K., Bender, D.A., Botham, K., M., Kennelly, P.J., Rodwell V.W. and Well, P.A., (2012). Harper's Illustrated Biochemistry (29th Edition) McGraw-Hill Medical
2. Devlin T.M. (2010) Textbook of Biochemistry with Clinical Correlation 7th Edition. JohnWiley & Sons Inc.
3. Nelson, D.L. and Cox M.M., (2009) Lehninger Principles of Biochemistry 4th edition

UNIT THREE - WATER, ACIDS, BASES AND BUFFER

CONTENT

1.0 Introduction

Water is the most abundant matter on earth and also in all living creatures. Typically, organisms are constituted of 70 to 90 % water. Water must be present before any metabolic activity can take place in the cell. It is referred to as a weak electrolyte because it can undergo partial dissociation to two ions made up of a proton (H^+) and hydroxyl ion (OH^-).

Acids and Bases are defined with respect to their ability to gain or loss protons. Buffer is a solution that resists change in the pH of a solution when proton or hydroxyl ions are suddenly

added. Concentration of these ions determines the degree of acidity or alkalinity of solutions, including body fluids.

2.0 Objectives

At the end of this unit, you should be able to:

i. Explain the properties of water
ii. Describe the importance of water as the major component of living organisms.
iii. Define acid, base and buffer
iv. Calculate the pH, pOH and pKa of a given solution
v. Explain the biological importance of buffer

3.0 Main Content

3.1 The properties of Water

i. Water is the predominant chemical component of all living organisms.
ii. Most chemical reactions in the cell take place in aqueous environment.
iii. Hydrogen bonds hold the oxygen and hydrogen atoms together in a water molecule.
iv. The oxygen of water is very electronegative, while hydrogen is electropositive, as a result water is dipolar and exhibit slight tendency to dissociate.

3.2 Biological importance of water

i. A molecule with electrical charge distributed unequally about its structure is referred to as a dipole. $H_3O = H^+ + OH^-$
ii. The strong dipole and high dielectric constant of water enables it to dissolve large quantities of charged compounds.
iii. Presence of hydrogen bond also enables water to dissolve many organic molecules that contain functional groups.
iv. Water provide environment for macromolecules to achieve stable structure in solution

3.3 Acid, Base and Buffer

Acid is a compound that dissociates in aqueous solution to produce proton (H^+) and a conjugate base (A^-). $HA = H + A$

i. Acid may dissociate partially (weak acid) or completely (strong acid) in solution. In solution, weak acid establishes equilibrum between the proton and its conjugate base.
ii. The equilibrum constant is called the acid dissociation constant (Ka) where K is the constant and a is the acid.

$$Ka = = \frac{[H^+][A^-]}{[HA]}$$

iii. Base is a compound that accepts proton in aqueous environment, for example ammonia reacts with a proton to produce an ammonium ion.

Calculation of pH, pOH and pKa

i. The pH of a solution is simply defined as the negative logarithm of hydrogen ions concentration, pH = - log [H+] and
    a. pOH = - log [OH-].

Example 1: If the H+ concentration of a solution is 4.2 x 10-3 calculate the pH of the solution.

Solution:
pH = - log [H+] , log[4.2 x 10-3] = log 4.2 + log 10-3 = 0.62 -3 = -2.38.
Substitute for log [H+] in the equation.
pH = -(-2.38), the two negative values canceled out, pH = 2.38

Example 2: Calculate the [H+], [OH-] and pH of 0.01M ethanoic acid, given that (Ka = 1.76 x 10-5).

Solution:
Note that ethanoic acid is a weak acid, it dissociates partially in solution, therefore HA = H+ + A- , if the conjugates are represented by x, then HA = 0.1- x ≈ 0.1(value of x is negligible)

$$Ka = \frac{[H+][A-]}{[HA]}$$

Ka = 1.76 x 10-5 = x2/0.1
x2 = 1.76 x 10-6 , x is equal to the square root of 1.76 x 10-6
this is equal to 1.33 x 10-3, therefore,
[H+] = 1.33 x 10-3

pH = -log 1.33 x 10-3 = 0.12-3, if you take away 3 from 0.12, this will give you a negative value (-2.88).
pH = 2.88 .

To calculate the pOH, the dissociation of pure water will be considered. H3O = H+ + OH-,
[H+] + [OH-] = 1.0 x10-14,
[OH-] = 1.0 x 10-14/ [H+]
= 1.0 x 10-14/1.33 x 10-3,
[OH-] =7.52 x 10-12.
But pOH = -log 7.52 x 10-12
This is equal to 0.88 – 12,
pOH = 11.12

Buffer is a solution that resists change in pH when acid or base is added. When either acid or base is added, there will be temporary change in pH, but the pH is quickly restored. A buffer contains a weak acid and its conjugate base. Examples of buffer solutions are: Acetate buffer (acetic acid and acetate salt), bicarbonate buffer (carbonic acid and bicarbonate salt).

i. How buffers regulate the pH of solution

If hydrogen ions are added to a buffer solution, the conjugate base react with the excess hydrogen ions to form the acid.

If OH- ions are added, they react with the acid present in the buffer to produce water and conjugate base.

ii. Preparation of buffer

To prepare buffer, Henderson Hasselbalch equation is used to calculate the concentrations of acid and base components.

The equation is $pH = pKa + \log \frac{[A^-]}{[HA]}$

Biological importance of buffer

i. Body fluids such as blood, cerebrospinal fluid, saliva etc. have constant pH under normal physiological conditions.
ii. This is possible due to the presence of buffer in these fluids. Hydrogen and hydroxyl ions are constantly added to the body fluids as products of metabolism.
iii. Approximate pH of some body fluids are : blood - 7.4, cerebrospinal fluid - 7.30 to 7.50, saliva – 6.5 to 7.5.

4.0 Conclusion

Water is life and a core component of all other fluids that make chemical reactions possible in the body. Fluid of different chemical media can be prepared at different concentrations. Acids, bases and buffers serve difference purposes in the body.

5.0 Summary

In study unit, you have learnt about the properties of Water, the biological importance of water and the structures of acid, base and buffers.

6.0 Tutor Marked Assignments

6.1 Activity – See Laboratory practical manual

6.2 Answer the following questions

i. What are the qualities that make water a universal solvent
ii. Define acid and base
iii. Define buffer and explain how they regulate the pH of solution
iv. Calculate the pH of a solution of weak acid whose Molarity is 0.0008.
v. Calculate the [H+], [OH-] and pH of 2.5 x 10-3 M ethanoic acid, given that (Ka = 1.48 x 10-5).
vi. State the Henderson Hasselbalch equation and its importance

7.0 References and other resources

1. Murray, R.K., Bender, D.A., Botham, K., M., Kennelly, P.J., Rodwell V.W. and Well, P.A., (2012). Harper's Illustrated Biochemistry (29th Edition) McGraw-Hill Medical
2. Devlin T.M. (2010) Textbook of Biochemistry with Clinical Correlation 7th Edition. JohnWiley & Sons Inc.
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UNIT FOUR - CHEMISTRY OF AMINO ACIDS AND PROTEINS 1

CONTENT

1.0 Introduction

Amino acids are the basic structural units of Proteins. Proteins in all species from bacteria to humans are constructed from the same set of twenty amino acids. Outside the formation of proteins, amino acids are involved in neurotransmitter transport and biosynthesis. An amino acid consists of amino group (NH2), a carboxylic group (-COOH), a hydrogen atom (H) and a distinctive R group which is specific to each amino acid. These 3 groups are bonded to a carbon atom, called the α- carbon. Amino acids take part in many types of reactions, but the most important of these is the formation of a peptide bond. This involves the joining of the α-carboxyl group of one amino acid to the α- amino group of another amino acid, with the loss of a water molecule. Amino acids are grouped according to the nature of their side chains .Since amino acids are weak acids, their strength is expressed as pKa(negative log of ionization constant). The net charge on an amino acid depends on the pKa of its functional groups and the pH of the surrounding medium. The isoelectric pH, also called pI is the pH midway between pKa values on either side of the isoionic species.

2.0 Objectives

At the end of this unit, you should be able to:

i. Describe the general structure of α- amino acids
ii. Identify the 20 amino acids which make up proteins
iii. Explain the relationship between R groups and the chemical properties of amino acids.
iv. Calculate the pI of a monoamino monocarboxylic amino acid
v. Describe how peptide bonds are formed and peptides named.

3.0 Main Content

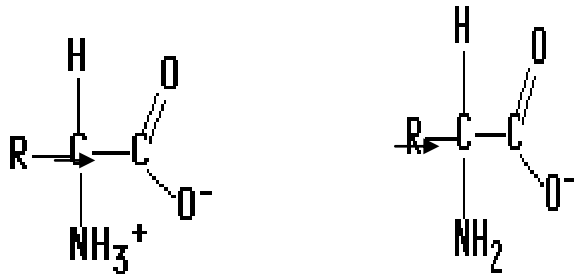
3.1 Chemical nature of amino acids

Amino acids are the basic structural units of proteins. An amino acid consists of amino group, a carboxyl group, a hydrogen atom and a distinctive R group bonded to a carbon atom, called the α- carbon. An R group is referred to as a side chain.

***Fig 1.1a* Amino acid structure**

Amino acids in solution at neutral pH are predominantly dipolar ions (or Zwitterions) rather than unionized molecules. In the dipolar form, the amino group is protonated (-NH3+) while the carboxyl group is dissociated (-COO-). The ionization state of an amino acid varies with pH. In acid solution e.g at pH 1, the carboxyl group is un-ionized (-COOH) and the amino group is ionized (-NH3+). In alkaline solution, the carboxyl group is ionized (-COO-) and the amino group is un-ionized (-NH2). At physiologic pH, carboxyl groups exist almost entirely as -COO- and amino groups predominantly as –-NH3+.

Fig 1.1b An amino acid in its uncharged and Dipolar forms.



pH 1 pH7 pH 11

Fig 1.1 c Ionization states of an amino acid as a function of pH

Twenty kinds of side chains varying in size, shape, charge, hydrogen bonding capacity and chemical reactivity are commonly found in proteins.. All proteins in all species from bacteria to humans are constructed from the same set of twenty amino acids. The different range of functions mediated by proteins results from the diversity and versatility of these twenty kinds of building blocks. The arrangement of four different groups about the α- carbon confers optical activity on amino acids. The 2 mirror images are called the L-isomer and the D-isomer. However, only L-amino acids are constituents of proteins. D- amino acids that occur naturally include free D-serine and D- aspartate in brain tissue, D-alanine and D-glutamate in the cell walls of gram + ve bacteria and D-amino acids in some non-mammalian peptides and certain anti biotics. Many proteins also contain derived amino acids , which are usually formed by enzymatic modification of an amino acid after it has been incorporated into a protein e.g collage n contains hdroxyproline (the –OH group serves to stabilize the collagen fibre), pro thrombin, a clotting protein contains γ-carboxyglutamate. Defective carboxylation of glutamate in this protein may lead to haemorrhage. The action of some hormones is also mediated by phosphorylation and dephosphorylation of specific serine residues in a variety of proteins.

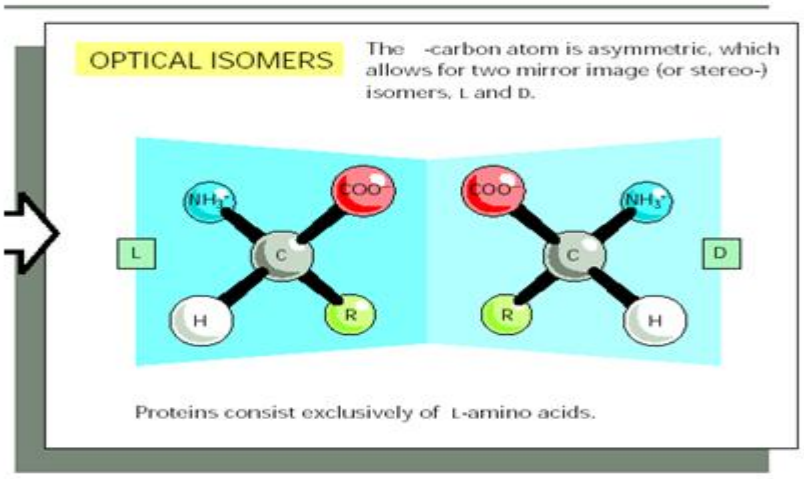


Fig 1.2 a Absolute configurations of the L- and D- isomers of amino acids.

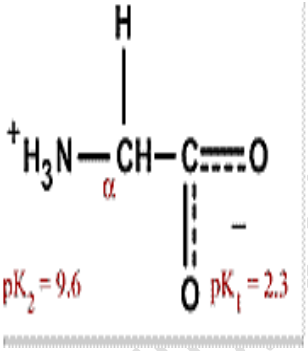
3.2 The 20 amino acids found in proteins

The simplest amino acid is glycine, which contains a hydrogen atom as side chain. It is the only non-chiral amino acid. Alanine has a methyl group as its side chain. Others that have hydrocarbon side chains include valine, leucine, isoleucine and proline (proline contains a secondary rather than a primary a7ino group and can be referred to as an imino acid .

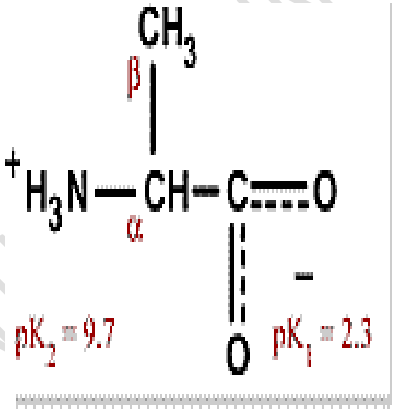
Serine and threonine contain aliphatic hydroxyl groups. Aromatic amino acids include Phe, Tyr and Trp. The side chains of these amino acids are uncharged at physiologic pH. Lysine and Arginine contain + vely charged side chains at neutral pH, while His is either +vely charged or neutral depending on its local environment. These 3 are called basic amino acids. Glu, Asp are called acidic a.as and are –vely charged at physiol. pH. The uncharged derivatives of Glu and asp are glutamine and asparagine. They contain a terminal amide group rather than a carboxylate. Two amino acids with sulfur containing side chains are methionine and cysteine. Cys plays a special role in some proteins by forming disulfide cross-links.

Table 1.2: The 20 Amino acids

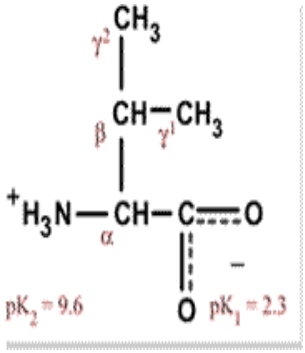
Amino acid	Three –letter abbreviation	One-letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V



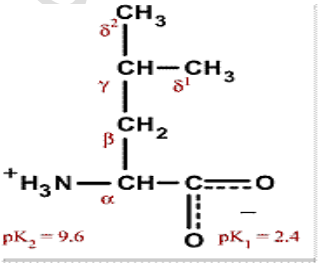
Gly



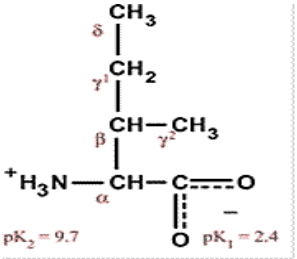
Ala



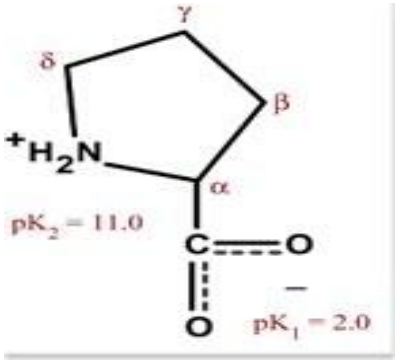
Val



Leu

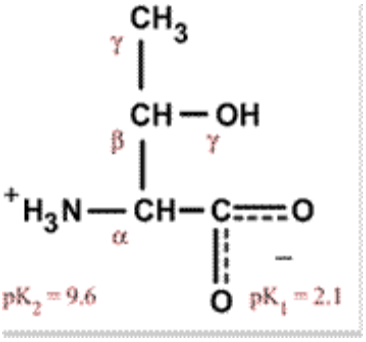


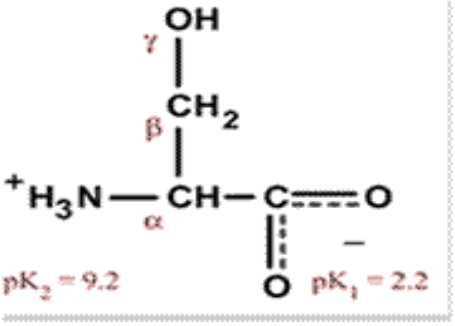
Ileu



Pro

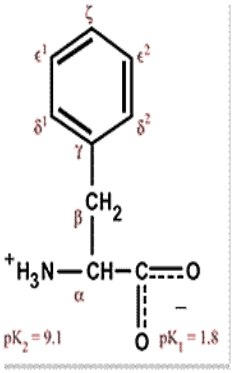
Amino acids with Hydrocarbon side chains

