Essential Physiological Biochemistry

An organ-based approach

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WILEY-BLACKWELL

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This edition first published 2009

Wiley-Blackwell is an imprint of John Wiley & Sons, formed by the merger of Wiley's global Scientific, Technical and Medical business with Blackwell Publishing.

Registered office: John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Other Editorial Offices:
9600 Garsington Road, Oxford, OX4 2DQ, UK
111 River Street, Hoboken, NJ 07030-5774, USA

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Library of Congress Cataloguing-in-Publication Data

Reed, Stephen, 1954-
Essential physiological biochemistry : an organ-based approach / Stephen Reed.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-0-470-02635-9 (cloth) – ISBN 978-0-470-02636-6 (pbk.)
1. Biochemistry. 2. Organs (Anatomy) 3. Metabolism. I. Title.
[DNLM: 1. Biochemical Phenomena. 2. Metabolism–physiology. QU 34 R326e 2009]
QP514.2.R44 2009
612'.015–dc22

2009021620

ISBN: 978 0 470 02635 9 (HB) 978 0 470 02636 6 (PB)

A catalogue record for this book is available from the British Library.

Set in 10.5/12.5 pt Minion by Thomson Digital, Noida, India
Printed in Singapore by Markono Pte. Ltd

First impression – 2009

To my parents, C and I, who made so many good things happen;
To Jessica who will be an excellent physician;
To Ele who will find success outside science.

Contents

Preface

My purpose in writing this book was not to produce a comprehensive textbook of human biochemistry; there are numerous excellent textbooks that meet that need. The impetus for writing this book was to present aspects of metabolism within an appropriate physiological context. Indeed the original working title for the project was a 'hitch-hiker's guide to metabolism', with a focus on important processes, integration and control aspects without delving too deeply into chemical mechanisms. Too often, students perceive learning biochemical pathways to be 'difficult' or 'boring', largely because they are presented with a sequence of named intermediates and enzymes which are to be learnt by heart. In fact, metabolism is neither difficult nor boring, if it is approached in the right way. This book aims to present a 'right way' and so make learning and, more importantly, understanding as easy as possible. To the reader, I say this: imagine you are about to take up a new sport or you may simply want to be a spectator. The excitement of the competition may in itself be adequate for you to derive some enjoyment but to fully appreciate the sport, you will need to learn the rules or laws of the game first. Similarly, taking time and trouble to learn the rules of metabolism and learning to recognize themes will require some intellectual application. Once mastered, appreciation of the beautiful, logical simplicity of intermediary metabolism will enhance your understanding.

Whilst it is inconceivable that any text on biochemistry could be devoid of chemistry, this book is aimed at student biologists for whom biochemistry is a component part, but not the principal part, of their studies, so word equations have been used to illustrate reactions wherever possible and chemical structures shown only where necessary. The student groups who will find *Essential Physiological Biochemistry* valuable are those following undergraduate courses in physiology, nutrition, sports science and biomedical science. This text will also act as useful primer and quick reference for medicine or for postgraduates who need a revision guide.

The chapters are arranged into two distinct sections, the first dealing with basic concepts of metabolism and its control whilst the second focuses on selected tissues, illustrating how biochemistry underpins the physiological activities of certain tissues and systems. Where pathways occur in two or more organs of the body, details are given in only one chapter with cross-referencing to other relevant sections. Decisions to describe common pathways in one chapter rather than another were pragmatic ones and doubtless some would argue, not entirely appropriate.

If, having read this text, students feel that biochemistry is accessible rather than intimidating; if they become aware that metabolism is flexible, integrated and 'logical' rather than a collection of apparently disparate reactions and pathways which must be memorized, and if they acquire even a small sense of wonderment and awe about the subtlety that the subject holds, the effort of writing the book will have been justified.

Acknowledgements

The realization of this book has been a long process and it would not have happened without the advice and support of several people. First, Nicky McGirr at Wiley-Blackwell for her unwavering encouragement and whose patience and belief in the project were crucial when my own were flagging. Secondly, Fiona Woods and Robert Hambrook also at Wiley-Blackwell for valuable help and advice in the latter stages of the project.

I am grateful to many colleagues at Westminster who offered critical and constructive comment on parts of the text during its compilation. Many of their views have been accommodated, but any errors are entirely my own.

Lastly, to Gill, MBL, for her understanding.

Stephen Reed

1

Introduction to metabolism

Overview of the chapter

In this chapter we will consider definitions of metabolism; the biochemistry–physiology continuum. The concept of metabolic pathways and their organization and control of metabolism are likened to a road map involving 'flow' of substrates but with mechanisms to accelerate or slow down pathways or to direct substrates through alternative routes.

Introductions to enzyme kinetics and bioenergetics are given with explanations of key terms such as K_m and V_{max}; coenzymes, cofactors and inhibitors; typical metabolic reactions; free energy; exergonic and endergonic reactions, catabolism and anabolism.

Guidance on how to study metabolic pathways is given using glycolysis as a model pathway.

1.1 Introduction

Movement; respiration; excretion; nutrition; sensitivity; reproduction. These are the six criteria often used by biologists to define 'life'. Whilst physiologists describe many processes of human biology at the tissue and organ level, a biochemist studies the same processes but at a 'higher magnification'. To a biochemist, the six features listed above can all be described in terms of chemical events, so a useful definition of biochemistry is 'the study of life at the molecular level'. The discipline of cell biology fits between physiology and biochemistry, but the three disciplines together form a continuum of knowledge and investigation.

