Long-Distance Electron Transfer in Proteins and Model Systems

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Just a few years ago, the idea of "long-distance" chemical reactions, in which the reactants are held fixed at distances (10–30 Å) that preclude collisions, was viewed with suspicion or disbelief by many chemists. Fortunately, nature is not so skeptical, and biological energy is channeled in the photosynthetic and respiratory electron transport chains via just such long-distance reactions. The biological matrix (e.g., proteins) encases reactive centers like metal ions, metalloporphyrins, or flavins so that the reactive groups are separated by many angstroms of "insulating" matrix.

In this Account, we briefly review recent attempts to clarify how these critical reactions occur in simple chemical systems, as well as in protein-based systems, with the ultimate aim of understanding, and perhaps emulating, the efficient biological electron transport systems.

Long-Distance Electron Tunneling: A Simple Theoretical Approach

The concept of "electron tunneling" (i.e., passage through a classically impenetrable barrier) has been well established in physics for over 50 years.[1,2] A number of elegant recent reviews of the theoretical concepts in electron tunneling exist, so we will restrict the present comments to a mathematically annotated descriptive framework.

A reaction at a fixed donor–acceptor distance, in which diffusion plays no part, might be formally considered as a unimolecular reaction within a "supermolecule" composed of donor, acceptor, and environment. For such a reaction, the rate constant can be written as $k_{et} = (2\pi/h)|V_{ab}|^2$·FCWD. While the first term is the familiar barrier crossing rate of transition state theory, the second term (the tunneling matrix element) describes the mixing of donor and acceptor wave functions (i.e., overlap) and the third term, a Franck–Condon-weighted density of states, describes the nuclear motions along the reaction coordinate that accompany electron transfer. Each of these latter terms is considered in more detail below.

An easy way to understand long-distance transfer is to realize that molecular wave functions can extend very far from their radial maximum, albeit with *low* probability (Figure 1). Two features are immediately apparent in Figure 1. First, there is some probability (given by the shaded area) that the wave functions of the donor (D) and acceptor (A) will mix. Second, the magnitude of this mixing (i.e., the interaction energy $|V_{ab}|$) depends strongly on donor–acceptor distance $R_{D\text{-}A}$; $|V_{ab}| \propto \exp(-\alpha R)$.

In a semiclassical picture, $|V|$ is related to the splitting between the reactant and product potential surfaces (Figure 2). Thus when $|V|$ is large (large overlap, small R), the reaction coordinate will proceed solely along the lower single surface. Thus, every trajectory that reaches the transition state will lead to products and we say the reaction is *a*diabatic.[4] If however, $|V|$ is small (Figure 2b), a given trajectory may easily "jump" over the small gap, remaining on the reactant surface without passing over to the product surface. Thus the reaction can reach the transition state many times without leading to products. (Such reactions are commonly called "nonadiabatic".[4])

The simple orbital overlap picture in Figure 1 neglects any possible role of the medium between D and A. A more realistic approach considers coupling between the donor and acceptor via appropriate orbitals in the intervening medium (Figure 3). This model can be viewed as a special application of the familiar principle of superexchange.[5] In this form we still obtain $|V_{ex}| \propto \exp(-\alpha R)$, with $\alpha \cong (1/d) \ln (B/\beta)$.

We now turn to the role of nuclear motion in electron transfer, focusing on the classical (in every sense) theory of Marcus.[6,7] A number of nuclear motions accompany electron transfer, either within the reactants (e.g., bond lengthening, as an electron is placed in a σ^* orbital) or in the surrounding medium (e.g., solvent repolarization to stabilize a charge center). Thus, in Figure 2, the nuclear minima for the reactant and product surfaces differ. Invoking the Franck–Condon principle, this nuclear motion (which is necessary for conservation of energy) occurs *prior* to the actual electron "jump".[2]

Referring back to Figure 2, we can characterize the "reorganization energy", λ, involved in moving an electron from D to A by considering λ as the total en-

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(1) Gamow, G. *Z. Phys.* **1978**, *51*, 204–212.

(2) Detailed overviews (far beyond the scope of the present personalized account) include: (a) Chance, B. et al. *Tunneling in Biological Systems*; Academic: New York, 1978. (b) Guarr, T.; McLendon, G. *Coord. Chem. Rev.* **1985**, *68*, 1–52.

(3) Hopfield, J. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3640–3644. This influential paper provides an interesting example of feedback between teaching and research: Hopfield developed the idea in order to create a lecture topic for his biophysics course!

(4) Adiabatic in this context means simply that the reaction occurs along the single (lower) surface as in Figure 2a, instead of involving two (diabatic) surfaces, as in Figure 2b ("nonadiabatic" is an unfortunate, but widespread, double negative).

(5) (a) Miller, J. R.; Beitz, J. *J. Chem. Phys.* **1981**, *74*, 6746–6753. (b) McConnell, H. *J. Chem. Phys.* **1961**, *35*, 508.

(6) An excellent discussion of reorganization energy is found in: Marcus, R.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265–312. Note that the E_a value calculated is an activation *free* energy and thus depends on $\Delta S°$. A detailed discussion is found in the above paper.

(7) Marcus, R. *J. Chem. Phys.* **1956**, *24*, 966–971.

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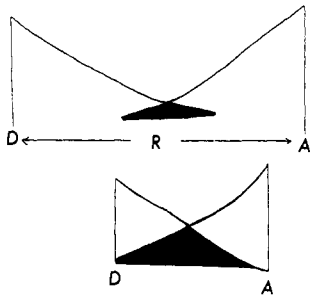

Figure 1. A simple graphical depiction of wave function mixing (which is reflected in the tunneling matrix element $|V|$) at long distance can be obtained by considering the overlap of (Slater H-like) wave functions. At long distance (R) the net mixing (black area) is very small, while as distance decreases, the overlap (and $|V|$) increases exponentially.

