Electron Transfer between Cytochrome *c* and Porphyrins

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Abstract: The electron transfer between cytochrome *c* and several water-soluble porphyrins (ZnTPPS, ZnTMPyP, ZnTPPC, H_2TPPS, and H_2TMPyP where TPPS is tetrakis(sulfonatophenyl)porphyrin, TMPyP is tetrakis(*N*-methylpyridyl)porphyrin, and TPPC is tetrakis(carboxyphenyl)porphyrin) has been studied by the method of photoexcitation. For each porphyrin, two rates corresponding to electron transfer from porphyrin triplet to ferricytochrome *c* and from ferrocytochrome *c* to porphyrin cation radical are determined. The second-order rate constant increases from 3.5×10^8 to 11×10^8 M^{-1} s^{-1} (25 °C, 0.5 M ionic strength, pH 7.0) as the driving force ΔE for the reaction increases from 0.54 to 1.06 V. A reorganization energy of 1.0 eV was deduced for the above electron-transfer reactions, when the rates (at infinite ionic strength) are analyzed according to the theories of Marcus and Hopfield.

An important aspect in the study of electron-transfer processes in biological systems is the elucidation of the relationship between the transfer rate and the driving force (ΔE) for the reaction.[2-7] According to the theories of Marcus[2] and Hopfield,[3] the transfer rate increases rapidly with increasing ΔE at low driving forces, attains a maximum at $\Delta E_{max} = \lambda$ (sum of molecular and solvent reorganization energies), and then drops as ΔE further increases. Such a ΔE dependence is unique to the above theories, and their validity can thus be tested by experimental determination of transfer rates over a wide ΔE range. After much effort,[8] the inverted region has been observed only recently by Miller et al.[9] in a pulse radiolysis experiment on a model system in solution with ΔE ranging from 0 to 2.4 V. For protein molecules, however, the situation is less certain since most existing data involve reactions at relatively low driving force ($\Delta E \leq 0.3$ V).[6,10,11]

High ΔE data have recently been obtained by the method of photoexcitation.[5,7,12,13] Meyer et al. studied the electron transfer in cytochromes and other proteins by employing laser-flash generated flavin semiquinones as reductants.[5,13] From measurements on a large number of homologous proteins, transfer rates were obtained over a wide range of driving forces and for ΔE as high as 1.0 V. Another scheme to initiate electron transfer is to use excited complexes as oxidants or reductants.[7,12,14] By this method, we have recently measured the transfer rates between ferrocytochrome *c* and several ruthenium and osmium complexes with ΔE varying from 0.56 to 1.0 V.[7] Although the inverted region has not yet been seen in both cases, the results so far indicate that the transfer rate for cytochrome *c* levels off at a $\Delta E \simeq 1.0$ V.

Another redox system which is amenable to similar studies is the porphyrin, as some of them, when in the triplet state, are strong reducing/oxidizing agents.[15,16] The results should be of particular interest, considering that most electron-transfer proteins have metalloporphyrins as active centers. In this work, we report the results on photoinduced electron transfer between cytochrome *c* and several water-soluble porphyrins including ZnTPPS, ZnTMPyP, ZnTPPC, H_2TPPS, and H_2TMPyP, where TPPS = tetrakis(sulfonatophenyl)porphyrin, TMPyP = tetrakis(*N*-methylpyridyl)porphyrin and TPPC = tetrakis(carboxyphenyl)-porphyrin.

Experimental Section

Horse-heart cytochrome *c* (Type VI, Sigma Chemical Co.) was used as received. The measurments were conducted on two separate batches of samples which contained respectively (11.5 ± 0.5)% and (10.3 ± 0.5)% of ferrocytochrome *c* as determined by the addition of a slight excess of $K_3Fe(CN)_6$ to several concentrations of protein and monitoring the optical density at 550 nm.

