Chem Factsheet

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Enzymes: Activity and Kinetics

After completing this Factsheet you will be able to:

- describe the features of enzyme structure.
- understand the function of enzymes,
- understand how the activity of enzymes is affected by changing conditions.
- understand the terms V_{max} and K_M.
- describe how the activity of enzymes is affected by inhibitors.

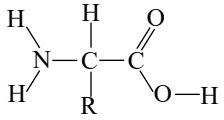
What are Enzymes?

Enzymes are biological catalysts. They are responsible for catalysing all the reactions that take place in living systems, from the replication of DNA and synthesis of proteins to respiration and transmission of nerve signals. Understanding the activity of enzymes can help understand diseases and can bring about the basis of treatments and even cures.

Enzyme structure

Enzymes are polymers of α-amino acids. There are 20 naturally-occurring α-amino acids in nature. They all have the same basic structure but vary in the group of atoms attached to the α-carbon (the R-group).

Fig. 1



Where R is any one of 20 different groups

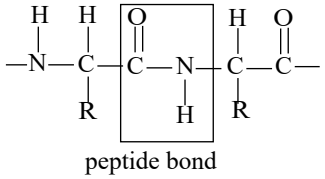
examples of R groups

-H	glycine
$-CH(OH)CH_3$	threonine
$-CH_2COOH$	aspartic acid
$-(CH_2)_4NH_2$	lysine
$-CH_2SH$	cysteine

The 20 R-groups vary from a single hydrogen atom to chains and rings of carbon atoms. These R-groups can have amino groups ($-NH_2$), hydroxyl groups (-OH), carboxylic acid groups (-COOH), and even thiol groups (-SH). As we shall see later, the nature of the R groups is fundamental to the catalytic activity of the enzyme.

Amino acids are joined together in a sequence determined by the DNA sequence in a gene. A condensation reaction happens, joining the amino group of one amino acid to the acid group of a second. The result is a peptide bond, also known as an amide linkage or carboxamide functional group. The polymer formed is a polypeptide.

Fig. 2



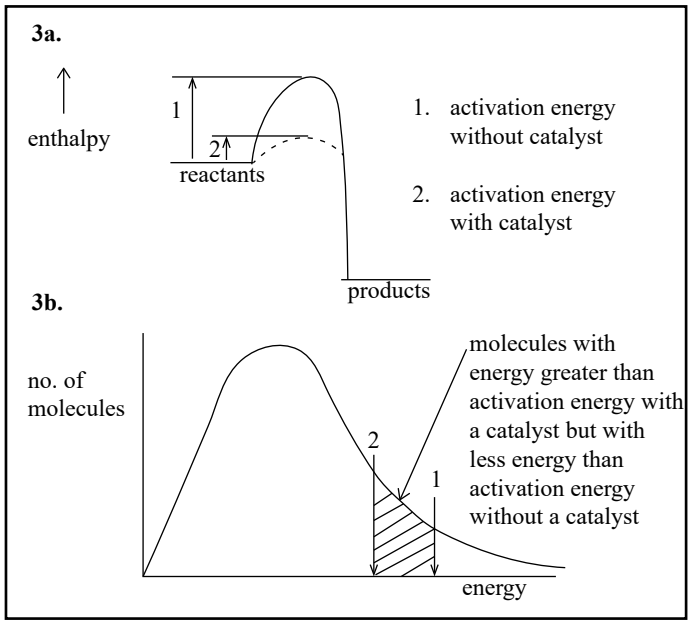
peptide bond

The interactions between peptide bonds at different parts of the chain, together with interactions between R-groups, stabilise the structure and therefore the three-dimensional shape of the enzyme. These intra-molecular bonds include weak van der Waals' forces (London dispersion forces and permanent dipole-permanent dipole interactions), hydrogen bonds, electrostatic interactions and even strong covalent bonds forming between two neighbouring thiol groups (a disulfide bridge).

