

Table of Contents

Preface	xix	to Understand the Detailed Mutual Changes in Both Substrate and Enzyme During Catalysis	34
Chapter 1. An Introduction to Enzyme Science	1		
1.1. Catalysis	5	1.6.2. We Need New Approaches for Determining the Channels Allowing Energy Flow During Enzyme Catalysis	37
1.1.1. Roots of Catalysis in the Earliest Chemical Sciences	5	1.6.3. We Need Additional Probes of Enzyme Catalysis	38
1.1.2. Synthetic Catalysts	7	1.6.4. We Need to Learn How Proteins Fold and How to Manipulate Protein Stability	38
1.2. Biological Catalysis	12	1.6.5. We Need to Develop a Deeper Understanding of Substrate Specificity	39
1.2.1. Roots of Enzyme Science	12	1.6.6. We Need to Develop the Ability to Design Entirely New Biological Catalysts	42
1.2.2. Enzyme Technology	13	1.6.7. We Need to Define the Efficient Routes for Obtaining High Potency Enzyme Inhibitors as Drugs and Pesticides	44
1.3. Development of Enzyme Kinetics	15	1.6.8. We Need to Learn More About <i>In Singulo</i> Enzyme Catalysis	45
1.4. The Concept of a Reaction Mechanism	19	1.6.9. We Need to Develop Comprehensive Catalogs of Enzyme Mechanisms and to Use Such Information in Fashioning New Metabolic Pathways	46
1.4.1. Chymotrypsin: The Prototypical Biological Catalyst	20	1.6.10. We Need to Understand How to Analyze the Kinetic Behavior of Discrete Enzyme-Catalyzed Reactions as Well as Metabolic Pathways in their Environment	48
1.4.2. Ribozymes	22	1.6.11. We Need to Develop Techniques that will Facilitate Investigation of Chromosomal Remodeling, Epigenetics, and the Genetic Basis of Disease and Cell Survival	49
1.4.3. Mechanoenzymes	23	1.6.12. We Need to Develop Effective Enzyme Preparations for Use in Direct Enzyme Therapy	50
1.5. Explaining the Efficiency of Enzyme Catalysis	25		
1.5.1. Stabilization of Reaction Transition States	26		
1.5.2. Electrostatic Stabilization of Transition States	27		
1.5.3. Intrinsic Binding Energy	28		
1.5.4. Reacting Group Approximation, Orientation and Orbital Steering	28		
1.5.5. Reactant State Destabilization	29		
1.5.6. Acid/Base Catalysis	30		
1.5.7. Covalent Catalysis	30		
1.5.8. Transition-State Stabilization by Low-Barrier Hydrogen Bonds	31		
1.5.9. Catalytic Facilitation by Metal Ions	32		
1.5.10. Promotion of Catalysis via Enzyme Conformational Flexibility	32		
1.5.11. Promotion of Catalysis via Force-Sensing and Force-Gated Mechanisms	33		
1.6. Prospects for Enzyme Science	34		
1.6.1. We Need Better Methods for Analyzing Enzyme Dynamics			

Chapter 2. Active Sites and their Chemical Properties	53		
2.1. Enzyme Active Sites	54	2.5. Metal Ions in Enzyme Active Sites	81
2.1.1. Most Enzymes are Proteins, which are Linear Polymers of α -Amino Carboxylic Acids	55	2.5.1. A Group of Biologically Significant Metal Ions is Essential for Catalysis by Some Enzymes	82
2.1.2. Active-Site Residues may be Classified with Respect to their Function(s)	57	2.5.2. Enzyme-Bound Metal Ion Complexes Share Structural and Chemical Features	84
2.1.3. Active Sites Typically Occupy only 2–3 per cent of the Total Volume of an Enzyme	59	2.5.3. The Chemistry of Metal Ion-Ligand Complexes is Dominated by the Nature of their Ligancy	85
2.1.4. Binding Energy Often Indicates the Strength of Enzyme Interactions with Substrates and Cofactors	60	2.5.4. Field-Effects Influence the Color and Magnetic Properties of Metal Ion Coordination Complexes	87
2.1.5. The Structural Organization of Enzymes can be Considered Hierarchically	61	2.5.5. The Reaction Mechanisms of Transition Metal Complexes are Determined by their Inner- and Outer-Sphere Coordination Behavior	89
2.1.6. Enzymes Often Occur in Multiple Molecular Forms	63	2.5.6. Metal Ions Form Complexes with Enzymes and/or their Substrates	93
2.2. Forces Affecting Enzyme Structural Stability and Interactions	64	2.5.7. Properties of Selected Active-Site Metal Ions	95
2.2.1. Electrostatic Interactions Influence Enzyme Structure and Interactions	64	2.5.8. A Survey of Metal Ion Complexes within Selected Enzymes Reveals Key Features of Binding-Site Organization	109
2.2.2. Ion–Dipole and Dipole–Dipole Interactions are Specialized Electrostatic Phenomena	66	2.6. Active Sites of Enzymes Acting on Polymeric Substrates	112
2.2.3. Hydrogen Bonding Mainly Plays a Compensatory Role in Stabilizing Proteins	66	2.6.1. Many Endonucleases Achieve their Remarkable Specificity by Means of Subsite Recognition	113
2.2.4. Hydrophobic Interactions Play a Dominant Role in Stabilizing Most Proteins	69	2.6.2. Proteases were the First Enzymes Shown to have Subsites for Interacting with their Polymeric Substrates	114
2.2.5. Although Individually Weak, van der Waals Interactions are so Numerous that they Contribute Significantly to Overall Protein Stability	70	2.6.3. Endo-Glycosidases also Exploit Subsites to Achieve Specificity	115
2.2.6. Some Proteins are Occasionally Stabilized by π -Cation Interactions	70	2.6.4. Subsites Facilitate Substrate Recognition by Signal-Transducing Protein Kinases	115
2.3. Active-Site Diversification	70	2.7. Basic Organic Chemistry of Enzyme Action	116
2.3.1. Enzyme Diversification can be Explained Structurally	71	2.7.1. There are Six Major Classes of Enzyme-Catalyzed Covalent Bond-Making/-Breaking Reactions	118