Biochemical studies follow several themes. For example, investigations can be focussed on the chemical *structures* of molecules, (for example the structure of glycogen, DNA or protein conformation) or the structural inter-relationship between molecules (e.g. enzymes with their substrates, hormones with their receptors). The other branch of biochemical enquiry is into those numerous '*dynamic*' events known collectively as 'metabolism', defined here as 'all of the chemical reactions and their associated energy changes occurring within cells'. The purpose of metabolism is to provide the

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energy and building materials required to sustain and reproduce cells and thereby the organism.

It is estimated that there are between 2000 and 3000 different types of metabolic reaction occurring, at various times, within human cells. Some of these are common to all cell types whilst others are restricted to one or two particular tissues whose specialized physiological functions reflect the specialized metabolic changes occurring within them. Metabolism is a fascinating, yet at first sight, complicated process apparently representing a daunting challenge for the learner. For most students, it is unnecessary to learn *every* reaction in *every* pathway; what is important is that there an *understanding of concepts* of metabolism so that what appears to be a complicated set of reactions and pathways can be seen in terms of relatively few chemical and thermodynamic (energetic) principles. Metabolism may be likened to a journey; there is a starting place and a destination and there will be some important intermediate stops, perhaps where a change of mode of transport will be necessary, there will be points of interest and also places *en route* which deserve little or none of our attention. This analogy will be further developed later in the text. Furthermore, it is vital to realize that metabolism is adaptable; changes in physiological situations, for example fed or fasting, resting or exercising, health or ill- health will result in changes in particular aspects of metabolism.

The purpose of this book is to present metabolism in an organ-based fashion to make clear the links between biochemistry and physiology. By presenting metabolism in an appropriate tissue-context, the significance of pathways and their inter-relationships should be more meaningful.

1.2 Metabolic pathways

The term 'intermediary metabolism' is used to emphasize the fact that metabolic processes occur via a series of individual chemical reactions. Such chemical reactions are usually under the control of enzymes which act upon a substrate molecule (or molecules) and produce a product molecule (or molecules) as shown in Figure 1.1. The substrates and products are referred to collectively as 'intermediates' or 'metabolites'. The product of one reaction becomes the substrate for another reaction and so the *concept* of a metabolic pathway is created.

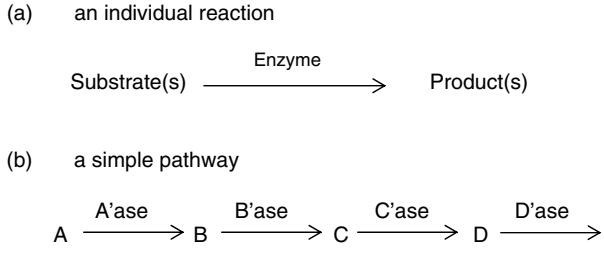


Figure 1.1 Simple representation of a metabolic pathway. 'Compound' B is the product of the first reaction and the substrate for the second reaction, and so on. Capital symbols represent metabolic intermediates and lower case letters with the suffix 'ase' represent enzymes

Metabolism and the individual reactions which comprise a pathway represent a dynamic process. Terms such as 'flow', 'substrate flux', 'rate' and 'turnover' are all used to communicate the idea of the dynamic nature of metabolism.

The student should be aware that a pathway is essentially a conceptual 'model' developed by biochemists in order to represent the flow of compounds and energy through metabolism. Such models are simply ways of trying to explain experimental data. A potential problem in representing metabolic pathways as in Figure 1.1 is that there is an implication that they are physically and/or topographically organized sequences. This is not necessarily true. With some exceptions (described in Section 1.3), most enzymes are likely to be found 'free' within the cytosol or a compartment of a cell where reactions occur when an enzyme and its substrate meet as a result of their own random motion. Clearly this would be very inefficient were it not for the fact that cells contain many copies of each enzyme and many molecules of each type of substrate.

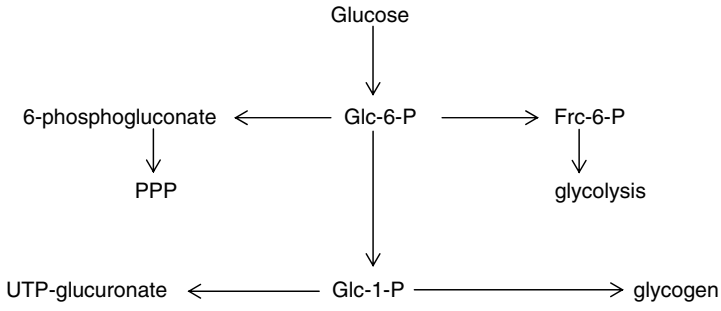
Think again about making a journey; a useful analogy is a road map of a city centre where there are main and subsidiary routes, one-way systems and interchanges, where traffic flow is controlled by signals and road-side signs. Very few people would attempt to learn, that is memorize, a complete route map, but learning the 'rules of the road' coupled with basic map reading skills and knowledge of main roads will enable most people to negotiate successfully a journey from one place to another. A complete diagram of intermediary metabolism appears to be as complicated as a road map of a city or region, that is a tangle of individual reactions with numerous substrates.

