Long-Range Electron Transfer in Heme Proteins

STEPHEN L. MAYO, WALTHER R. ELLIS, JR., ROBERT J. CRUTCHLEY, HARRY B. GRAY

Kinetic experiments have conclusively shown that electron transfer can take place over large distances (greater than 10 angstroms) through protein interiors. Current research focuses on the elucidation of the factors that determine the rates of long-range electron-transfer reactions in modified proteins and protein complexes. Factors receiving experimental and theoretical attention include the donor-acceptor distance, changes in geometry of the donor and acceptor upon electron transfer, and the thermodynamic driving force. Recent experimental work on heme proteins indicates that the electron-transfer rate falls off exponentially with donor-acceptor distance at long range. The rate is greatly enhanced in proteins in which the structural changes accompanying electron transfer are very small.

THE ELECTRON TRANSFERS THAT FIGURE PROMINENTLY IN oxidative phosphorylation (*1, 2*) and photosynthesis (*3, 4*) frequently involve protein-bound metal ions that are separated by relatively large distances (10 Å or more), as illustrated by the recent x-ray structural analysis of a bacterial photosynthetic reaction center (*5*). Although the interactions between the electron-exchanging sites are expected to be weak, owing to the long distances involved, the redox reactions are often surprisingly fast (*6*). This circumstance has led experimentalists and theoreticians to devote much attention to the factors that control the rates of long-range electron transfers (*7–15*). Of primary concern is the dependence of the kinetics on donor-to-acceptor distance (*16*), but most investigators recognize that inner-sphere reorganization (that is, changes in geometry of the donor and acceptor upon electron transfer), solvation (*17, 18*), the thermodynamic driving force ($\Delta G°$), the nature of the intervening medium (*19*), and the donor-acceptor orientation (*20, 21*) also play key roles.

Experiments that have provided useful information about long-range electron-transfer rates have involved proteins and protein complexes in which the donor-acceptor distances can be estimated from crystal structure analyses. In the best of cases the distance is reasonably well fixed during the course of electron transfer, but in other instances there is some uncertainty about the distance at the time the electron travels from the donor to the acceptor. For example, Isied and co-workers (*22*) have demonstrated that a polypeptide bridge is capable of mediating the passage of an electron between two redox centers at relatively short range, but the types of molecules they have used are not rigid enough to allow the determination of distance dependence of the transfer rate at long range.

S. L. Mayo is a graduate student, W. R. Ellis, Jr., is a research fellow, and H. B. Gray is the Arnold O. Beckman Professor of Chemistry in the Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125. R. J. Crutchley is assistant professor of chemistry, Carleton University, Ottawa, Canada K1S 5B6. Correspondence should be addressed to H.B.G.

Cytochrome *c*

One approach to the study of long-range electron transfer is to introduce by chemical modification a donor or acceptor site in a structurally characterized metalloprotein. The protein framework may be viewed from an inorganic chemist's point of view as that of a bridging ligand. The artificial donor-acceptor complex that has received most attention is $a_5Ru^{2+/3+}$ (a = NH_3), which covalently bonds to the surface histidines of proteins. This complex can be attached by the reaction of $a_5Ru(OH_2)^{2+}$ with native protein under mild conditions, and the "ruthenated" protein can be purified by ion-exchange chromatography.

Horse heart cytochrome *c* was selected as the first target for derivatization experiments, because it is commercially available and very stable. Additionally, more is known about the structure and reactivity of cytochrome *c* than about those of any other electron-transfer protein (*23–25*). In 1982 our group together with Worosila and Isied reported the preparation of a ruthenium derivative of cytochrome *c*, and we showed that the a_5Ru^{3+} moiety was coordinated to a nitrogen on the imidazole ring of histidine-33 in the protein (*26*). A computer-generated view (*27–30*) of the structures of the two redox centers in a_5Ru(His-33)Cyt *c* is shown in Fig. 1.

Rigid-geometry conformational searching on a_5Ru(His-33) tuna cytochrome *c* (where Trp-33 of the tuna structure was replaced with histidine) has shown that the His-33 side-chain conformation is not much affected by the a_5Ru group (*29*). And, importantly, spectroscopic measurements have confirmed that the conformation of a_5Ru(His-33)Cyt *c* is virtually the same as that of the native protein (*26, 31*). Electrochemical data further establish that the heme *c* is unperturbed in the ruthenated protein, because the thermodynamic parameters associated with heme *c* reduction are similar to those observed for the native protein (Table 1).

The rate of long-range (~12 Å) electron transfer from the a_5Ru(His-33)$^{2+}$ center to the ferriheme has been measured by two methods. One involves the reduction of a_5Ru(His-33)$^{3+}$Cyt *c*-(Fe^{3+}) by electronically excited $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine) to rapidly yield a mixed-valence species that subsequently decays to the fully reduced protein by slow intramolecular electron transfer (*32*). In the second, pulse radiolysis (*33*) is used to generate reducing agents that react with the fully oxidized ruthenated protein to produce a_5Ru(His-33)$^{2+}$Cyt *c*(Fe^{3+}). In both instances, the sequence of electron-transfer steps is given by Eq. 1:

$$a_5Ru(His\text{-}33)^{3+}Cyt\ c(Fe^{3+}) \xrightarrow{fast} a_5Ru(His\text{-}33)^{2+}Cyt\ c(Fe^{3+}) \xrightarrow{slow,\ k_1} a_5Ru(His\text{-}33)^{3+}Cyt\ c(Fe^{2+}) \quad (1)$$

The intramolecular (k_1) rate constants extracted from the two types of experiments are not substantially different; flash photolysis gives a rate of 30 sec^{-1} (25°C), whereas pulse radiolysis yields 50 sec^{-1}. At present one of the most important criteria for establishing conformity between theoretical models and experimental data is the temperature dependence of the electron-transfer rate. The long-range ($Ru^{2+} \rightarrow Fe^{3+}$) electron-transfer rate does not depend strongly on temperature; indeed, in the flash photolysis experiments there is little if any change in the 30 sec^{-1} rate from 0° to 80°C (*34*).

