BIOL 157: BIOLOGICAL CHEMISTRY

Lecture 12:

Introduction to Enzyme Chemistry

Lecturer:

Christopher Larbie, *PhD*

Introduction

- Living organisms seethe with metabolic activity.
- Thousands of chemical reactions are proceeding very rapidly at any given instant within all living cells.
- Virtually all of these transformations are mediated by **enzymes,** proteins (and occasionally RNA) specialized to catalyse metabolic reactions.
- The substances transformed in these reactions are often organic compounds that show little tendency for reaction outside the cell.
- An excellent example is glucose, a sugar that can be stored indefinitely on the shelf with no deterioration.

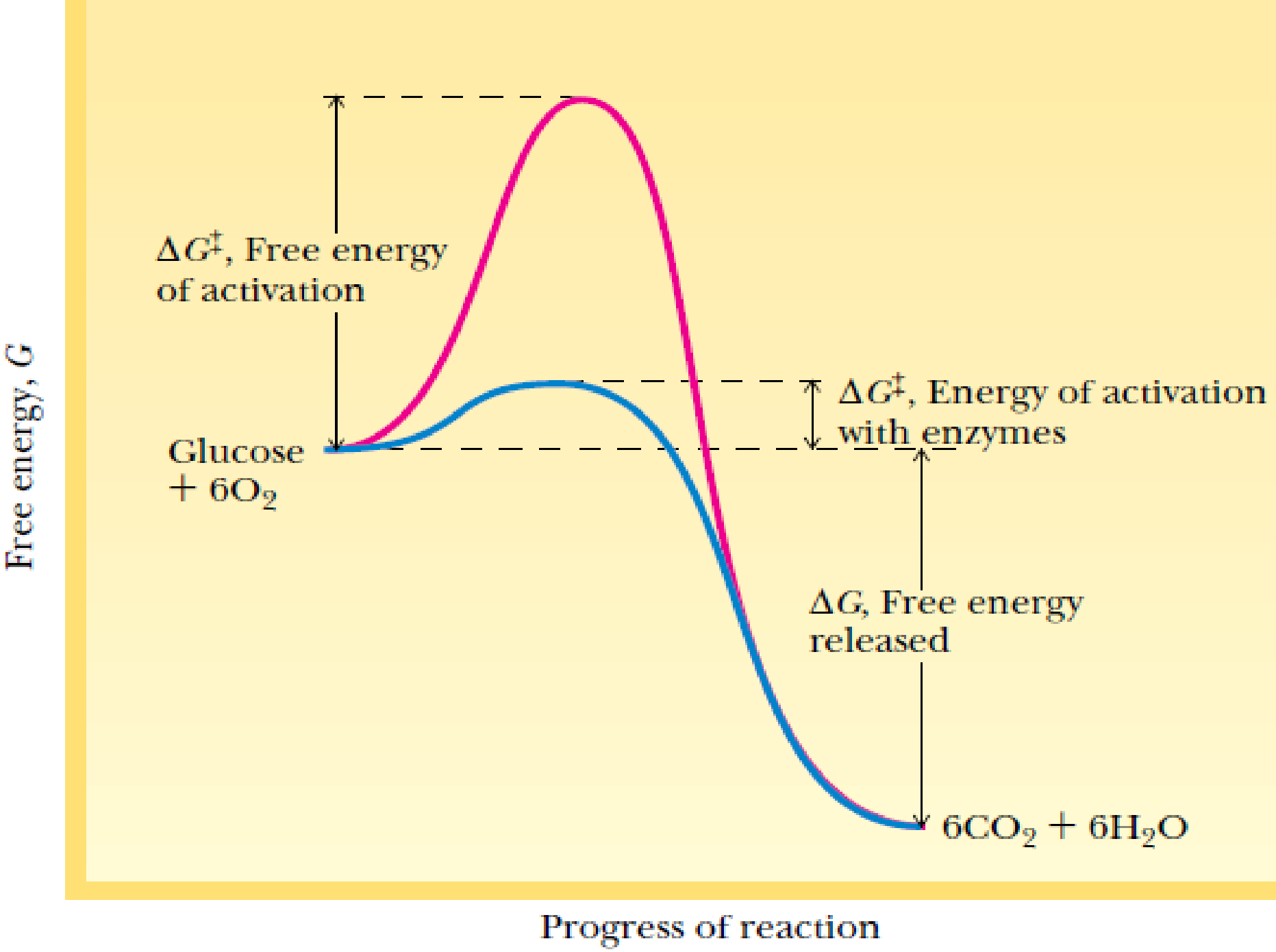
- Most cells quickly oxidize glucose, producing carbon dioxide and water and releasing lots of energy (-2870 kJ/mol is the standard free energy change [$\Delta G°$'] for the oxidation of glucose).

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O + 2870\ \text{kJ of energy}$$

- In chemical terms, 2870 kJ is a large amount of energy, and glucose can be viewed as an energy-rich compound even though at ambient temperature it is not readily reactive with oxygen outside of cells.

- Stated another way, glucose represents **thermodynamic potentiality:** its reaction with oxygen is strongly exergonic, but it just doesn't occur under normal conditions.

- On the other hand, enzymes can catalyse such thermodynamically favourable reactions so that they proceed at extraordinarily rapid rates.


Free energy, G
ΔG‡, Free energy of activation
Glucose + 6O2
ΔG‡, Energy of activation with enzymes
ΔG, Free energy released
6CO2 + 6H2O
Progress of reaction

- In glucose oxidation and countless other instances, enzymes provide cells with the ability to exert *kinetic control over thermodynamic potentiality.*

- That is, living systems use enzymes to accelerate and control the rates of vitally important biochemical reactions.

Enzymes as agents of metabolic function

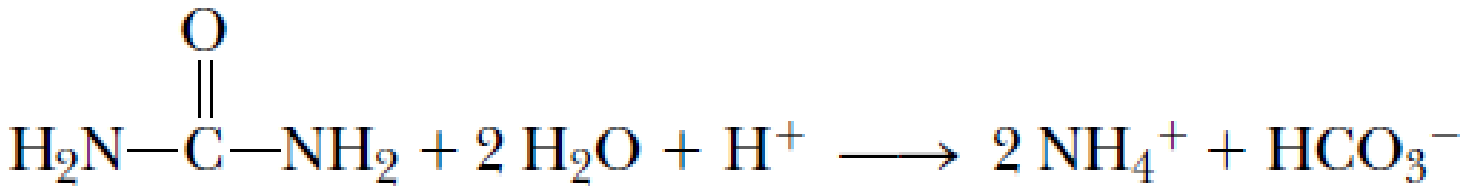
- Acting in sequence, enzymes form metabolic pathways by which nutrient molecules are degraded.
- Energy is released and converted into metabolically useful forms, and precursors are generated and transformed to create the literally thousands of distinctive biomolecules found in any living cell.
- Situated at key junctions of metabolic pathways are specialized **regulatory enzymes** capable of sensing the momentary metabolic needs of the cell and adjusting their catalytic rates accordingly.
- The responses of these enzymes ensure the harmonious integration of the diverse and often divergent metabolic activities of cells so that the living state is promoted and preserved.

Catalytic Power, Specificity, and Regulation

Catalytic Power

- Enzymes display enormous catalytic power, accelerating reaction rates as much as 10^{16} over uncatalysed levels.
- This is far greater than any synthetic catalysts can achieve.
- Enzymes accomplish these astounding feats in dilute aqueous solution under mild conditions of temperature and pH.

- For example, the enzyme jack bean *urease* catalyses the hydrolysis of urea:



- At 20°C, the rate constant for the enzyme-catalysed reaction is 3×10^{4}/sec; the rate constant for the uncatalysed hydrolysis of urea is 3×10^{-10}/sec.

- Thus, 10^{14} is the ratio of the catalysed rate to the uncatalysed rate of reaction.

- Such a ratio is defined as the relative **catalytic power** of an enzyme, so the catalytic power of urease is 10^{14}.

Specificity

- A given enzyme is very selective, both in the substances with which it interacts and in the reaction that it catalyses.
- The substances upon which an enzyme acts are traditionally called **substrates**.
- In an enzyme-catalysed reaction, none of the substrate is diverted into nonproductive side-reactions, so no wasteful by-products are produced.
- It follows then that the products formed by a given enzyme are also very specific.

- The selective qualities of an enzyme are collectively recognized as its **specificity**.