Understanding biochemical pathways is somewhat similar to map reading. The flow of traffic along roads and through the city is conceptually similar to the flow of substrates within the cell. Rather than visualizing cars, vans and trucks, think about the numerous carbon, hydrogen, nitrogen, phosphorus and oxygen atoms 'flowing' as component parts of substrate molecules, through pathways within the cell. Just as the traffic flow is regulated and directed with signals and restrictions, so too is the flow of substrates. Vehicles (metabolites) join or leave a particular traffic flow at intersections (converging or diverging pathways); the rate of flow is affected by traffic signals (enzymes), by road works or accidents (defective enzymes) and by the number of vehicles using the road (concentration of substrate molecules); they may need to take short-cuts or be diverted to avoid congested areas. Similarly, substrate molecules also may be routed via alternative pathways in a manner which best serves the physiological requirements of the cell at any particular moment. At times vehicles will need to take on fuel and some molecules need to be 'activated' by attachment to coenzyme A or uridine diphosphate (UDP), for example).

A note about terminology.

Glucose-1-phosphate (Glc-1-P) means that glucose has a phosphate attached at carbon 1 in place of a hydrogen atom.

Fructose-6-phosphate (Frc-6-P) means that fructose has a phosphate attached at carbon 6 in place of a hydrogen atom.



The relative activities of the enzymes which use glc-6-P as substrate determine the net flow.

Frc-6-P = fructose-6-phosphate
Glc-1-P = glucose-1-phosphate
PPP = pentose phosphate pathway
UTP = uridyl triphosphate

Figure 1.2 Glucose-6-phosphate is at a 'metabolic cross-roads'

1,3-bis phosphoglycerate (1,3-BPG) glyceric acid (glycerate) has 2 phosphate groups attached at carbons 1 and 3.

NB: 'bis' formerly designated as 'di'

Cells contain a large number of individual *types* of substrates; this is often referred to as the 'pool of intermediates'. One type of substrate may have a role to play in two or more pathways at different times according to the physiological demands being made on the cell. Metabolic regulation involves enzymes operating on substrates that occur at junctions of two or more pathways to act as flow-control points, rather like traffic signals. A good example of a substrate at a crossroads is glucose-6-phosphate (Glc-6-P), an intermediate that is common to glycolysis, glycogen turnover, the pentose phosphate pathway (PPP) and via UDP-glucose, the uronic acid pathway (Figure 1.2).

Clearly, substrates such as Glc-6-P do not 'belong' to a particular pathway but may occur within several routes. Channelling of the compound through a particular pathway will be determined by the relative activity of the enzymes using the substrate which in turn will be determined (regulated) by cellular requirements. Different pathways become more or less significant according to the physiological conditions (e.g. fed or fasting state, active or resting) in which the cell or organism finds itself.

1.3 Organization of pathways

Pathways can be illustrated in a metabolic map as linear, branched or cyclic processes (Figure 1.3) and are often compartmentalized within particular subcellular location: glycolysis in the cytosol and the Krebs tricarboxylic acid (TCA) cycle in

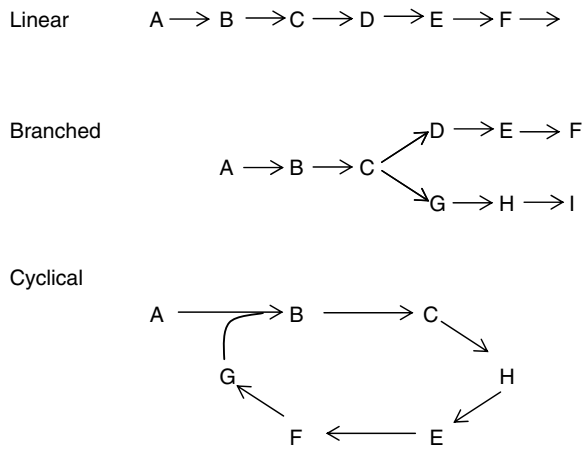


Figure 1.3 Conceptual arrangement of pathways

mitochondria are obvious examples. However, not all reactions of a particular pathway necessarily occur in the same organelle or location. Haem synthesis and urea synthesis (both described in Section 6.2) for example occur partly in the mitochondria and partly in the cytosol of liver cells.

Once an enzyme-catalysed reaction has occurred the product is released and its engagement with the next enzyme in the sequence is a somewhat random event. Only rarely is the product from one reaction passed directly onto the next enzyme in the sequence. In such cases, enzymes which catalyse consecutive reactions, *are* physically associated or aggregated with each other to form what is called a multi enzyme complex (MEC). An example of this arrangement is evident in the biosynthesis of saturated fatty acids (described in Section 6.30). Another example of an organized arrangement is one in which the individual enzyme proteins are bound to membrane, as for example with the ATP-generating mitochondrial electron transfer chain (ETC) mechanism. Intermediate substrates (or electrons in the case of the ETC) are passed directly from one immobilized protein to the next in sequence.

Biochemical reactions are interesting but they are not 'magic'. Individual chemical reactions that comprise a metabolic pathway obey, obviously, the rules of organic chemistry. All too often students make fundamental errors such as showing carbon with a valency of 3 or 5, or failing properly to balance an equation when writing reactions. Furthermore, overall chemical conversions occur in relatively small steps, that is there are usually only *small* structural changes or differences between *consecutive* compounds in a pathway.

To illustrate this point, consider the following analogy. The words we use in everyday language are composed from the same alphabet of letters. Changing even one letter within a word changes the meaning. Try converting the word WENT into COME by changing *only one* letter at a time. Each intermediate must be a meaningful English word.