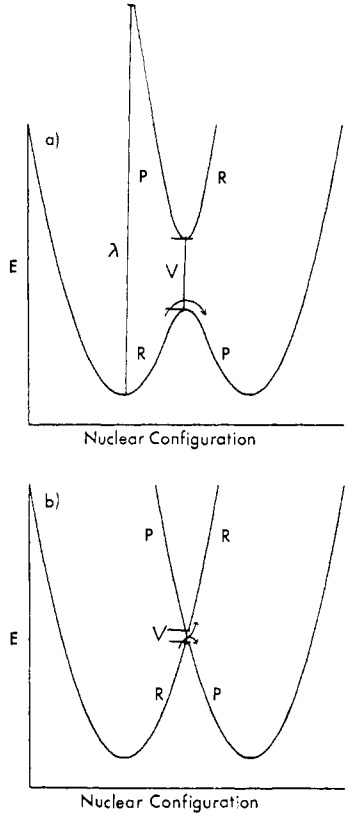

Figure 2. Simple two-dimensional reaction coordinate diagram emphasizes some key parameters in electron-transfer theories. The matrix element $|V|$ is quantitatively related to interaction of the reactant (R) and product (P) surfaces. When $|V|$ is large, all reaction trajectories remain in a single lower (adiabatic) surface. When $|V|$ is small, there is a high probability that a trajectory along R may remain on the (upper) reactant surface without crossing to the product well. Thus reaction trajectories can cross the turning point (transition state) without leading to product formation. (Such reactions are called nonadiabatic.) The reorganization energy, λ, can be viewed as the energy necessary to move an electron from donor to acceptor *without* allowing any concomitant nuclear motion.

ergy required for a (Franck–Condon) transition between the reactant → product surfaces, *without* allowing nuclear motion. Clearly, λ incorporates the fundamental structural information inherent in the potential surface (i.e., bond frequencies and displacements): $\lambda = {}^1/_2\sum_j(h\omega)_j^2(\Delta l_j)^2$.[6] (There can also be a contribution from nonvibrational terms.[6]) At this stage, we realize from high school geometry that for equivalent parabolas as in Figure 2, the activation energy (E_a) can be obtained directly from λ: $E_a = \lambda/4$. In the more general and interesting case where $\Delta G_{products} - \Delta G_{reactants} < 0$ (Figure 4), it is clear that E_a depends on ΔG: as ΔG becomes more negative, E_a decreases. Again using geometric arguments, one obtains $E_a = (\Delta G - \lambda)^2/4\lambda$ as first derived by Marcus.[7] Note this simple expression makes the surprising prediction of an *optimal* ΔG for electron transfer; k_{et} maximizes when $\Delta G = \lambda$.

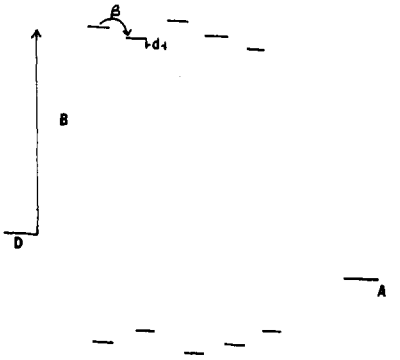

Figure 3. A simple "superexchange" model for long-distance electron transfer includes a small mixing of donor (D) and acceptor (A) wave functions with intervening medium wave functions (via superexchange).[5] Interaction may proceed via "electron" (conduction band) states or "hole" (valence band) states. Here B is the difference in donor and medium ionization potential, β is an exchange integral, and d is the spacing between molecules in the intervening conducting medium.

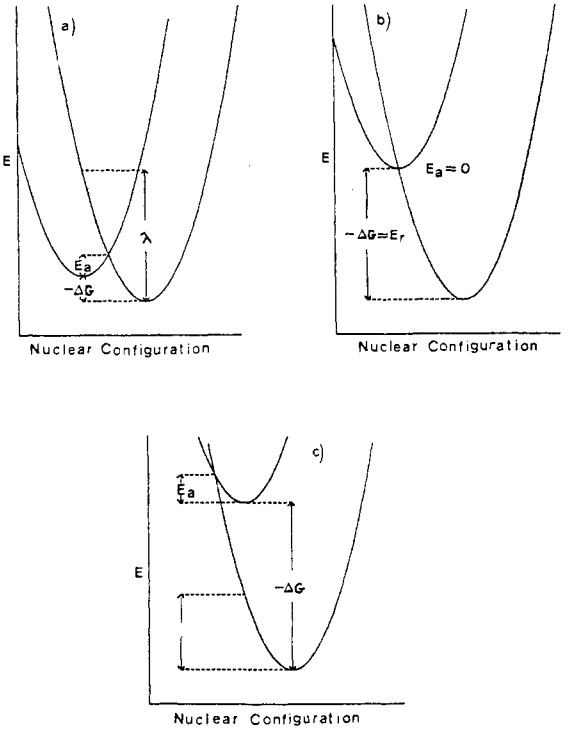

Figure 4. The reaction free energy, ΔG, can compensate for the reorganization energy, λ, to lower the overall activation energy. According to Marcus' theory, $E_a = (\Delta G - \lambda)^2/4\lambda$. Thus when $\Delta G < \lambda$, rate increases as ΔG increases, reaching an *optimum* when $\Delta G = \lambda$; when $\Delta G > \lambda$, rate *decreases* as ΔG increases!

In sum, then, theory makes two fundamental predictions. First, electron-transfer rates should decrease rapidly with increasing distance: $k \propto \exp(-\alpha R_2)$. The precise value of α, however, might depend markedly on the medium. Second, at a given distance, rates should depend significantly on ΔG, reaching a maximum when $\Delta G = \lambda$.

