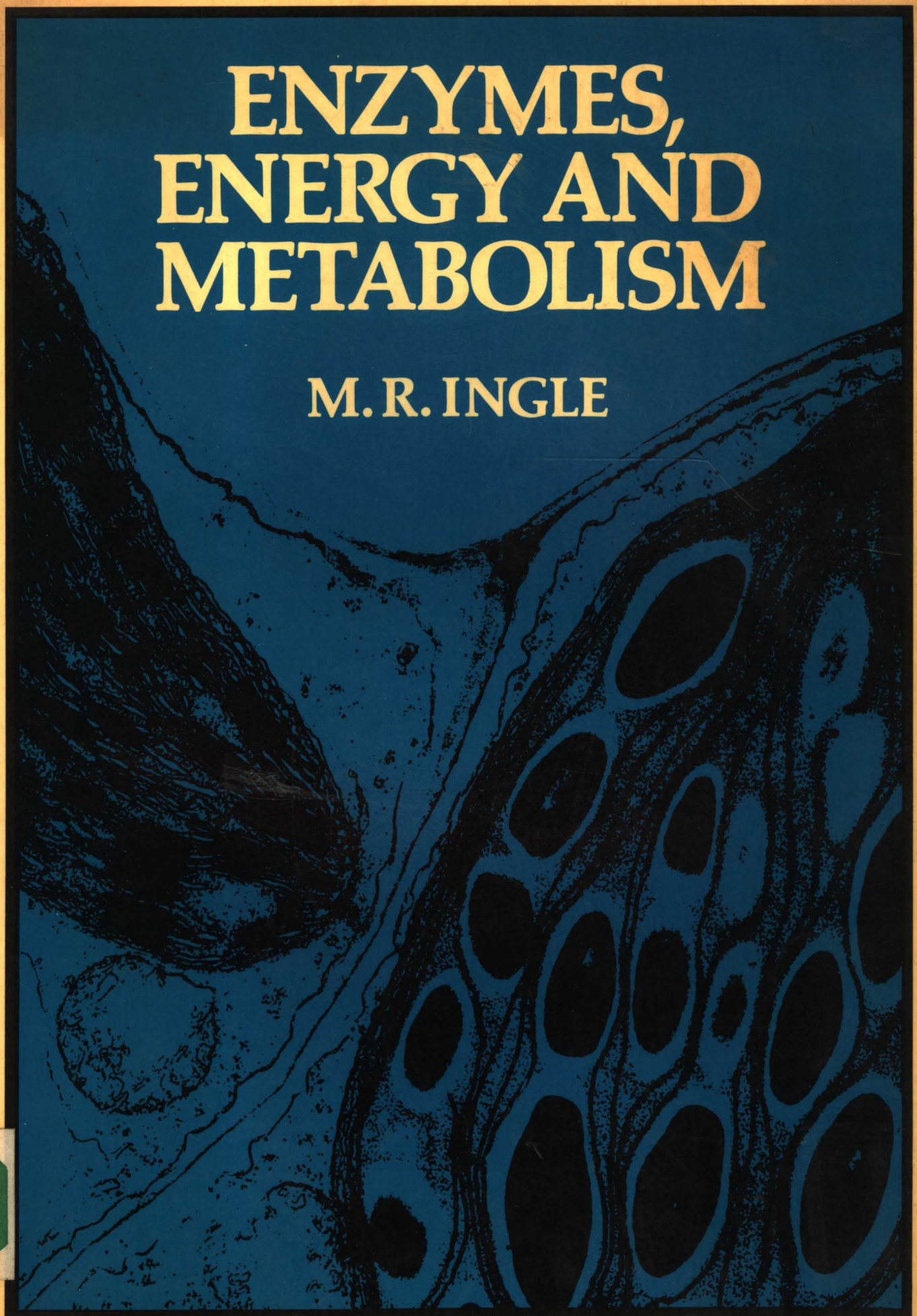
ENZYMES, ENERGY AND METABOLISM

M. R. INGLE



STUDIES IN ADVANCED BIOLOGY 3

ENZYMES, ENERGY AND METABOLISM

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PREFACE

Most A-level students have access to a general text which provides a framework on which their course can be constructed. This series attempts to build on that framework by examining defined areas of the syllabus more closely. It looks especially at those parts of the subject which have recently undergone the most change, and attempts to bring together new concepts which are presently widely scattered throughout the available literature. In doing so, it tries to avoid simply substituting new dogmatic assertions for old, and to show how today's concepts have evolved from previous ones. A glossary is provided to avoid interrupting the text with too many asides.

This particular title posed the dilemma that a significant number of biology students may not have taken a formal course in chemistry and yet face examination questions which can be extremely taxing even to those who have (all the topics included have recently appeared in examinations in some form). I have tried to write the book making minimal assumptions about the reader's previous knowledge of chemistry, and to deal with specific issues when they arise. I have also attempted to write the book through a biologist's eyes rather than a biochemist's (at the risk of incurring the derision of both!), and so bridge the gap between the many excellent general biology and specifically biochemistry texts that are available.

MRI
1986

Acknowledgements

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The author and publishers wish to thank the following for permission to reproduce material:
Fig 3.1 Koch-Light Ltd.; Fig 4.5 Professor Ruth Bellaires, University College London; Fig 5.6 Science Photo Library; Fig 5.9 (2) Dr Steve Long, University of Essex; Fig 5.10 Dr Jean Whatley, Botany Department, University of Oxford; Fig 5.12 Biophoto Associates.

Chemical nomenclature

The International Union of Physics and Chemistry (IUPAC) and International Union of Biochemists (IUB) have laid down precise rules for the naming of chemical compounds. Systematic names are used for those substances which the student is likely to meet on a concurrent chemistry course. However, many of the more unwieldy systematic names are not in common usage, and in these cases the trivial name is employed. Thus the text uses the term 'citric acid' rather than 2 hydroxypropane, 1.2.3 carboxylic acid, and 'lactose' rather than 4-0-(β-D-galactopyranosyl)-β-D-glucopyranose.

Abbreviations

E	redox potential (V)
F	Faraday's constant (96.5 kJ mole^{-1} V^{-1})
ΔG^{o}	Gibbs standard free energy change (kJ mole^{-1})
ΔG	actual free energy change (kJ)
h	Planck's constant (6.626×10^{-34} J s)
λ	wavelength of light (nm)
ln	natural logarithm ($2.303 \log_{10}$)
M	moles per dm^3
mole	the amount of a substrate which contains as many ions, atoms or molecules as there are atoms in 0.012 kg^{12}C
N	Avogadro's number (the number of molecules, ions etc. in one mole: 6.023×10^{23})
R	universal gas constant (8.31×10^{-3} kJ mole^{-1} K^{-1})
T	absolute temperature in Kelvin (K)
z	number of electrons

C3	an organic molecule containing three carbon atoms
C-3	the *third* carbon atom in an organic molecule containing an unspecified number of carbon atoms
C3—COOH	an organic molecule containing three carbon atoms (one of which has a carboxyl group), i.e. total number of carbon atoms = 3 not 4
citrate/citric acid pyruvate/pyruvic acid	the terms for the dissociated and undissociated forms of organic acids are used interchangeably

Contents

1

Energy and Organisms

SUMMARY

The fundamental laws of physics and chemistry operate in living organisms and explain, at a molecular level, how organisms operate. Energy is needed to enable all chemical reactions to proceed. Organisms have developed mechanisms for storing chemical energy and using it to drive energy-consuming reactions.

