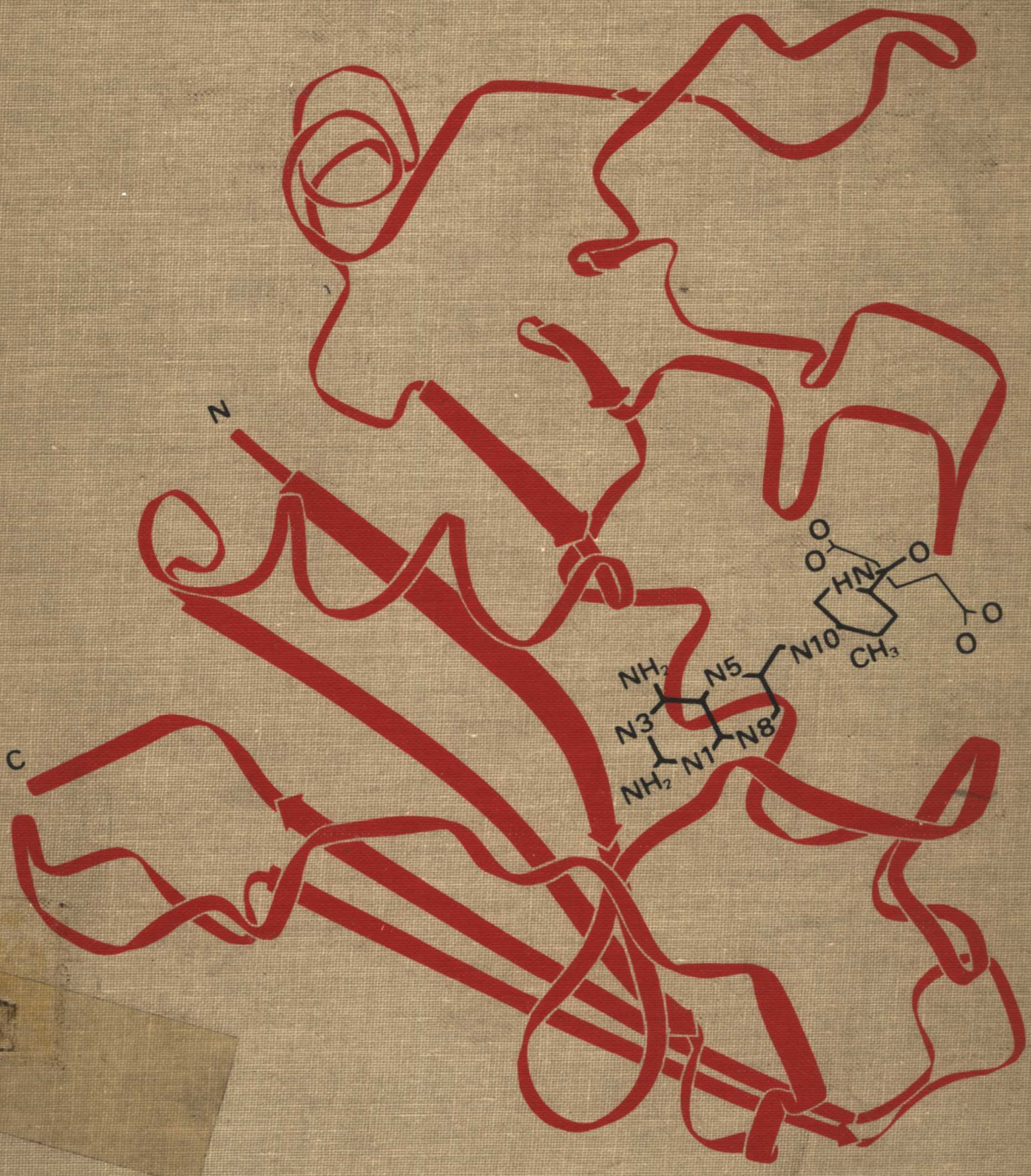
Enzymatic Reaction Mechanisms

CHRISTOPHER WALSH



Enzymatic Reaction Mechanisms

CHRISTOPHER WALSH
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

W. H. FREEMAN AND COMPANY
San Francisco

Cover art redrawn after D. Matthews, R. Alden, J. Bolin, S. Freer, R. Hamlin, N. Xuong, J. Kraut, M. Poe, M. Williams, and K. Hoogsteen, 1977, *Science* 197:452. Copyright © 1977 by the American Association for the Advancement of Science.

