

Published on Web 04/06/2007

## 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<sub>2</sub> structure and reactivity to coordination number, geometry, and bonding atoms).<sup>1,2</sup> Several enzymes—PHM, D $\beta$ H, and CcO<sup>3</sup>—possess active site Cu ions bound by contiguous histidine residues, a binding motif unique to these redox/O<sub>2</sub>-processing enzymes.<sup>4,5</sup>

We hypothesized that HisHis ligation of copper ion, in particular Cu<sup>I</sup> (the reduced form of the Cu<sup>II</sup>/Cu<sup>I</sup> 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<sup>I</sup> complexes of monodentate ligands (also see later).<sup>6–10</sup> 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<sup>I</sup> geometries are observed.

Dipeptides with regioselectively substituted imidazole sidechains (N<sub>e</sub> vs N<sub>o</sub>) have been synthesized by modifications of literature procedures and standard solution-phase techniques.<sup>11</sup> Tautomeric preferences appear in Cu enzymes:<sup>12–14</sup> Cu ion binds to N<sub>o</sub>N<sub>o</sub> of the HisHis fragment of the PHM Cu<sub>H</sub> site,<sup>5,15,16</sup> but N<sub>e</sub>N<sub>e</sub> at the cyt. *c* oxidase Cu<sub>B</sub> site.<sup>4,12,17,18</sup> The ligands synthesized for study (diagram) were chosen in order to elucidate the implications of these binding modes.



Cu<sup>I</sup> complexes of  $\mathbf{L}_{\delta}$ ,  $\mathbf{L}_{\epsilon}$ , and  $\mathbf{L}_{\mathbf{H}}$  were synthesized in either CH<sub>2</sub>-Cl<sub>2</sub> or acetone using [Cu<sup>I</sup>(MeCN)<sub>4</sub>]Y salts (Y = ClO<sub>4</sub><sup>-</sup>, B(C<sub>6</sub>F<sub>5</sub>)<sub>4</sub><sup>-</sup>).<sup>11</sup> 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 [LCu<sup>I</sup>]<sup>+</sup> cation formulations and small shifts in the imidazolyl C-*H* resonances are observed by <sup>1</sup>H NMR spectroscopy of [LCu<sup>I</sup>]<sup>+</sup> compared to that of the free ligand.<sup>11</sup>

EXAFS analysis of the solid complexes provides unequivocal evidence for a near-linear 2-coordinate geometry, with His ligation<sup>19</sup> in *all* cases. For  $[L_{\delta}Cu^{I}]^{+}$ , the Fourier transform and the results/ data for all complexes are given in Figure 1.<sup>11</sup> Fits to two N<sub>His</sub>-



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

ligand scatterers with Cu–N  $\approx 1.86-1.87$  Å are indicative of linear 2-coordinate Cu<sup>I</sup>. (Cu–N does *not* deviate from 1.86 to 1.88 Å for known chemical examples.<sup>6–9,20</sup>) 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 [L<sub>0</sub>Cu<sup>I</sup>]<sup>+</sup> (1) near-edge absorption is intense compared to 3-coordinate analogues (vide infra).<sup>21,22</sup>

DFT geometry optimization (B3LYP/6-311G<sup>\*\*</sup>) supports the experimental (i.e., EXAFS) structure analysis (Figure 1).<sup>11</sup> A Cu-His<sub>N∂</sub>His<sub>N∂</sub> model minimizes to near-linear 2-coordinate geometry with Cu–N bonds within 0.002–0.005 Å of the EXAFS values.<sup>23</sup> In contrast, molecular mechanics and DFT calculations suggest that an *intra*molecular 2-coordinate structure bound solely by the N<sub>ε</sub> of His imidazoles is thermodynamically disfavored, requiring severe strain in the ligand. A dimeric Cu<sub>2</sub>L<sub>2</sub> structure, that is, with *inter*molecular Cu-His<sub>Nε</sub> 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.<sup>24</sup> However, the slope for the  $\mathbf{L}_{\epsilon}$  complex indicates a 2:1 electrolyte behavior; that is, a dimer,  $[(\mathbf{L}_{\epsilon})_{2}Cu^{I}_{2}](ClO_{4})_{2}$ , 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<sup>I</sup>-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  $N_{\delta}$  nitrogens of adjacent His residues.

