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Effects of Isotope Substitution on Intermolecular Electron Transfer in Biological Systems

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Isotope effects on electron transfer reactions are discussed in terms of the multiphonon radiationless transition theory. The D_2O substitution effect on the oxidation of cytochrome *c* (Kihara & McCray, 1973) is analyzed. On the basis of these results the molecular mechanism for cytochrome *c* oxidation is proposed.

1. Introduction

For many years the mechanisms of intermolecular electron transfers (ET) have been the subject of much research in theoretical biophysics. Since the initial formulation, by Szent–Györgyi, 40 years ago of the semiconductive principle of electron migration between biological molecules many investigators have focused attention on this problem. Without going into details concerning the possible role of this mechanism in electron transport processes, we would like to emphasize that to all likelihood, the light-induced oxidation of cytochrome *c* by the reaction center bacteriochlorophyll dimer which is considered in this paper does not occur by the semiconducting mechanism. The temperature dependence of the rate of cytochrome *c* oxidation observed by De Vault & Chance (1966) can be accommodated within the general tunneling framework. However, the concept of an "elastic" tunneling advanced by Chance and co-workers is inadequate to account for the activation energy (about 0·1 eV) observed at temperatures above 130 K. A reasonable explanation of the rate constant temperature dependence $K_{i,f}(T)$ has been given in terms of non-elastic electron tunneling involving conformational rearrangements and relaxation processes (Blumenfeld & Chernavskii, 1973; Chernavskaja & Chernavskii, 1978; Dogonadze, Kuznetsov & Ulstrup, 1977; Grigorov & Chernavskii 1972; Hopfield 1974; Jourtner 1976; Kuznetsov, Sondergard & Ulstrup 1978; Vol'kenstein 1969). The formalisms of these works rests on a theory of multiphonon radiationless processes (Bixon & Jourtner, 1978; Freed &

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Jourtner, 1970; Robinson & Frosh, 1962) and on a theory of oxidation-reduction reactions in solution (Dogonadze & Kuznetsov, 1973; Kesther, Logan & Jourtner, 1974; Levich & Kuznetsov, 1970).

From analysis of the temperature dependence of the cytochrome c oxidation rate constant $K_{i,f}(T)$ (Hopfield, 1974; Jourtner, 1976) some important conclusions can be drawn. First, the activation pattern of $K_{i,f}(T)$ at temperatures above 130 K suggests that the transition probability sharply increases with increasing vibration excitation in the initial electronic state. This implies that in the system of interest there is a strong electron-conformation coupling at least with one vibrational mode R_m. Figure 1 shows a schematic representation of a section (along the co-ordinate R_m) of the initial (i) and final (f) nuclear potential surfaces. The strong coupling situation occurs, whenever $\Delta_m = \Delta R_m / a_m \gg 1$.

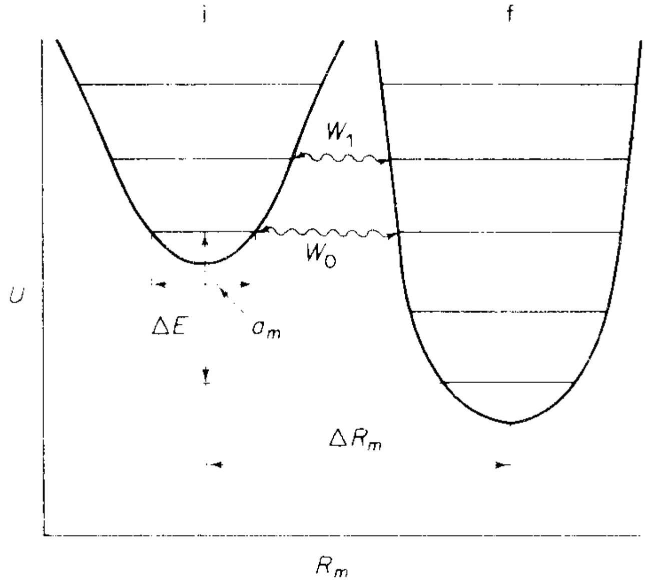

FIG. 1. A sectional view of the potential surfaces in the initial and final state (along the R_m co-ordinate) R_m, the accepting mode.

As the transition probability is proportional to the overlap of the nuclear wavefunctions in the initial (i) and final (f) states, it progressively increases with vibrational level number as it is seen from Fig. 1 i.e. $W_1 \gg W_0$. Basing on this inequality, one can easily see the required temperature dependence $K_{i,f}(T)$, since by definition

$$K_{i,f}(T) = (W_0 + W_1\, e^{-\hbar\omega_m/kT} + \ldots)/(1 + e^{-\hbar\omega_m/kT} + \ldots).$$

The mode R_m in this case is an accepting mode, since the excess energy ΔE (or the thermal effect) is largely transformed into its vibration excitation.

The term "accepting mode" is applied to vibrations on the case when $\Delta_m \gg 1$ and $\Delta E = 0$.

Table 2(*d*) represents dimensionless displacement and frequency for the accepting mode R_m obtained by theoretical treatments of the rate temperature dependence $K_{i,f}(T)$ for cytochrome *c* oxidation in *Chromatium*. There are some variations in values of Δ_m^2, ω_m and $V_{i,f}$ derived from different theoretical estimations (which differ only in details), but all estimates are of the same order of magnitude (Hopfield, 1974, 1977; Jourtner, 1976; Kuznetsov *et al.*, 1978). The value of $\omega_m = 300\text{–}400\ \text{cm}^{-1}$ is close to the totaly symmetric metal-porphyrin vibrational frequency in cytochrome and chlorophyll. This mode is likely to be accepting (Jourtner, 1976). This question will be considered in greater detail in section 5, on the basis of the deuteration effects.

The deuterium isotope effect on the oxidation of cytochrome *c* observed by Kihara & McCray (1973) is convincing evidence for a close correlation between electron transfer and configuration changes. Upon heavy water (D_2O) substitution the T dependence of $K_{i,f}$ exhibits a 1·2–1·4-fold decrease (Table 1, Fig. 2) while the activation energy remains almost unchanged. The effect cannot be explained by proton (nor electron) migration, for otherwise the mass m_H dependence of $K_{i,f}$ would have an exponential, instead of a root pattern at low temperature (Bell, 1973). To all appearance, the deuteration effect arises from the involvement of hydrogen atom vibrations in electron transfer.

