Principles of Biochemistry I

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1.0 Biochemistry and the Language of Chemistry

Objectives

1. Know the properties of life
2. Know the macromolecules and their monomeric components
3. Understand the hierarchy of biomolecules
4. Know the differences between prokaryotes and eukaryotes

1.1 The Science of Biochemistry

The roots of biochemistry can be traced thousands of years into human history, long before we understood the scientific mechanisms. Before the work of Friedrich Wohler, it was believed that substances were either of biological or nonliving origins, and that the two were fundamentally different. Wohler's 1828 breakthrough was to synthesize urea from ammonium cynate, proving that the difference between types of substances may be less than previously thought.

A second important development in the field of biochemistry was that of Louis Pasteur, who devised methods for excluding bacteria in fermentation mixtures. Though still believing in "vitalism", the view that biological reactions require a supernatural force, Pasteur recognized that certain organisms provide different products in fementation, such as yeast's ability to convert sugar into alcohol in winemaking.

Through the early work in genetics by Gregor Mendel, and later the advances in our knowledge of DNA by James Watson and Francis Crick, it was recognized that DNA provided a storage mechanism for an enormous amount of genetic information.

Biochemistry is largely a science that depends on results of experiments, unlike more theoretical sciences such as physics. Biochemistry has benefited greatly from technological advances that allow more information to be elucidated in experiments. For example, X-ray diffraction was key to the discovery of DNA's double helix structure.

1.2 The Elements and Molecules of Living Systems

An astounding fact of biochemistry is that it is only possible through the death of stars. The early universe was abundant in only the lightest elements, which condensed into stars and formed into heavier elements through thermonuclear reactions in stars. These elements are released when the star dies and recondenses into planets and smaller stars.

Life depends heavily on elements that are only created in these stellar forges, such as Carbon, Oxygen, Nitrogen, and Phosphorous. These elements existed in a "primodial soup" for billions of years on Earth, before some event was able to assemble these simple molecules into a self-replicating structure. It is believed RNA may be based on these early stuctures.

Simple molecules are usually combined into macromolecules in lifeforms, either in biopolymers or lipids. Biopolymers are carbohydrates, proteins, and nucleic acids that have assembled into larger chain-like structures. Lipids, or fats, are hydrogen-carbon chains that are important in cellular functioning.

1.3 Distinguishing Characteristics of Living Systems

What is life? The answer is debated between biochemists even today, but one biochemist, Daniel Koshland, suggests there are seven "pillars" of life. The pillars, such as "improvisation" and "regeneration", provide a framework for determining life. However, these pillars are not agreed on by all biochemists, and viruses pose an interesting example where not all scientists agree are alive.

1.4 The Unit of Biological Organization: The Cell

In 1665, Robert Hooke indentified a cellular structure in plants that we know today as cell walls. When Theodor Schwann discovered evidence that animals are also made up of cells, it was theorized that all organisms are either cells or groups of cells, which we know is true today.

The main types of cells are prokaryotes and eukaryotes. Prokaryotic cells lack internal subdivisions, or organelles, that eukaryotes posesses. Organisms can be single cells, or multicellular, consisting of many different types of cells.

1.5 Biochemistry and the Information Explosion

Technology and information have been very beneficial for biochemistry. Through knowledge of chemical reactions and biological processes, biochemists have been able to identify important pathways of metabolism.

Other related fields have also benefited, such as bioinformatics, genomics, and proteomics. The future for biochemistry is bright, with cutting-edge technology such as synthetic biology on the horizon.

2.0 The Chemical Foundation of Life

Objectives

1. Understand the importance of water
2. Know the physical properties of water
3. Understand the hydrophobic effect in thermodynamic terms
4. Know weak interactions in biomolecules
5. Understand the basic acid-base chemistry
6. Know the Henderson-Hasselbalch equation and be able to use it in buffer systems

2.1 The Importance of Noncovalent Interactions in Biochemistry

While covalent interactions are absolutely needed for many biological processes, noncovalent bonds are extremely important as well. For example, the sequence of DNA is maintained by covalent bonds, while the structure itself is a result of noncovalent forces.

Noncovalent interactions are substantially weaker than covalent bonds, usually on the order of 10 to 100 times weaker. However, this weakness is essential for life, as it allows bonds to be formed, broken, and reformed without a huge input of energy.

2.2 The Nature of Noncovalent Interactions

Noncovalent interactions are electrostatic in nature, depending on electric charges to determine attractive and repulsive forces. There are three main types of noncovalent bonds:

1. Charge-Charge Bonds (13-17 kJ/mol)
2. Dipole and Induced Dipole Bonds (0.4-0.8 kJ/mol)
3. Hydrogen Bonds (2-21 kJ/mol)

Charge-charge bonds, also know as ionic bonds or salt bridges, are present in molecules with net electrical charges. A molecule with a net positive electrical charge is attracted to molecules with net negative electrical charges, and this interaction is common in biological environments.