- Intimate interaction between an enzyme and its substrates occurs through molecular recognition based on structural complementarity; such mutual recognition is the basis of specificity.

- The specific site on the enzyme where substrate binds and catalysis occurs is called the **active site**.

Regulation

- Regulation of enzyme activity is achieved in a variety of ways, ranging from controls over the amount of enzyme protein produced by the cell to more rapid, reversible interactions of the enzyme with metabolic inhibitors and activators.

- Because most enzymes are proteins, we can anticipate that the functional attributes of enzymes are due to the remarkable versatility found in protein structures.

Enzyme Nomenclature

- Traditionally, enzymes often were named by adding the suffix *-ase* to the name of the substrate upon which they acted.

- **Examples**: *urease* for the urea-hydrolyzing enzyme and *phosphatase* for enzymes hydrolyzing phosphoryl groups from organic phosphate compounds.

- Other enzymes acquired names bearing little resemblance to their activity

- **Examples**: the peroxide-decomposing enzyme *catalase* or the proteolytic enzymes (*proteases*) of the digestive tract, *trypsin* and *pepsin*.

- Because of the confusion that arose from these trivial designations, an International Commission on Enzymes was established in 1956 to create a systematic basis for enzyme nomenclature.

- Although common names for many enzymes remain in use, all enzymes now are classified and formally named according to the reaction they catalyse.

- Six classes of reactions are recognized. Within each class are subclasses, and under each subclass are subsubclasses within which individual enzymes are listed.

- Classes, subclasses, subsubclasses, and individual entries are each numbered, so that a series of four numbers serves to specify a particular enzyme
- A systematic name, descriptive of the reaction, is also assigned to each entry

Systematic Classification of Enzymes According to the Enzyme Commission

E.C. Number	Systematic Name and Subclasses
1	*Oxidoreductases* (oxidation–reduction reactions)
2	*Transferases* (transfer of functional groups)
3	*Hydrolases* (hydrolysis reactions)
4	*Lyases* (addition to double bonds)
5	*Isomerases* (isomerization reactions)
6	*Ligases* (formation of bonds with ATP cleavage)

Coenzymes

- Many enzymes carry out their catalytic function relying solely on their protein structure.

- Many others require nonprotein components, called **cofactors** which may be metal ions or organic molecules referred to as **coenzymes**.

- Cofactors, because they are structurally less complex than proteins, tend to be stable to heat (incubation in a boiling water bath).

- Many coenzymes are vitamins or contain vitamins as part of their structure.

- Usually coenzymes are actively involved in the catalytic reaction of the enzyme, often serving as intermediate carriers of functional groups in the conversion of substrates to products.

- In most cases, a coenzyme is firmly associated with its enzyme, perhaps even by covalent bonds, and it is difficult to separate the two.

- Such tightly bound coenzymes are referred to as **prosthetic groups** of the enzyme.

- The catalytically active complex of protein and prosthetic group is called the **holoenzyme**.

- The protein without the prosthetic group is called the **apoenzyme** and is catalytically inactive.

Enzyme Cofactors: Some Metal Ions and Coenzymes and the Enzymes with Which They Are Associated

Metal Ions and Some Enzymes That Require Them		Coenzymes Serving as Transient Carriers of Specific Atoms or Functional Groups		Representative Enzymes Using Coenzymes
Metal Ion	Enzyme	Coenzyme	Entity Transferred	
Fe^{2+} or Fe^{3+}	Cytochrome oxidase	Thiamine pyrophosphate (TPP)	Aldehydes	Pyruvate dehydrogenase
	Catalase	Flavin adenine dinucleotide (FAD)	Hydrogen atoms	Succinate dehydrogenase
	Peroxidase	Nicotinamide adenine dinucleotide (NAD)	Hydride ion (H^-)	Alcohol dehydrogenase
Cu^{2+}	Cytochrome oxidase			
Zn^{2+}	DNA polymerase	Coenzyme A (CoA)	Acyl groups	Acetyl-CoA carboxylase
	Carbonic anhydrase	Pyridoxal phosphate (PLP)	Amino groups	Aspartate aminotransferase
	Alcohol dehydrogenase	5′-Deoxyadenosylcobalamin (vitamin B_{12})	H atoms and alkyl groups	Methylmalonyl-CoA mutase
Mg^{2+}	Hexokinase			
	Glucose-6-phosphatase	Biotin (biocytin)	CO_2	Propionyl-CoA carboxylase
Mn^{2+}	Arginase	Tetrahydrofolate (THF)	Other one-carbon groups	Thymidylate synthase
K^+	Pyruvate kinase (also requires Mg^{2+})			
Ni^{2+}	Urease			
Mo	Nitrate reductase			
Se	Glutathione peroxidase			

Enzyme Kinetics

- **Kinetics** is the branch of science concerned with the rates of chemical reactions.

- The study of **enzyme kinetics** addresses the biological roles of enzymatic catalysts and how they accomplish their remarkable feats.

- In enzyme kinetics, we seek to determine the maximum reaction velocity that the enzyme can attain and its binding affinities for substrates and inhibitors.

- Coupled with studies on the structure and chemistry of the enzyme, analysis of the enzymatic rate under different reaction conditions yields insights regarding the enzyme's mechanism of catalytic action.

- Such information is essential to an overall understanding of metabolism.

- Significantly, this information can be exploited to control and manipulate the course of metabolic events.

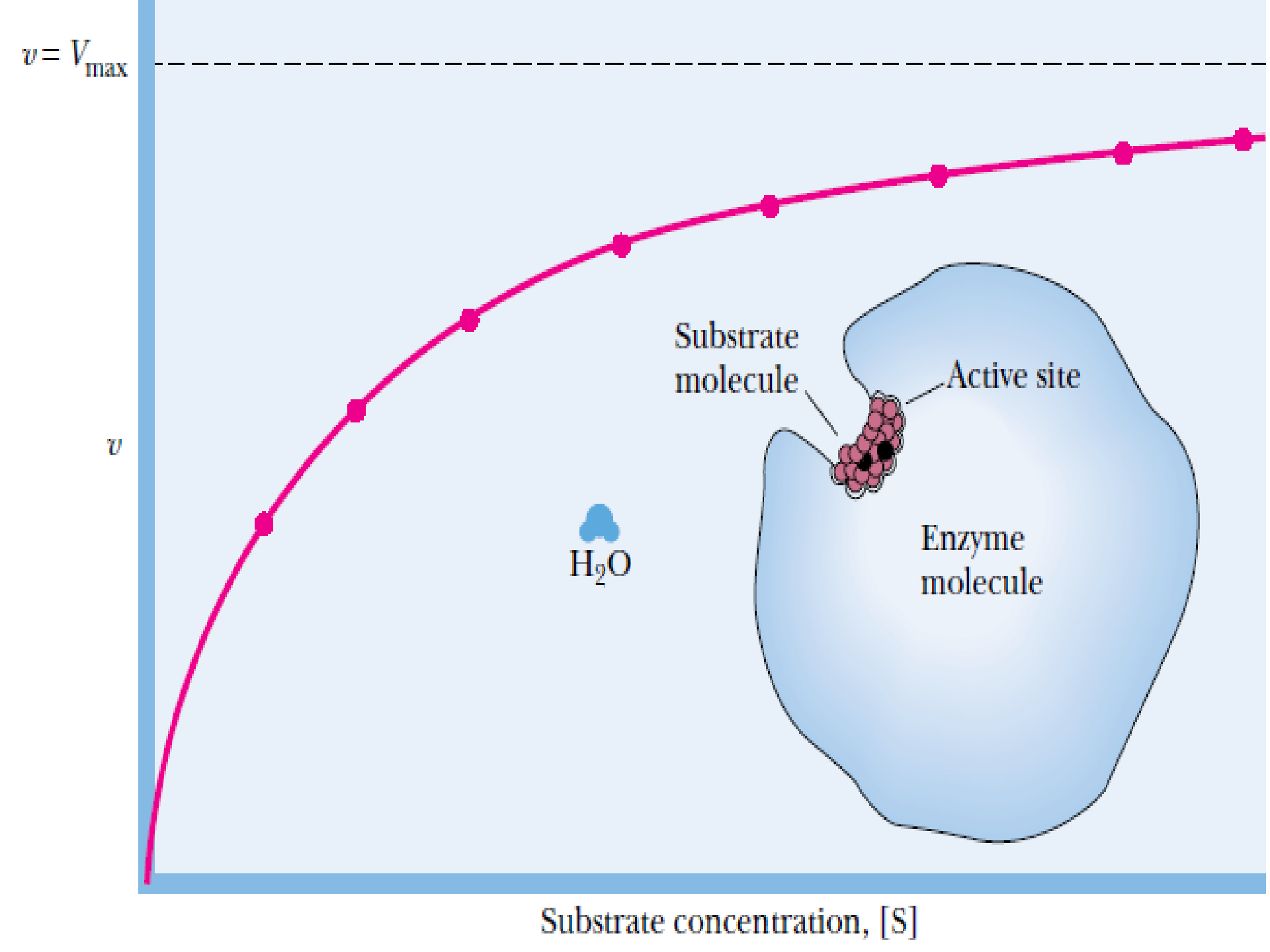
- The science of pharmacology relies on such a strategy. **Pharmaceuticals,** or **drugs,** are often special inhibitors specifically targeted at a particular enzyme in order to overcome infection or to alleviate illness.

