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Electron transfer between cytochrome *c* and metal hexacyanide complexes. Effect of thermodynamic driving force on the electron transfer rate

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The electron transfer reactions between ferrocytochrome *c* and three isomorphic hexacyanide complexes, $[Fe(CN)_6]^{3-}$, $[Os(CN)_6]^{3-}$ and $[Ru(CN)_6]^{3-}$, have been studied using the method of photoexcitation. The transfer rates for $[Os(CN)_6]^{3-}$ and $[Ru(CN)_6]^{3-}$ are, respectively, about 45- and 200-times higher than that of $[Fe(CN)_6]^{3-}$. A reorganization energy of approx. 0.8 eV was found for the cytochrome *c*-hexacyanide system when the data were analyzed according to the theory of Marcus.

Introduction

The electron transfer reactions between metalloproteins and small inorganic complexes have been extensively studied so as to elucidate the mechanism of electron transfer in biological molecules [1–6]. Among the numerous reactions examined, that between cytochrome *c* and iron hexacyanide is unique because it is the first example demonstrating that these reactions occur, in general, via a precursor complex [7,8], i.e.

$$P + S \rightleftharpoons P \cdot S \rightleftharpoons P^+ \cdot S^- \rightleftharpoons P^+ + S^-$$

where P and S stand for protein and small complex, respectively.

In a previous work, we used the method of photoexcitation to investigate in detail the kinetics of electron transfer between ferrocytochrome *c* and ferrihexacyanide [9]. The results confirmed that there is a strong binding site for ferricyanide on the protein surface through which electron is transferred from cytochrome *c* to ferricyanide at a rate of about 46000 s^{-1}. In addition, our study also reveals that there is one (or more) weak binding site via which electron transfer can occur. The relative contribution of the weak site to the overall transfer rate becomes significant at high ionic strength.

In this report, we have extended the study to two isomorphous analogs of $Fe(CN)_6^{3-}$, $Os(CN)_6^{3-}$ and $Ru(CN)_6^{3-}$, to elucidate the relationship between the electron transfer rate and the driving force (ΔE) for the reaction. Experimentally, since $Os(CN)_6^{3-}$ and $Ru(CN)_6^{3-}$ are difficult to isolate, they are generated by photoionization of the complexes in the reduced form [10,11]. The results are analyzed according to the electron transfer theories of Hopfield [12] and Marcus [13], and a reorganization energy of approx. 0.77 eV was found for the cytochrome *c*-hexacyanide system.

Materials and Methods

Horse-heart cytochrome *c* (Type VI, Sigma Chemical Company) was used as received; $K_4Fe(CN)_6$ and other chemicals were of reagent grade. The $K_4Os(CN)_6$ complex was prepared by the method of Krauss and Schrader [14]; $K_4Ru(CN)_6$ was prepared by the method of Krause and Violette [15]. All samples were prepared in 1 mM (pH 7) phosphate buffer. The ionic concentration was adjusted by the addition of NaCl. The overal ionic strength I of the sample was computed from

$$I = \tfrac{1}{2}\sum_i C_i Z_i^2$$

where C_i and Z_i are the molarity and the charge of each species, respectively. The concentration of the protein and hexacyanide complexes were determined by absorption spectrophotometry.

The electron-transfer rates were measured using a conventional flash photolysis set-up with the 266 nm

Abbreviation: Cyt, cytochrome.

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output of a Quanta-Ray DCR-2 Nd: YAG laser (pulse width ≈ 5 ns) as the excitation source. Hexacyanide complexes in the oxidized form were first generated by photoionization of the reduced form [10,11] in a sample containing Cyt^{II} and a given hexacyanide, $M(CN)_6^{4-}$ (M = Fe, Os or Ru). The subsequent electron transfer between Cyt^{II} and $M(CN)_6^{3-}$ was followed by monitoring the absorbance change of the protein at 550 nm and 570 nm. The reactions can be described by the following equations

$$M(CN)_6^{4-} + h\nu \longrightarrow M(CN)_6^{3-} + e_{aq}^-$$

$$M(CN)_6^{3-} + Cyt^{II} \longrightarrow M(CN)_6^{4-} + Cyt^{III}$$

Typically, the laser energy per pulse was 100 mJ/cm^2. The signals were usually averaged over 10 shots, and the sample was well stirred between shots. The measurement was conducted at room temperature which was about 20 °C.

