COMPREHENSIVE BIOCHEMISTRY

EDITED BY

MARCEL FLORKIN

AND

ELMER H. STOTZ

VOLUME 3

METHODS FOR THE STUDY OF MOLECULES

COMPREHENSIVE BIOCHEMISTRY

EDITED BY

MARCEL FLORKIN

Professor of Biochemistry, University of Liège (Belgium)

AND

ELMER H. STOTZ

Professor of Biochemistry, University of Rochester, School of Medicine and Dentistry, Rochester, N.Y. (U.S.A.)

VOLUME 3

METHODS FOR THE STUDY OF MOLECULES

ELSEVIER PUBLISHING COMPANY

AMSTERDAM · NEW YORK

1962

CONTRIBUTORS TO THIS VOLUME

L. J. BELLAMY
Explosives Research and Development Establishment, Ministry of Aviation, Waltham Abbey, Essex (Great Britain)

G. J. BULLEN, B.Sc., PH.D., A.R.I.C.
Crystallography Laboratory, Department of Physics, Birkbeck College, University of London, W.C.1 (Great Britain)

MICHEL DAUNE
Maître de Conférences à la Faculté des Sciences de Strasbourg, Centre de Recherches sur les Macromolécules, 6, rue Boussingault, Strasbourg (France)

ANDERS EHRENBERG
Biochemical Department, Karolinska Institutet, Stockholm (Sweden)

CHRISTINE D. JARDETZKY, PH.D.
Biological Laboratories, Harvard University, Cambridge 38, Mass. (U.S.A.)

OLEG JARDETZKY, M.D., PH.D.
Department of Pharmacology, Harvard Medical School, Boston 15, Mass. (U.S.A.)

RICHARD ALAN MORTON, PH.D., D.Sc., F.R.I.C., F.R.S.
Johnston Professor of Biochemistry, Department of Biochemistry, The University, Liverpool 3 (Great Britain)

NORMAN H. NACHTRIEB, PH.D.
Department of Chemistry and Institute for the Study of Metals, University of Chicago, 5640 Ellis Avenue, Chicago 37, Ill. (U.S.A.)

CHARLES SADRON
Professeur à la Faculté des Sciences de Strasbourg, Directeur du Centre de Recherches sur les Macromolécules, 6, rue Boussingault, Strasbourg (France)

HUGO THEORELL
Biochemical Department, Karolinska Institutet, Stockholm (Sweden)

S. I. WEISSMAN, PH.D.
Department of Chemistry, Washington University, Saint Louis 30, Mo. (U.S.A.)

GENERAL PREFACE

The Editors are keenly aware that the literature of Biochemistry is already very large, in fact so widespread that it is increasingly difficult to assemble the most pertinent material in a given area. Beyond the ordinary textbook the subject matter of the rapidly expanding knowledge of biochemistry is spread among innumerable journals, monographs, and series of reviews. The Editors believe that there is a real place for an advanced treatise in biochemistry which assembles the principal areas of the subject in a single set of books.

It would be ideal if an individual or small group of biochemists could produce such an advanced treatise, and within the time to keep reasonably abreast of rapid advances, but this is at least difficult if not impossible. Instead, the Editors with the advice of the Advisory Board, have assembled what they consider the best possible sequence of chapters written by competent authors; they must take the responsibility for inevitable gaps of subject matter and duplication which may result from this procedure.

Most evident to the modern biochemist, apart from the body of knowledge of the chemistry and metabolism of biological substances, is the extent to which he must draw from recent concepts of physical and organic chemistry, and in turn project into the vast field of biology. Thus in the organization of Comprehensive Biochemistry, the middle three sections, Chemistry of Biological Compounds, Biochemical Reaction Mechanisms, and Metabolism may be considered classical biochemistry, while the first and last sections provide selected material on the origins and projections of the subject.

It is hoped that sub-division of the sections into bound volumes will not only be convenient, but will find favour among students concerned with specialized areas, and will permit easier future revisions of the individual volumes. Toward the latter end particularly, the Editors will welcome all comments in their effort to produce a useful and efficient source of biochemical knowledge.