- A detailed knowledge of the enzyme's kinetics is indispensable to rational drug design and successful pharmacological intervention.

Kinetics of Enzyme-Catalysed Reactions

- Examination of the change in reaction velocity as the reactant concentration is varied is one of the primary measurements in kinetic analysis.

- Analyses of enzyme-catalysed reactions involving only a single substrate yield remarkably different results.


$v = V_{max}$
v
Substrate molecule
Active site
H_2O
Enzyme molecule
Substrate concentration, [S]

- At low concentrations of the substrate S, *v* is proportional to [S], as expected for a first-order reaction.
- However, *v* does not increase proportionally as [S] increases, but instead begins to level off.
- At high [S], *v* becomes virtually independent of [S] and approaches a maximal limit.
- The value of *v* at this limit is written V_{max}. Because rate is no longer dependent on [S] at these high concentrations, the enzyme-catalysed reaction is now obeying **zero-order kinetics**.
- That is, the rate is independent of the reactant (substrate) concentration

- This behaviour is a **saturation effect:** when *v* shows no increase even though [S] is increased, the system is saturated with substrate.

- Such plots are called **substrate saturation curves**.

- The physical interpretation is that every enzyme molecule in the reaction mixture has its substrate-binding site occupied by S.

The Michaelis–Menten Equation

- Lenore Michaelis and Maud L. Menten proposed a general theory of enzyme action in 1913 consistent with observed enzyme kinetics.

- Their theory was based on the assumption that the enzyme, E, and its substrate, S, associate reversibly to form an enzyme-substrate complex, ES:

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES}$$

- This association/dissociation is assumed to be a rapid equilibrium, and *K*s is the *enzyme : substrate dissociation constant.*

- At equilibrium,

$$k_{-1}[\mathrm{ES}] = k_1[\mathrm{E}][\mathrm{S}] \quad \textbf{and} \quad K_\mathrm{S} = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]} = \frac{k_{-1}}{k_1}$$

- Product, P, is formed in a second step when ES breaks down to yield E + P.

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

- E is then free to interact with another molecule of S.

The Michaelis Constant, *Km*

- The ratio of constants is itself a constant and is defined as the **Michaelis constant, *Km***

$$K_m = \frac{(k_{-1} + k_2)}{k_1}$$

- Substituting this relationship into the expression for *v* gives the **Michaelis–Menten equation**

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

- This equation says that the rate of an enzyme-catalysed reaction, *v,* at any moment is determined by two constants, *Km* and *V*max, and the concentration of substrate at that moment.

Enzyme Units

- In many situations, the actual molar amount of the enzyme is not known.
- However, its amount can be expressed in terms of the activity observed.
- The International Commission on Enzymes defines **One International Unit** of enzyme as *the amount that catalyses the formation of one micromole of product in one minute.*
- Because enzymes are very sensitive to factors such as pH, temperature, and ionic strength, the conditions of assay must be specified.

- Another definition for units of enzyme activity is the **katal**

"One katal is *that amount of enzyme catalysing the conversion of one mole of substrate to product in one second*"

- Thus, one katal equals $6x10^7$ international units

Turnover Number

- The **turnover number** of an enzyme, *k*cat, is a measure of its maximal catalytic activity.

*k*cat is defined as “the number of substrate molecules converted into product per enzyme molecule per unit time when the enzyme is saturated with substrate”

- The turnover number is also referred to as the **molecular activity** of the enzyme.

- For the simple Michaelis–Menten reaction under conditions of initial velocity measurements, k_2=*k*cat

- Provided the concentration of enzyme, [E_T], in the reaction mixture is known, *k*cat can be determined from *V*max
- At saturating [S], $v = V\text{max} = k_2[ET]$. Thus,

$$k_2 = \frac{V_{\max}}{[\mathbf{E}_T]} = k_{\text{cat}}$$

- The term *k*cat represents the kinetic efficiency of the enzyme.

- Catalase has the highest turnover number known; each molecule of this enzyme can degrade 40 million molecules of H_2O_2 in one second.

- At the other end of the scale, lysozyme requires 2 seconds to cleave a glycosidic bond in its glycan substrate.

Effect of pH on Enzymatic Activity

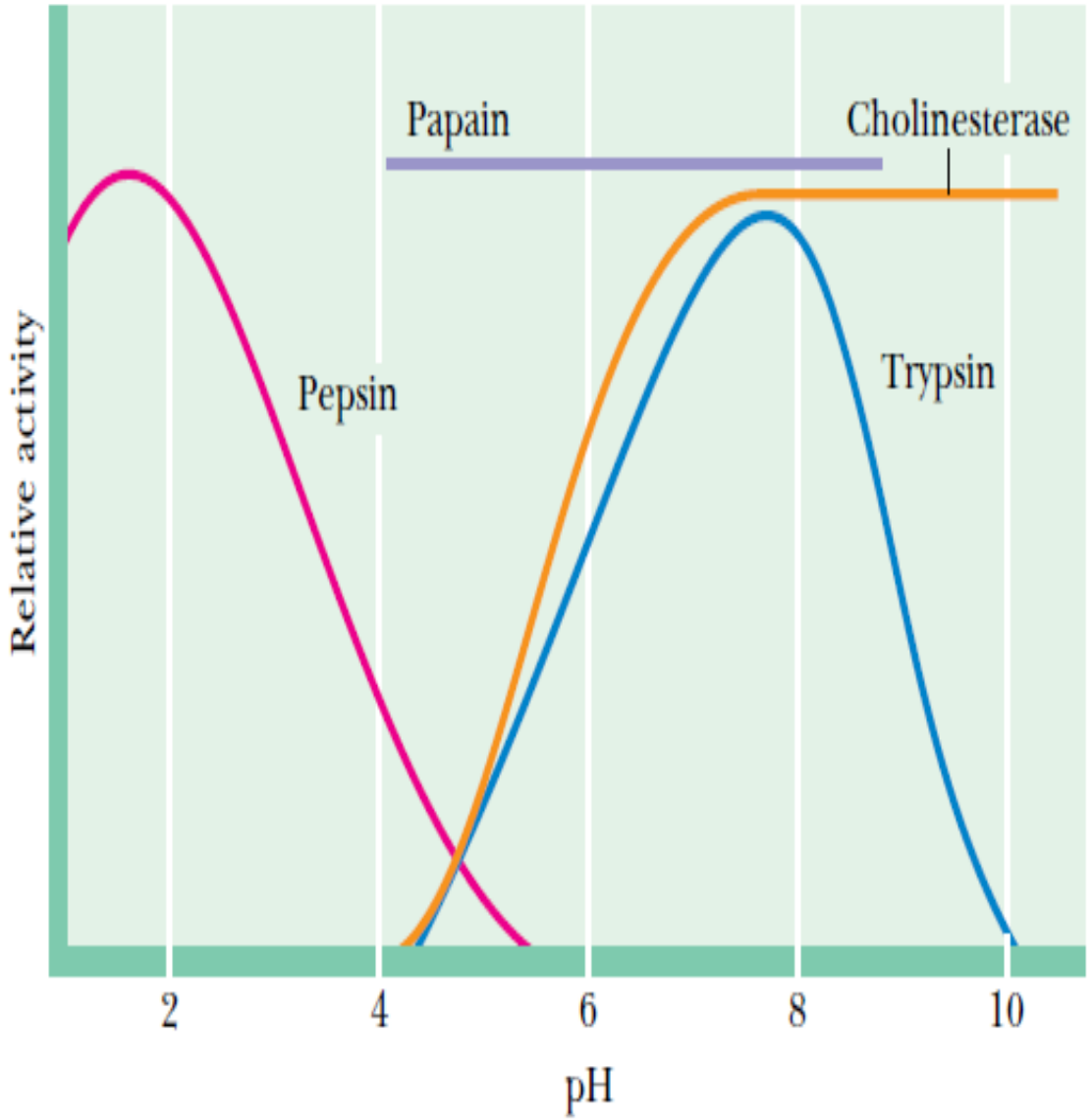
- Enzyme-substrate recognition and the catalytic events that ensue are greatly dependent on pH.

