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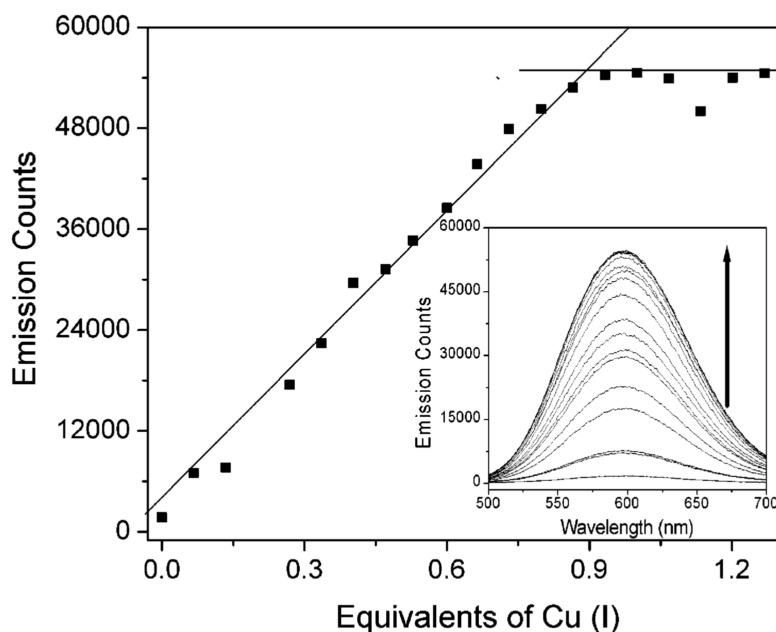

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Cu(I) Luminescence from the Tetranuclear Cu_4S_4 Cofactor of a Synthetic 4-Helix Bundle

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The rational design of synthetic metalloproteins having specific chemical functions and/or interesting physical properties holds the promise of delivering important advances to science and medicine. However, this goal not only demands the ability to synthesize protein structures capable of binding specific transition metal ions, but also requires insight into how such properties can be influenced by the interplay between inorganic coordination chemistry and the protein environment. Thus, the emerging field of metalloprotein design seeks to develop new types of synthetic metalloproteins in order to better understand the nature of this important relationship.[1-3] Within this context, it is shown here that the addition of Cu(I) to a cysteine-containing random-coil peptide results in the formation of a 4-helix bundle metalloprotein that displays a strong room-temperature luminescence from an embedded Cu_4S_4 cofactor.

The peptide C16C19-GGY having the sequence, Ac-K(IEALEGK)$_2$(CEACEGK)(IEALEGK)GGY-amide, was prepared by solid-phase methods.[4] This peptide is similar to that extensively used by our group to prepare two-stranded coiled-coil proteins for use in long-range electron-transfer studies,[5,6] but the Cys-X-X-Cys metal-binding motif was introduced into positions 16−19 of the sequence. The anaerobic addition of $[Cu(CH_3CN)_4]^+$ to samples of C16C19-GGY results in significant changes to the far UV circular dichroism spectrum of the peptide (Supporting Information). The spectrum of the apopeptide displays a negative maximum at 195 nm, indicating the existence of a random coil. However, the addition of copper ion produces negative maxima at 208 and 222 nm with an ellipticity ratio of $[\theta_{222}]/[\theta_{208}] = 1.0$, which indicates that the metal peptide exists as a coiled-coil.[7] These results show that, as with the case of Cd(II) addition,[4] the C16C19-GGY peptide undergoes a metal-induced folding process when it binds Cu(I).

Sedimentation equilibrium analysis was used to determine the oligomerization state of the Cu−C16C19-GGY adduct. The data were fit to both a two-component ideal noninteracting model and a monomer−tetramer model (Supporting Information). Both analyses indicated the presence of two species that correspond to a peptide monomer and tetramer, respectively. These results were not anticipated from the sequence of the apopeptide, which was based on a heptad repeat known to form two-stranded coiled-coils.[5] These results thus illustrate how the structures of metalloproteins can be controlled by the directional bonding properties of their inorganic cofactors.

