

Preface

Rapid-equilibrium enzyme kinetics has several advantages over steady-state enzyme kinetics. That is not to say that it is always applicable, but rapid-equilibrium enzyme kinetics yields simpler rate equations than steady-state enzyme kinetics. Since rapid-equilibrium rate equations are the simplest possible, they are the place to start. They are actually widely used. When rapid-equilibrium rate equations are applicable, Michaelis constants are equilibrium constants. For simple mechanisms, rapid-equilibrium rate equations are readily derived by hand, but, for more complicated mechanisms, they can be derived using a computer without having to write a computer program. This is especially useful when pH effects are involved, or there are many reactants, as for random $A + B + C \rightarrow$ products, or multiple forms of inhibition, activation, and modification. When the rapid-equilibrium assumption is applicable, all the reactions up to the rate-determining reaction are at equilibrium. The expression for the equilibrium concentration of the enzyme-substrate complex that yields products can be derived with a computer using Solve, which is an operation that solves a set of simultaneous polynomial equations. This operation is available in *Mathematica*^R, *Maple*^R, and *MatLab*^R. The input for Solve includes equilibrium expressions for an independent set of reactions and a conservation equation. The use of a computer has the additional advantage that the rapid-equilibrium rate equation is obtained in computer-readable form and does not have to be typed into the computer. Rapid-equilibrium rate equations can be derived with a computer for very complicated mechanisms.

Solve can also be used to calculate the values of the kinetic parameters in a rate equation from the minimum number of velocity measurements. This calculation can be made using steady-state rate equations as well as rapid-equilibrium rate equations. When rapid-equilibrium rate equations do not represent the experimental data, steady-state rate equations can be used, or empirical rate equations can be used.

There are two ways to organize a book on rapid-equilibrium kinetics. There could be chapters on deriving rate equations, estimating kinetic parameters, effects of pH, effects of inhibitors, etc. Or there could be chapters on various types of reactions with derivations of rate equations for different mechanisms, estimation of kinetic parameters, effects of pH, etc. This second option is followed here by treating different kinds of mechanisms separately so that a user can study various aspects of the kinetics of a certain type of enzyme-catalyzed reaction in a single chapter. The exceptions of this organizational plan are Chapter 1 on biochemical thermodynamics that is the foundation for the rapid-equilibrium assumption, the three chapters on inhibition, activation, and modification, and the last chapter on systems of enzyme-catalyzed reactions.

Biochemical thermodynamics is based on the Legendre transformed Gibbs energy G' that brings in the pH as an independent variable. This development has brought in new ideas about the use of thermodynamics in biochemistry. Enzyme kinetics necessarily contains biochemical thermodynamics because rate equations including the reverse reaction must yield the equilibrium composition at long times. When rate equations are derived on the assumption that there is a rate-determining reaction, the reactions before the rate-determining reaction are treated with biochemical thermodynamics. For example, the equilibrium composition of substrates, enzymatic sites, enzyme-substrate complexes, hydrogen ions, inhibitors, activators, and modifiers is calculated using an independent set of reactions. The relationship between biochemical thermodynamics and rapid-equilibrium enzyme kinetics is made evident by the Haldane relation that expresses the apparent equilibrium constant K' for the reaction that is catalyzed in terms of kinetic parameters in the rate equation. The existence of the Haldane relation means that the thermodynamic effects of temperature, pH, and ionic strength on equilibrium are all in the kinetic parameters. Of course biochemical thermodynamics is also built into transient kinetics, but that is not discussed in this book.

This book is concerned with six levels of understanding the kinetics of an enzyme-catalyzed reaction. (1) The first level is the understanding of the thermodynamics of the enzyme-catalyzed reaction. When an enzyme-catalyzed reaction occurs, the

composition changes until equilibrium is reached. The equilibrium concentrations of the substrates can be calculated using the apparent equilibrium constant, which is a function of temperature, pH and ionic strength. The apparent equilibrium constant may also be a function of $[\text{Mg}^{2+}]$, for example. This thermodynamic information does not tell us anything about the mechanism or rate constants because the apparent equilibrium constant depends entirely on the thermodynamic properties of the substrates. (2) The second level of understanding is to be sure that the velocity at specified temperature, pH, and ionic strength is directly proportional to the total enzyme concentration. This is true for all the mechanisms discussed here. (3) The third level of understanding involves the determination of the rate equation at a particular temperature, pH, and ionic strength. There are two types of rapid-equilibrium rate equations: rate equations for the forward reaction and complete rate equations that include the reverse rate equation as well. In this book, when the catalyzed reaction is represented by $A + B \rightarrow \text{products}$, only the forward reaction is discussed, and when the catalyzed reaction is represented by $A + B = P + Q$, the rate equation includes the parameters for the reverse reaction, as well as the forward reaction. This book is concerned with rapid-equilibrium rate equations, but there is the possibility that a steady-state rate equation is required. Even an empirical rate equation may be required. A complete rate equation can be tested by using the Haldane relation to calculate the apparent equilibrium constant at the experimental temperature, pH, and ionic strength. (4) The fourth level of understanding involves the study of the effects of pH on the kinetics. Even when the substrates do not have pKs, velocities will in general depend on the pH. These effects are determined by pKs of the enzymatic site, the enzyme-substrate complexes, and the substrates. It is possible that one or more hydrogen ions may be consumed in the mechanism. When a steady-state rate equation is required, these effects become much more complicated, but when the rapid-equilibrium assumption is applicable, all of these effects can be represented by pKs and the number of hydrogen ions consumed in the mechanism. When these effects can be determined quantitatively for both the forward and reverse reactions, the Haldane relation must yield the correct dependence of the apparent equilibrium constant on the pH and ionic strength. It is important to distinguish between the number n of hydrogen ions consumed in the mechanism and the change in binding of hydrogen ions determined by thermodynamic measurements. The number n of hydrogen ions consumed in a reaction in a mechanism is an integer, but the change in binding of hydrogen ions in an enzyme-catalyzed reaction depends on the pH. (5) When the rapid-equilibrium assumption applies, Michaelis constants are equilibrium constants, and so the study of the effect of temperature can yield standard transformed Gibbs energies of reaction, standard transformed enthalpies of reaction, and standard transformed entropies of reactions in the mechanism. (6) The sixth level of understanding involves inhibition, activation, and modification by binding at the catalytic site or other sites. Modifiers can be inhibiting or activating, but they provide additional pathways to products. This book deals with all six levels of understanding.

