This chapter is designed to enable students to:

- recognise that enzymes play an essential role as biological catalysts
- develop awareness of the distinguishing characteristics of enzymes
- distinguish between monomeric and oligomeric enzymes
- understand the mode of operation of enzymes
- recognise the factors that affect enzyme activity
- gain knowledge and understanding of the concept of enzyme inhibition, both reversible and irreversible
- become familiar with the role of cofactors, coenzymes and prosthetic groups in enzyme activity.

**Figure 3.1** The search for enzymes begins within days of birth. A blood sample from this baby is tested for the presence of several key enzymes. If the test reveals a missing enzyme, treatment is begun. Treatment can prevent the destructive mental retardation of phenylketonuria (PKU) that can result when the enzyme phenylalanine hydroxylase is missing. Treatment can prevent the serious consequences of galactosemia when the enzyme galactose-1-phosphate uridylyltransferase is missing. In this chapter, we will explore enzymes, their structure and their function in life-sustaining reactions.
The plague of the sea

As the long ocean voyage continued with no landfalls, the majority of the ship’s crew became increasingly ill and exhibited a range of unpleasant symptoms — their gums became swollen and purple in colour, teeth became loose in their sockets, skin discoloured and spontaneous haemorrhages occurred. The suffering of the sailors was compounded because even minor wounds failed to heal. Then followed swelling, ulceration and, eventually, death. This illness described as ‘the plague of the sea’ came into prominence in the fifteenth century when explorers first ventured on extended ocean voyages or on expeditions to remote terrestrial regions in high latitudes. We now know this disease as scurvy.

Read a description of scurvy written by Jacques Cartier, an expedition leader who sailed from France in 1535 to present-day Canada and travelled up the Saint Lawrence River: ‘Some could not stand on their feet. Others also had their skins spotted with spots of blood of a purple colour then it did ascend up their ankles, knees, thighs, shoulders, arms and necks. Their mouths became stinking, their gums so rotten, that all their flesh did fall off, even to the roots of their teeth which did also almost all fall out.’

What is scurvy?

Scurvy is a deficiency disease, resulting from a lack of vitamin C (ascorbic acid).

However, most animals can produce their own supplies of vitamin C by a multistep biochemical pathway that converts glucose to ascorbic acid. In contrast, human beings and some other primates, guinea pigs and a few other animals cannot synthesise their own supplies of vitamin C. Instead, these animals must acquire vitamin C through their dietary intake. So, while the sailors on diets of dry beef and biscuits on long voyages during the fifteenth to the eighteenth centuries were at extreme risk of death by scurvy, the rats on these ships were scurvy-free because they could synthesise their own supplies of vitamin C.

Figure 3.2 shows the multistep pathway from glucose to ascorbic acid that occurs in the liver of almost all mammals (and in the kidneys of birds).

**Figure 3.2.** Multistep biochemical pathway from glucose to vitamin C (ascorbic acid). Each step in this pathway is catalysed by a specific enzyme. Almost all mammals can synthesise vitamin C by this pathway, except for human beings and some other primates, guinea pigs, and bats of the genus *Pteropus.*
Those few mammals that cannot synthesise their own vitamin C are missing the terminal enzyme in the biosynthetic pathway of glucose to ascorbic acid. The gene encoding this enzyme, *gulono-lactone oxidase*, has undergone substantial mutation so that it no longer produces a protein that can function as an enzyme. This missing enzyme is the reason that sailors on long sea voyages during the fifteenth to the eighteenth century suffered from and were at risk of death from scurvy.

James Lind (1716–1794), a Scottish physician, was the first to recognise that scurvy could be prevented by the juice of citrus fruits. In his *Treatise of the Scurvy*, published in 1753, Lind recommended that citrus juice be distributed to sailors to prevent scurvy. In 1795, when the Royal Navy finally adopted the practice of issuing lime juice to sailors, scurvy disappeared from the crews of all their ships.

Many fresh fruits and vegetables are rich sources of vitamin C, as, for example, cantaloupe, cherries, kiwi fruit, and cabbage, cauliflower, bean sprouts, red and green peppers, and potatoes (see figure 3.3).

The missing enzyme in scurvy

Those few mammals, including humans, that cannot synthesise their own vitamin C are unable to produce an enzyme called *gulono-lactone oxidase* (*GULO*). This enzyme is needed to catalyse the last step in the pathway that manufactures ascorbic acid from glucose. In humans, the *GULO* gene, located on the number-8 chromosome, is defective.

When the defective *GULO* gene is compared to that of the rat, several major differences are seen: the functional *GULO* gene of the rat consists of a long sequence of DNA that includes 12 exons. In contrast, the homologous *GULO* gene in humans is missing most of these exons; it has only five. Not surprisingly, the major deletions of DNA coding sequences mean that the protein product translated from this gene is non-functional as a GULO enzyme. Figure 3.4 shows a diagrammatic representation of the rat *GULO* gene and the corresponding gene in humans. In addition, compared to the rat *GULO* gene, the DNA of the remaining five exons in the human gene shows other mutations including two single nucleotide deletions, one triple nucleotide deletion, and one single nucleotide insertion.

![Figure 3.4 Diagram showing a comparison of (a) the GULO gene that encodes the active GULO enzyme in rats, and (b) the defective GULO gene of humans, which encodes a non-functional protein. The loss of exons in humans is also seen in the genome of most primates.](image-url)
Scurvy is just one example of the critical role of enzymes in maintaining the life of an organism. Every living organism has a myriad of enzymes that operate continuously to ensure that the cellular processes essential for life occur at rates sufficiently fast to maintain the living state.

At every moment, enzymes are jump-starting essential chemical reactions in cells and organisms, as, for example, the chemical reactions that:

- enable some microbes to convert nitrogen in the air to essential amino acids
- enable plants to convert the energy of sunlight to sugars
- supply the energy needs of all cells, through cellular respiration
- break down food of animals and fungi to forms that can be absorbed and utilised through digestion
- synthesise macromolecules that are essential to the structure and function of mammals, including:
  - proteins, such as contractile proteins that enable movement, antibody proteins that provide immunity against infectious diseases, signalling proteins that enable communication between cells, proteins that transport oxygen, and proteins that form the structure of bones, cartilage and connective tissue
  - nucleic acids that determine aspects of heredity.

Table 3.1 gives some examples of enzymes essential to these life-maintaining processes.

**TABLE 3.1** Examples of some enzymes involved in various biological processes. Processes involve multistep biochemical pathways, with each step catalysed by a specific enzyme.