Note that this effect fundamentally differs from a more drastic effect of deuteration which has been observed in radiationless process in aromatic molecules (Siebrand, 1967). In the latter case, reduced displacements in equilibrium of the nuclear potential surfaces are small, i.e. $\Delta^2 \ll 1$ (weak coupling), and the probability of a radiationless transition has an exponential dependence on the number of excited vibrational quanta $W \sim e^{-\gamma E/\hbar\omega}$ (Englman & Jourtner, 1970). In this case the high-frequency hydrogen mode is an important sink for the electron energy E ($E \gg \hbar\omega$).

TABLE 1

Experimental rate constants of cytochrome c *oxidation in* Rhodopseudomonas *sp. NW at various conditions (Kihara & McCray,* 1973)

T K	80	298	$K(298\ \text{K})/K(80\ \text{K})$
$K_{H_2O} \times 10^6\ \text{s}^{-1}$	0·23 ± 0·02	1·89 ± 0·07	8·24 ± 0·7
$K_{D_2O} \times 10^6\ \text{s}^{-1}$	0·165 ± 0·08	1·43 ± 0·02	8·67 ± 0·5
K_{H_2O}/K_{D_2O}	1·39 ± 0·12	1·32 ± 0·07	—

TABLE 2

Relative changes of the cytochrome c *oxidation rate constant* $K_{i,f}(T)$ *over temperatures from* 0 *to* 300 *K at various parameters of the accepting mode*

q or q'	0†					
S	6·6[†, a]					9
ω_m cm^{-1}	310[b]	330†	350	389[c]	400	400
$\frac{K_{i,f}(300\text{ K})}{K_{i,f}(0\text{ K})}$	10·2	9·1	8·25	6·8	6·75	23·4

q or q'	1		2			3	
S	7·55[a]	9	8·5[a]	9	20[d]	9[a]	
ω_m cm^{-1}	310	400	310	400	400	310	400
$\frac{K_{i,f}(300\text{ K})}{K_{i,f}(0\text{ K})}$	5	6·3	3	3·16	10^3	1·7[e]	1·74

[a] The values of S_{H_2O} specified in the Table correspond (at given q's) to the observed low temperature isotope effect.

[b,c] The frequencies of water molecule vibrations about the Mg and Fe ions, respectively in $Mg(H_2O)_6^{2+}$ and $Fe(H_2O)_6^{2+}$ (Nakamoto, 1963; Nakagawa & Shimanouchi, 1964).

[d] Jourtner (1976) for *Chromatium*. The value of q is erroneous, as in *Chromatium* $\Delta E =$ 0·45 eV (see Hopfield, 1977).

[e] The ratio $K_{i,f}(274\text{ K})/K_{i,f}(0\text{ K})$.

[†] Parameters of the system that are quantitatively consistent with both the observed isotope effect and temperature dependence of cytochrome c oxidation in *Rds*.sp., *NW*.

Deuteration results in a $\sqrt{2}$-fold decrease of the quantum $\hbar\omega$ and in an exponential decline of the transition probability. In biological systems thermal effects do not exceed the energy quantum of the high-frequency hydrogen atom vibration (0·4–0·5 eV). Hence, deuteration cannot critically influence energy sink processes, even if hydrogen atom modes are implicated in the process. The deuteration effect in biological systems is the result of a delicate interplay between the components involved and for this reason seems to be a promising tool to use for gaining more useful information on the subject.

The goal of our present work is to analyze the possible effects of deuteration on ET processes. The analysis below leads us to conclude that the deuteration isotope effect observed in the cytochrome c oxidation

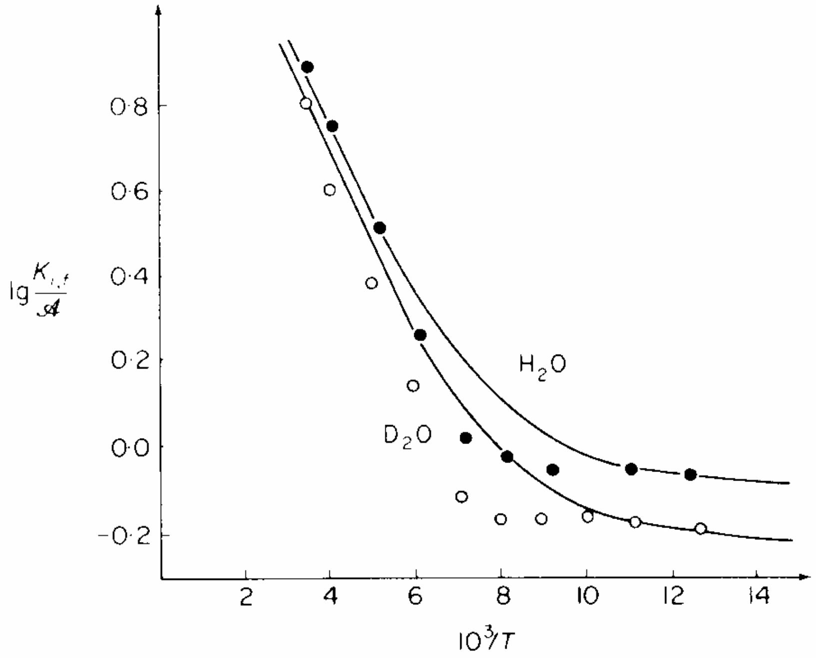

FIG. 2. The temperature dependence of and the isotope effect on the rate constant of cytochrome *c* oxidation in *Rhodopseudomonas* sp., *NW*; ——— equation (21) (corresponding parameters are given in Table 2) ●, ○, experimental results of Kihara & McCray (1973).

reaction (Kichara & McCray, 1973):

(1) cannot be explained in terms of hydrogen atom vibrations in the accepting mode, should this be the case, the deuteration would produce an exponential effect on the transition rate at low temperatures;

(2) is not associated with the vibrational relaxation of the hydrogen atoms in the system, for in its presence fortuitous effects and functional instability would take place at small variations in the redox potentials of the donor and acceptor;

(3) seems most probably to be associated with considerable configurational changes in the first hydration layer of the porphyrin complexes.

This interpretation of the deuterium isotope effect in the cytochrome *c* oxidation reaction gives some clue to better understanding of the mechanism of this process and provides a framework for understanding of the relation between this mechanism and the structure of the reaction center.

