ENZYME INHIBITORS AS SUBSTRATES

Interactions of esterases with esters of organophosphorus and carbamic acids

W. N. ALDRIDGE
Biochemical Mechanisms Section, Toxicology Unit, M.R.C. Laboratories, Woodmansterne Road, Carshalton, Surrey, England

and

ELSA REINER
Institute for Medical Research, Yugoslav Academy of Sciences and Arts, Moše Pijade 158, Zagreb, Yugoslavia

1972

NORTH-HOLLAND PUBLISHING COMPANY
AMSTERDAM • LONDON
Contents

Editors' preface V
Authors' preface VII
Abbreviations 1

Chapter 1. Definitions and nomenclature 3
1.1. Introduction 3
1.2. Kinetic constants 4
1.3. Types of inhibition 5
1.4. Nomenclature of esterases 6
1.5. Nomenclature of inhibitors 7

Chapter 2. Reaction of B-esterases with acylating inhibitors 8
2.1. Introduction 8
2.2. General features of kinetics of acylation of B-esterases 13
   2.2.1. Acylated enzyme 14
   2.2.2. Inhibitors resembling substrates 18
   2.2.3. Michaelis complex 25
   2.2.4. Inhibition of B-esterases by di-esters of phosphoric acids 28
   2.2.5. Comparison of substrates and inhibitors 28
2.3. Experimental procedures and the interpretation of results 30
   2.3.1. Impurities in inhibitors 30
   2.3.2. Solubility of inhibitors limiting their rate of reaction 33
   2.3.3. Impure enzyme preparations 34
   2.3.4. Presentation and evaluation of raw data 34

Chapter 3. Kinetics of reaction of B-esterases with organophosphorus compounds 37
3.1. Rate of phosphorylation when intermediate complexes are not considered 37
3.2. The influence of Michaelis complex on the rate of acylation 41
   3.2.1. Rate of phosphorylation in the absence of substrate 43
Chapter 4. Deacylation of phosphorylated B-esterases

4.1. Phosphorylated B-esterases – reaction with water
   4.1.1. Instability of inhibited enzymes
   4.1.2. Hydrolysis of phosphorylated B-esterases
   4.1.3. Properties of the spontaneous reactivation process

4.2. Deacylation by nucleophilic reagents
   4.2.1. Introduction
   4.2.2. Structure–activity relationship
   4.2.3. Mechanism of reaction of nucleophilic reagents with organophosphorus compounds
   4.2.4. Mechanism of reaction of nucleophilic reagents with phosphorylated B-esterases
   4.2.5. Kinetics of reactivation
   4.2.6. Phosphorylated oximes
   4.2.7. Reactivation by fluoride and molybdate

4.3. Dealkylation (aging)
   4.3.1. The aging phenomena
   4.3.2. Dealkylation and structure of the inhibited enzymes
   4.3.3. Properties of the aging reaction
   4.3.4. The mechanism of dealkylation
   4.3.5. The present position

Chapter 5. Effect of substrate on reaction of B-esterases with organophosphorus compounds

5.1. Introduction
5.2. Rate of phosphorylation
5.3. Rate of dephosphorylation
5.4. Dissociation constants
5.5. Inhibition by excess substrate
5.6. Concentration of active centres

Chapter 6. Effect of pH on reaction of B-esterases with organophosphorus compounds

6.1. Introduction
6.2. Theoretical considerations
6.3. Effect of pH on enzyme–substrate and enzyme–inhibitor reactions

Chapter 7. Effect of temperature on reactions of B-esterases with organophosphorus compounds

7.1. Effect of temperature on rate constants – theory
7.2. Effect of temperature on equilibrium constants—theory 113
7.3. Effect of temperature on the Michaelis constant and the maximum rate of substrate hydrolysis 114
7.4. Effect of temperature on equilibrium constants 118
7.5. Effect of temperature on inhibition and spontaneous reactivation 120

Chapter 8. Kinetics of reaction of B-esterases with carbanates 123
8.1. Introduction 123
8.2. General features of kinetics 130
8.2.1. Structure–activity relationships for inhibition 130
8.2.2. Stability to hydrolysis and inhibitory potency 134
8.2.3. Kinetics of carbamylation of B-esterases 135
8.2.4. The stability of carbamylated enzymes 137
8.2.5. Time course of the reaction and steady-state inhibition 140
8.2.6. The effect of pH, temperature and ions on carbamylation and decarbamylation 142
8.2.7. Comparison between carbanates and organophosphorus compounds 145

Chapter 9. Reaction of B-esterases with organosulphur compounds 146

Chapter 10. Interaction of inhibitors with enzymes analogous to inhibition by substrate 152
10.1. Introduction 152
10.2. Inhibition of acetylcholinesterase by haloxon 155
10.3. Relationship between reversible and progressive inhibition 159
10.4. Influence of substrate on reversible inhibition 162
10.5. The influence of substrate on progressive inhibition 164
10.6. Implications for future experimental procedures 166
10.7. Structural requirements for reaction with site 2 168

Chapter 11. Acylated amino acids in inhibited B-esterases 170

Chapter 12. A-esterases 176
12.1. Distribution 176
12.2. Specificity 178
12.2.1. Summation test 179
12.2.2. Ratios of activities 180
12.2.3. Heat inactivation 182
12.2.4. Effect of ions (activation and inhibition) 182
12.3. Structure-specificity relationships 184
12.4. Mechanism of action and comparison with B-esterases 186
Chapter 13. Acylating inhibitors as tools in the study of biological processes

