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REORGANIZATION ENERGIES FOR PROTEIN-TO-PROTEIN ELECTRON TRANSFER AND INTERFACIAL DYNAMICS: "PLAYING FAST AND LOOSE"

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Abstract Recent work on electron transfer in protein-protein complexes is summarized. Detailed measurements of binding equilibria and binding dynamics have been obtained for several systems: cytochrome c:cytochrome c peroxidase (CCP), cytochrome c:cytochrome b_5, and hemoglobin:hemoglobin reductase. For the cytc:ccp system binding and subsequent electron transfer have ben probed using site-directed mutagenesis. These results suggest that binding and recognition involve several, overlapping binding sites. Furthermore, sites exist for binding cytc(II) vs. cytc(III). Thus, electron transfer must involve diffusion along the protein surface which connects the oxidized and reduced sites. Those single site mutants which bind less strongly have a lower diffusion barrier and thus can undergo more rapid electron transfer. This coupling of interfacial motion to reaction may offer a natural explanation for the large reorganization energies observed in biological systems.

Keywords: Electron transfer, protein, cytochrome c, dynamics, reorganization energy

Over the last decade, the study of biological electron transfer has been transformed by the application of ideas and techniques developed in physicochemical studies of electron transfer between small molecules[1-3] to the study of electron transfer between macromolecular donor-acceptor pairs.[4]

For example, John Miller's pioneering experiments[3,5,6] at Argonne clearly revealed several key aspects of the molecular determinants of electron transfer rate. First, in accord with theoretical predictions, it was shown that electron transfer rates depend strongly on donor-acceptor distance: $k_{et} \alpha \exp -\beta R$ where R is the donor-acceptor distance (Å) and $0.8Å \leq \beta \leq 1.2Å^{-1}$. By studying rate at fixed distance, the full free energy dependence of electron transfer rates also was revealed.[3,6] In particular, an optimal exothermicity for electron transfer rates was observed when $\Delta G^\circ = \lambda$, the "reorganization energy" which accompanies charge induced atomic displacements in the reactants and surrounding medium. The rate decreases not only when $\Delta G < \lambda$, but also when $\Delta G > \lambda$, the famous "inverted region" first predicted by Marcus thirty years ago,[1b] but previously masked in experimental studies by diffusional effects, which are

obviously eliminated in "fixed distance" studies. A schematic of the parameters relevant to Marcus theory is shown in Figure 1.

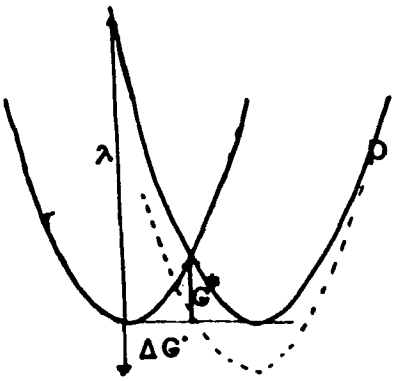

FIGURE 1 Predicted dependence of electron transfer rate (ket) in free energy (ΔG) by the theory of Marcus, outlined in reference 1. Points show data from reference 3.

Over the last few years, these same concepts and techniques used in defining the chemical physics of small molecule electron transfer have been applied to protein electron transfer. This has resulted in both new insights and new puzzles. For example, inorganic biochemists have used metal substitution in heme proteins to vary ΔG over a wide range. The application of physicochemical techniques like pulse radiolysis and laser photolysis to these derivatives showed that for protein-protein adducts, as for small molecules, electron transfer rates depend strongly on $\Delta G°$, and reach a maximum when $\Delta G = \lambda$[2,3] (Figure 2). The puzzle in these data lies in the magnitude of the reorganization energy, λ. The reorganization energy, λ, measures the displacements associated with the change in oxidation state of the donor and acceptor and their surrounding medium. (For proteins, this medium is largely the protein matrix itself.) If there is little change in protein geometry upon oxidation-reduction, then λ should be small. Proteins like cytochrome c and cytochrome b_5 indeed show very little change in structure with oxidation or reduction. Thus, Warshel[7] carefully estimated that the reorganization energy for cytochrome c should be ~0.1 V and even less for cytochrome b_5. In contrast, the measured reorganization energy is ca. 0.8 V for electron transfer in the cytochrome c/cytochrome b_5 complex and is even higher for other protein-electron transfer systems.[4]