- An enzyme possesses an array of ionizable side chains and prosthetic groups that not only determine its secondary and tertiary structure but may also be intimately involved in its active site.

- Further, the substrate itself often has ionizing groups, and one or another of the ionic forms may preferentially interact with the enzyme.

- Enzymes in general are active only over a limited pH range and most have a particular pH at which their catalytic activity is optimal.

- These effects of pH may be due to effects on *Km* or *V*max or both



Optimum pH of Some Enzymes	
Enzyme	Optimum pH
Pepsin	1.5
Catalase	7.6
Trypsin	7.7
Fumarase	7.8
Ribonuclease	7.8
Arginase	9.7

- Although the pH optimum of an enzyme often reflects the pH of its normal environment, the optimum may not be precisely the same.

- This difference suggests that the pH-activity response of an enzyme may be a factor in the intracellular regulation of its activity.

Effect of Temperature on Enzymatic Activity

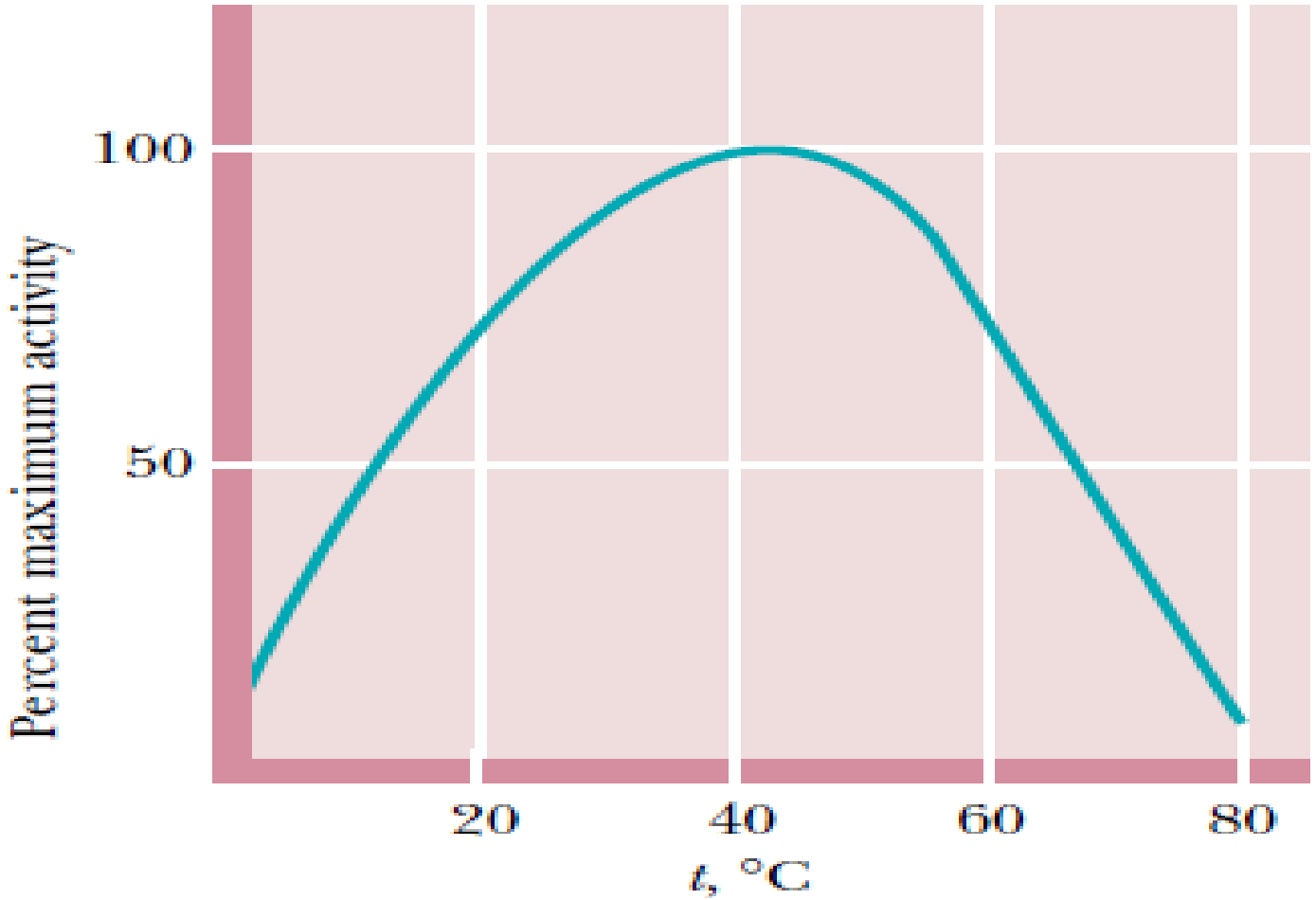
- Like most chemical reactions, the rates of enzyme-catalysed reactions generally increase with increasing temperature.
- However, at temperatures above 50° to 60°C, enzymes typically show a decline in activity.
- Two effects are operating here: (a) the characteristic increase in reaction rate with temperature, and (b) thermal denaturation of protein structure at higher temperatures.

- Most enzymatic reactions double in rate for every 10°C rise in temperature (that is, $Q_{10}=2$, where Q_{10} is defined as *the ratio of activities at two temperatures 10° apart*) as long as the enzyme is stable and fully active.

- Some enzymes, those catalysing reactions having very high activation energies, show proportionally greater Q_{10} values.

- The increasing rate with increasing temperature is ultimately offset by the instability of higher orders of protein structure at elevated temperatures, where the enzyme is denatured and inactivated.

- Not all enzymes are quite so thermally labile. For example, the enzymes of thermophilic bacteria found in geothermal springs retain full activity at temperatures in excess of 85°C.



Enzyme Inhibition

- If the velocity of an enzymatic reaction is decreased or **inhibited,** the kinetics of the reaction obviously have been perturbed.

- Systematic perturbations are a basic tool of experimental scientists; much can be learned about the normal workings of any system by inducing changes in it and then observing the effects of the change.

- The study of enzyme inhibition has contributed significantly to our understanding of enzymes.

Reversible Versus Irreversible Inhibition

- The inhibitor may interact either reversibly or irreversibly with the enzyme.
- **Reversible inhibitors** interact with the enzyme through noncovalent association/dissociation reactions.
- In contrast, **irreversible inhibitors** usually cause stable, covalent alterations in the enzyme; that is, the consequence of irreversible inhibition is a decrease in the concentration of active enzyme.

Reversible Inhibition

- Reversible inhibitors fall into two major categories: **competitive** and **noncompetitive**.

- ***Competitive inhibitors*** are characterized by the fact that the substrate and inhibitor compete for the same binding site on the enzyme. The **active site** or **S-binding site.**

- Thus, increasing the concentration of S favours the likelihood of S binding to the enzyme instead of the inhibitor, I.

- That is, high [S] can overcome the effects of I. The other major type, ***noncompetitive*** inhibition, cannot be overcome by increasing [S].

Irreversible Inhibition

- If the inhibitor combines irreversibly with the enzyme—for example, by covalent attachment—the kinetic pattern seen is like that of noncompetitive inhibition, because the net effect is a loss of active enzyme.

- Usually, this type of inhibition can be distinguished from the noncompetitive, reversible inhibition case since the reaction of I with E (and/or ES) is not instantaneous.

- Instead, there is a *time-dependent decrease in enzymatic activity* as E + I $\rightarrow$ EI proceeds, and the rate of this inactivation can be followed.

- Also, unlike reversible inhibitions, dilution or dialysis of the enzyme:inhibitor solution does not dissociate the EI complex and restore enzyme activity.

Suicide Substrates—Mechanism-Based Enzyme Inactivators

- **Suicide substrates** are inhibitory substrate analogs designed so that, via normal catalytic action of the enzyme, a very reactive group is generated.

