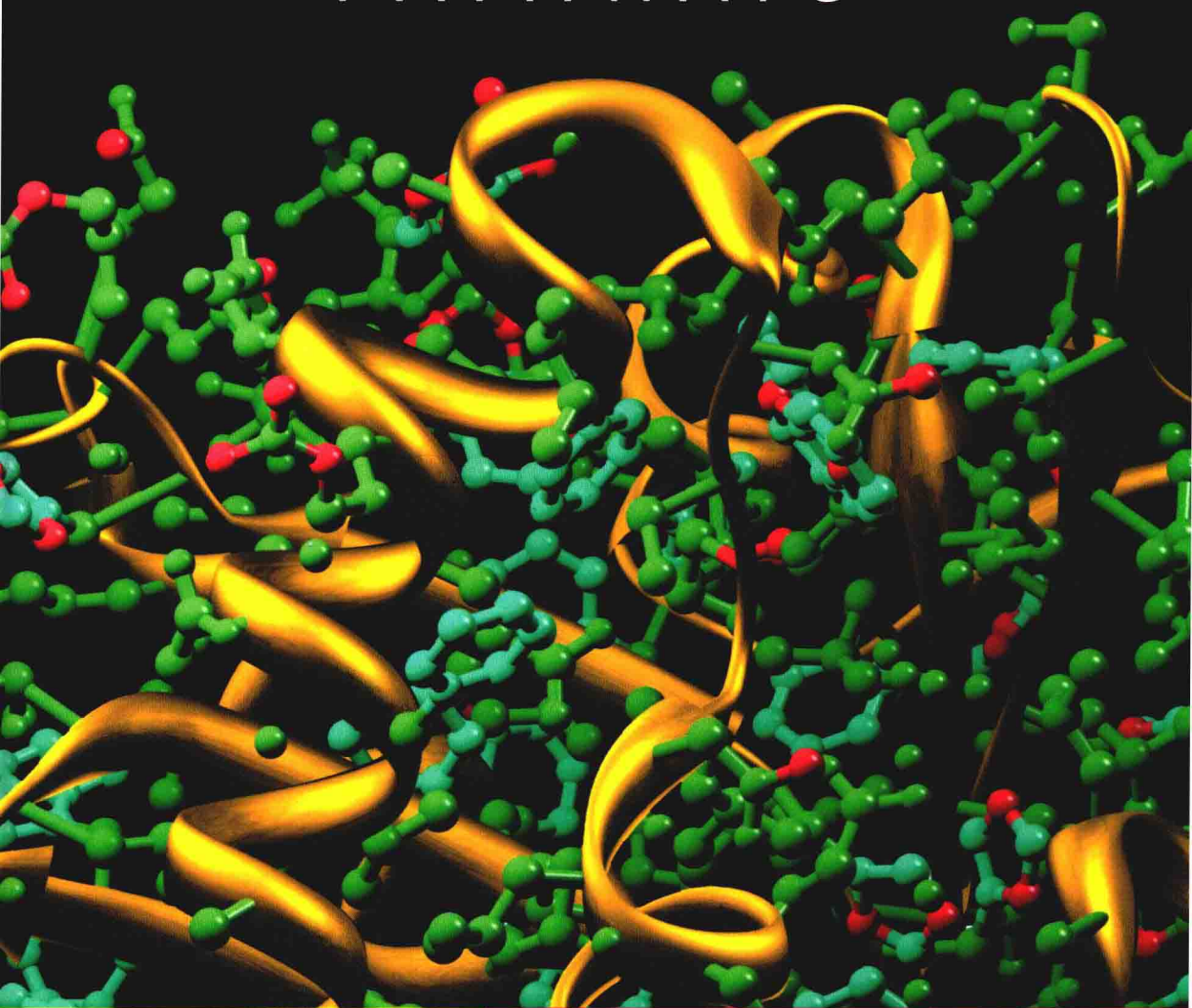
Lloyd Wolfinbarger, Jr.

