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A POSSIBLE NEW MECHANISM OF TEMPERATURE DEPENDENCE OF ELECTRON TRANSFER IN PHOTOSYNTHETIC SYSTEMS

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Summary

A new theory for the electron transfer by the non-adiabatic process is formulated taking into account the origin shift and the frequency change of the vibration. The resultant formulas are quite similar to those of Jortner (Jortner, J. (1976) J. Chem. Phys. 64, 4860—4867) except that the free energy gap ΔG is used instead of the energy gap ΔE. By applying this theory to the photosynthetic electron transfer, the role of the remarkable temperature dependence of the electron transfer from cytochrome to P^+ in *Chromatium vinosum* and the experimental data were reproduced very well using a small value of the coupling strength in contrast with the previous theory. This implies that proteins play a role to exclude many of the solvent molecules from the region of the electron transfer reaction between the donor and acceptor molecules. The negative activation process in the back electron transfer from Q_A^- to P^+, the very slow back electron transfer from I^- to P^+ and the solvent isotope effect on the cytochrome oxidation are also successfully explained by this new theory. It is shown that even a qualitative conclusion as to the molecular parameters obtained from the temperature dependence of the electron transfer is different between the present theory and that of Jortner.

Introduction

The low temperature experiment by DeVault and Chance [1] of the electron transfer from cytochrome in *Chromatium vinosum* suggested the quantum

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mechanical tunneling mechanism of electron transfer in biological systems. That is, the electron transfer rate decreased by lowering the temperature from 300 K to about 90 K, and it became constant below 90 K. After this pioneering work, many investigators measured the temperature dependence of the electron transfer rate in various photosynthetic primary processes. All of the results showed a constant electron transfer rate at low temperatures. Those data are summarized in recent reviews [2,3]. Hopfield [4] formulated the electron transfer rate semiclassically by the thermally activated electron tunneling mechanism. That is, following the concept of the excitation energy transfer by Förster [5] and Dexter [6], he evaluated the temperature dependence of the overlap integral between the electron removal spectrum of a donor molecule, D, and the electron insertion spectrum of an acceptor molecule, A, by taking account of the origin shift of the normal coordinate vibrations. Jortner [7] imporved Hopfield's theory by treating the vibration quantum-mechanically, and pointed out that the correction to Hopfield's theory is considerable at low temperatures. Although these theories apparently explained the experimental curve very well, they adopted some unknown parameters, some values of which (a large value of the coupling strength and relatively small value of the average frequency) seem to be unreasonable as pointed out by Blankenship and Parson [2]. Problems in the current theory when applied to the electron transfer in the various steps of the primary process of photosynthesis were also discussed in detail by Blankenship and Parson [2]; the review article by DeVault [3] covers a wide scope of electron and nuclear tunneling phenomena which are seen in many biological systems as well as chemical and physical systems, and it treats the tunneling mechanisms in a unified manner.

In this paper, we investigate the possibility that the remarkable temperature dependence of the photosynthetic electron transfer rate might emerge from the vibrational frequency change between the initial and final state. Thus, we formulate the electron transfer rate by the non-adiabatic process, taking account of both the origin shift and the frequency change of the vibration due to the electron transfer. It will be shown that the resultant formule are similar to those derived by Jortner [7] but that the free energy gap should be used instead of the usual energy gap. This theory is extensively applied to the electron transfers in the various steps of the primary process of photosynthetis. It will be shown that many of these experimental results could be satisfactorily reproduced by this theory using reasonable values of parameters.

Theory

We assume the Born-Oppenheimer approximation at the starting point. The initial vibronic state $\langle iu|$ denotes the electronic state of DA and the vibrational state u. The final state $\langle fv|$ denotes the electronic state of D^+A^- and the vibrational state v. These vibrations include intra- and intermolecular vibration modes of donor, acceptor, proteins, membrane and solvent.

Using the time dependent first-order perturbation theory, the electron transfer rate W is written as

$$W = Z^{-1} \sum_u \exp(-E_u/kT) \sum_v \frac{2\pi}{\hbar} |\langle iu|H'|fv\rangle|^2 \, \delta(E_u - E_v + \Delta E) \qquad (1)$$

where

$$E_u = \sum_j (n_j + \tfrac{1}{2})\, h\nu_j \tag{2}$$

$$E_v = \sum_j (m_j + \tfrac{1}{2})\, h(\nu_j - \Delta\nu_j) \tag{3}$$

$$Z = \sum_u \exp(-E_u/kT) \tag{4}$$

where ΔE is the energy gap between the electronic energies of the initial and final states. H' is the perturbation Hamiltonian. Its explicit form was given by Kestner et al. [8]. n_j and m_j are vibrational quantum numbers of mode j in the initial and final states, respectively. u and v are sets of vibrational quantum numbers $(n_1, n_2, \ldots, n_N)$ and $(m_1, m_2, \ldots, m_N)$, respectively. We neglect the mode mixing of the vibration between the initial and final states. The displacement of the origin of the normal coordinate Δ_j and the frequency change $\Delta\nu_j$ are taken into account.