This exercise is conceptually similar to biochemical conversions. One of the skills of the experimental biochemist is to identify metabolic intermediates and then to

arrange them in a chemically sensible sequence to represent the pathway, that is develop the model to explain the experimental results. A model answer to the word puzzle cited above is given at the end of the chapter.

1.4 Enzymes and enzyme-mediated reactions

This section deals with the nature of enzymes and their importance in metabolic control is discussed more fully in Chapter 3. Enzymes are biocatalysts whose key characteristics are as follows;

Enzymes are:

- Proteins;
- Chemically unchanged at the end of the reaction they catalyse and so are reusable;
- Required in small amounts because they are recycled;
- Able to act upon a specific substrate or structurally very similar substrates;
- Able to act on a particular part (functional group) within the substrate;
- Able to catalyse a specific type of chemical reaction;
- Able to operate under mild conditions of pH, temperature and pressure (if gases are involved).

1.4.1 Equilibrium or steady state?

The majority of biochemical reactions are *reversible* under physiological conditions of substrate concentration. In metabolism, we are therefore dealing with chemical *equilibria* (plural). The word equilibrium (singular) signifies a balance, which in chemical terms implies that the *rate* of a forward reaction is balanced (i.e. the same as) the *rate* of the corresponding reverse reaction.

$$r \rightleftarrows p \text{ which may also be written as } r \leftrightarrow p$$

r = reactant(s)

p = product(s)

$$r \rightarrow p \text{ is the forward reaction}$$

and

$$p \rightarrow r \text{ is the reverse reaction}$$

Many chemical reactions (especially those occurring within cells) are theoretically reversible under reasonable conditions of pressure (when gasses are involved, which is rare), temperature and concentration.

In a closed system, that is one in which there is no addition of 'r' nor any removal of 'p', the reaction will come to a perfect balance; 'the point of equilibrium'. A common misunderstanding of the concept of this point of equilibrium is that it implies an *equal concentration* of r and p. This is not true. The point of equilibrium defines the relative concentrations of r and p when the *rate* of formation of p is exactly equal to the *rate* of formation of r. The point of equilibrium value for a chemical reaction can be determined experimentally. If the starting concentration of the reactant is known, then it follows that the *relative* concentrations of r and p when equilibrium has been reached must reflect the *relative* rates of the forward and reverse reactions. For a given reaction, under defined conditions, the point of equilibrium is a constant and given the symbol K_{eq}.

Thus

$$K_{eq} = \frac{[p]}{[r]}$$

where [ ] indicates molar concentration

When the equilibrium concentration of p is *greater* than the equilibrium concentration of r, we can say that the forward reaction is favoured (faster) and $K_{eq} > 1$;

$$r \rightleftharpoons p \qquad \text{so at equilibrium, } [p] > [r]$$

NB: the weight and size of the arrows represents the relative rates of reaction

The higher the value of K_{eq}, the more difficult it is for that reaction to 'go backwards' so effectively it becomes unidirectional.

Conversely, if [r] is greater then [p], the reverse reaction is favoured and $K_{eq} < 1$ because $[p] < [r]$ signifies that the forward reaction becomes increasing less likely and the value becomes smaller. It could be argued that a 'true' equilibrium occurs only when $[r] \approx [p]$, but K_{eq} is a measure of the relative *rates* of the forward and reverse reactions. An important consequence of the magnitude of K_{eq} is that the further away a reaction is from a true equilibrium, the greater the energy change involved in that reaction. This is explained in more detail later in this chapter and also in Chapter 2.

Most individual biochemical reactions are reversible and are therefore quite correctly considered to be chemical equilibria, but cells are not closed systems; fuel (e.g. a source of carbon and, in aerobic cells, oxygen) and other resources (e.g. a source of nitrogen and phosphorus) are continually being added and waste products removed, but their relative concentrations within the cell are fairly constant being subject to only moderate fluctuation. Moreover, no biochemical reaction exists in isolation, but each is part of the overall flow of substrate through the pathway as a whole.

Stated simply, biochemical reactions never reach a true equilibrium because the product of one reaction is the substrate for the next and so the reaction is 'pulled' towards completion achieving net formation of product. Indeed, if reactions inside a cell were *true equilibria*, there would be no net flow of substrate, no formation of end

products and therefore no metabolic pathway. Biologically, this would not be very desirable! The situation which exists within cells is better described as a *steady state*. In this condition, there *is* net flow of matter but the instantaneous concentrations of intermediates fluctuate relatively little, unless a 'stress' for example the need to respond to a physiological challenge, is placed on the system.

Although there is a bewildering array of individual reactions occurring within cells they can be classified into a small number of groups. Learning the types of reactions and then identifying particular examples as and when they arise is easier than trying simply to memorize a sequence of chemical changes. Typical biochemical reactions include the following (Figures 1.4 to 1.17).

