Bio Factsheet

Number 43

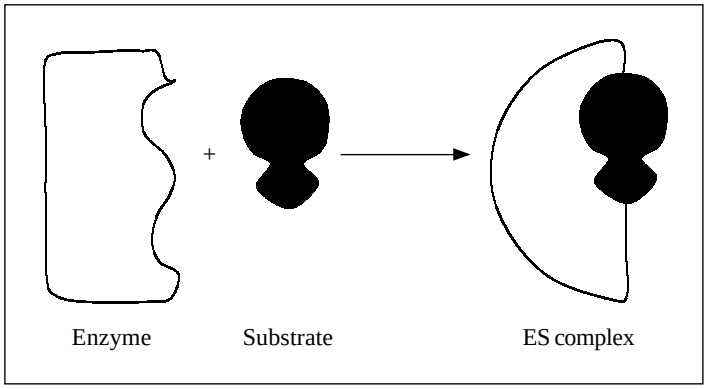
Factors Affecting Enzyme Activity

Enzymes are globular proteins which act as biological catalysts. This means that they speed up the rate of reaction by lowering the activation energy, that is the energy required to break bonds. Enzymes are a complex tertiary and sometimes quaternary shape and catalyse reactions by forming a complex (known as the enzyme substrate complex) at a specific region of the enzyme called the active site.

Enzyme + substrate → enzyme substrate complex → product

Enzymes are specific; any individual enzyme can usually only catalyse one particular reaction. The **induced fit hypothesis** has been put forward to explain how enzymes work. The key points of the induced fit hypothesis are as follows (Fig1):

Fig 1. Induced fit hypothesis



1. Substrate approaches the active site of the enzyme.
2. The shape of the active site then changes to fit precisely around the substrate – in other words, the substrate **induces** the active site to change shape.
3. The reaction is catalysed and products form.
4. The products are a different shape from the substrate and therefore diffuse away from the active site. As they do, the active site reverts to its original shape.

Factors affecting enzyme activity

1. Temperature

Enzymes have an optimum temperature – this is the temperature at which they work most rapidly. Below the optimum temperature, increasing temperature will increase the rate of the reaction. This is because temperature increases the kinetic energy of the system, effectively increasing the number of collisions between the substrate and the enzyme's active site.

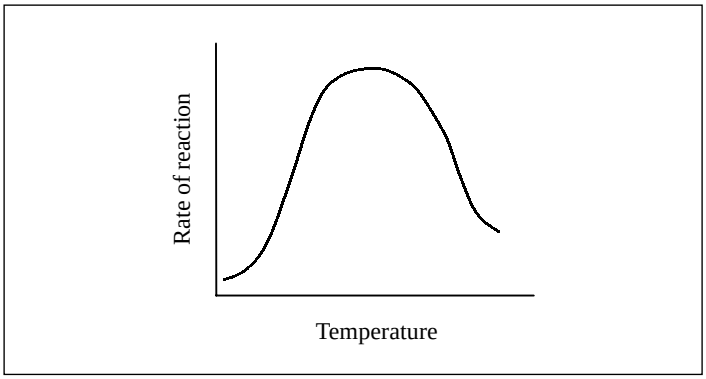
Temperatures above the optimum will lead to **denaturation**. This occurs because the hydrogen bonds and disulphide bridges which maintain the shape of the active site are broken. Thus, enzyme substrate complexes can no longer be formed.

The effect of temperature on the rate of a chemical reaction is described by the term "temperature coefficient" (Q_{10}).

$$Q_{10} = \frac{\text{rate of reaction at T + 10°C}}{\text{rate of reaction at T°C}}$$

Many enzymes have a Q_{10} of between 2 and 3. In other words, provided that the temperature is not so high that it causes denaturation, an increase in temperature of 10°C will speed up the reaction by a factor of 2-3, that is it will double or treble it (Fig 2).

