also be pH dependent. Figure 7 demonstrates the validity of this interpretation. At pH 7.0 (Figure 7A), Hb is converted to Hb^+ and HbNO and, subsequently, Hb^+ is reduced to HbNO. In the initial stages of this transformation, the characteristic isosbestic point for the conversion of Hb to Hb^+ and HbNO is evident at 536 nm, but as the reaction continues and Hb^+ is reduced to HbNO, a new isosbestic point is observed at 540 nm. The comparable reaction at pH 8.0 (Figure 7B) is distinctly different. The kinetic order with respect to [Hb] and $[HN_2O_3^-]$ at pH 8.0 is the same as that determined at pH 7.0.[1,12] However, the rate of reaction is approximately 10 times slower than the corresponding reaction at pH 7.0, an isosbestic point is observed at 544 nm, and the spectral display of Figure 7B does not match that of Figure 6A at any time during the reaction. Part of this spectral difference may be due to a complex between Hb and nitroxyl, but we do not have independent evidence for this association.

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Communications to the Editor

Free Energy Dependence of the Rate of Long-Range Electron Transfer in Proteins. Reorganization Energy in Ruthenium-Modified Myoglobin

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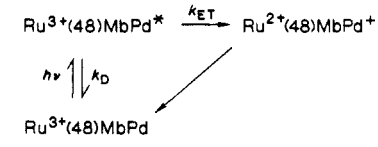
Long-range electron-transfer (ET) reactions in biological systems are under intense study.[1-8] One way to probe the factors that influence ET is through studies on two-site fixed-distance donor–acceptor systems consisting of a redox-active metal complex attached to the surface of a structurally characterized metalloprotein.[5-8] By using this approach we have demonstrated[6a] that the rate of long-range ET in sperm whale myoglobin (Mb) modified at histidine-48 with pentaammineruthenium (a_5Ru) and *trans*-tetraamminepyridineruthenium ($a_4$pyRu) depends on reaction free energy as predicted by Marcus theory.[2] These low driving force results (ΔG° = 0.020–0.275 V) indicated that the reorganization energy (λ) for ET might be 2 eV or even higher. We now report an investigation covering a wide range of reaction free energies that places the reorganization energy for ruthenium-modified myoglobin between 1.90 and 2.45 eV.

Our strategy involves replacing the heme in a_5Ru(48)Mb and $a_4$pyRu(48)Mb with a photoactive porphyrin that possesses a highly reducing excited state. With this approach we significantly increase the overall ET driving force while maintaining the same well-defined ΔG° step between the a_5Ru and $a_4$pyRu derivatives (0.26 V) as in the heme systems.[6a] The photoactive porphyrin is Pd(mesoporphyrin IX) (PdP), which has an excited-state reduction potential of −0.64 (10) V vs NHE.[9] PdP was inserted into native and ruthenium-modified Mb via reaction with apoprotein (12 h, 5% DMSO/phosphate, pH 7). The native and ruthenium-labeled palladium Mbs (MbPd and Ru(48)MbPd) were purified by gel-filtration chromatography and ultrafiltration.

The MbPd system is particularly attractive because the ET rates can be determined directly by monitoring the quenching of the PdP emission. Electronically excited MbPd (MbPd*) was produced via pulsed laser excitation (10 ns pulse, 532 nm), and the emission intensity was monitored at 670 nm. Kinetic analysis yields an observed first-order rate constant for the decay of the MbPd* emission intensity: $k_{obsd} = k_D + k_{ET}$.[10,11]



The decay in emission intensity for native MbPd closely follows first-order kinetics with a k_D of 1.0 (5) × 10^3 s^{-1}. Both a_5Ru^{3+}(48)- and a_4pyRu^{3+}(48)-modified MbPd exhibit enhanced emission quenching as expected for ET from the PdP excited state; the Ru^{3+}(48)MbPd* → Ru^{2+}(48)$MbPd^+$ driving forces are 0.72 (10) and 0.98 (10) V, respectively. The quenching of a_5Ru(48)MbPd* closely follows first-order kinetics with an ET rate of 9.1 (5) × 10^3 s^{-1} (Figure 1a). The kinetics for $a_4$pyRu(48)MbPd* are biphasic; an ET rate of 9.0 (5) × 10^4 s^{-1} was determined for the major (fast) component (Figure 1b). The minor (slow) component is probably due to residual native MbPd.

The ET results for Ru(48)MbM (M = Fe,[6a] Zn,[12] Pd) are shown in Figure 2.[13] The upper and lower curves represent the

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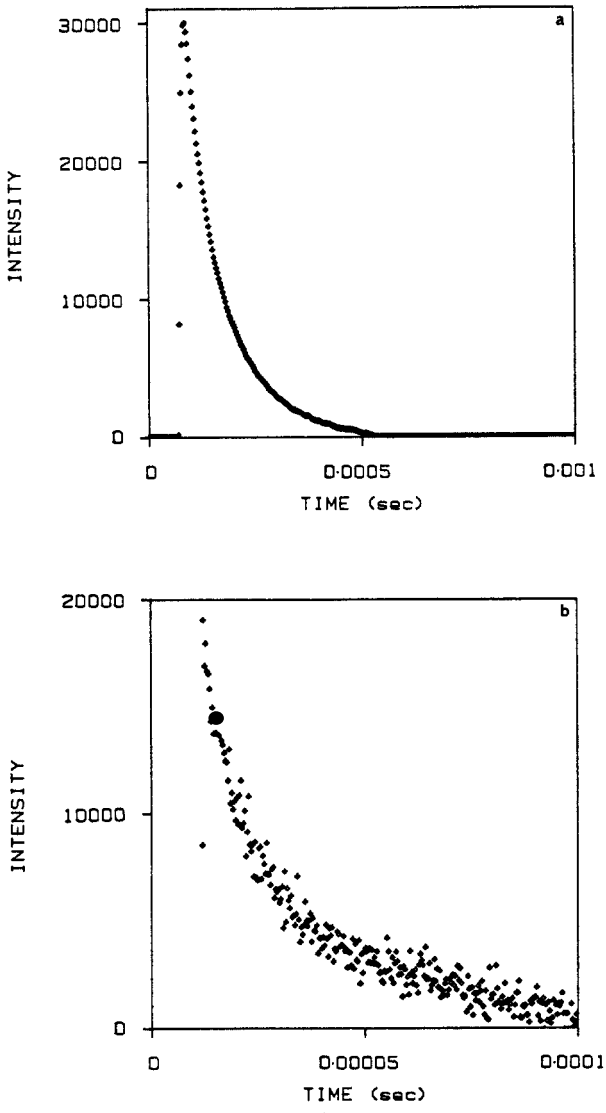