<table>
<thead>
<tr>
<th>Process</th>
<th>Example of enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>cellular respiration</strong></td>
<td></td>
</tr>
<tr>
<td>glycolysis</td>
<td>hexokinase, phosphofructokinase</td>
</tr>
<tr>
<td>Krebs cycle</td>
<td>succinic dehydrogenase</td>
</tr>
<tr>
<td>electron transport chain</td>
<td>cytochrome c oxidase</td>
</tr>
<tr>
<td><strong>nucleic acid functions</strong></td>
<td></td>
</tr>
<tr>
<td>DNA replication</td>
<td>DNA polymerase</td>
</tr>
<tr>
<td>transcription of mRNA from DNA</td>
<td>RNA polymerase</td>
</tr>
<tr>
<td>mRNA translation</td>
<td>amino acyl tRNA synthetase</td>
</tr>
<tr>
<td>joining DNA segments</td>
<td>ligase</td>
</tr>
<tr>
<td>cutting DNA</td>
<td>endonucleases</td>
</tr>
<tr>
<td><strong>digestion</strong></td>
<td></td>
</tr>
<tr>
<td>breakdown of starch</td>
<td>amylase</td>
</tr>
<tr>
<td>breakdown of proteins</td>
<td>trypsin, pepsin</td>
</tr>
<tr>
<td>breakdown of lipids</td>
<td>lipase</td>
</tr>
<tr>
<td>breakdown of galactose</td>
<td>galactose-1-phosphate uridylyltransferase</td>
</tr>
<tr>
<td>breakdown of lactose</td>
<td>lactase</td>
</tr>
<tr>
<td><strong>metabolic pathways</strong></td>
<td></td>
</tr>
<tr>
<td>formation of lactose</td>
<td>lactose synthase</td>
</tr>
<tr>
<td>formation of uric acid (as in gout)</td>
<td>xanthine oxidase</td>
</tr>
<tr>
<td>formation of melanin (pigment)</td>
<td>tyrosinase</td>
</tr>
<tr>
<td>formation of collagen</td>
<td>lysyl oxidase</td>
</tr>
<tr>
<td>formation of ascorbic acid (vit C)</td>
<td>gulono-lactone oxidase</td>
</tr>
</tbody>
</table>
What is an enzyme?

The key features of enzymes may be summarised as follows:

- **Enzymes are biological catalysts:** Catalysts are substances that speed up the rate of chemical reactions without themselves being used up in the reactions. The basic function of enzymes is to act as catalysts by increasing the rate of almost all the chemical reactions in living organisms, and to do this within the prevailing conditions of temperature and pH within cells. Later in this chapter (see page 88), we will explore how enzymes manage to speed up the rates of chemical reactions in cells.

  Why is there a need to speed up chemical reactions in living organisms? You cut your hand on a piece of jagged metal and start bleeding. Enzymes are immediately activated, causing the blood in the damaged region to clot, plugging the wound and stopping the bleeding. Without the action of these enzymes, bleeding could continue, creating a life-threatening situation. The steak that you recently ate was broken down in the acidic conditions of your stomach at body temperature over a period of several hours. This breakdown was the result of the catalytic action of digestive enzymes such as pepsin and trypsin acting on the protein of the steak. To do the same without enzymes also requires acidic conditions, but would require a few days (not hours) and a temperature of 100 °C (not body temperature). These two examples give a clue to the importance of enzymes in maintaining life. Without enzymes, the speed of biochemical reactions would be far too slow to sustain the living state.

- **Enzymes are proteins:** Almost without exception, enzymes are soluble globular proteins (in contrast to insoluble fibrous protein, such as collagen and silk). Globular proteins are spherical or globe-like in overall shape, and are typically water-soluble. Water solubility results from the fact that, in a globular protein, the amino acids with polar side chains are exposed at the surface of the protein where they can interact with nearby polar water molecules and dissolve. In contrast, amino acids with nonpolar side chains tend to point inwards. (Refer to the Appendix to check out the different side chains of amino acids.)

- **Enzymes consist of one or more polypeptide chains:** A minority of enzymes are termed monomeric enzymes, with each enzyme consisting of a single polypeptide chain formed of amino acids joined by peptide bonds. The typical number of amino acids in the polypeptide chain is in the range of 100 to 300. The folding and twisting of the polypeptide chain of such an enzyme produces its tertiary structure, which is stabilised by the formation of other bonds, including disulfide bonds, ionic bonds and hydrogen bonds.

  Examples of monomeric enzymes include lysozyme (in saliva), trypsin (in the gastric juices of the stomach), papain (in pineapple) and ribonuclease. Figure 3.5 shows a ribbon model of the lysozyme enzyme with 12 different colours denoting the various amino acids, either singly (grey = gly) or a pair (red = asp and glu, and blue = lys and arg) or, in one case, a trio (green = leu, val and ile).

  The majority of enzymes are oligomers, with each enzyme consisting of two or more polypeptide chains that, in this context, are called sub-units. In some oligomeric enzymes, the sub-units or polypeptide chains are identical, while, in others, the sub-units differ. Examples of oligomeric enzymes include lactose synthase (in mammary glands) with two different sub-units, and phosphofructokinase with four identical sub-units (see figure 3.6).

  Because they consist of two or more sub-units, oligomeric enzymes have a quaternary structure that is stabilised by covalent bonds. Oligomeric enzymes with their multiple sub-units have properties that are not present...
in monomeric enzymes, as, for example, the presence of multiple binding sites.

The functional enzyme lactate dehydrogenase (LDH) consists of four sub-units, each of which may be either an H or an M sub-unit. LDH present in heart muscle consists of four H sub-units, while the LDH in skeletal muscle consists of four M sub-units. Other tissues have other forms of LDH, including H₃M, H₂M₂, and HM₃. These five forms of LDH are termed isozymes, and each has slightly different properties. Note that just two sub-units can produce five different isozymes.

- **Every enzyme has an active site**: Typically, a small pocket or cleft in an enzyme is an **active site**, also known as the **substrate binding site**, which is essential for the enzyme to function. The **substrate** of an enzyme is the molecule that the enzyme acts on or the input to an enzyme-catalysed reaction. The output of an enzyme-catalysed reaction is the **product** or products. The active site of the enzyme that comes into contact with the substrate is small in comparison to the rest of the enzyme.

The active site of each enzyme is formed by the folding of the polypeptide chain(s) to form a small region with a unique stereospecific 3D shape. The active site can recognise and bind to its specific substrate because of the complementary shapes of the active site and the substrate.

Figure 3.7a shows a diagrammatic representation of a substrate interacting with the active site of an enzyme. Typically, an active site consists of three to twelve amino acids organised to form a particular shape. If the shape of the active site of an enzyme is altered by any chemical or physical treatment, the ability of the enzyme to function is reduced or permanently or temporarily lost. Figure 3.7b shows the active site of the enzyme aspartate transaminase, with six amino acids involved in the active site. The cytoplasmic aspartate transaminase enzyme is an oligomer with the functional enzyme consisting of two identical sub-units, each containing 412 amino acids.

