Published in final edited form as:

J Biol Inorg Chem. 2012 February ; 17(2): 285–291. doi:10.1007/s00775-011-0849-9.

Type 1 copper site synthetic model complexes with increased redox potentials

Lei Yang and

Department of Chemistry and Center for Metals in Biocatalysis, University of Minnesota, 207 Pleasant St. SE, Minneapolis, MN 55455, USA

William B. Tolman

Department of Chemistry and Center for Metals in Biocatalysis, University of Minnesota, 207 Pleasant St. SE, Minneapolis, MN 55455, USA

William B. Tolman: wtolman@umn.edu

Abstract

Reactions of NaSCPh_3 with $(\text{R}_3\text{tacn})\text{Cu}(\text{OTf})_2$ (R is Me, iPr; tacn is 1,4,7-triazacyclononane; OTf is CF_3SO_3^-) yield blue complexes identified as $((\text{R}_3\text{tacn})\text{CuSCPh}_3)(\text{OTf})$ on the basis of UV–vis, resonance Raman, and electron paramagnetic resonance (EPR) spectroscopy and electrospray ionization mass spectrometry. These complexes exhibit spectroscopic properties typical of type 1 copper sites in proteins, including diagnostic $\text{S}\pi \rightarrow \text{Cu}(d_{x^2-y^2})$ ligand-to-metal charge transfer transitions at approximately 610–630 nm and small $A_{\parallel}$ values in EPR spectra of less than $100 \times 10^{-4} \text{ cm}^{-1}$. Cyclic voltammetry experiments revealed redox potentials for the complexes similar to those of several low-potential type 1 copper proteins (e.g., azurin, stellacyanin) and approximately 0.5 V higher than those of previously reported model compounds. Thus, the new complexes mimic key aspects of *both* the structure and the function of type 1 copper sites.

Keywords

Type 1 copper; Blue copper; Spectroscopy; Electron transfer; Electrochemistry

Introduction

Type 1 copper sites (e.g., in cupredoxins) play a central role in biological electron transfer [1] and exhibit unusual active site structural and spectroscopic properties [2–5]. As a result, efforts to understand structure/function relationships in such sites have been extensive, and have led to detailed knowledge of type 1 copper active site electronic structures and the factors that underlie their widely differing redox potentials (for a recent, lead reference, see [6]). Studies of synthetic models of the type 1 copper active sites have been informative [7–16], in particular for obtaining insights into the nature of the strong, highly covalent $\text{Cu}(\text{II})$ –thiolate interaction that is responsible for the distinct absorption feature

Correspondence to: William B. Tolman, wtolman@umn.edu.

Electronic supplementary material The online version of this article (doi:10.1007/s00775-011-0849-9) contains supplementary material, which is available to authorized users.

[approximately 600 nm, $S\pi \rightarrow \text{Cu}(d_{x^2-y^2})$ ligand-to-metal charge transfer] and small copper hyperfine coupling ($A_{\parallel} \sim 60 \times 10^{-4} - 70 \times 10^{-4} \text{ cm}^{-1}$) in the respective UV-vis and electron paramagnetic resonance (EPR) spectra of these sites. The few synthetic model complexes that exhibit these key features are shown in Fig. 1 [7, 9, 13, 14]. All contain sterically hindered, monoanionic supporting nitrogen-donor ligands that yield neutral Cu(II)–SR complexes with biologically relevant distorted tetrahedral or trigonal planar (nontetragonal) geometries.

Notwithstanding their structural and spectroscopic similarities to the biosites, **1–3** (see Fig. 1) exhibit Cu(II)/Cu(I) redox potentials that are disappointingly low compared with those of the type 1 proteins, with the exception of **1** (R is C_6F_5), which features a poorly electron donating thiolate donor (Table 1). We hypothesize that the low potentials arise primarily from stabilization of the Cu(II) center by the monoanionic supporting ligands. In addition, the overall neutral charge of the oxidized complexes is favored in the relatively nonpolar organic solvents used in the electrochemistry experiments. Accordingly, by using neutral nitrogen-donor supporting ligands that would yield cationic Cu(II)–SR species, one could potentially obtain model complexes that exhibit increased, more biologically relevant redox potentials. On the other hand, such a strategy would also exacerbate problems often encountered in attempts to synthesize Cu(II)–SR complexes that involve the formation of Cu(I) complexes and RSSR from reactions of Cu(II) precursors with thiolates [17].

