The Dependence of Biological Electron Transfer Rates on Exothermicity: The Cytochrome c/Cytochrome b_5 Couple

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Abstract: Rates of electron transfer within the noncovalently bound cytochrome c/cytochrome b_5 complex are reported for several derivatives, including $Fe^{II}cytb_5/Fe^{III}cytc$ ($1.6 \pm 0.7 \times 10^3$ s^{-1}), $^3(Zn(porph))$*cytc/$Fe^{III}cytb_5$ ($5 \pm 1 \times 10^5$ s^{-1}), $^3(H_2(porphcytc))$*/$Fe^{III}cytb_5$ ($5 \pm 0.5 \times 10^4$ s^{-1}), and ($porphH_2$)cytc/$Fe^{III}cytb_5$ ($8 \pm 1 \times 10^3$ s^{-1}). These data show a strong dependence of rate on exothermicity, and are consistent with Marcus theory and related theories of electron transfer. The results suggest that reorganization energies for protein–protein reactions may be about 0.7 eV in these reactions, which is higher than commonly supposed.

Despite intense theoretical[1] and practical interest in electron transfer reactions of proteins, very few of the studies available[2] provide insight into the specific factors which control biological electron transfer. Experiments on electron transfer (ET) reactions in small molecules have demonstrated that when the donor and acceptor are held at fixed distances a rich dependence on distance, exothermicity ($-\Delta G$), and solvent emerges for ET rates.[3] In contrast to the rapidly growing literature on fixed distance reactions involving small molecules,[3,4] few examples exist of fixed distance ET in proteins,[2,5] and no systematic study of rate vs. ΔG is available at fixed distance.[7]

We now wish to report studies of electron transfer reactions which occur at a single fixed distances within the noncovalent complex formed by two proteins, cytochrome c (cytc) and cytochrome b_5 ($cytb_5$). In this complex, the two heme centers are believed to lie in parallel planes separated by a closest approach of 8.5 Å (edge–edge) (Figure 1).[8] We report for the first time the ET rate of the bound physiological $Fe^{II}cytb_5/Fe^{III}cytc$ couple. We also have prepared two well-characterized[9b,10] metal-substituted derivatives of cytc, Zn(cytc), and free base H_2 porphyrin cytochrome c (porph(cytc)), which provide a wide range of reaction exothermicities in either ground state or excited state reactions (see Scheme I).

These studies are directed at two questions: (1) How does the rate of intramolecular electron transfer in a bound protein–protein complex depend on reaction exothermicity: e.g., what is the effective reorganization energy, λ, for a protein–protein electron transfer reaction? (2) If λ can be so determined, what is the effective electronic coupling parameter for this protein–protein couple?

Materials and Methods

Cytochrome c (type VI) was purchased from Sigma and further purified by chromatography on DEAE cellulose.

Cytochrome b_5 was isolated from calf liver by the method of Mauk.[8b] It was purified to a purity index (A_{410}/A_{280}) of $\cong$ 5.8.

Porphyrin cytc and Zn(cytc) were prepared by the basic method of Vanderkooi[11] with an all kel F line for manipulation of HF, as shown in Figure 2.

The crude porphyrin cytc was purified by chromatography on CM cellulose, followed by chromatofocusing. Purity was established by the presence of a single band on G-75 sephadex, a single isoelectric focusing component, visible spectroscopy, and NMR.

Water was triply glass distilled. All other chemicals were reagent grade.

Solutions were prepared for pulse radiolysis experiments by gently degassing with highly purified He or Ar. Solutions contained 1–2 mM phosphate, pH 7, and protein concentrations ranging from 1×10^{-6} to 1×10^{-5} M. Identical ET rates were found in the cytc/$cytb_5$ complex for these two concentrations.

tert-Butyl alcohol (0.01 or 0.05 M) was added to scavenge all OH· produced on radiolysis. Pulse radiolysis experiments were carried out with 4-ns pulses of 20 MeV electrons from the Argonne linear accelerator. A complete description of the system is published elsewhere.[3] Conditions for the other reducing systems were the following: isopropyl alcohol (0.01 M), pH 7, 10^{-3} M phosphate solutions were saturated with N_2O; benzophenone 10^{-4} M, pH 7, 10^{-3} M phosphate solutions were degassed by gentle bubbling with He gas.

Generally, 4-ns pulses were used, which resulted in a concentration of e_{aq}^- of $\sim 10^{-6}$ M, as determined by NCS^- dosimetry. Specific dose levels were chosen so that <15% of the total protein was reduced on

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(1) Recent theoretical treatments of the dependence of rates of (biological) electron transfer on distance and exothermicity include the following: (a) Hopfield, J. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3640–3644. (b) Jortner, J. *J. Am. Chem. Soc.* **1980**, *102*, 6676–6680. (c) Marcus, R. *Faraday Soc. Discuss.* **1982**, *74*, 1–10.

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(3) (a) Miller, J.; Closs, G.; Calcaterra, L. *J. Am. Chem. Soc.* **1984**, *106*, 3047–3049. (b) Beitz, J.; Huddleston, K.; Miller, J. *J. Am. Chem. Soc.* **1984**, *106*, 5057. (c) Miller, J. *Science* **1975**, *189*, 221–224. (d) Guarr, T.; McGuire, M.; McLendon, G. *J. Am. Chem. Soc.* **1983**, *105* 616–619.

