THEORY AND EXPERIMENT IN PHOTOSYNTHETIC ELECTRON TRANSFER

Rudolph A. Marcus, Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, CA 91125
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1. INTRODUCTION (1)

The recent determination of the crystallographic structure of a bacterial photosynthetic reaction center has provided an important framework for treating electron transfers in these systems. As in electron transfers in simpler systems, a knowledge of both the structure and the thermodynamics is needed for understanding the experimental results on reaction rates at the fundamental level. In this lecture we first describe the theory of simple electron transfer reaction rates, and then consider how the resulting interaction between theory and experiment may assist us in treating electron transfers in the photosynthetic reaction center.

In the final portion of this talk we summarize a view of the current state of knowledge in this electron transfer complex, and some of the questions which remain. We include in our discussion the very recent and dramatic results of Zinth and coworkers on the role of the bacteriochlorophyll monomer at room temperature (2), presented also at this Congress. Ultimately, all of the evidence for the mechanism should fit. In bacterial photosynthesis we have not yet reached that state, but several recent developments have perhaps begun to simplify the picture, examined in this lecture.

2. THEORETICAL OVERVIEW (3)

In studies of the simple electron transfer systems two approaches have been adopted in relating theory to experiment. One of these is to proceed in a more or less *ab initio* manner, attempting to calculate the absolute reaction rate constants. Sometimes this calculation can be made, but often the necessary data are incomplete. A second procedure is to explore, instead, theoretically based relationships among various reaction rate constants. The reactions whose rates are compared may all be occurring in solution. Or, with judicious use of theory, rates of electron transfers in solution may be compared with those across various types of interfaces. This second alternative, of relating reaction rates to each other, has been the most common one adopted in the literature, several examples being given in the next section. It also entails fewer theoretical assumptions: Many of these relationships were derived theoretically using a molecular statistical mechanical treatment of the problem. The relations were established because of large cancellations, when ratios of various rate constants (relationships) were calculated, ratios of quantities whose individual numerical values were only imperfectly known. The overall theory emphasizes thermodynamic (ΔG^0), "intrinsic" (λ) and electronic (H_{DA}) factors as influencing the electron transfer rate, and provides a way of sorting out these factors.

In an electron transfer reaction a reorganization of the solvent and of the intramolecular coordinates occurs, until some configuration is reached such that during the electron transfer no change occurs in the values of the

coordinates or momenta of the atoms (Franck-Condon principle). Typically, this reorganization occurs prior to and after the actual electron transfer (Fig. 1).