Free-base porphyrins tetrapyridylporphyrin (H_2TPyP), tetrakis(sulfonatophenyl)porphyrin (H_2TPPS), and tetrakis(carboxyphenyl)-porphyrin (H_2TPPC) were obtained from Strem Chemicals and other porphyrins were prepared as follows.[16,17] Free-base porphyrin tetrakis-(*N*-methylpyridyl)porphyrin (CH_2TMPyP) was prepared by methylation of H_2TPyP with methyl iodide and was purified by successive reprecipitations from NaI solutions. The chloride salt of H_2TMPyP was obtained by passing an aqueous solution of the corresponding iodide complex through a Dowex 2 anion exchange column in the Cl^- form. Zn-(TPyP) was prepared by refluxing zinc acetate (0.10 g) with H_2TPyP (0.1 g) in glacial acetic acid. The tetramethylpyridinio derivative, NTMPyP, was prepared and purified by essentially the same procedure as described for H_2TMPyP. Insertion of Zn(II) into H_2TPPS was carried out by refluxing zinc acetate (0.1 g) with H_2TPPS (0.1 g) in DMF for 10 min. When the reaction was completed, the solvent was subsequently removed on a rotatory evaporator under vacuum. The substance was purified by passing it through a Dowex 50 cation exchange column in the Na^+ form. Zn(TPPC) was prepared according to the procedures of Adler et al.[17] and subsequently purified on a Sephadex column.

The electron-transfer rate was studied by using a conventional transient absorption setup with the 355-nm output of a Quanta-Ray DCR-2 Nd:YAG laser as the excitation source. Typically, the laser energy used was 5 mJ/cm^2 per pulse. The kinetics was monitored by following the absorbance change at selected wavelengths between 500 and 650 nm, depending on the porphyrin used. The signals were usually averaged for 100 laser shots. For each porphyrin, the reaction rate was measured as a function of protein concentration (10-100 μM) at a constant porphyrin

(1) (a) The Chinese University of Hong Kong. (b) The University of Hong Kong.
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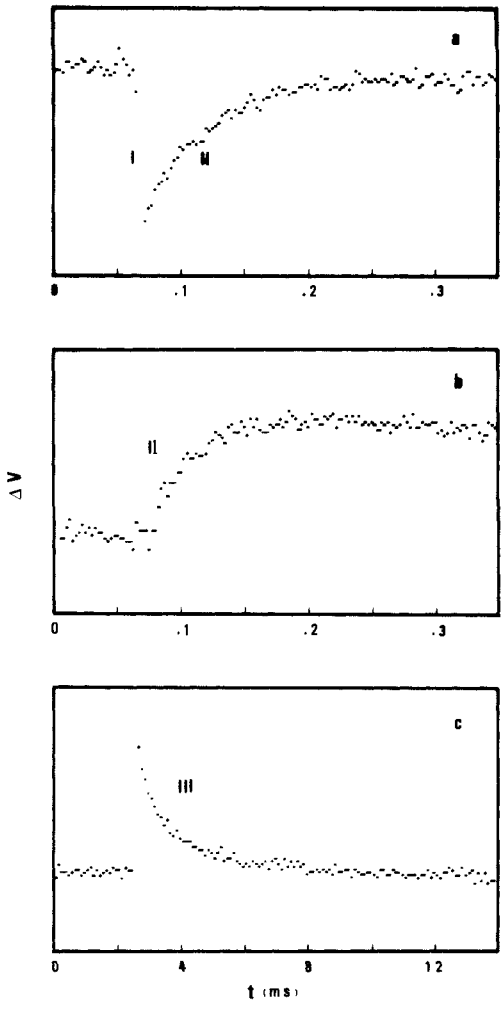

Figure 1. Typical transients obtained for the (ZnTPPS–cytochrome *c*) system at (a) 553 nm, (b) 605 nm, and (c) 605 nm. I, II, and III correspond to three different processes observed following excitation (see text for details). The sample contained 20 μM (oxidized + reduced) cytochrome *c* in 0.5 M NaCl and 5 mM (pH 7) phosphate buffer.

concentration (typically 10 μM for H_2TPPS, 50 μM for all other porphyrins) in 5 mM pH 7 phosphate buffer under anaerobic conditions. Studies of the effect of porphyrin concentration show that the rates are not affected by a threefold variation in concentration. For all systems, except (H_2TMPyP–cytochrome *c*), the protein used contained approximately 10% of reduced species. For (H_2TMPyP–cytochrome *c*) the protein was 100% oxidized, prepared by addition of a slight excess of $K_3Fe(CN)_6$ and then followed by extensive dialysis. The ionic strength of the sample was adjusted by the addition of NaCl.

