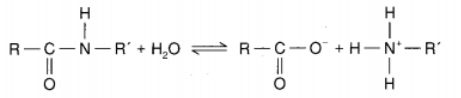
2: The Reaction Pathways of Zinc Enzymes and Related Biological Catalysts

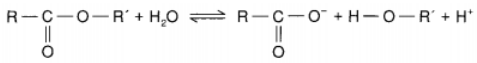
I. Introduction

This chapter deals with metalloenzymes wherein the metal acts mainly as a Lewis acid; i.e., the metal does not change its oxidation state nor, generally, its protein ligands. Changes in the coordination sphere may occur on the side exposed to solvent. The substrate interacts with protein residues inside the active cavity and/or with the metal ion in order to be activated, so that the reaction can occur. Under these circumstances the catalyzed reactions involve, as central steps with often complex reaction pathways, the following bond-breaking and/or formation processes:



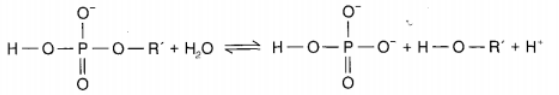
(2.1)

Peptide Hydrolysis



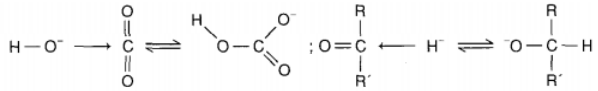
(2.2)

Carboxylic Ester Hydrolysis



(2.3)

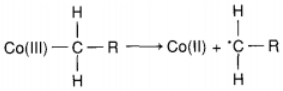
Phosphoric Ester Hydrolysis



(2.4)

Nucleophilic Addition of OH^- and H^-

Scheme (2.3) also pertains to the reactions which need ATP hydrolysis to promote endoenergetic reactions. We will also briefly deal with coenzyme B_{12}; this is a cobalt(III) complex that, by interacting with a number of proteins, produces an R-CH_2 radical by homolytic breaking of the Co-C bond as follows:



(2.5)

After an R-CH_2 radical is formed, it initiates a radical reaction. This is the only system we treat in which the oxidation state changes.

II. The Natural Catalysts

III. Strategies for the Investigation of Zinc Enzymes

A. Why Zinc?

1. The Groups to Which Zinc(II) is Bound
2. The Reactivity of Zinc(II) in Cavities
3. The Investigation of Zinc Enzymes

B.

VI. Perspectives

Although a great deal is known about the biophysical characteristics of the various enzyme derivatives mentioned in this chapter, we are still far from a clear understanding of their mechanisms of action, especially if we take into consideration the role of each amino-acid residue inside the active-site cavity. Although we can successfully discuss why certain metal ions are used in certain biological reactions, we still do not know why nickel(II), for example, is involved in the enzymatic hydrolysis of urea.[199,200] If we are content with the explanations given in Sections III.A or V.D, we would need model compounds that are good catalysts and perform the job in several steps. This latter requirement would make the various models much more interesting, and would represent a new objective in the investigation of the structure-function relationship of catalytically active molecules. Indeed, the synthesis of large polypeptides may in principle provide such models. In this respect we need to know more about protein folding, for which emerging techniques like protein computer graphics and molecular dynamics are very promising.

Chemical modifications of proteins like the alkylation of carboxylate[124,201] or histidine[202] residues have been performed for a long time. A newer approach toward modeling the function of a protein, and understanding the role of the active site, involves cleaving part of a naturally occurring protein through enzymatic or chemical procedures, and then replacing it with a synthetic polypeptide. The use of modem techniques of molecular genetics has allowed site-directed mutagenesis to become in principle a very powerful technique for changing a single residue in a cavity. Site-directed mutagenesis is a very popular approach, and its principal limitation with respect to the synthetic polypeptide route is that only natural amino acids can be used (aside from the technical difficulties in both approaches). Small quantities of site-directed mutants have been obtained for CPA[125-127] and AP,[203] whereas the expression of CA[204,205] is now satisfactory.