Fig 2. Effect of temperature on enzyme activity

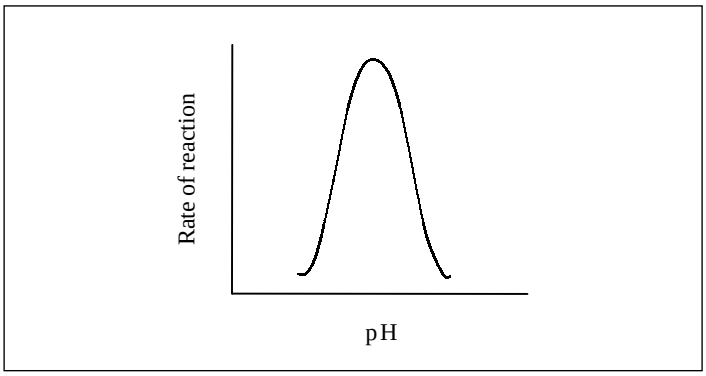


2. pH

The effect of a change in pH on enzyme activity is shown in Fig 3. As with temperature, each enzyme has an optimum pH. If pH increases or decreases much beyond this optimum, the ionisation of groups at the active site and on the substrate may change, effectively slowing or preventing the formation of the enzyme substrate complex. At extreme pH, the bonds which maintain the tertiary structure – hence the active site – are disrupted and the enzyme is irreversibly denatured.

Since most human enzymes are intracellular, most have a pH optimum of 7.3-7.4. However, pepsin, which works in the acidic environment of the stomach, has an optimum of 2.4 (Fig 3).

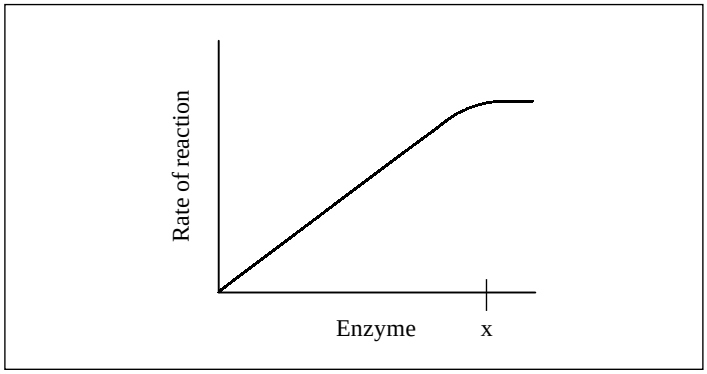
Fig 3. Effect of pH on enzyme activity



3. Enzyme concentration

The effect of enzyme concentration on the rate of reaction is shown in Fig 4. At low enzyme concentrations there are more substrate molecules than there are available active sites. Increasing the number of active sites by increasing the concentration of the enzyme, therefore, effectively increases the rate of the reaction. Eventually, at point x, increasing the enzyme concentration has no effect on the rate of reaction. This is because it is now the number of substrate molecules which has become the limiting factor.

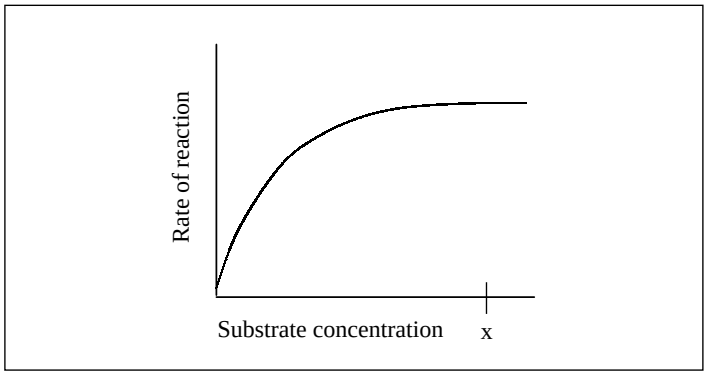
Fig 4. Effect of enzyme concentration on enzyme activity



4. Substrate concentration

Fig 5 shows the effect of substrate concentration on the rate of reaction.

Fig 5. Effect of substrate concentration on enzyme activity



At low substrate concentration the reaction proceeds slowly. This is because there are not enough substrate molecules to occupy all of the active sites on the enzyme. As substrate concentration increases, the rate increases because there are more enzyme substrate complexes formed. At point x, however, increasing the substrate concentration will have no further effect on the rate of reaction. This is because all of the enzyme's active sites are now occupied by substrate molecules – increasing the substrate concentration further will have no effect, because no more enzyme substrate complexes can form. The rate of reaction now depends on the turnover rate of the enzyme, i.e. the number of substrate molecules transformed by one molecule of enzyme per second. Carbonic anhydrase has the highest turnover rate of any known enzyme (Table 1).

