# Contents

Preface xi Symbols and Abbreviations xiii Introduction and Definitions xv

- 1 Multiple Equilibria, Principles, and Derivations 1
- 1.1 General Considerations 1
- 1.2 Diffusion 2
- 1.3 Modes of Ligand Binding 4
- 1.4 Interaction between Macromolecules and Ligands 6
- 1.4.1 Binding Constants 6
- 1.4.2 Binding to a Single Site 7
- 1.5 Binding to Identical Independent Sites 7
- 1.5.1 General Binding Equation 7
- 1.5.2 Graphic Representations of the Binding Equation 13
- 1.5.2.1 Direct and Linear Diagrams 13
- 1.5.2.2 Analysis of Binding Data from Spectroscopic Titrations 15
- 1.5.3 Binding of Different Ligands, Competition 18
- 1.5.4 Noncompetitive Binding 21
- 1.6 Binding to Nonidentical, Independent Sites 23 References 25

### 2 Cooperativity and Allosteric Enzymes 27

- 2.1 Binding to Interacting Sites 27
- 2.1.1 The Hill Equation 27
- 2.1.2 The Adair Equation 29
- 2.1.3 The Pauling Model 32
- 2.2 Allosteric Enzymes 32
- 2.2.1 The Symmetry or Concerted Model 33
- 2.2.2 The Sequential Model and Negative Cooperativity 38
- 2.2.3 Analysis of Cooperativity 42
- 2.2.4 Physiological Aspects of Cooperativity 44



ν

- vi Contents
  - 2.2.5 Examples of Allosteric Enzymes 46
  - 2.2.5.1 Hemoglobin 46
  - 2.2.5.2 Aspartate Transcarbamoylase 48
  - 2.2.5.3 Aspartokinase 49
  - 2.2.5.4 Phosphofructokinase 50
  - 2.2.5.5 Allosteric Regulation of the Glycogen Metabolism 50
  - 2.2.5.6 Membrane-Bound Enzymes and Receptors 50
  - 2.3 Binding to Nonidentical, Interacting Sites 51 References 52
  - 3 From Reaction Order to the Michaelis-Menten Law: Fundamental Relationships of Enzyme Kinetics 55
  - 3.1 Reaction Order 55
  - 3.1.1 First-Order Reactions 56
  - 3.1.2 Second-Order Reactions 57
  - 3.1.3 Zero-Order Reactions 58
  - 3.2 Steady-State Kinetics and the Michaelis–Menten Equation 58
  - 3.2.1 Derivation of the Michaelis–Menten Equation 58
  - 3.3 Analysis of Enzyme Kinetic Data 62
  - 3.3.1 Graphic Representations of the Michaelis–Menten Equation 62
  - 3.3.1.1 Direct and Semilogarithmic Representations 62
  - 3.3.1.2 Direct Linear Plots 68
  - 3.3.1.3 Linearization Methods 70
  - 3.3.2 Analysis of Progress Curves 72
  - 3.3.2.1 Integrated Michaelis–Menten Equation 73
  - 3.3.2.2 Determination of Reaction Rates 75
  - 3.3.2.3 Graphic Methods for Rate Determination 77
  - 3.3.2.4 Graphic Determination of True Initial Rates 79
  - 3.4 Reversible Enzyme Reactions 80
  - 3.4.1 Rate Equation for Reversible Enzyme Reactions 80
  - 3.4.2 Product Inhibition 82
  - 3.4.3 The Haldane Relationship 84 References 85

### 4 Enzyme Inhibition and Related Mechanisms 87

- 4.1 Unspecific and Irreversible Inhibition 87
- 4.1.1 Unspecific Inhibition 87
- 4.1.2 Irreversible Inhibition 88
- 4.1.2.1 General Features of Irreversible Inhibition 88
- 4.1.2.2 Suicide Substrates 90
- 4.1.2.3 Transition-State Analogs 91
- 4.1.2.4 Analysis of Irreversible Inhibition 92
- 4.2 Reversible Inhibition 94
- 4.2.1 General Rate Equation 94