2. Intermolecular Transition Rate Mathematical Formulation

The formulation of a theoretical description of the intermolecular transition rate rests upon the following general formalism (Landau & Lifshits, 1959):

(1) The initial and final states of a system consisting of a donor molecule (D) and acceptor molecule (A^+) and their surrounding are characterized in a one-electron approximation by the wavefunctions

$$\begin{aligned} \Psi_{i,n} &= \varphi_0(r, R_A, R_D)X_D(R_D)X_{A^+}(R_A)X_i(R_s) \\ \Psi_{f,l} &= \varphi_A(r, R_A, R_D)X_{D^+}(R_D)X_A(R_A)X_f(R_S) \end{aligned} \tag{1}$$

φ_D and φ_A are the electronic wavefunctions of the donor and acceptor which generally depend on the nuclear co-ordinates of D and A. The X terms represent the wavefunctions of the intramolecular vibrational modes of the donor and acceptor centers and of the vibrational modes of the solvent (R_S) at a given electronic density distribution; "n" and "l" are the generalized indices of vibrational states.

(2) The transition probability in unit time can be adequately expressed in terms of the Fermi golden rule (first-order perturbation theory):

$$W_{i,n}^{f,l} = \frac{2\pi}{\hbar}|\langle\Psi_{f,l}|V|\Psi_{i,n}\rangle|^2\delta(E_{i,n} - E_{f,l}) \tag{2}$$

where E represents the energy of the respective state; "V" is a perturbation causing a transition. Equation (2) is valid, if at least one of the states lies in a continium spectrum. For a transition without involvement of long-wave modes of the medium (in weak coupling situation with the solvent)

$$W_{i,n}^{f,l} = \frac{1}{\hbar}\left|V_{i,n}^{f,l}\right|^2 \frac{\Gamma}{(E_{i,n} - E_{f,l})^2 + \Gamma^2/4}, \tag{3}$$

where Γ denotes the widths of the vibrational levels due to interactions with the medium modes (Kaizer, 1974; Dogonadze *et al.*, 1975; Djidjoev *et al.*, 1978). Equation (3) also holds for a case where quasilocal modes of the solvent are present.

(3) Since electron transition rate is in fact by far slower than the rate of vibrational relaxation (Lin, 1967; Robinson & Frosh, 1962), the distribution of vibrational energy levels may be regarded as being in equilibrium. The rate constant for the reaction in question can then be given by

$$K_{f,i} = Q^{-1} \sum_{l,n} W_{i,n}^{f,l}\, \mathrm{e}^{-\hbar\omega_n/kT}, \tag{4}$$

where Q is the statistical sum; $\hbar\omega_n$ is the energy of the nth vibrational level.

(4) In an harmonic oscillator approximation, equation (4) can be rewritten as (Jourtner, 1976; Pekar, 1951):

$$K_{i,f}(T) = \frac{|V_{i,f}|^2}{\hbar^2}\,\mathrm{e}^{-G}\int_{-\infty}^{+\infty} \mathrm{d}t\,\exp\left[-i\frac{\Delta E}{\hbar}t + G_t(t) + G_-(t)\right] \tag{5}$$

where

$$G_+(t) = \tfrac{1}{2}\sum_j \Delta_j^2(\bar{n}_j+1)\,\mathrm{e}^{i\omega_j t},$$

$$G_-(t) = \tfrac{1}{2}\sum_j \Delta_j^2 \bar{n}_j\,\mathrm{e}^{-i\omega_j t}, \tag{6}$$

$$G = \tfrac{1}{2}\sum_j \Delta_j^2(2\bar{n}_j+1),$$

$$\bar{n}_j = [\mathrm{e}^{\hbar\omega_j/kT}-1]^{-1}.$$

$\Delta E = E_{f,0} - E_{i,0}$ corresponds to the energy gap between the minima of the initial and final nuclear potential surfaces.

$$\Delta_j^2 = M_j\omega_j(\Delta R_j)^2/\hbar.$$

ΔR_j represents the nuclear displacement of the equilibrium configuration for the jth mode; M_j, ω_j are the mass and frequency of the jth oscillator. The index j refers to all normal modes. $\bar{n}_j$ denotes the mean thermal population of the jth mode.

The electron exchange matrix element $V_{i,f}$ between D and A is defined as

$$|V_{i,n}^{f,l}|^2 = |V_{i,f}|^2 F_{l,n} \tag{8}$$

where $F_{l,n}$ is a product of the Franck–Condon factors for all normal modes.

We shall restrict ourselves to consideration of vibrations with approximately similar frequencies ω_m. The rate constant (Jourtner, 1976) can then be given by

$$K_{i,f}(T) = \mathscr{A} I_q\left(\frac{S}{sh\,\hbar\omega_m/2kT}\right)\exp\left(q\frac{\hbar\omega_m}{2kT} - S\,cth\frac{\hbar\omega_m}{2kT}\right), \tag{9}$$

where $I_q(z)$ is the modified Bessel function

$$S = \sum_{\omega_j \approx \omega_m} \Delta_j^2/2. \tag{10}$$

Here "q" is the integral number closest to $\Delta E/\hbar\omega_m$. The pre-exponential factor A is proportional to $|V_{i,f}|^2$ and is dependent on the vibrational spectrum of the oscillator (see the Appendix):

$$\mathscr{A} = \frac{2\pi}{\hbar^2\omega_m}|V_{i,f}|^2\rho(\Delta E - q\hbar\omega_m;\Gamma). \tag{11}$$

"Γ" is the sum of the energy level widths. The form of the spectral function $\rho(E, \Gamma)$ will be given below.

As mentioned, equation (9) provides qualitative and quantitative account for the temperature dependence of cytochrome c oxidation which has been observed by De Vault and Chance, on the assumption that there is a strong coupling situation, i.e. $S \gg 1$. If such, the influence of the hydrogen atom mass, m_H, on $K_{i,f}$ which has been observed in the experiment cannot be explained by a direct relation between the accepting mode and hydrogen oscillations. Should this be the case $\Delta_m^2 \sim m_H^{1/2}$; $\omega_m \sim m_H^{1/2}$ and $K_{i,f}$ would have an exponential behavior pattern at low temperatures. On the other hand, the presence of strong coupling with the hydrogen atom modes is inconsistent with electron vibration spectroscopic observations (McCoy & Ross, 1962; Byrne, McCoy & Ross, 1965) which have demonstrated that Δ_H for hydrogen atom stretches roughly equals 0·1 and Δ_H for deformation bends is zero. The value of $\omega_m \simeq 400\ \text{cm}^{-1}$ deduced by Jourtner (Table 2) will reflect the metal-ligand vibrational frequency of the porphyrin complex rather than hydrogen atom vibrations. Some more delicate effects resulting from the influence of the water molecule mass on Δ_m and ω_m will be considered below (see section 5).