In fact, the active site of an enzyme is a 3D space. Figure 3.8a shows a space-filling model of the enzyme hexokinase, which is involved in the glycolysis pathway. The active site of hexokinase is at the bottom of the cleft that can be
seen on the left-hand side of the model. Figure 3.8b shows a stylised diagram of an active site of an enzyme with its substrate identifying the weak bonds that hold the substrate and the active site together. These weak bonds are hydrogen bonds (H—H), and ionic bonds between groups on the substrate and groups on the active site that carry opposite charges. These weak bonds are sufficient to stabilise the enzyme-substrate (E-S) complex, but they can easily be broken, allowing the products of the reaction to be released, and freeing the active site to be occupied by another substrate molecule. (Later in this chapter we will see that, if a molecule forms a strong covalent bond with a group that is part of the active site of an enzyme, the enzyme will be inactivated.)

**Figure 3.8** (a) Space-filling model of a hexokinase enzyme with its active site at the base of the cleft shown at the left-hand side of the model. Different colours denote the various amino acids that form the single polypeptide chain of this enzyme. (Source: Feng, J., Zhao, S., and Liu, L., RCSB Protein Data Bank) (b) Schematic diagram showing the weak bonds, such as hydrogen bonds and ionic bonds, that temporarily stabilise the enzyme-substrate (E-S) complex at the active site of an enzyme.

- **Each enzyme has a specific activity:** The specificity of enzymes is demonstrated in various ways, including:
  - absolute or substrate specificity, meaning that an enzyme can act on one substrate only; for example, the enzyme lactase can only hydrolyse the milk sugar lactose to form glucose and galactose.
  - bond specificity, meaning that an enzyme can act on one kind of chemical bond; for example, peptidase enzymes act specifically on the peptide bonds between any two amino acids, and the enzyme amylase acts specifically on the 1,4 glycosidic bonds that link the glucose monomers of starch and glycogen.
  - group specificity, meaning that an enzyme can only act on molecules with particular functional group(s) surrounding a bond; for example, the digestive enzyme trypsin can only act on peptide bonds that are adjacent to amino acids with basic side chains, namely lysine and arginine.

**A time and place for enzyme action**

Where and when does enzyme action occur? Most enzymes act on substrates in solution within cells of particular human tissues. So, most enzymes are intracellular.

However, some enzymes take action in extracellular locations, such as lysozyme in tears, which are part of the body’s first line of defence against microbial infection (see chapter 7, page 287), the digestive enzymes such as amylase, which are produced in salivary glands, and the clotting enzyme thrombin. The digestive enzymes of the stomach, the pancreas and the small intestine are also examples of extracellular enzymes.
Many enzymes exist in cells as inactive **proenzymes** (also termedzymogens), which are only activated in response to relevant signals. This strategy prevents enzyme activity occurring at inappropriate times or places and causing damage. For example:

- The proteolytic enzyme pepsin is secreted by stomach cells as an inactive proenzyme, called pepsinogen. Once in the extracellular space of the stomach cavity, the pepsinogen molecules are activated by the acidic conditions (pH 2 to 3) in the stomach to form pepsin. This activation involves the removal of a peptide (44 amino acids long) from pepsinogen that masks the active site. Once this is removed, active pepsin is produced (see figure 3.9).

![Diagram showing the activation of the inactive proenzyme pepsinogen to the active proteolytic enzyme pepsin.](image)

**FIGURE 3.9** Diagram showing the activation of the inactive proenzyme pepsinogen to the active proteolytic enzyme pepsin, which is initiated by the low pH conditions of the stomach. These low pH conditions cause the removal of a masking sequence of a 44 amino-acid peptide from the active site, transforming pepsinogen into pepsin.

- The clotting enzyme thrombin is normally present as an inactive proenzyme called prothrombin. Prothrombin is converted to active thrombin only when blood vessels are damaged, bleeding occurs, and there is a need for clot formation. In the bloodstream is a soluble monomeric protein called fibrinogen. Thrombin catalyses the conversion of the soluble blood protein fibrinogen to the insoluble polymeric protein fibrin, which aggregates and forms a clot, stopping the loss of blood (see figure 3.10).

![An extract of part of the clotting cascade that shows the conversion of inactive prothrombin to active thrombin.](image)

**FIGURE 3.10** An extract of part of the clotting cascade that shows the conversion of inactive prothrombin to active thrombin, which, in turn, catalyses the conversion of soluble fibrinogen to insoluble fibrin aggregates, forming a clot and trapping platelets and blood cells.
**NAMING ENZYMES**

Usually an enzyme has two names, as follows:

1. a recommended or **trivial name** that is short, often ending in -ase; in many cases, the substrate of the enzyme is included in this name: for example, tyrosinase, lactase, sucrase, creatine kinase. (Recommended names are the enzyme names that are used throughout this chapter.) What reaction do you think the enzyme succinic dehydrogenase carries out?

2. a **systematic name** that is long and identifies the exact reaction that is catalysed by the enzyme; for example:
   - amylase, present in human saliva, has the systematic name 4-alpha-D-glucan glucanohydrolase
   - glucose oxidase has the systematic name β-D-glucose:oxygen 1-oxidoreductase
   - aspartate aminotransferase has the systematic name L-aspartate:2-oxoglutarate aminotransferase
   - tyrosinase has the systematic name L-tyrosine, L-dopa:oxygen oxidoreductase.

In addition, each enzyme has an EC (Enzyme Commission) number. The EC numbers group enzymes into one of six groups identifying the types of enzyme-catalysed reaction that are carried out. Table 3.2 shows these six groups. Each enzyme has an EC identifier that consists of four numbers, such as:

Tyrosinase is identified as EC 1.14.18.1; glucose isomerase is identified as EC 5.3.1.5; aspartate aminotransferase is identified as EC 2.6.1.1.

The first number identifies the major reaction type that the enzyme can catalyse. So, any enzyme with the first EC number of 2 must be a transferase enzyme, which catalyses a particular transferase reaction.