13.1. Introduction 190
13.2. Detection and separation of esterases 191
   13.2.1. A-esterases and B-esterases 191
   13.2.2. Separation of B-esterases 192
   13.2.3. The design of experimental methods 197
13.3. The use of organophosphorus compounds in the study of immunological phenomena 201
   13.3.1. Introduction 201
   13.3.2. Complement-dependent chemotaxis of polymorphonuclear leucocytes 202
   13.3.3. Cell-dependent inhibition of chemotaxis 203
   13.3.4. Chemotactic factor-dependent inhibition of chemotaxis 206
   13.3.5. Deactivation of rabbit polymorphonuclear leucocytes by chemotactic factor 207
   13.3.6. The activatable esterase and the hydrolysis of acetyl DL-phenylalanine \( \beta \)-naphthylester 208
   13.3.7. Other systems which have been studied 210
   13.3.8. General comment 211
13.4. Delayed neurotoxicity caused by organophosphorus compounds 212
   13.4.1. Introduction 212
   13.4.2. Delayed neurotoxicity 213
   13.4.3. The 'enzyme-inhibition' hypothesis 213
   13.4.4. Measurement of relevant phosphorylated proteins 215
   13.4.5. The phosphorylation site and esterase activity 217

Chapter 14. General conclusions

14.1. Three-step reaction sequence 221
14.2. Michaelis constant and catalytic centre activity 223
14.3. Structure–activity relationships 226
14.4. Design of inhibitors 229
14.5. A-esterases and B-esterases 230
14.6. Interaction of inhibitors or substrates with two different sites on an enzyme 231
14.7. Conclusion 234

Appendices

Appendix 1. Steady-state kinetics of substrate hydrolysis. Derivation of the Michaelis constant and the catalytic centre activity 236
Appendix 2. Kinetics of substrate hydrolysis, when the concentration of the Michaelis complex is negligible 238
Appendix 3. Time course of progressive inhibition: derivation of \( K_a \)
   A3.1. General considerations 241
   A3.2. Derivation of \( K_a \) and \( k_{+2} \) 243
Appendix 4. Time course of progressive inhibition when enzyme and inhibitor form a complex which is not a Michaelis complex 244
Appendix 5. Time course of progressive inhibition of an enzyme with two binding sites for the inhibitor 246
Contents

Appendix 6. Effect of pH on progressive inhibition 249
Appendix 7. Inhibition of a mixture of enzymes by one acylating inhibitor 251
Appendix 8. Effect of substrate on reversible inhibition of an enzyme with two binding sites. Substrate binds to site 1; inhibitor binds to both sites 253
Appendix 9. Effect of substrate on reversible inhibition of an enzyme with two binding sites. Substrate binds to both sites; inhibitor binds to site 1 255
Appendix 10. Effect of substrate on reversible inhibition of an enzyme with two binding sites. Substrate binds to site 1; inhibitor binds to site 2 257
Appendix 11. Effect of substrate on reversible inhibition of an enzyme with two binding sites. Substrate binds to both sites; inhibitor binds to site 2 259
Appendix 12. Effect of substrate on reversible inhibition of an enzyme with two binding sites. Substrate binds to both sites; inhibitor binds to both sites 261
   A12.1. General considerations 261
   A12.2. Derivation of the equation 262
   A12.3. Comparison between mechanisms discussed in appendices 8–12 265
Appendix 13. Effect of substrate on progressive inhibition. Michaelis complex between enzyme and inhibitor not considered 265
Appendix 14. Effect of substrate on progressive inhibition. Michaelis complex between enzyme and inhibitor taken into account 267
Appendix 15. Effect of substrate on progressive inhibition of an enzyme with two binding sites. Substrate binds to both sites; inhibitor binds to both sites 268
   A15.1. General considerations 268
   A15.2. Derivation of the equation 269
Appendix 16. Effect of substrate on progressive inhibition of an enzyme with two binding sites. Substrate binds to both sites; inhibitor binds to site 1 and/or site 2 271
   A16.1. No binding of inhibitor to site 2 272
   A16.2. No binding of inhibitor to site 2; the proportion of enzyme with inhibitor on site 1 is small 272
   A16.3. The proportion of enzyme with inhibitor on site 1 is small 272
Appendix 17. Effect of substrate on progressive inhibition of an enzyme with two binding sites. Substrate binds to site 1; inhibitor binds to site 1 and/or site 2 273
   A17.1. No binding of substrate to site 2 273
   A17.2. No binding of substrate or inhibitor to site 2 274
   A17.3. No binding of substrate to site 2; the proportion of enzyme with inhibitor on site 1 is small 274
   A17.4. No binding of substrate or inhibitor to site 2; the proportion of enzyme with inhibitor on site 1 is small 275
   A17.5. Comparison between mechanisms discussed in appendices 15–17 275
Appendix 18. Comparison between mechanisms of inhibition discussed in appendices 3, 4, and 5 276
   A18.1. General consideration 276
   A18.2. Mechanism discussed in appendix 3 276
   A18.3. Mechanism discussed in appendix 4 277
   A18.4. Mechanism discussed in appendix 5 278
   A18.5. The effects of substrate 279
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgments</td>
<td>280</td>
</tr>
<tr>
<td>Recommended reference works</td>
<td>281</td>
</tr>
<tr>
<td>1. Mechanism of enzyme action</td>
<td>281</td>
</tr>
<tr>
<td>2. Enzyme kinetics</td>
<td>282</td>
</tr>
<tr>
<td>3. Chemical modification of catalytic proteins</td>
<td>282</td>
</tr>
<tr>
<td>4. Mechanism of action of toxic substances on enzymes</td>
<td>282</td>
</tr>
<tr>
<td>References</td>
<td>284</td>
</tr>
<tr>
<td>Formula index</td>
<td>296</td>
</tr>
<tr>
<td>Subject index</td>
<td>301</td>
</tr>
</tbody>
</table>