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Application of Electron-Transfer Theory to Several Systems of Biological Interest

R.A. Marcus
Arthur Amos Noyes Laboratory of Chemical Physics*,
California Institute of Technology, Pasadena, CA 91125, USA

N. Sutin
Department of Chemistry, Brookhaven National Laboratory,
Upton, NY 11973, USA

Abstract. Electron-transfer reaction rates are compared with theoretically calculated values for several reactions in the bacterial photosynthetic reaction center. A second aspect of the theory, the cross-relation, is illustrated using protein-protein electron-transfers.

I. Introduction

Studies of electron-transfer reactions for a variety of inorganic and organic reactions have established various factors as influencing the reaction rates. In particular, quantities such as the standard free-energy of reaction, ΔG°, the reorganization parameters for the vibrational (λ_i), and solvational (λ_o) changes, the separation distance r, and the work terms (e.g., coulombic interactions), affect the reaction rate. The experimental evidence and comparisons with theoretical relationships and calculations were presented in a recent review [1]. In addition, other factors, such as the mutual orientation of the reactants, when the latter are nonspherical, the nature of the material between the reactants, and the electronic energy levels of the donor and acceptor, are expected to influence the rate in the case of "nonadiabatic" [1] reactions (defined later). Various theoretical calculations on some or all of these other factors (many referred to in [1]) have been made, though the experimental data on them are, for the most part, presently scanty. Another factor influencing the electron-transfer rate is present in sufficiently viscous media, more precisely, in media with slow dielectric relaxation times. It has been found for reactions which are intrinsically very fast and for which, at the same time, the ratio λ_i/λ_o is sufficiently small [2]. (References to the data are cited in [1]. A detailed treatment, which includes the dependence on λ_i/λ_o is given in [2].)

The article mentioned earlier [1] included a review of various electron-transfer reactions of biological interest. In the present communication we shall describe some of the results for the rates of reactions in the photosynthetic bacterial reaction center, and for protein-protein electron-transfers. In particular, a comparison is made of predicted and experimental rate-constants. We consider, first, an example of the application of the theoretical cross-relation [3] to reactions of metalloproteins, in order to illustrate an aspect of the theory which has been frequently tested with purely inorganic or oryanic systems.

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II. Application of the Cross-Relation to Protein-Protein Electron-Transfers

In the cross-relation [3] the rate-constant k_{12} for the reaction between two different redox species,

$$red_1 + ox_2 \xrightarrow{k_{12}} ox_1 + red_2 \qquad (1)$$

is related to two self-exchange rate-constants k_{11} and k_{22}:

$$red_1 + ox_1 \xrightarrow{k_{11}} ox_1 + red_1 \qquad (2)$$

$$red_2 + ox_2 \xrightarrow{k_{22}} ox_2 + red_2 \quad . \qquad (3)$$

The relation is

$$k_{12} = (k_{11}k_{22}K_{12}f_{12})^{1/2} \qquad (4)$$

where K_{12} is the equilibrium-constant for reaction (1), f_{12} is a known function of k_{11}, k_{22} and K_{12} given, for example, in [1] (frequently close to unity), and the k's appearing in (4) are corrected for the various 'work' terms, by a factor labelled W_{12} in [1]. In the case of metalloproteins, the self-exchange rate-constants k_{11} and k_{22} are typically determined by NMR or EPR line-broadening studies. The cross-relation, (4), is compared in Table I with data in the literature, taken from [1]. (Extensive references to the literature for such studies and tests of the cross-relation are also given in [1].)

