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Biochemistry Level III

The M & E TECbook Series

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Preface

This book has been written to represent the Technician Education Council's Level III Unit in Biochemistry. A student following programmes of study in the natural or chemical sciences, or in medical laboratory sciences, will need to have a knowledge of biochemistry and of the place biochemistry has in his particular field of interest. Alternatively, if he wishes to be a biochemist and work in one of those areas usually regarded as the domain of biochemistry a familiarity with the content of this book will be essential.

Each unit of a Technician Education Council course of study is built around specific "learning objectives". These have been written for two reasons: (*a*) as guidance to the topic coverage of the unit, and (*b*) as guidance for the student as to what is expected of him and to the depth each topic should be taken. The word "guidance" here is important. The reader should not regard the learning objective as his obligatory prescription for success. This would be far too daunting an approach. Instead, each learning objective should be read as a help to the student to know how best to learn the subject. His assessment, in any case, will be largely based on these objectives.

As important, of course, are those things the student is not expected to do with the body of knowledge. If the learning objectives do not suggest that you should be able to accurately draw all the structural formulae for the Krebs cycle components, described in Chapter 8—and they don't—then it would be unproductive at this level to try to do so.

This book has been written around the package of learning objectives written by a panel of biochemists, which included the author for the Technician Education Council. Several objectives have been reworded from the original draft, however, but only where, in the author's opinion, it clarifies to the reader what is expected of him. In addition, one or two further objectives have been included in order to give the subject coverage more coherence. Furthermore, several of the objectives in Chapter 5, dealing with hydrogen ion concentration and buffering in relation to amino acids and proteins, will be seen to overlap with objectives from the Chemistry II Unit, as do certain objects in Chapter 1 on Biological Stereochemistry. This has been done on purpose and without apology, in the belief that overlaps and associations between the unitised subject areas

should be looked for, encouraged and is essential in any unitised scheme of study. Other notable overlaps with the Cell Biology II Unit will be noticed in Chapter 6 and Chapter 8 on the chemistry of nuclear material and metabolism.

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Acknowledgments

The cover shows a series of peaks which represent ultra-violet absorbing metabolites and nucleotides from umbilical cord blood which have been separated by high pressure liquid chromatography. This is reproduced with the kind permission of Dr. R. A. Harkness. This chromatogram overlays a photograph of a squash preparation of giant chromosomes from the salivary gland of *Simulium* sp. My thanks are extended to Mrs. Nora Dean and Mrs. Ann Falvey for their skill and time preparing this.

My wife, Shirley, typed and re-read the manuscript and Mr. L. North and Dr. E. Kerr offered their useful and constructive criticism in an editorial capacity. All deserve my special thanks, as do Julie Nelson and Ros Woodward of Macdonald & Evans Ltd. for their work on the manuscript. Responsibility for remaining imperfections is, of course, taken by myself, the author, to whom any comments as to the progress of the TEC units in Biochemistry in relation to the text would be welcomed.

Introduction

THE SCOPE OF THE BIOCHEMIST

The scope of biochemists' interests is not limited by the traditional subdivisions within the biological, nor even the chemical sciences. Biochemistry is the study of the chemical nature of living organisms and the chemical transformations which take place within the cell. Any modern definition of biochemistry must also include a study of the molecular mechanisms (including those involving hormones) by which these transformations are controlled and integrated in such a way as to produce a living organism capable of adapting to changes in its environment, be that organism a bacterium or a mammal. So within the field of biochemistry there is room for both the chemist, who is interested in natural products such as proteins, enzymes, lipids or carbohydrates, their chemistry and metabolism, and the biologist who wishes to study life at the molecular level and how it is organised and controlled.

Yet biochemistry is a subject in its own right. The questions which biochemists ask and the techniques they use to investigate and measure biomolecular phenomena are, on the whole, different from those of a traditional organic chemist, physiologist, microbiologist, botanist or zoologist. Figure 1 illustrates those areas of the biological and chemical sciences in which biochemists may find suitable employment finding the answers to a realm of fascinating and sometimes life-saving problems. It also serves to remind us that in spite of differences in emphasis and techniques used between different branches of the chemical and biological sciences, there are common areas of interest shared by the biochemist and his colleagues working in their different areas.

Table I shows four areas which broadly define the scope of biochemists' interests. As the new student of biochemistry gains more knowledge and understanding of the subject, this categorisation may prove useful.

TABLE I. THE SCOPE OF BIOCHEMISTRY.

1. The chemistry and assay of natural products and cell components.
2. The biochemical fate of molecules and their enzyme catalysed transformations.
3. Energy transfer processess.
4. Molecular control mechanisms which enable the organism and its metabolism to adapt.