The following terms are used:

aerobic respiration anaerobic respiration enzyme glycolysis hydrolysis kilojoule (kJ) mole nucleotide pH

The cell is a highly organised biochemical machine inside which most of the materials for an organism are processed, and most of an organism's energy is utilised and dissipated. In a single cell, scores and sometimes hundreds of reactions may be occurring simultaneously. In this book we shall consider why these reactions occur at all, why they happen at the speed they do, and how they are organised and regulated. In order to illustrate the discussion we shall examine those processes which contribute most substantially to the essential flow of energy through an organism.

1.1 THERMODYNAMICS

Organisms obey the laws of physics and chemistry. Indeed those characteristics which we call 'life' are not just consistent with such laws, but explained by them. Two laws of thermochemistry have outstanding significance in this context.

The first law of thermodynamics

This law, which is also known as the **Principle of Conservation of Energy**, may already be familiar to the reader:

> Energy cannot be created or destroyed, but it may be converted from one form into another.

By energy we mean the ability to do work – any kind of work – though in the context of this book we shall generally mean energy involved in converting one chemical substance into another.

From the first law it follows that one would have to express grave doubts if anyone claimed after a race to have 'used up a lot of energy'. They may have converted energy from one form to another, but they have not 'used it up' in the sense of destroying it.

Q1 What form(s) would the energy take before and after a race?

More on energy. Energy can come in many forms, but here it will be heat, light and chemical energy which concern us most.

The last of these is especially important. The energy of a molecule is made up of several components. First, and most important, there is the energy of the electrons arising from their positions relative to the nucleus. In addition, there is the energy of the molecules as they move through space, rotate and vibrate. These forms of energy are intrinsic, i.e. they are an inherent property of matter.

The second law of thermodynamics

This law may be stated in various ways, such as:

> Processes involving the transformation of energy (i.e. work) will not take place spontaneously unless the total products of the transformation are more random than their precursors.

Our hypothetical athlete would be fully justified in saying that some *potential energy* (the capacity to do useful work, e.g. run) had been used up. During the race, the respiration of his/her blood sugar would not only have provided heat and chemical energy for muscle contraction, but would also have been converted into other forms. Among these other forms would be energy dissipated by increasing the purely random movement of molecules in and around the runner. Energy in this form, called **entropy**, is unusable.

As chemical reactions are a form of work, the laws of thermodynamics ought to have an important impact on the way in which we think about the many reactions occurring in a living organism. It follows from these laws that usable energy must be available for all chemical reactions to occur. Chemical reactions fall into two broad categories: those which occur of their own accord, without being supplied with external energy (**exergonic reactions**); and those which only occur if they are pushed along by an external energy supply (**endergonic reactions**).

1.2 EXERGONIC REACTIONS

Reactions in a living organism which involve breaking down complex organic molecules into simple ones (**catabolism**) are typically exergonic. The aerobic respiration of glucose is a classic example:

reactants form products

oxygen + glucose → carbon dioxide + water

To accommodate the first law of thermodynamics we must assert that the left-hand side of the reaction exactly balances the right-hand side in terms of energy. Yet this reaction occurs without any outside energy being supplied, and the second law predicts that the products must somehow contain *less* intrinsic energy than the reactants. Are the laws contradicting each other? No, not really, because during the reaction some energy is released in various forms such as heat.

Chemists usually represent the difference between the energy content of reactants and products by the term ΔG^{o} (**Gibbs standard free energy change**). It is the energy difference between *one mole* of reactants and products at *equilibrium*, and is measured in kilojoules per mole (kJ $mole^{-1}$). By convention, if energy is released during a reaction it is given a negative sign. A complication arises if H^{+} ions are involved, because ΔG^{o} would then be measured in a solution containing 1.0 mole H^{+} per dm^{3} solution. This solution would be pH 0! To allow for this the term $\Delta G^{o'}$ is normally used in biology. $\Delta G^{o'}$ is the same as ΔG^{o} but assumes pH 7: a value which approximates rather better to the aqueous conditions in the cell. A more adequate representation of aerobic respiration would therefore be

$$6O_2 + C_6H_{12}O_6 \rightarrow 6CO_2 + 6H_2O$$
$$(\Delta G^{o'} = -2870 \text{ kJ mole}^{-1})$$

$\Delta G^{o'}$ (or ΔG^{o}) is helpful because it enables the energy changes of different reactions to be compared under a set of standard conditions. However, it has two very serious limitations:

(i) Reactants are never 1.0 M in living cells. They usually vary between 0.01 M (10^{-2} M) and 0.00001 M (10^{-5} M).

(ii) Reactions rarely reach equilibrium in living organisms. Frequently equilibrium is prevented by various cellular activities.

A knowledge of the actual free energy change, ΔG, is therefore really more useful when trying to understand how organisms work. This takes into account the actual concentrations of materials, the pH, and does not assume equilibrium. It is, however, exceedingly difficult to measure.

1.3 ENDERGONIC REACTIONS

Reactions in the body involving the synthesis of complex molecules from simple ones (**anabolism**) are typically endergonic. Photosynthesis is a classic example:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$
$$(\Delta G^{o'} +2870 \text{ kJ mole}^{-1})$$

Q2 What information is provided in the equation which tells us that the above reaction cannot occur of its own accord?

Q3 Which law of thermodynamics tells us that the *energy* changes involved in aerobic respiration and photosynthesis are equal (but opposite)?

Q4 In photosynthesis, what form does the energy take (2870 kJ $mole^{-1}$) initially? Ultimately?

Endergonic reactions are driven in the body by coupling them to exergonic reactions, so that the energy change for the overall (coupled) reaction is favourable (ΔG^{o} or $\Delta G^{o'}$ becomes negative). Thus, suppose in an endergonic reaction:

$A \rightarrow B$ ($\Delta G^{o'} = +20$ kJ $mole^{-1}$)

whilst in an exergonic reaction:

$X \rightarrow Y$ ($\Delta G^{o'} = -33$ kJ $mole^{-1}$).

If the two are coupled:

$A+X \rightarrow B+Y$ ($\Delta G^{o'} = (+20-33) = -13$ kJ $mole^{-1}$).

The coupling makes the overall $\Delta G^{o'}$ negative, so the reaction can proceed. The ultimate source of energy for the vast majority of endergonic reactions in organisms is light energy which is absorbed during photosynthesis. (The only exceptions are chemosynthetic bacteria, which substitute chemical energy derived from their environment for light.) These external forms of energy are used either directly or indirectly (via food which photosynthesis produces) to generate high energy intermediates. The latter are the *immediate* power supply for endergonic reactions.

Organisms are remarkably conservative in their choice of high energy intermediates, relying principally on just two main categories: **phosphorylated nucleotides** and **reduced dinucleotides**.

