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The influence of electrostatic interactions and intramolecular dynamics on electron transfer from the cytochrome subunit to the cation - radical of the bacteriochlorophyll dimer in reaction centers from Rps. viridis

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Abstract Interheme electrostatic interaction can explain the acceleration of the electron transfer (ET) rate from the highest potential heme (C_{380}) to the photooxidized bacteriochlorophyll dimer (P^+) which takes place after the reduction of neighbouring heme(s) of the cytochrome subunit in the reaction center of Rps. viridis. The electrostatic interaction energies calculated for neighbouring hemes, 7.0 Å apart (edge-to-edge), and for two high potential hemes, 21.5 Å apart are found to be 0.110 eV and 0.040 eV respectively. The reorganisation energy of the $C_{380}-P^+$ transition of about 0.290±0.030 eV is calculated using the Marcus theory of electron tunneling. An empirical relation for the rate of ET is given. The low temperature restriction of the $C_{380}-P^+$ transition is caused by an energetic inhibition which originates from an opposite shifting of the energy levels of C_{380} and P^+ due to the freezing of protein dynamics and protein-bound water mobility. The freezing of the protein dynamics is revealed by the Mössbauer effect and correlates with the efficiency of the ET.

Key words Reaction center · Cytochrome · Electron tunneling · Dynamics · Mössbauer spectroscopy

Abbreviations RC, reaction center · P^+, cation-radical of bacteriochlorophyll dimer · C_{380}, C_{20}, C_{310}, C_{-60}, hemes, indexed by the values of their individual redox potentials (in mV) · ET, electron transfer.

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Introduction

In photosynthesis the primary charge separation and the subsequent electron transfer across the membrane occur in a protein called the reaction center (RC). For the RC from Rps. viridis a detailed picture of the position and the orientation of the electron donor and acceptor groups is available (Deisenhofer et al. 1985). Therefore, this molecule appears to be an ideal model for studying the mechanism of the electron transfer in biological and chemical systems.

The RC contains a dimer of bacteriochlorophyll as a primary donor of electrons (P), and subsequent chains of acceptors and donors of electrons. The acceptor chain is formed by the following molecules: a monomeric bacteriochlorophyll (B), a bacteriopheophytin (I), and the primary and the secondary quinones (Q_A, Q_B). A chain of secondary electron donors is formed by the four hemes of the tightly bound cytochrome subunit. The hemes (C_i), indexed by the values of their individual redox potentials (in mV), are located according to their increasing distance from P in the sequence C_{380}, C_{20}, C_{310} and C_{-60} along the chain of reducers (Deisenhofer et al. 1985, Fritzsch et al. 1989). The sequence of donors and acceptors can be represented by the scheme:

$$C_{-60}-C_{310}-C_{20}-C_{380}-P-B-I-Q_A-Q_B$$

The edge-to-edge distance between P and the nearest heme C_{380} equals 12.3 Å.

The kinetics of electron transfer between different donor and acceptor groups in the RC have been characterized in detail, including the temperature dependence. The analysis of the kinetic data and theoretical computations give rise to several questions concerning the electron transfer mechanism of redox reactions in the RC. Some of these questions related to the influence of electrostatic interactions and intramolecular dynamic properties of protein are discussed in this paper.

The peculiarities of the C_{380}–P^+ electron transfer at low temperature

After the absorption of a photon the electron from the excited level of P moves rapidly along the acceptor chain B, I, Q_A, Q_B. As result a cation radical P^+ with a redox potential of 520 mV (Dohse et al. 1995) is formed. The subsequent conversion of P^+ to P depends on the redox potential of the medium in which the experiment is performed. The consequent decrease of the redox potentials is accompanied by the consecutive reduction of the hemes C_{380}, C_{310}, C_{20}, which – as was shown by Dracheva et al. (1988); Kaminskaya et al. (1990); Shopes et al. (1987) and recently, more precisely, by Ortega and Mathis (1993) – has some paradoxical influence on the kinetics of the regeneration process. This will be discussed in the following.

The C_{-60} heme can be reduced at low redox potentials (E_h <–60 mV) but under these conditions the Q_A is simultaneously reduced because its E_m is about 0.0 mV (Shopes et al. 1987). After a light flash the electron transfer (ET) cannot proceed to Q_A and instead of the C_{380}–P^+ transition a faster electron back transfer from I to P^+ takes place.

If at the moment of P^+ formation the C_{380} is the only reduced heme, the C_{380}–P^+ transition takes place with a rate constant $k_1 = 3.0 \cdot 10^6\ s^{-1}$ at room temperature. In the following we take into account the dominant component of the P^+ reduction by C_{380} oxidation as described by Ortega and Mathis (1993) as "very fast component" while the other slower components are treated as a product of the heterogeneities in the RC. When dealing with the rate k of the electron transfer (ET) we use the indices 1, 2, 3 to show if one, two or three hemes are reduced.

