COMPREHENSIVE BIOCHEMISTRY

EDITED BY

MARCEL FLORKIN

AND

ELMER H. STOTZ

VOLUME 2

ORGANIC AND PHYSICAL CHEMISTRY

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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
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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.

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Chapter I

Mechanisms of Organic Reactions

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1. Introduction

During the last 25 years organic chemistry has increasingly turned from investigations based solely on the synthetic approach to investigations of the mechanisms of organic reactions. These investigations encompass the kinetics of the reaction, the possible intermediates in the process, the effect of structure on reactivity, and the effect of various external factors on reactivity such as temperature, catalysis and solvent. Mechanism studies have provided a collision by collision account of the paths of a large number of organic reactions, together with a rather precise description of the transition states of these reactions. The results of such studies have been fruitful in explaining the chemical behavior of organic molecules and in predicting new processes.

Biochemical processes (or enzymatic processes) can be shown to involve many of the common organic reactions that have been investigated in detail from a mechanistic viewpoint. Obviously the pathway of the enzymatic reaction must differ in some respects from simple organic reactions, for the former in general proceed much more readily than the latter. But the similarities between organic and enzymatic reactions are so great, as will be shown as this chapter proceeds, that the utilization of the vast store of knowledge of organic reaction mechanisms for the proper consideration of enzymatic processes would seem to be mandatory. For the proper interpretation of a complex enzymatic reaction, it is essential that the background of its simpler organic counterparts be understood and used.

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The chemical transformations involved in enzymatic processes can in general be thought to proceed either via covalent interaction of the substrate with the enzyme with the formation of enzyme–substrate intermediates or via some non-covalent interaction without the formation of an intermediate. These possibilities have been termed "double" and "single" displacement reactions[1].

If an enzymatic process proceeds by means of a single displacement mechanism, the correlation between the mechanism of the organic process and the enzymatic process should be especially high since presumably the enzyme is not changing the pathway of the reaction but only its energetics. Enzymatic reactions which proceed by means of double displacement mechanisms need not bear any resemblance to the organic reaction containing the same reactants and products, but in many cases organic reactions have been studied mechanistically which proceed by "double" displacement mechanisms and which can be considered analogs of an enzymatic process. These analogies are especially close in organic reactions carried out with coenzymes which have qualitatively (although not quantitatively) duplicated enzymatic processes. Also, analogies between organic and enzymatic processes involving double displacement reactions have been demonstrated with molecules which have been designed to reproduce a portion of the protein part of the enzyme. In many cases, therefore, fruitful comparison between organic and enzymatic systems exists.

It is the aim of this chapter to present a background in organic reaction mechanisms which will enable the person interested in biochemistry to bring to the field of enzymology the fruits of organic chemistry. It is obviously impossible to cover the entire field of organic reaction mechanisms in a limited chapter. Selection has therefore been made of those topics which it is felt bear most closely on enzymatic reactions. Furthermore, it is the aim of this chapter to present the mechanisms of a number of organic processes which can be considered to serve as model systems of corresponding enzymatic processes.

In order to select topics for discussion, it is well to consider enzymatic reactions from the point of view of organic reaction mechanisms. When this is done, the scheme in Table I emerges.

It appears from the rather brief analysis of the main enzymatic reactions that the chemistry involved falls into only a few categories and further that the reaction classes are mainly those which have been studied in detail in simple organic systems. It is of course recognized that the similarities in the overall reactions between organic and enzymatic systems does not imply similarities in the pathway of the reactions. But, as a suitable starting point for consideration of enzymatic reactions, the corresponding overall organic reaction is the obvious choice.

TABLE I

AN ANALYSIS OF ENZYMATIC REACTIONS FROM THE VIEWPOINT OF ORGANIC REACTION MECHANISM

1. Nucleophilic substitution reactions in aliphatic systems

a. Reactions of sulfonium ions and nucleophiles
   1. S-adenosylmethionine
   2. Dimethylthetin
b. Reactions of disulfides and mercaptide ion
c. Reactions of ammonium ions and nucleophiles
   1. Diphosphopyridine nucleotide
   2. Thiamine
d. Reactions of acetals and nucleophiles
   1. Glycosides
   2. Glycosyl phosphates
e. Reactions of glycosylamines and nucleophiles
f. Electrophilic substitution of OH^+

2. Addition and elimination involving carbon–carbon unsaturation

a. Dehydration of alcohols to olefins
   1. Enolase, fumarase, crotonase, aconitase
   2. Pyridoxal activated enzymes
b. Deamination
   1. Aspartase
c. Removal of the elements of hydrogen sulfide or mercaptan
d. Isomerism of isopentenol pyrophosphate
e. Polymerization of olefins
   1. Dimerization or trimerization of isopentenol pyrophosphate
   2. Cyclisation of squalene to triterpenes