(4) Other recent examples of fixed distance electron transfer reactions include the following: (a) Passman, P.; Verhoeven, J.; DeBoerth, *Chem. Phys. Lett.* **1978**, *57*, 530. (b) Creutz, C.; Kroger, P.; Matsubara, T.; Netzel, T.; Sutin, N. *J. Am. Chem. Soc.* **1979**, *101*, 5442–5443. (c) Kira, S.; Nosaka, Y.; Imamura, M. *J. Phys. Chem.* **1980**, *84*, 1882. (d) Moore, T.; Gust, D.; Mathis, P.; Mialocq, J.; Chachaty, C.; Bonsasson, V.; Land, E.; Doizi, D.; Liddell, P.; Lehman, W.; Neweth, G.; Moore, A. *Nature (London)* **1984**, *307*, 630. (e) Wasielewski, M.; Niemczyk, M. *J. Am. Chem. Soc.* **1984**, *106*, 5043. Wasielewski, M.; Niemczyk, M.; Suec, W.; Pewitt, E. B., submitted. A more detailed bibliography is available in ref 3b.

(5) Poulos, T.; Finzel, B. *Peptide Protein Revs.* **1984**, *4*, 115–171.

(6) See, for example: Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259.

(7) Important data are available, however, for the dependence of rate on ΔG in diffusional systems involving small molecule donors. These results are complicated by the distance dependence of electron transfer, see: Mauk, G.; Scott, R.; Gray, H. *J. Am. Chem. Soc.* **1980**, *102*, 4360–4363 and references therein.

(8) (a) Salemme, F. R. *J. Mol. Biol.* **1976**, *102*, 563–568. (b) Mauk, M.; Mauk, A. G. *Biochemistry* **1982**, *21*, 1843–1846. (c) For benzophenone, only one experiment was performed, thus no standard deviation is reported. Independent experiments demonstrated benzophenone anion radical was stable under our conditions for a few microseconds.

(9) (a) Moore, G.; Williams, R. J. P.; Chien, J. C. W.; Dickinson, L. C. *J. Inorg. Biochem.* **1980**, *13*, 1–15. (b) Fisher, W.; Taniuchi, H.; Anfinsen, C. B. *J. Biol. Chem.* **1973**, *248*, 3188–95. (c) Smith, M.; McLendon, G. *J. Biol. Chem.* **1978**, *253*, 4004. (d) Sandberg, K.; McLendon, G. *J. Biol. Chem.* **1978**, *253*, 3913.

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Scheme I

$$Fe^{III}cytc/Fe^{III}cytb_5 \xrightarrow{e_{aq}^-} Fe^{III}cytc/Fe^{II}cytb_5 \xrightarrow{k_{et}} Fe^{II}cytc/Fe^{III}cytb_5 \quad (a)$$

$$porph(cytc)/Fe^{III}cytb_5 \xrightarrow{e_{aq}^-} porph^-cytc/Fe^{III}cytb_5 \xrightarrow{k_{et}} porph(cytc)/Fe^{II}cytb_5 \quad (b)$$

$$^3(porph)^*cytc/Fe^{III}cytb_5 \rightarrow porph^+cytc/Fe^{II}cytb_5 \quad (c)$$

Figure 1. Proposed structure of the cytochrome *c*/cytochrome b_5 complex based on computer modeling experiments. (Courtesy T. Poulos, Genex.)

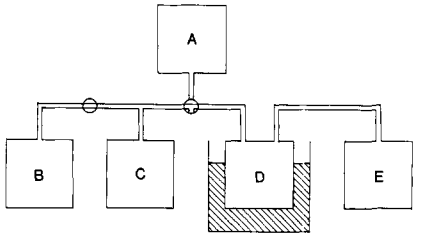

Figure 2. Schematic of Teflon-Kel F line used for preparation of porphyrin cytc: (A) N_2 tank; (B) HF tank; (C) CoF_3 scrubber; (D) cytc container in liquid N_2 bath; (E) $Ca(OH)_2$ solution.

radiolysis in order to eliminate the possibility of multiple radical formation. Note that, for bimolecular reactions, these conditions dictate that rate will be dose independent, since unreacted reagent is always present in excess. Under these pseudo-first-order conditions, the rate will, of course, depend on the concentration of the excess component. For unimolecular reactions, rate is predicted to be independent of both dose and protein concentration.

Flash photolysis experiments were carried out as described previously.[2b,c] Solution conditions were identical with those for pulse radiolysis, except that *tert*-butyl alcohol was omitted. The rates of triplet decay of Zn(cytc) or porph(cytc) were monitored at 460 nm. Within experimental error, identical results were obtained for single shot or multiply averaged experiments. Therefore, rate constants were obtained from the more precise signal-averaged data. Excitation at 355 or 532 nm produced identical results. The kinetics were also shown to be independent of excitation power over the range 10 to 150 mJ/pulse.

All radiolysis and photolysis experiments were repeated on several occasions, using independent preparations of both cytc and $cytb_5$. Uncertainties in rate constants are based on standard deviations from multiple independent determinations. The least-squares uncertainties for individual determinations of k_{et} were generally $\leq 10\%$.

