Enzymes

University of Anbar/College of Pharmacy

Second semester 2021-2022 / Biochemistry I / 3rd stage

References :

1- Harper’s Illustrated Biochemistry
2- Lehninger Principles of Biochemistry

By
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Enzymes :

Enzymes are biocatalysts Life is possible due to the coordination of numerous metabolic reactions inside the cells. Proteins can be hydrolyzed with hydrochloric acid by boiling for a very long time; but inside the body, with the help of enzymes, proteolysis takes place within a short time at body temperature. Enzyme catalysis is very rapid; usually 1 molecule of an enzyme can act upon about 1000 molecules of the substrate per minute. Deficiency of enzymes will lead to block in metabolic pathways causing inborn errors of metabolism. The substance upon which an enzyme acts, is called the substrate. The enzyme will convert the substrate into the product or products.

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Characteristics of Enzymes

- Almost all enzymes are proteins. Enzymes follow the physical and chemical reactions of proteins.

- They are heat labile (a molecule that is heat-labile means it can exist transiently in a particular conformation by means of heat before assuming a lower energy or stable conformation).

- They are water-soluble. iv. They can be precipitated by protein precipitating re agents (ammonium sulfate or trichloroacetic acid).

- They contain 16% weight as nitrogen.

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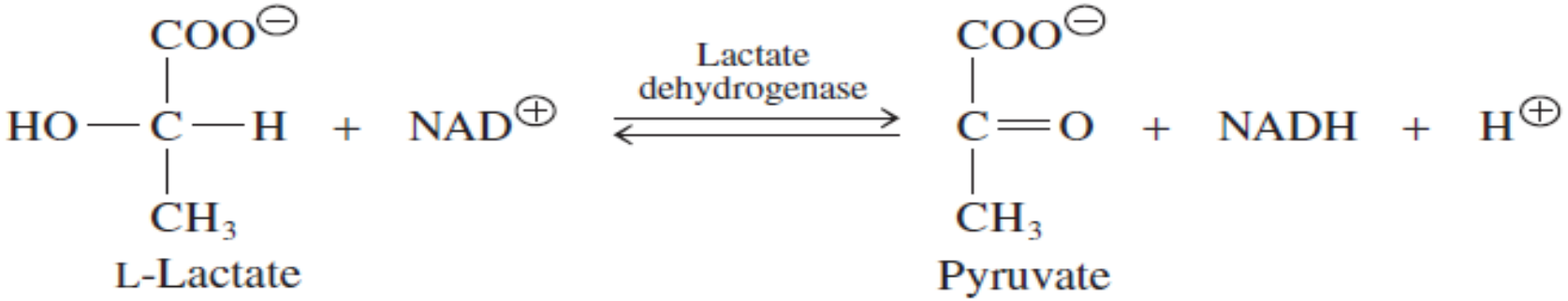
Nomenclature the Six Classes of Enzymes

Most of the classical metabolic enzymes are named by adding the suffix *-ase* to the name of their substrates or to a descriptive term for the reactions they catalyze For

1. Oxidoreductases catalyze oxidation–reduction reactions. Most of these enzymes are commonly referred to as **dehydrogenases.** Other enzymes in this class are called oxidases, peroxidases, oxygenases, or reductases. One example of an oxidoreductase is lactate dehydrogenase (EC 1.1.1.27) also called lactate:NAD oxidoreductase.



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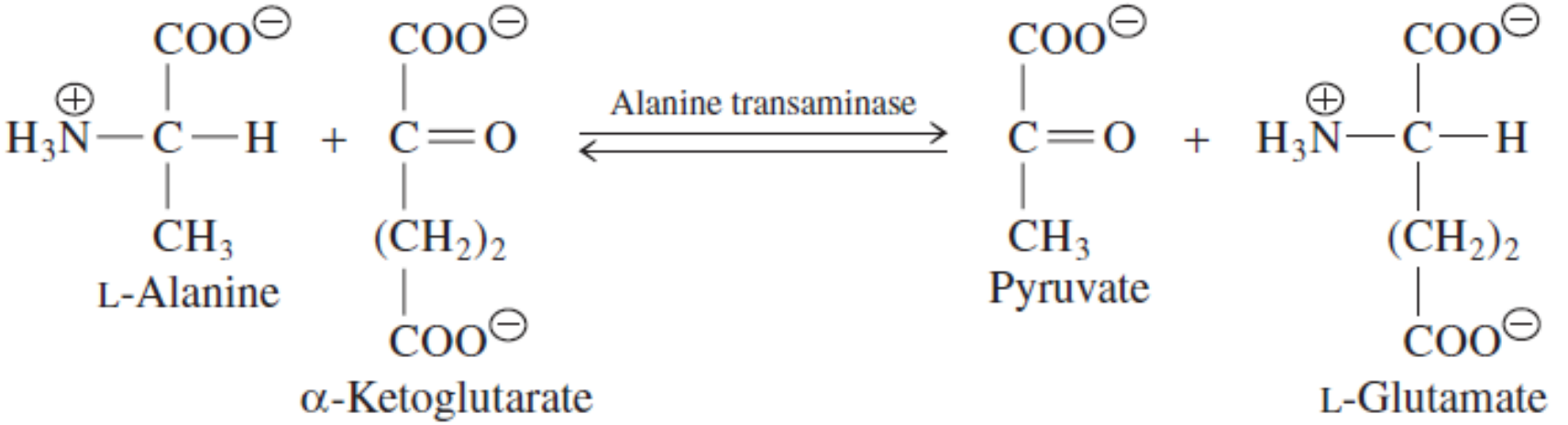
2. Transferases catalyze group-transfer reactions, and many require the presence of coenzymes. In group-transfer reactions, a portion of the substrate molecule usually binds covalently to the enzyme or its coenzyme. This group includes kinases, enzymes that catalyze the transfer of a phosphoryl group from ATP. Alanine transaminase, whose systematic name is L-alanine:2-oxyglutarate aminotransferase (EC 2.6.1.2), is a typical example of this class.



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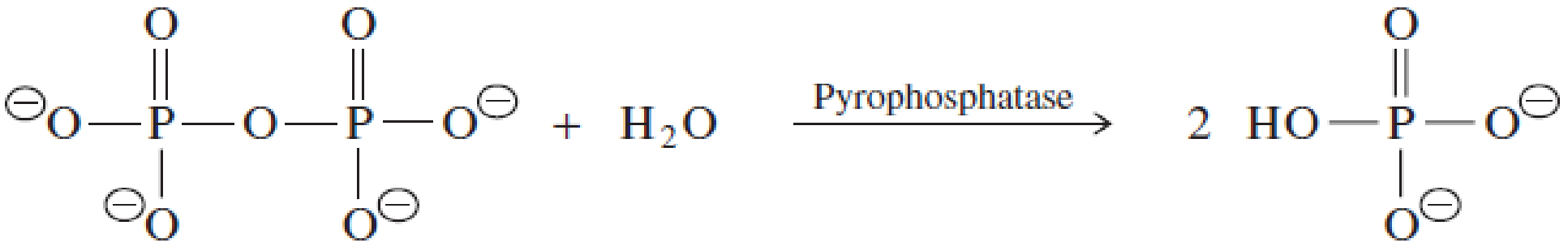
3. Hydrolases catalyze hydrolysis. They are a special class of transferases, with water serving as the acceptor of the group transferred. Pyrophosphatase is a simple example of a hydrolase. The systematic name of this enzyme is diphosphate phosphohydrolase (EC 3.6.1.1).



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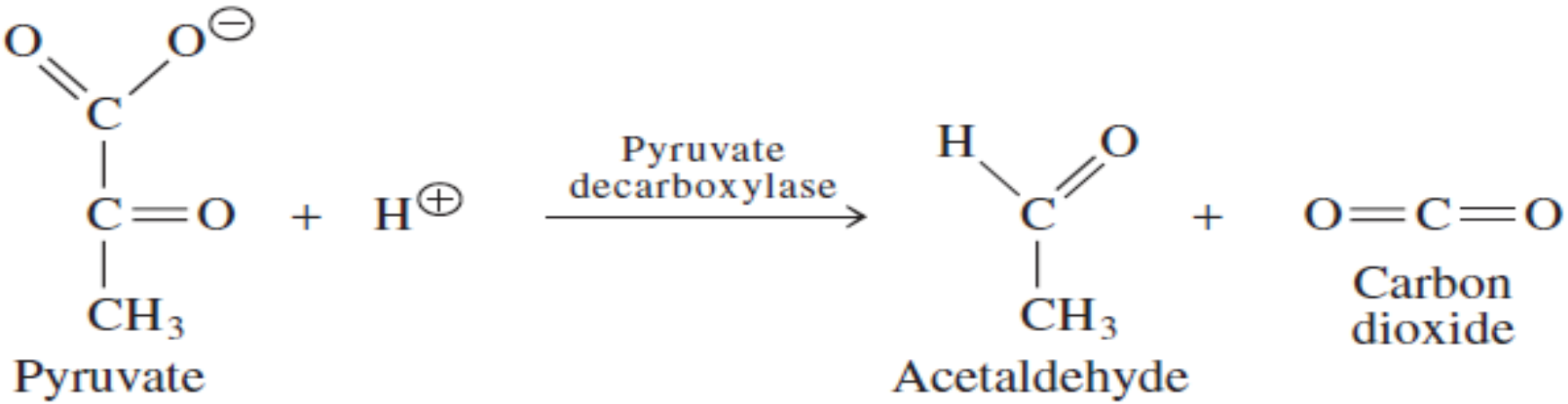
4. Lyases catalyze lysis of a substrate, generating a double bond; these are nonhydrolytic, nonoxidative, elimination reactions. In the reverse direction, lyases catalyze the addition of one substrate to a double bond of a second substrate. A lyase that catalyzes an addition reaction in cells is often called a **synthase.** Pyruvate decarboxylase belongs to this class of enzymes since it splits pyruvate into acetaldehyde and carbon dioxide. The systematic name for pyruvate decarboxylase, 2-oxo-acid carboxy-lyase (EC 4.1.1.1), is rarely used.