The weak temperature dependence of the long-range electron-transfer rate in a_5Ru(His-33)Cyt *c* suggests that the energy for reorganization of the heme *c* in the protein is relatively small (~0.3 eV), a finding that is in accord with Warshel's theoretical analysis based on the structures of oxidized and reduced tuna cytochrome *c* (*35*). An even smaller value (~0.1 eV) is indicated by recent resonance Raman scattering measurements (*36*). Evidently, a low-spin heme encapsulated in the hydrophobic interior of a protein can perform its redox function efficiently, because in this environment the structural changes that are required for the oxidation or reduction of the site are very small. In an aqueous medium, the electron-transfer reorganization energy is expected to be much greater (*35*).

It is clear from the a_5Ru(His-33)Cyt *c* experiments that an electron can be transferred over a relatively long distance (~12 Å) through a protein in a fraction of a second. The transfer time is fast in comparison with results obtained in some model systems (*37*), but it is much slower than the rates of 10^6 to 10^9 sec^{-1} observed by Calcaterra, Closs, and Miller (*38, 39*) in their work on long-range electron transfer between a biphenyl radical anion and various organic acceptors (for example, naphthyl) held ~10 Å apart by a steroid frame. Vast differences in the reduction potentials of the donors and acceptors, solvents, and "through-bond" distances in the protein and hydrocarbon systems suggest that different mechanisms may operate in the two cases. In a_5Ru(His-33)Cyt *c*, the electron transfer from Ru^{2+} to Fe^{3+} is most likely forced to occur through the ~12 Å of "space" that includes two amino acid residues (Fig. 2)

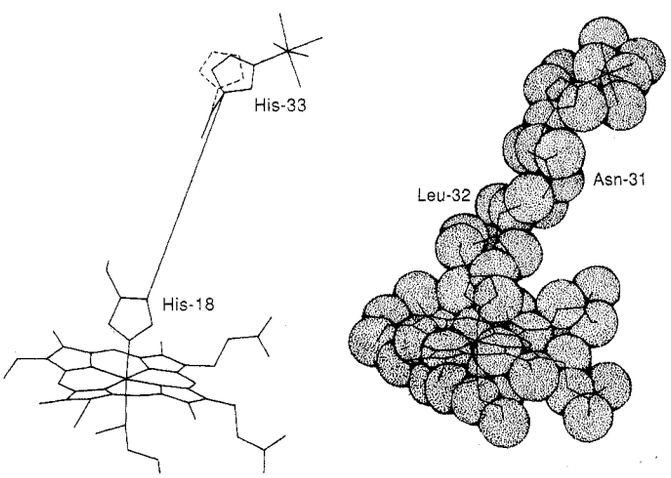


Fig. 1 (left). Computer-generated view of the two redox units in a_5Ru(His-33)Cyt *c*. The heme and its ligands are as in the tuna cytochrome *c* structure (*28, 44*) and the conformation of a_5Ru(His-33)(Trp-33 of tuna cytochrome *c* replaced by histidine) is as predicted by rigid-geometry conformational energy searching (*29*). The dashed lines show the position of the histidine side chain before optimization with a_5Ru. The calculated deviation in the 11.7-Å edge-to-edge distance is less than 0.1 Å, thereby indicating that a_5Ru does little to perturb the structure of the protein. Fig. 2 (right). Computer-generated view of the two redox units in a_5Ru(His-33)Cyt *c* and the residues comprising the intervening medium. The dots represent the van der Waals surfaces of the displayed atoms and show that there is van der Waals contact over the "through-space" electron-transfer pathway.

Table 1. Thermodynamic parameters (*p*H 7.0) for the reduction of the heme *c* in native and $a_5Ru^{2+/3+}$ modified cytochrome *c*. See (*34*). Abbreviations: $E°$, reduction potential; NHE, normal hydrogen electrode; $\Delta G°$, free energy change; $\Delta S°$, entropy change; $\Delta H°$, enthalpy change.

Measure	a_5Ru(His-33)Cyt *c*	Native Cyt *c*
$E°$ (mV) versus NHE*	270 ± 2	260 ± 2
$\Delta G°$ (kcal mol^{-1})*	−6.22 ± 0.05	−6.00 ± 0.05
$\Delta S°$ (cal deg^{-1} mol^{-1})	−29.2 ± 0.8	−28.5 ± 1.2
$\Delta H°$ (kcal mol^{-1})*	−14.9 ± 0.3	−14.5 ± 0.4

*At 25°C.

(*40*), whereas the transfer from biphenyl anion to naphthyl may be through-bond. The latter proposal is supported by recent theoretical work that indicates that electron transfer across the steroid system is facilitated by the network of carbon-carbon single bonds (*41*). The question of through-bond (*42*) versus through-space electron transfer is an important one. Recent theoretical studies (*15, 43*) indicate that through-bond transfer is more efficient in certain cases. Nevertheless, in cytochrome *c*, there is no evidence that a through-bond mechanism operates at long range.