Predictions of the changes in structure needed to affect the reaction pathway can nowadays be made with the aid of computers. The occurrence of the predicted change can be checked through x-ray analysis and NMR. The latter spectroscopy is today well-recognized as being able to provide structural information on small ($\leq$20 kDa) proteins through 2- or 3-dimensional techniques.[206-208] These techniques are increasingly being applied to paramagnetic metalloproteins such as many of those discussed here.[208,209] The advantage of handling a paramagnetic metalloprotein is that we can analyze signals shifted far away from their diamagnetic

positions, which correspond to protons close to the metal ion,[69] even for larger proteins. It is possible to monitor the distances between two or more protons under various conditions, such as after the addition of inhibitors or pseudosubstrates, chemical modification, or substitution of a specific amino acid.

VII. References

1. H. Sigel and A. Sigel, eds., *Metal Ions in Biological Systems*, Dekker, **26** (1990).
2. R. K. Andrews, R. L. Blakeley, and B. Zemer, in Reference 1, **23** (1988).
3. K. Doi, B. C. Antanaitis, and P. Aisen, *Struct. Bonding* **70** (1988), I.
4. M. M. Werst, M. C. Kennedy, H. Beinert, and B. M. Hoffman, *Biochemistry* **29** (1990), 10526, and references therein.
5. A. G. Orpen *et al.*, *J. Chem. Soc., Dalton Trans.* **S1** (1989).
6. F. H. Westheimer, *Spec. Publ. Chem. Soc.* **8** (1957), I.
7. F. Basolo and R. G. Pearson, *Mechanisms of Inorganic Reactions*, Wiley, 2d ed., 1967.
8. L. G. Sillen and A. E. Martell, *Stability Constants of Metal-Ion Complexes*, Spec. Publ. Chern. Soc., London, **25**, 1971.
9. E. J. Billo, *Inorg. Nucl. Chem. Lett.* **11** (1975), 491.
10. I. Bertini, G. Canti, C. Luchinat, and F. Mani, *Inorg. Chem.* **20** (1981), 1670.
11. S. Burki, Ph.D. Thesis, Univ. Basel, 1977.
12. P. Wolley, *Nature* **258** (1975), 677.
13. J. T. Groves and R. R. Chambers, *J. Am. Chem. Soc.* **106** (1984), 630.
14. J. T. Groves and J. R. Olson, *Inorg. Chem.* **24** (1985), 2717.
15. L. J. Zompa, *Inorg. Chem.* **17** (1978), 2531.
16. E. Kimura, T. Koike, and K. Toriumi, *Inorg. Chem.* **27** (1988), 3687; E. Kimura *et al.*, *J. Am. Chem. Soc.* **112** (1990), 5805.