Results

I. Assignment of Absorbance Changes. The absorbance changes following the laser flash occur in three separate stages. The nature of each stage is identified by examining the signal as a function of wavelength. The features seen for the various porphyrins are essentially the same, and only those for the (ZnTPPS–cytochrome *c*) system are shown as illustrations. The instantaneous change after excitation (process I, Figure 1a) corresponds to the generation of the excited triplet state (P*) of the porphyrin (P).

$$P + h\nu \rightarrow P^* \qquad (1)$$

The subsequent change in absorbance (process II, Figure 1a,b) reflects the decay of P* and the concomitant reduction of ferricytochrome *c* (which we denote as the forward reaction).

$$P^* + \text{cyt-}c^{3+} \xrightarrow{k_f} P^+ + \text{cyt-}c^{2+} \qquad (2)$$

The identification is obtained by studying the signal amplitude vs. wavelength at a time after process II has been completed. As shown in Figure 2, the spectrum thus obtained for the

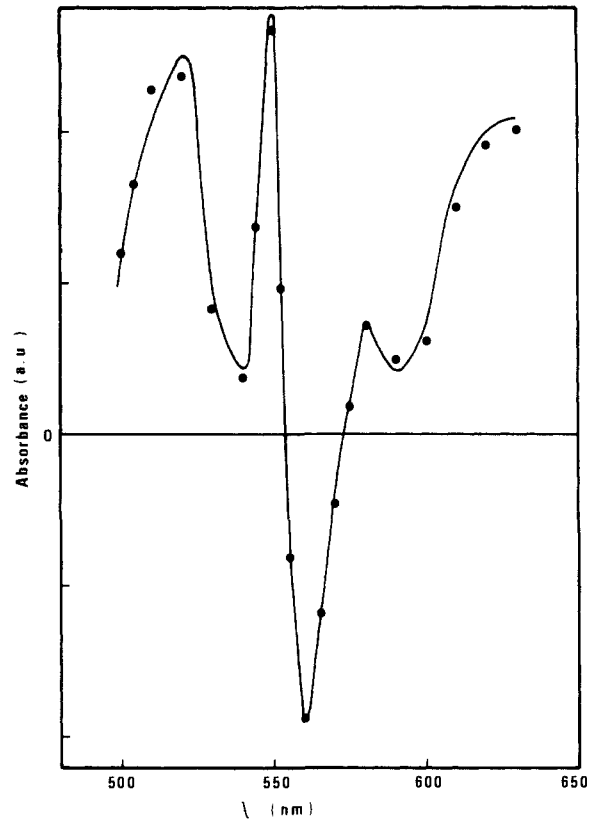

Figure 2. Difference spectrum obtained for the (ZnTPPS–cytochrome *c*) system by monitoring the absorbance at a time after process II (Figure 1) has been completed. The solid line denotes the spectrum calculated from a linear combination of (cyt-c^{3+}–cyt-c^{2+}) and (ZnTPPS–$ZnTPPS^+$).[18] The experimental conditions are the same as in Figure 1.

(ZnTPPS–cytochrome *c*) system agrees well with that calculated from a linear combination of the difference spectra of (cyt-c^{3+}–cyt-c^{2+}) and (ZnTPPS–$ZnTPPS^+$).[18] This assignment is further corroborated by the fact that the decay of P* (process II, Figure 1a) and the production of P^+ and cyt-c^{2+} (process II, Figure 1b) are simultaneous.[19] The final decay (process III, Figure 1c) corresponds to the back reaction

$$P^+ + \text{cyt-}c^{2+} \xrightarrow{k_b} P + \text{cyt-}c^{3+} \qquad (3)$$

which is expected to occur since P^+ is strongly oxidizing. This identification is supported by the fact that the absorbance returns to its preflash base line and that the rate of conversion from P^+ to P and from cyt-c^{2+} to cyt-c^{3+} as determined at different wavelengths are equal.

II. Quenching (Forward Reaction) Rate Constants. The decay of the porphyrin triplet is best monitored by its transient absorption as it is only weakly phosphorescent.[16] Within experimental errors, the decay follows a single exponential over 3–4 half-lives. In all cases, a good linear relationship between τ_0/τ and cyt-c^{3+} concentration was observed (τ and τ_0 being the triplet lifetime with and without cyt-c^{3+} present) (Figure 3), , and the bimolecular quenching rate constant k_q can be determined from the slope of such a plot. As seen in Figure 3, all porphyrins, except, H_2TMPyP, quench cyt-c^{3+} efficiently. The quenching rate k_q will be equal to the electron-transfer rate k_f in eq 2 only if quenching of P*

(18) Difference spectrum of (cyt-c^{3+}–cyt-c^{2+}) is obtained from the following reference: Margoliash, E.; Frohwirt, N. *Biochem. J.* **1959**, *71*, 570–572. Difference spectrum of (ZnTPPS–$ZnTPPS^+$) is determined by ourselves using the transient absorption setup with $K_3Fe(CN)_6$ (which has essentially no absorption between 500 and 600 nm for both the oxidized and reduced form) as the electron acceptor.

