Copper–Peptide Complex Structure and Reactivity When Found in Conserved His-Xaa-His Sequences

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Supporting Information

ABSTRACT: Oxygen-activating copper proteins may possess His-Xaa-His chelating sequences at their active sites and additionally exhibit imidazole group δN vs εN tautomeric preferences. As shown here, such variations strongly affect copper ion’s coordination geometry, redox behavior, and oxidative reactivity. Copper(I) complexes bound to either δ-HGH or ε-HGH tripeptides were synthesized and characterized. Structural investigations using X-ray absorption spectroscopy, density functional theory calculations, and solution conductivity measurements reveal that δ-HGH forms the CuI dimer complex [Cu₂(δ-HGH)]₂⁺ (1) while ε-HGH binds CuI to give the monomeric complex [Cu(ε-HGH)]⁺ (2). Only 2 exhibits any reactivity, forming a strong CO adduct, [Cu(ε-HGH)(CO)]⁺, with properties closely matching those of the copper monoxygenase PHM. Also, 2 is reactive toward O₂ or H₂O₂, giving a new type of O₂-adduct or CuI −OH complex, respectively.

The study of peptide complexation to copper ions has been of great interest to (bio)chemists since the most common ligands at copper active sites in proteins are amino acids, most often histidine.1 A survey of His imidazole group binding to copper proteins involved in redox chemistry, including O₂ reactivity, indicates that the His-Xaa-His (Xaa = amino acid) tripeptide motif is a frequently observed sequence, including, for example, His-Thr-His in PHM, and D/J/M,4 His-Val-His in SODS5 and pMMO,6 and His-Gln-His in APLP2 and LYOX.7 Four highly conserved His-Xaa-His sequences exist in a bridging fashion in the trinuclear copper ion cluster of MCOs (Figure 1).8 Also, a similar motif appears in pentapeptide domains (HLHWH) present in the amyloid precursor protein (APP) associated with the development of Alzheimer’s disease.7,9 The imidazole group of histidine ligands can bind to CuI ion through either the δN or εN site, and tautomeric preferences occur in different classes of copper proteins.10 The variations most certainly are critical in determining the functions and properties of the enzymes because of differences in decisive steric/electronic effects imparted to the copper ion center, for example controlling the exact nature of O₂ binding and consequent structure—reactivity and the specificity of substrate approach. For example, in PHM, O₂ binds at the CuM site, which is close to where the substrate docks; CuM is ligated by two εN sites of His’s in an HTH sequence. CuM, which is ∼11 Å away, facilitates electron transfer, but it binds to three δNHisi sites (in fact where two of the His residues are adjacent in the overall peptide sequence).3 These observations raise basic questions relevant to PHM active-site structure and function: how do these specific tautomeric imidazole N atom configurations imposed by nature control (i) copper coordination number and geometry, (ii) CuI/CuII redox potential, (iii) electronic structure/bonding and associated spectroscopic properties, and (iv) exogenous ligand preferences?

We have previously reported studies of CuI complexes of modified histidylhistidine (HisHis) peptides11 where imidazole N atoms were specifically blocked, allowing study of δ-HH (δN of both His available for metal coordination) or ε-HH (εN of both His available for metal coordination). Significantly, both dipeptides adopt a linear two-coordinate NHis-CuI=–NHis environment. In the present work, we aimed to understand why the unique His-Xaa-His sequence is particularly “selected” in nature by generating CuI complexes of His-Gly-His tripeptides1h with varying δN versus εN atom availability and investigating their structural features and chemical properties.

The tripeptides δ-HGH and ε-HGH (Chart 1) were synthesized by modifications of literature procedures and standard solution-phase peptide synthesis techniques.12 The
N-/C-tripeptide terminal groups were also “protected” using either fluorenlymethoxy carbonyl (Fmoc), tert-butyloxycarbonyl (Boc), or benzyl groups to avoid any likelihood of terminal-group Cu coordination. δ-HGH and ε-HGH were metallated with [Cu(CH3CN)3]ClO4 in CH2Cl2. Solid complexes were isolated by precipitation and purified by recrystallization from CH2Cl2/Et2O; their elemental analysis and electrospray ionization mass spectrometry envelope isotope patterns were consistent with the [ligand−Cu+] cation formulations.14