17. R. S. Brown *et al.*, *J. Am. Chem. Soc.* **104** (1982), 3188.
18. I. Bertini *et al.*, *Inorg. Chem* **29** (1990), 1460.
19. R. J. P. Williams, *Coord. Chem. Rev.* **100** (1990), 573.
20. D. W. Christianson, *Adv. Prot. Chem.* **42** (1991), 281.
21. I. Bertini, C. Luchinat, and M. S. Viezzoli, in I. Bertini *et al.*, eds., *Zinc Enzymes*, Birkhauser, 1986, p.27.
22. D. S. Auld and B. L. Vallee, in Reference 21, p. 167.
23. G. Formicka-Kozlowska, W. Maret, and M. Zeppezauer, in Reference 21, p. 579.
24. I. Bertini and C. Luchinat, in Reference 30, p. 101.
25. I. Bertini and C. Luchinat, *Adv. Inorg. Biochem.* **6** (1984), 71.
26. H. Sigel, ed., *Metal Ions in Biological Systems*, Dekker, **12** (1981).
27. T. G. Spiro, ed., *Copper Proteins*, Wiley, 1981.
28. J. E. Coleman and P. Gettins, in Reference 21, p. 77.
29. J. E. Coleman and D. P. Giedroc, in Reference 1, **25** (1989).
30. H. Sigel, ed., *Metal Ions in Biological Systems*, Dekker, **15** (1983).
31. N. U. Meldrum and F. J. W. Roughton, *J. Physiol.* **75** (1932), 4.
32. D. Keilin and T. Mann, *Biochem. J.* **34** (1940), 1163.
33. S. Lindskog, in T. G. Spiro, ed., *Zinc Enzymes: Metal Ions in Biology*, Wiley, **5** (1983), 78; D. N. Silverman and S. Lindskog, *Acc. Chem. Res.* **21** (1988), 30.
34. J.-Y. Liang and W. N. Lipscomb, *J. Am. Chem. Soc.* **108** (1986), 5051.
35. K. M. Merz, *J. Am. Chem. Soc.* **112** (1990), 7973.
36. (a) G. Sanyal, *Ann. N. Y. Acad. Sci.* **429** (1984), 165; (b) G. Sanyal and T. H. Maren, *J. Biol. Chem.* **256** (1981), 608.
37. T. Kararli and D. N. Silverman, *J. Biol. Chem.* **260** (1985), 3484.
38. A. Liljas *et al.*, *Nature* **235** (1972), 131.
39. K. K. Kannan *et al.*, *Proc. Natl. Acad. Sci. USA*, **72** (1975), 51.
40. E. A. Eriksson *et al.*, in Reference 21, p. 317.
41. S. Lindskog in Reference 21, p. 307.
42. E. Clementi *et al.*, *FEBS Lett.* **100** (1979), 313.
43. B. P. N. Ko *et al.*, *Biochemistry* **16** (1977), 1720.
44. A. Ikai, S. Tanaka, and H. Noda, *Arch. Biochem. Biophys.* **190** (1978), 39.
45. I. Bertini, C. Luchinat, and A. Scozzafava, *Struct. Bonding* **48** (1982), 45.
46. A useful review by W. W. Cleland on pH-dependent kinetics can be found in *Methods Enzymol.*, 1987, 390.

