Enzymes: Mechanism of Action

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BIOMEDICAL IMPORTANCE

Enzymes are biologic polymers that catalyze the chemical reactions which make life as we know it possible. The presence and maintenance of a complete and balanced set of enzymes is essential for the breakdown of nutrients to supply energy and chemical building blocks; the assembly of those building blocks into proteins, DNA, membranes, cells, and tissues; and the harnessing of energy to power cell motility and muscle contraction. With the exception of a few catalytic RNA molecules, or ribozymes, the vast majority of enzymes are proteins. Deficiencies in the quantity or catalytic activity of key enzymes can result from genetic defects, nutritional deficits, or toxins. Defective enzymes can result from genetic mutations or infection by viral or bacterial pathogens (eg, *Vibrio cholerae*). Medical scientists address imbalances in enzyme activity by using pharmacologic agents to inhibit specific enzymes and are investigating gene therapy as a means to remedy deficits in enzyme level or function.

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. Like all catalysts, enzymes are neither consumed nor permanently altered as a consequence of their participation in a reaction.

In addition to being highly efficient, enzymes are also extremely selective catalysts. Unlike most catalysts used in synthetic chemistry, 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 only of specific stereoisomers of a given compound—for example, D- but not L-sugars, L- but not D-amino acids. Since they bind substrates through at least "three points of attachment," enzymes can even convert nonchiral substrates to chiral products. Figure 7–1 illustrates why the enzyme-catalyzed reduction of the nonchiral substrate pyruvate produces L-lactate rather a racemic mixture of D- and L-lactate. The exquisite specificity of enzyme catalysts imbues living cells with the ability to simultaneously conduct and independently control a broad spectrum of chemical processes.

ENZYMES ARE CLASSIFIED BY REACTION TYPE & MECHANISM

A system of enzyme nomenclature that is comprehensive, consistent, and at the same time easy to use has proved elusive. The common names for most enzymes derive from their most distinctive characteristic: their ability to catalyze a specific chemical reaction. In general, an enzyme's name consists of a term that identifies the type of reaction catalyzed followed by the suffix *-ase.* For example, dehydrogen*ases* remove hydrogen atoms, prote*ases* hydrolyze proteins, and isomer*ases* catalyze rearrangements in configuration. One or more modifiers usually precede this name. Unfortunately, while many modifiers name the specific substrate involved (xanthine oxidase), others identify the source of the enzyme (pancreatic ribonuclease), specify its mode of regulation (hormone-sensitive lipase), or name a distinguishing characteristic of its mechanism (a cysteine protease). When it was discovered that multiple forms of some enzymes existed, alphanumeric designators were added to distinguish between them (eg, RNA polymerase III; protein kinase Cβ). To address the ambiguity and confusion arising from these inconsistencies in nomenclature and the continuing discovery of new enzymes, the International Union of Biochemists (IUB) developed a complex but unambiguous system of enzyme nomenclature. In the IUB system, each enzyme has a unique name and code number that reflect the type of reaction catalyzed and the substrates involved. Enzymes are grouped into six classes, each with several subclasses. For example, the enzyme commonly called "hexokinase" is designated "ATP:D-hexose-6-phosphotransferase E.C. 2.7.1.1." This identifies hexokinase as a member of class 2 (transferases), subclass 7 (transfer of a phosphoryl group), sub-subclass 1 (alcohol is the phosphoryl acceptor). Finally, the term "hexose-6" indicates that the alcohol phosphorylated is that of carbon six of a hexose. Listed below are the six IUB classes of enzymes and the reactions they catalyze.

1. **Oxidoreductases** catalyze oxidations and reductions.

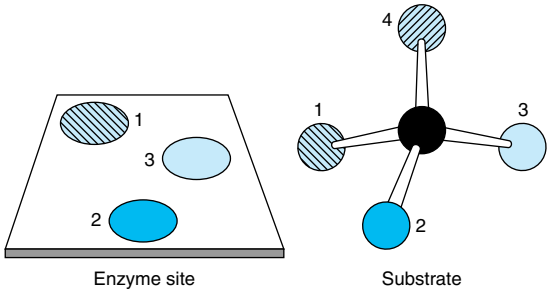

Figure 7–1. Planar representation of the "three-point attachment" of a substrate to the active site of an enzyme. Although atoms 1 and 4 are identical, once atoms 2 and 3 are bound to their complementary sites on the enzyme, only atom 1 can bind. Once bound to an enzyme, apparently identical atoms thus may be distinguishable, permitting a stereospecific chemical change.

2. **Transferases** catalyze transfer of groups such as methyl or glycosyl groups from a donor molecule to an acceptor molecule.
3. **Hydrolases** catalyze the hydrolytic cleavage of C—C, C—O, C—N, P—O, and certain other bonds, including acid anhydride bonds.
4. **Lyases** catalyze cleavage of C—C, C—O, C—N, and other bonds by elimination, leaving double bonds, and also add groups to double bonds.
5. **Isomerases** catalyze geometric or structural changes within a single molecule.
6. **Ligases** catalyze the joining together of two molecules, coupled to the hydrolysis of a pyrophosphoryl group in ATP or a similar nucleoside triphosphate.

Despite the many advantages of the IUB system, texts tend to refer to most enzymes by their older and shorter, albeit sometimes ambiguous names.