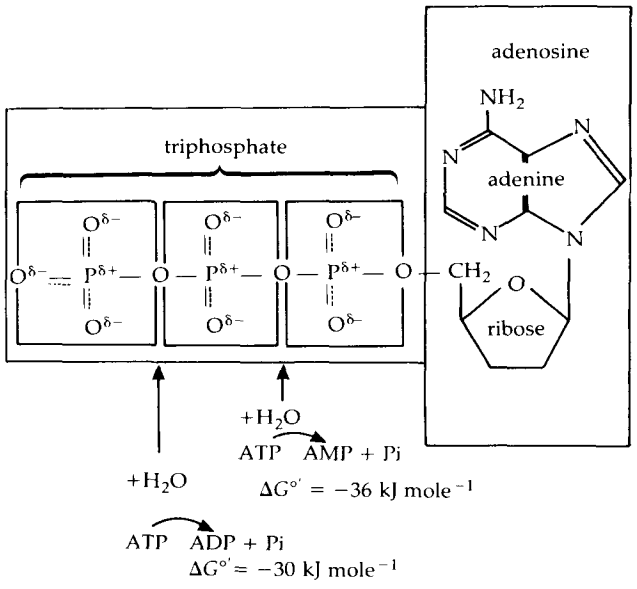
1.3.1 Phosphorylated nucleotides

The majority of endergonic reactions are powered by the phosphorylated nucleotide **adenosine triphosphate (ATP)**. Hydrolysis of the third phosphate group yields energy which can be made to do useful work ($\Delta G^{o'} = -30$ kJ $mole^{-1}$). Adenosine diphosphate (ADP) and inorganic phosphate (Pi) are byproducts of the reaction. Alternatively, ATP may be hydrolysed to adenosine monophosphate and pyrophosphate (AMP + PP) yielding a similar amount of energy. Hydrolysis of the remaining phosphate (AMP → adenosine + Pi) yields only a small amount of energy and is biologically insignificant (Fig. 1.1).

The first reaction is very common and is usually abbreviated to

ATP → ADP + Pi + energy

as shown in Fig. 1.1 (inset), but this can be very misleading. Energy is never given out by breaking a



Energy is released when the last, or last two, phosphates are hydrolysed by an enzyme such as ATPase. Such enzymes normally require Mg^{2+} in order to function. ATP is often loosely complexed to this ion in the cell.

$ATP^{4-} + H_2O \xrightarrow{ATPase} ADP^{3-} + Pi^{2-} + H_2O$
$(\Delta G^{\circ\prime} = -30 \text{ kJ mole}^{-1})$

glucose + phosphate → glucose phosphate
$(\Delta G^{\circ\prime} = +16 \text{ kJ mole}^{-1})$

Since ΔG° is positive (i.e. the reaction is energetically unfavourable), glucose phosphate cannot be synthesised by the above reaction.

ATP → ADP + Pi $(\Delta G^{\circ\prime} = -30 \text{ kJ mole}^{-1})$

However, the energy released during ATP hydrolysis is considerable, and can be used to drive the above, thus:

glucose + ATP → glucose phosphate + ADP
$(\Delta G^{\circ\prime} = -14 \text{ kJ mole}^{-1})$

Note that the energy released in the last reaction is the nett quantity left over from the first two reactions.

Inset: Coupling of ATP to an endergonic reaction

Fig. 1.1 *Adenosine triphosphate.* ATP is made from an organic base (adenine), a C5 sugar (ribose) and three phosphates. The significance of the small negative and positive charges (δ^- and δ^+) is discussed at the end of Section 1.3.1. Various values for $\Delta G^{\circ\prime}$ are given in the literature, mostly between 30 and 33 kJ $mole^{-1}$.

chemical bond, as the abbreviation implies: in fact, energy is always required. The 30 kJ $mole^{-1}$ produced by ATP hydrolysis is the nett amount left over as a result of

energy *released* during the formation of new bonds	minus	energy *required* to break existing bonds

During respiration and photosynthesis, ATP is resynthesised from ADP and Pi. Thus the same molecules can be used over and over again; they can be made in one part of the cell and consumed in another.

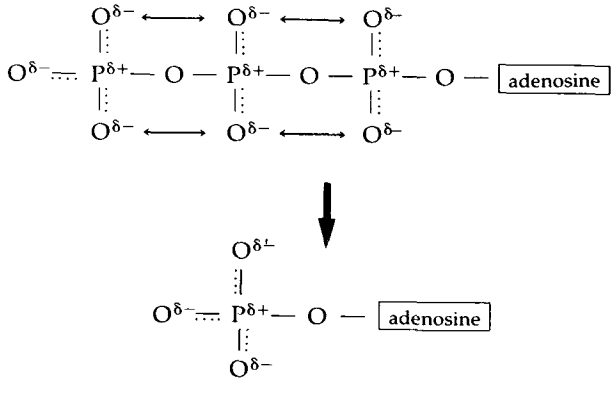
Other phosphorylated nucleotides may be used to drive some endergonic reactions. Thus, whereas ATP is used for the synthesis of starch, protein and fat from their component parts, **guanidine triphosphate (GTP)** builds cellulose, and **uridine triphosphate (UTP)** builds glycogen, both from glucose. All the phosphorylated nucleotides yield a similar amount of energy when hydrolysed. Why ATP should be used so frequently as a power supply seems rather odd, and the energy content alone obviously cannot be the reason. Perhaps ATP is stable enough to prevent accidental wastage, but unstable enough to be broken down under appropriate conditions; perhaps enzymes using it can recognise it more easily.

The squiggle and the energy in ATP

The chemical energy potentially available in ATP is sometimes represented by allocating a 'squiggle' (= high energy bond) to the terminal phosphate, thus: ADP ~ Pi.
This has led to two misconceptions:

(i) *There is energy in the bond which is released when it is broken.* There is not. As stated above, energy is *always* required to break chemical bonds but if *more* energy is released when new (and more stable) bonds form as a result of a reaction then, on balance, energy will be released.

(ii) *There is something unique about this particular covalent bond.* There is not. It is likely, however, that in the cell the phosphate groups in ATP ionise, and therefore the molecule as a whole must contain more energy to maintain its integrity than in the absence of such charges. These charges are relieved on hydrolysis of the last (or last two) phosphate groups, resulting in the formation of more stable (lower energy) structures.



⟷ opposed charges which are relieved on hydrolysis

Hence it is incorrect to imagine that the energy in ATP is 'stored' in the terminal phosphate bond. For these reasons the term 'high energy bond' and the squiggle notation will not be used in this text.

1.3.2 Hydrogen (electron) carriers

Many biologically important reactions involve the gain or loss of electrons. These are called **redox reactions** (reduction–oxidation reactions). The substance which gains an electron is said to be reduced, and the substance which loses one is said to be oxidised. In cells the movement of an electron is usually accompanied by the movement of a proton (hydrogen ion) so that in most cases reduction means the gain of hydrogen, and oxidation the loss of hydrogen. Redox reactions in cells normally involve hydrogen (electron) carriers. Those which are encountered most often are **nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP)** and **flavin adenine dinucleotide (FAD)**.

$$\underset{\text{oxidised form}}{NAD} + 2H \underset{\text{oxidation}}{\overset{\text{reduction}}{\rightleftharpoons}} \underset{\text{reduced form}}{NADH_2}$$

The ability of chemicals to accept or donate electrons varies. Substance A might be able to donate electrons to substance B but not to substance C. Indeed C might donate electrons to A (and B). Hence we can arrange chemicals in a sequence according to their ability to donate electrons. In this example the sequence would be C → A → B. This ability may be quantified under standard conditions of concentration, temperature and pH. Electron-donating ability is described in terms of the **redox potential**, the units being volts. Table 1.1 lists some biologically important redox potentials. What the table means is that substances at the top can reduce (donate electrons or hydrogen to) those lower down. Thus $NADH_2$ can pass hydrogen to oxygen, forming water, but water cannot spontaneously pass hydrogen back to NAD to re-form $NADH_2$.

$$\underset{\text{reduced}}{NADH_2} + \underset{\text{oxidised}}{\tfrac{1}{2}O_2} \longrightarrow \underset{\text{oxidised}}{NAD} + \underset{\text{reduced}}{H_2O}$$

From the laws of thermodynamics this must mean that the oxidation of $NADH_2$ by oxygen has a negative $\Delta G^{o'}$. Indeed, whenever an electron passes 'down' the table, $\Delta G^{o'}$ must be negative.