2.3.2. Catalytic Promiscuity may Explain the Emergence of Catalytically Diversified Enzymes	74	2.7.2. Carbon has Several Reactive Forms in Enzymatic Mechanisms	119
2.4. Additional Functional Groups in Enzyme Active Sites	77	2.7.3. Many Enzymes Use the Same General Reaction Mechanisms	
2.4.1. Vitamin-Based Coenzymes Increase the Chemical Versatility of Enzyme Active Sites	77		

First Discovered by Physical Organic Chemists	120	Coordinated Electron Transfer Reactions	158
2.7.4. Nucleophilic Substitution is a Widely Used Reaction Mechanism in Enzyme Catalysis	121	2.10.4. Enzyme-Catalyzed Electron Transfer may be analyzed by Marcus Theory	160
2.7.5. Enzyme-Catalyzed Elimination Reaction Mechanisms have Many Precedents in Organic Chemistry	125	2.10.5. Simple Kinetic Models can Account for the Behavior of Biological Electron Transfer Reactions	161
2.7.6. Enzymes are Highly Effective in Forming, Stabilizing, and Utilizing Carbanion Intermediates During Catalysis	125	2.10.6. Several Prototypical Redox Enzymes Provide Valuable Insights into Electron Transfer Kinetics and Mechanisms	162
2.7.7. Free Radicals are Formed in a Surprising Number of Enzyme-Catalyzed Reactions	131	2.10.7. Enzyme Electrodes Combine the Specificity of Biological Catalysis with the Versatility of Potentiometry or Amperometry	164
2.7.8. The Versatility of Enzymes can be Illustrated by Considering a Selected Group of Reaction Mechanisms	134	Appendix	168
2.8. Detecting Covalent Intermediates in Enzyme Reactions	137	Chapter 3. Fundamentals of Chemical Kinetics	171
2.8.1. Enzymes Form a Wide Range of Enzyme-Substrate Covalent Compounds, and Many are Catalytically Competent	137	3.1. Timescale of Chemical Processes	171
2.8.2. Side-Reactions Often Provide Invaluable Clues About Mechanisms of Enzyme Catalysis	141	3.2. The Empirical Rate Equation	172
2.8.3. Some Enzyme-Substrate Covalent Compounds can be Chemically Trapped	143	3.3. Reaction Rate, Order and Molecularity	174
2.9. Basics of Enzyme Stereochemistry	145	3.3.1. Reaction Rate	174
2.9.1. Definitions	145	3.3.2. Reaction Order	175
2.9.2. The Cahn-Ingold-Prelog System Allows One to Assign the Absolute Stereochemical Configuration of Chiral Compounds	146	3.3.3. Molecularity	176
2.9.3. The Prochirality of Molecules may also be Specified Systematically	147	3.3.4. Zero-Order Kinetics	177
2.9.4. The Stereochemistry of Methyl Transfer Reactions may be Analyzed Using Enzymes of Known Stereochemistry as Reference Reactions	149	3.3.5. First-Order Kinetics	177
2.10. Electron Transfer Reactions	150	3.3.6. Second-Order Kinetics	180
2.10.1. The Thermodynamic Properties of Oxidation-Reduction Reactions are Defined by Redox Potentials	154	3.3.7. Pseudo First-Order Kinetics	180
2.10.2. The Redox Behavior of Complex Metalloenzymes can be Evaluated Spectroscopically by Stoichiometric Titration Techniques	157	3.4. Basic Strategies for Evaluating Rate Processes	181
2.10.3. Respiratory Chains are Comprised of Highly		3.4.1. Initial-Rate Method	181
		3.4.2. Progress Curve Analysis	182
		3.5. Composite Multi-stage (Multi-step) Mechanisms	184
		3.5.1. Series First-Order Kinetics	185
		3.5.2. Reversible First-Order Kinetics	186
		3.5.3. Reversible Second-Order Kinetics	186
		3.5.4. Rapid-Equilibrium and Steady-State Treatments	186
		3.5.5. Rate-Controlling Steps	188
		3.5.6. Principles of Detailed Balance	189
		3.5.7. Thermodynamic Cycles for Evaluating Detailed Balance	190
		3.5.8. Kinetic Equivalence and Mechanistic Ambiguity	192
		3.6. Thermal Energy: The Boltzmann Distribution Law	192
		3.7. Solution Behavior of Reacting Molecules	194
		3.7.1. Water: A Unique Solvent for Biochemical Processes	194
		3.7.2. Diffusion Limitations on Chemical Processes Occurring in Water	196

3.7.3. Electrostatic Effects on Magnitude of Bimolecular Rate Constants	199	4.4.2. Beer's Law is a Quantitative Expression Linking Absorbance to Concentration	240
3.7.4. Reactant Desolvation	200	4.4.3. Some Enzyme Assays Use Alternative Substrates that are Chromogenic	243
3.8. Transition-State Theory	201	4.5. Basic Fluorescence Spectroscopy	243
3.9. Chemical Catalysis	203	4.5.1. Fluorescence Spectra Depend on Excited-State Relaxation	244
3.9.1. Accelerating Rate without Altering the Equilibrium Poise	203	4.5.2. Features of a Research-Grade Spectrophotofluorimeter	244
3.9.2. Nucleophilic and Electrophilic Facilitation	205	4.5.3. The Concentration of Various Metabolites may be Quantified Through Fluorescence Spectrometry	246
3.9.3. Buffer Catalysis	206	4.5.4. Biological Molecules may Contain Intrinsic or Extrinsic Fluorescent Reporter Groups	247
3.9.4. Autocatalysis	206	4.5.5. Fluorescence Anisotropy is a Powerful Technique For Quantifying Binding Interactions	250
3.10. Reaction Coordinate Diagrams	207	4.5.6. Förster (Fluorescence) Resonance Energy Transfer (FRET) is an Exquisitely Distance-Sensitive Probe of Enzymes	252
3.11. Thermodynamic Principles	210	4.5.7. Continuous Fluorescence Assays are Now Available for Pi- and PPi-Producing Reactions	253
3.11.1. Chemical Equilibrium	210	4.5.8. Chemiluminescence is a Photoemissive Process Often Exploited in Enzyme Rate Assays	254
3.11.2. Direction and Extent of Chemical Reaction	210	4.6. Measuring Reaction Rates with Isotopes	255
3.11.3. Using $\Delta\Delta G$ to Define Binding Energetics	211	4.6.1. Stable Isotopes are Versatile Probes in Enzyme Kinetics	255