47. Y. Pocker and S. Sarkanen, *Adv. Enzymol.* **47** (1978), 149.
48. I. Bertini and C. Luchinat, *Acc. Chern. Res.* **16** (1983), 272.
49. S. Lindskog and I. Simonsson, *Eur. J. Biochem.* **123** (1982), 29.
50. B.-H. Jonsson, H. Steiner, and S. Lindskog, *FEBS Lett.* **64** (1976), 310.
51. H. Steiner, B.-H. Jonsson, and S. Lindskog, *Eur. J. Biochem.* **59** (1975), 253.
52. D. N. Silverman *et al.*, *J. Am. Chem. Soc.* **101** (1979), 6734.
53. S. H. Koenig *et al.*, *Pure Appl. Chem.* **40** (1974), 103.
54. I. Simonsson, B.-H. Jonsson, and S. Lindskog, *Eur. J. Biochem.* **93** (1979), 4.
55. T. J. Williams and R. W. Henkens, *Biochemistry* **24** (1985), 2459.
56. I. Bertini, C. Luchinat, and M. Monnanni, in Reference 99, p. 139.
57. I. Bertini *et al.*, in Reference 21, p. 371.
58. I. Bertini *et al.*, *Inorg. Chem.* **24** (1985), 301.
59. I. Bertini *et al.*, *J. Am. Chem. Soc.* **100** (1978), 4873.
60. R. C. Rosenberg, C. A. Root, and H. B. Gray, *J. Am. Chem. Soc.* **97** (1975), 21.
61. B. Holmquist, T. A. Kaden, and B. L. Vallee, *Biochemistry* **14** (1975), 1454, and references therein.
62. M. W. Makinen and G. B. Wells, in H. Sigel, ed., *Metal Ions in Biological Systems*, Dekker, **22** (1987).
63. M. W. Makinen *et al.*, *J. Am. Chem. Soc.* **107** (1985), 5245.
64. A. E. Eriksson, Uppsala Dissertation, Faculty of Science, n. 164, 1988.
65. A. E. Eriksson, A. T. Jones, and A. Liljas, *Proteins* **4** (1989), 274.
66. A. E. Eriksson *et al.*, *Proteins* **4** (1989), 283.
67. I. Bertini *et al.*, *Inorg. Chem.*, **31** (1992), 3975.
68. P. H. Haffner and J. E. Coleman, *J. Biol. Chem.* **248** (1973), 6630.
69. I. Bertini and C. Luchinat, *NMR of Paramagnetic Molecules in Biological Systems*, Benjamin/Cummings, 1986.
70. R. D. Brown III, C. F. Brewer, and S. H. Koenig, *Biochemistry* **16** (1977), 3883.
71. I. Bertini, C. Luchinat, and M. Messori, in Reference 62, vol. 21.
72. I. Solomon, *Phys. Rev.* **99** (1955), 559.
73. I. Bertini *et al.*, *J. Magn. Reson.* **59** (1984), 213.
74. L. Banci, I. Bertini, and C. Luchinat, *Magn. Res. Rev.*, **11** (1986), 1.
75. I. Bertini *et al.*, *J. Am. Chem. Soc.*, **103** (1981), 7784.
76. T. H. Maren, A. L. Parcell, and M. N. Malik, *J. Pharmacol. Exp. Theor.* **130** (1960), 389.
77. J. E. Coleman, *J. Biol. Chem.* **243** (1968), 4574.
78. S. Lindskog, *Adv. Inorg. Biochem.* **4** (1982), 115.
79. G. Alberti *et al.*, *Biochim. Biophys. Acta* **16** (1981), 668.
80. J. I. Rogers, J. Mukherjee, and R. G. Khalifah, *Biochemistry* **26** (1987), 5672.
81. C. Luchinat, R. Monnanni, and M. Sola, *Inorg. Chim. Acta* **177** (1990), 133.
82. L. Morpurgo *et al.*, *Arch. Biochem. Biophys.* **170** (1975),360.
83. I. Bertini and C. Luchinat, in K. D. Karlin and J. Zubieta, eds., *Biological and Inorganic Copper Chemistry*, vol. 1, Adenine Press, 1986.
84. I. Bertini *et al.*, *J. Chem. Soc., Dalton Trans.* (1978), 1269.
85. P. H. Haffner and J. E. Coleman, *J. Biol. Chem.* **250** (1975), 996.
86. I. Bertini *et al.*, *J. Inorg. Biochem.* **18** (1983), 221.
87. I. Bertini, E. Borghi, and C. Luchinat, *J. Am. Chem. Soc.* **102** (1979), 7069.
88. I. Bertini *et al.*, *J. Am. Chem. Soc.* **109** (1987), 7855.
89. P. Yeagle, Y. Lochmtiller, and R. W. Henkens, *Proc. Natl. Acad. Sci. USA* **48** (1975), 1728.
90. P. J. Stein, S. T. Merrill, and R. W. Henkens, *J. Am. Chem. Soc.* **99** (1977), 3194.
91. P. S. Hubbard, *Proc. Roy. Soc. London* **291** (1966), 537.
92. A. Lanir and G. Navon, *Biochemistry* **11** (1972), 3536.
93. J. J. Led and E. Neesgard, *Biochemistry* **26** (1987), 183.
94. N. B.-H. Johnsson *et al.*, *Proc. Natl. Acad. Sci. USA* **77** (1980), 3269.
95. J. L. Evelhoch, D. F. Bocian, and J. L. Sudmeier, *Biochemistry* **20** (1981), 4951.
96. J.-Y. Liang and W. N. Lipscomb, *Proc. Natl. Acad. Sci. USA* **87** (1990), 3675.
97. K. M. Merz, *J. Mol. Biol.* **214** (1990), 799.