3. Nucleophilic reactions of carboxylic acid derivatives

a. Reactions of thiol esters with nucleophiles such as mercaptan, carboxylate ions, amines, alcohol, water, phosphate, amino acids
   1. Acyl coenzyme A reactions
   2. Acyl lipoic acid reactions
   3. Acyl glutathione reactions
b. Reactions of acyl phosphates with water, arsenate, hydroxylamine, alcohol, mercaptan, amine and amino acid
   1. Acyl phosphate reactions
   2. Acyl adenylic acid reactions
   3. Aminoacyl adenylic acid reactions
   4. Carbamyl phosphate reactions
c. Reactions of esters with water, alcohol and hydroxylamine
   1. Amino acid ester reactions
   2. Aminoacyl RNA reactions
d. Reactions of amides with water, amines, hydroxylamine and amino acids
   1. Amide reactions
   2. Peptide reactions
   3. Hydrazide reactions
   4. Hydroxamic acid reactions
e. Reactions of carboxylic acids with water, alcohol, amine, amino acids and mercaptan
   1. Amino acid reactions
   2. Carboxylic acid reactions

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TABLE I (*continued*)

4. Nucleophilic reactions of phosphoric acid and sulfuric acid derivatives
- a. Reactions of adenosine triphosphate with various nucleophiles
  - 1. Water
  - 2. Alcohols, mercaptans and amines
  - 3. Phosphate ions
  - 4. Carboxylate ions
  - 5. Sulfate ions
- b. Reactions of phosphoric acid esters with various nucleophiles
  - 1. Water
  - 2. Alcohols
- c. Reactions of sulfuric acid derivatives with various nucleophiles
  - 1. Sulfate esters
  - 2. Sulfuric–phosphoric anhydrides

5. Addition to carbonyl groups
- a. Hemiacetal formation and decomposition
  - 1. Mutarotase
- b. Reaction with amino groups
  - 1. Schiff base formation in pyridoxal catalyzed reactions
  - 2. Various aminations in purine biosynthesis
  - 3. Probable condensation with enzymatic amino groups in acetoacetic acid decarboxylation
  - 4. Condensations involving tetrahydrofolic acid
- c. Addition to α,β-unsaturated carbonyl compounds

6. Participation of enols and enolate ions
- a. Enolization–ketonization equilibria
  - 1. Triosephosphate isomerase
  - 2. Phosphoglucose isomerase
  - 3. Phenylpyruvate tautomerase
- b. The aldol condensation and related reactions
  - 1. Aldolase
  - 2. Condensing enzyme (acetyl CoA plus oxaloacetate)
- c. The Mannich reaction
  - 1. The postulated biogenesis of alkaloids
- d. Pyridoxal catalyzed reactions
  - 1. Amino acid racemases
  - 2. Transaminases
  - 3. Enzymes catalyzing various amino acid condensations and other related reactions
- e. The Claisen condensation
  - 1. Acetyl-CoA transacetylase
  - 2. Other fatty-acid transacylases
- f. Reactions related to the benzoin condensation
  - 1. Transketolase
  - 2. Other condensation reactions requiring thiamine pyrophosphate as cofactor

7. Carboxylation and decarboxylation
- a. β-Ketoacids and related compounds
  - 1. Acetoacetate decarboxylase
  - 2. Acetolactate decarboxylase
  - 3. Malonyl-CoA decarboxylase
- b. Pyridoxal catalyzed reactions and related cases
  - 1. Amino acid decarboxylases
  - 2. (Non-enzymatic) ninhydrin reactions

TABLE I (*continued*)

c. α-Ketoacids and related compounds
   1. α-Carboxylase
   2. Benzoylformate decarboxylase
   3. Oxalate decarboxylase
d. Oxidative decarboxylation
   1. Probable prior decarboxylation reactions involving thiamine pyrophosphate as a cofactor (*e.g.* pyruvate oxidase)
   2. Probable direct oxidative decarboxylations (*e.g.* oxalate oxidase)

8. Aromatic substitution

a. Electrophilic aromatic substitution
   1. Tyrosine iodinase
b. Homolytic aromatic substitution
   1. (Probably) phenylalanine-4-hydroxylase
c. Nucleophilic aromatic substitution
   1. Numerous aminations in purine and pyrimidine metabolism
   2. (Nonenzymatic) reactions of dinitrofluorobenzene

9. Oxidation–reduction reactions

a. Ionic (two electron) redox reactions
   1. Internal isomerizations, such as the glyoxalase reaction
   2. Intermolecular hydride transfers, such as the alcohol dehydrogenase reactions involving DPN
b. Free radical oxidation
   1. Numerous reactions involving flavoproteins
   2. Reactions involving interaction with oxygen
   3. Aromatic (*e.g.* tryptophan) and aliphatic (*e.g.* steroid) hydroxylases and peroxidases
   4. Oxidative coupling of phenols in alkaloid synthesis and probably in thyroxine synthesis
c. Photochemical oxidation
   1. Primary step in photosynthesis

It should be noted that the nomenclature in the classification scheme of Table I corresponds not to the usual biochemical customs, but rather to organic chemical conventions. In certain instances these conventions are identical, in other cases the two diverge. Choice has been made on the basis of the authors' habits and on the basis that such nomenclature will enable the reader to better familiarize himself with the organic literature. That the organic nomenclature is most descriptive of the actual chemical occurrences is of course obvious to an organic chemist.