Sponsoring Editor: Arthur C. Bartlett
Manuscript Editor: Lawrence W. McCombs
Designer: Robert Ishi
Production Coordinator: Linda Jupiter
Illustration Coordinator: Batyah Janowski
Artist: Eric G. Hieber, EH Technical Services
Compositor: The Universities Press Limited
Printer and Binder: The Maple-Vail Book Manufacturing Group

Library of Congress Cataloging in Publication Data

Walsh, Christopher.
Enzymatic reaction mechanisms.

Bibliography: p.
Includes index.
1. Enzymes. I. Title.
QP601.W23 574.1'925 78–18266
ISBN 0–7167–0070–0

Printed in the United States of America

9 8 7 6 5 4 3 2 1

PREFACE

This book has developed from a chemistry course on enzymatic reaction mechanisms that I have taught to chemistry and biology undergraduate and graduate students at M.I.T. The basic approach of analyzing catalysis in biological systems according to the type of chemical reaction involved has in turn derived from the biochemistry course taught primarily by Professors Abeles and Jencks at Brandeis University during my postdoctoral stay there from 1970 through 1972.

The basic premise in this approach is that most of the transformations undergone by specific metabolites in biological systems can be collected into a small number of types of chemical reactions. With a feeling for the underlying patterns of the ways certain functional groups are enzymatically processed, one can see the chemical logic of metabolic sequences and interconversions.

Because enzymes are catalysts, the study of enzymatic reactions must deal both with the kinetics and with the nature and scope of the chemistry that occurs. There are several recent texts, at both beginning and advanced levels, that focus on kinetic mechanisms of enzymatic catalysis. Although kinetic questions are repeatedly considered qualitatively in this book, that subject is not its major focus. Rather, a complementary approach is taken to analyze the kinds of chemical mechanisms that are likely to proceed by low activation-energy barriers (and therefore rapidly) in enzymatic systems. This book includes relatively few kinetic constants and relatively many structures analyzing patterns of electron flow as bonds break and reform during the course of the enzyme-mediated transformation. To this end, much of the book is devoted to coenzyme-dependent enzymatic reactions, analyzing how the particular chemistry open to the specific coenzyme is used to direct flux of reaction molecules to a single set of products and to accelerate the rate of such reactions.

The organization of this book also differs somewhat from many texts on enzymology, which have introductory chapters on structure, several chapters on kinetics, and a concluding chapter or two on specific enzymes, invariably focusing on a few standbys such as chymotrypsin, carboxypeptidase, lysozyme, and alcohol dehydrogenase. Instead, although this book uses hydrolytic enzymes that work on acyl-group–containing substrates as a jumping-off point, the focus here is on a thorough examination of the scope and diversity of enzymatic catalysis. It would be nice to have high-resolution X-ray maps available for each category of enzymes, but that would entail a long wait, especially for some enzymes that carry out the most interesting chemistry. In fact, I began writing this book in 1973 largely because I felt there was no coherent, comprehensible treatment available in any text of the pattern of enzymatic redox reactions, the heart of cellular energetics.

Major sections deal with (1) enzymes that carry out group transfers (of electrophilic substrate fragments to nucleophilic acceptors), (2) enzymes that accelerate oxidation–reduction chemistry, particularly reductive oxygen metabolism, (3) enzymes that promote eliminations, isomerizations, and rearrangements of substrate skeletons, and (4) enzymes that make and break carbon–carbon bonds (the reactions that are the building blocks of biosynthesis).

The pace of new discoveries in biochemistry is encouraging to those engaged in research, but it is a bit dismaying to the author attempting to gauge the state of knowledge in a given area and to provide some interpretations. I have tried to emphasize concepts and ideas about enzymatic chemistry that should have reasonable time constants and that should not rise or fall on the basis of the last (or next) experiment.

The level of the book should make it suitable both as a text and as a reference work (albeit with some selectivity in topics covered) in enzymology. It should be usable by and useful to both advanced undergraduate and graduate students in chemistry, biology, biochemistry, medicinal chemistry, and pharmacology departments, as well as to professional scientists in those areas. Each of the four major sections (and separate chapters within them) can be taught essentially independently, although the group-transfer section is the likely starting place because it contains much basic information. Extensive cross-referencing will aid teacher and student in locating relevant material elsewhere in the text. Most classes should be able to deal comfortably with three of the four sections in a one-semester course. The final chapter in the book can follow any combination of preceding chapters because it attempts to analyze and integrate the chemical logic (and underlying strategies) of metabolic pathways.