The inset to Figure 1 shows that the addition of Cu(I) to C16C19-GGY results in the appearance of an intense ($\phi = 0.053$) room temperature luminescence that is centered at 600 nm and is stable

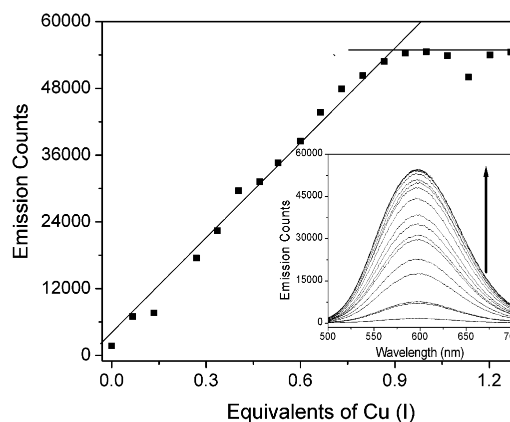

Figure 1. Emission titration of C16C19-GGY by $[Cu(CH_3CN)_4]PF_6$. Inset: Emission spectra obtained upon addition of Cu(I) to the peptide solution. Conditions: 120 μM C16C19-GGY in 0.2 M acetate buffer (pH 5.4) containing 730 μM tris-(2-carboxyethyl)phosphine (TCEP) as a reducing agent. The emission spectrum of the Cu(I) adduct formed in the absence of TCEP is identical to that shown in the inset.

upon standing overnight under ambient conditions. This luminescence has an excitation spectrum with a maximum at 275 nm and can be quenched by the addition of either ferricyanide, oxygen, or urea, which indicates that the emitting species is associated with the reduced Cu(I) state, has significant triplet character, and is quenched upon exposure to bulk solvent. Similar properties have been reported for Cu(I) derivatives of the metal-binding protein, metallothionein,[8,9] as well as to those of the copper responsive transcription factors, ACE1, AMT, and CopY, which all contain embedded thiolato metal-binding domains.[10]

Figure 1 shows that the emission intensity of Cu(I)−C16C19-GGY increases as increasing amounts of Cu(I) are titrated into the peptide solution, but saturates after ca. 0.9 equiv of metal ion has been added. These results indicate that four Cu(I) centers have been incorporated into the peptide tetramer. This conclusion is consistent with earlier observations that luminescent Cu(I) compounds contain polynuclear metal clusters in which metal−metal interactions play an important role in stabilizing the emissive photoexcited state.[11] The metal binding stoichiometry was further studied by UV titrations, as the binding of Cu(I) to cysteine residues is known to produce CysS to Cu(I) ligand-to-metal charge transfer (LMCT) and metal-localized transitions in the UV region of the spectrum.[12,13] The addition of Cu(I) to C16C19-GGY produces a new absorption band having a maximum at 236 nm and a shoulder at 296 nm, the intensity of which scales with added Cu(I) until saturating after ca. 1 equiv of copper has been added to the peptide solution (Supporting Information). CD titrations provided somewhat similar

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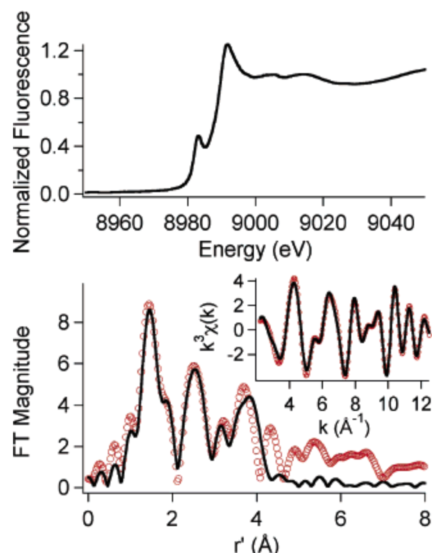

