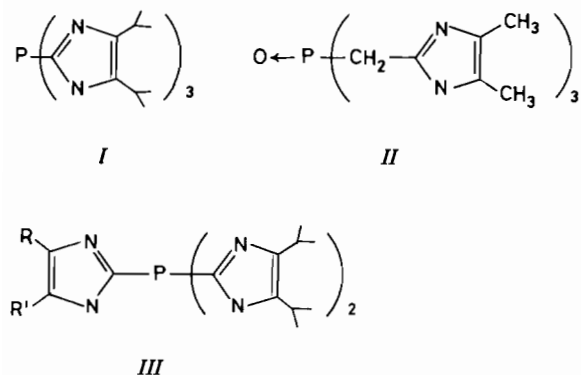of these (*I* and *II*) have been shown to have several features in common with CA including low coordination numbers, for the Zn(II) and Co(II) complexes,

I   *II*

III

pH-dependent visible absorption spectra of the Co(II) complexes and anion dependent Co(II) absorption spectra [3]. Importantly, the Zn(II) complexes of *I* and *II* show modest catalytic activity toward the interconversion of CO_2 and HCO_3^-. As well, monovalent anions appear to inhibit the catalysis similar to the situation in the enzyme [3].

Although these previous studies indicate that small chelates such as *I*:M(II) or *II*:M(II) offer an effective approach to understanding various facets of the native enzyme, neither of these displays the phenomenal catalytic prowess of CA. We believe that when bound to Zn(II) in a tridentate fashion, the *iso*propyl groups of *I* encapsulate the metal in a restrictive way such that at most one additional ligand can easily be bound. If, during the catalytic sequence, the metal is required to be 5-coordinate to accommodate both CO_2 and an activated H_2O (OH^-), then perhaps the modest catalytic ability of *I*:Zn(II) is related to steric encumbrance of this 5-coordinate state.

With this premise in mind, the new work to be presented will focus on the synthesis and physical studies of complexes of *III*. While *III* still provides the requisite tridentate coordination of M(II), two sets of *iso*propyl groups protect the metal ion from 2:1 L:M(II) chelation but offer a greater accessibility of reactants to the metal surface. Groups R and R' are appended in such a way as to provide approximations of other groups in the active site of CA (like the threonine OH group) which are believed to be important in catalysis.

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G6

The Investigation of Cobalt(II) Substituted Carbonic Anhydrase and Carboxypeptidase A

I. BERTINI*, C. LUCHINAT and G. LANINI

*Institutes of General and Inorganic Chemistry, University of Florence, Florence, Italy*

It is generally accepted that information obtained on cobalt(II) complexes can be transferred to the analogous zinc compounds. Therefore substitution of zinc(II) with cobalt(II) in zinc containing enzymes allows one to investigate these systems through spectroscopic techniques. The data reported here are concerned with carbonic anhydrase (CA) and carboxypeptidase A (CPA).

^1H NMR data in D_2O on CoCA reveal a relatively sharp signal assigned to the β proton of the histidine bound to the metal. Its T_1^{-1} value is constant with pH for the bovine CA isoenzyme B (BCAB) whereas it is lower at low pH in the case of human CA isoenzyme (HCAB). These data parallel the pH dependence of the water ^1H NMR data at every magnetic field [1, 2]. It is proposed that in the latter case (HCAB) a change in coordination chemistry occurs at low pH, and in particular that five coordinate species are obtained, whereas CoBCAB at every pH and CoHCAB at high pH are pseudotetrahedral. The general equilibrium is
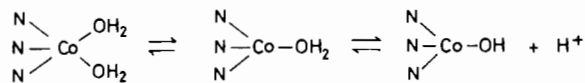
Model compounds show that nuclear longitudinal relaxation decreases from tetra- to five- to six-coordination in cobalt(II) complexes. The above equilibrium is consistent with the difference in the electronic spectra and in the pK_a between the two isoenzymes.

Water ^1H NMR data, coordinated histidine ^1H NMR data, electronic spectra and EPR data are in our opinion consistent with five-coordination in CoCPA [3]. We believe that five coordination is reached through two water molecules. N_3^- is found to bind
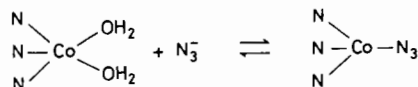
cobalt(II) providing a derivative the electronic spectrum of which can be interpreted as being due to tetra-coordination. The affinity of N_3^- for the enzyme decreases with pH, the pK_a being around 9. This behavior of N_3^- is analogous to that shown with CoHCAB both with respect to change in coordination number and pH dependence of the affinity constants.

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G7

Catalysis by Zn²⁺-Metalloenzymes Proceeds via a Penta-Coordinate Metal–OH₂ Complex

MARVIN W. MAKINEN

Department of Biophysics and Theoretical Biology, The University of Chicago, Chicago, Ill. 60637, U.S.A.

The active site of a variety of metalloenzymes contains a catalytically essential tetra-coordinate Zn^{2+} ion to which a water molecule is a ligand. It is generally assumed that the metal-bound water molecule has no role in catalysis. However, for both the native Zn^{2+}- and Co^{2+}-reconstituted forms of carboxypeptidase A (CPA) and liver alcohol dehydrogenase (LADH), we have identified metal ion dependent ionizations that govern steady state kinetic parameters and can be ascribed only to a metal-bound water molecule in catalytically competent reaction intermediates [1, 2]. To assign the coordination environment of the Co^{2+} ion in these reaction intermediates, we have identified spectroscopic parameters of the high-spin Co^{2+} ion that serve as a direct index of coordination number.

Our studies [3] show on the basis of a group theoretical treatment of spin–orbit coupling, together with relationships derived through second-order perturbation theory, that the magnitude of the zero-field splitting (ZFS) of the high-spin Co^{2+} ion is a direct monitor of crystal field symmetry and that it follows the order $ZFS_4 < ZFS_5 < ZFS_6$ where the subscript indicates the coordination number. Measurement of the ZFS in structurally defined coordination complexes confirms this relationship and indicates that the magnitude of $2|D|$ is $0-13$ cm⁻¹ for tetra-coordinate complexes, $20-60$ cm⁻¹ for penta-coordinate complexes, and $90-130$ cm⁻¹ for hexa-coordinate complexes. Application of this theory to measurements of the ZFS of CoCPA and CoLADH shows that the active site metal ion is tetra-coordinate in the free enzyme and is altered to a penta-coordinate complex in true reaction intermediates. In these intermediates the metal ion coordinates both the substrate and a water molecule simultaneously. Together with kinetic studies identifying ionizations of a metal-bound water molecule and showing that the reaction pathway is identical for Zn^{2+}- and Co^{2+}-enzymes, the results require that, upon formation of a penta-coordinate Zn^{2+}–OH_2 complex, breakdown of the acyl-enzyme (mixed anhydride) reaction intermediate of CPA occurs via metal–hydroxide nucleophilic attack on the carbonyl carbon of the scissile bond while in the physiologically active, ternary enzyme–NAD⁺– alcohol complex of LADH a neutral metal-bound water molecule serves as the base catalyst for proton abstraction. The results of these studies show that the metal-bound water has an integral catalytic role in CPA and LADH. It is probable that the metal–OH_2 complex has similarly a functional role in other Zn^{2+}-metalloenzymes.

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