Enzymes: pt 1 Chapter 19

Controlling Reactions in Organisms

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Enzymes

- Common Enzymes
- How Enzymes Work: Chymotrypsin
- Classes of Enzymes
- Cofactors
- Rates of Reaction: Environment
  - Temperature
  - pH
- Rates of Reaction: Inhibition and Activation
  - Allosteric Control
  - Inhibition
  - Feedback
  - Competative
  - Noncompetative
  - Irreversible

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19.1 Catalysis by Enzymes

- **Enzyme:** A protein that acts as a catalyst for a biological reaction.
- Used with in organisms but humans make use of them outside their natural environment.
  - Detergents and Stain Removers
  - Meat Tenderizers
  - In Medicine

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Detergents and Stain Removers

- **Amylase** is frequently used to remove starchy stains and residues.
- Amylase is used to break down carbohydrates (polymer chains of sugars) in our bodies, principally in our mouths.
- Amylase works on only works to break down only some types bonds found in carbohydrates.
- It breaks the starches down into the sugars maltose and maltotriose
- It has the same purpose in organisms.

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Papain

- **Papain** is an enzyme that breaks down proteins, by breaking the bonds between certain types of amino acids.
- Traditionally papain is the enzyme used in meat tenderizers.
- In medicine this same enzyme is used to clean away dead tissue.
- It is also used to clean teeth when treating for cavities.

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Substrate-defined

The specific molecule that an enzyme acts on is called that enzyme's **substrate**.

- For amylase it is starches
- For Papain it is proteins
- Enzymes are categorized based on their substrates and the type of reaction that they catalyze.

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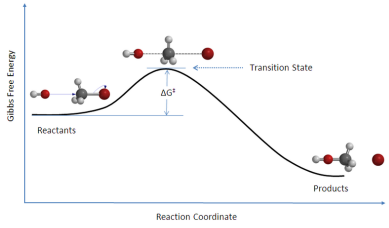
Catalysts Reviewed

Catalysts speed up reactions that are spontaneous, by lowering the activation energy (Ea) of the reaction.



Reaction: $HO^- + CH_3Br \rightarrow [HO{\cdots}CH_3{\cdots}Br]^{\ddagger} \rightarrow CH_3OH + Br^-$

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Chymotrypsin

- We will be looking at **chymotrypson** as an enzyme model.
- Like papain chymotrypsin is a enzyme that breaks down proteins.
- The polypeptide is broken down by a process called **hydrolysi**s.
- This reaction is spontaneous (G<0) but it does not happen very quickly, chymotrypsin speeds it along by lowering the activation energy of the reaction.

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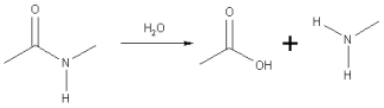
Hydrolysis of Peptide Bonds

- Bonds between amino acids are called peptide bonds. When peptide bonds are broken the reaction appears to be addition of a water molecule, so the reaction is called hydrolysis.



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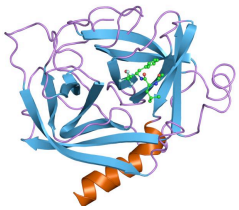
Chymotrypsin + Peptide



- The peptide fits into a particular site in chymotrypsin.
- Hydrophobic forces have a significant role in attracting the peptide into this site.
- Only peptides with amino acids that have a phenyl group are drawn into this site.

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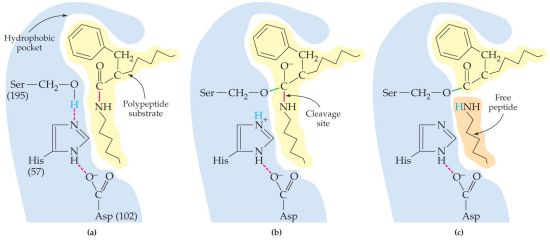
Hydrolysis of a peptide bond by chymotrypsin. (a) The polypeptide enters the active site (b) Hydrogen transfer allows formation of a strained intermediate (c) The peptide bond is broken.