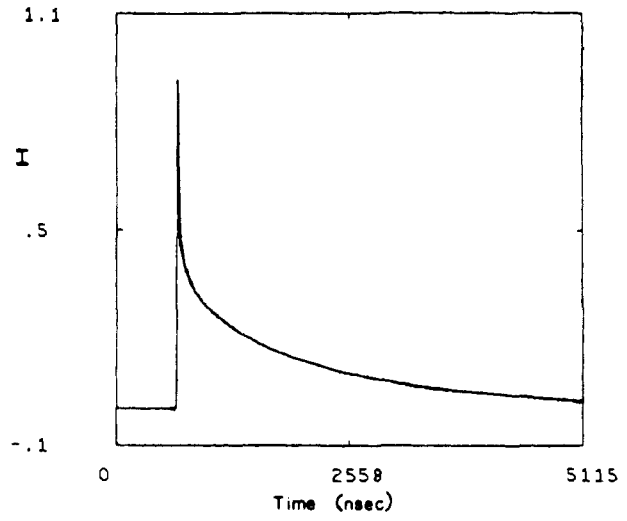

Figure 5. The range of donor–acceptor distances in a random ensemble results in a range of rates characterized by a highly nonexponential line shape. The data shown are for the reaction of excited Ru(4,4′-(carboxyethyl ester)bipyridine)$_3$[9c] with tetramethylphenylenediamine in polycarbonate.

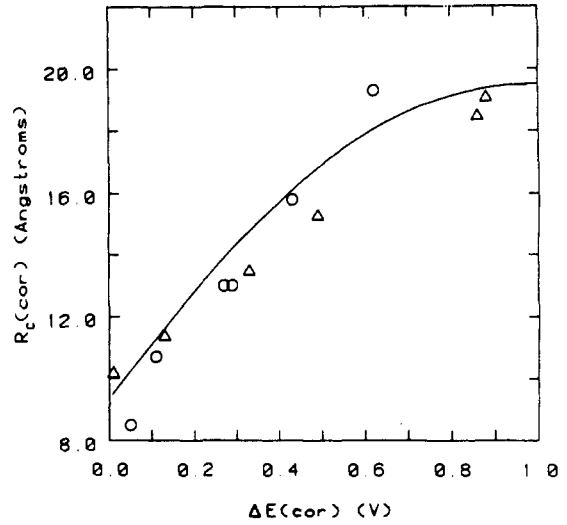

Figure 6. Plot of R_c (the accessible distance for electron transfer in 10^{-6} s) vs free energy for reaction of Ru(bpy)$_3^{2+*}$ (and its homologues) with *N,N*-tetramethylphenylenediamine and its homologues (from ref 9c).

Experimental Tests of Long-Distance Electron Transfer

For chemical reactions in solution, the pioneering work on long-distance electron transfer was reported by Miller.[8] Over 10 years ago, he first reported pulse radiolysis studies of collisionless electron transfer between donors and acceptors randomly doped into rigid glasses. This work first showed that electron-transfer rates do depend exponentially on distance, as postulated above. Our own work is similar in concept, using photochemistry to study donor–acceptor reactions, rather than radiolysis.[9] Thus when a donor (e.g., Ru(bpy)$_3^{2+}$) and acceptor (e.g., tetramethylphenylenediamine) are randomly dispersed in a rigid matrix (glycerol, polycarbonate, etc.), a wide dispersion in donor–acceptor distances is obtained, with a corresponding dispersion in rates. When reaction is initiated (by a short laser pulse), those donor–acceptor pairs that are in close proximity will react quickly, while the further separated pairs will react slowly, leading to a characteristic line shape (Figure 5). For a random ensemble, at a known concentration, the radial distribution function is known,[10] and we rapidly found that such reactions were best described by the form $k \propto \exp[(-1.1 \pm 0.2)R]$, in good agreement with both the radiolytic approach and theoretical predictions.

In related experiments, it was possible to vary ΔG by varying the nature of either the donor or the acceptor. These experiments demonstrate that Marcus' theory is equally applicable to fixed-distance electron transfer as to collisional systems. In the simplest such experiments, one simply measures how (static) emission intensity depends on acceptor concentration. Since at *fixed* distance, transfer rates depend exponentially on distance, there exists (in glasses) a critical distance, R_0, and corresponding concentration ($C_0 \propto R_0^3$) such that $k_{et}(R_0) = k_{em} = 1/\tau_0$, where τ_0 is the donor lifetime in the absence of acceptor. When $R < R_0$ i.e., $(C) > (C_0)$, $k_{et}(R) \gg k_{em}$ and emission is largely quenched, while when $R > R_0$, $k_{et}(R) \ll k_{em}$ and emission is unaffected. Under these constraints $I/I_0 \propto \exp(-C_0)$. Thus a plot of ln (I/I_0) vs acceptor concentration gives the critical distance R_0; i.e., "How far can an electron transfer in time τ_0?" As Figure 6 shows, rapid rates (10^6 s^{-1}) can be obtained at quite long distance (>12 Å edge–edge) *if* Franck–Condon factors are optimal (i.e., when $\Delta G = \lambda$).

More recent work in several laboratories has focused on donor–acceptor pairs connected by rigid bridging frameworks (e.g., steroids).[11-15] One striking result of these experiments has been the observation[11] of "exothermic rate restriction", first predicted by Marcus. Thus, as ΔG increases, k_{et} increases, reaching a maximum when $\Delta G = \lambda$. Further increases in ΔG *decrease* the rate. Such an effect had not been observed in fluid solution, leading some to question the basic theory. In retrospect, it is clear that diffusional processes mask the interesting dependence of rate on both distance *and* free energy, so that the observed rate plateaus at $k_{et} \approx k_{diffusion}$.

Finally, we should briefly mention a potentially interesting effect of solvent dynamics on rate. The foregoing (transition state) theory implicitly assumes that equilibrium is attained at every point along the reaction coordinate. However, since electron transfer can be an inherently fast process, it is possible that electron

(8) Miller, J. R. *Science (Washington, D.C.)* **1975**, *189*, 221–224.