Results

Fig. 1 shows a typical signal at 570 nm obtained by photoexication of a sample of ferrocytochrome *c* and $Os(CN)_6^{4-}$. The initial small jump was found to result from a laser-induced change in the absorbance of the protein, and this part of the signal remained unchanged in the time scale of the experiment. The subsequent increase in absorption reflects the oxidation of the protein from Cyt^{II} to Cyt^{III} by the $Os(CN)_6^{3-}$ generated from photoionization of $Os(CN)_6^{4-}$. The absorption change with time follows a single exponential, since the reaction is pseudo first-order ($[Cyt^{II}] \gg [Os(CN)_6^{3-}]$). The stability of the $Os(CN)_6^{3-}$ (and $Ru(CN)_6^{3-}$) was monitored by repeating the transient experiment in the wavelength region of 300–400 nm in the absence of Cyt^{II}. These transient signals remained essentially constant in the time scale of the electron transfer, indicating that the observed transfer rate is not limited by the stability of the oxidized complexes generated. In the reaction, hydrated electrons were generated as by-products. They probably decay by reducing O_2 in the sample to O_2^- radicals and by interacting with some amino acid residues of the protein. The signal in Fig. 1

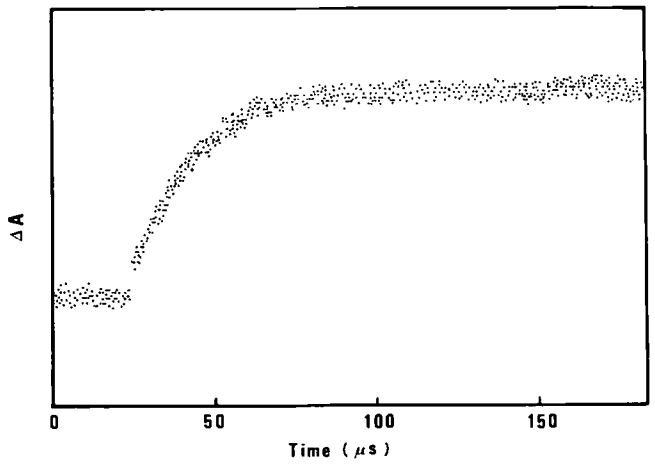

Fig. 1. Typical transient obtained for the $Os(CN)_6^{3-}$-Cyt^{II} system at 570 nm which illustrates the oxidation of the protein to Cyt^{III}. The sample contained 100 μM Cyt^{II} and 1 mM $Os(CN)_6^4$ in 1 mM (pH 7) phosphate buffer. The ionic strength was 60 mM.