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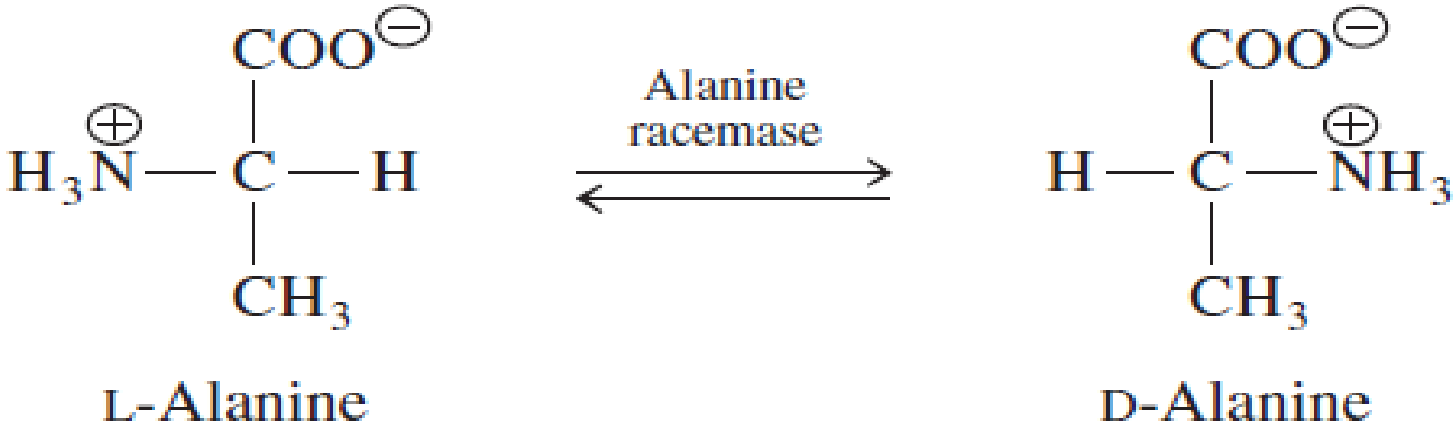
5. Isomerases catalyze structural change within a single molecule (isomerization reactions). Because these reactions have only one substrate and one product, they are among the simplest enzymatic reactions. Alanine racemase (EC 5.1.1.1) is an isomerase that catalyzes the interconversion of L-alanine and D-alanine.



L-Alanine D-Alanine

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6. Ligases catalyze ligation, or joining, of two substrates. These reactions require the input of the chemical potential energy of a nucleoside triphosphate such as ATP. Ligases are usually referred to as **synthetases.** Glutamine synthetase, or L-glutamate: ammonia ligase (ADP-forming) (EC 6.3.1.2), uses the energy of ATP hydrolysis to join glutamate and ammonia to produce glutamine.

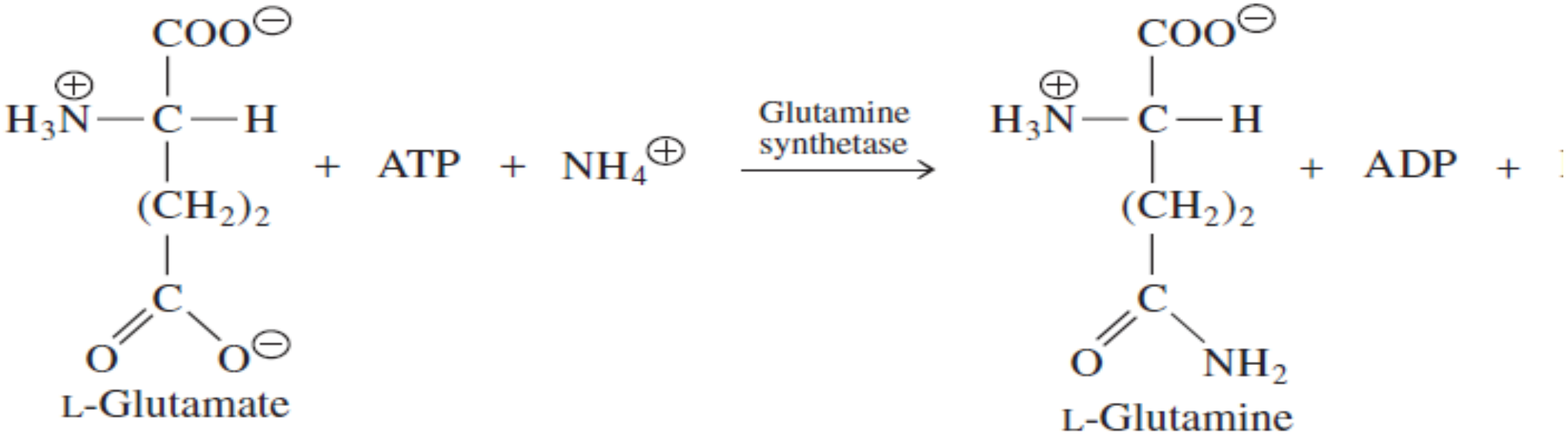

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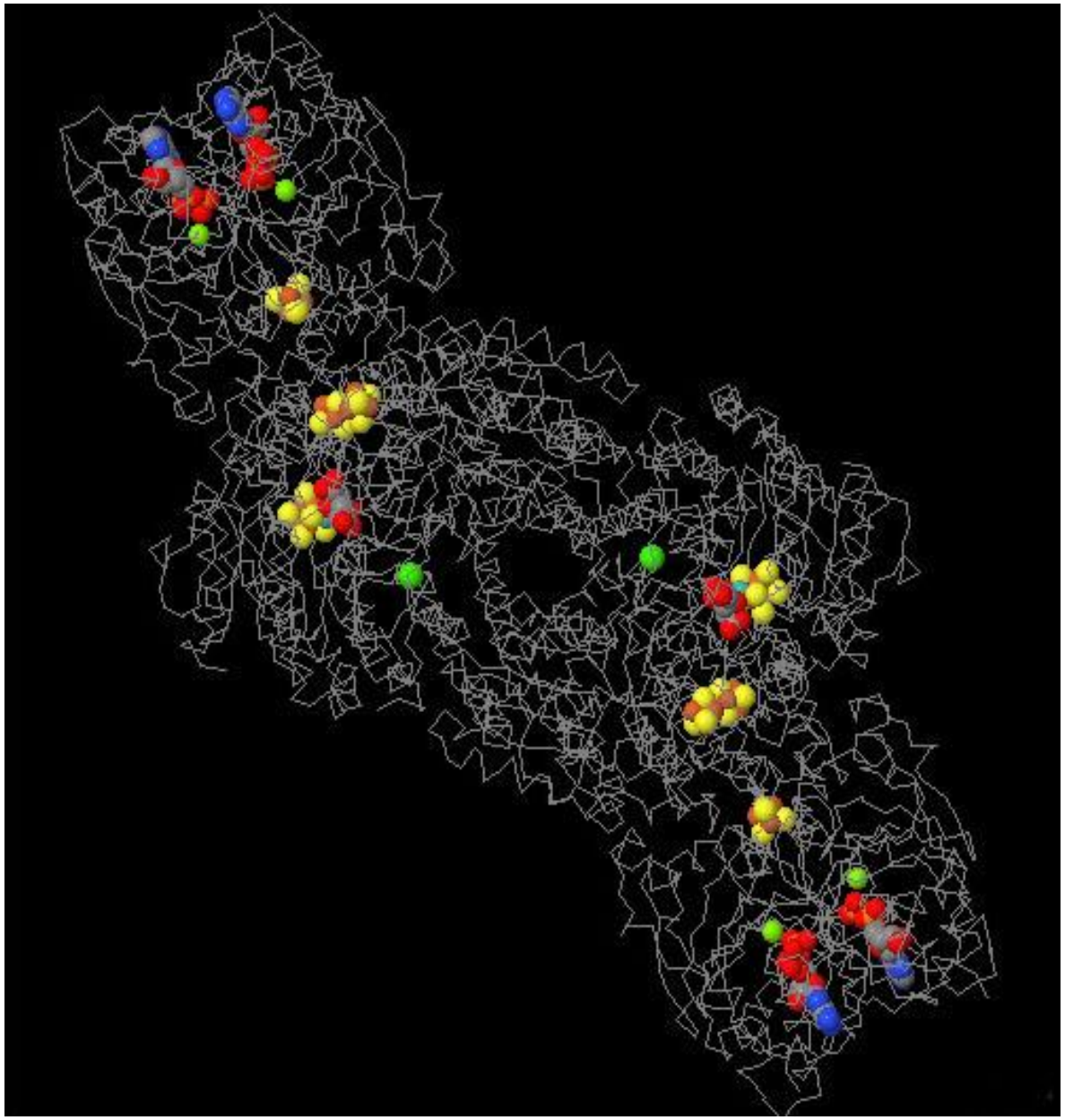
TABLE 6-3 International Classification of Enzymes

Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

Summery of Cofactors

- An additional non-protein molecule that is needed by some enzymes to help the reaction.
- coenzymes are organic molecules that are required by certain enzymes to carry out catalysis.
- Many vitamins are coenzymes.
- Cofactors are often classified as inorganic substances that are required for, or increase the rate of, catalysis. Fe^{+3},Fe^{+2}, Zn^{+2}



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Systematic Classification of Enzymes According to the Enzyme Commission

E.C. Number	Systematic Name and Subclasses
1	*Oxidoreductases* (oxidation–reduction reactions)
1.1	Acting on CH—OH group of donors
1.1.1	With NAD or NADP as acceptor
1.1.3	With O_2 as acceptor
1.2	Acting on the $\diagup C{=}O \diagdown$ group of donors
1.2.3	With O_2 as acceptor
1.3	Acting on the CH—CH group of donors
1.3.1	With NAD or NADP as acceptor
2	*Transferases* (transfer of functional groups)
2.1	Transferring C-1 groups
2.1.1	Methyltransferases
2.1.2	Hydroxymethyltransferases and formyltransferases
2.1.3	Carboxyltransferases and carbamoyltransferases
2.2	Transferring aldehydic or ketonic residues
2.3	Acyltransferases
2.4	Glycosyltransferases
2.6	Transferring N-containing groups
2.6.1	Aminotransferases
2.7	Transferring P-containing groups
2.7.1	With an alcohol group as acceptor
3	*Hydrolases* (hydrolysis reactions)
3.1	Cleaving ester linkage
3.1.1	Carboxylic ester hydrolases
3.1.3	Phosphoric monoester hydrolases
3.1.4	Phosphoric diester hydrolases
4	*Lyases* (addition to double bonds)
4.1	C—C lyases
4.1.1	Carboxy lyases
4.1.2	Aldehyde lyases
4.2	C—O lyases
4.2.1	Hydrolases
4.3	C—N lyases
4.3.1	Ammonia-lyases
5	*Isomerases* (isomerization reactions)
5.1	Racemases and epimerases
5.1.3	Acting on carbohydrates
5.2	Cis-trans isomerases
6	*Ligases* (formation of bonds with ATP cleavage)
6.1	Forming C—O bonds
6.1.1	Amino acid–RNA ligases
6.2	Forming C—S bonds
6.3	Forming C—N bonds
6.4	Forming C—C bonds
6.4.1	Carboxylases

- prosthetic group: A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a holoenzyme. The protein part of such an enzyme is called the apoenzyme or apoprotein.

- Enzymes, like other proteins, have molecular weights ranging from about 12,000 to more than 1 million. Some enzymes require no chemical groups for activity other than their amino acid residues. Others require an additional chemical component called a cofactor—either one or more inorganic ions, such as Fe 2+ , Mg 2+ , Mn 2+ , or Zn 2+, or a complex organic or metalloorganic molecule called a coenzyme.