### TABLE 3.2
The Enzyme Commission (EC) groups. Every enzyme belongs to one of these six groups, which identify the types of chemical reactions that are catalysed by enzymes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Reaction type catalysed</th>
<th>Example of enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC 1 Oxidoreductases</td>
<td>oxidise/reduce molecules by transfer of O and H atoms</td>
<td>succinic dehydrogenase</td>
</tr>
<tr>
<td>EC 2 Transferases</td>
<td>transfer a group from substrate to product</td>
<td>aspartate transaminase</td>
</tr>
<tr>
<td>EC 3 Hydrolases</td>
<td>cleave chemical bonds by hydrolysis</td>
<td>amylase</td>
</tr>
<tr>
<td>EC 4 Lyases</td>
<td>cleave bonds in a substrate (not by hydrolysis)</td>
<td>aldolase</td>
</tr>
<tr>
<td>EC 5 Isomerases</td>
<td>change arrangement of atoms in a molecule</td>
<td>glucose isomerase</td>
</tr>
<tr>
<td>EC 6 Ligases</td>
<td>join two molecules by forming a new bond</td>
<td>glutamate-cysteine ligase</td>
</tr>
</tbody>
</table>

**KEY IDEAS**

- Enzymes are biological catalysts composed of protein.
- Catalysts speed up the rate of chemical reactions.
- Some enzymes are monomeric and consist of a single polypeptide chain, while most are oligomers.
- The active site of an enzyme has a 3D shape that is complementary to the shape of its specific substrate.

**ODD FACT**

The enzyme known as pepsin (EC 3.4.23.2) was given its name in 1836 by Theodor Schwann (1810–1882), who, along with Matthias Schleiden (1804–1881), formulated the Cell Theory in 1839.
QUICK CHECK

1. Briefly explain the meaning of the following statement: Enzymes are biological catalysts.
2. Identify a key difference between the members of the following pairs:
   a. monomeric enzyme and oligomeric enzyme
   b. proenzyme and enzyme
   c. substrate specificity and bond specificity
   d. hydrolase and transferase.
3. Identify the following statements as true or false:
   a. The typical shape of an enzyme is globular.
   b. The enzyme lysozyme consists of a single polypeptide chain.
   c. Most of the structure of an enzyme forms part of its active site.
   d. Most enzymes are oligomers.
   e. Enzymes speed up the rate of chemical reactions in the body.
   f. Each enzyme can catalyse just one specific reaction involving a single substrate.
   g. The E-S complex is stabilised by strong covalent bonds.

Explaining enzyme specificity

Let’s now look at why enzymes have a high level of specificity for one substrate, or for a functional group or side chain, or for a bond between particular groups. To do this, let’s use models. What is a model? Models are used to represent biological entities, such as biological processes, in a way that makes them conceptually easier to understand or enables predictions to be made about their operation. A model may be an analogy, such as the lock-and-key model of enzyme-substrate interactions described below. A model may be a mathematical representation, such as the Hardy-Weinberg equation to represent a population gene pool.

Lock and key or induced fit?

The essential prelude to the catalytic action of an enzyme is the binding of the substrate(s) to the active site of the enzyme. Two models have been put forward in an attempt to explain this specificity:
1. the lock-and-key model
2. the induced fit model.

The earliest explanation for the specificity of enzymes for particular substrates was proposed by Emil Fischer (1852–1919) in 1894. Fischer explained the specificity of enzymes using the analogy of a lock and key. In this lock-and-key model, the enzyme acts as the lock and the substrate as the key. Only the correctly shaped key (substrate) can fit the lock (the active site of the enzyme). This model correctly identified the importance of the complementary shapes of the active site of an enzyme and its specific substrate and recognised that enzyme specificity is determined by the active site of the enzyme. The lock-and-key model assumes that the active site of an enzyme (the lock) is rigid and fixed.

A second model was premised on the active site of an enzyme having a defined shape, but one that has a degree of flexibility. This is the induced fit model, which was proposed by Daniel Koshland in 1958. Koshland proposed that, just as a glove changes its shape when a hand slips into it, so does the active site change its shape to fit tightly around its substrate. In this model, the binding site of an enzyme is not viewed as being exactly complementary to the substrate. When the reaction is complete, the empty active site returns to its relaxed resting state.

Figure 3.11 shows the difference between these two models.
Mode of action of enzymes

At any minute, hundreds of chemical reactions are taking place in living organisms, and this multitude of linked chemical reactions comprises the metabolism of an organism. Most of these chemical reactions occur inside cells, but a few are extracellular in location. Almost without exception, every step in a metabolic pathway is catalysed by a specific enzyme. In a person, enzyme-managed reactions are involved in growth, repair, cellular respiration, reproduction, digestion, breathing, clotting of blood, and immunity, with several thousand different enzymes involved.

In metabolism, some compounds are broken down with an associated release of energy, while other compounds that are needed by cells are built up or synthesised in a process that requires an input of energy. These two aspects of metabolism are recognised in the following classification:

- **catabolism** = series of cellular reactions that releases useful energy from the breakdown of complex molecules
- **anabolism** = series of cellular reactions in which the complex molecules required by cells are synthesised from simpler building blocks, a process that requires an input of energy.

Metabolic reactions that release energy are said to be **exergonic**, while reactions that require energy are termed **endergonic**. Figure 3.12 shows how the energy released in catabolic reactions drives the energy-requiring anabolic reactions. This illustrates the principle of **coupling** of exergonic and endergonic reactions.
Metabolic reactions are neither random nor unrelated. Instead, each reaction is part of a coordinated sequence of enzyme-driven reactions or pathways. Several mechanisms operate to ensure that the chemical reactions operate in orderly and balanced sequences. For example, end-product inhibition (see pages 94 and 95) prevents the build-up of an excess product of a reaction and ensures that product(s) of reactions are formed only as and if required. (In bacteria, another way of achieving this is through operons and their regulation — refer to chapter 2.) Interference between biochemical pathways is minimised by having particular pathways occurring within different membrane-bound compartments in a cell. We have already seen how the production of inactive proenzymes, which must be activated to functional enzymes, can also control when and where enzymes are active.

Let’s look now at how enzyme-catalysed reactions take place.

**Mode of enzyme action**

Enzymes are biological catalysts that accelerate chemical reactions in cells. The molecules that are inputs to enzymic action are termed substrates, and the outputs of enzymic action are termed products. We can show a general enzyme-catalysed reaction as follows:

\[ E + S \rightleftharpoons ES \rightleftharpoons P + E \]

that is: **Enzyme + Substrate \rightleftharpoons Enzyme-Substrate complex \rightleftharpoons Product + Enzyme**
Figure 3.13 is a diagram illustrating the critical step in an enzyme-catalysed reaction, namely the formation of the enzyme-substrate complex at the active site of the enzyme.

![Diagram](image)

Look at the equation and diagram, and note that:
1. The reaction involves the formation of an enzyme-substrate complex (E-S). The formation of the E-S complex means that the enzyme specifies the substrate it will bind to, the reaction it will catalyse, and the product(s) that will be formed.
2. The enzyme is not consumed in the reaction. Once the product is formed, the enzyme is free to interact and bond with another substrate molecule. A consequence of this is that only a small amount of enzyme is needed to catalyse a reaction and produce large quantities of product.