Results

Metal-Substituted Cytochromes. Metal substitution provides a simple approach to modulating the redox potential within a protein–protein couple. We assume metal substitution does not affect the structure of the proteins. Because small differences in structure might cause significant changes in rates, this assumption requires careful consideration. Changes in protein properties on changing a heme metal center are well documented.[9c,d] For example, metal-substituted cytochromes are clearly less *stable* to denaturation than Fe^{III}cytc. However, this may merely indicate an expected difference in the relative energies of the "native" conformations for Fe(cytc) vs. Zn(cytc), since the Fe structure is stabilized by one extra bond: the Fe–Met80 coordinate bond. Such effects are fully documented for metal-substituted myoglobins.[9d] This effect would be analogous to the difference in stability of Fe^{II}cytc vs. Fe^{III}cytc.[9c]

The following section describes experimental tests and published reports which suggest that cytochrome *c* and the cytc/$cytb_5$ complex do not undergo significant structural changes on metal removal or replacement.

Anfinsen and co-workers showed that the structurally sensitive circular dichroism spectra, intrinsic viscosity, and tryptophan emission of native cytc and porph(cytc) are similar or identical, implying similar or identical tertiary structures are found in both systems.[9b] Subsequently, Moore and Williams showed,[9a] and we have confirmed,[2c] that the NMR spectra of Zn(cytc) and Fe^{II}cytc are superimposable for a wide range of amino acid resonances, consistent with similar or identical conformations for both proteins.

Furthermore, in previous work with the Zn(cyt)/$Fe^{III}cytb_5$ couple, we reported dipolar energy transfer measurements, which support Salemme's structural model for the Fe(cytc)/Fe($cytb_5$) complex.[2c]

Finally, in a particularly stringent test, we have measured the binding constants of Fe^{III}cytc/$Fe^{III}cytb_5$, Zn^{II}cytc/$Fe^{III}cytb_5$, and porph(cytc)/$Fe^{III}cytb_5$. Within experimental error (20%), identical binding constants were found for all three systems.[10] The binding constant was similarly unaffected by addition of 0.01 M *tert*-butyl alcohol (this measurement was made only for the iron-containing cytochromes).

A primary reason for preparing metal-substituted cytochromes is to provide a range of redox potentials for electron transfer. In independent work,[10] Magner has estimated these potentials by photochemical excited-state quenching experiments using graded series of electron donors or electron acceptors. The potentials so obtained are

$$\begin{aligned} ^3*Zn(cytc)/Zn(cytc)^+ \quad & E^\circ = 0.75 \pm 0.1\ V \\ ^3*porph(cytc)/porph(cytc)^+ \quad & E^\circ = 0.35V \pm 0.1\ V \\ porph(cytc)/porph(cytc)^- \quad & E^\circ = -1.1V \pm 0.2\ V \end{aligned} \quad (1)$$

Pulse Radiolysis: Bimolecular Kinetics and Controls

The pulse radiolysis of Fe^{III}cytc has been examined by several previous workers, and our results are in general agreement with previous reports.[12] The solvated electron e_{aq}^- rapidly reduces cytc to produce a stable Fe^{II} product (Figure 3).

Similar reactivity is found for reduction of $cytb_5$ (Figure 4). Thus the key question is how this reactivity is altered in a mixture of cytc and $cytb_5$. Fortunately, convenient wavelengths are available to allow $Fe^{II}cytb_5$ ($\lambda = 428$ nm, $\epsilon_{b5} \gg \epsilon_{cytc}$) and Fe^{II}cytc ($\lambda = 416$ nm, isosbestic for $Fe^{II}/Fe^{III}b_5$) to be investigated independently.

As a check on the radiolysis technique, we first examined the reaction of $Fe^{II}cytb_5$ with Fe^{III}cytc under conditions previously studied by stopped flow.[13] At [cytc] = [$cytb_5$] $\leq 5\ \mu M$ and ionic strength = 0.1 M no complex is formed (complex formation is decreased strongly at high ionic strength), and the reaction (viz. Scheme Ia) occurs by a simple bimolecular path. We find k_{bi} =

(12) Klapper, M. H.; Faraggi, M. *Q. Rev. Biophys.* **1979**, *12*, 465–519.
(13) Strittmatter, P. In "Rapid Mixing and Sampling in Biochemistry", Chance, B., Eisenhardt, R., Gibson, Q., Lundber Holm, K., Eds.; Academics: New York, 1964; pp 71–84.

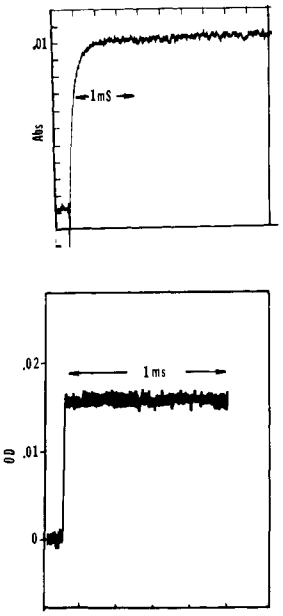

Figure 3. (a, top) Kinetic trace for reduction of Fe^{III}cytc by e_{aq}^-; [cytc] = 20 μM, $[e_{aq}^-]$ = 1 μM, 0.05 M tert-butyl alcohol, pH 7 phosphate (1 mM) λ_{obsd} = 416 nm. (b, bottom) Kinetic trace for reduction of $Fe^{III}cytb_5$ by e_{aq}^- conditions as in Figure 2, λ_{obsd} = 428 nm.

