Minisymposium: Models for Metal Ion Binding Sites and Metal Catalysis in Enzymes

Convener: RONALD BRESLOW; New York, N.Y., U.S.A.

G1

Studies on Zinc Enzymes and Models

RONALD BRESLOW*, JIK CHIN and DONALD HILVERT

Department of Chemistry, Columbia Univeristy, New York, N.Y. 10027, U.S.A.

Carboxypeptidase A catalyzes the hydrolysis of peptides and related esters. For one special type of ester an anhydride intermediate has been suggested [1], but our work on ^{18}O exchange reactions [2] indicated that normal peptide substrates do not use a mechanism involving an anhydride intermediate but instead proceed by direct hydrolysis. A loophole in this proof involves the 'trapped water' problem: ^{18}O exchange with solvent might not be observed if the water molecule produced in one step is enzyme bound, and not equilibrated with solvent. Kinetic ^{18}O isotope effect studies have now been performed to close this loophole and establish the true enzymatic mechanism.

In kinetic isotope effect studies a reaction partitions between heavy and light water. Another partitioning of interest is between water and methanol. We find that even the unusual substrate which reportedly [1] forms an anhydride with carboxypeptidase A will not incorporate methanol. By contrast, model systems [3] for the zinc-catalyzed cleavage of an anhydride show a preference for methanolysis over hydrolysis. This contrasting behavior suggests that the enzymatic reaction involves *two* proton transfers.

Carbonic anhydrase is a zinc enzyme catalyzing a deceptively simple hydration reaction. A variety of ligands have been prepared [4] which form zinc complexes related to the active site of the enzyme, but few of the complexes show striking catalytic ability. The special requirements for catalysis will be discussed.

1 M. W. Makinen, K. Yamamura and E. T. Kaiser, *Proc. Nat. Acad. Sci. USA, 73,* 3882 (1976).
2 R. Breslow and D. L. Wernick, *ibid., 74,* 1303 (1977).
3 e.g. R. Breslow, D. E. McClure, R. S. Brown and J. Eisenach, *J. Am. Chem. Soc., 97,* 194 (1975).
4 For an early report, see C. C. Tang, D. Davalian, P. Huang, and R. Breslow, *J. Am. Chem. Soc., 100,* 3918 (1978).

G2

Metal Ion Effects in the Hydrolysis of Esters and Anhydrides

THOMAS H. FIFE

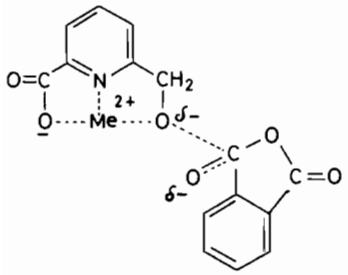
Department of Biochemistry, University of Southern California, Los Angeles, Calif., U.S.A.

Carboxypeptidase A is a Zn(II) metalloenzyme which catalyzes the hydrolysis of ester and peptide substrates [1]. X-ray crystallographic analysis at 2-Å resolution has shown the zinc ion to be chelated to the carbonyl oxygen of poor peptide substrates [1–3]. The carboxyl group of glutamic acid-270 has also been implicated in the catalytic process, and mechanisms have been suggested involving nucleophilic attack and classical general base catalysis [2, 3]. Evidence has been presented for a nucleophilic mechanism in the enzyme catalyzed hydrolysis of esters at low temperatures [4], the reaction presumably proceeding *via* an anhydride intermediate. It has therefore been of great importance to determine the effects of metal ions in ester hydrolysis reactions involving carboxyl group participation and in the hydrolysis of anhydrides.

Divalent metal ions (Cu^{2+}, Zn^{2+}, Ni^{2+}, and Co^{2+}) at saturating concentrations produce large rate enhancements in the hydrolysis of esters (ranging up to 10^8) via a metal ion promoted attack of hydroxide ion [5–7]. When the leaving group is phenolic, mechanisms involving intramolecular carboxyl group participation, either as a nucleophile or a general base, cannot compete effectively with the metal ion promoted hydroxide ion reaction [5, 6]. This is because carboxyl group participation in these systems is not appreciably enhanced by the metal ions. However, when the leaving group is an aliphatic alcohol of high pK_a, then significant rate enhancements are observed in carboxyl nucleophilic reactions [7, 8]. In the hydrolysis of 2-(6-carboxypyridyl)methyl hydrogen phthalate, saturating concentrations of Co^{2+} and Ni^{2+} enhance the rate of intramolecular carboxyl attack over 100 fold while with Cu^{2+} the rate enhancement is 10^4 [7]. In these reactions the metal ion exerts its effect through a transition state effect in which the leaving group is stabilized. This appears to be a general mechanism in reactions in which C–O bond breaking is rate determining. *(See next column)*

Both metal ion promoted water and OH^- catalyzed reactions are observed in the hydrolysis of cinnamic (6-carboxy)picolinic monoanhydride [9]. Rate en-

Inorganica Chimica Acta, 79 (1983)



hancements in the former reaction are greater than 100 fold at saturating concentrations of Ni^{2+}, Co^{2+}, and Zn^{2+}. The rate constants in these reactions are only approximately 100 fold less than in comparable reactions in the Zn(II), Ni(II), and Co(II) carboxypeptidase A catalyzed hydrolysis of the ester substrate O-(*trans*-cinnamoyl)-L-β-phenyllactic acid.