98. C. A. Fierke, T. L. Calderone, and J. F. Krebs, *Biochemistry* **30** (1991) 11054.
99. M. Aresta and G. Forti, eds., *Carbon Dioxide as a Source of Carbon*, Reidel, 1987.
100. M. Aresta and J. V. Schloss, eds., *Enzymatic and Model Carboxylation and Reduction Reactions for Carbon Dioxide Utilization*, Kluwer, 1990.
101. C. K. Tu and D. N. Silverman, *J. Am. Chem. Soc.* **108** (1986), 6065.
102. E. Chaffee, T. P. Dasgupta, and J. M. Harris, *J. Am. Chem. Soc.* **95** (1973), 4169.
103. J. B. Hunt, A. C. Rutenberg, and H. Taube, *J. Am. Chem. Soc.* **74** (1983), 268.
104. R. S. Brown, N. J. Curtis, and J. Huguet, *J. Am. Chem. Soc.* **103** (1981), 6953.
105. I. Tabushi and Y. Kuroda, *J. Am. Chem. Soc.* **106** (1984), 4580.
106. I. Bertini *et al.*, *Gazz. Chim. Ital.* **118** (1988), 777.
107. L. Meriwether and F. H. Westheimer, *J. Am. Chem. Soc.* **78** (1956), 5119.
108. D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *J. Am. Chem. Soc.* 92 (1970), 6151.
109. M. L. Bender, R. J. Bergeron, and M. Komiyama, *The Bioorganic Chemistry of Enzymatic Catalysis*, Wiley, 1984.
110. B. Anderson *et al.*, *J. Am. Chem. Soc.* **99** (1977), 2652.
111. M. L. Bender and B. W. Tumquest, *J. Am. Chem. Soc.* **77** (1955), 4271.
112. D. L. Miller and F. H. Westheimer, *J. Am. Chem. Soc.* **88** (1966), 1514.
113. H. Kroll, *J. Am. Chem. Soc.* **74** (1952), 2036.
114. D. A. Buckingham, D. M. Foster, and A. M. Sargeson, *J. Am. Chem. Soc.* **90** (1968), 6032.
115. R. Breslow, in R. F. Gould, ed., *Bioinorganic Chemistry (Advances in Chemistry Series*, vol. **100**), American Chemical Society, 1971; Chapter 2.
116. A. Schepartz and R. Breslow, *J. Am. Chem. Soc.* **109** (1987), 1814.
117. J. T. Groves and R. R. Chambers, Jr., *J. Am. Chem. Soc.* **106** (1984), 630.
118. M. A. Wells and T. C. Bruice, *J. Am. Chem. Soc.* **99** (1977), 5341.
119. H. Sigel, ed., *Metal Ions in Biological Systems*, Dekker, **5** (1976).
120. N. E. Dixon and A. M. Sargeson, in Reference 33.
121. R. W. Hay, G. Wilkinson, R. D. Gillard, and J. A. McCleverty, eds., in *Comprehensive Coordination Chemistry*, Pergamon Press, 1987.
122. D. C. Rees, M. Lewis, and W. N. Lipscomb, *J. Mol. Biol.* **168** (1983), 367.
123. D. C. Rees *et al.*, in Reference 21, p. 155.
124. D. S. Auld, K. Larson, and B. L. Vallee, in Reference 21, p. 133.
125. W. J. Rutter, personal communication.
126. D. Hilvert et al., *J. Am. Chem. Soc.* **108** (1986), 5298.
127. S. J. Gardell *et al.*, *J. Biol. Chem.* **262** (1987), 576.
128. D. S. Auld *et al.*, *Biochemistry*, **31** (1992), 3840; W. L. Mock and J. T. Tsay, *J. Biol. Chem.* **263** (1988), 8635.
129. G. Shoham, D. C. Rees, and W. N. Lipscomb, *Proc. Natl. Acad. Sci. USA* **81** (1984), 7767.
130. K. F. Geoghegan *et al.*, *Biochemistry* **22** (1983), 1847.
131. I. Bertini *et al.*, *J. Inorg. Biochem.* **32** (1988), 13.
132. C. Luchinat *et al.*, *J. Inorg. Biochem.* **32** (1988), 1.
133. R. Bicknell *et al.*, *Biochemistry* **27** (1988), 1050.
134. I. Bertini *et al.*, *Biochemistry* **27** (1988), 8318.
135. M. E. Sander and H. Witzel, in Reference 21, p. 207.
136. M. W. Makinen, in Reference 21, p. 215.
137. D. W. Christianson and W. N. Lipscomb, *Acc. Chem. Res.* **22** (1989), 62.
138. D. W. Christianson *et al.*, *J. Biol. Chem.* **264** (1989), 12849.
139. B. W. Matthews, *Acc. Chem. Res.* **21** (1988), 333, and references therein.
140. B. S. Cooperman, in Reference 119.
141. D. M. Blow, J. J. Birktoft, and B. S. Hartley, *Nature* **221** (1969), 337.
142. R. C. Nordlie, in P. D. Boyer, ed., *T he Enzymes*, Academic Press, 3d ed., **4** (1975), 543.
143. R. Breslow *et al.*, *Proc. Natl. Acad. Sci. USA* **80** (1983), 4585.
144. H. W. Wyckoff *et al.*, *Adv. Enzymol.* **55** (1983), 453.
145. E. E. Kim and H. W. Wyckoff, *J. Mol. Biol.* **218** (1991), 449.
146. L. Banci *et aI., J. Inorg. Biochem.* **30** (1987), 77.