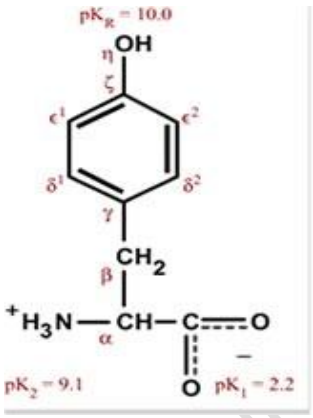


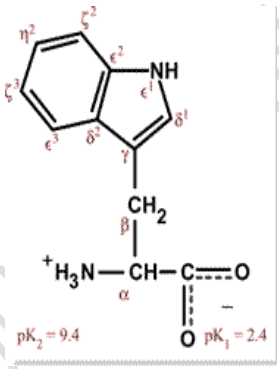


Ser Thr

Amino acids with side chains containing hydroxyl (OH) groups

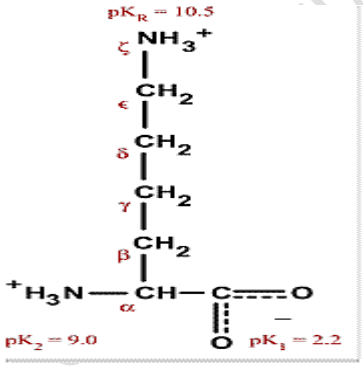


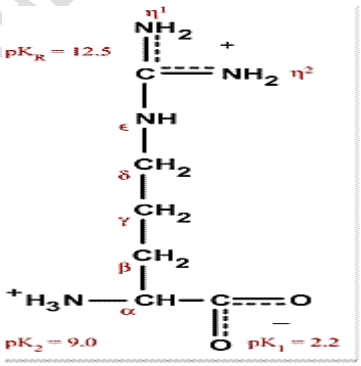


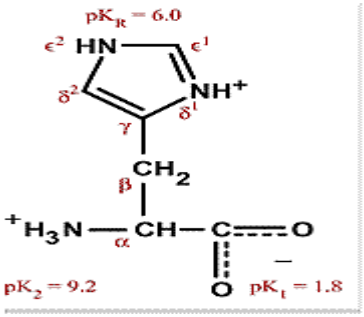


Phe Tyr Trp

Amino acids with aromatic side chains

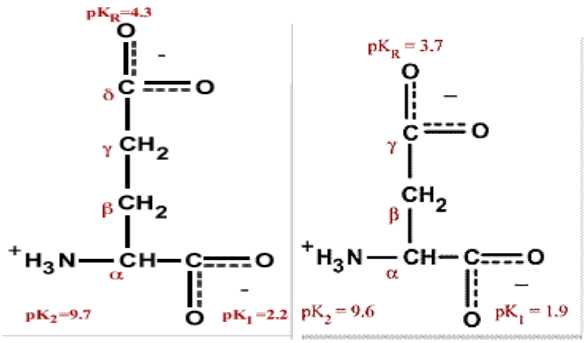






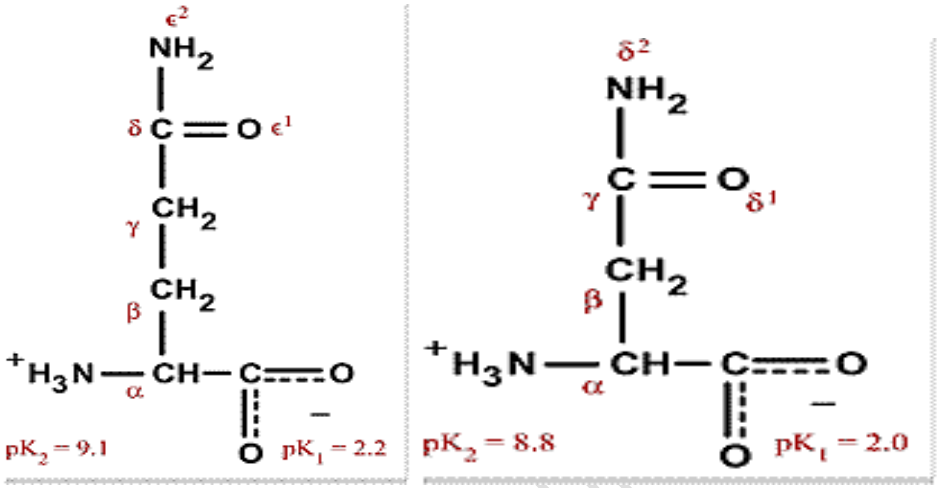
Lys Arg His

Basic Amino acids



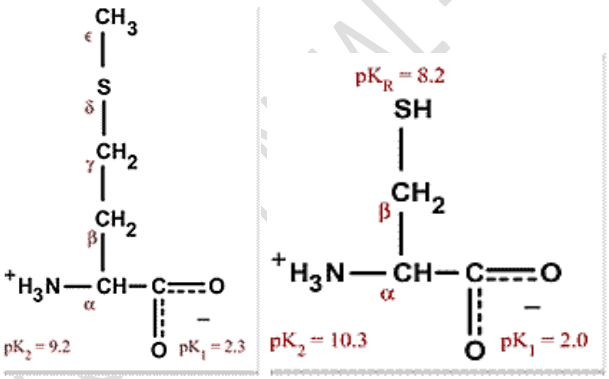
Glu Asp

Acidic amino acids



Gln Asn

Amino acids with carboxamide groups



Met Cys

Sulphur-containing amino acids.

Fig *1.3:* Structures of Amino acids

3.3 R Groups and the chemical properties of amino acids.

The charged functional groups of some amino acids ensure that they are readily soluble in polar solvents such as water and ethanol but insoluble in nonpolar solvents such as benzene or ether. Similarly, the high amount of energy required to disrupt the ionic forces that

stabilize the crystal lattice account for the high melting poits of amino acids. Amino acids do not absorb visible light and are therefore colourless. However, Tyr,Phe and Trp absorb high wavelength UV light. They contribute majorly to the ability of most proteins to absorb light in the region of 280nm. The respective R groups of amino acids determine their properties e.g the hydrophobic R groups of Ala, Val, Leu and Ileu as well as the aromatic R groups of Phe, Tyr and Trp typically occur in the interior of cytosolic proteins. The charged R groups of basic and acidic amino acids stabilize specific protein conformation via ionic interactions or salt bonds. Each functional group of an amino acid exhibits all its characteristic chemical reactions. For carboxylic acid groups, such reactions include formation of esters, amides and acid anhydrides while those for amino groups include acylation, amidation and esterification.

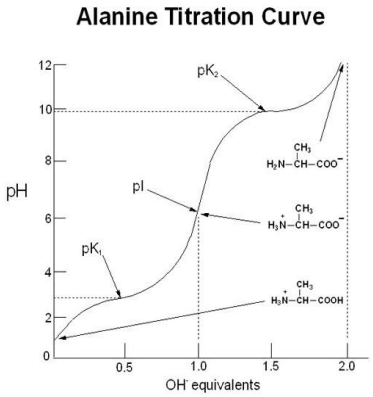
3.4 pI of Amino acids

The net charge on an amino acid i.e the sum of all the +vely and –vely charged groups present depend on the pK values of its functional groups and the PH of the surrounding medium. Altering the charge on amino acids and their derivatives by varying the pH facilitates the physical separation of amino acids, peptides and proteins. The iso electric pH , called the pI is the pH midway between pK values on either side of the isoelectric species. For an amino acid such as alanine that has only 2 ionizable groups, the isoelectric pH is
pI= Pk1 + pK2/2= 2.35+ 9.69/2= 6.02

For polyfunctional acids, pI is the pH midway between the pka values on either side of the isoionic species e.g for aspartic acid, pI = 2.09+ 3.96/2, = 3.20

Table 1.3: pK values of some amino acids

	pK values at 25^{o}C		
Amino acid	A-COOH group	α-NH^{3+} group	Side Chain
Valine	2.3	9.6	
Aspartic acid	2.0	10.0	3.9
Glutamic acid	2.2	9.7	4.3
Histidine	1.8	9.2	6.0
Cysteine	1.8	10.8	8.3
Tyrosine	2.2	9.1	10.9
Lysine	2.2	9.2	10.8
Arginine	1.8	9.0	12.5



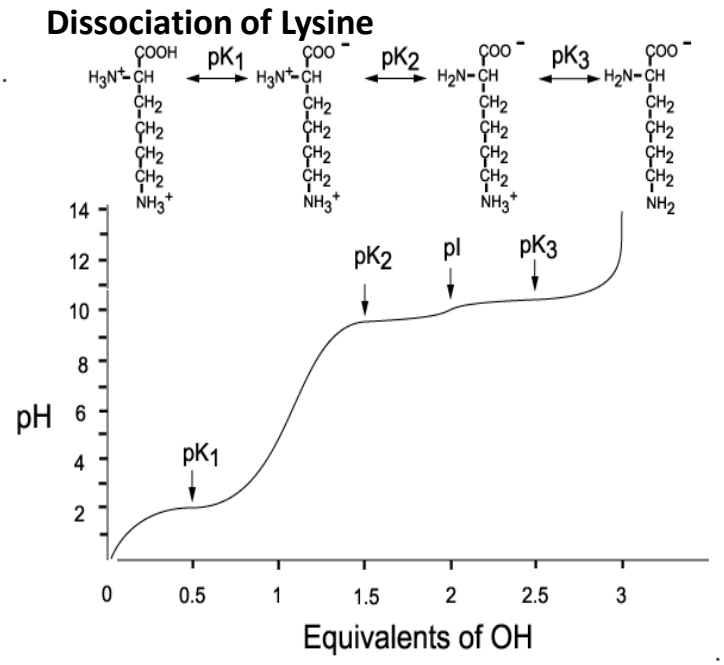


Fig 1.4: Determination of isoelectric point of Ala and Lys

3.5 Formation of peptide bonds

The most important reaction of amino acids is the formation of a peptide bond. This involves the joining of the α- carboxyl group of one amino acid to the α- amino group of another amino acid, with resultant loss of a water molecule. The biosynthesis of peptide bonds requires an input of free energy, whereas their hydrolysis is thermodynamically favourable. Many amino acids (usually > 100) are joined by peptide bonds to form a polypeptide chain

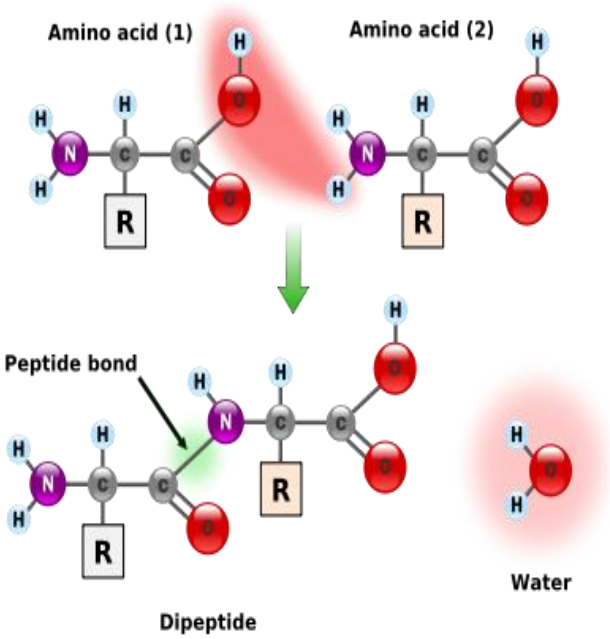


Fig 1.5: **Formation of peptide bond**

An amino acid unit in a polypeptide is called a residue. A PP chain has different ends- the α-amino and the α- carboxyl groups. The amino end is taken to be the beginning of the chain e.g in the tripeptide ala-gly-trp, alanine is the amino-terminal residue and trp is the carboxyl terminal residue. A PP chain consists of a regularly repeating part, called the main chain (also called the backbone), and a variable part comprising the distinctive side chains. In some proteins, a few side chains are cross-linked by disulfide bonds. These cross links are formed by the oxidation of cysteine residues. The resulting disulfide is called cystine. Amino acids present in peptides are called aminoacyl residues and are named by replacing the-ate or –ine suffix of free a.as with ---yl e.g aspartyl, alanyl etc. Peptides are then named as derivatives of the carboxyl terminal aminoacyl residue e.g Lys-Leu-Tyr-Gln is called Lysyl-Leucyl-Tyrosyl-Glutamine. This indicates that the α- carboxyl group of glutamine is not involved in peptide bond formation. Many proteins consist of a single polypeptide chain e.g myoglobin. Others contain 2 or more chains which may be either identical or different e.g Hemoglobin is made up of 2 chains of one kind and 2 of another kind of pp chain (α and β) , held together by non covalent forces. The PP chains of some multichain proteins are linked by disulfide bonds e.g insulin.

5.0 Summary

In this unit, you have learnt about the chemical nature of amino acids, the 20 amino acids found in proteins, the R Groups and the chemical properties of amino acids, the pI of Amino acids as well as formation of peptide bonds

6.0 Tutor Marked Assignments

6.1 Activity – As specified in the manual

6.2 Answer the following questions

i. Describe the general structure of α- amino acids
ii. Identify the 20 amino acids which make up proteins
iii. Explain the relationship between R groups and the chemical properties of amino acids.
iv. Calculate the pI of a monoamino monocarboxylic amino acid
v. Describe how peptide bonds are formed and peptides named

7.0 References and other resources

1. Murray, R.K., Bender, D.A., Botham, K., M., Kennelly, P.J., Rodwell V.W. and Well, P.A., (2012). Harper's Illustrated Biochemistry (29th Edition) McGraw-Hill Medical
2. Devlin T.M. (2010) Textbook of Biochemistry with Clinical Correlation 7th Edition. JohnWiley & Sons Inc.
3. Nelson, D.L. and Cox M.M., (2009) Lehninger Principles of Biochemistry 4th edition

UNIT FIVE - CHEMISTRY OF AMINO ACIDS AND PROTEINS (II)

CONTENT

1.0 Introduction

We continue with our study of Amino acids and proteins. In this session, you will learn the various methods of determining the amino acid components of peptides and proteins and the principles on which these methods are based. You will also learn how amino acids in a given sample can be quantified. A man called Fredrick Sanger was the first scientist to determine the amino acid sequence of a protein. He determined the amino acid sequence of insulin in 1953 and was given the 1958 nobel prize in chemistry in recognition of his work. Today, methods for this procedure have been automated and made simple.The amino acid sequence of many proteins are now known and this has assisted Biochemists in tracing molecular events in evolution. Alterations in amino acid sequence can produce abnormal function and disease e.g. sickle cell anaemia results from replacement of a single amino acid, Glu with Val in the B chain of Haemoglobin. The 4 levels of protein structure are also discussed as well as the various functions of proteins in Biological systems.

2.0 Objectives

At the end of this unit, you should be able to:

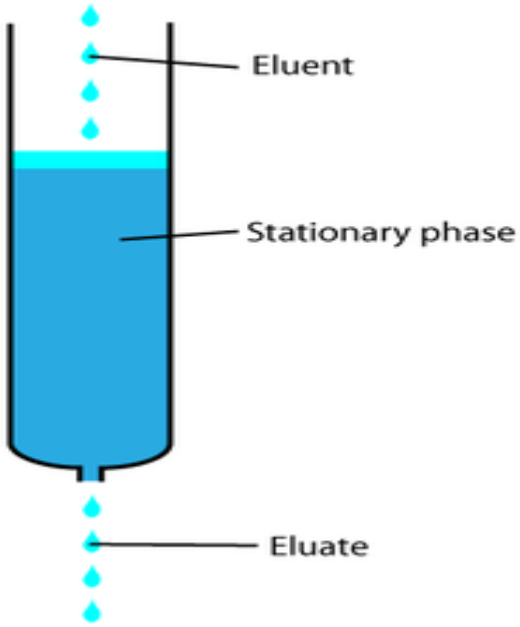
i. Explain the process of Protein purification
ii. Discuss how amino acid composition is determined
iii. Discuss how amino acid sequence is determined
iv. Explain the different levels of protein structure
v. State the roles of proteins in Biological processes
vi. Explain how amino acid sequence of a protein is determined

3.1 Purification of Proteins

Each protein has a unique, precisely defined amino acid sequence. In 1953, Frederick Sanger determined the amino acid sequence of insulin. Since then, hundreds of other proteins have been sequenced. This is an important procedure as it provides insight into its biological activity and function. Much of the evolutionary history of the protein can also be known from its aa sequence and composition as proteins resemble one another in their amino acid sequences only if they have a common ancestor. Consequently, molecular events in evolution can be traced from amino acid sequences. Alterations in amino acid sequence can produce abnormal function and disease e.g. sickle cell anaemia results from replacement of a single amino acid, Glu with Val in the chain of Hae. The first step is to purify the protein, after which its amino composition is determined

A protein must be purified prior to determination of its chemical composition, structure and function. The first task is to develop an assay procedure for the protein. This utilizes specific properties of the protein such as the rate of transformation of substrate to product (in the case of an enzyme), antibody-antigen reaction or a physiological response that gives a quantitative measure of activity per unit protein concentration. This is known as the protein's specific activity. The purpose of a purification procedure is to increase a protein's specific activity to the value expected for the pure protein. Initial purification of a soluble cellular protein involves disruption of the cell membranes, followed by differential centrifugation in a density gradient to isolate the protein from sub cellular particles and high molecular weight aggregates. Further purification may utilize selective precipitation by inorganic salts (salting out) or by organic solvents. Final purification includes a combination of techniques that separate based on molecular charge, molecular size, affinity or a combination of two or more of these.. Techniques based on charge include electrophoresis, isoelectric focusing and ion exchange chromatography. Those based on molecular mass or size include ultracentrifugation, molecular exclusion chromatography/gel filtration while affinity chromatography is based on the affinity of the protein for its substrate, membrane receptors or antibodies. Polyacrylamide gel electrophoresis is based on size and charge.

Ion Exchange column

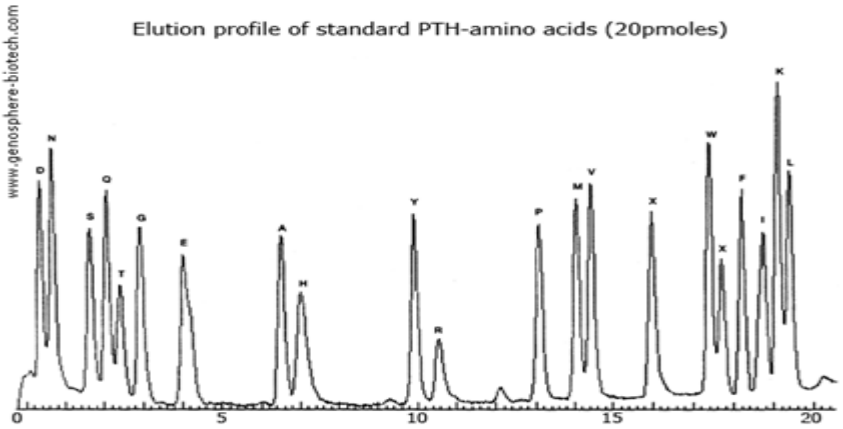


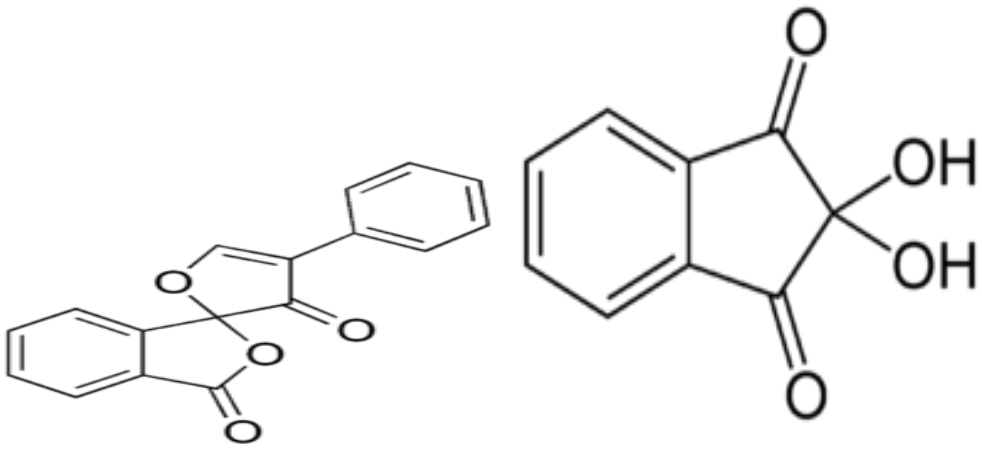
Running a protein through a column

Fig 5.1: Tools for protein purification

Determination of amino acid composition of a peptide/ protein

The peptide is hydrolyzed into its constituent amino acids by heating it in 6N HCl at 110^{o}C for 24 hours. The amino acids in the hydrolysate are separated by ion exchange chromatography on a column of sulfonated polystyrene. The separated amino acids are detected by the colour produced when they are heated with ninhydrin: α- amino acids give an intense blue colour while imino acids such as proline give a yellow colour. The quantity of amino acids is proportional to the optical absorbance of the solution after heating it with ninhydrin. Fluorescamine can also be used, which reacts with its α- amino group to form a highly fluorescent product. The identity of the amino acid is revealed by its elution volume, i.e the vol of buffer used to remove the amino acid from the column. The chromatographic pattern of the hrdolysate is then compared with that of a standard mixture of amino acids.



