Electron self-exchange in azurin: Calculation of the superexchange electron tunneling rate
(biological electron transfer/many-electronic wave function)

KURT V. MIKKELSEN*, LARS K. SKOV*, HERBERT NAR†, AND OLE FARVER‡§

*Institute of Chemistry, The H. C. Ørsted Institute, University of Copenhagen, Denmark; †Max-Planck-Institut für Biochemie, Martinsried b. München, Germany; and ‡Institute of General Chemistry, Royal Danish School of Pharmacy, Copenhagen, Denmark

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ABSTRACT Electronic coupling between the copper atoms in an azurin dimer has been calculated in this conformationally well-defined system by using many-electronic wave functions. When one of the two water molecules forming intermolecular hydrogen bonds between the copper-ligating His-117 of the two azurins is removed, the calculated coupling element is reduced from $2.5 \times 10^{-4}$ to $1.1 \times 10^{-5}$ eV (1 eV = $1.602 \times 10^{-19}$ J). Also, the effects of the relative orientations of the two water molecules have been analyzed. The results show that water molecules may play an important role as switches for biological electron transfer. The rate of electron self-exchange between two azurins has been calculated, and the result is in very good agreement with the rate found experimentally.

Long-range electron transfer (ET) plays a major role in many biological processes—e.g., in photosynthesis and other energy conversion systems (1, 2). Many recent reports have documented that ET can take place over large distances in both native and modified protein systems and investigations were made on the effects of driving force, reorganization energy, distance, and the nature of the intervening medium on the rate of long-range ET (3–8).

Azurins are blue single copper proteins found in many bacterial systems, where they function as mobile electron carriers in the respiratory system. The three-dimensional structure is now available for a number of both natural (wild type) and single-site mutated azurins (9–12), which makes this group of proteins highly interesting for studies of the different factors that control the rate of long-range ET (13). The rate of self-exchange reactions in both Pseudomonas aeruginosa azurin (14–16) and Alcaligenes denitrificans azurin (17) have been determined by $^1$H NMR line broadening and by rapid-freeze ESR as a function of temperature, pH, and ionic strength. The structure of the binary complex in the self-exchange reaction is unknown, but the NMR studies described above as well as mutational studies implicate the hydrophobic surface patch surrounding the Cu ligand His-117 (12). The association along this patch would minimize the distance between the two metal centers. Crystallographic data support this notion, since in several azurin crystal polymorphs the molecules are pairwise packed in this manner (9–12). The three-dimensional structure of P. aeruginosa azurin shows that the imidazole rings of His-117 in the azurin dimer are interconnected via hydrogen-bond formation to two water molecules (10). NMR $^1$H/$^2$H exchange experiments (18) indicate that these water molecules are also tightly bound in solution. On the basis of these findings, an involvement of the water molecules in the ET pathway of the self-exchange reaction as well as the cross-exchange reactions with cytochrome c and nitrite reductase has been suggested (10).

Procedures for calculation of electronic coupling using many-electronic wave functions have been developed for ET systems (19–27). Consequently, with the above structural information we have now undertaken a theoretical analysis of the Cu(II)/Cu(I) self-exchange ET system in azurin and present a direct calculation of the intermolecular coupling element for ET.

Theory

The initial and final states of the ET process are characterized in terms of diabatic surfaces and corresponding electronic wave functions ($\Psi_i$ and $\Psi_f$). The electronic wave functions are in these calculations given as semipirical intermediate neglect of differential overlap wave functions at the self-consistent field level of theory and the ET coupling elements are calculated according to refs. 19–22, 28–32. The initial (final) electronic wave function corresponds to having the electron on the donor (acceptor) site. These two electronic wave functions were obtained by taking advantage of the possibility of obtaining broken symmetry solutions, in which the odd electron is localized on either the “left” or “right” half of the system.

The nuclear configuration was given as the one determined by x-ray crystallographic experiments on wild-type P. aeruginosa azurin (11) except for H atoms, for which the positions were calculated.