Considering the various approximations made in this application of (4), and various uncertainties in the data themselves, discussed in [1], the agreement in Table I can be regarded as quite reasonable. In making such

TABLE I. Comparison of Observed and Calculated Rate-Constants for Protein-Protein Reactions

Oxidant	Reductant	k_{12},obsd [M^{-1} s^{-1}]	k_{12},calcd[a] [M^{-1} s^{-1}]
Cytochrome c	Azurin	1.6×10^3	3×10^4
Plastocyanin	Cytochrome c	1.0×10^6	5×10^5[b]
Stellacyanin	Cytochrome c	3.5×10^2	4×10^3
Azurin	Cytochrome c-551	6.0×10^6	8×10^5[c]
Plastocyanin	Cytochrome c-551	7.5×10^5	5×10^5[c]
Cytochrome c	Cytochrome c-551	6.7×10^4	1×10^5[c]

[a] Unless otherwise indicated, the calculated rate-constants do not contain the W_{12} correction. References for the results are given in [1].

[b] The W_{12} correction is considerably larger for this reaction than for the other systems considered, and was included in the calculation of k_{12}.

[c] Calculated using 1.2×10^7 M^{-1} s^{-1} for the self-exchange rate-constant and +0.28 V for the reduction potential of the cytochrome c-551 couple using data cited in [1], where it is also noted that the net charge on the protein is -2/-3.

an application, the rates of both the self-exchanges and the cross-reactions should be determined under pH conditions, where both redox forms of each protein have the same number of protons. (This may not have been the case for all of the reactions in Table I, and, if so, the experimental rate-constants in those cases should be corrected accordingly.)

As a preamble to the consideration of absolute rate constants for reactions between reactants fixed in a membrane, we consider first some available data on the dependence of the electron-transfer rate on the separation distance between the reactants.

III. Distance-Dependence of the Rate-Constant

A knowledge of the dependence of the rate-constant on the separation distance between fixed redox sites is needed for understanding their electron-transfer rate-constants. Information on this distance-dependence comes from several sources: (i) the unusual time-dependence of reaction rates when there is a distribution of separation distances of the reactants in frozen or other solid or highly viscous media, e.g., [4,5], (ii) the conductivity of fatty acid salt layers of varying chain length between two electrodes, e.g. [6,7], (iii) intramolecular electron-transfer rates in model compounds containing a pair of fixed sites separated by more or less rigid organic bridges, e.g., [8-11], and (iv) intramolecular transfers between the metal center of a metalloprotein and an inorganic redox complex attached to various sites in the protein [12,13]. Of these sources, there are fairly extensive published data on (i) and (ii), while data are beginning to appear on (iii) and (iv). The published data for the latter two are too few to estimate a distance-dependence, although this situation will undoubtedly be remedied soon.

Experimental data for the rate-constant or, in the case of method (ii), for the conductivity are usually represented by an exponential dependence on the separation-distance r, e.g., [4-7],

$$k(r) = \exp[-\beta(r-r_0)] \qquad (5)$$

TABLE II. Some Tentative Values of β in the Literature

System	β [$\AA^{-1}$]	Comment
biphenyl-aromatics[a]	1.2	frozen media
biphenyl$^-$-naphthalene	1.2	frozen media
biphenyl$^-$-phenylethylene	1.0	frozen media
biphenyl$^-$-acridine	1.1	frozen media
pyrene$^+$-TMPD[b]	1.15	frozen media
biphenyl$^+$-TMPD[b]	1.15	frozen media
biphenyl$^-$-cinnamoyl grp.	(< 1.0)	steroid bridged
Al/C_n/Hg, Al or Au	1.5	conduction
Al/C_n/Mg	1.0	conduction

[a] Many aromatic acceptors were used, and gave an average value of $\beta = 1.2\ \AA^{-1}$.

[b] TMPD denotes tetramethylphenylenediamine.

Values of β are given in Table II. A typical value is seen to be in the range 1.0 to 1.2 $Å^{-1}$, and we shall use a value of 1.2 $Å^{-1}$ when, as in Table I, the intervening material between the two redox sites is "saturated", i.e., nonconjugated. When a portion of the intervening material is conjugated (e.g., aromatic) the value of β for that part of the separation-distance is expected to be lower.

A functional form for k(r), which takes into account the various nodal properties of the electronic wave functions, as well as the three-dimensional geometry, is expected to be somewhat more complicated than that given by (5) and to include, for example, a pre-exponential term which depends on r, e.g., [14]. However, (5) has been frequently used in the literature, since the presently available data do not yet document the need to use a more complicated expression.