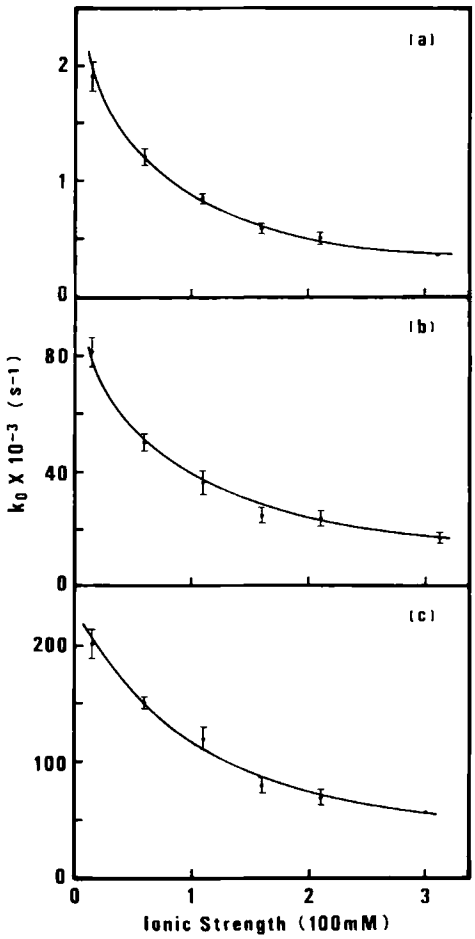

Fig. 2. Plots of k_0 vs. ionic strength for the oxidation of Cyt^{II} by (a) $Fe(CN)_6^{3-}$ (b) $Os(CN)_6^{3-}$ and (c) $Ru(CN)_6^{3-}$. The total Cyt^{II} and hexacyanide concentrations were 100 μM and 1 mM, respectively.

TABLE I

Bimolecular electron-transfer rate constants, k_{12}, between ferrocytochrome c and hexacyanicde complexes. Typical accuracies are ± 10%.

Ionic strength (mM)	$Fe(CN)_6^{3-}$ ($M^{-1}\cdot s^{-1}$)	$Os(CN)_6^{3-}$ ($M^{-1}\cdot s^{-1}$)	$Ru(CN)_6^{3-}$ ($M^{-1}\cdot s^{-1}$)
15	$1.9\cdot10^7$	$8.1\cdot10^8$	$2.0\cdot10^9$
60	$1.2\cdot10^7$	$5.0\cdot10^8$	$1.5\cdot10^9$
110	$8.5\cdot10^6$	$3.6\cdot10^8$	$1.2\cdot10^9$
160	$5.9\cdot10^6$	$2.5\cdot10^8$	$7.9\cdot10^8$
210	$5.1\cdot10^6$	$2.4\cdot10^8$	$6.9\cdot10^8$
310	$3.7\cdot10^6$	$1.7\cdot10^8$	$5.6\cdot10^8$

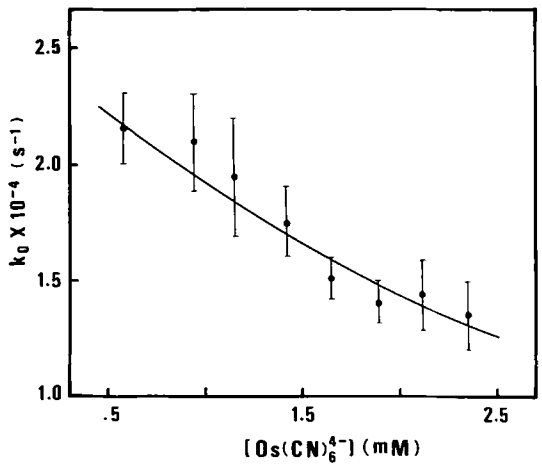

Fig. 3. Plots of k_0 vs. $Os(CN)_6^{4-}$ concentration for the oxidation of Cyt^{II} by $Os(CN)_6^{3-}$. The Cyt^{II} concentration was 30 μM and the ionic strength was kept at 30 mM.

decays very slowly, in a time scale of seconds, as a result of the reduction of Cyt^{III} back to Cyt^{II} by the O_2^- generated. This re-reduction is usually not complete and to minimize this effect, we have ensured that the samples were well stirred between shots and new samples were used after more than five shots.

Apart from a difference in the time constant of the exponential shown in Fig. 1, the results obtained for all the hexacyanides are qualitatively the same. Fig. 2 shows a plot of the rate of oxidation of Cyt^{II} by the three hexacyanides at different ionic strengths. The rate constants decrease monotonically with ionic strength, in accord with that the binding constant between Cyt^{II} and the complexes becomes weaker at higher values of I. Table I summarizes the bimolecular rate constants obtained for the three hexacyanides. These values give the effect of the driving force on the electron transfer rate (see Discussion below).