Liège/Rochester
July 1962

M. FLORKIN
E. H. STOTZ

PREFACE TO SECTION I

(VOLUMES 1–4)

Students and teachers of Biochemistry would not deny the importance of a sound understanding of at least certain areas of organic and physical chemistry in the comprehension of modern biochemistry. Toward this end the Editors have constituted the first section of Comprehensive Biochemistry. This section is intended neither as a textbook of organic nor of physical chemistry, but rather as a collection of chapters which seem generally pertinent in the interpretation of biochemical techniques and in the understanding of the chemistry of biological compounds and reaction mechanisms. Certain areas of organic and physical chemistry have been reserved for later presentation in context with specific biochemical topics, but the material of Section I seems to the authors to underlie all of modern biochemistry. The choice of material for Section I may well not agree with that of individual readers, and comments toward the construction of future volumes will be appreciated.

Section I has been subdivided into groups of topics designated as Atomic and Molecular Structure (Volume 1), Organic and Physical Chemistry (Volume 2), Methods for the Study of Molecules (Volume 3), and Separation Methods (Volume 4). It is hoped that all may find general favour, and that the individual volumes will find a special place on the shelf of the specialist.

Liège/Rochester
July 1962

M. FLORKIN
E. H. STOTZ

CONTENTS

VOLUME 3

METHODS FOR THE STUDY OF MOLECULES

Chapter I. Crystallography

by G. J. BULLEN

Chapter II. X-Ray Diffraction

by G. J. BULLEN

Chapter III. Analysis by Emission Spectroscopy

by N. H. NACHTRIEB

Chapter IV. Spectrophotometry in the Ultraviolet and Visible Regions

by R. A. MORTON

Chapter V. Infrared Spectra of Compounds of Biological Interest

by L. J. BELLAMY

Chapter VI. Fluorescence

by A. EHRENBERG AND H. THEORELL

Chapter VII. Electronic Paramagnetic Resonance

by S. I. WEISSMAN

Chapter VIII. Nuclear Magnetic Resonance

by C. D. JARDETZKY AND O. JARDETZKY

Chapter IX. Determination of Mass, Form and Dimensions of Large Particles in Solution

by CH. SADRON AND M. DAUNE

Chapter I

Crystallography

G. J. BULLEN

Birkbeck College, Crystallography Laboratory, University of London (Great Britain)

1. Introduction

The aim of the two chapters *Crystallography* and *X-ray Diffraction* is to acquaint the reader with the information, useful to him as a biochemist, that can be obtained from crystallographic studies. The former chapter deals with the crystal lattice and the terminology used to describe it and with methods of determining crystal structures from the diffraction data; in the latter are discussed the principles of X-ray diffraction and the experimental techniques used to record the diffraction pattern. We shall be concerned not only with deriving a detailed picture of the atomic and molecular arrangement from this pattern but also with the briefer measurements of unit cell dimensions and optical and other physical properties which can provide useful information quickly.

The fact that crystals diffract X-rays (and also electron and neutron beams) shows that they are made up of a regular three-dimensional arrangement of atoms or molecules in which a motif containing a relatively small number of molecules is repeated parallel to itself many thousands of times. It is this regularity of structure which is responsible for the regular external shape of the crystal though the latter is not the most characteristic manifestation of the structural regularity. Of more importance is anisotropy of properties. A crystal is homogeneous, *i.e.* it has identical properties at all points within it, but it is, in general, anisotropic; directed properties such as thermal conductivity or the speed of propagation of light depend upon direction in the crystal. The external regularity may be removed, *e.g.* by unequal growth of faces or by dissolving faces away, but the anisotropy remains.

References p. 32

2. The crystal lattice

As the internal structure of a crystal is periodic it can be discussed in terms of a lattice, which is an array of points in three dimensions such that the surroundings of each point are identical. We can choose any point in the motif as a lattice point; the array of similarly situated points in all other motifs then constitutes the lattice. The size and shape of a lattice are specified by

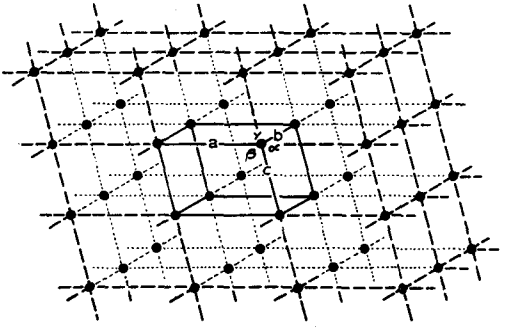

Fig. 1. A crystal lattice with one unit cell outlined. (Courtesy of K. LONSDALE, *The Centennial Review*, II (1958) 275.)

six parameters, the three primitive translations a, b and c (Fig. 1) and the three angles between them α, β and γ. The parallelepiped with sides a, b, and c, which may be taken as the motif that is repeated to form the crystal, is called the *unit cell*. The translations a, b and c are the axes of a co-ordinate system (not necessarily Cartesian) used to describe the geometry of the molecular arrangement.

