Rates of Intramolecular Electron Transfer in Ru(bpy)$_2$(im)(His83)-Modified Azurin Increase below 220 K

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One of the many remarkable aspects of electron transfer (ET) in the photosynthetic reaction center is the finding that the rate of the primary step is virtually independent of temperature.[1] Upon cooling from 300 to 4 K, the ET rate *increases* by more than a factor of 3. Weakly temperature-dependent rates are typical of ET steps in the photosynthetic reaction center but not of protein ET reactions in general.[1−9] In the other proteins that have been studied over a wide temperature range, ET rates tend to decrease dramatically at cryogenic temperatures (Figure 1).[4,5,8,9] Similar observations have been made with synthetic donor−acceptor complexes.[10]

Pseudomonas aeruginosa azurin is a blue copper protein with a $Cu^{2+/+}$ reduction potential of 0.31 V vs NHE.[11] Both the structure[12] and the ET reactions[13−19] of azurin have been investigated extensively. In one series of experiments, we found that the rate of $Cu^+ \rightarrow Ru^{3+}$ ET in Ru(bpy)$_2$(im)(His83)azurin (bpy = 2,2′-bipyridine; im = imidazole) is independent of temperature between 308 and 276 K.[18] Here we describe an examination of ET in Ru(His83)azurin down to cryogenic temperatures: the rate of $Cu^+ \rightarrow Ru^{3+}$ ET at 170 K is slightly greater than that measured at room temperature.

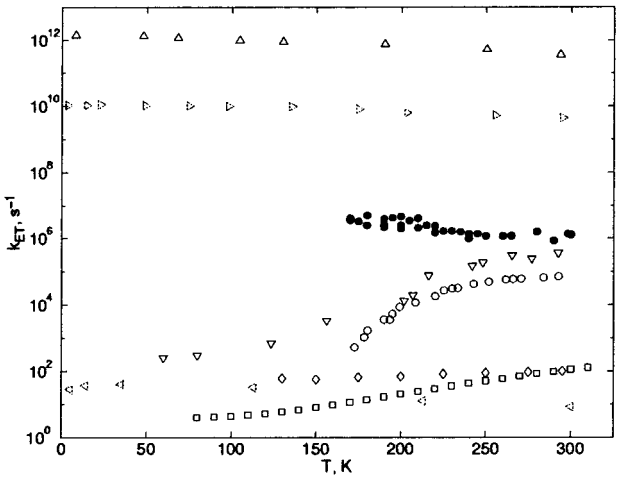
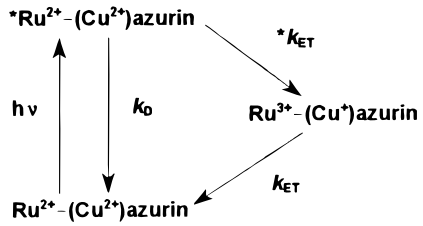


Figure 1. Temperature dependences of intramolecular ET rate constants in proteins: (△) primary charge separation in the *Rhodopseudomonas viridis* photosynthetic reaction center;[1] (triangle pointed to the right) ET from reduced bacteriopheophytin to quinone in the *Rhodopseudomonas sphaeroides* reaction center;[2] (▽) oxidation of cytochrome *c* in *Chromatium vinosum* reaction centers;[4] (triangle pointed to the left) reduced quinone to oxidized special pair ET in the *Rps. sphaeroides* reaction center;[3] (□) ET from triplet-excited Zn-porphyrin (*ZnP) to a ferriheme in a metal-substituted hybrid hemoglobin;[5] (◊) ET from a cyanoferroheme to a Mg-porphyrin radical cation in a metal-subsituted hybrid hemoglobin;[6] (○) *ZnP→Ru(NH$_3$)$_5$(His48)$^{3+}$ ET in Zn-substituted myoglobin;[8] and (●) $Cu^+ \rightarrow Ru^{3+}$ ET in Ru(His83)-azurin.