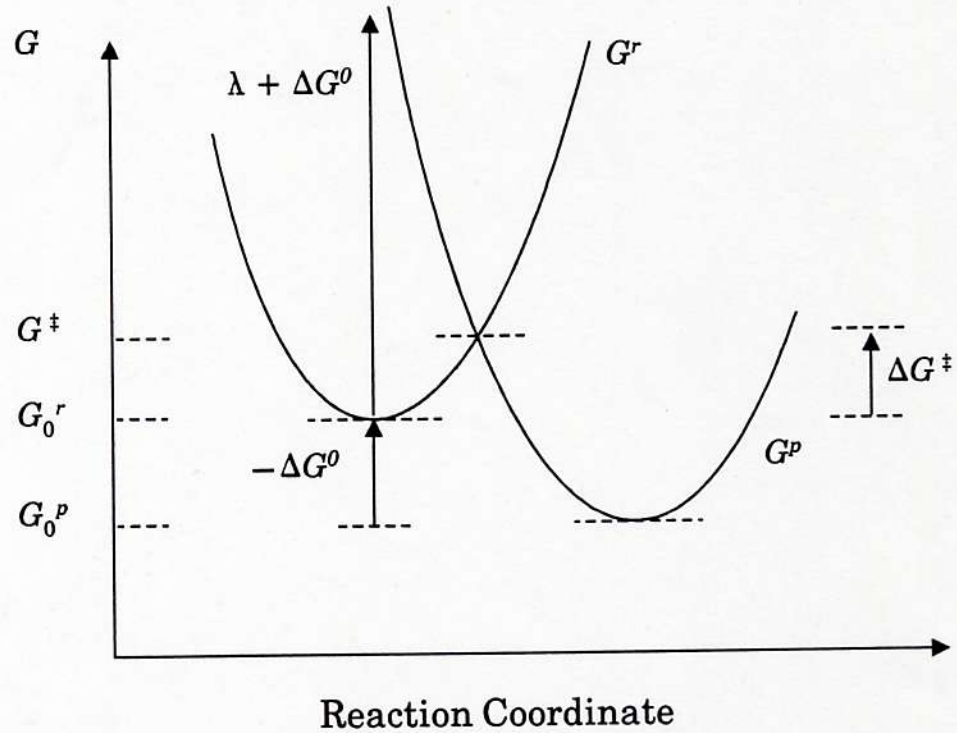

Figure 1. Free energy curves for reactants plus environment G^r and for products plus environment G^p.

In Fig. 1, plots of G^r, the free energy of the reactants plus surrounding environment, and G^p, the free energy of the products plus environment, are given. The details of the relationship between these approximately parabolic plots and the much more complicated $\sim 10^{23}$-dimensional plots of the corresponding two potential energy surfaces vs. the reaction coordinate are discussed elsewhere (3,4). The electron transfer satisfies the Franck-Condon principle by occurring at the intersection of the two many-dimensional potential energy surfaces and, thereby, it was shown, at the intersection of the G^r and G^p curves. In Fig. 1 the value of G at the intersection is denoted by $G^{\ddagger}$. At any temperature T the reacting system undergoes fluctuations, moving randomly on the G^r curve. The reorganization before electron transfer involves a fluctuation from the region near the minimum $G_0{}^r$ of the G^r curve so as to reach the intersection. After the system moves from the G^r to the G^p curve as a result of an electron transfer, it eventually reaches the region near the minimum $G_0{}^p$ of the G^p curve after suitable fluctuations. One of the main aims of the theory was to obtain a practical method of calculating the activation free energy $(G^{\ddagger} - G_0{}^r)$ $(\equiv \Delta G^{\ddagger})$ and with it, after introducing dynamical concepts, the reaction rate constant.

An expression for the rate constant $k(r)$ for an ET reaction from a donor D to an acceptor A at a fixed separation distance r can, for purposes of the present

discussion, be written as (3),

$$k(r) = \frac{2\pi}{\hbar} \frac{|H_{DA}|^2 \, e^{-\Delta G^{\ddagger}/k_B T}}{(4\pi\lambda k_B T)^{\ddagger}}, \qquad \Delta G^{\ddagger} = (\Delta G^0 + \lambda)^2 / 4\lambda \tag{2.1}$$

in the case of a nonadiabatic reaction (i.e., weak electronic coupling of D and A), omitting any work terms in the interests of brevity. The latter are absent in the intramolecular case. The free energy barrier $\Delta G^{\ddagger}$ in (2.1) depends on the reorganization parameter λ depicted in Fig. 1, and on the "standard" free energy of reaction ΔG^0 at the given r, also depicted there. H_{DA} is the magnitude of the electronic interaction between the donor D and the acceptor A. For application to electron transfer reactions at electrodes, an electrochemical analog occurs instead of ΔG^0. λ is the sum of vibrational (λ_i) and environmental or solvational (λ_0) contributions. If the equilibrium bond lengths in the redox states before and after reaction (e.g., using EXAFS in solution or X-ray diffraction in crystals) and if the relevant normal mode frequencies are known (from vibrational spectra) λ_i can be estimated including, if necessary, any other geometrical changes. For the past thirty-odd years λ_0 has been estimated from dielectric continuum arguments, but now is just beginning to be calculated from numerical (molecular dynamics) simulations of which there are one or two realistic examples (5). Quantum effects on the nuclear motion can also occur, particularly when the reduced vibrational frequency $\hbar\omega/2k_BT$ becomes significant, and then eq. (2.1) is replaced by an appropriate quantum expression (cf 3 for refs.), e.g., when $-\Delta G^0 \simeq \lambda$ and $\hbar\omega/2k_BT \gg 1$, the $\Delta G^{\ddagger}/k_BT$ in (2.1) is removed and the $4\pi\lambda k_BT$ is replaced by $2\pi|\Delta G^0|\hbar\omega$, for a one-vibrational frequency model.