By making the decoupling $<\mathrm{i}u|H'|\mathrm{f}v> = <\mathrm{i}|H'|\mathrm{f}><u|v>$, we can write W as

$$W = AZ^{-1} \sum_u \exp(-E_u/kT) \sum_v |\langle u|v\rangle|^2\, \delta(E_u - E_v + \Delta E) \tag{5}$$

where

$$A = \frac{2\pi}{\hbar} |\langle \mathrm{i}|H'|\mathrm{f}\rangle|^2 \,. \tag{6}$$

The summation over u and v in a case similar to Eqn. 5 was made by Freed and Jortner [9]. However, its result is too complex to allow insight on the temperature dependence of the transfer rate. Hence we will confine ourselves to the case in which the frequency change $\Delta\nu_j$ is small compared with ν_j, and derive formulae accurate to the first order in $\Delta\nu_j/\nu_j$. Under such a condition, Lin [10] already derived the formula of the transition rate in the case of the nonradiative transition. It is straightforward to modify his theory to our case. The electron transfer rate W can be written as

$$W = \bar{A} \exp\left(- \sum_j (\Delta^2/2) \coth(h\nu_j/2kT)\right) \cdot I_{|p|}(S \operatorname{csch}(h\langle\nu\rangle/2kT))$$

$$\times \exp(ph\langle\nu\rangle/2kT)\,, \tag{7}$$

where

$$S = \sum_j (\Delta_j^2/2)\,, \tag{8}$$

$$p = \left(\Delta E + \sum_j (h\,\Delta\nu_j/2) \coth(h\nu_j/2kT)\right)/h\langle\nu\rangle\,, \tag{9}$$

$$\bar{A} = 4\pi^2 |\langle \mathrm{i}|H'|\mathrm{f}\rangle|^2/h^2\langle\nu\rangle\,. \tag{10}$$

The definition of the averaged frequency $<\nu>$ is somewhat obscure. But, taking as our basis the theory of Lin, we tentatively give a formula as follows,

$$\sum_j (\Delta_j^2/2)\,\mathrm{csch}(h\nu_j/2\boldsymbol{k}T) = S\,\mathrm{csch}(h\langle\nu\rangle/2\boldsymbol{k}T)\,. \tag{11}$$

Thus defined, $<\nu>$ would in general be temperature dependent. $<\nu>$ is averaged as follows: at high temperatures

$$\frac{S}{\langle\nu\rangle} \cong \sum_j (\Delta_j^2/2)/\nu_j \tag{12}$$

and at low temperatures

$$S\exp(-h\langle\nu\rangle/\boldsymbol{k}T) \cong \sum_j (\Delta_j^2/2)\exp(-h\nu_j/\boldsymbol{k}T)\,. \tag{13}$$

In both cases, for equal values of $\Delta_j^2/2$, those terms for smaller values of ν_j dominate. Thus, the averaging procedure for $<\nu>$ is similar between high and low temperatures. Then, we can expect that the temperature dependence of $<\nu>$ will be small except for the special case that Δ_j^2 becomes large with a large value of ν_j. In this paper, we treat $<\nu>$ as constant for simplicity.

Eqn. 7 can be rewritten in a familiar form as follows:

$$W = \bar{A}\exp(-S(2\bar{v}+1)\cdot I_{|p|}(2S(\bar{v}(\bar{v}+1))^{1/2})\cdot((\bar{v}+1)/\bar{v})^{p/2}\,, \tag{14}$$

with

$$\bar{v} = (\exp(h\langle\nu\rangle/\boldsymbol{k}T) - 1)^{-1}\,. \tag{15}$$

In Eqn. 14, we have assumed $\sum_j(\Delta_j^2/2)\coth(h\nu_j/2\boldsymbol{k}T) \simeq S\coth(h<\nu>/2\boldsymbol{k}T)$, which holds true for $h\nu_j >> \boldsymbol{k}T$. Eqn. 9 can be rewritten as

$$p = \left(\Delta E + \sum_j (h\,\Delta\nu_j/2) + \sum_j \bar{n}_j h\,\Delta\nu_j\right)/h\langle\nu\rangle \tag{16}$$

with

$$\bar{n}_j = \exp(h\nu_j/\boldsymbol{k}T) - 1)^{-1}\,. \tag{17}$$

The meaning of Eqn. 16 is clear. $\sum_j(h\Delta\nu_j/2)$ is the difference of the zero-point vibrational energy, which will be designated as $\Delta E_{\mathrm{z.p.}}$. As shown in the following, $\sum_j\bar{n}_j h\Delta\nu_j$ is the contribution from the free energy change due to the frequency change. The partition function Z_{v} of the vibration is

$$Z_{\mathrm{v}} = \prod_j \sum_{n_j} \exp(-n_j h\nu_j/\boldsymbol{k}T)\,. \tag{18}$$

The vibrational free energy is

$$G_{\mathrm{v}} = -\boldsymbol{k}T\ln Z_{\mathrm{v}} = \sum_j \boldsymbol{k}T\ln(1-\exp(-h\nu_j/\boldsymbol{k}T))\,. \tag{19}$$

Then, the vibrational free energy change due to the frequency change is given to the first order of $\Delta\nu_j/\nu_j$ as follows,

$$\Delta G_v = \sum_j \frac{\partial G_v}{\partial \nu_j} \Delta\nu_j = \sum_j \bar{n}_j h \, \Delta\nu_j \, . \tag{20}$$

From these conditions, it is found that p is written as

$$p = \Delta G/h\langle\nu\rangle \tag{21}$$

where ΔG is the total free energy gap between the initial and final staes. Eqns. 14 and 21 are correct to the first order of $\Delta\nu_j/\nu_j$. Usually the frequency change is less than 10%. Thus, the criterion for the approximation is satisfied in most cases.

It should be noticed that Eqn. 14 is the same form as that of Jortner [7] except that ΔE is replaced by $\Delta G(\Delta G = \Delta E + \Delta E_{\text{z.p.}} + \Delta G_v)$. This replacement is important at high temperature where ΔG_v is expressed by

$$\Delta G_v = \sum_j kT(\Delta\nu_j/\nu_j) \, . \tag{22}$$

In the photosynthetic system, molecules are large and so the number of vibrational modes is very large. Thus, ΔG_v can be comparable with ΔE even if $|\Delta\nu_j/\nu_j| << 1$ holds.