- The reaction only takes place when there is an accessible peptide bond that joins an amino acid with a phenyl group to the other amino acid.
- The reaction is speeds up because the side group on the amino acid serine, binds to the carbon with the double bond to the oxygen, and provides the hydrogen that will stabilize the nitrogen when the C-N bond is broken.
- Since the reaction involved adding a water to the peptide bond, the active site will need to be replenished with water.

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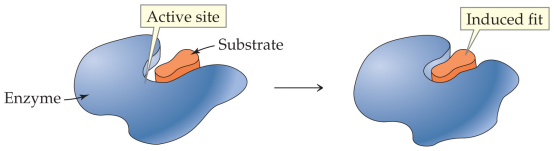
Enzymes in General

In all enzymes the the substrate is held at a specific location where the reaction takes place. This region is called the active site of the enzyme. Enzyme molecules are flexible, even though they have a defined shape, the active site of the enzyme moves to accommodate the substrate and facilitate the reaction.



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Catalysis in Enzymes

Enzymes act as catalysts because of their ability to:

- Bring substrate(s) and catalytic sites together (proximity effect).
- Hold substrate(s) at the exact distance and in the exact orientation necessary for reaction (orientation effect).
- Provide acidic, basic, or other types of groups required for catalysis (catalytic effect).
- Lower the energy barrier by inducing strain in bonds in the substrate molecule (energy effect).

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Classes of Enzymes

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How Enzymes Are Grouped

- Enzymes are grouped into classes based on the chemistry that occurs on them.
  - Chymotrypsin is one of a class of enzymes that break bonds in biological systems by adding a water molecule to the atoms on either side of the bond.
  - This class of enzymes are called hydrolases.
- Often, in a more detailed description of an enzyme, the organism that the enzyme was taken from, or where its from in the organism, is mentioned
- The same enzymes are used in many parts of the body and in quite varied organisms so these characteristics of an enzyme are less useful.

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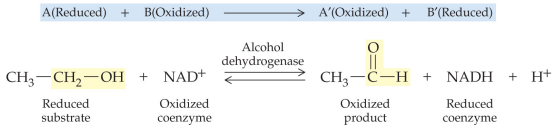
TABLE 19.2 Classification of Enzymes

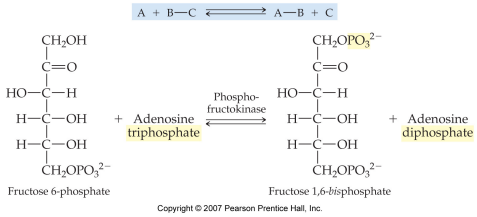
MAIN CLASS AND SUBCLASS	EXAMPLES OF REACTION TYPES CATALYZED
Oxidoreductases	**Oxidation–reduction reactions**
Oxidases	Addition of O_2 to a substrate
Reductases	Reduction of a substrate
Dehydrogenases	Removal of 2 H's to form a double bond
Transferases	**Transfer of functional groups**
Transaminases	Transfer of amino group between substrates
Kinases	Transfer of a phosphoryl group between substrates
Hydrolases	**Hydrolysis reactions**
Lipases	Hydrolysis of ester groups in lipids
Proteases	Hydrolysis of peptide bonds in proteins
Nucleases	Hydrolysis of phosphate ester bonds in nucleic acids
Isomerases	**Isomerization of a substrate**
Lyases	**Group elimination to form double bond or addition to a double bond**
Dehydrases	Removal of H_2O from substrate to give double bond
Decarboxylases	Replacement of a carboxyl group by a hydrogen
Synthases	Addition of small molecule to a double bond
Ligases	**Bond formation coupled with ATP hydrolysis to provide energy**
Synthetases	Formation of bond between two substrates
Carboxylases	Formation of bond between substrate and CO_2 to add a carboxyl group ($-COO^-$)

19.3 Enzyme Classification

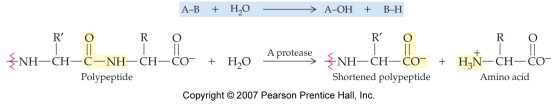
- Enzymes are divided into six main classes according to the general kind of reaction they catalyze.
- **Oxidoreductases** catalyze redox reactions of substrates, most commonly addition or removal of oxygen or hydrogen. These enzymes require additional molecules that are reduced or oxidized as the substrate is oxidized or reduced.



