Factors that Affect the Rate of Biological Electron Transfer

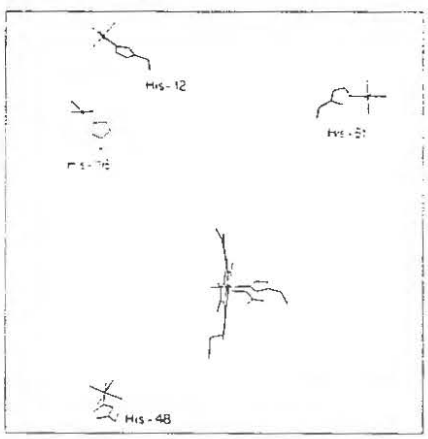
Brandon J. Cruickshank Literature Seminar February 16, 1989

Biological electron transfer has been studied extensively in recent years [1]. In this process, electrons are transferred between metal sites or organic prosthetic groups within a single protein or complex of proteins such that the electron must traverse a considerable amount of intervening protein matrix [1b]. Electron transfer between sites separated by up to 20-30 Å is known in diverse biological electron transfer reactions including energy channeling in the photosynthetic and respiratory electron transport chains [1c].

Four factors which have been shown to affect the rates of biological electron transfer are: (1) the intersite separation distance [2-4], (2) the nature of the intervening medium [5], (3) the orientation or conformation of the protein [6-8], and (4) the driving force for the reaction [9]. Each of these four areas has received increased attention recently, although current efforts appear to be concentrating on the effects of orientation and driving force on electron transfer.

In general, research in biological electron transfer follows two schemes. The first scheme involves the covalent binding of small redox active inorganic molecules to specific residues on the protein surface. Secondly, a physiological couple, either electrostatic or covalent in nature, can be formed between two proteins. Flash photolysis or pulse-radiolysis is used to initiate the electron transfer between the small molecule and the active site or between the protein partners, and then the electron transfer process is monitored by spectroscopic techniques. Via computer modeling and the known binding site, the distance between the metal center and the orientation of the redox partners can be determined fairly accurately. Site-specific mutagenesis and metal substitution can be used to vary the pathway and the driving force for the electron transfer reaction, respectively.

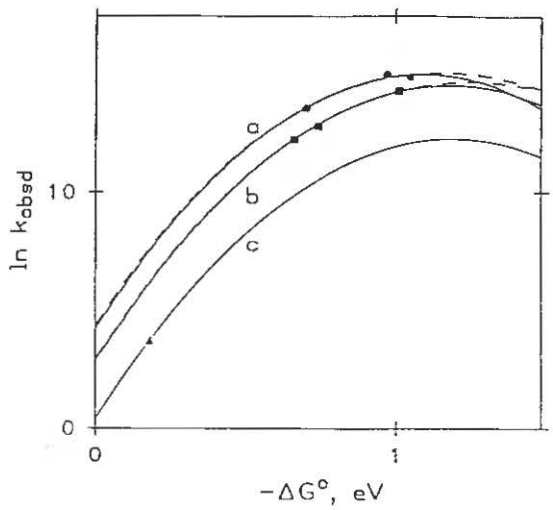
Early work by Gray on the effect of distance on the rate of electron transfer involved attempting to analyze bimolecular protein/small inorganic molecule electron transfer rate constants in terms of the acceptor/donor distance in an assumed precursor complex [3]. Differences in rates were attributed solely as increases in the extent of penetration of the redox agent into the protein, thereby reducing the separation distance. Recent work has focused on electron transfer between sites that are separated by a known, fixed distance. In fact, Gray and coworkers have been able to covalently attach $Ru a_5$ (where a = NH_3) to four surface accessible histidine imidazoles of myoglobin, each of which is a different distance from the metal center [4].



Site-specific mutagenesis has been used as a tool to vary the electron transfer pathway [5]. Hoffman and coworkers have applied this method to change the phenylalanine residue at position 82 of cytochrome c (in a preformed cytochrome c/Zn cytochrome c peroxidase complex) which is known to be involved in the electron transfer pathway [5a]. They have found a 10^4 increase in rate for aromatic residues compared to aliphatic residues. There are two explanations for this observed difference in rate. The most likely explanation involves hole transfer from $ZnCcP^+$ to $Fe^{II}Cc$ being facilitated because the heme π-electron systems are coupled through the intervening aromatic rings of cytochrome c residue 82 and His-181 of cytochrome c peroxidase. Another alternative for the observed difference in rates could be explained by conformational "gating" [6]. For the aliphatic derivatives the rate limiting step might be a conformational conversion to a protein orientation that undergoes rapid electron transfer.

McLendon and coworkers have observed conformational "gating" in a cytochrome c/cytochrome b_2 complex in which the heme of cytochrome c has been substituted with Zn porph, and porph [7]. Within these three complexes, the driving force varies by approximately 0.60 eV. Marcus theory predicts that the electron transfer rate should increase with driving force until $-\Delta G > \lambda$ (reorganization energy) [10]. Considering the variation in driving force, a large difference in electron transfer rates should be observed; however, the rates are essentially equivalent. The rate limiting step is most likely a conformational conversion to a protein orientation which is capable of electron transfer. This conversion is unaffected by a change in driving force. Recent research by Kostic and coworkers has focused on the electron transfer rate difference observed between electrostatic and covalent complexes of cytochrome c and platocyanin [8]. The electrostatic complex has a $k_{et} \approx 1000\ s^{-1}$, while the covalent complexes have electron transfer rate constants of approximately $0\ s^{-1}$. The covalent isomers are "locked" in the wrong orientation for electron transfer. And, due to the multiple bond linking the proteins, they cannot relax into an appropriate orientation required for intracomplex electron transfer.