Extended X-ray absorption fine structure (EXAFS) spectroscopy (Figure 2)14 of LCu1 complex solids and accompanying computational analyses (Figure 3) provide strong evidence that Cu complexes of both δ-HGH and ε-HGH possess two-coordinate NHis−Cu−NHis geometries. Multiple scattering definitively reveals the patterns known for NHis−Cu coordination. For the Cu1 complex of δ-HGH, the data given in Figure 2a display the best fit to two δNHis-ligand scatterers with Cu−N = 1.867 Å, indicative of linear two-coordinate Cu1, as observed earlier with the HisHis peptides.11 These very short Cu−N bonds are characteristic of this very low coordination, being significantly shorter than those found in three-coordinate Cu2−N3 compounds.15

The EXAFS data for the solid complex of Cu1 with ε-HGH are extremely similar. The best and only fit was found with two-His ligation (Figure 2b) and a Cu1−NHis bond length of 1.878 Å, also indicating two-coordinate Cu1. The only significant difference is a small decrease in the intensity of the 8983 eV pre-edge transition found in the X-ray absorption near-edge structure (XANES) spectroscopic data, which may suggest some deviation from a strictly linear geometry.11,16 As described below, this deviation seems to directly relate to this Cu1 compound’s remarkably different (compared with the δ-HGH Cu1 complex) electrochemical and CO-binding behavior and its reactivity toward O2 and H2O2.

Density functional theory (DFT) structural analyses and supporting solution conductivity measurements lead to differing formulations for δ-HGH and ε-HGH in comparison with our previous findings for HisHis dipeptides. The EXAFS data indicate near-perfect linear two-coordination for Cu1 in the δ-HGH complex. Solution conductivity data in dimethylformamide (DMF) provide an Onsager plot for a Cu1−δ-HGH complex with a slope in the range expected for 2:1 electrolyte behavior, thus indicating a dimer formulation, [[Cu1(δ-HGH)]]+ (1). We note that Figure 3a is a geometry optimization assuming a dimer formulation. In fact, higher-level computations and energy comparisons (in vacuum) reveal that a monomeric formulation and structure are slightly favored (by 11.5 kJ/mol).14 The stronger solution experimental evidence thus points to the dimer formulation; apparently, intramolecular two-coordination leads to an excessively strained structure. By contrast, structural energy minimization for a Cu2−ε-HGH complex leads to a preferred mononuclear formulation, [Cu1(ε-HGH)]+ (2) (Figure 3b), on the basis of electronic energies corrected for zero-point energy; a dimer structure as in 1 is thermodynamically disfavored by 44.0 kJ/mol. Also, a dimer is ruled out by solution conductivity measurements showing that this complex behaves as a 1:1 electrolyte.14,17 Notably, the DFT-derived structure for complex 2 reveals a significant bending in the two-coordinate Cu1 coordination, with εN−Cu−εN = 160.2°, as suggested by the XANES data and the unexpected oxidative reactivity (vide infra); nevertheless, short Cu1−εN bond distances are present that are typical of this coordination number for Cu1 and much shorter than those observed in three-coordinate Cu2−N3 compounds (vide supra).

The features observed here for Cu1 binding to His-Xaa-His peptides contrast greatly with those observed for the previously studied HisHis peptides,12 where the [Cu1(δ-HH)]+ complex showed monomeric behavior (DFT and solution conductivity) while [Cu1(ε-HH)]+ is a 2:1 solution electrolyte with a dimeric structure. Just inserting a Gly amino acid between two His residues leads to significant changes in the Cu coordination environment. Do these alterations affect other physical/spectroscopic properties or reactivity patterns?

To address such questions, we first examined the CO binding behavior of the new Cu1−peptide complexes, as CO is a Cu1−specific ligand (and more generally an O2 surrogate) and can provide insights into coordination number and ligand donation ability. CO adducts of acetone solutions (under Ar) of 1 and 2 were generated by direct CO bubbling. As previously established for near-linear two-coordinate [Cu1(HisHis)]+ complexes, CO binding is very weak, and high-frequency stretching vibrations (νCO = 2110–2122 cm−1) of low intensity are observed.11b,15c,18 This is also the case here, as the IR spectrum of 1−CO exhibits νCO = 2103 cm−1 (Table 1). By contrast, [Cu1(ε-HGH)(CO)]+ (2−CO) displays a high-intensity absorption at lower frequency (νCO = 2092 cm−1).14 This observation suggests that there is a significant geometric-coordinative effect leading to stronger ligation of CO to Cu2− and better back-donation from Cu1 when it is bound to the ε-HGH ligand rather than to either the δ-HGH or HisHis system (Table 1). This νCO of 2092 cm−1 for 2−CO in fact compares very well with that observed for the enzyme Cu1M sites in PHM (2093 cm−1)19 and D/M (2089 cm−1),20 which are ligated by two histidyl εN atoms of the His-Thr-His active-site tripeptide sequence (Figure 1a). Thus, 2−CO possesses a
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Table 1. Comparison of Properties of Cu(I)–Peptide Complexes