Figure 1. Time-resolved decay of the emission intensity for (a) a_5Ru^{3+}(48)MbPd* and (b) a_4pyRu^{3+}(48)MbPd* monitored at 670 nm following 532 nm pulsed laser excitation. The observed decay rates (k_D) are 1.0 (5) × 10^4 and 9.1 (5) × 10^4 s^{-1}, respectively. Conditions: 25 °C, 0.01 M pH 7 phosphate, O.D. (390 nm) = 0.5–1.0.

limits we place on the reorganization energy in Ru(48)MbM.[14] The relatively large λ (1.90–2.45 eV) is similar to those reported for ET in Ru(33)cyclochrome *c*[Zn][11] and in hemoglobin [Zn,-Fe].[15] The high reorganization energy in these protein systems probably can be attributed to reorganization of the aqueous medium around the redox sites. The faster ET rates observed for rigid aliphatic systems[16] may be due to the low λ(~1 eV) in nonaqueous media and not an enhanced donor–acceptor electronic coupling. In fact, the β of 0.91 $Å^{-1}$ determined for Mb[12] is nearly the same as the 0.85 $Å^{-1}$ decay factor reported[16b] for one of the organic donor–acceptor systems.

Our work indicates that very high ET rates in proteins could be realized at relatively small driving forces if λ were reduced to

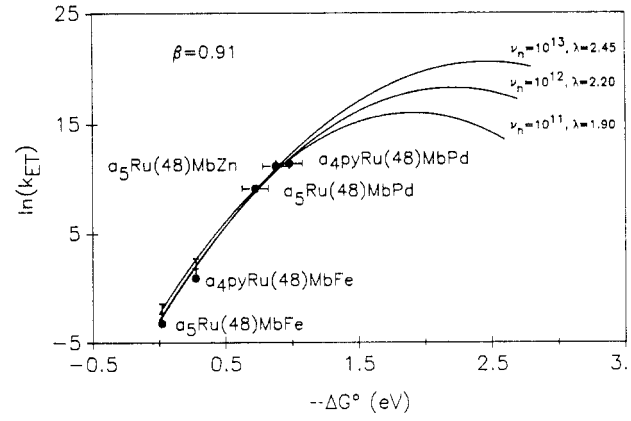

Figure 2. Free energy dependence of ln k_{ET}. The ln k_{ET} (Fe) error bars correspond to a 1 (1) Å uncertainty in the ET distance; this uncertainty arises because of differences in the delocalization of the MP ET orbital (Fe is more localized than Zn or Pd).

1 eV or less. (At a driving force of 0.1 V with λ = 1 eV and β = 0.9 $Å^{-1}$, for example, the rate calculated for 15 Å ET is 8 × 10^4 s^{-1}.) It would be surprising if biological systems did not use such reductions in reorganization energy to achieve specificity.

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Carbanion Intermediates in the Photodecarboxylation of Benzannelated Acetic Acids in Aqueous Solution

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Organic photochemical reactions proceeding via carbanion intermediates are not common. Several photodecarboxylations[1,2] and a photoretro-aldol type reaction[3] have been reported as proceeding via carbanions. On the other hand, the photochemistry of carbanions has been well-documented.[2a,4] Essentially nothing is known about the ability of cyclic hydrocarbon π-systems in stabilizing carbanions in the excited state, whereas Hückel's 4*n* + 2 rule works well for the corresponding ground-state systems.[5] One method[6] to probe the relative stability of charged cyclic π-systems in the excited state is to study the reactivity of suitably designed molecules capable of producing such intermediates on photolysis. We have thus reported results which suggest that cyclic 4π-electron carbocations are better accommodated than cyclic 6π-electron carbocations in the excited state, via study of photosolvolysis of benzannelated alcohols.[7,8] We now report results which show that the photodecarboxylation of several benzannelated acetic acids proceed via carbanion intermediates and in addition,

(13) The curves in Figure 2 were calculated by using a Marcus expression[2] for the ET rate, $k = \nu_n \exp[-\beta(d-3)] \exp[-(\Delta G° + \lambda)^2/4\lambda RT]$. The values of β (0.91 $Å^{-1}$) and *d* (13.2 Å) have been determined previously for RuMbZn.[12] The curves were fit to the experimental data by varying λ, with ν_n fixed at 10^{11}, 10^{12}, or 10^{13} s^{-1}.

(14) The finding that all five rates can be fit satisfactorily with a single λ indicates that outer-sphere contributions (λ_o) dominate, because λ_i is expected[6] to be larger for Fe than for Zn or Pd.

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