ENZYMES: Catalysis, Kinetics and Mechanisms

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For
Sandhya, Jahnavi, and Chaitanya

Preface

Any living being is a reflection of its enzyme arsenal. We are and do what our enzymes permit.

Christian de Duve

Enzymes are the lead actors in the drama of life. Without these molecular machines the genetic information stored in DNA is worthless. With rising attention to the fashionable fields like molecular biology, genetic engineering, and biotechnology, the techniques to manipulate DNA have occupied center stage. Being popular, many concepts of molecular biology/genetic engineering are now introduced to undergraduates. Unfortunately, this has happened at the cost of other fundamental facets of biology, including enzymology. In the excitement to collate volumes of data for Systems Biology (and the various "Omics" fashions), the beauty and vigor of careful analysis – one enzyme at a time – is neglected. It is an intellectual challenge to assay individual enzymes while avoiding complications due to others – an almost forgotten activity in modern biology. Many in the present generation assume that performing one standard assay will tell you everything about that enzyme. While biochemists spent lifetimes on a single native enzyme, the notion today is that one can characterize a mutant in the morning! Over the last three decades devoted enzymologists have become a rare breed. Many Biology teaching programs have expanded in the areas of molecular and cellular biology while they manage with a makeshift enzymology instructor. New students who are attracted to the study of enzymes do exist, but they find themselves in a very bleak teaching environment. Not surprisingly their numbers are dwindling. Reservoirs that are not replenished may soon run dry.

Purpose of This Book

Genes for enzymes are routinely fished out, cloned, sequenced, mutated, and expressed in a suitable host. Characterizing the mutant enzyme, however, requires a thorough mechanistic study – both chemical and kinetic. It is thus an exciting time to do enzymology. Hopefully, this book provides enough basic exposure to make this happen.

The ease with which sophisticated data are collected nowadays has dispirited the slow and burdensome approach of resolving and reconstituting a complex enzyme system. Micro-arrays that measure the transcription of many genes at a time disclose neither the abundance nor any attributes of the enzymes/proteins they encode. As F.G. Hopkins wrote in 1931 "*..the biochemist's word may not be the last in describing life, but without his help, the last word will never be said.*" This is true of enzymology as well. While the interest and expertise in teaching/learning enzymology has declined exponentially, working knowledge of enzymology remains indispensable. Enzymes have come to occupy vast areas of modern biology research and the biotechnology industry. Enzymes whether used as popular kits, mere research tools, or for their own sake require a minimal appreciation of their workings. A tome on enzymology that focuses and logically connects theory of enzyme action to actual experimentation is desirable. One objective of this book is to bridge this gap and enable students to understand, design, and execute enzyme experiments on their own.

Enzyme study can range from the simple to the most complicated. Approaches that can be performed in a modest laboratory setup and with no fancy equipment are needed. Conveying the excitement of enzymology within a modest budget and with few experiments is desirable. And hence, equipment intensive approaches – such as structural enzymology, sophisticated techniques like X-ray, NMR, ESR, fast reactions, and isotope effects – have received a somewhat limited coverage. Readers interested in them will yet find sufficient background material here.

Audience and Their Background

Reasons for the cursory coverage of enzymology in most contemporary biology academic programs are twofold. Over-emphasis and glamorization of molecular biology (later genetic engineering!) in the last few decades has captured a disproportionately large allocation of resources and time. Secondly, as a cumulative effect of this attitude, very few well-trained specialists in enzymology are available today. Therefore, study material that encourages students/researchers to understand, design, and execute experiments involving enzymes on their own is needed. The contents of the present book are expected to serve this purpose.

Most biochemistry and molecular biology students are introduced to enzymes as commercial reagents and as faceless as buffers and salts. This has led to inadequate appreciation of enzymology and its practices. Standards for reporting enzymology data (STRENDA; available at http://www.strenda-db.org) are a recent effort to prescribe the best approaches to generate and report enzyme data. With an ever-increasing reliance on genomics and proteomics, enzymes are no longer isolated and/or assayed for activity. Often their role is inferred from sequence data alone. "*Molecular biology falters when it ignores the chemistry of the products of DNA blueprint – enzymes – the protein catalysts of the cellular machinery.*" This philosophy was beautifully reiterated by Arthur Kornberg in his "Ten Commandments of Enzymology" (*J Bacteriol.* (2000) 182:3613–3618; *TIBS* (2003) 28:515–517). The

present book is an attempt to sift through chemical sophistication and simplify it for an audience with a biology background. It will serve the curricular needs of senior undergraduates and postgraduates in Biochemistry, Biotechnology, and most branches of modern biology.