When the reactants (or reactant in the electrochemical or intramolecular case) have a distribution of r's, the $k(r)$ given by (2.1) is weighted by this distribution, in order to obtain the observed rate constant k_r:

$$k_r = \langle k(r) \rangle_r \; . \tag{2.2}$$

Some of the applications of eqs. (2.1)-(2.2) which have found widespread use in the literature – the calculation of certain ratios of rate constants k_r or of the effect of various variables on k_r – are considered next. Later we explore whether such applications can also be made for electron transfers in the photosynthetic reaction center.

The theoretically derived relationships which have been tested extensively in the case of simple electron transfer systems include (3)

1. The effect of ΔG^0 on $\Delta G^{\ddagger}$, and the analogous electrochemical effect of the metal-solution potential difference on k. The effect of ΔG^0 on $\Delta G^{\ddagger}$ also includes the "inverted" effect, predicted from eq. (2.1) in 1960 and confirmed experimentally in 1985 at Argonne and Chicago, and at other laboratories since.
2. The "cross-relation" which relates the rate constant k_{12} for an electron transfer reaction between two different redox species $A_{ox} + B_{red}$, to the rate constant k_{11} for the self-exchange reaction (isotropic exchange) $A_{ox} + A_{red}$, and to that of B, k_{22}, and the equilibrium constant K_{12}

$$k_{12} \simeq (k_{11}k_{22}K_{12}f_{12})^{\frac{1}{2}} \quad , \tag{2.3}$$

where f_{12} is a known function of k_{11}, k_{22} and K_{12}. This relation has also been applied to protein-protein electron transfers (3).

3. The relationship between rate constants k_{11} of electron transfer in self-exchange reactions and the rate constants k_{el} for the corresponding electron transfers across electrode-solution interfaces, $(k_{11}/Z_{11})^{\frac{1}{2}} \gtrsim k_{el}/Z_{el}$, where the Z's are the appropriate collisional numbers.

A comparison of these and other predictions with experiment has been given in a review (3) in 1985.

In summary, the principal experimental tests of eqs. (2.1)-(2.2), of which the above are some examples, have been in comparing predicted ratios of rate constants, so as to avoid the uncertainties present in calculating only the *absolute* values of the k_r's, as interesting as the results for such calculations are. Examples of absolute k_r calculations are given in ref. (3).

There have been other developments recently, particularly in the field of solvent dynamics, stimulated in part by the introduction of very fast (picosecond, femtosecond) experimental techniques for studying electron transfers, techniques which have now recently been applied to photosynthetic systems. In one approximation (Debye "solvent"; single exponential regime; λ_i/λ_o small) the observed rate k_{obs} is given by

$$\frac{1}{k_{obs}} = \frac{1}{k_{relax}} + \frac{1}{k_r} \tag{2.4}$$

where k_{obs} is the experimental rate constant, k_r is the activation controlled rate constant given by eqs. (2.1)-(2.2), and k_{relax} is a quantity which depends on the solvent relaxation time τ_L, $\Delta G^{\ddagger}$ and λ_i/λ_o (6). When $\Delta G^{\ddagger}$ and λ_i/λ_o tend to zero and $\tau_L k_r \gg 1$, $1/k_{obs}$ tends to τ_L (eq. (9.2) for τ_b in ref. 6b).

3. PHOTOSYNTHETIC SYSTEMS

a. Early Steps

We have noted in the previous section that tests, correlations, or predictions of relationships based on eqs. (2.1)-(2.2) have required a knowledge of thermodynamic properties, such as the ΔG^0 in eq. (2.1), an electrode potential (in excess of the standard potential) in the electrode-liquid case, or K_{12} in eq. (2.3). We consider next how much of the relationship-type of approach, particularly the ratios of various k_r's, can be carried over in the photosynthetic case.

Some of the reactions observed in the in bacterial reaction center include (7-9) (3.1)-(3.9), writing (3.1)-(3.2) for the moment as two separate steps,

$$BChl_2^* \; BChl \; BPh \xrightarrow{k_1} BChl_2^+ \; BChl^- \; BPh \tag{3.1}$$

$$BChl_2^+ \; BChl^- \; BPh \xrightarrow{k_2} BChl_2^+ \; BChl \; BPh^- \tag{3.2}$$

$$BChl_2^+ BChl \; BPh^- \longrightarrow BChl_2^* \; BChl \; BPh \tag{3.3}$$

$$BChl_2^+ BChl\, BPh^- \longleftrightarrow (BChl_2^+ BChl\, BPh^-)^T \quad (3.4)$$