- This reactive group then forms a covalent bond with a nearby functional group within the active site of the enzyme, thereby causing irreversible inhibition.

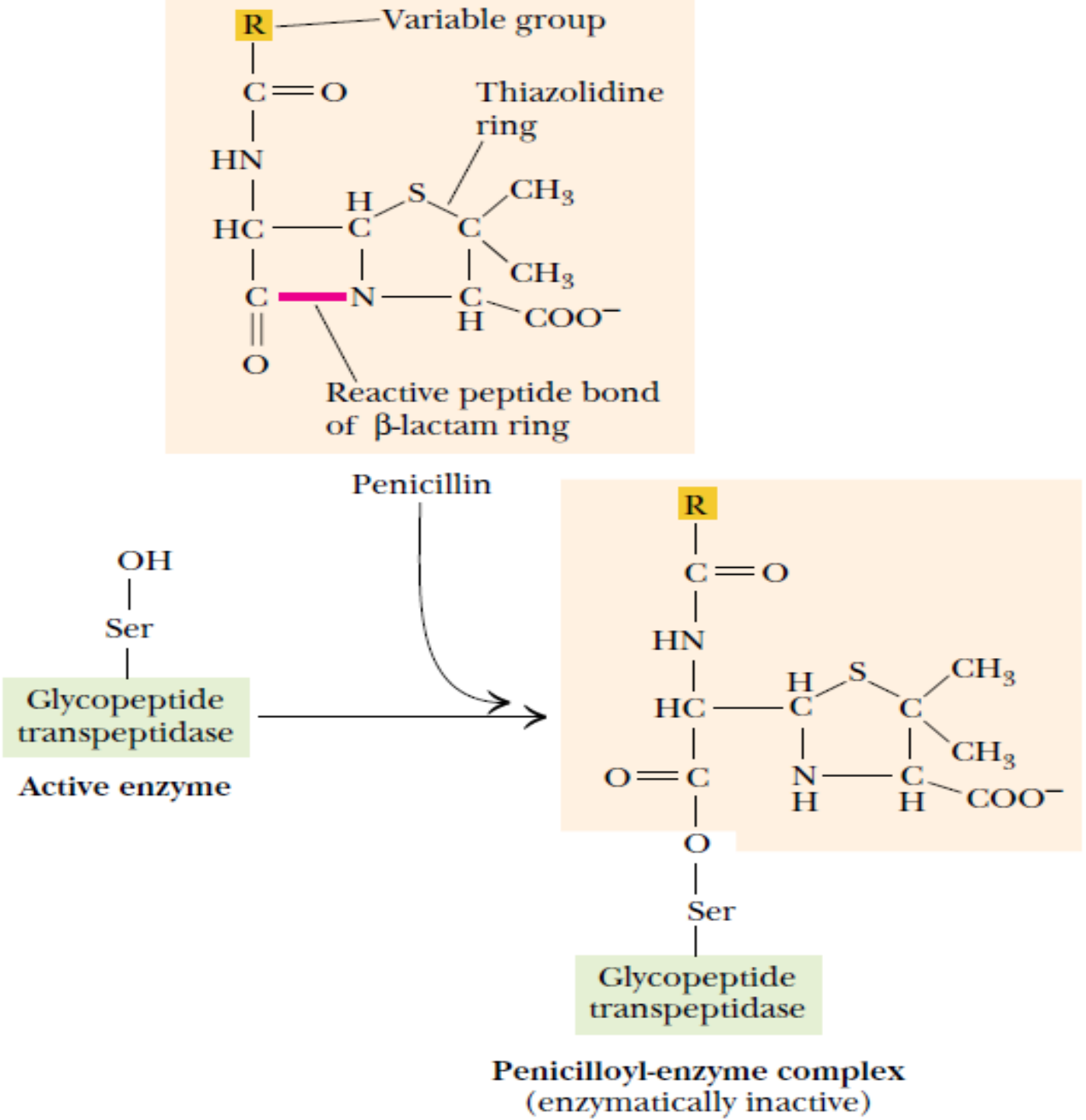
- As substrate analogs, they bind with specificity and high affinity to the enzyme active site; in their reactive form, they become covalently bound to the enzyme.

- This covalent link effectively labels a particular functional group within the active site, identifying the group as a key player in the enzyme's catalytic cycle.

- Several drugs in current medical use are mechanism-based enzyme inactivators.

- For example, the antibiotic **penicillin** exerts its effects by covalently reacting with an essential serine residue in the active site of *glycoprotein peptidase,* an enzyme that acts to cross-link the peptidoglycan chains during synthesis of bacterial cell walls.

- Once cell wall synthesis is blocked, the bacterial cells are very susceptible to rupture by osmotic lysis, and bacterial growth is halted.


R
Variable group
C=O
Thiazolidine ring
HN
HC
H
S
CH3
C
C
CH3
C
N
C
H
COO−
O
Reactive peptide bond of β-lactam ring
Penicillin
OH
Ser
Glycopeptide transpeptidase
Active enzyme
R
C=O
HN
HC
H
S
CH3
C
C
CH3
O=C
N
C
H
COO−
H
O
Ser
Glycopeptide transpeptidase
Penicilloyl-enzyme complex
(enzymatically inactive)

ENZYME SPECIFICITY AND REGULATION

- The extraordinary ability of an enzyme to catalyse only one particular reaction is a quality known as **specificity**.
- Specificity means an enzyme acts only on a specific substance, its substrate, invariably transforming it into a specific product.
- That is, an enzyme binds only certain compounds, and then, only a specific reaction ensues. Some enzymes show absolute specificity, catalysing the transformation of only one specific substrate to yield a unique product.
- Other enzymes carry out a particular reaction but act on a class of compounds.

- For example, *hexokinase* (ATP: hexose-6-phosphotransferase) will carry out the ATP-dependent phosphorylation of a number of hexoses at the 6-position, including glucose while *glucokinase* only phosphorylates glucose.

Specificity as a Result of Molecular Recognition

- An enzyme molecule is typically orders of magnitude larger than its substrate. Its active site comprises only a small portion of the overall enzyme structure.

- The active site is part of the conformation of the enzyme molecule arranged to create a special pocket or cleft whose three-dimensional structure is complementary to the structure of the substrate.

- The enzyme and the substrate molecules “recognize” each other through this structural complementarity.

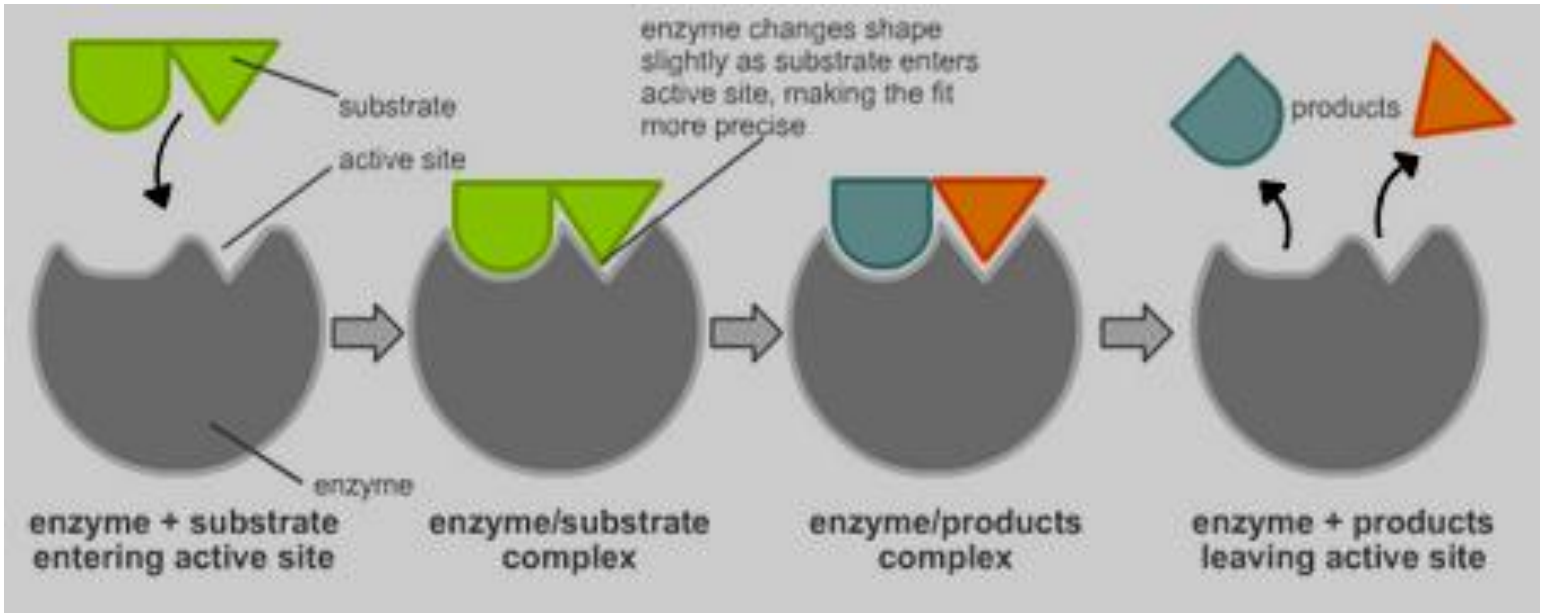
- The substrate binds to the enzyme through relatively weak forces—H bonds, ionic bonds (salt bridges), and van der Waals interactions between sterically complementary clusters of atoms.