Dealing with reaction rates, enzymology is a quantitative and analytical facet of biological understanding. Appreciation of rate equations and their meaning therefore becomes important. Minimal competence with algebra, logarithms, exponential relationships, equations to fit straight lines, and simple curves is crucial. While one need not be scared of fearsome equations, the essence of the physical models they represent (or do not represent!) ought to be understood. To an extent, this book is my response to oust the fear of the quantitative in the students of Biology. Because enzymes catalyze chemical reactions, chemical mechanisms are of great concern. They are best understood with adequate preparation in concepts like valency, movement of electrons and charges in molecules, acids and bases, etc. The study of mechanistic enzymology is meaningless without this background. We may recall from Emil Fischer's Faraday Lecture to the Chemical Society in 1907: "*... the separation of chemistry from biology was necessary while experimental methods and theories were being developed. Now that our science is provided with a powerful armoury of analytical and synthetic weapons, chemistry can once again renew the alliance with biology, not only for the advantage of biology but also for the glory of chemistry.*" Enzymology without Chemistry (physical ***and*** organic) is a limited descriptor of surface (superficial!) phenomena. This requirement obviously puts some burden on students who have lost touch with chemistry for few years in the pursuit of "Biology Only" programs.

Basic knowledge on amino acids, their reactivity, and protein structure is a prerequisite to study enzymes. Protein (and hence enzyme) purification methods/tools like various fractionation/separation techniques and chromatographies are not explicitly covered here. Also, essential techniques of protein structure determination do not find a dedicated treatment in this book. One may find such background material in the standard text books of biochemistry. Lastly, the reader is expected to be familiar with the concepts of concentrations, ionic strength, pH, etc. and exposure to biochemical calculations is essential.

Organization

This book endeavors to synthesize the two broad mechanistic facets of enzymology, namely, the chemical and the kinetic. It also attempts to bring out the synergy between enzyme structures and mechanisms. Written with self study format in mind, the emphasis is on how to begin experiments with an enzyme and subsequently analyze the data collected. Individual concepts are treated as stand-alone short sections, and the book is largely modular in organization. The reader can focus on a concept (with real examples) with minimal cross-referencing to the rest of the book. Many attractive enzymes were consciously passed up in order to suit the "Biology" audience. This error of omission painfully belongs to the author. A

limited treatment on applied aspects of enzymes is deliberate as one fully subscribes to Louis Pasteur's dictum – "*There are no applied sciences. . . .The study of the applications of science is easy to anyone who is master of the theory of it.*" The book then would also have become unmanageably long.

Individual concepts (as chapters) are conveniently grouped into five broad parts. It all begins with an overview of enzyme catalysis (Part I) followed by a section (Part II) on kinetic practices and measurement of enzyme activity. Two major themes of mechanistic enzymology, namely, the kinetic (Part III) and the chemical (Part IV) occupy bulk attention. A short piece on integrating enzyme kinetic and chemical mechanisms (in Part IV) is a novelty and should be of value. Aspects of enzymology in vivo and frontier research themes form the last section (Part V).

The original literature for this book was collected up to year 2016. Fresh research material, constantly being added to many topics, made it hard to draw this boundary. Otherwise, the book would have been always under preparation! Besides listing select text books and original publications, references to recent reviews on most topics are provided. Wherever possible, literature is cited from easily available and open-access resources.

How to Use This Book

The book contains a balance of physical and chemical fundamentals. Students of modern biology come from many different backgrounds. Hopefully, those from more physical and chemical background will enjoy the material as is. Many of the physico-chemical concepts and mathematical material may be difficult to students narrowly exposed to biological sciences alone. The essential theory to help such audience is presented in Chaps. 9 and 10 (covering chemical kinetics) and 29, 30, and 31 (covering organic reaction mechanisms). It is highly recommended that the uninitiated read these chapters first. Chapter 24 arrives before a primer on acid-base chemistry in Part IV; hence, it is suggested to read Chap. 30 before approaching the material in Chap. 24. A complete mechanistic understanding of enzyme action is possible only through a variety of experimental approaches. How these bits of information are combined to arrive at the final description may be found in Chaps. 28 and 36. Inclusion of regulation of enzyme activity (Chap. 37) under Frontiers of Enzymology (in Part V) may not be such a revelation since novel regulatory features are being discovered with remarkable regularity.

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Useful Constants and Conversion Factors

Calorie (cal):
(Heat required for raising the temperature of 1 g water from 14.5 °C to 15.5 °C)
1 cal = 4.184 J
1 kcal = 1000 cal = 4184 J

Joule (J):
$1\ J = 0.239\ cal = 1\ kg \times m^2 \times s^2 = 2.624 \times 10^{19}\ eV$

Coulomb (C):
$1\ C = 6.242 \times 10^{18}$ electron charges

Avogadro's number (N):
$N = 6.022 \times 10^{23}\ mol^{-1}$

Faraday constant (***F***):
$F = 23.063\ kcal \times V^{-1} \times mol^{-1} = N$ electron charges $= 96{,}480\ C \times mol^{-1}$

Boltzmann constant (k_B):
$k_B = 1.381 \times 10^{-23}\ J \times K^{-1} = 1.38 \times 10^{-16}\ cm^2 \times g \times s^{-2} \times K^{-1}$