For any chemical reaction to occur, the reactants must be in an activated state. The energy level that must be attained is termed the **activation energy**. If the reactant molecules do not have sufficient energy, no reaction can occur.

**Enzymes lower the energy level that is needed to activate reactant molecules.** Enzymes do this by binding to the reactant molecules and positioning or bending them in a way that enables the breaking of existing bonds and the formation of new ones to occur more readily. As a result, more reactant molecules have sufficient energy to react, and so the reaction proceeds much more rapidly than a non-catalysed reaction.

In reality, enzymes speed up chemical reactions in the body by a factor of between $10^6$ to $10^{12}$ times so that essential chemical reactions can occur at body temperature.

By providing an alternative reaction pathway with a lower activation energy requirement, enzymes produce reaction rates that are much faster than those for the non-catalysed pathway. Figure 3.14 shows a graphical representation of an enzyme-catalysed reaction and a non-catalysed reaction.

![Graph](image)
Factors affecting enzyme activity

Various factors that have an impact on enzyme activity include:
- temperature
- enzyme concentration
- pH
- substrate concentration.

Effect of temperature on reaction rate

For most chemical reactions, an increase in temperature causes an increase in the reaction rate. As the reactants are heated, the particles move faster and are more likely to collide with sufficient energy to overcome the activation energies of the reactants. However, beyond a certain point, that relationship no longer holds.

Each enzyme has a temperature at which it operates most effectively, and, for most human enzymes, that optimum temperature is about 37 °C or normal body temperature. On either side of that optimum is a range of temperatures at which enzymes will still operate, but at decreasing effectiveness.

As the temperature increases further, thermal denaturation of proteins begins to occur. Heat denaturation permanently changes the tertiary shape of enzyme molecules so that the shape of the active site is altered and the enzyme is inactivated. Fevers occur when the body temperature rises above normal. A high fever, defined as a core body temperature of 41.5 °C and higher, is extremely dangerous in part because of the harmful effect of this temperature on the thousands of enzymes in the human body.

If the temperature falls too low, the enzyme and substrate molecules do not have sufficient energy to interact. Enzymes that are inactivated because of a low temperature can become active again when the temperature rises. Because low temperatures do not permanently inactivate enzymes, enzymes are often stored in laboratories in the cold; for example, enzymes can be stored in buffered 50 per cent glycerol, which remains liquid at temperatures down to –35 °C.

Consider the following experiment: tubes containing the same amount of enzyme and the same volume of solutions of different concentrations of substrate (glucose) were incubated under identical conditions. The rate of breakdown of glucose was measured and plotted against the original concentration of the substrate. What was the maximum rate of the reaction?

Not all organisms have enzymes with optimal temperatures similar to that of the enzymes of the human body. Table 3.3 shows some temperature minima, maxima and optima for the growth of some microbes, both bacteria and archaeans. While these figures relate to growth and not to a particular enzyme, they are indicators of the temperature tolerance of the enzymes of these various microbes.

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Minimum</th>
<th>Optimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listeria monocytogenes</td>
<td>1</td>
<td>30–37</td>
<td>45</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>10</td>
<td>30–37</td>
<td>45</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>20</td>
<td>37</td>
<td>40</td>
</tr>
<tr>
<td>Thermus aquaticus</td>
<td>40</td>
<td>70–72</td>
<td>79</td>
</tr>
<tr>
<td>Sulfolobus acidocaldarius</td>
<td>70</td>
<td>75–85</td>
<td>90</td>
</tr>
<tr>
<td>Pyrobacterium brackii</td>
<td>80</td>
<td>102–105</td>
<td>115</td>
</tr>
</tbody>
</table>
Enzymes synthesised by bacteria and archaea with optimal growth temperatures greater than 80°C are termed hyperthermophilic enzymes. In contrast to the enzymes of most organisms, these enzymes are resistant to irreversible denaturation at high temperatures, and are said to be thermostable.

**Effect of pH on reaction rate**

The pH (hydrogen ion concentration) at which enzymes can operate varies, typically according to the environment in which the enzyme normally operates (see figure 3.16). Unlike the normal human core body, which is regulated within a narrow range, various compartments of the human body have very different prevailing pH values, such as the stomach with a pH of 2 to 3, and the small intestine with a pH around 6.

Enzymes can operate as catalysts within a range that is defined by a maximum pH and a minimum pH, with the highest activity (in terms of substrate molecules catalysed per unit time) occurring at the optimal pH for the enzyme concerned. What is the pH optimum for the enzyme alkaline phosphatase?

Usually, an enzyme works within quite a narrow pH range. At the optimal pH, the reaction rate is greatest. Changes in pH can alter intra- and intermolecular bonds, changing the shape of the enzyme and affecting its efficiency.

The pH of a solution is a measure of the concentration of hydrogen ions per litre of solution. The pH of a neutral solution, such as pure water, is 7.0. A pH value below 7 indicates an acid, the lower the number, the more acidic the solution. A pH value above 7 indicates a basic solution. Most biological fluids have a pH between 6 and 8; for example, human blood is maintained at about pH 7.4. There are a few extremes beyond this range, such as gastric juice in the stomach, which has a pH of about 2.

Each enzyme acts best at a particular pH, which is known as the optimal pH for the enzyme (see figure 3.16). Pepsin, which is secreted in the human stomach, has an optimal pH of 2. Trypsin, secreted in the human small intestine, has an optimal pH of about 8.0. Carbonic anhydrase, an enzyme found in human blood, has an optimal pH of 7.4. Enzymes that act within human cells generally have an optimal pH of about 7.6. A change in pH from the optimum can change the shape of an enzyme and affect its ability to combine with its substrate. Because the enzyme is less able to combine with its substrate, it is unable to act and the rate of the metabolic reaction declines. An enzyme becomes less efficient if the variable value is greater or less than the optimal.

**Effect of substrate concentration on reaction rate**

The addition of more substrate to an enzyme solution initially increases the rate of the reaction, provided that some active sites of the enzyme are not yet occupied. However, a set amount of enzyme molecules is present, and the rate of the reaction tapers off once all the active sites of the enzyme molecules become occupied.

So, for a given enzyme concentration, the rate of reaction increases with increasing substrate concentration. But only up to a point. Beyond this, any further increase in substrate concentration produces no significant change in reaction rate (see figure 3.17). At this point, all the active sites of the enzyme molecules at any given moment are occupied by substrate molecules. The E-S complexes (enzyme/substrate complexes) have to dissociate before the active sites are free to accommodate more substrate.