1. **Atomic and molecular rearrangements**
   Isomerization involving (a) a change in functional group or (b) the repositioning of atoms within the same molecule, for example

   a. ribose-5-P $\xrightarrow[\text{(aldehyde to ketone)}]{}$ ribulose-5-P

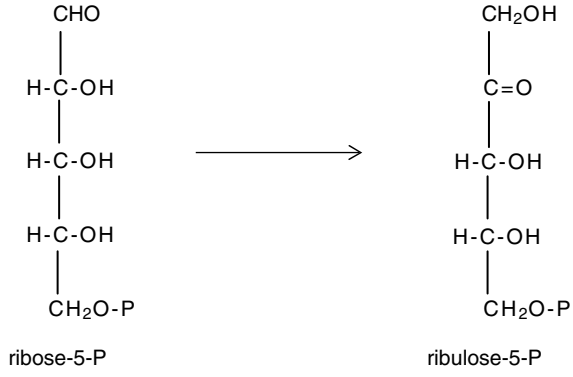


Figure 1.4 Enzyme: phosphoriboisomerase

   b. 2-phospho glycerate $\xrightarrow[\text{(repositioning of the P group from carbon 2 to carbon 3)}]{}$ 3-phospho glycerate

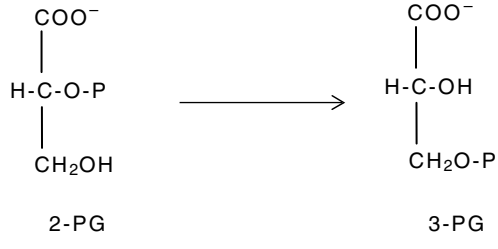


Figure 1.5 Enzyme: phosphoglyceromutase

2. **Substitution**
Replacement of one atom or group with another, for example, a hydrogen atom is replaced by a methyl group;

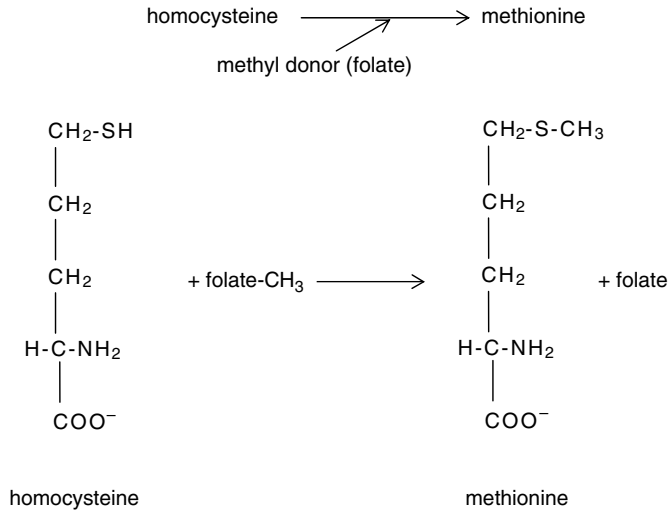


Figure 1.6 Enzyme: methionine synthase

3. **Redox reactions** ***These Are Very Important***
Oxidation and reduction reactions always occur together and are usually easily spotted because of the involvement of a coenzyme.

$$\text{A-H}_{(\text{red})} + \text{coenz(ox)} \leftrightarrow \text{B}_{(\text{ox})} + \text{coenz-H(red)}$$

Coenz = coenzyme;
(red) = reduced form;
(ox) = oxidized form (i.e. fewer hydrogen atoms/electrons than the reduced form);
for example, lactate dehydrogenase

$$\text{lactate} + \text{NAD}^+ \leftrightarrow \text{pyruvate} + \text{NADH} + \text{H}^+$$

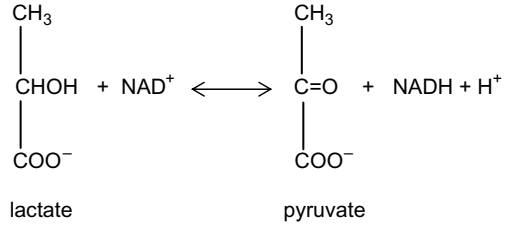


Figure 1.7 Enzyme: lactate dehydrogenase

The lactate is oxidized (two hydrogen atoms removed) and the NAD^+ is reduced to $NADH + H^+$

Oxidation sometimes occurs simultaneously with another chemical change. For example, oxidative decarboxylation or oxidative deamination.

a. Oxidative decarboxylation; CO_2 released

$$\text{pyruvate} + NAD^+ + \text{CoASH} \rightarrow \text{acetyl CoA} + CO_2 + NADH + H^+$$
$$(\text{CoASH} = \text{co enzyme A})$$

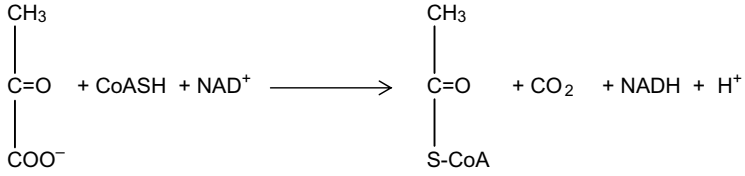


Figure 1.8 Enzyme: pyruvate decarboxylase

b. Oxidative deamination; NH_3 released

$$\text{glutamate} + NAD^+ + H_2O \rightarrow NH_3 + \text{2-oxoglutarate} + NADH + H^+$$

