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Electronic Structure Description of the μ_4-Sulfide Bridged Tetranuclear Cu_Z Center in N_2O Reductase

Peng Chen,[†] Serena DeBeer George,[†] Inês Cabrito,[‡] William E. Antholine,[§] José J. G. Moura,[‡] Isabel Moura,*[,‡] Britt Hedman,[†,||] Keith O. Hodgson,[†,||] and Edward I. Solomon*[,†]

Department of Chemistry, Stanford University, Stanford, California 94305, Departamento de Química, CQFB, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2825-114 Caparica, Portugal, Biophysics Research Institute, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, Stanford Synchrotron Radiation Laboratory, SLAC, Stanford University, Stanford, California 94309

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Nitrous oxide reductase (N_2OR) catalyzes the two-electron reduction of the green house gas N_2O (produced by denitrification and other processes) to N_2 and H_2O in the terminal step of anaerobic metabolism and is involved in ATP synthesis.[1] The crystal structures of N_2OR from *Pseudomonas nautica* (Pn) and *Paracoccus denitrificans* (Pd) were recently solved.[2,3] The dimeric protein contains a Cu_A center and a Cu_Z center in each subunit. The Cu_Z center has a μ_4-sulfide bridged tetranuclear Cu cluster with seven His ligands and a water-derived ligand (the water position is uncertain in the Pn crystal structure, vide infra), and shows unusual electronic[4,5] and vibrational[6] spectral features. The purpose of this study is to use a combination of saturation magnetic circular dichroism (MCD), Cu K-edge X-ray absorption spectroscopy (XAS), multifrequency EPR, and density functional theory (DFT) calculations to determine the spin state, oxidation states, electron spin distribution, and the ground-state wave function of the Cu_Z center in the dithionite reduced form of N_2OR from Pn where the Cu_A center is completely reduced,[7] and to correlate the ground-state wave function to reactivity.

Variable-temperature, variable-field saturation (VTVH) MCD was used to determine the spin state of the Cu_Z center, where the MCD intensity of different possible spin states will show different saturation behavior.[8] Figure 1 inset shows the 5 K 7 T MCD spectrum of Cu_Z, similar to that reported in the literature.[5] The saturation data of the Cu_Z MCD intensity at 620 nm at various temperatures are plotted along with simulated saturation curves for $S = {}^1/_2$, 1, and ${}^3/_2$ systems assuming isotropic polarization and g values (Figure 1). The data clearly indicate that the Cu_Z center has an $S_{Total} = {}^1/_2$ ground state.[9,10]

With four Cu atoms having an $S_{Total} = {}^1/_2$, the Cu_Z center oxidation state can be one Cu^{II} and three Cu^{I}, or three Cu^{II} and one Cu^{I}. Cu K-edge XAS was used to distinguish between these possibilities, where Cu^{I} complexes have a characteristic feature at ~8984 eV while Cu^{II} complexes have no peak maximum below 8985 eV (except for a weak Cu 1s → 3d transition at 8979 eV).[11] The Cu K-edge spectrum of the Cu_Z center is shown in Figure 2.[12] The data were simulated with use of Cu^{II} to Cu^{I} ratios of 3:1 and 1:3 (Figure 2).[13] The simulations clearly show that the $1Cu^{II}/3Cu^{I}$ model is far better than the $3Cu^{II}/1Cu^{I}$ alternative, thus indicating that there is only one oxidized Cu in the Cu_Z center and that the Cu oxidation states are $1Cu^{II}/3Cu^{I}$.[14]

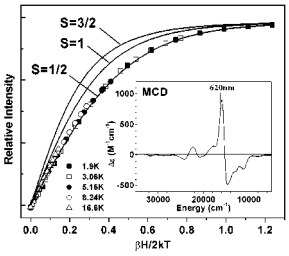

Figure 1. Saturation MCD (points) of Cu_Z along with simulated saturation curves (solid lines). Inset: 5 K 7 T MCD spectrum of Cu_Z.

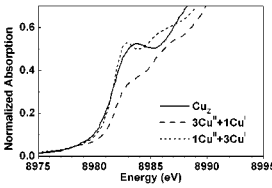

Figure 2. Cu K-edge XAS spectrum of Cu_Z and simulated spectra assuming $3Cu^{II} + 1Cu^{I}$ and $1Cu^{II} + 3Cu^{I}$.

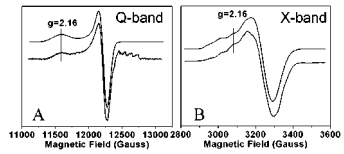

Figure 3. Experimental (lower) and simulated (upper) EPR spectra of Cu_Z: (A) Q-band, 35.001 GHz, 15 K; (B) X-band, 9.319 GHz, 77 K.