Dipole and induced dipole interactions are much weaker forces present in molecules with no net electrical charge, but have asymmetric internal charge distributions. An example is water, a polar molecule with no net charge. These forces are also known as Van Der Waals interactions, or London Dispersion forces.

The last noncovalent bond is the hydrogen bond, a typically weak interaction that can become very powerful in certain molecule structures. Hydrogen bonds are the most important factor for explaining the unusual features of water compared to similar molecules.

2.3 The Role of Water in Biological Processes

Water is a unique substance among similar molecules, primarily due to hydrogen bonding. Water remains liquid at much higher temperatures than other small molecules that rapidly turn into gases in typical environments on Earth.

Water also has a higher heat capacity, and is less dense when solid, all due to the effects of hydrogen bonds. These properties are very important for life. For example, if solid water was more dense than liquid water, like most substances, the polar ice caps would not float, which could cause extreme cooling of the planet as ice would be insulated under water.

Water is also an excellent solvent for ionic compounds due to its polarity and ability to act as both an acid and base. It is no surprise that living organisms are largely made of water due to its versatility as an ionic solvent.

2.4 Acid-Base Equilibria

Using the Bronsed-Lowry definition of acids and bases as proton donors and acceptors, respectively, water can act as both an acid and base under different conditions.

Most biochemical processes take place in a fairly narrow range, defined by a pH in the range of 6.5-8.0, known as the "physiological pH range". To maintain this narrow range, lifeforms use chemical buffers to reduce changes in pH.

Buffers are combinations of weak acids and their conjugate bases in ratios appropriate for absorbing ions from the environment while reducing the change in pH.

2.5 Interactions Between Macroions in Solution

Macroions are large polyelectrolytes, such as nucleic acids, that may carry net electrostatic charges. Changes in pH can influence these forces and allow molecules to interact as they get closer together.

These interactions can be strongly influenced by small ions such as salts. For this reason, biochemists must pay close attention to both ionic strength and pH.

3.0 Free Energy

Objectives

1. Know the types of systems and how it applies to living system
2. Understand “high-energy” compounds and coupled reactions
3. Know how to use free energy equations and predict the direction of the reactions
4. Know how to calculate free energy for redox reactions
5. Understand the concept and the process of equilibration

3.1 Free Energy

Biochemical processes are driven by thermodynamic forces that govern the chemical reactions. There are two important laws of thermodynamics that are especially relevant to this branch of biochemistry (bioenergetics):

1. Energy is conserved inside an isolated system
2. Systems proceed towards maximum entropy

Thermodynamic systems can be defined as isolated, open, or closed,and consists of the system itself and its surroundings.

Energy is defined as the ability to do work in a system, and Free Energy (ΔG) is defined as the _available_ energy in a system. From ΔG we can determine if a process requires energy or releases it, and how much energy is released.

Enthalpy (ΔH) is the measure of heat released by a reaction under constant pressure:

$$\Delta H = H * final - H * initial$$

Entropy is a measure of the disorder in a system, or stated another way, how randomly arranged its components are:

Enthalpy and entropy are the primary state functions involved in bioenergetics and can tell us if a reaction is favorable, the direction of the reaction, and the energy required or released.

3.2 Free Energy: The Second Law In Open Systems

In organisms, energy and matter are constantly exchanged with the environment, and so are not isolated systems. Living organisms must maintain a complex set of reactions that require ongoing energy input from the environment. In turn, organisms release heat that increases the entropy of the universe.

Reactions that are favorable at a constant temperature and pressure have negative free energy, and are said to be _exergonic_ reactions. In contrast, _endergonic_ reactions have a positive ΔG and are not favorable at the given conditions.

3.3 The Relationships Between Free Energy, the Equilibrium State, and Non-Equilibrium Concentrations of Reactants and Products

Equilibrium is a condition where a system has achieved a state of minimal energy, and the system will tend to remain at equilibrium until an external force is applied. Even with outside influence, systems will eventually tend towards the equilbrium state again, known as Le Chatelier's Principle.

Changes in equilbrium can be caused by changes in concentration gradients, which will influence the value of ΔG. Many biological processes work by maintaining for altering a concentration gradient, such as sodium and potassium transport in neurological processes.

3.4 Free Energy in Biological Systems

Every biological process must be thermodynamically favorable. Often, reactions are not favorable at biochemical standard conditions, and so a process is needed to modify the conditions of the reaction or the environment. Coupling an unfavorable reaction to a highly favorable one can allow the reaction to proceed under standard conditions.

ΔG can also be calculated for a class of reactions known as reduction and oxidation reactions (redox).

$$\Delta G^{\circ\prime} = -nF\Delta E^{\circ\prime} = -nF[E^{\circ\prime}_{(e^- acceptor)} - E^{\circ\prime}_{(e^- donor)}]$$

4.0 Nucleic acids

Objectives

1. Know functions and structures of nucleotides
2. Know functions and structures of nucleic acids
3. Understand nucleic acid sequencing
4. Understand cloning, PCR, and DNA fingerprinting

4.1 Nucleic Acids - Informational Macromolecules

Deoxyribonucleic acid (DNA) and Ribonucleic Acid (RNA) are some of the most important molecules in biochemistry. These molecules are know as polynucleotides, polymers linked by phosphodiester bridges, and form the basis for all genetic information in organisms.