Herein we report the successful preparation of thermally unstable cationic LCuSR^+ complexes supported by trialkyl-substituted 1,4,7-triazacyclononane (tacn) species (L is Me_3tacn or iPr_3tacn). These compounds exhibit key spectroscopic characteristics akin to those exhibited by type 1 active sites and **1–3**. In addition, the complexes exhibit Cu(II)/Cu(I) redox potentials approximately 0.5 V higher than those reported for **1–3** (R is CPh_3) and similar to values reported for several type 1 copper centers. These new complexes thus exhibit both essential spectroscopic signatures and accurate functional characteristics of the type 1 copper biosites.

Materials and methods

General considerations

All solvents and reagents were obtained from commercial sources and used as received unless otherwise noted. The solvents dichloromethane, acetonitrile, pentane, and Et_2O were dried over CaH_2 and distilled under a vacuum or passed through solvent purification columns (Glass Contour, Laguna, CA, USA). Tetrahydrofuran (THF) was dried over sodium/benzophenone and distilled under a vacuum, and the d_8 -THF and CD_2Cl_2 used in NMR measurements were degassed by the freeze–pump–thaw technique. NaS-CPh_3 was prepared according to published procedures and recrystallized from Et_2O at 253 K [13]. The copper(II) complex $(\text{iPr}_3\text{tacn})\text{Cu}(\text{OTf})_2$ (OTf is CF_3SO_3^-) was prepared according to published procedures [22–24]. All copper complexes were prepared and stored in a Vacuum Atmospheres glove box under dry nitrogen. NMR spectra were recorded with a Varian VI-300 spectrometer. Chemical shifts (δ) were referenced to residual solvent signal and integrated intensities were compared with the intensity of 1,3,5-trimethoxybenzene added as an internal standard. UV-vis spectra were recorded with a Hewlett-Packard 8453 (190–

1,100 nm) diode-array spectrophotometer. Low-temperature UV–vis experiments were performed using a Unisoku low-temperature UV–vis cell holder. Elemental analyses were performed by Atlantic Microlab (Norcross, GA, USA). Electrospray ionization (ESI) mass spectra were recorded with a Bruker BioTOF II instrument; the blue THF product solutions for the reactions of Cu(II) complexes with NaSCPh₃ (see later) were quickly transferred using a precooled (dry ice) syringe under protection of argon at 193 K and injected into the inlet tube. IR spectra were obtained using a Thermo Nicolet Avatar 370 Fourier transform (FT) IR instrument. Resonance Raman spectra were collected with an Acton 506 spectrometer using a Princeton Instruments LN/CCD-11100-PB/UBAR detector and ST-1385 controller interfaced with the program Winspec. The spectra were obtained at 77 K using a backscattering geometry. Excitation at 568.2 nm was provided by a Spectra-Physics BeamLok 2060-KR-V krypton ion laser. Raman shifts were externally referenced to liquid indene and internally referenced to solvent (THF). X-band EPR spectra were recorded with a Bruker E-500 spectrometer with an Oxford Instruments EPR-10 liquid-helium cryostat (4–20 K, 9.61 GHz). Simulations were performed using Bruker SimFonia (version 1.25). Electro-chemical experiments were performed under argon using a three-chamber cell with platinum working and auxiliary electrodes, an Ag/AgNO₃ (10 mM in CH₃CN) reference electrode, and a BAS Epsilon potentiostat. Experiments were performed using analyte concentration of 1 mM in THF or CH₂Cl₂ with 0.4 M tetrabutylammonium hexafluorophosphate (Bu₄NPF₆) (sample volumes of approximately 5 mL), at 273 K. The ferrocene/ferrocenium (Fc/Fc⁺) couple was recorded for reference.

(Me₃tacn)Cu(OTf)₂

A solution of Me₃tacn (116 mg, 0.676 mmol) in THF (2 mL) was added to a suspension of CuCl₂ (89 mg, 0.66 mmol) in THF (2 mL). A green precipitate formed, which was dissolved by adding acetonitrile (1 mL) to the reaction solution. The green solution was stirred for 1 h, after which a solution of AgOTf (340.6 mg, 1.33 mmol) in CH₃CN (2 mL) was added dropwise, causing the immediate precipitation of AgCl and a change of the color of the solution from green to blue. The mixture was stirred for 3 h, and then filtered through Celite[®]. The solvent was removed from the filtrate under reduced pressure and the resulting blue–green solid was crystallized by diffusing Et₂O into a concentrated CH₂Cl₂ solution at room temperature (328 mg, 91%). UV–vis [λ_{max} , nm (ϵ , M^{−1} cm^{−1}) in CH₂Cl₂]: 291 (4,900), 671 (130). FT-IR (cm^{−1}): 3,269.9, 1,472.6, 1,247.2, 1,224.8, 1,158.3, 1,028.8, 1,008.8, 894.4, 787.1, 744.7. Anal. Calcd for C₁₁H₂₁CuF₆N₃O₆S₂: C, 24.79; H, 3.97; N, 7.88. Found: C, 24.80; H, 4.35; N, 8.35.