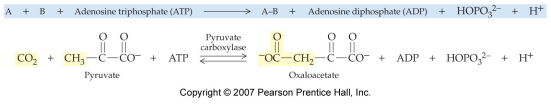
Transferases catalyze transfer of a group from one molecule to another. Kinases transfer a phosphate group from ATP to give ADP and a phosphorylated product. There are at least 518 different kinases. They are important for wide variety of purposes.



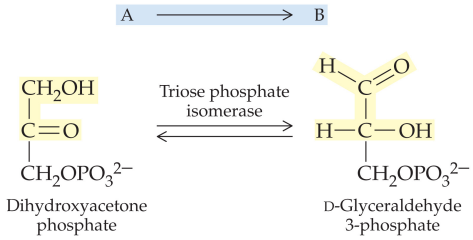
- **Hydrolases** catalyze the breaking of bonds with addition of water.
- The digestion of carbohydrates and proteins by hydrolysis requires these enzymes.
- Enzymes in this class also repair DNA when a mutation has occurred.
- And, as seen in the case of chymotrysin, hydrolases break down the bonds that hold the amino acids in a protein together.



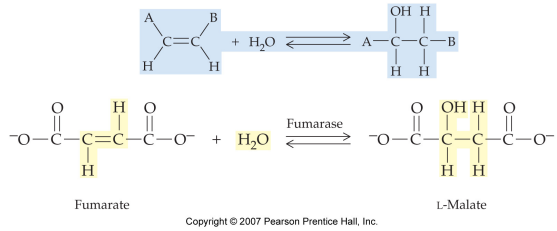
- **Ligases** catalyze the bonding together of two substrates. Such reactions are generally not favorable and require energy from ATP hydrolysis.
- ATP, adenine triphosphate, is a molecule that has a very significant role in energy transfer in cells.
- ATP makes energy available when it is converted to ADP, adenine diphosphate.



- **Isomerases** catalyze the isomerization (rearrangement of atoms) of a substrate in reactions that have but one substrate and one product.
- **Isomers** are molecules that have the same chemical formulas but not the same structure.



Lyases catalyze the addition of a molecule to a double bond or the reverse reaction in which a molecule is eliminated from a double bond.

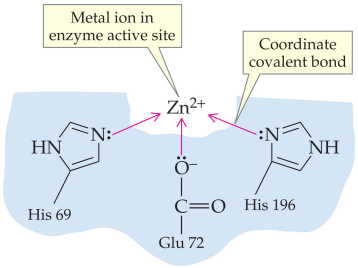


- Enzymes have the family-name ending -ase.
- Exceptions occur for enzymes such as papain and trypsin, which are still referred to by older common names.
- Modern systematic names always have two parts:
  - the first identifies the substrate on which the enzyme operates
  - the second part is an enzyme subclass name like those shown on the next slide.
- Example: Pyruvate carboxylase is a ligase that acts on the substrate pyruvate to add a carboxyl group.

19.2 Enzyme Cofactors

- **Cofactor:** A nonprotein part of an enzyme that is essential to the enzyme's catalytic activity; a metal ion or a coenzyme.
- **Coenzyme:** A molecule that is composed mainly of carbons, hydrogens, and oxygens, that acts as an enzyme cofactor.
- Why are cofactors necessary? The functional groups in proteins are limited to those of the amino acid side chains (-OH, -COOH, -NH2, -SH). By combining with cofactors, enzymes acquire chemically reactive groups not available as side chains.

Trace minerals and certain vitamins are a dietary necessity because they function as building blocks for cofactors and we cannot synthesize them.