We need a simple practical way to estimate the variation of ΔG_v with temperature. A possibility is to define

$$\Delta E_1 = \sum_j h \, \Delta\nu_j \tag{23}$$

and then $\bar{n}_a$ such that

$$\Delta G_v = \Delta E_1 \cdot \bar{n}_a \, . \tag{24}$$

ΔE_1 has a meaning of the sum of the energy difference of fundamental vibrations between the initial and final states. Eqns. 20, 23 and 24 require that

$$\bar{n}_a = \sum_j \bar{n}_j h \, \Delta\nu_j / \Delta E_1 = \sum_j \bar{n}_j \, \Delta\nu_j / \Sigma_j \, \Delta\nu_j \, . \tag{25}$$

ΔE_1 should be temperature-independent and the temperature dependence of $\bar{n}_a$ is seen to be a composite of the temperature variations of $\bar{n}_j$ weighted according to the frequency shifts of the modes involved. If the ν_j values of these modes are sufficiently homogeneous and close to each other we can expect $\bar{n}_a$ to vary in a way similar to the variation of the $\bar{n}_j$. Thus, in such cases we can expect the temperature dependence of $\bar{n}_a$ to be similar to that of Eqn. 17 and we define a term, ν_a, to characterize it as follows:

$$\bar{n}_a = (\exp(h\nu_a/kT) - 1)^{-1} \tag{26}$$

where ν_a is a certain averaged frequency. At the extreme of high temperature ν_a is given accurately by

$$\nu_a = \sum_j \Delta\nu_j \left[\sum_j \Delta\nu_j/\nu_j\right] .$$

In such a treatment ν_a is the characteristic frequency determining the boundary between temperature dependence at high temperature and temperature independence at low temperature. It serves the same purpose for the effects of frequenct shift as does the frequency, $<\nu>$, which characterizes the effect of equilibrium position displacement.

Eqn. 14 may not be valid in all cases. For example, since $\Delta\nu_j$ can be either positive or negative, it is possible that ΔE_1 could be zero while ΔG_v could be finite. In such a case $\bar{n}_a$ would be infinite. This is the result of the artificiality of Eqns. 24 and 26. In such a case it will be better to define $\Delta G_v = \Delta E_1^+ \cdot \bar{n}_a^+ + \Delta E_1^- \cdot \bar{n}_a^-$ where each term is due to the group of modes with positive or negative value of $\Delta\nu_j$. When the temperature dependence of the electron transfer exhibits very complicated behavior, it may become necessary to consider ΔG_v as composed of more than two groups of modes. However, in this paper, we assume the form of Eqn. 26 as the first step of such a study.

From definition, it is evident that ΔE_1 is twice that of $\Delta E_{z.p.}$. Then, the total free energy gap can be written as

$$\Delta G = \Delta E + \Delta E_{z.p.} + \Delta G_v = \Delta E + \Delta E_1(1/2 + \bar{n}_a)\,. \tag{27}$$

When $h\langle\nu\rangle >> kT$ holds, Eqn. 14 can be simplified as

$$W = \bar{A}\,\exp(-S)(S^p/\Gamma(p+1))\,, \tag{28}$$

where Γ is the Gamma function. It is obvious that Eqn. 28 is temperature-independent as long as p is assumed to be constant, as usually assumed in the current theories. However, in our theory p and then W can vary with temperature through the temperature dependence of ΔG_v. As we see in the following, the condition $h<\nu> >> kT$ seems to hold in the photosynthetic systems and the simple Eqn. 28 is usable.

Numerical calculations

(a) Electron transfer from cytochrome to P^+

In reproducing the temperature dependence of the electron transfer rate from cytochrome to the primary electron donor cation P^+ (bacteriochlorophyll dimer cation [11]) in *C. vinosum*, Jortner [7] used the same formula as Eqn. 14 but with ΔE instead of ΔG. The temperature dependency arises in his theory through the vibrational population $\bar{v}$. $\Delta E = 0.1$ eV was assumed. (In Hopfield's theory [4], $\Delta E = 0.05$ eV was assumed.) The value of $<\nu>$ was almost uniquely determined by the critical temperature, T_c, below which the electron transfer rate becomes constant. The parameters so obtained are listed in Table I. The value of S used by Jortner [7] is similar to that of Hopfield [4].

Blankenship and Parson [2] also made the similar curve-fitting with the use of a somewhat different value of ΔE, 0.45 eV, which was estimated from the difference of the midpoint redox potential $\Delta E_m'$ between P^+ and cytochrome under the assumption that the net entropy change in the reaction would be negligible. In such a manner, they must choose a quite large value of S(44—48). These results are listed in the middle column of Table I. It is seen that the values of S and $\bar{A}$ vary considerably changing ΔE from 0.10 to 0.45 eV. The

TABLE I

PARAMETERS USED IN THE ANALYSIS OF THE ELECTRON TRANSFER FROM CYTOCHROME TO THE PRIMARY ELECTRON DONOR CATION IN *C. VINOSUM*

	Hopfield [a]	Jortner [b]	Blankenship and Parson [c]	This work
$\langle\nu\rangle/c(\mathrm{cm}^{-1})$ [d]	245	400	400	1000
S	33	20	44	0.5
ΔG(eV) [d]	0.05	0.10	0.45	0.45 [e]—0.88 [f]
$\bar{A}(\mathrm{s}^{-1})$	$5.1 \cdot 10^{10}$ [g]	$1.0 \cdot 10^{9}$	$2.0 \cdot 10^{12}$	$2.7 \cdot 10^{8}$
p	1.6	2.0	9.0	3.6 [e]—7.0 [f]

[a] Ref. 4.

[b] Ref. 7.

[c] Ref. 2. The above values are from one of the three plausible sets.

[d] In the cases of Hopfield, Jortner, and Blankenship and Parson, ΔG should be replaced by ΔE.

[e] The value at room temperature.

[f] The value at 0 K.

