Clinical Diagnosis of Trace elements and Enzymes

Enzymes

Enzymes are specific biologic proteins that catalyze biochemical reactions without altering the equilibrium point of the reaction or being consumed or changed in composition. The other substances in the reaction are converted to products.

In addition to the basic enzyme structure, a nonprotein molecule, called **activators**.

Prosthetic Groups

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.**

Cofactors

Cofactors serve functions similar to those of prosthetic groups but bind in a transient, dissociable manner either to the enzyme or to a substrate 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

A **coenzyme** is an organic cofactor, such as nicotinamide adenine(dinucleotide NAD).

ENZYME CLASSIFICATION

1. **Oxidoreductases.** Catalyze an oxidation–reduction reaction between two substrates

2. **Transferases.** Catalyze the transfer of a group other than hydrogen from one substrate to another

3. **Hydrolases.** Catalyze hydrolysis of various bonds

4. **Lyases.** Catalyze removal of groups from substrates without hydrolysis; the product contains double bonds

5. **Isomerases.** Catalyze the interconversion of geometric, optical, or positional isomers

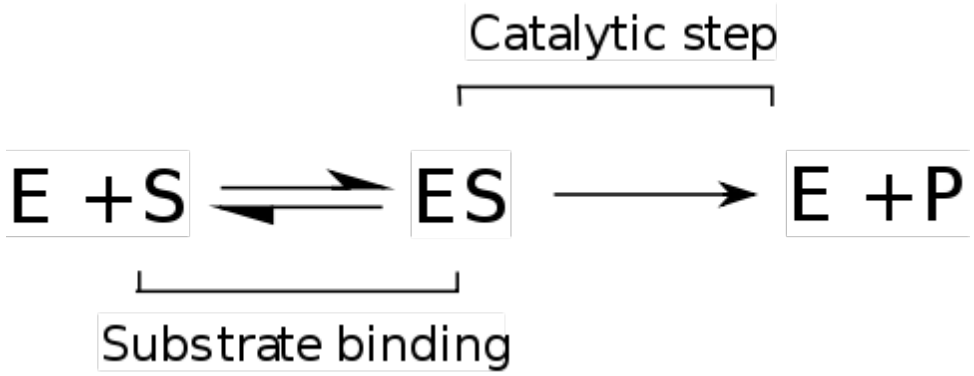
6. **Ligases.** Catalyze the joining of two substrate molecules, coupled with breaking of the pyrophosphate bond in adenosine triphosphate (ATP) or a similar compound.

TABLE 12-1 CLASSIFICATION OF FREQUENTLY QUANTITATED ENZYMES

CLASS	RECOMMENDED NAME	COMMON ABBREVIATION	STANDARD ABBREVIATION	EC CODE NO.	SYSTEMATIC NAME
Oxidoreductases	Lactate dehydrogenase	LDH	LDH	1.1.1.27	L-Lactate:NAD^+ oxidoreductase
	Glucose-6-phosphate dehydrogenase	G-6-PDH	G-6-PD	1.1.1.49	D-Glucose-6-phosphate:$NADP^+$ 1-oxidoreductase
	Glutamate dehydrogenase	GLD	GLD	1.4.1.3	L-glutamate:NAD(P) oxidoreductase, deaminase
Transferases	Aspartate amino-transferase	GOT (glutamate oxaloacetate transaminase)	AST	2.6.1.1	L-Aspartate:2-oxaloglutarate aminotransferase
	Alanine amino-transferase	GPT (glutamate transaminase)	ALT	2.6.1.2	L-Alanine:2-oxaloglutarate aminotransferase
	Creatine kinase	CPK (creatine phosphokinase)	CK	2.7.3.2	ATP:creatine *N*-phosphotransferase
	γ-Glutamyl-transferase	GGTP	GGT	2.3.2.2	(5-Glutamyl)peptide: amino acid-5-glutamyltransferase
	Glutathione-*S*-transferase	α-GST	GST	2.5.1.18	Glutathione transferase
	Glycogen phosphorylase	GP	GP	2.4.1.1	1,4-α-D-Glucan: orthophosphate α-D-glucosyltransferase
	Pyruvate kinase	PK	PK	2.7.1.40	Pyruvate kinase
Hydrolases	Alkaline phosphatase	ALP	ALP	3.1.3.1	Orthophosphoric monoester phosphohydrolase (alkaline optimum)
	Acid phosphatase	ACP	ACP	3.1.3.2	Orthophosphoric monoester phosphohydrolase (acid optimum)
	α-Amylase	AMY	AMS	3.2.1.1	1,4-D-Glucan glucanohydrolase
	Cholinesterase	PCHE	CHE	3.1.1.8	Acylcholine acylhydrolase
	Chymotrypsin	CHY	CHY	3.4.21.1	Chymotrypsin
	Elastase-1	E1	E1	3.4.21.36	Elastase
	5-Nucleotidase	NTP	NTP	3.1.3.5	5′-Ribonucleotide phosphohydrolase
	Triacylglycerol lipase		LPS	3.1.1.3	Triacylglycerol acylhydrolase
	Trypsin	TRY	TRY	3.4.21.4	Trypsin
Lyases	Aldolase	ALD	ALD	4.1.2.13	D-D-Fructose-1, 6-bisdiphosphate D-glyceraldehyde-3-phosphate-lyase
Isomerases	Triosephosphate isomerase	TPI	TPI	5.3.1.1	Triose-phosphate isomerase
Ligase	Glutathione Synthetase	GSH-S	GSH-S	6.3.2.3	Glutathione synthase

