Active-Site Electronic Structure Contributions to Electron-Transfer Pathways in Rubredoxin and Plastocyanin: Direct versus Superexchange

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Plastocyanin (Pc), a blue copper protein,[1,2] and rubredoxin (Rd), a single iron-sulfur protein,[3] are important biological electron-transfer (ET) agents. In this study, we have used self-consistent-field Xα scattered-wave (SCF-Xα-SW) molecular orbital calculations, calibrated to charge-transfer (CT) absorption data, to determine the electronic contributions of the Pc and Rd active sites to ET pathways. Though much attention has been given to the long-range mediation of protein ET, this work focuses on the potentially important role of active-site metal-ligand covalency in the determination of effective ET pathways. In both proteins the redox active orbital is dominated by metal-cysteine π-bonding interactions. The CT spectra of Pc and Rd, however, reveal a striking difference in the extent of π-bonding covalency. In the Pc active site, the HOMO is dominated by an exceptionally strong covalent Cu-S(Cys)pπ bond which activates the cysteine (Cys) residue for efficient long-range ET. In the Rd active site, Fe-S(Cys)pπ bonding in the HOMO is found to be extremely weak so that the rapid electron-self-exchange (ese) rate[4] (~10^9 M^{-1} s^{-1}) is not consistent with an extended superexchange pathway. Therefore, a direct ET mechanism involving overlap with the FeS_4 active site is predicted.

Electronic structure calculations were performed using the QCPE release[5] of the SCF-Xα-SW code.[6] The Pc site was modeled in C_s symmetry by $[Cu(SCH_3)(C_3H_4N_2)_2\{S(CH_3)_2\}]^+$, and the Rd active site was modeled in D_{2d} symmetry by $[Fe(SCH_3)_4]^{1-}$. Sphere radii[7,8] were optimized by reproducing the experimental CT spectra.

In Pc, the trigonally distorted tetrahedral blue copper active site is buried ~6 Å from the protein surface and consists of a copper atom strongly coordinated to three nearly coplanar atoms ($N_{\delta 1}$ of histidines (His) 37 and 87 and S_γ of Cys 84) and has an additional weak bond to S_δ of methionine (Met) 92.[9] Two ET pathways have been reported for Pc.[9] The shorter "adjacent" path (~6 Å) proceeds from the Cu atom through the surface-exposed His 87 residue and is involved in ese reactions and ET with anionic redox partners. The longer "remote" path (~13 Å) involves the Cys 84 and tyrosine (Tyr) 83 residues. Tyr 83 lies on the protein surface in a negatively charged region and favors ET reactions with cationic redox partners. A similar (Cys-His) ET pathway is present in the multicopper oxidases and in nitrite reductase.[1]

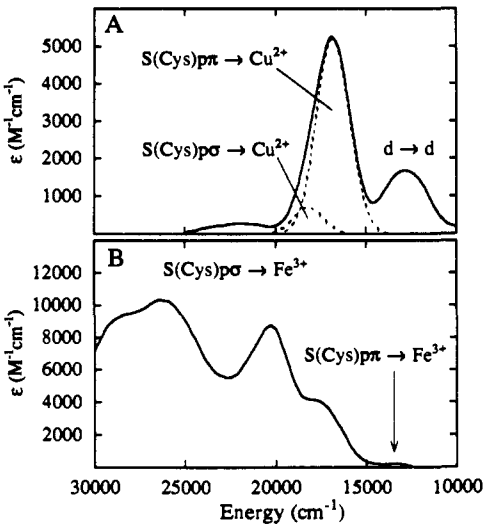

Figure 1. Electronic absorption spectra of (A) plastocyanin[7] and (B) rubredoxin.[3]

Both pathways in Pc exhibit comparable ET rates of 10^4-10^5 M^{-1} s^{-1}.[10] However, for tunneling through a structureless one-dimensional barrier, the rate of ET (k_{ET}) is expected to decay exponentially with the distance (r_{DA}) between the donor (D) and acceptor (A) sites and can be expressed as:[11-13]

$$k_{ET} = A^2 \exp(-\beta r_{DA})(FC) = (2\pi\hbar)|T_{DA}|^2(FC) \qquad (1)$$

where T_{DA} is the electronic tunneling matrix element connecting the donor and acceptor states and (FC) is the Franck-Condon factor. Using eq 1 with r_{DA} = 6 Å for the adjacent path and 13 Å for the remote path,[9] k_{ET} is calculated to be ~1500× more rapid through the adjacent path. Beratan has developed a pathway model which estimates T_{DA} as a function of the number of bonds and contacts connecting the donor and acceptor sites.[11] For Pc, considering only covalent linkages between Cu-His(87) (4 bonds) and Cu-Cys(84)-Tyr(83) (12 bonds), the pathway model predicts that the adjacent path is ~3000× more rapid for ET. The observed ET rates, however, do not show this great disparity. The apparent near equality of k_{ET} for the two ET paths can be related to the anisotropic covalency of the HOMO as outlined below.

