On the mechanism of cytochrome oxidation in bacterial photosynthesis

Quantum tunnelling effects revisited

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We propose that the unique temperature dependence of the Chance-DeVault cytochrome oxidation reaction in *Chromatium* is not due to a transition from low-temperature nuclear tunnelling to a high-temperature activated electron transfer (ET), but rather originates from two parallel ET processes from two distinct low-potential cytochromes to the bacteriochlorophyll dimer cation. These involve a slow activationless process, which dominates at low temperatures ($T \leqslant 120$ K) and an activated process, which is practically exclusive at high temperatures. This conjecture provides plausible nuclear and electronic coupling terms and structural data for the two cytochrome oxidation reactions.

Bacterial photosynthesis *Cytochrome c* *Electron transfer* *Quantum tunneling*

1. INTRODUCTION

Chance and DeVault discovered [1] a unique temperature dependence of the rate constant, k, for electron transfer (ET) from the low-potential cytochrome c (cyt c) to the bacteriochlorophyll dimer $(BChl)_2$ cation in the photosynthetic bacterium *Chromatium vinosum*. A sharp transition is exhibited from a high-temperature activated region, where k drops by 3 orders of magnitude (i.e. from $k \simeq 10^6\ s^{-1}$ at 300 K to $k \simeq 5 \times 10^2\ s^{-1}$ at 120 K) to a temperature-independent k at low temperatures ($T < 120$ K). Subsequent studies [2,3] on the same system revealed the same general behaviour with an even sharper transition of k between the two regions. The characteristics of this reaction [1–3] were interpreted within the general framework of the nonadiabatic multiphonon ET theory [4–8]. It was proposed [4,5] that the temperature-independent region manifests a nuclear tunnelling process for ET, while the temperature dependence of this reaction reflects a transition from tunnelling at low temperatures to an activated rate process at high temperatures. Although the qualitative traditional interpretation [4,5] of the temperature dependence of the Chance-DeVault reaction is very plausible and appealing, the quantitative physical information, which emerges from such an analysis [4–8], raises serious conceptual difficulties. The nuclear and electronic parameters, which are deduced from such an analysis, are unreasonably large. In particular we note that:

(i) The high-transition temperature $T_0 = 120$ K [1–3] implies that the characteristic vibrational mode, which is effectively coupled to the electronic process, i.e. undergoes a large configurational change during ET, has a frequency of $\hbar\omega = 400–500\ cm^{-1}$ [4–8]. Such a high frequency must correspond to intramolecular vibrational modes of the porphyrin rings in the cytochrome c and/or in the $(BChl)_2$. The effective coupling of high-frequency intramolecular vibrational modes to this ET process seems to contradict the results of a recent analysis [9] of the temperature dependence of two ET reactions in the reaction centres of

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bacteria, i.e. the activationless reduction of the quinone [10] and the back recombination between the quinone anion and $(BChl)_2^+$ [11], both of which are characterized [9] by a low effective frequency of $\hbar\omega = 100\ cm^{-1}$, and which correspond to coupling with the exterior polar protein medium modes.

(ii) A large nuclear reorganization energy $E_c = 18500\ cm^{-1}$ in conjunction with the high characteristic frequency ($\hbar\omega = 500\ cm^{-1}$), which emerges from the quantitative analysis of the temperature dependence of the ET rates [8], manifests huge intramolecular configurational changes which accompany the ET process. This large value of E_c implies a change of 0.7 Å in the nuclear equilibrium configuration of four intramolecular vibrational modes. This conclusion is in contrast with the available structural kinetic and spectroscopic data. X-ray crystallographic analysis for the two oxidation states of tuna cyt c [12] reveals small configurational changes in the heme groups. Reorganization energy calculations [13] for the cyt c electron exchange reaction, which are based on the crystallographic data [12], result in a low value of $E_c = 350\ cm^{-1}$ for the intramolecular reorganization energy. The contribution of the intramolecular configurational changes within $(BChl)_2$ accompanying ET is also expected to be small, as can be inferred from the kinetics of ET from electronically excited $(BChl)_2$ to bacteriopheophytin [14]. The weak temperature dependence of this reaction between 4 and 300 K implies that it is activationless [15]. Accordingly, the reorganization energy is equal to the free-energy ΔE of this reaction [15]. Taking $\Delta E = 1200\ cm^{-1}$ [14], we infer that for this reaction $E_c \leqslant 1200\ cm^{-1}$, which is 1 order of magnitude lower than E_c deduced [8] for cytochrome oxidation. Furthermore, spectroscopic studies of chlorophylls [16] reveal that the 0-0 vibrationless, pure electronic transition dominates both in absorption and in emission, which proves that the configurational changes in chlorophylls upon electronic excitation are minor. A similar situation is expected to prevail for ET.