In principle, the binding between Cyt^{II} and the hexacyanides is best demonstrated by examining the variation of the electron transfer rate with Cyt^{II} concentration. However, it is difficult to conduct our experiment at high concentrations (up to the millimolar range mM) of Cyt^{II} because the absorption of the protein would be too high to yield an acceptable signal-to-noise ratio. For the limited range of protein concentration in which measurements could be carried out (100–400 μM), the observed rate is proportional to [Cyt^{II}] at all ionic strengths investigated. An indirect demonstration of the binding between Cyt^{II} and the complexes was achieved by studying the electron transfer rate as a function of the complex concentration. Taking the case of $Os(CN)_6^{3-}$ as an illustration (Fig. 3), it is seen that, at low ionic strength, the transfer rate decreases with increasing concentrations of $Os(CN)_6^{4-}$. This indicates that the free Cyt^{II} concentration is reduced because of binding between Cyt^{II} and $Os(CN)_6^{4-}$. At higher ionic strength ($I > 100$ mM), the complex concentration no longer has any effect on the transfer rate.

Discussion

The kinetics of electron transfer between cytochrome c and one of the hexacyanide complexes, $Fe(CN)_6^{3-}$, has been studied extensively [7–9,16–32]. Our previous work has shown that the electron transfer for this system occurs via two (or more) sites of vastly different binding affinites [9]. The binding constant for the strong binding site decreases from about 1600 M^{-1} to 80 M^{-1} as I increases from 15 mM to 140 mM. For the weak site, the binding constant is less than 100 M^{-1} even at low ionic strength. Similar conclusions have been reached by the NMR study of Eley et al. [31]. As explained above, because of technical difficulty, we were not able to verify experimentally the kinetic scheme for the two other hexacyanide complexes. The results obtained for the complex concentration dependence (Fig. 3) serve only to indicate the presence of binding at low ionic strength, but cannot be used to derive the binding constants for $Os(CN)_6^{3-}$ and $Ru(CN)_6^{3-}$. However, since these two complexes are isomorphic to $Fe(CN)_6^{3-}$, it is reasonable to assume that the kinetic results obtained in our previous work should hold for all the complexes.

A quantitative analysis of our data can be performed by assuming that the electron transfer reaction occurring at a given site on the protein can be described by the following equations

$$M(CN)_6^{3-} + Cyt^{II} \underset{d_{32}}{\overset{a_{32}}{\rightleftharpoons}} M(CN)_6^{3-} \cdot Cyt^{II} \xrightarrow{k_e} M(CN)_6^{4-} \cdot Cyt^{III} \quad (1)$$

$$M(CN)_6^{4-} + Cyt^{II} \underset{d_{22}}{\overset{a_{22}}{\rightleftharpoons}} M(CN)_6^{4-} \cdot Cyt^{II} \quad (2)$$

In Eqn. 1, we have assumed that the complex-bound protein, $M(CN)_6^{3-} \cdot Cyt^{II}$, does not undergo any large structural rearrangements prior to electron transfer. This is reasonable since the size of the hexacyanides is not sufficiently large and the binding, electrostatic in nature, is not sufficiently strong. We have also omitted the backward electron-transfer reaction, since its rate is negligible compared with the forward rate k_e for all the complexes. The analysis of the reaction can be further simplified by recognizing that since the concentration of the $M(CN)_6^{3-}$ generated is much less than of Cyt^{II} initially in the sample, the electron transfer reaction (Eqn. 1) has essentially no effect on the concentration of free Cyt^{II}. Thus Eqns. 1 and 2 in practice are

decoupled and, since $[M(CN)_6^{4-}] \gg [Cyt^{II}]$, the total free Cyt^{II} concentration $[Cyt^{II}]_f$ is given by Eqn. 2 to be

$$[Cyt^{II}]_f = \frac{[Cyt^{II}]_t}{1 + K_{22} \cdot [M(CN)_6^{4-}]} \tag{3}$$

where $K_{22} = a_{22}/d_{22}$ is the binding constant of Cyt^{II} for $M(CN)_6^{4-}$ and $[Cyt^{II}]_t$ is the total Cyt^{II} concentration in the sample. Under such an approximation, the observed electron transfer rate, k_0, is given by

$$k_0 = \frac{k_e a_{32} [Cyt^{II}]_f}{a_{32}[Cyt^{II}]_f + d_{32} + k_e} \tag{4}$$