Scheme 1



We have employed laser-flash transient spectroscopy to measure $Cu^+ \rightarrow Ru^{3+}$ ET rates (k_{ET}) in Ru(His83)azurin.[13,14,16] The oxidation state of the Cu site is probed by transient absorption at 633 nm following 480-nm excitation of the Ru chromophore. The ET rate constant measured in aqueous solution (sodium phosphate buffer μ = 0.1 M, pH 7.0) at 298 K is 1.2(1) × 10^6 s^{-1}, and, in the 308−276 K temperature range, k_{ET} varies by less than the experimental uncertainty.[18] We have employed a water/glycerol cryosolvent (65% v/v) in order to extend these measurements to lower temperatures.[20] Luminescence-decay and ET kinetics were measured at 10-K intervals over the 300−170 K temperature range (Figure S1). The Ru(bpy)$_2$(im)(His83)$^{2+}$ luminescence lifetime behaves predictably between 300 and 170 K; the k_D doubles with each 75-K decrease in temperature. $Cu^+ \rightarrow Ru^{3+}$ ET rates extracted from the transient absorption measurements, however, are unaffected by temperature between 300 and 220 K; interestingly, these rates *increase* from 1.2(2) × 10^6 s^{-1} at 220 K to 4(1) × 10^6 s^{-1} at 170 K (Figure 2).

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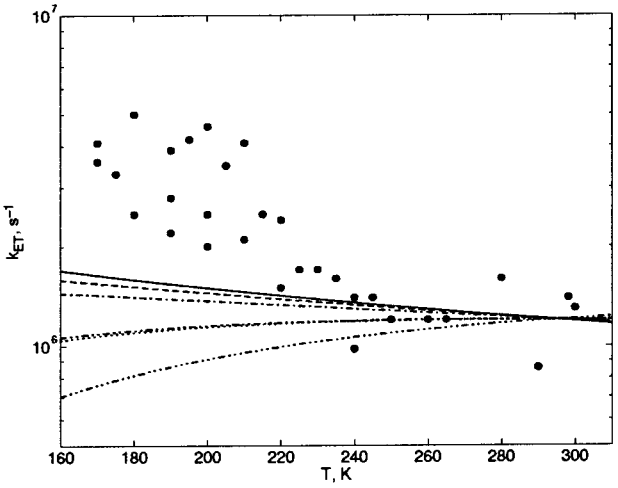

Figure 2. Variation of k_{ET} with temperature in Ru(His83)-azurin. The smooth lines were generated using eq 1 with $H_{AB} = 0.07$ cm^{-1} and λ values of 0.5 (···), 0.6 (− − −), 0.7 (—), 0.8 (−·−), 0.9 (−··−), and 1.0 eV (−···−).

Semiclassical theory (eq 1) predicts that the rates of intramolecular ET reactions depend on the driving force ($-\Delta G°$), the reorganization energy (λ), and the reactant−product electronic

$$k_{ET} = \sqrt{\frac{4\pi^3}{h^2\lambda k_B T}}\, H_{AB}^2 \exp\left[\frac{-(\Delta G° + \lambda)^2}{4\lambda k_B T}\right] \qquad (1)$$

coupling (H_{AB}).[21] The negligible variation of the $Cu^+ \rightarrow Ru^{3+}$ ET rate in the 308−220 K temperature range is consistent with $\lambda = 0.7(1)$ eV and $H_{AB} = 0.070(5)$ cm^{-1} (Figure 2).[22] This temperature independence arises from the near equality of the reaction driving force and reorganization energy, combined with the small entropy change for $Cu^+ \rightarrow Ru^{3+}$ ET.[18] Reorganization energies less than 0.6 eV and greater than 0.8 eV would lead to a significant decrease in ET rate at 170 K (Figure 2).

The increase in k_{ET} below 220 K, however, cannot be accommodated by the exponential term in the semiclassical rate expression. The alternative possibility is that the low-temperature behavior is the result of enhanced electronic coupling. Rates of ET in Ru-modified azurin exhibit an exponential dependence on donor−acceptor separation (R) with a decay constant of 1.1 Å^{-1}.[13−16] A 1-Å decrease in R, then, could produce the 3-fold increase in k_{ET} found upon cooling from 300 to 170 K. However, even if the thermal compressibility of azurin is comparable to that of myoglobin,[23] it is unlikely that the Ru−Cu distance would contract by 1 Å upon cooling the protein to 170 K.