If the molecules in the unit cell are arranged symmetrically, *i.e.* related in position and orientation by symmetry operations, the lattice will also possess symmetry. It is the symmetry of internal structure that is responsible for the external symmetry of the crystal faces. Crystal symmetry is described using the following symmetry elements:

(a) Rotation axes which may be 2-, 3-, 4-, or 6-fold

A figure is said to possess an X-fold rotation axis if it is brought into self-coincidence by a rotation of $360°/X$ about that axis. The regular tetrahedron (Fig. 2a), for example, has a 3-fold axis passing through each vertex perpendicular to the opposite face. When the tetrahedron has been rotated through 120° about one of these axes it presents the same appearance to the

observer as it did before. The rotation axes are also termed diad, triad, tetrad, and hexad respectively. A 1-fold axis is equivalent to no symmetry at all, *i.e.* to identity. Lattices do not exhibit 5-fold axes or axes of order higher than 6.

(b) Rotation–inversion axes

The operation of an X-fold rotation–inversion, symbol $\bar{X}$, is one of rotation through 360°/X about the axis followed by inversion through a centre (X = 1, 2, 3, 4, or 6). The regular tetrahedron has $\bar{4}$ axes passing through the mid-points of opposite edges. The methane molecule (Fig. 2b) has tetrahedral symmetry, containing $\bar{4}$ and triad axes. The description of

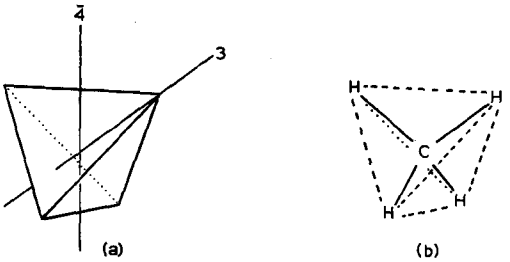

Fig. 2. (a) Symmetry axes of the regular tetrahedron; (b) The methane molecule.

all symmetry elements as either rotation axes or rotation–inversion axes has the merit of being systematic, although some rotation–inversion axes are equivalent to more familiar concepts. Thus the $\bar{1}$ axis is equivalent to a centre of symmetry, $\bar{2}$ to a mirror plane placed perpendicular to the $\bar{2}$ axis, and $\bar{6}$ to a triad axis together with a mirror plane perpendicular to it.

A given crystal may exhibit several symmetry elements passing in different directions. One in the shape of a regular tetrahedron, for example, has three $\bar{4}$ axes like that shown in Fig. 2a, four triad axes through the four vertices, and six mirror planes ($\equiv \bar{2}$) each containing an edge and bisecting the opposite edge. A combination of symmetry elements like this is called a *point group*; thirty-two point groups can be constructed using the symmetry elements described above and crystals are accordingly classified as belonging to one of thirty-two *crystal classes*. Two systems of symbols are used for point groups. In the Hermann–Mauguin system, usually preferred by crystallographers, the symbol comprises the symmetry elements required to specify the point group. For example the symbol for the symmetry of the tetrahedron is $\bar{4}3m$. The basic notation is

References p. 32

Rotation axis X, Inversion axis $\bar{X}$

Rotation axis with mirror plane normal to it X/m

Rotation (inversion) axis with mirror plane parallel to it Xm ($\bar{X}$m)

Rotation (inversion) axis with diad axis normal to it X2 ($\bar{X}$2)

Rotation axis with mirror planes both normal and parallel to it X/mm

Examples: 4/m, 4mm (two sets of mirror planes parallel to 4), $\bar{4}$2m, 4/mmm.

For a full explanation of point-group symbols the reader should consult Volume I of *International Tables for X-ray Crystallography*[1].

The shape of the lattice, *i.e.* the relative lengths of a, b and c, and the values of the interaxial angles, depends upon the symmetry of the arrangement of the molecules in the unit cell. For example, if the point group of the crystal is one containing a tetrad axis, the lattice must have a square cross-section, *i.e.* $a = b$ (taking c parallel to the tetrad) and $\alpha = \beta = \gamma = 90°$. The crystal classes are arranged in seven systems each characterized by the possession of a certain minimum symmetry and a consequent shape of lattice. The systems are listed in Table I with the minimum symmetry required for each and the corresponding relations between the axial parameters.