The fourth factor that affects the rate of electron transfer is the driving force for the reaction. Originally, it was thought that proteins intrinsically had small reorganization energies (λ), so that the inverted region could be observed at small free energy (ΔG) values. It is now known that the reorganization energies for proteins are much larger than predicted [1c]. Recent efforts by Gray [9a] and McLendon [9b] have claimed to fit Marcus theory, although their data could also fit a straight line.



There is a need for additional ways to vary the driving force within a particular protein couple to verify a decrease in electron transfer rate once $-\Delta G$ exceeds λ.

References

1. Recent Reviews:
   (a) Gray, H. B.; Mauk, A. G.; Scott, R., "Experimental Approaches to Studying Biological Electron Transfer," J. Chem. Ed., 1985, 62, 932.
   (b) Isied, S. S., "Long-Range Electron Transfer in Peptides and Proteins," Prog. Inorg. Chem. 1984, 32, 443.
   (c) McLendon, G., "Long-Distance Electron Transfer in Proteins and Model Systems," Acc. Chem. Res. 1988, 21, 160.
   (d) McLendon, G., "Quantum Mechanical Effects in Inorganic and Bioinorganic Electron Transfer," Coor. Chem. Rev. 1985, 68, 1.

2. McLendon, G., personal communication.

3. Gray, H. B.; Mauk, A. G.; Scott, R., "Distances of Electron Transfer to and from Metalloprotein Redox Sites in Reactions with Inorganic Complexes," J. Am. Chem. Soc. 1980, 102, 4360.

4. (a) Gray, H. B., "Long-Range Electron Transfer in Myoglobin," Annals New York Academy of Sciences, in press.
   (b) Gray, H. B., "Distance Dependence of Photoinduced Long-Range Electron Transfer in Zinc/Ruthenium-Modified Myoglobins," J. Am. Chem. Soc. 1988, 110, 435.

5. (a) Hoffman, B. M.; Mauk, A. G., "Regulation of Interprotein Electron Transfers by Residue 82 of Yeast Cytochrome c," Science 1988, 240, 311.
   (b) Hoffman, B. M.; Mauk, A. G., "Yeast cytochrome c with phenylalanine or tyrosine at position 87 transfers electrons to (zinc cytochrome c peroxidase)$^+$ at a rate ten thousand times that of the serine-87 or glycine-87 variants," Proc. Natl. Acad. Sci. USA 1987, 84, 1249.
   (c) McLendon, G., "Effects of Amino Acid Replacements in Yeast Iso-1 Cytochrome c on Heme Accessibility and Intracomplex Electron Transfer in Complexes with Cytochrome c Peroxidase," Biochemistry 1988, 27, 4445.
   (d) McLendon, G., "Formation of Electrostatically-Stabilized Complex at Low Ionic Strength Inhibits Interprotein Electron Transfer Between Yeast Cytochrome c and Cytochrome c Peroxidase," Biochem. Biophysics. Res. Comm. 1988, 151, 429.
   (e) Tollin, G., "Kinetics of Reduction by Free Flavin Semiquinones of the Components of the Cytochrome c-Cytochrome c Peroxidase Complex and Intracomplex Electron Transfer," Biochemistry 1987, 26, 2836.

6. (a) Hoffman, B. M.; Ratner, M. A. "Gated Electron-Transfer: When are Observed Rates Controlled by Conformational Interconversion?," J. Am. Chem. Soc. 1987, 109, 6237.
   (b) Hoffman, B. M., personal communication.

7. McLendon, G., "Electron Transfer in the Cytochrome c/Cytochrome b_2 Complex: Evidence for "Conformational Gating" J. Am. Chem. Soc. 1987, 109, 7540.

8. (a) Kostic, N. M., "Oxidoreduction Reactions Involving the Electrostatic and the Covalent Complex of Cytochrome c and Plastocyanin: Importance of the Protein Rearrangement for the Intracomplex Electron-Transfer Reaction," Biochemistry 1989, in press.
(b) Kostic, N. M., personal communication.
(c) Northrup, S. H. "Brownian Dynamics of Cytochrome c and Cytochrome c Peroxidase Association," Science 1988, 241, 67.

9. (a) Gray, H. B., "Driving-Force Effects on the Rate of Long-Range Electron Transfer in Ruthenium-Modified Cytochrome c," submitted.
(b) McLendon, G., "Free Energy Effects on Biological Electron Transfer: Reactions of Iron(IV) Cytochrome c Peroxidase (ES) with Metallocytochromes c," J. Am. Chem. Soc. 1988, 110, 3345.

10. Marcus, R. A., "Exchange Reactions and Electron Transfer Reactions Including Isotope Exchange," Discuss Faraday Soc. 1960, 29, 21.

11. General Electron Transfer Reviews:
(a) Sutin, N., "Nuclear, Electronic, and Frequency Factors in Electron-Transfer Reaction," Acc. Chem. Res. 1982, 15, 275.
(b) Ratner, M. A., "Electron Tunneling in Solid-State Electron-Transfer Reactions," Chem. Rev. 1987, 87, 113.