DNA and RNA are formed from nucleotides, which are formed from nucleosides linked to a phosphate group. All nucleotides contain three main features:

1. A purine or pyrimidine base
2. A deoxyribose or ribose sugar
3. A phosphate bond

Purine Bases

- Adenine
- Guanine

Pyrimidine Bases

- Cytosine
- Thymine
- Uracil

4.2 Primary Structure of Nucleic Acids

Polynucleotide chains have some distinct and important primary features, including *directionality* and *individuality*.

Directionality refers to the fact that the phosphodiester link is always between 3' and 5' carbons, which gives the chain two distinguishable ends.

Individuality refers to the specific sequence of nucleotide bases, also known as the primary structure of the nucleic acid.

4.3 Seconday and Tertiary Structures of Nucleic Acids

The secondary structure of a nucleic acid is the three-dimensional structure of the individual nucleotide bases, while the tertiary structure is the description of longer-range interactions such as supercoiling.

In DNA, the secondary structure is the famous double helix, deduced by scientists Watson and Crick using data such as the statistical distribution of base pairs and X-ray analysis.

The DNA commonly found in cells is known as B form, or B Helix, and is the form Watson and Crick originally studied. However, another tertiary form exists, A form, seen in double-stranded RNA molecules and DNA-RNA hybrids. DNA also often forms structures, and tightly coiled states known as *supercoiled* molecules.

4.4 Alternative Secondary Structures of DNA

Both DNA and RNA have additional alternative secondary structures. Left-handed DNA (Z-DNA) is a form of DNA that forms a zig-zag structure instead of the typical double-helix. Other atypical forms include double hairpins (cruciforms), triple helices, and g-quadruplexes.

4.5 The Helix-to-Random Coil Transition

Environmental conditions such as temperature can cause loss of secondary structure in nucleic acids, a process called denaturation. This change is often reversible, and under controlled conditions can reform in a process called renaturation, or annealing.

4.6 The Biological Functions of Nucleic Acids

DNA and RNA are required for three major biological processes, *replication*, *transcription*, and *translation*. In replication, DNA molecules are copied when a cell divides, providing each with a high-fidelity transmission of genetic information.

RNA is used in transcription in the form of messenger RNA (mRNA) to create complementary bases from DNA molecules. These mRNA molecules are then processed in translation, a process that creates amino acids from specific three-base sequences known as codons.

Cloning is a process that extracts DNA sequences from a parent cell, processes it and links it together with additional manufactured fragments, and introduces it into a host cell. In this way, genes, or even entire genomes, can be copied.

When a single strand of genetic material needs to be copied, biochemists can rely on a process known as polymerase chain reaction (PCR). This technique combines short DNA strands (primers) and a DNA polymerase in a repeated heating and cooling cycle to encourage formation of specific complementary sequences.

Since the advent of cheaper and more reliable DNA sequencing techniques, genetic material is now often used to identify individuals and unique DNA characteristics. This widely used laboratory technique is known as DNA fingerprinting.

5.0 Introduction to Proteins

Objectives

1. Know the structures and be able to draw and name all AA's
2. Know the pKa ranges of all AA's
3. Understand ionization and be able to calculate isoelectric points (pI's) of oligopeptides
4. Read how the nonstandard AA's are made and used
5. Know the polypeptide formation and understand the polypeptide diversity
6. Understand protein purification techniques
7. Understand protein sequencing
8. Read about the protein evolution

5.1 Amino Acids

During the cellular translation process, mRNA is decoded into a sequence of amino acids, which may go on to polymerize and form full proteins. These 20 common (and 2 rare) amino acids are coded for in our genetic code. Amino acids come in three predominate forms, nonpolar, polar-charged, and polar-uncharged:

	Name	Abbreviations	pKa1 (-COOH)	pKa2 (-NH3)	pI
Nonpolar Aliphatic	Glycine	Gly, G	2.34	9.60	5.97
	Alanine	Ala, A	2.23	9.69	6.00
	Valine	Val, V	2.32	9.62	5.96
	Leucine	Leu, L	2.36	9.60	5.98
	Isoleucine	Ile, I	2.36	9.60	6.02

	Name	Abbreviations	pKa1 (-COOH)	pKa2 (-NH3)	pI
Nonpolar	Proline	Pro, P	1.99	10.60	6.30
	Methionine	Met, M	2.28	9.21	5.74

	Name	Abbreviations	pKa1 (-COOH)	pKa2 (-NH3)	pI
Nonpolar Aromatic	Phenylalanine	Phe, F	1.83	9.13	5.48
	Tyrosine	Tyr, Y	2.20	9.11	5.66
	Tryptophan	Trp, T	2.83	9.39	5.89