- Specificity studies on enzymes entail an examination of the rates of the enzymatic reaction obtained with various **structural analogs** of the substrate.

The “Lock and Key” Hypothesis

- Pioneering enzyme specificity studies at the turn of the century by the great organic chemist Emil Fischer in 1897 led to the notion of an enzyme resembling a **“lock”** and its particular substrate the **“key.”**

- This analogy captures the essence of the specificity that exists between an enzyme and its substrate, but enzymes are not rigid templates like locks.



The “Induced Fit” Hypothesis

- Enzymes are highly flexible, conformationally dynamic molecules, and many of their remarkable properties, including substrate binding and catalysis, are due to their structural flexibility.

- Realization of the conformational flexibility of proteins led Daniel Koshland to hypothesize that the binding of a substrate (S) by an enzyme is an interactive process.

- That is, the shape of the enzyme’s active site is actually modified upon binding S, in a process of dynamic recognition between enzyme and substrate aptly called **induced fit**.

- In essence, substrate binding alters the conformation of the protein, so that the protein and the substrate “fit” each other more precisely.

- The process is truly interactive in that the conformation of the substrate also changes as it adapts to the conformation of the enzyme.

- In enzyme catalysis, precise orientation of catalytic residues comprising the active site is necessary for the reaction to occur; substrate binding induces this precise orientation by the changes it causes in the protein’s conformation.

“Induced Fit” and the Transition-State Intermediate

- The catalytically active enzyme:substrate complex is an interactive structure in which the enzyme causes the substrate to adopt a form that mimics the transition-state intermediate of the reaction.

- Thus, a poor substrate would be one that was less effective in directing the formation of an optimally active enzyme:transition-state intermediate conformation.

- This active conformation of the enzyme molecule is thought to be relatively unstable in the absence of substrate, and free enzyme thus reverts to a conformationally different state.

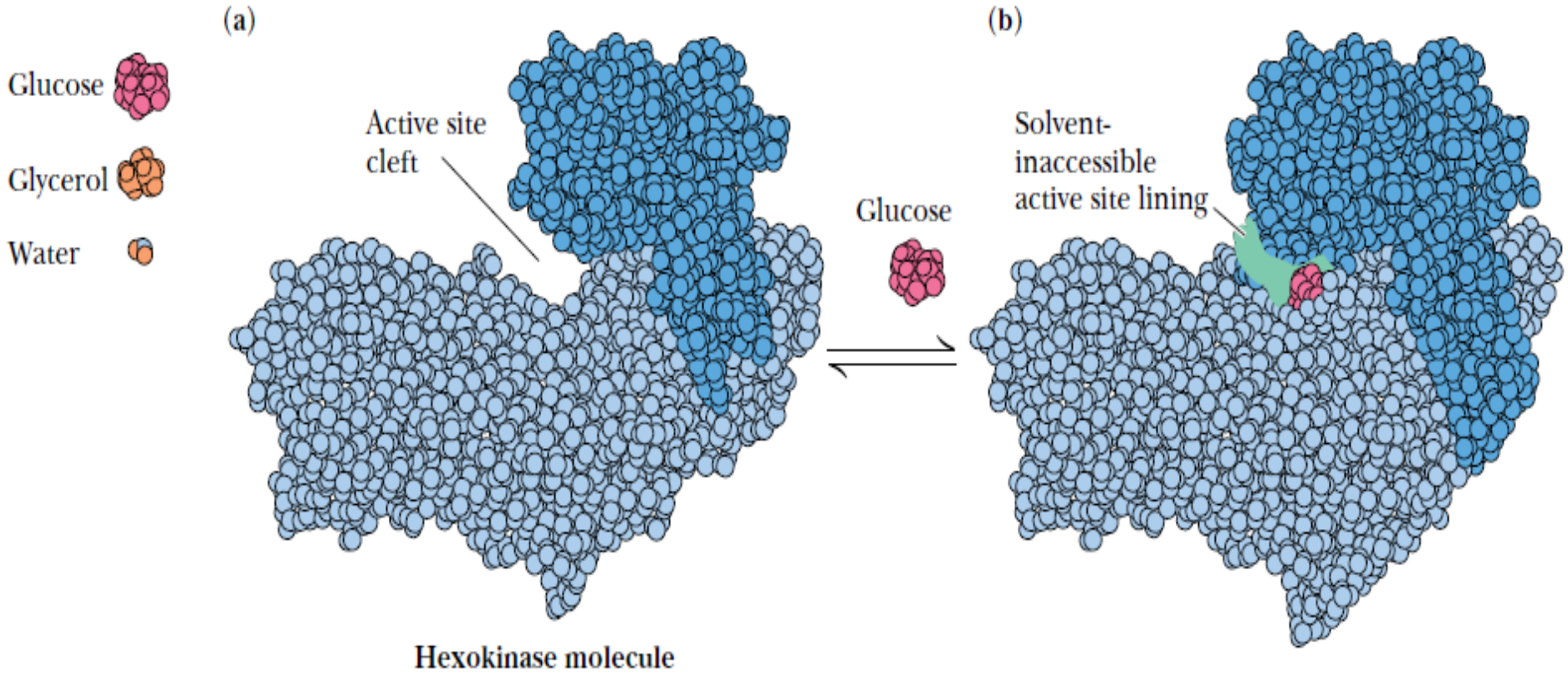
Specificity and Reactivity

- Consider, for example, why hexokinase catalyses the ATP-dependent phosphorylation of hexoses but not smaller phosphoryl-group acceptors such as glycerol, ethanol, or even water.

- Surely these smaller compounds are not sterically forbidden from approaching the active site of hexokinase.

- Indeed, water should penetrate the active site easily and serve as a highly effective phosphoryl-group acceptor.

- Accordingly, hexokinase should display high ATPase activity. It does not. Only the binding of hexoses induces hexokinase to assume its fully active conformation.


Glucose
Glycerol
Water
(a)
Active site cleft
Glucose
(b)
Solvent-inaccessible active site lining
Hexokinase molecule

Controls Over Enzymatic Activity—General Considerations

- The activity displayed by enzymes is affected by a variety of factors, some of which are essential to the harmony of metabolism.

1. The enzymatic rate, “slows down” as product accumulates and equilibrium is approached

- The apparent decrease in rate is due to the conversion of P to S by the reverse reaction as [P] rises. Once [P]/[S] = *K*eq, no further reaction is apparent.

- *K*eq defines thermodynamic equilibrium. Enzymes have no influence on the thermodynamics of a reaction.

- Also, product inhibition can be a kinetically valid phenomenon: Some enzymes are actually inhibited by the products of their action (Feed-back inhibition).

2. The availability of substrates and cofactors will determine the enzymatic reaction rate

- In general, enzymes have evolved such that their *Km* values approximate the prevailing *in vivo* concentration of their substrates.

