Influence of Copper−Sulfur Covalency and Copper−Copper Bonding on Valence Delocalization and Electron Transfer in the Cu_A Site of Cytochrome *c* Oxidase[||]

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Cytochrome *c* oxidase (C*c*O) participates in cellular respiration by coupling the four-electron reduction $O_2 \rightarrow H_2O$ with transmembrane proton pumping.[1] The Cu_A center of C*c*O is the first destination in electron transfer (ET) from cytochrome *c* to the Cu_B-heme a_3 pair, where O_2 reduction occurs. Several similarities exist between Cu_A and blue-copper sites, including their analogous cupredoxin folds, copper-thiolate ligation, and ET function. In contrast with the monomeric blue-copper sites, however, EPR,[2] EXAFS,[3] and recent X-ray crystal structures[4] have shown that Cu_A is a delocalized mixed-valence Cu dimer bridged by two cysteine sulfurs (S(Cys)) and, in this regard, more closely resembles the mixed-valence iron−sulfur ET clusters. In this communication, we explore the contributions of Cu−ligand and Cu−Cu bonding to valence delocalization and ET in the Cu_A site of C*c*O. Sulfur K-edge X-ray absorption spectroscopy (XAS) provides the first direct experimental probe of copper−sulfur covalency in the half-occupied highest-energy molecular orbital (HOMO) of Cu_A and two structurally-defined dithiolate-bridged models, delocalized mixed-valence **1**[5a] and dicupric **2**.[5b] The Cu coordination environment of each system is shown in Chart 1. Electronic absorption (Abs) and magnetic circular dichroism (MCD) spectroscopies are used to measure the total inter-ion electronic coupling in Cu_A and **1** through identification of the Class III mixed-valence $\psi \rightarrow \psi^*$ transitions.[6] The results from these techniques combine to define the pathways for delocalization in Cu_A and **1** and to describe the individual Cu−S and Cu−Cu contributions to this process. The S K-edge data additionally reveal significant anisotropic Cu−ligand covalency, permitting comparison of competing N- and S-based ET pathways to and from the Cu_A site.

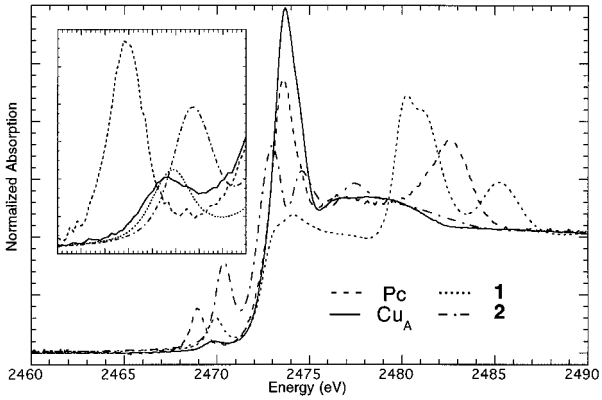
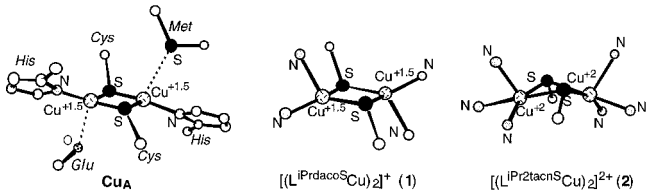


Figure 1. S K-edge XAS spectra of Pc,[8] Cu_A, and models **1** and **2**. Inset: Renormalized pre-edge intensities shown from 2467.5 to 2471.5 eV.

Chart 1



The normalized S K-edge XAS spectra of **1**, **2**, Cu_A, and oxidized plastocyanin (Pc) are shown in Figure 1 with the renormalized pre-edge features displayed in the inset.[7] As previously described,[8] the intensity of a S pre-edge absorption feature is directly related to the %S character, α'^2, in the half-occupied HOMO, $\Psi^* = (1 - \alpha'^2)^{1/2}(\text{Cu 3d}) - \alpha'(\text{S 3p})$. S K-edge and Xα-SW results[8] indicate that Pc has a S(Cys) covalency of 38%. Using the Pc covalency as a reference, the %S covalency is found to be 13% in Cu_A, 14% in **1**, and 25% in **2**. The value obtained for **2** reflects the covalency for each Cu site since both have one d-orbital hole. As there is only one hole shared between the two Cu centers in both Cu_A and **1**, their covalencies obtained above must be doubled to determine the total HOMO S covalencies, giving congruent values of 26 and 28%, respectively.

Abs and MCD spectra of Cu_A sites are dominated by three strong features below 25 000 cm^{-1}.[2b,11,14] The lowest energy

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(1) (a) Babcock, G. T.; Wikström, M. *Nature* **1992**, *356*, 301−307. (b) Ramirez, B. E.; Malmström, B. G.; Winkler, J. R.; Gray, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 11949−11951.