	Name	Abbreviations	pKa1 (-COOH)	pKa2 (-NH3)	pI
Polar	Serine	Ser, S	2.21	9.15	5.68
	Cysteine	Cys, C	1.96	10.28	5.07
	Threonine	Thr, T	2.09	9.10	5.60
	Asparagine	Asn, N	2.02	8.80	5.41
	Glutamine	Gln, Q	2.17	9.13	5.65

	Name	Abbreviations	pKa1 (-COOH)	pKa2 (-NH3)	pI
Polar (+)	Histidine	His, H	1.82	9.17	7.59
	Lysine	Lys, K	2.18	9.21	5.74
	Arginine	Arg, R	2.17	9.04	10.76

	Name	Abbreviations	pKa1 (-COOH)	pKa2 (-NH3)	pI
Polar (-)	Aspartic Acid	Asp, D	1.88	9.60	2.77
	Glutamic Acid	Glu, E	2.19	9.67	3.22

In the table above, pI represents the isoelectric point, the pH at which the molecule has a neutral overall charge and the zwitterion form is dominant.

The isoelectric point can be calculated for oligopeptides by assuming each peptide is fully protonated (pH=0) and incrementing charges on each group as the pH is raised and groups are deprotonated until the net charge is zero.

In addition to the amino acids listed above, there is a group of "nonstandard" amino acids that can be created in other organisms, as well as post-translational modification of the standard amino acids.

5.2 Peptides and the Peptide Bond

Amino acids may be convalently bonded to each other via amide, or peptide bonds, in structures known as polypeptides. These bonds form between the α-carboxylic acid group in one amino acid and the α-amino group in another.

5.3 Proteins: Polypeptides of Defined Sequence

When polypeptides reach a defined length and sequence they're referred to as proteins. This sequence of peptides is defined as the primary structure of the protein.

Determining proteins requires analysis which was tranditionally accomplished using protein purification methods such as anion exchange chromatography (IEC) and size exclusion chromatography (SEC). These techniques use features of the proteins, such as pI, polarity, solubility, and more, to purify proteins for further analysis.

5.4 From Gene to Protein

Proteins are ultimately determined by genetic sequences in a series of well-known steps. DNA is transcribed into sequences of mRNA, which is then translated into amino acids. These amino acids form peptides and polypeptides, eventually forming proteins in the body.

5.5 From Gene to Sequence to Protein Function

Though biochemistry has been historically more concerned with studying functions and structures of macromolecules, disciplines such as systems biology have recently started delving deeper into the function of proteins.

In the body, thousands of molecules may form complex interactions. In contemporary biochemistry, computers are being used to create simulations of these interactions to help determine the functions of proteins and gene sequences.

5.6 Protein Sequence Homology

Protein sequencing is a process that evaluates the total amino acid composition and other metrics to determine relationships between proteins and genetic sequences. Sequences are reconstructed from polypeptides that have been degraded using Edman Degradation followed by a polypeptide cleavage procedure.

Protein sequences are also used to determine evolutionary relationships between organisms. By comparing distributions of proteins, genetic similarities can be deduced that may provide information about evolutionary ancestry. Phylogenetics is the science concerned with mapping these relationships.

6.0 The Three-Dimensional Structure of Proteins

Objectives

1. Know the 4 levels of structural complexity of proteins
2. Understand the properties of a peptide bond
3. Know the a helix and b sheet structures
4. Know the structures of a keratin, b silk fibroin, and collagen
5. Be familiar with coils, bulges, turns, loops, and cross-overs
6. Read about X-ray crystallography and NMR
7. Be familiar with common supersecondary motifs
8. Understand the forces holding 3D & 4D structures
9. Understand protein folding

6.1 Secondary Structure

Proteins have four levels of structural complexity:

- **Primary (1°)** structure is the amino acid sequence
- **Secondary (2°)** structure is local areas of protein chain structure
- **Tertiary (3°)** structure is the arrangement of secondary structural elements
- **Quaternary (4°)** structure is the arrangement of polypeptides to form multisubunit complexes

Common secondary protein structures:

- α helices
- β sheets
- Reverse turns
- β bulges
- Coils
- Ω loops

Both α helices and β sheets are important structural units that together form more complex arrangements of proteins. In fact, these arrangements are the most commonly observed secondary structures in proteins.

Reverse turns are regions where a polypeptide makes a sharp turn, and are common at the surface of globular proteins. β bulges are areas of dense Hydrogen bonding of β sheets.

Coils are secondary structures where polypeptides wrap around themselves or other polypeptides. Ω loops are strands of amino acid sequences that loop back on themselves, forming a shape similar to the Greek letter Omega (Ω). These structures may then go on to form supersecondary structures called motifs.

6.2 Fibrous Proteins

Some proteins, known as "fibrous" proteins, are remarkable for their filamentous forms. These proteins form many of the hard, elastic, and connective tissues in animals and other organisms.

Keratins

Keratins are classes of proteins with long peptide sequences that make up many animal tissues. α keratins in particular are common in hair, horns, nails, and feathers, and are distinguished by a 3.6 residue/turn α-helix, which tends to create coiled-coil structures where the inside of the coils form a hydrophobic interface.