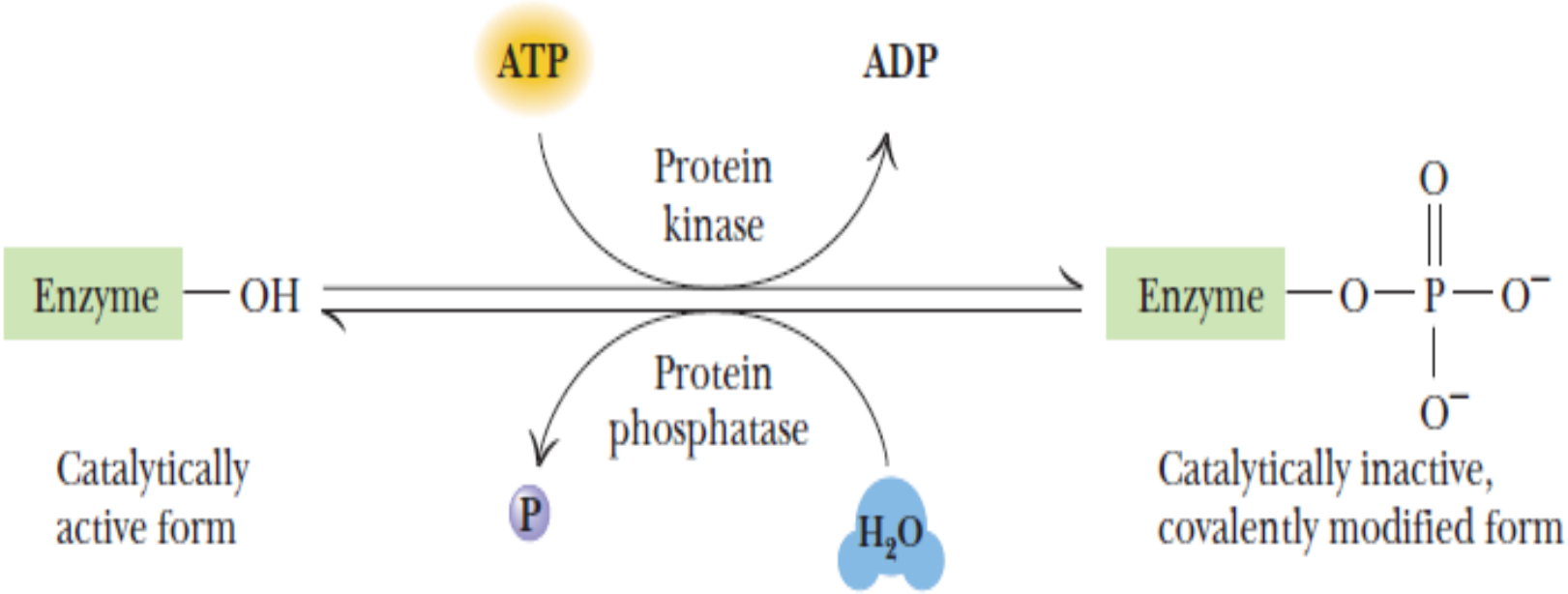
- It is also true that the concentration of some enzymes in cells is within an order of magnitude or so of the concentrations of their substrates.

3. There are genetic controls over the amounts of enzyme synthesized (or degraded) by cells

- If the gene encoding a particular enzyme protein is turned on or off, changes in the amount of enzyme activity soon follow.
- **Induction,** which is the activation of enzyme synthesis, and **repression,** which is the shutdown of enzyme synthesis, are important mechanisms for the regulation of metabolism
- By controlling the amount of an enzyme that is present at any moment, cells can either activate or terminate various metabolic routes.
- Genetic controls over enzyme levels have a response time ranging from minutes in rapidly dividing bacteria to hours (or longer) in higher eukaryotes.

4. Enzymes can be regulated by **covalent modification,** the reversible covalent attachment of a chemical group.

- For example, a fully active enzyme can be converted into an inactive form simply by the covalent attachment of a functional group, such as a phosphoryl moiety.



- Alternatively, some enzymes exist in an inactive state unless specifically converted into the active form through covalent addition of a functional group.

- Covalent modification reactions are catalysed by special **converter enzymes,** which are themselves subject to metabolic regulation.

- Although covalent modification represents a stable alteration of the enzyme, a different converter enzyme operates to remove the modification.

- By so doing the conditions that favoured modification of the enzyme are no longer present, the process can be reversed, restoring the enzyme to its unmodified state.

5. Enzymatic activity can also be activated or inhibited through noncovalent interaction of the enzyme with small molecules (metabolites) other than the substrate

- This form of control is termed **allosteric regulation,** because the activator or inhibitor binds to the enzyme at a site *other* than the active site.

- Further, such allosteric regulators, or **effector molecules,** are often quite different sterically from the substrate.

- Because this form of regulation results simply from reversible binding of regulatory ligands to the enzyme, the cellular response time can be virtually instantaneous.

6. Specialized controls:

- Enzyme regulation is an important matter to cells, and evolution has provided a variety of additional options, including zymogens, isozymes and modulator proteins.

Zymogens

- Most proteins become fully active as their synthesis is completed and they spontaneously fold into their native, three-dimensional conformations.

- Some proteins, however, are synthesized as inactive precursors, called **zymogens** or **proenzymes,** that only acquire full activity upon specific proteolytic cleavage of one or several of their peptide bonds.

- Unlike allosteric regulation or covalent modification, zymogen activation by specific proteolysis is an irreversible process.

- Activation of enzymes and other physiologically important proteins by specific proteolysis is a strategy frequently exploited by biological systems to switch on processes at the appropriate time and place.

- ***Insulin*:** Is an important metabolic regulator, and is generated by proteolytic excision of a specific peptide from **proinsulin**.

Proteolytic Enzymes of the Digestive Tract:

- Enzymes of the digestive tract that serve to hydrolyse dietary proteins are synthesized in the stomach and pancreas as zymogens

Pancreatic and Gastric Zymogens

Origin	Zymogen	Active Protease
Pancreas	Trypsinogen	Trypsin
Pancreas	Chymotrypsinogen	Chymotrypsin
Pancreas	Procarboxypeptidase	Carboxypeptidase
Pancreas	Proelastase	Elastase
Stomach	Pepsinogen	Pepsin

- Only upon proteolytic activation are these enzymes able to form a catalytically active substrate-binding site.

- ***Blood Clotting*****:** The formation of blood clots is the result of a series of zymogen activations.

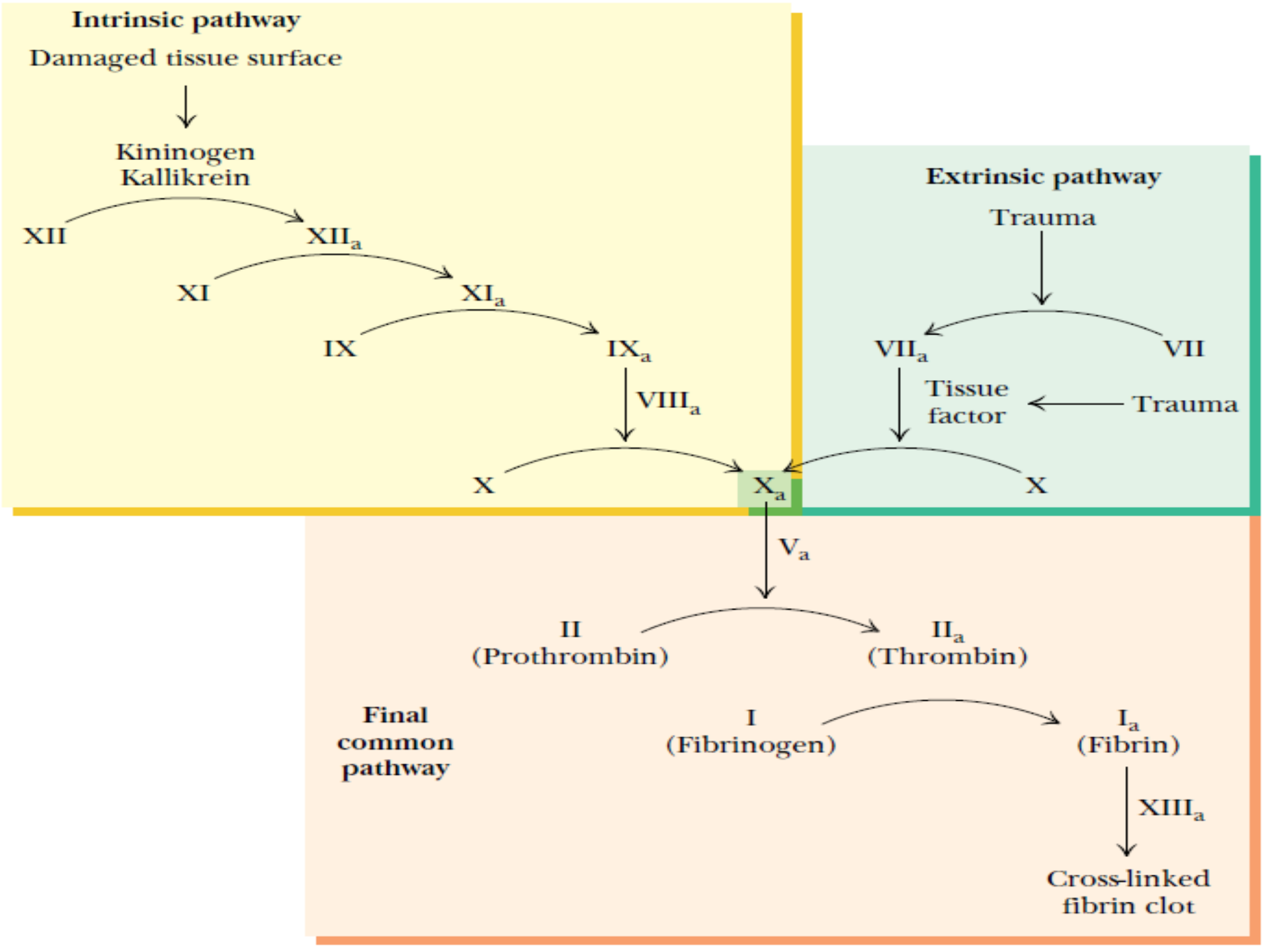
- The amplification achieved by this cascade of enzymatic activations allows blood clotting to occur rapidly in response to injury.