The approximate value of the association rate constant, a_{32}, may be obtained from our previous study of the electron transfer between cytochrome c and zinc porphyrins, $ZnTPPS^{4-}$, where TPPS is tetrakis-(sulfonatophenyl)porphyrin [33]. From the observed bimolecular transfer rate, we estimate a_{32} to vary from $6 \cdot 10^9$ $M^{-1} \cdot s^{-1}$ ($I \approx 20$ mM) to $2 \cdot 10^9$ $M^{-1} \cdot s^{-1}$ ($I \approx$ 300 mM). For the strong binding site, the dissociation rate constant may then be estimated to vary from $4 \cdot 10^6$ s^{-1} to $5 \cdot 10^7$ s^{-1} as I increases from 10 mM to 300 mM. Thus, it is seen that $d_{32} \gg a_{32}[Cyt^{II}]_t$ for $[Cyt]_t \approx$ 100 μM which is the concentration used in this experiment. Similarly, for the weak site, d'_{32} should be larger than $1 \cdot 10^8$ s^{-1} at all ionic strengths used in this experiment. Such d'_{32} values are much greater than a'_{32} $[Cyt^{II}]_t$ as well as the intramolecular electron transfer rates k'_e (see below) for all the complexes. Taking all approximations into account, the total contribution of the two sites to the observed bimolecular rate $k_{12} = k_0/[Cyt^{II}]_t$ may then be represented as

$$k_{12} = \frac{k_e a_{32}}{(d_{32} + k_e)(1 + K_{22}[M(CN)_6^{4-}])} + k'_e K'_{32} \tag{5}$$

where $K'_{32} = a'_{32}/d'_{32}$ is binding constant for the weak site.

In the following, we discuss the electron transfer rates obtained for the various complexes based on the above kinetic scheme (Eqn. 5). First let us consider the simple case of high ionic strength. For example, at $I = 300$ mM, the binding constant for the strong binding site should be quite small (say approx. 20 mM^{-1}), indicating that $d_{32} \approx 1 \cdot 10^8$ s^{-1}. Such a d_{32} value is sufficiently large compared to k_e for all the complexes (see below) so that Eqn. 5 can be further simplified to

$$k_{12} = k_e K_{32} + k'_e K'_{32} \tag{6}$$

where $K_{32} = a_{32}/d_{32}$ is the binding constant for the strong site. According to the electron transfer theories of Hopfield [12] and Marcus [13]

$$k_e = \frac{2\pi}{\hbar} \frac{1}{(4\pi\lambda kT)^{1/2}} |T_{ab}|^2 e^{-(\Delta E - \lambda)^2/4\lambda kT} \tag{7}$$

where T_{ab} is the electron transfer matrix element, ΔE and λ are the overall potential and the reorganization energy for the reaction, respectively. ΔE and λ should be the same for the two sites and Eqn. 6 becomes

$$k_{12} = \frac{2\pi}{\hbar} \frac{1}{(4\pi\lambda kT)^{1/2}} e^{-(\Delta E - \lambda)^2/4\lambda kT} (|T_{ab}|^2 K_{32} + |T'_{ab}|^2 K'_{32}) \tag{8}$$