# ENZYME

## REGULATION IN METABOLIC PATHWAYS



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# ENZYME REGULATION IN METABOLIC PATHWAYS

Enzymology is the study of enzymes as protein molecules, with specific folding patterns of the amino acid polymer and unique binding sites wherein intra- and inter-molecular distances define the specificity of the molecule to attract, bind to, and change some substrate molecule.

*Enzyme Regulation in Metabolic Pathways* shows the reader how to understand the roles of enzymes and their kinetic constants in intermediary metabolism. It provides a means of correlating data obtained in experimental studies to multiple possible mechanisms through which some enzyme may catalyze the conversion of a substrate to a product. Quasi-equilibrium assumptions are used throughout the book, keeping the rate equation derivations simple. Actual metabolic pathways with known (presumed) positive and negative regulation events are linked to these potential kinetic mechanisms using both rate equation derivations and data plots illustrating how the rate equation derivations can be used to explain the data plots.

This book will be a valuable reference for students in biological sciences and biochemistry, taking a core course in enzymology.

## About the Author

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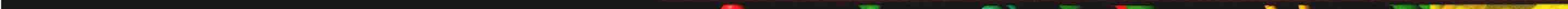

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# Enzyme Regulation in Metabolic Pathways

*Lloyd Wolfinbarger, Jr.*

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Enzyme Regulation in Metabolic Pathways

# Preface

This book was developed from courses in biochemistry and enzymology at the upper undergraduate and lower graduate level. I found that my students were quick at learning structures and metabolic pathways in biochemistry and at deriving rate equations for single-substrate/single-product as well as two-substrate/two-product enzyme-catalyzed reactions, but neither group of students was getting a good understanding of how enzymes directed the flow(s) of metabolites through metabolic pathways and how these metabolites regulated which pathway managed to metabolize the most metabolites to some end product(s). All too often, they were too focused on passing the tests rather than trying to understand and to challenge what I was teaching them.

Enzymology is frequently taught in a standard biochemistry textbook, and the course in biochemistry using that book takes a fairly standard approach in covering energies of activation, steady state versus quasi-equilibrium kinetics, and modes of modifier inhibition/activation of an enzymatic reaction. A typical enzymology course tends to focus on the differing kinds of enzymes and the kinds of reactions they catalyze, kinetic rate equation derivations, data plots obtained with different enzyme modifiers, and, in the more extensive texts, kinetic mechanisms by which an enzyme may catalyze a chemical reaction.

The basic premise of this book will be that there is a need for an easy means of correlating the data one actually obtains in experimental studies with multiple possible mechanisms through which some enzyme may catalyze the conversion of a substrate to a product. Although not the most appropriate means of determining some potential kinetic mechanism, quasi-equilibrium assumptions will be used throughout the book in that these keep the rate equation derivations simple. Actual metabolic pathways with known (presumed) positive and negative regulation events are linked to these potential kinetic mechanisms using both rate equation derivations and data plots illustrating how the rate equation derivations can be used to explain the data plots.

Finally, I want to emphasize that it is not the purpose of this book to be technically accurate with respect to enzyme kinetics, nor is it the purpose of this book to teach you about metabolic pathways or the traditional ways in which metabolic pathways are thought to be regulated. I do not profess to be a hard core enzymologist devoted to teaching enzymology. Rather I propose to show you how I came to understand the roles of enzymes and their kinetic constants in intermediary metabolism, and thus the purpose of this book is to challenge you

to think about what I convey to you and hopefully to get you to challenge my teachings. In challenging my teachings, you will achieve the purpose of this book. You will learn to think rather than learn to take notes so you can pass the next test.

The level and contents of this book should make it suitable as a reference text in biochemistry courses at the undergraduate level and as a textbook for a course in basic enzymology taken prior to a more advanced course in enzymology at the graduate level.

I acknowledge substantial help from my former students who over my 30 odd years as a professor constantly challenged my ideas and interpretation of data, and particularly to one of my former employees, Katrina Ruth, who challenged me to stay on track with what I wanted to write and greatly assisted me in writing what I wrote in a more clear and concise manner.