Fibroin

The β sheet structure can be arranged in stacked, antiparallel forms that yield both flexibility and strength. The strong Van der Waals interactions of β sheets are what give spider silk its impressive tensile strength, in a structure called β silk fibroin.

Collagen

Collagen is another fibrous protein, a triple helix structure found in skin, tendons, cartilage, bone, and teeth. Like keratin, collagen's multiple-helix structure gives it ample flexibility and strength.

6.3 Globular Proteins

Groups of folded α-helices and β-sheets can form higher-level structures known as globular proteins. These proteins form when secondary structures pack closely together to create more stable structures.

6.4 Factors Determining Secondary and Tertiary Structure

The final structure of a protein is determined by a complex set of variables ranging from temperature, pH, and influence from other molecules. Primary thermodynamic factors can be grouped into three categories:

1. Intramolecular Interactions (charge-charge, H-bonds, Van der Waals)
2. Conformational Entropy
3. Solvent Entropy

Chaperones are molecules that promote folding of proteins in a specific way that increases the likelihood of a particular final product. By using chaperones, the time and difficulty of folding proteins can be greatly reduced.

6.5 Dynamics of Globular Protein Structure

When proteins condense,they go through a process known as "folding," where the molecule twists and turns itself as it descends down an energy gradient towards a local energy minimum.

Simulating protein folding is difficult due to the sheer number of possible conformations for a given protein. Each conformation represents a potential combinatorial state and, assuming even a modest simulation rate, one of millions of potential states that may take years to simulate.

Due to the sensitive, stochastic nature of protein folding, it's sometimes possible for proteins to misfold. In animals, protein misfolding can lead to life-threatening diseases. Alzheimer's, Parkinson's, and ALS are a few of many diseases thought to be caused by or related to protein misfolding.

6.6 Prediction of Protein Secondary and Tertiary Structure

As one might expect, predicting higher-level structures in proteins is difficult due to the large number of complex interactions involved. Some properties of amino acid sequences provide hints about the secondary structure of proteins. Computational simulation is the current best method for predicting tertiary protein structures, and is unfortunately only about 60% accurate.

6.7 Quaternary Structure of Proteins

When tertiary protein structures interact, they may form quaternary structures, the highest protein structure level. These structures are created and repaired piece-by-piece due to their size. Quaternary structures have some advantages over tertiary structures, such as increased genetic efficiency and stability. Quaternary structures are said to have varying degrees of α-helix or β-sheet character.

7.0 Protein Function and Evolution

Objectives

1. Know the structure and action of antibodies.
2. Understand the multiple functions of proteins.
3. Know how structure and function relate in myoglobin and hemoglobin.
4. Know the effects of pH, [CO2], BPG (adult vs. fetal), and E to V substitution on hemoglobin and its O2 binding.
5. Understand both cooperativity models.
6. Understand the structure of a sarcomere.
7. Know the mechanism and regulation of muscle contraction.

7.1 Structure and Action of Antibodies

Antibodies, or immunoglobulins, are proteins produced by an animal's immune system. These molecules bind to foreign substances during an immune response to protect the host organism. In an adaptive immune response, lymphatic cells called B lymphocytes secrete antibodies designed to bind to specific invading substances.

Epitopes, or antigenic determinates, are sites on the surface of foreign substances where antibodies may bind, and usually consist of carbohydrate or amino acid groups. After initial exposure to an antigenic substance, some B lymphocyte cells known as memory cells will presist long after the antigen is no longer present. This ensures rapid immune response to future infections.

In B lymphocyte cells, structures known and light and heavy chain sequences are combined to form the structure of the antibody. These polypeptide chains can be rearranged to form a variety of sequences, and therefore, a variety of antibodies to bind with many different foreign substances.

7.2 Functions of Proteins

In the body, proteins serve a wide variety of purposes and important functions. Some proteins function as enzymes to catalyze biochemical reactions, such as ribonuclease A. Other proteins serve as regulatory proteins such as insulin, transport proteins such as myoglobin and hemoglobin, storage proteins such as ovalbumin.

Structural proteins are found throughout the body in collagen, while contractile proteins form the muscles and power movement. Adaptor proteins such as SH3 and AKAP serve as chemical messengers, and protective proteins such as immunoglobulins protect the host from foreign substances. Finally, proteins can be found in exotic forms, such as antifreeze and glue.

7.3 How Structure and Function Relate in Myoglobin and Hemoglobin

Myoglobin is a monomeric heme, a structural motif consisting of a polypeptide chain wrapped around a heme group. The heme group contains an O_2 binding site that allows myoglobin to bind and release oxygen in body tissues.

Likewise, hemoglobin is a heme, but consists of a tetrameric motif. Hemoglobin is used to transport oxygen from the lungs to the rest of the body, and also carbon dioxide back to the lungs to be exhaled. Hemoglobin has two states, a T state (low oxygen affinity), and an R state (high oxgygen affinity), which it oscillates between during the Perutz mechanism. In this mechanism, bound oxygen alters the structure of the hemoglobin due to steric strain.

