3 Enzymes: structure and function

In this chapter we discuss the structure and function of enzymes. Drug action at enzymes is discussed in Chapter 7 and in other chapters throughout the text.

3.1 Enzymes as catalysts

Enzymes are proteins which act as the body's catalysts—agents that speed up a chemical reaction without being consumed themselves. Without them, the cell's chemical reactions would either be too slow or not take place at all. An example of an enzyme-catalysed reaction is the reduction of **pyruvic acid** to **lactic acid**, which takes place when muscles are over-exercised, and is catalysed by an enzyme called **lactate dehydrogenase** (Fig. 3.1).

Note that the reaction is shown as an equilibrium. It is, therefore, more correct to describe an enzyme as an agent that speeds up the approach to equilibrium, because the enzyme speeds up the reverse reaction just as efficiently as the forward reaction. The final equilibrium concentrations of the starting materials and products are unaffected by the presence of an enzyme.

How do enzymes affect the rate of a reaction without affecting the equilibrium? The answer lies in the existence of a high-energy **transition state** that must be formed before the starting material (the **substrate**) can be converted to the product. The difference in energy between the transition state and the substrate is the **activation energy**, and it is the size of this activation energy that determines the rate of a reaction, rather than the difference in energy between the substrate and the product (Fig. 3.2). An enzyme acts to lower the activation energy by helping to stabilize the transition state. The energy of the substrate and products are unaffected, and therefore the equilibrium ratio of substrate to product is unaffected. We can relate energy to the rate and equilibrium constants with the following equations:

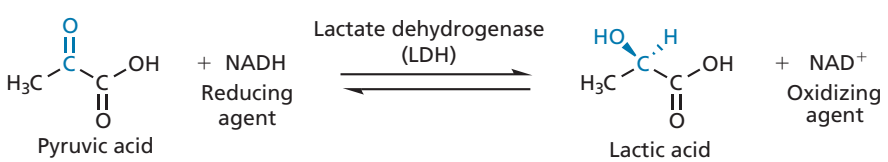


FIGURE 3.1 Reaction catalysed by lactate dehydrogenase.

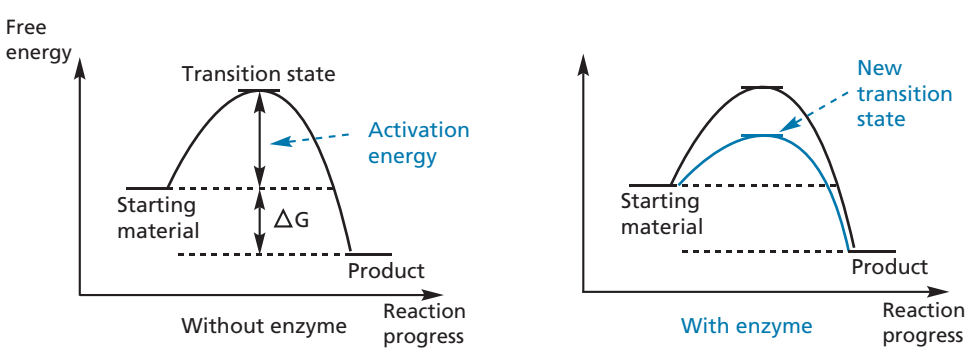


FIGURE 3.2 Graphs demonstrating the stabilization of a reaction's transition state by an enzyme.

$$\text{Energy difference} = \Delta G = -RT \ln K$$

where K is the equilibrium constant (= [products]/[reactants]), R is the gas constant (= 8.314 J mol^{-1} K^{-1}), and T is the temperature.

$$\text{Rate constant} = k = Ae^{-E/RT}$$

where E is the activation energy and A is the frequency factor.

Note that the rate constant k does not depend on the equilibrium constant K.

We have stated that enzymes catalyse reactions, but we have still to explain how.

3.2 How do enzymes catalyse reactions?

The factors involved in enzyme catalysis are summarized below and will be discussed in more detail in sections 3.2–3.5.

- Enzymes provide a reaction surface and a suitable environment.
- Enzymes bring reactants together and position them correctly so that they easily attain their transition-state configurations.
- Enzymes weaken bonds in the reactants.
- Enzymes may participate in the reaction mechanism.
- Enzymes form stronger interactions with the transition state than with the substrate or the product.

An enzyme catalyses a reaction by providing a surface to which a substrate can bind, resulting in the weakening of high-energy bonds. The binding also holds the substrate in the correct orientation to increase the chances of reaction. The reaction takes place, aided by the enzyme, to give a product which is then released (Fig. 3.3). Note again that it is a reversible process. Enzymes can catalyse both forward and backward reactions. The final equilibrium mixture will, however, be the same, regardless of whether we supply the enzyme with substrate or product. Substrates bind to, and react at, a specific area of the enzyme called the **active site**—usually quite a small part of the overall protein structure.

3.3 The active site of an enzyme

The active site of an enzyme (Fig. 3.4) has to be on or near the surface of the enzyme if a substrate is to reach it. However, the site could be a groove, hollow, or gully allowing the substrate to sink into the enzyme. Normally, the active site is more hydrophobic in character than the surface of the enzyme, providing a suitable environment for many reactions that would be difficult or impossible to carry out in an aqueous environment.

Because of the overall folding of the enzyme, the amino acid residues that are close together in the active site may be far apart in the primary structure. Several amino acids in the active site play an important role in enzyme function, which can be demonstrated by comparing the primary structures of the same enzyme from different organisms. Here, the primary structure differs from species to species as a result of **mutations** happening over millions of years. The variability is proportional to how far apart the organisms are on the evolutionary ladder. However, there are certain amino acids that remain constant, no matter the source of the enzyme. These are amino acids that are crucial to the enzyme's function and are often present in the active site. If one of these amino acids is altered through mutation, the enzyme could become useless and the cell bearing this mutation would have a poor chance of survival. Thus, the mutation would not be preserved. The only exception to this would be if the mutation introduced an amino acid which could either perform the same task as the original amino acid or improved substrate binding. This consistency of amino acids in the active site can often help scientists determine which amino acids are present in an active site, if this is not known already.