(iii) A large electronic coupling $V = 90\ cm^{-1}$ is required for a quantitative fit of the ET data [8]. Such a large interaction can only arise from close contact between cyt c and $(BChl)_2$, which contradicts all the available structural information. ESR data give a centre-to-centre distance of ~25 Å for the separation between these prosthetic groups in *Chromatium* [17], which is close to the distance of 23 Å obtained from crystallographic studies for *Rhodopseudomonas viridis* [18]. At such distances one expects [4,5,15] V to be lower than $1\ cm^{-1}$.

(iv) A close examination of the fit of the experimental data for *Chromatium* [1–3] to the multiphonon ET theory [4–8] clearly indicates the inadequacy of the theory in the vicinity of the transition temperature around 120 K (see e.g. fig.4 of [8]). The experimental data correspond to a sharp break rather than a smooth change. The only way to achieve a better agreement between theory and experiment is to increase the parameters E_c and V even further, which contradicts both facts (see (ii) and (iii)) and intuition.

(v) Some experimental data, which have accumulated regarding analogous ET processes in other photosynthetic bacteria [19–22], reveal that the temperature dependence of the cyt c oxidation reaction in *Chromatium* is by no means universal, as other systems reveal a broad spectrum of temperature dependences of the ET rates. For example, ET between the high-potential cyt c and the primary donor in *Ectothiorodospira shaposhnikovii* [21] is characterized by a fast ($\sim 10^6\ s^{-1}$) temperature-independent rate, while ET in *Rps. gelatinosa* reveals a weak temperature dependence ($k(298\ K)/k(80\ K) \sim 5$) [22]. A somewhat larger temperature dependence ($k(298\ K)/k(80\ K) \sim 8$) is exhibited in *Rhodopseudomonas* sp. NW [22], which shows a very sharp transition at 150 K from a temperature-dependent to a temperature-independent ET rate.

This information provides compelling evidence that it is highly improbable that a tunnelling process prevails at temperatures around 100 K for the ET between cyt c and the primary donor. The nature of the Chance-DeVault reaction [1–3], which served as a touchstone [4,5] for the applicability of the ET theory to biological systems, has to be re-examined.

2. A CONJECTURE ON THE MECHANISM OF CYTOCHROME OXIDATION IN *CHROMATIUM*

We assert that cyt c oxidation in *Chromatium* does not exhibit a transition from low-temperature

nuclear tunnelling to a high-temperature activated process, which involves ET from a single cyt c to the dimer cation. Rather, we propose that the unique temperature dependence for this reaction originates from the combination of several parallel ET reactions with different activation energies, each of them occurring from a different cytochrome molecule. At least for three photosynthetic bacteria, i.e. *Chromatium* [17], *Rps. viridis* [18] and *E. shaposhnikovii* [21], it is known that the reaction centre includes four cyt c molecules involving two high-potential and two low-potential cyts c. Some evidence for parallel ET reactions is available for *Chromatium* at room temperature, where the ET rate from the low-potential cytochrome and the high-potential cytochrome are $(1\ \mu s)^{-1}$ and $(2\ \mu s)^{-1}$, respectively [17], while at low temperatures oxidation by the high-potential cytochrome is 10-fold slower than that by the low-potential cytochrome [2]. Another example for parallel reactions is provided by the high-potential cytochromes in *Thiocapsa pfennigii* [23] in which one of the cytochromes is oxidized more rapidly than the second. We propose that both the two low-potential cytochromes in *Chromatium* can be operative in ET and (at least) two ET processes occur: (I) a moderately slow activationless process; (II) an activated process.