3. Effects Arising from Changes in the Energy Level Widths

A possible m_H dependency of the pre-exponential factor A could arise from changes in widths "Γ" due to deuteration. From the ratio $K_H/K_D = 1{\cdot}2\text{–}1{\cdot}4$ and equation (9) the relationship below can be derived

$$K_{i,f} \sim \mathscr{A} \sim m_H^{1/2}. \tag{12}$$

It is shown in the Appendix that with the Lorentzian spectral line shape of energy levels

$$\rho(E, \Gamma) \simeq 1; \quad \text{at } \pi\Gamma \gg \omega_m \tag{13}$$

and

$$\rho(E, \Gamma) \simeq \frac{1}{2\pi} \frac{\omega_m \Gamma}{(\Gamma/2)^2 + \left(\dfrac{\omega_m}{\pi}\right)^2 \sin^2 \dfrac{\pi E}{\hbar\omega_m}}; \quad \text{at } \pi\Gamma \ll \omega_m. \tag{14}$$

It is obvious from equations (13) and (14) that Γ-variations affects the spectral function $\rho(\Delta E, \Gamma)$ (11) when $\Gamma \ll \hbar\omega_m$. For the cytochrome c oxidation process, this corresponds to $\Gamma < 100\ \text{cm}^{-1}$. Examples in which small values of Γ were found ($\Gamma \sim 10\text{–}20\ \text{cm}^{-1}$) are the C—H vibration modes of chloroform and benzene, arising from weak intermolecular interactions (Schroeder *et al.*, 1977; Tanable & Jonas, 1977). The formation of H-bonds should lead to an increase in the width of the vibrational levels. If the width "Γ" is largely determined by the rate of the phase relaxation of hydrogen atom vibrations, $\rho(\Gamma)$ is sensitive to the hydrogen atom mass in accordance with the relationship $\Gamma \sim m_H^{-1}$ (Wertheimer, 1978). From equation (14) one can see that at small Γ's when $|\sin(\pi \Delta E/\hbar\omega_m)| \sim 1$, the deuterium isotope effect will be nearly the same as in the cytochrome c oxidation reaction. In this connection, some comments are in order. First, there is little probability that the width of the vibrational levels in the final state is determined predominantly by the hydrogen atom relaxation. Secondly, measurements of the level width in metal-porphyrins gave rather large values $\frac{1}{2}\Gamma \sim 250\,\mathrm{cm}^{-1}$) (Shelnutt *et al.*, 1977). Thirdly, in view of an oscillating resonance-like pattern of the ΔE dependence of $\rho(\Delta E, \Gamma)$ the question arises whether biological systems can stably operate at small Γs. In fact, at small widths, varying the redox potential over a rather narrow region (about 20 mV) would lead to an one-order-of-magnitude change in the value of $K_{i,f}$. On the other hand, the relation of equation (14) incorporates factors that may have regulatory functions. Meanwhile, no justification has been obtained to date for recognizing this relation as being realistic.

Considering all the above facts, it is concluded that the deuteration effect on vibrational relaxation may be neglected and $\rho(\Delta E, \Gamma)$ can be taken as unity.

For further analysis of the pre-exponential factor A it is appropriate to treat here the matrix element $V_{i,f}$.

4. Electron Exchange Distances and Associated ET Mechanisms

Let us suggest that the separation between D and A is large and the wavefunction of the electron localized, for example, on D is not correlated with the nuclear vibrations of the acceptor A. Then it is adequate to assume that the wavefunctions φ_D and φ_A are molecule functions of isolated D and A. In such a case, the perturbation V can be treated in terms of an interaction between the electron and the acceptor molecule $-V_A(r, R_A)$ (Kestner *et al.*, 1974). Since the amplitude of atom vibrations, ΔR_A, is much smaller than the separation between the donor and acceptor, L, the predominant contribution to V_A comes from the static part of $V_A(r, R_A)$ (R_A here is the mean co-ordinate of the atoms within the molecule A). It is evident that within the non-adiabatic mechanism the matrix element $V_{A_{i,f}}$ is independent of the hydrogen atom mass.

On the other hand, evidence to date indicates that the separation between the cytochrome c heme and the reaction center bacteriochlorophyll dimer is about 20–30 Å (Tiede *et al.*, 1978). At such a large L the value of the tunneling factor is negligibly small. From general considerations, it follows that

$$V_{A_{i,f}} \simeq \frac{e^2}{L}\,\mathrm{e}^{-L/a} \tag{15}$$

where

$$a = \hbar/\sqrt{2m_e\varepsilon}, \tag{16}$$

"a" represents the effective decay distance of the electron wavefunction, m_e is the mass of an electron; ε is the energy of electron binding. For aromatic Fe-organic compounds the value of ε is on the scale of 6 eV (taking into account the electron affinity of the surrounding), i.e. $a \sim 0{\cdot}75$ Å. Hence, at $L = 20$ Å $V_{i,f} \sim 10^{-12}$ eV, a value 7–8 orders of magnitude smaller than that deduced from the experiment (Hopfield, 1974; Jourtner, 1976). In this physical situation tunneling with virtual excitation or superexchange mechanism (Jourtner, 1976; Kuznetsov *et al.*, 1978) seems most probable for electron transfers. The effectiveness of a radiationless electron transition is determined by a non-adiabaticity operator (Nikitin, 1970; Freed & Jourtner, 1970):

$$C_{i,n}^{i^*,n^*} = -\hbar^2 \sum_j \frac{1}{M_j} \left\langle \mathrm{X}_{i^*,n^*} \left| \left(\varphi_{i^*} \left| \frac{\partial}{\partial R_j} \right| \varphi_i \right) \frac{\partial}{\partial R_j} \right| \mathrm{X}_{i,n} \right\rangle. \tag{17}$$

Symbols $(|\;|)$ and $\langle|\;|\rangle$ denote the integration over electronic and all nuclear co-ordinates, respectively.