IV. Expressions for the Rate Constant

In applying (5), or an equation based on it, to the experimental data for reactions between fixed sites, some cognizance should be taken of the nonspherical nature of the reactants. A distance of particular interest is that between the closest atoms (of the two reactants) that are strongly coupled to their respective redox sites. This distance, denoted by d, is used in the following approximate expression for the reaction-rate constant k, an expression discussed more fully in [1]:

$$k_{cl} = 10^{13}\, e^{-\beta d}\, e^{-\Delta G^*/RT}\ s^{-1} \quad . \tag{6}$$

When the motion of the nuclei is treated classically ($k = k_{cl}$), we have [1]:

$$\Delta G^* = \frac{\lambda}{4}\left[1 + \frac{\Delta G^\circ}{\lambda}\right]^2 \tag{7}$$

where ΔG^* and ΔG° are the free energy barrier and the "standard" free energy of reaction when the reactants are a distance r apart. Strictly speaking, the pre-exponential factor in (6) depends weakly on the temperature, being inversely proportional to $\sqrt{T}$ in the case of a "nonadiabatic" reaction (a reaction for which $\exp(-\beta d) \ll 1$), e.g., discussed in [1].

When the motion of the nuclei is treated, instead, quantum mechanically, the following expression for the ratio of the quantum to the classical rate-constants can be used [1]. It is based on now standard expressions in the literature for k_q and k_{cl} that are cited, for example, in [1]:

$$\frac{k_q}{k_{cl}} = \frac{\sqrt{4\pi\lambda RT}}{h\nu}\,\frac{e^{vy-S\coth y}\, I_v(S\,\mathrm{cosech}\, y)}{e^{-\Delta G^*/RT}} \quad , \tag{8}$$

where ν is some mean vibration frequency for the relevant nuclear motion (a one-reactive-mode model has been used for the quantum rate-constant in (8)), v is $-\Delta G^\circ/h\nu$, S is $\lambda/h\nu$, y is $h\nu/2RT$, and I_v is a modified Bessel function. The ratio k_q/k_{cl} reduces to unity when $h\nu/2RT$ becomes small, and is of the order of unity even when $h\nu/2RT = 1$. For example, when $-\Delta G^\circ/h\nu$ and $\lambda/h\nu$ are, respectively, (1,2), (3,6), (5,10) and (10,20), the values k_q/k_{cl} are 1.1, 1.1, 1.2 and 1.5 in the case of $y = 1$. For $\Delta G^\circ = 0$ and the above values of $\lambda/h\nu$, the corresponding values of

k_Q/k_{Cl} are 1.3, 1.4, 1.6 and 2.3, respectively. Finally, when $-\Delta G^\circ = \lambda$, the values of k_Q/k_{Cl} are all equal to 0.87 for the above values of $\lambda/h\nu$. (It can be shown that, when $\nu = S$ and provided that ν is not too small, the ratio k_Q/k_{Cl} is equal to $\sqrt{(\tanh y)/y}$ which is equal to 0.87 for $y = 1$.)

It is characteristic of both the classical and the quantum expressions for the rate-constant that a maximum appears in k_{Cl} and k_Q as a function of increasing $-\Delta G^\circ$, when $-\Delta G^\circ = \lambda$ [1]. Further, the activation energy for the classical expression and, in effect, for the quantum expression (given by (72) of [1]) is zero when $-\Delta G^\circ = \lambda$. We use this result later.

The distance d appearing in (6) was taken in [1] to be the actual separation distance of the centers of the two closest (in the case of aromatics) carbon atoms of the two reactants, minus some amount to allow for the extension of the π-electronic orbitals beyond the carbon nuclei. For concreteness a value of d = 3 Å was used. Thereby, when the frequency of motion along the reaction coordinate is about 10^{13} s^{-1} the reaction is "adiabatic" when these aromatic carbons are a distance 3 Å or less apart.

