Enzymes (A2)

Enzymes are catalysts that calalyse chemical reactions taking place within living organisms.

In common with inorganic catalysts, enzymes:

- speed up a reaction without being used up.
- provide an alternative reaction pathway with lower activation energy.

Catalytic efficiency

Enzymes are very effective catalysts, catalytic efficiency of some enzymes are as follow:

enzyme	turnover number
carbonic anhydrase	36 000 000
catalase	5 600 000
β-amylase	1 100 000
β-galactosidase	12 500
phosphoglucose isomerase	1 240
succinate dehydrogenase	1 150

Note:
The catalytic activity of enzymes is measured 2 ways:

- enzyme activity: the number of moles of substrate converted to product per minute
- turnover number: the number of substrate molecules reacted per enzyme per minute.

Specificity of enzymes

Enzymes are very specific, generally catalysing only one particular reaction.

The specific substance (metabolite) that fits on the enzyme surface and is converted to product(s) is called a substrate.

Carbonic anhydrase, for instance, is an enzyme in red blood cells that catalyses the reaction:

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

Carbon dioxide is the substrate in this reaction because it fits onto the surface of the enzyme. Water reacts with the carbon dioxide, but only when the carbon dioxide is bound to the enzyme surface.

In a cellular environment, this specificity is absolutely essential. An enzyme must be able to distinguish its substrate molecules from other molecules.

Each enzyme has a specific substrate, the substrate being the target molecule acted upon during the enzyme-catalysed reaction.

Enzymes are functioning within the rules that define catalytic activity, they differ from ordinary chemical catalysts in several aspects:

- Enzymes are all large protein molecules.
- Higher reaction rates – the rates of enzyme catalysed reactions are often increased by factors of 10^6 to 10^{12} times compared to the uncatalysed reaction and are several orders of magnitude greater than those of the corresponding chemically catalysed reaction.
- Milder conditions – enzyme catalysed reactions occur under relatively milder conditions: temperature around 37°C, atmospheric pressure and pH around neutrality.
- Greater reaction specificity – enzymes are much more choosy with regard to their substrate and products. Enzyme-catalysed reactions are 'clean', the reactions do not produce side products.
- Capacity for regulation – the catalytic activities of many enzymes can be regulated by the concentrations of substrates other than the substrate (non-competitive inhibition)

Shapely molecules

The complicated folding of the protein chain to form the tertiary structure gives rise to 'clefts' or 'crevices' of precise geometric shape on the surface of the enzyme.

The precise shapes of these clefts have evolved to 'recognise' and hold in place a particular substrate molecule while it reacts.

This substrate-binding site has a shape that matches the shape of the substrate.

This region is where the enzyme-catalysed reaction takes place; it is known as the active site of the enzyme.

The catalytic properties and specificity of an enzyme are determined by the chemical nature of the amino acid R-groups located at the active site.

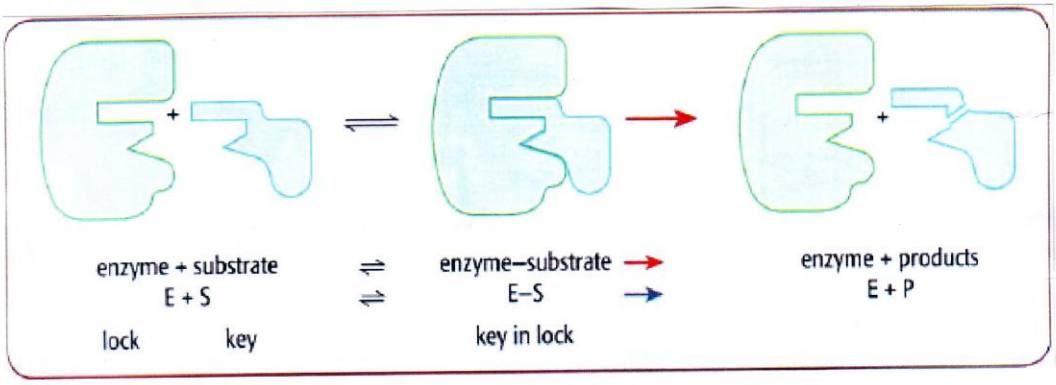
The active site usually occupies less than 5% of an enzyme's surface area and involves only a small number (3 to 12) of amino acids.