THE DEVELOPMENT OF BIOCHEMISTRY

Table II gives the reader a selection, albeit incomplete, of historical milestones in biology and biochemistry, beginning with Wohler's synthesis of urea, 150 years ago. This ended the so-called "vitalist" theory in which living matter was endowed with an intangible quality and was not expected to be composed of "ordinary" chemical

TABLE II. SOME IMPORTANT LANDMARKS IN THE HISTORY OF BIOCHEMISTRY.

1828	Wöhler	Synthesised urea from the inorganic compounds, potassium cyanate and ammonium sulphate. Ended vitalist theory.
1837	Schwann	Demonstrated alcohol production by living yeasts.
*c.*1850	Bernard	Introduced the concept of homeostasis, the constancy of the internal environment of an organism.
1868	Miescher	Isolated nucleic acid material from pus cells.
*c.*1880	Fischer	Derived the chemical structure of many natural products, particularly the sugars.
1897	Buchner	Showed that alcohol could be produced by a cell-free preparation of yeasts, i.e. from isolated enzymes.
1900	Mendel	Described plant breeding experiments from which he deduced that hereditary material was passed to subsequent generations by both male and female.
1906	Hopkins	First described vitamins as essential dietary substances. He also described the dynamic nature of metabolism.
*c.*1920	Warburg	Measured respiration in living organisms. He isolated a coloured substance, "atmungsferment", later shown to be cytochrome oxidase.
1920	Meyerhof	Demonstrated the breakdown of glycogen to lactic acid in muscle. He also described the breakdown of glucose to simpler substances.
1921	Banting and Best	Demonstrated that a pancreatic ex-

		tract containing insulin when injected into a diabetic patient caused glucose uptake in the tissues and relief of symptoms.
1937	Krebs	Elucidated a cyclic series of reactions in which a C_2 fragment of glucose breakdown (acetyl coenzyme A) is lost as two CO_2 molecules (Krebs citric acid cycle).
1953	Watson and Crick	Proposed the double helix molecular structure of DNA for the first time. This paved the way to an understanding of how DNA, the cell's hereditary material, isolated in 1868 by Miescher, could transmit genetic information and control the cells activities.
1956	Sutherland	Described the substance cyclic AMP (adenosine monophosphate) and introduced the idea that it acted within the cells of target organs for those hormones which cannot enter the cells, in order to bring the biochemical action of the hormone, i.e. the secondary messenger concept.
1956	Sanger	Published the amino acid structure of insulin, the first time this had ever been done for a protein.
1960	Yalow and Berson	Described the technique of radioimmunoassay, which could measure accurately and specifically amounts of hormones (and other substances) in biological fluids of as little as 10^{-12} g.
1961	Mitchell	Proposed the chemiosmotic theory of the mechanism of energy transfer between the electron transport chain and ATP formation, in mitochondria.
1978	Khorana	Continued to refine the newly found ability to synthesise a complete gene in the laboratory, implant it into a micro-organism and demonstrate that it could be faithfully reproduced in subsequent generations and be biologically active.

learning objectives. These questions are mainly of the "multiple choice" type, but there are also "matching block" and "assertion-reason" questions together with some calculations on pH and energetics.

In multiple choice tests, you are required to identify an "odd man out" statement out of five. Sometimes there will be one correct response out of five, in others one incorrect response out of five correct. For example, identify the incorrect statement. Which of the following animals is not a mammal?

A. Dog
B. Cat
C. Earthworm
D. Human
E. Elephant

In "matching block" tests you are given two columns of statements which are required to match up into five pairs. For example, match each symbol, A–E with the chemical element, 1–5.

A. H	1. Nitrogen
B. C	2. Hydrogen
C. O	3. Sulphur
D. N	4. Oxygen
E. S	5. Carbon

In "assertion-reason" questions, two statements are given, each of which may be correct or incorrect. If the two statements are correct, you then have to decide whether the second statement is a correct explanation of the first.

This type of question should be answered with a letter A–E, according to the following convention.

Answer	*First Statement (Assertion)*	*Second Statement (Reason)*
A	True	True and reason
B	True	True, not reason
C	True	False
D	False	True
E	False	False

e.g. Roses are red

because

Violets are blue.

the techniques at his disposal allow him. Tswett, in 1904, for example, demonstrated the separation of leaf pigments into three or four separate green and yellow bands, on a column of calcium carbonate. Today, using the technique of High Pressure Liquid Chromatography (H.P.L.C.), the effective separation, identification and accurate measurement of levels of 10^{-12} g of a substance can be made easily. Tswett's four bands could now be resolved into several hundred compounds. This simple example illustrates how new techniques can open doors previously closed and how biochemists need to ask new questions with the evolution of new technology such as what the function of the newly discovered compounds in the leaf is.