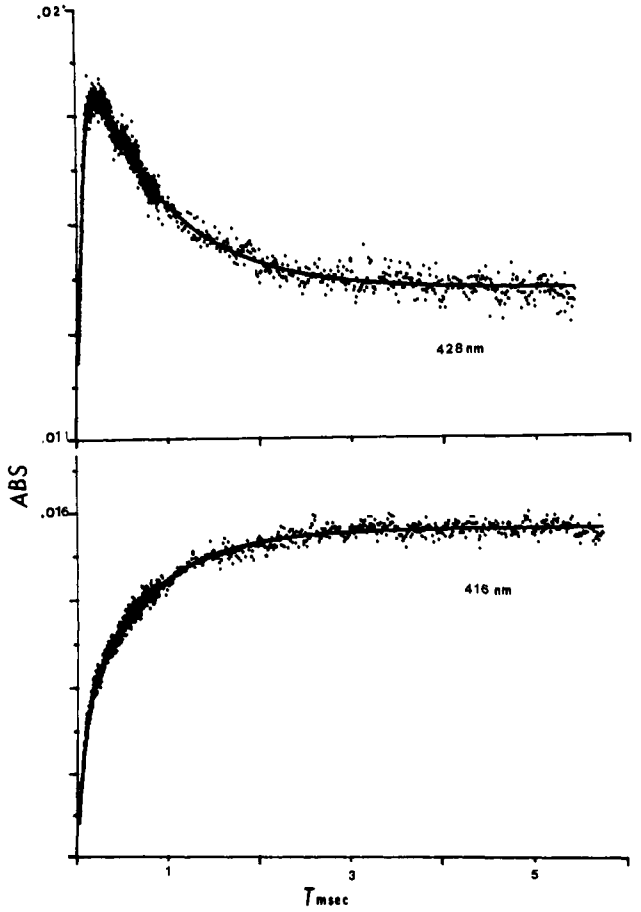

Figure 4. Kinetic trace for reaction of e_{aq}^- with the Fe^{III}cytc/$Fe^{III}cytb_5$ complex. Note initial rapid capture by both the cytc and $cytb_5$ (λ = 428 nm, top) sites (λ = 416 nm, bottom) is followed by first-order transfer from the $cytb_5$ site to the cytc site. Concentrations: [complex] = 20 μM, $[e_{aq}^-]$ = 4 μM, 1 mM phosphate, pH 7, 0.05 M *tert*-butyl alcohol. The observed rate, k_{obsd} = 1400 s^{-1} by least-squares fit (shown), is independent of [cyt] and $[e_{aq}^-]$.

3.9×10^7 M^{-1} s^{-1}, compared with the previous report of $k_{bi} = 4 \times 10^7$ M^{-1} s^{-1} under identical conditions.[13] As expected for a bimolecular process, the rate depends linearly on [cytc] and is independent of dose. The coincidence between the pulse radiolysis result and the stopped flow data indicates that radiolysis with e_{aq}^- is not complicated by any radical intermediates.

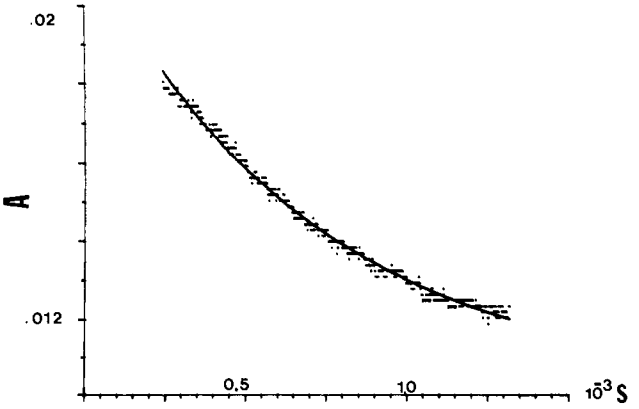

Figure 5. Kinetic trace for reaction of e_{aq}^- with a previously pulsed sample of cytc/$cytb_5$ complex. Note the kinetics of reaction do not change, but the amplitude does change since some of the cytc sites are already reduced. Reaction conditions are identical with those in Figure 4.

This conclusion was double checked with CO_2^-· as the reductant (10^{-2} M formate N_2O saturated) rather than e_{aq}^-, with identical results. On the other hand, the rate of intramolecular electron transfer in the c/b_5 complex showed some dependence on the method of reduction of the complex, as described in the following section.

Unimolecular Kinetics

With these controls in hand, reaction conditions were chosen to ensure complete complex formation. All subsequent experiments were carried out at pH 7, 1 mM inorganic phosphate, with [cytc] = [$cytb_5$] $\leq$ 3 μM. Under these conditions, >90% of the $cytb_5$ is bound to cytc.[8b]

In contrast to the data at 0.1 M ionic strength, the rate of electron transfer from $cytb_5$ to cytc under these conditions is *independent* of the concentrations of cytochromes, over the range 3–20 μM, and is also independent of radiation dose, consistent with *intra*molecular electron transfer within the bound complex. The rate constant is independent of concentration (to ±2%), which means that the bimolecular rate constant for *complexed* cytochromes is less than 2×10^7 M^{-1} s^{-1} (vs. 3.9×10^7 for uncomplexed cytochromes). The actual bimolecular rate constant is plausibly much smaller than this limit, so we made no attempt to measure it. A simple intramolecular transfer is further indicated by the coincidence of the rate of decay of $Fe^{II}cytb_5$, measured at 428 nm, and the rate of growth of Fe^{II}cytc, measured at 416 nm (Figure 5). From these data, we derive a rate constant for the intramolecular electron transfer reaction (Scheme Ia) of k_{ET} = 1400 ± 100 s^{-1}. Experiments were also conducted with isopropyl alcohol radical k_{ET} = 2500 ± 500 s^{-1} or benzophenone radical anion k_{ET} = 1100 s^{-1}.[8c] These differences are repeatable and were obtained with the same protein preparation, but the source of the differences is not known. We report the average value of k_{ET} = 1660 ± 700 s^{-1}.

