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Engineering protein structure for electron transfer function in photosynthetic reaction centers

Christopher C. Moser and P. Leslie Dutton

The Johnson Research Foundation, Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA (USA)

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A basic relationship is defined that incorporates the three parameters that effectively modulate the rate of intraprotein electron transfer, namely distance, free energy and reorganization energy. This empirically validated relationship is used to explore the minimal requirements for protein-catalyzed conversion of excited electronic states into stable charge separated states, the essence of photosynthesis.

Introduction

The goal of relating structure to bioenergetic function has stimulated many avenues of research that have contributed to an increasingly detailed description of the electron transfer proteins essential to life. Bacterial photosynthetic reaction center proteins have always played a significant role, not only by illustrating the basic mechanisms by which light energy is converted to electrical and chemical energy, but also by providing general principles that have been directly relevant to respiratory electron transfer. With the resolution of the crystal structures of the bacterial reaction centers in *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides* [1–3] and the large scale modification of the free energies of the photosynthetic electron transfer reactions [4–6], it has become possible to describe a general picture of the factors that determine intraprotein electron transfer [7]. Of the several factors that significantly modify electron transfer in chemical systems, only three appear to be effectively modified in biological systems, namely distance, free energy and reorganization energy. Electron transfer theory based on these three variables condenses into a simple relationship that adequately embraces a wide range of electron transfer reactions. This relation permits us to readily visualize the basic structural and energetic requirements for the construction of functional electron transfer proteins that can operate within the constraints imposed by a chemiosmotic mechanism. In this report we explore the minimal requirements for protein-catalyzed conversion of excited electronic states into stable charge separated states, the essence of photosynthesis.

Correspondence to: C.C. Moser, The Johnson Research Foundation, Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104, USA.

General electron-transfer theory

It appears that intraprotein electron transfer generally takes place by a tunneling mechanism over distances that are relatively large on an atomic scale. Thus, the interaction between the donor and acceptor redox centers is usually quite small. Under these conditions Fermi's Golden Rule provides a good first-order description of the rate of non-adiabatic electron transfer (see Devault [8]).

$$k_{et} = 2\pi / \hbar V^2 FC \quad (1)$$

Two parameters determine the rate k_{et}. The quantum mechanical matrix element V^2 couples the reactant and product electronic state, and is critically dependent on the extent of overlap of the reactant and product electronic wavefunctions. The Franck-Condon weighted density of states, FC, reflects the integrated overlap of the reactant and product nuclear wavefunctions.

For biological systems the most significant variation of V^2 can be understood as the continuously decreasing overlap of electronic wavefunctions of the reduced donor and acceptor redox centers with increasing distance. A simple approximation based on the tunneling between two narrow potential wells representing the redox centers, through a barrier of constant height over

a distance R, yields an exponential decay of the wavefunction overlap and hence V^2.

$$V^2_{(R)} = V^2_{(0)} \exp(-\beta R) \quad (2)$$

The exponential coefficient β is essentially a description of how well the electronic wavefunctions penetrate the medium between the redox centers.

Franck-Condon factors have been described by Marcus [9,10] in a simple model that approximates reactant and product nuclear wavefunctions as harmonic oscillations with identical frequencies ($\hbar\omega$) but with different equilibrium nuclear positions and free energies. In this description the overlap of the nuclear wavefunctions and hence the rate will have an overall Gaussian dependence on the free energy of the reaction.

$$FC = (4\pi\lambda kT)^{-1/2} \exp[-(\Delta G - \lambda)^2/4\lambda kT] \quad (3)$$

Thus, when all other variables are held constant, the Marcus relation predicts that the rate will rise as the free energy ($-\Delta G^\circ$) of the reaction increases from zero to some maximal value. The free energy associated with the maximal rate provides the experimental definition of the reorganization energy (λ). Theoretically, the reorganization energy reflects the energy required to distort the equilibrium nuclear geometry of the reactant state into the geometry of the product state while constraining the electron to remain on the donor. As the free energy is increased beyond the reorganization energy, the overlap of the harmonic oscillator nuclear wavefunctions becomes poor and the rate is expected to fall. This behavior of increasing reaction free energy and decreasing rate of electron transfer defines the Marcus 'inverted region' and has been confirmed experimentally [11].