(9) (a) Guarr, T.; Maguire, M.; McLendon, G. *J. Am. Chem. Soc.* **1983**, *105*, 616–619. (b) Strauch, S.; Guarr, T.; McLendon, G.; Maguire, M. *J. Phys. Chem.* **1983**, *87*, 3575–3581. (c) Guarr, T.; Maguire, M.; McLendon, G. *J. Am. Chem. Soc.* **1985**, *107*, 5104–5111.

(10) Electron-exchange emission quenching in a random donor–acceptor (D–A) ensemble was considered in detail by Inokuti and Hirayama (Inokuti, M.; Hirayama, F. *J. Chem. Phys.* **1965**, *4*, 1978) and independently by Thomas et al. (Thomas, D.; Hopfield, J.; Augustiniak, M. *Phys. Rev. A* **1965**, *140*, 202–220. As written by Inokuti, the emission intensity $I(t) = I_0 \exp\{t/\tau_0\}[R_1^{R_2} \exp\{-[kn(R)]\}\omega(R)](4\pi R^2)\, dR]^N$. The number of rigid D–A systems seems to be growing exponentially: a random sampling is given in several of the following references.

(11) Miller, J.; Closs, G.; Calcaterra, L. *J. Am. Chem. Soc.* **1984**, *106*, 3047–3049.

(12) Passman, P.; Verhoeven, J.; DeBoerth, J. *Chem. Phys. Lett.* **1978**, *57*, 530.

(13) Wasielewski, M.; Niemczyk, M. *J. Am. Chem. Soc.* **1984**, *106*, 5043.

(14) Creutz, C.; Kroger, P.; Matsubara, T.; Netzel, T.; Sutin, N. *J. Am. Chem. Soc.* **1979**, *101*, 5442.

(15) Heiler, D.; Rogalsky, P.; McLendon, G. *J. Am. Chem. Soc.* **1987**, *109*, 604–606.

transfer may become fast with respect to the *rate* of solvent repolarization which defines the nuclear reaction coordinate. In such a case, solvent fluctuations can control the rate of barrier crossing. Detailed treatments of this solvent dynamical effect have recently been published, with the general result that $k_{et} \leq 1/\tau$(solvent).[16] Some interesting data from several laboratories (including our own) support the general outlines of these theories.[17] In general, the simple theory should be further modified by effects of nonadiabaticity and free energy, both of which will tend to decrease the solvent dynamic effects. Recent steps in this direction have been taken.[17] In principle, dynamic medium effects could be quite important when repolarization is slow, as in polymers (and proteins).

In summary, experiments on fixed-distance electron transfer between small molecules, from a variety of laboratories using different techniques, establish the following basic predictions.

1. Rates depend exponentially on distance: $k_{et} \propto \exp-[(\alpha R)]$. For the donors we examined $\alpha \approx 1.1$ and depends only weakly on donor ionization potential, consistent with a superexchange mechanism.

2. The dependence of rate on ΔG is quite consistent with predictions of Marcus' theory. Particularly noteworthy is the elegant demonstration by Closs and Miller[11] of the inverted region, in which rates decrease when $\Delta G > \lambda$.

3. Solvent dynamic effects can be observed,[17] but corrections to the simplest theories[16] are probably in order.

Electron Transfer in Biological Systems: Structural Considerations

Given this background, we now focus on determinants of *biological* electron-transfer rates to attempt to learn how the specific structure(s) of biological (protein) redox systems modulate rates and specificities of physiological redox processes.

Our own work largely focuses on electron transfer between proteins that are physiological partners. In a complementary approach pursued by Gray, Isied, and others,[18] redox-active groups (e.g., $Ru(NH_3)_5^{2+}$) are covalently attached to specific protein sites. Comparative rate studies of such systems may ultimately provide details on preferred *pathways* for electron transfer since the precise distance for electron transfer is best defined in such systems.

In our studies, the two reacting proteins bind to form a well-defined complex, which is stabilized by both electrostatic and hydrophobic interactions.[19] (Hoffman's pioneering work on electron redistribution in hybrid hemoglobins can be viewed as a special case of such interactions.[20]) At low ionic strength (μ), the

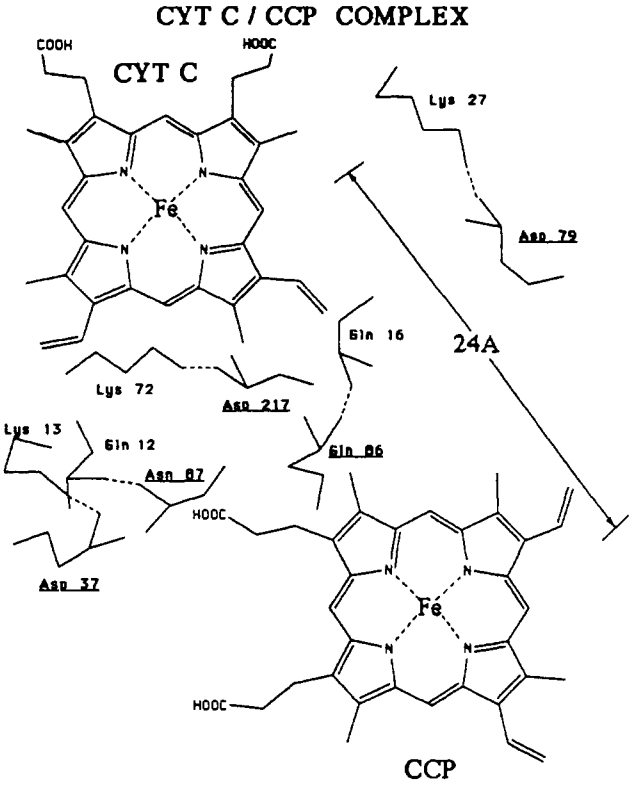

Figure 7. Model of the complex formed between cyt *c* and ccp based on the model of Poulos.[19a] In this model the Fe–Fe distance is ca. 22 Å.

binding constant for binding of physiological protein partners can be quite large. For example, for the cytochrome *c* (cyt *c*)/cyt *c* peroxidase (ccp) system, $K = 10^7\ \mu^{-1}$ at $\mu = 1$ mM.[19] At higher ionic strength, K decreases, but a bound complex may still exist as a (necessary) transient intermediate along the reaction pathway. It is important to note that the binding constant can also be quite sensitive to the oxidation states of the reactant proteins,[21] suggesting that motion at the interface likely occurs in going from reactants to products.