Since the quantities in the bracket $(|T_{ab}|^2 K_{32} + |T'_{ab}|^2 K'_{32})$ are identical for all isomorphic complexes, the large variations found in the electron transfer rates of the various hexacyanides must result from the differences in their redox potential $E°$. As can be seen from Table I, for an increase in ΔE of about 0.5 eV from $Fe(CN)_6^{3-}$ to $Ru(CN)_6^{3-}$ (see below), k_{12} increases by about 150-fold ($I = 310$ mM). It should be noted that, in fact, this factor of 150 is probably a lower limit, since the bimolecular rate obtained for $Ru(CN)_6^{3-}$ is not much less than the association constant a_{32}. Taking into account the diffusion controlled effect, a more accurate value k_{12}^a can be deduced from k_{12} as follows [34]

$$\frac{1}{k_{12}} = \frac{1}{a_{32}} + \frac{1}{k_{12}^a}$$

Assuming $a_{32} = 2 \cdot 10^9$ $M^{-1} \cdot s^{-1}$, k_{12}^a for $Ru(CN)_6^{3-}$ is found to be $7.8 \cdot 10^8$ $M^{-1} \cdot s^{-1}$ ($I = 310$ mM). Since k_{12} at high ionic strength (see Table I) and k_e exhibit the same ΔE dependence (Eqns. 7 and 8), using the obtained k_{12}^a values, k_e for $Os(CN)_6^{3-}$ and $Ru(CN)_6^{3-}$ can be deduced to be about 45- and 210-times, respectively, higher than that of $Fe(CN)_6^{3-}$. In our previous work, the intramolecuar electron transfer rate for the strong binding site k_e for $Fe(CN)_6^{3-}$ was found to be $4.65 \cdot 10^4$ s^{-1} [9], thus k_e for $Os(CN)_6^{3-}$ and $Ru(CN)_6^{3-}$ are equal to $2.0 \cdot 10^6$ s^{-1} and $9.8 \cdot 10^6$ s^{-1}, respectively.

At low ionic strength, the ratio of k_{12} between $Fe(CN)_6^{3-}$ and $Ru(CN)_6^{3-}$ decreases to about 100 (see Table I). This can be easily understood in terms of the above kinetic scheme (Eqn. 5). For $I = 10$ mM, $K_{32} \approx$ 1500 M^{-1} and k_e for $Ru(CN)_6^{3-}$ is now comparable to d_{32}. Hence, as can be seen from Eqn. 5, k_{12} for $Ru(CN)_6^{3-}$ will be reduced relative to that for $Fe(CN)_6^{3-}$. In fact, if we take $a_{32} = 6 \cdot 10^9$ $M^{-1} \cdot s^{-1}$ and use the kinetic constants obtained in our previous work, the smaller ratio can be reasonably well accounted for quantitatively.

The reorganization energy λ for the reaction can be deduced by fitting the k_e derived above for the various

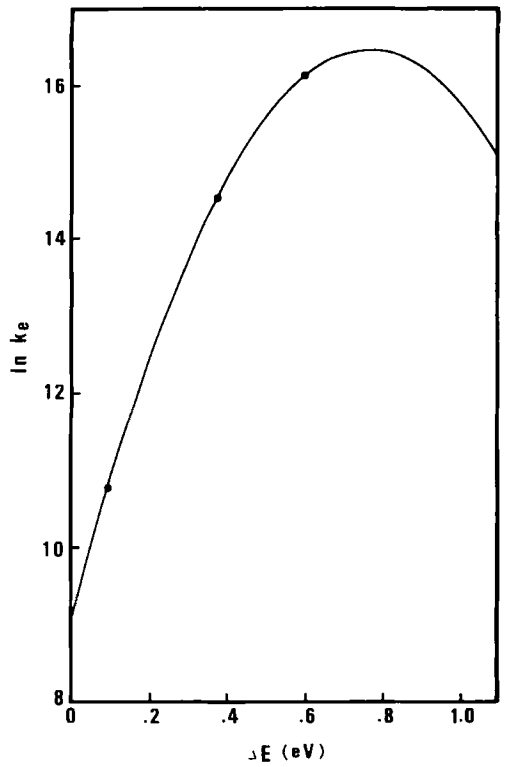

Fig. 4. Plots of $\ln(k_e)$ vs. the driving force ΔE for the electron transfer reaction between Cyt^{II} and hexacyanide complexes. The solid line is a theoretical curve calculated using $k_e = Ae^{-(\Delta E-\lambda)^2/4\lambda kT}$, with $A = 1.4 \cdot 10^7\ s^{-1}$ and $\lambda = 0.77$ eV.