(19) The two processes can be observed separately at selected isosebestic points. For example, for ZnTPPS, the P* decay rate can be monitored at 553 nm which corresponds to the isosebestic point of the (cyt-c^{3+}–cyt-c^{2+}) + (P–P^+) difference spectra, whereas the electron transfer rate can be determined at the P* – P isosebestic point at 605 nm.

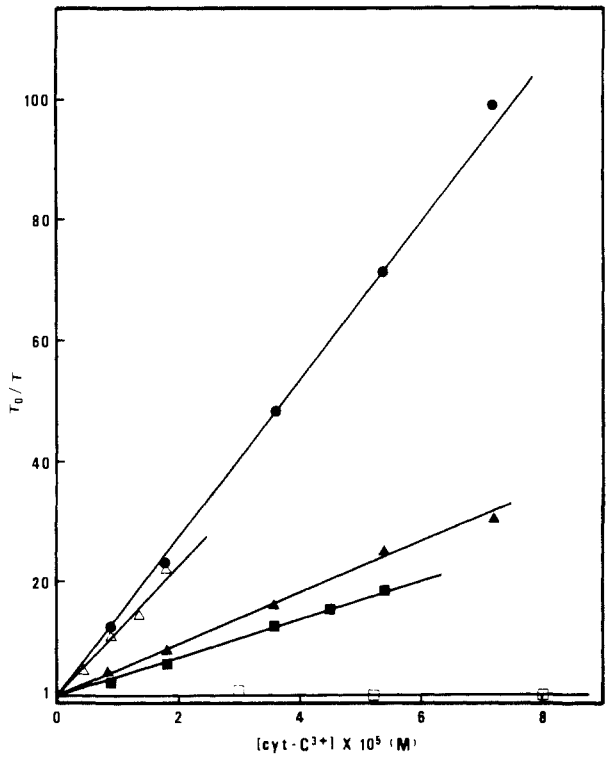

Figure 3. Stern–Volmer plots of quenching of ZnTPPS (●), ZnTPPC (■), ZnTMPyP (▲), H_2TPPS (△), and H_2TMPyP (□) by ferricytochrome *c*. The ionic strength is 0.51 M except for reaction involving H_2TPPS which is 0.01 M.

by energy-transfer processes is small. As will be shown in the discussion, this is true for our present case.

The variation of the quenching rate constants with ionic strength I depends on the porphyrins used. As the ionic strength increases from 0.01 to 0.5 M, k_q for ZnTPPS and ZnTPPC decrease by a factor of 5.5, while that for ZnTMPyP increases by a factor of 4.2 (Figure 4a). The quenching rate for H_2TPPS cannot be determined over a wide range of ionic strength, as this porphyrin dimerizes, especially at high salt concentration.[20] As a result, we have only performed measurement on this prophyrin at I = 0.01 M using a low porphyrin concentration of ~10 μM, and a k_q of 3.3×10^9 M^{-1} s^{-1} was obtained.[21]

III. Back Reaction Rate Constants. The analysis of the back reaction is greatly simplified by the presence of approximately 10% of cyt-c^{2+} in the protein sample. Under this condition, the concentration of cyt-c^{2+} throughout the experiment (>1 μM) is much greater than that of P^+ (estimated to be <0.1 μM), and so the reaction is pseudo-first-order. The plots of the decay rate vs. cyt-c^{2+} concentration are linear in all cases (Figure 5), the slopes of which give the bimolecular electron-transfer rate k_b (eq 3). For H_2TMPyP, we have already seen that little cyt-c^{2+} was generated in the forward reaction, and thus no back reaction could be observed. Figure 4b shows the ionic strength dependence of k_b for the three Zn porphyrins, and the results are essentially the same as those observed for k_q in the forward reaction. For H_2TPPS, $k_b = 2.8 \times 10^9$ M^{-1} s^{-1} at I = 0.01 M.