An example of a *structural model* for one such complex, the cyt *c*/ccp complex, is shown in Figure 7. Such models are based on a variety of data, including chemical modification, computer modeling, and various physical measurements.[19a] In turn, they suggest certain other experiments that can be used to test and refine the models. For example, we used dipolar energy transfer to measure the heme–heme distance in the cyt *c*/cyt b_5 complex;[23] Vanderkooi has reported similar measurements in related systems.[24] However, given the *motion* of the complex that accompanies electron transfer, as noted above, any structural model can provide only a starting point for understanding the

(16) (a) Calef, D.; Wolynes, P. *J. Chem. Phys.* **1983**, *78*, 470–482. (b) Zusman, L. *Chem. Phys.* **1980**, *49*, 295–304. (c) VanderZwan, G.; Hynes, J. T. *J. Chem. Phys.* **1982**, *76*, 2993–3001.

(17) (a) McGuire, M.; McLendon, G. *J. Phys. Chem.* **1986**, *90*, 2549. (b) Weaver, M.; Gennett, T. *Chem. Phys. Lett.* **1985**, *113*, 213–218. (c) Kosower, E. *Acc. Chem. Res.* **1982**, *15*, 259–266. (d) Grampp, G. *Z. Phys. Chem.* **1986**, *148*, 53–63. (e) Nadler, R.; Marcus, R. *J. Chem. Phys.* **1984**, *84*, 4894. (f) Sparpaglione, M.; Mukamel, S. *J. Phys. Chem.*, in press.

(18) (a) Winkler, J.; Nocera, D.; Yocum, K.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1982**, *104*, 5798–5800. (b) Isied, S.; Kuehn, C.; Worosilia, G. *J. Am. Chem. Soc.* **1982**, *104*, 7659.

(19) For reviews, see: (a) Poulos, T.; Finzel, B. *Pept. Protein Rev.* **1984**, *4*, 115–179. (b) Margoliash, E.; Bosshard, R. *Trends Biochem. Sci.* *8*, 316–320. (c) A good example of a (nondynamic) binding study is provided by: Mauk, M.; Mauk, A. G. *Biochemistry* **1982**, *21*, 1843.

(20) McGourty, J.; Blough, N.; Hoffman, B. *J. Am. Chem. Soc.* **1983**, *105*, 4470–4472.

(21) Some data, primarily from steady-state measurements, suggest k_B may depend significantly in oxidation state. For an example, see: Sligar, S.; Gunsalus, I. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1078–1082.

(22) (a) Hazzard, J. T.; McLendon, G.; Cusanovich, M.; Tollin, G. *Biochem. Biophys. Res. Commun.*, in press. (b) Hazzard, J. T.; Tollin, G. *Biochemistry* **1987**, *26*, 2836.

(23) (a) McLendon, G.; Miller, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 7811. (b) McLendon, G.; Winkler, J.; Nocera, D.; Mauk, A.; Mauk, M. *J. Am. Chem. Soc.* **1985**, *107*, 739.

(24) Vanderkooi, J.; Adam, F.; Erickinska, M. *Eur. J. Biochem.* **1975**, *60*, 199.

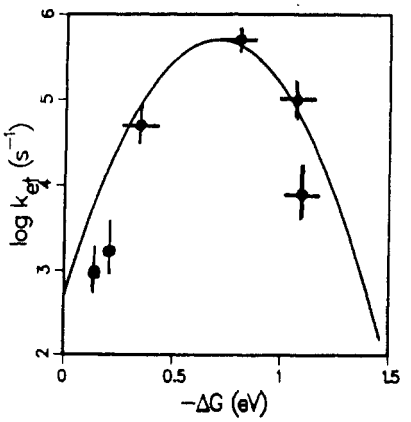

Figure 8. Plot of intracomplex electron-transfer rate between cyt *c* and cyt b_5 as a function of free energy. Solid line is fit to Marcus' theory, $\lambda = 0.8v$.

dynamics of protein–protein electron transfer. A recent example of the effect of interface structure on reaction kinetics comes from collaborative studies that I carried out with Hazzard, Tollin, and Cusanovich.[22] We studied electron-transfer reactions within the cyt *c*/ccp (ES) complex. At low ionic strength, Fe^{II}(cyt *c*) transfers an electron to Fe^{IV}(ccp) with $k_{et} \cong 150\ s^{-1}$. At high ionic strength, the complex is largely dissociated, so simple (preequilibrium) kinetics predicts that the observed rate should decrease. In fact, k_{obsd} increases to a limit of $1800\ s^{-1}$. Such data suggest that the stable reactant complex must rearrange to a structure of optimal reactivity; such rearrangement is facilitated by loosening the complex. A similar situation is found for the cyt *c*/cyt b_5 couple and likely for other systems as well. Finally, we note that the *rate* of such motion might become rate limiting in protein complexes.[34] We believe we have found such an example in the cyt *c*/cyt b_2 complex, where the rate is limited to ca. $150\ s^{-1}$ by a conformational change.