3.11.4. Alberty Treatment of Biochemical Thermodynamics	211	4.6.2. Radioisotopes Provide Extremely Sensitive Assays of Enzyme Rate Processes	260
3.11.5. Some Reacting Systems are Best Analyzed by Principles of Non-Equilibrium Thermodynamics	212	4.7. Multisubstrate Kinetics and Inhibitor Kinetics	264
3.12. Concluding Remarks	214	4.8. Analysis of Enzyme Rate Data	265
Chapter 4. Practical Aspects of Measuring Initial Rates and Reaction Parameters	215	4.8.1. Enzyme Rate Data must be Appropriately Weighted	266
4.1. Design of Initial-Velocity Enzyme Assays	215	4.8.2. Quantitative Analysis of Reaction Progress-Curves can be Used to Evaluate Rate Parameters	268
4.1.1. "Activity Purity" is Sufficient in Most Initial-Rate Studies	216	4.8.3. Global Analysis Offers Added Advantages in Statistical Analysis	270
4.1.2. Discontinuous and Continuous Rate Measurements	217	4.9. Working with ATP-Dependent Enzymes	272
4.1.3. Each Enzyme Rate Assay has Its Own Special Set of Requirements	220	4.10. Regenerating Nucleoside 5'-Triphosphate Substrates	275
4.2. Enzyme Purification	232	4.10.1. Protein and Enzyme Concentration	276
4.2.1. While Time-Consuming, the Task of Enzyme Purification is Often Well Founded	232	4.10.2. Total Protein Concentration can be Determined Quantitatively	276
4.2.2. Biochemists have Developed a Powerful Battery of Techniques for Purifying Enzymes	234	4.10.3. Active Enzyme Concentration can be Quantified by Several Techniques	276
4.3. Coupled (or Auxiliary) Enzyme Assays	235		
4.3.1. A Simple Kinetic Treatment Explains the Lag-Phase in Coupled Enzyme Assays	238		
4.3.2. The Auxiliary Enzyme and Assay Conditions must be Suited to the Primary Enzyme Reaction	239		
4.4. Basic UV/Visible Absorption Spectroscopy	240		
4.4.1. Absorption Spectra Depend on the Quantum States of Electron Orbitals	240		

4.11. Equilibrium Constant Determinations	278	5.3. Catalysis Involving Two or More Intermediates	298
4.11.1. Equilibrium Constants can be Evaluated in a Variety of Ways	279	5.3.1. Derivation of the Two-Intermediate Case Illustrates Why this Treatment is a More Realistic Representation of an Enzyme Mechanism	298
4.11.2. The Determination of the Arginine Kinase Reaction Equilibrium Constant is an Excellent Example of a Well-Designed and Well-Executed Determination	281	5.3.2. A Shortcut can be Taken to Derive the Steady-State Rate Equation for the Reverse Two-Intermediate Reaction Scheme	298
4.12. Concluding Remarks	284	5.3.3. Multiple Internal Isomerizations are without Effect on the General Form of the Final Steady-State Rate Equation	298
Chapter 5. Initial-Rate Kinetics of One-Substrate Enzyme-Catalyzed Reactions	287	5.3.4. The Haldane Relationship also Constrains the Relative Magnitudes of Key Rate Parameters in the Two-Intermediate Scheme	300
5.1. Michaelis-Menten Treatment	287	5.4. Additional Comments on Fundamental Kinetic Parameters	301
5.1.1. Derivation of the Michaelis-Menten Equation Reveals how Key Assumptions Define an Enzyme's Initial-Rate Behavior	288	5.4.1. The <i>Michaelis Constant</i> has Several Important Implications, with Regard to Both "Substrate Affinity" and Substrate Specificity	301
5.1.2. K_S , V_m , V_m/K_S , and $[S]/K_S$ are Rate Parameters Defining an Enzyme's Initial-Rate Behavior	289	5.4.2. The <i>Turnover Number</i> k_{cat} Indicates Number of Substrate Molecules Converted to Product per Enzyme Active Site per Second	303
5.1.3. Several Methods for Plotting Initial-Rate Data are Quite Useful but have Inherent Limitations	290	5.4.3. The " <i>Specificity Constant</i> " V_{max}/K_m or k_{cat}/K_m Indicates the Efficiency of Substrate Capture by an Enzyme	304
5.1.4. The Michaelis-Menten Equation Predicts a Linear Dependence of Reaction Rate on the Concentration of Active Enzyme	292	5.4.4. The <i>Commitment to Catalysis</i> Measures an Enzyme's Ability to Convert the E·S Complex to E·P, as Compared to Reconversion of E·S to a Prior Enzyme Form	307
5.1.5. The Quadratic Formula is Required When the Enzyme Concentration Approaches Substrate Concentration	292	5.4.5. Evolution of Catalytic Proficiency	308
5.1.6. Nonproductive Substrate Binding Cannot be Detected by the Michaelis-Menten Treatment	293	5.4.6. Internal Equilibria and Energetics of Perfected Enzymes	309
5.2. The Briggs-haldane Steady-State Treatment	293	5.5. Reaction Progress Curve Analysis	310
5.2.1. Derivation of this Rate Equation Reveals Key Features of Steady-State Processes	294	5.6. Ribozyme Kinetics	311
5.2.2. Reaction Energetics Determine the Effect of Increasing Substrate Concentration on the Conversion of E + S to E·S Complex	295	5.7. Proteasome Kinetics	313
5.2.3. The Corresponding Reverse-Reaction Rate Equation can now be Written	295	5.8. Isomerization Mechanisms	314
5.2.4. The <i>Haldane Relationship</i> Constrains the Values of Key Rate Parameters for Reversible Enzyme-Catalyzed Reactions	296	5.9. Simultaneous Action of an Enzyme on Different Substrates	315
5.2.5. The Briggs-Haldane Equation Requires that an Enzyme System Satisfies the Steady-State Assumption	296	5.10. Enantiomeric Enrichment and Anomeric Specificity	316
		5.11. Simultaneous Action of Two Enzymes on the Same Substrate	318
		5.12. Induced-Fit Mechanisms	319