Enzyme function

Like any catalyst, enzymes provide alternative pathways for a reaction with lower activation energy.

Fig. 3a and 3b

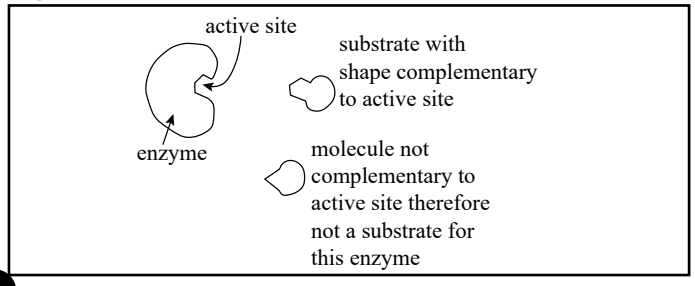


Also, like any catalyst, enzymes are not permanently changed by the reaction and can be reused.

Enzymes achieve this lower activation energy by providing, in the form of precisely positioned R-groups, nucleophiles, and electrophiles to attack the reactant. A few of the amino acids in the polypeptide will have R-groups that take part in the reaction. This is described as the active site of the enzyme. The active site has a shape that is complementary to that of the molecule the enzyme is acting on (the substrate).

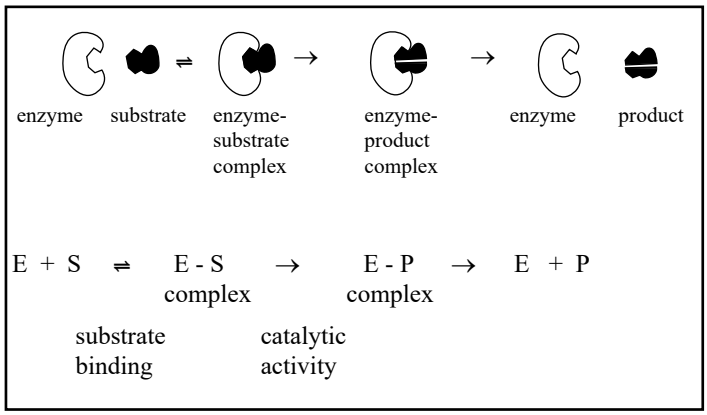
Some enzymes may ensure that two reactants are precisely aligned for a reaction to occur. This can only happen because the shape of the enzyme is carefully attained. The majority of amino acids in the enzyme are needed to achieve the precise shape of the active site. Of course, this does mean that for every different reaction in the cell, a different enzyme is needed since the active site of one enzyme will not be precisely complementary to the molecules involved in a different reaction.

Fig. 4



Enzyme reactions can be summarised in the figure below.

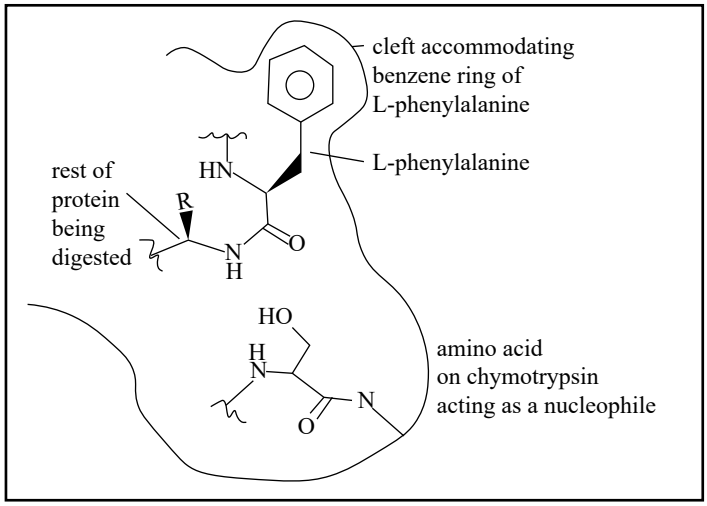
Fig. 5



The substrate must first bind to the enzyme at the active site. This forms an enzyme-substrate complex. The reaction takes place and the substrate is converted into a product which is then released.