$$(BChl_2^+ BChl\, BPh^-)^T \xrightarrow{k_T} BChl_2^T BChl\, BPh \quad (3.5)$$

$$BChl_2^+ BChl\, BPh^- \xrightarrow{k_S} BChl_2\, BChl\, BPh \quad (3.6)$$

$$BChl_2^+ BChl\, BPh^- Q_A \xrightarrow{k_3} BChl_2^+ BChl\, BPh\, Q_A^- \quad (3.7)$$

$$BChl_2^+ BChl\, BPh\, Q_A^- \xrightarrow{k_4} BChl_2\, BChl\, BPh\, Q_A \quad (3.8)$$

$$BChl_2^+ BChl\, BPh\, Q_A^- \longrightarrow BChl_2^*\, BChl\, BPh\, Q_A \quad . \quad (3.9)$$

The superscript T in (3.4)-(3.5) denotes a triplet state. Reaction (3.4) is treated in a coherent manner (hyperfine-induced) rather than with the usual chemical kinetics rate constant formalism. Reactions (3.3) - (3.6) have also been investigated in quinone-free reaction centers, to remove the complications of extra interactions of the Q^- spin with those of the other radicals. We focus later on whether (3.1)-(3.2) involves one or two steps.

b. Remarks on ΔG^0's and on k's

Most of the k_r's of the above reactions have been measured, in some cases spectroscopically. Rate constants such as k_T in (3.5) and k_S in (3.6) have been inferred from a magnetic field analysis mentioned below.

Several of the ΔG^0's are known, but that of (3.1) is not. Studies of magnetic field-dependent recombination rates, together with magnetic field-dependent $BChl_2^T$ lifetimes, have led to the determination of ΔG^0 for reaction (3.5), ~ -0.165 eV for *Rb. sphaeroides* (10). From it and from the energy difference (0.4 eV) of $BChl_2^*$ and $BChl_2^T$, the sum of the ΔG^0's of reactions (3.1) and (3.2) has been determined as ~ -0.26 eV at room temperature (10, 11). The ΔG^0 for reaction (3.8) has been estimated from delayed fluorescence and also from *in situ* titrations to be about 0.5 eV (12). The ΔG^0 for (3.1) - (3.2) inferred from delayed fluorescence, perhaps for forming an unrelaxed state of $BChl_2^+$ BChl BPh^-, is ~ -0.15 eV (12a).

The study of the effect of magnetic fields on reactions in the $BChl_2^+$ BChl BPh^- system also led to a measurement of ΔE_{ST}, the singlet-triplet energy difference of this radical pair, which is also the energy of reaction (3.4) (e.g., 13). (ΔE_{ST} is usually written in the literature as J or 2J.)

The principal unknown ΔG^0's are the individual ΔG^0's of (3.1)-(3.2), only their sum being known. In comparison with the study of electron transfers of smaller systems (section 2) not only is the ΔG^0 for (3.1) not known but also, apart from a study of the Q_A reactions such as (3.8) with systematic replacement of Q_A, no systematic investigation of the effect of the ΔG^0's on the k_r of each reaction step has been possible. The only systematic type of study of varying ΔG^0 on a k_r has been through the effect of external electric fields (e.g., 14,15). The interpretation of those effects (the coupling to the field is via the

dipole change accompanying reaction) is not simple. The modification of the external field by the system itself ("the internal field") is imperfectly known.

It has not been possible, of course, to determine any self-exchange k's in the reaction center and so pursue tests such as that of the cross-relation, eq. (2.3). Effects of temperature on some of the k's and on ΔE_{ST} have been determined and provide useful information. Reactions such as (3.1)-(3.2), (3.5), (3.7) and (3.8) are "activationless", and so their rates are, interestingly enough, "maximized" in nature, for the given separation distances.

Other relevant studies of the reaction center include spectroscopic determinations of the absorption spectra of the $BChl_2$, BChl and BPh, the effect of electric fields on the spectra, the fluorescence, the effect of the electrical field on the fluorescence yield (14), and an electric field-induced fluorescence anisotropy (16). We return to the latter two phenomena later.

c. Recent History

The interpretation of data relevant to reactions (3.1)-(3.2) is currently in a state of flux. Experimental data (2) presented at this Congress on the initial step in the *Rb. sphaeroides* bacterial photosynthetic reaction center at room temperature suggests two steps, (3.1)-(3.2), instead of the recently accepted one-step model (cf 17). Because of what *may* be a rapidly changing picture it is useful to summarize first the recent history and then the current results, the deductions from them, and questions which remain.

