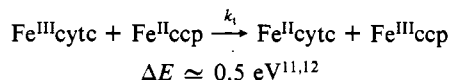


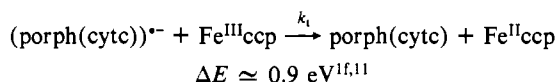
Photoinduced Long-Range Electron Transfer in the Zinc-Substituted Cytochrome *c*/Cytochrome *c* Peroxidase Complex

Sir:

Some understanding of biological electron transfer is developing as a result of intensive kinetic studies of metalloproteins.¹ A particularly attractive system for such detailed studies is the cytochrome *c* (cytc)/cytochrome *c* peroxidase (ccp) couple. The crystal structures of both proteins² and a computer model of the complex³ have been published. In addition, it is possible to employ metal substitution to systematically vary parameters such as reaction free energy, which play an important role in controlling electron-transfer rates.⁴ Previously, we reported the rates of ground-state electron transfer for ccp/Mcytc couples, where M = Fe and H₂porphyrin (porph).⁵ For the reaction with Fe(cytc)



the electron-transfer rate, k_t , is $0.23 \pm 0.025 \text{ s}^{-1}$. Likewise, for the reaction with H₂porph(cytc)



the rate of electron transfer, k_t , is $150 \pm 20 \text{ s}^{-1}$. Interestingly, as the reaction free energy for the Mcytc/Fe^{III}ccp couple was increased, a corresponding increase in electron-transfer rate was observed.

In a similar vein, Hoffman and co-workers independently reported elegant studies of photoinduced electron transfer within the ³(Zn(ccp))*/Fe^{III}cytc system, which proved to be quite sensitive to structural features of the complex.⁵

In order to better understand the reaction chemistry and observed dependence of rate on reaction free energy of this protein complex, we have studied and here report the electron transfer rates for the photochemical reaction in which Zn- and H₂porph-substituted cytochrome *c* react with Fe^{III}ccp within the cytc/ccp complex.

CCP was isolated by the method of Nelson et al.⁷ with the following modifications: cross-linked DEAE agarose was substituted for DEAE cellulose; cytochrome *c* peroxidase was extracted in 0.05 M sodium acetate, pH 5.0, thereby allowing the enzyme to be loaded directly onto the DEAE column; the enzyme was concentrated by ultrafiltration, using an Amicon cell and a 10 000 Da cut-off filter rather than a second DEAE column. The

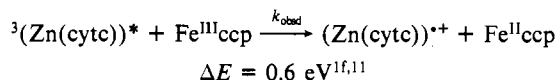
enzyme was then crystallized by dialysis against twice distilled water and stored at $-196 \text{ }^\circ\text{C}$. The enzyme prepared in this manner had a purity index of 1.2 as determined by the ratio of absorbance at 407 nm to that at 280 nm. When assayed according to the procedure of Yonetani,⁸ the cpp had a specific activity of 0.52 (ΔOD at 550 nm per 10 s per 10 μL of enzyme solution/absorbance of enzyme at 407 nm).

Zn(cytc) was prepared by the addition of 100 mM zinc acetate to an acidic, aqueous solution of porphyrin cytochrome *c*. (Porph(cytc) was obtained from Sigma type VI cytc by the method of Vanderkoöi et al.⁹) The mixture was then heated and maintained at $40 \text{ }^\circ\text{C}$ for 1 h. An ultraviolet-visible spectrum recorded on a Perkin-Elmer $\lambda 3$ spectrophotometer confirmed the presence of Zn(cytc). Excess zinc acetate was removed by ultrafiltration, and final protein purification was completed by using exclusion chromatography (Sephadex G-25). All sample handling was done in the dark to prevent photodegradation of Zn(cytc) in the presence of oxygen.

The second harmonic (532 nm) of Quanta-Ray DCR-2 pulsed Nd:Yag laser (7 ns pulse width) was used to photoexcite the samples. Changes in the Soret band (470 nm) of the cytochrome *c* derivatives following the flash were monitored by using an Oriol No. 6333 tungsten-halogen lamp. After leaving the sample, the monitoring light passed through a Bausch & Lomb Model No. 42-250471-1 monochromator and an RCA IP28 photomultiplier. The output from the photomultiplier was digitized by a Gould Biomation 6500 waveform digitizer and transferred to an MF-211 Charles River Data System (LSI-11/03) computer. After analysis the data were plotted by using a Hewlett-Packard 7470A plotter.