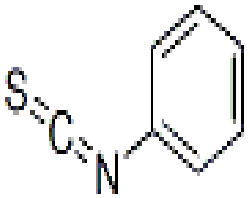


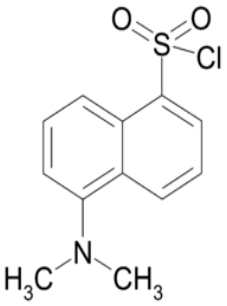
Fluorescamine Ninhydrin

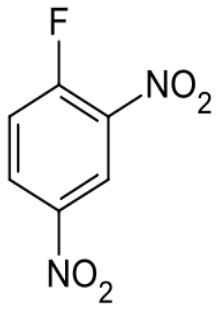
Fig 5.2: Reagents for Quantifying proteins

Determination of Amino Acid Sequence of a Peptide/Protein

Sequencing of a protein was first carried out by Frederick sanger in 1953. He determined the amino acid sequence of insulin. Mature insulin consists of the 21-residue A chain and the 30-residue B chain linked by disulfide bonds. Fred Sanger reduced the disulfide bonds using performic acid and thus separated the 2 chains. He then cleaved each chain into smaller peptides using trypsin, chymotrypsin and pepsin. The resulting peptides were then precipitated with 6N HCl to hydrolyze peptide bonds and generate peptides with as few as two or three amino acids. He then reacted each peptide with 1-fluoro-2,4-dinitrobenzene (Sanger's reagent) which derivatizes the exposed α amino group of amino acid residues. Working backwards to larger fragments enabled Sanger to determine the complete sequence of Insulin. Dansyl Chloride is also used to identify amino-terminal residues. It reacts with amino groups to form highly fluorescent and stable sulfonamide derivatives. Although the DNP and Dansyl methods are powerful, they cannot be used repetitively on the same peptide because the peptide is totally degraded in the acid-hydrolysis step. Pehr Edman introduced Phenylisothiocyanate (Edman's reagent) to selectively label the amino- terminal residue of a peptide. This generates a PTH (phenylthiohydantoin) derivative . in contrast to the Sanger's reagent, the PTH derivative can be removed under mild conditions to generate a new amino terminal residue. Successive rounds of derivatization with Edman's reagent can therefore be used to sequence many residues of a single sample of peptide. Edman sequencing has been automated, using a thin film or solid matrix to immobilize the peptide and HPLC to identify PTH amino acids. The Edman method determines the first 20-30 residues of a peptide. Since most pps contain several hundred aas, it is necessary to first cleave the pp into small peptides prior to Edman sequencing. Reagents for the chemical or enzymatic cleavage of proteins include CNBr, Trypsin etc. Following cleavage, the resulting peptides are purified and sequenced.







Phenyl isothiocyanate (Edman's Reagent)

Dansyl chloride

1-fluoro 2,4-dinitrobenzene

Fig 5.3a Reagents used for sequencing proteins/peptides. They label the exposed α-amino group of amino acid residues.

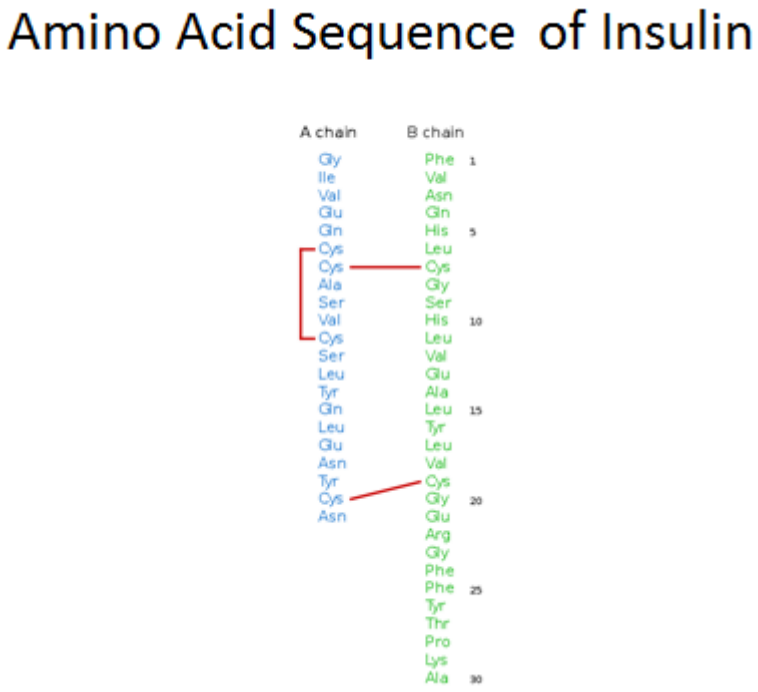


Fig 5.3.1 . Amino acid sequence of Insulin

Table 5.1 : Reagents used in the cleavage of polypeptides

Reagent	Cleavage site
Chemical cleavage	
Cyanogen Bromide	Carboxyl side of Met residues
Hydroxylamine	Asparagine-glycine bonds
2-Nitro-5-thiocyanobenzoate	Amino side of cysteine residues
Enzymatic cleavage	
Trypsin	Carboxyl side of Lys and Arg
Clostripain	Carboxyl side of Arg residues
Staphylococcal protease	Carboxyl side of Asp and Glu

3.1 Purification of Proteins

There are 4 levels of protein structure:

Primary structure refers to the sequence of amino acids and location of disulfide bridges if there are any. It is a complete description of the covalent connections of a protein.

Secondary structure refers to the steric relationship of amino acid residues that are close to one another in the linear sequence. Some of these relationships are of a regular kind, giving rise to a periodic structure. The α helix, the β- pleated sheet and the collagen helix are examples of 2^0 structure. The helix is a rod like structure. The tightly coiled polypeptide main chain forms the inner part of the rod, and the side chains extend outward in a helical array. The stability of an α helix arises primarily from hydrogen bonds formed bw the NH and CO groups of the main chain. The Co group of each amino acid is hydrogen bonded to the NH group of the amino acid that is situated 4 residues ahead in the linear sequence. Each residue is related to the next one by a translation of 1.5 Å along the helix axis and a rotation of 100^o, which gives 3.6 amino acids per turn of helix. Thus a.as that are spaced 4-5 apart in the linear sequence are spatially close to one another in an α helix while those 2 apart in the linear sequence are situated on opposite sides of the helix and so are unlikely to make contact. The pitch of the α helix is 5.4 Å (the product of the translation and no of residues per turn).The α helix can be right handed (clockwise) or left-handed (anticlockwise). Only right handed helices are found in proteins. Since the peptide bond of proline lacks a H atom to contribute a H bond, proline can only be stably accommodated within the first turn of an α helix. When present elsewhere, proline disrupts the conformation of the helix, producing a bend.. Because of its small size, glycine also often induces bends in α helices. The β sheet differs markedly from the α helix in that it is a sheet rather than a rod. The pp in the β pleated sheet is fully extended rather than being tightly coiled. The axial distance bw adjacent amino acids is 3.5Å. The β sheet is stabilized by hydrogen bonds bw NH and CO groups in different pp strands, whereas in the α helix, the H bonds are bw NH and CO groups in the same PP chain.

The collagen helix is responsible for the high tensile strength of collagen, the major component of skin, bone and tendon.

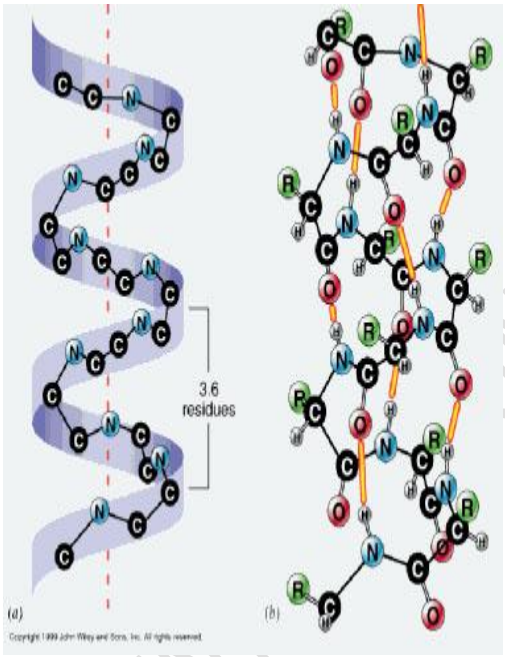
Tertiary Structure

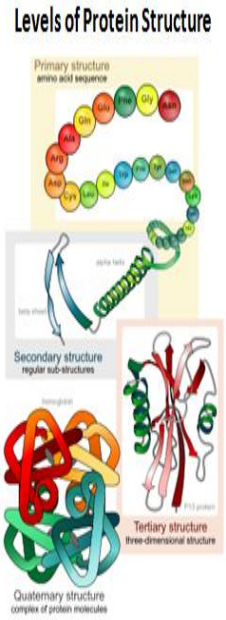
This refers to the entire 3-dimensional conformation of a protein. It is the steric relationship of amino acid residues that are far apart in the linear sequence. It includes the geometric relationship between distant segments of primary structure and the positional relationship of

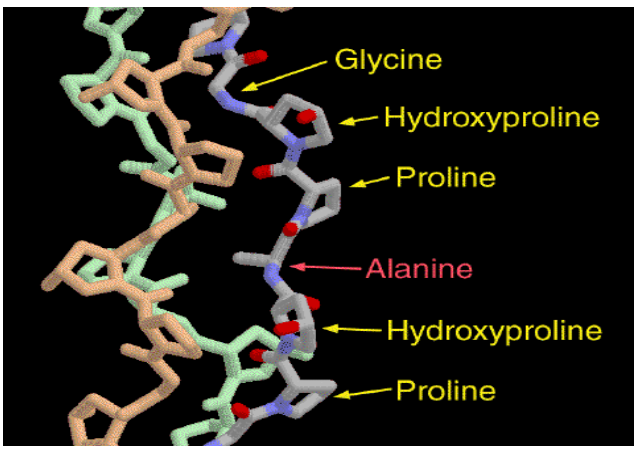
the side chains with one another. It indicates, in 3- dimensional space how secondary structural features (Helices & sheets) assemble to form domains.

Quartenary Structure

This refers to the arrangement of polypeptide chains in a multichain protein. The subunits in a 4^0 structure are associated non-covalently. It should be noted that a protein having only 1 pp chain e.g myoglobin , as well as one in which the PP chains are covalently bonded together e.g chymotrypsin cannot have 4^0 structure. Each PP chain in a protein with 4^0 is referred to as a subunit e.g Hae has 4 pp chains(α2β2) held together non covalently in a specific conformation as required for its function. A protein domain refers to a compact, semi-independent globular unit of protein structure. It is a section of protein structure capable of performing a particular chemical or physical task.3^0 and 4^0 structures are stabilized by non covalent interactions which include hydrophobic interactions, hydrogen bonds, salt bridges and intra polypeptide disulfide bonds.







Collagen helix

Fig 5.4: Levels of Protein Structure

3.3 Roles of Proteins in Biological processes

Proteins play crucial roles in virtually all Biological processes. Some of these roles include

Enzymatic catalysis: Nearly all chemical reactions in Biological systems are catalyzed by enzymes.Chemical transformations rarely occur at perceptible rates in vivo in the absence of enzymes.All known enzymes are proteins. Thus, proteins play the unique role of determining the pattern of chemical transformations in biological systems.

Transport and storage: Many small molecules and ions are transported by specific proteins e.g Hae transports oxygen in erythrocytes while myoglobin transports oxygen in muscle. Transferrin carries iron in the plasma of blood to the liver where it is stored as a complex with ferritin, another protein.

Co-ordinated motion: proteins are the major components of muscle. Muscle contraction is accomplished by the sliding motion of two kinds of protein filaments.On the microscopic scale, coordinated motion such as the mvmt of chromosomes in mitosis and the propulsion of sperm by their flagella are also produced by contractile assemblies consisting of proteins.

Mechanical Support: The high tensil strength of skin and bone is due to the presence of collage, a fibrous protein

Immune protection: Antibodies are highly specific proteins that recognize and combine with foreign substances such as viruses, bacteria and cells from other organisms.

Generation and transmission of nerve impulses: The response of nerve cells to specific stimuli is mediated by receptor proteins e.g rhodopsin is the photoreceptor protein in retinal rod cells.

Control of Growth and Differentiation: Controlled sequential expression of genetic information is essential for the orderly growth and differentiation of cells. Repressor proteins are important control elements that silence specific segments of the DNA of a cell. Nerve growth factor, a protein complex serves to guide the formation of neutral networks in higher organisms.

4.0 Conclusion

Proteins are essential nutrients for tissue growth, repairs and replacement.

5.0 Summary

In this unit, you have learnt about the following:

i. Purification of Proteins
ii. Purification of Proteins
iii. Roles of Proteins in Biological processes

6.0 Tutor Marked Assignments

6.1 Activity

6.2 Answer the following questions:

i. Explain the process of Protein purification

ii. Discuss how amino acid composition is determined
iii. Discuss how amino acid sequence is determined
iv. Explain the different levels of protein structure
v. State the roles of proteins in Biological processes
vi. Explain how amino acid sequence of a protein is determined

7.0 References and other resources

1. Murray, R.K., Bender, D.A., Botham, K., M., Kennelly, P.J., Rodwell V.W. and Well, P.A., (2012). Harper's Illustrated Biochemistry (29th Edition) McGraw-Hill Medical
2. Devlin T.M. (2010) Textbook of Biochemistry with Clinical Correlation 7th Edition. JohnWiley & Sons Inc.
3. Nelson, D.L. and Cox M.M., (2009) Lehninger Principles of Biochemistry 4th edition

UNIT SIX - CLASSIFICATION OF LIPIDS

CONTENT

1.0 Introduction

Lipid is another important macro molecule found in all living cells. It is the only macromolecule without a specific monomeric unit. The word lipid was derived from Greek word *lipos*, the meaning of *lipos* in greek is fats. This is why lipid and fat are used interchangeably in many literatures. Lipids are described as a group of heterogeneous compounds that are not readily soluble in water or polar solvents but are readily soluble in organic solvents such as chloroform, hydrocarbons etc. Examples of lipids include fats, oils steroids, waxes and related compounds. Lipids are usually hydrophobic or amphipathic molecules, the most common lipids in nature consist of fatty acids linked by an enter bond to glycerol or to other alcohol such as cholesterol. In addition to fatty acids and alcohol they may contain other compounds such as phosphoric acid, organic bases and carbohydrates. All the components of lipids can be released or separated by various hydrolytic procedures.

The study of lipid biochemistry is important for thorough understanding of causes, effects and management of metabolic disorders of lipid metabolism such as obesity and

cardiovascular diseases. Measurement of lipoproteins and cholesterol in the blood can serve as early warning for impending danger to our health.

2.0 Objectives

At the end of this unit, you should be able to:

i. List the biological functions of lipids
ii. Classify lipids to different groups based on their chemical composition
iii. Describe the classes of Lipoproteins

3.1 Biological functions of lipids

Lipids have various important functions in animals,some of the functions are:

i. Lipids are important dietary constituents of food and have high energy value.
ii. Dietary lipids are the sources of fat soluble vitamins and essential fatty acids. They also help in transportation of many water insoluble molecules.
iii. Lipids serve as energy stores, fats stored in animals as triacylglycerol or triglyceride in the adipose tissue is a potential source of energy.
iv. Fats present in the subcutaneous tissues act as an insulating material.
v. Lipids are important component of plasma membranes.
vi. Lipids are involved in various intra and intercellular signaling processes.

3.2 Classification of Lipids

Lipids are classified into three groups based on the number and types of components present in the lipid. The three groups are simple lipids, complex or compound lipids and derived lipids.

Simple Lipids

They are the simplest form of lipids and the ester of fatty acids with various types of alcohols. When simple lipids are hydrolyzed, they yield just two types of products. (Fatty acids and alcohol or glycerol). Examples of simple lipids are fats, oils and waxes.

(a) Fats – Fats are solid at room temperature because their fatty acids are saturated. Fats are obtained naturally from animal sources; example of natural fats is lard. Butter and margarine are artificial fats produced by hydrogenation of oils (conversion of unsaturated).

Fats play vital role in maintaining healthy skin and hair, insulating body organs against shock and maintaining body temperature. In animals, fats are stored in the white adipose tissue, or fatty tissue. Adipocyte store fat derived from the diet and those derived from metabolism in the liver. *Visceral fat* is located within the abdominal wall i.e. beneath the wall of abdominal muscle) whereas *subcutaneous* fat is located beneath the skin.

(b) Oils- Oils are liquid lipids at room temperature due to the presence of unsaturated fatty acids. Oils are obtained from plant seeds or through the melting of animal fats.

(c) Waxes - They are also solid at room temperature due to the presence of saturated fatty acids and high molecular weight monohydric alcohol.

Complex Lipids

Complex lipids contain other amphipathic compounds in addition to fatty acids and alcohol present in simple lipids. The compounds include phosphoric acid, various sugars, sphingosine, ethanolamine and serine. Generally, complex lipids yield three or more primary products when they are hydrolyzed. Examples of complex lipids include phospholipids, glycolipids and sphingolipids. Sphingophospholipids are made up of fatty acids, sphingosine, phosphoric acid and choline.

Sulpholipids (sulphur containing lipids), and lipoproteins also belong to the group of complex lipids.

Derived Lipids

These lipids are derived from the hydrolysis of simple and complex lipids. They are mainly fatty acids, glycerols and steroids or sterols.