Figure 2. Copper K-edge XAS of Cu−C16C19-GGY. The XANES spectrum (top), Fourier-transformed (FT window = 2−12.5 $Å^{-1}$, uncorrected for phase shifts, bottom), and Fourier-filtered (back transform window = 1−4 Å, insert) EXAFS spectrum (data shown as circles and fit as a solid line). The fit shown was obtained for 1 N @ 1.89(1) Å (σ^2 = 0.001(1) $Å^2$) + 2 S @ 2.22(2) Å (σ^2 = 0.013(2) $Å^2$) + 2 Cu @ 2.88(1) Å (σ^2 = 0.009-(1) $Å^2$) + 2 S @ 3.54(3) Å (σ^2 = 0.006(2) $Å^2$) +1 Cu @ 3.91(1) (σ^2 = 0.001(1) $Å^2$) and had a GOF value of 0.37.

results, as the intensity of the signal at 222 nm was seen to increase upon successive additions of Cu(I) and reach a plateau after ca. 1 equiv of added metal.

The Cu K-edge XANES spectrum of Cu−C16C19-GGY (Figure 2) shows a Cu edge with an energy appropriate for a Cu(I) center (E = 8987.0 eV) that lacks the 1s → 3d transition typical of Cu(II) sites and exhibits a pre-edge peak at 8983.5 eV consistent with a 1s → 4p transition observed for Cu(I) centers.[14] The shape of the peak observed for Cu−C16C19-GGY indicates a three-coordinate site, as the intensity is too weak for a linear two-coordinate geometry and the energy is too low for a four-coordinate site.[14]

EXAFS analysis (Figure 2) is consistent with a Cu(I) site having a $N(O)S_2$ ligand donor set. The best fit for the data over the range of 1−4 Å (uncorrected for phase shifts) consists of one N- and two S-donors at distances of 1.89(1) and 2.22(2) Å, respectively. Fits obtained including an N/O-donor had GOF values that were markedly improved over the corresponding fits lacking the N/O-donor (see Supporting Information). Fits calculated for two S-donors represented the best compromise between a low GOF and a high σ^2 value (see Supporting Information) and gave a coordination number in agreement with the XANES analysis. The data also reveal the presence of additional scatterers in the second and third coordination spheres of the Cu centers, indicating the presence of a Cu cluster. These data can be fit with two Cu atoms at a distance of 2.88(1) Å, and with Cu and S atoms at 3.91(1) and 3.54(3) Å, respectively.

The results are consistent with the formation of a Cu(I) cluster containing a Cu_4S_4 ring, where each Cu is bridged by the side chains of two cysteine residues and has terminal N/O ligation. However, such a model indicates that only half of the available cysteine residues are ligated to metal atoms. This prediction is verified by the titration of free thiol groups with DTNB, which confirms the presence of one free thiol per peptide chain.

The proposed Cu(I) cofactor strongly resembles the structure of a recently reported synthetic Cu_4S_4 cluster containing Cu(I) in a trigonal NS_2-donor environment.[15] The Cu−S distances (2.22(2) Å) are similar to the average Cu−S distances in the synthetic cluster (2.21(2) Å) and are significantly longer than those for linear two-coordinate CuS_2 complexes found in transcription factors (~2.15 Å[10]). The Cu−N(O) scatterer observed in Cu−C16C19-GGY may be derived from glutamate residues found in the peptide or from coordination of solvent. The Cu−N(O) distance is shorter than that in the synthetic cluster (2.09(1) Å) but similar to that found in the trigonal model compound, $[Cu(1,2\text{-}Me_2Im)_3]PF_6$ (1.89 Å).[16] The Cu−Cu distances (2.88(1) and 3.91(1) Å) found for Cu−C16C19-GGY are also similar to those observed in the synthetic cluster (avg. 2.70(4) and 3.81(3) Å). The two Cu−Cu distances observed are appropriate for a dihedral angle formed by intersecting Cu_3 planes that is ~134°, which describes a Cu_4 configuration that is distorted 18% from square planar (180°) toward tetrahedral (70°) geometry.

In summary, the results show that the addition of Cu(I) to the random coil C16C19-GGY peptide produces a self-organized 4-helix bundle which is likely driven by the formation of the luminescent Cu_4S_4 cluster.

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Supporting Information Available: CD, UV, AUC, and XAS results. This material available free of charge via the Internet at http://pubs.acs.org.

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