Myoglobin

The importance of donor and acceptor structural changes in long-range electron transfer is underscored by recent work on ruthenated sperm whale myoglobin. Unlike cytochrome *c* (*23, 44*), the heme in myoglobin (Mb) is high-spin in both the Fe^{2+} and Fe^{3+} states (*45*), and a water ligand is lost when $Mb(Fe^{3+})$ is reduced to $Mb(Fe^{2+})$ (*46*). The electron-transfer reorganization energy for myoglobin is therefore expected to be relatively large (*47*).

Computer graphics analysis indicates that each of the four surface histidines shown in Fig. 3 should be able to bind to $a_5Ru^{2+/3+}$ without altering the protein conformation. Experimental work in our laboratory has confirmed this prediction; the four singly modified proteins, a_5Ru(His-12)Mb, a_5Ru(His-48)Mb, a_5Ru(His-81)Mb, and a_5Ru(His-116)Mb, have been prepared and fully characterized (*48, 49*). Of the four ruthenated derivatives, the electron-transfer distance from $a_5Ru(His)^{2+/3+}$ to heme ($Fe^{3+/2+}$) is shortest in a_5Ru(His-48)Mb (14.6 Å; see Fig. 3).

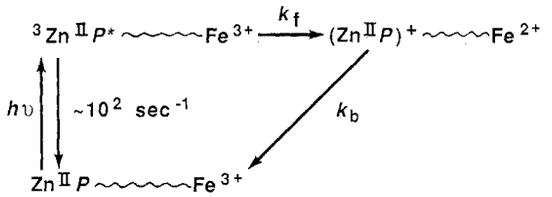
Electrochemical data confirm that the myoglobin structure is not perturbed by the a_5Ru^{3+} group that is bonded to His-48 (Table 2). In this case, what is observed kinetically is an approach to equilibrium (Eq. 2)

$$a_5Ru(His\text{-}48)^{3+}Mb(Fe^{3+}) \xrightarrow{fast} a_5Ru(His\text{-}48)^{2+}Mb(Fe^{3+}) \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} a_5Ru(His\text{-}48)^{3+}Mb(Fe^{2+}) \quad (2)$$

Both the forward (k_1) and reverse (k_{-1}) electron-transfer rates have been determined over a 5° to 45°C temperature range by the flash photolysis technique; at 25°C, the forward rate constant is 0.019 sec^{-1}, whereas the back–electron transfer proceeds at 0.041 sec^{-1}. Both reactions are strongly temperature-dependent, with $\Delta H^{\ddagger}$ = 7.4 and 19.5 kcal mol^{-1}. Based on the thermodynamic and kinetic data for long-range electron transfer in a_5Ru(His-48)Mb, the reorganization energy of the myoglobin high-spin heme has been calculated to be 0.87 eV (*49, 50*), which, as expected, is much larger than that for the low-spin heme in cytochrome *c*. This result is in accord with earlier studies of myoglobin (*51*), cytochrome *c* (*52*), and iron porphyrin (*53, 54*) cross-reactions, which suggest that electron transfers involving high-spin hemes are more sluggish than those of low-spin analogs.

Excited-State Zinc Porphyrins as Electron Donors

Independently, Hoffman and co-workers (*55–59*) and McLendon and colleagues (*60–63*) have shown the utility of an electronically excited zinc porphyrin as an electron donor in long-range electron-transfer experiments. This excited triplet species, $^3ZnP^*$, is a good reducing agent and lives long enough (~20 msec at 25°C in nonaqueous solvents) to undergo electron transfer to a distant acceptor in a protein (Eq. 3)



(3)

The $(Zn^{II}P)^+$ cation radical product of the *kf* step is a strong oxidant and back–electron transfer is observed to produce the ground state and complete the cycle. For example, Hoffman's work on a zinc-iron hybrid hemoglobin, [Zn,Fe]Hb, has shown that an electron can be transferred ~20 Å from $^3Zn^{II}P^*$ to a high-spin ferriheme at a rate of 100 sec^{-1} at room temperature (*55, 56*).

McLendon and co-workers have measured the rates of long-range electron transfer in several protein-protein complexes (*60–64*) whose structures have been modeled by optimizing certain surface interactions between the two partners (*65–67*). A case in point is the complex between zinc-substituted hemoglobin and cytochrome *b*5, Hb(Zn*P*)/Cyt *b*5(Fe^{3+}). Photoproduction of $^3ZnP^*$ is followed by long-range $^3ZnP^* \rightarrow Fe^{3+}$ electron transfer in the Hb/Cyt *b*5 complex at a rate of 8000 sec^{-1} at room temperature (*60*). This rate is rather slow for a transfer that is believed to involve a relatively short (~10 Å) donor-acceptor distance (*65*); however, analogous experiments on the zinc-cytochrome *c* complex with cytochrome *b*5 (^3Zn*P* Cyt *c*)/Cyt *b*5(Fe^{3+}), where the estimated donor-acceptor distance is ~8 Å, have yielded a rate of 5×10^5 sec^{-1} at room temperature (*61*).