[g] This value is obtained by equating the tunneling matrix element T_{ab} to $\langle \mathrm{i}|H'|\mathrm{f}\rangle$ in our theory.

significant facts in those results are that $\langle\nu\rangle/c$ is fairly small (400 cm^{-1}) and S is very large. Transition metal ions such as Fe^{2+} have metal-ligand vibrations with such a magnitude of wavenumber [8]. If a large S value is due to the movement of the Fe^{2+} to the heme plane in the cytochrome upon a change in redox state, the evaluated parameters are considered to be consistent. (If we assume that the effective mass of Fe^{2+}-ligand vibration is 56 dalton, its frequency is $1.2 \cdot 10^{13}$ s^{-1} and Fe^{2+} displaces by 0.4 Å due to the electron transfer, we obtain $\Delta = 10$ and then $S = 50$.) Such a large movement might occur in hemoglobin, but not in soluble high-potential *c*-type cytochromes [12]. Whether it occurs in the membrane-bound low-potential cytochrome of *C. vinosum* is problematic [2]. As an actual situation, we observe similar critical temperatures, T_{c}, among electron transfer processes which do not include the liganded Fe^{2+} [2,3]. (An example is the back electron transfer from $\mathrm{Q}_{\mathrm{A}}^{-}$ to P^{+} as shown in this paper.) Furthermore, the change of W with temperature (0—300 K) in the case of the cytochrome oxidation in *Rhodopseudomonas* sp. N.W. is much smaller than in *C. vinosum*. If Fe^{2+} motion is predominantly effective, the temperature dependence should be quite similar among various species because the electronic property of Fe heme will not change among species. Thus, the movement of Fe^{2+} seems to be unimportant for the electron transfer in the photosynthetic system. The short-range specific hydrational modes of solvent may have the vibrational frequency 300—500 cm^{-1} [8]. However, it seems to be improbably that those modes contribute very much to the large value of S in the photosynthetic system by a large rearrangement of the hydration. The hydrated solvent will be frozen when temperature is lowered. If the solvent is frozen, the large rearrangement of the hydration is prohibited and the hydrational mode cannot contribute to the large value of S. Thus, the electron transfer should be decreased drastically below the freezing temperature. In contrast with this, the experimental curve of the electron transfer rate is smooth with temperature. Thus, it is imagined that proteins to which donor and acceptor molecules are bound will exclude many of solvent molecules from the region of the electron transfer reaction, and that solvent molecules cannot couple with the electron transfer reaction dominantly in the photosynthetic system. Our above conjecture is supported by the experiment of solvent isotope effect on the electron transfer rate, suggesting that ν_a is not effectively affected by the deuterium exchange as we discuss later. The above conjecture is also supported by hydrophobicity of the reaction center components. The cytochromes donating to the reaction center in *C. vinosum* are water insoluble [13—15].

The resonance Raman spectrum of cytochrome *c* [16] revealed that the scattering amplitude and thus the displacement Δ_j due to the excitation was large for those modes of 1000—1600 cm^{-1} and small for those modes of 600—800 cm^{-1}. (The relative intensity of the scattering amplitude is proportional to Δ_j^2 in the case of the strongly allowed transition [17].) The Δ_j value due to the change of the redox state is considered to be similar to that due to the photoexcitation if the solvent modes are not significant. Thus, ν_j/c with large value of Δ_j^2 must be much larger than 400 cm^{-1}. In all of the following, we tentatively assume $<\nu>/c = 1000$ cm^{-1}.

According to our molecular orbital calculations (Kakitani et al., unpublished data) of the molecular conformation and the vibration, the value of S due to the addition or removal of an electron is about 0.2—0.4 for porphin and bacteriochlorophyll and 0.5—1.0 for semiquinone. If we take into account the possible small contribution from protein vibrations, a reasonable value of S might be 0.5—1.0.

In our parametrization, $h<\nu> \, >> kT$ holds in the temperature range with which we are concerned. Thus, we can adopt Eqn. 28 for our analysis of the temperature dependence of the electron transfer rate in photosynthetic systems.

By fitting the theoretical temperature dependent curve calculated from Eqn. 28 to the experimental curve of electron transfer of *C. vinosum*, we evaluate the parameters as follows: $<\nu>/c = 1000$ cm^{-1}, $S = 0.5$, $\Delta E + \Delta E_{z.p.} = 0.88$ eV, $\Delta E_1 = -0.98$ eV, $\nu_a/c = 250$ cm^{-1} and $\bar{A} = 2.7 \cdot 10^8$ s^{-1}. (These parameter values are also listed in the second column of Table II.) In our evaluation of parameters, ΔG becomes 0.45 eV at room temperature in agreement with the difference of the midpoint redox potential. The value of ν_a was determined predominantly by T_c.

Our theoretical curve is plotted in Fig. 1 together with the theoretical curves by Jortner and by Blankenship and Parson. All the three theoretical curves can reproduce the experimental data very well in spite of the fact that the chosen values of the parameters vary considerably between ourselves and others. Our chosen value of S is very small and $S << p$ holds while the values of S of Jortner and of Blankenship and Parson are very large and $S >> p$ holds. The electron transfer rate W using Eqn. 28 is plotted in Fig. 2 as a function of p for S = 0.5, 1, 20 and 40. As $S << p$ holds in our calculation, the slope at the right side of the maximum of the sharp curve (S = 0.5) was used. The increase of W with temperature was obtained by decreasing p. In the calculation by Jortner and by Blankenship and Parson, $S >> p$ holds. Thus, the slope at the left-hand side of the maximum of the curve (S = 20 or 40) was used. The increase of W with temperature was obtained by broadening the curve of the Franck-Condon factor. From this it will be seen that our mechanism of the temperature depen-