The experiments were run with samples prepared in 10 mM potassium phosphate buffer pH 7.0 at $22 \text{ }^\circ\text{C}$; conditions that promote formation of a stable 1:1 complex between ccp and cytochrome *c*.¹³ Since the excited-state porphyrin triplets can be quenched by oxygen, extreme care was used in degassing the samples. Both the ccp and cytc samples were degassed in the dark by very slowly bubbling He through the samples for several hours. To insure that the samples were properly degassed, the lifetime of the cytc samples (10 μM) was obtained in the absence of ccp. The samples were then degassed for twice the amount of time. If the same rate was obtained, then the sample was thoroughly degassed. Upon titration of the cytc samples with degassed ccp, the lifetimes remained exponential but the rates increased linearly with added ccp until a 1:1 ratio was reached. Titration of additional ccp did not change the rate, indicating a 1:1 complex had been reached.

Reaction within the Zn(cytc)/ccp complex was initiated by flash photoexcitation, which formed the slowly decaying zinc protoporphyrin triplet state of Zn(cytc). The ³(Zn(cytc))* then decays and can reduce ferric ccp. The reaction proceeds as follows:



The observed rate constant (k_{obsd}) is a sum of the intrinsic triplet decay rate constant for ³(Zn(cytc))* (k_p) and that for other decay processes (k_d), $k_{\text{obsd}} = k_p + k_d$. The observed rate constant $k_{\text{obsd}} = 80 \pm 2 \text{ s}^{-1}$ (Figure 1b). The triplet decay curve of ³(Zn(cytc))* is exponential with a rate constant $k_p = 48 \pm 2 \text{ s}^{-1}$ (Figure 1a). Therefore, from the measured k_{obsd} and k_p one obtains the decay rate within the Zn(cytc)/ccp complex, $k_d = 32 \pm 3 \text{ s}^{-1}$.

A variety of pathways are available to account for the observed decay within the complex. These include (1) electron transfer, (2) dipolar energy transfer, (3) electron exchange or magnetic

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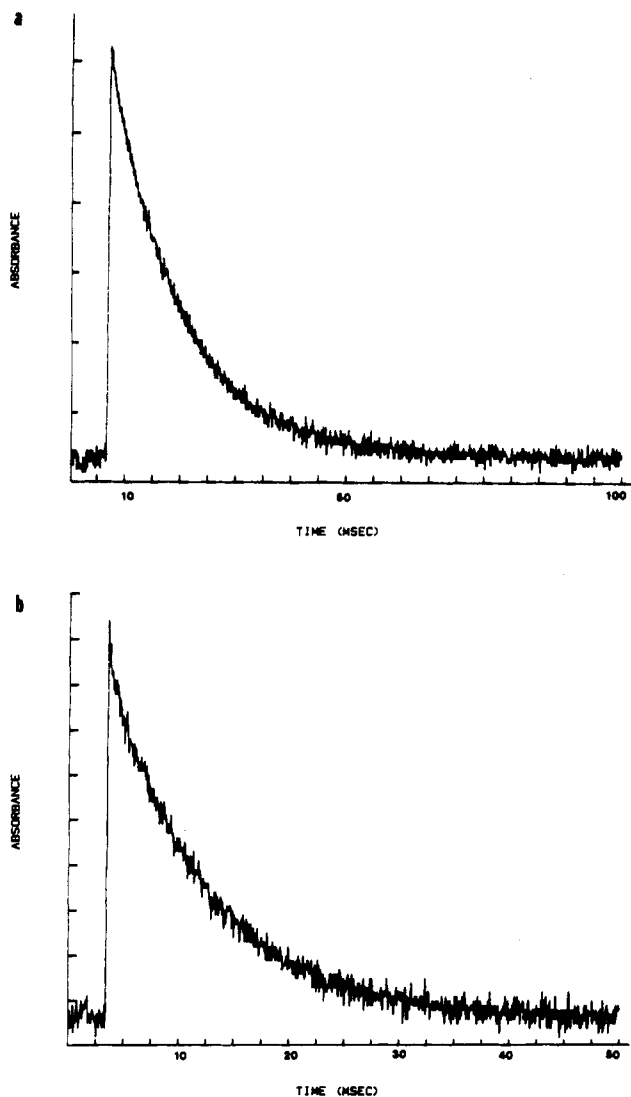