- some enzyme proteins are modified covalently by phosphorylation, glycosylation, and other processes. Many of these alterations are involved in the regulation of enzyme activity.

TABLE 6-1 Some Inorganic Ions That Serve as Cofactors for Enzymes

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease

TABLE 6-2 Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Allosteric Enzymes:

Allosteric enzymes are enzymes whose properties are affected by changes in structure. The structural changes are mediated by interaction with small molecules. Allostery in the previous when we examined the binding of oxygen to hemoglobin. Allosteric enzymes often do not exhibit typical Michaelis–Menten kinetics due to cooperative binding of substrate, as is the case with the non-enzyme hemoglobin. the figure next slide shows a versus [S] curve for an allosteric enzyme with cooperative binding of substrate.

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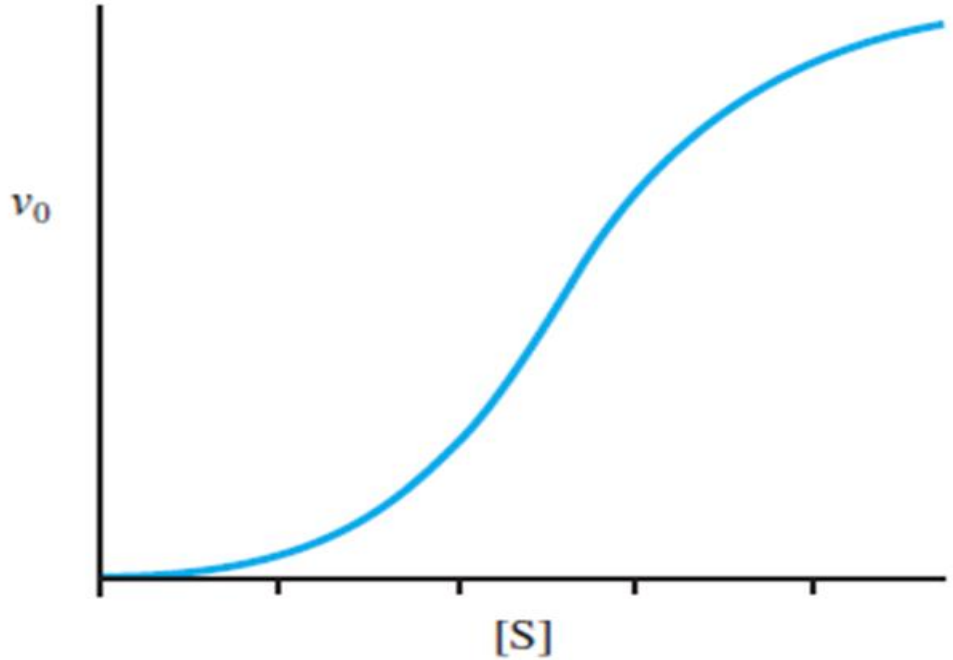
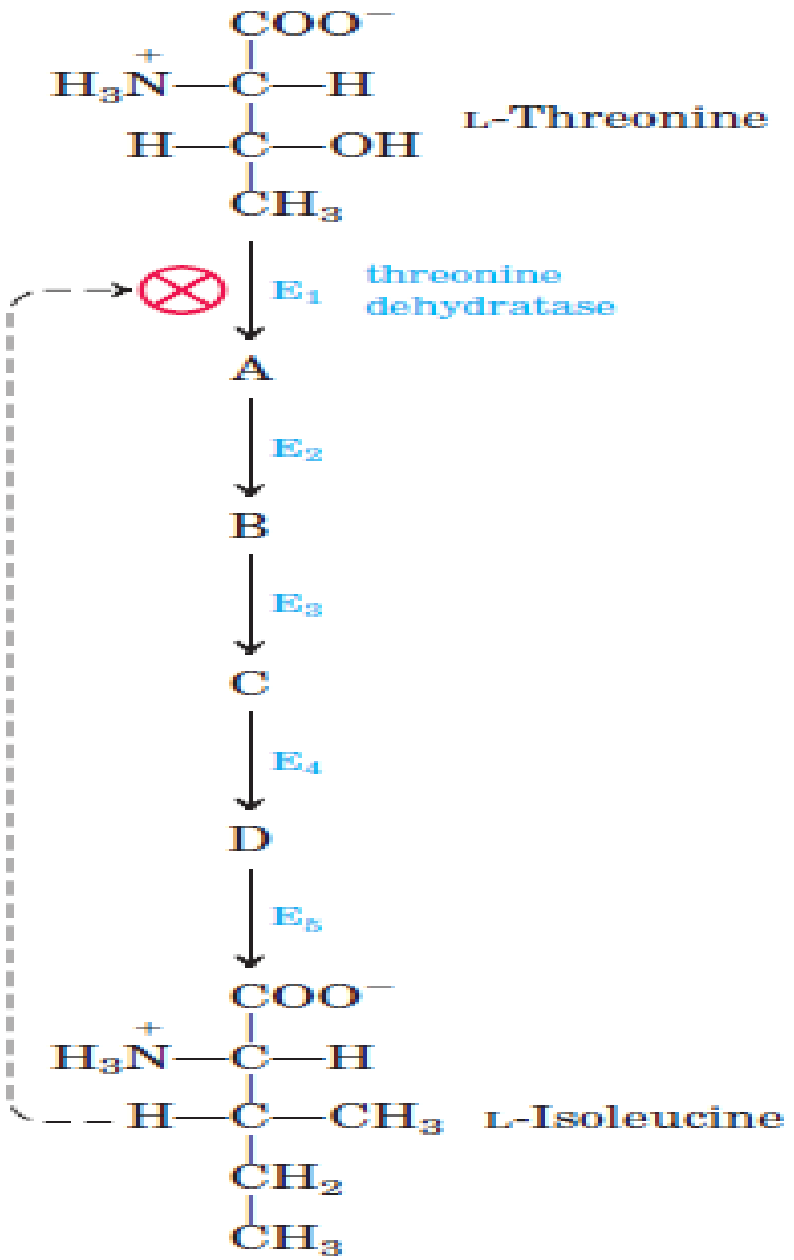
Sigmoidal curves result from the transition between two states of the enzyme. In the absence of substrate, the enzyme is in the T state. The conformation of each subunit is in a shape that binds substrate inefficiently and the rate of the reaction is slow.





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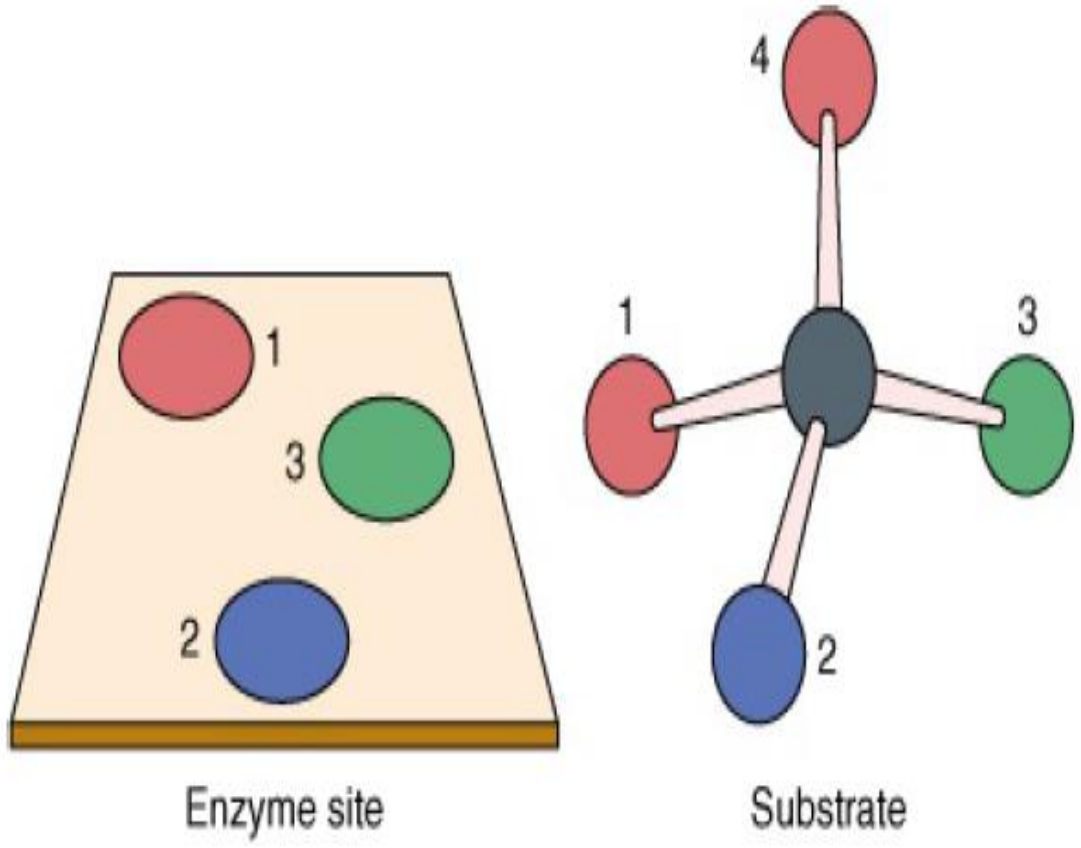
ENZYMES ARE EFFECTIVE & HIGHLY SPECIFIC CATALYSTS:

The enzymes that catalyze the conversion of one or more compounds **(substrates)** into one or more different compounds **(products)** enhance the rates of the corresponding noncatalyzed reaction by factors of at least 10^6.

- Enzymes are extremely selective catalysts.
- Enzymes are specific both for the type of reaction catalyzed and for a single substrate or a small set of closely related substrates.
- Enzymes are also stereospecific catalysts and typically catalyze reactions of only one stereoisomer of a given compound—for example, D- but not L-sugars, L- but not D-amino acids.



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An imaginary enzyme (stickase) designed to catalyze breakage of a metal stick:

(a) Before the stick is broken, it must first be bent (the transition state). In both stickase examples, magnetic interactions take the place of weak bonding interactions between enzyme and substrate.

(b) A stickase with a magnet-lined pocket complementary in structure to the stick (the substrate) stabilizes the substrate. Bending is impeded by the magnetic attraction between stick
and stickase.

(c) An enzyme with a pocket complementary to the reaction transition state helps to destabilize the stick, contributing to catalysis of the reaction. The binding energy of the magnetic interactions compensates for the increase in free energy required to bend the stick.