(2) (a) Kroneck, P. M. H.; Antholine, W. E.; Riester, J.; Zumft, W. G. *FEBS Lett.* **1988**, *242*, 70−74. (b) von Wachenfeldt, C.; deVries, S.; van der Oost, J. *FEBS Lett.* **1994**, *340*, 109−113.

(3) Blackburn, N. J.; Barr, M. E.; Woodruff, W. H.; van der Oost, J.; de Vries, S. *Biochemistry* **1994**, *33*, 10401−10407.

(4) (a) Iwata, S.; Ostermeier, C.; Ludwig, B.; Michel, H. *Nature* **1995**, *376*, 660−669. (b) Tsukihara, T.; Aoyama, H.; Yamashita, E.; Tomizake, T.; Yamaguchi, H.; Shinzawa-Itoh, K.; Nakashima, R.; Yaono, R.; Yoshikawa, S. *Science* **1995**, *269*, 1069−1074. (c) *Ibid.* **1996**, *272*, 1136−1144.

(5) (a) Houser, R. P.; Young, V. G., Jr.; Tolman, W. B. *J. Am. Chem. Soc.* **1996**, *118*, 2101−2102. (b) Houser, R. P.; Halfen, J. A.; Young, V. G., Jr.; Blackburn, N. J.; Tolman, W. B. *J. Am. Chem. Soc.* **1995**, *117*, 10745−10746.

(6) Gamelin, D. R.; Bominaar, E. L.; Mathonière, C.; Kirk, M. L.; Wieghardt, K.; Girerd, J.-J.; Solomon, E. I. *Inorg. Chem.* **1996**, *35*, 4323−4335.

(7) S K-edge XAS spectra of the *B. subtilis* C*c*O Cu_A fragment,[2b] **1**, and **2** were obtained at Stanford Synchrotron Radiation Laboratory (SSRL) as described previously.[8] The monochromator was fully tuned, and the data were collected at 4 °C for Cu_A and 25 °C for **1** and **2**. The pre-edge intensities have been rescaled by factors of 3 for Pc, 4 for Cu_A, 1.5 for **1**, and 1 for **2** based on the ratio of total sulfurs present versus the number that contribute to pre-edge intensity. Note that, as with Pc, the long axial S(Met) ligand in Cu_A has negligible overlap with the equatorial HOMO and therefore does not contribute to pre-edge intensity. The intensities of normalized pre-edge features were quantitated by fits to the data using EDG_FIT, written by Dr. Graham N. George of SSRL. On the basis of Pc, the error associated with the covalencies is ∼3%.[8] 4.2 K Abs and MCD spectra of Cu_A (50% glycerol-d_3 by volume) and **1** (mull) were collected as previously described.[9] Xα-SW calculations were performed using the published crystallographic coordinates of **1**[5a] and atomic sphere sizes adapted from experimentally-calibrated Pc calculations.[10]

(8) Shadle, S. E.; Penner-Hahn, J. E.; Schugar, H. J.; Hedman, B.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1993**, *115*, 767−776.

(9) Gamelin, D. R.; Bominaar, E. L.; Kirk, M. L.; Wieghardt, K.; Solomon, E. I. *J. Am. Chem. Soc.* **1996**, *118*, 8085−8097.

(10) Gewirth, A. A.; Solomon, E. I. *J. Am. Chem. Soc.* **1988**, *110*, 3811−3819.

(11) Farrar, J. A.; Lappalainen, P.; Zumft, W. G.; Saraste, M.; Thompson, A. J. *Eur. J. Biochem.* **1995**, *232*, 294−303.

(12) Wallace-Williams, S. E.; James, C. A.; de Vries, S.; Saraste, M.; Lappalainen, P.; van der Oost, J.; Fabian, M.; Palmer, G.; Woodruff, W. H. *J. Am. Chem. Soc.* **1996**, *118*, 3986−3987.

(13) (a) Hush, N. S. *Prog. Inorg. Chem.* **1967**, *8*, 391−444. (b) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265−322. (c) Newton, M. D. *Chem. Rev.* **1991**, *91*, 767−792.