When the energy of the harmonic oscillator vibrational frequencies is large compared to twice the Boltzmann thermal energy, $2kT$, then quantum corrections must be added [10,12,13]. Nevertheless, under many circumstances, these quantum expressions have a nearly Gaussian form. Indeed, the expression of Hopfield [14] represents the same Gaussian form as the expression of Marcus, except that the $2\lambda kT$ terms expressing the variance in the Marcus expression are replaced by $\lambda\ \hbar\omega \coth(\hbar\omega/2kT)$. At low temperatures, the coth term reduces to a value of 1, and the rate becomes temperature independent, while at high temperatures the term reduces to $2kT/\hbar\omega$ to reproduce the Marcus expression. When multiple vibrations are coupled to the electron transfer, the individual Gaussian free energy vs. rate relations for each vibration can be convoluted into an overall rate dependence similar in form to the quantum corrected version of Eqn. 3, but with the single oscillator frequency replaced by a 'characteristic frequency'. This characteristic frequency essentially represents a reorganization energy weighted average of all the individual vibrational energies.

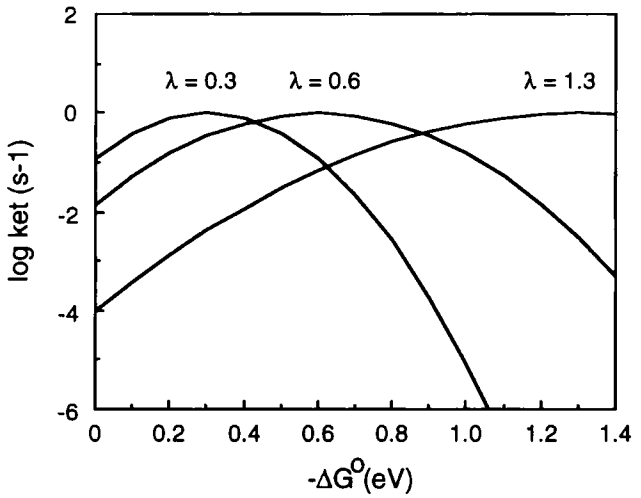

Fig. 1. Representative free energy dependencies of the rate of intraprotein electron transfers. The Gaussian relation of Eqn. 4, closely related to the Marcus relation of Eqn. 3, appear parabolic on a logarithmic rate scale. Examples of the range of reorganization energies believed to be relevant for intraprotein electron transfer, 300 to 1300 meV, are shown for the same optimal rate.

Empirical relation for intraprotein electron transfer

In Moser et al. [7], we found that an examination of the free energy dependence of intraprotein electron transfer for physiological reactions and non-physiological reactions in native or modified proteins is adequately modeled by an approximately Gaussian dependence, with a reorganization energy ranging from about 300 to about 1300 meV. Fig. 1 provides examples of rate vs. free energy relationships for several values of reorganization energy. We also showed that for reactions in which rates have been measured over an extensive free energy range, the breadth of the approximately Gaussian free energy curves appear, at physiological temperatures, to be largely due to differences in the value of λ rather than to variations in $\hbar\omega$. The characteristic frequency associated with the width of the Gaussian appears to be about 70 meV, similar to the value found for electron transfer between chemically synthesized organic redox centers dispersed in MTHF glass [11] and only slightly larger than $2kT$ at physiological temperatures. Thus the classical Eqn. 3 of Marcus is relatively accurate for room temperature intraprotein transfer.

The Franck-Condon factors are least affected by particular values of λ, $\hbar\omega$ and T at the rate optima, where the exponential term disappears and only a very modest square root dependence remains. Thus, an appreciation of the non-Franck-Condon factors that influence electron transfer rates is best gained by com-