The Zn(II), Ni(II), and Co(II) carboxypeptidases give similar plots of k_{cat}, K_m, and k_{cat}/K_m *vs.* pH in hydrolysis of O-(*trans*-cinnamoyl)-L-β-phenyllactic acid in H_2O at 30 °C. The k_{cat} *vs.* pH profiles all show a sigmoidal region in which pK_{app}^{ES} values are closely similar (Zn(II) 6.2; Ni(II) 6.2., and Co(II) 5.7). At pH > 9 apparent OH^- catalyzed reactions occur with rate enhancements of 10^7–10^8 over nonenzymatic OH^- catalyzed hydrolysis of the ester. These reactions very likely represent metal ion promoted OH^- catalyzed breakdown of the anhydride intermediate similar to the reactions observed in the model studies. Modification of the carboxyl group of Glu-270 to the methoxyamide by the method of Petra [10] leads to loss of activity at all pH values including pH > 9. It is probable that breakdown of an anhydride intermediate is rate determining at all pH values greater than 6. Both formation and breakdown of the anhydride intermediate are very likely facilitated by the metal ion.

Acknowledgement. This work was supported by research grants from the National Institutes of Health and the National Science Foundation.

1 J. A. Hartsuck and W. N. Lipscomb, 'The Enzymes', 3rd ed., P. D. Boyer, Ed., Academic Press, New York, 1971, Vol. 3.
2 M. A. Ludwig, and W. N. Lipscomb, In 'Inorganic Biochemistry', G. Eichorn, Ed., American Elsevier, New York, 1973, pp. 438–487.
3 W. N. Lipscomb, *Acc. Chem. Res., 3,* 81 (1970).
4 M. W. Makinen, K. Yamamura and E. T. Kaiser, *Proc. Natl. Acad. Sci. U.S.A., 73,* 3882 (1976).
5 T. H. Fife and V. L. Squillacote, *J. Am. Chem. Soc., 100,* 4787, (1978).
6 T. H. Fife, T. J. Przystas, and V. L. Squillacote, *J. Am. Chem. Soc., 101,* 3017 (1979).
7 T. H. Fife and T. J. Przystas, *J. Am. Chem. Soc., 104,* 2251 (1982).
8 T. H. Fife and T. J. Przystas, *J. Am. Chem. Soc., 102,* 7297 (1980).
9 T. H. Fife and T. J. Przystas, *J. Am. Chem. Soc.*, in press.
10 P. H. Petra, *Biochemistry, 10,* 3163 (1971).

G3

Synthetic Iron Chelating Agents as Probes of the Iron Coordination Site and Metal Ion Exchange Kinetics of Transferrin and Lactoferrin

KENNETH N. RAYMOND* and T. D. Y. CHUNG

Department of Chemistry, University of California, Berkeley, Calif. 94720, U.S.A.

Transferrin, the serum glycoprotein of molecular weight 81,000 which transports iron in human serum, has two similar, but non-equivalent, binding sites for high-spin ferric ion. Coordination of a metal ion requires (bi-)carbonate or a functional analogue as a synergistic anion. One of the major questions regarding the binding of ferric and other metal ions by transferrin has concerned the number of tyrosine phenolate groups coordinated to the metal and whether this changes from one metal to another. We have shown, using different ultraviolet spectroscopy of metal ion binding of transferrin and the model ligand EHPG [ethylene-bis(*o*-hydroxyphenylglycine)], that for *all* metal ions there are two tyrosine phenolate groups coordinated per ion (see Fig. 1). For ions above a certain critical size, such as Pr(III), coordination of the metal becomes incomplete as only one of the binding sites is less able to accommodate such large ions. In the case of Fe(III) we have proposed that the third proton which is released upon the binding of ferric ion by transferrin is due to a hydrolysis of a coordinated water molecule. The results of continuing experiments in this area and studies for transferrin and its close relative lactoferrin will be described.

Circulating transferrin in normal human beings has only about one-third of its iron binding sites occupied by Fe(III). Thus transferrin acts as an iron buffer for serum, maintaining a chemical activity of free ferric ion that corresponds to 10^{-24} *M* [Fe^{3+}].

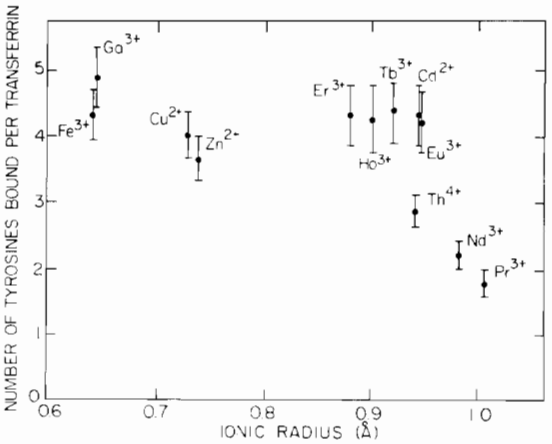


Fig. 1. Ability of transferrin to engage in tyrosyl coordination of metal ions as a function of ionic radius.