At high temperatures reaction (II) is much faster, while at low temperatures reaction (I) predominates. The low-temperature nuclear tunnelling contribution from reaction (II) is negligible.

Fig.1 portrays schematic nuclear potential energy curves for the two parallel ET processes. It is important to emphasize that the nuclear coordinate in fig.1 represents the configurations of the exterior protein medium as, on the basis of a recent analysis of ET processes [9], we assert that the dominating nuclear contribution to such ET processes involves the coupling with the low-frequency medium modes. As is apparent from fig.1, the activationless reaction (I) involves the crossing of the potential surfaces at the minimum of the initial state, while reaction (II) is characterized by a nuclear barrier. The qualitative differences between the dynamics of reactions (I) and (II), which are reflected in the distinct temperature dependence of their rates, originate from different medium reorganization energies of the protein

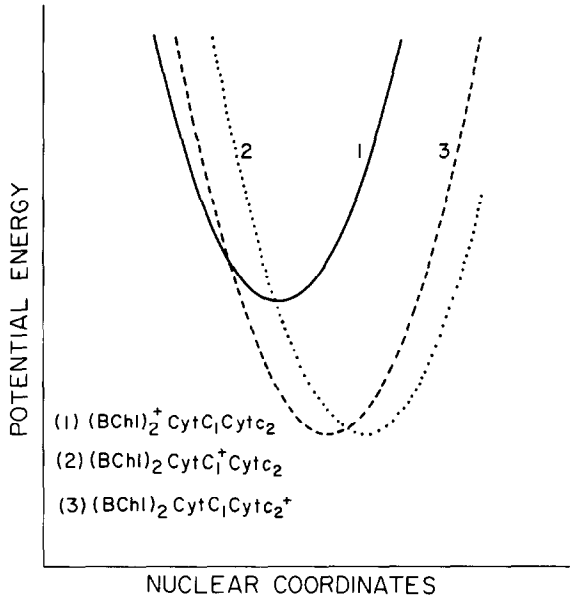

Fig.1. A schematic representation for the nuclear potential surfaces for the two parallel ET cytochrome oxidation reactions in *Chromatium*. Two low-potential cytochromes, labelled cyt c_1 and cyt c_2, can transfer an electron to $(BChl)_2^+$. The activationless reaction (I) involves the transition from the potential surface (1) to the potential surface (2), while the activated reaction (II) corresponds to the transition between the potential surfaces (1) and (3).

low-frequency modes. These distinct medium reorganization energies, E_m, for the parallel ET reactions from the cyt c molecules are due to: (i) different local protein configurations of polar groups around the two cytochromes; (ii) different donor-acceptor distances, which provide different electrostatic contributions to E_m. We shall now proceed to analyse the temperature dependence of reactions (I) and (II) in terms of the nonadiabatic multiphonon ET theory [4–8,15].

3. APPLICATION OF THE ELECTRON TRANSFER THEORY

The ET rate constant, k, can be expressed in the well-known form [4–8,15]:

$$k = (2\pi/\hbar)|V|^2 F, \qquad (1)$$

where V is the two-centre, one-electron exchange (or superexchange) integral and F is the thermally averaged nuclear overlap factor. The latter nuclear

term involves two contributions [13]; the medium reorganization energy E_m and the intramolecular reorganization energy E_c, with the total nuclear reorganization energy being $E_r = E_m + E_c$. It has been demonstrated that [9] the major contribution to E_r originates from the changes in the equilibrium configurations of the medium modes, i.e. $E_m/E_c \ll 1$. Under these circumstances a single-mode approximation to the ET rate is applicable and eqn 1 reduces to [15]

$$k = \frac{2\pi |V|^2}{\hbar^2\omega}\left(\frac{\bar{\nu}+1}{\bar{\nu}}\right)^{p/2} \exp[-S(2\bar{\nu}+1)] \times I_p(2S\sqrt{\bar{\nu}(\bar{\nu}+1)}), \quad (2)$$

where ω is the (average) effectively coupled vibrational frequency, $\bar{\nu} = [\exp(\hbar\omega/kT) - 1]^{-1}$ is the thermal population of that mode, $S = E_r/\hbar\omega$ is the nuclear reorganization energy in frequency units and $p = \Delta E/\hbar\omega$ is the energy gap ΔE, i.e. the free energy of the reaction, in frequency units. Regarding the parameters required for the quantitative description of reactions (I) and (II), we chose the energy gap $\Delta E = 3500\ cm^{-1}$ [1] for both reactions.

