Communications to the Editor

Electron Transfer and Energy Transfer in the Hb:Hb Reductase (cyt b_5) System

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Despite intensive and ongoing studies of biological electron transfer,[1-3] only a few physiological (protein–protein) systems have been examined in sufficient detail to permit analysis of key molecular parameters like the reorganization energy and electronic couplings. The Hb/cyt b_5 complex is an important system to study for several reasons.[4] Both proteins are well characterized, including high-resolution crystal structures which allow for computer modeling and aid in understanding of experimental results.[5] Furthermore, a testable model of the Hb/cyt b_5 complex exists, and the driving force of the electron-transfer reaction can be varied over a wide range via metal substitution of Hb. In previously studied protein systems, the rate constant of electron transfer has been shown to be a function of driving force (ΔG), distance (r), and environmental effects like ionic strength (μ).[1,2] The present work seeks to determine the role of these parameters for the Hb/cyt b_5 complex by combining energy-transfer studies of distance with electron-transfer kinetic studies of native and metal-substituted Hb, for which ΔG_{et} can be systematically varied.[3] Transient absorbance is used to study the photoinduced intracomplex electron transfer over a range of driving forces and ionic strengths, and static and dynamic fluorescence techniques are used to measure the distance between metal centers within the bound Hb/cyt b_5 complex.

In earlier work, it was shown that electron transfer could occur from a photoexcited Zn heme in Hb to Fe(III)b_5, producing a (directly observed) Fe(II)b_5 product.[4] In the present work, we have measured electron-transfer rate constants for a variety of hemoglobin derivatives including the thermal reaction of Fe(II)b_5 with Fe(III)Hb and the photochemical electron transfer

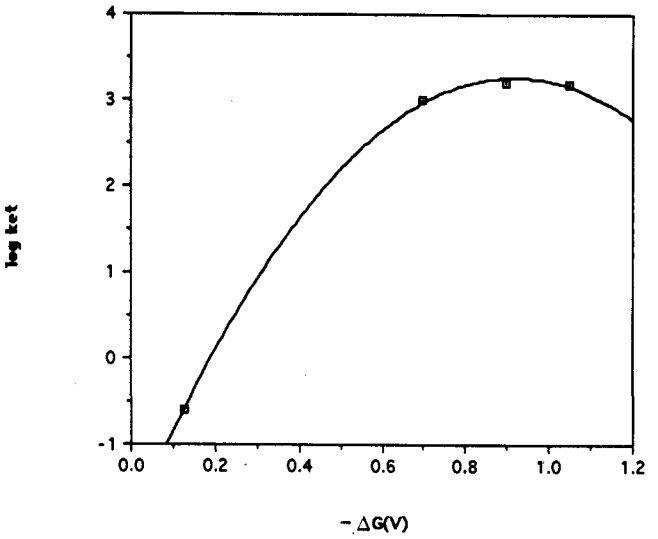

Figure 1. Plot of electron-transfer rate constant (k_{et}) versus reaction free energy (ΔG). Experiments at 25 °C, pH = 6.2, 1 mM phosphate buffer. For the excited-state values, ΔG was estimated as outlined in ref 3.

Table I. Electron-Transfer Rates (k_{et}) for Cyt b_5/Hb Systems

system			
cyt b_5/Fe_4Hb	cyt b_5(H_2porph)*Hb	cyt b_5/Zn_4*Hb	cyt b_5/Mg_4*Hb
0.25 ± 0.1 s^{-1}	1000 ± 100 s^{-1}	1600 ± 100 s^{-1}	1500 ± 200 s^{-1}

to Fe(III)b_5 from the metal-substituted derivatives[3] (ZnHb), (H_2-porphHb), and (MgHb). These differences may affect the estimated τ value, but such effects are between ground and excited state, which is expected to be relatively small by analogy to other systems.[3-8] The thermal rates were measured by directly mixing Fe(III)Hb with Fe(II)b_5, prepared in degassed buffer (pH 7.2 phosphate, μ = 1–100 mM) by dithionite titration of Fe(III)b_5. The photochemical rates were measured as previously described.[4,6,9] Kinetics were measured at pH 6.2 phosphate in buffers ranging from 1×10^{-3} to 1×10^{-1} M phosphate. The results are summarized in Figure 1 and Table I. In going from Fe(III)Hb ($\Delta G \approx -0.12$ V) to ZnHb ($\Delta G \approx -0.9$ V),[3] the observed rate increases approximately 10^3-fold. This change is consistent with a reorganization energy of ca. $\lambda = 0.9$ V.[4c] By itself, this value is unremarkable and in line with values observed for other protein electron-transfer systems.[7-10] However, the maximum rate seems quite low given the relatively short distance (8 Å heme edge to heme edge) which had been postulated by the current model of the Hb/b_5 complex.[5] We therefore reexamined the heme-heme distance, using fluorescence energy transfer [from ZnHb to Fe(III)b_5] as a direct measure of the distance. Both steady-state and time-resolved (single photon counting) measurements were made and agreed well.