Similar differences in intramolecular rates have been observed before. Studies of intramolecular electron transfer in the His 33-$Ru^{III}(NH_3)_5$/cytc system give rate variations from 30 s^{-1} for photoinitiated electron transfer[2a] to 50–65 s^{-1} for radiolysis induced electron transfer.[2e]

To our knowledge, this measurement of k_{ET} is the first direct determination of the rate of intramolecular electron transfer for a bound physiological protein–protein complex. In this context, it is noteworthy that reaction of the *bound* complex is too fast to be allowed by stopped flow and underscores the potential utility of pulse radiolysis for such investigations.

An interesting sidelight of the reaction within the bound complex emerges from analysis of the rate of *formation* of the reduced

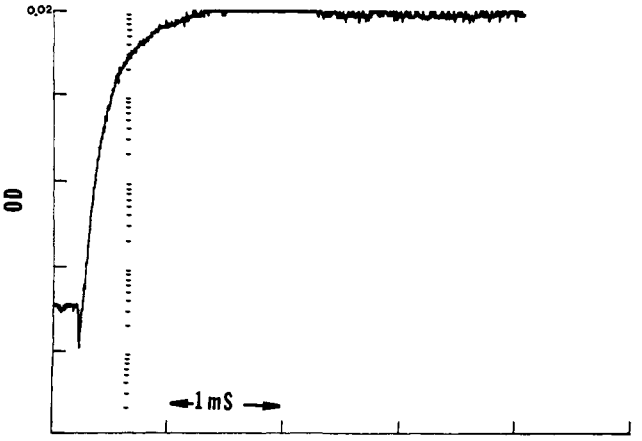

Figure 6. Kinetic trace for reaction of e_{aq}^- with a sample of Fe^{II}cytc/$Fe^{III}cytb_5$ complex. Here the reduced $cytb_5$ is stable, since intramolecular transfer is excluded. This result obviates any possibility that radical reactions are responsible for the kinetics observed in Figures 4 and 5.

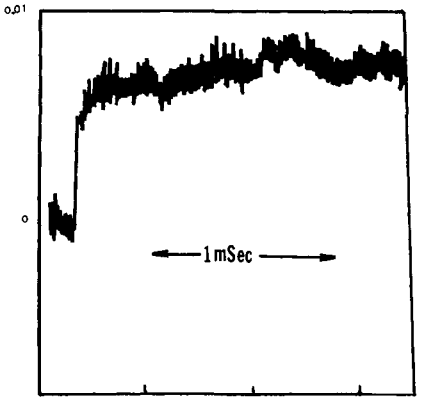

Figure 7. Kinetic trace for reaction of e_{aq}^- with porph(cytc) monitored at 436 nm. Other conditions are the same as in Figure 3.

$Fe^{II}cytb_5$ kinetic intermediate. Within the complex, <20% of the $Fe^{II}cytb_5$ is formed concomitantly with the decay of the e_{aq}^- species. Instead, most $Fe^{II}b_5$ is formed in a delayed reaction, with $k_{ET} \sim 10^6\ s^{-1}$.

This side reaction is fortuitous in ensuring a high yield for $cytb_5$ reduction. However, we were concerned that such radical reactions *might* lead to complex kinetics. Two control experiments strongly argue that radical intermediates do not complicate the observed kinetics. First, it is possible to pulse the same solution several times. In this case, the amplitude of the observed reaction changes but the *rate* does not (Figure 6).

A simple related control shows that when $Fe^{III}cytb_5$ is reduced in the presence of Fe^{II}cytc, the $Fe^{II}cytb_5$ produced is stable over the entire time of observation (Figure 7). Thus, the reactivity of $Fe^{II}cytb_5$ within the c/b_5 complex is determined solely by the presence or absence of a (bound) Fe^{III}cytc moiety. This result is not surprising, since the Fe center is obviously at much lower energy than any other radical species.

Porphyrin cytc$Fe^{III}cytb_5$

A key question raised in this work is the following: How does the rate of electron transfer within a protein–protein complex depend on reaction exothermicity? In order to examine a maximal range of ΔG values, we studied the generation of the reactive anion radical of porphyrin cytc and its electron transfer to $Fe^{III}cytb_5$.

The reduction of porphyrin cytc by e_{aq}^- has previously been reported by van Gelder et al.[14] In agreement with their work, we find that reduction of porph(cytc) produces a stable porphyrin

(14) Veerman, E.; Van Leuwen, J.; Van Buren, K.; van Gelder, B. *Biochim. Biophys. Acta* **1982**, *680*, 134–141.