## Author's Review

Yes, I know that this book is repetitive, redundant, and tedious. I also know, and suggest to you, that if you expected this book to teach you about basic enzymology, you picked up the wrong book. I was a professor at a major State University for some 30 years over a time where computers were first beginning to make their appearance, and I retired when computers had pretty much taken over the thinking process once reserved for people. When I was a young Assistant Professor, I ran across a small paperback book entitled *To Know a Fly* written by Vincent G. Dethier. The book was easy to read, with cartoons no less, and described how the author learned how to ask the right questions in his conduct of research using the common housefly. He asked how did flies know when something was sweet (food), how did they land upside down on the ceiling, and other equally important questions. The answers, at least for me however, were not about learning how flies know when something is food, but rather about how to ask the right questions and come up with the proper experiments to answer a question. This is, I hope, what this book is about.

You will be dragged through pseudo-thermodynamics, complex enzyme mechanisms, rate equation derivations that go nowhere, and through seemingly endless repetition to answers that are not absolute (only possible). But, somewhere in all of this, you will hopefully begin to think about what questions to ask when it comes to the study of enzyme function(s) and how to think about the vast numbers of possibilities you can devise in the way of answers (none, of course, that will be verifiable). If you are successful, you will realize that in all of this repetition, redundancy of writing, and tedious attention to details, it is not the answers that are important in life, it is the questions that you will learn to ask.

I quickly learned to use computers and to incorporate them into my teaching. However, I never lost sight of the importance of getting a small group of people together in a classroom where the sharing of creative (and frequently "ridiculous") ideas led to understanding and knowledge with respect not only to biochemistry or enzymology, but also to all those other bits and pieces of information floating around in our heads that led us to new ideas and understanding.

I will leave you with the admonition that you should ignore the tedious, the repetition, and the redundancy and forge ahead into the book. Ignore the failings of the author and focus on creating those visual images of the "possibles" and how you arrived at those "possibles." As is always said, it is the journey that is important, not the destination.

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# Part I

*Beyond this point there be dragons.*
Admonition on old seafaring maps

# 1

# Characteristics of Enzymes

At university, Enzymology was the class that most biochemistry or biology majors dreaded taking. Those students who liked the class were typically math majors who took the class for the thrill of solving complex rate equation derivations. Those students who had to take the class against their will were those who might need to understand the role of enzymes as they pertained to other aspects of biochemistry, but otherwise had little desire to sit through boring lectures involving lots of equations and the occasional molecular structures on the white board. As a professor teaching biochemistry to undergraduate and graduate students the task fell to me to keep my students' attention, so they didn't fall asleep, yet challenge them to understand why what I was teaching them could be both fun and useful.

I started teaching traditional enzymology as it was presented in the textbooks of the day (and I'm sorry to say is still being presented today). I found my students were passing the tests, but failing to understand how to interpret data and more importantly how to fit the data they were obtaining in their research into something meaningful and exciting. I eventually adopted a strategy of engaging the minds of my students with challenging, but improbable, enzymatic mechanisms and found that steady state kinetics, while more relevant, hindered the understanding of some of the more basic principles associated with enzyme kinetics. I finally hit upon the use of quasi-equilibrium assumptions and my students began to question and challenge my lectures—I had finally arrived as a professor.

Enzymology can be the study of enzymes as protein molecules with specific folding patterns of the amino acid polymer and unique binding sites wherein intra- and inter-molecular distances define the specificity of the molecule to attract, bind to, and change some substrate molecule. Computer-aided molecular modeling is a wonderful aspect of both biochemistry and enzymology in providing visuals essential to understanding, but does little to help with data analysis. Alternatively, enzymology can be the study of how these protein molecules control and mediate the flow of metabolites through intermediary metabolism affecting what we call metabolic viability to life forms. It is this latter study of enzymes that will be the focus of this book.