Long-range electron-transfer experiments on the complex between cytochrome *c* and zinc-substituted yeast cytochrome *c* peroxidase, (^3Zn*P* C*c*P)/yeast Cyt *c*(Fe^{3+}), have been reported by Hoffman and co-workers (*58*). The rate of transfer from $^3ZnP^*$ to Fe^{3+} in the yeast protein-protein complex is 120 sec^{-1}, whereas the corresponding rate for yeast (^3Zn*P* C*c*P)/horse heart cytochrome *c*(Fe^{3+}) is only 10 sec^{-1}. The reduced rate in the yeast-horse complex could be due to a medium effect (that is, the yeast-yeast complex could exhibit a medium-enhanced rate that operates over the estimated 17-Å donor-acceptor distance). Alternatively, the donor-acceptor distance may be slightly greater in the yeast-horse complex.

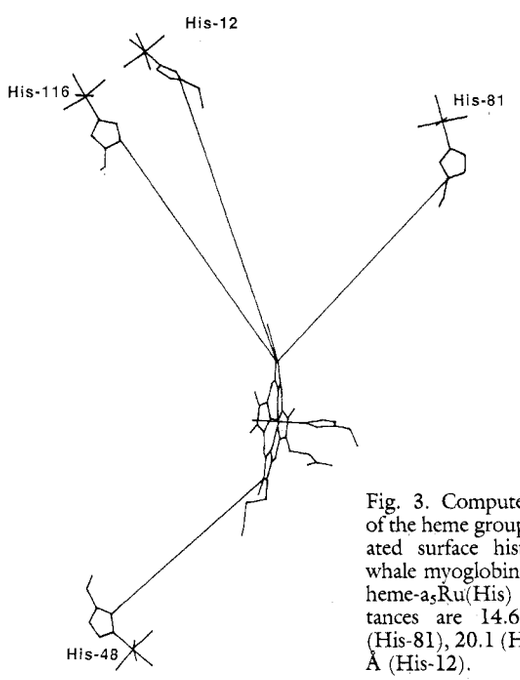


Fig. 3. Computer-generated view of the heme group and four ruthenated surface histidines in sperm whale myoglobin (*29*). The closest heme-a_5Ru(His) edge-edge distances are 14.6 (His-48), 19.1 (His-81), 20.1 (His-116), and 22.1 Å (His-12).

Distance Dependence

Kinetic and thermodynamic data for long-range electron transfer from $^3ZnP^*$, Fe^{2+}, or Ru^{2+} to Fe^{3+} acceptors in various proteins and protein-protein complexes are collected in Table 3. Since the driving forces are comparably large in all cases in which $^3ZnP^*$ is the donor, it is likely that the rate variations are due mainly to differences in the donor-acceptor distance and the nature of the intervening medium. In the three examples with relatively low driving forces (~0.2 to ~0.4 V), the acceptor is the low-spin heme *c*(Fe^{3+}) of horse heart cytochrome *c*. Thus, in both sets of experiments, if one assumes weak donor-acceptor electronic coupling, the rate of electron transfer should fall off exponentially with distance (*R*) between donor and acceptor,

$$k = k_0 \exp(-\beta R) \quad (4)$$

where β reflects the extent of electronic coupling through the protein medium (*11, 12*).

Plots of ln *k* versus *R* based on the data (Table 3) for small and large ΔG° experiments indicate that β is 0.7 to 0.9 Å^{-1} (Fig. 4). Both β values are surprisingly small, thereby raising the possibility that the intrinsically weak donor-acceptor electronic coupling is propagated through a protein more effectively than commonly assumed (*11, 12*). (Alternatively, the small values may signal that the computer-generated structures of the protein complexes are not good indicators of the electron-transfer distances.) There is a hint that β may depend on the donor energy, with the smaller value associated with the more energetic donor. In this connection, studies of long-range electron transfer in blue copper proteins with donor reduction potentials between 0.2 and 0.4 V versus normal hydrogen electrode (NHE) have been interpreted in terms of β values of ~1.2 to 1.4 Å^{-1} (*68, 69*). Of course, blue copper centers are electronically distinctive (*69, 70*), and comparisons with hemes may not be valid.

Future Directions

Although much valuable information has been obtained from both intermolecular and intramolecular studies, no biological system studied to date addresses the issue of distance dependence on electron-transfer rates in a definitive fashion. Even in systems such as myoglobin, where four singly ruthenated proteins have been characterized (see Fig. 3), and where preliminary measurements of $^3ZnP^* \rightarrow Ru^{3+}$ electron-transfer rates in the a_5RuMb(Zn*P*) derivatives indicate that β is about 1.4 Å^{-1} (*71*), the issue of distance dependence is clouded by the nonconstancy of factors such as donor-acceptor orientation and the nature of the intervening medium.

In recent months we have started to explore the possibility of using site-directed mutagenesis to build a suitable system for determining how electron-transfer rates change with distance. The idea, which could be called nonperturbative site-directed mutagene-

sis, is to introduce ligands at sites remote from the native electron-transfer center. The ligands would then provide a means of probing that center, without perturbing it in the process.

Specifically, we intend to introduce histidine residues onto the surface of yeast iso-1 cytochrome *c* and to study the electron-transfer kinetics from these remote sites to the heme iron, using the ruthenium method. Yeast iso-1 cytochrome *c* is particularly attractive for this purpose because of the extensive genetic work done on the system (*72*) and the availability of the gene (*73–78*). And, in general, the wealth of sequence and structural data available for *c*-type cytochromes (*79*) enhances the potential of designing isofunctional mutants. By judiciously choosing the new histidine positions, we should be able to generate a series of proteins that will allow a direct examination of the effect of distance on intraprotein electron-transfer rates.