TABLE II

SUMMARY OF THE PARAMETER VALUES OBTAINED IN THIS PAPER

	Cyt.$c \rightarrow P^+$ *C. vinosum*	$Q_A^- \rightarrow P^+$ *Rps.* sp. N.W.	$P^*I \rightarrow P^+I^-$ *Rps.* sp. N.W.	$P^+I^- \rightarrow PI$ *Rps.* sp. N.W.	Cyt.$c \rightarrow P^+$ *Rps.* sp. N.W.	
					H_2O	^2H_2O
$<\nu>/c(\mathrm{cm}^{-1})$	1000	1000	1000	1000	1000	1000
S	0.5	1.0	1.0	1.0	1.0	1.0
$\Delta E + \Delta E_{\mathrm{z.p.}}(\mathrm{eV})$	0.88	0.49	0.46	0.98	0.64	0.66
$\nu_a/c(\mathrm{cm}^{-1})$	250	300	250	250	300	300
$\Delta E_1(\mathrm{eV})$	—0.98	0.36	—0.14	—0.17	—0.44	—0.41
$\bar{A}(\mathrm{s}^{-1})$	$2.7 \cdot 10^8$	$2.1 \cdot 10^3$	$1.1 \cdot 10^{13}$	$1.4 \cdot 10^{12}$	$7.3 \cdot 10^7$	$7.3 \cdot 10^7$
$\Delta G(0$—$300\ \mathrm{K})\ (\mathrm{eV})$	0.88—0.45	0.49—0.60	0.46—0.40	0.98—0.90	0.64—0.45	0.67—0.49
$W(0$—$300\ \mathrm{K})\ (\mathrm{s}^{-1})$	$2.5 \cdot 10^2$ —10^6	36—9.3	$2.8 \cdot 10^{11}$ —$5 \cdot 10^{11}$	$2 \cdot 10^7$ —$7 \cdot 10^7$	$1.5 \cdot 10^5$ —$2 \cdot 10^6$	10^5 —$1.3 \cdot 10^6$

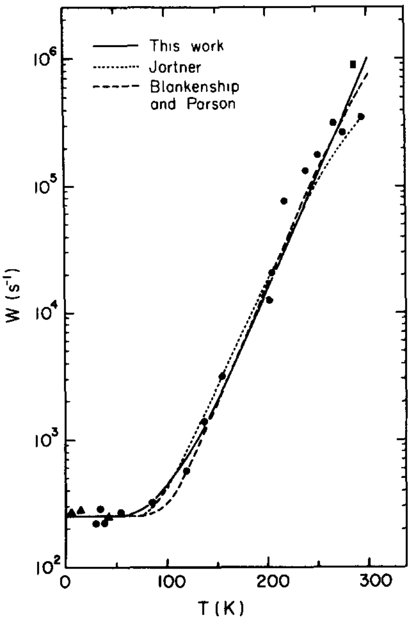


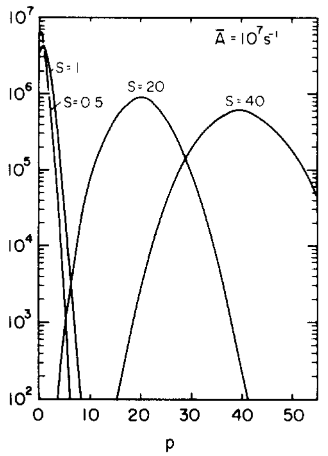
Fig. 1. The electron transfer rate constant W for cytochrome *c*-552 oxidation in *C. vinosum* vs. temperature. ●, DeVault and Chance [1]; ▲, DeVault et al. [18]; ■, Parson and Case [19] and Seibert and DeVault [20]. This graph is taken from Blankenship and Parson [2] except for the theoretical curves of Jortner and of results from this work.

dence is quite different from the previous ones.

Our chosen value of $|\Delta E_1|$ is a reasonable one. (This value becomes smaller when we use a smaller value of $<\nu>$; e.g., ΔE_1 is about 0.5 eV when $<\nu>$ is 800 cm^{-1}.) Indeed, we should realize that $\Delta E_1 = 2\Delta E_{\mathrm{z.p.}}$ and that it is possible for $|\Delta E_{\mathrm{z.p.}}|$ to amount to about 0.5 eV. For example, the difference between the zero-point energy of the lowest triplet and the ground state for aromatic hydrocarbons was estimated to be about 0.5 eV [9]. In our systems of parameters, the value of ΔE_1 is negative. This means that the averaged vibrational frequency increases by the electron transfer from cytochrome to P^+. Our value of ν_a is smaller than the averaged frequency $<\nu>$ chosen by Jortner and others but similar to $<\nu>$ chosen by Hopfield [4]. Our value of $\bar{A}$ is similar to that given by Jortner (see Table I).

(b) Back electron transfer from Q_A^- to P^+

Probably the greatest usefulness of the present theory is that the negative activation process of the back electron transfer in photosynthetic system can be easily explained if ΔE_1 has a positive value. The main example will be the back reaction between P^+ and the quinone acceptor anion Q_A^- as shown in Fig. 3. We can reproduce this experimental results by choosing the parameters such as those in the third column of Table II. In this case, ΔG was fitted to be 0.60 eV at room temperature, in agreement with the experimental data of the midpoint potential change, ΔE_m [2]. The values of $\bar{A}$ and ΔE_1 are small. The theoretical curve obtained using Eqn. 28 is plotted in Fig. 3, and is found to be



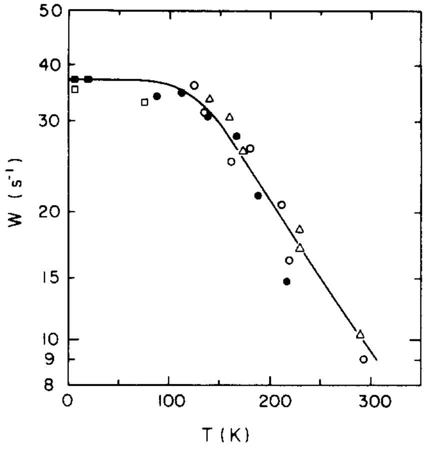


Fig. 2. The graph of variation of W with p for some values of S as calculated using Eqn. 28. $\bar{A}$ is chosen as 10^7 s^{-1}. W becomes maximum at $p \simeq S$.