19 of the 20 naturally-occurring amino acids exhibit chirality around the α-carbon (glycine is achiral). All naturally-occurring enzymes are made from L-amino acids. This means that enzymes are chiral catalysts and therefore only one enantiomer of a molecule may act as a substrate for a particular enzyme. For example, the protease enzyme chymotrypsin hydrolyses proteins during digestion by attacking the peptide bond next to the amino acid phenylalanine. Only the correct enantiomer of this amino acid will result in a reaction. The active site of the enzyme has a cleft that fits the benzene ring of the L-form of the amino acid only. The D-form (mirror-image of L-form) does not fit into the active site.

Fig. 6



The chirality of enzymes has huge implications in pharmaceutical industry; different enantiomers of a drug may elicit very different responses in an organism. In the worst cases, one enantiomer may be toxic whilst the other may be the active agent.

Enzyme activity

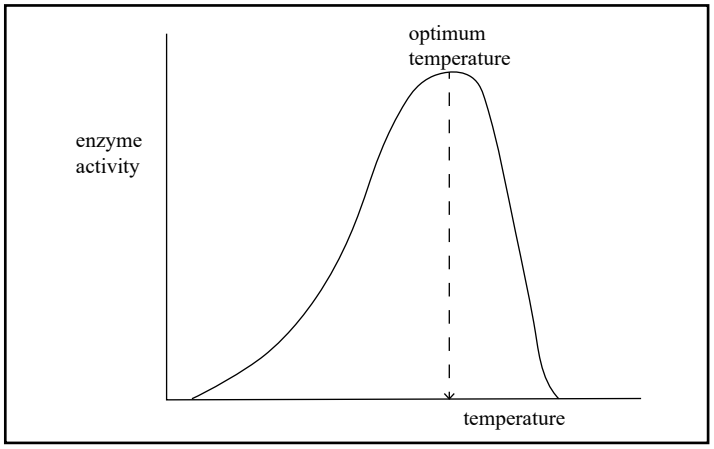
The rate of an enzyme-catalysed reaction can be followed in the same way as any chemical reaction – i.e. by measuring the formation of product or the use of reactant (substrate). In enzymology, the maximum number of conversions of substrate to product per unit time by a single active site is termed the turnover number. The turnover number may be several thousand molecules a second for some enzymes.

The conditions that lead to maximum enzyme activity are called optimum conditions and these vary depending on the site of enzyme action in the body and the organism in which the enzyme normally works. Since the shape of the active site of the enzyme is critical to its activity, any conditions that disrupt the shape will reduce the activity of the enzyme.

Effect of Temperature

In humans, the typical optimum temperature for an enzyme is 37 °C (body temperature).

Fig. 7



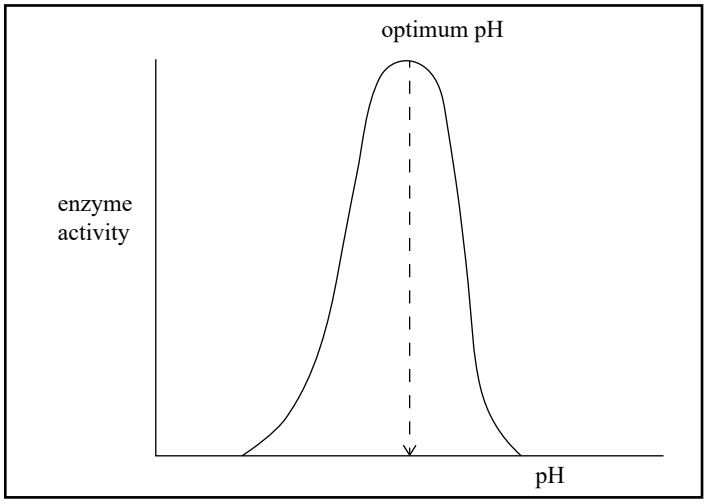
At temperatures below optimum, the rate of enzyme catalysed reactions decreases. Molecules have less kinetic energy and therefore fewer molecules collide with energy greater than the activation energy. As temperature rises beyond optimum, the kinetic energy of the enzyme increases to such an extent that the intramolecular forces responsible for maintaining the shape of the enzyme—and therefore its active site—are broken.