The results are unexpected. Energy transfer from Hb to b_5 is remarkably inefficient (ca. 10%), corresponding, in the simplest analysis,[6] to a distance of ~25 Å between the heme metal centers.

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(4) (a) Simolo, K.; McLendon, G.; Mauk, A. G.; Mauk, M. *J. Am. Chem. Soc.* **1984**, *106*, 5012. (b) The thermal reaction rate for Fe(II)b_5 + Fe(III)Hb has been independently measured by Hultquist *et al.* with similar results ($k_1 \approx 1$ s^{-1}) to those obtained in our lab: Hultquist, D.; Sames, L.; Juckett, D. *Curr. Top. Cell. Reg.* **1984**, *24*, 287. (c) We note that in order to obtain a wide ΔG range, both the thermal Fe(II) b_5/Fe(III)Hb reaction and the excited-state reactions are combined in the same data analysis. This may introduce some additional uncertainty in the λ value so derived.

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(6) (a) Winkler, J.; Nocera, D.; McLendon, G.; Gray, H. B. *J. Am. Chem. Soc.* **1985**, *107*, 739. (b) Vanderkooi, J.; Adar, F.; Erickinska, M. *Eur. J. Biochem.* **1976**, *64*, 381. (c) The energy-transfer rate is assumed as $k(s^{-1}) = (J_n {}^4\kappa^2\lambda_{donor}R^6)(8.7 \times 10^{23})$. These calculations assume a value of the angle orientation factor $K^2 = 1$ (of the refractive index $n = 1.4$) and spectral overlap integral $J = 1 \times 10^{-38}$ cm^6, calculated from the overlap of donor emission and acceptor absorbance. Similar measurements (McLendon, G., *et al. J. Am. Chem. Soc.* **1993**, in press) reproduce the observed distance in the cyt c:ccp complex: Pelletier, H.; Kraut, J. *Science* **1992**, *258*, 1748.

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At this distance, the relatively slow transfer ($\sim$1500 s^{-1}) falls well in line with values observed for other intracomplex protein electron-transfer systems.[10a,b]

Finally, we address the question of complex dynamics in this system. In many previously studied protein-to-protein electron-transfer reactions (cyt c:ccp,[7] cyt c:plastocyanin,[8] cyt b_2:cyt c[9]), conformational rearrangements have been shown to play an important role. Thus, for example, the bimolecular rate constant k_{bi} cannot (in these systems) directly predict the intracomplex rate constant (k_t) using the simple preequilibrium expression $k_{b_i} = k_t K_c$, where K_c is the complex formation constant. Indeed, for the cyt c:ccp system, the observed value of k_{b_i} increases as K_c decreases![7b]

For the Hb/b_5 system, however, simple preequilibrium behavior *is* observed, at least under these conditions. By varying the ionic strength, one can readily vary the amount of complex formation from $\geq$90% (at μ = 1 mM) to $\leq$10% (at μ = 12 mM). This change is clearly reflected in the quenching kinetics, which are cleanly second order at μ = 12 mM and cleanly first order at μ = 1 mM (concentrations of quencher ranged from 0.5–12 mM). Most interestingly, unlike the other systems cited,[7-9] the Hb/b_5 system *does* exhibit simple "preequilibrium" kinetics. Under bimolecular conditions, the bimolecular rate constant is correctly predicted from the measured equilibria[11] and the limiting value of k_t (1500 s^{-1}) over the range from $K_c = 3 \times 10^5$ to 6×10^3 M^{-1}. [At lower values of K (higher μ), the quenching rate constant cannot be measured accurately, as it approaches the value for spontaneous triplet decay.] Thus, the Hb:b_5 system appears to be characterized by a single "reaction site" over a range of ionic strengths, apparently uncomplicated by the kind of interfacial dynamics observed in other protein-protein systems.[7-9] As such, the Hb:b_5 system appears to be well suited for detailed studies of electron transfer within a physiological protein-protein system.

In summary, electron transfer in the physiological Hb:b_5 system is characterized by a strong dependence of the rate constant on reaction free energy (consistent with Marcus's theory). The maximum rate, $k_c \approx 1500$ s^{-1}, occurs when $\Delta G \approx \lambda \approx 0.9$ V. This relatively slow rate is explained by the long distance (25 Å) between the heme centers inferred from energy-transfer measurements.

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