Intracomplex Electron Transfer: Dependence on Free Energy

With this background, we set out to learn how rate depends on ΔG (thus obtaining implicit information on protein reorganization energies). It was widely believed that protein reorganization energies were likely to be low,[25] so that rates would approach maximal values at biologically relevant free energies (i.e., $\Delta G \approx \lambda$). If so, any changes in ΔG away from the biological value should *decrease* the rates of electron transfer. Since biology provides only *one* free energy value per protein pair, we decided to vary ΔG by the method of metal substitution, which had proved useful in other mechanistic problems in bioinorganic chemistry.[26] Metal substitution can change ΔG and also introduces such possibilities as direct photoinduced reactions. However, such substitutions require a demonstration that substitution has not radically altered the structure of the substituted protein. A variety of experiments in various laboratories, ranging from high-resolution NMR[27] to

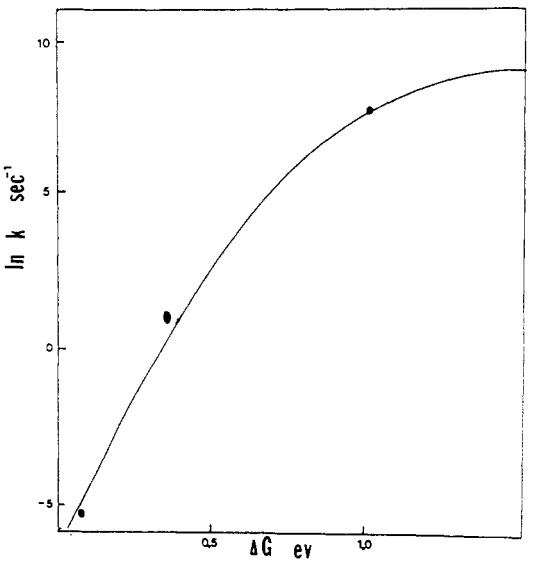

Figure 9. Plot of intracomplex electron-transfer rate between cyt *c* and ccp as a function of free energy. Solid line assumes Marcus' theory; $\lambda = 1.5\ v$.

binding studies between protein partners,[28] assured us that, at least in the case of metal-substituted cytochromes and hemoglobins, the native structure was maintained.[23,28,29] Thus, we could with reasonable assurance compare intracomplex rates between, for example, Fe^{III}(cyt *c*)/Fe^{II}(cyt *b*) vs Fe^{III}(cyt *b*)/Zn^{II}(cyt *c*) or Fe^{II}(cyt *c*)/Fe^{IV}(ccp) vs Zn^{II}(cyt *c*)/Fe^{IV}(ccp). Such comparisons, as illustrated in Figures 8 and 9 show that intracomplex electron-transfer rates generally increase with increasing ΔG, reaching maximal values (i.e., when $\Delta G \cong \lambda$) around 0.8 V for the cyt *c*/cyt b_5 system to 1.5 V for the cyt *c*/ccp system. Clearly, such results do *not* support the suggestion that protein reorganization energies are unusually small for biological couples. Similarly large values were obtained in Hoffman's elegant studies of electron transfer in Zn/Fe hybrid hemoglobins.[20]

It seems then, at least in those protein systems that have been examined to date, rate is not necessarily maximized for physiological electron transfer. This is not unreasonable when we consider that even the "slow" rates at physiological ΔG valus are quite competent and are generally *not* rate determining within metabolic pathways. Indeed, under these circumstances, one may consider that for biological electron transfer, the *rate* of transfer may be less critical than the *specificity* of that transfer. Furthermore, in the simplest possible model, the quantities of rate and specificities can be thermodynamically linked. Consider the hypothetical reaction between an oxidized protein P_1^{ox} and reduced protein P_2^{red}

$$P_1^{ox} + P_2^{red} \overset{K_1}{\rightleftharpoons} P_1^{ox}P_2^{red} \overset{\Delta G_r}{\rightleftharpoons} P_1^{red}P_2^{ox} \overset{K_2}{\rightleftharpoons} P_1^{red} + P_2^{ox}$$

$$\Delta G^\circ$$

ΔG_r = free energy in complex

ΔG° = free energy for uncomplexed species

As already noted, in general $K_1 \neq K_2$. The electron-transfer rate constant (k_{et}) depends on ΔG_r, which in turn depends on K (as K increases, ΔG_r decreases).

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(29) We note that small differences in structure might produce significant rate differences, as indicated by studies with cyt *c* mutants.

Thus, one may ensure high reaction specificity via a high value of K_1, but only at the expense of a decrease in ΔG_r, with a coupled decrease in k_{et}. The fact that $K_1 \neq K_2$ further suggests a possible source for the large λ values inferred for protein electron transfer. That $K_1 \neq K_2$ suggests that the interfacial structures of the reactant and product complexes might differ. Motion at the interface thereby can contribute to, or even dominate, the reorganization energy λ. Any such motion will effect electron-transfer rates, even when $K_1 \approx K_2$. The *dynamics* of such motion might also be quite important. In one limit, such conformational motions can become rate limiting.[34] Although the rates of electron transfer in the cyt b/cyt c complexes are nominally "fast", they are much slower than might be expected from studies of small molecules. For example, we synthesized and studied electron transfer in the bisporphyrin molecule shown in Figure 10, which has a similar structure, distance, and orientation as the cyt c/cyt b_5 system. This model gives a rate constant of $>10^9$ s^{-1} at optimal ΔG.[15] Studies in glasses and of Ru–protein adducts provide similar rates at a similar distance.

It appears then that the rate of interprotein transfer from cyt b_5 to cyt c may be limited by the rate of motions within the complex. Some support for this idea comes from the sensitivity of rate to small structural perturbations. For example, the cyt b_5/bovine cyt c complex reacts more rapidly (6000 s^{-1}) than does the cyt b_5/yeast cyt c complex ($k \sim 900$ s^{-1}). The *medium* dependence can also be important: in the ^{3}(Zn(cyt c))/cyt b_5 reaction the rate constant ranges from 4×10^4 s^{-1} to 2×10^5 s^{-1}, depending on specific medium, even under conditions where the complex is fully bound (i.e., rate is independent of protein concentration). Finally, a limiting example of such conformational control might be provided by the cyt b_2/cyt c system, which gives a rate of 150 s^{-1}, *independent* of the reaction ΔG (i.e., the rate is identical for ^{3}(Zn(cyt c))/cyt b_2, ^{3}(prophcyt c)/cyt b_2, and Fe^{II}(cyt b_2)/Fe^{III}(cyt c). Such control of rate by conformational "gating"[34] could provide another level of biological control.