Reaction Rate

Catalytic activity is measured by **turnover number**, the maximum number of substrate molecules acted upon per enzyme per unit time. Most enzymes turn over 10–1000 molecules per second.

TABLE 19.1 Turnover Numbers for Some Enzymes

ENZYME	REACTION CATALYZED	TURNOVER NUMBER (MAXIMUM NUMBER OF CATALYTIC EVENTS PER SECOND)
Papain	Hydrolysis of peptide bonds	10
Ribonuclease	Hydrolysis of phosphate ester link in RNA	10^2
Kinases	Transfer of phosphoryl group between substrates	10^3
Acetylcholinesterase	Deactivation of the neurotransmitter acetylcholine	10^4
Carbonic anhydrase	Converts CO_2 to HCO_3^-	10^6
Catalase	Decomposition of H_2O_2 to $H_2O + O_2$	10^7

What Changes the Rate of Reaction

- Enzymes speed reactions by reducing the activation barrier of the reaction, but some of the events that would speed up a non-enzyme catalyzed reaction will sometimes speed an enzyme catalyzed reaction and sometimes slow it down.

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Increasing the Concentration of the Substrate

- This is like increasing the concentration of the reactants, in a non enzyme catalyzed reaction.
- This does increase the rate of the reaction because it increases the frequency of enzyme substrate collisions.
- But eventually there is so much substrate available that the rate of reaction is no longer limited by the rate of collision, but rather by the amount of time required for the reaction to take place on the enzyme.

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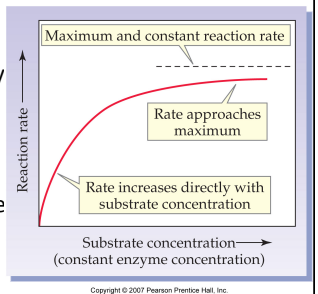
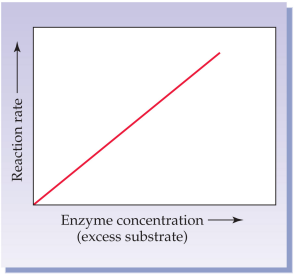
Substrate concentration

- At low substrate concentration, the reaction rate is directly proportional to the substrate concentration.
- With increasing substrate concentration, the rate drops off as more of the active sites are occupied.
- All of the active sites are converting substrate to product.



Increasing the Amount of the Enzyme

- Organisms can also increase the rate of an enzyme catalyzed reaction by increasing the concentration of the enzyme.
- This way of speeding things up is limited by the amount of substrate available.
- If the concentration of substrate is unlimited than the reaction rate would be expected to increase linearly with the enzyme concentration.



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Increasing the Temperature

- In general increasing temperature will increase the rate of a reaction.
- Enzymes catalyzed reactions are temperature dependent but increasing temperature does not necessarily mean an increase in reaction rates.
- The temperature at which the enzyme functions best generally matches the usual temperature of the organism.

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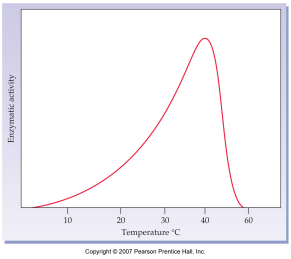
Temperature and Reaction Rate

- The data at the right indicates that it is likely the organism that produces and uses this enzyme has a body temperature a little less than 40 C.
- What do you think the steep drop off in enzyme activity* indicates?
- Why does the rate seem to increase with temperature between 4 and 38 C?

*Enzyme Activity is a way of describing the rate of reaction at the enzyme site.



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Effect of Concentration on Enzyme Activity

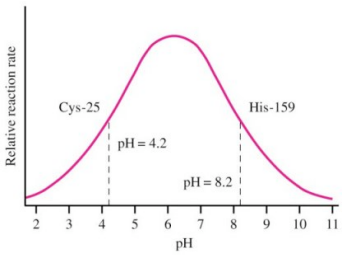
- Variations in the concentration of the enzyme or the substrate alters the rate of enzyme catalyzed reaction.