Results and Discussion

The self-exchange ET rate for Pseudomonas azurin has been determined by both $^1$H NMR and rapid-freeze ESR, and the rate constant at 298 K is in the range 0.4–1.4 $\times$ $10^6$ M$^{-1}$s$^{-1}$ (14–17). The rate is fairly insensitive to pH and ionic strength and the activation entropy is relatively favorable (15). In azurin, the copper atoms are buried in the protein interior $\approx 7$ Å below the hydrophobic surface patch around the Cu ligand His-117. In several crystal forms, two azurin monomers are associated via their hydrophobic patches, which isolate the Cu centers from the bulk solution (Fig. 1). Two water molecules are connected by hydrogen bonds to His-117 N$^\alpha$ and Val-43 O on the monomers. In addition, in P. aeruginosa azurin crystals, the dimer packing is such that the water molecules form a hydrogen bond with each other. This results in a continuous electron density bridge between the redox centers, which we propose as a possible “through hydrogen bond” pathway in the azurin electron self-exchange reaction (Fig. 2). The separation distance between the Cu atoms of 14.9 Å is close to the minimal value for an azurin dimer. Removal of the two intercalating water molecules does not

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Abbreviation: ET, electron transfer.

‡To whom reprint requests should be addressed.

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allow for a reduction of the Cu–Cu distance but will only cause a break in the ET pathway.

We have now performed a theoretical study of this symmetric, two-site electron exchange system in *P. aeruginosa* azurin: Az[Cu⁺]–His-117–H₂O–H₂O–His-117–Az[Cu²⁺] (Fig. 2). By using the approach outlined above, we have directly calculated the coupling elements for the initial and final states of the ET process occurring over this conformationally well-defined system.

The coupling element with both water molecules present is calculated to be \( V_F = 2.5 \times 10^{-6} \text{ eV} (2.0 \times 10^{-2} \text{ cm}^{-1}) \) (1 eV = 1.602 \times 10^{-19} \text{ J}). When removing one of the water molecules, the electronic coupling decreases considerably to \( V_F = 1.1 \times 10^{-7} \text{ eV} \). It should be stressed that these coupling constants have not been subject to any scaling but are the direct result of the calculations. At the given nonadiabatic limit, the rate constant for ET (\( k_{ET} \)) at fixed distance is (13)

\[
k_{ET} = \frac{2\pi}{\hbar} V_F^2 (FC),
\]  

where \( V_F \) is the electronic coupling between the two states, \( \hbar \) is the Planck constant, and \( (FC) \) is the Franck–Condor factor describing the nuclear motion, which in the high-temperature limit is given by

\[
(FC) = (4\pi\kappa_kB^T)^{-1/2} \exp[-(\Delta G^0 + \lambda)^2/4\kappa_kB^T].
\]  

In the present case of self-exchange, the standard free energy of the reaction \( \Delta G^0 = 0 \), and thus the only contribution to the \( (FC) \) term is the reorganization energy, \( \lambda \). Eq. 1 then becomes

\[
k_{ET} = \frac{2\pi}{\hbar} V_F^2 (4\pi\kappa_kB^T)^{-1/2} \exp\left( -\frac{\lambda}{4\kappa_kB^T} \right).
\]  

X-ray crystallographic data have shown that reorganization around the copper site is minimal when the redox state changes between Az[Cu²⁺] and Az[Cu⁺] (33), and this has also been confirmed in studies of intramolecular ET in azurin, with \( \lambda = 0.25 \text{ eV} \) (4, 34). Inserting the values of \( V_F \) and \( \lambda \) in Eq. 3, we calculate an expected rate of intramolecular ET in the dimeric association complex \( k_{ET} = 2 \times 10^8 \text{ s}^{-1} \).