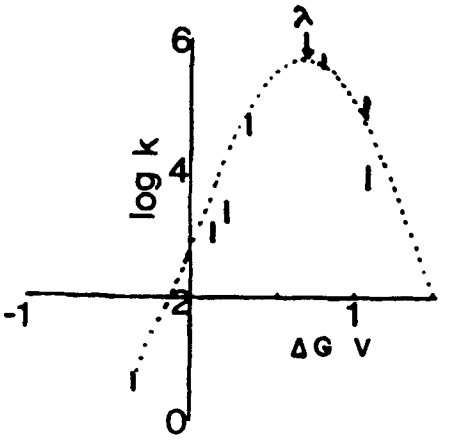

FIGURE 2 Dependence of ket on ΔG for the cytochrome c:cytochrome b_5 complex in which ΔG was varied by changing the oxidation state, T = 298°C, pH 7, or central metal, of the heme (from reference 4). (The point at ΔG, -0.2 v, was obtained by a magnetic resonance relaxation experiment: Concar, Whitford and McLendon, submitted.)

While the reasons for this discrepancy are unclear, the simplest possibility is that one precept of "relative" Marcus theory is not obeyed. It is generally true for small molecules, which do not strongly interact or bind, that the observed reorganization energy for cross reaction $D + A \rightarrow D + A^-$, can be estimated from the reorganization energies for the exchange reactions:

$$\text{e.g., } A^- + A \qquad A + A^- (\lambda_A), \text{ so that } \lambda_{DA} = \lambda_{A/2} + \lambda_{D/2}$$

The situation for specific protein partners can be different. In order to ensure biological specificity of electron transfer, the donor protein and acceptor protein must recognize and bind to one another. The binding must be strong enough to ensure that a complex lasts long enough to permit efficient electron transfer (ca. 10^{-3} sec for cytochrome:cytochrome c peroxidase), but not so long as to interfere with turnover. This recognition is accomplished by a combination of hydrophobic patches and complementary charge-charge "patches" (e.g., the invariant lysines of cytochrome C) which occur on the partner proteins (Figure 3). Both of these types of interactions

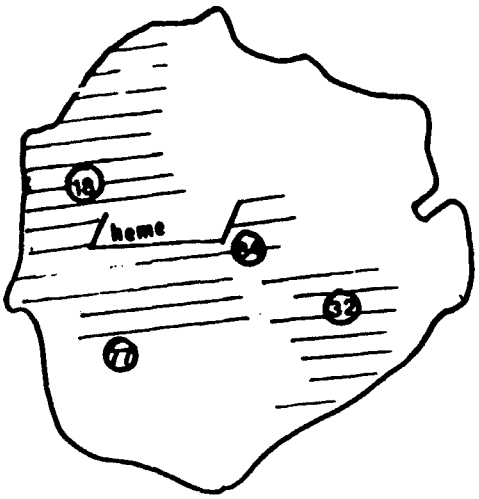

FIGURE 3 Schematic of the "binding patch" of cytochrome c, identified by NMR experiments and mutagenesis. The patch includes both hydrophobic residues (Phe 82) and a ring of lysines, which we call Tolkein's Ring ("one ring to bind them all").

are short range and directional. Thus, if redox changes at the active site affects the protein surface, this linkage could give rise to relatively large effective reorganization energies.