EPR spectroscopy was used to determine the unpaired electron spin distribution (i.e. of one Cu^{II}) of this Cu_Z center. The X-band EPR spectrum of Cu_Z has been reported[4] and shows metal hyperfine structure in the $g_{||}$ region (Figure 3B, lower). The analysis of the hyperfine coupling is not clear due to the broad signal. Consequently the system g values could not be accurately determined. Therefore Q-band EPR was used to map the system g values. The EPR spectrum of Cu_Z at Q-band (Figure 3A, lower) is very close to that of an axial system with $g_{||} \approx 2.16 > g_{\perp} \approx 2.04 > 2.0$, indicating that the metal 3d electron hole resides in a Cu $d_{x^2-y^2}$ orbital.[15,16] Given these g values, simultaneous fitting of the EPR spectra at Q- and X-band requires at least a second Cu to account for the metal hyperfine pattern observed at X-band (Figure 3A,B, upper), with one Cu center dominating the hyperfine splitting ($A_{||} = 61 \times 10^{-4}$ cm^{-1}) and a second Cu contributing another ~30% of the spin ($A_{||} = 24 \times 10^{-4}$ cm^{-1}).[17,18] Thus the single electron spin of the Cu_Z center partially delocalizes over at least two Cu centers with a ~5:2 spin distribution. The ground state is a class II partially

* To whom correspondence should be addressed.
[†] Department of Chemistry, Stanford University.
[‡] Universidade Nova de Lisboa.
[§] Wisconsin Medical College.
[||] Stanford Synchrotron Radiation Laboratory.

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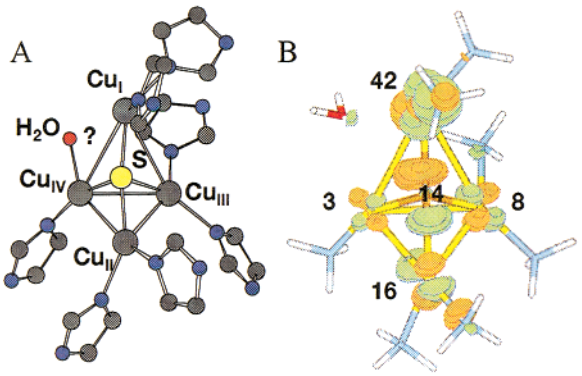

Figure 4. (A) Crystal structure of the Cu_Z center of N_2OR from Pn. (B) DFT calculated lowest unoccupied spin-down molecular orbital. Spin distributions (%) are labeled on the Cu and S atoms. Contours are drawn at ±0.05, 0.10, 0.15, 0.20 [e/bohr^3]$^{1/2}$.

delocalized mixed-valent system[19] with the single 3d electron hole dominantly in a $d_{x^2-y^2}$ orbital on one Cu center.

The crystal structure (Figure 4A),[20,21] spin state ($S = {}^1/_2$), and Cu oxidation states ($1Cu^{II}/3Cu^{I}$) were input into a spin-unrestricted DFT calculation to obtain a description of the ground-state wave function of the Cu_Z center.[22] The histidine ligands were modeled as NH_3. Hybrid DFT calculations have been widely used in the study of Cu sites in proteins. The calculations are found to be very sensitive to the amount of Hartree−Fock exchange included in the hybrid functional. A spectroscopically calibrated hybrid functional is used here (38% Hartree−Fock exchange added to the BP86 functional).[23] Figure 4B gives the lowest unoccupied spin down molecular orbital of the Cu_Z center, which is equivalent to the singly occupied molecular orbital in a spin-restricted formalism and reflects the ground state wave function and its spin distribution. The calculated wave function is partially delocalized and the Cu_I is the dominantly oxidized copper center (42%) while Cu_{II} is the second Cu, which has significant spin density (16%). The spin distribution has a ~5:2 ratio on these two Cu's, consistent with the Q-/X-band EPR results. There is also some spin delocalization over Cu_{III} (8%) and Cu_{IV} (3%). The μ_4-bridging sulfide also has significant contribution to the ground-state wave function (14%) and forms a dominant σ-bonding interaction (Cu_I-S-Cu_{II} angle ≈ 165°) with the $d_{x^2-y^2}$ orbitals of Cu_I and Cu_{II},[24] which constitutes an excellent σ superexchange pathway between Cu_I and Cu_{II} for electron delocalization.

In summary, the μ_4-sulfide bridged tetranuclear Cu_Z center is an $S_{Total} = {}^1/_2$ system with one oxidized Cu and the spin mainly residing on Cu_I with significant contribution from Cu_{II} and the approximately linear bridging sulfide.[25] This ground-state wave function of Cu_Z provides initial possible insight into the enzymatic reactivity of N_2O reduction. The substrate binding edge[2] (involving both Cu_I and Cu_{IV}) of Cu_Z has one dominantly oxidized Cu_I and one dominantly reduced Cu_{IV} center. This should favor the N_2O interaction (possibly bridging) and oxo transfer, while providing the capability for overcoming the reaction barrier[26] by simultaneous two-electron reduction of N_2O. One electron can be donated directly from Cu_{IV} and the other from Cu_{II} through the $Cu_{II}-S-Cu_I$ σ superexchange pathway. Additionally, the Cu_{IV} and Cu_{II} centers have good electron-transfer pathways from the two bridging thiolates of the neighboring Cu_A center in the dimeric protein, allowing rapid rereduction in turnover.[27,28]

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Supporting Information Available: Coordinates for Cu_Z model calculation and additional simulation of Cu_Z K-edge (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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