Figure 1. (a) Excited-state decay of $^3(\text{Zn}(\text{cytc}))^*$ measured at 470 nm: $[\text{Zn}(\text{cytc})] = 10 \mu\text{M}$, 10 mM potassium phosphate buffer, pH 7.0, 22 °C. (b) Excited-state decay of the $^3(\text{Zn}(\text{cytc}))^*/\text{Fe}^{\text{III}}\text{ccp}$ complex measured at 470 nm: $[\text{Zn}(\text{cytc})] = 10 \mu\text{M}$, $[\text{ccp}] = 15 \mu\text{M}$, 10 mM potassium phosphate buffer, pH 7.0, 22 °C. Note: The shape of the decay curve remains exponential upon addition of ccp; however, the lifetime of the sample increases from 48 s^{-1} (curve a) to 80 s^{-1} (curve b) as evidenced by the change in time scale.

dipole energy transfer, and (4) enhanced, intramolecular radiationless decay. As described previously,^{1j} the only effective pathways are electron transfer and (Förster) dipole-dipole energy transfer. To evaluate the contribution of energy transfer to the total decay rate, we calculated the rate constant for long-range energy transfer. The rate constant, k_e may be written¹⁰

$$k_e = \frac{(8.8 \times 10^{-28})\kappa^2}{n^4\tau R^6} F_D(\lambda) E_A(\lambda) \lambda^4 d\lambda$$

where κ = dipole-dipole orientation factor, n = refractive index of the medium, τ = natural lifetime of donor, and R = center-to-center distance between donor and acceptor (cm). $F_D(\lambda) E_A(\lambda) \lambda^4 d\lambda$ = overlap integral ($\text{cm}^6 \text{ mol}^{-1}$) between donor emission and acceptor absorption bands where $F_D(\lambda)$ is the normalized donor emission probability and $E_A(\lambda)$ is the molar decadic extinction spectrum of the acceptor ($\text{cm}^2 \text{ mol}^{-1}$). We use $R = 25 \text{ \AA}$, (from computer modeling),^{2b} $n = 1.4$, $\tau = 75 \text{ ns}$, and $\kappa^2 = 1$. We assume 100% overlap between donor emission, which occurs from 720 to 740 nm,¹⁴ and weak acceptor absorption $\epsilon_{\text{max}}(730) = 30 \text{ M}^{-1}$

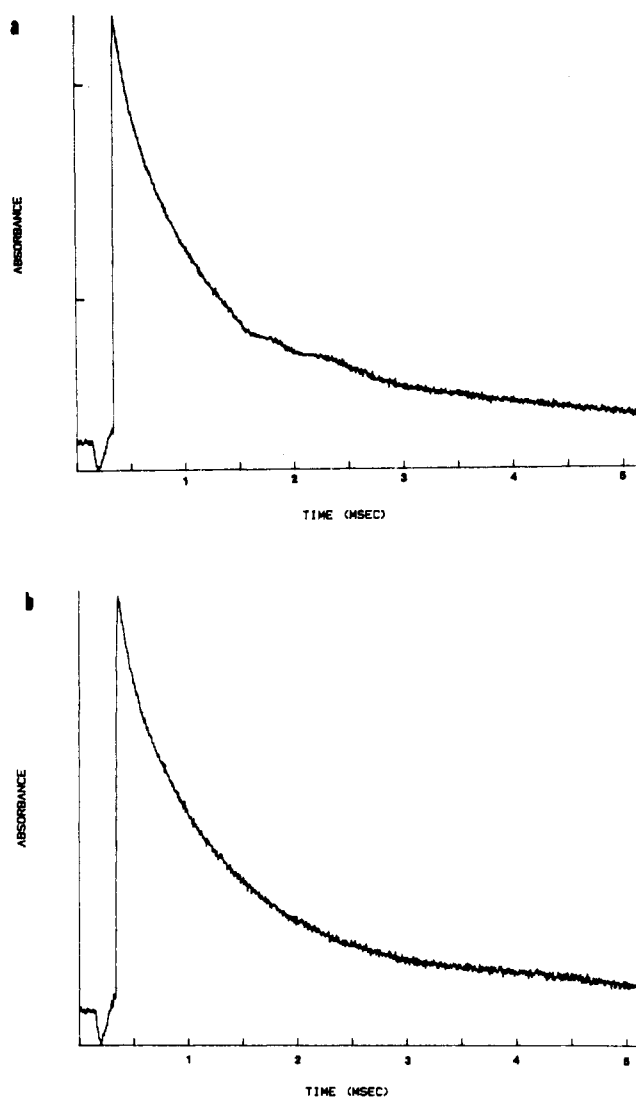
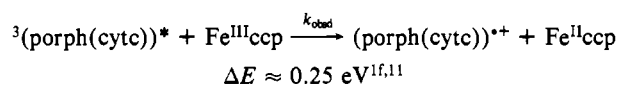