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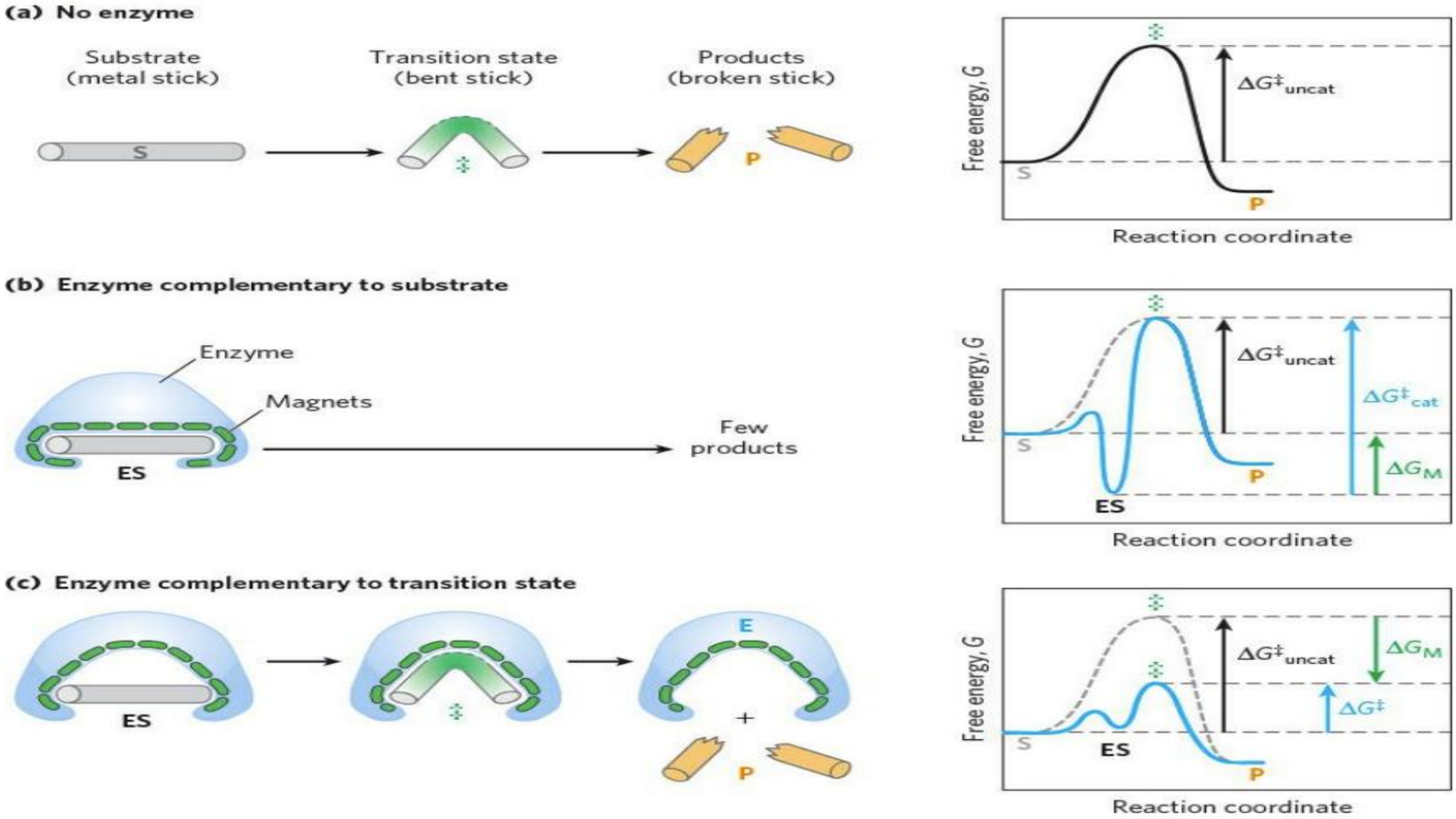
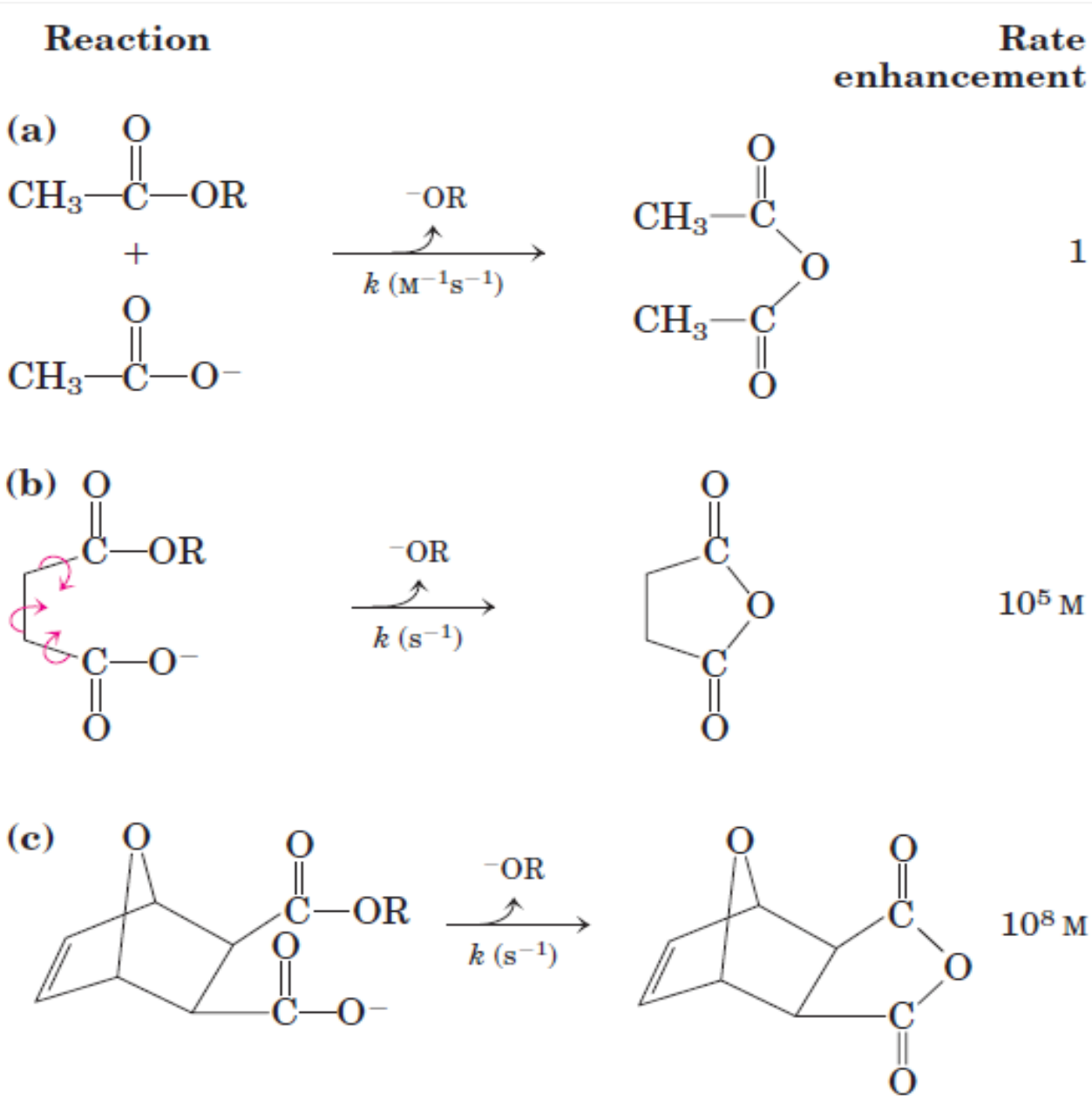

(a) No enzyme
Substrate (metal stick)
Transition state (bent stick)
Products (broken stick)
S
P
Free energy, G
$\Delta G^{\ddagger}_{uncat}$
Reaction coordinate
(b) Enzyme complementary to substrate
Enzyme
Magnets
ES
Few products
$\Delta G^{\ddagger}_{cat}$
ΔG_M
(c) Enzyme complementary to transition state
E
+
$\Delta G^{\ddagger}$

Important notes:

Binding energy makes an important, and sometimes the dominant, contribution to catalysis. Consider what needs to occur for a reaction to take place. Prominent physical and thermodynamic factors contributing to $\Delta G^{\ddagger}$, the barrier to reaction, might include

(1) A reduction in entropy, in the form of decreased freedom of motion of two molecules in solution.



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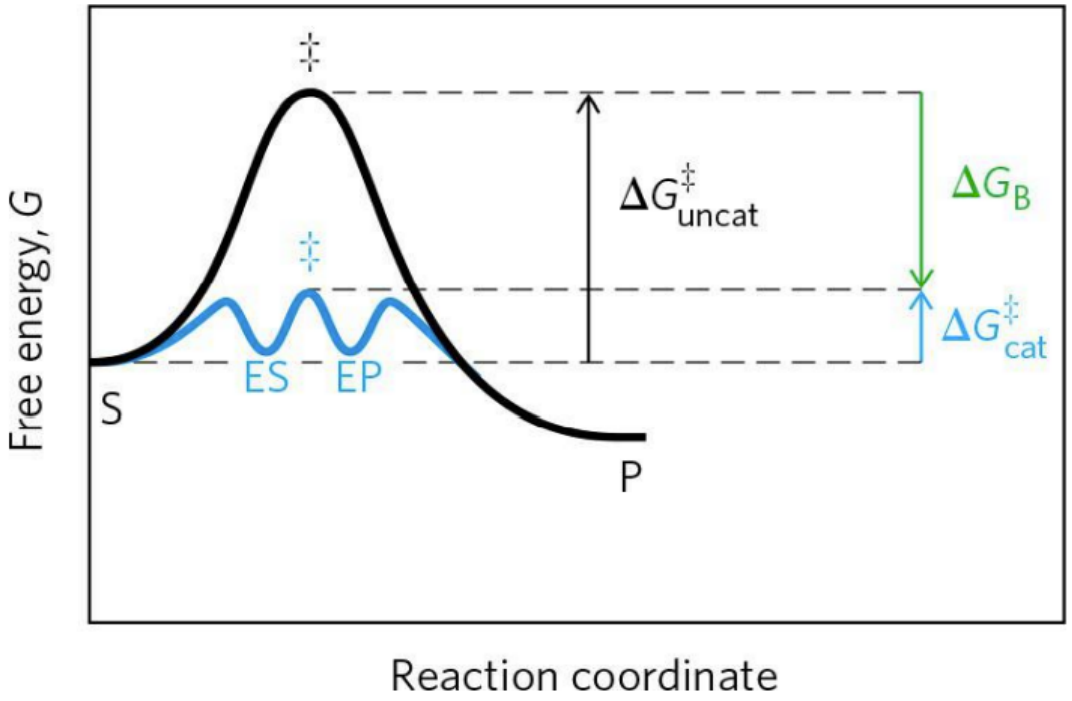
(2) The solvation shell of hydrogen-bonded water that surrounds and helps to stabilize most biomolecules in aqueous solution.

(3) The distortion of substrates that must occur in many reactions.

(4) The need for proper alignment of catalytic functional groups on the enzyme.



