Methods in Enzymology

Volume 64

Enzyme Kinetics and Mechanism

Part B
Isotopic Probes and Complex Enzyme Systems

EDITED BY

Daniel L. Purich

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Preface

In the early years of chemistry the identification of new reactions preceded serious consideration of reaction kinetics, and it was not until Berthelot derived the bimolecular rate equation in 1861 that chemical kinetics offered any real value to the practicing chemist. Fortunately, biochemistry, which had its roots in the late nineteenth century, experienced the benefit of developments in kinetic theory. In fact, kinetic arguments have played a major role in defining the metabolic pathways, the mechanistic action of enzymes, and even the processing of genetic material. Nevertheless, it is amusing to witness the disdain of many investigators toward mechanistic conclusions drawn from kinetic data. After all, kinetic arguments are frequently tediously detailed with algebra and calculus, and so many refuse to believe that such abstract constructs truly apply to real systems. For those of us who derive much fascination, excitement, and satisfaction from the combination of chemical and kinetic probes of enzyme mechanism and regulation, the statement that "kinetics never proves anything" is especially amusing. When one views the definition of the word "proof" as an operation designed to test the validity of a fact or truth, the preceding statement serves only to demonstrate that we have failed to communicate the power and scope of kinetic arguments. The purpose of this volume is to initiate those who are interested in an advanced treatment of enzyme kinetic theory and practice. Indeed, this area of biochemistry is rich in information and experimental diversity, and it is the only means to examine the most fundamental characteristic of enzymes—catalytic rate enhancement.

Part A (Volume 63) and Part B (Volume 64) are the first of a series of volumes to treat enzyme kinetics and mechanism, and the chapters presented have been written to provide practical as well as theoretical considerations. However, there has been no attempt on my part to impose a uniform format of symbols, rate constants, and notation. Certainly, uniformity may aid the novice, but I believe that it would also present a burden to those wishing to examine the literature. There, the diversity of notation is enormous, and with good reason, because the textural meaning of particular terms must be considered. In this respect, the practice of utilizing a variety of notations should encourage the student to develop some flexibility and thereby ease the entry into the chemical literature of enzyme dynamics and mechanism. Each of the contributors is an expert in the literature, and I have been especially pleased by the constant reference to key sources of experimental detail.

I wish to acknowledge with pleasure and gratitude the cooperation and ideas of these contributors, and I am indebted in particular to Professors

Fromm and Cleland for many suggestions during the initial stages of developing the scope of this presentation. My students, certainly R. Donald Allison, also deserve much praise for surveying the literature and convincing me that a balanced view of the field could be presented in the confines of this series. The staff of Academic Press has also provided great encouragement and guidance, and to them I am deeply indebted. Finally, I wish to acknowledge the wisdom and friendship offered to me by Sidney Colowick and Nathan Kaplan.

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Edited by ANTHONY SAN PIETRO

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Section I

Isotope Probes of Mechanism

[1] Isotope Exchange Methods for Elucidating Enzymic Catalysis

By DANIEL L. PURICH and R. DONALD ALLISON

Isotope exchange methods constitute an entire domain of enzyme kinetics that until 20 years ago was largely concerned with net velocity measurements. The availability of isotopes and appropriate sensing devices (e.g., mass spectroscopy and scintillation counting) has encouraged the development of new approaches to understand enzyme catalysis and regulation. Among the early investigators concerned with exchange studies,[1-3] Boyer was probably the first to recognize their power and scope,[4,5] and his recent review outlines many of the major findings.[6] At one time, equilibrium exchange studies were employed almost exclusively as an adjunct to initial-rate studies of the ordering of substrate binding and product release.[7-10] Now, the approach has been considerably extended to involve rate measurement of loss, or exchange, of essentially all possible substrate atoms or functional groups of atoms, the determination of kinetic isotope effects, the definition of hitherto hidden stereochemical processes, and the examination of the interference of regulatory activators, inhibitors, and interconverting enzymes with the detailed chemical steps in the catalytic process. It is also interesting to note that oxidative phosphorylation and photophosphorylation, while outside the scope of this chapter, have also yielded important information through exchange studies.

To thoroughly examine each of the above aspects of exchange studies would require excessive space, and the reader is referred to several additional sources.[6,11,12] Generally, we will deal with representative applica-

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tions of isotopic exchange at, or near, equilibrium. Here, one may find information on the binding and release of substrates, the rapidity of certain exchanges relative to each other and to the rate-limiting or rate-determining steps, the occurrence of abortive complexes, the likelihood of substrate synergism and possibly cooperativity, and the validity of the rapid-equilibrium assumption. We shall also examine exchange processes away from equilibrium, a condition of obvious importance when one studies irreversible or essentially irreversible processes. Again, valuable information frequently obtained in such cases includes the order of substrate binding and product release, the participation of covalent enzyme–substrate intermediates, and the nature of the irreversible step.

Systems at Equilibrium

Even at equilibrium enzymes relentlessly shuttle substrates and products forth and back, and the flux in each direction is essentially constant and balanced over the time period used in measurements. Inasmuch as the rate of enzymic catalysis depends upon the concentration of enzyme–substrate(s) and enzyme–product(s) complexes, the enzyme's behavior at equilibrium is a complex composite of the net reaction rates in the forward and reverse directions observed away from equilibrium. It is also true that the equilibrium flux in each direction depends upon enzyme concentration, and one may adjust the enzyme level to suit the limitations on the exchange experiment.

As noted earlier, introduction of a labeled substrate or product may be utilized to trace the course of the reaction quantitatively. Depending upon the position of the isotopic atom(s) in the labeled substrate or product, various exchanges may be examined. The types of exchanges subject to measurement can be illustrated by considering a bisubstrate reaction in the following form:

$$A—X + B—Y \rightleftharpoons A—Y + B—X \qquad (1)$$

In all, one might expect that there are a number of exchange reactions to be examined. However, not all hypothetical exchanges actually involve the transfer of atoms (or functional groupings of atoms) between the various substrate–product partners; thus, only three exchanges occur. For example, in the hexokinase reaction it is possible to observe exchange reactions between glucose and glucose 6-phosphate, ADP and ATP, ATP and glucose 6-phosphate, but not glucose and ADP. Likewise, NAD-dependent dehydrogenases will never undergo exchange between the oxidized substrate and the oxidized coenzyme. Nonetheless, more complicated enzyme systems may have a number of exchange processes