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Conformational Dynamics and Temperature Dependence of Photoinduced Electron Transfer within Self-Assembled Coproporphyrin:Cytochrome *c* Complexes

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ABSTRACT The focus of the present study is to better understand the complex factors influencing intermolecular electron transfer (ET) in biological molecules using a model system involving free-base coproporphyrin (COP) complexed with horse heart cytochrome *c* (Cc). Coproporphyrin exhibits bathochromic shifts in both the Soret and visible absorption bands in the presence of Cc and an absorption difference titration reveals a 1:1 complex with an association constant of $2.63 \pm 0.05 \times 10^5$ M^{-1}. At 20°C, analysis of time-resolved fluorescence data reveals two lifetime components consisting of a discrete lifetime at 15.0 ns (free COP) and a Gaussian distribution of lifetimes centered at 2.8 ns (representing ^{1}COP → Cc ET). Temperature-dependent, time-resolved fluorescence data demonstrate a shift in singlet lifetime as well as changes in the distribution width (associated with the complex). By fitting these data to semiclassical Marcus theory, the reorganizational energy (λ) of the singlet state electron transfer was calculated to be 0.89 eV, consistent with values for other porphyrin/Cc intermolecular ET reactions. Using nanosecond transient absorption spectroscopy the temperature dependences of the forward and thermal back ET originating from triplet state were examined (^{3}COP → Cc ET). Fits of the temperature dependence of the rate constants to semiclassical Marcus theory gave λ of 0.39 eV and 0.11 eV for the forward and back triplet ET, respectively ($k_f = (7.6 \pm 0.3) \times 10^6$ s^{-1}, $k_b = (2.4 \pm 0.3) \times 10^5$ s^{-1}). The differing values of λ for the forward and back triplet ET demonstrate that these ET reactions do not occur within a static complex. Comparing these results with previous studies of the uroporphyrin:Cc and tetrakis (4-carboxyphenyl)porphyrin:Cc complexes suggests that side-chain flexibility gives rise to the conformational distributions in the ^{1}COP → Cc ET whereas differences in overall porphyrin charge regulates gating of the back ET reaction (reduced Cc → COP^+).

INTRODUCTION

Electron transfer (ET) reactions play a pivotal role in the catalytic cycles of a wide range of biologically important processes including nitrogen fixation, photosynthesis, and respiration (Barbara et al., 1996; Boxer, 1990; Marcus and Sutin, 1985; McLendon and Hake, 1992; Winkler and Gray, 1992). Biological ET reactions occur between electrostatically stabilized protein:protein complexes and between various redox-active cofactors embedded within a single protein complex. Intramolecular ET rates are modulated by donor-acceptor distance, thermodynamic driving force, donor-acceptor orientation, and the nature of the intervening medium (Cave et al., 1986; Gaines et al., 1991; Isied et al., 1988; Nocek et al., 1990; Nuevo et al., 1993; Osuka et al., 1990; Siddarth and Marcus, 1990; Siders et al., 1984; Wuttke et al., 1992). The rates of intermolecular ET reactions require additional mechanistic steps described as: 1), formation of the protein:protein complex; 2), ET between the redox centers of each protein within the complex; and 3), dissociation of the protein:protein complex. Thus, the rate of ET in such complexes may have an additional component to the overall reorganizational energy required for intermolecular ET which influences protein:protein recognition and docking (i.e., gating effects; see also Clark-Ferris and Fisher, 1985; Corin et al., 1991; Fox et al., 1990; Marcus, 1956; Mauk et al., 1994; McLendon and Miller, 1985; Miller et al., 1984; Wallin et al., 1991; Zhou et al., 1990; Zhou and Kostic, 1992, 1993a).

Conformational dynamics associated with the interface between the donor and acceptor proteins have been suggested for complexes involving cytochrome *c* (Cc) and either cytochrome *c* peroxidase (CCP) or plastocyanin (PC) (Zhou and Kostic, 1992; Mei et al., 1999; Zhou and Hoffman, 1993). In the case of intermolecular ET between ^{3}ZnCc and PC, or ^{3}SnCc and PC, the kinetic data could be explained using a model in which rearrangement of the proteins within the complex occurs subsequent to docking, but before ET (i.e., conformational gating; see also Zhou and Kostic, 1993b). The corresponding back ET reactions were not found to be gated. In the Cc/CCP system, it has been suggested that two docking sites are available on CCP for Cc which differ in their reactivity (Stemp and Hoffman, 1993; Zhou and Hoffman, 1993). A model in which interconvertible conformational substates within the complex regulates ET between the two proteins has been proposed to account for the presence of multiphasic kinetic data (Mei et al., 1999; Wallin et al., 1991).

An alternative approach to study intermolecular ET in proteins involves the use of small photoactive molecules

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electrostatically bound to the docking region of redox proteins. Previous studies by Clark-Ferris and Fisher (1985) and Zhou et al. (1990) have demonstrated that anionic porphyrins can form complexes with Cc by forming electrostatic contacts with exterior lysines associated with the protein. The lysine residues believed to participate in complex formation are Lys 13, 27, 72, and 86. In addition, Zhou et al. (1990) and Zhou and Rodgers (1991) have shown that excitation of bound photoactive uroporphyrin results in fixed-distance ET with rates that depend on solution ionic strength. Interestingly, the variation in ET rate with reaction driving force is consistent with semiclassical Marcus theory for the thermally activated back ET reaction, whereas the photoinitiated forward reaction showed no inverted region with the same range of driving force. The lack of an inverted region was attributed to contributions from the coordinate solvent mode or to conformational gating.