Role of binding energy in catalysis. To lower the activation energy for a reaction, the system must acquire an amount of energy equivalent to the amount by which ΔG ‡ is lowered. Much of this energy comes from binding energy, ΔGB, contributed by formation of weak noncovalent interactions between substrate and enzyme in the transition state. The role of ΔGB is analogous to that of ΔGM

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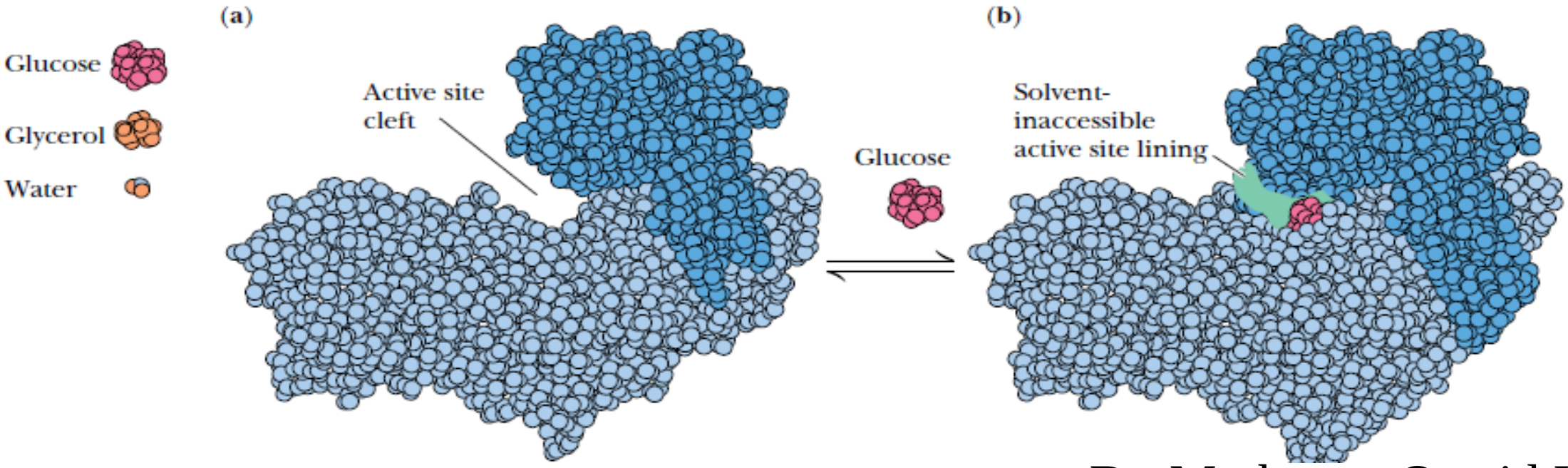
Enzyme work theories

1- 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 pliancy. 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 nzyme's active site is actually modified upon binding S, in a process of dynamic recognition between enzyme and substrate fittingly called **induced fit.**



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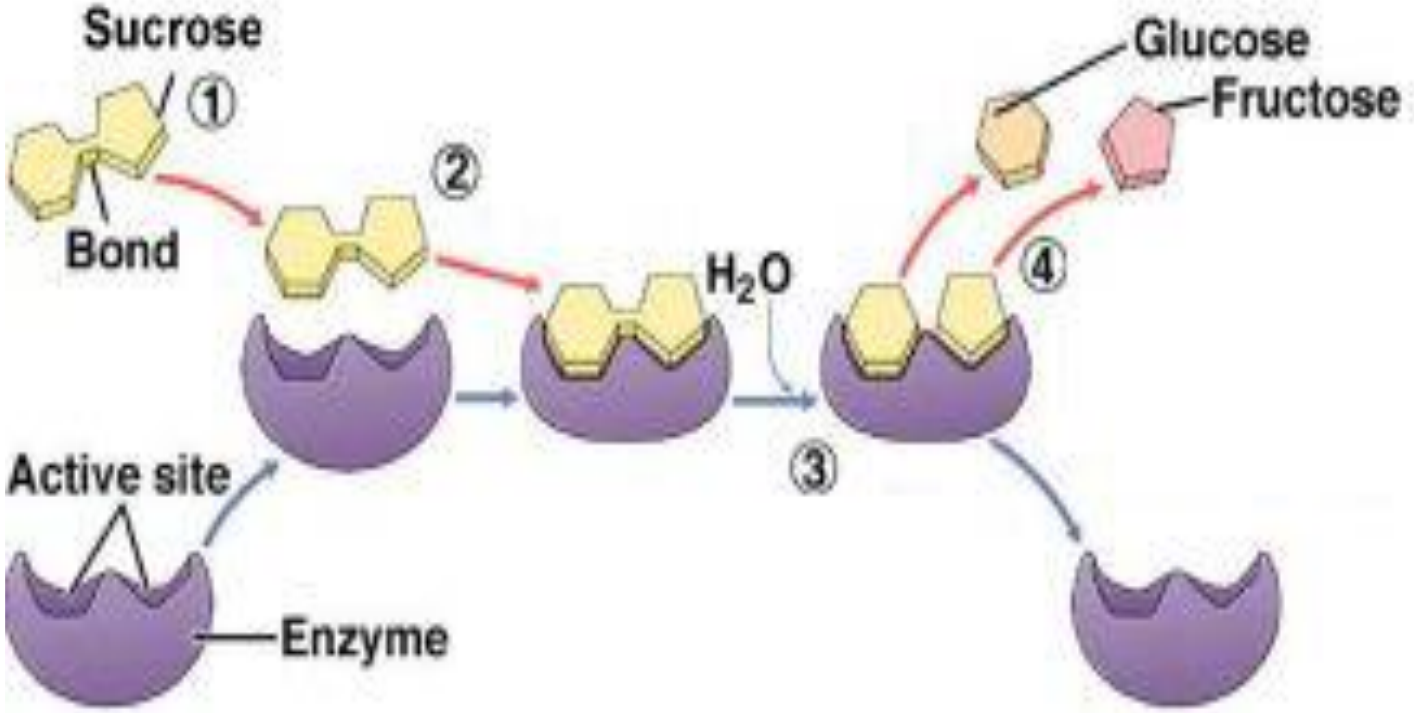
2- Lock and Key Hypothesis:

Pioneering enzyme specificity studies at the turn of the century by the great organic chemist Emil Fischer 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. Enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another.



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ENZYMES EMPLOY MULTIPLE MECHANISMS TO FACILITATE CATALYSIS:

Enzymes use various combinations of many general mechanisms to achieve dramatic catalytic enhancement of the rates of chemical reactions.

Acid–Base Catalysis:

The ionizable functional groups of aminoacyl side chains and (where present) of prosthetic groups contribute to catalysis by acting as acids or bases. Acid-base catalysis can be either specific or general. By "specific" we mean only protons (H_3O^+) or OH^- ions. In **specific acid catalysis** or **specific base catalysis,** the rate of reaction is sensitive to changes in the concentration of protons of but *independent* of the concentrations of other acids (proton donors) or bases (proton acceptors) present in the solution or at the active site. Reactions whose rates are responsive to *all* the acids or bases present are said to be subject to **general acid catalysis** or **general base catalysis.**

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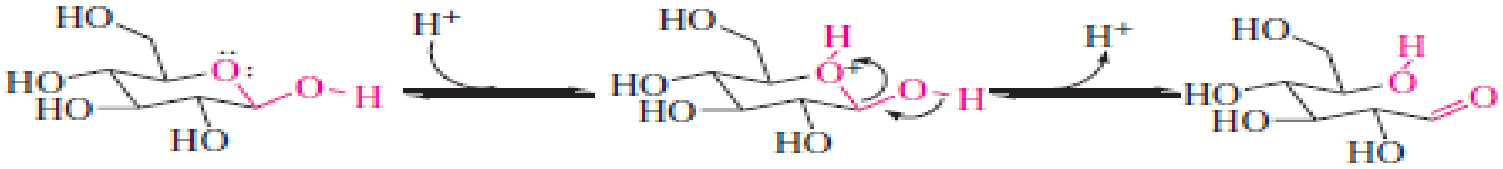
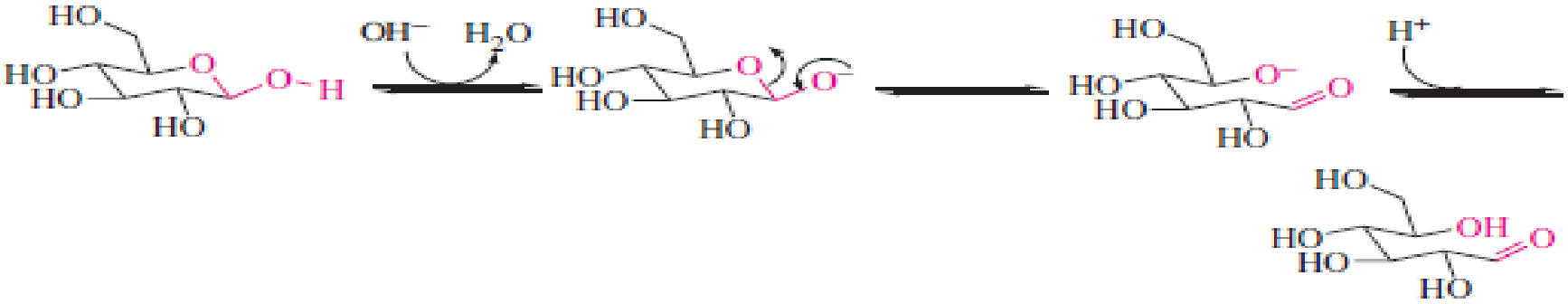
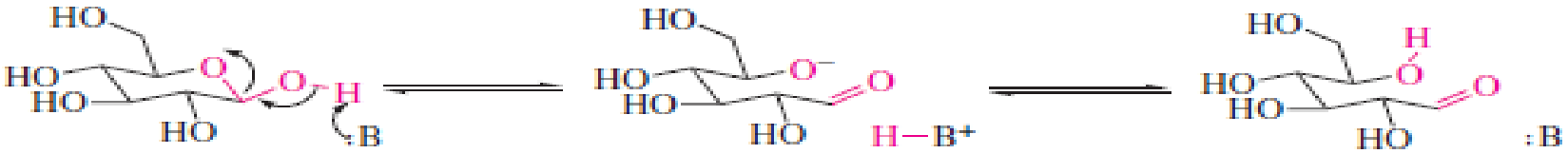
Specific acid catalyzed