Fig. 3. The back electron transfer rate constant between P^+ and Q_A^- vs. temperature. ●, *Rps. sphaeroides* strain R-26 reaction centers; ○, R-26 reaction centers +3 mM *o*-phenanthroline; △, *Rps. sphaeroides* wild-type chromatophores +3 mM *o*-phenanthroline. All taken from Hsi and Bolton [21]. □, ■, R-26 reaction centers (McElroy et al. [22,23]). This graph is taken from Blankenship and Parson [2] except for the theoretical curve depicted by the solid line.

in good agreement with the experimental data. Such a successful explanation has not been given before [2] except in qualitative discussion [24] using the soft mode of vibrations or the postulation that P and Q_A move slightly closer at low temperatures [8,25,26].

(c) Relation between electron transfers $P^*I \rightarrow P^+I^-$ and $P^+I^- \rightarrow PI$

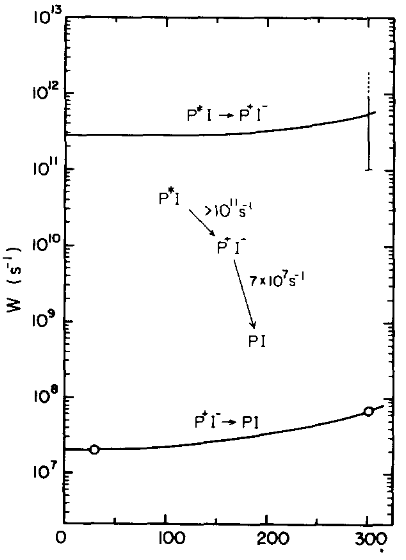
Picosecond kinetic measurements have shown that the photo-oxidation of P and reduction of the initial electron acceptor I (bacteriopheophytin [11]) are completed within 10 ps [27—32]. The fluorescence yield from P^* increases by a factor of about 2 as the temperature is decreased through the region between 180 and 220 K [33]. This could mean that the rate of electron transfer decreased by 50% in this range [2]. The rate constant of the back reaction from P^+I^- to PI is about $7 \cdot 10^7$ s^{-1} at room temperature and about $2 \cdot 10^7$ s^{-1} at 30 K [32, 34,35]. The back reaction is slower than the former reaction by about 10^4 times. The free energy difference ΔG is estimated to be about 0.40 eV for $P^*I \rightarrow P^+I^-$ and about 0.90 eV for $P^+I^- \rightarrow PI$ at room temperature [2].

In the following we try to reproduce these rate constants while satisfying the above values of ΔG. For the $P^*I \rightarrow P^+I^-$ and $P^+I^- \rightarrow PI$, we choose the parameter values as those in the forth and fifth columns of Table II, respectively. Theoretical curves obtained using these parameters and Eqn. 28 are plotted in Fig. 4. The result is very satisfactory.

It should be mentioned that the difference of four orders of magnitude between the forward and the back reaction was obtained by using the very sharply decreasing region of the Franck-Condon factor curve for $S = 1$ in Fig. 2. When S is large (20—40), the Franck-Condon factor curve changes gradually with the energy difference, and so one cannot explain this large difference between the forward and back rate constants consistently [2].

The remarkable difference between the forward and back reaction rates appears to be crucial for efficient primary photooxidation in a photosynthetic system, and can be contrasted with the similar process in solution where the two rates are similar in magnitude [32]. A possible explanation for this difference has been discussed recently by assuming a proper arrangement of donor and acceptor molecules in the biological system [32,36,37]. Regarding this problem, we consider, however, that the above remarkable properties are due to the small value of *S* in biological systems and due to the large value of *S* in model systems in solution. That is, in solution, the rearrangement of the solvent will be very large accompanying the electron transfer and *S* becomes large. However, in biological systems the solvent molecule may be excluded from the near region of the donor and acceptor molecules by the protein moiety and then *S* will have a small value which is due to the conformation change of the pigment (bacteriochlorophyll).

Our value of ΔE_1 obtained for the electron transfer of $P^*I \rightarrow P^+I^-$ is small and similar to that of $P^+I^- \rightarrow PI$. This will mean that the change of the force constants due to electron transfer is small and similar between the two processes. In other words, the vibrational property of P^+I^- is intermediate between P^*I



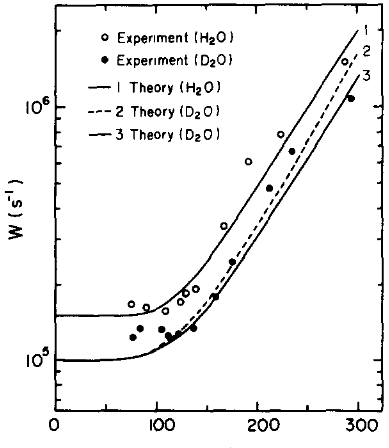


Fig. 4. The temperature dependence of the forward electron transfer ($P^*I \rightarrow P^+I^-$) and the back electron transfer ($P^+I^- \rightarrow PI$) in bacterial reaction centers. The rate of the forward electron transfer is approx. 10^4 times as large as that of the back electron transfer. The solid curve is the calculated value. ○ and ├· · · · denote the experimental values. Typical values of the electron transfer rates at room temperature are written in the graph.

Fig. 5. The temperature dependence of electron transfer rate from cytochrome in *Rps.* sp. N.W. ○, H_2O as solvent; ●, ^2H_2O as solvent. Experimental points are from Kihara and McCray [38]. The theoretical values are plotted by the solid curves. 1, H_2O solvent; 2, ^2H_2O solvent ($\langle\nu\rangle$ is decreased by 4% from that of H_2O solvent), and 3, ^2H_2O solvent ($\Delta E_{z.p.}$ is increased by 0.015 eV from that of H_2O solvent).

and PI. By this, we may conjecture that molecular conformation of P^+I^- will be intermediate between P^*I and PI. If so, we can ascertain that the value of S in both electron transfers is small, because the conformation change between P^* and P, which can be evaluated from its optical absorption band shape, is small ($S < 1$).

