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Mechanistic Studies of Synthetic and Naturally Occurring Hydroxamic Acid Complexes of Iron(III) Relating to Biological Iron Transport

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Iron is an essential nutrient for microorganisms and higher forms of life. Due to the insolubility of iron(III) in aqueous medium at physiological pH, aerobic and facultative aerobic microorganisms require iron transport compounds, called siderophores. The specific function of these siderophores is to solubilize the iron by forming an iron(III) complex, and then transport it to the cell where the iron is released. The siderophores may be divided into two classifications based on the nature of the iron binding site: catechols and hydroxamic acids. A series of eighteen monohydroxamic acids, $R_1C(O)N(OH)R_2$, have been synthesized with various R_1 and R_2 groups. Kinetic data will be presented for the complexation and dissociation of iron(III) with this homologous series of synthetic hydroxamic acids. The relative influence of the R_1 and R_2 substituent on the kinetic and thermodynamic stability of the complex will be discussed. Relative rates, linear free energy relationships, and activation parameters will be used to support an associative interchange (I_a) mechanism for iron(III) complex formation, with initial bond formation at the carbonyl oxygen atom. A limited comparison between the kinetic complexation behavior of thiohydroxamic acids, $R_1C(S)N(OH)R_2$, relative to their oxygen counterparts will be presented. These data and the corresponding mechanistic interpretations will be used as a model for iron transport by hydroxamic acid based siderophores. The specific case of the hexadentate siderophore ferroxamine B will be presented. Data to illustrate the catalyzed removal of iron(III) from ferrioxamine B will be presented and discussed as a possible model for iron bioavailability.

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Structures of Cu(II)–(Histidine)$_2$ in Solution

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We have examined the structure of Cu(II)–(histidine)$_2$ in solution using optical, electron paramagnetic resonance (EPR) and electron spin-echo (ESE) spectroscopies. Histidine is a potential tridentate ligand with three groups capable of binding Cu(II): 1) carboxyl oxygen, 2) imidazole nitrogen, and 3) amino nitrogen. Histidine is involved in the coordination of metal ions in a number of copper proteins, including superoxide dismutase, ceruloplasmin, ascorbate oxidase, galactose oxidase, *etc.* In addition, histidine has been implicated in the *in vivo* transport of copper between albumin and cells.

Previously the structure of Cu(II)–(histidine)$_2$ was determined using proton NMR and optical spectroscopies, and X-ray crystallography. Two structures were suggested. In the first, the Cu(II) is coordinated by two imidazole and by two amino nitrogen atoms. In the second structure the Cu(II) is ligated to a single imidazole nitrogen, and an amino nitrogen from one of the histidines and an amino nitrogen and a carboxyl oxygen from the second histidine.

Computer aided analysis of the optical pH titration of Cu(II)–(histidine)$_2$, at 25 °C, shows two transitions, with pK's of 3.6 and 5.5. Both titrations have n = 1. An EPR pH titration at 77 K, shows 2 transitions with pK's of 2.8 and 4.4 and n values of 2 and 1, respectively. The lower pK represents the complexation of Cu(II)-aquo ($g_\parallel$ = 2.417, $A_\parallel$ = 14.1 mK) by histidine to form an intermediate complex ($g_\parallel$ = 2.306, $A_\parallel$ = 18.6 mK). The second pK represents the conversions of this intermediate to the form of the complex present at physiological pH ($g_\parallel$ = 2.242, $A_\parallel$ = 18.8 mK). The changes in $g_\parallel$ suggest the binding of an additional equatorial nitrogen when the intermediate is converted to the neutral pH form.

In a separate study, we used ESE spectroscopy to determine the number of imidazole nitrogen atoms equatorially coordinated to Cu(II) in the bis histidine complex. The observed periodicities in the ESE decay envelope are due to interactions of the unpaired electron with the remote, protonated ^{14}N of bound imidazole. Directly coordinated ^{14}N is not observed in these experiments. Fourier transformation of the modulation pattern for the complex prepared at pH 7.6 shows frequencies at 0.7, 1.5, and 4.0 MHz, which are characteristic of Cu(II) coordinated by imidazole.