I acknowledge substantial intellectual debt to Professors R. H. Abeles and W. P. Jencks of Brandeis University and Professors F. Lipmann and L. B. Spector

of The Rockefeller University for both facts and philosophy. I am indebted to the M.I.T. students in Chemistry 5.50 (Enzymatic Reaction Mechanisms) over the past five years for their comments, positive and negative, as they used two stages of course notes (efficiently printed by M.I.T. Graphic Arts Services) that preceded this manuscript. I owe thanks to my colleagues in my research group during this period who carefully read various portions of the manuscript and repeatedly—if somewhat gleefully—pointed out ways of improving the presentation. Michael Johnston deserves particular thanks for reading galley and page proofs. I am delighted to acknowledge the generous support of the Alfred P. Sloan Foundation (1975–1977) and the Camille and Henry Dreyfus Foundation (1976–1978) for fellowships without which the manuscript would not have been completed. Finally, I acknowledge that it would have been no fun at all without Diana and Allison.

November 1978 *Christopher Walsh*

CONTENTS

Section I

INTRODUCTION

This brief section contains two chapters. The first outlines the scope and organization of the book. The second chapter summarizes some basic concepts about enzymes and enzymatic catalysis—themes that introduce and will underlie later discussions throughout the text.

Chapter 1

Introduction

This introductory chapter is divided in two parts. The first part is a brief discussion of the philosophical orientation and the purpose of this book. It delineates the approach used, explains why the principles are apt for describing enzyme-catalyzed reactions, and sets forth the overall goal in terms of insights into how enzymes work and what reactions they catalyze.

The second part of this chapter summarizes the general outline of the four major sections of the text. It sets out the major premises around which the individual chapters of each section are grouped. It may be useful to review this first chapter again for general orientation as you go through the four major text sections on (1) enzymatic group transfers, (2) oxidation–reduction reactions, (3) eliminations, isomerizations, and rearrangements, and (4) reactions that make or break carbon–carbon bonds.

1.A ORIENTATION AND PURPOSE OF THIS BOOK

The intent of this book is to provide a simple chemical framework for the study and analysis of enzyme-catalyzed reactions. Enzymes are macromolecular protein catalysts, consisting of linear condensed polymers of a small set of α-amino acids joined in amide linkages. Each enzyme has a genetically mandated and unique primary sequence, and it folds in three dimensions into a precise orientation with remarkable catalytic activity. Enzymes are the major agents that effect controlled chemical changes in biological systems. It is fair to say that, if one wishes to

understand the chemistry of living systems and the kinds of molecular transformations that occur in them, one must understand both the nature of enzymatic catalysis and the scope of the reactions encountered. This, in turn, entails knowledge of (1) the composition, size, and structure of the subset of proteins that display enzymatic activity and (2) the chemistry of the enzymes' functional groups that actually engage in catalysis. These investigations provide insights into the two most distinguishing characteristics of enzymes as catalysts: the remarkable specificity and rate accelerations of enzyme-mediated transformations.

We shall touch on all of these elements in more or less detail. However, although this book can by no means be considered brief, it has a somewhat narrow focus. The focus is not on protein chemistry per se, nor on three-dimensional protein structure, nor even on the chemistry of catalysis itself. Rather, the purpose of this text is to group the almost bewildering array of enzyme-catalyzed processes into a few major reaction types common in organic chemistry, to develop some simple chemical intuitions about how these reactions occur, and then to see how enzymes modulate and control the chemical paths open to their specific reactants. The focus is primarily on the nature of the chemical changes undergone by substrates as they are converted to products, and on the nature and chemistry of the active-site groups on the enzyme catalyst.

The focus is on the mechanisms by which some stable substrate species—in aqueous solution at neutral pH (for the most part) and room temperature—is converted into some stable product species. Therefore, we shall continually analyze biochemical mechanisms in terms of their two components: (1) the *kinetics* of the process, and (2) the *structural changes in chemical bonds* during the process.

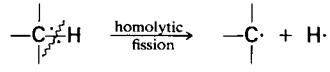
The kinetic question in chemical or biochemical mechanistic study is essentially a question of timing. In what temporal sequence do bonds break and form? Do changes occur sequentially or simultaneously? Because enzymes are such efficient catalysts, and because they always combine with substrates in a specific binding step as a prelude to chemistry, we shall examine how fast both chemical and physical steps occur.

The structural-change question devolves to a question of how chemical bonds break and form in these biological systems. What is the nature of the enzymatic transition state? What kinds of intermediates, with finite lifetimes, form? Do these intermediates involve *covalent bonds* between enzyme and some fragment of a substrate? What catalytic advantage do these mechanisms possess, and how are transition states selectively stabilized? The ultimate goal is to use mechanistic intuition derived from consideration of specific enzyme systems to gain predictive ability—not only to forecast what mechanism will be operant in a given instance, but also to say how a biological system can and will process a substrate with

certain structural elements, how it will cope with certain kinds of functional groups.