Definitions of subject boundaries, therefore, need constantly reviewing as new discoveries are made. The dotted lines in Fig. 1 which separate biochemistry from the other subject areas shown, are meant to represent the diffuse and ill-defined nature of these boundaries. In fact, any of the subjects shown in Fig. 1 could exchange with biochemistry in the centre, making the point even more strongly. Nature, of course, knows no subject boundaries. Frequently such definitions are more value to those who teach than those who either practice or learn the subject.

Further study of biochemistry beyond the objectives of the present text will appropriately include such subjects as vitamin and mineral metabolism, a study of techniques of particular interest to the biochemist, a further study of the metabolism of carbohydrates, lipids and nitrogen-containing compounds, such as glycogen, steroids and porphyrins, the biochemistry of the immune system, a review of the variety of biochemical systems exhibited by bacteria, a further study of enzymes and the role of hormones in metabolism.

The order in which the chapters appear in the book and that in which they are read may differ. However, Chapter 1 should be read before Chapters 2, 3 and 6; Chapter 4 before Chapters 5 and 7 and a study of Chapters 8 and 9 should wait until last.

LEARNING OBJECTIVES

At the beginning of each chapter is given an over-all objective in terms of the learning outcome on the part of the reader. Following this is a more detailed list of performance objectives which the reader should aim to be able to do after studying the chapter.

SELF-ASSESSMENT QUESTIONS

At the end of each chapter is a list of objective tests which are designed to help the reader assess whether or not he has realised the

Here, both statements are true but the second is not a correct reason for the first, so the answer, according to the table above is "B".

In addition to assessing whether the chapter objectives have been reached, the self-assessment questions also have an important teaching and reinforcement function. They are designed to highlight the more important concepts and draw distinctions and comparisons by the choice of alternative answers ("distractors"). For this reason, the reader is encouraged to consider carefully these alternatives and why they are wrong as well as why the correct one is correct. The answers to the self-assessment questions are given in the Appendix.

Contents

CHAPTER ONE

Biological Stereochemistry

CHAPTER OBJECTIVES

After studying this chapter you should be able to:

* regard all molecules as three-dimensional and understand that enzymes can "recognise" subtle differences in the chemical structure of organic molecules in the cell;
* explain why single bonded carbon atoms have a tetrahedral shape;
* explain why double bonded carbon atoms are planar;
* use Fischer Projection Formulae to represent carbon-containing molecules;
* understand the meaning of the symbols *"D"*, *"L"*, (+), and (−);
* know what is meant by the term "isomerism" and give examples of stereoisomers found as natural products;
* state the criterion for optical activity in organic molecules;
* recognise *"D"* and *"L"* series monosaccharides and amino acids.

INTRODUCTION

This chapter begins by attempting to explain to the new student of biochemistry the importance of the three-dimensional shape of molecules to the organisation within a living cell. Some of the properties of the carbon atom are discussed and, in particular, how these give rise to shape and isomerism in organic molecules found in nature. Finally, at the end of the chapter, we are reminded of the precise and intimate binding which must occur between naturally occuring molecules and enzymes and that this is one important feature of the control of reactions in the cell.

SHAPES OF MOLECULES IN THE CELL

There are all sorts of shapes and sizes of molecules to be found in the cells of animals, plants and micro-organisms. This is not an accident of nature. The shape of a molecule is one of the ways by which an enzyme can "recognise" it and therefore distinguish it from all the other hundreds of molecules in the cell. This is an aspect of the specificity between an enzyme and its substrate and

without such specificity enzymes could not exert order and control over the chemical reactions which take place in the cell. Instead of a highly organised metabolism, we would have a chaotic jumble of random reactions incompatible with life. The aim of biochemistry is to unravel this complex system of reactions in order to see how it is controlled to give what we recognise as "life" and to study the chemical components of these metabolic reactions.

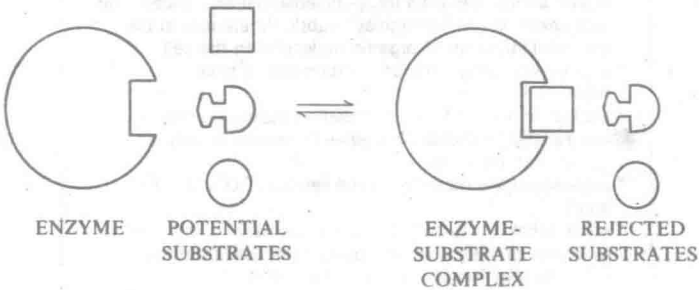

Fig. 2. *Diagrammatic representation of enzyme–substrate binding.*

Enzymes provide us with the key to how reactions in a cell are controlled, whether a reaction will take place or not, and, if so, how fast. We shall be looking at enzymes in more detail in a later chapter. They are all proteins and have a particular *binding site* for chemically binding to the specific molecule known as the substrate of the enzyme. If a molecule is not the right shape, it will not bind to the enzyme and will not, therefore, be accepted as its substrate. Figure 2 summarises this idea. An enzyme presented with a range of molecules all having different shapes and chemistries, will be able to bind only to those which "fit" its active site. The enzyme urease, for example, will only bind to the compound urea, its specific substrate. No other molecule will fit.