Amino acids present in the active site can have one of two roles:

- binding—the amino acid residue is involved in binding the substrate or a cofactor to the active site;

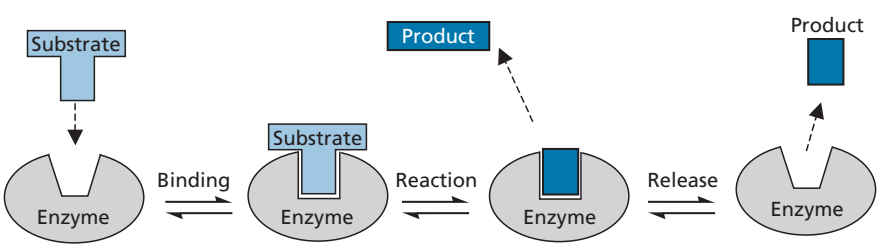


FIGURE 3.3 The process of enzyme catalysis.

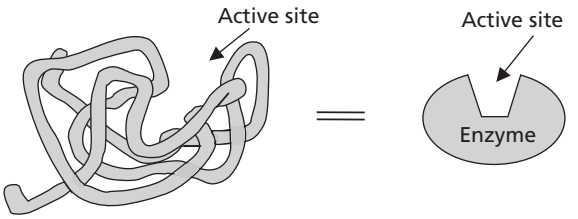


FIGURE 3.4 The active site of an enzyme.

- catalytic—the amino acid is involved in the mechanism of the reaction.

We shall study these in turn.

3.4 Substrate binding at an active site

The interactions which bind substrates to the active sites of enzymes include ionic bonds, hydrogen bonds, dipole–dipole, and ion–dipole interactions, as well as van der Waals and hydrophobic interactions (section 1.3). These binding interactions are the same bonding interactions responsible for the tertiary structure of proteins, but their relative importance differs. Ionic bonding plays a relatively minor role in protein tertiary structure compared with hydrogen bonding or van der Waals interactions, but it can play a crucial role in the binding of a substrate to an active site.

As intermolecular bonding forces are involved in substrate binding, it is possible to look at the structure of a substrate and postulate the probable interactions that it will have with its active site. As an example, consider **pyruvic acid**—the substrate for **lactate dehydrogenase** (Fig. 3.5).

If we look at the structure of pyruvic acid, we can propose three possible interactions by which it might bind to its active site—an ionic interaction involving the ionized carboxylate group, a hydrogen bond involving the ketonic oxygen, and a van der Waals interaction involving the methyl group. If these postulates are correct, it means that within the active site there must be **binding regions** containing suitable amino acids that can take part in these intermolecular interactions. Lysine, serine, and phenylalanine residues respectively would fit the bill. A knowledge of how a substrate binds to its active site is invaluable in designing drugs that will target specific enzymes (Chapter 7).

3.5 The catalytic role of enzymes

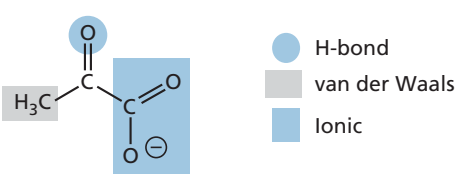
We now move on to consider the mechanism of enzymes and how they catalyse reactions. In general, enzymes catalyse reactions by providing binding interactions, acid/base catalysis, nucleophilic groups, and cofactors.

3.5.1 Binding interactions

In the past, it was thought that a substrate fitted its active site in a similar way to a key fitting a lock (**Fischer's lock and key hypothesis**). Both the enzyme and the substrate were seen as rigid structures, with the substrate (the key) fitting perfectly into the active site (the lock) (Fig. 3.6). However, this scenario does not explain how some enzymes can catalyse a reaction on a range of different substrates. It implies, instead, that an enzyme has an optimum substrate that fits it perfectly, whereas all other substrates fit less perfectly. This, in turn, would imply that the catalysed reaction is only efficient for the optimum substrate. As this is not the case for many enzymes, the lock and key analogy must be invalid.

It is now proposed that the substrate is not quite the ideal shape for the active site, and that it forces the active site to change shape when it enters—a kind of moulding process. This theory is known as **Koshland's theory of induced fit** as the substrate induces the active site to take up the ideal shape to accommodate it (Fig. 3.6).

For example, a substrate such as **pyruvic acid** might interact with specific binding regions in the active site of lactate dehydrogenase via one hydrogen bond, one ionic bond, and one van der Waals interaction (Fig. 3.7).



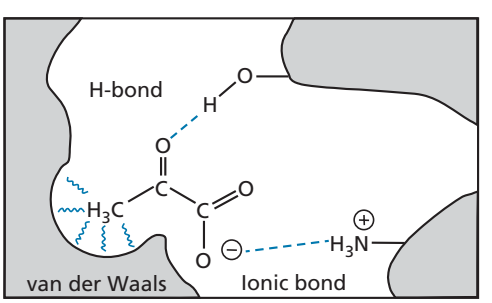


FIGURE 3.5 Binding interactions between pyruvic acid and lactate dehydrogenase.

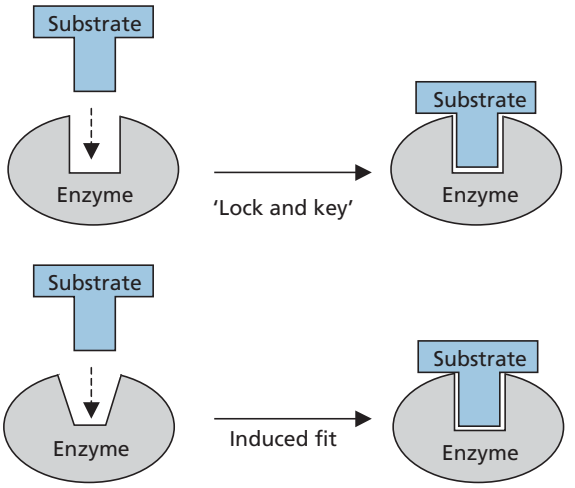


FIGURE 3.6 The 'lock and key' and 'induced fit' hypotheses for substrate–enzyme binding.

However, if the fit is not perfect, the three bonding interactions are not ideal either. For example, the binding groups may be slightly too far away from the corresponding binding regions in the active site. In order to maximize the strength of these bonds, the enzyme changes shape such that the amino acid residues involved in the binding move closer to the substrate.

This theory of induced fit helps to explain why some enzymes can catalyse reactions involving a wide range of substrates. Each substrate induces the active site into a shape that is ideal for it and, as long as the moulding process does not distort the active site so much that the reaction mechanism proves impossible, the reaction can proceed. The range of substrates that can be accepted depends on the substrates being the correct size to fit the active site and having the correct binding groups in the correct relative positions.