7.4 Effects of pH, [CO2], BPG, and E to V Substitution on Hemoglobin

Hemoglobin function is driven by environmental conditions such as pH, temperature, and pressure. The pH of the environment plays in important role during physical exercise, where exhausted muscles secrete lactic acid as a response to an oxygen deficit in the surrounding tissues. The acid lowers the pH of the tissues, which acts as a signal to deliver more oxygen via the Bohr Effect.

Carbon dioxide can lower the oxygen binding affinity of hemoglobin via the carbamation reaction. When carbon dioxide concentration increases in tissues, some may bind to the N-terminal of hemoglobin, releasing hydrogen ions that contribute to the Bohr effect. These hemoglobin molecules then transport the carbon dioxide back to the lungs to be exhaled.

The molecule 2,3-Bisphosphoglyceric acid (BPG) can be used to lower the oxygen affinity of hemoglobin. It binds with deoxygenated hemoglobin more readily and promotes the release of oxygen molecules, which allows red bloods cells to release oxygen near the tissues that need it most.

7.5 Cooperativity Models

The unique constraints for an oxygen-transporting molecule require specific properties and tolerances. In hemoglobin, the molecule must be saturated at a specific temperature and pressure, and must reserve oxygen for periods of high physical demands. Hemoglobin accomplishes this through cooperative interactions at the oxygen bind sites.

The exact mechanism of this cooperation is still unknown, but there are several leading theories. In sequential models, oxygenation progresses by changing conformations in sequence, while concerted, multistate, and dynamics models allow for more variability in the mechanism's progression.

7.6 Sarcomere Structure

Sarcomeres are the basic structures of muscle tissue, separated in muscle fibers by the Z lines. Between these lines lie two types of filaments, thin filaments and thick filaments, composed of the proteins myosin and actin, respectively.

Actin molecules bind to the Z line, while myosin is interwoven between two actin filaments, holding them together as a single unit, the sarcomere. The region where the myosin and actin filaments intersect is called the A band, and the sections between A bands are called I bands.

7.7 Mechanism and Regulation of Muscle Contraction

During muscle contraction, binding sites on the actin molecules are exposed by removing the protein tropomyosin. This action is facilitated by changing the structure of the protein with calcium ions in a process called calcium-induced calcium release (CICR).

Once the binding sites are exposed, the myosin protein heads bind and perform a muscle contraction. After contraction, the myosin heads relax, releasing ADP and a phosphate ion. When a new ATP molecule binds to the myosin, the heads release from the binding sites, and a muscular recovery stroke occurs.

8.0 Enzymes: Biological Catalysis

Objectives

1. Know the general properties of enzymes.
2. Know various catalytic mechanisms.
3. Know the transition state diagram.
4. Understand the transition state inhibitors and catalytic antibodies.
5. Understand the Philip's mechanism of lysozyme action.
6. Understand the catalytic triad mechanism of serine proteases.
7. Know Michaelis-Menten kinetics.
8. Understand how to analyze kinetic data.
9. Know three types of enzyme inhibition.
10. Understand regulation of enzyme activity

8.1 General Properties of Enzymes

Enzymes are biological catalysts used to increase the velocity of biochemical reactions. Unlike chemical catalysts, enzymes have different catalytic power, specificity, reaction conditions, and ability to be regulated. These properties give cells the ability to modify kinetic properties of reactions in the body.

Biochemists classify enzymes by the type reaction they catalyze. There are six major types of enzymes:

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Ligases
6. Isomerases

8.2 Catalytic Mechanisms

Enzymes catalyze reactions using one or more mechanisms in the body. Some mechanisms facilitate acid/base reactions, such as peptide hydrolysis by chymotrypsin. Chymotrypsin also participates in covalent catalysis.

In electrostatic stabilization, enzymes serve to stabilize molecules in their transition states, which lowers the required activation energy for the reaction. Metal ion catalysis assist in several ways or can inhibit reactions. Other enzymes catalyze reactions by physically orienting molecules to initiate the reaction.

In general, enyzmes bind more tightly to transition states. On a reaction coordinate diagram, enzyme activity is often reflected by stabilization of the transition state. Steric strain, desolvation, and entropy loss can be used to destabilize the catalyzed energy state.

8.3 Transition State Inhibitors and Catalytic Antibodies

Enzymes are not used up during catalysis, so regulating enzymatic activity requires deactivation of the enzyme. In transition state inhibitors, a molecule that mimics the reactant enters the active site on an enzyme and binds to it. This causes the enzyme to change its shape and transition state, preventing its enzymatic activity.

8.5 Philip's Mechanism

The enzyme lysozyme is used to hydryolyze glycosidic bonds between N-acetylmuramic acid (NAM) and N-acetylglucosmine (NAG) in bacterical cell walls.

8.6 Catalytic Triad Mechanism

The body contains a number of enzymes known as serine proteases. These enzymes are used to cleave polypeptides at specific sites, depending on the enzyme structure. One of these proteases, chymotrypsin, a digestive enzyme used to break down proteins in food, uses a catalytic triad to perform its catalysis.