On such a graph, two values are typically noted:

\[ V_{\text{max}} = \text{maximum reaction rate or velocity} \]

\[ V_{\text{max}} \text{ is reached when all the active sites of the enzyme are saturated with substrate molecules.} \]

\[ V_{\text{max}} \text{ is directly proportional to enzyme concentration.} \]
**Effect of enzyme concentration on reaction rate**

Enzymes are not consumed in reactions and so are usually needed in small amounts only. For this reason, the question of the effect of increasing enzyme concentration on reaction rate is not a common concern. Provided that the substrate concentration is high and that temperature and pH are kept constant, the rate of reaction is proportional to the enzyme concentration, as shown in figure 3.18. In the most unusual situation of a very high concentration of enzyme, the substrate concentration may become rate-limiting and, if so, the reaction rate will stop increasing and will flatten out.

**Enzymes can be inhibited**

An enzyme inhibitor is a molecule that binds to an enzyme, reducing its activity by interfering with the enzyme in some way.

**Inhibition can be irreversible or reversible**

Irreversible inhibition of an enzyme occurs when a compound binds covalently to one or more amino acids and alters the structure of the enzyme, affecting its active site. This permanently inhibits the enzyme because the inhibitor-enzyme bond is so strong that the inhibition cannot be reversed by the addition of excess substrate. Not surprisingly, compounds that are irreversible inhibitors of enzymes are termed poisons. Examples of enzyme inhibitors that are irreversible include cyanide and nitrogen mustard gas.

- Cyanide is an irreversible inhibitor of cytochrome c oxidase, a key enzyme in the electron transport chain. Inhibition of this enzyme stops the production of ATP by aerobic respiration, resulting in death.
- Another irreversible inhibitor is the poison malonate, which binds covalently to the active site of the Krebs cycle enzyme, succinate dehydrogenase.
- DFP (diisopropylfluorophosphate), a nerve gas, is an irreversible enzyme inhibitor. DFP binds to a serine amino acid at the active site of the enzyme, acetylcholine esterase (ACE) (see figure 3.19). ACE plays an essential role at nerve synapses (see figure 5.22 on page 177) in deactivating neurotransmitters after a nerve impulse has passed. By inhibiting the action of ACE, DFP is a powerful neurotoxin, and small quantities of this poison cause continuous muscle contraction, constriction of the bronchial tubes of the lungs, seizures and death. Irreversible inhibitors have applications in medicine. The drug penicillin acts as an irreversible inhibitor of the enzyme transpeptidase, which is needed by bacteria to form their cell walls, and so prevents them from reproducing. The anti-inflammatory and analgesic properties of aspirin are due to the fact

![Graph showing reaction rate plotted against increasing substrate concentration.](image)

**FIGURE 3.17** Graph showing reaction rate plotted against increasing substrate concentration.

![Graph showing provided substrate concentrations are high and conditions are kept constant, increases in enzyme concentration are expected to produce an increase in reaction rate.](image)

**FIGURE 3.18** Provided substrate concentrations are high and conditions are kept constant, increases in enzyme concentration are expected to produce an increase in reaction rate.

that this drug inhibits the activity of the platelet enzyme cyclooxygenase (COX) by reacting, chemically changing an amino acid at the active site.

In **reversible inhibition**, the enzyme is not permanently inhibited or damaged. Reversible inhibitors inactivate enzymes through noncovalent interactions that can be reversed. In contrast to the situation with an irreversible inhibitor, an inhibitor that is reversible can dissociate from the enzyme. Removal of the inhibitor reverses the inhibition.

Two types of reversible inhibition are competitive inhibition and non-competitive inhibition.

### Competitive inhibition

**Competitive inhibition**: Competitive enzyme inhibitors have a **similar shape** to the usual substrate molecule and so can bind to the active site of the enzyme, preventing the formation of the enzyme-substrate (E-S) complexes. As a result, over a given period of time, fewer substrate molecules bind to the active site of the enzyme so that the rate of the reaction is decreased (see figure 3.20).

**Figure 3.20** (a) In competitive inhibition, inhibitor molecules compete with the substrate molecules for access and bind to the active site of the enzyme. (b) Graph showing the rate of enzyme action versus substrate concentration in the absence of a competitive inhibitor and in the presence of a competitive inhibitor.
Eventually, the inhibitor moves from the active site, so the competitive inhibition is usually temporary.

Because the substrate and the competitive inhibitor are competing for the same active sites on enzyme molecules, the degree of inhibition produced depends on the relative concentrations of substrate and inhibitor molecules. For example, adding more substrate molecules increases the chance that a random collision with the active site will be that involving a substrate molecule rather than that involving a molecule of the competitive inhibitor.

**Non-competitive inhibition**

Non-competitive inhibitors do not bind to the active site, but bind to another site on the enzyme molecule called the *allosteric site*. The binding of the non-competitive inhibitor distorts the 3D shape of the enzyme so that it can no longer bind to its substrate and, therefore, can no longer catalyse the usual reaction (see figure 3.21). Adding more substrate molecules does not have any effect on this non-competitive inhibition. Can you suggest why?

![Figure 3.21](image)

**Figure 3.21** (a) Graph showing rates of enzyme action versus substrate concentration in the absence of inhibitor (black line), in the presence of a non-competitive inhibitor (red line), and in the presence of a competitive inhibitor (blue line). These two different inhibitors bind to different sites on the enzyme molecule, with (b) showing the case for non-competitive inhibitors. Only in one case do the inhibitor molecules compete with substrate molecules for the same site on the enzyme. Which type of inhibition is that?

In summary, enzymes may have several binding sites, the most well known being their active site or substrate-binding site, while others may be present, including allosteric sites where binding inhibits enzyme function.

The presence of an allosteric binding site on an enzyme enables regulation of metabolic pathways to occur through a process termed **end-product inhibition**. Figure 3.22 shows an example of end-product inhibition where the end product of a metabolic pathway binds non-competitively to the first enzyme in the pathway, stopping the action of the enzyme. Regulation of this type means that products will not continue to be produced regardless of the need for them.
Enzymes contain a protein backbone. For some enzymes, the protein makes up their entire structure. However, for many enzymes, additional non-protein components are present that are important contributors to both the activity and the stability of these enzymes. These additional non-protein groups needed for enzymes to function properly are termed **cofactors**.
Cofactors include both inorganic and organic molecules:

1. **Inorganic cofactors** include metal ions such as Mg$^{2+}$, Cu$^{2+}$, Mn$^{2+}$, or clusters of several ions.

   Examples of enzymes with a metal (inorganic) cofactor are shown in Table 3.4.