((Me₃tacn)Cu(NCCH₃)₂)(SbF₆)₂

A solution of Me₃tacn (69 mg, 0.4 mmol) in CH₃CN (1 mL) was added to a solution of CuCl₂ (52 mg, 0.39 mmol) in CH₃CN (2 mL). The clear deep-green solution was stirred for 80 min, after which a solution of AgSbF₆ (278 mg, 0.81 mmol) in CH₃CN (2 mL) was added dropwise, causing immediate precipitation of AgCl and a color change of the solution from green to bright blue. The mixture was stirred overnight, filtered through Celite[®], and the filtrate evaporated under reduced pressure. The blue residue was washed with Et₂O (3 × 6 mL) and then crystallized by diffusion of Et₂O into a concentrated CH₃CN solution at room temperature (257 mg, 86%). UV–vis [λ_{max} , nm (ϵ , M^{−1} cm^{−1}) in CH₂Cl₂]: 294

(4,400), 622 (230). FT-IR (cm^{-1}): 2,320.5, 2,296.9, 1,480.8, 1,301.1, 1,068.3, 1,056.5, 1,003.5, 835.5, 785.5. Anal. Calcd for $\text{C}_{13}\text{H}_{27}\text{CuF}_{12}\text{N}_5\text{Sb}_2$: C, 19.80; H, 3.45; N, 8.88. Found: C, 19.37; H, 3.60; N, 8.74.

$((\text{iPr}_3\text{tacn})\text{Cu}(\text{NCCH}_3)_2)(\text{SbF}_6)_2$

This compound was prepared similarly to the Me_3tacn analog described in the previous section, except with use of solutions of iPr_3tacn (89 mg, 0.35 mmol) and CuCl_2 (46 mg, 0.34 mmol) in THF (2 mL) with CH_3CN (1 mL) added to dissolve the initially precipitated $(\text{iPr}_3\text{tacn})\text{CuCl}_2$. The product was isolated as blue crystals (267 mg, 92%). UV-vis [λ_{max} , nm (ϵ , $\text{M}^{-1} \text{cm}^{-1}$) in CH_2Cl_2]: 320 (3,100), 669 (170). FT-IR (cm^{-1}): 2,977.4, 2,363.1, 1,495.5, 1,470.2, 1,378.4, 1,169.4, 1,147.3, 1,074.4, 1,052.2, 966.7, 833.8, 779.9. Anal. Calcd for $\text{C}_{19}\text{H}_{39}\text{CuF}_{12}\text{N}_5\text{Sb}_2$: C, 26.15; H, 4.50; N, 8.03. Found: C, 25.86; H, 4.39; N, 7.79.

Reactions of Cu(II) complexes with NaSCPh_3

The following is an illustrative procedure. In an inert atmosphere, solutions of $(\text{Me}_3\text{tacn})\text{Cu}(\text{OTf})_2$ (21 mg, 0.039 mmol) in 1 mL THF and NaSCPh_3 (12.4 mg, 0.039 mmol) in 1 mL THF were loaded into two separate Schlenk tubes, the tubes were sealed with septa, and were cooled to 193 K in a dry ice/acetone bath under positive argon pressure. The NaSCPh_3 solution was then quickly transferred to the stirred $(\text{Me}_3\text{tacn})\text{Cu}(\text{OTf})_2$ solution under argon by a cannula, causing instant formation of a clear, deep-blue solution (Fig. S1). The solution was stirred for 30 min prior to subsequent characterization/experiments (EPR, resonance Raman, UV-vis, cyclic voltammetry, ESI-MS). To identify the product of decomposition, after the solution had been warmed to room temperature, the solvent was removed under a vacuum and the resulting residues were washed with aqueous ammonia (approximately 5 mL), the aqueous layers were extracted with CH_2Cl_2 (3×5 mL), and the extracts were dried with Na_2SO_4 . After filtration and removal of solvent from the filtrate, the residue was analyzed by ^1H NMR spectroscopy using 1,3,5-trimethoxybenzene as an internal standard, which showed the presence of $\text{Ph}_3\text{CSSCPh}_3$ in 84% (R is Me) and 79% (R is iPr) yields, respectively [25].

