to transferrin. Other coordination properties and relative iron transfer kinetics of these two proteins will be discussed.

TABLE I. pM Values of Selected Fe(III) Sequestering Agents.

Ligand	pM[a] ($-\log[Fe^{3+}_{aq}]$)
Enterobactin	35.5
HBED[b]	31.0
MECAM	29.4
MECAMS	29.1
3,4-LICAMS	28.5
Me_3MECAMS	26.6
Ferrioxamine B	26.6
EHPG[c]	26.4
TRIMCAMS	25.1
NAcMECAMS	25.0
DTPA[d]	24.7
Transferrin	23.6
EDTA[e]	22.2
Tiron[f]	19.5

[a]Calculated for 10 *M* ligand, 1 *M* Fe^{3+}, pH 7.4. [b]N,N-bis (2-hydroxybenzyl)-ethylenediamine-N,N'-diacetic acid. [c]Ethylene-1,2-bis(2-hydroxyphenylglycine). [d]Diethylene-triaminepentaacetic acid. [e]Ethylenediaminetetraacetic acid. [f]1,2-Dihydroxy-3,5-disulfobenzene.

G4

Synthetic Models of Metalloenzymes

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Metal ions play an important role in the enzymic catalysis of many metalloproteins. The molecular details of the catalytic cycle are often obscured by the complexity of the biological system. It has been the goal of our research for the past few years to elucidate the mechanisms of metalloenzymes through the synthesis of simple metal complexes that mimic the structure of the active sites. The reactivity of such metal complexes have provided insights into the enzyme mechanism. Further, successful enzyme models have provided a rational basis for the construction of synthetic, biomimetic catalysts. In this lecture, recent advances in the study of active site models of carboxypeptidase A, CPA, a zinc-containing protease, and cytochrome P-450, a heme-containing monooxygenase will be described.

The role of zinc in the peptidase activity of CPA has been ascribed to coordination of the substrate amide carbonyl, coordination of a nucleophilic hydroxide or even to a less specific structural role. To choose among these possibilities we have synthesized a family of metal-complexing amides which does not allow a metal-carbonyl interaction. Large zinc- and copper-mediated rate enhancements (10^4–10^7) for amide hydrolysis are observed with these compounds. Kinetic and titrimetric measurements indicate that the deprotonation of a metal-bound water is a component of this catalysis. A mechanism for amide hydrolysis involving nucleophilic attack of a metal hydroxide is consistent with the observed results.

The catalytic cycle of cytochrome P-450 has been suggested to involve a reactive oxo-iron intermediate which is responsible for oxygen transfer to the substrate. We have prepared the first synthetic example of an iron(IV)-porphyrin cation radical complex (*1*). This species has been shown to be extraordinarily reactive toward hydrocarbons. The physico-chemical characterization of *1* and the elucidation of the mechanism of olefin epoxidation and alkane hydroxylation will be described.

G5

Trisimidazolylphosphine: M(II); Models for the Metal-Binding Site in Carbonic Anhydrase

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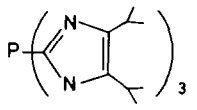
The apparently simple processes of CO_2 hydration and HCO_3^- dehydration (eqn. 1) play a key role in several diverse physiological processes such as gas balance, photosynthesis, shell formation and pH control [1]. So important is this reaction to living systems that Nature has provided virtually all

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \quad (1)$$

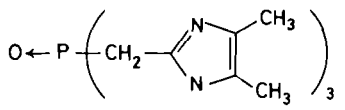
organisms with an enzyme whose only known physiological role is to facilitate the interconversion of CO_2 and HCO_3^-. According to X-ray crystallographic determinations [2] the active site of carbonic anhydrase consists of an essential Zn(II) ion held in the protein by three histidine imidazole units in a distorted tetrahedral fashion: the remaining Zn(II) ligand positions are said to be occupied by H_2O and/or OH^-, these being important for the catalytic events. Although many studies with the enzymes isolated from human and bovine erythrocytes have been undertaken, the mechanism by which CA catalyses the process in eqn. 1 remains elusive [1].

As an alternative approach to studying the catalysis of CO_2 hydration and HCO_3^- dehydration, we have initiated a program of synthesizing and evaluating simple *tris*-imidazolyl containing phosphines as approximations for the metal-binding sites in CA. Two

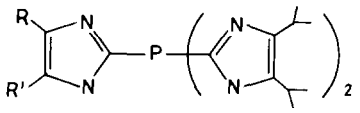
of these (*I* and *II*) have been shown to have several features in common with CA including low coordination numbers, for the Zn(II) and Co(II) complexes,



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.

1 S. Lindskog in 'Advances in Inorganic Biochemistry', G. L. Eichhorn and L. G. Marzilli, Eds., Elsevier Biomedical, New York, 1982, pp. 115–170.
2 K. K. Kannan, B. Notstrand, K. Fridborg, S. Lövgren, A. Ohlsson and M. Petef, *Proc. Natl. Acad. Sci. U.S.A., 72,* 51 (1975).
3 (a) R. S. Brown, D. Salmon, N. J. Curtis and S. Kusuma, *J. Am. Chem. Soc., 104,* 3188 (1982).
(b) R. S. Brown, N. J. Curtis and J. Huguet, *J. Am. Chem. Soc., 103,* 6953 (1981).

G6

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

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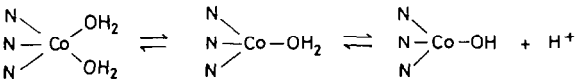
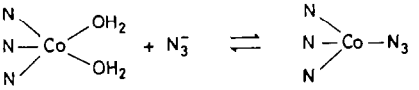
It is generally accepted that information obtained on cobalt(II) complexes can be transferred to the analogous zinc compounds. Therefore substitution of zinc(II) with cobalt(II) in zinc containing enzymes allows one to investigate these systems through spectroscopic techniques. The data reported here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