In terms of second-order perturbation theory (Landau & Lifshits, 1959), the matrix element for electron exchange with virtual excitation is displayed in the form

$$\tilde{V}_{i,n}^{f,l} = C_{i,n}^{i^*,n^*} V_{Ai^*,n^*}^{f,l}/\varepsilon \tag{18}$$

where i^*, n^* represent the excited state of the donor molecule, with the binding energy, ε^*, being much smaller than $\varepsilon: \varepsilon^* \ll \varepsilon$. The electron exchange matrix element $V_{i,f}$ under those conditions is proportional to e^{-L/a^*}, where $a^* = a(\varepsilon/\varepsilon^*)^{1/2}$. At large distances L it may occur that with increasing "a" the increment of $V_{i^*,f}$ will exceed $C_{i,n}^{i^*,n^*}/\varepsilon$ and this channel will become dominant. The matrix element of the non-adiabaticity operator, $C_{i,n}^{i^*,n^*}$, is on the scale of 10^{-2}–10^{-3} eV. Calculations yield that electron transfer with virtual excitation are probable at $L > 10$ Å.

By assuming that upon excitation dimensionless displacements of the equilibrium atomic configurations are small (Byrne *et al.*, 1965) and by confining equation (17) to the contribution from one promoting mode "p" only, the matrix element of equation (18) can be recast in the form

$$\tilde{V}_{i,n}^{f,l} = \frac{\hbar^{3/2}\omega_p^{1/2}}{\varepsilon(2M_p)^{1/2}} C'_{i,i^*} V_{A_{i^*f}} [(n_p + 1)\delta_{n_p^*-1,n_p} + n_p \delta_{n_p^*+1,n_p}] F_{n,l} \tag{19}$$

where

$$C'_{i,i^*} = \left(\varphi_{i^*} \left| \frac{\partial}{\partial R_p} \right| \varphi_i \right) \qquad V_{A_{i^*,f}} = (\varphi_A | V_A | \varphi_{D^*})$$

$F_{n,l}$ is the Franck–Condon factor for the accepting modes. Using equation (19), an expression below can be derived, describing the rate constant for electron tunneling with virtual excitation:

$$K^*_{i,f}(T) = \frac{\pi \hbar \omega_p}{M_p \varepsilon^2 \omega_m} |C'_{ii^*} V_{A_{i^*,f}}|^2 \, \mathrm{e}^{-Scth\, \hbar\omega_m/2kT}$$
$$\times \left[(\bar{n}_p + 1) I_{q'}\left(\frac{S}{sh\, \hbar\omega_m/2kT}\right) \mathrm{e}^{q'\hbar\omega_m/2kT} + \bar{n}_p I_{q''}\left(\frac{S}{sh\, \hbar\omega_m/2kT}\right) \times \mathrm{e}^{q''\hbar\omega_m/2kT} \right], \tag{20}$$

where $q' = (\Delta E - \hbar\omega_p)/\hbar\omega_m$; $q'' = (\Delta E + \hbar\omega_p)/\hbar\omega_m$. The first term in equation (20) arises from the excitation of a vibrational quantum in the promoting mode. The second term is a contribution due to the uptake of a quantum, $\hbar\omega_p$, from this mode. The quantum of intramolecular vibrations, $\hbar\omega_p$, equals 0·1–0·4 eV. For $T < 300$ K the mean thermal phonon population $\bar{n}_p \ll 1$ and the rate constant $K^*_{i,f}$ can be defined as a function of the form

$$K^*_{i,f}(T) \approx \frac{2\pi}{\hbar^2 \omega_m} |\tilde{V}_{i,f}|^2 I_{q'}\left(\frac{S}{sh\, \hbar\omega_m/2kT}\right) \mathrm{e}^{-Scth(\hbar\omega_m/2kT) + q'(\hbar\omega_m/2kT)}. \tag{21}$$

Compared with equation (9) describing a direct non-adiabatic process, equation (21) has a different matrix element $\tilde{V}_{i,f}$ and $q' = (\Delta E - \hbar\omega_p)/\hbar\omega_m$, instead of $V_{i,f}$ and $q = \Delta E/\hbar\omega_m$.

For example the promoting modes in aromatic systems may be out-of-plane bends of hydrogen atoms (Hung & Henry, 1978). Physically, they may originate from a deformation of the π-orbitals, when the vibrations distort the plane symmetry of the aromatic cycle (Coulson, 1961). In the case of cytochrome *c* oxidation there is little probability that the isotope effect is related to variations in the C–H vibrations. In fact, D_2O substitution does not affect the C–H groups. If the deuteration had taken place, the value of "q" in equation (21) would have changed and as a consequence the amount of deuteration effect would have been far from the observed one (1, 4).

It is possible that the observations of Kihara & McCray (1973) can virtually be explained by some small variations in the electron–phonon coupling S.

5. Molecular Mechanism of Cytochrome *c* Oxidation, Discussion

The temperature dependence of the cytochrome *c* oxidation rate in *Rhodopseudomonas* sp., *NW* and *Rps.gelatinosa* is quantitatively different from that of *Chromatium* (De Vault & Chance, 1966). In the case of *Chromatium* the rate of the reaction in the temperature range 100–300 K increases by a factor of 10^3. Table 1 below represents estimates of the rate of this reaction in *Rhodopseudomonas* sp., *NW*, deduced from Kihara's and McCray's experimental results (see also Fig. 2). As is seen, the D_2O substitution leads to a 1·4-fold slowing of the reaction at low temperatures. The slowing is somewhat less pronounced at room temperature.