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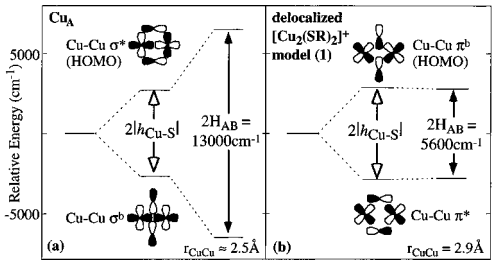

Figure 2. D_{2h}-idealized MO splittings in (a) Cu_A and (b) **1**, showing the separate contributions of Cu−S (h_{Cu-S}) and Cu−Cu (h_{Cu-Cu}) bonding interactions to $2H_{AB}$.[21] σ and π labels refer to the symmetries of Cu−Cu interactions only. The specific energies shown result from $|h_{Cu-S}|$ α $(\alpha'(S))^2$ and $|[h_{Cu-Cu}(\pi, 2.9\ Å)]/[h_{Cu-Cu}(\sigma, 2.5\ Å)]| = 0.01$. The nonzero value for h_{Cu-Cu} (π, 2.9 Å) is included in b to illustrate that this interaction opposes the effect of superexchange in this splitting.

of these, occurring at 13 000 cm^{-1} in *Bacillus subtilis* C*c*O,[7] is assigned as the Class III mixed-valence $\psi \rightarrow \psi^*$ transition[6,9] on the basis of the large change in Cu···Cu separation observed to accompany this excitation in near-IR resonance Raman experiments.[6,12] This transition involves electron promotion between related symmetric and antisymmetric MOs of the delocalized dimer, which leads to resonance enhancement of totally-symmetric Raman vibrations that reflect these bonding changes.[6] In the Class III delocalization limit, this transition energy relates directly to the ground-state electronic-coupling matrix element, H_{AB}, which is responsible for valence delocalization: $E_{\psi\rightarrow\psi^*} = 2H_{AB}$.[13] $2H_{AB}$ in Cu_A is therefore experimentally determined to be 13 000 cm^{-1}. A similar isolated low-energy feature is observed at 5600 cm^{-1} in Abs and MCD spectra of **1**[14] and is assigned as the $\psi \rightarrow \psi^*$ transition in this dimer. This assignment is supported by Xα-SW calculations,[5a,7,14] which indicate ∼23% HOMO S covalency and predict this transition to occur at 4800 cm^{-1}, with no other dipole-allowed electronic transitions in this region.

From the Abs, MCD, and S K-edge results, MO splitting diagrams are constructed for Cu_A (Figure 2a) and **1** (Figure 2b) that describe the independent contributions, $|h|$, of Cu−S and Cu−Cu bonding interactions to inter-ion electronic coupling. Xα-SW calculations on **1** predict a HOMO involving an in-plane π-bonding combination of Cu monomeric orbitals (π^b with respect to the metals), the π-antibonding combination (π^*) being filled and at deeper energy (Figure 2b). Significantly, the greater energy of the π^b combination than its π^* counterpart indicates that this splitting in **1** is dominated by Cu−S interactions (since dominant Cu−Cu interactions would result in $\pi^* > \pi^b$) and demonstrates that valence delocalization in **1** is mediated by the bridging ligation rather than by direct Cu−Cu overlap. On the basis of the large Cu−Cu separation in **1** ($r_{Cu-Cu} = 2.9$Å) we assume that only ligand contributions to H_{AB} are significant in this dimer, such that $2|h_{Cu-S}| \approx 2H_{AB} = 5600\ cm^{-1}$ (Figure 2b). The similar Cu−S HOMO covalencies measured for **1** (28% S) and Cu_A (26% S) (Figure 1) further suggest that the increase of $2H_{AB}$ in Cu_A to 13 000 cm^{-1} is not due to a large increase in $|h_{Cu-S}|$ but is instead due to a direct Cu−Cu bonding interaction ($|h_{Cu-Cu}|$) resulting from the significantly-shorter Cu−Cu separation in Cu_A ($r_{Cu-Cu} \approx 2.5$Å). These observations experimentally exclude a $\pi^b-\pi^*$ delocalization pathway in Cu_A analogous to that in **1** (since increasing $2|h_{Cu-Cu}|$ in a $\pi^b-\pi^*$ pathway (Figure 2b) would decrease the experimental $2H_{AB}$) and implicate instead a $\sigma^b-\sigma^*$ pathway, for which h_{Cu-S} and h_{Cu-Cu} contribute with the same sign to H_{AB} (Figure 2a). On the basis of the similar Cu−S covalencies in Cu_A and **1**, the value of $2H_{AB}$ observed in **1** provides an estimate of the superexchange contribution to $2H_{AB}$ in Cu_A ($2|h_{Cu-S}|$ in Figure 2a), while the remaining ∼7400 cm^{-1} is then attributable to direct Cu−Cu $\sigma^b-\sigma^*$ splitting of these orbitals.[15] Therefore, in contrast with delocalization in **1**, which is mediated exclusively by the sulfur bridges (Figure 2b), the pathway for delocalization in Cu_A is seen to contain comparable contributions from both Cu−Cu and Cu−S interactions. These results provide a basis for understanding the origin of valence delocalization in Cu_A and **1**: The delocalization observed in **1** indicates that the value of $2H_{AB} = 5600\ cm^{-1}$ is large relative to the vibronic trapping energetics of this dimer. Comparing the delocalization pathway of **1**, which contains significant S covalency but little or no direct Cu−Cu interaction, to that in the structurally-similar valence-trapped $S = {}^1/_2$ $[Fe_2(\mu\text{-}S)_2]^+$ ferredoxins ($2H_{AB} \approx 2000\ cm^{-1}$), which involves predominantly a direct Fe−Fe interaction and little or no Fe−S covalency,[9,16] shows that electronic coupling in **1** is indeed large and emphasizes that bridging superexchange contribution can be an efficient mediator of valence delocalization. Direct 2.5 Å Cu−Cu bonding interactions in Cu_A more than double the magnitude of $2H_{AB}$ relative to the already large value found in **1**, thus stabilizing the delocalization of Cu_A despite its lower-symmetry protein environment.