Table 1. Enzyme turnover rates

Enzyme	Turnover rate
Carbonic anhydrase	36×10^6
Catalase	5.6×10^6
Lysozyme	60

5. Cofactors

Many enzymes require cofactors to function properly. There are three main types of cofactor; co-enzymes, inorganic ions and prosthetic groups.

1. **Coenzymes** are organic molecules which often contain a vitamin molecule as part of their structure. Coenzymes become loosely bound to the enzyme and move away from the enzyme once the reaction is completed. One coenzyme, e.g. NAD^+ may react with many different enzymes in many different types of reaction. NAD^+ transfers hydrogen in reactions involving dehydrogenase enzymes.

2. **Inorganic** metal ions are also known as enzyme activators. They change the charge in the active site, enabling the enzyme substrate complex to form. Some become intimately bound to the enzyme, e.g. Fe^{2+} in catalase. Most others accelerate the binding between the enzyme and the substrate, e.g. Mg^{2+} in phosphotransferases.

3. **Prosthetic** groups are coenzymes that bind permanently to the enzyme molecule and remain there even after the reactions are complete, e.g. FAD (flavin adenine dinucleotide). Like NAD^+ it carries hydrogen atoms, this time with oxidase enzymes.

6. Inhibitors

Inhibitors slow down the rate of reaction. As such, they are an essential form of cellular control, allowing enzyme reaction rate to be slowed when necessary. Some enzymes are inhibited by the end product of the reaction they catalyse (see Factsheet 31 Enzyme control of metabolic pathways).

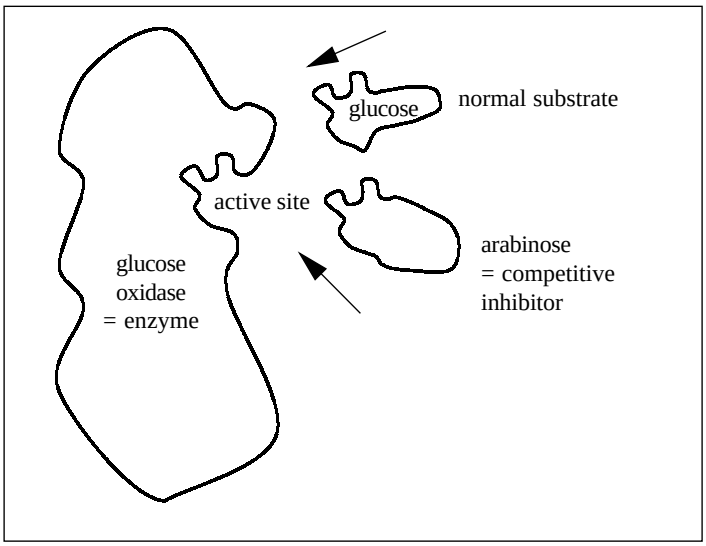
(a) Reversible inhibitors

There are two types of reversible inhibitor:

- competitive reversible inhibitor
- non-competitive reversible inhibitor

Competitive reversible inhibitors are structurally similar to the normal substrate and compete with the normal substrate for the active sites (see Fig 6).

Fig 6. Competitive reversible inhibition

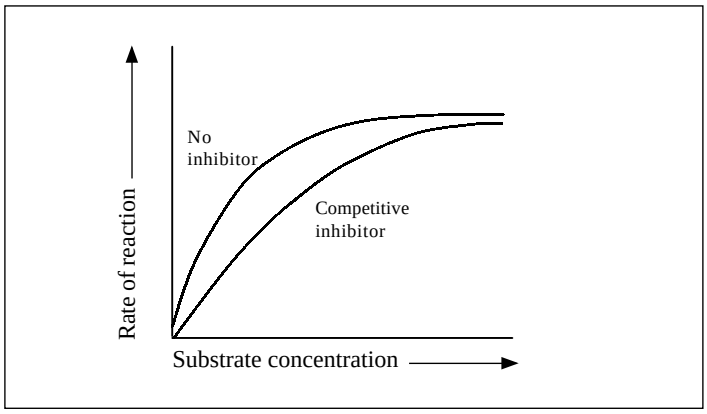


Typical Exam Questions

1. Describe and explain the effect of pH, temperature, enzyme concentration etc. on rate of reaction
2. Explain the induced fit hypothesis
3. Explain the role of cofactors

However, if the concentration of the normal substrate is increased, reversible inhibitors are displaced from the active site and the normal enzyme substrate complex can form (Fig 7).