5.12.1. There are Many Outstanding Examples of Induced-Fit Binding Behavior	320	6.2.4. Theorell and Chance Defined a Special <i>Ordered Binary Complex Mechanism</i> for Two-Substrate Enzyme Catalyzed Reactions	347
5.12.2. Induced-Fit Energetics may be Analyzed with Thermodynamic Cycles	324	6.2.5. The <i>Steady-State Random Kinetic Mechanism</i> is Far Too Complicated for the Unambiguous Experimental Determination of Key Rate Parameters	348
5.12.3. Induced-Fit Behavior and Enzyme Specificity	324	6.2.6. The Cha Method Assumes that Certain Reaction Mechanism Segments are at Thermodynamic Equilibrium	349
5.12.4. Induced-Fit Behavior Represents a Considerable Challenge for Computer-Based Ligand-Docking	327	6.3. Derivation of Rapid Equilibrium Bisubstrate Rate Equations	349
5.13. Kinetics of Enzymes Acting on Polymeric Substrates	327	6.3.1. The Rapid-Equilibrium Assumption Greatly Facilitates the Derivation of Rate Laws for Bisubstrate Random Kinetic Mechanisms	350
5.13.1. Processive <i>versus</i> Distributive Mechanisms	327	6.3.2. The Rapid Equilibrium Treatment of the Ordered Sequential Kinetic Mechanism Gives Rise to What is Probably the Simplest of Multi-Substrate Rate Laws	350
5.13.2. Random Scission Kinetics of Endo-Depolymerases	329	6.4. Ping Pong Bi Bi Mechanism	352
5.13.3. Some Depolymerizing Enzymes Show Evidence of Substrate-Assisted Catalysis	330	6.4.1. Ping-Pong Mechanisms have a Distinctive Steady-State Rate Equation that Gives Rise to Parallel-Line Patterns in $1/v$ -versus- $1/[A]$ and $1/v$ -versus- $1/[B]$ Plots	352
5.13.4. Microarray and Phage-Display Profiles of Enzyme Specificity	331	6.4.2. Ping Pong Enzymes Catalyze <i>Partial-Exchange</i> Reactions	353
5.13.5. "Hidden" Nonproductive Interactions in Steady-State Treatments of Enzyme Acting on Polymeric Substrates	332	6.4.3. Some Partial Exchange Reactions can be Mechanistically Ambiguous	354
5.14. Concluding Remarks	333	6.4.4. Burst Kinetics Provide Information About Rate-Contributing Steps in Enzyme-Catalyzed Reaction Mechanisms	356
 Chapter 6. Initial-Rate Kinetics of Multi-Substrate Enzyme-Catalyzed Reactions	 335	6.4.5. Certain Hydrolase/Transferase-Type Enzymes have Distinctive Kinetic Properties	356
6.1. Bisubstrate Kinetic Mechanisms	335	6.4.6. Substrate Inhibition Offers Insights About Ping-Pong Reactions	357
6.1.1. Cleland's Notation Conveniently Represents Multi-Substrate Kinetic Mechanisms	336	6.4.7. Multi-Site Ping Pong Kinetic Mechanisms Account for the Transfer of Reactant Moieties Between Substrate-Binding Pockets within Topologically Complex Active Sites	358
6.1.2. There are Numerous Examples of Well-Characterized Bisubstrate Enzyme Kinetic Mechanisms	338	6.5. Graphical and Quantitative Analysis of Bisubstrate Kinetics	359
6.2. Derivation Steady-State Bisubstrate Rate Equations	341	6.5.1. Re-Plotting Bisubstrate Experimental Rate Data is a Useful Way to Derive Key Rate Parameters	359
6.2.1. <i>Fromm's Systematic Method</i> for Deriving Rate Equations is a Simple and Reliable Alternative to the More Confusing King-Altman Approach	341		
6.2.2. The <i>Two-Step Computer-Assisted Method</i> is a Rapid, Automatic Way to Obtain Enzyme Rate Laws	343		
6.2.3. <i>Cleland's Net Reaction Rate Method</i> is a Simple, Elegant, and Reliable Way to Derive Rate Equations for <i>Unbranched</i> Kinetic Mechanisms	345		

6.5.2. Haldane Relations can be Used to Distinguish Kinetic Mechanisms of Bisubstrate Enzyme-Catalyzed Reactions	359	7.1.9. 3',5'-cyclic AMP Phosphodiesterase Activation by Ca^{2+} -Calmodulin: A Thorough Kinetic Analysis	388
6.5.3. The Dalziel Phi Method is a Quantitative Approach for Distinguishing Rival Bisubstrate Kinetic Mechanisms	360	7.1.10. The Method of Continuous Variation Analysis may be Used to Determine Activator Binding Site Number and Affinity	389
6.5.4. Fromm's Point-of-Convergence Method is Another Method for Distinguishing Bisubstrate Kinetic Mechanisms	362	7.1.11. Time-Dependent Enzyme Activation Requires Special Treatment	390
6.5.5. Crossover-Point Analysis also Allows Discriminates Bisubstrate Enzyme Kinetic Mechanisms	363	7.1.12. Some Agents Exhibit Biphasic Activation and Inhibition Effects	391
6.5.6. Some Multi-Substrate Initial-Rate Kinetic Data can be Ambiguous	364	7.2. Metal-Nucleotide Complexes as Substrates	391
6.6. Three-Substrate Enzyme Kinetics	366	7.2.1. Most ATP-Dependent Enzyme-Catalyzed Reactions Require Complexation of ATP^{4-} with a Divalent Metal Ion	393
6.6.1. There are Numerous Kinetic Schemes for Three-Substrate Enzyme Catalyzed Reactions	366	7.2.2. Certain Mechanoenzymes Use Metal-Free ATP^{4-} and GTP^{4-} , Albeit Slowly	394
6.6.2. There are Several General Strategies for Reducing the Complexity of Three-Substrate Initial-Velocity Experiments	368	7.2.3. Exchange-Inert Metal-Nucleotide Complexes are Powerful Mechanistic Probes	395
6.7. Multisubstrate "ISO" Mechanisms	370	7.3. pH Effects on Enzyme Kinetics	397
6.8. Kinetic Properties of Enzymes Exhibiting Branched Transfer Pathways	372	7.3.1. Many Enzymes Exhibit a Characteristic pH Optimum	397
6.9. Concluding Comments	373	7.3.2. Enzymes Often <i>Display pH-Dependent Changes in Activity</i>	398
Appendix	374	7.3.3. Several Methods may be Employed to Estimate Catalytic pK_a Values	401