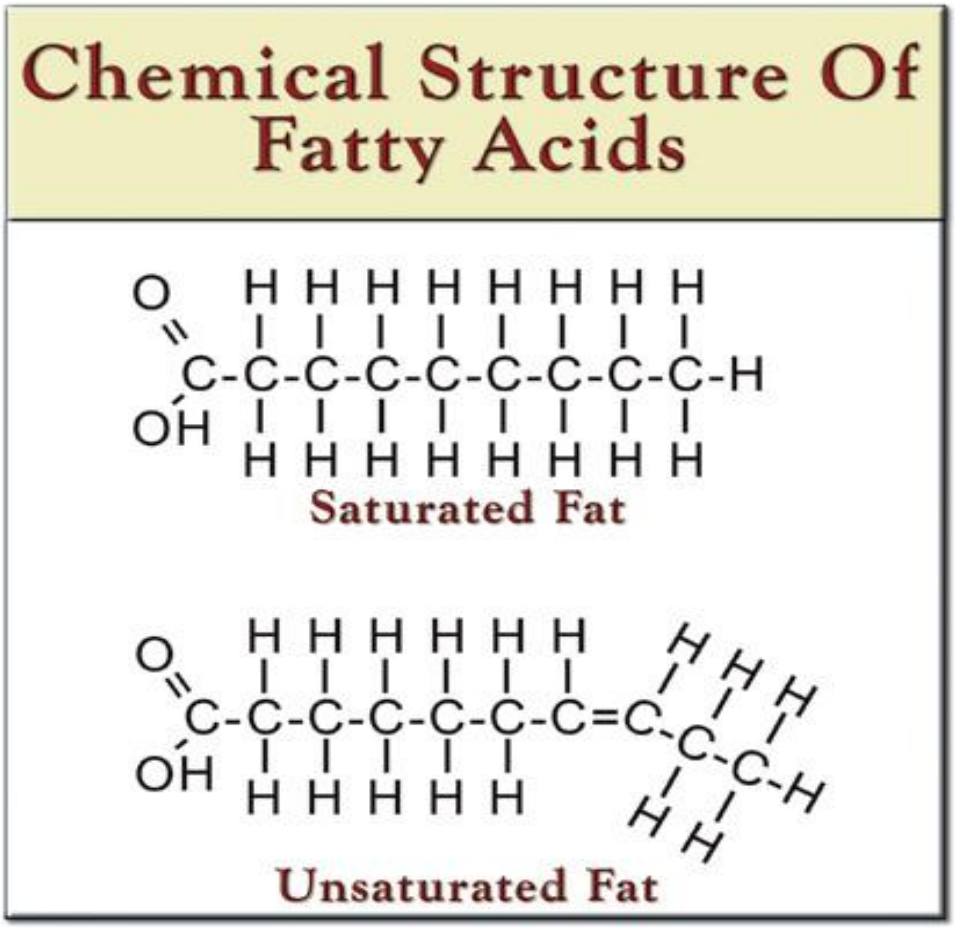


Figure 6.1: Chemical structures of fatty acids (Source google images)

- Fatty Acids

Fatty acids are the carboxylic acids with a long hydrocarbon chain. Fatty acids in animals occur mainly as esters in natural fats and oils (they exist as components of triglycerides)

Fatty Acids

Fatty acids occur as free fatty acids in the plasma where it acts as transporter of various biological molecules, especially plasma albumin. Fatty acids that occur in natural fats usually contain an even number of carbons between 11 and 24 carbon atoms. The 16 and 18 carbon fatty acids are the most common FA in animals. They may be saturated or unsaturated.

(a) Saturated fatty acids

Saturated fatty acids are the fatty acids without double bonds, examples of such fatty acids are palmitic acid (C16), stearic acid (C18) and arachidonic acid (C20). Palmitic acid, myristic

acid and stearic acid are the most abundant saturated fatty acids in human. Lauric acid (C12) is present in coconut oil.

Unsaturated fatty acids are the FA that contains one or more double bonds. Unsaturated FA can be divided into three groups based on the number of double bonds present. Most naturally occurring unsaturated FAs have a cis-configuration i.e. the hydrogen atoms are on the same side of the chain while FAs containing trans-double bonds (hydrogen atoms are on the opposite sides) are found in small amounts in natural fats and in greater amounts after processes involving catalytic hydrogenation.

1. Monounsaturated Fatty Acids (MUFA): They contain only one double bond. *Oleic* acid is the most abundant monounsaturated FA in nature, Palmitoleic acid is another example of MUFA, and it is present in nearly all fats.
2. Polyunsaturated fatty acids (PUFA) – These are the FAs obtained from plant seeds. They usually contain 2 or more double bonds. In PUFA, double bonds are usually separated by a methylene (CH_2) group. PUFA are present in oils such as soyabean oil, groundnut oil, sunflower, benin-seed oil etc. Examples of PUFA are *linoleic* and *linolenic* acids; they are also called omega 6 and omega 3 FAs respectively. These two PUFA are also referred to as essential PUFA (EFA). They are essential because animals cannot synthesize them, therefore they must be supplied to the body in the diet.

Linoleic acid- it is also known as omega-6 fatty acid (ω_6)

Linolenic acid– it is known as omega-3 fatty acid (ω_3)

Why are these two PUFAs essential to animals? Linoleic and linolenic fatty acids are the biosynthetic precursors of 20 and 22 carbons polyunsaturated FAs, with 3 to 6 double bonds. Enzymes which insert double bonds into unsaturated fatty acids are known as desaturases. Desaturases in animals can insert a double bond on the carboxyl side of existing double bonds only, they are called Δ^9 desaturases. Δ^9 desaturases are the only desaturase present in animals; hence they can synthesize only oleic acid and members of its family. The FAs derived from linoleic acid, especially arachidonic acids, are the precursors of the prostaglandins and steroids.

Symptoms of essential fatty acids deficiency

When essential fatty acids (omega-3 and omega-6 fatty acids) are not present in our diets, our body will not be able to produce prostaglandins and sterols (this is just one of many functions of these FA). We know that hormones are very important for reproduction in man and woman, deficiency of material required to synthesize them will result in inadequate or complete absence of the hormones. The result is infertility. So, foods that are rich in essential fatty acids are good for our health, examples of such foods are fish, poultry products, fruits, vegetables, nuts (especially walnuts), legumes (especially soyabeans), beef etc. The symptoms of essential fatty acids deficiency are:

i. Growth retardation
ii. Poor wound healing
iii. Dermatitis, and hair loss
iv. Kidney and liver diseases
v. Infertility
vi. Depression

The most noticeable symptoms of EFA deficiency are skin disorders such as scaly dermatitis. It can show up another on the body, but usually occur on the hands, shoulders, forearms and face. When EFA are included in the diets, these symptoms disappear within 7days.

Eicosanoid

The third group of unsaturated FA is the family of PUFA known as eicosanoids. The eicosanoids are a complex family of bioactive lipid messengers, generated by oxygenation of 20-carbon polyunsaturated fatty acids, primarily arachidonic acid. Eicosanoids are local-acting hormones that stimulate cells adjacent to their site of synthesis. In general, eicosanoids have a short half-life, usually on the order of minutes. They are not stored in cells but instead are released as soon as they are synthesized. Eicosanoids fall into two main classes;

i. Prostanoids that have a ring structures including prostaglandins, thrombaxanes and prostacyclins.
ii. Linear eicosanoids consisting of leukotrienes, lipoxins and hydroxyl eicosa tetraenoid acids (HETE).

Prostaglandins

Prostaglandins exist in virtually every mammalian tissue. They are synthesized from arachidonic acid. Prostaglandins act to modulate many physiological functions including blood pressure, contraction of uterus, and inflammatory signals. Prostaglandins are designated PGA, PGD, PGE and PGF based on the functional groups on the cyclopentane ring. Prostaglandin H_2 and prostacyclin are synthesized from PG H_2.

Leukotrienes and lipoxins are linear eicosanoids unlike the prostanoids, which contain a ring in their structure, the leukotrikenes are linear molecules. The terms leukotriene was derived from their cell of origin (Leukocytes) and the fact that their structures contain three carbon-carbon double bonds in conjugation. The most important Leukotrienes in humans are: LTA_4 and LTB_4.

9.2.4. Triacylglycerols

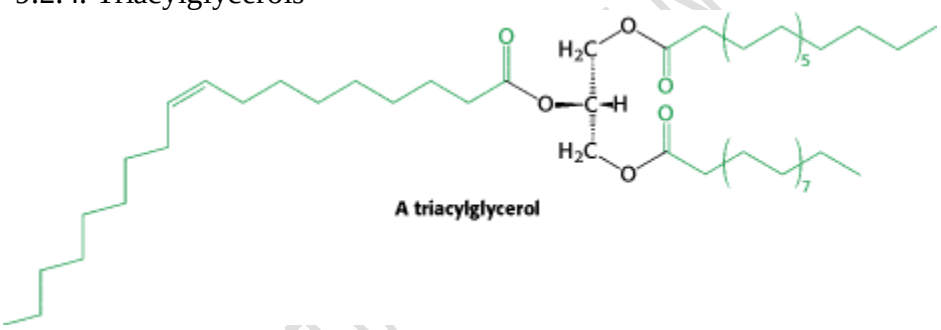


Figure 6.2: Structure of triacylglycerol

Triacylglycerols are esters of the alcohol called glycerol and fatty acids. They represent the major form of fat found in nature and their primary function is to provide energy for the cell. The cell degrades fatty acids to Co_2 and water at the expense of molecular oxygen. One gram of fatty acid liberates about 9 kilocalories (38 KJ) of energy, this is about 2.5 times higher than that of the other foodstuffs i.e. protein and carbohydrate which yield about 4 kilocalories/g (17KJ) when catabolized. The human body stores large amounts of fatty acids as triglyceride in adipose tissue, and this is the storage form of energy in all animals. Fatty acids in the form of triglycerides are in anhydrous form, whereas, carbohydrates and proteins are stored in an aqueous environment. It is evident that, in terms of the energy to mass ratio, fat is a much more efficient means of storing energy than carbohydrate or protein. Mammalian tissues also contain some diglycerides and menoglyceriles, but they occur in very small quantity when compared with triglycerides.

Naturally occurring fats and oils develop unpleasant smell and taste if stored for long under moist conditions, the chemical reaction responsible for the odour and taste as called rancidity.

Rancidity is due to partial hydrolysis of TAG and the subsequent oxidation of the hydrolysed fatty acids to aldehyde and ketone

Steroids

Cholesterol is a monohydric alcohol; it is classified as a devived lipid because it is a component of other complex lipids. It is also a precursor of two important classes of molecules; bile acids and steroid hormones; cholesterol is probably the best-known steroid because of its association with atherosclerosis and heart disease.

All steroids have a similar cyclic nucleus resembling phenanthrene (rings A, B and C) to which a cyclopentane ring Δ is attached.

The five classes of steroids are

i. Glucocorticoids]
ii. Minerals corticoids] 21- carbon structures
iii. Progesterone
iv. Testosterone] 19 carbon structure
v. Androgens]

Bile acids or bile salts play a major role in the digestion and absorption of triacylglycerols and cholesterolyl esters. Formation of bile salts represents the only significant metabolic mechanism for eliminating excess cholesterol from the body.

3.3 Lipoproteins

Lipids are not soluble in aqueous solution such as the blood. Since lipids are not soluble in water, how are they transported in the body? This problem was solved by the presence of a lipid carrier called lipoproteins. Lipoprotein is made up of lipid and protein in such a way that the lipids components are packed at the center while the protein components serve as the coat. The lipoprotein coat also contains amphipathic molecules such as phospholips, cholesterol, and specially synthesized protein called apoproteins. The polar ends of the amphipathic lipids face the surface of the particles, while the hydrophobic portions are oriented towards the centre of the particle.

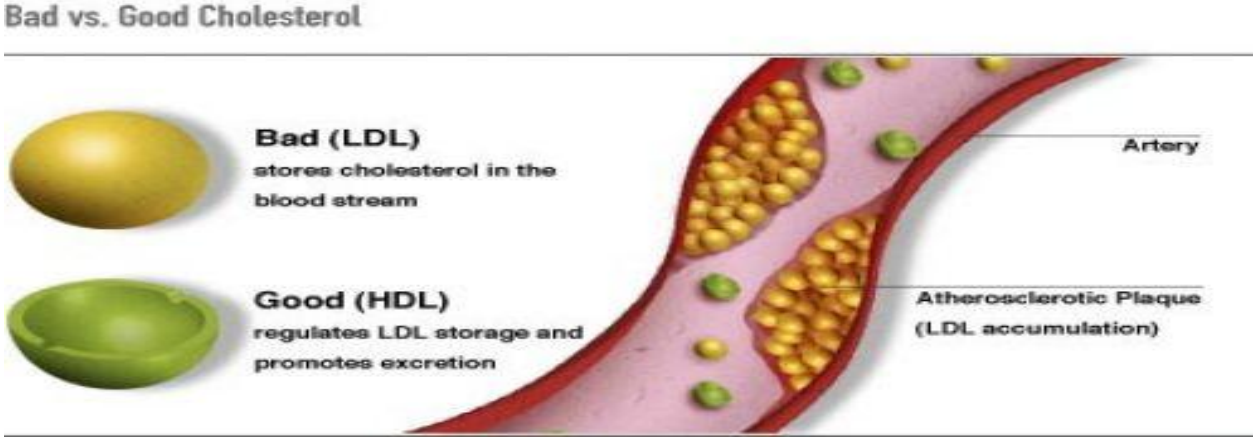


Figure 6.3: Effects of LDL and HDL on the artery (source: google images)

Classification of lipoproteins

i. High Density Lipoprotein (HDL): The most dense lipoprotein class is called high density lipoprotein. It contains about 50% lipids and 50% protein. The major function of HDL is to collect cholesterol from peripheral tissues and transport it back to the liver where it is converted to bile acids and excreted. This function is referred to as reverse cholesterol transport (RCT). HDL is usually referred to as good cholesterol because of its ability to reduce the body's cholesterol content; high concentration of HDL in the blood is good for the prevention of cardiovascular diseases.

ii. Low Density Lipoproteins (LDL): They are cholesterol rich particles, LDL carries about 75% of the total cholesterol in human plasma. Its major function is the transport of cholesterol to various extrahepatic tissues. They are usually referred to as bad cholesterol because of their role in cholesterol distribution to body cells. So, low concentration of LDL in the plasma is beneficial to the body than high concentration.

iii. Very low Density lipoprotein (VLDL): They are secreted to the blood plasma by the hepatocytes. Only 50-60% of their mass is triacylglycerols and they contain relatively more cholesterol esters than the chylomicrons.

iv. Chylomicrons: These particles have the lowest density among the lipoproteins. About 90% of their mass is made up of triglyceride, cholesterol esters and free cholesterols.

5.0 Summary

In this unit, you have learnt about the biological functions of lipids, classification of Lipids and lipoproteins.

6.0 Tutor Marked Assignments

6.1 Activity – See Laboratory practical manual

6.2 Answer the following questions:

i. Describe the group of macromolecules called lipids and give 3 examples
ii. Lipids are classified to 3 groups, name them and give 2 examples of each group
iii. Describe the biological importance of lipids
iv. Write short notes on the following : Waxes, Fats and Oils
v. Differentiate between saturated and unsaturated fatty acids, give two examples of each
vi. List the two essential fatty acids and explain the reason why they are essential to human beings.
vii. If there is deficiency of essential fatty acids, what are the symptoms the person will feel or observe?
viii. Describe the Eicosanoids and their biochemical reactions
ix. Explain why the triglycerides store energy better than proteins and carbohydrates
x. How are lipids transported in the blood bearing in mind that lipids are not soluble in polar solution such as blood?

7.0 References and other resources

1. Murray, R.K., Bender, D.A., Botham, K., M., Kennelly, P.J., Rodwell V.W. and Well, P.A., (2012). Harper's Illustrated Biochemistry (29th Edition) McGraw-Hill Medical

2. Devlin T.M. (2010) Textbook of Biochemistry with Clinical Correlation 7th Edition. JohnWiley & Sons Inc.
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UNIT SEVEN – INTRODUCTION TO NUCLEIC ACID BIOCHEMISTRY-I

CONTENT

1.0 Introduction

Nucleic acids are the most important macromolecules in all living things because it is the genetic material responsible for the transfer of genetic information from one generation of organisms to another. The traits we shared with our parents are due to the genes inherited from them. These include but not limited to physical appearance, colour of skin, size of eye balls, intelligence etc.

2.0 Objectives

At the end of this unit, you should be able to:

i. Describe the chemical components of nucleic acids
ii. Describe the formation of nucleosides
iii. Describe the formation of nucleotides
iv. Describe Deoxyribonucleic Acids (DNA)
v. Describe Ribonucleic Acids (RNA)
vi. Explain the following terms:
    a. (a) Replication (b) Transcription (c) Translation (d) DNA Denaturation and Renaturation
vii. State the importance of some synthetic nucleotides
viii. The chemical components of nucleic acids

3.0 Main Content

3.1 Nucleic acids

Nucleic acids are polymers of nucleotides i.e. nucleotides are the monomeric units of nucleic acids. Each nucleotide has three components: a five-carbon monosaccharide, a nitrogen-containing cyclic compound (base) and a phosphate group.

Five carbon Sugars – The five carbon sugars are known as pentoses. Two different five-carbon sugars are employed in the construction of nucleic acids; they are ribose and deoxyribose sugars. The only difference between them is the absence of oxygen on the

2'carbon atom of deoxyribose sugar. The structure shown in figure 10.1 is ribose sugar while the structure in figure 10.2 is deoxyribose sugar.

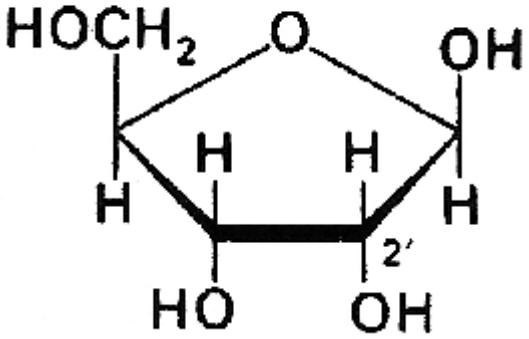


Figure 7.1 The structure of ribose sugar (source: google images)

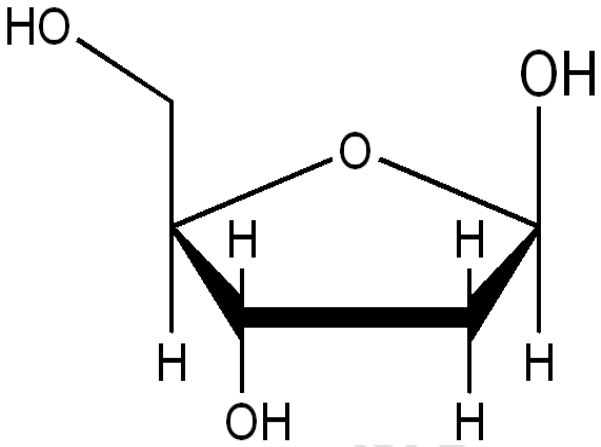


Figure 7.2: The structure of deoxyribose sugar (source: google images)

1. **Nitrogenous bases –** The nitrogenous bases employed in the construction of nuclei acids are members of two classes of nitrogen containing compounds known as *purines and pyrimidines.* Purine has 2 derivatives in nucleic acids (Adenine and Guanine) usually represented by letters A and G. Pyrimidine has 3 derivatives in nucleic acids (cytosine, uracil and thymine) usually represented by capital letters C,U,T. Thymine is present only in DNA molecules while uracil is present only in RNA. Adenine, Gnanine and cytosine bases are present in both DNA and RNA.