From equations (9) and (21) it follows that at low temperatures

$$K_{i,f}(0) \sim \frac{S^q}{q!} e^{-S} \tag{22}$$

where $q = \Delta E/\hbar\omega_m$ or $(\Delta E - \hbar\omega_p)/\hbar\omega_m$. From the reasoning above one may expect that in this case the deuteration effect is related to some small variation in the parameter S (10). Since D_2O substitution does not result in entire deuteration, it seems reasonable to suppose that "S" is directly related to structural alterations of the interfacial hydration layers of the porphyrin complexes involved in the reaction. Calculations of the frequency of the accepting mode, ω_m [on the basis of the temperature of the inflexion point of the $K_{i,f}(T)$ curve] yielded a value which is close to the vibration frequencies of the Mg–O and Fe–O bonds in the $Mg(H_2O)_6^{2+}$ and $Fe(H_2O)_6^{2+}$ ions (Table 2). On the other hand, changes in the charged state of metal ions in aqueous solution are attended, as a rule, by noticeable variations of the ion radius and, hence, of the length of the metal–H_2O bond (Kestner *et al.*, 1974). For instance, for the oxidation of $Fe(H_2O)_6^{2+}$ to $Fe(H_2O)_6^{3+}$ the electron–phonon coupling strength per 1 H_2O molecule is

$$\tfrac{1}{2}\Delta^2_{Fe\text{-}OH_2} = (\Delta R_{Fe\text{-}OH_2})^2 M_{H_2O}\omega_{Fe\text{-}OH_2}/2\hbar \simeq 2,4. \tag{23}$$

This value is in the same order of magnitude to those specified in Table 2. Recent dehydration experiments (Knox *et al.*, 1977) have demonstrated a significant influence of water and its state on electron transport activity.

If it is true that the value of S is in direct relation with structural changes in the hydration layers, then according to equations (7) and (10)

$$S_{H_2O}/S_{D_2O} = (m_{H_2O}/m_{D_2O})^{1/2} \tag{24}$$

whence, using equation (22) one can easily determine, at a given ΔE (or q), the coupling constant S_{H_2O} for the physical situation observed by Kihara and McCray at low temperatures [Table 2(a)].

In this case ΔE, determined on the basis of the corresponding redox potentials, equals $0{\cdot}1 - 0{\cdot}4$ eV (Hopfield 1974, 1977; Jourtner 1976; Dutton, Jackson, 1972). It is seen from Table 2 that at $q \geq 2$ and S_{H_2O}

corresponding to the isotope effect the temperature dependence of $K_{i,f}$ is quantitatively inconsistent with the experiment. This seeming contradiction is not hard to reconcile with the experiment upon a closer consideration of the mechanism of the reaction.

As mentioned above, the separation between heme and the pigment is about 20–30 Å. At such a large L the mechanism with virtual excitation is to be dominant [equation (20)]. A promoting mode energy quantum is equal to 0·1–0·4 eV. In this physical situation almost all energy ΔE is put into the promoting mode. We may, therefore, take q as zero. With this, there will be a good agreement between theoretical and experimental ratios $K_{i,f}(298°)/K_{i,f}(80\ \mathrm{K})$. The best fit will be at $\omega_m = 330\ \mathrm{cm}^{-1}$, a frequency close to that of the Mg–OH_2 vibrations (Table 2).

Upon D_2O substitution, ω_m will reduce to $314\ \mathrm{cm}^{-1}$ and $S_{D_2O} = 6{\cdot}93$. Figure 2 shows that at the above values of ω_m and S a satisfactory fit is provided between the corresponding theoretical and experimental curves: $K_{H_2O}(T)$ and $K_{D_2O}(T)$.

The pre-exponential factor A $(0{\cdot}17 \times 10^9\ \mathrm{s}^{-1})$ is similar in order of magnitude to that for *Chromatium* (Jourtner, 1976), indicating the similarity of the electronic structures in the two cases. At $L = 20$–30 Å (Tiede *et al.*, 1978) the tunneling factor (at $a \sim 1$ Å) is negligibly small and a value of 10^8–$10^9\ \mathrm{s}^{-1}$ for A can be explained in terms of the superexchange mechanism (via virtual states). Taking the non-adiabaticity operator $C_{i,n}^{i^*,n^*}$ as 10^{-3} eV, $V_{A_{i^*},f}$ as $e^2/L = 0{\cdot}7$ eV (at $L = 20$ Å) and ε as 7 eV, we obtain $\tilde{V}_{i,f} \approx 10^{-4}$ eV; whence $A \approx 1{\cdot}5 \times 10^9\ \mathrm{s}^{-1}$. This is an upper limit of A, since the effectiveness of excited electron migration has been taken as unity (see also Kharkyanen *et al.*, 1978).

Let us consider some structural aspects of the cytochrome c oxidation reaction. The variation in the length of the metal–H_2O bond, when the ion charge is changed by unity is typically 0·1–0·2 Å (Kestner *et al.*, 1974). Comparison of this value with $S_{H_2O} = 6{\cdot}6$ suggests that 2–3 molecules of water are involved in the reaction. On the other hand it has already been demonstrated (Katz *et al.*, 1976) that the reaction center bacteriochlorophyll dimer includes two water molecules, those in the co-ordination bond to the Mg atom and in the H–bond to the C = O group of the $\bar{V}$ ring [Fig. 3(a)]. Changes in the charged state of the dimer appear concomitant with changes in effective charges on the Mg atoms and on the C = O groups (δ_1 and δ_2, respectively). This has to cause the equilibrium position of the H_2O dipole to change too (Fig. 3(b)]. Since $S_{H_2O} = 6{\cdot}6$, it is clear that the equilibrium position will be shifted by 0·2 Å. If the heme of cytochrome c is co-ordination-bonded by a water molecule, the latter will give its contribution (23) to S_{H_2O} too. The displacement of the water molecules of the

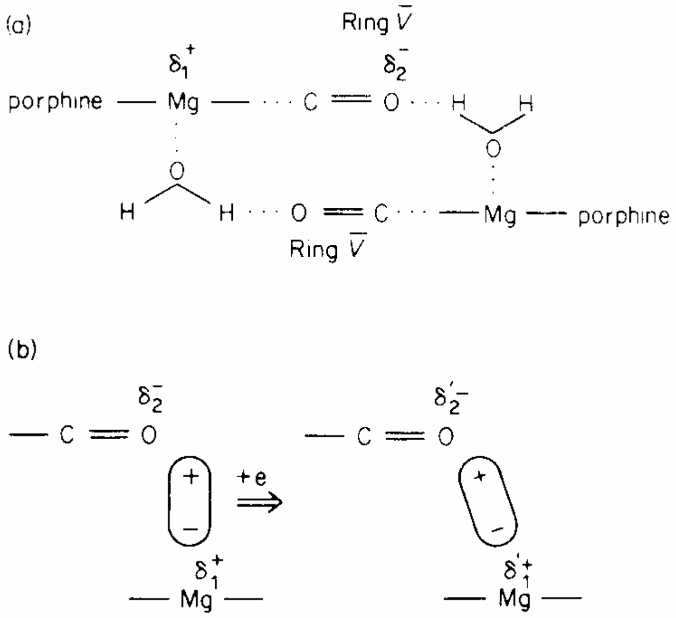

FIG. 3. A possible model of the accepting mode in the cytochrome *c* oxidation reaction. (a) The structure of the reaction center bacteriochlorophyll dimer (Katz *et al.*, 1976). (b) Reorientation of the H_2O molecule dipole which is taking place, when the charged state of the reaction center is changed; δ_1 and δ_2, the effective charges on the Mg and O atoms.