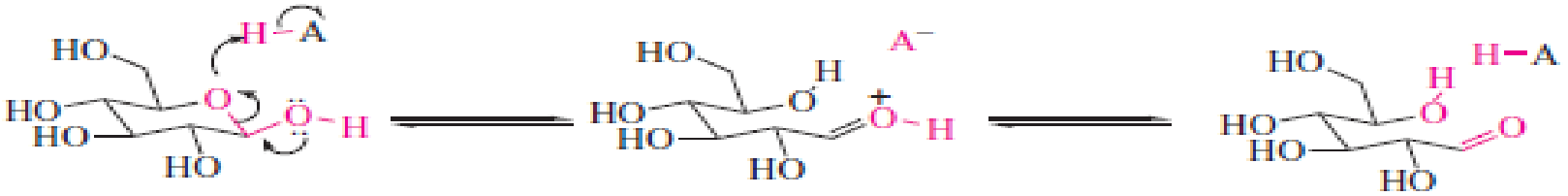
Specific base catalyzed

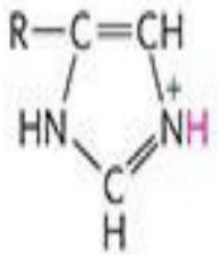
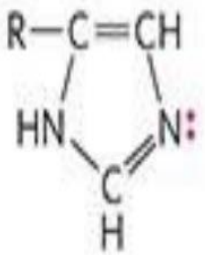
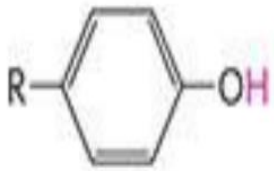
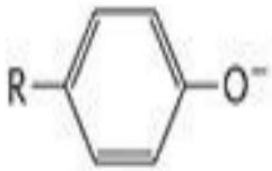


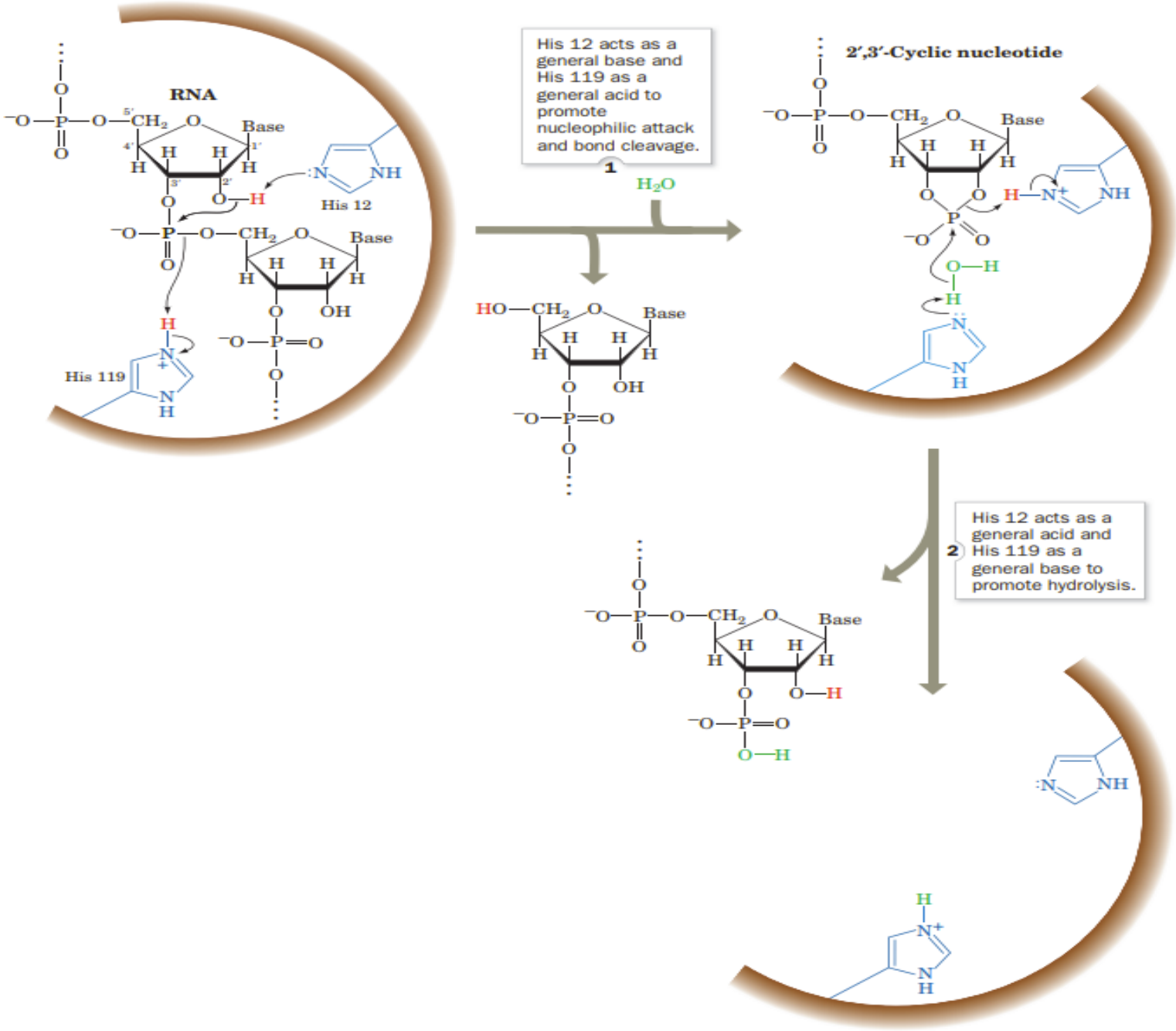
General base catalyzed



General acid catalyzed



Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	R—COOH	R—COO⁻
Lys, Arg	R—⁺NH(H)—H	R—NH₂
Cys	R—SH	R—S⁻
His		
Ser	R—OH	R—O⁻
Tyr		



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Catalysis by Strain:

Enzymes that catalyze *lytic* reactions that involve breaking a covalent bond typically bind their substrates in a confirmation that is somewhat unfavorable for the bond that will undergo cleavage. This conformation mimics that of the **transition state intermediate,** a transient species that represents the transition state, or half-way point, in the transformation of substrates to products. The resulting strain stretches or distorts the targeted bond, weakening it and making it more weak to cleavage.

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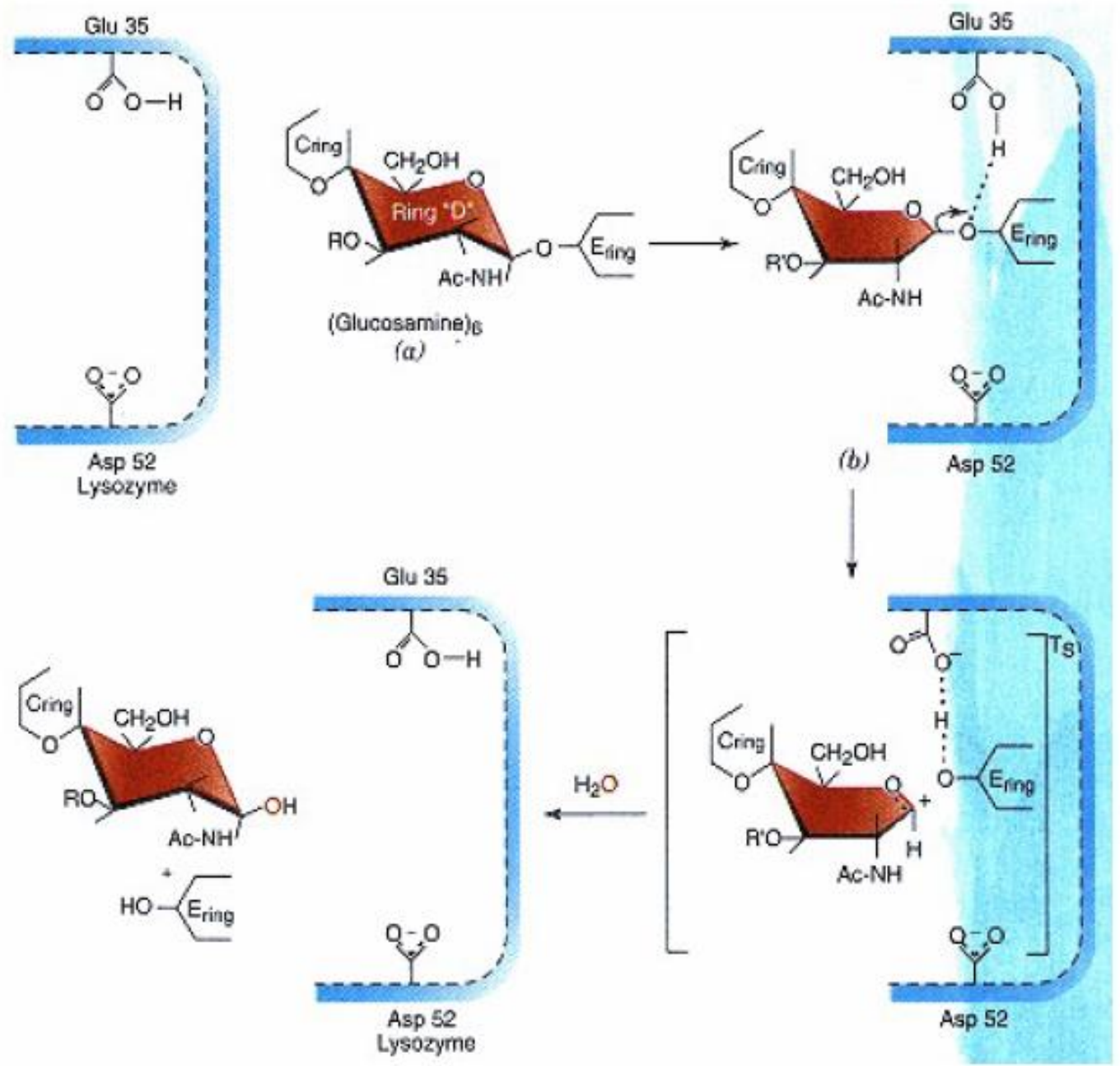
Catalysis by Strain:

For catalysis of lytic reactions, which involve breaking a covalent bond, enzymes typically bind their substrates in a conformation that weakens the bond targeted for cleavage through physical distortion and electronic polarization. This strained conformation mimics that of the transition state intermediate, a transient species that represents the midway point in the transformation of substrates to products.