Discussion

Consider first the quenching reactions. There are two possible quenching mechanisms for the porphyrin triplet P*: (1) energy transfer via dipolar and via exchange interactions, and (2) electron transfer. In the present case, dipolar energy transfer is expected to be small since the phosphorescent quantum yield of the porphyrin is extremely low (<10^{-4}), and this weak emission does not overlap with the cytochrome *c* absorption spectrum.[16] Thus energy transfer can only proceed via the exchange mechanism, which can also be shown to be unimportant as follows. Since the triplet

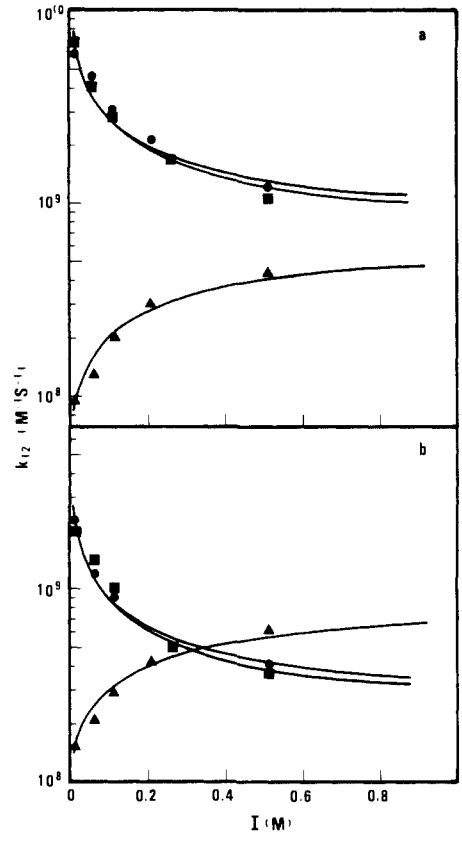

Figure 4. Plot of second-order rate constants for the forward (a) and back (b) reactions vs. ionic strength for ZnTPPS (●), ZnTPPC (■), and ZnTMPyP (▲). Solid lines are calculated curves (see text for details).

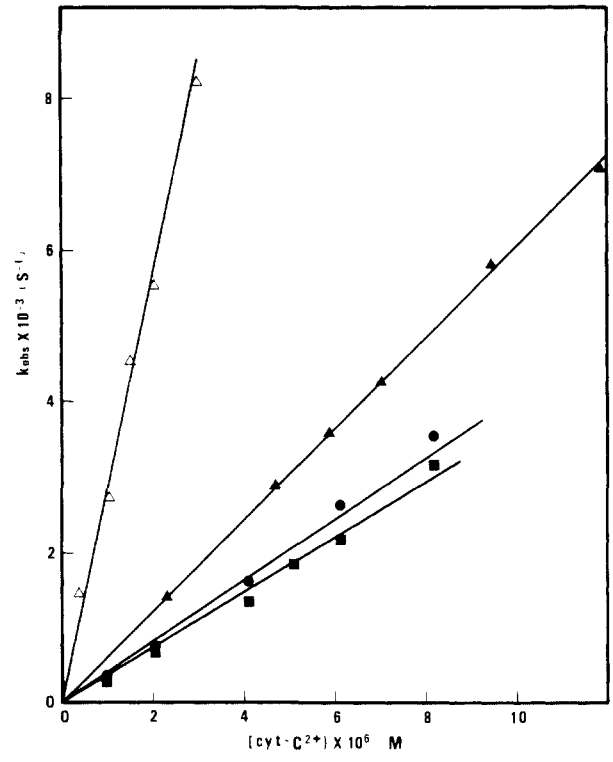

Figure 5. Plot of k_{obsd} vs. cyt-c^{2+} for the oxidation of ferrocytochrome *c* by $ZnTPPS^+$ (●), $ZnTPPC^+$ (■), $ZnTMPyP^+$ (▲), and H_2TPPS^+ (△). The ionic concentration is 0.51 M except for reaction involving H_2TPPS^+ which is 0.01 M.

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(21) H_2TPPS should be chiefly monomeric under our experimental conditions as verified by the following observations: Beer's law holds for H_2TPPS concentration as high as 20 μM; the measured τ_0 agrees with literature values;[16] k_q does not vary for H_2TPPS concentration between 5 and 20 μM.

energy level of all porphyrins studied is similar, the energy contribution to the observed k_q for all of them should be roughly the same. This contribution can be estimated by considering the porphyrin H_2TMPyP which has the lowest k_q of $<2 \times 10^7$ M^{-1} s^{-1}. The quenching rate by energy transfer for all porphyrins thus cannot exceed this value of 2×10^7 M^{-1} s^{-1}, which is more than an order of magnitude smaller than the k_q observed for other porphyrins. Electron-transfer quenching is therefore predominantly responsible for the quenching process in those cases, and the observed k_q can be equated with the forward electron-transfer rate k_f defined in eq 2. The low k_q observed for H_2TMPyP can also be understood because ΔE for its forward reaction (eq 2) with cytochrome *c* is <0.39 V[22] which is the lowest among the porphyrins studied.