Once the shape of the enzyme has been lost it cannot be regained, and is said to have been denatured. The active site of a denatured enzyme is no longer complementary to its substrate and enzyme activity is permanently lost.

Effect of pH

pH optima in humans vary; stomach enzymes works best at low pH (1-2) whilst salivary enzymes work most efficiently around pH 7.

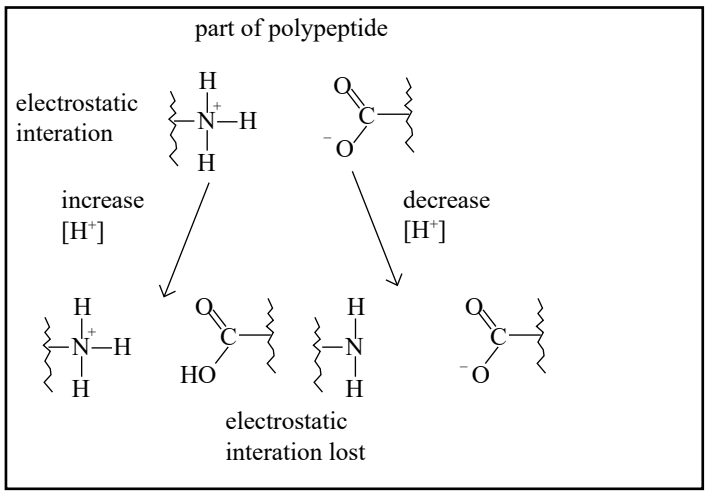
Fig. 8



The shape of the graph is similar to that of the temperature graph. However, the loss of activity either side of optimum pH is sharper. As pH decreases and the concentration of H^+ ions increases, R groups can become protonated ($NH_2 \rightarrow NH_3^+$). Increases in pH may lead

to deprotonation of R groups (COOH → COO^-). At optimum pH, R-groups will be charged such that electrostatic attractions between neighbouring amino acids will help maintain the structure of the enzyme. Variations in H^+ concentration will change the charges of these R-groups and the interactions will be lost. Even if the pH is returned to optimum, the chances of the correct interactions reforming are low and the enzyme remains denatured and inactive.

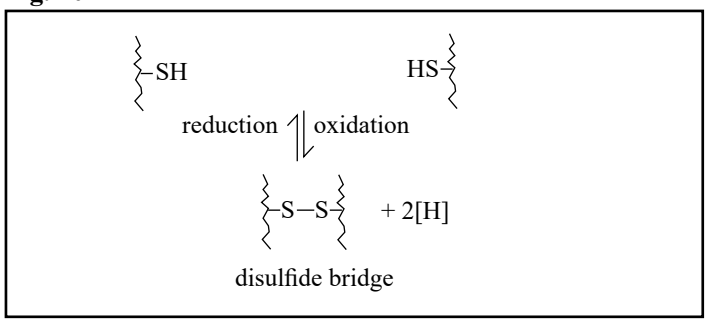
Fig. 9



Effect of Redox Conditions

The formation of a covalent bond between two R-groups containing thiol groups (–SH) is an oxidation reaction. Consequently the breaking of this covalent bond is a reduction reaction. These strong covalent bonds are called disulfide bridges.

Fig. 10



Changes in redox conditions can break disulfide bridges or form ones that do not normally occur under optimum conditions. In either case, the enzyme's shape and activity can be lost.

