Electron Transfer in Metalloproteins: Models and Experiment

Christopher Ziegler Literature Seminar November 16, 1993

Long range electron transfer is a central and ubiquitous process in all biological systems [1,2]. Primary metabolic pathways such as photosynthesis and oxidative phosphorylation depend on the successful transport of electrons across membranes. Electron transfer systems are very often comprised of metalloproteins, and the electrons must traverse large distances (~10- 15Å) between the metal centers. Recently, several theoretical and experimental approaches to this problem have thrown light on the nature of electron transport in metalloproteins, and have enabled the electron transfer rates in these systems to be predicted with fair accuracy.

Several theoretical and experimental systems have been created to approximate electron transfer in biological systems [3-7]. One of the most thoroughly examined model and experimental systems involves a donor (or acceptor) embedded in a protein, and an acceptor (or donor) covalently attached to the periphery of the protein (Figure 1). In such a system, the rate constant for electron transfer is expressed by Fermi's Golden Rule:

$$k_{ET} = \frac{2\pi}{\hbar}\left|H_{DA}\right|^2 FC$$

The Franck-Condon factor (FC) is an expression of the nuclear contributions (a weighted density of vibrational states) of the protein system, and thus the rate is dependent on the electronic nature of the protein medium separating the donor and acceptor sites (H_{DA}) [8].

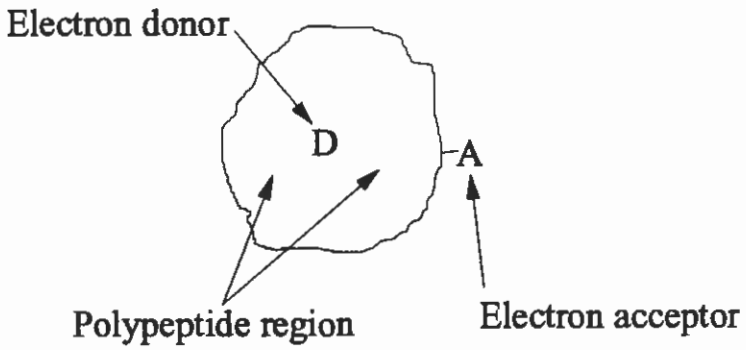


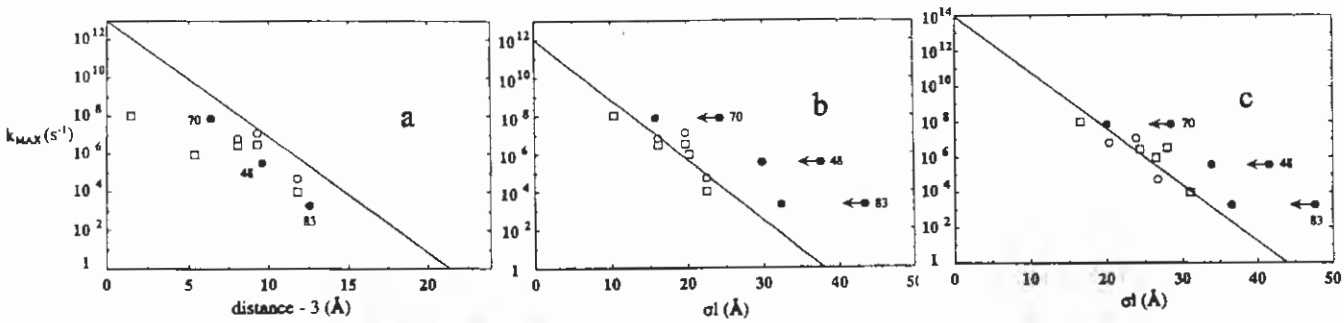
Figure 1

Two empirical methods for approximating the electronic nature of the protein medium have been developed over the past two years. The simplest of these empirical models was formulated by Dutton and coworkers [9]. This model is based on experimental data obtained from ET systems such as photosynthetic reaction centers, semisynthetic Ru modified proteins, and covalently linked systems. The Dutton model assumes that the protein is a homogeneous system, and that the decay rate parameter for a wavefunction in this medium is 1.4 $Å^{-1}$, between that of a vacuum and a covalent bond. Thus, according to the Dutton model, the donor-acceptor distance is the only factor that affects the ET rates. In contrast to the Dutton methodology, Beratan and Onuchic have developed an empirical calculative method based on a heterogeneous protein representation [10-16]. In this approach, covalent bonds, hydrogen bonds, and van der Waals interactions all contribute differently to wavefunction decay in a ratio of approximately 1:3:10. Thus, according to the pathways model,

electrons can take various paths from donor to acceptor, and the structure of the protein medium between the donor and acceptor does play a role in ET rates.