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Scheme 1



The complexes' properties were probed via reactivity with small molecules (O<sub>2</sub>, CO) and electrochemistry (CV). All three complexes are unreactive toward O2 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<sup>I</sup> complexes studied by Sorrell, Karlin, and others.<sup>6-8</sup> The HisHis [LCu<sup>I</sup>]<sup>+</sup> complexes bind CO (as an O<sub>2</sub>-surrogate) weakly, with highfrequency stretching vibrations ( $\nu_{\rm CO} = 2110 - 2105 \text{ cm}^{-1}$ ) of low intensity.8,25 The complexes display irreversible redox behavior, to be expected for two-coordinate copper, and a high  $E_{pa}$  value ([L<sub> $\delta$ </sub>-Cu<sup>I</sup>]<sup>+</sup> in DMF: 325 mV vs Fc<sup>+</sup>/Fc) is consistent with resistance to oxidation.

Three-coordinate derivatives, formed by the addition of Nmethylimidazole (MeIm) to the parent CuI-HisHis complexes, exhibit starkly contrasting behavior (Scheme 1).  $[L_{\delta}Cu^{I}(MeIm)]^{+}$ (4), characterized by C,H,N analysis and <sup>1</sup>H NMR spectroscopy,<sup>11</sup> for example, oxidizes rapidly with added O2.26 The complex binds CO in CH<sub>2</sub>Cl<sub>2</sub> solution with more pronounced backbonding (and thus lowered  $v_{\rm CO} = 2075 \text{ cm}^{-1}$ ) and higher intensity compared to 2-coordinate  $[L_{\delta}Cu^{I}]^{+}$ . Complex 4 exhibits reversible redox behavior,  $E_{1/2} \simeq -275$  mV vs Fc<sup>+</sup>/Fc (DMF solvent).

EXAFS and XANES data obtained for  $[(L_{\delta})Cu^{I}(MeIm)]$  confirm a 3-coordinate structure (Scheme 1); the near-edge absorption is of characteristically lower intensity compared to the 2-coordinate parent (Figure 1).<sup>21</sup> The complex adopts a distorted T-shaped geometry, in which the Cu-N<sub>His</sub> 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<sup>I</sup>.

In conclusion, we have synthesized a series of Cu<sup>I</sup> 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<sub>H</sub> (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<sub>H</sub> electron-transfer site of PHM. Changes in Cu<sub>H</sub> coordination are known to occur upon oxidation of the enzyme.5 Cu<sub>H</sub> coordination and CO-binding characteristics are also influenced if substrate is added (and binds to the Cu<sub>M</sub> site  $\sim 11$  Å away),<sup>27,28</sup> or when Met<sup>314</sup> at this Cu<sub>M</sub> site is mutated.<sup>29</sup> Further, enzyme activity and possibly its mechanism are altered by mutations of His<sup>172</sup> (the third ligand).<sup>30</sup>

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<sub>H</sub> site (e.g, what subtle tuning of CuI-coordination facilitates electron-transfer chemistry). In addition, these results have encouraged us that studies of CuI-model peptide complexes may yield insights into Cu-redox properties and Cu<sup>I</sup>-O<sub>2</sub> reactivity that have not been forthcoming with studies of other model systems. Future investigations include CuI oxidative chemistry in these unique HisHis Cu-binding environments, such as occurs in CcO (vide supra) and also in the copper binding portion of the amyloid beta (A $\beta$ ) peptide involved in Alzheimer's Disease.31

Acknowledgment. We gratefully acknowledge financial support of this work (Grants NIH GM28962, K.D.K.; NIH Postdoctoral Fellowship, R.A.H.; NIH NS27583, N.J.B.).

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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JA0708013