complexes to Eqn. 7. The ΔE values are obtained from the following free molecular redox potentials E° ($I \approx 0$) assuming that the potentials of the protein and the hexacyanides are not greatly affected by the binding; $E^\circ(Cyt^{III}/Cyt^{II}) = 0.26$ V [1], $E^\circ(Fe(CN)_6^{3-}/Fe(CN)_6^{4-}) = 0.356$ V [35], $E^\circ(Os(CN)_6^{3-}/Os(CN)_6^{4-}) = 0.634$ V [36] and $E^\circ(Ru(CN)_6^{3-}/Ru(CN)_6^{4-}) = 0.86$ V [37]. The λ decuced from the best fit is 0.77 eV (Fig. 4). This value is somewhat lower than that of 1.1 eV obtained for the cytochrome *c*-porphyrin system [33], but is very similar to that of 0.7 eV deduced for the cytochrome *c*-cytochrome b_5 couple by McLendon and Miller [38]. The higher λ found for the case of the porphyrin is likely to arise from the outer sphere solvent reorganization term [39]. According to the additivity rule of Marcus [40], the reorganization energies for the protein and hexacyanide self-exchange reactions, λ_{11} and λ_{22}, are related to λ by $\lambda = (\lambda_{11} + \lambda_{22})/2$. The value of λ_{22} should be about 1 eV as estimated from the peak wavelength of the $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ charge-transfer band [29]. Thus, λ_{11} for cytochrome *c* is approx. 0.55 eV, which is a factor of 2 higher than that computed by Churg et al. from the observed X-ray structural changes between reduced and oxidized cytochrome *c* [39].

In conclusion, this study has provided important information on the effect of thermodynamic driving force on biological electron transfer reactions. At present, most of the data are obtained at relatively low driving forces ($\Delta E \leqslant 0.3$ V) [1–4]. As shown in this and other studies [5,6,33,38], high ΔE data can be readily obtained by the method of photoexcitation. They are important for testing the applicability of existing theories to complex protein molecules. The results of this experiment are consistent with the theories of Hopfield [12] and Marcus [13], and a reorganization energy of approx. 0.8 eV is found for the cytochrome *c*-hexacyanide system. We are currently conducting similar studies on other heme proteins such as myoglobin which also undergo a reversible redox reaction. These studies are useful for identifying the various factors which facilitate the physiological function of electron transfer proteins such as cytochrome *c*.