Plank's constant (h):
$h = 6.626 \times 10^{-34}\ J \times s = 6.626 \times 10^{-27}\ cm^2 \times g \times s^{-1}$

Gas constant (R):
$R = N\ k_B = 1.987\ cal \times mol^{-1} \times K^{-1} = 8.315\ J \times mol^{-1} \times K^{-1}$

Absolute temperature (degree Kelvin, K):
0 K = absolute zero = −273 °C; 25 °C = 298 K

RT at 25 °C:
$RT = 2.478\ kJ \times mol^{-1} = 0.592\ kcal \times mol^{-1}$

Units for ΔG, ΔH, and ΔS:
For ΔG and ΔH: cal $\times$ mol^{-1} (or J $\times$ mol^{-1})
For ΔS: cal$\times$$mol^{-1}$ $\times$ K^{-1} (or J $\times$ mol^{-1} $\times$ K^{-1})

Enzyme catalytic unit:
1 U = 1 μmol $\times$ min^{-1} = 16.67 nkatal
1 katal = 1 mol $\times$ $s-^{1}$

Curie (Ci):
Quantity of a radioactive substance that decays at a rate of 2.22×10^{12} disintegrations per minute (dpm)

Acknowledgments

In the era of molecular biology, genetic engineering, and genomics, enzymology is often deemed unglamorous. In this backdrop, it is my good fortune to have benefited from the wisdom of a few enzymology stalwarts. I was initiated into research on enzymes at the Indian Institute of Science, Bangalore. But after a stint at Institute for Enzyme Research, UW-Madison, I was consumed by this evergreen subject. I owe much to these two great institutions in whetting my appetite for enzymology and the preparation for this book. Being a postdoctoral fellow in Prof. Henry Lardy's group and taking a course on enzymes with Prof. WW Cleland were invaluable. Much of the ground covered in this book was developed while teaching the "Molecular Enzymology" course at IIT Bombay, over 25 years. It was exciting to teach and learn about enzymes with so many bright and committed graduate students. Those indifferent to enzymology (and there were many!) helped me evolve a few tricks to get them interested – I am grateful to them. Any good feature of this book is clearly a result of such an exposure. I thank my colleagues in the department, particularly, Professors K.K. Rao, P.J. Bhat, and P.V. Balaji, who presumed my competence in the subject; this pushed me to exert more and do better. Thanks to Prof. P Bhaumik for enriching me with the structural aspects of enzymology.

The material and the organization of this book evolved over the years. The work was initiated during 2007 while on sabbatical leave from IIT Bombay. The financial support for book writing from Continuing Education Program (CEP) cell at IIT Bombay is gratefully acknowledged. The inputs of four anonymous reviewers improved the quality of this book and for this I am indebted to them. Encouragement and generous support of Ms. Suvira Srivastav, Dr. Bhavik Sawhney, and Ms. Saanthi Shankhararaman from Springer Nature was valuable in bringing this book to fruition.

Salvador Dalí once said – "*Have no fear of perfection – you'll never reach it.*" Surely, this book has its own share of glitches. All those errors and limitations are mine alone; I will be very grateful to the readers for pointing them out to me (nsp@iitb.ac.in) for rectification.

This book would not have been possible without the academic spirit inculcated in me by my father. I am deeply indebted to three women for inspiration – my mother

(Akka) for always believing in me, my wife (Sandhya) for the constant reminder that in the race for quality there is no finish line, and my daughter (Jahnavi) for allowing me to dream even the impossible. I particularly thank my wife Sandhya for her continued support during the longer than anticipated gestation period of this book.

Contents

About the Author

N. S. Punekar is currently working as a Professor at prestigious Indian Institute of Technology (IIT) Bombay, Mumbai, India. He obtained his Ph.D. from the coveted Indian Institute of Sciences, Bangalore, India, in the year 1984 and subsequently worked as postdoctoral fellow at University of Wisconsin, Madison, USA, till 1988. He joined IIT Bombay as Assistant Professor in 1988 and subsequently got elevated to the rank of Professor in 2001.

Dr. Punekar's major research interest lies in microbial biochemistry and molecular enzymology, microbial metabolic regulation, understanding metabolism through biochemical and recombinant DNA techniques, and fungal molecular genetics and its applications to metabolic engineering. Dr. Punekar has published around 50 papers in peer-reviewed reputed journals and more importantly has 4 patents to his credit. He has been an excellent teacher of Enzymology and Industrial Microbiology, which is evident by the fact that he has received the "Excellence in Teaching Award" at IIT Bombay in the years 2000, 2012 and 2018. He possesses more than two decades of experience of teaching Enzymology at IIT Bombay.

He is associated with various societies and committees of Government of India as an expert member. He is also in editorial board of journals like *Indian Journal of Biochemistry and Biophysics*, *Indian Journal of Biotechnology*, *Indian Journal of Experimental Biology*, etc.