PROSTHETIC GROUPS, COFACTORS, & COENZYMES PLAY IMPORTANT ROLES IN CATALYSIS

Many enzymes contain small nonprotein molecules and metal ions that participate directly in substrate binding or catalysis. Termed **prosthetic groups, cofactors,** and **coenzymes,** these extend the repertoire of catalytic capabilities beyond those afforded by the limited number of functional groups present on the aminoacyl side chains of peptides.

Prosthetic Groups Are Tightly Integrated Into an Enzyme's Structure

Prosthetic groups are distinguished by their tight, stable incorporation into a protein's structure by covalent or noncovalent forces. Examples include pyridoxal phosphate, flavin mononucleotide (FMN), flavin dinucleotide (FAD), thiamin pyrophosphate, biotin, and the metal ions of Co, Cu, Mg, Mn, Se, and Zn. Metals are the most common prosthetic groups. The roughly one-third of all enzymes that contain tightly bound metal ions are termed **metalloenzymes.** Metal ions that participate in redox reactions generally are complexed to prosthetic groups such as heme (Chapter 6) or iron-sulfur clusters (Chapter 12). Metals also may facilitate the binding and orientation of substrates, the formation of covalent bonds with reaction intermediates (Co^{2+} in coenzyme B_{12}), or interaction with substrates to render them more **electrophilic** (electron-poor) or **nucleophilic** (electron-rich).

Cofactors Associate Reversibly With Enzymes or Substrates

Cofactors serve functions similar to those of prosthetic groups but bind in a transient, dissociable manner either to the enzyme or to a substrate such as ATP. Unlike the stably associated prosthetic groups, cofactors therefore must be present in the medium surrounding the enzyme for catalysis to occur. The most common cofactors also are metal ions. Enzymes that require a metal ion cofactor are termed **metal-activated enzymes** to distinguish them from the **metalloenzymes** for which metal ions serve as prosthetic groups.

Coenzymes Serve as Substrate Shuttles

Coenzymes serve as recyclable shuttles—or group transfer reagents—that transport many substrates from their point of generation to their point of utilization. Association with the coenzyme also stabilizes substrates such as hydrogen atoms or hydride ions that are unstable in the aqueous environment of the cell. Other chemical moieties transported by coenzymes include methyl groups (folates), acyl groups (coenzyme A), and oligosaccharides (dolichol).

Many Coenzymes, Cofactors, & Prosthetic Groups Are Derivatives of B Vitamins

The water-soluble B vitamins supply important components of numerous coenzymes. Many coenzymes contain, in addition, the adenine, ribose, and phosphoryl moieties of AMP or ADP (Figure 7–2). **Nicotinamide** and **riboflavin** are components of the redox coenzymes

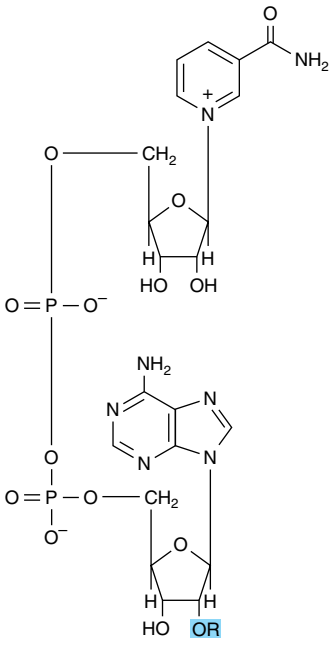

Figure 7–2. Structure of NAD^+ and $NADP^+$. For NAD^+, R = H. For $NADP^+$, $R = PO_3^{2-}$.

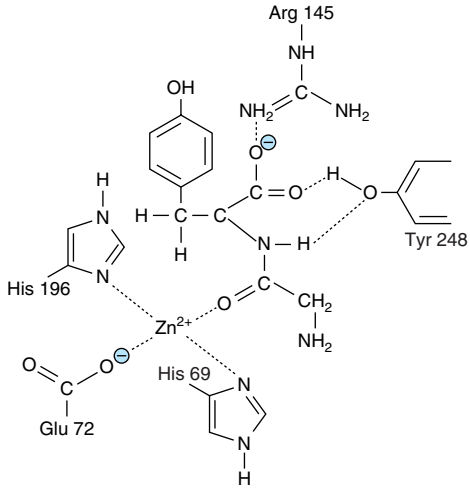

Figure 7–3. Two-dimensional representation of a dipeptide substrate, glycyl-tyrosine, bound within the active site of carboxypeptidase A.

NAD and NADP and FMN and FAD, respectively. **Pantothenic acid** is a component of the acyl group carrier coenzyme A. As its pyrophosphate, **thiamin** participates in decarboxylation of α-keto acids and **folic acid** and **cobamide** coenzymes function in one-carbon metabolism.

CATALYSIS OCCURS AT THE ACTIVE SITE

The extreme substrate specificity and high catalytic efficiency of enzymes reflect the existence of an environment that is exquisitely tailored to a single reaction. Termed the **active site,** this environment generally takes the form of a cleft or pocket. The active sites of multimeric enzymes often are located at the interface between subunits and recruit residues from more than one monomer. The three-dimensional active site both shields substrates from solvent and facilitates catalysis. Substrates bind to the active site at a region complementary to a portion of the substrate that will *not* undergo chemical change during the course of the reaction. This simultaneously aligns portions of the substrate that *will* undergo change with the chemical functional groups of peptidyl aminoacyl residues. The active site also binds and orients cofactors or prosthetic groups. Many amino acyl residues drawn from diverse portions of the polypeptide chain (Figure 7–3) contribute to the extensive size and three-dimensional character of the active site.