Both k_f and k_b vary with ionic strength, as a result of the electrostatic interactions between the porphyrins and the protein. For each porphyrin, k_f and k_b exhibit the same ionic concentration dependence (Figure 4), as they are both controlled by porphyrin–cytochrome interactions which depend mainly on the charges of the porphyrin and the protein side groups. For the various porphyrins, however, the dependence is different, with the electron-transfer rate increasing by a factor of 4.2 for ZnTMPyP and decreasing by a factor of 5.5 for ZnTPPS and ZnTPPC as the ionic strength increases from 0.01 to 0.5 M. This can be understood by noting that the charges of the three porphyrins ZnTMPyP, ZnTPPS, and ZnTPPC are 4+, 4−, and 4−, respectively, while that of cytochrome *c* is 6.5+.[10] For the ($ZnTMPyP^{4+}$–cytochrome $c^{6.5+}$) system, as ionic strength increases, the electrostatic repulsion between the two species is reduced because of charge-screening effects, thereby leading to an increase in transfer rate. Similar considerations apply to the ($ZnTPPS^{4-}$–cytochrome *c*) and the $ZnTPPC^{4-}$–cytochrome *c*) system.

To obtain a genuine ΔE dependence for the transfer rates, it is necessary to extrapolate our results to a condition in which electrostatic effects are negligible. This was performed by determining the rates at infinite ionic strength (k_{12}^{∞}) in the same way as Meyer et al.[13,23] and the results vs. ΔE are shown in Figure 6. According to the theories of Marcus[2] and Hopfield,[3] at low ΔE, the electron-transfer rate increases rapidly with increasing driving force. This increase becomes more gradual at higher ΔE, and the rate reaches a maximum at $\Delta E = \lambda$ (reorganization energy). The observed moderate increase in k_{12}^{∞} with increasing ΔE implies that the ΔE range covered in this experiment corresponds to the region near $\Delta E = \lambda$. A more quantitative analysis of our results was performed by fitting the data to a function of the form $k_{max} \exp[-(\Delta E - \lambda_{12})^2/4\lambda_{12}kT]$,[2-4] and the fit gives $k_{max} = 9.2 \times 10^8$ M^{-1} s^{-1}, $\lambda_{12} = 0.92$ eV.[24] From the calculated curve (Figure 6), it is evident that in order to see the inverted region predicted by the theories, it is necessary to have data with ΔE of 1.2 V or higher.[25] Unfortunately, we are unable to find at present any Zn or related porphyrins which can give such a high ΔE. In assessing the reliability of the obtained k_{max} and λ_{12}, it is relevant to note that the high k_{12}^{∞} ($\sim 9.0 \times 10^8$ M^{-1} s^{-1}) obtained at $\Delta E \sim 1$ V may not correspond to the true rates, as the observed values at such driving forces are near the limit set for diffusion-controlled reactions. If the diffusion-controlled limit k_{diff} is known, the actual transfer rate k_{act} can be deduced from[26,27]

$$\frac{1}{k_{act}} = \frac{1}{k_{12}^{\infty}} - \frac{1}{k_{diff}}$$

The higher k_{act} thus obtained will invariably lead to higher values for k_{max} and λ_{12}. For example, assuming $k_{diff} = 2.0 \times 10^9$ M^{-1} s^{-1}, the fit using the corrected data k_{act} gives $k_{max} = 1.5 \times 10^9$ M^{-1} s^{-1} nd $\lambda_{12} = 0.96$ eV. Similarly, for $k_{diff} = 1.0 \times 10^9$ M^{-1} s^{-1}, $k_{max} = 5 \times 10^9$ M^{-1} s^{-1} and $\lambda_{12} = 1.12$ eV are obtained. The fact that λ_{12} is not much altered by the correction indicates that the value of 1.0 eV is a good typical number for the reorganization energy of the present system.