These nitrogenous bases are the key components of nucleic acid because genetic information is stored in the sequence of bases along the DNA strands. Every time a cell divides, the information is passed along to the new cells.

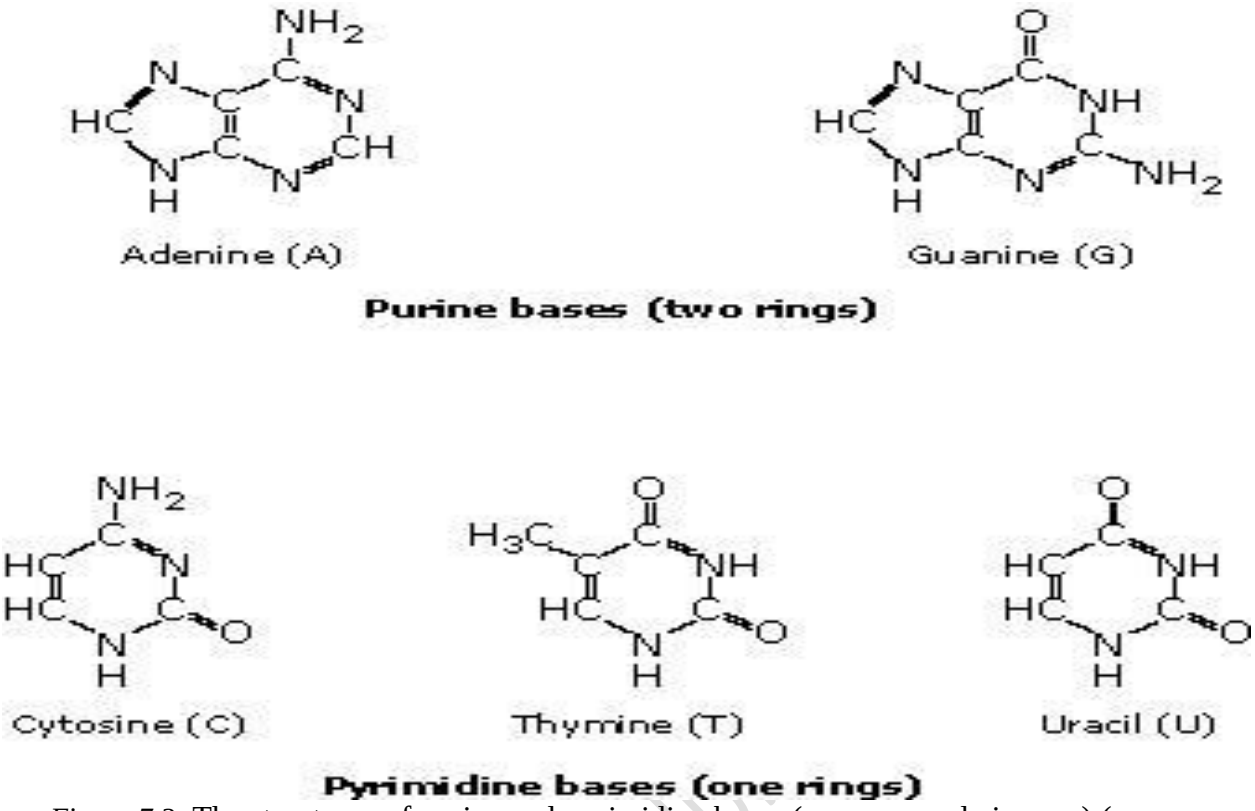


Figure 7.3. The structures of purine and pyrimidine bases (source google images) (source: google images)

2. **Phosphate Group** – It is a very important component of nucleic acids. It serves as the link between 2 sugar molecules or nucleotides in polynucleotides. The phosphate group is a strong acid in solution; this explains why DNA and RNA are called acids. It is also responsible for the negative charges on DNA and RNA at physiological pH.

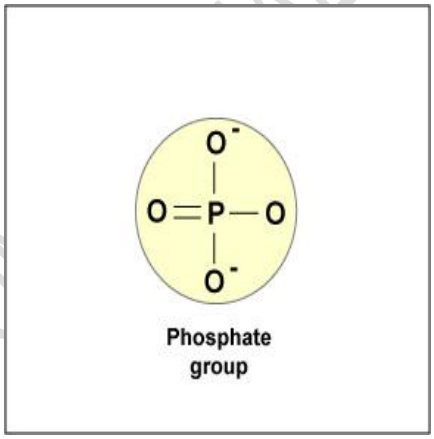


Figure 7.4: The phosphate group (source: google images)

3.2 The Nucleosides

Nucleosides – Ribose or deoxyribose Sugar +Nitrogenous Base

The combination of ribose or deoxyriboe and one of the 5 bases produces a nucleoside. They are linked via a covalent β-N-glycosidic bond. Nucleosides present in RNA are Adenosine, guanosine, cytidine and uridine.

Nucleosides present in DNA are deoxy adenosine, deoxygyanosine, deoxycytidine and deoxythymidine or thymidine usually abbreviated as A, G, C and T. While the nucleotides present in RNA are represented by A, G, C, U. The structures of uridine (a representative of RNA) and thymidine (a representative of DNA) are shown in figure 10.5

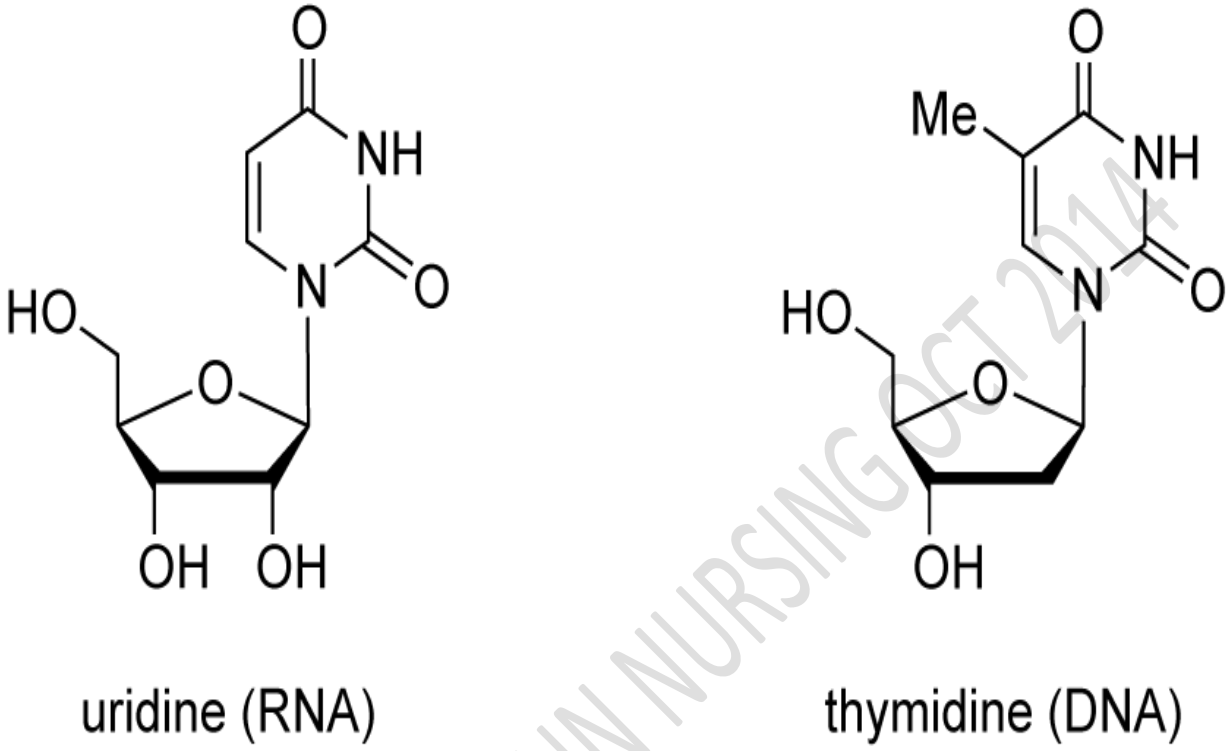


Figure 7.5: The structures of 2 nucleosides, uridine and thymidine (source: google images)

The Nucleotides

Nucleotides are phosphrylated nucleosides (nucleosides + phosphate) or sugar + base + phosphate. Each nucleotide is a 5-monophosphate ester of a nucleoside. The Po_4 group is attached to the hydroxyl group of pentose sugar by an ester linkage, usually OH group on carbon 5, 3 or 2.

3.3 Types of Nucleotides

i. **Monophosphate** – Nucleotides containing only one phosphate molecule
   a. AMP – Adenosine monophosphate
   b. GMP – Guanosine monophosphate
   c. UMP – Uridine monophosphate (the structure is shown in figure 7.6)

ii. Diphosphates and Triphosphates contain two and three molecules of phosphate respectively. Examples of diphosphate nucleotides are ADP, GDP, UDP while ATP, GTP and UTP are examples of triphosphate nucleotides.

iii. **Cyclic nucleotides –** In cyclic nucleotides, a Po_4 molecule esterifies two OH molecules on carbon 5 and carbon 3 or carbon 3 and 2 (RNA) small letter C is usually added to denote cyclic nucleotide e.g. 3; 5^1 cAMP (3^1, 5^1 cyclic Adenosine monophosphate),3^1, 5^1 c-GMP is called 3^1, 5^1 cyclic Guanosine monophosphate.

iv. **Polynucleotides-** The 3^1OH of the pentose of a mononucleotide esterifies the 5^1phosphoryl group of the 2^{nd} mononucleotide to form a dinucleotide. The bond between two nucleotides in polynucleotides is known as 3^1, 5^1 phosphodiester bond. The next nucleotide joins the existing polynucleotide through its free 3^1-OH group.

Each end of a nucleotide polymer (polynucleotide) is distinct; one end has a free 5^1 Phosphate while the other end has a free 3^1-OH group, by convention the ncuelotides or base sequence is written in 5^1 to 3^1 - direction, therefore polynucleotides are called directional macromolecules.

DNA and RNA are long linear polymers called nucleic acids that carry information in a form that can be passed from one generation to the next. Genetic information is stored in the sequence of bases along a nucleic acid chain.

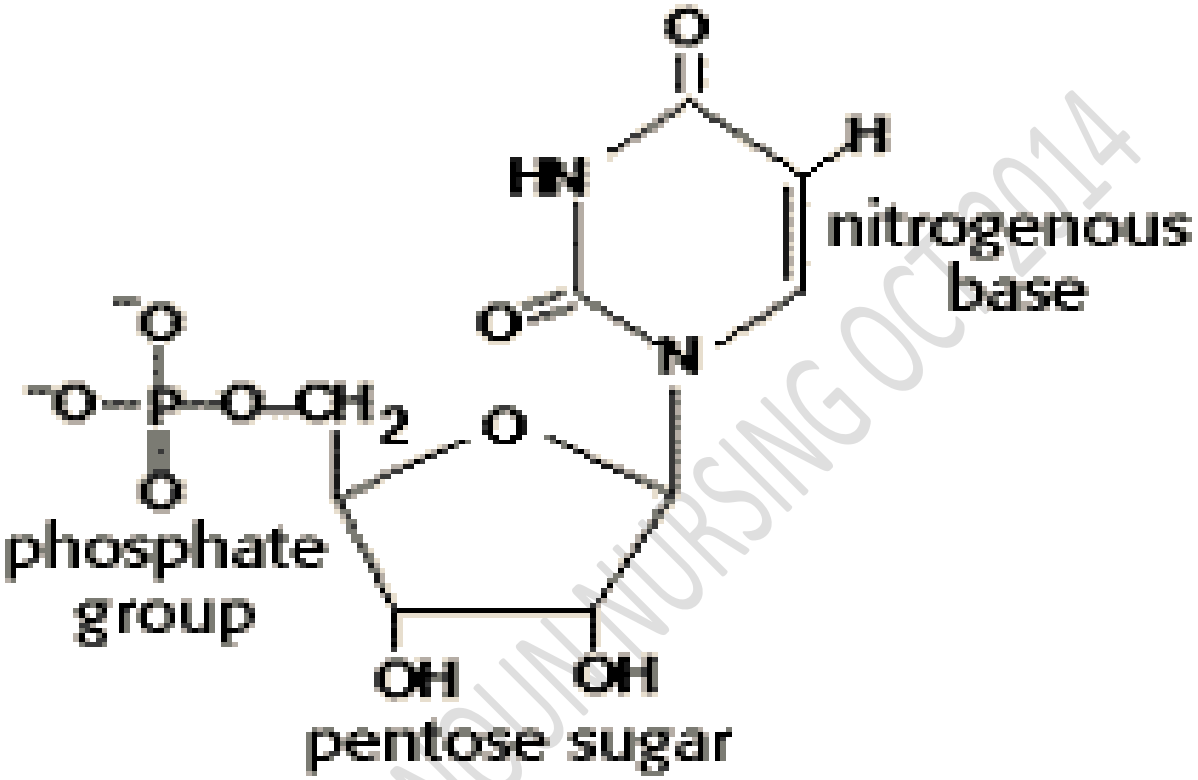


Figure 7.6: Structure of uridine monophosphate (source: google images)

5.0 Summary

In this unit, you have learnt about the nucleic acids and nucleotides

6.0 Tutor Marked Assignments

6.1 Activity – As given by the facilitator.

6.2 Answer the following questions:

i. Describe nucleic acids and explain their importance to all living organisms
ii. List the chemical components of nucleic acids and describe each component
iii. What are nucleotides? Give 4 examples of nucleotides

UNIT EIGHT - THE STRUCTURES OF DNA AND RNA

CONTENT

1.0 Introduction
2.0 Objectives

1.0 Introduction

Recollect that we discussed the chemical components of nucleic acids in study session 10. These components can combine in different ways to form two types of nucleic acids: deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA). These two molecules are the hereditary materials with different physical and chemical properties.

2.0 Objectives

At the end of this unit, you should be able to:

i. Describe the structure of DNA and RNA
ii. List the structural differences between DNA and RNA.
iii. Draw the structure of DNA
iv. Explain the meaning of (i) Transcription (ii) Replication (iii) Translation
v. List at least 3 biological functions of nucleotides apart from genetic functions.

3.0 Main Content

3.1 Structure of DNA

Deoxyribonucleic acid (DNA) is an example of polynucleotide and it is one of the two types of nucleic acids that made up the genetic materials. DNA exists as a double stranded molecule (figure 11.1b) in eukaryotes but single stranded DNA is common among the prokaryotes. The two helical polynucleotide chains usually coil around a common axis. DNA must undergo series of folding and super-folding to enable it fit into the little space available inside the nucleus of cells. This continuous folding finally give rise to a genetic material called chromosomes. Segment of a strand that contains sequence of nucleotides capable of producing a functional protein is refered to as a gene. The four nucleotide units present in DNA are deoxyadenylate, deoxythymidylate, deoxyguanylate and deoxycytidylate.

A unique characteristic of naturally occurring DNA molecules is their very long chain of nucleotides. A DNA molecule must comprise many nucleotides; this enables it to store sufficient genetic information necessary for even the simplest organism. For example the E.coli genome is a single DNA molecule consisting of two chains of 4.6 million nucleotides. The human genome (complete genetic information of an organism) comprises of about 3 billion nucleotides.

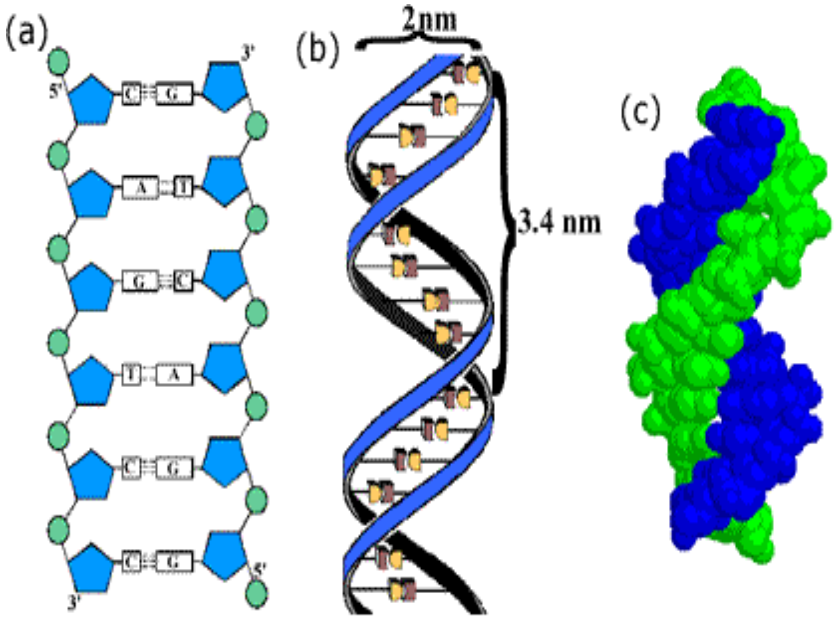


Figure 8.1: (a) Stacking of two chains (b) Double stranded structure of DNA (c) Beads representation of double stranded DNA (source: google images)

Ribonucleic acid is also a polynucleotide like DNA but it exist as a single stranded molecule (Figure 11.2). It is usually produced from the DNA in eukaryotes but it serves as the main genetic material in some viruses, especially the retroviruses such as HIV. The sequence of nucleotides in RNA is determined by the sequence of DNA present in the cell. This will be clearer section in section 7.6. There are some differences between RNA and DNA, I have just discussed one of them. Other differences are:

i. The sugar component of DNA is deoxyribose but in RNA we have ribose sugar
ii. The nucleotides are also different by just one; in place of thymine monophosphate in DNA, uridine monophosphate is present in RNA as shown in section 10.2.