If two hemes (C_{380} and C_{310}) are reduced the "normal" sequence of transitions is observed at room temperature: a slightly accelerated oxidation of the proximal C_{380} heme with the rate $k_2 = 3.6 \cdot 10^6\ s^{-1}$ and a reduction of the C_{380} heme by ET from C_{310} with the rate of $4.0 \cdot 10^5\ s^{-1}$ (Ortega and Mathis 1993). However, if at the instant of the photooxidation all three hemes are reduced, only the oxidation of the C_{20} heme can be observed experimentally. The reduction of P^+ occurs with the increased rate $k_3 = 6.0 \cdot 10^6\ s^{-1}$ which is the same as the oxidation rate of C_{20}. On the basis of these data one could come to the paradoxical conclusion that the rate of the ET from the distal C_{20} heme, – its distance from P is almost twice as large as that of the proximal C_{380} heme, – is nevertheless twice as fast as the rate of ET from the proximal C_{380} heme to P^+. However, such a conclusion contradicts well known theoretically and experimentally proven results that the rate of tunneling decreases exponentially with an increase of the distance of tunneling transfer (Markus and Sutin 1985; De Vault 1980).

As was pointed out quite early (Markus and Sutin 1985; De Vault 1980) this paradoxical observation can be explained by the assumption of two-electron correlated tunneling, with the accelerated C_{380}–P^+ transition as the first stage and very fast filling of the vacancy formed at C_{380} by the ET from C_{20} as the second stage. According to the calculation based on the structural and kinetic data (Moser et al. 1992), the C_{20}–C_{380} transition should be faster than $10^9\ s^{-1}$ (Ortega and Mathis 1993; Dalidchick et al. 1994). Therefore, the oxidation of C_{380} heme is difficult to observe experimentally because the population of this state is less than 10^{-3}.

The acceleration of the C_{380}–P^+ transition after reduction of the C_{310} heme or both the C_{310} and C_{20} hemes can be caused by the displacement, δE, of the energy level of the C_{380} heme due to interheme electrostatic interaction as illustrated in Fig. 1. The magnitude of this energy shift can be evaluated using recent experimental data (Ortega and Mathis 1993) and conventional ET theory (Markus and Sutin 1985; DeVault 1980).

The ET rate k is usually expressed by

$$k = 2\pi\hbar^{-1} |V_R|^2 FC \qquad (1)$$

where $|V_R|^2$ is the electronic coupling factor between the initial and final states, FC is the Franck–Condon weighted density of states which is related to the overlap of the nuclear wave functions of the initial and final states and $\hbar$ is Planck's constant. The electronic coupling, V_R, falls off exponentially with an increase of edge-to-edge distance R between donor and acceptor (Markus and Sutin 1985; DeVault 1980)

$$|V_R|^2 = |V_0|^2 \exp(-\beta R) \qquad (2)$$

The coefficient β describes the contribution made by the intervening medium in propagating the wave function. The analysis of the variety of ET reactions in proteins, including RC, reveals that the protein medium acts as a relatively uniform barrier to the electron tunneling, exhibiting a β value of about $1.4 Å^{-1}$ (Moser et al. 1992). A simple approximation based on the picture of tunneling between two narrow potential wells corresponds to

$$\beta = 2\hbar^{-1}(2\ mH)^{1/2} \qquad (3)$$

where m is the mass of the electron and H is the height of the potential barrier with respect to the ground level of C_{380}. This yields H = 1.9 eV, which can be considered as an ionization energy (Fig. 1). A value of 2 eV was suggested for H by DeVault (1980).

The magnitude of electrostatic displacement energy, δE_i, of the electron energy level of C_{380} heme with reduced C_{310} or reduced C_{310} and C_{20} hemes can be estimated as (Dalidchik et al. 1994)

$$\delta E_i = (\hbar/R)(H/2\ m)^{1/2} \ln(k_i/k_1) \qquad (4)$$

where k_1 is an initial rate when only the C_{380} heme is reduced (i = 1) and k_i is the rate after reduction of C_{310} (i = 2) or both C_{310} and C_{20} hemes (i = 3). δE_i causes a decrease in the barrier height, H (see Fig. 1). The results for k_i and δE_i are summarized in Table 1. The initial free energy of the reaction, G_1, calculated as the difference between the redox potentials of P^+ (+0.520 eV) and C_{380} heme (+0.380 eV) would thus be increased by 0.040 eV when the C_{310} heme is reduced and by 0.150 eV when both the C_{310} and C_{20} hemes are reduced (Table 1). A more precise estimation of δE_i must take into account the dependence

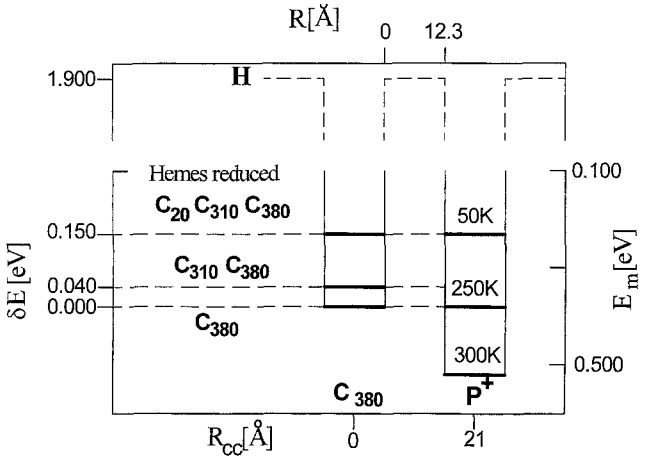

Fig. 1 The scheme of the electrostatic displacements of the energy level of heme C_{380} (δE) at the different redox states of the cytochrome subunit of the RC from Rps. viridis at room temperature (*left side*). The schematic representation of the redox potential (E_m) of P^+ and C_{380} (*right side*), the possible position of the energy level of P^+ at different temperatures is also shown. H is the barrier height for the electron tunneling. R is the edge-to-edge and R_{cc} the center-to center distance between P and the nearest heme C_{380}