2. Nucleophilic substitution reactions in aliphatic systems

Of the various classes of organic reactions, nucleophilic substitution reactions at a saturated carbon atom have been studied most extensively. These reactions belong to a family called displacement reactions, designated as in the following equation. The group A, the displacing or substituting agent, can be a nucleophile, electrophile or free radical. These reactions are known

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respectively as nucleophilic, electrophilic or radical substitution reactions. Of the three types of substitution reactions, nucleophilic substitution reactions are of greatest importance to biochemistry and have been studied in greatest detail.

$$A + B—C \longrightarrow A—B + C$$

The timing of the two processes necessary to complete the above equation, breaking the B—C bond and making the A—B bond, leads to the consideration of three simple mechanistic categories: (1) simultaneous bond-making and bond-breaking; (2) complete bond-breaking prior to bond-making; and (3) complete bond-making prior to bond-breaking. The first two possibilities appear to encompass the nucleophilic substitution reactions at a saturated carbon atom. The third possibility is in reality an addition followed by an elimination reaction, which requires that the group B be unsaturated (a carbonyl group or carbon–carbon double bond) or that it be an atom other than carbon such as silicon which can readily expand its valence shell. All three of these possibilities are of fundamental importance in explaining the reactions of organic compounds but it is the first two that are pertinent to a discussion of the nucleophilic substitution reactions at a saturated carbon atom.

The mechanism that a specific reaction follows will be the one which is energetically most feasible. In terms of the transition state theory, the pathway a reaction takes is that one containing the lowest energy barrier. For simple displacement reactions conforming to the above equations such as the $H\text{-}H_2$ reaction, the pathway of the reaction can be specified as a simultaneous bond-making and bond-breaking process which proceeds through an essentially linear transition state (the top of the energy barrier)[2]. In organic reactions the same process can be postulated.

In order to achieve reaction, the system must overcome a free energy barrier characterized by the standard free energy of activation, ΔF^*, which is a measure of the free energy difference between the ground state and the transition state. From the postulates of the absolute rate (or transition state) theory, the rate constant for the reaction in solution is given by

$$\text{rate constant} = \kappa(kT/h)\, e^{-\Delta F^*/RT}$$

in which κ, the "transmission coefficient", is usually taken as unity for the reactions being considered, k is Boltzmann's constant, and h is Planck's constant. In terms of the transition state theory, the mechanism of a substitution reaction is completely specified only by defining the complete free energy surface including all possible reaction paths. However, since ΔF^* is defined as the lowest energy barrier over which the reaction may pass, other

paths need not be considered. The reaction mechanism for a nucleophilic substitution reaction may then be defined in terms of the configuration of the transition state and its thermodynamic properties.

If bond-breaking occurs prior to bond-making, the energy profile will almost certainly contain an intermediate (defined as a minimum in a plot of standard free energy *vs.* reaction coordinate) as well as two transition states (Fig. 2).

The energy profiles illustrated in Figs. 1 and 2 imply that every thermal reaction that proceeds through a set of configurations must exhibit a reverse

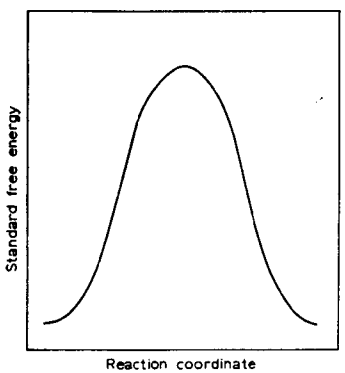

Fig. 1. A reaction involving only a transition state.

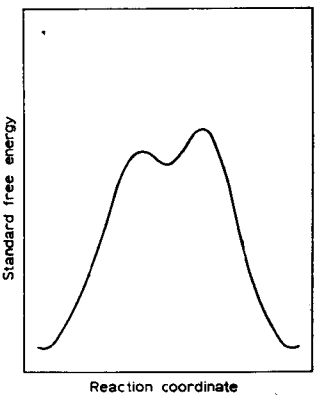

Fig. 2. A reaction involving an unstable intermediate which is formed in infinitesimal amount.

reaction which contains exactly the same set of configurations. This concept, termed the *principle of microscopic reversibility*, appears to hold in all cases known. This does not imply that there cannot be several paths from a reactant to a product, with the incursion of other species, as appears to be the case in many enzymatic systems.