Cyclic voltammetry

In a typical procedure, solutions of $(\text{R}_3\text{tacn})\text{Cu}(\text{OTf})_2$ (R is Me or iPr) (10 mM) in 0.5 mL THF and NaSCPh_3 (10 mM) in 0.5 mL THF were cooled to 193 K under argon in septum-sealed Schlenk tubes. The NaSCPh_3 solution was transferred into the solution of $(\text{R}_3\text{tacn})\text{Cu}(\text{OTf})_2$ under argon using a cannula. The resulting clear blue solution was stirred for 30 min and then quickly transferred into a three-chamber cell loaded with 0.5 M Bu_4NPF_6 solution in 4 mL THF, which was precooled in an ice bath (273 K) under argon. Measurements were taken under positive argon pressure after the mixture had been stirred for approximately 1 min at 273 K.

Results and discussion

Reaction of $(\text{R}_3\text{tacn})\text{Cu}(\text{OTf})_2$ with NaSCPh_3 in THF at 193 K resulted in the immediate development of a deep blue color (Fig. S1) and new features in UV-vis spectra (R is

iPr₃tacn, Fig. 2; R is Me, Fig. S2). The blue solutions exhibit similar UV–vis absorptions with λ_{max} 609 nm/740 (sh) nm (R is Me) or 630 nm/780 (sh) nm (R is iPr). For the system with R is iPr, these features remained unperturbed at 193 K for several hours, but decayed within 30 min at 273 K. Decreased stability was observed for R is Me; the absorption features decayed within 1 h at 193 K, 30 min at 233 K, and approximately 10 min at 273 K. We surmise that decay involves formation of Cu(I) species and Ph₃CSSCPh₃, of which the latter was identified in 84% (R is Me) and 79% (R is iPr) yields by ¹H NMR spectroscopy on the residues remaining after warming to room temperature.

The absorption wavelengths for the blue product solutions are similar to those reported for type 1 copper sites and complex **1** (R is CPh₃) (Table 1), although their extinctions are low. By analogy to published assignments [4, 5, 11, 15], we attribute the absorptions to $S\pi \rightarrow \text{Cu}(d_{x^2-y^2})$ ligand-to-metal charge transfer transitions and Cu $d-d$ transitions, respectively, and hypothesize that their low extinctions are at least partially due to incomplete formation of the product chromophore resulting from some reduction to Cu(I) species and Ph₃CSSCPh₃. In support of this hypothesis, EPR integration data indicate approximately 75% yield of Cu(II) products (see below). In addition, peaks are present in the diamagnetic region of ¹H NMR spectra (193 K, *d*₈-THF) attributable to (R₃tacn)Cu(I) and Ph₃CSSCPh₃, although we have not been able to unambiguously assign all of the features observed. We conclude that the extinctions reported in Table 1 are likely underestimates; assuming approximately 75% yield, we calculate values for the features at 609 and 630 nm of 2,200 and 2,000 M⁻¹ cm⁻¹, respectively.

To further probe the ligand-to-metal charge transfer assignment for the 609- and 630-nm bands and obtain structural information for the blue species, resonance Raman spectroscopy experiments were conducted. Using $\lambda_{\text{ex}} = 568$ nm on frozen solutions of the blue species (THF, 77 K), we observed peaks at 428 cm⁻¹ (R is iPr) or 426 cm⁻¹ (R is Me) (insets in Figs. 2, S2). On the basis of their similarities to peaks reported for type 1 copper proteins and **1–3**, and with the caveat that we lack data for isotope-substituted samples, these features may be attributed to $\nu(\text{Cu-S})$ vibrational modes.

X-band EPR spectra and simulations of the signals observed for the product solutions (THF, 2 K) are shown in Fig. 3. Integrations of these signals relative to an external Cu(II) standard indicated 75 and 77% yields from the R is Me and R is iPr starting materials, respectively; as mentioned above, this incomplete conversion likely arises from some reduction of Cu(II) by thiolate to yield Ph₃CSSCPh₃ (there is no evidence for the presence of the starting complexes) [26]. The complexes with R is Me and R is iPr both exhibit axial Cu(II) signals, but with significantly different $g_{\parallel}$ and $A_{\parallel}$ values (see the caption to Fig. 3). The small values of both parameters for the species with R is iPr match well with those observed for oxidized type 1 copper sites and Cu(II) models **1–3**, and are key characteristics of three- or four-coordinate Cu(II)–thiolate sites with highly covalent Cu–S bonding [4, 15, 16]. The larger values for the species with R is Me are more consistent with a tetragonal Cu(II) center with reduced Cu–S covalency, like in “red” copper proteins that contain five-coordinate Cu(II)–thiolate centers (cf. $g_z = 2.245$, $A_{\parallel} = 140 \times 10^{-4}$ cm⁻¹ for nitrosocyanin [27, 28]). Additional low intensity at low g values (indicated by asterisks in Fig. 3, not simulated) suggests the presence of a small amount of an additional Cu(II) species. We postulate that for the system

with the less sterically hindered Me₃tacn ligand, binding of the OTf[−] counterion to yield a five-coordinate Cu(II) site occurs upon freezing the solution; binding of solvent to other type 1 model complexes to yield five-coordinate sites has been reported [7, 8]. Consistent with this hypothesis of a reversible structural change upon freezing, we noticed that the color of the solution changed upon freezing in liquid N₂ from blue to green, and that this color change was reversed upon warming back to 193 K (Fig. S1). No such color changes were observed for the iPr₃tacn system, where the greater steric bulk of the ligand substituents apparently inhibits OTf[−] binding.