reaction center will be in this case somewhat smaller—about 0·15 Å. Such a scale of displacements (0·15–0·2 Å) are of comparable value with those taking place, whenever molecular charged states are changed, for instance, when the H_2 to H_2^+ transition ($\Delta R \approx 0{\cdot}3$ Å) occurs (Coulson, 1961).

Since within the proposed mechanism of cytochrome *c* oxidation the isotope effect is largely sensitive to the mass of the H_2O molecule, the validity of this mechanism can be varified by experiments involving H_2O^{18} substitutions. The H_2O^{18} effect must be the same as this of D_2O^{16}. The D_2O^{18} substitution should produce a more strong effect.

6. Conclusion

The deuterium isotope effects which have been observed indicate the involvement of electronic vibrational interactions in ET reactions. In a formal way, electron transfer can be described in terms of a transition of the system between two electronic vibration states (Fig. 1). However, in such a description, devoid of any structural predictions, many of the physical features cannot be made explicitly clear. Moreover, a great variety of structural and chemical factors which determine the functioning of the "protein machine" in whole remain beyond the scope of formal theories. In this regard, deuterium exchange experiments are most encouraging, since they may help get a feeling of the importance of water and the extent of its influence in elementary biological processes on the one hand (see Katz *et al.*, 1976; 1977; Knox *et al.*, 1977), and may, on the other hand, provide useful information for extending a formal theoretical treatment beyond the present scope.

The experimental results of Kihara & McCray (1973), if they are correct, imply the important role of water molecules in the accepting mode (Fig. 1). Using equation (22) for $T = 0$ K, the amount of isotope effect and the known number "q" of excitation quanta transmitted to the accepting mode, one can easily determine the value for the coupling constant S_{H_2O}. It appears that a good fit between theory and experiment is provided only at some particular value of q (Table 2). For *Rhodopseudomonas* sp., *NW* it is the case at $q = 0$ and at ω_m close to the frequency of the H_2O–Mg^{+2} vibrations. The thermal effect in this case, ΔE, is about 0·1–0·4 eV and, hence, the zero value of q is well consistent with the tunneling mechanism with virtual excitation [equation (20)]. One very strong supporting argument for this mechanism is a value of $10^9\,s^{-1}$ for the pre-exponential factor A, a value is hard to accomodate within ordinary tunneling providing electron transfers over distances (L) of 20–30 Å.

In light of our findings we predict that about 2–3 water molecules are involved in cytochrome *c* oxidation. Physically, the observed effect may be associated with the reorientation of the water molecules complexed with the bacteriochlorophyll dimer in response to a change in the charged state of the reaction center [Fig. 3(b)]. We do not rule out the implication of the H_2O molecule coordinated to the cytochrome *c* heme.

H_2O^{18} and D_2O^{18} substitution experiments would provide more useful information on the mechanism of the process in question.

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APPENDIX

(1) Let us consider the relation between the rate constant and width of vibrational levels. The integral of equation (5) can be rewritten as

$$J(\omega, \Gamma) = 2Re\int_0^\infty \mathrm{d}t \exp[-i\omega t + S(\bar{n}+1)\,\mathrm{e}^{i\omega_m^* t} + S\bar{n}\,\mathrm{e}^{-i\omega_m t}] \qquad (A1)$$

ω and ω_m are normally complex numbers. The real part of $\hbar\omega$ is the energy taken up by an oscillator having a frequency of $Re\,\omega_m$. Using the multinominal expansion, equation (A1) becomes:

$$J(\omega, \Gamma) = \sum_{\alpha=0}^{\infty} \sum_{\beta=0}^{\infty} \frac{[S(\bar{n}+1)]^{\alpha}[S\bar{n}]^{\beta}}{\alpha!\beta!} f[\omega - (\alpha-\beta)\omega_m]. \tag{A2}$$

The function $f(\Omega_{\alpha,\beta})$ here is determined by the imaginary parts of ω and ω_m.

(2) If $Im\omega_m = 0$, $f(\Omega_{\alpha,\beta})$ is a function of the difference $(\alpha-\beta)$ only. Then equation (2A) can be recast in the form:

$$J(\omega_0, \Gamma_0) = \sum_{q=-\infty}^{+\infty} \left(\frac{\bar{n}+1}{\bar{n}}\right)^{q/2} I_q\{2S[\bar{n}(\bar{n}+1)]^{1/2}\} f(\omega_0 - q\omega_m). \tag{A3}$$

Here $I_q(z)$ is the modified Bessel function; "f" has either a Lorentzian or Gaussian shape (Dogonadze *et al.*, 1975; Vorotyntsev & Itskovich, 1978), depending on the expression for the imaginary part of ω:

$$f(\omega_0 - q\omega_m) = \frac{\Gamma_0}{(\omega_0 - q\omega_m)^2 + \Gamma_0^2/4} \tag{A4}$$

for

$$\omega = \omega_0 - i\Gamma_0/2,$$

$$f(\omega_0 - q\omega_m) = \frac{2\sqrt{\pi}}{\Gamma_0} \exp\left[-(q\omega_m - \omega_0)^2/\Gamma_0^2\right] \tag{A5}$$

for

$$\omega = \omega_0 - it\Gamma_0^2/2.$$

When Γ_0 goes toward zero

$$f(\omega_0 - q\omega_m) \rightarrow 2\pi\delta(\omega_0 - q\omega_m). \tag{A6}$$

With some restrictions imposed on ω_0 (Dogonadze & Kusnetsov, 1973; Jourtner, 1976), the saddle point method can be applied for a calculation of equation (A3).