In an initial experiment aimed at improving the stability of the wild-type protein, we have replaced Cys-102 with Ser (*80*). This was done to prevent the formation of protein-protein dimers in the redox-active environment of the ruthenation reaction and to allow more convenient electrochemical analysis. The resulting mutant, as anticipated from computer graphics studies, is isofunctional with the wild-type protein. Preliminary spectroelectrochemical studies (*81*) indicate that the redox potential of the iron center is virtually the same (272 mV versus NHE; *p*H 6.8, ionic strength 0.1*M*, 25°C) as the potential of the wild-type protein (*82*).

Concluding Remarks

It is apparent that a key component of the activation energy for metalloprotein electron transfer is the inner-sphere reorganization. Metalloproteins that function solely as electron-transfer agents minimize inner-sphere reorganization by adopting coordination environments that are midway between those favored by the oxidized and reduced metal cofactors. These proteins could be called "long-range electron transferases."

Table 2. Thermodynamic parameters (*p*H 7.0) for the reduction of a_5Ru^{3+} and the heme site in native and modified myoglobin. See (*50*).

Measure	Native Mb $Fe^{3+/2+}$	Modified Mb	
		$Fe^{3+/2+}$	$a_5Ru^{3+/2+}$
$E°$ (mV) versus NHE*	58.8 ± 2	65.4 ± 2	85.8 ± 2
$\Delta G°$ (kcal mol^{-1})*	−1.36 ± 0.05	−1.51 ± 0.05	−1.98 ± 0.05
$\Delta S°$ (cal deg^{-1} mol^{-1})	−39.2 ± 1.2	−37.6 ± 1.2	4.2 ± 1.2
$\Delta H°$ (kcal mol^{-1})*	−13.0 ± 0.4	−12.7 ± 0.4	−0.7 ± 0.4

*At 25°C.

Table 3. Kinetic and thermodynamic data for long-range electron transfer in proteins and protein complexes. See (*16*). Abbreviations: *k*, electron-transfer rate; *R*, distance between donor and acceptor; $\Delta G°'$, free energy change.

Donor/acceptor	k (sec^{-1})	R (Å)	$\Delta G°'$ (V)	Reference
(^{3}Zn*P* Cyt *c*)/Cyt $b_5(Fe^{3+})$	$5 \pm 1 \times 10^5$	8	0.85	(*61*)
[^{3}Zn*P*/Fe^{3+}]Hb/Cyt $b_5(Fe^{3+})$	$8 \pm 2 \times 10^3$	10	0.80	(*60*)
(^{3}Zn*P* C*c*P)/yeast Cyt $c(Fe^{3+})$	138 ± 12	17	0.90	(*58*)
[^{3}Zn*P*/Fe^{3+}]Hb	100 ± 10	20	0.80	(*55, 56*)
Cyt $b_5(Fe^{2+})$/horse Cyt $c(Fe^{3+})$	$1.6 \pm 0.7 \times 10^3$	8	0.26	(*64*)
a_5Ru(His-33)$^{2+}$Cyt $c(Fe^{3+})$	30 ± 3	12	0.19	(*34*)
Yeast C*c*P(Fe^{2+})/horse Cyt $c(Fe^{3+})$	0.3 ± 0.1	17	0.40	(*63*)

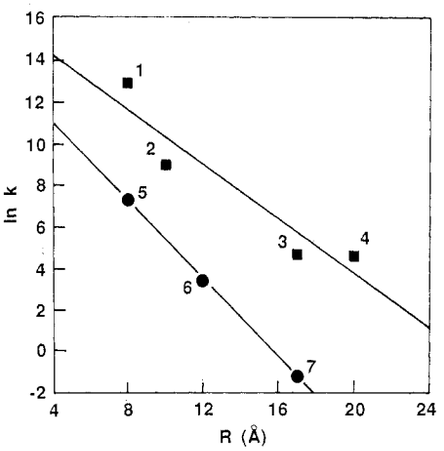


Fig. 4. Plot of ln *k* versus donor-acceptor distance (*R*) for large driving force (1–4) and small driving force (5–7) experiments. See Table 3.

Interpretations of the electron-transfer kinetic behavior of heme oxidase and oxygenase enzymes must take into account the role of different heme spin states (*83*). In some cases (for example, cytochrome cd_1, *Pseudomonas aeruginosa* cytochrome *c* peroxidase, and beef heart cytochrome *c* oxidase) both high-spin and low-spin hemes are found within the same protein molecule. In other cases (for example, cytochrome P-450) the heme exists as an equilibrium mixture of high-spin and low-spin forms. Although the spin state itself probably will not determine the redox kinetics of a heme site, a change in heme geometry (perhaps linked with a spin equilibrium) is expected to result in slow electron transfer (*84*). Provided that no major changes in heme geometry occur, it is possible that, other factors being equal, the different heme spin states will exhibit similar redox activities (*85*).

Although progress has been made in elucidating some of the factors that control long-range electron-transfer rates in proteins, there is much left to learn. An interesting possibility that has been raised by the experimental work, and one that has some theoretical support (*86*), is that β varies with donor reduction potential. But we must emphasize that it is not yet established that the rate constant, *k*, always depends exponentially on distance, $\exp(-\beta R)$, in a protein environment. In the few studies involving analogous donors and acceptors at different fixed distances, the ln *k* values appear to decrease linearly with *R*, but many more experiments of this type will be needed before we can consider the matter settled.