- 4.2.1.1 Noncompetitive Inhibition and Graphic Representation of Inhibition Data 97
- 4.2.1.2 Competitive Inhibition 102
- 4.2.1.3 Uncompetitive Inhibition 106
- 4.2.2 Partial Inhibitions 108
- 4.2.2.1 Partially Noncompetitive Inhibition 108
- 4.2.2.2 Partially Uncompetitive Inhibition 110
- 4.2.2.3 Partially Competitive Inhibition 111
- 4.2.3 Noncompetitive and Uncompetitive Product Inhibition 113
- 4.2.4 Substrate Inhibition 114
- 4.3 Enzyme Reactions with Two Competing Substrates 116
- 4.4 Different Enzymes Catalyzing the Same Reaction 118 References 119

### 5 Multi-Substrate Reactions 121

- 5.1 Nomenclature 121
- 5.2 Multi-Substrate Mechanisms 122
- 5.2.1 Random Mechanism 122
- 5.2.2 Ordered Mechanism 127
- 5.2.3 Ping-Pong Mechanism 129
- 5.2.4 Product Inhibition in Multi-Substrate Reactions 131
- 5.2.5 Haldane Relationships in Multi-Substrate Reactions 132
- 5.2.6 Mechanisms with More Than Two Substrates 133
- 5.2.7 Other Nomenclatures for Multi-Substrate Reactions 134
- 5.3 Derivation of Rate Equations of Complex Enzyme Mechanisms 135
- 5.3.1 King–Altmann Method 135
- 5.3.2 Simplified Derivations Applying Graph Theory 140
- 5.3.3 Combination of Equilibrium and Steady-State Approach 141 References 143
- 6 pH and Temperature Dependence of Enzymes 145
- 6.1 pH Optimum and Determination of pK Values 145
- 6.2 pH Stability 147
- 6.3 Temperature Dependence 148 References 152

### 7 Special Enzyme Mechanisms 153

- 7.1 Kinetic Treatment of Allosteric Enzymes 153
- 7.2 Hysteretic Enzymes 154
- 7.3 Kinetic Cooperativity, the Slow Transition Model 155
- 7.4 Ribozymes 156
- 7.5 Enzymes Reacting with Polymeric Substrates 159 References 160

- 8 Enzymes Bound to Artificial Matrices and to Membranes 163
- 8.1 Immobilized Enzymes 163
- 8.1.1 Kinetics of Immobilized Enzymes 163
- 8.1.2 External Diffusion Limitation 165
- 8.1.3 Internal Diffusion Limitation 166
- 8.1.4 Inhibition of Immobilized Enzymes 168
- 8.1.5 pH and Temperature Behavior of Immobilized Enzymes 169
- 8.2 Enzyme Reactions at the Membrane 169
- 8.2.1 Transport Processes 169
- 8.2.2 Enzyme Reactions at Membrane Interfaces *172* References *175*

## 9 Isotope Exchange and Isotope Effects 177

- 9.1 Isotope Exchange 177
- 9.1.1 Isotope Exchange Kinetics 177
- 9.2 Isotope Effects 181
- 9.2.1 Primary Kinetic Isotope Effect 181
- 9.2.2 Influence of the Kinetic Isotope Effect on V and  $K_m$  182
- 9.2.3 Other Isotope Effects 183 References 184

# 10 Related Subject Areas 185

- 10.1 Relationship between Enzyme Kinetics and Pharmacokinetics 185
- 10.2 Application of Statistical Methods in Enzyme Kinetics 189
- 10.2.1 General Remarks 189
- 10.2.2 Statistical Terms Used in Enzyme Kinetics *191* References *193*