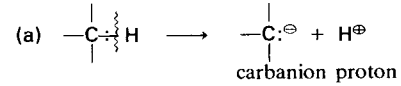
1.A.1 Modes of Bond Breaking

A major, pervasive didactic device in this book (borrowed from mechanistic organic chemistry) is the analysis of what happens to a shared electron pair in a covalent bond between two atoms as that bond breaks and then reforms with new partner atoms during enzymatic transformations. For example, hundreds of enzymatic reactions involve the fission of carbon–hydrogen bonds at some stage in the reaction. The C—H bond can be cleaved in only two ways. (1) *Homolytic* cleavage, with one electron remaining with carbon and one with hydrogen, produces carbon radicals and hydrogen radicals.

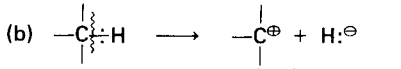


radicals

In general, radical species in biochemical reactions are unstable and not readily formed. (2) Reactions occur much more often by *heterolytic* cleavage of bonds, one atom retaining the two electrons. When a C—H bond cleaves heterolytically, there are two options: (a) the electrons can remain with carbon, yielding a carbanion intermediate (or a fleeting carbanionic transition state) and a proton, $H^{\oplus}$, or (b) electrons can depart with the hydrogen, producing an electron-deficient *carbonium ion* and a *hydride ion.*



carbanion proton



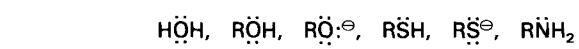
carbonium ion hydride ion

Carbon is a more electronegative atom than hydrogen and, in general, pathway (a) is preferred. Indeed, much of enzyme chemistry involves controlled generation of carbanions, as we shall see. But the fragmentation pattern (b), to yield the equivalent of a hydride ion, is a consistent explanation of the facts about dehydrogenases that use nicotinamide coenzymes as redox cofactors, as we note in Chapter 10.

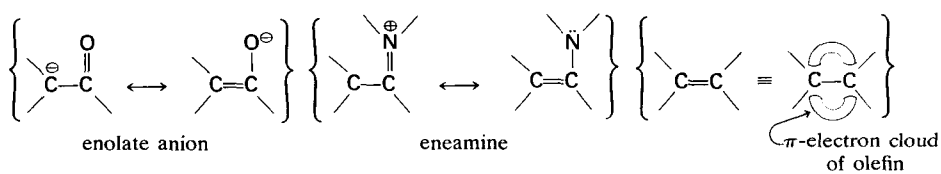
1.A.2 Nucleophiles and Electrophiles

Both of the heterolytic pathways of bond cleavage for C—H (or, more generally, between any two atoms) *involve ionic intermediates* or *transition states*, where full or partial positive or negative charges are developed on reacting atoms. Reactions involving such ionic species are predominant in organic chemistry and enzyme chemistry (we shall come back to unpaired electrons in Section III when we discuss redox catalysis). The predominance of these reactions has led to the broad categorization of various reagents or reactants or enzyme substrates into two broad classes: electron-rich or electron-deficient species.

Electron-rich species have been termed **nucleophiles** (from Greek, meaning "nucleus loving"). Because nuclei of atoms contain the positively charged protons, nucleophilic reagents or atoms are seeking positively charged or electron-deficient species to combine with and to give up electrons to. The recipients are defined as **electrophiles**, those that "love electrons." Nucleophiles, then, are often anionic (negatively charged) or contain a lone or unshared electron pair. They are the *attacking molecules* in chemical (and therefore in enzyme-catalyzed) reactions. Oxygen, sulfur, and nitrogen nucleophiles are important in biological reactions.



In reactions where carbon–carbon bonds are formed, carbanions act as nucleophiles, generally as enolate ions or eneamines or via the π-electrons of carbon–carbon double bonds (Chapter 26).



In the biological systems under consideration, there are only a few important electrophiles available to the various nucleophilic groups on substrates or enzymes for reaction. The electrophiles are electron-deficient, often cationic (i.e., positively charged) and/or with an unfilled valence electron shell (metal cations). Important biological electrophiles include *protons* and *metal cations* (from the +1 oxidation state of copper to the +6 oxidation state of molybdenum). Additionally, the coenzyme forms of vitamin B_1 and vitamin B_6 (thiamine pyrophosphate and pyridoxal phosphate, respectively) serve as electron sinks (electrophiles) during covalent-adduct formation with various substrates in certain kinds of enzymatic catalysis.