Distance and "Medium Conduction" Effects

With the Franck–Condon-dependent activation energies defined through the preceding experiments, in principle it becomes possible to evaluate the effect of electronic coupling on rate; i.e., how does rate depend on distance R? In this context, the most extensive data come from studies of RuN_5-modified proteins by Gray and co-workers. These data suggest rate scales as $\exp(-0.9R)$, in reasonable agreement with expectations from small molecules.[30]

For physiological couples, the number of direct measurements is too small to permit such a quantitative evaluation. Qualitatively, however, rates do decrease as distance increases. For example, the rate constant at an optimal ΔG value for Zn*/Fe(Hb) ($R \cong 20$ Å) is $k_{max} = 10^2$ s^{-1}, while for the cyt c/ccp complex ($R \cong 16$ Å) $k_{max} = 10^3$ s^{-1}, for the cyt c/cyt b_5 complex (at optimal ΔG) $R = 8$ Å and $k \cong 10^5$ s^{-1}. Specific influences of the protein medium in donor–acceptor coupling also remain essentially unexplored, yet the few existing data suggest interesting specific effects may exist. Consider, for example, photoinduced electron-transfer reactions exemplified by hemoglobin (Hb), which contains the donor and acceptor metals on the α and β subunits. The rate constant of the photodriven reaction

$$Zn^*Hb\alpha/Fe^{III}Hb\beta \xrightarrow{k_q} Zn^{+}Hb\alpha/Fe^{II}Hb\beta$$

is $k_q = 100$ s^{-1},[20] while the rate constant for the reaction

$$Zn^{+}Hb\alpha/Fe^{II}Hb\beta \xrightarrow{k_b} ZnHb\alpha/Fe^{III}Hb\beta$$

which occurs at a similar ΔG, is *much* larger: $k_b = 1000$ s^{-1}.[31] The result that $k_q \ll k_b$ is found in a variety of protein systems, including the cyt c/cyt b_5[23] complex,[32] the ZnHb/cyt b_5 complex, the Zn(ccp)/cyt c[33] and ccp/Zn(cyt c) complexes,[34] and a series of Ru-substituted Zn–cyt adducts. Since both k_q and k_b have similar, highly exothermic ΔG values, the inequality $k_b \gg k_q$ probably does not reflect a Franck–Condon term, but rather the electronic term. A possible rationalization involves superexchange through "hole" states of the protein medium (recall Figure 2). Such coupling would be more efficient for the back reactions, in which the hole donor was a porphyrin cation radical, than for the (reducing) forward reactions, in which the only possible hole donor was a far more stable Fe(III) porphyrin. Thus the forward reactions are likely to proceed via "electron" (lowest unfilled molecular orbital, or conduction band, in other vocabularies) states. If this rationale were correct, then changing the nature of the medium "valence band" should dramatically change rates. Recent elegant studies by Hoffman, Mauk, et al. show that when an evolutionarily invariant aromatic residue in cyt c (Phe-82) is mutated to an aliphatic one, k_q is minimally affected, but k_b decreases dramatically.[33]

Mutational Approaches

In the preceding discussion we showed that protein–protein electron transfer may be characterized by large reorganization energies, likely associated with motion at the protein interface. In order to investigate the role of specific interactions at the interface, we established a collaborative effort with Fred Sherman, using genetic engineering methods to selectively modify the binding interface of cyt c. By a combination of genetic analysis and chemical modification studies, previous workers had identified key evolutionarily invariant amino acids in cyt c which are believed to constitute part of the binding domain for the protein partners of cyt c.[19,29] These amino acids include Lys-8, -13, -27, -72, -87, and -88.

These lysines thus provide a target to apply genetic engineering techniques in order to map out the effect of mutations on the binding and reactivity of cyt c with other proteins. Although these studies are at an early stage, the results to date have been rather striking. Initially the most surprising result was that some mutations of invariant lysines did not *decrease* the rates of protein–protein electron transfer but actually *in-*