To test these ideas, we turned to a more poorly coordinating counterion, SbF₆[−], by preparing the new complexes ((R₃tacn)Cu(CH₃CN)₂)(SbF₆)₂ and treating these species with NaSCPh₃ in THF at 193 K. UV–vis spectra of the resulting blue solutions were identical to those observed for the species with OTf[−] counterions, and the EPR spectra for R is iPr₃tacn were closely similar for both OTf[−] and SbF₆[−] counterions (Fig. S3; parameters for the latter are $g_{\parallel} = 2.19$, $g_{\perp} = 2.07$, $A_{\parallel} = 84 \times 10^{-4} \text{ cm}^{-1}$). Importantly, for R is Me, the EPR spectra for the species with OTf[−] and SbF₆[−] counterions are quite different, with the spectrum for the latter having small values of $g_{\parallel} = 2.24$ and $A_{\parallel} = 94 \times 10^{-4} \text{ cm}^{-1}$ (Fig. S3). In addition, for the SbF₆[−] species, the blue color of the solution remains essentially unchanged upon freezing. These results support the notion that coordination of the counterion to yield a five-coordinate Cu(II) center occurs for the R is Me system upon freezing for OTf[−], but for SbF₆[−] such binding does not occur and a geometry is adopted that is similar to that seen for both counterions for R is iPr.

The positive ion ESI mass spectrum of the blue solution for R is iPr contains a major peak envelope with an isotope pattern that matches the formula C₃₄H₄₈CuN₃S, corresponding to (iPr₃tacn)Cu^{II}(SCPh₃)⁺ (Fig. S4). A similar peak envelope and assignment is seen for the case of R is Me at m/z 509, but this is a minor peak in the spectrum. The major peaks occur at higher m/z ~ 1,083 and 917, and are best modeled as [(Me₃tacn)₂(SCPh₃)₂Cu^I]₃⁺ (C₅₆H₇₂Cu₃N₆S₂) and [(Me₃tacn)₂(OTf)₃Cu^{II}]₂⁺ (C₂₁H₄₂Cu₂F₉N₆O₉S₃), respectively (Fig. S5). Clearly, decomposition occurs during the course of the ESI–MS experiment for R is Me, consistent with the aforementioned instability for this system in solution.

Taken together, the UV–vis, resonance Raman, EPR and ESI–MS data support formulation of the blue species for R is iPr as **4** (Fig. 4). The absorption, resonance Raman, and EPR features are closely similar to those of type 1 copper proteins and complex **1**, and support similarly strong, highly covalent Cu(II)–thiolate bonding in a four-coordinate geometry. The situation for R is Me is more complicated. The similarity of the UV–vis data for this system with those for R is iPr supports analogous formulation **5** (Fig. 4), but the EPR data and associated color changes upon freezing of the solutions suggest equilibration between **5** and five-coordinate **6** (Fig. 4) for X is OTf[−]. The R is Me system is also less stable in solution, as indicated by decay of the UV–vis absorption peaks and the higher nuclearity species seen in the ESI mass spectrum.

Taken together, the UV–vis, resonance Raman, EPR and ESI–MS data support formulation of the blue species for R is iPr as **4** (Fig. 4). The absorption, resonance Raman, and EPR features are closely similar to those of type 1 copper proteins and complex **1**, and support

similarly strong, highly covalent Cu(II)–thiolate bonding in a four-coordinate geometry. The situation for R is Me is more complicated. The similarity of the UV–vis data for this system with those for R is iPr supports analogous formulation **5** (Fig. 4), but the EPR data and associated color changes upon freezing of the solutions suggest equilibration between **5** and five-coordinate **6** (Fig. 4) for X is OTf[−]. The R is Me system is also less stable in solution, as indicated by decay of the UV–vis absorption peaks and the higher nuclearity species seen in the ESI mass spectrum.

