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Synthesis and X-ray Absorption Spectroscopy Structural Studies of Cu(I) Complexes of HistidylHistidine Peptides: The Predominance of Linear 2-Coordinate Geometry

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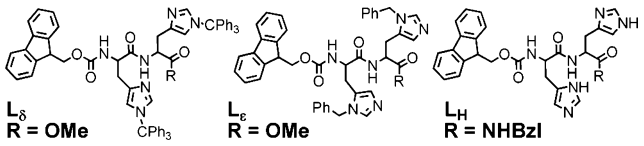
The importance of protein-based ligand tuning of copper active sites has been noted and recapitulated in model systems (e.g., sensitivity of Cu−O_2 structure and reactivity to coordination number, geometry, and bonding atoms).[1,2] Several enzymes—PHM, DβH, and C*c*O[3]—possess active site Cu ions bound by contiguous histidine residues, a binding motif unique to these redox/O_2-processing enzymes.[4,5]

We hypothesized that HisHis ligation of copper ion, in particular Cu^I (the reduced form of the Cu^{II}/Cu^I redox pair) may enforce structural/binding properties —in particular, a linear 2-coordinate geometry—that might also dictate particular redox properties and/or reactivity patterns for the system, as has been observed in two-coordinate Cu^I complexes of monodentate ligands (also see later).[6−10] Thus, we set out to investigate these biologically relevant coordination aspects of HisHis. In this report, we describe the generation of a series of such dipeptides and demonstrate via spectroscopic interrogation and chemical behavior that indeed, strong preferences for near-linear two-coordinate Cu^I geometries are observed.

Dipeptides with regioselectively substituted imidazole sidechains (N_ϵ vs N_δ) have been synthesized by modifications of literature procedures and standard solution-phase techniques.[11] Tautomeric preferences appear in Cu enzymes:[12−14] Cu ion binds to $N_\delta N_\delta$ of the HisHis fragment of the PHM Cu_H site,[5,15,16] but $N_\epsilon N_\epsilon$ at the cyt. *c* oxidase Cu_B site.[4,12,17,18] The ligands synthesized for study (diagram) were chosen in order to elucidate the implications of these binding modes.



Cu^I complexes of $\mathbf{L}_\delta$, $\mathbf{L}_\epsilon$, and $\mathbf{L_H}$ were synthesized in either CH_2Cl_2 or acetone using $[Cu^I(MeCN)_4]Y$ salts (Y = ClO_4^-, $B(C_6F_5)_4^-$).[11] Solid complexes were isolated by precipitation and purified by recrystallization; they give satisfactory C,H,N combustion analysis, ESI-MS mass envelope isotope patterns are consistent with the $[\mathbf{L}Cu^I]^+$ cation formulations and small shifts in the imidazolyl C-*H* resonances are observed by ^{1}H NMR spectroscopy of $[\mathbf{L}Cu^I]^+$ compared to that of the free ligand.[11]

EXAFS analysis of the solid complexes provides unequivocal evidence for a near-linear 2-coordinate geometry, with His ligation[19] in *all* cases. For $[\mathbf{L}_\delta Cu^I]^+$, the Fourier transform and the results/data for all complexes are given in Figure 1.[11] Fits to two N_{His}-ligand scatterers with Cu−N ≈ 1.86−1.87 Å are indicative of linear 2-coordinate Cu^I. (Cu−N does *not* deviate from 1.86 to 1.88 Å for known chemical examples.[6−9,20]) Three-coordinate complexes have significantly longer Cu−N bond distances. Near-edge (XANES) data (Figure 1) corroborate 2- and not 3-coordination, based on strong precedent; the 2-coordinate $[\mathbf{L}_\delta Cu^I]^+$ (**1**) near-edge absorption is intense compared to 3-coordinate analogues (vide infra).[21,22]

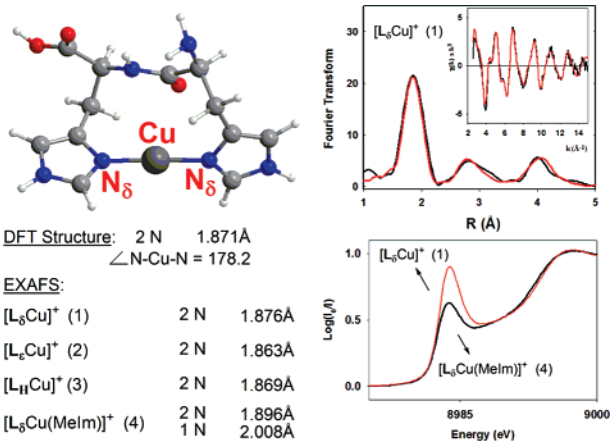


Figure 1. EXAFS and XANES data and results carried out on copper(I) complexes **1**−**4**, and results of DFT calculations related to $[\mathbf{L}_\delta Cu^I]^+$ (**1**).

DFT geometry optimization (B3LYP/6-311G**) supports the experimental (i.e., EXAFS) structure analysis (Figure 1).[11] A Cu-$His_{N\delta}His_{N\delta}$ model minimizes to near-linear 2-coordinate geometry with Cu−N bonds within 0.002−0.005 Å of the EXAFS values.[23] In contrast, molecular mechanics and DFT calculations suggest that an *intra*molecular 2-coordinate structure bound solely by the N_ϵ of His imidazoles is thermodynamically disfavored, requiring severe strain in the ligand. A dimeric $Cu_2\mathbf{L}_2$ structure, that is, with *inter*molecular Cu-$His_{N\epsilon}$ binding, is proposed to rationalize the EXAFS data (also see below).