In comparison with other experiments, it is seen that k_{max} determined for the present (porphyrin–cytochrome *c*) system is somewhat higher than that of 2.5×10^8 M^{-1} s^{-1} for the (ruthenium/osmium–cytochrome *c*) system.[7] This higher rate may result from the delocalization of the porphyrin electron density, which effectively leads to a reduction of the distance involved in the electron-transfer process. As to the reorganization energy λ_{12}, the values obtained for the two experiments are in good agreement with each other, which suggests that the porphyrins used have very similar reorganization energy (and self-exchange rates) for the self-exchange reaction as that of the ruthenium/osmium

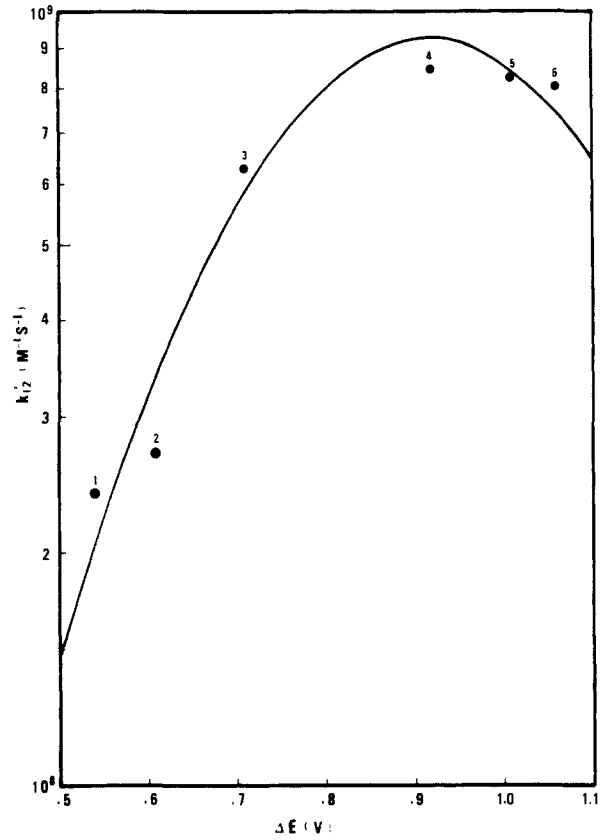

Figure 6. Plot of second-order rate constants (at infinite ionic strength) vs. the driving force ΔE for the electron-transfer reactions between cytochrome *c* and zinc porphyrins. 1, 2, 3, 4, 5, and 6 correspond to k_b for ZnTPPC, k_b for ZnTPPS, k_f for ZnTMPyP, k_b for ZnTMPyP, k_f for ZnTPPS, and k_f for ZnTPPC, respectively. ΔE values are obtained from literature.[10,16] The solid line is the theoretical curve based on the theories of Marcus[2] and Hopfield[3] for $k_{max} = 9.2 \times 10^8$ M^{-1} s^{-1} and $\lambda_{12} = 0.91$ eV (see text for details).

(22) This number is reached from $E(H_2TMPyP^+/H_2TMPyP^*) > -0.13$ V[16] and E(cyt-c^{3+}/cyt-c^{2+}) = 0.26 V.[10]

(23) Assume $\ln k_{12} = \ln k_{12}^{\infty} + [ae^{-b(I^{1/2})}/(1 + b(I^{1/2}))]$ where I is the ionic strength, a is a parameter which is proportional to the product of the effective charges of the two reactants, $b = 0.329\rho$, and ρ is the radius of the interaction domain of the reactants. We take $\rho = 4.5$ Å which is the value obtained for electron transfer between a number of *c*-type cytochromes with flavosemiquinones, and k_{12}^{∞} can be determined from the intercept of the plot of $\ln k_{12}$ vs. $e^{-b(I^{1/2})}/(1 + b(I^{1/2}))$. k_{12}^{∞} thus obtained differ from k_{12} at 0.5 M by about 35%.

(24) Note that the results for H_2TPPS were not used in the fitting because the free base porphyrins may have different molecular reorganization energy as that of the Zn porphyrins. Furthermore, the ionic strength dependence of H_2TPPS cannot be directly measured and thus its k_{12}^{∞} cannot be directly obtained.

(25) The recent work of Marcus and Siders (Siders, P.; Marcus, R. A. *J. Am. Chem. Soc.* **1981**, *103*, 748–752) has shown that quantum effects may be important in the "inverted region". Such effects will in general lead to a decrease in the drop of the electron-transfer rate with increase in driving force in the inverted region as compared to that predicted using the semiclassical theory (see Figure 6). Thus, in our case, one may need to have ΔE much greater than that 1.2 V in order to see the inverted behavior.