While it is clear that the basic folding pattern for a redox protein like cytochrome c does not change between Fe(II) cytochrome c and Fe(III) cytochrome c, it is quite possible that a number of changes may occur at the surface, which is relatively poorly defined in structural studies. Even a change in the mean displacements around the surface amino acids could propagate into a different preferred binding mode for the oxidized and reduced proteins. It is thus noteworthy that Fe(II) cytochrome c is known to have a few more "rigid" structures than does Fe(III) cytochrome c.

Quite recently, evidence suggests that Fe(II) cytochrome c and Fe(III) cytochrome c may indeed occupy slightly different sites in interacting with a protein partner like cytochrome c peroxidase. In studying the binding of cytochrome c to single-site charge mutants of cytochrome c peroxidase, Hake discovered that the pattern of mutational effect differs for Fe(II) cytochrome c than for Fe(III) cytochrome c (Figure 4). As the figure shows, the mutant K79 binds less well to cytochrome c (III) than does wild type cytochrome c peroxidase, while the mutant K217 surprisingly binds better. This

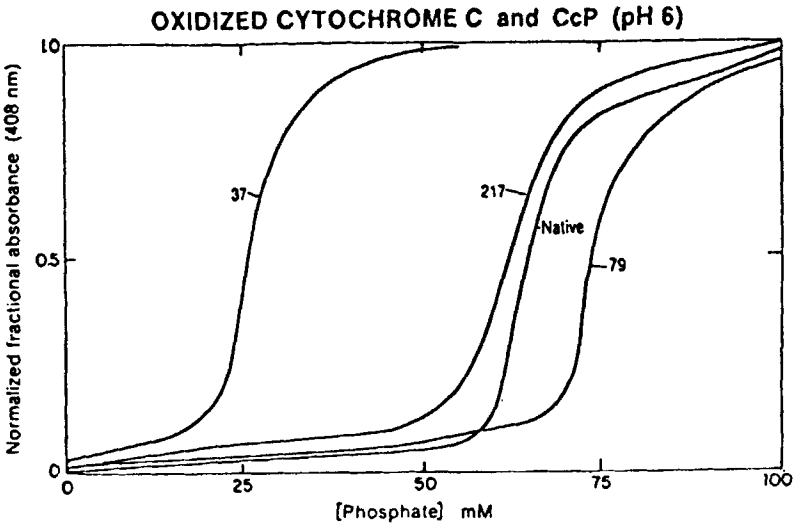

FIGURE 4 Relative binding profiles for cytochrome peroxidase to cytochrome c, measured by cytochrome c affinity chromatography (see Hake et al., this volume).

pattern is precisely reversed for binding to cytochrome c (II) which follows the order K79 > wt > K217.

While these data are somewhat complicated by the existence of specific anion binding sites on cytochrome c (III), the simplest explanation for these differences is that cytochrome c (II) and cytochrome c (III) bind to nearby spatially distinct sites[8] on cytochrome c peroxidase. These multiple electrostatic binding sites were identified by Northrup in his molecular dynamic simulations of cytochrome c:cytochrome c peroxidase bindings. This oxidation coupled site selection has several implications for biological electron transfer. The initial binding event between cytochrome c and cytochrome c peroxidase likely involves a charge-dipole directed approach of the two partners, as suggested by Koppenol and Margoliash,[9] so that cytochrome c can "stick" at a variety of charged sites This relative variability of binding has been called the "velcro" model of binding. This initial encounter complex subsequently equilibrates to produce a distribution of bound complexes. Some insight into both the static and dynamic nature of this complex has ben obtained by studying energy transfer within the cytochrome c:cytochrome c peroxidase complex, in which Fe(III) in the cytochrome c peroxidase has been replaced by Mg(II), giving a fluorescent heme as the energy donor, while the

cytochrome c heme acts as the energy acceptor. Energy transfer, like electron transfer, varies strongly with distance; the rate of dipolar energy transfer is proportional to R^{-6}.