   **TABLE 3.4** Examples of enzymes with the requirement for metal ion cofactors.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>catalase</td>
<td>Fe$^{2+}$ or Fe$^{3+}$</td>
</tr>
<tr>
<td>cytochrome oxidase</td>
<td>Cu$^{2+}$</td>
</tr>
<tr>
<td>glucose-6-phosphatase</td>
<td>Mg$^{2+}$</td>
</tr>
<tr>
<td>DNA polymerase</td>
<td>Zn$^{2+}$</td>
</tr>
</tbody>
</table>

2. **Organic cofactors** are sometimes divided into coenzymes and prosthetic groups, with prosthetic groups generally regarded as being those organic molecules that are bound tightly to particular enzymes.

   - **Prosthetic groups**: Some enzymes have a tightly bound heme group that is identified as a prosthetic group. These heme-containing enzymes include catalase and cytochrome c, and the heme molecule is a permanent part of these enzymes. Catalase is an oligomeric enzyme with four polypeptide chains and each chain has an associated heme molecule.

   - **Coenzymes**: Among the coenzymes are a number of key coenzymes that play a critical role in transferring functional groups in enzyme-catalysed reactions. These key coenzymes include NAD, NADP and ATP.

   Let’s look at NAD, which can exist in two forms:

   Nicotinamide adenine dinucleotide (NAD) is found in all living cells. It is present in a **loaded form** as NADH and is a source of electrons and a helper for any enzyme that catalyses a reaction in which its substrate is reduced. When NAD unloads its electrons, it is oxidised and the enzyme substrate is reduced. NAD is also present in cells in an **unloaded form** in which NAD is a receiver of electrons and is a helper for any enzyme that catalyses a reaction in which its substrate is oxidised. As NAD transitions between its loaded and unloaded forms, it switches from being a helper for the group of enzymes that catalyse reduction reactions to being a helper for those enzymes catalysing oxidation reactions.

   Figure 3.23 shows the loaded and unloaded (‘empty’) forms of the NAD coenzyme.

   So, NAD is a versatile molecule involved in a large number of redox reactions, sometime donating electrons and sometimes accepting them. It never rests . . . whatever form it is in, either loaded or unloaded, NAD can serve as a coenzyme for groups of enzymes involved in metabolic pathways. A similar story applies to the coenzyme NADP, which plays a comparable role in plants.

   Let’s now look at ATP as a coenzyme that transports chemical energy within cells for use in metabolic pathways. This coenzyme exists in the energy-rich form of ATP (adenosine triphosphate), which can provide energy to drive anabolic reactions when one of its phosphate groups is hydrolysed and it is converted to ADP (adenosine diphosphate).

   So, ATP is consumed in cells by energy-requiring (endergonic) processes, but ATP is regenerated by energy-releasing (exergonic) processes. Through these processes, ATP transfers energy between various metabolic pathways occurring in cells. There are many ATP-dependent enzymes in cells and these include ATP-dependent DNA ligases, ATP-dependent restriction enzymes, ATP-dependent deoxyribonucleases, and ATP-dependent phosphofructokinases.
Professor Leigh Ackland — molecular biologist

Professor Leigh Ackland is a research scientist and lecturer at the Centre for Cellular and Molecular Biology, School of Life and Environmental Sciences at Deakin University. Leigh writes:

‘As a research biologist, I have had a wonderful career working with stimulating and creative colleagues to unravel the mysteries of biological processes. One of my main interests is in the basic building blocks of biology: cells. To understand the functions of cells, we have developed specialised techniques to enable cells from different parts of the body to be grown outside the body: a technique termed tissue culture (see figure 3.24). Recent knowledge of the nutritional requirements of different types of cells has enabled us to grow cells taken from the skin, gut, breast and blood. Using this approach, we have learned much about the life of a cell, both in health and disease. In culture, we can make cells become specialised to carry out different functions, communicate with each other and their environment, and eventually die.

The technique of tissue culture has provided a wealth of information about the behaviour of cancer cells. Cancer cells start their lives as normal cells but undergo changes, causing them to grow out of control and to lose contact with each other and with the substrate to which they are attached. These changes can eventually lead to cells spreading around the body.

Tissue culture models and other studies indicate that breast cancer cells take on a mode of behaviour similar to that of developing cells that migrate during normal embryonic development. Cancer may thus be a recapitulation of some of the normal stages of development but is inappropriate in the context of adult function. The conversion of normal cells to those with abnormal behaviours characteristic of cancer cells is thought to occur through a process termed the epithelial-to-mesenchymal transition.

Our studies showed that cells that had undergone the epithelial-to-mesenchymal transition to become cancerous could not form proper contacts with each other or with the extracellular environment. They also had alterations in a cytoskeletal protein called vimentin. Vimentin is a hallmark of the epithelial-to-mesenchymal transition and is associated with the capacity of cells to invade or metastasise. Studies on cultured cells from breast cancer patients show increased levels of vimentin that correlates with the degree of invasiveness of the cancer.

The Australian Synchrotron, located in Melbourne, has provided us with novel imaging technologies for our research. This amazing facility generates accelerating electrons that produce beamlines with different properties to see inside cells. We have used the X-ray fluorescent beamline to detect differences between normal and diseased tissue.

My interest in science was kindled by my maternal grandfather, a chemistry teacher, who took me on expeditions to places such as museums and questioned the science behind what we saw. His inspiration stimulated me to study science at school and then at university.’

FIGURE 3.24 Professor Leigh Ackland grows cancer cells in a transparent flask so that the live cells can be seen under the phase contrast microscope. (Image courtesy of Professor Leigh Ackland, Deacon University.)

FIGURE 3.25 Cancerous breast cells viewed with a fluorescence microscope after staining for the presence of vimentin (green) and keratin (red). (Image courtesy of Professor Leigh Ackland, Deacon University.)
KEY IDEAS

- Two models to explain enzyme specificity have been proposed.
- Enzymes lower the energy level needed to activate reactant molecules as compared with the non-enzyme catalysed situation.
- Enzymes have an active site where their specific substrate binds, and typically have an allosteric site where inhibitors bind.
- The rate of an enzyme-catalysed reaction is affected by several factors, mainly temperature, pH and substrate concentration.
- Enzymes can be inhibited either irreversibly or reversibly.
- Reversible inhibition includes competitive and non-competitive inhibition.
- End-product inhibition is a form of non-competitive inhibition that is used to regulate the operation of a metabolic pathway.
- Most enzymes have non-protein components, termed cofactors, involved in their structure and/or function.
- One key group of cofactors includes the coenzymes NADH, NADP and ATP, which move functional groups and energy between metabolic pathways in cells.

QUICK CHECK

4 Briefly explain the meanings of the following terms:
   a activation energy
   b heat denaturation
   c pH optimum.

5 Identify one key difference between the members of the following pairs:
   a competitive and non-competitive inhibition
   b reversible and irreversible inhibition
   c loaded and unloaded forms of NAD
   d active site and allosteric site.