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(27) Sutin, N. In *Tunnelling in Biological Systems*; Chance, B., et al., Eds.; Academic Press: New York, 1979; pp 201–224.

complex. By using λ_{12} = 1.0 eV and taking λ_{22} for $Ru(bpy)^{2+/3+}$ self-exchange reaction to be 0.53 eV,[27,28] the reorganization energy λ_{11} for the cyt-c^{2+}/cyt-c^{3+} self-exchange reaction can be estimated from the additivity rule of Marcus[4] to be 1.5 eV. Another system which is related to the present one is the ($ZnP,Fe^{3+}P$) hybrid hemoglobin,[30,31] the reorganization energy λ_{12} of which for intramolecular electron transfer between the two metalloporphyrins has been determined recently to be about 2.3 eV.[31] The large difference (1.0 vs. 2.3 eV) found for the two systems may arise from the presence of an additional protein subunit in the hybrid hemoglobin, and/or that hemoglobin has an intrinsically large λ_{12} because it is not designed to carry out electron-transfer function. Further work along this line involving more proteins will be required before a more definite answer can be reached. Preliminery evidences also indicate that the transfer rate for the hybrid hemoglobin exhibits a much stronger ΔE dependence than what is presently observed within the same ΔE range.[31] This difference is not unexpected in view of the large λ_{12} found for the ($ZnP,Fe^{3+}P$) hybrid.

In conclusion, this work has demonstrated the usefulness of the porphyrin system in the study of the electron-transfer processes in proteins. Two transfer rates can be determined for each porphyrin as it goes through the $P \rightarrow P^* \rightarrow P^+ \rightarrow P$ cycle following the absorption of a photon. In the future, we hope to study several other proteins over a wide ΔE range in order to gain more insight into the electron-transfer mechanism in biological systems.

Registry No. ZnTPPS, 80004-36-0; ZnTPPC, 83294-30-8; ZnTMPyP, 40603-58-5; H_2TPPS, 39174-47-5; H_2TMPyP (chloride salt), 92739-63-4; CH_2TMPyP, 38673-65-3; Zn(TPyP), 31183-11-6; H_2TPyP, 16834-13-2; cyt-c, 9007-43-6.

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Infrared Multiphoton Isomerization Reactions of Alkenes and Dienes

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Abstract: The infrared multiphoton laser-induced unimolecular isomerization reactions of several simple alkenes, conjugated alkenes, and pentadienes have been investigated. Excitation of (*E*)-2-butene, pentene, and hexene results in contrathermodynamic $E \rightarrow Z$ isomerization and fragmentation, the isomerization/fragmentation ratio decreasing with increasing chain length and increasing laser fluence. These results are in qualitative agreement with RRKM calculations for average reactant energies of 75-85 kcal/mol. In addition to products of C-C homolysis observed with all three alkenes, 1-butene and 1,3-butadiene are formed from (*E*)-2-butene. (*E*)-Crotononitrile undergoes laser-induced isomerization without fragmentation, resulting in quantitative conversion to the *Z* isomer. In contrast, (*E*)-methyl crotonate undergoes both isomerization and fragmentation, while (*E*)-ethyl crotonate undergoes essentially quantitative elimination of ethylene. Irradiation of (*E*)- or (*Z*)-1,3-pentadiene at low laser fluences results exclusively in $E \rightleftarrows Z$ isomerization resulting in steady-state isomer ratios which depend upon the relative magnitude of the single photon cross sections of the two isomers. At higher fluences, both isomers are converted to cyclopentadiene and trace amounts of 1,4-pentadiene. Irradiation of 1,4-pentadiene results in efficient isomerization to (*E*)- and (*Z*)-1,3-pentadiene which reacts further to yield cyclopentadiene. The factors which govern these and related infrared multiphoton reactions are discussed.

The availability of high-powered pulsed infrared lasers has led to renewed interest in the unimolecular isomerization reactions of unsaturated hydrocarbons within the last decade.[1] The results of pulsed infrared multiphoton (IRMP) excitation of unsaturated hydrocarbons can differ markedly from those of conventional heating. Among the significant achievements of the IRMP method are (a) contrathermodynamic isomerization in a two-isomer system ($A \rightleftarrows B$),[2-4] (b) trapping of kinetically labile intermediates in consecutive reactions ($A \rightarrow B \rightarrow C$),[5,6] and control of branching ratios in competing reactions ($A \rightarrow B + C$).[7,8] These achievements are based on the wavelength selectivity and rapid heating provided by pulsed infrared lasers. Pulsed excitation of A under collision-free conditions at a frequency where B absorbs less strongly than A allows the formation of B even when it is less stable than A (contrathermodynamic) or C (trapping of kinetically labile intermediates). In cases where the product-forming channels have different activation energies and preexponential factors, the average energy of vibrationally excited A can be altered by varying the laser fluence, thereby changing the ratio of products B and C in competing reactions.

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