The depth of the modulation pattern has been shown to be a product function of the number of interacting nuclei and their distance. Thus if two imidazoles are coordinated to Cu(II), the depth of modulation is the square of that seen for a single coordinated imidazole provided that the Cu–N bond lengths are the same. Using Cu(II)–diethylenetriamine–imidazole, and Cu(II)–oxalate–(imidazole)$_2$ as single, and double imidazole models, we can quantitate the number of imidazoles bound to Cu(II) in Cu(II)–(histidine)$_2$. The depth of modula-

tion for Cu(II)–(histidine)$_2$ is comparable to that observed for Cu(II)–diethylenetriamine–imidazole. Therefore, at physiological pH, a single imidazole is equatorially coordinated to the metal ion. At pH 3.4, where the predominant species are Cu(II)-aquo and the low pH intermediate, the modulation pattern characteristic of coordinated imidazole is still observed. Thus imidazole remains bound to Cu(II) here as well.

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Resonance Raman Spectroscopy of Binuclear Iron Centers. Hemerythrin, Ribonucleotide Reductase and Iron Phenanthroline Complexes

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Binuclear iron centers are known to be present in the respiratory protein, hemerythrin [1], and in the enzyme, ribonucleotide reductase [2]. These centers are characterized by strong antiferromagnetic coupling ($-J \cong 100$ cm^{-1}) of the two ferric ions, and by one or more intense absorption bands between 320 and 380 nm ($\epsilon \cong 4000\ M^{-1}$ cm^{-1} per Fe atom). These properties have long been ascribed to the presence of a μ-oxo bridge between the iron atoms. Verification of such a bridge was obtained in the 2.2-Å resolution crystal structure of azidomethemerythrin [3].

Resonance Raman spectroscopy provides an additional valuable technique for the detection and characterization of binuclear iron centers. The Fe–O–Fe symmetric stretch, ν_s(Fe–O–Fe), is Raman active and the intensity of this vibration may be enhanced by excitation within the Fe–O–Fe charge transfer band in the near ultraviolet. As a model system, we have investigated the resonance Raman spectra of binuclear 1,10-phenanthroline (phen) complexes of iron(III) [4]. The complex $Fe_2O(phen)_4(NO_3)_4 \cdot 7H_2O$ has a Raman peak at 395 cm^{-1} which can be assigned to ν_s(Fe–O–Fe) on the basis of its frequency being appropriate to an Fe–O–Fe angle of 154°, its absence from the spectrum of mononuclear complex $Fe(phen)_3(ClO_4)_3 \cdot 3H_2O$, and its intensity being dependent upon excitation wavelength. The perchlorate and chloride salts of $[Fe_2O(phen)_4]^{4+}$ have similar resonance-enhanced modes close to 400 cm^{-1}, and in all three cases the enhancement is maximized using 363.8 nm excitation.

Confirmatory evidence for an Fe–O–Fe vibration can be obtained from oxygen isotope exchange with $H_2^{18}O$ solvent. For example, in the μ-oxo bridged dimer, $[Fe_2O(Cl)_6]^{2-}$, ν_s(Fe–O–Fe) at 458 cm^{-1} shifts to 400 cm^{-1} in $H_2^{18}O$ [5]. Similarly, the resonance-enhanced band at 507 cm^{-1} in the Raman spectrum of azidomethemerythrin shifts to 490 cm^{-1} for a sample which has been formed from oxyhemerythrin in $H_2^{18}O$ [6]. We have now found evidence for the corresponding Fe–O–Fe vibration at 489 cm^{-1} in the resonance Raman spectrum of oxyhemerythrin, through the use of near-ultraviolet excitation [7]. Furthermore, this band shows an isotope dependence on solvent ($H_2^{18}O$), and appears to be in resonance with the 360 nm electronic transition of oxyhemerythrin. The resonance enhancement of the ν_s(Fe–O–Fe) peak intensity with ultraviolet excitation has also been observed for azidomethemerythrin [7] and for ribonucleotide reductase [2]. In contrast to hemerythrin, however, where the oxo group only exchanges during exogenous ligand replacement, the oxo group in ribonucleotide reductase undergoes facile exchange with solvent ($k_{obs} = 8.3 \times 10^{-4}$ s^{-1}), indicating it is located in a more accessible site.

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SUPERQUAD – A New Computer Program for Determination of Stability Constants of Complexes by Potentiometric Titration

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We have developed, separately and together, a sequence of computer programs for the determina-