6 Identify the following statements as true or false:
   a Enzymes change the activation energy of a reaction by providing a different reaction pathway.
   b Non-competitive inhibition can be reduced by increasing the concentration of substrate.
   c Competitive inhibition can be reduced by increasing the concentration of substrate.
   d Irreversible inhibition typically involves the formation of covalent bonds between the inhibitor and the enzyme with a consequent change in the shape of the enzyme.
   e End-product inhibition works only when the end product has a shape that matches the active site of the first enzyme in the metabolic pathway.
   f When in its loaded form, NAD acts as a helper to any enzymes that catalyse reactions in which their substrate will be reduced.
The graph immediately below (figure 3.26) shows the behaviour of enzyme X under five different conditions.

a. What variable is plotted:
   i. on the vertical axis of this graph
   ii. on the horizontal axis?

b. How many experimental results are shown on this graph?
c. What condition was kept constant throughout the course of one experiment?

A dependent variable is the variable being tested in a scientific experiment and its value depends on the independent variable. As scientists change the independent variable, they observe and record the change in the dependent variable.

d. In these experiments, what is:
   i. the dependent variable
   ii. the independent variable?

e. What conclusion(s) about enzyme X can be drawn from these results?

Enzyme Y was isolated from extremophile microbes that live in hot volcanic waters.

f. Would you predict that enzyme Y would show similar behaviour to enzyme X when subjected to the same experimental conditions? Explain.

Examine the data shown in these graphical results and answer the following questions:

g. At what temperature did enzyme X lose about half its activity after just a few minutes?

h. After one hour at 60 °C, what percentage activity of enzyme X remained?
i. At 65 °C, about how long did it take for enzyme X to lose about 80% of its maximum activity level?
j. What happened to enzyme X during the first 10 minutes of its exposure at 70 °C?
k. Explain why the behaviour of enzyme X differs at 50 °C compared with its behaviour at 70 °C.

2. Do an internet search using the term ‘metabolic pathways’ and ask for images. You can expect to see some complex images that show the numerous metabolic pathways that operate in living organisms. Remember that the purpose of this activity is simply to give you a sense of the complexity of metabolic pathways and to alert you to the key role of enzymes in virtually every step in these pathways.

a. Examine several of these images to see some detail of metabolism. Is the scope of cellular metabolism what you expected?
b. One site states that these metabolic pathways are ‘daunting in their majesty beauty’. Do you agree?
c. Many of the images may show the various substrates and their products in metabolic pathways, but omit the enzymes involved in the various steps. Can you locate a metabolic map that also gives the enzymes involved?
Chapter review

Key words

activation energy  
active site  
allosteric site  
anabolism  
catabolism  
coenzyme  
cofactors  
coupling  
dergonomic

deroend  
end-product inhibition  
enzyme inhibitor  
enzyme-substrate (E-S) complex  
exergonic  
gulono-lactone oxidase (GULO)  
inactivated form  
inorganic cofactors  
irreversible inhibition  
loaded form  
lock-and-key model  
metabolism  
monomeric enzymes  
oligomers  
organic cofactors  
product  
proenzymes  
prosthetic group  
reversible inhibition  
scurvy  
similar shape  
substrate  
substrate binding site  
systematic name  
trivial name  
unloaded form

Questions

1 Making connections between concepts ➔ Use at least eight of the key words from this chapter to construct a concept map relating to enzymes.

2 Applying knowledge and understanding ➔ Examine figure 3.27. Compound A is a type of protein.
   a What is the general name given to the type of protein represented by Compound A?
   b What are the general names given to each of the parts labelled B, C, D and E?
   c Name the type of reaction shown in this diagram — is it catabolic or anabolic? Explain.
   d Is this reaction more likely to be endergonic or exergonic? Explain.

   ![Figure 3.27](image)

3 Applying knowledge and understanding to make predictions ➔ The graph below shows the effect of temperature on the activity of an enzyme from three different organisms.

![Graph](image)

   a What predictions, if any, might be made about the organisms that were the sources of these enzymes? Give a brief rationale for any predictions that you make.
   b Note that the shapes of the curves are different. Suggest a reasonable explanation for these differences.

4 Drawing conclusions ➔ Amino acids in a polypeptide chain are numbered from 1 to n, starting from the amino terminal; for example, the third amino acid might be Val 3 and the tenth in the sequence might be Leu 10, and so on . . .

   The active site of the enzyme trypsin, a digestive enzyme, consists of three amino acid as follows: Asp 102, His 57 and Ser 195, that is, these three amino acids are the 57th, the 102nd and the 195th amino acids in the primary structure of this enzyme.
   a Identify two events that could occur at the active site of an enzyme.
   b What stabilises a substrate in the active site of an enzyme?
One student suggested that the three amino acids at the active site of trypsin had been misnumbered. The student said that there is quite a distance separating them so that they cannot be close together at the active site.

Do you agree with the student? Give a clear explanation for the decision you have made.

5 Demonstrating knowledge and communication ➔ Identify a key difference between the members of the following pairs:
   a ‘lock-and-key’ model and ‘induced fit’ model
   b a prosthetic group and a coenzyme
   c loaded NAD and unloaded NAD
   d competitive inhibition and end-product inhibition
   e catabolism and anabolism
   f pepsinogen and pepsin.

6 Interpreting diagrammatic information ➔

Examine figure 3.28 above. What story does this diagram tell?

7 Demonstrating understanding and communication ➔ Consider the following figure, which shows the total amount of product formed during an enzyme-catalysed reaction.

Explain the following features of this graph:
   a the initial steep slope
   b the slowdown in the rate of product formation
   c the flattening of the curve to form a plateau.

8 Developing logical explanations ➔ Consider each of the following observations and formulate an explanation for the observation.
   a Salivary amylase enzyme breaks down starch. The process begins when saliva mixes with food in the mouth, but, when it reaches the stomach, the amylase enzyme no longer functions.
   b The jelly-like white of a raw hen’s egg consists principally of the protein albumin, but, after it is cooked, it forms a white solid.
   c When an inhibitor binds to an enzyme at a site other than the active site, this inhibition cannot be overcome by increasing the concentration of substrate molecules.
   d Enzyme-catalysed reactions operate at much faster rates than non-catalysed reactions.

9 Demonstrating knowledge and understanding ➔

Protein X is an enzyme.

a What is the general shape of protein X (as shown in the left-hand image)?

b When treated with a particular chemical, the shape of protein X was radically changed, as shown in the middle image. What specific effects has the chemical had on protein X?

c Describe what happened when the chemical was removed.

d What labels would you put on the action of the chemical on protein X?

e How, if at all, would the ability of protein X to catalyse its specific reaction be affected when it was in the ‘long snake form’?