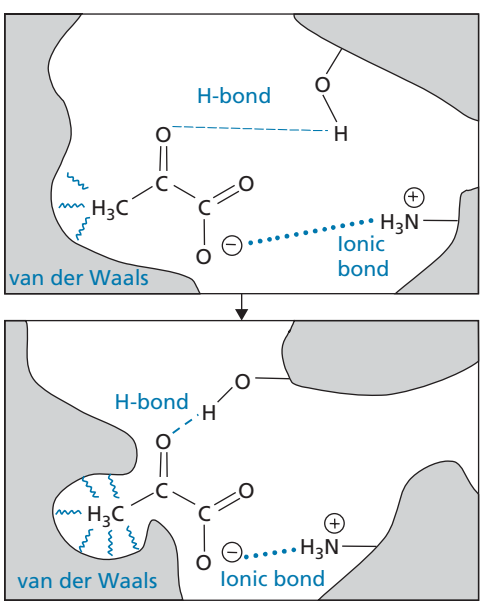


FIGURE 3.7 Example of an induced fit.

But note this. The substrate is not a passive spectator to the moulding process going on around it. As the enzyme changes shape to maximize bonding interactions, the same thing can happen to the substrate. It too may alter shape. Bond rotation may occur to fix the substrate in a particular conformation—and not necessarily the most stable one. Bonds may even be stretched and weakened. Consequently, this moulding process designed to maximize binding interactions may force the substrate into the ideal conformation for the reaction to follow and may also weaken the very bonds that have to be broken.

Once bound to an active site, the substrate is now held ready for the subsequent reaction. Binding has fixed the 'victim' (substrate) so that it cannot evade attack, and this same binding has weakened its defences (bonds) so that reaction is easier (a lower activation energy).

There is another point relating to substrate binding. The binding interactions with the active site must be sufficiently strong to hold the substrate for the subsequent reaction, but they cannot be too strong. If they were, the product might also be bound strongly and fail to depart the active site. This would block the active site of the enzyme and prevent it from catalysing another reaction. Therefore, a balance must be struck.

Finally, it is important to realize that the enzyme also binds the transition state involved in the enzyme-catalysed reaction. Indeed, the binding interactions involved are stronger than those binding the substrate, which means that the transition state is stabilized relatively more than the substrate. This results in a lower activation energy compared with the non-catalysed reaction.

3.5.2 Acid/base catalysis

Acid/base catalysis is often provided by the amino acid **histidine**, which contains an imidazole ring as part of its side chain. The imidazole ring acts as a weak base, which means that it exists in equilibrium between its protonated and free base forms (Fig. 3.8), allowing it to accept or donate protons during a reaction mechanism. This is important, as there are often very few water molecules present in an active site to carry out this role. Histidine is not the only amino acid residue that can provide acid/base catalysis. For example, a **glutamic acid** residue acts as a proton source in the reaction mechanism of the enzyme HMG-CoA reductase (Case study 1), while **aspartic acid** and **aspartate** residues act as proton donors and proton acceptors, respectively, in other enzyme-catalysed reactions (sections 7.4 and 20.7.4.1).

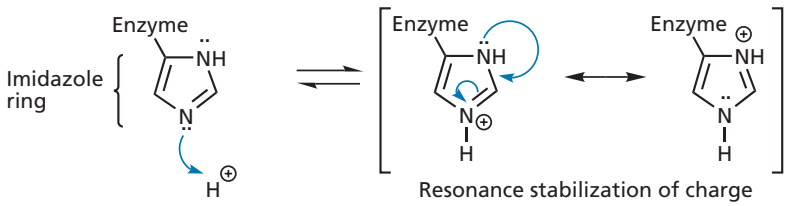


FIGURE 3.8 Histidine acting as a weak base.

Tyrosine acts as a proton source in the mechanism by which the enzyme 17β-hydroxysteroid type 1 catalyses the conversion of **estrone** to **estradiol**.

For additional material see Web article 1: steroids as novel anticancer agents.

3.5.3 Nucleophilic groups

The amino acids **serine** and **cysteine** are present in the active sites of some enzymes. These amino acids have nucleophilic residues (OH and SH respectively) which are able to participate in the reaction mechanism. They do this by reacting with the substrate to form intermediates that would not be formed in the uncatalysed reaction. These intermediates offer an alternative reaction pathway that may avoid a high-energy transition state and hence increase the rate of the reaction.

Normally, an alcoholic OH group, such as the one on serine, is not a good nucleophile. However, there is usually a histidine residue close by to catalyse the reaction. For example, the mechanism by which chymotrypsin hydrolyses peptide bonds (Fig. 3.9) involves a **catalytic triad** of amino acids—serine, histidine, and aspartic acid. Serine and histidine participate in the mechanism as a nucleophile and acid/base catalyst respectively. The aspartate group interacts with the histidine ring and serves to activate and orient it correctly for the mechanism.

The presence of a nucleophilic serine residue means that water is not required in the initial stages of the mechanism. This is important, firstly, because water is a