Previously our laboratory has utilized self-assembled uroporphyrin (URO)/Cc and tetrakis (4-carboxyphenyl)porphyrin (4CP)/Cc complexes to probe mechanisms of conformational gating (see Fig. 1 for porphyrin structures; see also Larsen et al., 1997). In both systems, complexation of the porphyrin to the protein results in bathochromic shifts in the absorption bands of the porphyrin. Interestingly, equilibrium circular dichroism data demonstrates that the transition dipoles of URO and 4CP that couple to the heme group of Cc are oriented 90° relative to each other. The effect of orientational differences on photoinduced electron transfer between the bound porphyrin and the heme group of Cc are demonstrated in the steady-state and time-resolved fluorescence and triplet-triplet transient absorption data obtained for the two complexes. In the case of the 4CP/Cc complex, the singlet state of the 4CP is significantly quenched by the heme group of the protein. Analysis of the time-resolved fluorescence data reveals two discrete lifetime components at 9.3 ns (free 4CP) and 1.27 ns (bound 4CP). Fluorescence lifetime analysis of URO complexed to Cc reveals two components consisting of a discrete component at 15.7 ns (free URO) and a Lorentzian distribution of lifetimes centered at 3.8 ns (Larsen et al., 1997). The efficient quenching was observed in both the Stern-Volmer analysis of the steady-state fluorescence and in the appearance of a shorter lifetime component of the time-resolved fluorescence analysis. Subsequently, the quenching of singlet state 4CP was significant enough that the intersystem crossing yield was diminished, resulting in very little triplet state formation and no triplet ET was observed. On the other hand, URO singlet state was less quenched by Cc such that a significant population of the triplet state was generated after photoexcitation. The URO triplet state quenching results in intercomplex electron transfer in which the observed forward and reverse rates are similar, at $(1.8 \pm 0.2) \times 10^6\ s^{-1}$ and $(1.6 \pm 0.4) \times 10^6\ s^{-1}$, respectively. The difference in ET mechanism (i.e., singlet versus triplet) can be rationalized in terms of distinct dipole orientations of the bound porphyrins relative to the heme group of the protein. It was further suggested that the distribution of lifetimes was a result of the flexibility of the alkyl carboxylic acid side chains of URO as compared to the more rigid phenyl groups of 4CP.

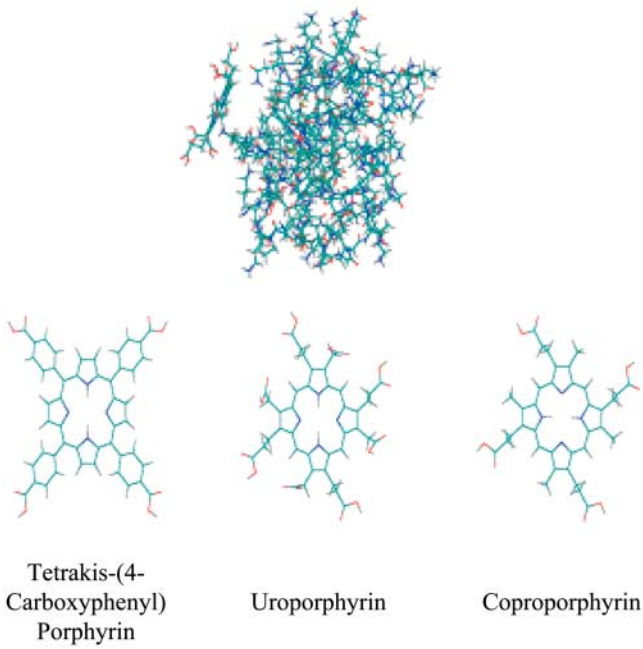

FIGURE 1 Structural diagrams of Tetrakis-(4-carboxyphenyl)porphyrin (*4CP*), Uroporphyrin (*URO*), and Coproporphyrin (*COP*). A model for the porphyrin:Cc complex is also shown.

Although the previous URO and 4CP studies established clear differences in both ^{1}Porphyrin → Cc and ^{3}Porphyrin → Cc ET mechanisms, the individual contributions of the charge and structure at the interface to the modulation of the ET reaction could not be unequivocally determined since URO differs from 4CP in both overall charge and peripheral structure. The present study extends the previous work with the aim of resolving the role of charge/structure in the modulation of the ET reaction by examining the temperature dependence of intramolecular ET within a self-assembled complex of coproporphyrin and Cc. Both singlet and triplet ET is examined. Coproporphyrin retains the charge of the 4CP (i.e., −4) but possesses the same flexible side chains as URO. The results presented here clearly demonstrate that the side-chain flexibility gives rise to conformational dynamics regulating singlet state ET. It is further demonstrated that the overall porphyrin charge significantly influences the back ET rate subsequent to triplet state ET.