Cyclic voltammograms were obtained for the blue products at 273 K by generating the solutions at 193 K and then adding them to a precooled (273 K) solution of electrolyte (Bu₄NPF₆) and waiting a short time (1 min) for temperature equilibration before making measurements. Data were acquired in both THF and CH₂Cl₂ in order to best draw comparisons with literature data for complexes **1–3** [7, 13, 14]. For R is iPr (Fig. 5), an electrochemically quasi-reversible ($E_{pc,pa} \sim 90$ mV at 20 mV/s) and chemically reversible ($i_{pa}/i_{pc} \sim 1$) wave was observed with $E_{1/2} = -458$ mV versus Fc/Fc⁺ in CH₂Cl₂ at 273 K (0.4 M Bu₄NPF₆).¹ In THF, $E_{1/2} = -487$ mV versus Fc/Fc⁺, although poorer behavior was observed in this solvent (larger $E_{pc,pa}$, more hysteresis; Fig. S6). For R is Me, irreversibility is indicated by $i_{pa} \ll i_{pc}$, so only approximate $E_{1/2}$ values of -430 mV (CH₂Cl₂) or -500 mV (THF) versus Fc/Fc⁺ can be extracted from the data (Fig. S6). To facilitate comparisons, the $E_{1/2}$ values are presented versus NHE in Table 1. The $E_{1/2}$ values for **4** and **5** are significantly more positive than those reported for **1–3** (R is CPh₃) [7, 13, 14]. Notably, the value for **4** is almost 0.6 V greater than that for **1** (R is CPh₃) in CH₂Cl₂ and almost 0.5 V greater than that for **2** in THF. Moreover, the $E_{1/2}$ values for **4** and **5** are similar to those seen in “low-potential” type 1 copper sites such as azurin and stellacyanin [19, 21]. We surmise that the increased potentials relative to **1–3** (R is CPh₃) are due to an electrostatic effect arising because **4** and **5** contain neutral ligands, just like the biosites, and are thus monocations, rendering reduction more favorable, whereas **1–3** (R is CPh₃) contain monoanionic ligands and are thus neutral, making reduction less favorable.

Conclusions

The reactions of NaSCPh₃ with (R₃tacn)Cu(OTf)₂ yield blue solutions, which for R is iPr contain the four-coordinate Cu(II)–thiolate complex **4** on the basis of UV–vis, resonance Raman, and EPR spectroscopy and ESI-MS. A similar complex **5** was identified for R is Me, but it is less stable and upon freezing of the solutions binds the counterion to form a five-coordinate tetragonal species tentatively formulated as **6** (Fig. 4). The spectroscopic data for **4** and **5** are in good agreement with those for type 1 copper proteins [4, 5] and previously reported models **1–3** [7, 13–16], supporting similarly strong and covalent Cu(II)–thio-late bonding interactions. On the other hand, the Cu(II)/Cu(I) redox potentials for **4** and **5** are approximately 0.5 V higher than for **1–3** (R is CPh₃) [7, 13, 14], and fall within the range exhibited by type 1 copper proteins [3]. Complexes **4** and **5** thus represent unique

¹This feature is not due to the Cu(I)/Cu(II) couple for (iPr₃tacn)-Cu(OTf)₂; independent measurement of the cyclic voltammogram of this complex in CH₂Cl₂ at room temperature (0.4 M Bu₄NPF₆) shows a quasi-reversible wave at $E_{1/2} \sim +0.48$ V vs. Fc/Fc⁺ (previously reported as +0.36 V vs. the saturated calomel electrode in CH₃CN [26]).

model compounds that replicate key aspects of both the structure and the function of type 1 copper proteins [3].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the NIH for financial support of this research (grant GM47365 to W.B.T.), Patrick Donoghue for assistance with EPR measurements, David Boyce for obtaining resonance Raman data, Jacqui Tehranchi for help with the electrochemistry experiments, and John Lipscomb and Lawrence Que Jr for access to EPR and resonance Raman facilities, respectively.