While no specific pathway for Cyt $c \rightarrow Cu_A$ ET has been identified, Cu_A to heme *a* ET has been proposed to involve Cu−N(His) ligation[1b,4c] since this accesses the shortest pathway. Previous studies have shown that the rate of ET, k_{ET}, between a donor and an acceptor is proportional to $(\alpha'^2)^2$.[13b,c,17] From this study, $\alpha'^2(Cys) \gg \alpha'^2(His)$ (13%/S(Cys) (Figure 1) *vs* ∼1−3%/N(His) from ENDOR[18]), from which $k_{ET}(S(Cys))/k_{ET}(N(His)) \approx 19-169$. From this ratio and estimation of an ET decay parameter of ∼0.6 per covalent bond,[19] a S(Cys) ET pathway could contain ∼5−10 covalent bonds more than a competing N(His) pathway and still achieve a comparable k_{ET}. Thus, the relatively high S(Cys) covalency in the Cu_A HOMO could contribute significantly to both Cyt $c \rightarrow Cu_A$ and $Cu_A \rightarrow$ heme *a* ET processes in C*c*O, analogous to the effects of anisotropic covalency observed in Pc ET kinetics.[17] It is interesting to note that Cu_A ET is faster than that of the blue copper center in the multicopper oxidases[20] despite Cu_A having reduced superexchange ligand covalencies and less efficient donor-to-acceptor ET pathways; this observation supports the possibility that the delocalized mixed-valence binuclear structure of Cu_A contributes significantly to its ET function.

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(14) Gamelin, D. R.; LaCroix, L. B.; Houser, R. P.; Tolman, W. B.; Mulder, T. C.; deVries, S.; Solomon, E. I. To be published.

(15) When compared to other first-row transition-metal dimers having unsupported M−M bonds, for which $\sigma-\sigma^*$ transition energies are typically >25 000 cm^{-1}, the ∼7400 cm^{-1} Cu−Cu $\sigma-\sigma^*$ bonding interaction in Cu_A is seen to be relatively weak. See, for example: Abrahamson, H. B.; Frazier, C. C.; Ginley, D. S.; Gray, H. B.; Lilienthal, J.; Tyler, D. R.; Wrighton, M. S. *Inorg. Chem.* **1977**, *16*, 1554−1556.

(16) Noodleman, L.; Baerends, E. J. *J. Am. Chem. Soc.* **1984**, *106*, 2316−2327.

(17) Lowery, M. D.; Guckert, J. A.; Gebhard, M. S.; Solomon, E. I. *J. Am. Chem. Soc.* **1993**, *115*, 3012−3013.

(18) Gurbiel, R. J.; Fann, Y.-C.; Surerus, K. K.; Werst, M. M.; Musser, S. M.; Doan, P. E.; Chan, S. I.; Fee, J. A.; Hoffman, B. M. *J. Am. Chem. Soc.* **1993**, *115*, 10888−10894.

(19) (a) Beratan, D. N.; Onuchic, J. N.; Betts, J. N.; Bowler, B. E.; Gray, H. B. *J. Am. Chem. Soc.* **1990**, *112*, 7915−7921. (b) Beratan, D. N.; Betts, J. N.; Onuchic, J. N. *Science* **1991**, *252*, 1285−1288.

(20) Solomon, E. I.; Sundaram, U. M.; Machonkin, T. E. *Chem Rev.*, **1996**, *96*, 2563−2605.

(21) The $[Cu_2(\mu\text{-}SR)_2]^+$ cores are depicted in the D_{2h} high-symmetry limit for clarity, although Xα-SW calculations indicate a significant dependence of the MO description on the S−C orientation.[14] Reduction from D_{2h} symmetry relaxes the rigorous $\sigma^b-\sigma^*$ and $\pi^b-\pi^*$ MO descriptions depicted and, in particular, allows for mixing of Cu−S σ, pseudo σ, and π bonding interactions.