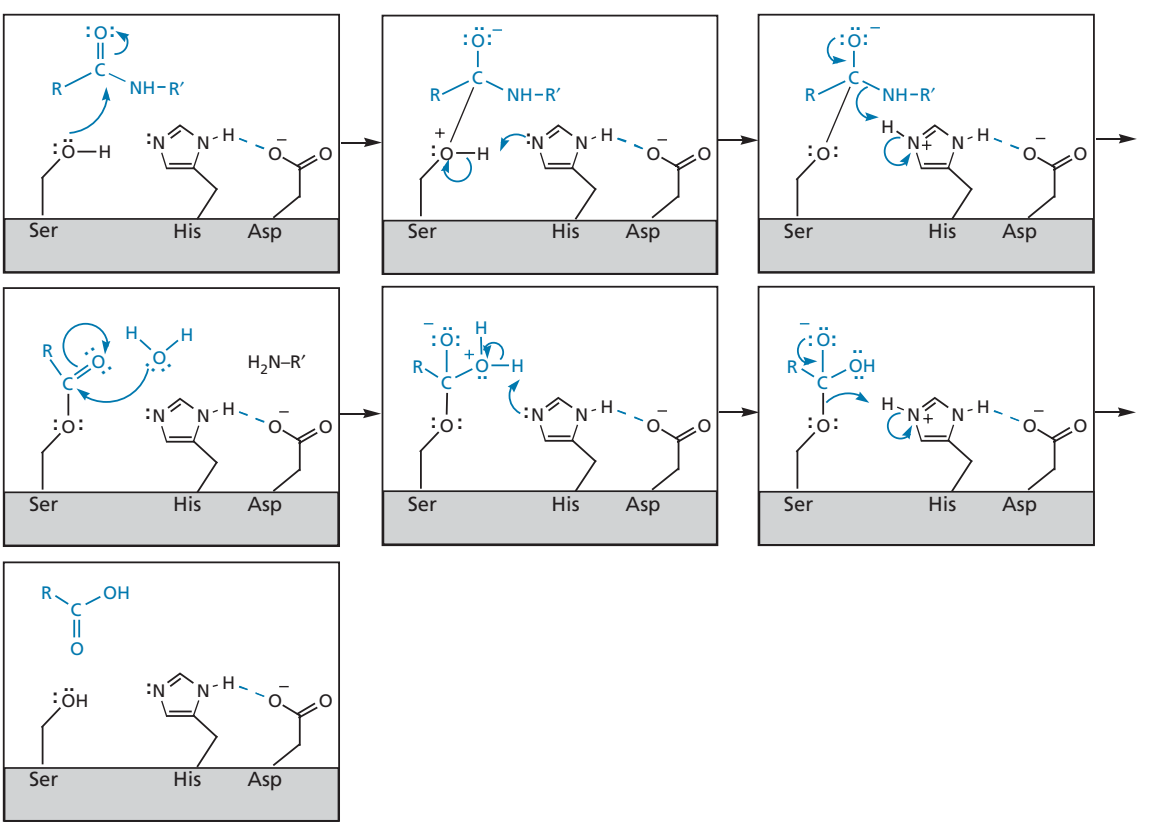


FIGURE 3.9 Hydrolysis of peptide bonds catalysed by the enzyme chymotrypsin.

poor nucleophile and may also find it difficult to penetrate the occupied active site. Secondly, a water molecule would have to drift into the active site and search out the carboxyl group before it could attack it. This would be something similar to a game of blind man's bluff. The enzyme, however, can provide a serine OH group positioned in exactly the right spot to react with the substrate. Therefore, the nucleophile has no need to search for its substrate: the substrate has been delivered to it.

Water is eventually required to hydrolyse the acyl group attached to the serine residue. However, this is a much easier step than the hydrolysis of a peptide link, as esters are more reactive than amides. Furthermore, the hydrolysis of the peptide link means that one half of the peptide can drift away from the active site and leave room for a water molecule to enter. A similar enzymatic mechanism is involved in the action of the enzyme **acetylcholinesterase** (section 22.12.3), **pancreatic lipase** (Box 7.2), and a viral protease enzyme carried by the hepatitis C virus (section 20.10).

The amino acid **lysine** has a primary amine group on its side chain which should make it a better nucleophilic group than serine or cysteine. However, the group is generally protonated at physiological pH, which precludes it acting as a nucleophile. Having said that, some enzymes have a lysine residue located in a hydrophobic pocket, which means that it is not protonated and can, indeed, act as a nucleophilic group.

3.5.4 Cofactors

Many enzymes require additional non-protein substances called cofactors for the reaction to take place. Deficiency of cofactors can arise from a poor diet resulting in the loss of enzyme activity and subsequent disease (e.g. scurvy). Cofactors are either metal ions (e.g. zinc) or small organic molecules called **coenzymes** (e.g. NAD^+, pyridoxal phosphate). Most coenzymes are bound by ionic bonds and other non-covalent bonding interactions, but some are bound covalently and are called **prosthetic groups**. Coenzymes are derived from water-soluble vitamins and act as the body's chemical reagents. For example, **lactate dehydrogenase** requires the coenzyme **nicotinamide adenine dinucleotide (NAD^+)** (Fig. 3.10) in order to catalyse the dehydrogenation of **lactic acid** to **pyruvic acid**. NAD^+ is bound to the active site along with lactic acid, and acts as the oxidizing agent. During the reaction it is converted to its reduced form (NADH) (Fig. 3.11). Conversely, NADH can bind to the enzyme and act as a reducing agent when the enzyme catalyses the reverse reaction.

$NADP^+$ and NADPH are phosphorylated analogues of NAD^+ and NADH, respectively, and carry out redox reactions by the same mechanism. NADPH is used almost exclusively for reductive biosynthesis, whereas NADH is used primarily for the generation of ATP.

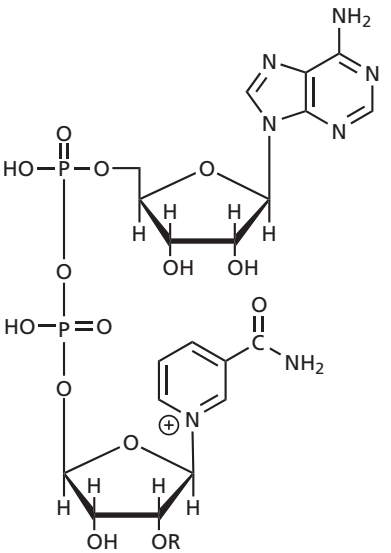


FIGURE 3.10 Nicotinamide adenine dinucleotide (R = H) and nicotinamide adenine dinucleotide phosphate (R = phosphate).

A knowledge of how the coenzyme binds to the active site allows the possibility of designing enzyme inhibitors that will fit the same region (see Case study 5 and section 21.6.2; see also Web article 1).

3.5.5 Naming and classification of enzymes

The name of an enzyme reflects the type of reaction it catalyses, and has the suffix '-ase' to indicate that it is an enzyme. For example, an **oxidase** enzyme catalyses an oxidation reaction. It is important to appreciate that enzymes can catalyse the forward and back reactions of an equilibrium reaction. This means that an oxidase enzyme can catalyse reductions, as well as oxidations. The reaction catalysed depends on the nature of the substrate, i.e. whether it is in the reduced or oxidized form.

Enzymes are classified according to the general class of reaction they catalyse and are coded with an EC number (Table 3.1).