Finally it should be mentioned that the electron transfer rate for $P^*I \rightarrow P^+I^-$ is very rapid. Corresponding to this, our value of $\bar{A}$ is very large. Thus our theory based on the nonadiabatic mechanism may not be applicable in such a case.

(d) Solvent isotope effect on cytochrome oxidation

Kihara and McCray [38] observed that when the solvent H_2O is replaced by ^2H_2O, the rate constant for the cytochrome oxidation in *Rhodopseudomonas* sp. N.W. is reduced by 1.4—1.5 times over the observed temperature range (77—300 K) as shown in Fig. 5. The theoretical explanation of this isotope effect was not successful until now with the parameter arrangement of $p < S$ [2]. In the following, we try to answer this problem using our formula. The substitution of H_2O by ^2H_2O will cause a large decrease of vibrational frequencies of hydrated solvent modes, if they exist. Furthermore, some of the H atoms bonded to oxygen or nitrogen atom in the protein molecule and donor or acceptor molecule will be replaced by ^2H atoms. Also in this case, those vibrational frequencies will be reduced a little.

We can choose the parameters for the H_2O solvent system as those in the sixth column of Table II, by fitting the theoretical curve to the experimental data. The curve 1 in Fig. 5 is that calculated for the H_2O solvent. Then, we first consider the case that $\langle\nu\rangle$ is predominantly changed by deuterium substitution. $\langle\nu\rangle$ is proportional to $\langle\mu\rangle^{-1/2}$ where μ is the effective mass of the vibration. p is proportional to $\langle\nu\rangle^{-1}$ and then to $\langle\mu\rangle^{1/2}$. S is proportional to $\langle\mu\nu\rangle$ because Δ_j is given by $2\pi\sqrt{\mu_j\nu_j/h}\,\Delta x_j$ with Δx_j being the displacement of the configuration coordinate of the atom. If we can put $\langle\mu\nu\rangle = \langle\mu\rangle \cdot \langle\nu\rangle$, S is proportional to $\langle\mu\rangle^{1/2}$. Thus, p and S increase in the same rate by the deuterium substitution. In such cases the change in W is very small. However, one must note that the averaging procedure for $\langle\nu\rangle$ is not the same as for S. Thus, it is doubtful that p and S increase at the same rate. It will be possible that S changes very little while p changes in proportion to $\langle\mu\rangle^{1/2}$. Tentatively we consider that the value of p is increased by the decrease of $\langle\nu\rangle$ by 4%, i.e., $\langle\nu\rangle/c = 960\ \text{cm}^{-1}$. The calculated W is plotted in Fig. 5 by the curve 2. The theoretical curve qualitatively explains the decrease of W by the deuterium substitution fairly well. It should be noticed that the decrease of W with increase of p is realized only if $p > S$ holds, as in our system of parameters.

Next we consider the role of the change in ν_a. In the case that $\langle\nu\rangle$ does not change, the value of p at very low temperature becomes the same for ^2H_2O solution and H_2O solution regardless of the value of ν_a. At higher temperatures, ΔG is decreased and then W increases with decrease of ν_a by the deuterium substitution in our parameter model. These tendencies are inconsistent with the experimental result. Thus ν_a should not play an important role in the deuterium substitution effect in our system. The hydrated solvent mode which possibly contributes to ν_a very much, if it exists, should play a minor role in the electron transfer of the primary process of photosynthesis.

Finally we consider a possible alternative mechanism of the decrease of electron transfer rate by the deuterium substitution. This is the change in the zero-point energy difference. In this, we assume that the change of $<\nu>$ and ν_a is small. If we put the parameter values as those in the last column of Table II, we obtaine the curve 3 in Fig. 5. The difference of our value of $\Delta E_{z.p.}$ between the deuterated and non-deuterated cases is 0.015 eV. This value is close to 0.025 eV, which is the change in the difference between the zero-point energies in the excited and ground states of aromatic hydrocarbons upon deuteration. The theoretical curve is quite satisfactory. Thus, the mechanism of the zero-point energy difference can explain the solvent deuterium effect on the photosynthetic electron transfer rate. In order to conclude this, however, it will be necessary to accumulate more experimental data on various aspects of the isotope effect.

Discussion

All the parameter values used in this paper are summarized in Table II. In all the cases $<\nu>/c$ is as large as 1000 cm^{-1}, S is as small as 0.5—1.0 and ν_a/c is 250—300 cm^{-1}. The rather small value of S and negatively large value of ΔE_1 for cytochrome $c \rightarrow P^+$ of *C. vinosum* are necessary to reproduce the great change of W in the temperature range 90—300 K. The very large values of $\bar{A}$ for $P^*I \rightarrow P^+I^-$ and $P^+I^- \rightarrow PI$ imply the close location of P to I, while the very small value of $\bar{A}$ for $Q_A^- \rightarrow P^+$ imply the remote location of P from Q_A. In the present model, $S < p$ holds. Thus, the negative activation is realized for the positive value of ΔE_1 as in the case of $Q_A^- \rightarrow P^+$. (In the system where $S > p$ holds, the negative activation is realized in the condition $\Delta E_1 < 0$). The solvent deuteration effect of W (approx. two thirds) is reproduced by the small change of $\Delta E_{z.p.}$. This result implies that the solvent vibrational modes do not contribute significantly to the electron transfer of cytochrome $c \rightarrow P^+$.

Although we reproduced some types of temperature dependence of W which were experimentally available now, we can predict the other type of the temperature dependency. If in the temperature range with which we are concerned p varies from a smaller (larger) value than S to a larger (smaller) value than S, W must have a maximum when the temperature is lowered (see Fig. 2). The maximum occurs at $p \simeq S$. Thus, this type of data will give an information as to the relation of p and S. It is also possible that p varies from a positive value to a negative value as the temperature is lowered. In such a case the electron transfer rate drastically decreases as soon as p becomes negative. Experimental evidence of such would be useful. When polar solvents can be found close to the region where the electron transfer reaction occurs, the coupling strength S becomes large and the average frequency $<\nu>$ must be small. Then the temperature dependency of W can be a contribution from both the temperature dependences of p and $\bar{\nu}$. If the temperature dependences of p and $\bar{\nu}$ contribute cooperatively, W must change drastically with the temperature. Instead, if the temperature effects of p and $\bar{\nu}$ work in opposition, a complex temperature dependence of W may be realized.