V. Reaction Rates in the Bacterial Photosynthetic Reaction Center

The reaction step involving the electron-transfer from an electronically-excited bacteriochlorophyll dimer $*(BChl)_2$ to a neighboring monomer BChl and pheophytin BPh has a lifetime of about 5 μs [15] at room temperature, while the transfer from BPh^- to the quinone has a lifetime of about 200 μs (about 100 μs below 100 K) [15]. Both reactions are essentially temperature-independent over this broad temperature-range, and we shall suppose, therefore, that $-\Delta G^\circ = \lambda$ for each. The values for d for the $*(BChl)_2$ + BChl and BPh^- + Q reactions are about 1 Å and 7 Å, respectively, obtained after subtraction of the 3 Å, in each case, from distances estimated from the crystal structure data (of Rps. viridis) [16]. Equation (6) then yields the lifetimes given in Table III.

The value for ΔG° appears to be quite small for the $*(BChl)_2$ + BChl and $BChl^-$ + BPh reactions, as is the λ for each reaction of these large relatively rigid molecules (data are summarized in [1]), perhaps 0.1-0.2 eV. The value of $-\Delta G^\circ$ for the BPh^- + Q reaction, on the other hand, is quite large (~ 0.61 eV for Rps. sphaeroides and ~ 0.47 eV for Rps. viridis), λ for a reaction involving Q is expected to be larger than λ's for reactions involving only the large molecules, partly because of the change in C-O bond length accompanying the $Q \rightarrow Q^-$ reaction and partly because of the smaller size of the Q molecule. (Cf Ref. [1] for factors influencing reaction rates, e.g., (2)-(6) there.) Calculated rates are given in Table III.

Variation of the Q caused ΔG° to vary by ~ 0.2 eV [17] and caused experimentally a variation in the lifetime for the BPh^- + Q reaction of a factor of two, with some randomness due to the somewhat different positions, shapes, or orientations of the different Q's. Again, when a second quinone was present (presumably as Q^-), creating a coulombic repulsion with the other Q^- newly formed in the BPh^- + Q reaction, the rate-constant for the latter reaction was smaller by almost a factor of two [18]. Theoretical calculations for this effect, taken from [1], are included in Table III.

The lifetime for the reduction of the $(BChl)_2^+$ by ferricytochrome c-558 is about 0.2 μs at room temperature [19]. The reduction of this $(BChl)_2^+$

TABLE III. Absolute Rates for Electron-Transfer Reactions in Photosynthetic Reaction Centers

Reaction[a]	$\Delta G°$ [b] [kcal mol^{-1}]	τ_{obsd}	τ_{calcd}
cyt c^{II} ⟿ $BChl_2^+$	-3.9	0.2 μs	0.02 μs
$^*BChl_2$ ⟿ BChl ⟿ BPh	-5(?)	~ 1 to 4 ps[c]	>0.3 ps[d]
BPh^- ⟿ Q	-12	100 to 200 μs[e]	140 μs
BPh^- ⟿ Q_A, Q_B^- present	~ 0.2 eV[f]	increases factor of 2	increases factor of 2
BPh^- ⟿ Q, Q varied	~ 0.2 eV[g]	changes factor of 2	changes factor of 2
*Porphyrin ⟿ Q	-λ	τ_{min} ~ 4 ps	τ_{min} ~ 9 ps

[a] Asterisks indicate electronically excited states.

[b] The $\Delta G°$ values are approximate.

[c] Assignment of time-constants is uncertain.

[d] The value of τ_{calcd} is larger than this value if smaller λ's are used, i.e., τ_{calcd} is about 1 ps when λ = 1.2 kcal mol^{-1}. (See also Appendix II of Ref. [1].)

[e] Value depends on temperature.

[f] This is the approximate decrease in $|\Delta G°|$ due to the presence of Q_B^-.