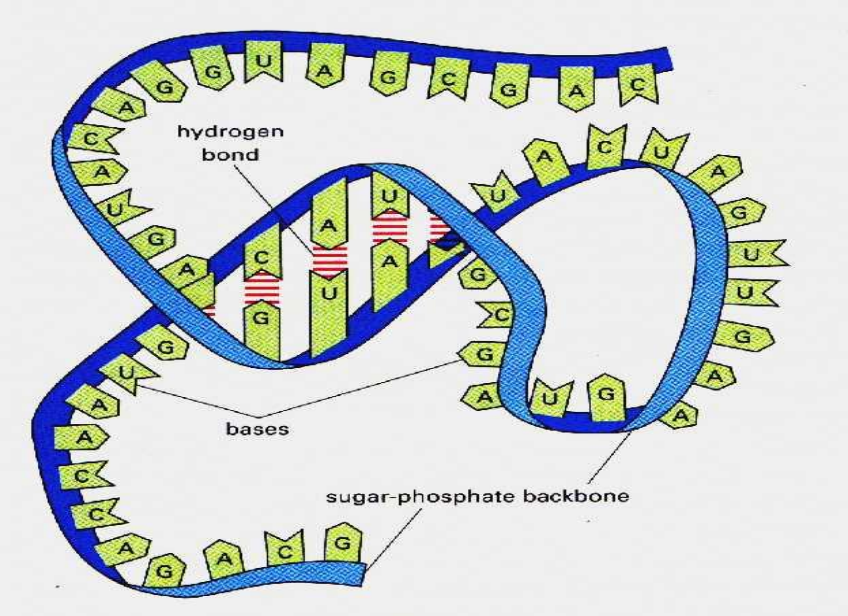


Figure 8.2: The structure of RNA molecule (source: google images)

3.2 Description of duplication and RNA synthesis in nucleic acid

(i) Replication (ii) Transcription (iii) Translation (iv) DNA Denaturation and Renaturation

i. Replication: This is also known as duplication, it is the process by which a replica or identical copy of DNA is made from the parent copy. This process occusr whenever a cell is preparing to divide. This process must take place so that the new cell will also contain the same genetic information present in the old cell. This is how the genetic information is preserved and passed from one generation to the next.
ii. Transcription: This is the process by which the genetic messages contained in the DNA molecule are read and copied correctly to produce ribonucleic acid (RNA). This process occurs whenever the cell or tissue require a particular protein to carry out a biochemical process, the protein required can be an enzyme, receptor or other forms of proteins.
iii. Translation: This is the process by which the genetic messages carried by RNA are decoded and used to build proteins (enzymes, receptors, hormones etc).
iv. DNA denaturation and renaturation: The process of separating the complementary strands of duplex DNA is called denaturation. The double stranded structure of DNA can be separated into two component strands in solution by increasing the temperature, decreasing the salt concentration and by extreme pH (strongly acidic or strongly basic solution). Because of the base stacking and the hydrogen bonding between the bases, the double stranded DNA molecule exhibits properties of a rigid rod and in solution; it is a viscous material that loses its viscosity upon denaturation as well as its biological properties.

The complete separation of DNA strands occur over a temperature range (5^0 range). The heating disrupts the hydrogen bonds between base pairs thereby causes the strands to separate. This process is also called **melting** of DNA. The melting temperature ™ of DNA molecule depends on the pH and ionic strength of the solvent. Purine and pyrimidine base composition also affect the melting temperature of DNA; the greater the content of G and C, the higher the melting temperature and vice-versa. There are 3 bonds between G and C while there are 2 bonds between A and T. The melting temperature ™ is defined as the temperature at which half of the helical structure is lost.

DNA solution absorbs maximally at a wavelength of 260mm, the absorbance increases as the double helix is separated into single strands (denaturation). This principle is also known as hypochronism.

Renaturation is the opposite of denaturation, it is the process of re-association of separated strands of DNA or any two strands of DNA that are complementary in base pairing. This process takes place only when the appropriate physiologic temperature and salt concentration are achieved. Re-naturation is also called **hybridization.**

3.3 Importance of some synthetic nucleotides

Synthetic nucleotides are used in chemotherapy of cancer and viral infections

Modified synthetic analog of purines, pyrimidines and nucleotides have useful applications in clinical medicine. These compounds are used to treat cancer because they can prevent DNA synthesis due to their toxicity to the enzymes involved in DNA synthesis.

Some of the synthetic nucleotides are also used to manage AIDS patients, because they are toxic to an important enzyme required for DNA synthesis in the virus. For example Azidothymidine (AZT) is a potent inhibitor of reverse transcriptase in HIV. Zidovudine is another drug for HIV control, its active component is 2^1- Azido – 3^1 deoxythymidine. (AZT inhibits HIV replication). AZT is not used alone but in combination with other drugs with different mechanism of activity. The combined therapy is called highly active antiretroviral therapy (HAART).

5.0 Summary

In this unit, you have learnt about the structure of DNA, description of duplication and RNA synthesis in nucleic acid and importance of some synthetic nucleotides.

6.0 Tutor Marked Assignments

6.1 Activity – As prescribed by the facilitator.

6.2 Answer the following questions:

i. Describe the structures of DNA and RNA
ii. What are the differences between DNA and RNA?
iii. Write short notes on each of the following: (i) Replication (ii) Transcription (iii) Translation (iv) DNA Denaturation and Renaturation

7.0 References and other resources

1. Murray, R.K., Bender, D.A., Botham, K., M., Kennelly, P.J., Rodwell V.W. and Well, P.A., (2012). Harper's Illustrated Biochemistry (29th Edition) McGraw-Hill Medical
2. Devlin T.M. (2010) Textbook of Biochemistry with Clinical Correlation 7th Edition. JohnWiley & Sons Inc.
3. Nelson, D.L. and Cox M.M., (2009) Lehninger Principles of Biochemistry 4th edition

Module 3 - Enzymology

Introduction:

The living cell is the site of tremendous biochemical activity (metabolism) where build up of new tissue, replacement of old tissue, conversion of food to energy, disposal of waste materials and all other activities characterized as life take place. However, majority of these biochemical reactions do not take place spontaneously but are made possible by the phenomenon of catalysis. (A catalyst accelerates a chemical reaction but undergoes no permanent chemical change in doing so). The catalysts of biochemical reactions are ENZYMES. They are responsible for bringing about all chemical reactions in living organism. Without them, these reactions will take place at a pace far too slow for the pace of metabolism. In this session, you will learn how the study of enzymes has evolved over the years, properties of enzymes and how this group of compounds are classified and named.

Module Objectives

At the end of this module, you should be able to:

Content

Unit 1: **Enzymology 1**
Unit 2: Enzyme Kinetics
Unit 3: Enzymology 3

UNIT ONE - ENZYMOLOGY 1

CONTENT

1.0 Introduction

You learnt about the roles of catalysts in chemical reactions in chemistry. In this unit, you will learn about the catalysts in human body, the enzymes.

2.0 Objectives

At the end of this unit, you should be able to:

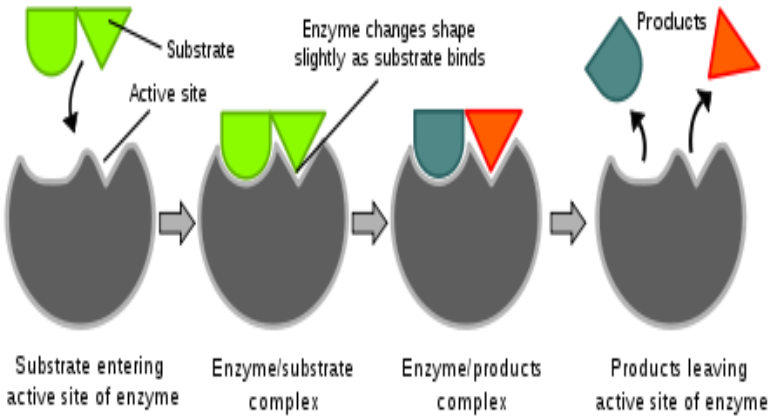
i. Define the role of enzymes as catalysts of biological systems.
ii. Outline and distinguish the 3 types of cofactors required by most enzymes.
iii. Explain how enzymes are named.
iv. Outline and explain the general properties of Enzymes.
v. Enumerate the different classes of enzymes and the types of reactions catalyzed by each class

3.0 Main Content

3.1 Enzymes as catalysts of Biological systems.

Although the phenomena of fermentation and digestion had long been known, the first clear recognition of an enzyme was made by Payen and Persoz when they found that an alcohol precipitate of malt extract contained a thermolabile substance that converted starch to sugar. This substance, now known as Amylase was at the time named diastase. The first enzyme to be obtained in pure form was urease, crystallized from Jack Beans in 1926. The work was carried out by James Sumner, for which he was awarded the 1946 Nobel Prize. Since them, thousands of different enzymes have been purified and their structures worked out.

With the exception of a few catalytic RNA molecules (ribozymes), all enzymes are proteins. They range in size from large multiple subunit complexes (called multimeric enzymes) to small single subunit forms. The region within the enzyme molecule that contains the chemical/functional groups to which the reactants (substrate) binds is called the active site / catalytic site / substrate binding site of the enzyme. The particular arrangement of an enzyme's amino acid side chains in the active site determines the type of molecules that can bind and react there.



The active site of an enzyme is usually found in a cleft or pocket that is lined by amino acid residues that participate in recognition of the substrate.

Fig 1.1: Active site of an enzyme.

3.2 Cofactors

Many enzymes have small non protein molecules associated with or near the active site that determine substrate specificity. These are called cofactors. A cofactor may be

i. A coenzyme- A non protein organic substance which is loosely attached to the protein part. Coenzymes are often derivatives of vitamins. They may or may not be modified

in the reactions. Those that are altered are also called co-substrates. The B vitamins supply important components of numerous coenzymes

ii. A prosthetic group-An organic substance which is tightly bound either covalently or non-covalently to the protein portion e. g flavin coenzymes, pyridoxal phosphate, biotin.

iii. A metal ion activator. These include most divalent metal ions as well as other ions e. g Cu^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+} and Mo^{3+}. Enzymes which require a metal ion for activity are called metal-activated enzymes. Metal ions are sometimes tightly bound to the protein portion. Enzymes containing such are called metalloenzymes.

The protein portion is called the apoenzyme while the entire active complex is called the holoenzyme i.e

Apoenzyme + Cofactor = Holoenzyme.

Table 1.1: Examples of Coenzymes, Metal ion activators and Non-vitamin coenzymes

Coenzymes	Vitamin	Reaction Mediated
Biotin	Biotin	Carboxylation
Cobalamin (B_{12})	B_{12}	Alkylation
Coenzyme A	Pantothenate	Acyl transfer
Flavin coenzymes	Riboflavin B2	Oxidation-reduction
Nicotinamide coenzymes	Nicotinamide	Oxidation-reduction
Pyridoxal phosphate	Pyridoxine B6	Amino group transfer
Tetrahydrofolate	Folic acid	One carbon group transfer
Thiamine pyrophosphate	Thiamine B1	Carbonyl transfer

Mg^{2+}	ATP-dependent reactions
Zn^{2+}	Carboxypeptidase, Alcohol dehydrogenase
K^{+}	Pyruvate kinase
Cu^{2+}, Fe^{2+}	Oxidases and hydroxylases
Non Vitamin Coenzymes	
Coenzyme	**Reaction Mediated**
ATP	Phosphate group transfer
Coenzyme Q	Oxidation- Reduction
S-Adenosyl methionine	Methyl group transfer
Glutathione	Oxidation-Reduction

3.3 Naming and classification of Enzymes

Except for some of the earliest studied enzymes e.g pepsin, rennin and trypsin, most other enzymes are known by common names obtained by adding the suffix-ase to the name of the substrate or to the reaction that they catalyze e.g glucose oxidase, transaminases etc. A systematic scheme for naming/ classifying enzymes was adopted in 1972 by IUB. In this system, each enzyme has a unique name and code number that reflects the type of reaction catalyzed and the substrates involved. The code number is made of 4 figures which indicate

the main class, sub class, sub sub class and the serial number of the enzyme in its sub sub class e.g the enzyme commonly called hexokinase has the IUB designation ATP: D- hexose-6 phosphotransferase EC 2.7.1.1. This identifies the enzyme as a member of class 2 (tranferases), sub class 7(involves transfer of a phosphoryl group), Sub-sub class 1 (an alcohol is the phosphoryl acceptor) . The last digit indicates that the enzyme is the first in its sub sub class.

For Example, the enzyme HEXOKINASE is named as follows:
IUB Name: ATP: D-Hexose-6 phosphotransferase EC 2.7.1.1
This identifies the enzyme as follows:

i. a member of class 2(transferase),
ii. sub-class 7 (Reaction involves transfer of a phosphoryl group
iii. Sub sub class 1 (an alcohol is the phosphoryl acceptor)
iv. Serial No 1 (in its sub sub class)

General Properties of Enzymes

i. Enzymes possess enormous catalytic power. Most accelerate reactions by factors of at least 1 million.

Consider the reaction catalyzed by carbonic anhydrase

$CO_2 + H_2O \longleftrightarrow H_2CO_3$

The enzyme is responsible for effecting the transfer of CO_2 from the tissues into the blood and then to the alveoli. Each enzyme is capable of hydrating 10^5 molecules of CO_2 in one second. The catalyzed reaction is 10^7 times faster than the uncatalyzed reaction.

ii. They are highly specific both the reaction catalyzed and their choice of reactants. Four distinct types of enzyme specificity have been identified. They include

A . Absolute specificity, where the enzyme catalyzes only one reaction.

B. Group specificity. The enzyme acts only on molecules with specific functional groups.

C. Linkage specificity- The enzyme acts on a particular type of chemical bond regardless of the rest of the molecular structure. E.g Trypsin splits peptide bonds on the carboxyl side of lysine and arginine residues only while thrombin acts only when the side chain on the carboxyl side is arginine while the one on the amino side is glycine.

D. Stereochemical specificity. The enzyme acts on a particular steric or optical Isomer

iii. **They** do not alter reaction equilibria

The combination of substrate and enzyme creates a new reaction pathway whose transition state energy is lower than it would be if the reaction were taking place in the absence of an enzyme. However, in this process, both the forward and reverse reactions are accelerated by precisely the same factor. Thus enzymes accelerate the attainment of equilibria but do not shift their position

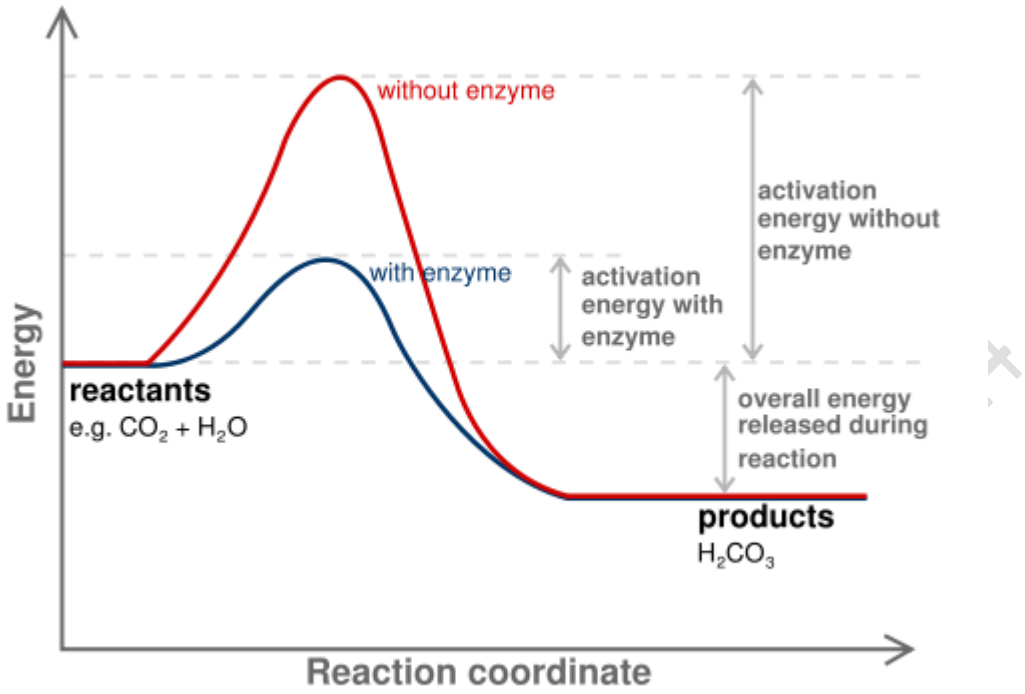


Fig 12.4a: Enzymes do not alter reaction

The enzyme stabilizes the transition state, reducing the energy needed to form products.

Enzymes are stable over only a limited range of Temperature

iv. Generally, a higher temperature brings about an increase in the movement of enzyme and substrate molecules, resulting in more collisions bw the molecules and an d formation of more products.(a 10^{o}C rise in temperature increases the activation energy of the molecules by about 12Kcal/Mol).However, if the temperature rises beyond a certain point (which differs for each enzyme), the enzyme activity levels out and then declines rapidly . This is because the enzyme is denatured by heat. The tertiary structure of the enzyme is altered and it can no longer function. Human enzymes generally exhibit stability at temperatures up to 45-55^{o}C. Thermophilic enzymes may be stable up to or above 100^{o}C.

For mammals and other homoeothermic organisms, changes in enzyme reaction rates with temperature assume physiologic importance only in circumstances such as fever or hypothermia.

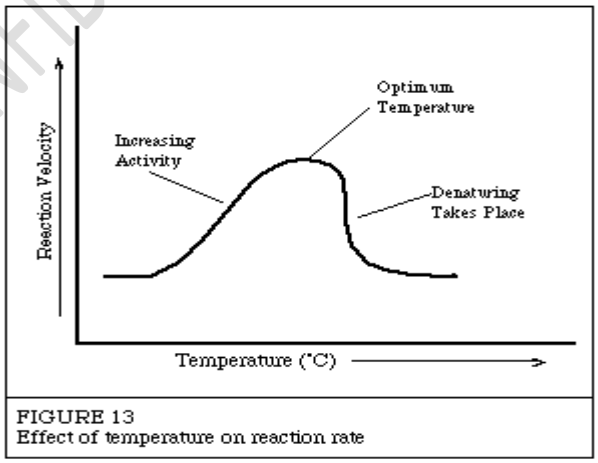


Fig 1.4b: Effect of temperature on reaction rate

V. Enzymes are stable over only a limited range of pH

Each enzyme has an optimal pH range that help maintain its normal configuration in the environment where it operates. Outside this range, changes in the charges on ionizable amino acid residues result in modification of the tertiary structure of the protein and eventually lead to denaturation.Examples of pH optima include the those of the digestive enzymes: Pepsin-2.0, Trypsin-10.0 and Chymotrypsin-8.0

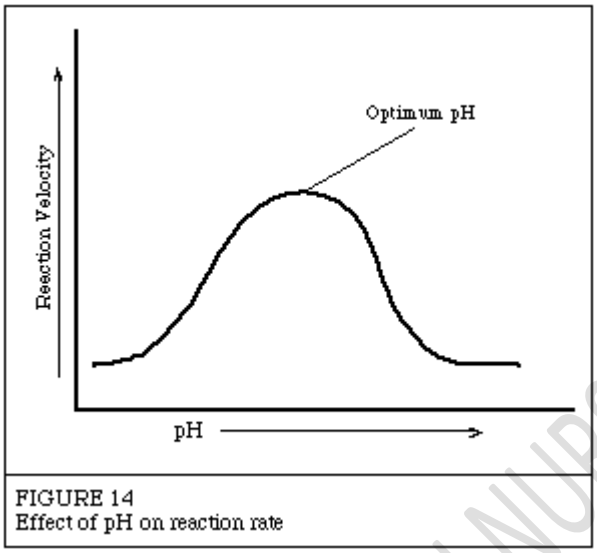


Fig 12.4c: Effect of pH on reaction rate.

