BOOK REVIEW

Enzyme kinetics and mechanism, by Paul F. Cook and W.W. Cleland. 2007. Garland Science, New York. 416 pp. \$70.00 (hardcover)

This 400-page volume successfully fills an important gap in the enzymology literature. A number of specialized texts on enzyme kinetics are available, but none has superseded Segal's indispensable 32-year old encyclopedic compilation that is on the bookshelf of most practicing enzyme kineticists. While the present text covers much of the theoretical content presented in Segal, the combined practical experience of the Cook and Cleland laboratories enables them to provide valuable guidance in, for example, experimental design and data interpretation.

Few will argue with the assertion that Cleland is nearly single-handedly responsible for the current codification of steady state enzyme kinetic analysis. Prior to the appearance of his *Biochimica et Biophysica Acta* papers of the early 1960s, only the kinetic mechanisms of single substrate/single product enzymes were easily tractable. More complex mechanisms, involving two or more substrates, required analysis by difficult kinetic methods such as that of Peller and Alberty (1959. *J. Amer. Chem. Soc.* **81**: 5907–5914). Cleland's colorful and appropriate representations of kinetic models (e.g., "ping-pong bi bi" to designate an enzyme converting two substrates to two products via a pathway where the first substrate, A, "pings" the enzyme to an alternate form, F, simultaneously releasing the first product, P, and the second substrate, B, "pongs" the enzyme back to the original state, E, while producing the second product, Q) were rapidly and broadly adopted, and became standard course material. Simple descriptions could be used to describe ordered or random addition and release for any number of substrates and products. The analyses were aided by the introduction of easily visualized linear or branched diagrams.

The text begins with a useful chapter (8 pages) that defines the common terms encountered in kinetic analyses including the important distinction between macroscopic and microscopic rate constants. The second chapter (7 pages) begins with four densely packed pages covering first-, *pseudo*-first-, and second-order kinetics. This naturally duplicates readily available treatments that are usually described more extensively in other texts. Readers

unfamiliar with at least these basic concepts of kinetic analysis are likely to struggle beyond this point. The next 18 pages extending to the end of Chapter 3 provide an introduction of the basic presentation of kinetic data and some essential guidance on how to assay enzymes. The important advantage of continuous over fixed time point assays is discussed. Coupled and total time course assays are considered briefly.

Chapter 4 packs an extraordinary quantity of important kinetic concepts and derivations. Included, *inter alia*, are the King-Altman and the net rate constant methods, equations for isotope exchange, and an introduction to data processing that includes error analysis. There is not a wasted word. All of these topics are discussed elsewhere, and the engaged reader will need to pursue the original references or other more in-depth treatments as necessary. The first 40 pages of Chapter 5 describe the methods developed by Cleland to analyze multi-reactant enzymes. The kinetic practitioner will readily discover the model most applicable to his/her own enzyme, and may or may not wish to understand the derivation. The chapter concludes with considerations of allostery, and contains a useful experimental flowchart designed to help identify kinetic mechanism.

The 83 pages of Chapter 6 present a comprehensive analysis of the various aspects of enzyme inhibition. This chapter will find an attentive audience in the pharmaceutical industry. The meaning of IC₅₀ values; the distinction between competitive, uncompetitive, and non-competitive inhibitors; and substrate and product inhibition are all well described. The best drug candidates are often slow or tight binding inhibitors, and this topic is carefully developed. The kinetic treatment of irreversible inhibitors is not given.

An introduction to pre-steady state and relaxation kinetics is provided in the 20 pages of Chapter 7, which will provide only initial guidance for those interested in this field. More comprehensive treatments such as those of Bernasconi, Caldin, or Eigen and deMaeyer will need to be consulted before conducting experiments with these techniques.

There is an important distinction to be made between kinetic and chemical mechanism. The portion of the book so far described deals almost exclusively with the former, which can mainly provide the investigator with information

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on the order of addition of substrate molecules, the composition of some intermediate complexes, and the order of product release. The chemical mechanism describes how the enzyme utilizes its endowed chemical and physical properties to effect the transformation of substrates to products. Probes of the latter usually require the combination of kinetic with other methods. Isotopes are widely used in enzymology, in large part because isotopic substitution in a substrate does not significantly perturb the chemical or physical properties of the molecule. Thus the free energy and site of association of a molecule with an enzyme active site is, to an excellent approximation, independent of isotopic substitution, and kinetic isotope effects thus interrogate changes in bonding between the ground and transition states almost exclusively. Cleland, in addition to his singular accomplishments in enzyme kinetics, has together with Cook and others, contributed prominently to the gleaning of chemical mechanisms from kinetic isotope effect experiments in enzymology.

Chapter 8 describes important widely used isotopic methods. These include isotope exchange at equilibrium to probe decisively the order of substrate addition, and positional isotope exchange to investigate bond formation processes in enzyme substrate intermediate complexes that would not be observed in the absence of the isotopic label. Chapter 9 describes the kinetics of enzyme reactions involving isotopes that are used mainly as probes of transition state structure and to deconvolute rate-determining steps in a complex reaction. The volume concludes with a chapter on the analysis of pH vs. kinetic parameter value profiles that are very helpful in assigning

Brønsted acid/base functionality in enzyme reaction mechanism.

The reader interested in examining the references must have a handy computer terminal. There is no list of references—only in-text citations of the form -B 10, 1947, referring to the article beginning on p. 1947 of Volume 10 of *Biochemistry*, are given. Names of senior authors are not uniformly provided to guide the reader to the literature. While this artifice saves paper, it does make searching the literature more problematic. I prefer to see the title, the names of the senior authors, and the year of publication to help to decide whether to consult the reference. It is to be hoped, therefore, that this protocol will not become widespread.

Sample reactions are cited in many cases, but the enzymes are often identified by name only. Scientists, whose main focus is not enzymology, might not be familiar with many of these. Drawings of substrates and products would have been useful.

This book should have extensive appeal to students as well as to practicing enzymologists. While the coverage is densely packed in some places, enough is given to familiarize the reader with nearly every aspect of modern enzyme kinetics. Industrial enzymologists may well find the book indispensable.

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