An important point raised by the plots in Fig. 4 is that the extrapolated electron-transfer rates near the van der Waals contact distance (~4 Å) fall orders of magnitude below the commonly assumed 10^{13} sec^{-1} value for the nuclear frequency factor even after allowance is made for the Franck-Condon term (containing ΔG^*). Points 1 to 4 in Fig. 4 are for systems that are estimated to be near the activationless regime. At relatively short range the donor-acceptor electronic coupling in these systems is extremely weak, corresponding to a small electronic matrix element T_{ab} (*12*). The exchange integral *J* is proportional to the square of T_{ab} (*87*) and hence magnetic measurements are relevant to this issue. Recent experimental determinations of *J* for iron porphyrins in van der Waals complexes in crystals have shown that there is very weak (*88*) ($|J| < 1$ cm^{-1})Fe^{3+}-Fe^{3+} antiferromagnetic coupling, which is consistent with heme-to-heme electron transfer at $R = 4$ Å (*16*) being orders of magnitude slower than 10^{13} sec^{-1}. At some point, as the distance decreases further, a donor-acceptor heme system should be able to couple strongly through the bonds of the porphyrin network. It may be in this region that the electron-transfer mechanism of a heme protein will change from through-space to through-bond.

REFERENCES AND NOTES

1. Y. Hatefi, *Annu. Rev. Biochem.* **54**, 1015 (1985).
2. B. P. S. N. Dixit and J. M. Vanderkooi, *Curr. Top. Bioenerg.* **13**, 159 (1984).
3. M. E. Michel-Beyerle, Ed., *Antennas and Reaction Centers of Photosynthetic Bacteria* (Springer-Verlag, Berlin, 1985).
4. Govindjee, Ed., *Photosynthesis: Energy Conversion by Plants and Bacteria* (Academic Press, New York, 1982), vol. 1.
5. J. Deisenhofer, O. Epp, K. Miki, R. Huber, H. Michel, *J. Mol. Biol.* **180**, 385 (1984); J. Deisenhofer, H. Michel, R. Huber, *Trends Biochem. Sci.* **10**, 243 (1985).
6. D. DeVault and B. Chance, *Biophys. J.* **6**, 825 (1966). This paper did much to stimulate further research on biological electron-transfer reactions.
7. J. L. Dreyer, *Experientia* **40**, 653 (1984).
8. S. Wherland and H. B. Gray, in *Biological Aspects of Inorganic Chemistry*, D. Dolphin *et al.*, Eds. (Wiley, New York, 1977), p. 289.
9. R. A. Scott, A. G. Mauk, H. B. Gray, *J. Chem. Educ.* **62**, 932 (1985).
10. S. S. Isied, *Prog. Inorg. Chem.* **32**, 443 (1984).
11. R. A. Marcus and N. Sutin, *Biochim. Biophys. Acta* **811**, 265 (1985).
12. J. J. Hopfield, in *Electrical Phenomena at the Biological Membrane Level*, E. Roux, Ed. (Elsevier, Amsterdam, 1978), p. 471; J. J. Hopfield, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3640 (1974).
13. B. Chance *et al.*, Eds., *Tunneling in Biological Systems* (Academic Press, New York, 1979).
14. D. DeVault, *Quantum Mechanical Tunnelling in Biological Systems* (Cambridge Univ. Press, Cambridge, ed. 2, 1984).
15. N. S. Hush, *Coord. Chem. Rev.* **64**, 135 (1985).
16. Donor-acceptor R values reported here are the closest edge-edge (porphyrin-porphyrin or imidazole-imidazole) distances.
17. A. Garg, J. N. Onuchic, V. Ambegaokar, *J. Chem. Phys.* **83**, 4491 (1985); J. N. Onuchic, D. N. Beratan, J. J. Hopfield, *J. Phys. Chem.*, in press.
18. E. M. Kosower, *J. Am. Chem. Soc.* **107**, 1114 (1985).
19. Aromatic ligands sometimes enhance electron-transfer rates between transition metal complexes in solution. Nothing is known about medium effects of this sort in proteins, however. Medium effects may be operative in cytochrome c_3 as there is direct structural evidence for heme-heme and heme–aromatic side chain interactions. Additionally, a large electrical conductivity is observed for cytochrome c_3 in the solid state. See K. Kimura, Y. Nakahara, T. Yagi, H. Inokuchi, *J. Chem. Phys.* **70**, 3317 (1979); M. Pierot, R. Haser, M. Frey, F. Payan, J.-P. Astier, *J. Biol. Chem.* **257**, 14341 (1982); Y. Higuchi, M. Kusunoki, Y. Matsuura, N. Yasuoka, M. Kakudo, *J. Mol. Biol.* **172**, 109 (1984).
20. P. Siders, R. J. Cave, R. A. Marcus, *J. Chem. Phys.* **81**, 5613 (1985). R. J. Cave, S. J. Klippenstein, R. A. Marcus, *ibid.* **84**, 3089 (1986).
21. M. Makinen, S. A. Schichman, S. C. Hill, H. B. Gray, *Science* **222**, 929 (1983).