ENZYMES EMPLOY MULTIPLE MECHANISMS TO FACILITATE CATALYSIS

Four general mechanisms account for the ability of enzymes to achieve dramatic catalytic enhancement of the rates of chemical reactions.

Catalysis by Proximity

For molecules to react, they must come within bond-forming distance of one another. The higher their concentration, the more frequently they will encounter one another and the greater will be the rate of their reaction. When an enzyme binds substrate molecules in its active site, it creates a region of high local substrate concentration. This environment also orients the substrate molecules spatially in a position ideal for them to interact, resulting in rate enhancements of at least a thousandfold.

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** or **specific base catalysis,** the rate of reaction is sensitive to changes in the concentration of protons but

independent of the concentrations of other acids (proton donors) or bases (proton acceptors) present in 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** or **general base catalysis.**

Catalysis by Strain

Enzymes that catalyze lytic reactions which involve breaking a covalent bond typically bind their substrates in a conformation slightly unfavorable for the bond that will undergo cleavage. The resulting strain stretches or distorts the targeted bond, weakening it and making it more vulnerable to cleavage.

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 then becomes a reactant. Covalent catalysis introduces a new reaction pathway that is energetically more favorable—and therefore faster—than the reaction pathway in homogeneous solution. The chemical modification of the enzyme is, however, transient. On completion of the reaction, the enzyme returns 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. Covalent catalysis often follows a "ping-pong" mechanism—one in which the first substrate is bound and its product released prior to the binding of the second substrate (Figure 7–4).

SUBSTRATES INDUCE CONFORMATIONAL CHANGES IN ENZYMES

Early in the last century, Emil Fischer compared the highly specific fit between enzymes and their substrates to that of a lock and its key. While the "lock and key model" accounted for the exquisite specificity of enzyme-substrate interactions, the implied rigidity of the enzyme's active site failed to account for the dynamic changes that accompany catalysis. This drawback was addressed by Daniel Koshland's **induced fit** model, which states that when substrates approach and bind to an enzyme they induce a conformational change, a change analogous to placing a hand (substrate) into a glove (enzyme) (Figure 7–5). A corollary is that the enzyme induces reciprocal changes in its substrates, harnessing the energy of binding to facilitate the transformation of substrates into products. The induced fit model has been amply confirmed by biophysical studies of enzyme motion during substrate binding.

HIV PROTEASE ILLUSTRATES ACID-BASE CATALYSIS

Enzymes of the **aspartic protease family,** which includes the digestive enzyme pepsin, the lysosomal cathepsins, and the protease produced by the human immunodeficiency virus (HIV), share a common catalytic mechanism. Catalysis involves two conserved aspartyl residues which act as acid-base catalysts. In the first stage of the reaction, an aspartate functioning as a general base (Asp X, Figure 7–6) extracts a proton from a water molecule, making it more nucleophilic. This resulting nucleophile then attacks the electrophilic carbonyl carbon of the peptide bond targeted for hydrolysis, forming a **tetrahedral transition state intermediate.** A second aspartate (Asp Y, Figure 7–6) then facilitates the decomposition of this tetrahedral intermediate by donating a proton to the amino group produced by rupture of the peptide bond. Two different active site aspartates thus can act simultaneously as a general base or as a general acid. This is possible because their immediate environment favors ionization of one but not the other.

CHYMOTRYPSIN & FRUCTOSE-2,6-BISPHOSPHATASE ILLUSTRATE COVALENT CATALYSIS

Chymotrypsin

While catalysis by aspartic proteases involves the direct hydrolytic attack of water on a peptide bond, catalysis

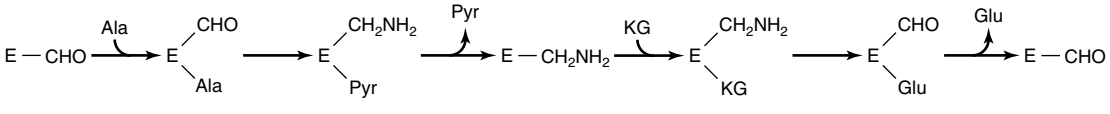

Figure 7–4. Ping-pong mechanism for transamination. E—CHO and E—CH_2NH_2 represent the enzyme-pyridoxal phosphate and enzyme-pyridoxamine complexes, respectively. (Ala, alanine; Pyr, pyruvate; KG, α-ketoglutarate; Glu, glutamate).

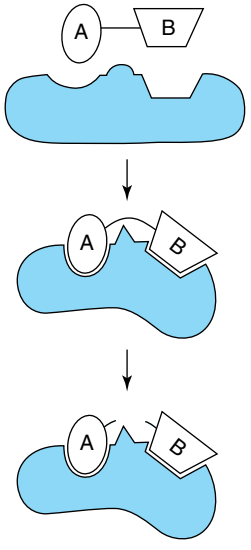

Figure 7–5. Two-dimensional representation of Koshland's induced fit model of the active site of a lyase. Binding of the substrate A—B induces conformational changes in the enzyme that aligns catalytic residues which participate in catalysis and strains the bond between A and B, facilitating its cleavage.

by the **serine protease** chymotrypsin involves prior formation of a covalent acyl enzyme intermediate. A highly reactive seryl residue, serine 195, participates in a charge-relay network with histidine 57 and aspartate 102. Far apart in primary structure, in the active site these residues are within bond-forming distance of one another. Aligned in the order Asp 102-His 57-Ser 195, they constitute a "charge-relay network" that functions as a "proton shuttle."

