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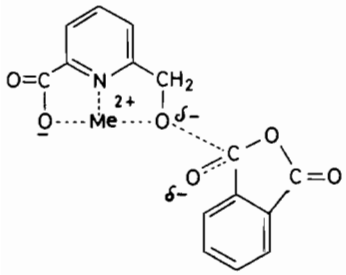

hancements in the former reaction are greater than 100 fold at saturating concentrations of Ni^{2+}, Co^{2+}, and Zn^{2+}. The rate constants in these reactions are only approximately 100 fold less than in comparable reactions in the Zn(II), Ni(II), and Co(II) carboxypeptidase A catalyzed hydrolysis 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.

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G3

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

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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+}].

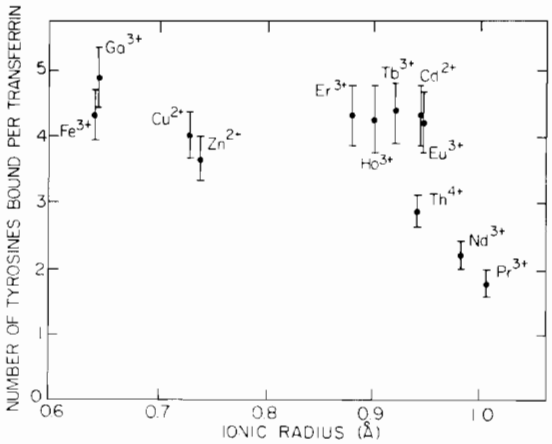

Fig. 1. Ability of transferrin to engage in tyrosyl coordination of metal ions as a function of ionic radius.

This very large thermodynamic stability of the transferrin iron complex makes it difficult for even the most powerful chelating agents to remove iron. Furthermore, the kinetics of iron release are extremely slow even for compounds with a much greater thermodynamic stability. Thus desferrioxamine B (DFO, Fig. 2), which has a stability about 10^6 times that of transferrin at millimolar concentrations and pH 7.4, removes iron from transferrin with a half life of several hours. However, the tricatecholate iron-chelating agent enterobactin (Fig. 2) or its synthetic analogues 3,4-LICAMS or MECAM (Fig. 3) take up iron from transferrin at rates approximately 200 times as fast as DFO.

For iron(III) removal by catecholate ligands, saturation kinetics are observed at higher ligand concentrations. The rate expression for iron removal from the protein, where L represents the tricatechol ligand and Tf represents the (bi-)carbonate complex of apotransferrin, gives the following rate expression:

$$d[FeL]/dt = k_{obs}[Fe(\text{transferrin-bound})] \quad (1)$$

where

$$k_{obs} = k_a[L]/(1 + k_b[L]) \quad (2)$$

This rate law has been explained by the following mechanism:

$$FeTf \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} FeTf^* \quad (3)$$

$$L + FeTf^* \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} LFeTf^* \quad (4)$$

$$LFeTf^* \xrightarrow{k_3} LFe + Tf^* \quad (5)$$

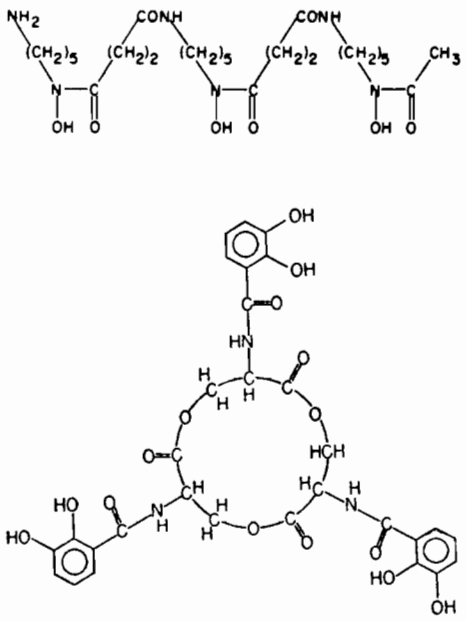

Fig. 2. Structural formulas of the naturally occurring chelating agents desferrioxamine B (top) and enterobactin (bottom).

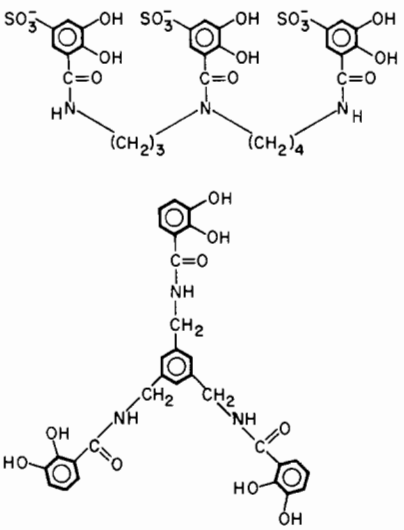

Fig. 3. Structural formulas of synthetic catecholate iron-chelating agents 3,4-LICAMS (top) and MECAM (bottom).

The application of the usual steady state approximations for the intermediates given above results in the following relationships between the experimental rate constants and those in the proposed mechanism:

$$k_a = k_3k_2k_1/[k_{-1}(k_{-2} + k_3)] \quad (6)$$

$$k_b = k_2/k_{-1} \quad (7)$$

The experimental values of these constants for the ligand 3,4-LICAMS at 0.2 mM concentration are presented in the table below for transferrin at 25 and 37 °C and for lactoferrin at 37 °C. If we make the assumption that the rate constant k_3 is much larger than the rate constant k_{-2}, the ratio of $k_a/k_b = k_1$. In other words, the ratio of these two experimental rate constants gives the rate for the first step of the reaction, which is assigned as a conformation change of the protein. In this conformation change the protein goes from a 'closed' stable form of the iron complex in which the iron center is buried 10 to 15 Å below the surface of the protein and hence inaccessible either to complexing or reducing agents, to an 'open' form which is the stable one for the apoprotein and in which the iron binding center is near the surface of the protein and hence accessible. It is this conformation change which becomes rate determining at high ligand concentrations for extraordinarily powerful iron chelating agents. The half life for this putative conformational change at 25 °C is 22 minutes, which decreases to 8.4 minutes at 37 °C. This corresponds to an enthalpy of activation of 14 kcal per mole for the iron centers. In contrast, the half life for this process at 37 °C for lactoferrin is 1100 minutes! This extremely slow rate of the conformation change of lactoferrin may by itself explain the approximately two order of magnitude greater stability it displays toward iron compared

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