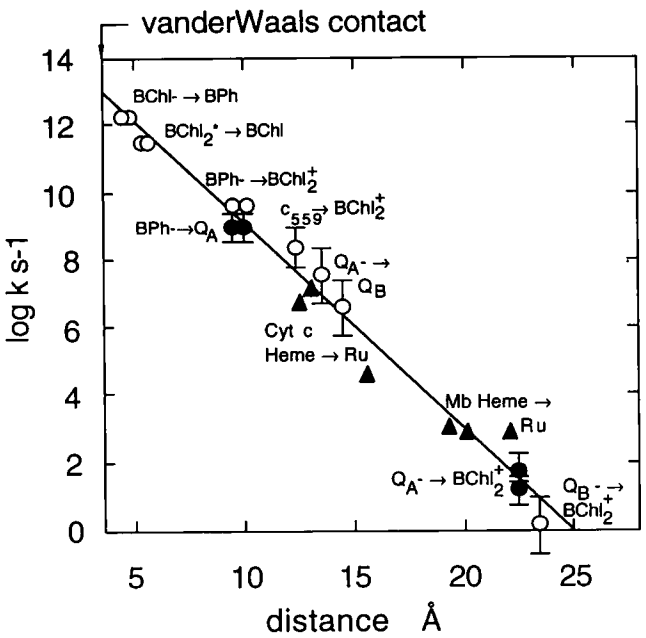

Fig. 2. The free energy optimized rate vs. edge-to-edge distance relationship for intraprotein electron transfer. Photosynthetic reaction center rates are shown as circles and excited heme-ruthenium electron transfers in modified myoglobin and cytochromes *c* are shown as triangles. Filled symbols are for reactions with extensive free energy dependence, while open circles are for reactions with a smaller experimental free energy dependence and more uncertain optimal rates, with uncertainties indicated by error bars. The fit line represents a simple exponential decay of Eqn. 2 with a β of 1.4 Å^{-1}. Distance, derived from crystal structures, is defined as center of edge atom of donor to center of edge atom of acceptor; thus the vertical line at 3.6 Å represents van der Waals contact.

paring electron transfer rates at the free energy optimum for each system. Fig. 2 shows that the logarithm of the optimal rates spanning 12 orders of magnitude for intraprotein electron transfer reactions as a function of the edge-to-edge distance generates a surprisingly linear relationship. It appears that the protein medium acts as a relatively uniform barrier to electron tunneling with an average β value of 1.7 Å^{-1}. Because physiological reactions, expected to feel the effects of natural selection, are indistinguishable in β from competing back reactions or indeed the non-physiological reactions of semi-synthetic systems, it appears that β is a property of the medium and may not be amenable to natural selection.

These observations allow us to develop a practical, empirical approximation for intraprotein electron transfer that has the simple Gaussian form of a Marcus-like expression and includes a log relationship between the optimal rate and the edge-to-edge distance using the apparently generic values of β and $\hbar\omega$.

$$\log k_{et} = 15 - 0.6R - 3.1(\Delta G - \lambda)^2/\lambda \qquad (4)$$

The coefficients of this empirical relation are derived with the following units: s^{-1} for the electron transfer rate k_{et}, Å for the edge-to-edge distance R, and eV for the free energy and reorganization energy. With this useful relation we can now entertain what engineering principles must be followed in the design of intraprotein electron transfer systems, including photosynthetic reaction centers.

Engineering constraints

One of the principal constraints on the design of a bioenergetic electron transfer protein will be the approx. 35 Å low dielectric membrane thickness that is a crucial component of any chemiosmotic system [15]. A related time constraint can be found in the typically microsecond to millisecond timescale required for diffusional reactions that connect integral membrane proteins to other redox agents. The particular dielectric thickness and diffusional rates found in natural systems may very well reflect the practical requirements of electrical energy storage and the leakiness of a membrane. These two constraints make it impossible to construct a transmembrane electron transfer protein system in which a single electron transfer between two redox centers can cross this barrier in the required time. Thus all electron transfer proteins that work within such a system must have at least two sequential electron transfer reactions to span the transmembrane distance, involving a minimum of three redox centers. Such is indeed the case with respiratory proteins including the bc_1 complex and cytochrome *c* oxidase.

Photosynthetic reaction centers are embedded in the same dielectric but face a much more severe time constraint. Excited singlet states that are a prelude to charge separation typically decay on a nanosecond timescale. With this time constraint, efficient electron transfer in any photosynthetic system must take place in at least 3 steps involving at least 4 redox centers. This is still one step and active redox center less than that used in the bacterial reaction centers of *Rb. sphaeroides* and *Rp. viridis*.

Although distance exerts an overwhelming control on electron transfer rates, Franck-Condon factors involving both free energy and reorganization energy can be critical. Generally speaking, natural systems will want to minimize the expenditure of free energy in forward reactions leading to stable charge separation in order to provide the maximum free energy for driving subsequent bioenergetic pathways.

A primary example of the influence of Franck-Condon factors on electron transfer engineering is found in the initial charge separation of any light induced electron transfer. These reactions will compete with a generally large free energy charge of recombination to the ground state, over the same distance as the charge

separation. Indeed, such rapid charge recombination limits the lifetime of charge separated states in many synthetic systems designed to study electron transfer. An elegant engineering solution, recognized by Miller [11] and Warshel [16], is to design the reorganization energy to be as small as practical, forcing the back reaction into the Marcus inverted region. This strategy appears to be used in the photosynthetic reaction centers. If a typical reorganization energy for intraprotein electron transfer is around 700 meV, then it would appear that a value of approx. 300 meV for the initial charge separation in reactions centers may partly reflect the pressure of a natural selection for a photosynthetic center with relatively delocalized charge (a dimer of chlorins) in a relatively rigid and non-polar protein environment.