Binding of substrate initiates proton shifts that in effect transfer the hydroxyl proton of Ser 195 to Asp 102 (Figure 7–7). The enhanced nucleophilicity of the seryl oxygen facilitates its attack on the carbonyl carbon of the peptide bond of the substrate, forming a covalent acyl-enzyme intermediate. The hydrogen on Asp 102 then shuttles through His 57 to the amino group liberated when the peptide bond is cleaved. The portion of the original peptide with a free amino group then leaves the active site and is replaced by a water molecule. The charge-relay network now activates the water molecule by withdrawing a proton through His 57 to Asp 102. The resulting hydroxide ion attacks the acyl-enzyme in-

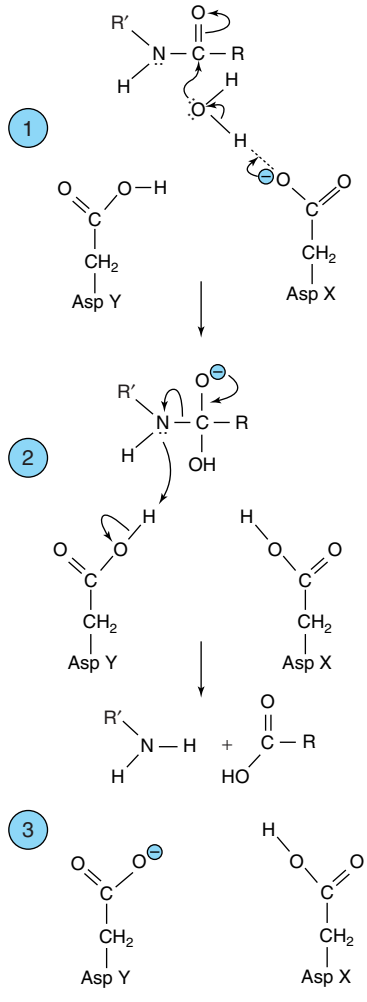

Figure 7–6. Mechanism for catalysis by an aspartic protease such as HIV protease. Curved arrows indicate directions of electron movement. ① Aspartate X acts as a base to activate a water molecule by abstracting a proton. ② The activated water molecule attacks the peptide bond, forming a transient tetrahedral intermediate. ③ Aspartate Y acts as an acid to facilitate breakdown of the tetrahedral intermediate and release of the split products by donating a proton to the newly formed amino group. Subsequent shuttling of the proton on Asp X to Asp Y restores the protease to its initial state.

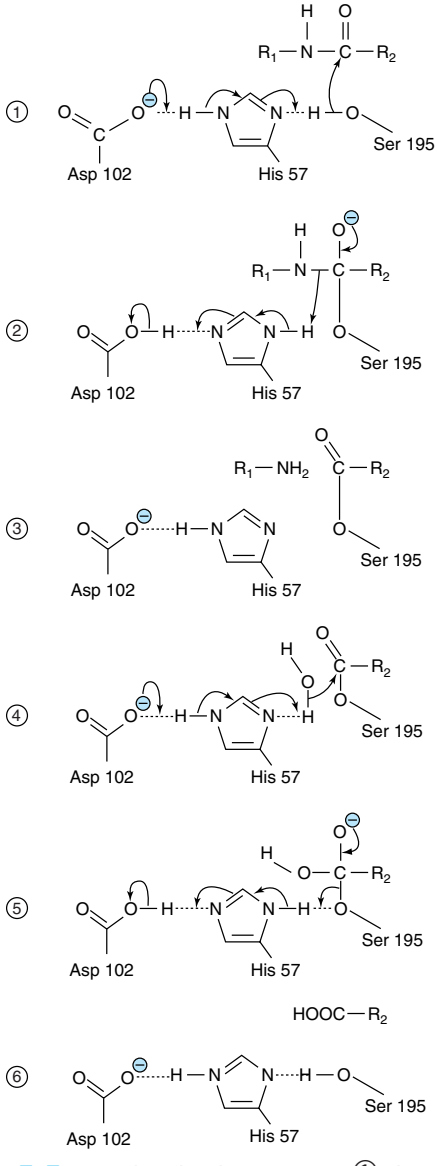

Figure 7–7. Catalysis by chymotrypsin. ① The charge-relay system removes a proton from Ser 195, making it a stronger nucleophile. ② Activated Ser 195 attacks the peptide bond, forming a transient tetrahedral intermediate. ③ Release of the amino terminal peptide is facilitated by donation of a proton to the newly formed amino group by His 57 of the charge-relay system, yielding an acyl-Ser 195 intermediate. ④ His 57 and Asp 102 collaborate to activate a water molecule, which attacks the acyl-Ser 195, forming a second tetrahedral intermediate. ⑤ The charge-relay system donates a proton to Ser 195, facilitating breakdown of tetrahedral intermediate to release the carboxyl terminal peptide ⑥.

termediate and a reverse proton shuttle returns a proton to Ser 195, restoring its original state. While modified during the process of catalysis, chymotrypsin emerges unchanged on completion of the reaction. Trypsin and elastase employ a similar catalytic mechanism, but the numbers of the residues in their Ser-His-Asp proton shuttles differ.