Enzymes are mostly proteins that are of variable length (with respect to amino acid sequences) and molecular weight. These amino acid polymers typically fold into some conformation that is most energetically favored based on the nature of

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the amino acids making up the protein and the aqueous environment in which they find themselves. For the most part, hydrophobic amino acids such as leucine or phenylalanine, as examples, are to be found in what might be called the hydrophobic core of the protein, whereas the hydrophilic amino acids such as histidine or aspartic acid, again as examples, will preferentially be found on those surfaces of the protein more exposed to an aqueous (hydrophilic) environment. The arrangement of hydrophobic and ionizable side groups of these hydrophilic amino acids is typically described as being present in some molecular organization that forms a region complementary to some low molecular weight solute. This region of the protein is generally regarded as constituting the substrate- (or modifier-) binding site. Whether this binding site tends to bind an unstable form of the substrate, stabilizing the unstable intermediate, and in so doing promoting its conversion to product; or whether this binding site tends to bind a stable form of the substrate and in so doing causes the substrate to shift into some less stable configuration promoting its conversion to product, will be discussed in detail. We will enter into this aspect of the basics of enzymology in detail in Chapter 2. For now, I only wish to stipulate that this enzyme with a substrate-binding site will be referred to as free enzyme (E) in subsequent sections. When free enzyme (E) binds with the substrate, it will be referred to as the enzyme/substrate complex (ES). As the enzyme facilitates the conversion of substrate to product via some unknown or unspecified mechanism, the product (P) released from the substrate-binding site will result in the (ES) complex reverting to free enzyme (E). Thus within the context of this book, the sum of the concentration of free enzyme (E) and enzyme/substrate complex (ES) will be referred to as the total amount (or quantity) of enzyme ($E_t$). When introducing modifiers of enzyme activity, I will use the simple connotation of a modifier (M) being either an activator (Ma) or an inhibitor (Mi). Modifiers will typically bind to free enzyme (E) to form a modified enzyme as either (MaE) or (MiE). Where substrate (S), enzyme (E), substrate/enzyme complex (ES), and so forth, are bracketed with square brackets, such as [S], the intent will be to express the molecule as some concentration. I will try to restate this point throughout the text, more to remind and help you than to irritate you with what will appear as my being overly redundant. Repetition is a good learning tool.

I would also like to emphasize one more point. I will make reference to "saturating" concentrations of substrate or modifier in the text. As you will see in later figures, as you add increasing concentrations of substrate (or modifier) to an enzymatic reaction, the rate of conversion of substrate to product will gradually increase until such time as that concentration approaches the capacity of that enzyme to bind to substrate converting it to product. At such a time where increasing the concentration of substrate no longer significantly increases the rate of conversion to product, it is generally assumed (described) as a saturation of enzyme by substrate. This will make more sense later, but I also want to emphasize that we will operate under the premise that the amount of substrate at any given concentration of that substrate will be inexhaustible. This means basically that you can crystallize salt out of sea water, but you will never run out of sea water where there is an infinite amount of salt. This is the difference between the concentration of salt in sea water and the amount of salt in the sea.

I shall take a rather simplistic approach to the overall mathematical equation subject with respect to enzymes by defining a few selected terms. As you get deeper into the study of enzymes and enzymology you will have an opportunity to learn that in seeking generalities, one must frequently stretch the truth a bit in order to understand the "why" when it comes to enzymes as mediators of intermediary metabolism. I will work almost exclusively under what is generally referred to as quasi-equilibrium assumptions, rather than the more probable and ultimately more useful steady-state assumptions to describe enzyme kinetic mechanisms and associated rate constants. Later on in your studies, you can move onto steady-state assumptions, but for now I will take a bit of poetic license and work under quasi-equilibrium assumptions.

## Thermodynamics

For now, let's think about the role of an enzyme and what we need to think about when it comes to an enzyme performing that role. Enzymes, as proteins in solution, have three simplistic energies. They have vibrational energy, which is simply the tendency of atoms and groups of atoms to present energy dissipation or collection as more or less a degree of stability/instability without presenting as either of the two other forms of energy. They also have rotational energy, which is simply the tendency of a molecule (collection of atoms) to "roll" or "spin" in place when in "solution." Finally, they have translational energy, which is simply the tendency of a molecule to move in some direction until events cause it to change that direction in favor of a second direction. Temperature has an impact on all three forms of energy in an enzyme, and we shall attempt to cover how all three forms of energy in an enzyme (as well as their substrates) factor into the role of an enzyme in speeding the rate of conversion of substrate to product without being consumed in the reaction. However, as I stated above, we will get more into this topic in Chapter 2. This chapter has more to do with trying to define terms than trying to explain how they help in describing how an enzyme functions.