The Effect of pH on Enzyme Activity

- Enzyme catalytic activity is highly dependent on pH.
- Optimum conditions vary slightly for each enzyme but are generally near the pH of the body fluid in which the enzyme functions.
- Pepsin, which initiates protein digestion in the highly acidic environment of the stomach, has its optimum activity in very acidic solutions, pH=2.
- Trypsin, another protease that is found in the small intestine, has optimum activity at pH 8. This would seem to match the basic environment of the small intestine.

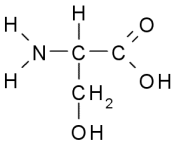
pH dependence of Reaction Rate

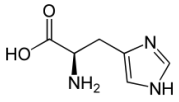
The pH dependence of the reaction rate can have multiple causes. Often the dependence is seen because the side chains that facilitate the reaction have been protinated or deprotinated.



Data is for papain
http://chemistry.umeche.maine.edu/CHY431/Peptidase10.html

pH Dependence of Reaction Rate





What else could cause the reaction rate to decrease?

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Controlled Enzyme Activity

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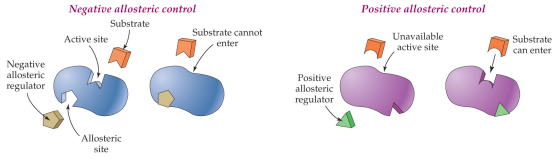
19.7 Enzyme Regulation: Feedback and Allosteric Control

- A variety of strategies are utilized to adjust the rates of enzyme-catalyzed reactions.
- Any process that starts or increases the action of an enzyme is an **activation**.
- Any process that slows or stops the action of an enzyme is an **inhibition**.
- Feedback and allosteric control are two strategies for enzyme regulation.

- **Allosteric control:** An interaction in which the binding of a regulator at one site on a protein affects the protein's ability to bind another molecule at a different site.
- Allosteric control can be either positive or negative.

- Binding a positive regulator changes the active sites so that the enzyme becomes a better catalyst and the rate accelerates.
- Binding a negative regulator changes the active sites so that the enzyme is a less effective catalyst and the rate slows down.



- **Feedback control:** Regulation of an enzyme's activity by the product of a reaction later in a pathway.
- If a product near the end of a metabolic pathway inhibits an enzyme that functions near the beginning of that pathway, the later products can act as a turn off switch to production of the product.
- http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120070/bio10.swf::Feedback%20Inhibition%20of%20Biochemical%20Pathways

Feedback Inhibition

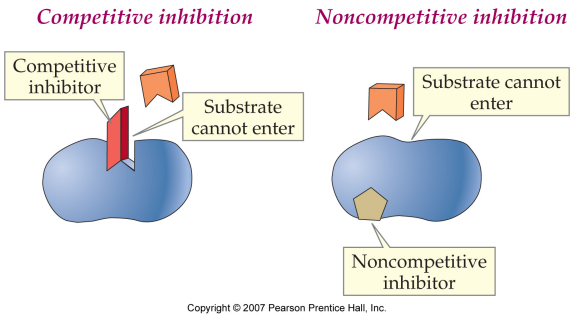
- Consider the metabolic pathway that converts the starting material A into the product D. There are 3 enzymes used to synthesize D. (E1, E2, E3)

  A + E1 → B +E2 → C +E3 →D

- The product D is able to interact with the first enzyme in the pathway, E1. When this happens E1 is no longer able to change A to B, and this means that less, or no more, D will be created.

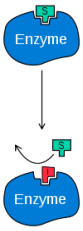
19.8 Enzyme Regulation: Inhibition

- Enzyme inhibition can be reversible or irreversible.
- In **reversible inhibition**, the inhibitor can leave, restoring the enzyme to its uninhibited level of activity.
- In **irreversible inhibition**, the inhibitor remains permanently bound and the enzyme is permanently inhibited.
- The inhibition can also be **competitive** or **noncompetitive**, depending on whether the inhibitor binds to the active site or elsewhere.