Chapter 7. Factors Influencing Enzyme Activity	379	7.3.4. Acetoacetate Decarboxylase Possesses a Catalytic Lysine Exhibiting an Atypical (or Perturbed) pK_a Value	404
7.1. Activator Effects on Enzyme Kinetics	379	7.3.5. pK_a Values may be Estimated on the Basis of Protein Structural Calculations	405
7.1.1. Definitions	381	7.3.6. Enzymes Exhibit a Wide Range of pH-Dependent Behaviors	406
7.1.2. Some Reversible Essential Activators Bind Before the Substrate	383	7.3.7. Some Enzymes Undergo pH-Dependent Changes in Mechanism	407
7.1.3. Some Reversible Essential Activators Bind After the Substrate	384	7.3.8. The pH Kinetics of Bisubstrate Enzymes can be Complex	408
7.1.4. Some Enzymes Randomly Bind Essential Activators and Substrate	385	7.3.9. Brønsted Theory Explains Important Aspects of Acid/Base Catalysis	409
7.1.5. Some Essential Activators Bind to an Otherwise Unreactive Substrate	385	7.4. Buffer Effects on Enzyme Kinetics	412
7.1.6. Some Activators Released During Catalysis Exhibit Rate-Limiting Rebinding	385	7.4.1. Many Factors Influence the Choice of a Buffer	413
7.1.7. Some Non-Consumed Substrates Behave as "Pseudo-Essential Activators"	386	7.4.2. Biochemists Exploit Various Properties of Selected pH Buffers	414
7.1.8. Enzyme Must Always have Basal Activity when a Nonessential Activator is Absent			

7.4.3. Some Buffers Actively Participate in Enzyme Catalysis	414	7.10. Non-Ideality Imposed by Molecular Crowding	444
7.4.4. Some Rate Studies may Require Buffers of Constant Ionic Strength	415	7.11. Enzyme Action on Sequestered Substrates	446
7.5. Ionic Strength Effects on Enzyme Kinetics	416	7.12. Interfacial Catalysis	447
7.5.1. Ionic Strength Defines a Solution's Ionic Nature	417	7.13. Proofreading Effects on Enzyme Catalysis	453
7.5.2. The Debye-Hückel Treatment Explains How Ions Alter the Thermodynamic Activity of Solutes	417	7.14. Kinetics of Crystalline Enzymes	457
7.5.3. Changes in Ionic Strength can Alter the Magnitude of Rate Constants	418	7.14.1. Accurate Activity Assays of Crystalline Enzymes can be Technically Challenging	458
7.5.4. Ionic Strength Alters Enzyme Catalysis Profoundly	419	7.14.2. Time-Resolved Laue X-Ray Crystallography is Quickly Becoming a Powerful Mechanistic Tool	459
7.5.5. There are Limits on the Applicability of Ionic Strength	421	7.14.3. Direct Measurement of Reactant Diffusion Rates in Enzyme Crystals can be Accomplished by Video Absorption Spectroscopy	459
7.6. Effect of Organic Solvents on Enzyme Activity	422	7.14.4. Cross-Linking can be an Effective Tool in Analyzing the Behavior of Crystalline Enzymes	459
7.7. Temperature Effects on Enzyme Kinetics	425	7.15. Probing Enzyme Catalysis Through Site-Directed Mutagenesis	460
7.7.1. Temperature Often Strongly Influences Enzyme Activity and Stability	425	7.15.1. Mutations – Particularly Long-Lived Naturally Occurring Mutations – are Intrinsically Interesting	460
7.7.2. The Kinetics of Thermal Inactivation can be Treated Phenomenologically	426	7.15.2. Early Mutagenesis Experiments Exploited Chemical Modification to Replace One Naturally Occurring Amino Acid with Another	461
7.7.3. Temperature Alters Both Equilibrium and Rate Constants	427	7.15.3. Alanine Scanning Mutagenesis Often Provides Useful Clues About Essential Functional Groups in Enzymes	462
7.7.4. Many Nonlinear Arrhenius Plots can be Explained in Terms of "Rate Compensation"	428	7.15.4. Enzyme Chemists have Adopted Efficient Strategies for Investigating Enzyme Catalysis by Site-Directed Mutagenesis	464
7.7.5. The Q_{10} Parameter is a Semi-Quantitative Measure of an Enzyme's Sensitivity to Changes in Temperature	429	7.15.5. Triose-Phosphate Isomerase: A Case Study in Directed Mutagenesis	472
7.7.6. Certain Organisms have their Own Characteristic Physiologic Temperature	429	7.15.6. Chemical Rescue is a Method for Restoring Activity in some Mutant Enzymes	479
7.7.7. Extremophilic Enzymes have Unusual Structural Stability	430	7.15.7. Site-Directed Mutagenesis Suffers Significant Limitations	480
7.7.8. Some Multi-Subunit Enzymes Exhibit the Phenomenon of Reversible Cold Inactivation	433	7.16. Concluding Remarks	483
7.7.9. Cryoenzymology Techniques Greatly Reduce the Rate of Enzyme Catalysis	435		
7.8. Pressure Effects on Enzyme Kinetics	438		
7.9. Effects of Immobilization on Enzyme Stability and Kinetics	439		
7.9.1. Kinetic Behavior of Matrix-Immobilized Enzymes can be Substantially Different than the Behavior of Solution-Phase Enzymes	442	Chapter 8. Kinetic Behavior of Enzyme Inhibitors	485
7.9.2. Enzymes Tethered with Flow Tubes have Special Kinetic Properties	443	8.1. Scope and Significance of Enzyme Inhibition	485
7.9.3. Enzyme Confinement may be Relevant to Cellular Conditions	443	8.1.1. Distinguishing Reversible and Irreversible Enzyme Inhibitors	485

8.1.2. Enzyme Inhibitors in Biomedicine	486	8.6. Transition-State Inhibitors	525
8.1.3. Broader Applications of Enzyme Inhibitors	489	8.6.1. The Energetics of Transition-State Stabilization Explains the Considerable Inhibitory Potency of Substances Resembling the Transition State	525
8.2. Reversible Enzyme Inhibition	489	8.6.2. There are Numerous Examples of Naturally Occurring and Synthetically Transition-State Analogs	527