22. S. S. Isied and A. Vassilian, *J. Am. Chem. Soc.* **106**, 1732 (1984); ———, R. H. Magnuson, H. A. Schwarz, *ibid.* **107**, 7432 (1985).
23. R. Timkovich, in *The Porphyrins*, D. Dolphin, Ed. (Academic Press, New York, 1979), vol. 7, p. 241.
24. G. R. Moore, C. G. S. Eley, G. Williams, *Adv. Inorg. Bioinorg. Mech.* **3**, 1 (1984).
25. G. Williams, G. R. Moore, R. J. P. Williams, *Comments Inorg. Chem.* **4**, 55 (1985).
26. K. M. Yocom *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7052 (1982).
27. All figures and calculations were done with BIOGRAF/III. BIOGRAF/III was designed and written by S. L. Mayo, B. D. Olafson, and W. A. Goddard III for use in molecular modeling applications that have particular emphasis on design, display, and analysis of biological molecules.
28. Protein coordinates were obtained from the Brookhaven Protein Data Bank [F. C. Bernstein *et al.*, *J. Mol. Biol.* **112**, 535 (1977)].
29. The rigid-geometry conformational searches were performed by successively incrementing the two histidine side-chain dihedral angles by 3.6° through the range 0° to 360°. Only residues within 8 Å of the "native" histidine position were included in the calculation and only the van der Waals energy was used to position the ruthenated histidine. The van der Waals energy was calculated with a Lennard-Jones 6–12 potential function with parameters that were obtained by fitting ab initio potential energy surfaces (*30*).
30. M. J. Brusich, B. D. Olafson, W. A. Goddard III, in preparation.
31. K. M. Yocom, J. R. Winkler, D. G. Nocera, E. Bordignon, H. B. Gray, *Chem. Scr.* **21**, 29 (1983).
32. J. R. Winkler, D. G. Nocera, K. M. Yocom, E. Bordignon, H. B. Gray, *J. Am. Chem. Soc.* **104**, 5798 (1982).
33. S. S. Isied, C. Kuehn, G. Worosila, *ibid.* **106**, 1722 (1984).
34. D. G. Nocera, J. R. Winkler, K. M. Yocom, E. Bordignon, H. B. Gray, *ibid.*, p. 5145.
35. A. K. Churg, R. M. Weiss, A. Warshel, T. Takano, *J. Phys. Chem.* **87**, 1683 (1983).
36. K. T. Schomacker, O. Bangcharoenpaurpong, P. M. Champion, *J. Chem. Phys.* **80**, 4701 (1984).
37. Several research groups are studying intramolecular electron transfer in model systems. Reports from two research groups of results for binuclear transition metal complexes containing nonconjugated bridging ligands have appeared [B. Anderes and D. K. Lavallee, *Inorg. Chem.* **22**, 2665 (1983); M. J. K. Geno and J. H. Dawson, *ibid.* **24**, 1731 (1985)]. The large reorganization energies associated with cobalt(III/II) couples in these systems are most likely responsible for the slow transfer rates.
38. L. T. Calcaterra, G. L. Closs, J. R. Miller, *J. Am. Chem. Soc.* **105**, 670 (1983).
39. J. R. Miller, L. T. Calcaterra, G. L. Closs, *ibid.* **106**, 3047 (1984).
40. Through-bond transfer is unlikely because the donor-acceptor distance along the polypeptide backbone (His-33 to His-18) is over 70 Å [A. A. S. da Gama, *Theor. Chim. Acta* **68**, 159 (1985)].
41. K. Ohta, G. L. Closs, K. Morokuma, N. Green, *J. Am. Chem. Soc.* **108**, 1319 (1986).
42. For other recent work on intramolecular electron transfer in fixed-distance systems, see J. R. Bolton *et al.*, *J. Chem. Soc. Chem. Commun.* **1985**, 559 (1985); N. S. Hush *et al.*, *Chem. Phys. Lett.* **117**, 8 (1985); M. R. Wasielewski, M. P. Niemczyk, W. A. Svec, E. B. Pewitt, *J. Am. Chem. Soc.* **107**, 1080 (1985); C. Franco and G. McLendon, *Inorg. Chem.* **23**, 2372 (1984); B. A. Leland *et al.*, *J. Phys. Chem.* **89**, 5571 (1985).
43. D. N. Beratan, J. N. Onuchic, J. J. Hopfield, *J. Chem. Phys.* **83**, 5325 (1985); S. Larsson and A. Volosov, in preparation.
44. T. Takano and R. E. Dickerson, *J. Mol. Biol.* **153**, 79 and 95 (1981).
45. E. Antonini and M. Brunori, Eds., *Hemoglobin and Myoglobin in Their Reactions with Ligands* (North-Holland, Amsterdam, 1971); J. M. Rifkind, in *Inorganic Biochemistry*, G. L. Eichhorn, Ed. (Elsevier, Amsterdam, 1973), vol. 2, p. 832.
46. T. Takano, *J. Mol. Biol.* **110**, 537 and 569 (1977).
47. Large changes in metal-ligand bond lengths have been directly associated with slow electron-transfer rates. See B. S. Brunschwig, C. Creutz, D. H. Macartney, T.-K. Sham, N. Sutin, *Faraday Discuss. Chem. Soc.* **74**, 113 (1982).