In general a catalytic system such as an enzyme can facilitate a reaction in two ways. The catalyst can lower the energy barrier for the process without changing the overall pathway or alternatively the catalyst can change the pathway completely. In the first instance the catalyst can be thought of as an energy source of some kind, which reduces the normal thermal energy necessary to carry out a reaction. In the second instance the catalyst must participate directly in the chemical transformations through the formation of covalent bonds. This covalent interaction between substrate and enzyme may take different forms in a set of forward and reverse reac-

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tions. Thus, for example, it is feasible that the enzymes, the additional components, and the pathways are completely different in the hydrolysis of proteins to amino acids than in the synthesis of proteins from amino acids.

(a) Bimolecular substitution reactions

(i) Kinetics and stereochemistry

A typical bimolecular nucleophilic substitution reaction is the hydrolysis of methyl iodide by hydroxide ions.

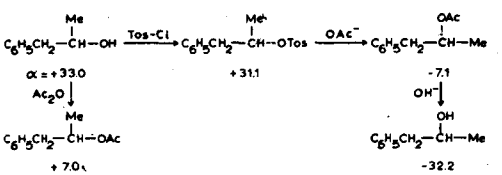
$$CH_3I + OH^- \longrightarrow CH_3OH + I^-$$

The kinetics of the forward reaction

$$\text{rate} = k[CH_3I]\,[OH^-]$$

indicates kinetic dependences on both the methyliodide and hydroxide ion concentrations. By analogy with the $H\text{-}H_2$ reaction which was analyzed in terms of a linear three-centered transition state, the methyl iodide–hydroxide ion reaction can be discussed in terms of a linear three-centered transition state in which the breaking of the carbon–iodine bond is facilitated by the making of the carbon–oxygen bond. The stereochemistry of a

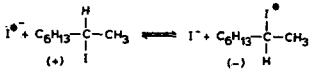
bimolecular nucleophilic substitution reaction at an asymmetric carbon atom could *a priori* result in retention, inversion, or loss of configuration. The picture of the transition state given above specifically predicts the possibility of inversion of configuration as the stereochemical result. This transition state has been eminently successful in explaining the Walden inversion reaction, a cycle of reactions in which an optically active substance is converted to its enantiomorph. A classical example of this process is the conversion of dextrorotatory benzylmethylcarbinol to its enantiomorph[3].



Both the carbinol and the acetate produced in the reaction of the tosylate (*p*-toluenesulfonate) with acetate ion have rotations opposite in sign but

nearly equal in magnitude to those of the original carbinol and of the acetate prepared from it. It can be demonstrated that the only reaction of the four which directly affects the asymmetric carbon atom is the reaction of acetate ion with benzylmethylcarbinyl tosylate. Therefore in this nucleophilic substitution reaction an inversion of configuration must have taken place. A similar sequence of reactions has demonstrated the conversion of the optically active primary alcohol, CH_3–CHDOH, into its enantiomorph.

While the above experiments demonstrate that the bimolecular nucleophilic substitution reaction may result in inversion of configuration, they do not necessitate that every substitution reaction involves an inversion. This conclusion can, however, be reached on the basis of a kinetic investigation of the simultaneous isotopic exchange and racemization of optically active 2-octyl iodide with radioactive iodide ion. Both isotopic exchange and racemization are second order kinetic processes. If each individual



displacement of organic iodide by iodide ion results in inversion, the rate of racemization should be exactly twice the rate of radioactive iodide exchange (as indicated by the incorporation of radioiodine in the organic molecule), since every conversion of a dextorotatory iodide to a levorotatory iodide leads to two molecules of racemized iodide. The ratio of racemization to exchange was found to be 1.93 ± 0.16, making it obligatory that every act of bimolecular substitution entails inversion of configuration[4].

(ii) Description of the transition state

The stereochemical experiments described above specify the geometry of the transition state for S_N2 (S = substitution, N = nucleophilic, 2 = bimolecular)* reactions which has important consequences with regard to the effect of structure on reactivity in these systems. The structure of the transition state may be regarded as a resonance hybrid of structures I, II, and III. The hybrid structure may be written as IV in which the dotted lines represent partial covalent bonds with the charge distribution as indicated. Alternatively the transition state may be represented by the molecular orbital picture V. These representations imply a rehybridization of the four original sp^3 orbitals of the central carbon atom. The three bonds not undergoing reaction are coplanar and are composed of three sp^2 orbitals of the central carbon atom. The remaining p orbital, perpendicular to the plane of

* This nomenclature, as well as much of the subsequent nomenclature, is that of Hughes and Ingold (see Ingold[6]).

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