The characteristic average phonon frequency was chosen as $\hbar\omega = 100\ cm^{-1}$, which constitutes a reasonable average of the frequency spectrum of typical proteins [24].

Reaction (I) is attributed to an activationless process, whose reorganization energy $E_r^{(I)}$ is equal to the energy gap, i.e., $E_r^{(I)} = \Delta E = 3500\ cm^{-1}$, so that $p = S = 35$. Eqn 2 for an activationless process in the strong coupling limit, i.e. $S \gg 1$, reduces to

$$k = k(T=0)\left[\frac{\exp(\hbar\omega/kT) - 1}{\exp(\hbar\omega/kT) + 1}\right]^{1/2} \quad (3)$$

where

$$k(T=0) = \frac{2\pi |V|^2}{\hbar^2\omega(2\pi p)^{1/2}} \quad (4)$$

is the low-temperature ($kT \ll \hbar\omega$) rate. The low-temperature data for the Chance-DeVault reaction can fit eqn 3 well with $k_I(T=0) = 500\ s^{-1}$ and $\hbar\omega = 100\ cm^{-1}$ (fig.2). The value of $k_I(T=0)$ results in the low value of the electronic coupling $|V_I| = 8 \times 10^{-4}\ cm^{-1}$.

Reaction (II) is an activated process, for which eqn 1 reduces in the high-temperature limit ($kT \gg \hbar\omega$) to the well-known form [4–8]

$$k = \frac{2\pi |V|^2}{\hbar(4\pi E_r kT)^{1/2}} \exp\left[-\frac{(\Delta E - E_r)^2}{4E_r kT}\right] \quad (5)$$

The high-temperature ($T > 120$ K) data (fig.2) were fitted by eqn 2 using $\hbar\omega = 100\ cm^{-1}$ and $\Delta E = 3500\ cm^{-1}$ and adjusting the values of $S(E_r/\hbar\omega)$ and $|V|$. The best fit is obtained by using $E_r = 900\ cm^{-1}$ and $|V_{II}| = 1.8\ cm^{-1}$. The low-temperature tunnelling limit of reaction (II) should be exhibited below 20 K. This tunnelling process corresponds, according to eqn 2, to the rate $k_{II}(T=0) = 1\ s^{-1}$, which is negligible relative to the experimental low-temperature rate ($500\ s^{-1}$).