On the other hand, it is also possible to circumvent a rapid recombination reaction by breaking up the initial charge separation into two steps, with the first step slower than the second. This may be a strategy that is also used in the bacterial reaction centers where a rapid electron transfer between BChl and BPh could assure a charge separation of 10 Å with nanosecond stability. A biomimetic, but covalently linked series of redox centers synthesized by Gust et al. [17] appears to provide another example where the second electron transfer appears to be more rapid and boosts quantum yield.

In the bacterial reaction center, Franck-Condon factors may also be important in assuring that the excited singlet state $BChl_2$ transfers an electron through only one of the similarly close BChls on either side of the approximate symmetry axis of the reaction center. The function of the BChl and BPh on the M subunit side of the reaction center is unknown, but the evidence for asymmetric electron transfer is clear. Electron transfer to the functionally inactive side may be made unfavorable by permitting λ to rise to 600 meV [7]. Similarly, an inactive side endothermic free energy [18] of 200 meV is sufficient to assure that electron transfer to the active site will take place with better than 99% efficiency.

Franck-Condon factors also appear to play a large role at the quinone binding sites of the bacterial reaction centers. The distances and free energy for the return of the electron from Q_A^- or Q_A^- to $BChl_{2+}$ are quite similar, yet the Q_A^- state can be stabilized for seconds if the reorganization energy for this reaction is made large [19], perhaps as large as 1300 meV. A large reorganization energy could be associated with a relatively polar Q_A site that is designed to couple protonation to electron transfer. Alternatively, the presence of protonation reactions at Q_A may make this reaction adiabatic and outside the scope of the non-adiabatic tunneling analysis presented here. Clearly adiabatic reactions are found in the relatively slow reactions involving diffusion, such as the interaction of soluble cytochrome *c* with reaction center, which can stabilize separated charges essentially indefinitely, by physically translating them.

Alternative photosynthetic charge separating designs

The similar reaction center structures resolved from X-ray diffraction studies of *Rp. viridis* and *Rb. sphaeroides* represent just one solution to the problem of converting light energy into stable charge separation within the engineering constraints that we have outlined. No doubt these structures reflect important concerns beyond that of electron transfer and function, including requirements for proper protein folding and stability. These structures may also include remnants of the evolutionary history of the proteins; for example the symmetry of the reaction center and the presence of apparently auxiliary redox centers may reflect gene duplication. Yet, as far as the basic rules of intraprotein electron transfer are concerned, it is clearly possible to construct an efficient artificial reaction center that uses fewer redox centers and conserves more energy.

In considering alternative reaction center design, we will insist on an engineering efficiency that is at least as good as the native reactions centers. Thus we will require the charge separation to take place over a distance of 48 Å, the center-to-center distance between Q_A and the cytochrome *c* heme in *Rp. viridis*. Similarly, we will require that the charge separation take place faster than the minimum $10^3\ s^{-1}$ respiratory rate and that charge separation be stable on this same millisecond timescale. We will require that the quantum efficiency be at least 99% and that the residual free energy stored as a redox potential difference for dark reactions be at least the approx. 0.4 eV found in native systems. For simplicity, we will use as terminal redox centers molecules the size of the Q_A and heme found in photosynthetic bacteria and use molecules the size of chlorins as intermediate redox centers. We will also assume that such a reaction center obeys our generic parameters for electron transfer with a 1.7 $Å^{-1}$ β and a 70 meV characteristic frequency, and we will choose as a typical absorbed photon energy 1.4 eV, corresponding to the long wavelength absorption maximum of *Rb. sphaeroides*.