The physical event leading to a bimolecular collision between two molecules in solution involves a random diffusion process of the molecules, forming an encounter complex. In this case, the second-order exchange rate constant, \( k_{EX} \) is

\[
k_{EX} = K_A \times k_{ET},
\]  

where \( K_A \) is the association constant and \( k_{ET} \) is the rate constant for the intercomplex ET. NMR studies have demonstrated that minimal association takes place in aqueous solutions of 1–2 mM azurin, and from the experimental data a \( K_A \) value of 1 M⁻¹ was determined (15). We may now calculate the self-exchange rate constant from Eq. 4 and find \( k_{EX} = 2 \times 10^9 \text{ M}^{-1}\text{s}^{-1} \). This is 50-fold less than that found experimentally at 298 K: 0.4–1.4 \times 10^9 \text{ M}^{-1}\text{s}^{-1} (14–16). This discrepancy, however, is not unacceptably large. One should bear in mind that the theoretical calculation of the electronic

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**Fig. 1.** Azurin crystal Cu sites with bridging water molecules. Electron density map showing the two Cu centers and the two imidazole rings of His-117 (10). The cavity formed by the hydrophobic patch residues is filled by two water molecules.

**Fig. 2.** Details of the suggested pathway for electron exchange between Cu(I) and Cu(II) in the azurin dimer. The x and y axes referred to in the text are defined by a normal coordinate system in the paper plane, with the origin in the center of mass of the lower left water molecule.
coupling is based on the x-ray crystallographic data, with calculated H atom positions that do not necessarily represent a configuration where the orientation of the water molecules is optimal with respect to electronic coupling with the aromatic imidazole ring systems. Therefore, a more detailed analysis calculating the coupling element as a function of different orientations of the water molecules has been carried out. In these calculations, the position of one of the two water molecules was changed systematically by rotating it in 36° steps around the x and y axes as defined in Fig. 2, which causes a variation of the electronic coupling $V_{d}$ between 2.5 $\times$ 10^{-6} and 1.8 $\times$ 10^{-5} eV. The position of the two protein molecules was not perturbed. Calculations show that due to the relatively large distance between the two His-117 side chains, the dihedral angle between the imidazole rings has only a minor influence on electronic coupling. Since nothing is known about the position of the water molecules during the instant of intramolecular ET, we do not claim that the above rotations represent any true conformation but merely show that rotation of just one of the water molecules, keeping the overall Cu–Cu distance constant, may alter the coupling constant by 1 order of magnitude. The result demonstrates how important a role water may play in biological ET (see below). A variation in $V_{d}$ between 2.5 $\times$ 10^{-6} and 1.8 $\times$ 10^{-5} eV brings $k_{ET}$ in the range from 2 $\times$ 10^{10} to 1 $\times$ 10^{10} M^{-1}s^{-1}, which is in agreement with the experimental value.

The absolute values of the coupling elements, using semiempirical electronic wave functions, are of course rather approximate. Still, as illustrated previously, semiempirical electronic structure approaches give good and reliable results when comparing ET pathways within the same ET system (35, 36).

Theoretical and computational investigations of the effects of bridging water molecules between organic donor and acceptor systems have clearly shown the implications of an on–off switching mechanism for the ET reactions (29–32). These effects are governed by variations of the electronic coupling element, the donor and acceptor energy levels, and the angular velocity of the bridging water molecule. The probability for ET, as also shown here, depends strongly on the orientation of the bridging system between the donor and the acceptor. For some orientations and angular velocities, the probability is insignificant, whereas for other configurations and velocities, the probability for ET may be many orders of magnitude larger.

When one of the two water molecules bridging the pair of azurin molecules is removed while maintaining the original configuration, our calculations show a decrease in electronic coupling from 2.5 $\times$ 10^{-6} to 1.1 $\times$ 10^{-7} eV. Since the rate constant depends on the square of the coupling, $V_{d}^{2}$, the rate of ET decreases by almost 3 orders of magnitude. This interesting result demonstrates that water molecules intercalated between electron donor and acceptor may play key roles as switches for biological ET.

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