Q5 Chemists have derived an equation which makes it possible to quantify the relationship between $\Delta G^{o'}$ of oxidation and redox potentials:

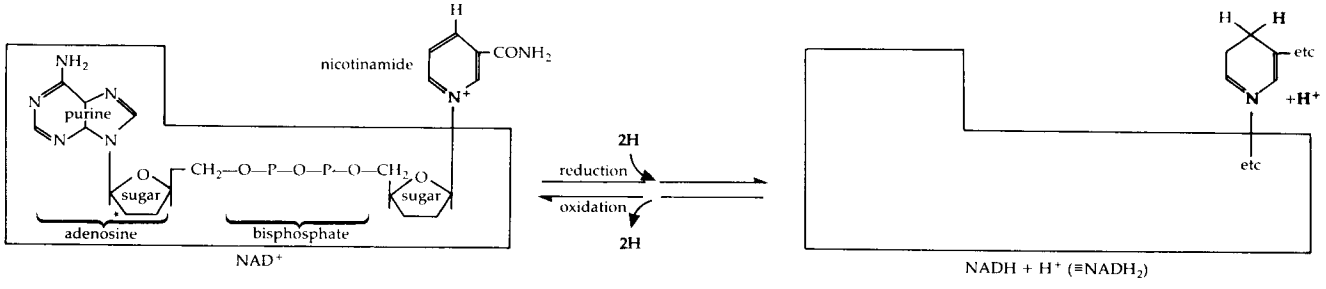
$$\Delta G^{o'} = -zFE$$

where z = the number of electrons involved
F = Faraday's constant (96.5 kJ mole^{-1}V^{-1}) and
E = the redox potential.

The values of $\Delta G^{o'}$ given in the table were calculated using this equation. Can you work out the value for the oxidation of $NADH_2$ by oxygen? Proceed as follows:

(i) If electrons are passing from $NADH_2$ to $\frac{1}{2}O_2$, through how many volts in *total* (Table 1.1) do the electrons move? (What is E?)

(ii) From $\Delta G^{o'} = -zFE$, calculate $\Delta G^{o'}$ for this reaction. Remember that *two* electrons are involved, i.e. $z = 2$.



Ignore the detail in the left-hand drawing: it is only there to show how the component parts join together (adenine, ribose (twice), diphosphate and nicotinamide). Notice how NAD can pick up two hydrogens (H) and so function as a 'hydrogen' carrier. Strictly speaking, it is better to describe it as an *electron* carrier since, as the diagram indicates, N^+ is actually converted to N, not NH^+.

Some electron (hydrogen) carriers	Full name	Comment
NAD	Nicotinamide adenine dinucleotide	Used in both anaerobic and aerobic respiration. In the latter, hydrogen is transferred from food materials to oxygen, forming water, and ATP is formed in the process (see Chemiosmosis, Chapter 4.4). NAD also substitutes for NADP during bacterial photosynthesis.
NADP	Nicotinamide adenine dinucleotide phosphate (same as NAD, with extra phosphate at position marked * in above figure)	Used in synthetic reactions such as photosynthesis and fatty acid synthesis where some of the intermediate compounds in these pathways are reduced. In photosynthesis, the ultimate source of the hydrogen is water, and its ultimate destination is organic acids, which are thus reduced to aldehydes (sugars).
FAD	Flavin adenine dinucleotide	Used at some stages of aerobic respiration in place of NAD.

Fig. 1.2 *Common hydrogen carriers.* Nicotinamide is made from **nicotinic acid (niacin)**, one of the vitamin B group. Flavin is made from **riboflavin** (Vitamin B_2). The deficiency symptoms which appear when such substances are missing from the diet are essentially outward signs of inadequate quantities of NAD, NADP and FAD.

Table 1.1 *Redox potentials of some biologically important compounds.* The redox potential (E'), which is measured in volts, varies with pH. The values given assume biological conditions, i.e. pH 7.

E' (V)	Oxidised → Reduced	$\Delta G^{o'}$ of oxidation by O_2	$\Delta G^{o'}$ of oxidation by NO_3^-
−0.43	$CO_2 \rightarrow CH_2O$; $Fd_{ox} \rightarrow Fd_{red}$	−239	−164
−0.32	$NADP \rightarrow NADPH_2$; $NAD \rightarrow NADH_2$	(See Q5 Chapter 1)	(See Q14 Chapter 4)
−0.22	$FAD \rightarrow FADH_2$	−199	−124
−0.1	Range for most cytochromes and other components of cellular electron transport systems		
+0.38	$PSI^+ \rightarrow PSI$	(Not biologically significant)	
+0.42	$NO_3^- \rightarrow NO_2^-$	−75	—
+0.81	$O_2 \rightarrow H_2O$	—	—
+0.9	$PSII^+ \rightarrow PSII$	—	—

Energy is released (ΔG is negative) if an electron moves from a (reduced) substance higher up the table to an (oxidised) substance lower down the table.

Key

Fd ferredoxin (a chloroplast component: Chapter 5)
CH_2O carbohydrate
PSI, PSII photosystems I and II (Chapter 5)
Other abbreviations are defined in the text

The amount of energy which is released by the oxidation of reduced dinucleotides can, as your answer to Q5 shows, be considerable. It can be used in a variety of ways. The energy released during the oxidation of $NADH_2$ itself, for example, is used to build up ATP from ADP and Pi. Indeed it is by far the most important mechanism of ATP synthesis in most organisms. Similarly, during photosynthesis, $NADPH_2$ (which is built up by light energy from NADP and H_2O) provides about 80% of the energy needed to convert CO_2 into carbohydrate:

$$CO_2 \xrightarrow[2NADPH_2 \rightarrow 2NADP]{\text{photosynthesis}} C(H_2O) + H_2O$$
carbohydrate

(ATP is also consumed in this reaction, and provides the remaining 20% of the energy required.)

1.3.3 Other high energy intermediates

The box in Section 1.3.1 emphasised that the energy content of ATP was a function of the electrical charges distributed throughout the phosphate groups. One might therefore expect to find that other phosphorylated compounds are also 'energy rich'. Table 1.2 confirms that this is indeed the case. Indeed, some micro-organisms even use polymers of *in*organic phosphate as energy reserves.

Table 1.2 *Miscellaneous 'high energy' compounds.* ΔG^o for ATP hydrolysis is given in Fig. 1.1.