These different complexes, which have different donor-acceptor distances, should give observably different rates. If the different complexes interconvert rapidly on the energy transfer time scale ($\leq 10^{-8}$ sec), then energy transfer will be described by pre-equilibrium kinetics and only a single decay rate will be observed. If interconversion is slow, then multiple decays should be observed. This condition of slow interconversion should be obtained at 77°K, while any diffusive motion should be frozen out. Indeed, although magnesium cytochrome c peroxidase alone shows essentially a single exponential decay at 77°K, the magnesium cytochrome c peroxidase:cytochrome c adduct shows a complex multi-exponential decay, which is most simply described by a distribution of complexes centered about 20Å distance, with an RMS deviation of ca. 6Å. By contrast, at room temperature, this complex decay collapses into essentially a single exponential decay.[10] This suggests that at room temperature the multiple cytochrome c: cytochrome c peroxidase complexes equilibrate within a few nanoseconds. This provides a good estimate of the time scale for evolution of the "velcro" complexes, involving two-dimensional diffusion to the most favored reactant [Fe(II) cytochrome c] site, fluctuation toward the Fe(III) site, with concomitant electron transfer, and, finally, dissociation of the Fe(III) product, completing one catalytic turnover. Since the electron transfer reactant (cytochrome c:cytochrome c peroxidase) complex and product complex have a different structure, electron transfer requires that the complex change structure during reaction. This conformational change will contribute to the observed reorganization energy. Since the forces (e.g., charge-charge interaction) which are involved in complex stabilization occur over a rather short range, small displacements may produce rather large reorganization energies. The postulate that interfacial dynamics are closely linked to electron transfer dynamics, both through reorganization energy and through the distance dependence of electron transfer, leads to an interesting prediction. Those mutations which facilitate interfacial motion should also facilitate electron transfer by reducing λ. This prediction appears to be correct. Recent studies have shown that the electron transfer reactivity of a cytochrome c:cytochrome c peroxidase complex increases when the precursor complex is slightly weakened, either by raising the ionic strength,[11] or by single-site mutagenesis on cytochrome c[12] or cytochrome c peroxidase.[13] For example, taking the mutants in Figure 4, K79 which binds most strongly, reacts most slowly, while K217, which binds most weakly, reacts correspondingly more rapidly. Of course, the situation is complicated, as noted above, by the fact that interfacial dynamics also affect the donor-acceptor distance and thereby the electron transfer rate(s). For this reason, studies of the temperature dependence of

electron transfer, which normally might provide an independent estimate of λ, are difficult to interpret, since temperature can affect both $\Delta H^{\pm}$ and the prefactor (through the distance dependence).

Finally, we note the reasons behind this "fast and loose" mode of protein binding and recognition have been discussed elsewhere. In brief, the strategy employed by nature seems geared to avoiding adventitious "short circuits" by maximizing the kinetic probability of successful complex formation between physiological partners. A dynamic recognition model not only maximizes the interaction surface but also allows for ready product release. The mean residence time ($1/k_{off} \approx 10^{-3}$ sec for cytochrome c: cytochrome c peroxidase) is conveniently coupled to the rate of electron transfer $k_{et} \approx 10^3$-10^4 s^{-1}: the proteins are bound just long enough to ensure productive electron transfer. In addition, this multiple site strategy allows a "mediator" protein like cytochrome c to service several different partners: cytochrome c peroxidase: cytochrome c oxidase, cytochrome b_2, cytochrome c reductase, etc. This binding mode does apparently lead to rather large λ's with an associated decrease in rate, but this is a small price to pay for biological specificity. This argument has been earlier codified in the so-called "biological redox laws". Apparently, nature plays "fast and loose" for good reason.

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14. Also called "Donna's Laws," they summarize some basic basics of biological electron transfer (and may perhaps be applicable to other biological processes): (1) longer is better, (2) quicker is not necessarily better and (3) motion at the interface is important.