147. P. Gettins and J. E. Coleman, *J. Biol. Chem.* **259** (1984), 11036.
148. I. Bertini *et al.*, *Inorg. Chem.* **28** (1989), 352.
149. A. Chaidaroglou *et al.*, *Biochemistry* **27** (1988), 8338.
150. E. C. Dinovo and P. D. Boyer, *J. Biol. Chem.* **246** (1971), 4586.
151. H. Dutler, A. Ambar, and J. Donatsch, in Reference 21, p. 471.
152. H. Eklund and C.-I. Bränden, in *Biological Macromolecules and Assemblies*, Wiley, 1985.
153. C.-I. Branden *et al.*, *The Enzymes* **11** (1975), 104.
154. E. S. Cedergren-Zeppezauer, in Reference 21, p. 393.
155. H. Theorell, *Feder. Proc.* **20** (1961), 967.
156. M. W. Makinen and W. Maret, in Reference 21, p. 465.
157. J. Kvassman and G. Pettersson, *Eur. J. Biochem.* **103** (1980), 565.
158. P. F. Cook and W. W. Cleland, *Biochemistry* **20** (1981),1805.
159. I. Bertini *et al.*, *J. Am. Chem. Soc.* **106** (1984), 1826.
160. W. Maret *et al.*, *J. Inorg. Biochem.* **12** (1980), 241.
161. H. B. Gray and E. I. Solomon, in Reference 27, p. 1.
162. J. S. Valentine and M. W. Pantoliano, in Reference 27, p. 291.
163. M. F. Dunn, A. K. H. MacGibbon, and K. Pease, in Reference 21, p. 486.
164. I. Bertini *et al.*, *Eur. Biophys. J.* **14** (1987), 431.
165. W. Maret and M. Zeppezauer, *Biochemistry* **25** (1986), 1584.
166. C. Sartorius, M. Zeppezauer, and M. F. Dunn, *Rev. Port. Quim.* **27** (1985), 256; C. Sartorius *et al.*, *Biochemistry* **26** (1987), 871.
167. G. Pettersson, in Reference 21, p. 451.
168. E. Garces and W. W. Cleland, *Biochemistry* **8** (1969), 633.
169. B. Edlund *et al.*, *Eur. J. Biochem.* **9** (1969), 451.
170. R. K. Crane, in M. Horkin and E. H. Stotz, eds., *Comprehensive Biochemistry*, Elsevier, **15** (1964), 200.
171. B. M. Babior and J. S. Krouwer, *CRC Crit. Rev. Biochem.* **6** (1979), 35.
172. C. Brink-Shoemaker *et al.*, *Proc. Roy. Soc. London, Ser. A* **278** (1964), 1.
173. B. T. Golding and P. J. Sellars, *Nature* (1983), p. 204.
174. D. Lexa and J. M. Saveant, *Acc. Chem. Res.* **16** (1983), 235.
175. R. A. Firth *et al.*, *Chem. Commun.* (1967), 1013.
176. G. N. Schrauzer and L. P. Lee, *J. Am. Chem. Soc.* **90** (1968), 6541.
177. R. A. Firth *et al.*, *Biochemistry* **6** (1968), 2178.
178. V. B. Koppenhagen and J. J. Pfiffner, *J. Biol. Chem.* **245** (1970), 5865.
179. H. A. O. Hill, in G. L. Eichhorn, ed., *Inorganic Biochemistry*, Elsevier, **2** (1973), 1067.
180. J. Halpern, *Pure. Appl. Chem.* **55** (1983), 1059.
181. J. Halpern, S. H. Kim, and T. W. Leung, *J. Am. Chem. Soc.* **106** (1984), 8317.
182. R. G. Finke and B. P. Hay, *Inorg. Chem.* **23** (1984), 3041; B. P. Hay and R. G. Finke, *Polyhedron* **4** (1988), 1469; R. G. Finke, in C. Bleasdale and B. T. Golding, eds., *Molecular Mechanisms in Bioorganic Processes*, The Royal Society of Chemistry; Cambridge, England (1990).
183. B. P. Hay and R. G. Finke, *J. Am. Chem. Soc.* **108** (1986), 4820.
184. B. P. Hay and R. G. Finke, *J. Am. Chem. Soc.* **109** (1987), 8012.
185. J. M. Pratt, *Quart. Rev.* (1984), 161.
186. J. Halpern, *Science* **227** (1985), 869.
187. B. M. Babier, *Acc. Chem. Res.* **8** (1975), 376.
188. B. T. Golding, in D. Dolphin, ed., B_{12} , Wiley, **2** (1982), 543.
189. N. Bresciani-Pahor *et al.*, *Coord. Chem. Rev.* **63** (1985), 1.
190. J. M. Pratt, *J. Mol. Cat.* **23** (1984), 187.
191. S. M. Chennaly and J. M. Pratt, *J. Chem. Soc., Dalton Trans.* (1980), 2259.
192. *Ibid.*, p. 2267.
193. *Ibid.*, p. 2274.
194. L. G. Marzilli *et al.*, *J. Am. Chem. Soc.* **101** (1979), 6754.
195. J. Glusker, in Reference 188, **1** (1982), 23.
196. G. De Alti *et al.*, *Inorg. Chim. Acta* **3** (1969), 533.