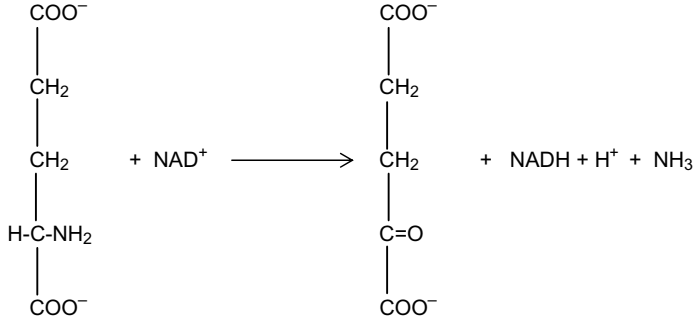


Figure 1.9 Enzyme: glutamate dehydrogenase

4. **Cleavage**

a. Hydrolysis, if water is used to break a bond

$$\text{Glucose-6-P} + H_2O \rightarrow \text{Glucose} + \text{Pi}$$ Pi symbolises free inorganic phosphate

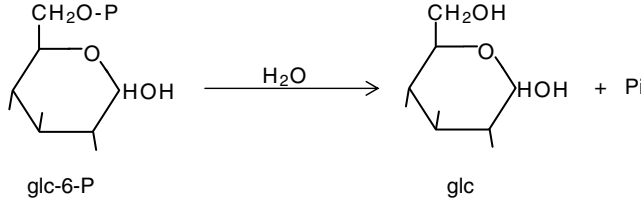
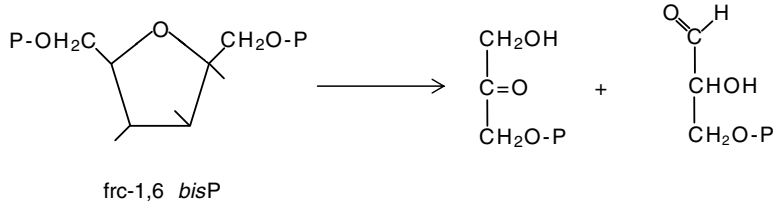


Figure 1.10 Enzyme: glucose-6-phosphatase

b. One molecule is split into two

$$\text{F-1, 6-}\textit{bis}\text{ phosphate} \rightarrow \text{glyceraldehyde-3-P} + \text{dihydroxyacetone-P}$$



frc-1,6 *bis*P

Figure 1.11 Enzyme: aldolase

5. **Condensation**

Two molecules join together with the elimination of a H_2O. Condensation reactions are used when macromolecules are being formed. Amino acids are joined via peptide bonds and monosaccharides via glycosidic bonds, both of which are condensation reactions.

$$2\ \text{glucose} \rightarrow \text{maltose} + H_2O$$

$$2\ \text{amino acids} \rightarrow \text{dipeptide} + H_2O$$

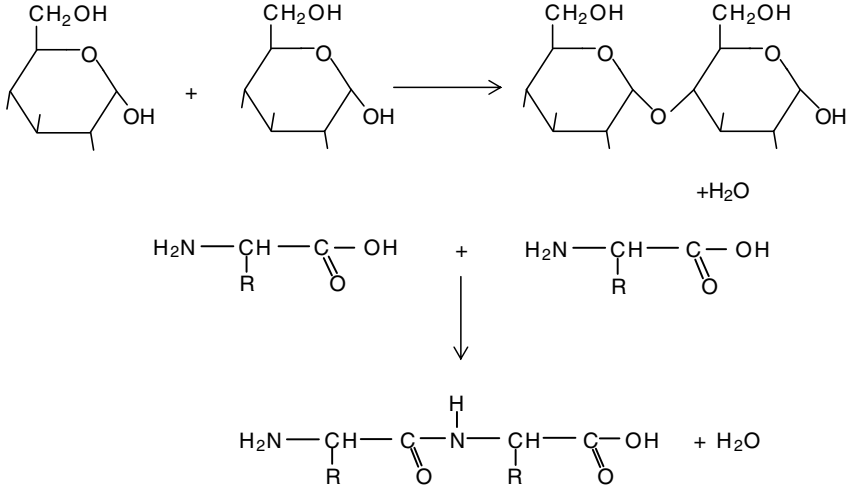


Figure 1.12 Enzymes: synthases

6. **Addition**

a. Two molecules are joined together but water is not eliminated. Often ATP is used to provide energy.

$$\text{glutamate} + NH_3 + \text{ATP} \rightarrow \text{glutamine} + \text{ADP}$$

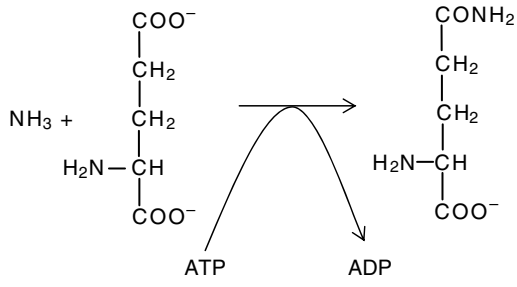


Figure 1.13 Enzyme: glutamine synthase

b. Alternatively, addition may across a double bond

$$\text{fumarate} + H_2O \rightarrow \text{malate}$$

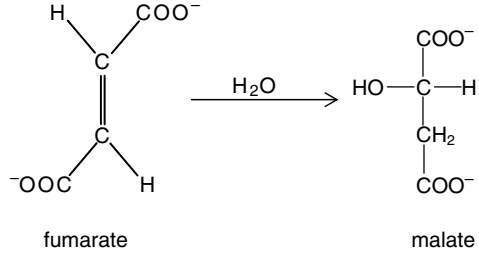


Figure 1.14 Enzyme; fumarase

7. **Transfer**

a. A phosphate group may be transferred from ATP to a substrate;

$$\text{glucose} + \text{ATP} \rightarrow \text{G-6-P} + \text{ADP}$$

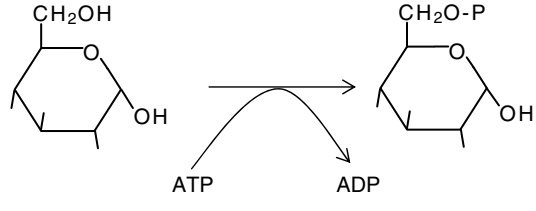


Figure 1.15 Enzyme: glucokinase or hexokinase

b. A functional group may be 'swapped' between two molecules

$$\text{glutamate} + \text{oxaloacetate} \rightarrow \text{2-oxoglutarate} + \text{aspartate}$$