Enzymes and their substrates are not the only natural products whose molecular architecture is important to the life of the organism to which they belong. Molecular structure has been selected for and designed by nature for a particular function as clearly as the nose on your face has. For example, the plant cell wall polysaccharide, cellulose, has a stringy, long structure and is insoluble, ideally suited for its structural role in the plant. The long, helical, interwoven structure of the animal protein, collagen, is strong and insoluble for its structural role in bone and connective tissue. The protein haemoglobin is roughly spherical in shape. It has ionic groups on its surface which make it soluble and what is more, has

the remarkable ability to actually change its shape after it has bound to an oxygen molecule. This has the surprising effect of making the haemoglobin molecule bind to yet another oxygen molecule with a greater affinity than for the first. "To him that hath shall be given even more", has been said of the haemoglobin molecule and it is all due to a question of shape! It does mean, of course, that the haemoglobin molecule is beautifully adapted to its main physiological role of transporting oxygen.

Over-all molecular shape, however, is only the sum of all the individual atoms and chemical bonds which make up the molecule. The carbon atom is the one common feature of all organic and biochemical compounds. We shall now go on to examine some of the unique chemical properties of carbon to see how these particularly suit it for its role in biochemistry.

THE CARBON ATOM

Carbon is in Group Four of the Periodic Table and has an atomic number of six. Its valency is four. Two electrons are in a 1*s* orbital, two in a 2*s* orbital and two are unpaired in 2*p* orbitals as illustrated in Fig. 3.

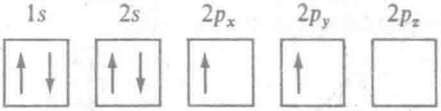

Fig. 3. *Ground electronic state of carbon.*

When a chemical bond forms between carbon and another element, one of the 2*s* electrons is "promoted" to the $2p_z$ orbital to give the electronic state represented in Fig. 4.

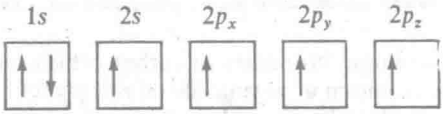

Fig. 4. *Promoted electronic state of carbon.*

After this promotion, the four electrons, 2*s*, $2p_x$, $2p_y$ and $2p_z$ are considered to assume the same "character" by a process known as *hybridisation* and are then called sp^3 electrons. Figure 5 helps to represent this hybridised condition in which the carbon atom is now ready to form a single bond with another atom by sharing each of these unpaired, hybridised electrons.

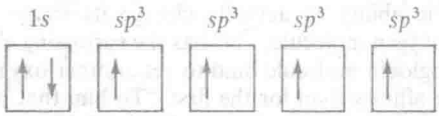

Fig. 5. *sp^3 hybridised state of carbon.*

Each of these unpaired sp^3 electrons occupies space around the carbon atom called an orbital. In an attempt to get as far away from each other as possible, these orbitals point to the four corners of a tetrahedron. Single bonded carbon compounds always possess these tetrahedrally shaped carbon atoms for this reason.

BONDING

Single bonds

When a bond forms between an atom of carbon and another atom, one of the sp^3 electrons "overlaps" or is "shared" with an electron from the other atom to form a covalent bond. Single bonds formed in this way are called σ-bonds (sigma bonds). Each σ-bond, therefore, is a pair of electrons. When an sp^3 hybridised carbon atom forms four single σ-bonds, they actually point in this tetrahedral manner as represented in Fig. 6 (*a*) and (*b*).

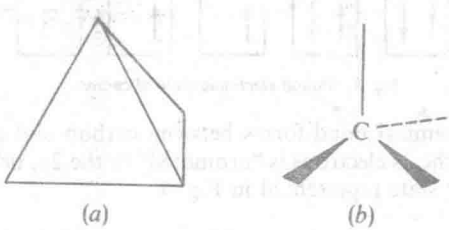

Fig. 6. (a) *A tetrahedron.* (b) *Four carbon bonds pointing to the four corners of a tetrahedron.*

One of the unique properties of carbon which has made it so central in the evolution of organic life on this planet, is its ability to form stable covalent bonds with such a variety of elements. The electropositive hydrogen on the one hand and the electronegative halogens, oxygen and sulphur, on the other, all form stable bonds with carbon. Furthermore, carbon has the rare ability to form long chains. Two naturally occuring compounds which illustrate this versatile bonding capability are the amino acid, *L*-serine, and the fatty acid, stearic acid, $CH_3(CH_2)_{16}COOH$. In serine, a carbon atom is bonded to oxygen, hydrogen, nitrogen and another carbon