Figure 2. (a) Excited-state decay of $^3(\text{porph}(\text{cytc}))^*$ measured at 470 nm: $[\text{porph}(\text{cytc})] = 10 \mu\text{M}$, 10 mM potassium phosphate buffer, pH 7.0, 22 °C. (b) Excited-state decay of the $^3(\text{porph}(\text{cytc}))^*/\text{Fe}^{\text{III}}\text{ccp}$ complex measured at 470 nm: $[\text{porph}(\text{cytc})] = 10 \mu\text{M}$, $[\text{ccp}] = 15 \mu\text{M}$, 10 mM potassium phosphate buffer, pH 7.0, 22 °C. Note: The lifetimes of the sample are identical (500 s^{-1}) in the absence (curve a) or the presence of ccp (curve b).

cm^{-1} .⁸ We calculate the energy transfer rate to be $k_e \approx 2 \text{ s}^{-1}$.

Since k_e is small, the major component of the decay rate can be attributed to long-distance electron transfer. Therefore, the electron-transfer rate within the $\text{Zn}(\text{cytc})/\text{ccp}$ complex $k_t \approx 30 \pm 3 \text{ s}^{-1}$.

To further investigate this reaction, we studied the $\text{porph}(\text{cytc})/\text{ccp}$ complex. Again, by flash photoexcitation the reaction was initiated by formation of the protoporphyrin triplet state which can reduce ccp. The reaction proceeds as follows:



The observed rate constant, k_{obsd} , is approximately $500 \pm 10 \text{ s}^{-1}$ (Figure 2b). The intrinsic decay rate of $^3(\text{porph}(\text{cytc}))^*$ we measured to be $k_p \approx 500 \pm 10 \text{ s}^{-1}$ (Figure 2a). Therefore, very little if any electron transfer occurs with the $^3(\text{porph}(\text{cytc}))^*/\text{ccp}$ complex. As in the $\text{Zn}(\text{cytc})$ system, energy transfer is not an efficient decay pathway since at $\lambda = 780 \text{ nm}$ $^3(\text{porph}(\text{cytc}))^*$

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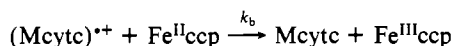
Table I

reaction	$-\Delta G_{\text{est.}}$ eV	$k_{\text{obsd.}}$ s ⁻¹	$^*k_{\text{pred.}}^a$ s ⁻¹	ref
(porph(cytc)) ⁺ /Fe ^{III} cpcp → porph(cytc)/Fe ^{II} cpcp	0.9	150 ± 2.0	200	4
³ (Zn(cytc)) [*] /Fe ^{III} cpcp → (Zn(cytc)) ⁺ /Fe ^{II} cpcp	0.6	30 ± 2	18	b
Fe ^{III} cytc/Fe ^{II} cpcp → Fe ^{II} cytc/Fe ^{III} cpcp	0.5	0.23 ± 0.2	3	4
³ (porph(cytc)) [*] /Fe ^{III} cpcp → (porph(cytc)) ⁺ /Fe ^{II} cpcp	0.25	~0	0.003	b

^a k_{pred} calculated by assuming $\lambda = 1.5$ eV and $A = 2 \times 10^3$ s⁻¹.
^b This work.

emission maximum) the acceptor extinction coefficient $\epsilon_A \leq 30$ M⁻¹ cm⁻¹.⁸

Interestingly in our photoexcited systems (³(Zn(cytc))^{*}/cpcp and ³(porph(cytc))^{*}/cpcp) as well as those of others,^{1c,16} transient redox products generally were not directly observed, for the following reason. It has been well established that in such systems formation of ground-state products



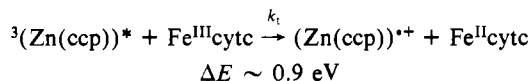
$k_b \gg k_t$. Consequently, no significant concentration build up of product transients occurs. This commonly observed phenomenon that $k_b \gg k_t$ is not well understood, but could be rationalized if "hole" transfer via a superexchange mechanism¹⁵ is particularly common in proteins. However, redox products *can* be demonstrated by trapping of the Fe^{II}cpcp product by CO, following the approach of Hoffman.^{1c,8} Thus, when Zn(cytc)/Fe^{III}cpcp is irradiated under CO, a difference spectrum assignable to Fe^{II}CO(cpcp) grows in. Details of these experiments will be presented elsewhere.