The rest of the enzyme structure functions as the scaffolding that maintains the shape of the active site.

The lock and key model

The precise specificity shown by enzymes can be explained by a model of enzyme activity as the 'lock and key' mechanism.

Enzymes catalysed reactions by binding to substrates in a manner similar to how a key (the substrate) fits into a lock (the enzyme active site).



The 'lock and key' mechanism

Locks and keys are complementary structures and this would also explain enzyme specificity.

Only one substrate will fit into the active site of the enzyme, just as only one key fits a lock.

The substrate must have the correct shape and size to fit the active site of the enzyme.

It must also have the necessary functional groups to form weak bonds with the amino acid side-chains lining the active site.

For example, when the substrate molecule fits into the active site:

- if a non-polar part of the substrate molecule is next to a non-polar part of enzyme, interactions involving van der Waals' forces is established.
- if a slightly positively charged part of the substrate molecule is next to a slightly negatively charged part of the enzymes, interactions involving dipole forces is established.

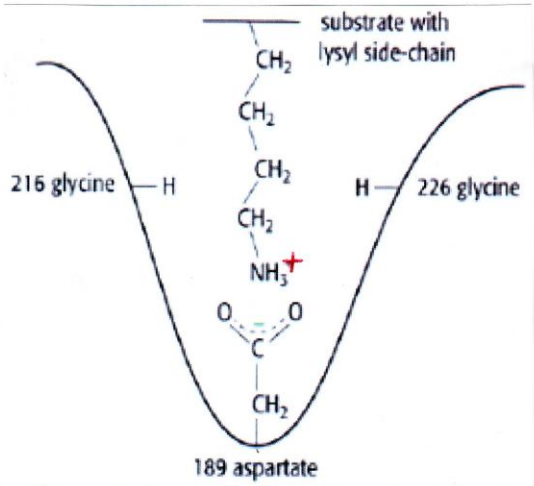
The sequence of reactions is:

- the substrate diffuses to the active site
- the substrate binds to the active site, forming an enzyme-substrate complex.
- amino acid side-chains (and other small molecules or ions) at the active site catalyse the reaction; in this process certain amino acid residues may act as proton donors/acceptors or nucleophiles.
- the products diffuse away from the active side.

Example:

Trypsin is a digestive enzyme which hydrolyses peptides derived from proteins in our food.

Trypsin is very specific and only breaks peptide bonds next to lysine and arginine residues in peptides.



The active site of trypsin will only bind amino acid side-chains which are relatively long and have a positively charged NH_3^+ group.
The numbers show the position of amino acid residues in the polypeptide chain.

The 'pocket' at the active site only allows amino acid side-chains of certain size and charge to enter.
The $-NH_3^+$ group, which forms when the side-chain of lysine (or arginine) accepts a proton, can form weak bonds with the aspartate residue at the active side.