Compound hydrolysed	$\Delta G^{o'}$ (kJ mole^{-1})	Comments
Phosphoenolpyruvate → pyruvate + phosphate	−54	ATP-generating steps during anaerobic respiration
Diphosphoglyceric acid → phosphoglyceric acid + phosphate	−51	
Creatine phosphate → creatine + phosphate	−43	Energy-generating mechanism in vertebrate muscle
Acetyl coenzyme A → acetate + coenzyme A	−31	Acetyl CoA is a source of organic carbon and energy for fatty acid synthesis, oxidative respiration and the synthesis of some amino acids
Sucrose → glucose + fructose	−29	Energy-generating mechanism during inulin synthesis
Glucose-6-phosphate → glucose + phosphate	−16	G6P is an activated form of glucose used in respiration

The only biologically significant molecule in Table 1.2 which is not phosphorylated, but which nevertheless yields useful amounts of energy on hydrolysis, is **acetyl coenzyme A**. More will be said about this substance later.

1.3.4 Coupling in series

It was noted in Section 1.2 that in biology the term $\Delta G^{o'}$ had severe practical and theoretical limitations, and that ΔG was more useful. The two can be related thus:

$$\Delta G = \Delta G^{o'} + RT \ln \left(\frac{\text{product concentration}}{\text{substrate concentration}} \right)$$

where R = Universal gas constant (8.31×10^{-3} kJ mole^{-1} K^{-1})

T = temperature in Kelvin

ln = 2.303 $\log_{10}$

Assuming organisms are at 25 °C, this equation simplifies down to

$$\Delta G = \Delta G^{o'} + 8.31 \times 10^{-3} \times 298 \times 2.3 \log_{10} \left(\frac{\text{product concentration}}{\text{substrate concentration}} \right)$$

$$\Delta G = \Delta G^{o'} + 5.7 \log_{10} \left(\frac{\text{product concentration}}{\text{substrate concentration}} \right)$$

Q6 shows why the equation is so useful.

> **Q6** Suppose that for a given reaction $\Delta G^{o'} = +8$ kJ mole^{-1}, but that in the conditions found in the cell the substrate concentration is 0.04 M, and the product concentration is 0.001 M. Calculate ΔG, and state whether or not, under these conditions, the reaction can proceed.

The equation shows that a reaction can be made possible (i.e. have a negative ΔG) if the substrate concentration is kept very high, and the product concentration is kept very low. This is possible if a highly exergonic reaction either precedes or follows an endergonic one: the two become **sequentially coupled**, or linked 'in series'. It is a relatively common phenomenon. About half the steps in anaerobic respiration are endergonic, but these are coupled in series to others which are strongly exergonic, and for the pathway as a whole $\Delta G^{o'} = -65$ kJ mole^{-1}.

1.4 THERMODYNAMICS AND LIFE

W. B. Yeats, the Irish poet, put the second law of thermodynamics in a nutshell. 'Things', he said, 'fall apart'. Continents drift, galaxies explode and dead organisms decay. There is nothing special in these examples; they are simply different expressions of how the amount of disorder (entropy) in the universe inevitably tends to increase. Let us imagine a very simple universe indeed, one consisting entirely of a metal rod, hot at one end and cold at the other. Heat will gradually move from the hot to the cold end, and during this time the transfer of heat could, in principle, be made to do useful work. Eventually, however, the heat will spread throughout the rod until the temperature is uniform, after which no further change will take place. Our model universe has come to an end of its life. In a similar way the 'real' universe is, according to the second law of thermodynamics, irrevocably 'running down'. Energy is *not* being lost (first law) but useful (potential) energy is being converted into non-useful forms. Entropy is increasing.

Living organisms are a peculiar localised reversal of the natural order. This reversal is expressed in organised growth, in the molecular architecture of tissues and cells, and in the synthesis of complex organic molecules. It is true that relatively disorganising events occur as well, but on balance entropy is *decreasing* during life. This localised reversal of the general trend towards increasing disorder is only possible at the expense of the rest of the universe. The maintenance of biological order – homeostasis in its broadest sense – requires a constant external supply of materials and energy.

Materials can be used and discarded, to be re-used by other organisms, so resulting in the endless recycling of carbon, nitrogen and other elements. In sharp contrast there is a one-way flow of energy through the living world, beginning with photosynthesis and ending, for example, in its dissipation as heat by carnivorous mammals. Organisms are rather like a huge, complex, endergonic reaction, in which ΔG can only be made favourable by coupling them to an external energy supply (light). It is this external supply of energy which results, in the case of every living organism, in a localised reversal of the second law of thermodynamics.

Study guide

Vocabulary

Distinguish between the following pairs of terms:

endergonic and exergonic reactions
reduction and oxidation
$\Delta G^{o'}$ and ΔG

Review Question

Relate the structure and properties of ATP to its function in living organisms. Illustrate your answer by reference to its involvement in *one* of the following:
(i) muscle contraction
(ii) the sodium pump
(iii) photosynthesis
(iv) starch synthesis.

Extension Question

Why does a biologist need to know about the second law of thermodynamics?

2

Enzymes

SUMMARY

Enzymes are protein catalysts which increase the rates of chemical reactions to the speeds needed to sustain life. They work by reducing the amount of energy which reactant molecules must possess before they can undergo a chemical change. Their unique properties are exploited by organisms for the regulation of metabolism.

The text itself defines several new terms, but the following are assumed (see Glossary if required):

equilibrium product substrate isomer
reactant X-ray crystallography pH

An appreciation of energetics tells us why some chemicals *can* react together and others cannot, but nothing whatever about how *fast* a reaction will proceed. The questions *'Is it possible?'* and *'How quickly?'* are quite different. The fact that the oxidation of cellulose is thermodynamically extremely favourable, for example, does not mean that this book will immediately burst into flames. To understand what governs the speed of a reaction we must turn to **enzymes** and a concept called **activation energy**.

2.1 CHARACTERISTICS OF ENZYMES

In 1897 the Buchner brothers showed that sugar could be fermented by the juices extracted from ground-up yeast cells. They called the active juice '**enzyme**' ('in yeast'; Gk.). Soon other extracts with the same ability to speed up chemical reactions were discovered. The term 'enzyme' was given to all these, and a new name, *zymase*, was given to the mixture of substances isolated by the Buchners. The chemical nature of enzymes was uncertain until Sumner (1926) first purified and crystallised the enzyme *urease*. It was a protein. Over 250 enzymes have since been purified, and without exception they have been proteins. The vast majority of enzymes are **intracellular** (operate inside cells). The more familiar digestive enzymes of the gut are therefore rather atypical, since these are all **extracellular**.

A **catalyst** is a substance which speeds up a chemical reaction. Enzymes are, by definition, organic catalysts. They resemble inorganic catalysts, such as platinum, in several important respects. For example:

(i) they remain unaltered at the end of the reaction;
(ii) they are not used up during the reaction;
(iii) small quantities are extremely effective;
(iv) neither alters the end products of the reaction;
(v) neither alters the equilibrium position of a reaction. The amount of product at the *end* of a reaction is the same whether or not a catalyst is present.

However, enzymes differ from inorganic catalysts in several important respects. Thus, enzymes are:

(i) affected by pH;
(ii) **denatured** (destroyed) by high temperatures;
(iii) affected by the presence of other substances such as coenzymes, cofactors and inhibitors;
(iv) highly specific, only catalysing one reaction, or one type of reaction. An enzyme which catalyses a single reaction is said to show **absolute specificity**. An enzyme which attacks one type of chemical bond in a variety of substrates, e.g. a peptide bond, is said to show **group specificity**.