Temperature has an obvious role in enzyme activities and a very complex role. Temperature changes directly impact on the vibrational energy of molecules such as substrates of enzymatic reactions. Using the brief description of vibrational energy in the previous paragraph, it is easy to suggest that as the temperature in which a substrate molecule (as well as an enzyme, but let's leave the enzyme out for now) finds itself, the increased vibrational energy will tend to present as increased movement of atoms relative to their covalent bonds, as movements of electrons in possible orbits around their nuclei, and/or as overall changes in the structural conformation of the molecule (substrate in this instance). In some respects, increases in vibrational energy may represent the more significant aspect of what has been described as the "energy of activation" of some molecule necessary for that molecule to undergo a spontaneous chemical reaction becoming another molecule (perhaps a "product" for sake of my keeping in focus with this book). As a molecule becomes "activated" through the introduction of energy—in the form of increased temperature(s)—more of the

substrate molecules will possess sufficient energy to acquire that "energy of activation"; and since the spontaneous chemical reaction will be defined as a concentration times some rate constant, the higher concentration of "activated" (energized) substrate will result in a faster rate of chemical change of that substrate into a product. You will encounter this issue again in Chapter 2 and Figure 2.4. However, this is a book about enzymes, and I would be remiss if I left the rather loose definition of "energy of activation" to apply only to "vibrational energy" of a molecule (substrate). Temperature also has effects on rotational and translational energies of molecules involved in some enzymatic reaction. As temperatures increase in some enzymatic reaction, molecules will tend to rotate and translate more freely, and while such rotational and translational energies may have less to do with the "energy of activation" component of a spontaneous chemical reaction, they most likely have more of an effect on the "energy of activation" associated with the enzyme-driven spontaneous chemical reaction than vibrational energy. So, how to define "energy of activation" for how we wish it to be used in the context of this book? The first thing we have to understand is that the energy of activation of a molecule that will undergo a spontaneous chemical reaction is not the same energy of activation of a molecule that will undergo a spontaneous chemical reaction where the rate of that spontaneous chemical reaction is enhanced through mediation of an enzyme (catalyst?). The energy of activation of the latter reaction should thus include roles for temperature, solution effects, enzyme, substrate, vibrational energies, rotational energies, and translational energies (note the use of "energies" here in that both the substrate and the enzyme possess these characteristics).

Like temperature, the solution in which an enzyme (and its substrates) is dissolved has an impact on all three forms of energy in an enzyme, but this impact is far more complicated than the role of temperature, although as we will see later, temperature has considerable impact on the nature of the solution and how that nature of the solution bears on the three forms of energy and subsequent interactions of the enzyme with its substrate(s) (and formed products). Enzymes function naturally in an aqueous solution of water and various ions (and/or other solutes). Water consists of a unique molecule consisting of one oxygen and two hydrogen atoms. The hydrogen atoms are situated on one side of the oxygen atom, and both kinds of atoms share electrons such that the electrons tend to spend more time with the oxygen atom than with the corresponding hydrogen atom(s) giving the whole molecule a dipole moment. This dipole moment imparts a slight negative nature to the oxygen side of the water molecule and a slight positive nature to the hydrogen side of the water molecule. It is this dipole moment that gives the water solution characteristics relevant to the energies of the enzyme, the energies of the substrate (and formed product), and directly impacts on the energy of activation and the kinetics of the enzymatic reaction in the conversion of substrate to product by that enzyme.

Normally water molecules are oriented rather randomly (with respect to their dipole moments) in some aqueous solution. However, lowering the temperature begins to remove energy from those water molecules, and at some sufficiently low temperature the water molecules will begin to lose rotational and translational energy and begin to align themselves according to their

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