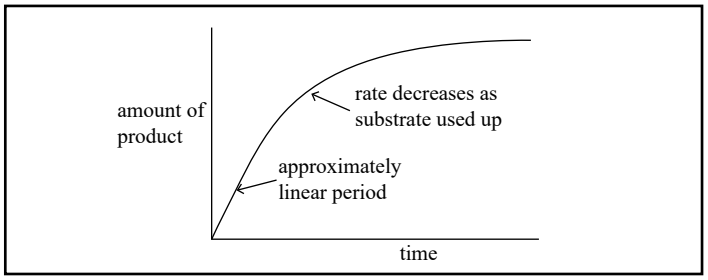
Effect of Heavy Metal Ions

Heavy metal ions such as mercury (Hg^{2+}) are poisonous since they act as non-competitive inhibitors (see later) of enzymes. The mercury ion bonds to the sulphur atoms of thiol groups in cysteine amino acids. Consequently, disulfide bridges may be disrupted and the shape of the enzyme will be lost. Silver, copper, and lead ions can form similar interactions with thiol groups and can also lead to the disruption of enzyme structure and loss of enzyme activity.

Effect of Substrate Concentration

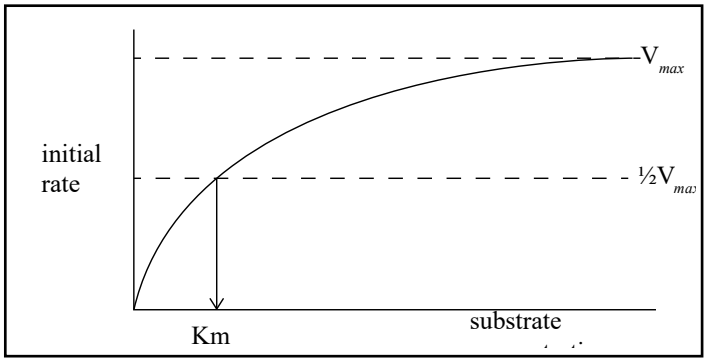
One of the best-known models of enzyme kinetics is named after the German biochemist Leonor Michaelis and the Canadian physician Maud Menten—Michaelis-Menten kinetics. It describes the rate of enzyme reactions by relating reaction rate to the concentration of the substrate.

Fig. 11



The initial rate of the reaction is the maximum rate that an enzyme can achieve under the tested conditions, i.e., temperature, pH and initial substrate concentration.

Fig. 12



In non-enzyme catalysed reactions, an increase in the concentration of a reactant will result in an increase in rate. However the figure above shows that as substrate concentration is increased, the initial reaction rate curves to a maximum. The enzyme active site is said to be saturated with substrate. The rate of reaction is limited by the number of active sites available for substrates to bind. A new substrate molecule cannot bind to the enzyme and react until the active site has released the products of the previous reaction. The maximum initial rate is called V_{max}.

The Michaelis constant (K_M) is the concentration of substrate needed for the enzyme to reach 50% of its maximum rate (½V_{max}).

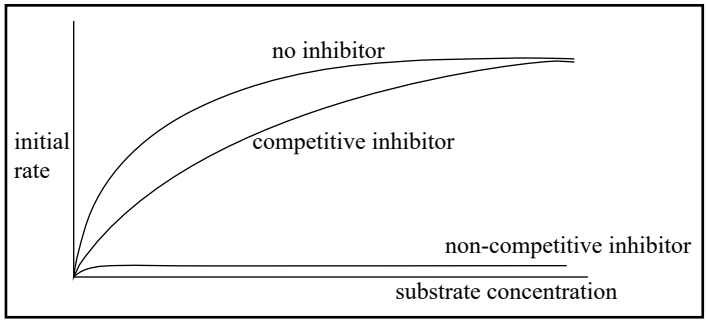
Effect of Inhibitors

Enzyme activity can be controlled in biological systems by the presence of inhibitors. These molecules reduce enzyme activity and can then control metabolic pathways in the cells.