How enzymes work

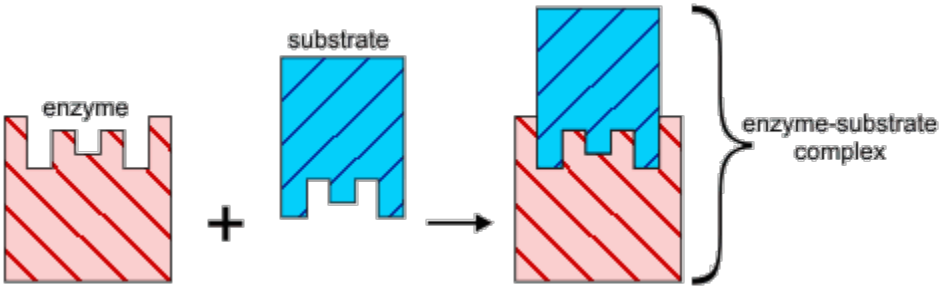
Enzymes have an **active site**. This is part of the molecule that has just the right shape and functional groups to bind to one of the reacting molecules. The reacting molecule that binds to the enzyme is called the **substrate**.



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

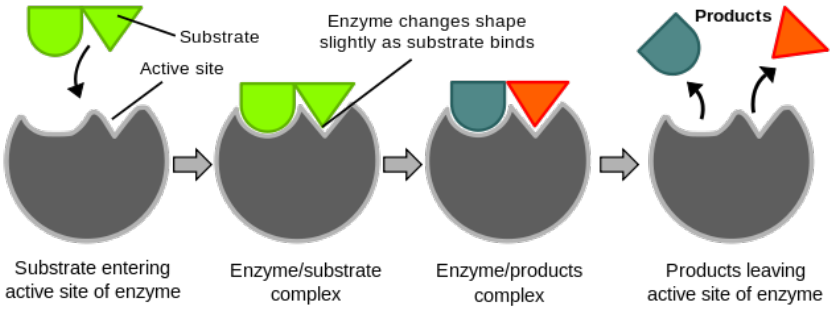
Lock and key model

Enzymes are very specific, and it was suggested by Emil Fischer in 1894 that this was because both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. This is often referred to as "the lock and key" model. However, while this model explains enzyme specificity, it fails to explain the stabilization of the transition state that enzymes achieve.



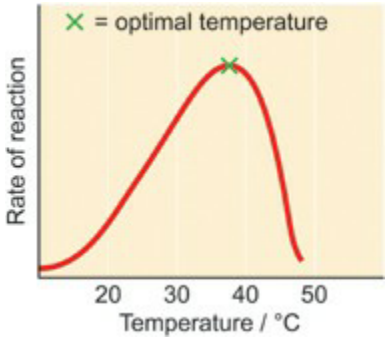
Induced fit

In this model the enzyme molecule changes shape as the substrate molecules gets close. The change in shape is 'induced' by the approaching substrate molecule. This more sophisticated model relies on the fact that molecules are flexible because single covalent bonds are free to rotate.



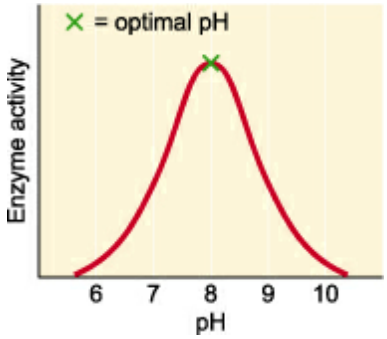
Factors affecting catalytic activity of enzymes

Temperature:



As the temperature rises, reacting molecules have more and more kinetic energy. This increases the chances of a successful collision and so the rate increases. There is a certain temperature at which an enzyme's catalytic activity is at its greatest (see graph). This optimal temperature is usually around human body temperature (37.5 ^{o}C) for the enzymes in human cells. Above this temperature the enzyme structure begins to break down (denature) since at higher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy.