8.2.1. Competitive Inhibition Requires a Substance to Bind to the Same Enzyme Form as the Substrate	489	8.6.3. High-Affinity Binding of Certain "Pro-Transition-State Analogs" is Triggered by Some Enzymes	528
8.2.2. Noncompetitive Inhibition Requires an Inhibitor to Bind to Both E and E·S Forms	501	8.7. Tight-Binding Reversible Inhibitors	531
8.2.3. Uncompetitive Inhibition Occurs when an Inhibitor Only Binds to E·S in One-Substrate Mechanisms	502	8.7.1. Reversible Tight-Binding Inhibitors Undergo Slow Inhibitor-Induced Enzyme Conformational Changes	531
8.2.4. Inhibition can be Linear or Non-Linear	504	8.7.2. Slow-Binding Inhibitors and Slow, Tight-Binding Inhibitors are Time-Dependent	533
8.2.5. Cleland Developed Useful Rules for Analyzing Reversible Dead-End Inhibition	505	8.7.3. Dihydrofolate Reductase Inhibition by Methotrexate Illustrates Key Features of Time-Dependent Reversible Inhibitors	535
8.2.6. Some Inhibitors Act Synergistically	505	8.7.4. β -Site Amyloid Precursor Protein-Cleaving Enzyme Undergoes Time-Dependent Inhibition by a Statine-Based Peptide	536
8.3. Substrate Inhibition	506	8.8. Measures of Reversible Inhibitor Potency	537
8.3.1. Excess Substrate can Give Rise to Nonlinear Inhibition	506	8.8.1. Percent Inhibition and Degree of Inhibition	537
8.3.2. Fromm's Alternative Substrate Inhibition Method Distinguishes Rival Multi-Substrate Kinetic Mechanisms	508	8.8.2. The IC_{50} Parameter	538
8.3.3. Huang's Constant-Ratio Alternative Substrate Inhibition Method Distinguishes Multi-Substrate Kinetic Mechanisms	510	8.9. Irreversible Enzyme Inhibition by Affinity Labeling Agents	539
8.3.4. Induced Substrate Inhibition is a Type of abortive Complex Inhibition	511	8.9.1. Baker Advanced the Rational Design of Active-Site Directed Irreversible Inhibitors	539
8.4. Product Inhibition	512	8.9.2. A Simple Rate Equation Explains Affinity-Labeling Kinetics	540
8.4.1. The Alberty/Fromm Strategy Uses Product Inhibition Patterns to Distinguish Rival Multi-Substrate Kinetic Mechanisms	512	8.9.3. The Presence of Substrate can Retard, but Not Block, Irreversible Inhibition	544
8.4.2. Product Inhibition Equations for Various Two-Substrate Kinetic Mechanism Indicate Potentially Unique Inhibition Patterns	513	8.9.4. Site-Directed Irreversible Inhibitors may be Distinguished from Tightly Bound Reversible Inhibitors	544
8.4.3. Abortive Complex Formation Alters Idealized Product Inhibition Patterns for Two-Substrate Kinetic Mechanisms	516	8.9.5. pH Often Strongly Influences the Action of Irreversible Inhibitors	544
8.4.4. A Foster-Neimann Plot Permits the Analysis of Progress Curves for Enzyme in the Presence of Product Inhibition	520	8.9.6. Unstable Affinity Reagents Frequently Undergo Time-Dependent Deactivation	545
8.4.5. Product Inhibitors can Provide Valuable Clues About Multisubstrate "Iso" Mechanisms	521	8.9.7. Quiescent Enzyme Inactivators are Special Irreversible Inhibitors	546
8.4.6. The Metabolic Significance of Product Inhibition Merits Greater Consideration	521	8.9.8. Syncatalytic Affinity-Labeling Agents React Synchronously with Catalysis	546
8.5. Multi-Substrate Geometric Inhibitors	523		

8.10. Photoaffinity Labeling of Enzyme Active Sites	547	Suggested Reading	573
8.10.1. Westheimer First Recognized the Power and Range of Photoaffinity Enzyme Reagents	548	Other Authoritative Readings from <i>Methods in Enzymology</i>	574
8.10.2. Photochemical Reactions Exhibit Distinctive Properties	548		
8.10.3. Even Photoaffinity Reagents Can Suffer Major Limitations	549		
8.11. Mechanism-Based Inhibition	550	Chapter 9. Isotopic Probes of Biological Catalysis	575
8.11.1. Mechanism-Based Inhibitors Proceed Along Parallel First-Order Paths	552	9.1. Utility of Isotopes in Defining Enzyme Stereochemistry	576
8.11.2. Mechanism-Based Inhibitors are Highly Versatile	556	9.1.1. Vennesland and Westheimer Established the Stereochemistry of NADH-Dependent Hydride Transfer Reactions	576
8.11.3. Some Noncovalent Enzyme Inhibitors Resemble Mechanism-Based Inhibitors	557	9.1.2. The Stereochemistry of Nucleotide-Dependent Reactions Provides Valuable Insights into the Chemical Mechanisms of Phosphoryl and Nucleotidyl Transfer Reactions	579
8.12. Designing Highly Effective Enzyme-Directed Drugs	558	9.2. Labeling Substrates for Isotopic Experiments	585
8.12.1. Drug Discovery Focuses on “Druggable” Enzyme Targets and Selection/Evaluation of their Inhibitors	558	9.3. Isotope Exchange at and Away from Equilibrium	586
8.12.2. Identifying and Perfecting Inhibitory Potency has Become a Well-Practiced Art	561	9.3.1. All Exchange Processes Obey Simple First-Order Kinetics, Regardless of the Number or Nature of Intermediate Steps in the Overall Chemical Reaction	586
8.12.3. Development of Mechanism-Based Inhibitors Remains a Powerful Approach	563	9.3.2. The Basic Experimental Strategy Focuses on the Atoms (or Groups of Atoms) Undergoing Exchange	587
8.12.4. Schramm’s Drug Design Strategy Focuses on Discerning Subtle Differences in Enzyme Transition States and Replicating Them When Designing Inhibitors	563	9.3.3. Equilibrium Exchange Rate Equations may be Derived Using Equilibrium or Steady-State Approximations	589
8.12.5. Pro-Drug Development is another Viable Approach in Rational Drug Design	566	9.3.4. Boyer’s Strategy for Examining Equilibrium Isotopic Exchanges can Define the Order of Substrate Addition and Product Release	592