It is possible that the electronic coupling increases upon cooling because of specific interactions in the ET coupling pathway. The ET coupling pathways between the Cu site and His83 of azurin have been analyzed in great detail.[19] The pathways in the best tunneling tube between Cu and Ru in Ru(His83)azurin are perpendicular to the intervening β strands, and several hydrogen bonds mediate the coupling (Figure 3). The best coupling path between the Cu site and the Ru(His83) complex involves two hydrogen bonds (Cys112:Sγ−Asn47:N and Trp48:N−Thr84:Oγ); the next best path couples across the Trp48:O−Thr84:N hydrogen bond. Additional tubes containing several more hydrogen-bonded interactions contribute to the Cu−Ru coupling. Beratan and Onuchic have suggested that the exponential distance decay for ET across hydrogen bonds is 3.4 Å^{-1}, substantially greater than the 1.1-Å^{-1} average decay that we have found in Ru-modified azurin.[24] Thus, the pathway model predicts that a cumulative 0.3-Å reduction in hydrogen-bond lengths can account for the observed increase in H_{AB} at low temperature. If this is the case, then coupling pathways in which hydrogen bonds do not play a significant role (e.g., Ru(His109)→Cu)[16] are not expected to exhibit increased couplings at low temperature.

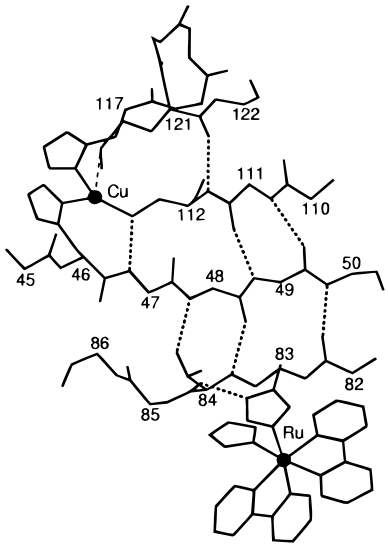

Figure 3. Structure of Ru(His83)azurin in the region between the two metal centers. The best tunneling tube between Ru and Cu is approximately perpendicular to the intervening β strands. Hydrogen bonds that connect the pathways that make up the tunneling tubes[19] are shown as dotted lines; the Cu-Met121(S) vector appears as a dashed line; amino acid α-carbon atoms are indicated by residue number.

The data demonstrate that Ru(His83)azurin is one of very few electron donor−acceptor complexes in which the rate of electron transfer does not drop precipitously at cryogenic temperatures. The near activationless $Cu^+ \rightarrow Ru^{3+}$ ET rate suggests that the nuclear potential surfaces for reactants (Ru^{3+}-Cu^+) and products (Ru^{2+}-Cu^{2+}) intersect near the minimum of the reactant surface ($-\Delta G° = \lambda$) and that the reaction driving force is not strongly dependent on temperature (i.e., $\Delta S° \approx 0$).[21,25] These properties are consistent with independent determinations of λ and $\Delta G°(T)$.[18] A modest reduction in hydrogen-bond lengths may be responsible for the slight increase in rate at low temperature.

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Supporting Information Available: Temperature dependence of the *Ru luminescence-decay rate constant (Figure S1) and transient absorption measurements of $Cu^+ \rightarrow Ru^{3+}$ ET (Figure S2) (2 pages). See any current masthead page for ordering and Web access instructions.

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(20) Ruthenium-modified azurin in sodium phosphate buffer, pH 7, was mixed with glycerol to give a ~65% (v/v) solution (final protein concentration ≈ 50 μM, final buffer concentration = 0.1 M). To ensure that the protein was fully oxidized throughout the measurement a small amount of $Fe(CN)_6^{3-}$ was also added to the solution (final $[Fe(CN)_6^{3-}] \approx 0.2$ mM). This addition has no influence on the very fast rate of intramolecular ET investigated here. The viscous solution (0.5 mL) was deaerated thoroughly on a vacuum line and transferred to a 3 × 10 mm quartz cuvette in a glovebox. The closed cuvette was placed on the coldfinger of a closed cycle refrigerator (CTI Cryogenics). Temperature stability was better than ±0.5 K. The kinetic measurements were performed using excitation at 480 nm generated by a XeCl excimer-pumped dye laser. Transient absorption was measured at 632.8 nm using the output of a HeNe laser for probe light.

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