References

1. Dennison C. *Coord Chem Rev.* 2005; 249:3025–3054.
2. Adman ET. *Adv Protein Chem.* 1991; 42:145–197. [PubMed: 1793005]
3. Gray HB, Malmstrom BG, Williams RJP. *J Biol Inorg Chem.* 2000; 5:551–559. [PubMed: 11085645]
4. Solomon EI, Szilagyi RK, DeBeer George S, Basumallick L. *Chem Rev.* 2004; 104:419–458. [PubMed: 14871131]
5. Solomon EI. *Inorg Chem.* 2006; 45:8012–8025. [PubMed: 16999398]
6. Marshall NM, Garner DK, Wilson TD, Gao Y-G, Robinson H, Nilges MJ, Lu Y. *Nature.* 2009; 461:113–116. [PubMed: 19890331]
7. Thompson JS, Marks TJ, Ibers JA. *J Am Chem Soc.* 1979; 101:4180–4192.
8. Kitajima N, Fujisawa K, Moro-oka Y. *J Am Chem Soc.* 1990; 112:3210–3212.
9. Kitajima N, Fujisawa K, Tanaka M, Moro-oka Y. *J Am Chem Soc.* 1992; 114:9232–9233.
10. Qui D, Kilpatrick L, Kitajima N, Spiro TG. *J Am Chem Soc.* 1994; 116:2585–2590.
11. Matsunaga Y, Fujisawa K, Ibi N, Miyashita Y, Okamoto K-i. *Inorg Chem.* 2005; 44:325–335. [PubMed: 15651879]
12. Fujisawa K, Fujita K, Takahashi T, Kitajima N, Moro-oka Y, Matsunaga Y, Miyashita Y, Okamoto K-i. *Inorg Chem Commun.* 2004; 7:119–1188.
13. Holland PL, Tolman WB. *J Am Chem Soc.* 1999; 121:7270–7271.
14. Holland PL, Tolman WB. *J Am Chem Soc.* 2000; 122:6331–6332.
15. Randall DW, George SD, Hedman B, Hodgson KO, Fujisawa K, Solomon EI. *J Am Chem Soc.* 2000; 122:11620–11631.
16. Randall DW, DeBeer S, Holland PL, Hedman B, Hodgson KO, Tolman WB, Solomon EI. *J Am Chem Soc.* 2000; 122:11632–11648.
17. Mandal S, Das G, Singh R, Shukla R, Bharadwaj PK. *Coord Chem Rev.* 1997; 160:191–235.
18. Connelly NG, Geiger WE. *Chem Rev.* 1996; 96:877–910. [PubMed: 11848774]
19. Ainscough EW, Bingham AG, Brodie AM, Ellis WR, Gray HB, Loehr TM, Plowman JE, Norris GE, Baker EN. *Biochemistry.* 1987; 26:71–82. [PubMed: 3030404]
20. Peisach J, Levine WG, Blumberg WE. *J Biol Chem.* 1967; 242:2847–2858. [PubMed: 4290867]
21. Nersissian AM, Immoos C, Hill MG, Hart PJ, Williams G, Herrmann RG, Valentine JS. *Protein Sci.* 1998; 7:1915–1929. [PubMed: 9761472]
22. Bar-Nahum I, York JT, Victor Young GJ, Tolman WB. *Angew Chem Int Ed.* 2008; 47:533–536.
23. Haselhorst G, Stoetzel S, Strassburger A, Walz W, Wieghardt K, Nuber B. *J Chem Soc Dalton Trans.* 1993:83–90.
24. Cahoy J, Holland PL, Tolman WB. *Inorg Chem.* 1999; 38:2161–2168. [PubMed: 11671001]
25. Cochran JC, Friedman SR, Frazier JP. *J Org Chem.* 1996; 61:1533–1536.

26. Halfen JA, Mahapatra S, Wilkinson EC, Gengenbach AJ, Young VG Jr, Que L Jr, Tolman WB. *J Am Chem Soc.* 1996; 118:763–776.
27. Basumallick L, Sarangi R, DeBeer George S, Elmore B, Hooper AB, Hedman B, Hodgson KO, Solomon EI. *J Am Chem Soc.* 2005; 127:3531–3544. [PubMed: 15755175]
28. Siluvai GS, Mayfield M, Nilges MJ, DeBeer George S, Blackburn NJ. *J Am Chem Soc.* 2010; 132:5215–5226. [PubMed: 20232870]

Fig. 1.

Previously reported Cu(II)-SR model complexes of type 1 copper sites [7, 9, 13, 14]

Fig. 2. UV-vis spectra (tetrahydrofuran, THF, 193 K) of 0.5 mM (iPr₃tacn)Cu(OTf)₂ (tacn is 1,4,7-triazacyclononane; OTf is CF₃SO₃⁻) (*blue line*) and the product of reaction of 0.5 mM (iPr₃tacn)Cu(OTf)₂ with 1 equiv NaSCPh₃ (*black line*). The *inset* shows the resonance Raman spectrum of the product solution (10 mM, λ_{ex} 568 nm, 77 K)

Fig. 3.