The catalytic triad mechanism requires three amino acids in a specific structure at the enzyme's binding site to work together. The serine residue is used to cleave peptide bonds, while histidine and aspartic acid work together to deprotanate serine, giving it the charge necessary to briefly form a covalent bond with the peptide bond. A water molecule is then activated by histidine, and the oxgygen of the water attacks the serine carbonyl, releasing the protein fragment and regenerating the serine hydroxyl group.

8.7 Michaelis-Menten Kinetics

Enzyme kinetics can be described using Michaelis-Menten kinetics, a system of relating reaction rate to substrate concentration. The model makes three key assumptions:

- Assumption of equilibrium - This assumption states that a reversible enzyme-substrate complex is formed, which then dissociates further.
- Steady state assumption - The steady state assumption states that the enzyme-substrate complex forms at the same rate that it disappears.
- Initial velocity assumption - The formation of products should be very energetically favored, implying that products do not readily reform the original reactants.

The Michaelis-Menten equation describes the relationship between reaction rate and substrate concentration:

$$v = \frac{V_{max}[S]}{K_M + [S]}$$

8.8 Analyzing Kinetic Data

Enzyme kinetics are described by several features that are used to relate reaction velocity to the concentrations of enzyme and substrate:

- Km - The Michaelis constant, derived from the rate constants $(k_{-1} + k_2)/k_1$
- Vmax - Theoretical maximum rate of the reaction, when the reactants and products are balanced under optimal conditions
- Kcat - Turnover rate, equal to the number of reactions per unit of time
- Catalytic Efficiency - K_{cat}/K_M, an estimate of how effective the enzyme is

8.9 Three Types of Enzyme Inhibition

Since enzymes are not consumed, they must be inhibited when their catalytic mechanisms are not necessary. There are two main types of enzymatic inhibition in the body, irreversible and reversible. There are also three subtypes of reversible inhibition in the body, competitive, uncompetitive, and noncompetitive.

Competitive

In competitive inhibition, the inhibitor I binds only to the enzyme E. This binding can prevent or reduce enzymatic activity by blocking the active site or by changing it to be less catalytic.

Uncompetitive

Uncompetitive inhibition is a hypothetical type of inhibition where the inhibitor I binds to the enzyme-subtrate complex.

Noncompetitive

Also called mixed inhibition, in noncompetitive inhibition the inhibitor I binds to either the enzyme E and/or to the enzyme-substrate complex.

8.10 Understand regulation of enzyme activity

Enzyme activity is regulated by a variety of factors in the body, some due to the reactions themselves, others due to intentional influence from the body or medications. For example, as product accumulates, the reaction rate slows. Other influences include substrate availability and genetic controls.

Effectors can alter enzyme activity through covalent modification or through allosteric effects, altering the enzymes shape and structure. Finally, other molecules such as zymogens, isozymes, and modulator proteins can change enzyme activity.

9.0 Carbohydrates

Objectives

1. Know classification of monosaccharides and their general names
2. Understand the properties and know structures of some monosaccharides
3. Know the reactions of hemiacetal and hemiketal formation
4. Recognize reducing sugars and the a and b glycosidic linkages
5. Know structural and storage polysaccharides
6. Understand the functions of glycoproteins

9.1 Classification of monosaccharides and their general names

Monosaccharides are carbohydrates, simple sugars, containing a single saccharide unit that cannot be easily broken down. They are the most basic units of carbohydrates, with a general formula $C_nH_{2n}O_n$.

Monosaccharides are classified by the number of carbons atoms they contain. Some examples of monosaccharide classes are triose, tetrose, pentose, hexose, heptose, etc., with the prefix denoting the number of carbon atoms. Monosaccharides containing more then eight carbons are rare due to their molecular instability.

9.2 The properties and structures of some monosaccharides

One of the most important monosaccharides, glucose, is an example of a hexose. Like many monosaccharides, glucose is found in both a linear form and a cyclic form. The cyclic form of glucose is more stable, and therefore much more common than the linear form.

Monosaccharides can contain aldehyde groups (aldoses) or ketone groups (ketoses). Aldoses and ketoses can contain chiral carbons, meaning they may form enantiomers, diastereoisomers, and epimers. Chirality is denoted using **D** or **L**, which indicates the configuration of the stereogenic carbon furthers from the aldehyde or ketone groups.

9.3 The reactions of hemiacetal and hemiketal formation

Both aldoses and ketoses often react intramolecularly to form cyclic versions of these monosaccharides. When an aldose cyclizes it forms a hemiacetal group between a hydroxyl group and a carbon at the other end of the linear form, while ketoses form hemiacetal groups when cyclizing.

The cyclic forms of these monosaccharides are far more common than the linear configurations due to increased stability. When the aldose or ketose cyclizes, a new bond is formed via nucleophilic addition, it can form either an alpha or beta configuration.

9.4 Recognize reducing sugars and the α and β glycosidic linkages

Sugars with free aldehyde or ketone groups are known as reducing sugars, and can reduce oxidizing agents such as peroxides.