Fructose-2,6-Bisphosphatase

Fructose-2,6-bisphosphatase, a regulatory enzyme of gluconeogenesis (Chapter 19), catalyzes the hydrolytic release of the phosphate on carbon 2 of fructose 2,6-bisphosphate. Figure 7–8 illustrates the roles of seven active site residues. Catalysis involves a "catalytic triad" of one Glu and two His residues and a covalent phosphohistidyl intermediate.

CATALYTIC RESIDUES ARE HIGHLY CONSERVED

Members of an enzyme family such as the aspartic or serine proteases employ a similar mechanism to catalyze a common reaction type but act on different substrates. Enzyme families appear to arise through gene duplication events that create a second copy of the gene which encodes a particular enzyme. The proteins encoded by the two genes can then evolve independently to recognize different substrates—resulting, for example, in chymotrypsin, which cleaves peptide bonds on the carboxyl terminal side of large hydrophobic amino acids; and trypsin, which cleaves peptide bonds on the carboxyl terminal side of basic amino acids. The common ancestry of enzymes can be inferred from the presence of specific amino acids in the same position in each family member. These residues are said to be **conserved residues.** Proteins that share a large number of conserved residues are said to be **homologous** to one another. Table 7–1 illustrates the primary structural conservation of two components of the charge-relay network for several serine proteases. Among the most highly conserved residues are those that participate directly in catalysis.

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

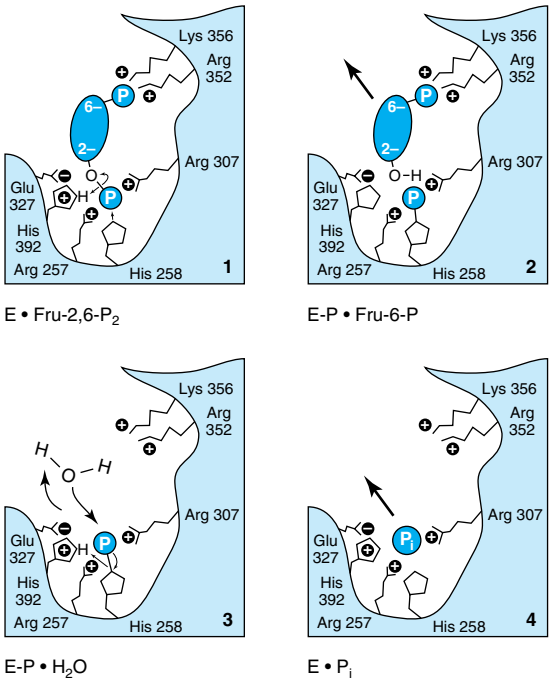

Figure 7–8. Catalysis by fructose-2,6-bisphosphatase. (1) Lys 356 and Arg 257, 307, and 352 stabilize the quadruple negative charge of the substrate by charge-charge interactions. Glu 327 stabilizes the positive charge on His 392. (2) The nucleophile His 392 attacks the C-2 phosphoryl group and transfers it to His 258, forming a phosphoryl-enzyme intermediate. Fructose 6-phosphate leaves the enzyme. (3) Nucleophilic attack by a water molecule, possibly assisted by Glu 327 acting as a base, forms inorganic phosphate. (4) Inorganic orthophosphate is released from Arg 257 and Arg 307. (Reproduced, with permission, from Pilkis SJ et al: 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase: A metabolic signaling enzyme. Annu Rev Biochem 1995;64:799.)

particular regulatory factors (Chapter 9) or substrate affinity (eg, hexokinase and glucokinase) that adapt them to specific tissues or circumstances. Some isozymes may also enhance survival by providing a "back-up" copy of an essential enzyme.

THE CATALYTIC ACTIVITY OF ENZYMES FACILITATES THEIR DETECTION

The minute quantities of enzymes present in cells complicate determination of their presence and concentration. However, the ability to rapidly transform thousands of molecules of a specific substrate into products imbues each enzyme with the ability to reveal its presence. Assays of the catalytic activity of enzymes are frequently used in research and clinical laboratories. Under appropriate conditions (see Chapter 8), the rate of the catalytic reaction being monitored is proportionate to the amount of enzyme present, which allows its concentration to be inferred.

Enzyme-Linked Immunoassays

The sensitivity of enzyme assays can also be exploited to detect proteins that lack catalytic activity. **Enzyme-linked immunoassays** (ELISAs) use antibodies covalently linked to a "reporter enzyme" such as alkaline phosphatase or horseradish peroxidase, enzymes whose products are readily detected. When serum or other samples to be tested are placed in a plastic microtiter plate, the proteins adhere to the plastic surface and are immobilized. Any remaining absorbing areas of the well are then "blocked" by adding a nonantigenic protein such as bovine serum albumin. A solution of antibody covalently linked to a reporter enzyme is then added. The antibodies adhere to the immobilized antigen and these are themselves immobilized. Excess free antibody molecules are then removed by washing. The presence and quantity of bound antibody are then determined by adding the substrate for the reporter enzyme.