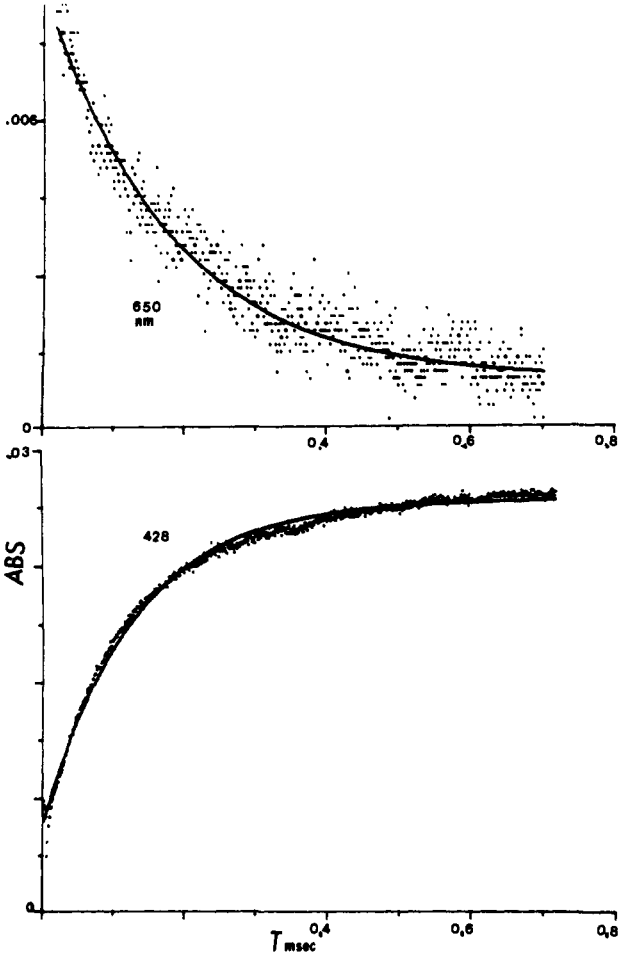

Figure 8. Kinetic trace from reaction of e_{aq}^- with the porph(cytc)/$Fe^{III}cytb_5$ complex. Top panel: decay of $porph^{-\cdot}$ radical measured at 700 nm. Bottom panel: formation of $Fe^{II}cytb_5$ measured at 428 nm. k_{obsd} = 12 600 ± 1200 s^{-1} by least-squares fit (shown).

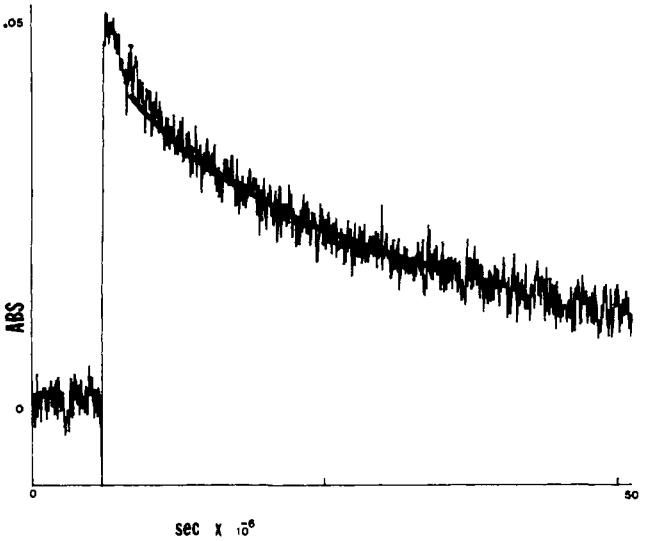

Figure 9. Excited-state decay of the ^{3}*(porphyrin cytc)/cyt $Fe^{III}b_5$ complex.

radical characterized by a broad absorbance between 600 and 700 nm (Figure 8). Preliminary attempts to reduce porphc with other reductants proved unsuccessful.

When radiolysis is carried out on solutions of the bound porph(cytc)/$Fe^{III}b_5$ complex, the porphyrin radical is again produced. However, it rapidly decays by a first-order process (k = 8 ± 1 × $10^3\ s^{-1}$) with concomitant reduction of $Fe^{III}cytb_5$ (Figure

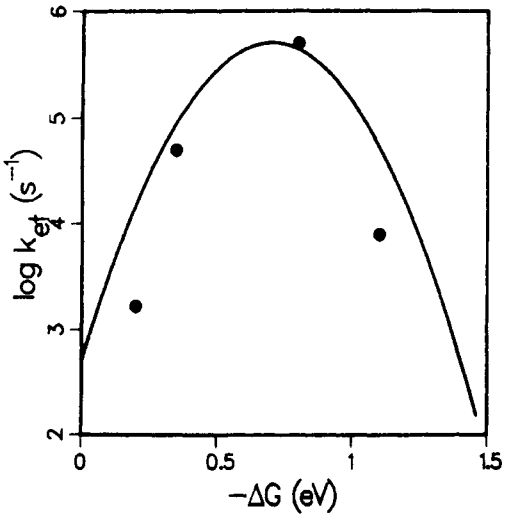

Figure 10. Plot of log (electron transfer rate constant) vs. ΔG for $Fe^{II}b_5/Fe^{III}c$), ^{3}*(porph(cytc))/$Fe^{III}b_5$, ^{3}(Zn(cytc))*/$Fe^{III}b_5$, and porph(cytc)/$Fe^{III}b_5$. The solid line is the theoretical fit assuming $\lambda = 0.8$ V and $k = A \exp(-(\Delta G + \lambda)2/4\lambda kT)$ (classical Marcus theory).