There is a possibility that the temperature dependence of $<\nu>$ is significant for the temperature dependence of W in some systems. If we adopt more detailed and complicated form of $\bar{n}_a$ than Eqn. 26, it may affect our numerical results to some extent, and it may be useful in explaining more complicated behavior of the temperature dependence of W in some systems. Such an advanced study will be made at a later time.

Next, we are going to discuss a formal property of our new formula of W. We designate the W given by Eqn. 14 as the forward electron transfer rate, W_f. The backward electron transfer rate, W_b, can be given by the same formula as Eqn. 14 but p and $\bar{v}$ should be replaced by

$$p = -\Delta G/h(<\nu> + \Delta\nu) \tag{29}$$

$$\bar{v} = (\exp(h(<\nu> + \Delta\nu)/kT) - 1)^{-1}, \tag{30}$$

where $<\nu> + \Delta\nu$ is the averaged vibrational frequency of the final state. Using the condition $|\Delta\nu_j/\nu_j| << 1$, one can approximate $<\nu> + \Delta\nu \simeq <\nu>$ in Enqs. 29 and 30. This treatment is based on the same order (first order) of approximation which we used in obtaining Eqn. 14. Then the ratio W_f/W_b can be written as

$$\frac{W_f}{W_b} = \left(\frac{\bar{v}+1}{\bar{v}}\right)^{\Delta G/h\langle\nu\rangle} = \exp(\Delta G/kT) \tag{31}$$

The formula 31 is the equilibrium constant for the electron transfer reaction and is, of course, required by the principle of microscopic reversibility. (It should be noticed that in our formulation ΔG is defined as $G_i - G_f$ where G_i and G_f are the free energy of the initial and final states, respectively.) It is quite reasonable that the same equilibrium constant is obtained irrespective of the reaction's being adiabatic or nonadiabatic. It should also be noticed that our ΔG in this paper is that obtained to the first-order approximation of $\Delta\nu_j/\nu_j$ and that Eqn. 31 holds only when all the quantities are obtained to the same order of approximation. A more exact derivation of Eqn. 31 starting with Eqn. 1 and an exact definition of ΔG is possible (Kakitani, H., unpublished data).

At the extremely high temperature ($kT >> h<\nu>$), Eqn. 14 is reduced to

$$W = \bar{A}\sqrt{h\langle\nu\rangle/2\pi SkT}\,\exp(-(\Delta G - Sh\langle\nu\rangle)^2/4Sh\langle\nu\rangle kT)\,. \tag{32}$$

This equation is the same form as that given by Marcus [19] based on the absolute rate theory, except the pre-exponential factor (the work, W^r, that is required to bring the reactants together to a certain separation, r, is not necessary in our case because the reactants are considered to be fixed). The intrinsic free energy barrier λ defined by Marcus corresponds to $Sh<\nu>$ in our theory. The physical meaning of the nonadibatic rate theory leading to the absolute rate theory at high temperatures will be discussed in detail elsewhere (Kakitani, H., unpublished data).

According to theories such as those of Jortner [7] and Hopfield [4] using ΔE instead of ΔG, the condition that W is independent of temperature (activationless) around room temperature means that $S \simeq p$ holds and electron transfer rate is maximized [2,40]. In contrast with this, in our theory, the condition of the activationless means simply $h\langle\nu\rangle \gg kT$ and the smallness of ΔE, independent of the relation between S and p. Thus, it should be emphasized that the activationless phenomena do not necessarily imply the matching condition $p \simeq S$. So, even a qualitative conclusion differs between our theory and the current theories.

Recently Sarai [24] proposed a possible role of protein using a soft mode and gave a qualitative explanation for the slow back reaction rate ($P^+I^- \rightarrow PI$) and the negative activation electron transfer in $P^+Q_A^- \rightarrow PQ_A$, and so forth. He claimed that if the coupling of protein mode with electron is not very strong, the rate of electron transfer has an oscillatory dependence on the value of the energy gap [40]. When a variety of modes is considered, interference among the quantum modes with high vibrational frequencies or among the soft modes tends to smear out the oscillatory energy gap dependence of the Franck-Condon factor. So, for the electron transfer in the protein environment, the number of good accepting modes of protein must be limited and the frequency of protein mode is well separated from that of a quantum mode. Thus, in the model of Sarai, the protein plays a role to select very little numbers of soft modes of vibrations to couple with the transferred electron. (Our present model contrasts strongly with this; the protein mode does not play any significant role in the Franck-Condon factor through Δ_j but a variety of protein modes contribute significantly to the entropic term of the free energy.) From the resonance Raman spectra of cytochrome *c* [16] it is suggested that many quantum modes with a large variety of frequencies ν_j have considerable values of Δ_j and contribute significantly to the Franck-Condon factor. Thus, considerable interference among quantum modes is expected in the electron transfer photosynthetic systems. In such cases, it will be rare that the oscillatory dependence of the Franck-Condon factor on the value of energy gap is really obtained by introducing a soft mode. Further theoretical investigations are necessary.

In summary, we derived new formulas for the electron transfer rate by the non-adiabatic mechanism and we could reproduce the remarkable temperature dependence of the electron transfer rate from cytochrome *c* to P^+ in *C. vinosum* using a small value of the coupling strength and a rather large value of $\langle\nu\rangle$. Furthermore, temperature dependencies of electron transfers including negative activation processes and the deuterium exchange effect were successfully explained by a suitable choice of parameter. Thus, one cannot neglect the possible role of the frequency change in the electron transfer rate in the biological system. It is a future task to extend the present theory to electron transfer in solution.

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