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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-