The co-ordinates of the saddle points t_k of the integrand of equation (A1) can be found from an equation of the form:

$$\omega = \omega_m S(\bar{n}+1)\, e^{i\omega_m t_k} - \omega_m S\bar{n}\, e^{-i\omega_m t_k}. \tag{A7}$$

We take that $\omega = \omega_0 - i\Gamma_0/2$. Since the two terms in (A7) have the same period of oscillations: $(2\pi/\omega_m)$, the resolution of (A7) can be expressed as

$$t_k = t_0 + \frac{2\pi}{\omega_m} K; \qquad K = 0, 1, 2 \ldots \tag{A8}$$

where t_0 represents the co-ordinate of the first saddle point. The summation over t_k gives

$$J(\omega_0, \Gamma_0) = Re\ \Lambda(t_0) cth\left(\frac{\pi i \omega}{\omega_m}\right). \tag{A9}$$

Here $\Lambda(t_0)$ stands for the contribution from the first saddle point. Equation (9A) can be re-written in the form (compare with Medvedev *et al.*, 1977):

$$J(\omega_0, \Gamma_0) \simeq \frac{\frac{1}{2} sh \frac{\pi\Gamma_0}{\omega_m} Re\,\Lambda(t_0) + \frac{1}{2}\sin\frac{2\pi\omega_0}{\omega_m} Im\ \Lambda(t_0)}{sh^2\frac{\pi\Gamma_0}{2\omega_m} + \sin^2\frac{\pi\omega_0}{\omega_m}}. \tag{A10}$$

For $kT \ll \hbar\omega_m$

$$\Lambda(t_0) = (\pi/2\omega_m|\omega|)^{1/2} \exp\left\{\frac{\Gamma_0}{2\omega_m}\varphi - \frac{\omega_0}{\omega_m}\ln\left|\frac{\omega}{\omega_m Se}\right| + \right.$$

$$\left. + i\left[\frac{\Gamma_0}{2\omega_m}\ln\left|\frac{\omega}{\omega_m Se}\right| + \left(\frac{1}{2} + \frac{\omega_0}{\omega_m}\right)\varphi\right]\right\} \tag{A11}$$

with $\varphi = \arcsin \Gamma_0/2|\omega|$.

Equation (11A) implies that at low temperatures the second term in (10A) may be neglected only when $\Gamma_0 > \omega_m$. For $\Gamma_0 \ll \omega_m$, the contributions from both terms are of the same order of magnitude. For high temperatures $kT \gg \hbar\omega_m (S\bar{n} \gg 1)$, one finds that

$$\Lambda(t_0) = \frac{(2\pi)^{1/2}}{\omega_m[S(2\bar{n}+1)]^{1/2}} \exp\left[S(2\bar{n}+1) - \frac{(\omega - S\omega_m)^2}{2S\omega_m^2(2\bar{n}+1)}\right]. \tag{A12}$$

When $\Gamma_0 \ll \omega_m$, the imaginary part of equation (12A) may be neglected. Since $\Gamma_0 > \omega_m$ we have $sh(\pi\Gamma_0/\omega_m) \gg 1$, then for high temperatures equation (10A) may be reduced to the first term only.

(3) Physically, the application of the condition $Im\omega_m = 0$ implies that vibrational level widths are independent of level number, n. In practice it seems more appropriate to introduce a linear level width dependence on its number, i.e.:

$$\Gamma_n = n\Gamma_1 \tag{A13}$$

where Γ_1 is the width of the first vibration level. This relation will implicitly enter the integrand of (1A), if ω_m has the form

$$\omega_m = \omega_m^0 - i\Gamma_1/2 \tag{A14}$$

ω_m^0 here represents the frequency of an accepting mode. In this case $f(\Omega_{\alpha,\beta})$

is a function of both α and β and equation (3A) does not hold for $J(\omega, \Gamma)$. However the application of the saddle point method may yield the required results. For this physical situation the co-ordinates of the saddle points are given by

$$\omega = \omega_m^* S(\bar{n}+1)\,\mathrm{e}^{i\omega_m^* t} - \omega_m S\bar{n}\,\mathrm{e}^{-i\omega_m t} \tag{A15}$$

ω_m^* here is a complex variable, conjugate to ω_m. ω may be complex to. Γ_0 in this case may reflect, for instance, the level width of the promoting oscillator [see equation (20)].

The difference between equations (A15) and (A7) is that equation (A8) is valid only at two ultimate conditions, namely, when (1) $kT \ll \hbar\omega_m$ and (2) $|\omega| \ll S\bar{n}|\omega_m|$.

For the low temperature region equation (A9) holds when $\omega_m = \overset{0}{\omega}_m + i\Gamma_1/2$.

By denoting the real part of $c\mathrm{th}[(\pi i\omega/\omega_m^*)]$ as $\rho(\tilde{\omega}, \Gamma)$, one obtains

$$\rho(\tilde{\omega}, \Gamma) = \tfrac{1}{2} sh\frac{\pi\Gamma}{\overset{0}{\omega}_m} \Big/ \left[sh^2\frac{\pi\Gamma}{2\overset{0}{\omega}_m} + \sin^2\frac{\pi\tilde{\omega}}{\overset{0}{\omega}_m} \right] \tag{A16}$$

where

$$\begin{aligned} \Gamma &= (\omega_0\Gamma_1 + \overset{0}{\omega}_m\Gamma_0)\frac{\overset{0}{\omega}_m}{(\overset{0}{\omega}_m)^2 + \Gamma_1^2/4} \\ \tilde{\omega} &= (\omega_0\overset{0}{\omega}_m - \Gamma_0\Gamma_1/4)\frac{\overset{0}{\omega}_m}{(\overset{0}{\omega}_m)^2 + \Gamma_1^2/4} \end{aligned} \tag{A17}$$

At small values of Γ_1 and large values of ω_0 equation (A16) significantly differs from the case described in point (2). From equation (A17) it is evident that for $\Gamma_1 \ll \omega_m$ and $\omega_0 \gg \omega_m$ the effective vibrational level Γ is virtually equal to $q\Gamma_1$ (where $q = (\omega_0/\omega_m) \gg 1$). For $\Gamma > \overset{0}{\omega}_m$, $\rho(\tilde{\omega}, \Gamma) = 1$.