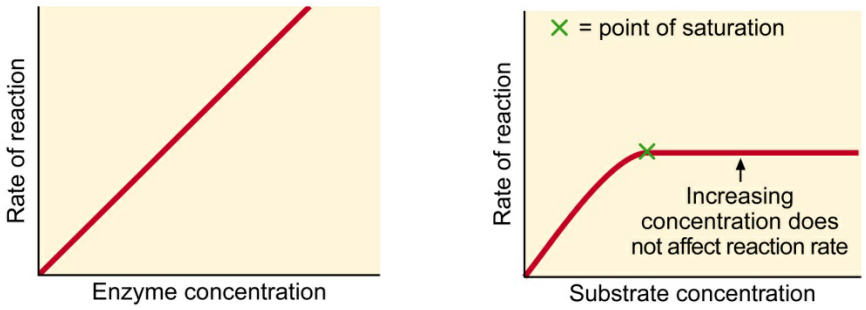
pH:



Each enzyme works within quite a small pH range. There is a pH at which its activity is greatest (the optimal pH). This is because changes in pH can make and break intra- and intermolecular bonds, changing the

shape of the enzyme and, therefore, its effectiveness.

Concentration of enzyme and substrate:

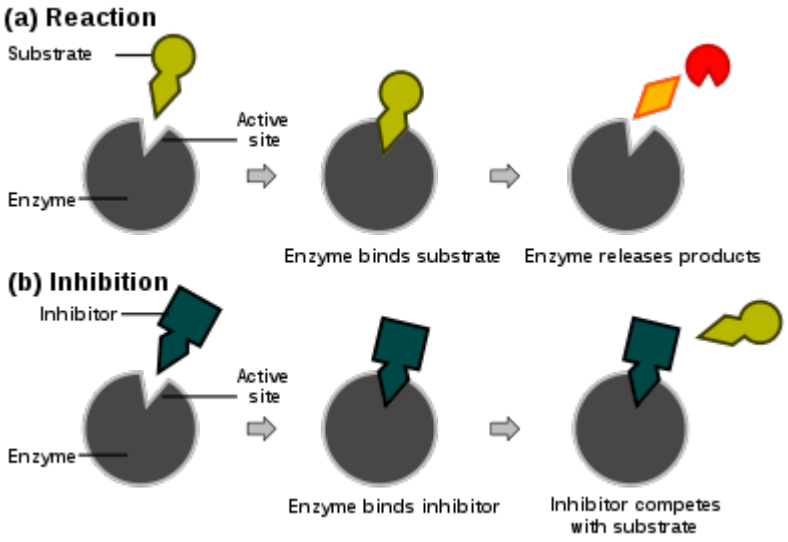


The rate of an enzyme-catalysed reaction depends on the concentrations of enzyme and substrate. As the concentration of either is increased the rate of reaction increases (see graphs).