8.12.6. Adaptive Inhibition is a New Approach for Designing Enzyme-Directed Drugs	567	9.3.5. Early Measurements Demonstrated Isotope Exchange, Even with Reversible Reactions Away from Equilibrium or with Virtually Irreversible Enzymes	596
8.12.7. Lupinski’s “Rule-of-Five Index” Predicts the Efficacy of Oral Drugs	568	9.3.6. A Fuller Range of Isotopic Exchange-Rate Behavior can be Revealed Using Britton’s Flux Ratio Method	596
8.12.8. Fragment-Based Lead Design	569	9.3.7. Isotopic Rate Measurements Provided the First Truly Comprehensive Analysis of Enzyme Transition-State Energetics	599
8.12.9. Distal-Site Drug Potentiation is Untested Approach for Improving Efficacy	571	9.4. Isotope Trapping Method: Enzyme-Bound Substrate Partitioning Kinetics	603
8.12.10. RNA Interference is an Under-Explored Way to Deplete Target Enzymes	571	9.5. Positional Isotope Exchange	606
8.12.11. Macromolecules also Offer Promise as Enzyme Inhibitors	572	9.6. Kinetic Isotope Effects	607
8.12.12. Metabolic Control Analysis may Facilitate the Evaluation of Drug Action	572		
8.13. Concluding Remarks	572		

9.6.1. Basic Definitions and Notation	608	10.2.7. Ribonucleotide Reductase Catalysis is Gainfully Examined by Rapid Freeze-Quench Techniques	654
9.6.2. Primary Kinetic Isotope Effects Reflect Differences in the Respective Zero-Point Energies of Isotopomers	609	10.2.8. "Burst-Phase" Kinetics also Reveal Key Features of Enzyme Action	655
9.6.3. Some Kinetic Isotope Effects are Measured by Equilibrium Perturbation	612	10.3. Relaxation Kinetics	656
9.6.4. Quantum Mechanical Hydrogen Tunneling Reveals Important Information about Reaction Barriers	613	10.3.1. Chemical Relaxation Encompasses a Robust Range of Techniques	658
9.6.5. The Magnitude of Secondary Kinetic Isotope Effects Distinguishes S_N1 - and S_N2 -type Nucleophilic Mechanisms	616	10.3.2. While Conceptually Straightforward, Relaxation Rate Analysis Offers Powerful Insight into Complicated Chemical Processes	658
9.6.6. Other Reaction Steps may Alter the Observed Kinetic Isotope Effects	619	10.3.3. The Temperature-Jump Method is Probably the Most Versatile Chemical Relaxation Technique	666
9.6.7. Multiple Isotopically Sensitive Steps may Influence the Magnitude of the Observed Kinetic Isotope Effect	622	10.4. Stopped-flow and Temperature-Jump Techniques Provide Powerful Insights into Enzyme Catalysis	669
9.6.8. Solvent Kinetic Isotope Effects (SIEs) Occur when Isotopically Labeled Solvent Molecules are Used in Rate Measurements	623	10.4.1. Ribonuclease	669
9.6.9. Other Cases Illustrating the Power of Kinetic Isotope Effect Measurements	627	10.4.2. Aminotransferase Catalysis: A Case Study in Temperature- Jump Kinetics	670
9.7. Determining the Rates of Enzyme Synthesis and Degradation	631	10.4.3. Dihydrofolate Reductase: Another Outstanding Example	671
9.8. Concluding Remarks Authoritative Readings from the "Enzyme Kinetics and Mechanism" volumes of <i>Methods in Enzymology</i>	634 635	10.5. Other Relaxation Techniques	672
		10.5.1. Pressure-Jump Methods Increase the Versatility of Relaxation Studies	672
		10.5.2. Concentration Analysis (CCA)	673
		10.6. Other Rapid Reaction Methods	674
		10.6.1. Flash Photolysis	675
		10.6.2. Pulsed Radiolysis	677
		10.7. Data Analysis	678
		10.8. Concluding Remarks	680
Chapter 10. Probing Fast Enzyme Processes	637		
10.1. Range of Fast Reaction Techniques	638		
10.2. Flow Techniques	641		
10.2.1. Rapid-Mixing Continuous-Flow Methods Permit the Detection of Reaction Intermediates	641		
10.2.2. Chance's Stopped-Flow Technique Revolutionized the Investigation of Moderately Fast Reactions	642		
10.2.3. NAD^+ Binding to Alcohol Dehydrogenase: A Case Study Illustrating the Utility of the Stopped-Flow Kinetic Measurements	645		
10.2.4. Rapid-Scan Devices Permit Spectroscopic Analysis During Stopped-Flow Experiments	647		
10.2.5. Rapid Mixing/Quenching Devices Permit Kinetic Analysis of a Wide Range of Chemical Reactions	649		
10.2.6. Freeze-Quench Approaches Rely on a Sudden Drop in Temperature to Arrest Otherwise Highly Dynamic Processes	653		
		Chapter 11. Regulatory Behavior of Enzymes	685
		11.1. Overview of Enzyme Regulation	685
		11.2. General Strategies for Measuring Ligand Binding	688
		11.3. The Hill Equation	691
		11.4. The Scatchard Equation	693
		11.4.1. A Modified Scatchard Equation Accounts for Steric Hindrance Amongst Sites	693
		11.4.2. The Scatchard Analysis may be Extended to Deal with Two Classes of Binding Interactions – One Strong and One Weak	694
		11.5. Wyman's Linked Function Analysis	694
		11.6. The Monod-Wyman-Changeux Model	695
		11.6.1. Several Key Properties of Allosteric Systems Suggested the Symmetry-Conserving MWC Model	695

11.6.2. MWC Ligand Saturation Functions are Simple Polynomials Accounting for Ligand Binding to One or Two Conformationally Distinct States of the Enzyme	696	11.12.4. Substrate Hydration may also Affect Channeling Measurements	722
11.6.3. The Saturation Function may be Generalized to Explain Ligand Binding to an Oligomer with n Symmetry-Conserved Sites	698	11.13. Metabolic Control Analysis	723
11.6.4. The MWC Model Also Accounts for the Effects of Positive and Negative Allosteric Modifiers	699	11.14. Concluding Comments	726