These new photochemical results can be combined with the thermal (radiolysis) data on the cytc/cpcp system to provide a more complete picture of how electron transfer varies with ΔG . The combined data suggest a relatively simple relationship between the intramolecular electron-transfer rate, k_t , and free energy, ΔG . As shown in Table I, the data for all four reactions are consistent with predictions using the classical Marcus theory for electron-transfer rates:⁴

$$k_t = A \exp(-(\Delta G + \lambda)^2 / 4\lambda kT)$$

where A = frequency factor related to donor-acceptor distance, $A \propto \exp(-\alpha R)$, and λ is the reorganization energy arising from all nuclear displacements between reactant and product states that accompany electron transfer.

Recently, Hoffman et al.⁵ studied a related photochemical electron transfer reaction. They observed the electron-transfer rate within the ³(Zn(cpcp))^{*}/cytc complex.



The measured electron-transfer rate within this complex $k_t = 17 \pm 3$ s⁻¹ at 20 °C. This rate cannot directly be compared to ours, since one involves reduction of Fe^{III}cytc while the other involves reduction of Fe^{III}cpcp. Significantly different reorganization energies are expected for these two couples. However, it is interesting to note that electron transfer is facile in *both* directions, in contrast to recent observations for Ru-substituted cytochrome *c*.¹⁶

These results demonstrate that the rate of electron transfer in the cytc/cpcp complex is quite sensitive to ΔG for the electron-transfer step. They also support the suggestion that a large reorganization energy, λ , is associated with protein repolarization around the developing charge in the cytc/cpcp complex. Similar trends seem to be emerging in other studies of electron-transfer reactions with protein/protein complexes.¹ For example, in the cytc/cyt *b*₅ complex the available data suggest $\lambda = 0.8$ eV,^{1f} and

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for the Zn/Fe hybrid of hemoglobin, temperature-dependence studies suggest $\lambda = 2.3$ eV.^{1g}

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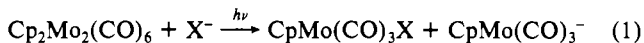
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Nineteen-Electron Adducts: Measurement of ΔG° for the Reactions of CpMo(CO)₃ with Halide Ligands (Cp = CH₃C₅H₄)

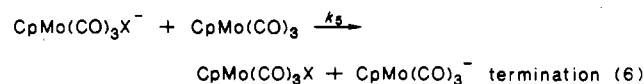
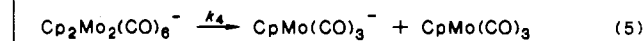
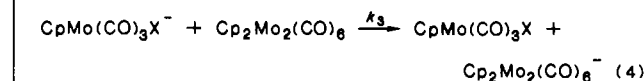
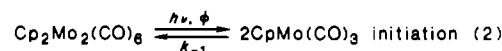
Sir:

In a series of recent papers, we demonstrated that 17-electron organometallic radicals react with Lewis bases to form 19-electron adducts.¹⁻³ In this communication, we present evidence showing that 19-electron adducts are, in some cases, thermodynamically downhill with respect to the 17-electron precursor and the Lewis base.

Halides and pseudohalides react photochemically with Cp₂Mo₂(CO)₆ according to the disproportionation reaction in eq 1.⁵ In our previous study of this reaction,² we showed that the



reaction followed the pathway in eq 2-6. Note that a key step



in the reaction pathway is eq 3, in which the 17-electron CpMo(CO)₃ species reacts with X⁻ to form the 19-electron adduct CpMo(CO)₃X⁻. In our previous study we also showed that the quantum yield for disappearance of Cp₂Mo₂(CO)₆ in eq 1 is given by eq 7, where $K' = K_{\text{eq}}k_3[\text{Cp}_2\text{Mo}_2(\text{CO})_6]$, $K = k_5K_{\text{eq}}$, and $I =$

$$\Phi = \phi \left(1 - \frac{k_{-1}}{k_{-1} + K[\text{X}^-]} \right) + \left(\frac{K'[\text{X}^-]}{(k_{-1} + K[\text{X}^-])^{1/2}} \right) (\phi/I)^{1/2} \quad (7)$$

the absorbed light intensity. This equation is useful in that it allows us to find K_{eq} from the dependence of Φ on [X⁻].

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