The dramatic contrast between the extremely rapid (3 ps at room temperature) formation of $BChl_2^+$ BChl BPh^- in the initial charge separation and the extremely small (10^{-3} cm^{-1}) singlet-triplet splitting ΔE_{ST} of the radical pair, $BChl_2^+$ BChl BPh^-, and the relatively small value of k_T ($\sim 5 \times 10^8$ s^{-1}) has been known for some time. To explain this difference we postulated that the initial charge separation occurred in two separate steps, reactions (3.1)-(3.2), but that the ΔE_{ST} and k_T involved a different mechanism, superexchange (18). The superexchange mechanism for reaction (3.1)-(3.2) was burdened, I felt, with various additional assumptions and adjustable parameters, all of which were not, to be sure, excluded. The two mechanisms for reaction (3.1)-(3.2), sequential and superexchange, differ in the value assumed for the unknown ΔG^0 for reaction (3.1). In the two-step mechanism the ΔG^0 for (3.1) is negative, while in a superexchange mechanism, it is substantially positive.

Subsequent experimental work not only appeared to rule out any detectable $BChl^-$ (17), but also placed such severe restrictions on the k_2 in reaction (3.2) as to make it too high to be consistent with a two-step mechanism (17). That left superexchange, with (in this author's opinion) its drawbacks of added parameters. An alternative mechanism, termed nonadiabatic/ adiabatic, was then considered (19), coherent like superexchange though with a positive ΔG^0 for (3.1). Calculations with R. Almeida (to be published) yielded too large a depletion of the BChl to be consistent with results in ref. (17a), where the depletion was reported as less than 2% at low temperatures. At room temperature the depletion in ref. (17b) was reported as less than 4%.

Other data besides the measurement of the rate constant of the primary process also appeared either to rule out the sequential mechanism, notably the electric field induced fluorescence anisotropy measurements (16), or to place such limits on the ΔG^0 for reaction (3.1) (inferred from the lack of temperature

dependence of ΔE_{ST}) that that ΔG^0 would be too small to permit a sequential mechanism (3.1)-(3.2) with reasonable λ's (20). Further, the calculated effect of an applied electric field on the k_r for (3.1) appeared to be too large for the sequential mechanism (3.1)-(3.2) to be tenable (21).

In summary, on one hand the sequential model seemed to be in conflict with the data, while the superexchange model contained so many adjustable parameters, such as an assumed cancellation as an explanation of the small ΔE_{ST}, that in this writer's opinion neither was particularly attractive.

During the past few weeks, however, several pieces of the evidence may have been modified. These modifications involve (*i*) the extent of transient $BChl^-$ formation at room temperature, (*ii*) the lower limit on $-\Delta G^0$ for reaction (3.1) inferred from $\Delta E_{ST}(T)$, and (*iii*) a recently suggested modified interpretation of the electric field-induced fluorescence anisotropy. In the next section we summarize the current situation and the questions.

d. Current Status

According to the new experimental results of Zinth and coworkers (2), the electron transfer from $BChl_2^*$ to BPh in *Rb. sphaeroides* at room temperature occurs sequentially via reactions (3.1)-(3.2) with $k_2 \simeq 4k_1$ and with values of $1/k_1$ of 3.5 ps and $1/k_2$ of 0.9 ps. In marked contrast, it is recalled, the $1/k_T$ for reaction (3.5) is about 2 ns, and the magnitude of the energy of reaction of (3.4), ΔE_{ST}, is also very small, $\sim 10^{-3}$ cm^{-1} (e.g., 13). This contrast of data involving the formation, recombination or interaction of the radical pair provided evidence for the suggestion that the formation of $BChl_2^+ BPh^-$ occurs sequentially, as in reactions (3.1)-(3.2), but that, because of the position of energy level of $BChl_2^+ BChl^- BPh$ (relative to that of $BChl_2^+ BChl BPh^-$ and of $BChl_2$ BChl BPh), the k_T for reaction (3.5) and the ΔE_{ST} of reaction (3.4) occur via a superexchange (18). Being a second order phenomenon, the latter would yield a relatively small k_T and ΔE_{ST}. We review next the quantitative aspects of this picture, to see whether the numerical values of k_1, k_T and ΔE_{ST} are internally consistent with it.