In the electronic absorption spectrum of Pc, Figure 1A, the dominant CT band at 598 nm (16 700 cm^{-1}) derives from a S(Cys)pπ → Cu^{2+} transition.[7] Normally, σ CT transitions are most intense, as is observed in Rd and other metal-thiolate systems.[14] The reversed CT intensity pattern results from the strong Cu-S(Cys) bond (2.13 Å[9]) which orients the half-occupied Cu $d_{x^2-y^2}$ orbital so that it is bisected by the Cu-S(Cys) bond and significantly overlaps the S(Cys)pπ orbital. Using SCF-Xα-SW calculations expanded to include imidizole ligation, the HOMO wave function (Ψ_{HOMO}) character is found to be 42% Cu $d_{x^2-y^2}$, 46% S(Cys)pπ, 2.3% N(His) (1.2% each), and ~0.0% S(Met), in excellent agreement with experimental results from Cu L-edge[15] and S K-edge[16] X-ray absorption spectroscopies. The dominant S(Cys)pπ character (γ_L^2 = 0.46, where γ_L is the ligand coefficient in Ψ_{HOMO}) activates the Cys ligand for ET as

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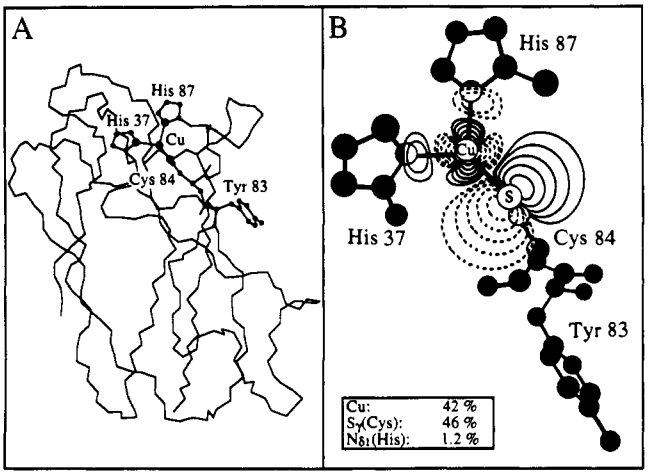

Figure 2. Ψ_{HOMO} wave function contour plot superimposed on the Pc structure. (A) Polypeptide backbone for Pc using the 1pcy coordinates[9] deposited in the Brookhaven protein data base.[25] Side groups of His 37, His 87, Cys 84, and Tyr 83 are included. (B) Expanded view of the proposed superexchange paths with Ψ_{HOMO} superimposed onto the crystal structure (viewed rotated 180° around the Cu–S (Cys) bond from A). Contour values are at ±0.16, ±0.08, ±0.04, ±0.02, and ±0.01 $(e/\mu_B^3)^{1/2}$, with negative values dashed.

shown in Figure 2, where the wave function contour for Ψ_{HOMO} has been superimposed onto the Pc structure. Newton[12,17] has shown that T_{DA} is proportional to γ_L^2, where γ_L is the ligand covalency in the redox active wave function. The ratio of the Cys/His ligand coefficients in Ψ_{HOMO} indicates that the anisotropic covalency enhances k_{ET} through the remote path by a factor of ~1500 over the adjacent path, making this a very effective ET route.[18] Thus, the unusual electronic structure of the blue copper active site makes a significant contribution to the ET reactivity of this protein.

The Rd active site[19] is situated at the surface of the protein and consists of an iron atom tetrahedrally coordinated by the S_γ atoms of four Cys residues, resulting in an effective active-site symmetry of D_{2d}. The extremely fast ese rate for Rd, estimated[4] to be ~10^9 M^{-1} s^{-1}, indicates that ET is nearly adiabatic and likely diffusion controlled; however, the path of ET is not known. From Mössbauer spectroscopy,[20] the π symmetry Fe d_{z^2} orbital is known to be the HOMO in reduced Rd. In strict D_{2d} symmetry, no S(Cys)pπ mixing is allowed in the d_{z^2} orbital; however, distortion from ideal D_{2d} geometry in the protein gives rise to some π mixing as evidenced in the CT spectrum of Rd (Figure 1B). In contrast to the very intense S(Cys)p$\pi \rightarrow Cu^{2+}$ transitions observed for Pc, the S(Cys)p$\pi \rightarrow$ Feπ transitions in Rd are very weak, nearly 2 orders of magnitude weaker than the S(Cys)p$\sigma \rightarrow$ Feσ CT bands. The weak S(Cys)p$\pi \rightarrow$ Feπ transitions reflect a limited amount of Fe–S(Cys) covalency in the HOMO, which will profoundly affect the ET kinetics in this protein.