Table 7–1. Amino acid sequences in the neighborhood of the catalytic sites of several bovine proteases. Regions shown are those on either side of the catalytic site seryl (S) and histidyl (H) residues.

Enzyme	Sequence Around Serine Ⓢ															Sequence Around Histidine Ⓗ										
Trypsin	D	S	C	Q	D	G	Ⓢ	G	G	P	V	V	C	S	G	K	V	V	S	A	A	Ⓗ	C	Y	K	S	G
Chymotrypsin A	S	S	C	M	G	D	Ⓢ	G	G	P	L	V	C	K	K	N	V	V	T	A	A	Ⓗ	G	G	V	T	T
Chymotrypsin B	S	S	C	M	G	D	Ⓢ	G	G	P	L	V	C	Q	K	N	V	V	T	A	A	Ⓗ	C	G	V	T	T
Thrombin	D	A	C	E	G	D	Ⓢ	G	G	P	F	V	M	K	S	P	V	L	T	A	A	Ⓗ	C	L	L	Y	P

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. The reduced coenzymes NADH and NADPH, written as NAD(P)H, absorb light at a wavelength of 340 nm, whereas their oxidized forms NAD(P)$^+$ do not (Figure 7–9). When NAD(P)$^+$ is reduced, the absorbance at 340 nm therefore increases in proportion to—and at a rate determined by—the quantity of NAD(P)H produced. Conversely, for a dehydrogenase that catalyzes the oxidation of NAD(P)H, a decrease in absorbance at 340 nm will be observed. In each case, the rate of change in optical density at 340 nm will be proportionate to the quantity of enzyme present.

Many Enzymes Are Assayed by Coupling to a Dehydrogenase

The assay of enzymes whose reactions are not accompanied by a change in absorbance or fluorescence is generally more difficult. In some instances, the product or remaining substrate can be transformed into a more readily detected compound. In other instances, the reaction product may have to be separated from unreacted substrate prior to measurement—a process facilitated by the use of radioactive substrates. An alternative strategy is to devise a synthetic substrate whose product absorbs light. For example, *p*-nitrophenyl phosphate is an artificial substrate for certain phosphatases and for chymotrypsin that does not absorb visible light. However, following hydrolysis, the resulting *p*-nitrophenylate anion absorbs light at 419 nm.

Another quite general approach is to employ a "coupled" assay (Figure 7–10). Typically, a dehydrogenase whose substrate is the product of the enzyme of interest is added in catalytic excess. The rate of appearance or disappearance of NAD(P)H then depends on the rate of the enzyme reaction to which the dehydrogenase has been coupled.

THE ANALYSIS OF CERTAIN ENZYMES AIDS DIAGNOSIS

Of the thousands of different enzymes present in the human body, those that fulfill functions indispensable to cell vitality are present throughout the body tissues. Other enzymes or isozymes are expressed only in specific cell types, during certain periods of development, or in response to specific physiologic or pathophysiologic changes. Analysis of the presence and distribution of enzymes and isozymes—whose expression is normally tissue-, time-, or circumstance-specific—often aids diagnosis.

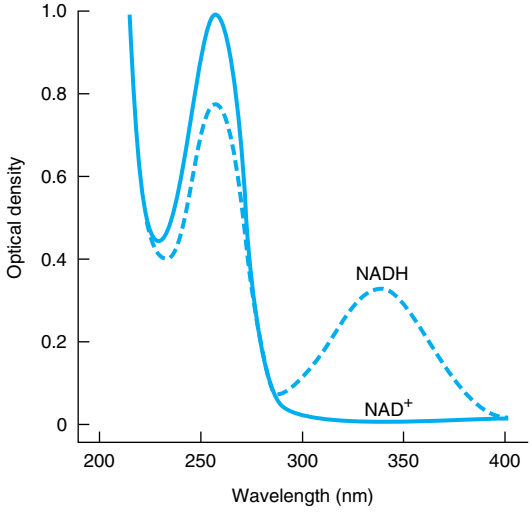

Figure 7–9. Absorption spectra of NAD$^+$ and NADH. Densities are for a 44 mg/L solution in a cell with a 1 cm light path. NADP$^+$ and NADPH have spectrums analogous to NAD$^+$ and NADH, respectively.

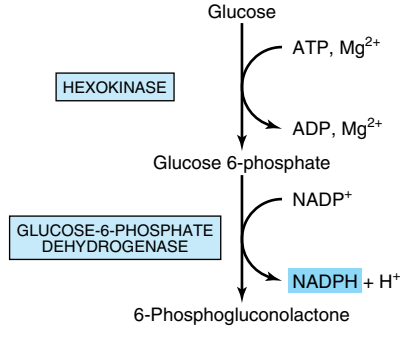

Figure 7–10. Coupled enzyme assay for hexokinase activity. The production of glucose 6-phosphate by hexokinase is coupled to the oxidation of this product by glucose-6-phosphate dehydrogenase in the presence of added enzyme and NADP$^+$. When an excess of glucose-6-phosphate dehydrogenase is present, the rate of formation of NADPH, which can be measured at 340 nm, is governed by the rate of formation of glucose 6-phosphate by hexokinase.