For the k_T in (3.5) and the ΔE_{ST} in (3.4), we suppose that any involvement of $(BChl_2^+ BChl^- BPh)^T$ in those two reactions is as a virtual state (superexchange), because of the relatively high energy of that state at the equilibrium geometry of the radical pair. Equation (2.1) can be used for k_T with $\lambda \simeq -\Delta G^0$, since k_T depends very little on temperature. The observed matrix element H_{DA}, denoted here by $\bar{H}_{13}^{rp,T}$, is found using (2.1), the known k_T and the known ΔG^0 to be ~ 1 cm^{-1} (22). An independent calculation of $\bar{H}_{13}^{rp,T}$ from the value of ΔE_{ST}, can also be made, making an approximation that the matrix element $\bar{H}_{13}^{rp,S}$ for interaction of $BChl_2^{*S}$ and BPh at the equilibrium geometry of the radical pair is approximately equal to $\bar{H}_{13}^{rp,T}$ for the same radical pair geometry. Denoting their common value by $\bar{H}_{13}^{rp}$, ΔE_{ST} is then given by (22)

$$\Delta E_{ST} \simeq |\bar{H}_{13}^{rp}|^2 \frac{H_{11}^S - H_{11}^T}{(H_{11}^T - H_{33})(H_{11}^S - H_{33})} , \qquad (3.10)$$

upon applying the partitioning method to obtain ΔE_{ST} (18a). In eq. (3.10) $H_{11}^S - H_{33}$ and $H_{11}^T - H_{33}$ are the "vertical" energies of reaction of (3.3) and (3.5), respectfully, i.e., calculated at the equilibrium geometry (vibrational and

protein coordinates) of the radical pair. Introducing approximate estimates for these energy differences, it was found from ΔE_{ST} and eq. (3.10) that $\bar{H}_{13}^{rp} \sim 1$ cm^{-1} (22), roughly the same as the $\bar{H}_{13}^{rp,T} \sim 1\ cm^{-1}$ just estimated from k_T. Thus, within the approximation $\bar{H}_{13}^{rp,T} \sim \bar{H}_{13}^{rp,S} \equiv \bar{H}_{13}^{rp}$ the results for k_T and ΔE_{ST} are seen to be internally consistent. Moreover, apart from changes in the energy differences in (3.10) from system to system it is seen from eq. (2.1), at $-\Delta G^0 \sim \lambda$, and from (3.10), that k_T and ΔE_{ST} should change in parallel from system to system (both are proportional to $|\bar{H}_{13}^{rp}|^2$). Their ratio would not be exactly constant, because of some variation in the energy differences in (3.10). Evidence for this parallel change has recently been offered (13a, b).

We next compare the matrix element H_{12}^{S} for reaction (3.1) with the value which, with some approximation, can be deduced from the 1 cm^{-1} value for $\bar{H}_{13}^{rp}$ above. The electronic interaction between $BChl_2$ and BPh via the BChl, obtained with a "partitioning" method, is (18,22)

$$\bar{H}_{13}^{rp} = \frac{H_{12}H_{23}}{H_{22}-H_{33}} \quad , \tag{3.11}$$

where $H_{22}-H_{33}$ is the "vertical" energy of reaction (3.1), evaluated at the equilibrium geometry of the radical pair. The simplest interpretation of the result, $k_2 \simeq 4k_1$, of Zinth and coworkers is that $H_{23} \sim 2H_{12}$ (18). Using an estimate of the equilibrium energy difference in $H_{22}-H_{33}$ equal to $-\Delta G^0 \sim -1400\ cm^{-1}$ and using $\lambda \sim -\Delta G^0$, this vertical difference in $H_{22}-H_{33}$ is about 2800 cm^{-1}. Then, the H_{12} estimated from (3.11) is about 35 cm^{-1}, which is within a factor of two of the value 25 cm^{-1} estimated independently from the k_1, rate constant of reaction (3.1) (22).