References

1 Wherland, S. and Gray, H.B. (1977) in Biological Aspects of Inorganic Chemistry (Addison, A.W. et al., eds.), pp. 289–368, Wiley, New York.
2 McArdle, J.V., Coyle C.L., Gray, H.B., Yoneda, G.S. and Holwerda (1977) J. Am. Chem. Soc. 99, 2483–2489.
3 Segal, M.G. and Sykes, A.G. (1978) J. Am. Chem. Soc. 100, 4585–4592.
4 Sisley, M.J., Segal, M.G., Stanley, C.S., Adzamli, I.K. and Sykes, A.G. (1983) J. Am. Chem. Soc. 105, 255–228.
5 Brunschwig, B.S., Delaive, P.J., English, A.M., Goldberg, M., Gray, H.B., Mayo, S.L. and Sutin, N. (1985) Inorg. Chem. 24, 3743–3479.
6 Cho, K.C., Che, C.M., Ng, K.M. and Choy C.L. (1987) J. Phys. Chem. 91, 3690–3693.
7 Stellwagen, E. and Shulman, R.G. (1973) J. Mol. Biol. 80, 559–573.
8 Stellwagen, E. and Cass, R.D. (1975) J. Biol. Chem. 249, 6788–6795.
9 Cho, K.C., Chu, W.F., Choy, C.L. and Che, (1988) C.M. Biochim. Biophys. Acta 934, 161–168.
10 Lachish, U., Shafferman, A. and Stein, G. (1976) J. Chem. Phys. 64, 4205–4211.
11 Vogler, A., Losse, W. and Kunkely, H. (1979) J. Chem. Soc. Chem. Commun. 187–188.
12 Hopfield, J.J. (1974) Proc. Natl. Acad. Sci. USA 71, 360–364.
13 Marcus, R.A. and Sutin, N. (1985) Biochim. Biophys. Acta 811, 265–322.
14 Krauss, F. and Schrader, G. (1928) J. Prakt. Chem. 119, 279–281.
15 Krause, R.A. and Violette, C. (1986) Inorg. Chim. Acta 113, 161–162.
16 Sutin, N. and Christman, J.R. (1961) J. Am. Chem. Soc. 83, 1773–1774.
17 Brandt, K.G., Parks, P.C., Czerlinski, G.H. and Hess, G.P. (1966) J. Biol. Chem. 241, 4180–4195.
18 Cassatt, J.C. and Marini, C.P. (1974) Biochemistry 13, 5323–5328.
19 Creutz, C. and Sutin, N. (1974) J. Biol. Chem. 249, 6788–6795.
20 Miller, W.G. and Cusanovich (1975) Biophys. Struct. Mech. 1, 97–111.
21 Ilan, Y. and Shafferman, A. (1979) Biochim. Biophys. Acta 548, 161–165.
22 McCray, J.A. and Kihara, T. (1979) Biochim. Biophys. Acta 548, 417–426.
23 Kihara, H. (1981) Biochim. Biophys. Acta 634, 93–104.
24 Ohno, N. and Cusanovich, M.A. (1981) Biophys. J. 36, 589–605.

25 Butler, J., Davies, D.M. and Sykes, A.G. (1981) J. Inorg. Biochem. 15, 41–53.
26 Ahmed, A.J. and Millet, F. (1981) J. Biol. Chem. 256, 1611–1615.
27 Hopfield, J.J. and Urgubil, K. (1982) in Electron Transport and Oxygen Utilization (Ho, C., ed.), pp. 81–84, Elsevier, New York.
28 Eley, C.G.S., Moore, G.R., Williams, G. and Williams, R.J.P. (1982) Eur. J. Biochem. 124, 295–303.
29 Chang, A.M. and Austin, R.H. (1982) J. Chem. Phys. 77, 5272–5283.
30 Ragg, E. and Moore, G.R. (1984) J. Inorg. Biochem. 21, 253–261.
31 Eley, C.G.S., Ragg, E. and Moore, G.R. (1984) J. Inorg. Biochem. 21, 295–310.
32 Cartling, B., Holtom, G.R. and Spiro, T.G. (1985) J. Chem. Phys. 83, 3894–3905.
33 Cho, K.C., Che, C.M., Ng, K.M. and Choy, C.L. (1986) J. Am. Chem. Soc. 108, 2814–2818.
34 Marcus, R.A. (1960) Discuss. Faraday Soc. 29, 129–130.
35 Hanania, G.I., Irvine, D.H., Eaton, W.A. and George, P. (1967) J. Phys. Chem. 71, 2022–2030.
36 Opekar, F. and Beran P. (1976) J. Electroanal. Chem. 71, 120–124.
37 Deford, D.D. and Davidson, A.W. (1951) J. Am. Chem. Soc. 73, 1469–1471.
38 McLendon, G. and Miller, J.R. (1985) J. Am. Chem. Soc. 107, 7811–7816.
39 Churg, A.K., Weiss, R.M., Warshel, A. and Takano, T. (1983) J. Phys. Chem. 87, 1683–1694.
40 Marcus, R.A. (1965) J. Chem. Phys. 43, 679–701.