MATERIAL AND METHODS

Bovine heart cytochrome *c* (Cc) (Sigma, St. Louis, MO) and free base coproporphyrin (COP) (Porphyrin Products) were used without further purification. Cc stock solutions were prepared in 5 mM potassium phosphate buffer, pH 7.0. COP stock solutions (~1 mM) were prepared in 0.1 N NaOH. The concentrations of the stock solutions were determined using ε_{550}

$= 19\ mM^{-1}\ cm^{-1}$ (Cc reduced minus oxidized), and $\varepsilon_{552} = 16.8\ mM^{-1}\ cm^{-1}$ (COP diluted in 0.1 N HCl). (Furhrhop and Smith, 1975; Margoliash and Frohwirt, 1959).

Optical absorption difference measurements were performed by diluting appropriate aliquots of the porphyrin and Cc stock solutions to a range of micromolar concentrations in 5 mM phosphate buffer, pH 7.0. One mL of each dilution was then placed in each side of a quartz tandem mixing cell (total path length was 1 cm), which was then sealed with a Teflon cover and the absorption spectrum recorded. The tandem cell was inverted to allow mixing of the porphyrin and protein. After a 10-min incubation period, the absorption spectrum of the combined protein:porphyrin solution was recorded. The optical difference spectrum is obtained by subtracting the spectrum before mixing from the spectrum after mixing.

Time-resolved fluorescence measurements were performed using an ISS-K2 multifrequency phase and modulation spectrofluorometer (ISS, Champaign, IL) equipped with an Argon ion laser (model 2045, Spectra-Physics, Mountain View, CA) as the excitation source. The sample chamber was temperature controlled to within ± 0.1°C. Samples were excited using the 488 nm Argon ion line and emission at wavelengths >530 nm was recorded through a Schott RG083 cut-on filter (for a description of phase and modulation time-resolved fluorescence methods, see Lakowicz, 1999; Valeur, 2002). Porphyrin sample concentrations were 15 μM; the samples were prepared to have a 30:70 ratio of complex to free porphyrin, to perform the temperature-dependent study. Fluorescein in 0.1 N NaOH was used as a lifetime reference standard ($\tau = 4.05$ ns). Lifetime data was analyzed using either software provided by ISS or Globals Unlimited (University of Illinois), using previously described fitting routines (Beechem et al., 1991; Jameson et al., 1984).

Triplet-state kinetics were examined using ns-transient absorption instrumentation (described in detail in Larsen et al., 1997). Briefly, COP/Cc samples were excited at 532 nm using a pulsed Nd:Yag laser (Continuum SureLite I, 7 ns FWHM and 7 mJ/pulse, Continuum, Santa Clara, CA). Changes in absorption were monitored using a Xe arc lamp (Oriel, Stratford, CT). The Xe probe light was overlapped with the pump laser in the sample cuvette and subsequently imaged onto the entrance slit of a SPEX 1680 1/4M double monochrometer (SPEX, Edison, NJ). The temperature of the sample cuvette was digitally controlled to within ± 0.1°C. The signal from the detector, a thermoelectrically cooled R928 (Hamamatsu, Bridgewater, NJ) photomultiplier tube, was amplified and then digitized using a Tektronix RTD710A 200 MHz transient digitizer (Tektronix, Beaverton, OR) and transferred to a 486-based microcomputer for further processing. Data fits were obtained using either Enzfitter (Biosoft, Ferguson, MO) or SigmaPlot (Chicago, IL) software.

RESULTS

Ground state complex

In both the URO/Cc and the 4CP/Cc systems, complexation of the porphyrin to the protein results in shifts in the porphyrin optical absorption bands. These shifts can be used to determine the binding constant of the complex. In the absence of Cc, the absorption spectrum of COP exhibits an absorption band with a maximum at 608 nm (Fig. 2 *I*). Upon addition of Cc, there is a bathochromic shift in the absorption band of COP (Fig. 2 *II*) proportional to the concentration of the complexed species. The bathochromic shift in the absorption bands results in a clear isosbestic point throughout the titration, indicating the presence of only two absorbing species in equilibrium. A shift in the absorption spectrum can be viewed more clearly by examining the complexed minus noncomplexed difference spectrum (Fig. 3).

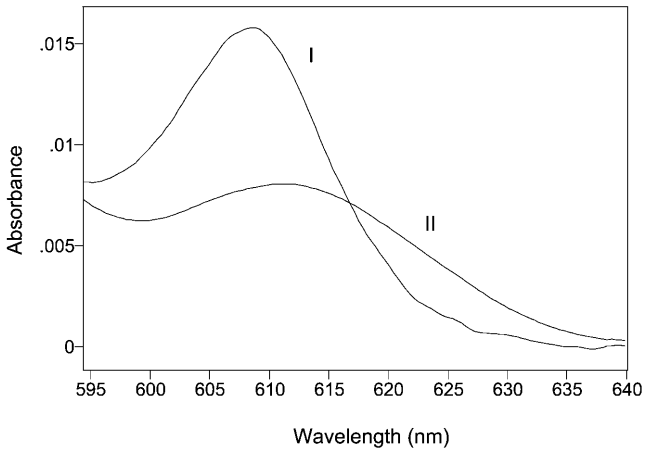

FIGURE 2 Optical absorption spectrum of COP (6 μM) in the absence (*I*) and presence (*II*) of Cc (15 μM).