In summary a sequential mechanism for (3.1)-(3.2) permits these pieces of data – the markedly large value of k_1 (if one assumes for the moment the correctness of Zinth and coworkers) and the small values of k_T and ΔE_{ST} – to be quite internally consistent. We consider next several remaining facts which militated against a sequential mechanism (3.1)-(3.2):

1. In an important work by Lockhart *et al.* (16), an electric field was applied and caused a field-induced anisotropy of the fluorescence in a sample of randomly oriented reaction centers. The angle between the transition dipole for the absorption $BChl_2 \rightarrow BChl_2^*$ and the direction of most enhanced fluorescence provided a measure of the direction of the dipole of the rate controlling step. The angle found experimentally appeared to eliminate the sequential mechanism, since it matched the direct transfer angle for BPh^- rather than that for reaction (3.1) (16). More recently, however, this analysis was extended by replacing a point dipole approximation by the actual charge distribution in the pigments, taking into account the marked asymmetry in the charge distribution of $BChl_2^+$ (23, Michel-Beyerle, M. E., Plato, M., and Ogrodnik, A., private communication). The result is that the observed angle for the anisotropy is consistent with the sequential mechanism (23).
2. An upper limit of 150 cm^{-1} for $-\Delta G^0$ for reaction (3.1) was inferred from the lack of dependence of ΔE_{ST} on temperature (20) in RYDMR data (24). The limit is so small that the high k_1 required (with eq. (2.1)) an abnormally small λ. Just recently, however, this picture may have altered: Measurements of k_T, ΔE_{ST} and the dependence, or lack of it, of

ΔE_{ST} on T, now all within the same laboratory, have now been interpreted as setting a much higher upper limit, 600 cm^{-1} or perhaps even 800 cm^{-1}, for $-\Delta G^0$, so permitting a more reasonable λ for (3.1) (13b).

3. The small $-\Delta G^0$ for (3.1) (#2) also led to too high a predicted effect of an applied electric field on the fluorescence yield, which competes with (3.1) (21). The modified analysis of the electric field induced anisotropy noted in #2 should be applied to this effect of the electric field also.

An important question concerns why the preferred electron transfer path is along the *L*-branch rather than the *M*-one in the protein. In terms of eq. (2.1) there are three possibilities, and the answer at this time is not known: the H_{DA}, ΔG^0 and/or λ for step (3.1) may be more favorable for the *L*-branch. For example, Professor Michel in his lecture mentioned that the *L*-side was more "rigid" – it had more aromatic residues. A smaller response of the protein by reorientation of dipolar groups or by volume changes to a change in the charge distribution accompanying reaction (3.1) leads to a smaller λ.

It will be interesting to see further the results of site-directed mutagenesis for changing the various parts of the protein and alter the rate constants and other properties. Regardless, however, whether the origin of the path preference *L* vs. *M* is identified, the correct mechanism should be one for which the remaining data are internally consistent. At present, this consistency appears to prevail for the sequential mechanism (3.1)-(3.2), as regards k_T and ΔE_{ST} and, if Zinth is correct for *Rb. sphaeroides* at room temperature, k_1. The field-induced fluorescence anistropy may also be consistent with this mechanism (23). The corresponding calculation on the effect of the electric field on the fluorescence quantum yield remains to be performed.

A number of other aspects remain to be interpreted in some detail, e.g., the k_S of reaction (3.6), both its numerical value and its temperature dependence, reconciling both with the large $-\Delta G^0$ (~1.19 eV for the recombination in *Rb. sphaeroides*). Information obtained about another reaction the recombination of Q_A^- and $BChl_2^+$, reaction (3.8), including its lack of temperature dependence, has provided information on the vibration frequencies (they can't be too small) and on the λ for that step (~0.5 to 0.8 eV) (25). This λ is substantially larger than those for reactions (3.1)-(3.2): The latter should have a $\lambda \simeq -\Delta G^0$ for any reaction step since the k's are so high, and the sum of the two $-\Delta G^0$'s for the (3.1)-(3.2) is only 0.26 eV. Gradually, then, some of the λ's or limits on them are also becoming known.

It is clear that a more detailed and consistent understanding of the elementary steps in the bacterial photosynthetic reaction center may have begun to emerge. However, further experiments, particularly from other laboratories and also of the Zinth type at low temperatures, are desirable.

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