Competitive Inhibition

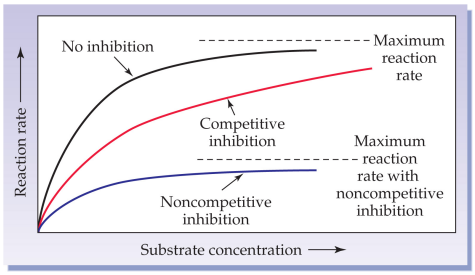
- In competitive inhibition the molecule that can inhibit the enzyme, binds to the enzyme active site.
- The decrease in enzyme activity is sensitive to the substrate concentration as well as the inhibitor concentration.
- Enough substrate can outcompete the inhibitor, as long as the inhibitor binds reversibly.



Noncompetitive Inhibition

The enzyme active site is changed when a molecule binds to another part of the protein.
When this happens the reaction is inhibited or slowed.
Increasing substrate concentration does not decrease the potency of this inhibitor.

A competitive inhibitor can eventually be overcome by higher substrate concentrations. With a noncompetitive inhibitor the maximum rate is lowered for all substrate concentrations.



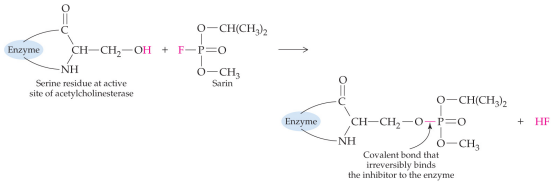
Irreversible Inhibition

Irreversible inhibition usually requires covalent bonds be formed between the inhibitor and the enzyme.

Heavy Metals

- Heavy Metals are typically nonreversible enzyme inhibitors (Heavy metals include arsenic, lead, mercury, copper and silver.)
- These metals can covalently bond to side groups of the amino acids that make up the enzymes, sometimes even interfering with the enzyme active site.
- Often these covalent bonds are made to –SH groups.

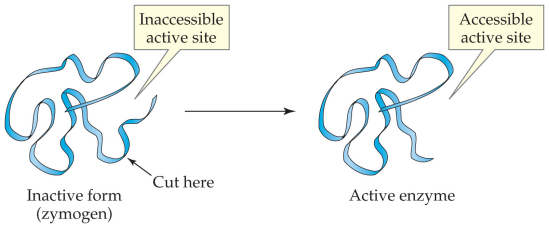
- Organophosphorus insecticides such as parathion and malathion, and nerve gases like Sarin are irreversible inhibitors of the enzyme acetylcholinesterase.
- Acetylcholinesterase breaks down acetylcholine immediately after it sends its message down the nerve.
- If the acetylcholine remains no further impulses can be sent and there is paralysis.



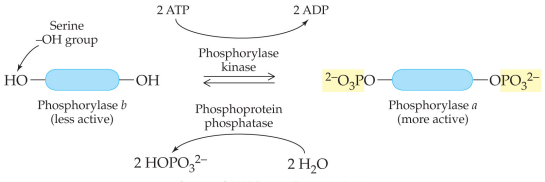
19.9 Enzyme Regulation: Covalent Modification and Genetic Control

- There are two general modes of enzyme regulation by **covalent modification,** removal of a covalently bonded portion of an enzyme, or addition of a group.
- Some enzymes are synthesized in inactive forms known as **zymogens** or **proenzymes**, activation requires a chemical reaction that splits off part of the molecule.
- **Genetic (enzyme) control:** Regulation of enzyme activity by hormonal control of the synthesis of enzymes is especially useful for enzymes needed only at certain times.

Enzymes that cause blood clotting or digest proteins are examples of enzymes that must not be active at the time and place of their synthesis.



Glycogen phosphorylase, the enzyme that initiates glycogen breakdown, is more active when phosphorylated. When glycogen stored in muscles must be hydrolyzed to glucose for quick energy, two serine residues are phosphorylated. The groups are removed once the need to break down glycogen for quick energy has passed.



Summary

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- Classes of Enzymes
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- Rates of Reaction: Environment
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