The change in absorbance at 622 nm was plotted versus the concentration of Cc and fit to a 1:1 binding equation:

$$\Delta Abs = K_a \Delta A_\infty [L]/(1 + K_a[L]), \quad (1)$$

where K_a is the association constant for the complex and ΔA_∞ is the absorbance of the complex at infinite Cc concentration (i.e., fully complexed coproporphyrin). An adequate fit to Eq. 1 (Fig. 3, *inset*) was obtained indicating 1:1 complex formation with an association constant of $2.63 \pm 0.05 \times 10^5\ M^{-1}$ in 5 mM phosphate buffer, pH 7.5. The value of the association constant is similar to the association constant of the URO\Cc complex, reported to be $\sim 9.5 \pm 10^5\ M^{-1}$ in 4 mM ionic strength buffer solution (Zhou et al., 1990).

Singlet electron transfer

At all temperatures, the fluorescence lifetime data for the COP/Cc system were best fit to two components (Fig. 4). The longer lifetime components were assigned to uncomplexed porphyrin, while the shorter components were attributed to the COP/Cc complex. At all temperatures studied, the short lifetime component did not fit well to a discrete lifetime and was best fit to a Gaussian distribution of lifetimes; at 20°C, for example, the short component fit best to a Gaussian distribution centered at 2.8 ns and a FWHM of 1.5 ns.

The observed distribution of lifetimes, attributed to Cc quenching of the COP singlet state, is concentration independent and assigned as ET between ^{1}COP and the heme group of Cc. Since COP has a similar peripheral substitution as URO (i.e., flexible carboxylic acids) and the same charge distribution of 4CP, the observation of a distribution of lifetimes suggests that the flexibility of the side chains is responsible for the singlet lifetime distribution in both the COP/Cc and URO/Cc complexes.

The temperature dependence of the singlet ET has been determined by studying the time-resolved fluorescence over a

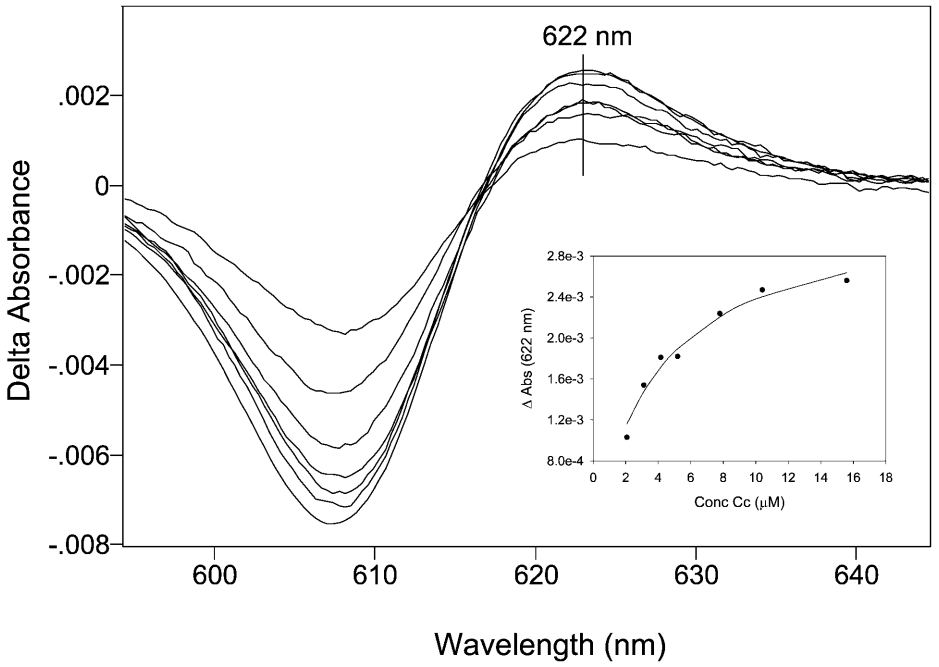

FIGURE 3 Absorption difference spectra of COP titrated with Cc; an isosbestic point is observed at 616 nm. (*Inset*) Depicting plot of $\Delta A_{622\ nm}$ versus [*COP*].

range of temperatures (~40 K). Fig. 4 shows the temperature dependence of the time-resolved fluorescence data acquired for COP in the presence of Cc (summarized in Table 1). The lifetime of the short component attributed to the complex decreases as the temperature increases, indicating an increase in ET rate constant. Similar to the URO/Cc system, the width of the distribution for the complex narrows, for COP/Cc, from 3.1 ns to ~1 ns as the temperature is increased.

The temperature dependence of the rate constants ($k_{ET} = 1/\tau_q - 1/\tau_o$) for ET between the ^{1}COP and the heme group of Cc can be fit to the semiclassical Marcus equation:

$$k_{ET} = \frac{2\pi}{\hbar}(H_{AB})^2 \frac{1}{\sqrt{4\pi\lambda k_B T}} e - \frac{(\Delta G^0 + \lambda)^2}{4\lambda k_B T}. \qquad (2)$$

In Eq. 2, H_{AB} is an electronic coupling factor that describes distance and orientation effects on the tunneling matrix element, λ is the reorganizational energy and is the sum of inner and outer sphere components, k_B is Boltzmann's constant, T is the temperature, and ΔG^o is the reaction free energy (Marcus, 1956). The ET rate constants were fit directly to Eq. 2 using nonlinear regression analysis. To fit the temperature-dependent data to the Marcus equation, the free energy (ΔG^o) for each of the reactions must be known. The free energy change associated with each of the photoinduced electron transfer reactions has been calculated. The Rehm-Weller equation was utilized to estimate the free energy changes for both types of the photoinduced electron transfer reactions (originating from ^{1}COP or ^{3}COP). The

