Antonio Baici

Kinetics of Enzyme-Modifier Interactions

Selected Topics in the Theory and Diagnosis of Inhibition and Activation Mechanisms

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To my students, Swiss Federal Institute of Technology and University of Zurich, 1972–2012

Foreword

Many drugs in current use owe their effectiveness to the fact that they are enzyme inhibitors. In addition, going back to the studies of invertase that Leonor Michaelis and his collaborators carried out at the beginning of the twentieth century, inhibitors have played a major role in efforts to understand the mechanisms that allow enzymes to fulfil their roles as catalysts. Bringing these two aspects together, we may note that understanding how inhibitors affect individual enzymes is a necessary step in understanding what happens when an enzyme that forms part of a metabolic pathway is inhibited in a living organism. Inhibitors and activators taken together are classified as enzyme modifiers, the subject of Antonio Baici's masterly book. Activators have been less intensively studied than inhibitors over the years, but they are also important and should not be forgotten.

It surely follows, therefore, that kinetic characterization of these effects is vital both for understanding enzyme mechanisms and for drug development. Yet the treatment in most general textbooks of biochemistry (with Henry Mahler and Eugene Cordes's *Biological Chemistry*, now more than 40 years old, as an honorable exception) is nearly always superficial and sometimes even misleading. More specialized books on enzymes mostly do little more than scratch on the surface, though again there is an honorable exception, in the form of Malcolm Dixon and Edwin Webb's *Enzymes*. Some of the books specifically devoted to enzyme kinetics, such as Irwin Segel's *Enzyme Kinetics*, include considerable detail about inhibition, though not all of them do. Few of the better books are very recent, however, and J. Leyden Webb's monumental treatise *Enzyme and Metabolic Inhibitors* dates from the 1960s. Many of today's readers are unlikely to know that it even exists, especially if they do all their reading on the web, or if they think that nothing published more than 6 months ago is worth reading.

The systematic treatment of inhibition and activation by Jean Botts and Manuel Morales [*Transactions of the Faraday Society* **49**, 696–707 (1954)] is even older, and the principles they set out are now rarely taught. In the distant past, when I used their theory as the basis of teaching about enzyme modifiers, it was obvious that it was very unpopular with students, being seen as highly complex and difficult. As Antonio Baici shows, however, it can be presented in a way that allows it to

be understood and applied. Moreover, it needed to be extended to take account of allosteric effects, which were essentially unknown in the 1950s.

Biochemists who believe in the comforting illusion that any inhibitor that has a structural resemblance to the substrate of the enzyme, and even one that does not, can be treated as a simple competitive inhibitor will find abundant evidence in the book that it is just that, an illusion. The author provides many examples of the kinds of behavior that people might prefer not to know about, and shows that building on Botts and Morales's pioneering work is necessary for an adequate understanding of enzyme modifiers. That in turn is necessary if one hopes to design inhibitors and activators that will be pharmacologically useful.

Marseille, France
March 2015

Athel Cornish-Bowden

Preface

This monograph is neither aimed at covering all technical aspects of enzyme kinetics nor at reviewing extensively enzyme inhibition and activation. Considering that fundamental aspects of enzyme kinetics have been competently treated in several books, while countless reviews and articles dealt with specific topics, I assume that readers of *Kinetics of Enzyme-Modifier Interactions* are already familiar with the basic principles of this discipline. My last thought while writing this book was to deal with *pièces de résistance* in the classical way found in existing excellent publications by copying and pasting established theories and methods, a bare nonsense. Rather, I felt that our knowledge of enzyme-modifier interactions could benefit from a scrutiny of existing but in a way buried concepts in need to be clarified and complemented by systematization using alternative methods. Therefore, the following chapters will examine under a magnifying glass selected topics and discuss less-known, neglected, or overlooked aspects by adding a pinch of new ideas. The goal is to support investigations in vitro by suggesting logical solutions to problems of various complexity and developing theoretical aspects of mechanisms beyond those already known but likely to exist in still unexplored niches. An extension to living organisms to include the flux control in whole systems, through modification of a particular enzyme, lies outside the objectives of this book.

To discredit the rumor about enzyme kinetics as an abstruse discipline, the students in my undergraduate courses were motivated by lectures in enzyme kinetics that included material present in this book. Students in master's degree programmes grasped quickly the various mechanisms of enzyme inhibition and activation, double inhibition, and slow-onset inhibition. Therefore, not being complicated as it may appear at first sight, this book is hoped to inspire teachers, students, and investigators in academia and industry interested in exploring the still partly uncharted territory of enzyme modification.

A possible novelty to students of kinetics is the systematic approach to enzyme modification mechanisms, which will be ranked taxonomically following criteria based on individual, unique characters that make them equal to *species*. Similar to plants and animals, to which systematic names are given to distinguish them from other related or unrelated species, also enzyme-modification mechanisms deserve

their own names. For users uncertain where to place their own experimental findings in the intricate labyrinth that leads from raw data to mechanisms and to the calculation of kinetic parameters, dichotomous keys will be provided as an analytical companion for the diagnosis of the basic enzyme-modification mechanisms and slow-onset enzyme-modifier interactions.