Monosaccharides can also join together to form polysaccharides using linkages known as alpha and beta glycosidic linkages. Alpha linkages point down, away from the aldehyde or ketone group, while beta linkages point up in the same direction.

9.5 Know structural and storage polysaccharides

Polysaccharides are found in plants and animals in a variety of roles, including structural and storage molecules. In plants, the main storage polysaccharide is starch, while glycogen is found in animals. Starch comes in two primary forms, amylose and amylopectin, where the less-common amylose contains branches and amylopectin does not.

The most important structural polysaccharide in plants is cellose, a polymer of glucose molecules. Cellulose provides structural strength in plants, but can also be softer like in cotton. In cellulose, the polysaccharide strands are parallel and can form ribbons from strong intermolecular interactions.

9.6 Understand the functions of glycoproteins

Glycoproteins are proteins that contain carbohydrate chains formed by either **N** or **O** glycosidic bonds. These proteins are commonly found on cellular membranes and perform a variety of functions, including receptor sites as in antibodies. For example, glycoprotein antibodies found on the surface of blood cells are the determinant for a person's blood type using the ABO blood type system.

10.0 Lipids, Membranes and Cellular Transport

Objectives

1. Know the classification of lipids.
2. Try drawing common triacylglycerols and glycerophospholipids.
3. Understand the biological membrane structure and the fluid mosaic model.
4. Know the types of membrane proteins.
5. Understand how lipid asymmetry is maintained.
6. Understand the thermodynamics of transport across the membranes.
7. Know the types of transport.
8. Understand how Na+/K+-ATPase work.

10.1 Know the classification of lipids.

Lipids, also known as fats, are important biological molecules found in many roles in the body. One example is the cell membrane in animal cells, formed from one or more layers of primarily lipid molecules.

Lipds are soluble in nonpolar solvents and insoluble in water and other polar solvents. Lipids are classified into groups of similar molecules, including fatty acids, glycerolipids, glycerophospholipids, sphingolipids, sterols, and many more.

10.3 Biological membrane structure and fluid mosaic model

As mentioned above, biological membrances are primarily composed of lipids and may form single layer membrances, micelles, or double-layer membranes called liposomes or vesicles.

In bilayer cell membranes, the fluid mosaic model is used to explain the structure and behavior of the lipid bilayer. In the fluid mosaic model, a lipid bilayer is embedded with proteins and other structures that can move laterally with ease due to the arrangement of lipid molecules. However, transverse movement requires more energy to flip between the outer membane to the inner or vice versa. In fact, proteins are often two large and require so much energy that they normally never flip. The fluidity of the membrane is determined by temperature, length of the lipid tails, the degree of saturation, and the presence of cholesterol.

10.4 Know the types of membrane proteins.

There are a variety of proteins commonly found embedded in cell membranes:

- Integral proteins
- Peripheral proteins
- Lipid-anchored proteins

Integral proteins (IMPs) are strongly embedded proteins found in membranes that can only be removed using detergents, nonpolar solvents, and denaturization. Many integral proteins are transmembrane, but not all. IMPs serve many functions in the membrane, including transport, channels, receptors, and more.

Peripheral proteins are weakly embedded proteins that are easier to remove, and therefore they are easier to study. They may attach to IMPs or they may be contained in the peripheral areas of the membrane.

Lipid-anchorage proteins, as the name implies, are anchored to the lipids that comprise the membrane bilayer.

10.5 Understand how lipid asymmetry is maintained.

The bilayer membrane is asymmetrical, meaning that the inner and outer membranes are structurally different. This asymmetry is important for the functionality of the membrane, as many reactions must happen outside the cell but not inside, or transport must occur in only one direction, etc.

Asymmetry is maintained through a number of mechanisms. Proteins for example, cannot rotate across the membrane. During membrane synthesis, new membranes are generated by expanding the existing asymmetric membranes.

10.6 Thermodynamics of transport across the membranes.

Transport across the membrane is generally driven by chemical potential and electrical potential. Energy reaches equilibrium across the membrane in the absence of active transport, and can be described using free energy and the concentrations on either side of the membrane.

10.7 Know the types of transport

Transport across lipid membranes occurs via multiple mechanisms:

- Non-mediated
- Mediated

In non-mediated transport (passive diffusion), ions and molecules can flow through the membrane due to difference in gradients across the membrane, such as electrochemical gradients.

Mediated, or active, transport (facilited diffusion) uses an active input of energy to aid the movement of ions and molecules, sometimes against gradients. Active transport mechanisms include ionophores, porins, and conformation-changing proteins.

10.8 Understand how Na+/K+-ATPase work.

One important active-transport protein is NA+/K+ ATPase, a protein found in cellular membranes that maintains concentrations of NA+ and K+ inside and outside the cell. This protein is found in all organs but is particularly important in the brain where it drives many neurochemical processes.

This protein requires an input of energy in the form of ATP hydrolysis, which pushes Na+ out of the cell and pulls K+ ions into the cell. This process accounts for a massive portion of the organism's energy requirements and is used throughout the body.