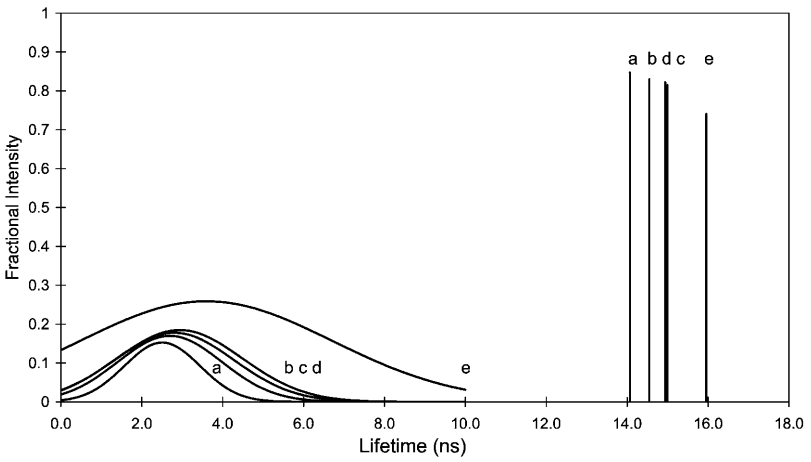

FIGURE 4 Fluorescence lifetime distributions for COP in the presence of Cc at various temperatures. The shorter lifetime component has been best fit to a Gaussian distribution and assigned to intercomplex ET quenching of ^{1}COP, whereas the longer lifetime (free COP) is fit to a discrete lifetime. Temperatures are: $a = 322.8$ K, $b = 313.4$ K, $c = 304.3$ K, $d = 293.7$ K, and $e = 284.5$ K.

TABLE 1 Lifetime distributed data as a function of temperature

Temp (K) ±0.1	τ_1 (ns) Gaussian*	Width (ns)	Fraction	τ_2 (ns) Discrete	Fraction
284.6	3.59	3.12	0.26	15.96	0.74
293.8	2.80	1.48	0.18	14.94	0.82
304.5	2.94	1.55	0.19	15.00	0.82
313.3	2.69	1.29	0.17	14.55	0.83
322.8	2.51	0.93	0.15	14.07	0.84

*Center of Gaussian distribution of lifetimes.

Rehm-Weller equation (Rehm and Weller, 1970) estimates the free energy change between a donor (D) and an acceptor (A) as:

$$\Delta G^{o} = e[E^{o}_{D} - E^{o}_{A}] - \Delta E^{*} + w, \quad (3)$$

where e is the unit electrical charge, E^{o}_{D} and E^{o}_{A} are the reduction potentials of the electron donor and acceptor, respectively, ΔE^{*} is the energy of the singlet or triplet excited state, and w is the work required to bring the donor and acceptor to within the ET distance. The work term in this expression can be considered to be '0' inasmuch as there exists an electrostatic complex before the electron transfer.

The estimated ΔG^{o} values were calculated to be $\Delta G^{o} = -142$ kJ/mole (-1.46 eV) for ^{1}COP to Cc, and $\Delta G^{o} = -104$ kJ/mole (-1.06 eV) for ^{3}COP to Cc kJ/mol (using 0.80 V for the oxidation potential of COP and 0.26 V for the reduction potential of Cc and singlet/triplet energies of 194 kJ/mol and 156 kJ/mol, respectively) (Armstrong et al., 1985; Kalyanasundaram et al., 1988). These estimated ΔG^{o} values predict both favorable singlet and triplet electron transfer within the complex. For the distribution of singlet lifetimes, the center represents the most populated or overall average conformation and the variation in this value as a function of temperature was used to make the plot of rate constant versus temperature shown in Fig. 5. By directly fitting the temperature-dependent rate data to the semiclassical Marcus equation using a nonlinear regression routine and the estimated ΔG^{0}, values of $\lambda = 0.89 \pm 0.01$ eV and $H_{AB} = (8.0 \pm 0.7) \times 10^{-4}$ eV were determined for the singlet ET reaction.

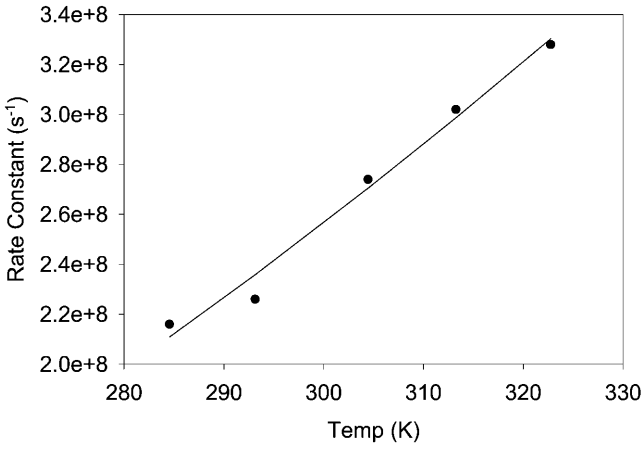

FIGURE 5 Plot of the rate constant (k_{ET}) versus temperature for the center of the ET Gaussian distribution of the COP/Cc complex. (*Solid line*) Represents the best fit to the semiclassical Marcus equation.

