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

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G1

Studies on Zinc Enzymes and Models

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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

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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-