9). As with the Fe/Fe reaction already discussed, this rate is independent of both radiation dose and total protein concentration. This result shows that changing from an Fe(II) reductant (with $\Delta G \approx -0.3$ eV) to a porphyrin radical anion reductant ($\Delta G \approx -1$ eV) increases the rate of electron transfer by ~7-fold. In order to examine the source of this rate increase, we compared these thermal reactions with analogous photochemical electron-transfer reactions, as discussed below.

Photochemical Electron Transfer

Detailed studies of the photoinduced electron transfer in the Zn(cytc)/Fe^{III}cytb_5 complex have already been reported.[2c] In brief, we showed that the photoexcited ^{3}(Z(cytc))* was quenched when bound to Fe^{III}cytb_3 with a rate constant $k_q = 5 \times 10^5$ s^{-1}. Detailed controls showed that this quenching is consistent with electron transfer but *not* with dipolar energy transfer, spin coupling, or other simple mechanisms for enhanced nonradiative decay. Entirely analogous results were found for triplet quenching in the ^{3}(porph(cytc))*/Fe^{III}cytb_5 complex. We find that the rate of quenching ($k_q = 5 \times 10^4$ s^{-1}) (Figure 10) is independent of [cytb_5] at low ionic strength ($\mu < 10$ mM) where all porphyrin cytc is bound but depends linearly on [cytb_5] at high ionic strength ($\mu > 100$ mM). As for the Zn(cytc) case previously reported, the rate is also independent of whether the porphyrin is excited at 532 or 355 nm and is also independent of the excitation power. *Unlike* the Zn(cyt) case, irradiation of porphyrin c/cytb_5 produces a permanent photoreduction of Fe^{III}cytb_5, albeit ion low quantum yield ($\phi \sim 0.02$). This result is analogous to previous studies of Zn hemoglobin,[2b,2d] where the cation radical produced on electron transfer is reduced by an internal oxidative protein degradation.

Discussion

The new radiolysis results, when combined with photochemical data on the cytc/cytb_5 system, suggest a surprisingly simple relationship between the intramolecular electron-transfer rate, k_{ET}, and reaction exothermicity, ΔG. As shown in Figure 10, this relationship is consistent with the classical (Marcus) theory for electron-transfer rates

$$k_{ET} = A \exp(-(\Delta G^\circ + \lambda)^2/4\lambda kT) \qquad (2)$$

where A is the frequency factor related to donor acceptor distance,[1-3] $A \propto \exp(-\alpha R)$, and λ is the reorganization energy arising from all nuclear displcements between the reactant and product states.[1] Such displacements may occur at the porphyrins themselves, or in the surrounding "medium" (in this case, the protein matrix surrounding the heme(s)).

The solid line in Figure 10 is predicted by eq 2, assuming $\lambda = 0.8$ eV. This value substantially exceeds some previous theoretical estimates of λ for cytochromes.[15]

Other explanations for the observed rate changes are, of course, possible. For example, conformational differences among the various metallocytochromes may dramatically affect the rates. We are particularly sensitized to this possibility by the fact that a seemingly small change in the method of reduction (e_{aq}^- vs. isopropyl alcohol radical) led to a factor of ≈2 change in ET rate. On the other hand, the relatively regular dependence of k_{Et} on ΔG seems an unlikely result of conformation changes, and the previously noted studies[9,10] on metallocytochrome conformations demonstrated strong similarities among Fe(cytc), Zn(cytc), and porph(cytc) and among their complexes with cytb_5.

Another possible explanation is that the prefactor, A, changes when the donor electron binding energy changes. Indeed, theory predicts such a change.[1] However, this hypothesis predicts the *highest* rate for porphy(cytc) radical and would predict a very different (and flatter) curve.[16] We have assumed that changes in the prefactor, A, are smaller than the changes due to requirements for nuclear reorganization upon electron transfer.

The classical version of Marcus theory (eq 2) is not a unique interpretation of our measurements; it is just the simplest interpretation. However, our principal conclusion, that $\lambda \cong 0.7$ eV, does not depend on our choice of this particular incarnation of ET theory.

In this content, it is noteworthy that recent data for electron transfer in other protein systems also suggest high values of λ. For example, Hofmann and co-workers have reported detailed temperature dependence studies of photoinduced electron transfer in Zn^{II}/Fe^{III} hybrid hemoglobins. These results are consistent with $\lambda \sim 2$ eV for this system.[17]

Limited data for the cytc/cytochrome *c* peroxidase system are also consistent with a value of $\lambda \sim 2$ eV for this system.[18]

Finally, with the Franck–Condon factors now experimentally estimated, it is possible to estimate the electronic coupling strength in the cytc/cytb_5 couple. First, we note that at the cytc/cytb_5 separation of ca. 16 Å (center–center separation of the hemes), the observed rate of 5×10^5 s^{-1} at optimal ΔG is similar to rates (10^6–10^8 s^{-1}) found at optimal ΔG for electron transfer betwen donors and acceptors dispersed in rigid glasses,[3] although a somewhat larger disparity would be found if edge-to-edge ditances could be compared. Thus, large rate enhancements or retardations are *not* required to understand electron transfer in the cytc/cytb_5 system. This contrasts with recent studies of Ru-substituted proteins,[2] in which observed intramolecular transfer rates are much slower than rates measured in model systems.