TABLE I

CRYSTAL SYSTEMS

System	*Minimum symmetry*	*Axial and angular relationships*
Triclinic	None *or* centre of symmetry only	$a \neq b \neq c$ $\alpha \neq \beta \neq \gamma \neq 90°$
Monoclinic	One 2-fold axis (rotation or inversion) usually placed parallel to b	$a \neq b \neq c$ $\alpha = \gamma = 90°, \beta \neq 90°$
Orthorhombic	Three mutually perpendicular 2-fold axes (rotation or inversion) parallel to a, b, c	$a \neq b \neq c$ $\alpha = \beta = \gamma = 90°$
Tetragonal	One 4-fold axis (rotation or inversion) placed parallel to c	$a = b \neq c$ $\alpha = \beta = \gamma = 90°$
Hexagonal	One 6-fold axis (rotation or inversion) placed parallel to c	$a = b \neq c$ $\alpha = \beta = 90°, \gamma = 120°$
Trigonal	One 3-fold axis (rotation or inversion) parallel to c (using hexagonal axes) or to [111] (using rhombohedral axes)	Hexagonal axes: $a = b \neq c$ $\alpha = \beta = 90°, \gamma = 120°$ Rhombohedral axes: $a = b = c$ $\alpha = \beta = \gamma < 120° \neq 90°$
Cubic	Four 3-fold axes each inclined at 54°44′ to the crystallographic axes	$a = b = c$ $\alpha = \beta = \gamma = 90°$

Note: The sign $\neq$ is used to mean "is not necessarily equal to"; equality may occur accidentally, though not as a consequence of symmetry.

A lattice may be primitive (symbol P), with only one lattice point in each unit cell (as in Fig. 1), or centred, with 2, 3 or 4 lattice points to each cell. For example Fig. 3 shows the cubic lattices. Besides the P lattice we can have a body-centred (I) lattice with a lattice point at the centre of the unit cell as well as at each corner, or an all-face-centred (F) lattice where the mid-points of the faces are lattice points (4 points per cell). In systems with lower symmetry there may be centring of only one pair of opposite faces of the cell (A, B, or C depending on which faces are centred). As trigonal and hexagonal lattices have the same shape, one system of axes (called hexagonal) is used for both. In the trigonal system the unit cell based on hexagonal axes can contain three lattice points, spaced equally along a

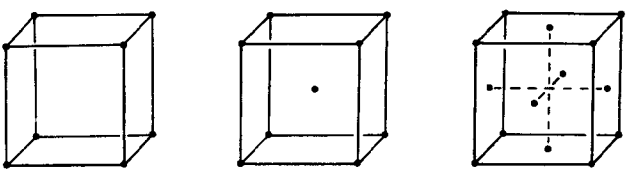

Fig. 3. The three cubic lattices.

body diagonal of the cell. The lattice is known as rhombohedral (R) because it can be described using the alternative system of rhombohedral axes. Note that the lattice becomes primitive if rhombohedral axes are used, but is centred when described in terms of hexagonal axes. In other words the rhombohedral unit cell has one-third the volume of the corresponding hexagonal cell and has lattice points only at its corners. The lattice symbol is R whichever system of axes is used. There are also trigonal crystals which have a unit cell based on hexagonal axes which is primitive. They have a P lattice and can in no circumstances be described using rhombohedral axes. In all there are 14 different lattices, known as Bravais lattices, distributed among the 7 systems.

For the purpose of interpreting X-ray diffraction by means of the Bragg equation (see Chapter II, p. 35) lattice points are considered as lying on sets of equidistant parallel planes (see Fig. 4 for a two-dimensional example). The orientation and spacing of a set of planes are specified by three indices h, k, l (called Miller indices) derived from the intercepts $a/h, b/k, c/l$, made on the crystallographic (lattice) axes by that plane in the set which is closest to but does not pass through the origin. As a consequence of the planes being drawn through lattice points, these intercepts will always be simple sub-multiples of a, b, c, so that the Miller indices will be positive or negative integers (except that when a plane is parallel to an axis, the intercept will

References p. 32

be infinite and the index zero). Defined in this way h, k, l cannot contain a common factor (but see Chapter II, p. 35 for a modification). The symbol (hkl) is used to denote the complete set of parallel planes. The distance between any two adjacent parallel planes or interplanar spacing d can be evaluated in terms of the Miller indices (the expression depends on the shape of the lattice, *e.g.* for an orthorhombic lattice, $1/d^2 = h^2/a^2 + k^2/b^2 + l^2/c^2$). In general as h, k, l increase, d decreases, *i.e.* the planes become closer together. Widely spaced planes such as (11) in Fig. 4 (two indices only are used for this two-dimensional example) are populated by many lattice points while planes with higher indices such as (23) contain fewer points. It is a fact of observation, expressed as the Law of Rational Indices, that the