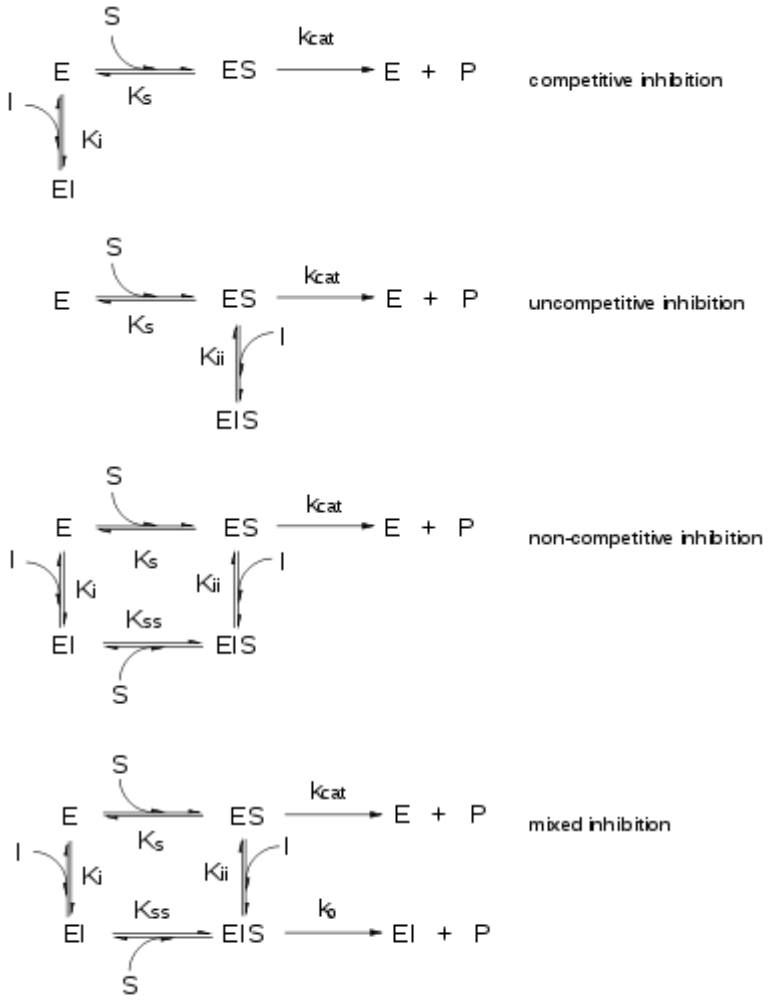
For a given enzyme concentration, the rate of reaction increases with increasing substrate concentration up to a point, above which any further increase in substrate concentration produces no significant change in reaction rate. This is because the active sites of the enzyme molecules at any given moment are virtually saturated with substrate. The enzyme/substrate complex has to dissociate before the active sites are free to accommodate more substrate .

Provided that the substrate concentration is high and that temperature and pH are kept constant, the rate of reaction is proportional to the enzyme concentration.

Inhibition



Competitive inhibitors bind reversibly to the enzyme, preventing the binding of substrate. On the other hand, binding of substrate prevents binding of the inhibitor. Substrate and inhibitor compete for the enzyme.



Types of inhibition.

Enzyme reaction rates can be decreased by various types of enzyme inhibitors.

Competitive inhibition

In competitive inhibition, the inhibitor and substrate compete for the enzyme (i.e., they can not bind at the same time). Often competitive inhibitors strongly resemble the real substrate of the enzyme. For example, methotrexate is a competitive inhibitor of the enzyme dihydrofolate reductase, which catalyzes the reduction of dihydrofolate to tetrahydrofolate. In competitive inhibition the maximal rate of the reaction is not changed, but higher substrate concentrations are required

to reach a given maximum rate.

Uncompetitive inhibition

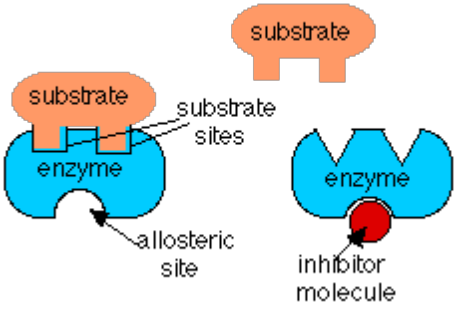
In uncompetitive inhibition, the inhibitor cannot bind to the free enzyme, only to the ES-complex. The EIS-complex thus formed is enzymatically inactive. This type of inhibition is rare, but may occur in multimeric enzymes.

Non-competitive inhibition

Non-competitive inhibitors can bind to the enzyme at the binding site at the same time as the substrate,but not to the active site. Both the EI and EIS complexes are enzymatically inactive. Because the inhibitor can not be driven from the enzyme by higher substrate concentration (in contrast to competitive inhibition

Allosteric enzyme:

Allosteric enzymes are enzymes that change their conformational ensemble upon binding of an effector, which results in an apparent change in binding affinity at a different ligand binding site. This "action at a distance" through binding of one ligand affecting the binding of another at a distinctly different site, is the essence of the allosteric concept. **Allostery** plays a crucial role in many fundamental biological processes, including but not limited to cell signaling and the regulation of metabolism. Allosteric enzymes need not be oligomers as previously thought. and in fact many systems have demonstrated allostery within single enzymes



Isoenzymes:

Isozymes (also known as **isoenzymes** or more generally as **Multiple forms of enzymes**) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. These enzymes usually display different kinetic parameters (e.g. different K_M values), or different regulatory properties. for example lactate dehydrogenase (LDH)). In biochemistry, isozymes (or isoenzymes) are isoforms (closely related variants) of enzymes. In many cases, they are coded for by homologous genes that have diverged over time.