A more quantitative analysis of the distance dependence requires several assumptions, as detailed below. We take $k_{ET} = k_0 F \exp(-\alpha(R-R_0))$ with $k_0 = 10^{13}$ s^{-1} for an adiabatic reaction (strong electronic coupling) and the relative Franck–Condon factor F being nearly unity at optimal exothermicity. We will assume that the reaction is marginally adiabatic with $k_0 = 10^{13}$ s^{-1} for the two porphyrins at van der Waals contact in a sandwich configuration but that k_0 is about 100 times smaller for the type of edge contact which would occur if the porphyrins in Figure 2 were moved 8.5 Å closer. This edge vs. sandwich comparison is based on simple model calculations of Case, Siders, and Marcus.[19] Thus, we estimate that $k_0(R_0) \cong 10^{11}$ s^{-1} at edge contact, so that $\exp(-\alpha(R-R_0)) \cong 2 \times 10^5/10^{11}$, giving $\alpha \cong 1.5$ Å^{-1}. This crudely estimated value for α is similar to values near 1.2 found in disordered polymers, glasses, and highly viscous solutions.[3b-d] However, with the limited data yet available, it is premature to

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assume that any single value of α will be generally applicable for protein electron-transfer reactions.

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Laser Flash Photolysis Study of the Hydrogen Atom Transfer Reaction from Triplet 1-Naphthol to Ground Benzophenone[1]

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Abstract: Laser flash photolyses at 337 nm have been carried out on methanol solutions of the 1-naphthol and benzophenone system. It is found that hydrogen atom transfer reaction from the triplet 1-naphthol ^{3}ROH* (produced by triplet sensitization of benzophenone) to the ground benzophenone BP occurs effectively through the triplet complex ^{3}ROH*···OC< to give the 1-naphthoxy radical RO· and the ketyl radical >ĊOH. The triplet-triplet energy transfer k_{ET} (4.1×10^9 M^{-1} s^{-1}) from ^{3}BP* to ROH is competitive with both the usual hydrogen atom abstraction k_{HA}' (7.4×10^8 M^{-1} s^{-1}) of ^{3}BP* from ROH and the quenching k_q' (2.2×10^9 M^{-1} s^{-1}) of ^{3}BP* induced by ROH at 290 K. These primary processes of ^{3}BP* are completed in 300 ns, and the equilibrium ^{3}ROH* + >CO $\rightleftarrows$ ^{3}ROH*···OC< is established very quickly. The equilibrium constant K^* for the ^{3}ROH*···OC< formation was obtained to be $16._7$ M^{-1} at 290 K ($\Delta H^* = -2.4$ kcal mol^{-1} and $\Delta S^* = -2.7$ eu). Then, the hydrogen atom transfer reaction takes place via the triplet complex with the rate constant k_{HT} (1.3×10^6 s^{-1} at 290 K, $A_{HT} = 3.7 \times 10^9$ s^{-1}; $\Delta E_{HT} = 4.6$ kcal mol^{-1}). Both RO· and >ĊOH produced by laser flash photolysis decay mainly via the radical reaction k_R between them with the rate constant 1.7×10^9 M^{-1} s^{-1} at 290 K. The reaction mechanism on the hydrogen atom transfer reaction from ^{3}ROH* (produced by triplet sensitization of BP) to yield RO· and >ĊOH is shown in detail.

Hydrogen atom transfer reactions of the triplet state of carbonyl compounds from a variety of substrates such as alcohols, hydrocarbons, and amines are well-known. The reaction proceeds by either hydrogen atom transfer or electron transfer followed by proton transfer. A large number of studies on intermolecular and intramolecular hydrogen-abstraction reactions of carbonyl triplets have been reported.[2-12] As for the photochemical features in the presence of phenols, reversible hydrogen abstraction by carbonyl triplets from phenols resulting in effective quenching of carbonyl triplets has been shown by Turro et al.[13,14] and Becker.[15] CIDNP studies have demonstrated by ketyl radical formation during the photolysis of acetophenone–phenol mixtures.[16,17] A laser flash photolysis study of the quenching of carbonyl triplets by phenols has been reported by Das et al.[18] showing that the quenching of aromatic carbonyl triplets by phenols is a very fast process for both n,π^* and π,π^* states.

However, little attention has been paid to hydrogen atom transfer from triplet aromatic compounds to the ground state of the aromatic carbonyl compounds until very recently. In a preliminary report,[19] we have demonstrated the hydrogen atom transfer reaction from triplet 2-naphthylammonium ion to ground benzophenone (or acetophenone) in a laser flash photolysis study, while in the excited singlet state of the ammonium ion proton transfer takes place effectively.[20]

In the present paper, we report the hydrogen atom transfer reaction from triplet naphthol (produced by triplet sensitization of benzophenone) to ground benzophenone by means of laser flash photolysis.

Experimental Section

Materials. Benzophenone and acetophenone used were the same as those reported elsewhere.[21] 1-Naphthol (G.R.grade, Wako) was purified by two recrystallizations from ethanol–water (1:1) mixtures. Methanol (Spectrosol, Wako) was used as a solvent.

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