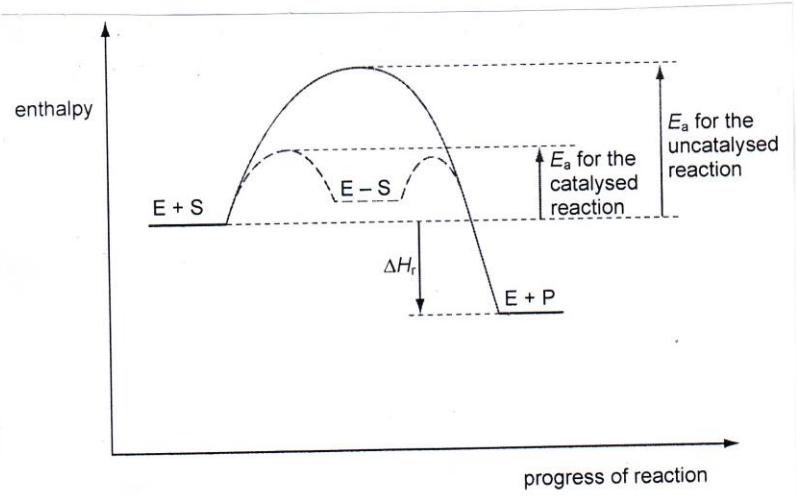
The rest of the pocket is lined with non-polar amino acid residues.
These form weak intermolecular attractions with the hydrocarbon part of the lysine (or arginine) side-chains.
When the lysine binds to the active site, other groups are brought up which catalyse the bond-breaking reaction.
The products then diffuse away.

Reaction kinetics involving enzyme

As homogeneous catalyst, enzymes function by providing an alternative reaction pathway that requires a lower Ea (activation energy).

Thus more molecular interactions possess sufficient energy to produce products.

The following energy profile diagram shows the formation of the enzyme – substrate complex reduces the energy requirement for the reaction to proceed.



energy profile for enzyme-catalysed and non-catalysed reaction.

The overall reaction between enzyme and its substrate can be represented by the following equation:

ENZYME + SUBSTRATE $\rightleftharpoons$ ENZYME-SUBSTRATE $\rightarrow$ ENZYME + PRODUCTS

(lock) (key) (key in lock)

E-S complexes are often almost as stable as the seperated enzyme + substrate, because of the extra bonding that binds the substrate to the active site.

The first stage of the reaction is reversible, since the Ea for the dissociation of the E-S complex back to E + S is about the same in size to that of the breakdown of E-S into E + P.

In some cases, the second stage is also reversible, making the whole enzyme catalysed process capable of proceeding in either direction depending on the cell's metabolic requirements.

Once the products have been formed, they leave the active site of the enzyme.

The enzyme is then free to combine with a new substrate molecule.

Like inorganic catalysts, enzymes are not used up in the reaction they catalyse so they can be used again and again.

Exercise 1.

Explain the terms:

i) active site

ii) lock and key mechanism

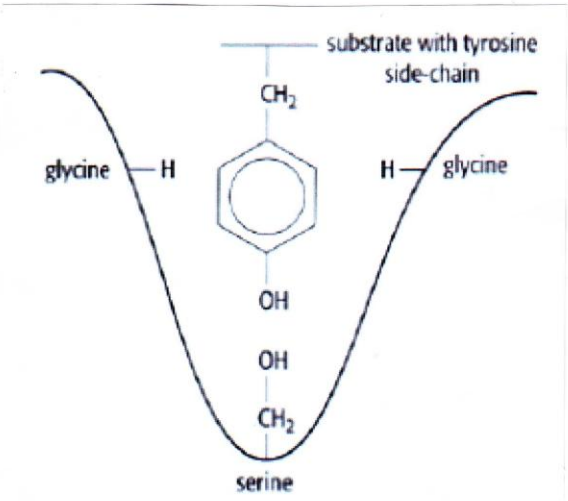
Workings

i) The substrate-binding site on the surface of the enzyme that matches the shape of the substrate. The enzyme-catalysed reaction takes place in this region.

ii) The substrate is complementary in shape to the active site of the enzyme.
It binds to the enzyme specifically because of the match in shape and also matching of the positions of polar and non-polar regions of the substrate and the active site (distribution of charge).
The substrate is said to be like a key in the lock, with the active site of the enzyme being the lock.

Exercise 2

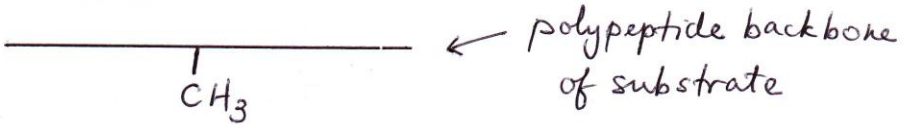
Chymotrypsin is an enzyme that catalyses the hydrolysis of peptide chains next to the amino acid tyrosine.