3.5.6 Genetic polymorphism and enzymes

There are often subtle differences in the structure and properties of an enzyme between different individuals. This is owing to the fact that the DNA that codes for proteins (Chapter 6) is not identical from person to person. On average, there is a difference of one base pair in every thousand between individuals. This is known as **genetic polymorphism**. As the nucleic acid bases act as

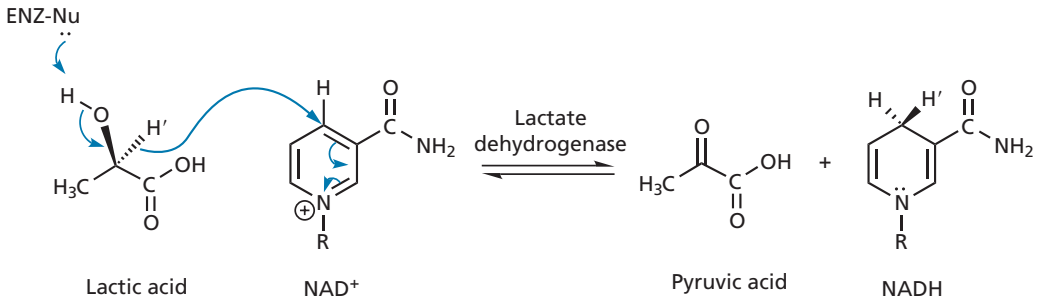


FIGURE 3.11 NAD^+ acting as a coenzyme.

TABLE 3.1 Classification of enzymes

EC number	Enzyme class	Type of reaction
E.C.1.x.x.x	Oxidoreductases	Oxidations and reductions
E.C.2.x.x.x	Transferases	Group transfer reactions
E.C.3.x.x.x	Hydrolases	Hydrolysis reactions
E.C.4.x.x.x	Lyases	Addition or removal of groups to form double bonds
E.C.5.x.x.x	Isomerases	Isomerizations and intramolecular group transfers
E.C.6.x.x.x	Ligases	Joining two substrates at the expense of ATP hydrolysis

Note: EC stands for Enzyme Commission, a body set up by the International Union of Biochemistry (as it then was) in 1955.

the code for amino acids in proteins, a difference at this level results in a different amino acid being introduced into the protein. Often, this has no observable effect on protein function, but not always. Some polymorphisms can adversely affect the proper functioning of an enzyme and lead to genetic disease. Others can have an influence on drug therapy. For example, individuals differ in their ability to metabolize drugs as a result of this phenomenon (section 11.5.6). Polymorphism can alter the sensitivity of an enzyme towards a drug, making the latter less effective. This is a particular problem in anticancer, antibacterial, and antiviral therapies where drug resistance can develop through the survival of cells containing less sensitive enzymes (Chapters 19–21).

3.6 Regulation of enzymes

Virtually all enzymes are controlled by agents which can either enhance or inhibit catalytic activity. Such control reflects the local conditions within the cell. For example, the enzyme **phosphorylase** *a* catalyses the breakdown of **glycogen** (a polymer of glucose monomers) to **glucose-1-phosphate** subunits (Fig. 3.12). It is stimulated by **adenosine 5′-monophosphate (AMP)** and inhibited by glucose-1-phosphate. Thus, rising levels of the product (glucose-1-phosphate) act as a self-regulating 'brake' on the enzyme.

But how does this control take place?

The answer is that many enzymes have a binding site which is separate from the active site called the **allosteric** binding site (Fig. 3.13). This is where the agents controlling the activity of the enzyme bind. When this occurs, an induced fit takes place which alters not only the allosteric binding site, but also the active site. Agents that inhibit the enzyme produce an induced fit that makes the active site unrecognizable to the substrate.

We might wonder why an agent inhibiting the enzyme has to bind to a separate, allosteric binding site and not to the active site itself. After all, if the agent could bind to the active site, it would directly block the natural substrate from entering. There are two explanations for this.

Firstly, many of the enzymes that are under allosteric control are at the start of a biosynthetic pathway (Fig. 3.14). A biosynthetic pathway involves a series of enzymes, all working efficiently to produce a final product. Eventually, the cell will have enough of the required material and will need to stop production. The most common control mechanism is known as **feedback control**, where the final product controls its own synthesis by inhibiting the first enzyme in the biochemical pathway. When there are low levels of final product in the cell, the first enzyme in the pathway is not inhibited and works normally. As the levels of final product increase, more and more of the enzyme is blocked and the rate of synthesis drops off in a graded fashion. Crucially, the final product has undergone many transformations from the original starting material and so it is no longer recognized by the active site of the first enzyme. A separate allosteric binding site is therefore needed which recognizes the final product. The biosynthesis of noradrenaline in section 23.4 is an example of a biosynthetic pathway under feedback control.

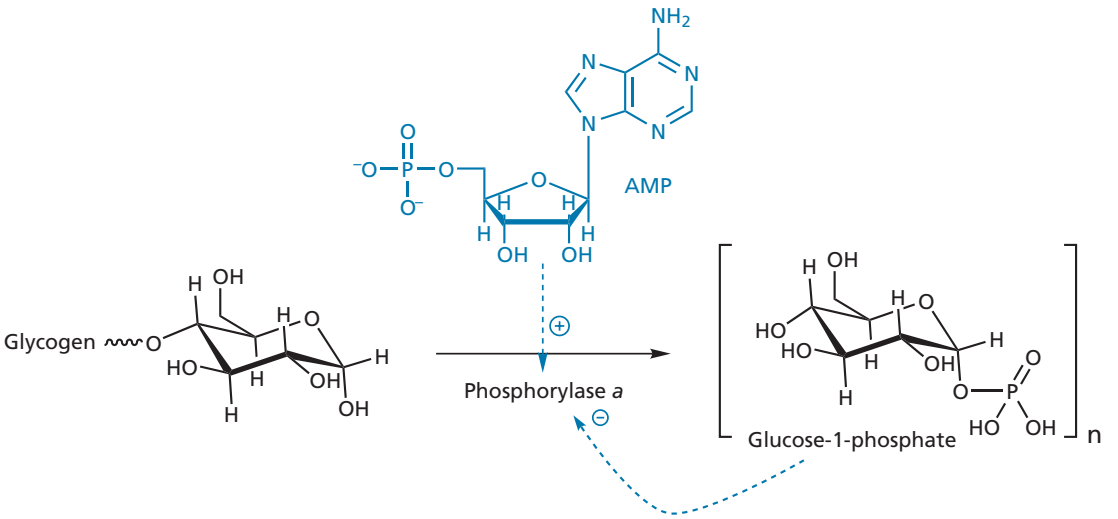


FIGURE 3.12 Internal control of the catalytic activity of phosphorylase *a* by glucose-1-phosphate and AMP.