- Seven of the clotting factors in their active form are serine proteases: **kallikrein, XIIa, XIa, IXa, VIIa, Xa,** and **thrombin.**

Two routes to blood clot formation exist

- The **intrinsic pathway** is instigated when the blood comes into physical contact with abnormal surfaces caused by injury.

- The **extrinsic pathway** is initiated by factors released from injured tissues.
- The pathways merge at Factor X and culminate in clot formation.
- Thrombin excises peptides rich in negative charge from **fibrinogen,** converting it to **fibrin,** a molecule with a different surface charge distribution .
- Fibrin readily aggregates into ordered fibrous arrays that are subsequently stabilized by covalent cross-links
- Thrombin specifically cleaves Arg-Gly peptide bonds and is homologous to trypsin, which is also a serine protease.


Intrinsic pathway
Damaged tissue surface
Kininogen
Kallikrein
XII
XIIa
XI
XIa
IX
IXa
VIIIa
X
Xa
Extrinsic pathway
Trauma
VIIa
VII
Tissue factor
Trauma
X
Va
II (Prothrombin)
IIa (Thrombin)
Final common pathway
I (Fibrinogen)
Ia (Fibrin)
XIIIa
Cross-linked fibrin clot

Isozymes

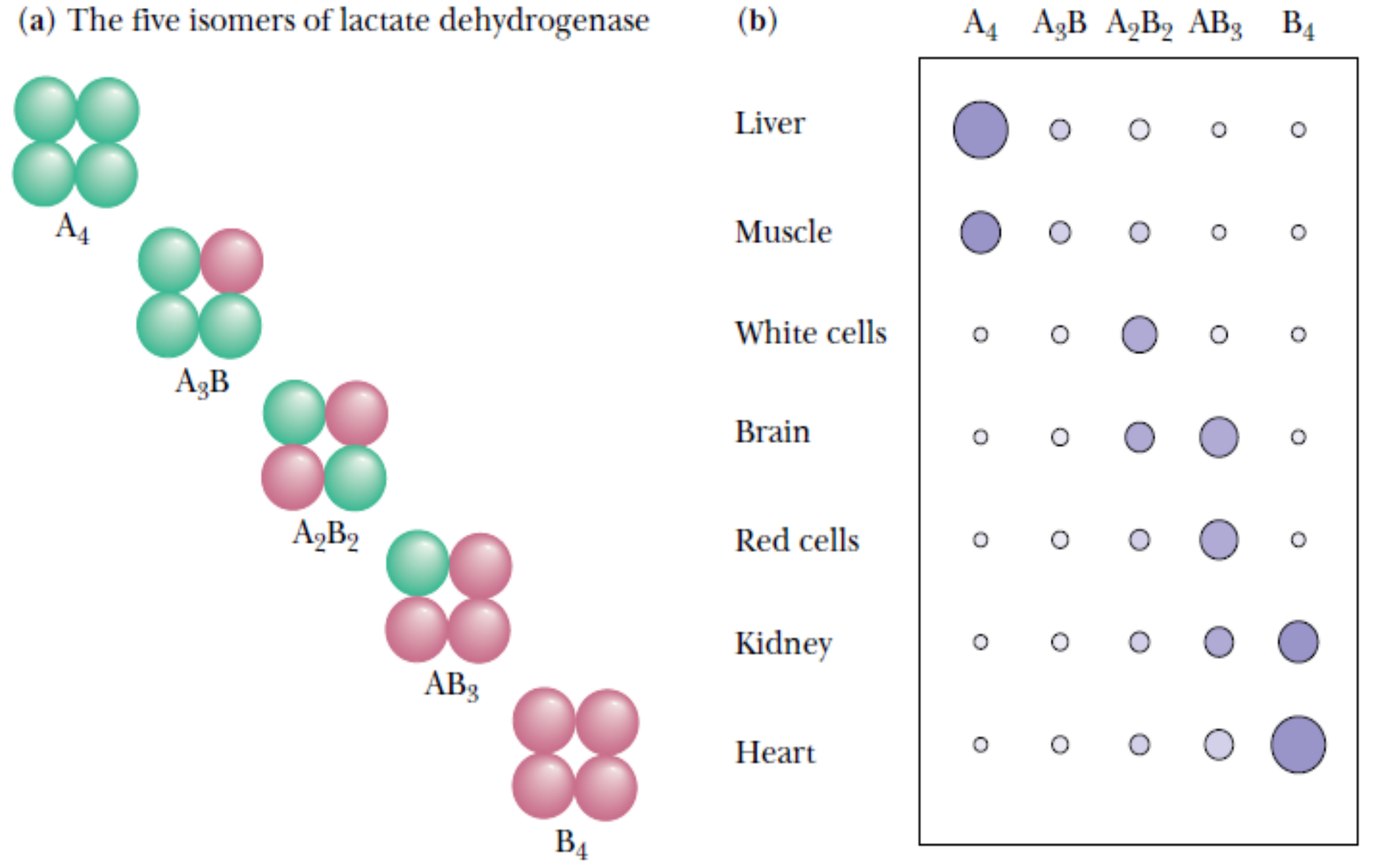
- A number of enzymes exist in more than one quaternary form, differing in their relative proportions of structurally equivalent but catalytically distinct polypeptide subunits.

- A classic example is mammalian **lactate dehydrogenase (LDH),** which exists as five different isozymes, depending on the tetrameric association of two different subunits, A and B: A4, A3B, A2B2, AB3, and B4

- The kinetic properties of the various LDH isozymes differ in terms of their relative affinities for the various substrates and their sensitivity to inhibition by product.

- Different tissues express different isozyme forms, as appropriate to their particular metabolic needs.

- By regulating the relative amounts of A and B subunits they synthesize, the cells of various tissues control which isozymic forms are likely to assemble, and, thus, which kinetic parameters prevail.


(a) The five isomers of lactate dehydrogenase
A4
A3B
A2B2
AB3
B4
(b)
A4
A3B
A2B2
AB3
B4
Liver
Muscle
White cells
Brain
Red cells
Kidney
Heart

Modulator Proteins

- **Modulator proteins** are proteins that bind to enzymes, and by binding, influence the activity of the enzyme.

- For example, some enzymes, such as *cAMP*-**dependent protein kinase**, exist as dimers of catalytic subunits and regulatory subunits.

- These regulatory subunits are *modulator proteins* that suppress the activity of the catalytic subunits.

- Dissociation of the regulatory subunits (modulator proteins) activates the catalytic subunits; reassociation once again suppresses activity.

- **Phosphoprotein phosphatase inhibitor-1 (PPI-1)** is another example of a modulator protein.

- When PPI-1 is phosphorylated on one of its serine residues, it binds to *phosphoprotein phosphatase*, inhibiting its phosphatase activity.

- The result is an increased phosphorylation of the interconvertible enzyme targeted by the protein kinase/phosphoprotein phosphatase cycle.