11.7. The Koshland-Némethy-Filmer Model	699	Chapter 12. Single-Molecule Enzyme Kinetics	729
11.7.1. The KNF Model is Rooted in Adair's Treatment of Polyvalent Ligand Binding Interactions	700	12.1. General Comments on Single-Molecule Enzyme Kinetics	729
11.7.2. The KNF Model Incorporates Elements of Pauling's Site Interaction Model	700	12.2. Demonstration of Single-Molecule Reaction Rates	730
11.7.3. The KNF Model Accounts for Both Positive and Negative Cooperativity	701	12.3. Kinetic Treatment of Single-Molecule Enzyme Behavior	733
11.7.4. While not an Enzyme, Hemoglobin Provided Many Clues About Allostery	702	12.4. Video Microscopy	737
11.7.5. Negative Cooperativity Distinguishes KNF Models from MWC Models	703	12.4.1. Kinesin Takes One 8-nm Step per ATP Molecule Hydrolyzed	737
11.7.6. Fraction-of-the-Sites Behavior: The Case of <i>Escherichia coli</i> Alkaline Phosphatase	705	12.4.2. <i>Dark-Field Microscopy</i> Affords Direct Observation of Microtubule Assembly/Disassembly Dynamics	738
11.8. Other Cooperativity Models	707	12.5. Optical Tweezers	739
11.8.1. Hybrid Cooperativity Models	707	12.5.1. Optical Tweezers Facilitated Single-Molecule Studies on RNA Polymerase	740
11.8.2. The Duke, Le Novère and Bray Conformational Spread Model	708	12.5.2. Optical Trapping Facilitates Single-Molecule Studies of RecA Polymerization on Double-Stranded DNA	742
11.8.3. V-Type Allosteric Systems	709	12.5.3. Actin-Based <i>Listeria</i> Motility Exhibits Monomer-Sized Stepping	742
11.9. Oligomerization-Dependent Changes in Enzyme Activity	709	12.6. Atomic Force Microscopy	744
11.9.1. Enzyme Self-Association can Alter Catalytic Activity	709	12.7. Near-Field Optical Microscopy	745
11.9.2. Some Substrates Alter Enzyme Oligomerization and Catalytic Activity	711	12.8. Fluorescence Microscopy	746
11.10. Hysteresis	712	12.8.1. Epifluorescence Permits Uniform Sample Illumination	746
11.11. Enzyme Amplification Cascades	713	12.8.2. Fluorescence Microscopy Permits Direct Observation of Rotatory Catalysis	748
11.12. Substrate Channeling	718	12.8.3. Total Internal Reflection Fluorescence Microscopy (TIRFM) Exploits Evanescent Wave Phenomena	749
11.12.1. Several Criteria Define Substrate Channeling	720	12.8.4. Single Dihydrofolate Reductase Molecules "Blink" During Catalysis	749
11.12.2. Tryptophan Synthase is an Outstanding Example of Substrate Channeling	721	12.8.5. Single-Molecule Fluorescence Facilitates Observation of Dextran Binding to Bacterial Glucosyltransferase	750
11.12.3. The Once-Confusing Story of NAD ⁺ Transfer Between Dehydrogenases Illustrates the Need for Careful Studies on Substrate Channeling	722	12.8.6. Single-Molecule Fluorescence also Provides a Way to Analyze the Conformational Dynamics of <i>Staphylococcal</i> Nuclease Catalysis	751

12.9. Fluorescence Correlation Spectroscopy (FCS)	751		
12.9.1. FCS Detects Emitted Light Fluctuations within Extremely Small Volumes	752	13.4.3. Motors “Walk” on the Potential Energy Surface During Chemical and Positional Transitions	778
12.9.2. One- and Two-Photon FCS Provides a Highly Versatile Enzyme Probe	753	13.5. Calcium Ion Pump: Chemical Specificity versus Vectorial Specificity	779
12.9.3. Basic Kinetic Theory	754	13.6. Actomyosin Mechanism	782
12.9.4. Proteolytic Cleavage may be Fruitfully Investigated by FCS	755	13.7. GTP-Regulatory Proteins	782
12.9.5. Endonucleolytic Cleavage has also been Examined by FCS	757	13.8. AAA⁺ Mechanoenzymes	783
12.10. “Zero-Mode” Waveguides for Single-Molecule Analysis	757	13.8.1. The AAA ⁺ ATPases Possess Common Structural Elements	784
12.11. Prospects	758	13.8.2. DNA Processivity Clamp Loader: An AAA ⁺ Mechanoenzyme	785
Chapter 13. Mechanoenzymes: Catalysis, Force Generation and Kinetics	761	13.9. Gradient-Driven Mechanoenzymatic Processes	788
13.1. Brief Overview of Energase-Type Reactions	761	13.10. ATP Synthase: Boyer’s Binding Change Mechanism	790
13.2. The Driving Force for Affinity-Modulated Molecular Motors	766	13.11. Role of ATP in Protein Folding	792
13.3. Qualitative Features of Force-Induced Noncovalent Bond Rupture	770	13.11.1. GroEL/GroES is a Model “Foldase” System	792
13.3.1. Bond Energetics may be Described as Potential Energy Functions	770	13.11.2. GroEL/GroES Mediates an Annealing/Folding Cycle	792
13.3.2. Kramers Developed an Insightful Bond Rupture Model	770	13.12. Actoclampin Molecular Motors	793
13.3.3. Noncovalent Bonding Interactions are Inherent to Mechanoenzyme Action	771	13.12.1. Hill-Type Mechanisms for Force Generation by Polymerizing Free-ended Filaments	794
13.3.4. Noncovalent Bonds may be Classified as <i>Ideal</i> , <i>Slip</i> , and <i>Catch Bonds</i>	773	13.12.2. The Actoclampin Hypothesis: Concerning the Existence and Action of Cytoskeletal Filament End-Tracking Motors	794
13.3.5. Green Fluorescent Protein Unfolding/Refolding is Force-Dependent	775	13.12.3. Processive Single-Filament End-Tracking by ActA-VASP Complex	795
13.4. Keller-Bustamante Treatment of Molecular Motor Behavior	776	13.12.4. Properties of Actoclampin Motors	798
13.4.1. Motor Molecule Motions are Analyzed in Terms of State Space and the Potential of Mean Force	777	13.13. Concluding Remarks and Prospects	802
13.4.2. Molecular Motors Operate Stochastically	778	References	807
		Appendix	845
		Glossary	847
		Index	865