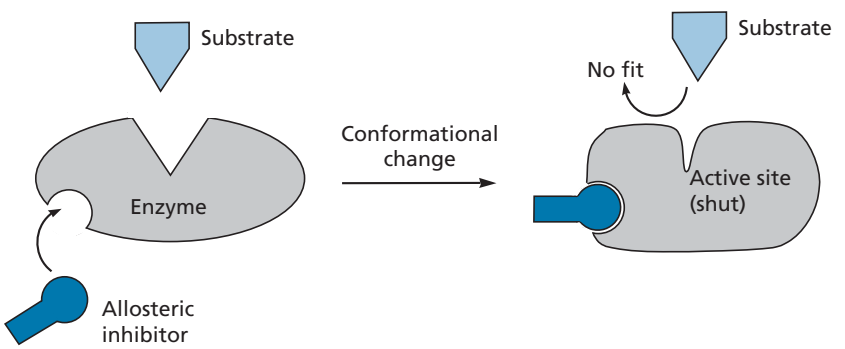


FIGURE 3.13 Allosteric inhibition of an enzyme.

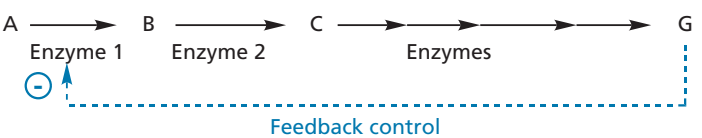


FIGURE 3.14 Feedback control of enzyme 1 by final product G.

Secondly, binding of the final product to the active site would not be a very efficient method of feedback control, as the product would have to compete with the enzyme's substrate. If levels of the latter increased, then the inhibitor would be displaced and feedback control would fail.

Many enzymes can also be regulated externally (Box 3.1). We shall look at this in more detail in Chapter 5, but, in essence, cells receive chemical messages from their environment which trigger a cascade of signals within the cell. In turn, these ultimately activate a set of enzymes known as **protein kinases**. The protein kinases play an important part in controlling enzyme activity within the cell by phosphorylating amino acids such as **serine, threonine**, or **tyrosine** in target enzymes—a covalent modification. For example, the hormone **adrenaline** is an external messenger which triggers a signalling sequence resulting in the activation of a protein kinase enzyme. Once activated, the protein kinase phosphorylates an inactive enzyme called **phosphorylase *b*** (Fig. 3.15). This enzyme now becomes active and is called **phosphorylase *a***. It catalyses the breakdown of glycogen and remains active until it is dephosphorylated back to phosphorylase ***b***.

In this case, phosphorylation of the target enzyme leads to activation. Other enzymes may be deactivated by phosphorylation. For example, **glycogen**

BOX 3.1 The external control of enzymes by nitric oxide

The external control of enzymes is usually initiated by external chemical messengers which do not enter the cell. However, there is an exception to this. It has been discovered that cells can generate the gas **nitric oxide** by the reaction sequence shown in Fig. 1, catalysed by the enzyme **nitric oxide synthase**.

Because nitric oxide is a gas, it can diffuse easily through cell membranes into target cells. There, it activates enzymes called **cyclases** to generate **cyclic GMP** from **GTP** (Fig. 2). Cyclic GMP then acts as a secondary messenger to influence other reactions within the cell. By this process, nitric oxide has an influence on a diverse range of physiological processes, including blood pressure, **neurotransmission**, and immunological defence mechanisms.

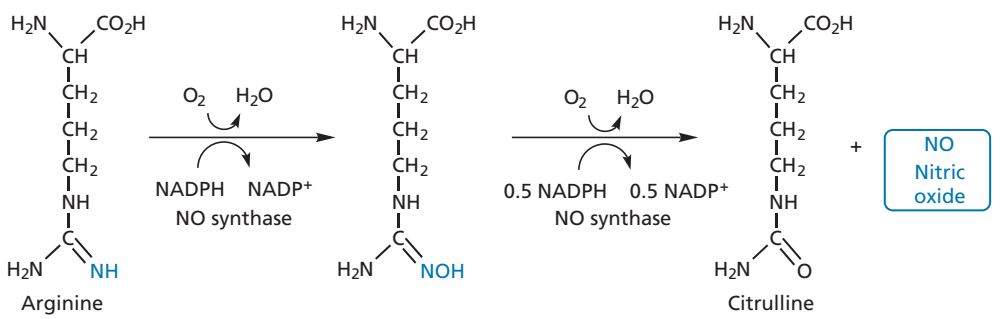


FIGURE 1 Synthesis of nitric oxide.

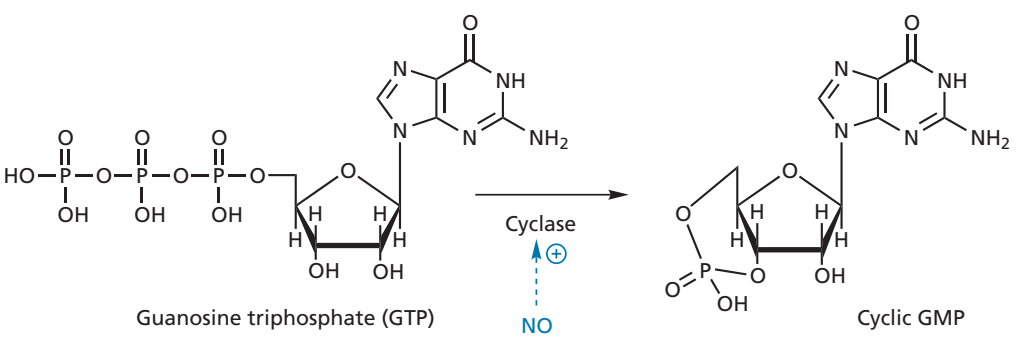


FIGURE 2 Activation of cyclase enzymes by nitric oxide (NO).