The extent of S(Cys)pπ covalency in the HOMO can be estimated from the magnetic circular dichroism (MCD) and electronic absorption spectra[21] of $[Et_4N][Fe(SR)_4]$ (R = 2,3,5,6-$(CH_3)_4C_6H$), an iron tetrathiolate model complex that exhibits band energies and intensities similar to those of Rd. From MCD data of the model complex, the spin–orbit coupling constant for the degenerate σ Fe^{3+} d_{xz} and d_{yz} orbitals is 65% of the free ion value, indicating 35% ligand character in dσ. The intensity of the Sp$\pi \rightarrow$ Fedπ CT transitions is only 3% that of the Sp$\sigma \rightarrow$ Fedσ transitions. As the intensity of a CT absorption band is directly proportional to the extent of ligand mixing in the accepting metal orbital,[22] the total Spπ character in the Fedπ orbitals is ~1%. Moreover, this value represents the maximum amount of Spπ mixing in the HOMO as the Sp$\pi \rightarrow$ Fedπ CT band involves transitions to the $d_{x^2-y^2}$ Feπ orbital as well as the redox-active d_{z^2} orbital. The lack of significant metal–Cys π-bonding in Rd relative to Pc is attributed to the longer Fe–S length (2.30 Å[19]) and contracted metal orbitals.

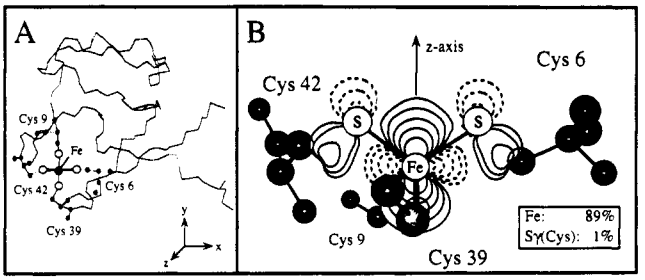

Figure 3. Ψ_{HOMO} wave function contour plot superimposed on the Rd structure. (A) Polypeptide backbone for Rd using the 4rxn coordinates[19] deposited in the Brookhaven protein data base.[25] Side groups of Cys 6, 9, 39, and 42 are included. (B) Expanded view of the proposed ET path (rotated 90° from A) with Ψ_{HOMO} superimposed onto the crystal structure (only S_γ and C_β of Cys 39 are shown for clarity). Contour values are the same as those in Figure 2.

Employing the relationship between γ_L and T_{DA} and Newton's[17] parameters for $Fe(H_2O)_6^{2+/3+}$, one can estimate T_{DA} for a direct outer-sphere ET mechanism in Rd via overlap with the Cys sulfurs. Using $(T_{DA})_{H_2O}$ = 25 cm^{-1}, $(\gamma_L)_{H_2O}$ = 0.06, and $(\gamma_L)_{S(Cys)}$ = 0.10, T_{DA} for ET through the Cys sulfurs in Rd is estimated to be 70 cm^{-1}. A small Fe–S bond distortion, <0.06 Å between oxidized and reduced Rd,[19,23] results in a low activation barrier to ET,[24] $\Delta G^\ddagger \approx 3$ kcal mol^{-1}. Assuming a nuclear vibration frequency, ν_n = 314 cm^{-1}, corresponding to the Fe–S breathing mode, and using the Landau–Zener expressions,[17] T_{DA} = 70 cm^{-1} corresponds to an electron transmission coefficient κ_{el} = 0.2 consistent with near adiabatic ET ($\kappa_{el} \approx 1$ corresponds to adiabatic behavior). An extension of the ET pathway beyond the Cys sulfurs would necessarily reduce the already small T_{DA}, thereby reducing k_{ET}. From eq 1, expansion of the ET route beyond S(Cys) lengthens r_{DA} by a minimum of 1.8 Å. Such an increase in r_{DA} substantially diminishes T_{DA} and leads to $\kappa_{el} < 0.01$, indicating nonadiabatic ET. Similarly, employing the pathway model of Beratan,[11] the inclusion in the ET path of only three additional covalent bonds beyond the Cys sulfurs also results in $\kappa_{el} < 0.01$. Therefore, the weak π covalency in the Rd HOMO results in extremely limited superexchange contributions to ET which would not sustain the high value of k_{ese} observed in this protein. As seen in Figure 3, the redox-active orbital of Rd is in fact well-oriented at the surface of the protein to allow direct access to the Cys sulfurs, and a direct outer-sphere ET mechanism would be expected.

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