These unique differences are all related to the fact that enzymes are proteins. The relationship between an enzyme and a substrate has been likened to that between a **lock** and a **key** (Section 2.2.2). In other words, the *shape* of an enzyme molecule is critically important for its normal functioning. The structure and shape depend on the various covalent and electrical bonds holding the molecule together (Fig. 2.1). It follows that factors which affect these bonds will affect the shape of the enzyme, and thus its activity. Heat, cofactors and pH (H^+ ions) are examples of such factors. The last of these also affects electrical charges on various ionised amino acids in the enzyme molecule. As we shall see (Section 2.2.3), having the right electrical charge in the right place at the right time is crucial for an enzyme's catalytic activity.

2.1.1 Types of enzymes

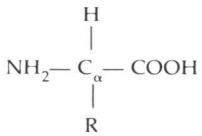
Over 90% of enzymes are simple **globular proteins**, the remainder being **conjugated proteins** with non-protein **prosthetic groups** (Fig. 2.1). For biological purposes, however, classifying enzymes by their physical characteristics is much less useful than classifying them by their function. The Enzyme Commission of the International Union of Biochemists (1964) opted for the latter approach, and listed six basic categories (Table 2.1).

Each of the six main categories is assigned a number: thus all hydrolases are in group 3. Each group is then subdivided several times and eventually the enzymes are individually numbered. The complete classification

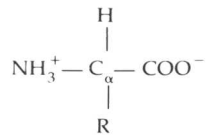
1

Generalised amino acid

non-ionised



both $-NH_2$ and $-COOH$ ionised



The degree of ionization on the amino ($-NH_2$) and carboxyl ($-COOH$) groups varies with pH.

About 20 types of amino acids occur in proteins. They differ from each other only by the variable side chain (R). Some of the broad categories of amino acids, together with specific examples, are:

Hydrophilic and acidic

Glutamate
$R = -(CH_2)_2COOH$

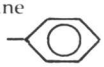
Hydrophilic and basic

Lysine
$R = -(CH_2)_4NH_2$

Sulphur containing

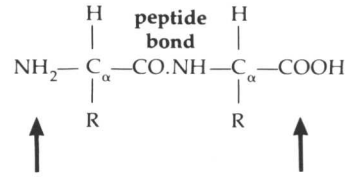
Cysteine
$R = -CH_2SH$

Hydrophobic

Phenylalanine
$R = -CH_2$ 

2

Amino acids combine by the formation of peptide bonds to form long chains called **polypeptides**. The number, type and arrangement of amino acids in a polypeptide depends on the protein, and is called the **primary structure** of the protein.

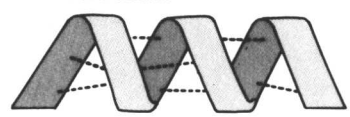


groups available for adding more amino acids

3

Between 20% and 80% of the polypeptide chain normally organises itself into configurations called α-helices or β-pleated sheets. These are called the **secondary structures** of the protein.

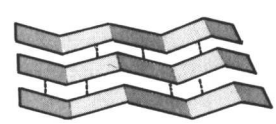
α-helix: more elastic



hydrogen bonds

In an α-helix, hydrogen bonds form between the $-NH_2$ group on the α-carbon of one amino acid, and the $-COOH$ group of the α-carbon four amino acids further along the polypeptide.

β-pleated sheet: greater tensile strength

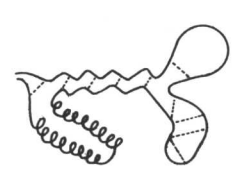


hydrogen bonds between polypeptides

In a β-pleated sheet, adjacent polypeptides are joined by hydrogen bonding between neighbouring peptide bonds. Some fibrous proteins, e.g. collagen, never form α-helices or β-pleated sheets.

4

Most of the remaining polypeptide now folds in a manner dictated by the properties of the constituent amino acids, to form the **tertiary structure**. The precise shape of the three-dimensional molecule which is formed plays a significant role in determining the properties and hence the function of the protein.



The tertiary structure is maintained by a few disulphide bridges (—S—S—) formed between sulphydryl (—SH)-containing amino acids, and a variety of weak electrical bonds such as hydrogen, dipole and electrostatic bonds. The latter are strongly affected by H^+ ions, and readily disrupted by temperature. Hence pH and heat markedly affect protein activity. Hydrophobic bonds are also present. These create small water-free zones in the protein.

5

Some proteins consist of a single polypeptide. In others, two or more polypeptides combine to form the **quaternary structure**. In many cases, such as haemoglobin, the constituent polypeptides are not identical.

6

The final product may take various forms.

Simple globular proteins: highly folded polypeptide chain(s). Over 90% of proteins are in this class.

Simple fibrous proteins: linear polypeptide chains. Keratin and collagen are in this class.

Conjugated proteins: mostly globular proteins, all combined to a non-protein component. If the compound is a tightly bound organic compound, it is called a **prosthetic group** (haem, of haemoglobin, being an example). If it is a small detachable molecule or ion it is usually called a **cofactor**.

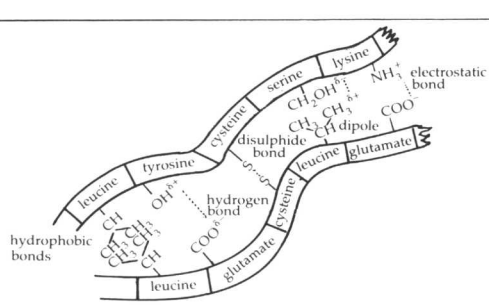


Fig. 2.1 *The structure of proteins*

Table 2.1 *Classification of Enzymes (IUB system; 1964)*

Number	Enzyme category	Type of reaction	Examples
1	Oxidoreductases	Oxidation/reduction reactions. Two types: oxidases and dehydrogenases	*Oxidases:* transfer hydrogen to oxygen, e.g. cytochrome oxidase $Cyt\ H_2 + \frac{1}{2}O_2 \rightleftharpoons Cyt + H_2O$ reduced cytochrome / oxidised cytochrome *Dehydrogenases:* transfer hydrogen to a molecule other than oxygen e.g. *lactate dehydrogenase* $C_3H_6O_3 + NAD \rightleftharpoons C_3H_4O_3 + NADH_2$ lactate / pyruvate This reaction occurs in liver when the oxygen debt is paid off following heavy exercise. e.g. *glutamate dehydrogenase* glutamate + H_2O → α-ketoglutarate + NH_3 (NAD → $NADH_2$) This reaction is an important mechanism for destroying excess amino acids. It occurs in liver cells, and is called *deamination*.
2	Transferases	Transfers a functionally important group from one molecule to another	*Transaminases*: transfer amino groups, making new amino acids from existing ones e.g. *aspartate transaminase* aspartate (amino $acid_1$) + α-ketoglutarate (carboxylic $acid_2$) ⇌ glutamate (amino $acid_2$) + oxaloacetate (carboxylic $acid_1$) Occurs in all cells. *Kinases*: transfer phosphate from (usually) ATP to another substance e.g. *hexokinase* ATP + glucose → ADP + glucose-6-phosphate This reaction 'activates' glucose prior to its breakdown in respiration. *Phosphorylases* (add inorganic phosphate without using ATP) are also in this category.
3	Hydrolases	Split molecules in two by the action of water	All digestive enzymes fall into this category: pepsin, trypsin etc e.g. *amylase* starch + H_2O → starch (one disaccharide shorter) + maltose (disaccharide) These reactions occur in the gut and transform large insoluble food molecules into smaller soluble ones which can be absorbed. Similar reactions also occur in the lysosomes of cells. *Phosphatases* (remove phosphate groups from organic molecules by hydrolysis) are also in group 3.