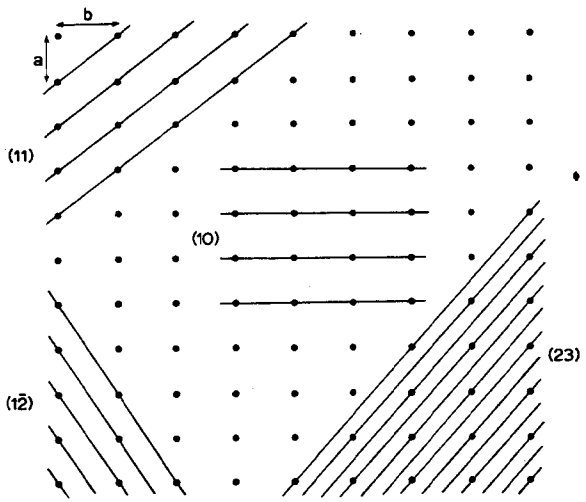

Fig. 4. Sets of planes in a crystal lattice.

external faces of a crystal are parallel to lattice planes with low indices (rarely greater than 3), *i.e.* planes clos ly populated by lattice points.

When a number of different lattice planes are parallel to a common direction, that direction, parallel to a row of lattice points, is called a zone axis. The most obvious zone axes are the crystallographic axes; for example, all (hko) planes will intersect in a zone axis parallel to c. If the co-ordinates of the first lattice point from the origin on the row of points parallel to a zone axis are ua, vb, wc (u, v, w being integers) the zone axis is given the symbol [uvw]. Co-ordinates of points in the unit cell are expressed as fractions of the cell edges a, b, c, *e.g.* the point (x, y, z) has actual co-ordinates (in Å say)

xa, yb, zc. Thus ($\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$) is the centre of the cell, ($\frac{1}{2}$, $\frac{1}{2}$, 0) the mid-point of the c face (the face parallel to a and b).

There are two further types of symmetry element which can occur in a crystal structure based on an infinite lattice but which cannot express the symmetry of a finite body such as a single crystal or a molecule. These are the screw axis and the glide plane. The operation of a screw axis X_n ($n < X$) is one of rotation through 360°/X followed by translation parallel to the axis by a fraction n/X of the lattice translation in that direction. A 2_1 screw, for example, involves rotation through 180° and translation by one half the lattice translation (Fig. 5) so that by two consecutive operations an atom is transferred to a similarly situated point in the next unit cell. Further opera-

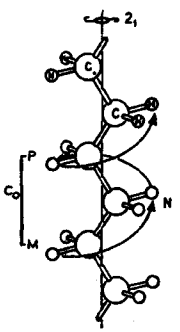

Fig. 5. A 2_1 screw axis relating the CH_2 groups of the polyethylene chain. Group M is brought successively to positions N, P, and so on, by rotation through 180° followed by translation along the axis by $\frac{1}{2}c_0$ (c_0 is the lattice translation). It is not necessary that, as here, the units (CH_2 groups) related by the screw be part of the same chemical molecule. Each could as well be an isolated molecule. (Courtesy of C. W. BUNN, *Chemical Crystallography*, Oxford University Press, 1945, p. 230.)

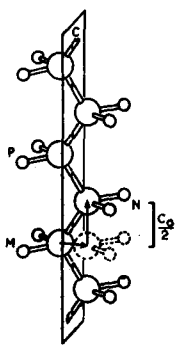

Fig. 6. Use of a c glide plane to relate the CH_2 groups of the polyethylene chain. Group M is moved to N by reflection in the plane followed by a translation of $\frac{1}{2}c_0$, and so on. (Courtesy of C. W. BUNN, *Chemical Crystallography*, Oxford University Press, 1945, p. 230.)

tions produce a screw motion through the lattice. It is obvious that the incorporation of translation prevents one applying this symmetry to a finite body. There are no inversion screw axes.

Glide planes a, b, c, n involve the operation of reflexion followed by a translation (parallel to the symmetry plane) of half a cell dimension (lattice translation) in the a, b, c or diagonal directions respectively (Fig. 6). When the lattice is non-primitive it is possible to have a d glide plane for which the translation is by one quarter of the diagonal.

A complete description of the symmetry of the atomic arrangement, *i.e.* of the space group, requires specification of the Bravais lattice and of the

References p. 32