[g] This is the approximate variation in $|\Delta G°|$ as Q is varied.

is presumably by the closest ferrocytochrome c molecule. In the calculations in Table III, an estimated edge-to-edge separation distance of $(BChl)_2^+$ to the closest heme ring of about 11 Å was used, estimated from the crystal structure data [16], and hence d = 8 Å, using the 3 Å correction mentioned earlier. The value of $-\Delta G°$ is about 0.17 eV, and a value of λ of about 13.5 kcal mol^{-1} was estimated† for this reaction, as discussed in [1]. The measured and calculated lifetimes for the reaction are given in Table III.

There is also a study of model porphyrin-quinone systems [20], for which a plot of rate-constant vs. $-\Delta G°$ gave a maximum rate, and for which d could also be estimated. The details are described in [1] and the result is included in Table III.

The experimental and theoretical results in Table III are seen to be in quite reasonable agreement, considering the various approximations and uncertainties present. Calculations utilizing the quantum expression in (8), rather than just the classical one in (7), give values [1] not too

†This rough estimate of λ was made [1] using the λ for the cytochrome c self-exchange reaction, the barrierless result for the $^*(BChl)_2$ - BChl - BPh reactions (with their λ then estimated from λ = $-\Delta G°$ for those steps) and an additivity rule for λ's [3].

different from those in Table III, at least for the approximate value of $h\nu$ assumed (~ 200 cm^{-1}). Recent calculations employing a frequency of 333 cm^{-1} (instead of the 200 cm^{-1} used in [1]) again show that the ratio of quantum to classical rate-constants at 25 °C is close to unity when $\lambda \leq 6$ kcal mol^{-1} (and $\Delta G° = -3$ kcal mol^{-1}) for the second entry in Table III. The classical rate-constant at 25 °C is typically a good approximation to the quantum rate-constant at that temperature for the frequencies used. The temperature coefficient of the reaction can therefore be found by considering the ratio k_{cl} (25 °C)/k_q (0 K), calculated from (14) and (71), respectively, in [1]. In the barrierless case ($-\Delta G° = \lambda$) this ratio is found to be $\sqrt{h\nu/2RT}$ when Stirling's approximation is introduced into (71) (T is the temperature of the classical measurement, 25°C in this case). For barrierless reactions the ratio k_{cl} (25 °C)/k_q (0 K) is typically close to unity for the vibrational frequencies used. The above considerations also show, we believe, the usefulness of the studies of the reaction rate-constant as a function of $-\Delta G°$. The latter permits one to locate the maximum in the plot, namely where $\Delta G° = -\lambda$, and thereby the $\Delta G°$ where the reaction is barrierless over a broad temperature-range. It thereby provides one way, for example, of estimating λ for the reaction, and provides also a direct measure of the factor 10^{13} exp($-\beta d$) in (7).

In the calculations in Table III we have adopted a value of β of about 1.2 $Å^{-1}$ as representing to a fair degree the data in Table II. A value of 1.0 $Å^{-1}$ would have altered the calculated results in Table III relatively little. There is some indication that β will depend on the orbital energy of the donor and of the acceptor, e.g. [21], and, in particular, that it may depend on whether or not a reactant is in an excited electronic state, e.g., [22]. There is some indication, for example, from data given in [1] (Table IV there), that β may indeed be less for photoexcited systems. However, in the case of the present Table III only one reaction involves a photoexcited molecule, and the value for its d is so small that calculated lifetime would be hardly affected by using a somewhat smaller value for β. Some discussion of the dependence of β on the orbital energies is given in (65)-(68) of [1].

A second aspect of β for which more experimental data is desirable concerns the role of the intervening material. Because of the delocalized nature assumed for an electronic orbital in an aromatic reactant in our computation of the distance d, it would be consistent not to include in the separation distance any length occupied by a conjugated molecule, at least as a first approximation.

In conclusion, we would like to note that the recent major breakthrough and focal point of the present conference - the determination of the crystal structure of Rps. viridis [16] - has provided an essential and previously missing ingredient required for the theoretical calculation of the reaction rates in the bacteriochlorophyll reaction center.

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