(continued)

Table 2.1 *Classification of Enzymes (IUB system; 1964) (continued)*

Number	Enzyme category	Type of reaction	Examples
4	Lyases	Add or remove groups without involving water	*Carboxylases*: add CO_2 e.g. *ribulose bisphosphate carboxylase* ribulose bisphosphate + CO_2 → 2 phosphoglyceric acid C5 sugar — 2 × C3 organic acid This occurs in the stroma of chloroplasts and is the principal mechanism by which atmospheric CO_2 is turned into organic material. *Decarboxylases*: remove CO_2 e.g. *pyruvate decarboxylase* pyruvic acid → ethaldehyde + CO_2 C3 — C2 — released Intermediate step in the conversion of sugars to ethanol during alcoholic fermentation.
5	Isomerases	Convert one isomer (form) of a compound into a different isomer by redistributing the atoms	*Mutases* e.g. *phosphoglucomutase* glucose-1-phosphate ⇌ glucose-6-phosphate This reaction occurs early in the respiration of sugars.
6	Ligases	Link together two molecules at the expense of ATP	*Synthetases* e.g. *aminoacyl synthetases* Join amino acids to tRNA during protein synthesis.

of α-*amylase*, for example, is 3.2.1.1. Table 2.1 is more than just a catalogue. It implies that all the biochemicals in a cell can be formed from six basic types of reaction, which is a considerable achievement considering the enormous diversity of substances present.

Most enzymes end in '-ase', as in α-*amylase*, above. The first part of the name often indicates the substrate on which the enzyme is acting: *amyl*ase (enzyme) acts on *amyl*ose (starch). Although the names of enzymes can be extraordinarily long, they are at least descriptive. Hence *'DNA-dependent RNA-polymerase'* indicates that this enzyme (*-ase*) makes RNA by polymerisation of nucleotides provided that DNA is present. Unfortunately there are some exceptions to these rules. For example, many digestive enzymes are known by their historical names, such as pepsin, trypsin etc. There is no alternative but to memorise these.

Look at Table 2.1.
Q1 Name the types of enzymes which:
(i) add CO_2
(ii) convert one isomer into another
(iii) remove hydrogen
(iv) break up large molecules into small molecules using water
(v) make new amino acids by transferring amino groups from existing ones
(vi) 'energise' molecules by adding Pi from ATP.
You will meet these enzymes several times in the following chapters.

2.2 THE MECHANISM OF ENZYME ACTION

2.2.1 Activation energy

If a reaction *can* take place (is exergonic) then it *will* take place, but it may do so very slowly. Paper does burn in air at room temperature, but extremely slowly. The brittle yellow pages of old manuscripts testify to a slow rate of combustion.

The rate of reaction is determined by how many molecules have the **activation energy** (E_A) to react together at any one moment. In a slow reaction only a small percentage of the molecules involved have the necessary amount. The activation energy therefore represents a kind of barrier which must first be overcome before the reaction can proceed (Fig. 2.2).

In principle, reactions can be speeded up in two ways:
(i) by supplying the reactants with more energy, for example by heating them;
(ii) by lowering the activation energy by means of a catalyst.

In organisms, there are limits to the amount of heat that can be supplied, because heat progressively denatures proteins. Among the proteins which could be damaged are, of course, the enzymes (Section 2.3.2). Enzymes lower activation energy barriers very dramatically. Without enzymes in your gut, it would take about 50 years instead of a few hours for your last meal to be hydrolysed (digested). Before we consider how enzymes lower the activation energy, it is necessary to take a closer look at the lock and key hypothesis.

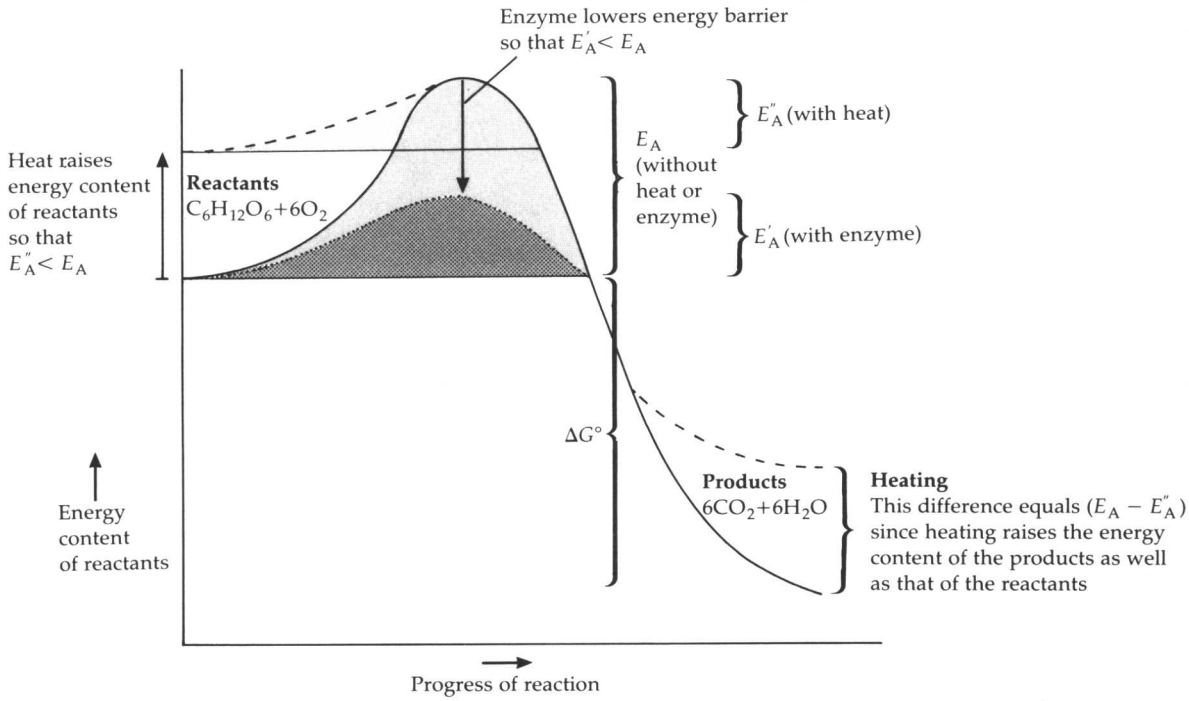


Fig. 2.2 *Activation energy: heat and enzyme action.* A secondary effect of heat is that it increases the *kinetic energy* of the reactants, so improving the chances of a collision. Although this contributes to an increase in rate, the effect is small.

2.2.2 The lock and key hypothesis

Emil Fischer's lock and key hypothesis (1894) has been an immensely useful concept. Fischer originally developed the idea in order to explain enzyme specificity. This it does admirably. For a key to work it must be provided with the right lock, and so it is with enzymes and substrates. Like all useful hypotheses, it makes testable predictions. In fact, it makes two.

The ES complex

The first prediction is that if enzymes and substrates really are analogous to locks and keys, then some reaction between an enzyme and a substrate must occur, however brief. We can therefore represent a reaction thus:

$$\underset{\text{enzyme substrate}}{E + S} \rightleftharpoons \underset{\text{complex}}{ES} \rightleftharpoons \underset{\text{enzyme product}}{E + P}$$

The most direct evidence for the formation of transient ES complexes is that they can actually be isolated from enzymes that work rather slowly. For example, under appropriate conditions chymotrypsin forms relatively stable chymotrypsin–protein complexes when the substrate (protein) is added. Other evidence comes from a technique called spectroscopy. Look at Fig. 2.3 and then Q2.