X-band EPR spectra (*black lines*) and simulations (*red lines*) of frozen THF solutions of the products of the reactions of NaSCPh₃ with (iPr₃tacn)Cu(OTf)₂ (*top*) and (Me₃tacn)Cu(OTf)₂ (*bottom*) (both measured at 2 K). The simulation parameters were as follows: *top* $g_{\parallel} = 2.20$, $g_{\perp} = 2.07$, $A_{\parallel} = 84 \times 10^{-4} \text{ cm}^{-1}$; *bottom* $g_{\parallel} = 2.31$, $g_{\perp} = 2.07$, $A_{\parallel} = 156 \times 10^{-4} \text{ cm}^{-1}$. The *asterisks* indicate signal intensity that is not simulated (see text)

Fig. 4.
Proposed Cu(II)-thiolate complexes

Fig. 5. Cyclic voltammogram of the blue product of the reactions of NaSCPh_3 with $(\text{iPr}_3\text{tacn})\text{Cu}(\text{OTf})_2$ in CH_2Cl_2 (complex **4**). The conditions were as follows: scan rate 20 mV/s, 0.4 M tetrabutylammonium hexafluorophosphate, 273 K

Table 1

Properties of selected type 1 copper active sites and model complexes

Complex or protein	UV-vis [λ_{max} , nm (ϵ , M ⁻¹ cm ⁻¹)]	$\epsilon_{\parallel}$	$A_{\parallel}$ ($\times 10^{-4}$ cm ⁻¹)	$\nu(\text{Cu-S})$ (cm ⁻¹)	Redox potential (mV vs. NHE) ^a
Azurin ^b	619 (5,100) 780 (sh, 1,040)	2.255	60	375–459 ^c	+276
Stellacyanin ^d	604 (4,000) 845 (sh, 700) ^e	NA ^f	NA ^f	–	+260 ^g
1 (R is CPh ₃)	625 (6,340) ^h	2.23	66 ^h	421 ^h	–343 ⁱ
1 (R is C ₆ F ₅) ^j	670 (5,960)	2.32	56	–	+243 ^k
2	749 (5,800) ^l	2.17	111 ^l	430 ^j	–180 ^{l,m}
3	691 (2,300) ⁿ	2.15	98 ⁿ	–	–203 ^{n,o}
((iPr ₃ tacn)CuSCPh ₃)(OTf) (4) ^p	630 (1,500) ^q 780 (sh)	2.20	84	428	+317 ^r , +246 ^s
((Me ₃ tacn)CuSCPh ₃)(X) (5) ^{p,t}	609 (1,600) ^q 740 (sh)	2.24	94	426	+299 ^u , +217 ^v

See Fig. 1 for the structures of **1–3** and Fig. 4 for the structures of **4** and **5**

NHE normal hydrogen electrode, NA not applicable, *tacn* 1,4,7-triazacyclononane, *OTf* CF₃SO₃[–]

^a All conversions between units required to report the data versus NHE were performed using [18] and/or <http://www.consultsr.com/resources>

^b From *Alcaligenes denitrificans* [19]

^c Multiple features observed in the resonance Raman spectrum due to kinematic coupling with a Cys residue

^d From *Rhus vernicifera*

^e From [20]

^f Rhombic electron paramagnetic resonance (EPR) spectrum

^g For cucumber stellacyanin [21]

^h From [15]

ⁱ Reported as –540 mV versus Ag wire at 253 K in CH₂Cl₂ [7]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

^jFrom [11]

^kMeasured in acetone, Bu₄NClO₄, reported at -468 mV versus ferrocene/ferrocenium (Fc/Fc⁺)

^lFrom [13]

^mMeasured in tetrahydrofuran (THF), 0.2 M tetrabutylammonium hexafluorophosphate (Bu₄NPF₆), reported as -980 mV versus Fc/Fc⁺

ⁿFrom [14]

^oMeasured in THF, 0.2 M Bu₄NPF₆, reported as -367 mV versus Ag/AgCl, with Fc/Fc⁺ at 636 mV versus Ag/AgCl, yielding the value shown versus NHE

^pThis work

^qExtinction coefficient based on 100% yield of the product from reaction of Cu(II) precursor with NaSCPh₃. Extinctions based on yields adjusted from EPR integrations are reported in the text

^rMeasured in THF, 0.4 M Bu₄NPF₆, -487 mV versus Fc/Fc⁺ at 273 K

^sMeasured in CH₂Cl₂, 0.4 M Bu₄NPF₆, -458 mV versus Fc/Fc⁺ at 273 K

^tX is OTf for UV-vis spectroscopy, Raman spectroscopy, and cyclic voltammetry; X is SbF₆ for EPR spectroscopy

^uMeasured in THF, 0.4 M Bu₄NPF₆, estimated as -505 mV versus Fc/Fc⁺ at 273 K because the wave is irreversible

^vMeasured in CH₂Cl₂, 0.4 M Bu₄NPF₆, estimated as -429 mV versus Fc/Fc⁺ at 273 K because the wave is irreversible (Fig. S6)