Fig 7. Effect of increased substrate concentration on reversible competitive inhibition



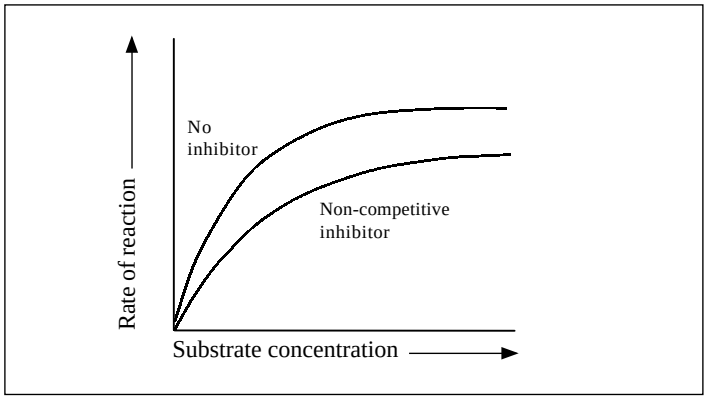
Example 1: arabinose competes with glucose for the active sites on glucose oxidase.

Example 2: oxaloacetate, malonate and pyrophosphate all compete with succinate for the active site of the enzyme succinate dehydrogenase.

Example 3: an individual who swallows methanol is in danger of becoming blind. This is because the methanol – which itself is not toxic – will be metabolised to formaldehyde which is extremely toxic and will cause blindness. At hospital, the individual will be treated with ethanol. The ethanol is structurally similar to methanol and will compete with methanol for the enzyme's active sites. Thus, the metabolism of methanol is slowed down.

Non-competitive reversible inhibitors react with the enzyme but not at the active site. They change the shape of the whole enzyme, including the shape of the active site, hence the reaction cannot proceed and no products are formed on those enzymes (Fig 8).

Fig 8. Effect of increased substrate concentration on non-competitive inhibition



(b) Irreversible inhibitors

Irreversible inhibitors bind covalently and permanently to the enzyme, preventing normal enzyme function. For example, Aspirin is an irreversible inhibitor of cycloxygenase, an enzyme involved in the synthesis of prostaglandins. Substances such as mercury, iron and arsenic bind irreversibly to the SH (sulphydryl) group on enzymes.

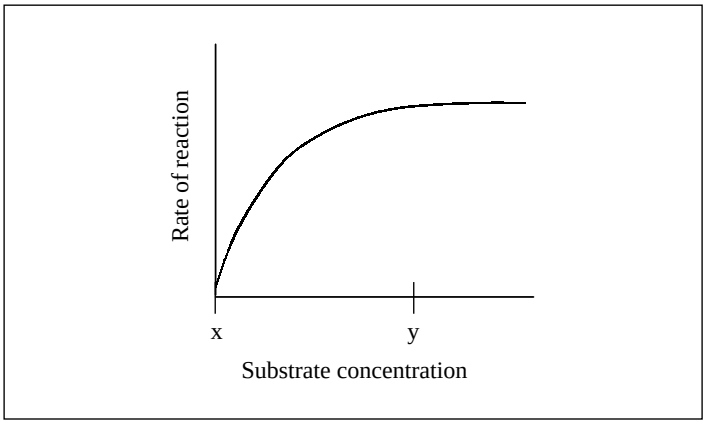
Practice Questions

1. Define the following terms:

   (a) induced fit hypothesis (3 marks)

   (b) denaturation (3 marks)

2. The graph show the effect of increasing substrate concentration on the rate of an enzyme controlled reaction.



   (a) Explain the shape of the curve between points x and y (2 marks)

   (b) Describe and explain the effect which a competitive reversible inhibitor would have on the rate of this reaction (2 marks)

Answers

Semicolons indicate marking points

1. (a) substrate approaches active site;
   causes shape of active site to change;
   allows formation of enzyme/substrate complex;
   products do not fit active site therefore diffuse away;

   (b) loss of quaternary/tertiary structure;
   loss of active site/permanent change in shape of active site;
   enzyme-substrate complex unable to form;
   caused by above optimum temperatures/pH above or below optimum;

2. (a) substrate concentration is limiting factor;
   as concentration increases more enzyme substrate complexes form;

   (b) slow it down;
   competitive inhibitor will occupy active sites;
   reducing number of enzyme-substrate complexes;

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