A competitive inhibitor competes with the substrate molecules to bind at the active site. Competitive inhibitors have shapes similar to the substrate and therefore fit well into the active site. However, unlike the substrate, inhibitors will not undergo a reaction. The presence of a competitive inhibitor reduces the chance of a substrate molecule binding to the enzyme. However, when substrate concentrations are much higher than inhibitor concentrations, the substrate will out-compete the inhibitor and V_{max} will eventually be reached, but at higher substrate concentrations than when the inhibitor is not present. As a consequence, K_M will increase, i.e. the concentration of substrate required to reach ½V_{max} will increase.

A non-competitive inhibitor will bind to the enzyme at a different site (sometimes called an allosteric site) and induce a shape change in the enzyme so that the active site is no longer complementary to the substrate or the substrate may still be able to bind, but may not be converted into product due to a change in a different part of the active site. V_{max} is reduced in both situations, though in the latter case, the binding of the substrate to the enzyme, and hence K_M, is unaffected.

Fig. 13

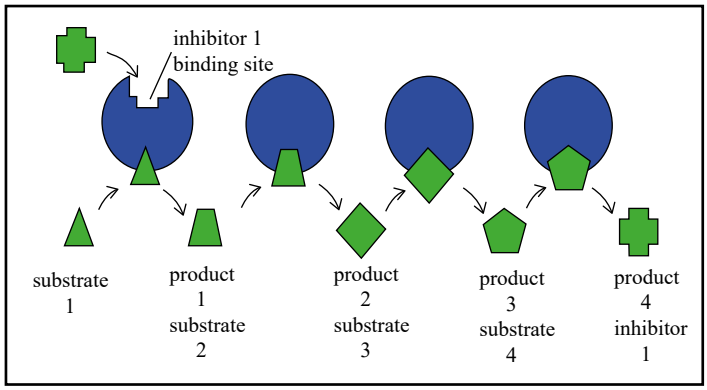


Both types of inhibition can be reversible or irreversible.

Product Inhibition

Product inhibition of enzyme activity is important in the regulation of metabolic pathways in biological systems. The product of an enzyme reaction (or a series of enzyme reactions) can bind to the enzyme and inhibit its activity. The metabolic pathway is therefore under a form of negative feedback control.

Fig. 14



For example, a metabolic pathway is catalysed by a series of enzymes (E1, E2, E3 and E4). The product of the first enzyme is the substrate for the second, the product of the second is the substrate for the third, and so on. The final product also acts as a non-competitive inhibitor for enzyme 1. When bound, the metabolic pathway is regulated.

Questions

1. Explain how the presence of an enzyme will increase the rate of a reaction.
2. Suggest how the intramolecular forces stabilising the shape of an enzyme from a hyperthermophilic bacterium (one that lives in very high-temperature conditions) may be different to those stabilising the shape of an enzyme from a human cell.
3. Describe the effect of temperature on enzyme-catalysed reactions.
4. Explain the effect on the rate of an enzyme-catalysed reaction if the concentration of enzyme is increased.
5. Explain why increasing the concentration of a substrate for an enzyme will never result in the enzyme reaching V_{max} if a non-competitive inhibitor is present.

Answers

1. Activation energy of reaction lowered; more molecules have $E>E_a$; number of successful collisions increases per unit time.
2. Enzymes in hyperthermophilic bacteria have stronger intramolecular forces within their enzymes because the enzymes are working in higher temperatures than human enzymes.
3. As temperature increases, the enzyme activity increases to a maximum. After optimum temperature, the enzyme activity decreases quickly.
4. $E + S \rightleftharpoons$ ES-complex equilibrium shifts right. Rate of the reaction will increase until the substrate concentration is limiting
5. Non-competitive inhibitors alter the shape of the active site. Enzyme-substrate complex cannot form no matter the concentration of substrate.

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