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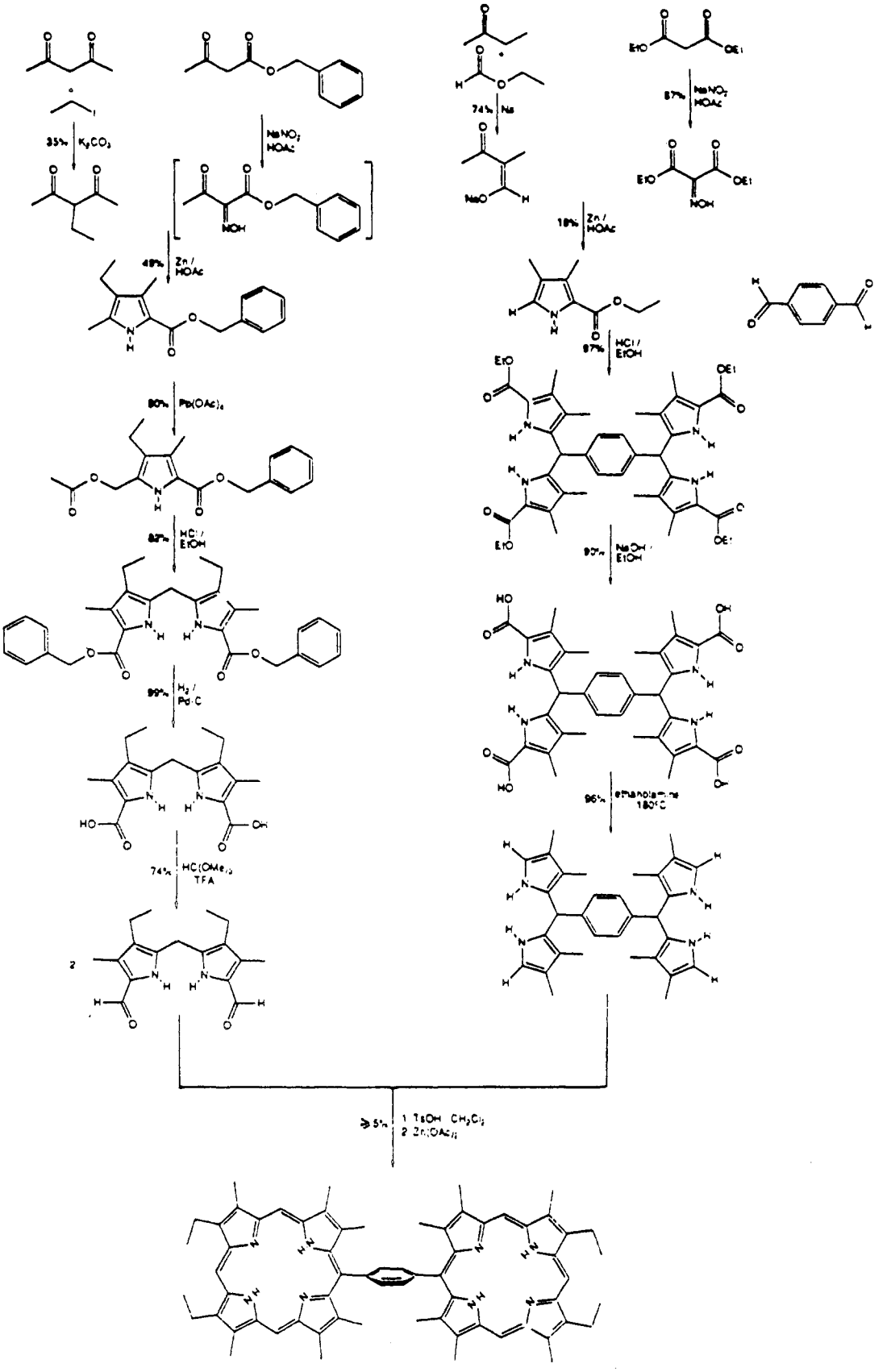

Figure 10. Synthesis of a model system for heme–heme electron transfer in protein complexes. For the molecule shown, $k_{et} = 2 \times 10^{10}$ s^{-1} at optimal free energy.

creased such rates, as judged both by steady-state kinetic measurements and by more exacting single-turnover measurements (see, e.g., Figure 11).

These data, then, offer a most compelling proof that maximal reaction rate, per se, is not the compelling evolutionary force for cyt *c* and related redox proteins.

Conclusions and Prospects

Over the past few years, it has been shown clearly that "long-distance" electron transfer is a common re-

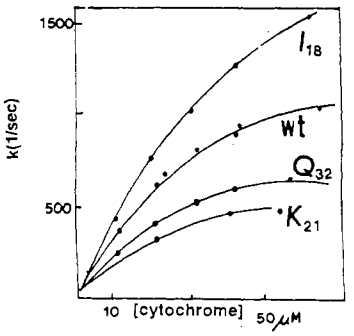

Figure 11. Variation in the observed rate of electron transfer from cyt *c* to ccp for various single-site mutants of cyt *c*. wt, "wild type" cyt *c*; I_{18}, arginine-18 → isoleucine; Q_{32}, lysine-32 → glutamine; K_{21}, glutamine-21 → lysine.

action mode in inorganic, organic, and biological chemistry. Rates generally decrease exponentially as distance increases and increase rapidly with increasing ΔG, reaching a maximum when the free energy and reorganization energy are equal. These same general trends are also found in protein systems: proteins show slower rates at longer distances, and rates increase markedly with increasing ΔG. However, the general trends may be significantly modulated by specific structural features in proteins. Many key questions remain; for example:

1. How does rate depend on distance? This question can only be reasonably addressed when the dependence of rate on free energy is known. A closely related question might be, what is the relevant distance anyway; is the *fastest* distance between two points always a straight line or can specific intervening residues promote donor–acceptor coupling? There are precious little data, but the preponderance of fast back reactions in protein photochemistry and the intriguing work on Phe-87 mutants suggest the possibility of important medium effects.

2. What determines the protein reorganization energy, λ? The growing body of evidence suggests that λ may be rather large for many protein couples. We have emphasized the possibility that interfacial motion may dominate λ, but other possibilities cannot be excluded: there are simply too few data to draw general conclusions. The *rates* of conformational reorganization also require careful examination: the limited studies with cyt *c*/cyt *b* and Hb suggest the possibility of conformationally "gated" rates.

3. Finally, and perhaps most importantly, how do proteins ensure biological specificity? The intrinsic reactivity of many redox protein active sites (e.g., Fe porphyrins, Fe–S clusters) is quite high. (Note, for example, the very fast electron-transfer rates obtained in bisporphyrin systems).[25] It is thus possible that proteins may even be designed to *minimize* these rapid rates, which might otherwise lead to disastrous biological "short circuits". If so, what is the structural basis for this modulation? It is clear that recognition occurs via the binding interface. The revolution in genetic engineering technology coupled with advances in structural techniques should help to map this interface. This would provide a first step in understanding the coupling of oxidation state to motion at the interface, which remains a major challenge to both theorists and experimentalists.

In each of these areas, the major challenges and opportunities lie ahead. The next major advances in this field will require the collaboration of chemists from a variety of backgrounds.

Were the editor to allow, this would be the longest section of this paper. I have been fortunate to work with a number of excellent students on this problem and to collaborate with some of the finest scientists I have known, including in particular John Miller, Harry Gray, Fred Sherman, Grant Mauk, Ann English, Mike Cusanovich, and Gordon Tollin. This Account has also benefited from detailed discussions with Brian Hoffman, Rudy Marcus, John Hopfield, and Dick Holm among many others. The work was supported by NIH, NSF, DOE, and the Sloan and Dreyfus foundations.