The fit of the experimental data in terms of reac-

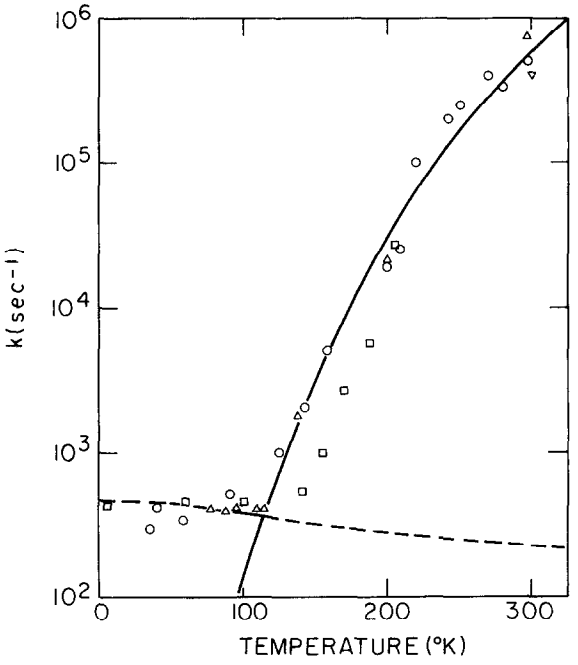

Fig.2. The temperature dependence of the rates of the Chance-DeVault cytochrome oxidation reactions in *Chromatium*. The points represent experimental data from the following sources: ○, [1]; △, [2]; □, [3]. Dashed and solid curves represent the rates of the two parallel ET reactions. Dashed curve corresponds to an activationless process, eqn 3, with $k(T=0) = 500\ s^{-1}$ and $\hbar\omega = 100\ cm^{-1}$. Solid curve corresponds to an activated process with $\Delta E = 3500\ cm^{-1}$, $E_r = 900\ cm^{-1}$ and $|V| = 1.8\ cm^{-1}$.

tions (I) and (II) (fig.2) is as good as can be expected. The validity of our new mechanistic description of cytochrome oxidation does not solely rest on the excellent fit of the experimental data, as this research area has previously been fraught with very convincing fits between theory and experiment for cytochrome oxidation [4–8], which resulted in unreasonable physical parameters. Rather, our model should be judged on the basis of the plausible physical parameters emerging from our analysis. In particular, we note that:

(i) The nuclear reorganization energies $E_r^{(I)} = 3500\ cm^{-1}$ and $E_r^{(II)} = 900\ cm^{-1}$ for the two parallel ET processes are reasonable. These are attributed to the protein medium reorganization energy, which involves the dislocation and rotation of polar groups of the protein around the prosthetic groups. These values of the medium reorganization energy are close to the corresponding values of E_r recently derived [9] for other ET processes in the reaction centre. The physically unreasonable huge intramolecular reorganization energy, which has emerged from previous analysis [8], has now been eliminated.

(ii) The electronic coupling terms for reactions (I) and (II) are widely different, i.e., $|V_I/V_{II}| \simeq 0.5 \times 10^{-3}$. This result implies a large separation between the two distinct low-potential cytochromes involved in the low-temperature and in the high-temperature ET processes. The low-temperature activationless ET occurs from a distant cytochrome, while the high-temperature activated ET proceeds from a cytochrome which is closer to the dimer. Invoking the primitive relation for the distance scale (R) of the electronic coupling [4,5,15] $V\alpha\exp(-\alpha R)$ with $\alpha \simeq 0.6\ Å^{-1}$ [4,5,25], we obtain a rough estimate of $R_I - R_{II} \sim 13$ Å for the differences in the distances of the two low-potential cytochromes from the special pair. This distance is consistent with information derived from ESR data [15], which implies that the separation between the two low-potential cytochromes in *Chromatium* exceeds 10 Å, while crystallographic data for *Rps. viridis* yield the centre-to-centre distance of ~14 Å between two cytochromes [18].

4. CONCLUDING REMARKS

Our new analysis of the celebrated Chance-DeVault cytochrome oxidation in *Chromatium* in terms of two parallel reactions resolves some of the mysteries which were prevalent in the theoretical interpretation of ET in bacterial photosynthesis. Low-temperature nuclear tunnelling is, of course, a perfectly acceptable physical process. However, we propose that for the cytochrome oxidation this process is masked by a parallel activationless ET reaction, which is efficient at low temperatures. We are aware of one additional report of low-temperature-independent ET rate in hybrid hemoglobin [26], which was attributed to nuclear tunnelling. However, the small low-temperature rate in [Zn Fe^{3+}] hybrid hemoglobin [26] was derived on the basis of the assumption that the triplet-ground state intersystem crossing is identical for the [Zn Fe^{3+}] and [Zn Fe^{2+}] systems. Obviously, further work is required to establish the occurrence of low-temperature nuclear tunnelling induced ET in [Zn Fe^{3+}] hybrid hemoglobin. Our analysis regarding the Chance-DeVault reaction accommodates the cytochrome oxidation within the framework of other multiphonon ET processes in the reaction centre of photosynthetic bacteria, which involve the protein modes to the nuclear reorganization energy [9] as the major ingredient. From the point of view of general methodology, our iconoclastic proposal regarding the Chance-DeVault reaction carries a disappointing message. It would appear that low-temperature tunnelling over a nuclear barrier for ET in biological systems has not yet been documented.

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