Vi . Enzymes are influenced by the concentration of substrate

At very low [S], collisions between enzyme and substrate are infrequent and the reaction proceeds slowly. As [S] increases, reaction rate increases proportionately as collisions become more frequent.

Rate α [A] [B]

When the enzyme begins to approach the maximum rate at which it can combine with substrate, the effect of increasing [S] diminish. At saturation point, the reaction is no longer affected by increase in [S].The rate of the reaction at this point is called maximum velocity. The [S] at which we have V_{max} is called K_m, (Michelis constant) for a particular enzyme.

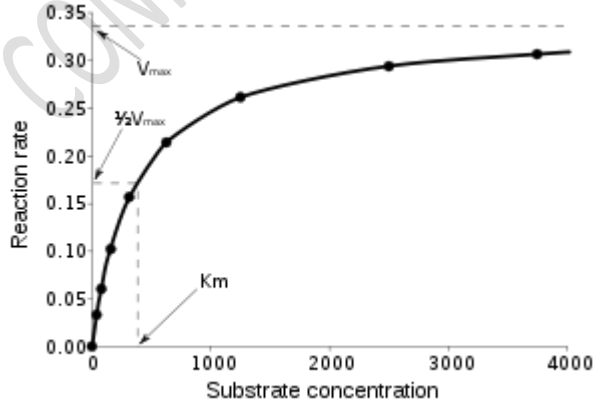


Fig 12.4d: Saturation curve for an enzyme reaction showing the relation between [S] and rate (v).

3.5 Classes of Enzymes

All known enzymes belong to one of 6 main classes:

- **Oxidoreductases**
- **Transferases**
- **Hydrolases**
- **Lyases/Synthases**
- **Isomerases**
- **Ligases/Synthetases**

i. Oxidoreductases

a. They catalyze oxidation – reduction reactions. This includes pairs of donors and acceptors such as saturated-unsaturated carbon-carbon bonds, alcohols-aldehydes, aldehydes-acids and amines-imines e. g alcohol dehydrogenase catalyzes the oxidation of an alcohol to an aldehyde by the removal of 2 electrons and 2 hydrogen atoms.Subclasses include oxidases, oxygenases and peroxidases. Oxidases transfer 2 electrons from a donor to oxygen, resulting in the formation of hydrogen peroxide e.g glucose oxidase.

b. Glucose + O_2→ Gluconolactone + H_2O_2

c. Oxygenases catalyze the incorporation of oxygen into a substrate e.g monooxygenases catalyze formation of a hydroxyl group and dioxygenases incorporate both atoms of O_2 into a substrate. Cytochrome P450 are an important group of enzymes that use oxygen in the metabolism of xenobiotics such as drugs and toxins. Peroxidases utilize H_2O_2 rather than oxygen as the oxidant e.g catalase which utilizes H_2O_2 as both donor and acceptor and functions in the cell to detoxify H_2O_2.

d. $H_2O_2 + H_2O_2 \leftrightarrow O_2 + 2H_2O$.

ii. Transferases

a. Members of this class catalyze a chemical group from one molecule to another, thus they have 2 substrates and 2 products. The major groups transferred include amino, acyl, phosphate and glycosyl. Subclasses include aminotransferases, kinases, glucosyl and phosphoryl transferases and phosphomutases.

b. Aminotransferases transfer an amino group from one amino acid to an α keto acid acceptor, resulting in the formation of a new amino acid and a new keto acid.

c. L-Glutamic acid + pyruvic acid

1. ↓

d. α ketoglutaric acid + L-Alanine

iii. Hydrolases

i. These catalyze hydrolysis reactions i.e the addition of water to cleave a chemical bond. Examples of such bonds include C-O, C-N, O-P and C-S. Subclasses include esterases, phosphatases and peptidases.

iv. Lyases

i. They catalyze reactions in which groups are either removed to form a double bond or are added to a double bond. Subclasses include decarboxylases and dehydratases. Some lyases require pyridoxal phosphate as the cofactor. An example is fumarase, an intermediate of the citric acid cycle.

v. Isomerases

i. Enzymes in this group are involved in moving a group or a double bond within the same molecule. These include cis-trans and aldose- ketose transformations. The enzyme is called a mutase when a phosphate is moved from one carbon to another e.g conversion of 2-phosphoglycerate to 3-phosphoglycerate by phosphoglycerate mutase. Epimerases and racemases change the stereochemistry at a carbon atom. The conversion of D-Lactate to L-Lactate is catalyzed by a racemase while conversion of D-xylulose 5-phosphate to D-ribulose 5-phosphate is brought about by epimerase.

vi. Ligases

i. These enzymes catalyze bond formation between two substrate molecules. Energy is required for the reaction to occur. Typically, the energy is supplied by ATP hydrolysis. They are also called synthetases. For example, to add CO_2 to pyruvate, CO_2 is incorporated into the coenzyme biotin, which requires hydrolysis of ATP. The CO_2 is transferred to pyruvate by the same enzyme.

Table 12.5 Major Enzyme Classes

First EC integer	Enzyme class	Type of reaction catalyzed
1	Oxidoreductases	Oxidation-reduction
2	Transferases	Chemical group transfer. A-X + B→ A+B-X
3	Hydrolases	Hydrolytic cleavages
4	Lyases	Non-hydrolytic cleavage leaving double bonds or addition of groups to a double bond
5	Isomerases	Change of geometric arrangement of a molecule
6	Ligases	Joining together of 2 molecules, accompanied by ATP hydrolysis

4.0 Conclusion

Enzymes of different classifications are involved in different types of chemical reactions in the body. Some factors make stability of the enzymes possible and these must be understood to be able to ensure that these are not contravened.

5.0 Summary

In this unit, you have learnt that enzymes are catalysts of biological systems. There are cofactors to efficiency of enzymes. Enzymes are classified into 6 types and are involved in different types of reactions in the body.

6.0 Tutor Marked Assignments

6.1 Activity – See the Laboratory manual.

6.2 Answer the following questions

i. Explain the role of enzymes as catalysts of biological systems.
ii. Distinguish the 3 types of cofactors required by most enzymes.
iii. Explain how enzymes are named.
iv. Discuss the general properties of enzymes.

v. Enumerate the different classes of enzymes and the types of reactions catalyzed by each class

7.0 References and other resources

1. Murray, R.K., Bender, D.A., Botham, K., M., Kennelly, P.J., Rodwell V.W. and Well, P.A., (2012). Harper's Illustrated Biochemistry (29th Edition) McGraw-Hill Medical
2. Devlin T.M. (2010) Textbook of Biochemistry with Clinical Correlation 7th Edition. JohnWiley & Sons Inc.
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UNIT TWO - ENZYME KINETICS

CONTENT

1.0 Introduction

This unit takes you through the chemical activity of an enzyme and how the activity can be measured using some equations.

2.0 Objectives

At the end of this unit, you should be able to:

i. Define basic terms related to enzyme kinetics
ii. Derive the Michelis- Menten rate equation
iii. State the linear forms of the Michelis- Menten rate equation
iv. Make linear plots and deduce kinetic parameters from a given set of data.

3.0 Main Content

3.1 Enzyme Activity

This can be defined as the amount of enzyme required to convert 1µmol of substrate to product per minute under specified assay conditions.

1U-1micromole/minute. This is the international unit. The SI unit of enzyme activity is the Katal, defined as the amount of enzyme required to convert 1mol of substrate to product per second.

$$1U = 1.67X\ 10^{-8}K = 16.7nK$$

Specific Activity: This is defined as the number of enzyme units per milligram of protein (µmol/min/mg).

Turnover number: This is equal to the units of activity per mole of enzyme. Also called the catalytic enzyme, it allows for direct comparison of catalytic ability between enzymes e. g the

constants for catalase and α amylase are 5 X 10^6 and 1.9 X 10^4 respectively. This indicates that catalase is about 2,500 times more active than α amylase.

Maximum velocity: This is the velocity obtained under conditions of substrate saturation of the enzyme and specified conditions of pH, temperature and ionic strength. V_{max} is a constant for a given enzyme.

3.2 Michelis-Menten rate equation

In 1903, French chemist Henri found that enzyme kinetics was initiated by a bond between enzyme and substrate. His work was taken up by Leonor Michaelis and Maud Menten. They proposed a mathematical model of the reaction. It involves an enzyme E binding to a substrate S to form a complex ES, which in turn is converted into a product P represented as follows:

$S+E \quad ES \rightarrow P+E$ (1)

At steady state, the rate of formation of the ES complex is the same as its rate of breakdown.

$V_{formation} = K_1[S][E]$

$V_{breakdown} = K_2[ES] + K_3[ES]$

$K_1[S][E] = [ES]\{K_2+K_3/K_1\}$ (2)

We can define the ratio of the rate constants as Km i.e $K_m = K_2+K_3/k_1$

K_m is the Michaelis constant

$[S][E] = [ES]K_m$ (3)

[E] is the concentration of free enzyme and is given by the difference between the total enzyme added to the system and any enzyme in the ES complex

i.e $[E] = \{[E_t] - [ES]\} = [ES]K_m$

Dividing through by [S] gives

$\{[Et] - [ES]\} = [ES]\ K_m/[S]$

Dividing thru by [ES] gives

$[E_T]/[ES] -1 = K_m/[S]$ or $[E_T]/[ES] = K_m/[S] +1 = K_m + [S]/[S]$ (4)

When the enzyme is saturated with substrate, all of it will be in the ES complex

$[E_T]$ will be = [ES]

Then, the velocity observed will be the highest possible

$V_{max} = K_3[E_T]$

$[E_T] = V_{max}/ K_3$

When [Et] is not equal to [ES], $V = K_3$ [ES], $[ES] = V/K_3$

$[E_T]/ [ES] = V_{max}/ K_3 / V/K_3 = V_{max}/ V$

$V_{max}/V = K_m + [S]/ [S]$

Or $v = V_{max}[S]/ K_m + [S]$.

This equation is called the Michaelis menten equation

Consider the M.m equation under 3 different conditions

1. When Km>>>[S],
   The equation becomes $V = V_{max}[S]/ Km$, $V = (V_{max}/K_m)$ [S]
   This implies that at low substrate concentrations, the rate is directly proportional to [S]
2. When K_m = [S]
   $V = V_{max}[S]/ 2[S]$ or $v = V_{max}/2$. i.e Km is the substrate concentration at which we have half the maximum velocity.
3. When Km<<<[S]
   $V = V_{max}$ i.e at high [S], $v = V_{max}$.

3.3 Linear forms of the Michelis-Menten Equation

The linear form of the equation is

$1/V = K_m/V_{max}[1/S] + 1/V_{max}$

A plot of 1/V vs 1/[S] yields a straight line whose slope is Km/V_{max} and whose y-intercept is $1/V_{max}$. The intercept on X-axis is $-1/K_m$.

This linear form is called the the LineWeaver-Burk equation and the plot the Lineweaver-Burk plot. It can be used to obtain values of the kinetic parameters for a given enzyme reaction.

K_m is an indication of the affinity of the enzyme for its substrate. Low K_m indicates high affinity and vice versa.

Other linear forms of the M.M equation are the Eadie-Hofstee equation and the Woolf equation.

Eadie-Hofstee- $V = -Km.\ V/[S] + V_{max}$

Woolf- $[S]/V = 1/V_{max}.[S] + Km/V_{max}$

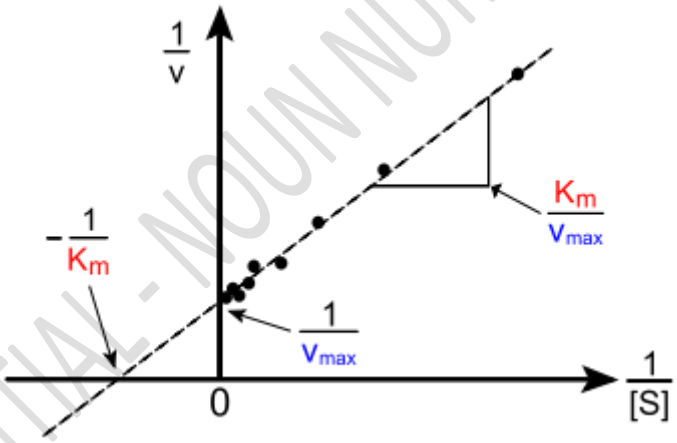

Fig 2.1: Lineweaver-Burk plot

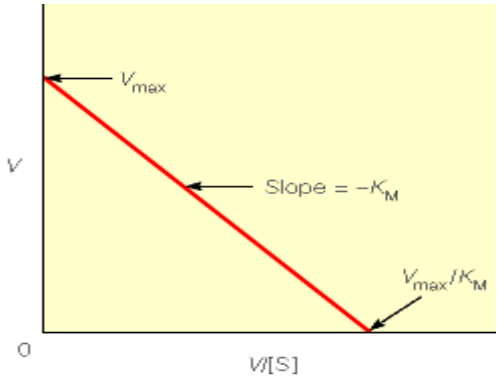

Fig 2.2: Eadie-Hofstee plot

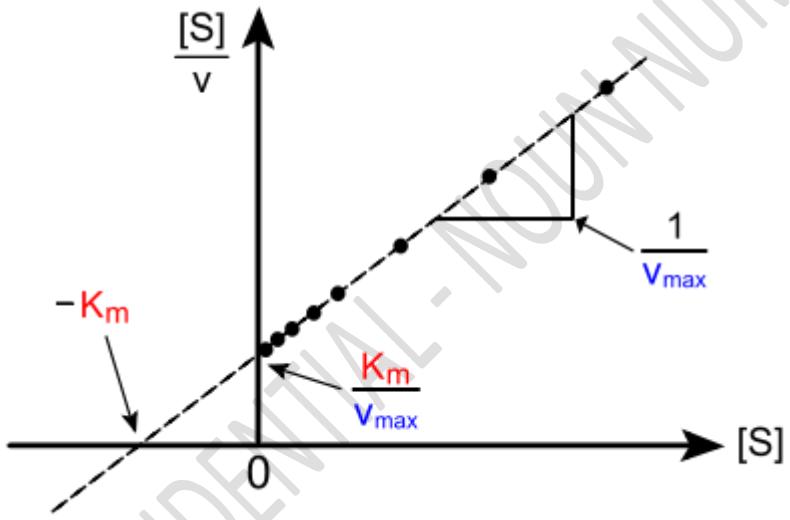

Fig 2.3: Woolf Plot

5.0 Summary

In this unit, you have learnt about the following:

i. Enzyme Activity
ii. Michelis-Menten rate equation
iii. Linear forms of the Michelis-Menten Equation

6.0 Tutor Marked Assignments

6.1 Activity – As prescribed by the facilitator

6.2 Answer the following questions:

i. Define basic terms related to enzyme kinetics
ii. Derive the Michelis- Menten rate equation
iii. State the linear forms of the Michelis- Menten rate equation
iv. Make linear plots and deduce kinetic parameters from a given set of data.

7.0 References and other resources

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UNIT THREE - ENZYMOLOGY 3

CONTENT

1.0 Introduction

Several compounds act as inhibitors of enzyme activity.The study of enzyme inhibitors has provided insight into the mechanism and pathway of catalysis e.g the types of functional group found in the active site and the roles played by these groups. Most pharmaceutical compounds are also designed as enzyme inhibitors. The different types of reversible inhibitors include competitive, uncompetitive and non-competitive inhibitors. They can be distinguished by their effects on the Lineweaver-Burk plot. Some enzymes play regulatory roles in metabolism. They include allosteric enzymes, covalently modulated enzymes and isozymes. Enzymes are very useful tools in clinical diagnosis.

2.0 Objectives

At the end of this unit, you should be able to:

i. Outline and distinguish the different types of reversible enzyme inhibition with specific examples and explain the use of enzyme inhibitors in drug design
ii. Enumerate the effects of the different types of inhibitors on Lineweaver-Burk plots
iii. Explain the regulatory roles of allosteric enzymes , covalently modulated enzymes and isoenzymes
iv. Give examples of enzymes used in clinical diagnosis and their diagnostic importance

3.1 Enzyme Inhibition

Much of the basic knowledge of metabolic pathways was determined by using inhibitors of specific enzymes. Also, through the study of enzyme inhibitors, valuable information has been obtained on the mechanism and pathway of enzyme catalysis, substrate specificity of enzymes, the nature of functional groups at the active site, and the participation of certain functional groups in maintaining the active conformation of the enzyme molecule. Pharmaceutical compounds are also mostly designed as inhibitors of specific enzymes. E.g. penicillin works by preventing completion of a cross link between 2 adjacent peptidoglycan chains in the cell wall. This process is carried out by the enzyme glycopeptide transpeptidase. Disulfiram is a drug used for the treatment of alcoholism. Alcohol is metabolized in 2 steps to acetic acid. The first enzyme, alcohol dehydrogenase yields acetaldehyde, which is then converted into acetic acid by aldehyde dehydrogenase. The latter enzyme has an active site cysteine residue that is irreversibly modified by disulfiram, resulting in accumulation of alcohol and acetaldehyde in the blood. People who take disulfiram become sick because of accumulation of acetaldehyde in blood and tissue, leading to alcohol avoidance.

Also, NSAIDs like Aspirin inhibit cyclooxygenase activity by reversibly blocking the channel for arachidonate in the enzyme. (compounds that inhibit cyclooxygenase have anti-inflammatory activity). Drugs designed to inhibit enzymes unique to a microorganism will produce few side effects in patients e.g sulfa drugs are used b.cos they inhibit folic acid synthesis in bacteria, while humans do not synthesize folic acid..

3 major types of reversible enzyme inhibition are competitive, uncompetitive and non competitive inhibition. They can be experimentally distinguished by the effects of the inhibitor on the reaction kinetics of the enzyme, which may be analyzed in terms of the basic Michaelis Menten rate equation.