Triplet electron transfer

Nanosecond transient absorption spectroscopy was used, *top* to probe the triplet state ET within the COP/Cc complex. Coproporphyrin exhibits a long-lived triplet state ($\tau = 2$ ms, Fig. 6, *top*) in the absence of Cc, similar to other porphyrins previously studied. Addition of Cc to a solution containing COP results in significant quenching of the triplet state (monitored at 434 nm, an isosbestic between reduced and oxidized Cc). The triplet state decay becomes biphasic as the concentration of Cc is increased (Fig. 6, *bottom*), similar to the previously studied URO/Cc complex (Larsen et al., 1997;

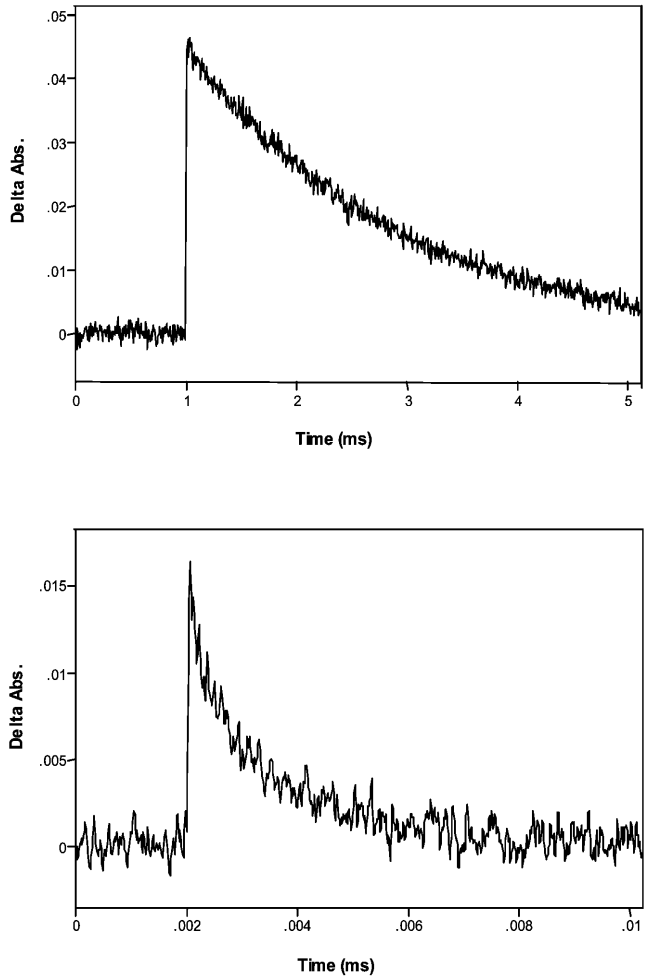

FIGURE 6 Transient absorption of COP (15 μM) at 434 nm in the absence (*top*) and presence (*bottom*) of Cc (45 μM). Samples were deaerated with argon. Traces represent the average of 100 shots on 5-ms (*top*) and 10-μs (*bottom*) timescales, respectively.

Zhou et al., 1990). Absorption changes at 550 nm (absorption maximum in Fe^{2+}Cc spectrum) were also examined to probe the ET rates between the COP and the heme of Cc.

Fig. 7 is a representative nanosecond transient absorption trace of the COP/Cc complex with a 550 nm probe. The biphasic trace represents the forward ET resulting in reduction of the Cc and subsequent reoxidation of the heme group by thermal back ET within the complex. The transient absorption traces could be fit to a double exponential decay revealing a forward ET rate constant of $(7.6 \pm 0.3) \times 10^6$ s^{-1} and a back ET rate constant of $(2.4 \pm 0.3) \times 10^5$ s^{-1} at 25°C. Previous studies (Larsen et al., 1997) have shown that the forward triplet rate constant for URO/Cc was similar to the corresponding thermal back ET rate constant. In contrast, the COP back ET rate constant is significantly smaller than the corresponding forward ET rate constant. Since URO and COP differ only in charge distribution, the triplet kinetic data demonstrates that the charge distribution of the porphyrin modulates the triplet ET reaction.

The triplet ET reaction rates (both forward and back) exhibit significant temperature dependences. The Marcus parameters have been extracted from the temperature-dependent data as described earlier. Fig. 8 displays a plot of the reaction rate versus temperature for both the forward ET reaction and the thermal back ET reaction. The temperature-dependent data has been fit to the semiclassical Marcus theory, represented in Fig. 8 by the solid line. The ΔG^os used in the fit were estimated to be -1.06 eV and -0.55 eV for the triplet forward ET and thermal back ET, respectively (as described earlier). Using these estimated ΔG^os, λ values of 0.39 ± 0.04 eV/0.11 ± 0.02 eV and $H_{AB} \sim 2 \times 10^{-3}$ eV/1×10^{-2} eV, were obtained for the forward and thermal back ET reactions, respectively.