Nonfunctional Plasma Enzymes Aid Diagnosis & Prognosis

Certain enzymes, proenzymes, and their substrates are present at all times in the circulation of normal individuals and perform a physiologic function in the blood. Examples of these **functional plasma enzymes** include lipoprotein lipase, pseudocholinesterase, and the proenzymes of blood coagulation and blood clot dissolution (Chapters 9 and 51). The majority of these enzymes are synthesized in and secreted by the liver.

Plasma also contains numerous other enzymes that perform no known physiologic function in blood. These apparently **nonfunctional plasma enzymes** arise from the routine normal destruction of erythrocytes, leukocytes, and other cells. Tissue damage or necrosis resulting from injury or disease is generally accompanied by increases in the levels of several nonfunctional plasma enzymes. Table 7–2 lists several enzymes used in diagnostic enzymology.

Isozymes of Lactate Dehydrogenase Are Used to Detect Myocardial Infarctions

L-Lactate dehydrogenase is a tetrameric enzyme whose four subunits occur in two isoforms, designated H (for heart) and M (for muscle). The subunits can combine as shown below to yield catalytically active isozymes of L-lactate dehydrogenase:

Lactate Dehydrogenase Isozyme	Subunits
I_1	HHHH
I_2	HHHM
I_3	HHMM
I_4	HMMM
I_5	MMMM

Distinct genes whose expression is differentially regulated in various tissues encode the H and M subunits. Since heart expresses the H subunit almost exclusively, isozyme I_1 predominates in this tissue. By contrast, isozyme I_5 predominates in liver. Small quantities of lactate dehydrogenase are normally present in plasma. Following a myocardial infarction or in liver disease, the damaged tissues release characteristic lactate dehydrogenase isoforms into the blood. The resulting elevation in the levels of the I_1 or I_5 isozymes is detected by separating the different oligomers of lactate dehydrogenase by electrophoresis and assaying their catalytic activity (Figure 7–11).

Table 7–2. Principal serum enzymes used in clinical diagnosis. Many of the enzymes are not specific for the disease listed.

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
γ-Glutamyl transpeptidase	Various liver diseases
Lactate dehydrogenase (isozymes)	Myocardial infarction
Lipase	Acute pancreatitis
Phosphatase, acid	Metastatic carcinoma of the prostate
Phosphatase, alkaline (isozymes)	Various bone disorders, obstructive liver diseases

ENZYMES FACILITATE DIAGNOSIS OF GENETIC DISEASES

While many diseases have long been known to result from alterations in an individual's DNA, tools for the detection of genetic mutations have only recently become widely available. These techniques rely upon the catalytic efficiency and specificity of enzyme catalysts. For example, the **polymerase chain reaction** (PCR) relies upon the ability of enzymes to serve as catalytic amplifiers to analyze the DNA present in biologic and forensic samples. In the PCR technique, a thermostable DNA polymerase, directed by appropriate oligonucleotide primers, produces thousands of copies of a sample of DNA that was present initially at levels too low for direct detection.

The detection of **restriction fragment length polymorphisms** (RFLPs) facilitates prenatal detection of hereditary disorders such as sickle cell trait, betathalassemia, infant phenylketonuria, and Huntington's disease. Detection of RFLPs involves cleavage of double-stranded DNA by restriction endonucleases, which can detect subtle alterations in DNA that affect their recognized sites. Chapter 40 provides further details concerning the use of PCR and restriction enzymes for diagnosis.

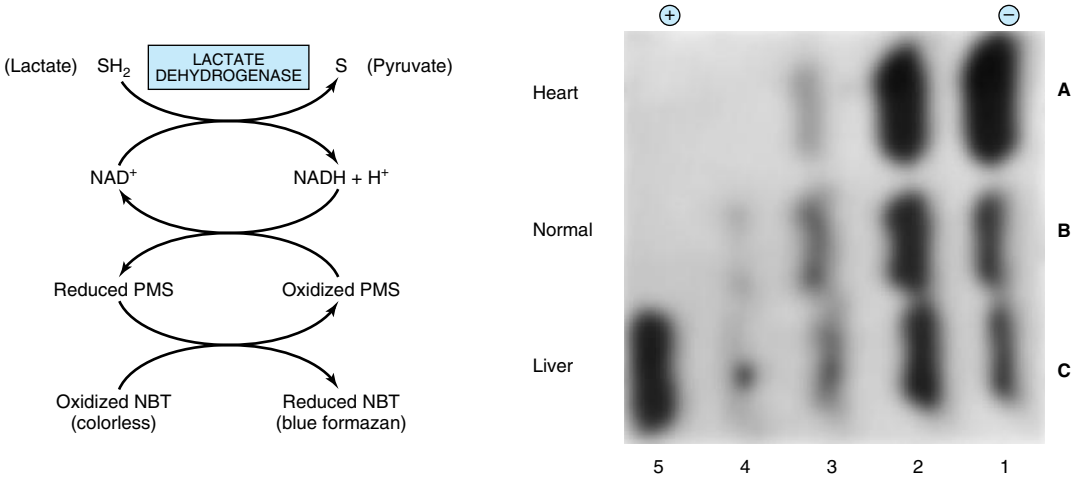

Figure 7–11. Normal and pathologic patterns of lactate dehydrogenase (LDH) isozymes in human serum. LDH isozymes of serum were separated by electrophoresis and visualized using the coupled reaction scheme shown on the left. (NBT, nitroblue tetrazolium; PMS, phenazine methylsulfate). At right is shown the stained electropherogram. Pattern A is serum from a patient with a myocardial infarct; B is normal serum; and C is serum from a patient with liver disease. Arabic numerals denote specific LDH isozymes.