In addition to empirical methods of calculation, several quantum mechanical models have also been proposed. It is important to note that since proteins are large many-bodied systems, they are analytically unsolvable, and thus assumptions must be made in order to approach this problem. One of the first calculative techniques used to estimate electron transport in metalloproteins was developed by Wolynes and Kuki [8, 17]. They used a path integral method with Monte Carlo biased sampling to simulate electron tunneling through protein media. Other frequently used methodologies include high order perturbation theory and extended Hückel calculations [18]. Marcus and Siddarth have used Hückel calculations in conjuction with algorithm searches and an AI program to determine the electronic contribution of the polypeptide region to ET rates [19-21]. Most recently, Kuki has developed an inhomogeneous aperiodic lattice (IAL) Hamiltonian to approximate the electronic coupling between donor and acceptor [22, 23].

Recent experimental work on Ru modified proteins by Gray and coworkers can be used to examine the validity of the various empirical and quantum methods for estimating ET rates [24-31]. Using sited directed mutagenesis and semisynthetic techniques, Gray and coworkers covalently attach ruthenium pentaamine or bipyridyl complexes to histidine residues on the periphery of myoglobin and cytochrome *c* proteins. In addition to attaching this donor/acceptor to the periphery of the protein, the heme group of myoglobin or cytochrome *c* can be replaced with a zinc protoporphyrin IX. Electron transfer can then be initiated in these modified proteins by exciting the zinc porphyrin, and rates can be determined by monitoring the lifetimes of the excited state ZnP* and resulting cation ZnP^+.



●: a_5Ru HuMb ○: a_5Ru Cyt *c* □: Ru(bpy)$_2$(im) Cyt *c*

Figure 2a, b, c

The empirical theories of Dutton and Beratan-Onuchic can be investigated by plotting the rates of ET versus either distance or effective path distance (σl) [31] (Figure 2a, b, c). As can be seen in Figure 2a, neither the myoglobin nor the cytochrome *c* data correspond well to Dutton's homogeneous decay parameter of 1.4$Å^{-1}$. However, the myoglobin data do fit linearly with distance. When rates are compared with l as in Figure 2b and c, the cytochrome *c* rates agree well with this parameter, but the myoglobin rates lie well off the line, even for a multipath approximation. It has been proposed that these results imply that myoglobin behaves like a Dutton type homogeneous system, whereas cytochrome *c* behaves like the Beratan-Onuchic model with a few dominant paths. It must be noted, however, that the experimental validity of Gray's technique has recently been questioned [32].

The predictive ability of the Marcus Hückel and the Kuki IAL Hamiltonian methods are compared in Figure 3 [21,22]. The rate constants predicted by the Marcus Hückel calcu-

lation do not agree with the literature, but their relative values do correlate with the relative experimental values. In contrast, the values predicted by the Kuki IAL Hamiltonian agree very well with Gray's experimental rates. Kuki also claims that his model can predict the relative importance of amino acids in the ET process, which would significantly advance the understanding of these systems. However, Kuki himself points out that his new Hamiltonian may not yet accurately describe the nature of ET in the polypeptide region [33].

Calculated Couplings for Cytochrome *c*

Derivative	R, Å	Calc.	Exp.	Rel. Calc.	Rel. Exp.
Hückel					
His 33	11.1	0.01	0.10	1	1
His 39	12.3	0.01	0.11	1	1.1
His 72	8.4	0.007	0.06	0.7	0.6
His 62	14.8	0.002	0.006	0.2	0.06
IAL					
His 39	13.0	0.20	0.21		
His 33	13.1	0.18	0.12		
His 62	15.6	0.018	0.012		

Figure 3

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