Mathematica is a wonderful application for rapid-equilibrium enzyme kinetics because *Solve* can be used to derive rapid-equilibrium rate equations and also to estimate the kinetic parameters using the minimum number of velocity measurements. *Mathematica* has the advantage of including a word processor so that a book with text and live equations can be written. In *Mathematica* the symbols for variables and kinetic parameters have to start with lower case letters because operations like *Solve*, *TableForm*, and *Round* start with capital letters. The names of programs also have to start with lower case letters. Names of reactants and functions cannot involve spaces, hyphens, dots or other mathematical symbols.

Each chapter in this book is a *Mathematica* notebook that includes text as well as calculations. These chapters include descriptions of the background for calculations, the calculations, and discussion of their significance. The CD at the back of the book contains the entire book in *Mathematica*. This CD can be downloaded into a personal computer with *Mathematica* installed, but it can also be read in a computer with *MathReader*, which is freely available from Wolfram Research, Inc. (100 Trade Center Drive, Champaign, IL 61820-7237, and www.wolfram.com). All the calculations in a chapter can be run by use of *Evaluate/Evaluate Notebook*. Programs and calculations can be copied and pasted in a new notebook. Calculations can be rerun with different input. The input to programs can be changed to apply these programs to other reactions and conditions.

Chapter 1

It is not necessary to be a programmer to use the programs in this book. More programs and calculations on rapid-equilibrium enzyme kinetics are available at *MathSource*. The references are at the end of the book, and this includes web URLs and notebooks in *MathSource*.

This book builds on my previous book "Biochemical Thermodynamics: Applications of *Mathematica*", Wiley, Hoboken, NJ (2006). That book provides a great deal of information on the thermodynamics of enzyme-catalyzed reactions that is relevant to enzyme kinetics. For example, it gives the apparent equilibrium constants for 229 enzyme-catalyzed reactions at 298.15 K, 0.25 M ionic strength, and pHs 5-9. More apparent equilibrium constants can be calculated for similar reactions.

This is the second book on enzyme kinetics written in *Mathematica*. The first is P. J. Mulquiney and P. W. Kuchel, *Modelling Metabolism with Mathematica*, CRC Press, Boca Raton, Florida (2003). Their book uses steady-state rate equations, and it gives a program in *Mathematica* to derive steady-state rate equations. This program is based on the method described by A. Cornish-Bowden (Biochem. J. 165, 55-59 (1977)). They give steady-state rate equations for about 30 enzyme-catalyzed reactions.

I am indebted to Professor William Martin McClain, Wayne State University, for catching some errors in my treatment of thermodynamic cycles, which have been corrected. He has written an excellent book "Symmetry Theory in Molecular Physics with *Mathematica*," Springer, 2009.

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Use of Mathematica

This book is written in *Mathematica* 7.0. Even if you are not familiar with *Mathematica* (Wolfram Research, Inc. 100 World Center Drive, Champaign, IL 61820-7237 and www.wolfram.com) you should be able to read this book. The concepts and calculations in biochemical kinetics are explained in words in the textual parts of the book. And the results of calculations are discussed in words. When *Mathematica* is used to make tables and figures, explanatory titles are given.

Since *Mathematica* is a high level language that uses commands like Solve and D (for differentiate), you can see what mathematical operations are involved in a program. Everything that is involved in making these calculations is shown. When a calculation is made, a semicolon is often put at the end of the input so that the result is not shown there. When calculations are performed in your computer, the semicolons can be omitted to see what the result is. Semicolons are used in the book to save space.

Each chapter is a *Mathematica* notebook. When a chapter has been downloaded, it can be run by using Evaluation/Evaluate Notebook. This will take a few seconds or a minute to evaluate the operations. Quit *Mathematica* and restart before opening another chapter. Calculations and programs can be copied and pasted into a notebook of your own. Then the input can be changed to solve your problem.

The page of Contents shows that this book contains 12 Chapters that are each a *Mathematica* notebook. These 12 notebooks are selfsufficient in the sense that they can each be run alone. As mentioned above, only one of these notebooks should be open at one time. Pages are numbered within each of the notebooks.

The rate equations in this book can be used to calculate velocities for different sets of kinetic parameters and substrate concentrations. This is a good way to learn about the behavior of a mechanism or to test the kinetic parameters that have been determined.

A number of books have been written to introduce *Mathematica* to new users. A list of books is available at the Wolfram Store. Wolfram offers free online seminars for new users: <http://www.wolfram.com/services/education/seminars/>.