A tyrosine residue bound to the active site of chymotrypsin

i) What features of the active site help to bind tyrosine?

ii) The structure of an alanine side-chain is show below:



Suggest why the alanine residue does not bind to the chymotrypsin active site.

Workings

i) Hydrogen bonding between the -OH of the tyrosine and the -OH at the bottom of pocket of the active site.

The small glycine side chains on the protein allow the large tyrosine molecules to fit into the active site where it also held by van der Waals' forces.

ii) The $-CH_3$ group on the alanine side-chain is quite small, so it does not get near enough to the polypeptide chain at the active site to be stabilised by van der Waals' forces.

It does not fit far enough into the pocket to be stabalised by side-chains of any other amino acid residues.

Exercise 3

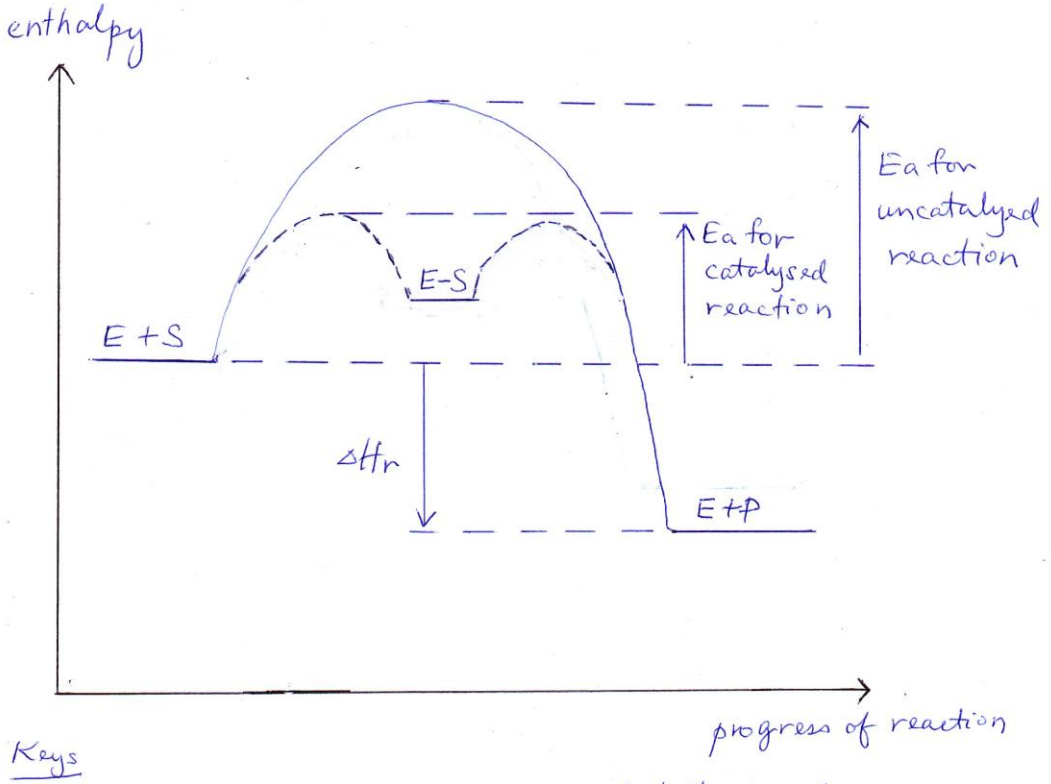
Draw an energy profile diagram to show a typical uncatalysed and enzyme-catalysed reaction.

On your diagram show:

i) the activation energy for the catalysed and uncatalysed reaction

ii) the enzyme, substrate and product and the enzyme-substrate complex.

Workings



Keys

E: Enzyme E-S: Enzyme-substrate complex

S: Substrate P: Product