If only one intermediate redox center is used, then even under the most favorable orientation of the redox centers and the most favorable free energies and reorganization energies, at least one of the electron transfers must take place over at least 16.3 Å at $2 \cdot 10^5\ s^{-1}$, and the quantum efficiency will be much less than 1%. With two intermediate redox centers, many geometries are successful, such as that indicated in Fig. 3. Terminal redox centers are labeled Q and C. An initial

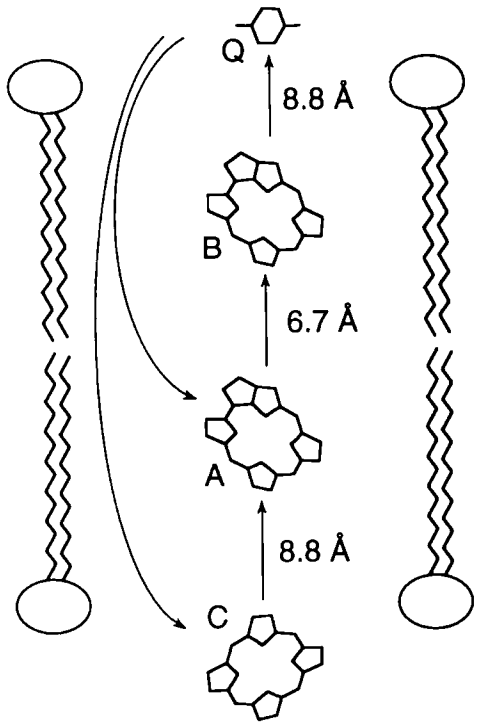

Fig. 3. A possible arrangement of redox centers for efficient charge separation in a hypothetical photosynthetic protein. The overall distance of charge separation between C and Q are represented as the same distance as the cytochrome *c* to Q_A electron transfer of the photosynthetic reaction center of *Rp. viridis*. A and B are represented as chlorin type redox centers which function as the absorber of light energy and as an electron-transfer intermediate, respectively. Distances and free energy of reactions are chosen according to the first-order description of intraprotein electron transfer presented in this report to achieve 99% quantum efficiency in charge separation across a low dielectric membrane with millisecond or better stability and minimum consumption of free energy.

charge separation between redox center A and B of 10^{11} s^{-1} would assure a quantum efficiency of 99%. This rate can be achieved while conserving free energy by using as small a reorganization energy as possible, say 0.3 eV, and by using a distance of 6.7 Å. The charge recombination rate to ground state is appropriately slow at $3 \cdot 10^4$ s^{-1}. With two more identical 8.8 Å edge-to-edge electron transfers, B to Q and C to A, each with as small a free energy as possible to avoid thermal back reactions, say 0.12 eV each, we anticipate rates of $2 \cdot 10^8$ s^{-1}. Charge recombination back to ground state from Q over 23.9 Å is less than 5 s^{-1} even for the worst case when the free energy is near optimal. The net result is rapid charge separation across the membrane (about 20 ns), with a consumption of only 0.54 eV of the photon energy to give a chemical potential of better than 0.8 eV compared to the native 0.4 eV.

With less favorable orientations of the cofactors, it is still possible to maintain a 99% quantum efficiency and 0.8 eV for dark reactions by adjusting cofactor distances. For example, if one cofactor is perpendicular to the transmembrane direction, distances of 6.7, 11.8 and 14.4 Å still permit efficient charge separation on a 700 ms timescale. If both redox centers A and B have orientations perpendicular to the transmembrane direction of electron transfer, then it will not be possible to achieve 99% quantum efficiency without adding an additional redox center. This would make the hypothetical photo-units formally similar to the reaction centers observed in nature.

We have considered charge separation from an initial excited singlet state, yet many absorbers will spontaneously decay to excited triplet states with a considerable loss of energy. In the face of an extremely fast singlet-triplet conversion, potential energetic losses through a triplet state can still be avoided by using favorable cofactor orientations and distances of 6.7, 6.7 and 10.8 Å, while still preserving a 99% quantum efficiency. Despite the initial energy loss, it is possible in principle to construct a photosynthetic reaction center that takes advantage of the long lifetime of an excited triplet state, beginning with a 15 Å, microsecond charge separation, provided the reorganization energy of this separation can be made small. However, the examples provided by protein systems suggest that slow and long distance electron transfers are associated with modest to large reorganization energies.

Finally, an estimate of the long wavelength limit for photosynthetically active photons must take into account the minimum energy required to drive subsequent dark reactions, which is about 0.4 eV in photosynthetic bacteria, and also consider the effect of a biological transmembrane electric field, typically around 250 mV. Using the favorable orientation of cofactors and increasing the free energy of individual steps to compensate for the electric field suggests that the lowest energy photon for efficient charge separation will be about 1.1 eV or about 1100 nm, somewhat longer than the low energy absorption of the reaction center of *Rp. viridis* at 960 nm, or even the low energy absorption of the antennae proteins in this species.

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