48. R. J. Crutchley *et al.*, in preparation.
49. R. J. Crutchley, W. R. Ellis, Jr., H. B. Gray, in *Frontiers in Bioinorganic Chemistry*, A. V. Xavier, Ed. (VCH Verlagsgesellschaft, Weinheim, Federal Republic of Germany, 1986), p. 679.
50. ———, *J. Am. Chem. Soc.* **107**, 5002 (1985).
51. A. G. Mauk and H. B. Gray, *Biochem. Biophys. Res. Commun.* **86**, 206 (1979).
52. K. M. Kadish and J. Jordan, *J. Electrochem. Soc.* **125**, 1250 (1978).
53. R. F. Pasternack and E. G. Spiro, *J. Am. Chem. Soc.* **100**, 968 (1978).
54. D. W. Dixon, M. Barbush, A. Shirazi, *Inorg. Chem.* **24**, 1081 (1985).
55. J. L. McGourty, N. V. Blough, B. M. Hoffman, *J. Am. Chem. Soc.* **105**, 4470 (1983).
56. S. E. Peterson-Kennedy, J. L. McGourty, B. M. Hoffman, *ibid.* **106**, 5010 (1984).
57. S. E. Peterson-Kennedy *et al.*, *Coord. Chem. Rev.* **64**, 125 (1985).
58. P. S. Ho, *et al.*, *J. Am. Chem. Soc.* **107**, 1070 (1985).
59. S. E. Peterson-Kennedy, J. L. McGourty, J. A. Kalweit, B. M. Hoffman, *ibid.* **108**, 1739 (1986).
60. K. P. Simolo, G. L. McLendon, M. R. Mauk, A. G. Mauk, *ibid.* **106**, 5012 (1984).
61. G. L. McLendon *et al.*, *ibid.* **107**, 739 (1985).
62. G. McLendon *et al.*, *Coord. Chem. Rev.* **64**, 113 (1985).
63. E. Cheung *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1330 (1986).
64. G. McLendon and J. R. Miller, *J. Am. Chem. Soc.* **107**, 7811 (1985).
65. T. L. Poulos and A. G. Mauk, *J. Biol. Chem.* **258**, 7369 (1983).
66. T. L. Poulos and J. Kraut, *ibid.* **255**, 10322 (1980).
67. F. R. Salemme, *J. Mol. Biol.* **102**, 563 (1976).
68. B. S. Brunschwig *et al.*, *Inorg. Chem.* **24**, 3743 (1985).
69. H. B. Gray, *Chem. Soc. Rev.* **15**, 17 (1986).
70. ——— and B. G. Malmström, *Comments Inorg. Chem.* **2**, 203 (1983).
71. A. W. Axup, R. J. Crutchley, M. Albin, H. B. Gray, in preparation.
72. F. Sherman and J. W. Stewart, in *The Molecular Biology of the Yeast Saccharomyces: Metabolism and Expression*, J. N. Strathern, E. W. Jones, J. L. Broach, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), p. 301.
73. F. Sherman, J. W. Stewart, E. Margoliash, J. Parker, W. Campbell, *Proc. Natl. Acad. Sci. U.S.A.* **55**, 1498 (1966); J. W. Stewart and F. Sherman, in *Molecular and Environmental Aspects of Mutagenesis*, L. Prakash, F. Sherman, M. W. Miller, C. W. Lawrence, H. W. Taber, Eds. (Thomas, Springfield, IL, 1974), p. 102.
74. S. Gillam, F. Rottman, P. Jahnke, M. Smith, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 96 (1977).
75. D. L. Montgomery, B. D. Hall, S. Gillam, M. Smith, *Cell* **14**, 673 (1978).
76. M. Smith *et al.*, *ibid.* **16**, 753 (1979).
77. D. W. Leung, K. Mehta, M. Smith, unpublished sequence of a 2.5-kb Bam HI to Hind III fragment containing the yeast iso-1 cytochrome c gene.
78. Yeast iso-1 cytochrome c gene, shuttle vectors, and yeast host were provided by M. Smith.
79. R. E. Dickerson and R. Timkovich, in *The Enzymes*, P. D. Boyer, Ed. (Academic Press, New York, 1975), p. 397.
80. S. L. Mayo, J. L. Campbell, J. H. Richards, H. B. Gray, in preparation.
81. W. R. Ellis, Jr., S. L. Mayo, J. L. Campbell, J. H. Richards, H. B. Gray, in preparation.
82. R. Margalit and S. Schejter, *Eur. J. Biochem.* **32**, 492 (1973).
83. T. L. Poulos and B. C. Finzel, in *Peptide and Protein Reviews*, M. T. H. Hearn, Ed. (Dekker, New York, 1984), vol. 4, p. 115.
84. Multiphase reduction kinetics have been attributed to a spin equilibrium for ferricytochrome P-450, with the high-spin ferriheme reducing faster than the low-spin ferriheme. The precise mechanism is not yet known, although a conformational change involving a Tyr residue is indicated. See W. L. Backes *et al.*, *Biochemistry* **24**, 5130 (1985); M. T. Fisher and S. G. Sligar, *ibid.*, p. 6696.
85. K. M. Kadish and C. H. Su, *J. Am. Chem. Soc.* **105**, 177 (1983).
86. D. N. Beratan and J. J. Hopfield, *ibid.* **106**, 1584 (1984); *J. Chem. Phys.* **81**, 5753 (1984).
87. P. Bertrand, *Chem. Phys. Lett.* **113**, 104 (1985) and references therein.
88. W. R. Scheidt, D. K. Geiger, Y. J. Lee, C. A. Reed, G. Lang, *J. Am. Chem. Soc.* **107**, 5693 (1985); G. P. Gupta, G. Lang, W. R. Scheidt, D. K. Geiger, C. A. Reed, *J. Chem. Phys.* **83**, 5945 (1985).

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