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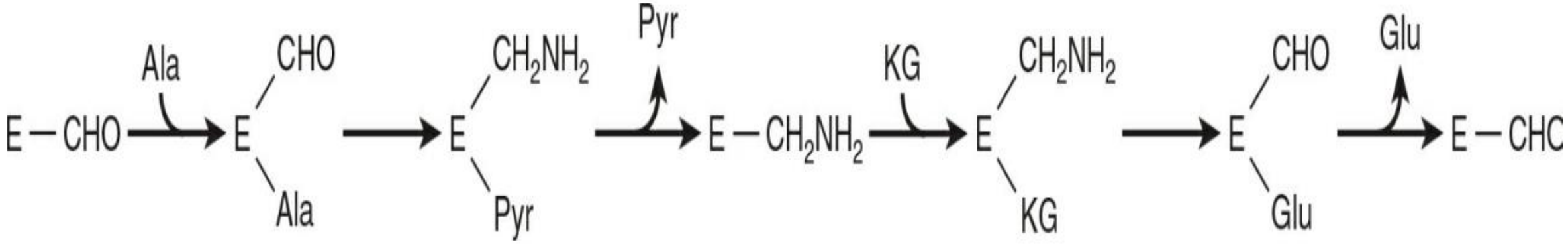
Covalent Catalysis:

The process of covalent catalysis involves the formation of a covalent bond between the enzyme and one or more substrates. The modified enzyme thus becomes a reactant. Covalent catalysis provides a new reaction pathway whose activation energy is lower—and rate of reaction therefore faster—than the pathways available in homogeneous solution. The chemically modified state of the enzyme is, however, transient. Completion of the reaction returns the enzyme to its original, unmodified state. Its role thus remains catalytic. Covalent catalysis is particularly common among enzymes that catalyze group transfer reactions. Residues on the enzyme that participate in covalent catalysis generally are cysteine or serine, and occasionally histidine.



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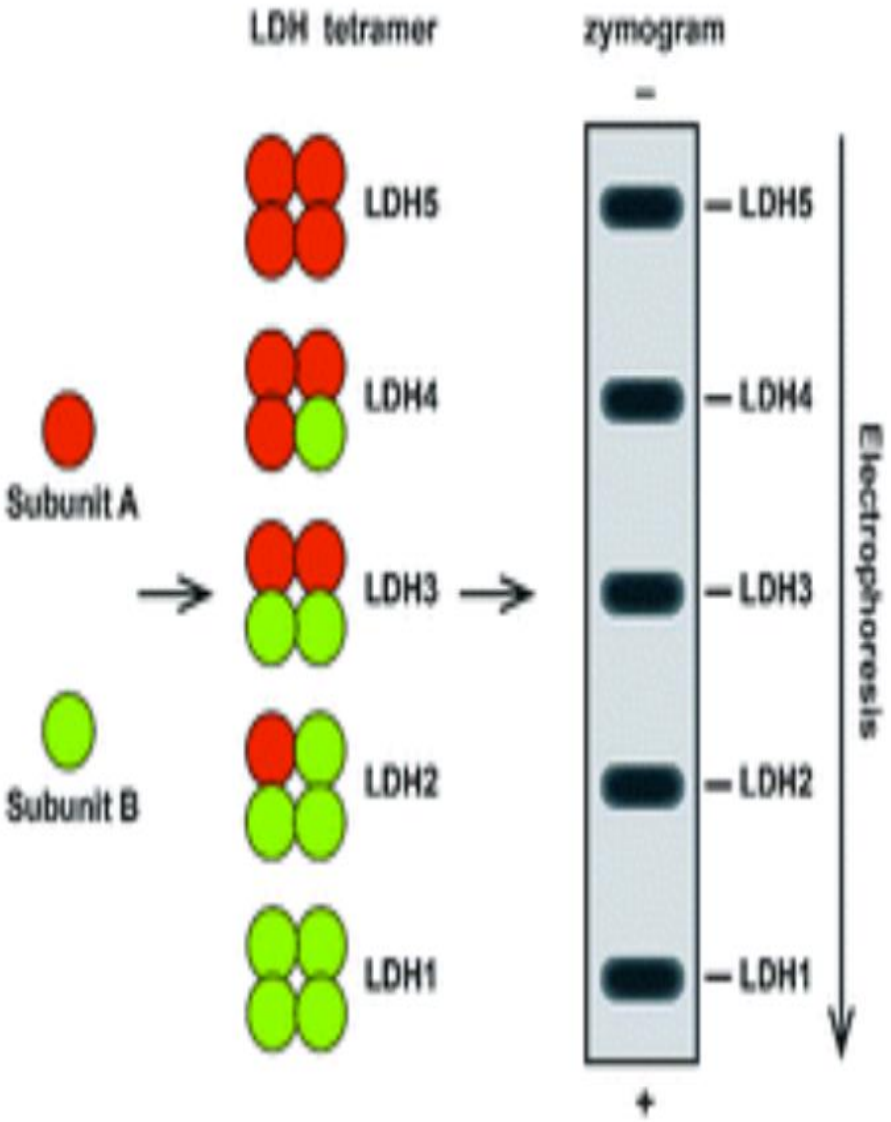
ISOZYMES ARE DISTINCT ENZYME FORMS THAT CATALYZE THE SAME REACTION

Higher organisms often elaborate several physically distinct versions of a given enzyme, each of which catalyzes the same reaction. Like the members of other protein families, these protein catalysts or **isozymes** arise through gene duplication. Isozymes may exhibit subtle differences in properties such as sensitivity to particular regulatory factors or substrate affinity (eg, hexokinase and glucokinase) that adapt them to specific tissues and lactate dehydrogenase (LDH) and its isoenzymes.



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THE CATALYTIC ACTIVITY OF ENZYMES FACILITATES THEIR DETECTION

Single-Molecule Enzymology:

The limited sensitivity of traditional enzyme assays necessitates the use of a large group, or ensemble, of enzyme molecules in order to produce measurable quantities of product.

Drug Discovery Requires Enzyme Assays Suitable for High-Throughput Screening:

Enzymes constitute one of the primary classes of biomolecules targeted for the development of drugs and other therapeutic agents. Many antibiotics, for example, inhibit enzymes that are unique to microbial pathogens. The discovery of new drugs is greatly facilitated when a large number of potential pharmacophores can be assayed in a rapid, automated fashion—a process referred to as high-throughput screening. High-throughput screening (HTS) takes advantage of recent advances in robotics, optics, data processing, and microfluidics to conduct and analyze many thousands of simultaneous assays of the activity of a given enzyme. Enzyme assays that produce a chromagenic or fluorescent product are ideal, since optical detectors are readily engineered to permit the rapid analysis of multiple samples.

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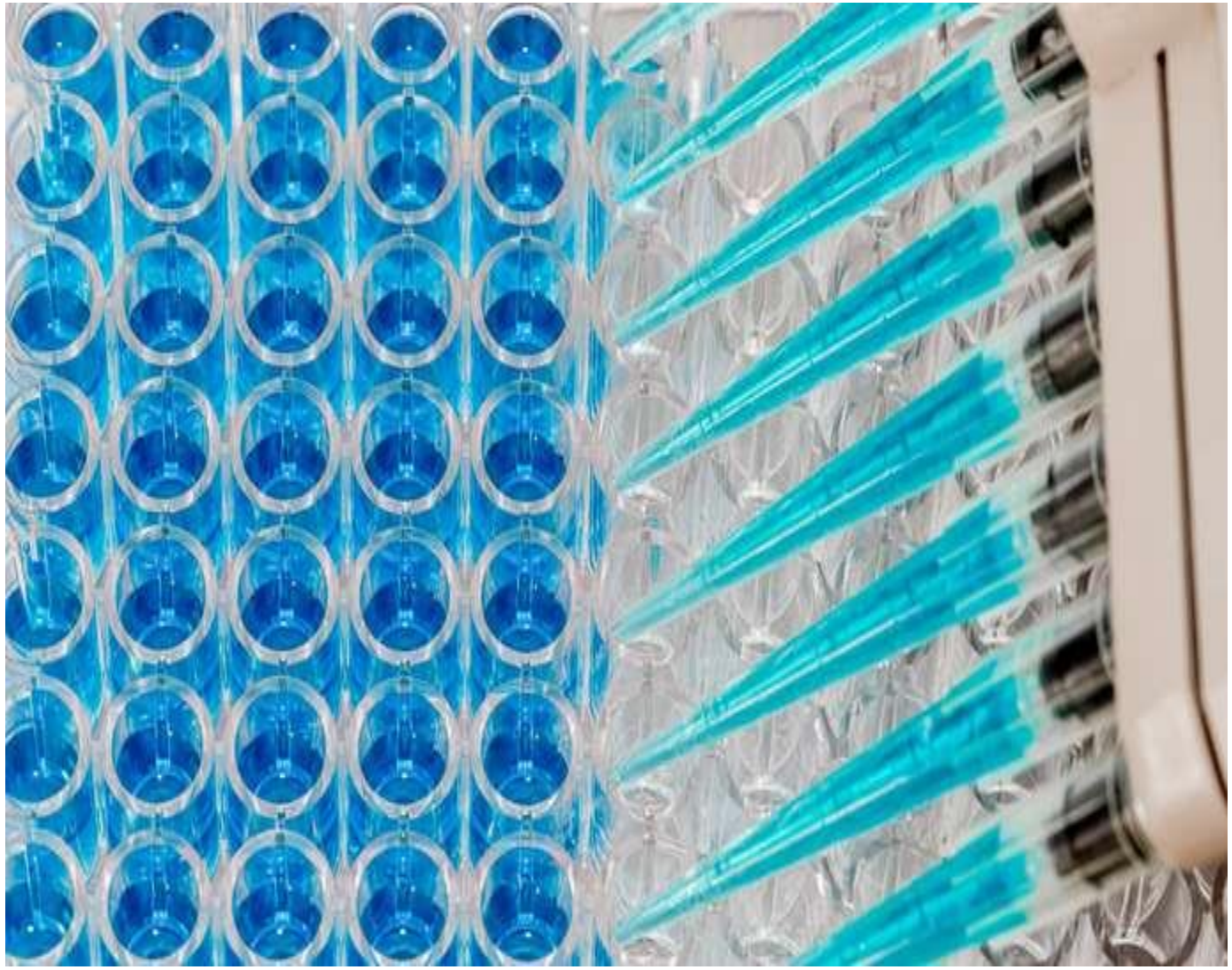
Enzyme-Linked Immunoassays:

The sensitivity of enzyme assays can be exploited to detect proteins that lack catalytic activity. Enzyme-linked immunosorbent assays (ELISAs) use antibodies covalently linked to a "reporter enzyme" such as alkaline phosphatase or horseradish peroxidase whose products are readily detected, generally by the absorbance of light or by fluorescence.

Enzyme-linked immunosorbent assays (ELISAs) typically are used to detect antigens, though they can also be used to detect other substances, including antibodies, hormones, and drugs.



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NAD(P)-Dependent Dehydrogenases Are Assayed Spectrophotometrically:

The physicochemical properties of the reactants in an enzyme-catalyzed reaction dictate the options for the assay of enzyme activity. Spectrophotometric assays exploit the ability of a Substrate or product to absorb light.

Involvement in disease:

The analysis of enzymes in blood plasma has played a central role in the diagnosis of several disease processes. Many enzymes are functional constituents of blood. Quantitative analysis of the activity of released enzymes or other proteins, typically in plasma or serum but also in urine or various cells, provides information concerning diagnosis, prognosis, and response to treatment.

