Mechanisms of Enzyme Regulation

- Substrate concentration
- Reversible inhibition by products or other compounds
- Allosteric activation or inhibition
- Proteolytic cleavage
- Covalent modification
- Modulator protein binding
- Amount of enzyme present

Substrate influence.

The rates of most enzymes are responsive to changes in substrate concentration because the intracellular level of many substrates is in the range of the K_m. Thus, an increase in substrate concentration prompts an increase in reaction rate, which tends to return the concentration of substrate toward normal.

Product inhibition. If the product accumulates, it can inhibit some enzymes. This form of control limits the rate of formation of the product when the product is underused. Besides you can remember that Enzymes do not affect equilibrium constants. It means that increasing product concentration causes to increasing reverse reaction and decreasing substrate formation.

<u>Allosteric regulation of metabolic pathways</u>. The activity of enzymes that catalyze key regulatory reactions **(committed steps)** of metabolic pathways are often subject to allosteric regulation. Their activity can be **modulated by the binding of allosteric effectors** to a site on the enzyme that is distinct from the active site (i.e., allosteric site).

1. Effectors are positive if they enhance the rate of a reaction (i.e., activators) and negative if they decrease the rate of reaction (i.e., inhibitors).

2. Feedback inhibition is negative modulation of the committed step of a metabolic pathway by its end product. This prevents unnecessary production of an excess of end product by shutting down the pathway until more is needed.

Covalent modification

1. Phosphorylation

a. **Effect on enzyme activity.** In certain enzymes, the addition of a phosphate group to a specific amino acid residue [usually serine (Ser), tyrosine (Tyr), or threonine (Thr)] by specific protein kinases dramatically enhances or depresses activity.
b. **This modification is reversible.** The phosphorylated enzyme may be dephosphorylated by specific phosphatases.

2. Nucleotidylation

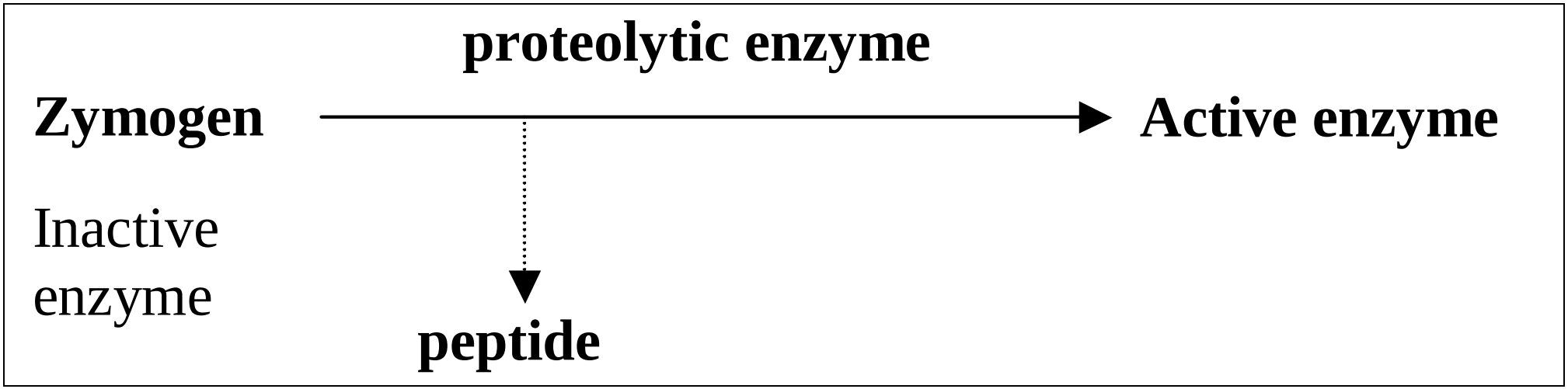
a. **Effect on enzyme activity.** The activities of certain enzymes are regulated by the reversible addition of a nucleotide (e.g., adenosine) to a specific amino acid.
b. **This modification is reversible.** For example, an adenylated enzyme may be deadenylated by a specific enzyme.

3. Proteolytic cleavage. Certain enzymes are synthesized as **proenzymes, or zymogens,** which are inactive forms of enzymes that become active only after being cleaved at a specific site in their polypeptide chain by specific **proteases.**

a. Many **digestive enzymes that hydrolyze proteins** (e.g., trypsin, pepsin) are synthesized as zymogens in the stomach and pancreas.

b. **Blood clotting** is mediated by a series of proteolytic zymogen activities of several serum enzymes

The partial cleavage of enzyme precursor
(proteolytic cleavage)



MECHANISMS FOR REGULATING ENZYME ACTIVITY

Regulator event	Typical effector	Results	Time required for change
Substrate availability	Substrate	Change in velocity	
Product inhibition	Product	Change in V_m and/or K_m	Immediate
Allosteric control	End product	Change in V_m and/or K_m	Immediate
Covalent modification	Another enzyme	Change in V_m and/or K_m	Immediate to minutes
Dissociation/association of regulatory protein (modulatory protein binding)	Second messengers	Change in V_m and/or K_m	Immediate
Synthesis or degradation of enzyme	Hormone or metabolite	Change in the amount of the enzyme	Hours to days

ISOENZYMES (ALSO CALLED ISOZYMES)

Definition. Isozymes are different molecular forms of enzymes that may be isolated from the same or different tissues.

Most isoenzymes are enzymes that catalyze the same reaction but differ in their physical properties because of genetically determined differences in amino acid sequence. Isoenzymes may contain different numbers of charged amino acids and may be separated from each other by elec-trophoresis. Different organs frequently contain characteristic proportions of different isoenzymes.

Clinical use. Analysis of the distribution of isozymes of particular enzymes is sometimes a useful tool in clinical diagnosis.

Lactate Dehydrogenase Isozyme	Subunits
l_1 (↑ under myocardial infarction)	HHHH
l_2	HHHM
l_3	HHMM
l_4	HMMM
l_5	MMMM

Serum enzyme levels

1. Description. Many enzymes are present in serum, and their activity can be easily assayed without purification.

2. Clinical use. Elevation or depression of the levels of activity of specific enzymes may indicate either the presence of a disease or damage to a specific tissue.

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 aminotransfer-ase (AST, or SGOT) Alanine aminotransferase (ALT, or SGPT)	Myocardial infarction Viral hepatitis
Amylase	Acute pancreatitis
Ceruloplasmin	Hepatolenticular degenera-tion (Wilson's disease)
Creatine kinase	Muscle disorders and myo-cardial 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, ob-structive liver diseases

Enzymes as diagnostic reagents

1. Description. Many purified enzymes are now commercially available for use in the determination of components in blood and tissues. Such enzymatic assays are usually more specific, more sensitive, and faster than chemical determinations.

2. Clinical use. Examples of clinically relevant compounds that can be determined enzymatically include glucose, urea, ethanol, and triglycerides.

Use enzymes as analytical reagents

Glucose oxydase	Determination of serum glucose level
Cholesterol oxydase	Determination of serum cholesterol level
Lipase	Determination of triacylglycerol level
Urease	Determination of serum urea level