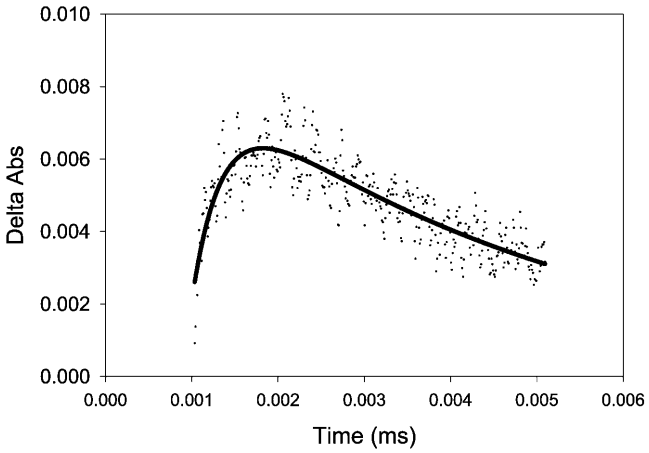

FIGURE 7 Representative transient absorption trace of the triplet ET within COP/Cc complex solubilized in a 5 mM phosphate buffer, pH 7.0, monitored at 550 nm. The sample contained 15 μM Cc and 45 μM COP. The trace is the average of 100 pulses.

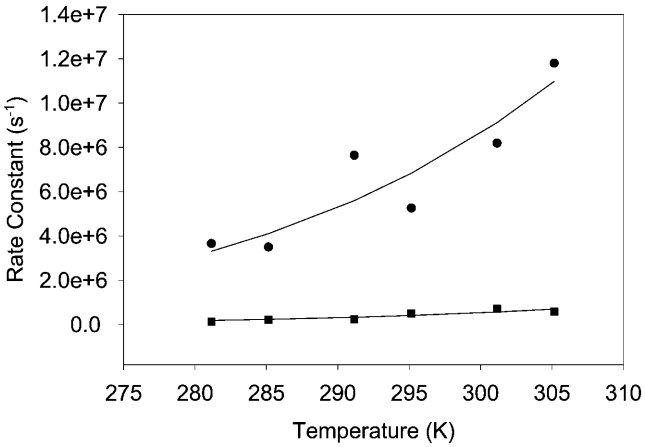

FIGURE 8 Plot of rate constant (k_{ET}) versus temperature for the COP/Cc electron transfer reaction, with the forward originating from the triplet state of COP and the thermal back transfer. (*Solid line*) Represents the best fit to the semiclassical Marcus equation.

DISCUSSION

Effect of porphyrin peripheral structure on singlet ET rates

Previous studies (Larsen et al., 1997) involving porphyrin: protein model complexes indicated significant differences in ET reaction rates depending on the nature of the bound porphyrin. The moderate quenching of the ^{1}URO allows formation of a significant triplet population that is further quenched through triplet ET, in contrast to the intense quenching of the ^{1}4CP and the observation of only singlet ET. The fluorescence lifetime data for the ^{1}4CP/Cc was best fit to a discrete lifetime, whereas the ^{1}URO/Cc was best fit to a distribution that was suggested to arise from the flexibility of the URO peripheral groups.

It was thought that the flexibility of the side chains can cause small variations in the intracomplex ET distance and/or electronic coupling factors, thus affecting the rate of the ET reaction. If the variations are on a timescale slower than the 3-ns range of the singlet lifetimes, then the singlet spectra would represent a snapshot of various porphyrin/Cc ET distances. Assuming the absence of an overwhelmingly preferential distance, a sampling of the distances at any time would be represented by a distance distribution. Subsequently, the ET rates would be expected to display a similar distribution.

In this study, it has been demonstrated that the singlet state lifetime of the COP/Cc complex is also best fit to a distribution of lifetimes. Since COP has the same overall charge as 4CP, the only difference is the flexibility of their peripheral groups. Therefore it can be concluded that the flexibility of the side chains is responsible for the interfacial dynamics of the singlet ET reaction, resulting in a distribution of lifetimes for both COP/Cc complex and the previous studied URO/Cc complex.

In addition, the temperature dependence of the singlet distribution of COP/Cc complex revealed similar behavior as the URO/Cc complex. This observation verifies that multiple conformations exist that can be represented by a potential energy surface where the surface should be made up of many shallow wells within an overall larger minimum (Croney et al., 2000; Jasuja et al., 2002). At high temperatures, the barrier of the shallow wells could be overcome creating an equilibrium or an average state that would be represented as a discrete lifetime. As the temperature is decreased, the interconversion rate will gradually reduce, such that multiple stable conformations can exist at any given time. The multiple stable conformations would then give rise to a broader distribution of lifetimes. Recent studies by Liang et al. (2002) also suggest ''dynamic docking'' within complexes between Zn-porphyrin reconstituted myoglobin and cytochrome b_5 in which a number of shallow energy wells make up an ''energy landscape'' for the complex. Within this landscape, only a small fraction of wells are active for ET. Our results suggest that for the COP:Cc system, the landscape is quite broad, and many more ET active states exist.

The reorganizational energy of the singlet ET was also calculated from the temperature-dependent data and found to be 0.89 eV. In the porphyrin:protein system Zhou and Rodgers (1991) obtained a value of 0.7 eV from a driving force dependence analysis. The protein:protein complexes modeled in this study have λ values reported between 0.6 and 1.2 eV.