RECOMBINANT DNA PROVIDES AN IMPORTANT TOOL FOR STUDYING ENZYMES

Recombinant DNA technology has emerged as an important asset in the study of enzymes. Highly purified samples of enzymes are necessary for the study of their structure and function. The isolation of an individual enzyme, particularly one present in low concentration, from among the thousands of proteins present in a cell can be extremely difficult. If the gene for the enzyme of interest has been cloned, it generally is possible to produce large quantities of its encoded protein in *Escherichia coli* or yeast. However, not all animal proteins can be expressed in active form in microbial cells, nor do microbes perform certain posttranslational processing tasks. For these reasons, a gene may be expressed in cultured animal cell systems employing the baculovirus expression vector to transform cultured insect cells. For more details concerning recombinant DNA techniques, see Chapter 40.

Recombinant Fusion Proteins Are Purified by Affinity Chromatography

Recombinant DNA technology can also be used to create modified proteins that are readily purified by affinity chromatography. The gene of interest is linked to an oligonucleotide sequence that encodes a carboxyl or amino terminal extension to the encoded protein. The resulting modified protein, termed a **fusion protein,** contains a domain tailored to interact with a specific affinity support. One popular approach is to attach an oligonucleotide that encodes six consecutive histidine residues. The expressed "His tag" protein binds to chromatographic supports that contain an immobilized divalent metal ion such as Ni^{2+}. Alternatively, the substrate-binding domain of glutathione S-transferase (GST) can serve as a "GST tag." Figure 7–12 illustrates the purification of a GST-fusion protein using an affinity support containing bound glutathione. Fusion proteins also often encode a cleavage site for a highly specific protease such as thrombin in the region that links the two portions of the protein. This permits removal of the added fusion domain following affinity purification.

Site-Directed Mutagenesis Provides Mechanistic Insights

Once the ability to express a protein from its cloned gene has been established, it is possible to employ **site-directed mutagenesis** to change specific aminoacyl residues by altering their codons. Used in combination with kinetic analyses and x-ray crystallography, this approach facilitates identification of the specific roles of given aminoacyl residues in substrate binding and catalysis. For example, the inference that a particular aminoacyl residue functions as a general acid can be tested by replacing it with an aminoacyl residue incapable of donating a proton.

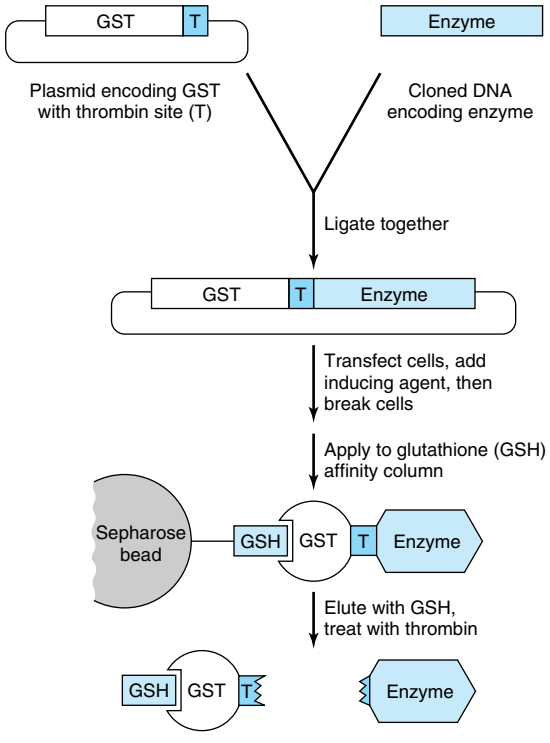

Figure 7–12. Use of glutathione S-transferase (GST) fusion proteins to purify recombinant proteins. (GSH, glutathione.)

SUMMARY

- Enzymes are highly effective and extremely specific catalysts.
- Organic and inorganic prosthetic groups, cofactors, and coenzymes play important roles in catalysis. Coenzymes, many of which are derivatives of B vitamins, serve as "shuttles."
- Catalytic mechanisms employed by enzymes include the introduction of strain, approximation of reactants, acid-base catalysis, and covalent catalysis.
- Aminoacyl residues that participate in catalysis are highly conserved among all classes of a given enzyme activity.
- Substrates and enzymes induce mutual conformational changes in one another that facilitate substrate recognition and catalysis.
- The catalytic activity of enzymes reveals their presence, facilitates their detection, and provides the basis for enzyme-linked immunoassays.
- Many enzymes can be assayed spectrophotometrically by coupling them to an $NAD(P)^+$-dependent dehydrogenase.
- Assay of plasma enzymes aids diagnosis and prognosis. For example, a myocardial infarction elevates serum levels of lactate dehydrogenase isozyme I_1.
- Restriction endonucleases facilitate diagnosis of genetic diseases by revealing restriction fragment length polymorphisms.
- Site-directed mutagenesis, used to change residues suspected of being important in catalysis or substrate binding, provides insights into the mechanisms of enzyme action.
- Recombinant fusion proteins such as His-tagged or GST fusion enzymes are readily purified by affinity chromatography.

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