## 11 Methods for the Study of Multiple Equilibria 195

- 11.1 General Aspects 195
- 11.2 Equilibrium Dialysis as an Example for the Performance of Binding Measurements 197
- 11.2.1 Principle of Equilibrium Dialysis 197
- 11.2.2 Control Experiments and Sources of Error 200
- 11.2.2.1 Dialysis Time 200
- 11.2.2.2 Concentration and Activity of the Macromolecule 200
- 11.2.2.3 Concentration of the Ligand 201
- 11.2.2.4 Donnan Effect 202
- 11.2.3 Continuous Equilibrium Dialysis 203
- 11.3 Ultrafiltration 206
- 11.4 Gel Filtration 208
- 11.4.1 Batch Method 208
- 11.4.2 The Method of Hummel and Dreyer 209
- 11.4.3 Other Gel Filtration Methods 210
- 11.5 Ultracentrifugation 212
- 11.5.1 Fixed-Angle Ultracentrifugation Methods 212

- 11.5.2 Sucrose-Gradient Centrifugation 215
- 11.6 Surface Plasmon Resonance 218 References 220

### 12 Manometric, Electrochemical, and Calorimetric Methods 223

- 12.1 Warburg's Manometric Apparatus 223
- 12.2 Electrochemical Methods 224
- 12.2.1 The Oxygen Electrode 224
- 12.2.2 The CO<sub>2</sub> Electrode 226
- 12.2.3 Potentiometry, Redox Potentials 227
- 12.2.4 The pH-Stat 227
- 12.2.5 Polarography 229
- 12.3 Calorimetry 230 References 232

### 13 Absorption and Fluorescence Spectroscopy 235

- 13.1 General Aspects 235
- 13.2 Absorption Spectroscopy 237
- 13.2.1 The Lambert–Beer Law 237
- 13.2.2 Spectral Properties of Enzymes and Ligands 238
- 13.2.3 Structure of Spectrophotometers 241
- 13.2.4 Double-Beam Spectrophotometer 245
- 13.2.5 Difference Spectroscopy 246
- 13.2.6 The Dual-Wavelength Spectrophotometer 249
- 13.3 Photochemical Action Spectra 250
- 13.4 Bioluminescence 251
- 13.5 Fluorescence Spectroscopy 251
- 13.5.1 Quantum Yield 251
- 13.5.2 Structure of Spectrofluorometers 252
- 13.5.3 Perturbations of Fluorescence Measurements 254
- 13.5.4 Fluorescent Compounds (Fluorophores) 255
- 13.5.5 Radiationless Energy Transfer 260
- 13.5.6 Fluorescence Polarization 262
- 13.5.7 Pulse Fluorometry 263
- 13.5.8 Fluorescence Correlation Spectroscopy 265 References 265

### 14 Other Spectroscopic Methods 269

- 14.1 Circular Dichroism and Optical Rotation Dispersion 269
- 14.2 Infrared and Raman Spectroscopy 274
- 14.2.1 IR Spectroscopy 274
- 14.2.2 Raman Spectroscopy 275
- 14.2.3 Applications 275
- 14.3 Nuclear Magnetic Resonance Spectroscopy 276
- 14.4Electron Paramagnetic Resonance Spectroscopy279References281

**x** Contents

### 15 Methods to Measure Fast Reactions 283

- 15.1 General Aspects 283
- 15.2 Flow Methods 284
- 15.2.1 The Continuous-Flow Method 284
- 15.2.2 The Stopped-Flow Method 287
- 15.2.3 Measurement of Enzyme Reactions by Flow Methods 291
- 15.2.4 Determination of the Dead Time 293
- 15.3 Relaxation Methods 294
- 15.3.1 The Temperature-Jump Method 294
- 15.3.2 The Pressure-Jump Method 297
- 15.3.3 The Electric Field Method 299
- 15.4 Flash Photolysis, Pico- and Femtosecond Spectroscopy 300
- 15.5 Evaluation of Rapid Kinetic Reactions (Transient Kinetics) 302 References 305

Index 307