Effect of porphyrin peripheral charge on triplet state ET

Transient absorption data obtained at 550 nm indicates ET arising from triplet state quenching within the COP/Cc electrostatic complex. It was expected that COP/Cc would exhibit triplet ET with rate constants for the forward and back reactions (^{3}COP $\rightarrow$ Cc and Cc $\rightarrow$ COP$^+$) similar to those obtained for the URO/Cc system. However, the triplet ET of the COP/Cc complex displays different characteristics. Specifically, distinct differences in the thermal back ET rates between the two complexes are observed. Since URO and COP only differ by the number of peripheral substituents, affecting the overall charge, then the charge must influence the triplet ET reaction. Subsequent to photoexcitation and intersystem crossing, the forward ET reaction arises from oxidation of the excited COP and subsequent reduction of the heme group of Cc. The corresponding thermal back ET reaction from the reduced heme of Cc to the COP π-cation radical returns the porphyrin to its ground state. Within the two systems, COP/Cc and URO/Cc, the forward ET reactions have similar rate constants on the order of 3×10^6 s^{-1}. In contrast, the thermal back ET rates are quite distinct for these systems, which can be seen by calculating the average ratio of the forward rate constant (k_f) to the thermal back rate constant (k_b) for each of the systems. In the URO/Cc system the average ratio of k_f/k_b is 6.9, whereas in the COP/Cc system the ratio k_f/k_b is 19. The difference in ET behavior must be a result of the difference in overall charge between the two porphyrins, since all other physical properties are similar.

The observation of distinct rate constants for the two back ET reactions that have the same driving force suggests that there may be another process involved that is gating the ET reaction. The first possibility is that there is a fast reorientation subsequent to the forward ET reaction resulting in a different back ET reaction in the COP/Cc system. In this case, the ET reaction originates from a different conformation that results in a slower rate. This ET reaction should yield reasonable Marcus parameters for fits of temperature-dependent rate data. The second possibility is that a slow reorientation takes place subsequent to the forward ET reaction followed by a reasonably fast ET reaction. In this case the slow reorientation would be the rate-limiting step and the observed rate would not represent a true ET reaction. The reaction rate will still vary with temperature, but the Marcus parameters obtained will be unrelated to the ET event (Davidson, 1996). Therefore, the effect of the charge at the interface can be understood by examining the Marcus parameters determined from analysis of the temperature-dependent rate data. If the Marcus parameters obtained from the temperature-dependent data are reasonable, then a fast reorientation model would be sufficient to describe the kinetics. On the other hand, if anomalous values are obtained, then a slow reorientation process most likely takes place.

Examination of Marcus parameters reveals differences in reorganizational energy of the triplet forward ET relative to the thermal back ET. The difference in λ between these two reactions is ~0.3 eV, which is identical to the difference previously observed in the URO/Cc system. However, the H_{AB} term for the back ET reaction within the COP/Cc system is on the order of 10^{-2} eV compared to10^{-4} eV for the other reactions. Typical H_{AB} values for nonadiabatic reactions are in the range of 10^{-3} to 10^{-5} eV (Davidson, 1996). The anomalous value of H_{AB} obtained for the COP/Cc back ET reaction indicates a gated reaction with a slow reorientation as the rate-limiting step. This slow reorientation could occur as a result of the π-cation radical having a smaller overall net charge than the triplet state, creating a less stable electrostatic interaction. This destabilization, in turn, could result in a conformation where the equilibrium heme-porphyrin distance has increased or the heme-porphyrin orientation has been altered giving rise to a smaller ET rate constant.

Typically in protein:protein systems the ET rate has been observed to fall off exponentially with distance:

$$k_{ET} = k_o \exp[-\beta(R - R_0)], \quad (4)$$

where β is a constant that determines the rate of fall off (1 Å^{-1}), R_0 is the van der Waals separation distance, R is the

actual ET donor/acceptor distance, and k_o is the rate constant at R_0. Using Eq. 4, a change in distance of ~3 Å is necessary to affect the rate constant of the reaction by an order of magnitude. For the porphyrin on the surface of the Cc, a 3 Å change in distance would be a significant perturbation in the electrostatic interactions, being almost twice the equilibrium distance between the bound porphyrin and the heme edge (2.8 Å). Therefore, it is unlikely that a change in distance alone is responsible for the observed modulation of the ET reaction.

A distinct change in the orientation of the bound porphyrin, due to a surface diffusion process that would be the rate-determining step in the thermal back ET reaction of the COP/Cc system, could account for the lowered back ET rate. Within the time immediately after the forward ET, the complex could undergo a conformational change, resulting in a different electronic coupling between the porphyrin and the heme of Cc. In the URO/Cc complex the eight peripheral carboxylic acid side chains of URO form electrostatic contacts with six lysine residues in what has been speculated to be a pseudostatic complex on the triplet timescale. Therefore, no apparent gating is observed. For COP complexes, on the other hand, formation of the π-cation radical may significantly lower the charge density below some threshold where surface diffusion occurs within the docking site, resulting in observed gating of the triplet ET.

CONCLUSION

In this study we have determined the temperature dependence of the rates of singlet and triplet ET between excited COP and the heme group of Cc and fit the data to the semiclassical Marcus equation to reveal the Marcus parameters for the ET reactions. The singlet ET within the COP/Cc complex is represented by a Gaussian distribution of singlet lifetimes. The distribution has been shown to represent multiple conformations within the complex, and the potential surface of the multiple conformations has been described. The triplet ET within the COP/Cc complex has been shown to have different λ for forward and back ET, supporting a conclusion that they are different reactions, as observed in the URO/Cc system. Further it is shown that the thermal back ET reaction of the COP/Cc is gated by apparent surface diffusion of the COP within the docking site of the Cc.

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