Competitive Inhibition

Here, the inhibitor combines with the free enzyme in such a way that it competes with the normal substrate for binding at the active site. It reacts reversibly with the enzyme to form an enzyme-inhibitor complex (EI), analogous to the ES complex.

$E+I \leftrightarrow EI$

A competitive inhibitor diminishes the rate of catalysis by reducing the proportion of enzyme molecules that have a bound substrate.

The inhibitor constant Ki can be defined as the dissociation constant of the EI complex i.e

$K_i = [E][I]/[EI]$

The classic example of competitive inhibition is the inhibition of succinate dehydrogenase by malonate and other dicarboxylate anions. SDH is one of the enzymes responsible for the reactions of the TCA cycle. It catalyzes the removal of 2 hydrogen atoms from the 2 methylene carbon atoms of succinate.The competitive inhibitor malonate resembles succinate in having 2 ionized carboxyl groups at PH 7.0.Besides malonate other dicarboxylate anions e.g oxalate and oxaloacetate may act as inhibitors of SDH.

The effect of a competitive inhibitor can be reduced or totally overcome by increasing [S].In this type of inhibition, Km is increased while Vmax remains the same.

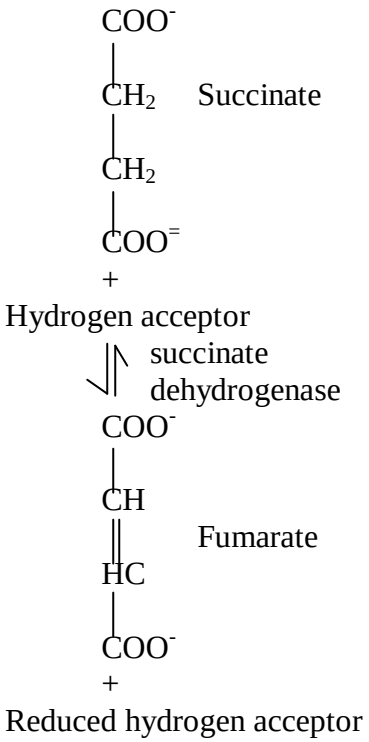


Fig 3.1.1 The Succinate dehydrogenase reaction

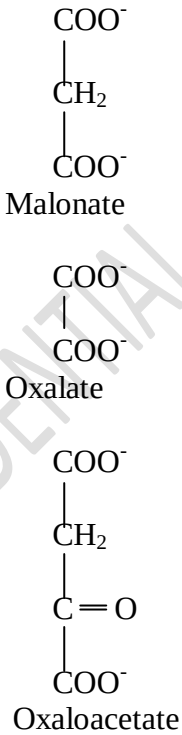


Fig 3.1.2: Other inhibitors of Succinate dehydrogenase

Uncompetitive inhibition

Here, the inhibitor does not combine with the free enzyme or affect its reaction with its normal substrate. Rather, it combines with the ES complex to give an inactive ESI complex which cannot undergo further reaction to yield the normal product

ES+ I↔ESI
Ki= [ES][I]/ [ESI]
Both Km and Vmx decrease in this type of inhibition. It is common in two-substrate reactions.

Non competitive inhibition

A non competitive inhibitor can combine with either the free enzyme or the ES complex, interfering with the action of both. Non competitive inhibitors bind to a site on the enzyme different from the active site, often to deform the enzyme so that it does not form the ES complex, and if formed, it does not decompose at the normal rate to yieldp roducts. These effects are not reversed by increasing the substrate concentration. In non competitive inhibition, the reaction with the inhibitor yields 2 inactive forms- EI and ESI

E+I↔EI
ES+I↔
For which there are 2 inhibitor constants
K^{EI}= [E][I]/ [EI]
K^{ESI}= [ES] [I]/ [ESI] which may or may not be equal.
Vmax is decreased while Km remains constant in this type of inhibition.
Examples include the inhibition of enzymes that require metal ions for activity by agents capable of binding the metal e.g EDTA reversibly binds Mg2+ and other divalent cations and thus noncompetitively inhibits some enzymes requiring such ions for activity

3.2 Effects of Inhibitors on Lineweaver-Burk plot

Effect of A Competitive Inhibitor
Lineweaver-Burk plot

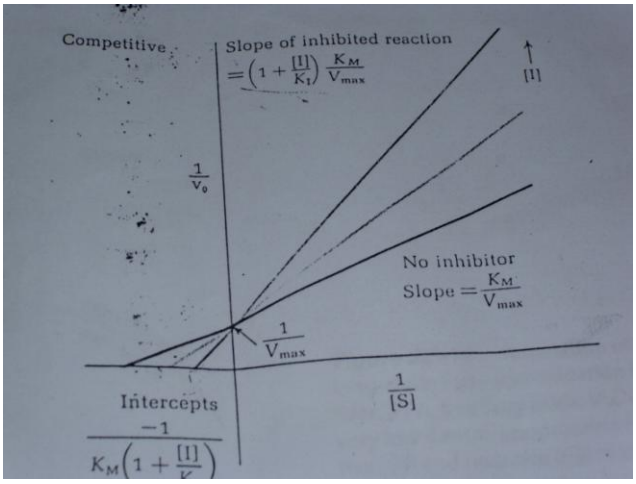
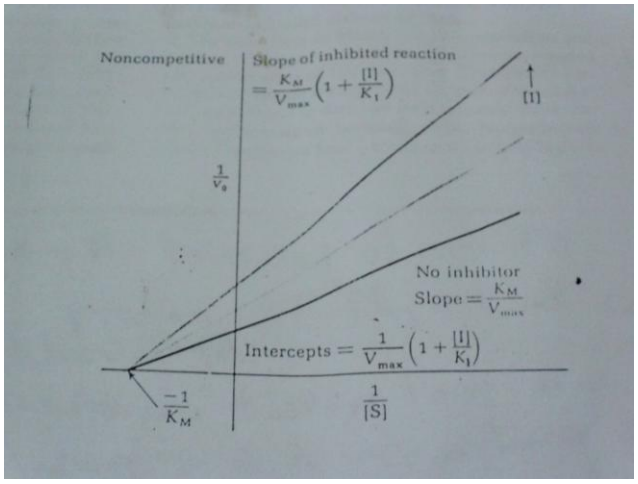


Fig 3.2:

Effect of a Non-Competitive Inhibitor on Lineweaver-Burk Plot



Effect of an Uncompetitive Inhibitor on Lineweaver-Burk Plot

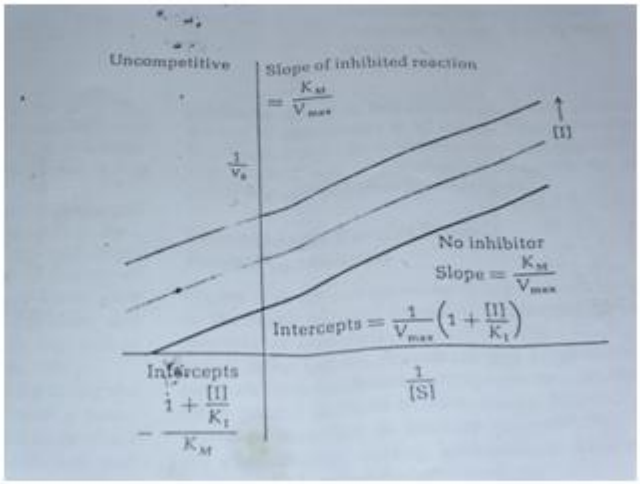


Fig 3.3:

Table 1.1: Summary of the effects of inhibitors on Lineweaver –Burk plots $1/V_0$ vs $1/[S]$

	Slope	Y-intercept
No inhibitor	K_m/V_{max}	$1/V_{max}$
Competitive	$K_m/ V_{max}(1+ [I]/K_i$	$1/V_{max}$
Uncompetitive	K_m/V_{max}	$1/V_{max}(1+[I]/K_i$
Noncompetitive	$K_m/ V_{max}(1+ [I]/K_i$	$1/V_{max}(1+[I]/K_i$

Irreversible Inhibition

Some enzymes undergo irreversible inactivation when they are treated with agents capable of covalently and permanently modifying a functional group required for catalysis, making the enzyme molecule inactive. This type of inhibition cannot be treated by M.M principles. Often, the inhibition sets in slowly compared with the normal reaction kinetics of the enzyme, so that the inhibition is incomplete at first but continuously increases with time because chemical modification of an increasing fraction of the enzyme molecule takes place. E.g alkylating agents such as iodoacetamide irreversibly inhibit the catalytic activity of some enzymes by modifying essential cysteine residues. Heavy metals, such as mercury and lead salts, also inhibit sulfhydryl enzymes. Eggs or egg-white are sometimes administered as an antidote for accidental ingestion of heavy metals: the egg white protein, ovalbumin is rich in sulfhydryl groups, traps the free metal ions and prevents their absorption from the GIT.

In many cases, irreversible inhibitors are used to identify active site residues involved in enzyme catalysis and to gain insight into the mechanism of enzyme action. By sequencing the protein, it is possible to identify the specific amino acid residue modified by the inhibitor and involved in catalysis.

3.3 Regulation of Enzyme Activity

Some enzymes possess special properties which endow them with regulatory roles in metabolism. Such enzyme forms are called regulatory enzymes. There are 2 major types of regulatory enzymes- allosteric enzymes and covalently modulated enzymes.

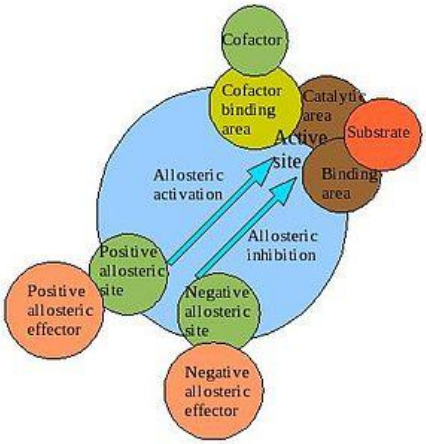
Allosteric regulation

Here, the catalytic site of the enzyme is modulated through the non covalent binding of a specific metabolite at a site on the protein other than the catalytic site. Most enzymes which are subject to such modification are rate-determining enzymes in metabolic pathways. Metabolites that bind at the allosteric site are called allosteric effectors, modifiers or modulators. Those that enhance the protein's activity are referred to as *allosteric activators*, whereas those that decrease the protein's activity are called *allosteric inhibitors*. The term *allosteric* comes from the Greek *allos* , "other", and *stereos* , "solid (object)", in reference to the fact that the regulatory site of an allosteric protein is physically distinct from its active site. The allosteric site at which the positive inh binds is referred to as an activator site while the negative inh binds at an inhibitory site. When an allosteric enzyme has only one specific modulator, it is said to be monovalent. Others which respond to 2 or more modulators, each bound to a specific site on the enzyme molecule are polyvalent. Most allosteric enzymes are oligomeric i.e they consist of several subunits. They consist of 2 or more polypeptide chain subunits, usually in an even number. Allosteric enzymes usually exhibit sigmoidal (S-shaped) kinetic curves rather than simple hyperbolic curves. Activators of allosteric enzymes shift the V vs S curve to the left, whereas allosteric inhibitors shift the curve to the right. A homotropic allosteric modulator is a substrate for its target enzyme, as well as a regulatory molecule of the enzyme's activity. It is typically an activator of the enzyme. On the other hand, a heterotropic allosteric modulator is a regulatory molecule that is not also the enzyme's substrate. It may be either an activator or an inhibitor of the enzyme.

End Product inhibition

There are many instances in which the final end product of a multienzyme metabolic pathway is an allosteric inhibitor of an enzyme that catalyzes an early and irreversible step of the pathway. This form of allosteric regulation prevents accumulation of additional end product and of metabolic intermediates once a cell has sufficient supplies of that metabolic end product. An example of this is Aspartate transcarbomylase, the enzyme that catalyzes the first reaction unique to pyrimidine biosynthesis.The enzyme is a multi subunit protein complex composed of 12 subunits (C6R6) forming 2 trimers of catalytic subunits and 3 dimers of regulatory subunits. It is feed-back inhibited by the end product of the pyrimidine pathway,

CTP . Following treatment with mercurials, ATCase loses its sensitivity to inhibition by CTP but retains its full activity for synthesis of carbamoyl aspartate. This suggests that CTP is bound at a different site from the substrate.



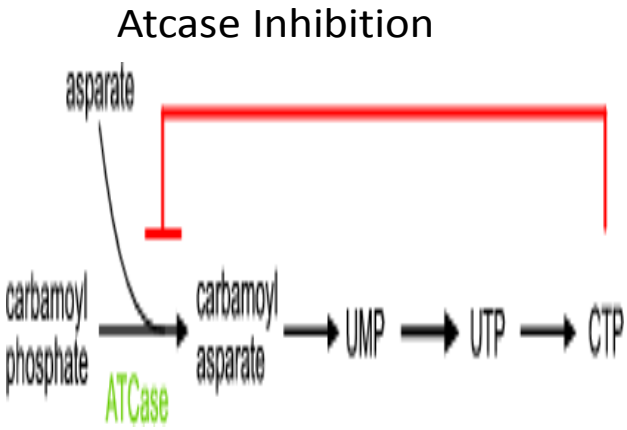


Fig 3.3: Allosteric Regulation

Covalently modulated regulatory enzymes

Here, active and inactive forms of the enzyme are interconverted by covalent modifications of their structure, catalyzed by other enzymes. A typical example is glycogen phosphorylase which catalyzes the breakdown of glycogen to yield Glucose 1 –phosphate

(Glucose)n + Pi ↔ (Glucose)n-1 + Glucose1 –Phosphate

The enzyme occurs in 2 forms- phosphorylase a, the more active form and Phosphorylase b, the less active form. A is converted to b through hydrolytic removal of phosphate groups attached to 4 serine residues contained in each of its subunits, brought about by the action of phosphorylase phosphatase. The b form can be reactivated to a form by the enzyme phosphorylase kinase. Thus, the activity of the enzyme is regulated by the action of 2 enzymes that shift the balance bw its active and inactive forms. Generally, many protein kinases regulate the activity of enzymes and other proteins by phosphorylation-dephosphorylation of serine, threonine or tyrosine residues on the proteins. Other groups

including sulphate and acetate can also be added to an enzyme to alter its activity. Covalent modification is an effective and rapid way of controlling the activity of a protein or enzyme.
Another way by which enzyme activity is regulated is the enzyme-catalyzed activation of inactive precursors of enzymes (zymogens) to yield the catalytically active forms as is found in the digestive enzymes pepsinogen, trypsinogen and chymotrypsinogen. These enzymes are converted into their active forms by the selective hydrolytic cleavage of one or more specific -`peptide bonds in the zymogen molecule as follows.
Pepsinogen→ Pepsin + Peptides (catalyzed by pepsin)
Trypsinogen →Trypsin + hexapeptides (catalyzed by enterokinase)
Chymotrypsinogen→ Chymotrypsin + 2dipeptides(catalyzed by trypsin)
This type of covalent regulation proceeds in only one direction i.e the enzymes cannot be converted back to their respective zymogens.

3.4 Clinical Applications of Enzymes

Many tissues produce enzymes that are relatively cell-specific. Because these enzymes are released into the circulation as a result of tissue damage, assays of the levels of certain enzymes in blood can provide useful diagnostic information.For example, ALT and AST are present in high concentrations in hepatocytes. When these cells are injured e.g by viral hepatitis or drug overdose, ALT and AST are released into the blood. Several other enzymes serve as tissue specific markers of cellular injury.
Isozymes are also very important in diagnosis. These are multiple forms of a given enzyme that occur within a single species of an organism or a single cell. They differ in amino acid composition and thus in their isoelectric properties. They can be separated from each other by electrophoresis. They also differ in their kinetic parameters and their physical properties. Isozymes with wide clinical applications include Lactate dehydrogenase, creatine kinase and Alkaline phosphatase. LDH occurs as 5 different isozymes in the tissues of vertebrates. They all catalyze the reaction
Lactate + NAD+ ↔Pyruvate + NADH + H^+
They have the same molecular weight and all contain 4 polypeptide chains. They consist of 5 different combinations of 2 types of PP chains designated M and H as follows

Type

Type	Composition	Location
LDH1	H4	Myocardium and Rbcs
LDH2	H3M	,, ,,
LDH3	H2M2	Brain and Kidney
LDH4	HM3	
LDH5	M4	Liver and skeletal muscle

Although they catalyze the same reaction, they differ significantly in their Km and Vmax values for substrates , particularly pyruvate . M4 has a relatively low Km for pyruvate while H4 has the highest Km for this substrate. The others have kinetic properties that are intermediate bw those of the M4 and H4 isozymes, in proportion to their relative content of M and H chains. These characteristics provide insight into the function of the isozymes. Skeletal muscle and embryonic tissue tend to utilize glucose anaerobically and break it down to form lactate. Thus, the M4 isozyme is well adapted to convert pyruvate rapidly to Lactate. Heart muscle on the other hand does not normally form Lactate from Glucose.LDH isozymes are important in the diagnosis of heart and liver disease. Normally, blood contains little LDH1, but the level increases after a myocardial infarction. Increase in LDH5 is indicative of a secondary liver damage in the patient. Thus, measurement of these isozymes allows monitoring of secondary complications of heart failure .

Table 12.4 Serum enzymes used in clinical diagnosis

Serum Enzyme	Major Diagnostic Use
AST	Myocardial infarction,
ALT	Viral Hepatitis
A-Amylase	Acute Pancreatitis
Ceruloplasmin	Hepatolenticular degeneration (Wilson's
Creatine Kinase	Muscle disorders and myocardial infarcti
γ-Glutamyl transpeptidase	Various Liver diseases
LDH isozymes	Myocardial infarction
Lipase	Acute Pancreatitis
Acid phosphatase	Prostate Cancer
Alkaline Phosphatase isozymes	Various bone disorders, obstructive liver

4.0 Conclusion

5.0 Summary

In this unit, you have learnt about the following:

i. Enzyme Inhibition
ii. Effects of Inhibitors on Lineweaver-Burk plot
iii. Regulation of Enzyme Activity
iv. Clinical Applications of Enzymes

6.0 Tutor Marked Assignments

6.1 Activity – As provided by the facilitator

6.2 Answer the following questions:

i. Distinguish the different types of reversible enzyme inhibition with specific examples and explain the use of enzyme inhibitors in drug design
ii. Enumerate the effects of the different types of inhibitors on Lineweaver-Burk plots

iii. Explain the regulatory roles of allosteric enzymes, covalently modulated enzymes and isoenzymes
iv. Give examples of enzymes used in clinical diagnosis and their diagnostic importance.

7.0 References and other resources

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