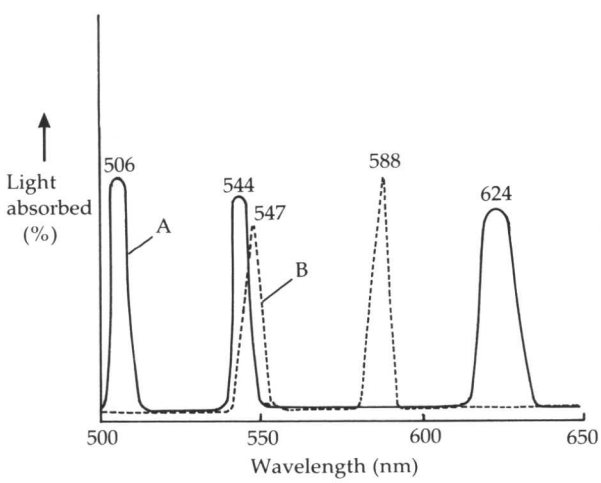


Fig. 2.3 *Spectroscopic evidence for an ES complex.* The graph shows the absorption spectrum for *catalase* before (—) and after (---) adding the substrate (H_2O_2).

Q2 A spectrophotometer is an instrument which measures the wavelengths of light absorbed by a substance. These wavelengths vary for different chemicals, and each chemical has a distinctive **absorption spectrum** (see Appendix). In the case of an enzyme, the wavelength it absorbs changes when a substrate is added, indicating a change in the enzyme. Thus in Fig. 2.3, graph A becomes graph B when equimolar concentrations of *catalase* and hydrogen peroxide are mixed. A moment later, graph B disappears and graph A reappears. What conclusions may be drawn from these results? (There are about four.)

The concept of the active site

A second prediction is that there must be one or more **active sites** on an enzyme which are the centres of

catalytic activity. Here we face a dilemma: enzymes are enormous, with a molecular mass of tens or hundreds of thousands, but substrates are often small. So are these (predicted) sites scattered all over the enzyme, or are there, say, just one or two at a fixed position? See if you can draw your own conclusions from Q3 and Q4.

Q3 *The number of substrate molecules which bind to an enzyme at any one moment in time.*

(i) What is suggested by the fact that *equimolar* concentrations of substrate and enzyme produce the changes shown in Fig. 2.3?

(ii) With a different enzyme and substrate, rather different results were obtained (Fig. 2.4). In this experiment, the wavelength of light absorbed by the substrate was measured at various concentrations of enzyme. What conclusions may be drawn from this graph?

Q4 *The position at which substrate molecules bind to an enzyme.*

A *substrate analogue* resembles a true substrate but binds very tightly to an enzyme, forming a stable ES complex. In one experiment an analogue always inactivated chymotrypsin when it bound *to one particular* site on the latter.

(i) What conclusion may be drawn from this experiment?

(ii) What assumption are you making in drawing this conclusion?

Experiments like those described in Q3 and Q4 support the view that substrate molecules bind only one or two at a time to specific points on an enzyme. They are consistent with the idea, if not direct proof, that enzymes possess specific active sites for catalysis, as implied by Fischer's lock and key hypothesis. **X-ray crystallography** is another powerful tool for determining the three-dimensional structure of an enzyme and its active site. Figure 2.5 shows the results of one such analysis on the enzyme *lactate dehydrogenase*.

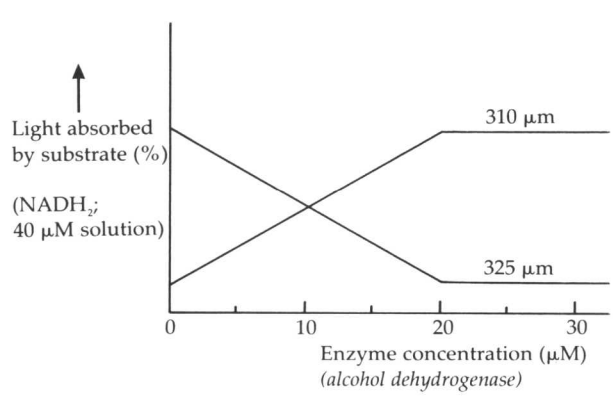


Fig. 2.4 *To determine the number of substrate molecules which bind to an enzyme at any one time: spectroscopic analysis of substrate ($NADH_2$) at increasing levels of enzyme (alcohol dehydrogenase)*

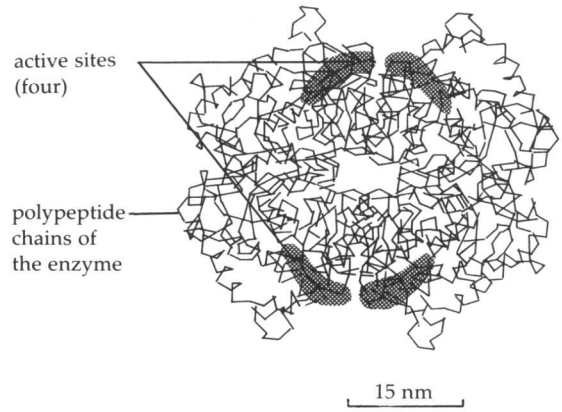


Fig. 2.5 *The structure of lactate dehydrogenase*

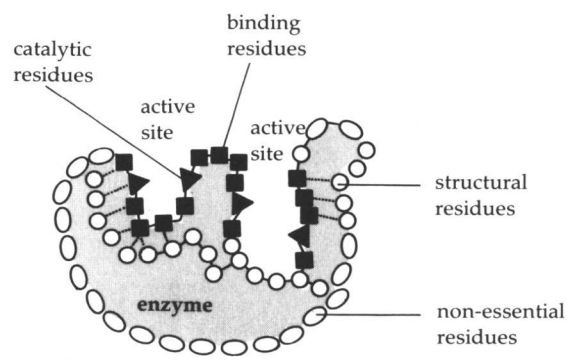


Fig. 2.6 *Amino acid residues in an enzyme*

If the active site is only a small part of the enzyme molecule, what is the rest of the molecule doing? Koshland (1963) suggested that an enzyme consists of essentially four categories of amino acids (Fig. 2.6):

(i) *Catalytic residues (catalytic site)*
These make and break chemical bonds. They are the basis of catalytic activity.

(ii) *Binding residues (binding site)*
These hold the substrate in place while catalysis is taking place.

The catalytic and binding residues together form the active site.

(iii) *Structural residues*
These hold the active site in the correct shape so that it can function properly.

(iv) *Non-essential residues*
These have no specific function. They are often near the surface of an enzyme and can be removed or replaced without loss of function.

Locks and keys reconsidered: the theory of induced fit

Evidence from protein chemistry suggests that a slight rearrangement of chemical groups occurs in both enzyme and substrate when an ES complex is formed. Enzymes are therefore best regarded as rather flexible molecules whose shape can change slightly under the influence of electrical charges present on the substrate during the formation of a complex. This idea, of an enzyme 'wrapping round' a substrate to form a more stable structure, is called the **induced-fit hypothesis**.