Since the customary vocabulary in enzyme inhibition and activation was insufficient in this novel perspective, the proposed nomenclature of mechanisms will follow their systematic ranking. In this respect, the most demanding group of enzyme modifiers was *mixed inhibition*, conventionally treated under this common heading, and now appearing as eight individual, well-distinguished entities flanked by five nonessential activation mechanisms and two hybrid species that have either inhibitor or activator character depending on substrate concentration. This group of fifteen mechanisms contains the strategic tools used by allosteric effectors in enzyme regulation. Knowing the details of the fine-tuning possibilities of this class of substances is not only important for interpreting physiological processes but also as support in emerging pharmacological approaches. Specific pharmacological targeting of unwanted enzyme activities deserves more attention than the estimation of a crude IC_{50} value.

In 2013 two important anniversaries have been celebrated: the 100 years of Michaelis and Menten's paper, considered the birth of modern enzyme kinetics, and the 50 years of Monod, Changeux, and Jacob's concept of allosteric regulation. A third event in 2013 passed unobserved, namely the 60th anniversary of the publication of Botts and Morales on the effects of modifiers upon the enzyme-catalyzed steady-state reaction rate. Although not highly regarded as the other mentioned illustrious publications, the work of Botts and Morales contains the basic elements for interpreting the interactions between enzymes and modifiers. The implications of their model are far-reaching and will be used in this book as the basis for a taxonomic ranking of inhibition and activation mechanisms.

Zurich, Switzerland Antonio Baici
March 2015

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In 2006, the Centenary year of *The Biochemical Journal*, I was invited to write a *classics* article on papers in enzyme kinetics published in the first 100 years of the same journal. The organizers gave me only one limit: to choose just three papers, a sort of judgement of Paris that made me uneasy. After realizing that my decision was unlikely to lead to a second Trojan War, I did not hesitate in choosing *The Direct Linear Plot* co-authored by Robert Eisenthal and Athel Cornish-Bowden as one of three classics published in *The Biochemical Journal*. Besides this method that I regularly taught in my lectures, I believe to have read all papers by Athel-Cornish Bowden and his books, tacitly nominating him my teacher in enzyme kinetics. I would like to thank Athel for his engagement and enthusiasm in this fascinating discipline.

Giorgio Semenza has been the first person with whom I had the privilege of talking about enzyme kinetics when, in 1972, I started in this field at the Swiss Federal Institute of Technology in Zurich. I admire Giorgio's immense culture, dear friend, medical doctor, and excellent biochemist who published rate equations for enzyme inhibition and activation in the *Journal of Theoretical Biology*.

Stephen Bearne was very kind and helpful in sharing with me his knowledge in kinetics and thermodynamics and supporting the construction of kinetic barrier diagrams with precious suggestions.

Brigita Lenarčič has been a long-term collaborator in projects focussed on peptidases involved in the degradation and remodeling of the extracellular matrix and their interactions with naturally occurring inhibitors. I always appreciated her profound experience in this challenging topic of enzymology.

Marko Novinec, from the laboratory of Brigita Lenarčič at the University of Ljubljana, spent more than 2 years in Zurich sharing with me joys and sorrows with bench work and publishing on the kinetics of cysteine cathepsins putting in focus

allosteric interactions. Marko's skill was fundamental in realizing a demanding project on the identification of allosteric sites in cathepsin K and the characterization of their interactions with a heap of amazing hyperbolic modifiers discussed in this book.

Supporting my request for help with handling multiple elementary steps between enzyme states, Feng Qi and Daniel Beard were kind enough to modify their useful algorithm KAPattern based on the method of King and Altman for the systematic generation of reaction patterns. The modified code, available online, is very helpful for deriving the rate equations of complex systems.

Patricia Schenker was unique among my students in choosing for her successful PhD work topics that were fully centered on enzyme kinetics. With her passion for mathematics, Patricia was not afraid of starting a difficult journey in the field of multiple enzyme modification or to combine molecular modeling with skillful and precise experimenting. Patricia carefully reviewed and gave constructive advice on technical details and on the structure of the entire book.

Thomas Hirt, Stefan Klauser, and Steve Rast, the information technology team at the Department of Biochemistry, University of Zurich, merit a sincere applause for supporting my demands with computer work and intervening within minutes for repairing my frequent mistakes. Thomas, who completed his Master in Biochemistry in my laboratory, actively participated in an interdisciplinary didactic project that is still used by students to simulate, by numerical integration methods, time-dependent phenomena in biochemistry, microbiology, and physics.

I thankfully acknowledge my colleagues at the Department of Biochemistry of the University of Zurich for fruitful collaborations, friendly relationships, and encouragement during my career: Hans Rudolf Bosshard, Amedeo Caflisch, Philipp Christen, Raimund Dutzler, Heinz Gehring, Sergio Gloor, Markus Grütter, Bernd Gutte, Ilian Jelezarov, Jeremias Kägi, Peter Lindner, Andreas Plückthun, Ben Schuler, Peter Sonderegger, and Milan Vašák. The scientific interaction with my colleagues and support given to their numerous students at various levels substantially contributed in enlarging my horizon in enzyme kinetics and macromolecular interactions in general.

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My wife Roberta and my sons Francesco, Federico, and Luca have always been very tolerant for the time I robbed with my research and teaching activity, including the time necessary to write this book after my retirement. I hope that the time I am now dedicating with much love to my grandchildren will partly compensate my sins of omission.

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