| Complex | Cu–N$_{His}$ (Å)$^c$ | ν$_{CO}$ (cm$^{-1})$ | Redox Behavior | O$_2$ /H$_2$O$_2$ Reactivity |
|---------|---------------------|-------------------|----------------|--------------------------|
| [Cu($\delta$-HGH)$_2$]$^{2+}$ | 1.867 | 2103 | Irreversible | No |
| [Cu($\epsilon$-HGH)]$^+$ | 1.878 | 2092 | Quasi-reversible | Yes |
| [Cu($\delta$-HH)]$^+$ | 1.876 | 2110 | Irreversible | No |
| [Cu($\epsilon$-HH)]$^{2+}$ | 1.863 | 2112 | Irreversible | No |

$^a$Determined by solution conductivity. $^b$Measured by XAS. $^c$IR stretching frequency.

Figure 4. (a) UV–vis spectra of [Cu$^{II}$(e-HGH)(OOH)]$^{+}$ generated by addition of 1.5 equiv of H$_2$O$_2$ to a 3.5 mM solution of 2 in acetone at 193 K. (b) EPR spectrum of [Cu$^{II}$(e-HGH)(OOH)]$^{+}$ at 77 K ($g_1=2.25$, $g_\perp=2.05$, $A_1=192$ G, $A_\perp=15$ G).

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OOH species. $^{24}$ [Cu$^{II}$(e-HGH)(OOH)]$^{+}$ (ClO$_4$) is presently characterized by (i) its UV–vis features ($\lambda_{\text{max}}=366$ nm ($\epsilon=2600$ M$^{-1}$ cm$^{-1}$)), assignable to a $\bullet$OOH $\rightarrow$ Cu$^{II}$ ligand-to-metal charge transfer absorption on the basis of the correspondence with a number of literature examples, and (ii) its distinctive mononuclear-type axial EPR spectrum at 77 K ($g_1=2.25$, $g_\perp=2.05$, $A_1=192$ G, $A_\perp=15$ G; Figure 4b).

In conclusion, we have generated new Cu(I) complexes with His-Gly-His tripeptides to probe fundamental aspects of Cu(I) chemistry with this particular histidine-containing sequence; we have also probed the presence of synthetically imposed tautomeric preferences (δ$\text{NIm}$ vs ε$\text{NHis}$ availability) for Cu(I). The dimer [Cu($\delta$-HGH)$_2$]$^{2+}$ (1) exhibits favorable near-linear twofold coordination via intermolecular Cu$^{II}$–δ$\text{NIm}$ binding. This complex is not redox-active and only weakly binds CO. Furthermore, it does not react with either O$_2$ or H$_2$O$_2$ (Scheme 1). However, [Cu($\epsilon$-HGH)]$^{+}$ (2) shows two-His ligation with deviation from linearity. The similarity of our synthetic construct to the protein is notable: the IR spectrum of the carbonyl adduct 2–CO matches that observed for the enzyme PHM Cu$_{His}$–CO adduct with its active-site eH-Xaa-eH chelating moiety. Also, 2 displays redox activity and readily reacts with O$_2$ and H$_2$O$_2$ to afford the first oxygen-intermediate species to be noted with Cu(I) ligated to biologically relevant His-containing peptides.

It is striking that a switch in the imidazole tautomer can radically influence the reactivity of a Cu(I) center. These results, in conjunction with our previous work with Cu(I)–HisHis complexes, highlight the manner in which nature exerts its control function. Even slight changes (dipeptide vs tripeptide; δ$\text{NIm}$ vs ε$\text{NHis}$ availability) can significantly affect an enzyme metal...
center’s structure and reactivity. Thus, our continuing research will add to an understanding of structure–function relationships in copper enzymes and the role of His binding motifs in facilitating Cu–O₂ (and even reactive oxygen species) intermediate formation.

■ ASSOCIATED CONTENT

* Supporting Information
Synthetic and analytical details; UV–vis, IR, and EPR spectra; cyclic voltammograms; and Onsager plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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(2) Abbreviations: PHM = peptidylglycine α-amidating monooxygenase; DPM = dopamine β-monooxygenase; SOD5 = superoxide dismutase; pMMO = particulate methane monoxygenase; APL2 = Arg-precursor-like protein 2; LYOX = l-lysyl oxidase; MCO = multicopper oxidase.

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