Solution (acetone) conductivity data for all three complexes were also acquired. Onsager plots for $[\mathbf{L}_\delta Cu^I]ClO_4$ and $[\mathbf{L_H}Cu^I]ClO_4$ have slopes in the range expected for 1:1 (monomeric) electrolytes, that is, consistent with the mononuclear complex formulation.[24] However, the slope for the $\mathbf{L}_\epsilon$ complex indicates a 2:1 electrolyte behavior; that is, a dimer, $[(\mathbf{L}_\epsilon)_2Cu^I_2](ClO_4)_2$, persists in acetone, as was formulated for the solid (vide supra). The preference for $\mathbf{L}_\epsilon$ to form a dimeric structure further demonstrates the favorability of the 2-coordinate near-linear geometry for these Cu^I-ligand systems. Additionally, $[\mathbf{L_H}Cu^I]^+$, with unblocked imidazole N-atoms, gives a 2-coordinate structure and 1:1 solution conductivity, suggesting it may preferentially bind to N_δ nitrogens of adjacent His residues.

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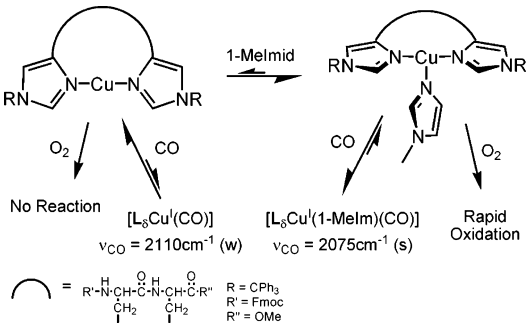
Scheme 1



The complexes' properties were probed via reactivity with small molecules (O_2, CO) and electrochemistry (CV). All three complexes are unreactive toward O_2 as solids or in solution below 0 °C (with only very slow oxidation occurring at room temperature). This behavior is analogous to that observed for linear 2-coordinate Cu^I complexes studied by Sorrell, Karlin, and others.[6−8] The HisHis $[\mathbf{L}Cu^I]^+$ complexes bind CO (as an O_2-surrogate) weakly, with high-frequency stretching vibrations (ν_{CO} = 2110−2105 cm^{-1}) of low intensity.[8,25] The complexes display irreversible redox behavior, to be expected for two-coordinate copper, and a high E_{pa} value ($[\mathbf{L}_\delta$-$Cu^I]^+$ in DMF: 325 mV vs Fc^+/Fc) is consistent with resistance to oxidation.

Three-coordinate derivatives, formed by the addition of *N*-methylimidazole (MeIm) to the parent Cu^I-HisHis complexes, exhibit starkly contrasting behavior (Scheme 1). $[\mathbf{L}_\delta Cu^I(MeIm)]^+$ (**4**), characterized by C,H,N analysis and ^{1}H NMR spectroscopy,[11] for example, oxidizes rapidly with added O_2.[26] The complex binds CO in CH_2Cl_2 solution with more pronounced backbonding (and thus lowered ν_{CO} = 2075 cm^{-1}) and higher intensity compared to 2-coordinate $[\mathbf{L}_\delta Cu^I]^+$. Complex **4** exhibits *reversible* redox behavior, $E_{1/2} \cong -275$ mV vs Fc^+/Fc (DMF solvent).

EXAFS and XANES data obtained for $[(\mathbf{L}_\delta)Cu^I(MeIm)]$ confirm a 3-coordinate structure (Scheme 1); the near-edge absorption is of characteristically lower intensity compared to the 2-coordinate parent (Figure 1).[21] The complex adopts a distorted T-shaped geometry, in which the $Cu-N_{His}$ bonds (presumably) have slightly lengthened (by 0.02 Å), and MeIm provides a Cu−N scatterer at a longer distance (2.008 Å) from Cu^I.

In conclusion, we have synthesized a series of Cu^I complexes of HisHis dipeptides showing that linear 2-coordinate $N_\delta N_\delta$ ligation is very favorable, but the site is NOT redox active. The geometry resembles that found by EXAFS for reduced PHM Cu_H (vide supra). We have here also demonstrated that addition of a third N-donor to these complexes activates the Cu ion for redox activity. This may have significance for a detailed understanding of the functioning of the Cu_H electron-transfer site of PHM. Changes in Cu_H coordination are known to occur upon oxidation of the enzyme.[5] Cu_H coordination and CO-binding characteristics are also influenced if substrate is added (and binds to the Cu_M site ∼11 Å away),[27,28] or when Met^{314} at this Cu_M site is mutated.[29] Further, enzyme activity and possibly its mechanism are altered by mutations of His^{172} (the third ligand).[30]

All these factors are relatively poorly understood; our new peptide models in further studies may shed light on the significance of the geometry and coordination of the PHM Cu_H site (e.g, what subtle tuning of Cu^I-coordination facilitates electron-transfer chemistry). In addition, these results have encouraged us that studies of Cu^I-model peptide complexes may yield insights into Cu-redox properties and Cu^I-O_2 reactivity that have not been forthcoming with studies of other model systems. Future investigations include Cu^I oxidative chemistry in these unique HisHis Cu-binding environments, such as occurs in C*c*O (vide supra) and also in the copper binding portion of the amyloid beta (Aβ) peptide involved in Alzheimer's Disease.[31]

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Supporting Information Available: Synthetic and computational details, spectroscopic methods and data, and EXAFS fitting methods are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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(3) Abbreviations: PHM = peptidylglycine α-hydroxylating monooxygenase; DβH = dopamine β-hydroxylase; C*c*O = cytochrome *c* oxidase.
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