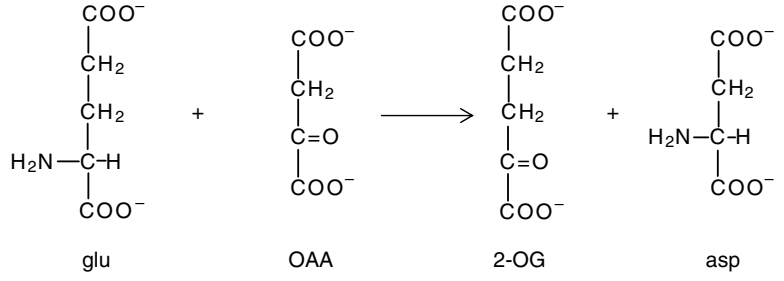


Figure 1.16 Enzyme: aspartate transaminase (= aspartate aminotransferase)

c. Quite complex chemical groupings may be transferred

sedoheptulose-7-P + glyceraldehyde-3-P → erythrose-4-P + fructose-6-P

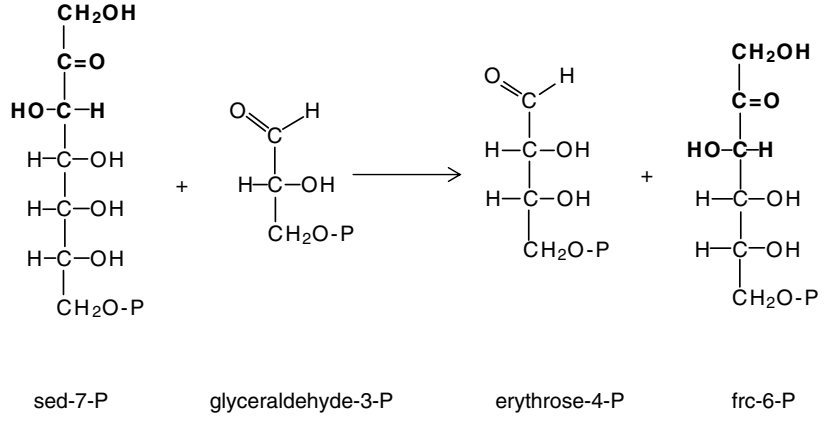


Figure 1.17 Enzyme: transaldolase

Here, a C3 unit (bold) has been transferred from sedoheptulose-7-P to glyceraldehyde-3-P.

The reactions given above illustrate the chemical changes that frequently occur in biochemistry. When you meet a reaction for the first time, it is a good idea to first of all identify the type of reaction occurring, and then look at the specific details.

Enzyme-mediated catalysis requires the breaking and making of chemical bonds between atoms; this involves changes in energy and is described by thermodynamics. Enzymes reduce the activation energy, that is make the reaction process easier to initiate but do not alter the overall energy change, which is determined by the free energy difference between the substrate(s) and the product(s). The change in free energy determines the *spontaneity* or likelihood of a reaction but the *speed* (kinetics) of an enzyme-catalysed reaction is governed by factors such substrate concentration,

enzyme concentration, pH temperature and the presence of activators or inhibitors. Principles of enzyme kinetics and thermodynamics as applied to biochemistry are dealt with in Sections 1.4.2 and 1.5 respectively, whilst a more detailed analysis and explanation of these topics can be found in Chapter 2.

1.4.2 Enzyme kinetics: an introduction

Kinetics is the study of the factors which influence reaction rates. Enzyme-catalysed reactions are subject to the same principles of rate regulation as any other type of chemical reaction. For example, the pH, temperature, pressure (if gases are involved) and concentration of reactants all impact on the velocity reactions. Unlike inorganic catalysts, like platinum for example, there is a requirement for the substrate (reactant) to engage a particular region of the enzyme known as the active site. This binding is reversible and is simply represented thus:

$$E + S \rightleftarrows [ES] \rightarrow P + E$$

Where,

E = enzyme

S = substrate

[ES] = enzyme-substrate complex

P = product

The relative rates of formation and dissociation of [ES] is denoted as K_m, the Michaelis constant. Each enzyme/substrate combination has a K_m value under defined conditions. Numerically, the K_m is the substrate concentration required to achieve 50% of the maximum velocity of the enzyme; the unit for K_m is therefore the same as the unit for substrate concentration, typically μmol/l or mmol/l. The maximum velocity the enzyme-catalysed reaction can achieve is expressed by the V_{max}; typical unit μmol/min. The significance of K_m and V_{max} will be discussed in greater detail in Chapter 2.