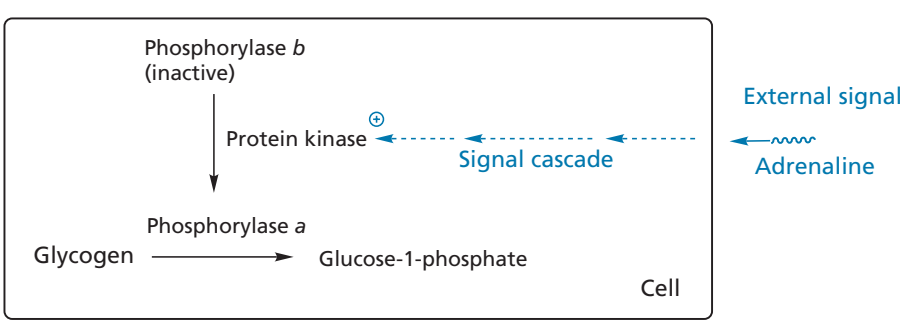


FIGURE 3.15 External control of phosphorylase *a*.

synthase—the enzyme that catalyses the *synthesis* of glycogen from glucose-1-phosphate—is inactivated by phosphorylation and activated by dephosphorylation. The latter is effected by the hormone **insulin**, which triggers a different signalling cascade from that of adrenaline.

Protein–protein interactions can also play a role in the regulation of enzyme activity. For example, signal proteins in the cell membrane are responsible for regulating the activity of membrane-bound enzymes (section 5.2).

3.7 Isozymes

Enzymes having a quaternary structure are made up of a number of polypeptide subunits. The combination of these subunits can differ in different tissues. Such variations are called **isozymes**. For example, there are five different isozymes of mammalian **lactate dehydrogenase** (LDH)—a tetrameric enzyme made up of four polypeptide subunits. There are two different types of subunits involved, which are labelled 'H' and 'M'. The former predominates in the LDH present in heart muscle, while the latter predominates in the LDH present in skeletal muscle. As there are two different types of subunit, five different isozymes are possible: HHHH, HHHM, HHMM, HMMM, and MMMM. Isozymes differ in their properties. For example, the M_4 isozyme in skeletal muscle catalyses the conversion of pyruvic acid to lactic acid and is twice as active as the H_4 isozyme in heart muscle. The H_4 isozyme catalyses the reverse reaction and is inhibited by excess pyruvic acid, whereas the M_4 isozyme is not.

KEY POINTS

- Enzymes are proteins that act as the body's catalysts by binding substrates and participating in the reaction mechanism.
- The active site of an enzyme is usually a hollow or cleft in the protein. There are important amino acids present in the active site that either bind substrates or participate in the reaction mechanism.
- Binding of substrate to an active site involves intermolecular bonds.
- Substrate binding involves an induced fit where the shape of the active site alters to maximize binding interactions. The binding process also orientates the substrate correctly and may weaken crucial bonds in the substrate to facilitate the reaction mechanism.
- The amino acid histidine is often present in active sites and acts as an acid/base catalyst. Glutamic acid, aspartic acid, and tyrosine also act as acid/base catalysts in some enzymes.
- The amino acids serine and cysteine act as nucleophiles in the reaction mechanisms of some enzymes. In some enzymes, lysine can act as a nucleophile.
- Cofactors are metal ions or small organic molecules (coenzymes) which are required by many enzymes. Coenzymes can be viewed as the body's chemical reagents.
- Prosthetic groups are coenzymes which are bound covalently to an enzyme.
- Enzymes are regulated by internal and/or external control.
- External control involves regulation initiated by a chemical messenger from outside the cell and which ultimately involves the phosphorylation of enzymes.
- Allosteric inhibitors bind to a different binding site from the active site and alter the shape of the enzyme such that the active site is no longer recognizable. Allosteric inhibitors are often involved in the feedback control of biosynthetic pathways.
- Isozymes are variations of the same enzyme. They catalyse the same reaction but differ in their primary structure, substrate specificity, and tissue distribution.
- The amino acid sequence in enzymes may differ between individuals as a result of genetic polymorphism. This may or may not result in a difference in enzyme activity.

3.8 Enzyme kinetics

3.8.1 The Michaelis-Menton equation

The Michaelis-Menten equation holds for an enzyme (E) which combines with its substrate (S) to form an enzyme–substrate complex (ES). The enzyme–substrate complex can then either dissociate back to E and S, or go on to form a product (P). It is assumed that formation of the product is irreversible.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

where k_1, k_2 *and* k_3 are rate constants.

For enzymes such as these, plotting the rate of enzyme reaction versus substrate concentration [S] gives a curve as shown in Fig. 3.16. At low substrate concentrations the rate of reaction increases almost proportionally to the substrate concentration, whereas at high substrate concentration the rate becomes almost constant and approaches a maximum rate ($rate_{max}$), which is independent of substrate concentration. This reflects a situation where there is more substrate present than active sites available; therefore, increasing the amount of substrate will have little effect.

The Michaelis-Menten equation relates the rate of reaction to the substrate concentration for the curve in Fig. 3.16.

$$\text{rate} = \text{rate}_{max} \frac{[S]}{[S] + K_M}$$

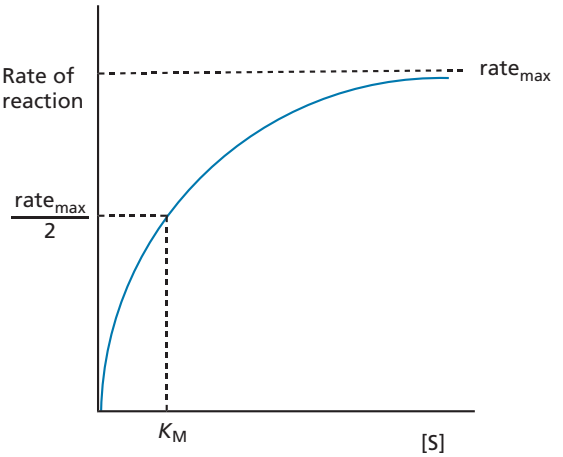


FIGURE 3.16 Reaction rate versus substrate concentration.

The derivation of this equation is not covered here, but can be found in most biochemistry textbooks. The constant K_M is known as the **Michaelis constant** and is equal to the substrate concentration at which the reaction rate is half of its maximum value. This can be demonstrated as follows. If Km = [S], then the Michaelis-Menten equation becomes:

$$\text{rate} = \text{rate}_{max}\frac{[S]}{[S]+[S]} = \text{rate}_{max}\frac{[S]}{2[S]} = \text{rate}_{max} \times \frac{1}{2}$$

The K_M of an enzyme is significant because it measures the concentration of substrate at which half the active sites in the enzyme are filled. This, in turn, provides a measure of the substrate concentration required for significant catalysis to occur.