197. K. Geno and J. Halpern, *J. Am. Chem. Soc.* **109** (1987), 1238.
198. C. Mealli, M. Sabat, and L. G. Marzilli, *J. Am. Chem. Soc.* **109** (1987), 1593.
199. *Nickel and its Role in Biology*, vol. 23 of Reference 62.
200. C. T. Walsh and W. H. Orme-Johnson, *Biochemistry* **26** (1987), 4901.
201. J. F. Riordan and H. Hayashida, *Biochem. Biophys. Res. Commun.* **41** (1970), 122.
202. R. G. Khalifah, J. I. Rogers, and J. Mukherjee, in Reference 21, p. 357.
203. A. Chaidaroglou *et al.*, *Biochemistry* **27** (1988), 8338.
204. C. Forsman *et al.*, *FEBS Lett.* **229** (1988), 360; S. Lindskog *et al.*, in *Carbonic Anhydrase*, F. Botre, G. Gros, and B. T. Storey, eds., VCH, 1991.
205. C. A. Fierke, J. F. Krebs, and R. A. Venters, in *Carbonic Anhydrase*, F. Botre, G. Gros, and B. T. Storey, eds., VCH, 1991.
206. K. Wüthrich, *NMR in Biological Research*, Elsevier-North Holland, 1976.
207. K. Wüthrich, *NMR of Proteins and Nucleic Acids*, Wiley, 1986.
208. I. Bertini, H. Molinari, and N. Niccolai, eds., *NMR and Biomolecular Structure*, Verlag Chemie, 1991.
209. J. T. J. LeComte, R. D. Johnson, and G. N. La Mar, *Biochim. Biophys. Acta* **829** (1985), 268.
210. Recently, ^{67}Zn has been used as a relaxing probe to monitor the binding of ^{13}C-enriched cyanide to zinc in carbonic anhydrase (see Section IV.C).
211. Recent work on HCA II has improved the resolution to 1.54 Å (K. Hakan *et al.*, *J. Mol. Biol.* **227** (1993), 1192). Mutants at positions 143 (R. S. Alexander, S. K. Nair, and D. W. Christianson, *Biochemistry* **30** (1991), 11064) and 200 (1. F. Krebs et al., Biochemistry 30 (1991),9153; Y. Xue *et al.*, *Proteins* **15** (1993), 80) also have been characterized by x-ray methods.
212. An x-ray study of the cyanate and cyanide derivatives of the native enzyme has shown that the anions sit in the cavity without binding to the metal ion (M. Lindahl, L.A. Svensson, and A. Liljas, *Proteins* **15** (1993), 177). Since NCO^- has been shown to interact with the paramagnetic cobalt(II) center, and ^{13}C-enriched cyanide has been shown to interact with ^{67}Zn-substituted CA (see Reference 67), it appears that the structures in the solid state and solution are strikingly different.
213. Recent x-ray data on the adduct of 1,2,4-triazole with HCA II confirm H-bonding with Thr-200 (S. Mangani and A. Liljas, *J. Mol. Biol.* **232** (1993), 9).
214. An HCO_3^--complex of the His-200 mutant of HCA II has been studied by x-ray methods. The data are consistent with the coordinated oxygen being protonated and H-bonded to Thr-199 (Y. Xue *et al.*, *Proteins* **15** (1993), 80).

Contributors and Attributions

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