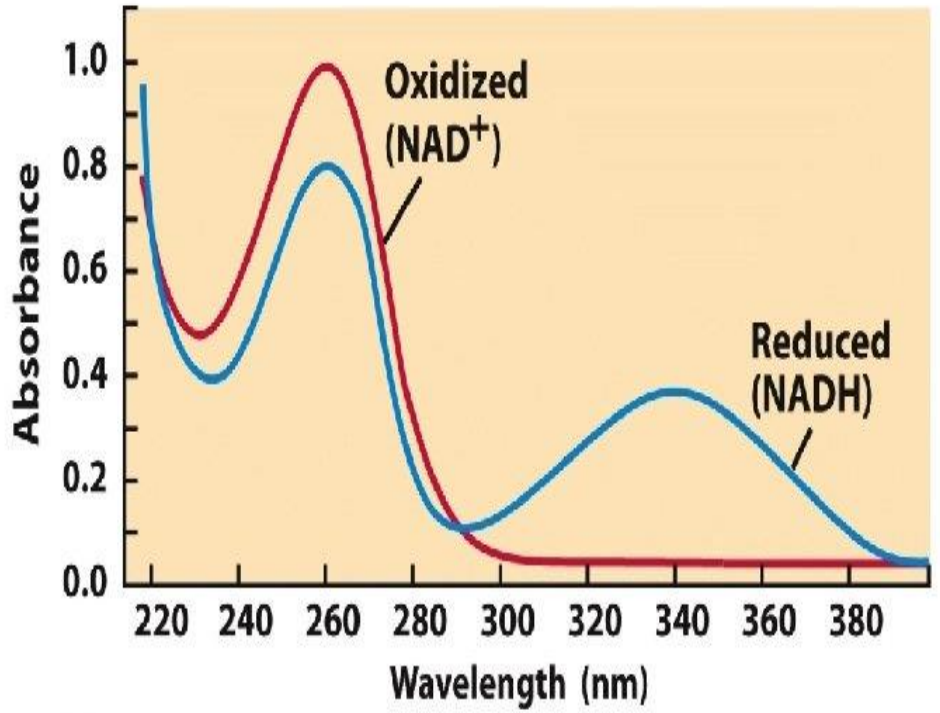

Figure 13-24b
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Table 7–2 Principal Serum Enzymes Used in Clinical Diagnosis

Serum Enzyme	Major Diagnostic Use
Aminotransferases	
Aspartate aminotransferase (AST, or SGOT)	Myocardial infarction
Alanine aminotransferase (ALT, or SGPT)	Viral hepatitis
Amylase	Acute pancreatitis
Ceruloplasmin	Hepatolenticular degeneration (Wilson's disease)
Creatine kinase	Muscle disorders and myocardial infarction
-Glutamyl transferase	Various liver diseases
Lactate dehydrogenase isozyme 5	Liver diseases
Lipase	Acute pancreatitis
Phosphatase, acid	Metastatic carcinoma of the prostate
Phosphatase, alkaline (isozymes)	Various bone disorders, obstructive liver diseases

ENZYMES FACILITATE DIAGNOSIS OF GENETIC AND INFECTIOUS DISEASES

Many diagnostic techniques take advantage of the specificity and efficiency of the enzymes that act on oligonucleotides such as DNA .Enzymes known as **restriction endonucleases,** for example, cleave double-stranded DNA at sites specified by a sequence of four, six, or more base pairs called **restriction sites.** Cleavage of a sample of DNA with a restriction enzyme produces a characteristic set of smaller DNA fragments . Deviations in the normal product pattern, called **restriction fragment length polymorphisms (RFLPs),** occur if a mutation renders a restriction site unrecognizable to its cognate restriction endonuclease or, alternatively, generates a new recognition site.

The **polymerase chain reaction (PCR)** employs a thermostable DNA polymerase and appropriate oligonucleotide primers to produce thousands of copies of a defined segment of DNA from a minute quantity of starting material. PCR enables medical, biological, and forensic scientists to detect and characterize DNA present initially at levels too low for direct detection.

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MULTIPLE FACTORS AFFECT THE RATES OF ENZYME-CATALYZED REACTIONS

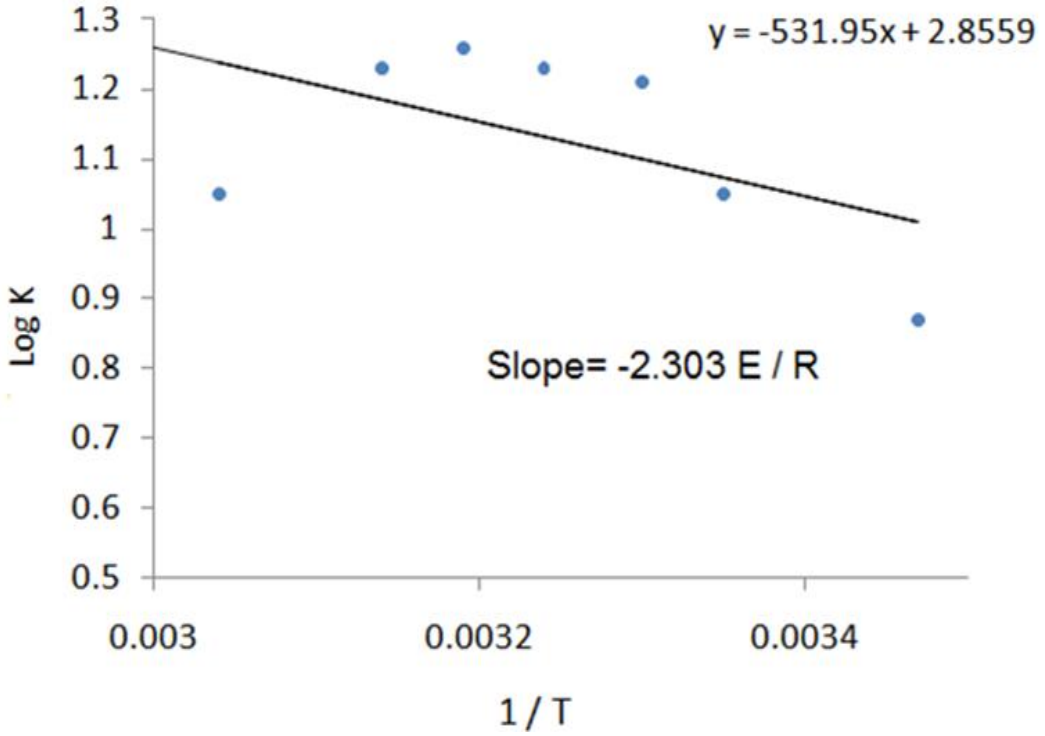
Temperature

Raising the temperature increases the rate of both uncatalyzed and enzyme-catalyzed reactions by increasing the kinetic energy and the collision frequency of the reacting molecules.

Enzymes from humans generally exhibit stability at temperatures up to 45–55°C. By contrast, enzymes from the thermophilic microorganisms that reside in volcanic hot springs or undersea hydrothermal vents may be stable at temperatures up to or even above 100°C.

Enzyme reactions as a function of temperature

((log K = -2.303 E/RT +log A))



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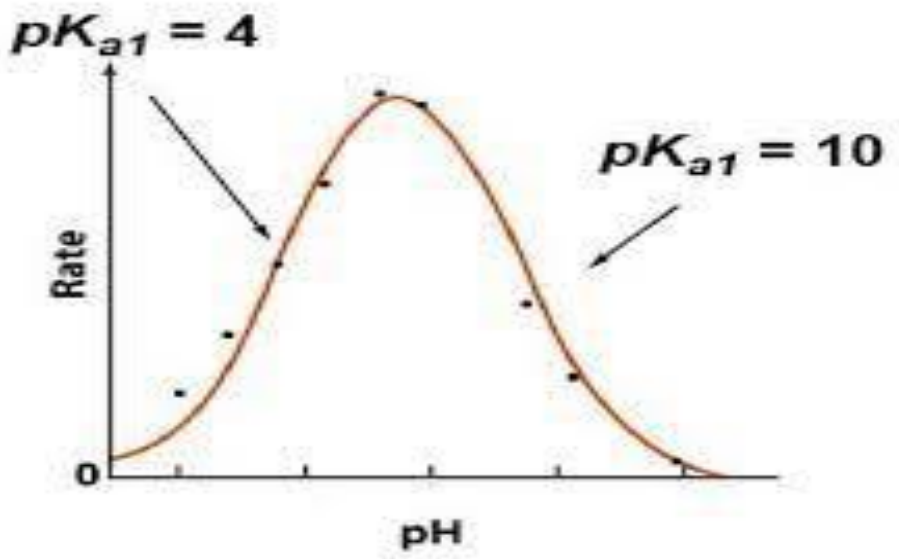
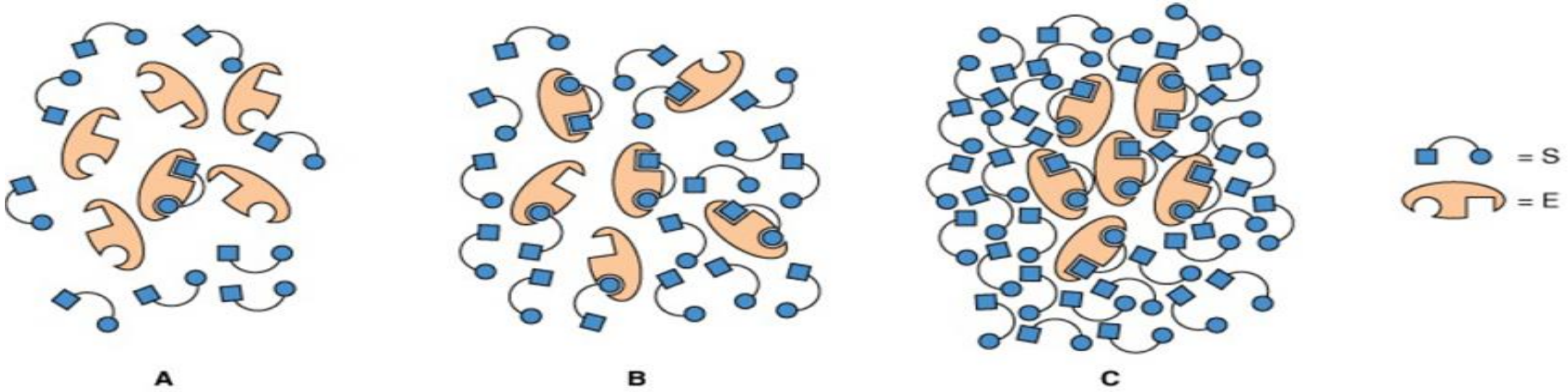
Hydrogen Ion Concentration:

The rate of almost all enzyme-catalyzed reactions exhibits a significant dependence on hydrogen ion concentration. Most intracellular enzymes exhibit optimal activity at pH values between 5 and 9.



SUBSTRATE CONCENTRATION AFFECTS THE REACTION RATE.



Thanks for listening