K_M is also related to the rate constants of the enzyme-catalysed reaction:

$$K_M = \frac{k_2 + k_3}{k_1}$$

Consider now the situation where there is rapid equilibration between S and ES, and a slower conversion to product P. This means that the substrate binds to the active site and departs several times before it is finally converted to product.

$$E + S \underset{k_2 \text{ fast}}{\overset{k_1 \text{ fast}}{\rightleftharpoons}} ES \xrightarrow[\text{slow}]{k_3} E + P$$

Under these conditions, the dissociation rate (k_2) of ES is much greater than the rate of formation of product (k_3). k_3 now becomes insignificant relative to k_2 and the equation simplifies to:

$$K_M = \frac{k_2 + k_3}{k_1} = \frac{k_2}{k_1}$$

In this situation, K_M effectively equals the dissociation constant of ES and can be taken as a measure of how strongly the substrate binds to the enzyme.

$$[ES] \rightleftharpoons [E] + [S] \qquad \text{dissociation constant} = \frac{[E][S]}{[ES]}$$

A high value of K_M indicates weak binding because the equilibrium is pushed to the right; a low K_M indicates strong binding because the equilibrium is to the left. K_M is also dependent on the particular substrate involved and on environmental conditions, such as pH, temperature, and ionic strength.

The maximum rate is related to the total concentration of enzyme ($[E]_{total} = [E] + [ES]$) as follows:

$$\text{rate}_{max} = k_3[E]_{total}$$

A knowledge of the maximum rate and the enzyme concentration allows the determination of k_3. For example, the enzyme **carbonic anhydrase** catalyses the formation of hydrogen carbonate and does so at a maximum rate of 0.6 moles of hydrogen carbonate molecules formed per second for a solution containing 10^{-6} moles of the enzyme. Altering the above equation, k_3 can be determined as follows:

$$k_3 = \frac{\text{rate}_{max}}{[E]_{total}} = \frac{0.6}{10^{-6}}\frac{\text{M s}^{-1}}{\text{M}} = 600\,000\,\text{s}^{-1}$$

Therefore, each enzyme is catalysing the formation of 600,000 hydrogen carbonate molecules per second. The turnover number is the time taken for each catalysed reaction to take place, i.e. 1/600 000 = 1.7 µs.

3.8.2 Lineweaver-Burk plots

A problem related to Michaelis-Menton kinetics is the fact that there may not be sufficient data points to

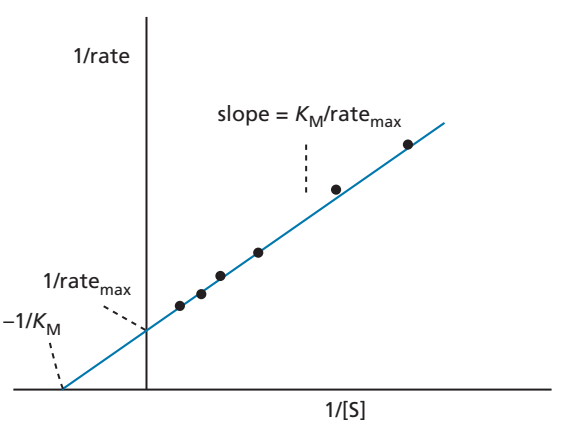


FIGURE 3.17 Lineweaver-Burk plot.

determine whether the curve of the Michaelis-Menton plot has reached a maximum value or not. This means that values for the maximum rate and K_M are likely to be inaccurate. More accurate values for these properties can be obtained by plotting the reciprocals of the rate and the substrate concentration to give a **Lineweaver-Burk plot** (Fig. 3.17):

$$\frac{1}{\text{rate}} = \frac{K_M}{\text{rate}_{max}} \cdot \frac{1}{[S]} + \frac{1}{\text{rate}_{max}} \qquad (y = m.x + c)$$

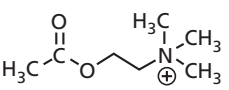
The maximum rate can then be obtained from the intersect of the line with the y-axis, while K_M can be obtained from the slope of the line or the intersect with the x-axis.

KEY POINTS

- The Michaelis-Menten equation relates the rate of an enzyme-catalysed reaction to substrate concentration.
- The Michaelis constant is equal to the substrate concentration at which the rate of the enzyme catalysed reaction is half of its maximum value.
- A Lineweaver-Burk plot provides more accurate values for the maximum rate and K_M.

QUESTIONS

1. Enzymes can be used in organic synthesis. For example, the reduction of an aldehyde is carried out using aldehyde dehydrogenase. Unfortunately, this reaction requires the use of the cofactor NADH, which is expensive and is used up in the reaction. If ethanol is added to the reaction, only catalytic amounts of cofactor are required. Why?
2. Acetylcholine is the substrate for the enzyme acetylcholinesterase. Suggest what sort of binding interactions could be involved in holding acetylcholine to the active site.



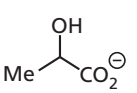
Acetylcholine

3. The ester bond of acetylcholine is hydrolysed by acetylcholinesterase. Suggest a mechanism by which the enzyme catalyses this reaction.
4. Suggest how binding interactions might make acetylcholine more susceptible to hydrolysis.
5. 17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) is an enzyme that catalyses the conversion of estrone to estradiol in the presence of the cofactor NADH. The initial rate data for the enzyme-catalysed reaction in the absence of an inhibitor is as follows:

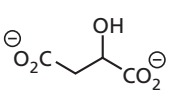
   Substrate concentration (10^{-2} mol dm^{-3}) 5 10 25 50 100

   Initial rate (10^{-1} mol dm^{-3} s^{-1}) 28.6 51.5 111 141 145

   Create a Michaelis Menton plot and a Lineweaver-Burk plot. Use both plots to calculate the values of K_M and the maximum rate of reaction. Identify which plot is likely to give the more accurate results and explain why this is the case.
6. Lactate dehydrogenase has a 1000-fold selectivity for lactate as a substrate over malate. However, if a mutation occurs that alters an active site glutamine residue to an arginine residue, the enzyme shows a 10,000-fold selectivity for malate over lactate. Explain this astonishing transformation.



Lactate



Malate

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