In a cell, enzymes do not always work at their V_{max}. The precise rate of reaction is influenced by a number of physiological (cellular) factors such as:

- [S]
- [coenzyme]
- presence of activators or inhibitors.

Because enzymes are proteins, they are subject to all of the factors (e.g. pH, temperature) which affect the three-dimensional integrity of proteins in general.

The ability of some organisms to control the pH and temperature of their cells and tissues represents a major biological development. Homeothermic animals (e.g. mammals) maintain a constant temperature of about 37 °C as this corresponds to the temperature of optimum activity of most enzymes. Poikilothermic or so-called cold-blooded animals (e.g. reptiles) have to sun themselves for sometime every morning in order to raise their body temperature in order to optimize enzyme activity within their cells.

Plants and single celled organisms have no means of autoregulating their operating temperature and thus their growth and replication are influenced by external conditions. Hence, we keep food at 4 °C in a refrigerator to prevent spoilage yet we incubate bacterial cultures at 37 °C and usually in a buffered medium when we wish to cultivate the cells for further study.

Homeostatic mechanisms also allow animals to control their intracellular pH very strictly. In humans for example, blood pH (usually taken as a reliable but indirect measure of cellular pH) is 7.4 ± 0.04. At 37 °C cytosolic pH is actually slightly lower at about 7.0 but different compartments within the eukaryotic cells may have quite different pH, for example, lysosomes have an internal pH of about 5; the inside of a mitochondrion is more alkaline than the outside whilst the inside of a phagosome in a white blood cell is more acidic than its surrounding cytosol, both situations arising due to proton pumping across a membrane.

Except in a few instances, the enzyme molecule is very much larger than the substrate(s) upon which it works. The reason for this great disparity in size is not entirely obvious, but the possibility of the enzyme binding with more than one small molecule (e.g. regulator molecules, see Section 1.4.3) arises when we are dealing with large structures.

1.4.3 Enzyme ligands: substrates, coenzymes and inhibitors

As we saw earlier in this chapter, substrates are the molecules which undergo chemical change as a result of enzyme activity. Many enzymes will only operate when in the presence of essential co-factors or coenzymes. The term 'coenzyme' is not entirely appropriate as it implies that, like enzymes themselves, these compounds do not undergo chemical change. This is not true and more accurate terminology would be co-substrate. Coenzymes are always much smaller than the enzymes with which they operate and are not heat sensitive as are the proteins.

Examples of coenzymes: vitamin-derived nucleotides; for example adenosine phosphates; ATP, ADP, AMP; nicotinamide derivatives; NAD^+, NADH, $NADP^+$, NADPH; flavin derivatives; FAD, $FADH_2$; coenzyme A (abbreviated to CoA, CoASH or CoA-SH).

Not all vitamin coenzymes need to be in the form of a nucleotide (base, sugar, phosphate). For example; thiamine; biotin; pyridoxine; vitamin B_{12}.

Some enzymes also require inorganic factors to achieve full activity. Such co-factors include metal ions, Mg^{2+}, Mn^{2+}, Zn^{2+} and non-metals, Cl^-.

Inhibitors are compounds which reduce the efficiency of an enzyme and are important in directing and regulating the flow (or flux) of substrates through a pathway. Inhibitors which bind strongly to the enzyme for example, poisons such as cyanide, cause irreversible effects, but inhibition is rarely 'all or nothing' in a cell. Most inhibitors bind reversibly (as does the substrate of course) to the enzyme. Inhibitors which are structurally very similar to the true substrate effectively 'block' the active site and are called competitive inhibitors, because they compete with the true substrate for binding to the enzyme. Here, the ratio of substrate [S] to inhibitor [I] is critical in determining the quantitative effect of the inhibitor. Non-competitive inhibitors are also act reversibly by preventing the release of the product or by distorting the shape of the enzyme so preventing the substrate accessing the active site.

1.5 Bioenergetics: an introduction to biological thermodynamics

Thus far, our discussion has considered the chemical changes which constitute metabolism. We must now introduce some fundamental ideas of bioenergetics. Further details can be found in Chapter 2.

All molecules have an amount of energy determined mainly by their chemical structure. Metabolism involves chemical change. Inevitably therefore, energy changes always accompany the chemical changes which occur in metabolism. Our understanding of bioenergetics arises from physics and the laws of thermodynamics.

The First Law of Thermodynamics states that energy can be neither created nor destroyed but different forms of energy can be interconverted. The three forms of energy which are important to us are enthalpy (heat or 'total energy', represented by the symbol H), free energy ('useful energy' symbol G, in recognition of Josiah Gibbs) and entropy ('wasted energy', symbol S). Free energy is termed 'useful' energy because it can bring about useful work such as biosynthesis, transmembrane secretion or muscle contraction. Entropy however is not available for work but is the energy associated with chaos, disorder, loss of organization or an increase in randomness. Imagine a building, a castle, a tenement, or an office block which has not been maintained and thus shows the ravages of time and neglect. The building has lost its initial organization and structure because insufficient energy has been expended on its upkeep. You are now imagining entropy.

These three energy terms we have met are related by the following equation:

$$\Delta H = \Delta G + \mathrm{T}\Delta S$$

where Δ indicates 'change in' and T is absolute temperature (Kelvin; °C + 273).

Rearranging this equation gives $\Delta G = \Delta H - \mathrm{T}\Delta S$, which shows that as entropy increases as a function of temperature, free energy decreases.