Table 1 C_{380}–P^+ electron transfer rates (k_i), electrostatic displacements of the energy level of heme C_{380} (δE_i), free energy of reaction (G_i) and reorganization energy (λ) for different reduced states of hemes in the cytochrome subunit of RC from Rps. viridis. k_i is taken from Ortega and Mathis (1993).

Index i	Reduced hemes	k_i [s^{-1}]	δE_i [eV]	G_i [eV]	λ [eV]
1	C_{380}	$3.0 \cdot 10^6$	0.000	0.140	0.290 ± 0.030
2	C_{380}, C_{310}	$3.6 \cdot 10^6$	0.040	0.180	0.290 ± 0.030
3	C_{380}, C_{20}, C_{310}	$6.10 \cdot 10^6$	0.150	0.290	0.290 ± 0.030

of k_i on the transition energy. But a rough determination of the time of the dissipation of the energy excess of about 0.150 eV considered as a sum of transitions between vibrational levels leads to a value of several ps at room temperature. This is negligibly small compared to the hundred ns time domain of C_{380} heme oxidation. The experimental confirmation of this estimation can be derived from the measured time of the transition of excited P – ground state P^+ transition which is 3.4 ps (Wolker et al. 1994).

The free energy of heme oxidation has been theoretically calculated recently as a pairwise interaction of heme iron with charged amino acids, heme propionic acids, neighbouring hemes and axial ligands (Churg and Warshel 1986; Gunner and Honig 1991). The calculated midpoints of the heme redox potentials (Gunner and Honig 1991) are in good agreement with experiments (Ortega and Mathis, 1993) within an accuracy of ±0.065 eV. This is indeed quite a good agreement when one takes into account the uncertainty of several parameters, e. g. the dielectric constant of the protein interior, assignment of the charges on ionizable residues etc. On the basis of these calculations it was concluded that neighbouring hemes, 14–16 Å apart (center-to-center distance), have an interaction energy of ca. 0.060 eV, while the electrostatic interaction energy between the two high potential or low potential hemes 28Å apart is about 0.010 eV (Gunner and Honig 1991). Within the mentioned error the calculated energies can be considered to be in a reasonable agreement with a magnitude of electrostatic displacement, δE_i, found with an approximation (Eq. (4)). The relationship between the ET rate, k_i, and the free energy of reaction, G_i, can be modelled using the classical Marcus expression for the high temperature limit

$$k_i = (2\pi/\hbar)(4\pi\lambda k_B T)^{-1/2} |V_0|^2 \exp(-\beta R) \exp[-(G_i-\lambda)^2/4\lambda k_B T] \quad (5)$$

where $|V_0|^2$ is the matrix element that couples the electronic wave functions (see Eq. (2)), G_i is the free energy difference with respect to P^+ (see Fig. 1), k_B is Boltzmann's constant, T is the absolute temperature and λ is the reorganization energy required to transform the equilibrium nuclear geometry of the reactant state into the geometry of the product state. Values for k_i can be taken from Ortega and Mathis (1993). This allows the calculation of λ with the assumption that $|V_0|^2$ and λ are not influenced by interheme electrostatic interaction. The data (Table 1) are fitted to Eq. (5) giving an empirical relation for the reaction rate:

$$\log k_i = 14.27 - 0.61\ R - 4.3(G_i-\lambda)^2/\lambda \quad (6)$$

where k_i is given in s^{-1}, R in Å, G and λ in eV, assuming T = 293 K and C_{380} – P^+ edge-to-edge distance R = 12.3 Å. Note, that this equation is rather similar to Eq. (4) in Moser and Dutton (1992) although the kinetics differ significantly in detail.

As was shown by Moser et al. (1992), an examination of the free energy dependence of the intraprotein electron transfer in native or modified proteins can be adequately described at room temperature by Eq. (5) with a β value of 1.4 $Å^{-1}$ and a λ from about 0.030 to about 1.300 eV.

The calculated average value of $\lambda = 0.290 \pm 0.030$ eV is relatively small. This is quite natural since the oxidation and reduction of the low–spin heme in cytochrome-c or of porphyrin species do not involve significant structural changes (Churg et al. 1983; Zheng et al. 1991) in contrast to the redox reaction in high-spin hemes. Moreover, hemes and, particularly, chlorophyll groups are enclosed in the hydrophobic interior of the protein with low permittivity. This can substantially decrease the reorganization energy (Moser et al. 1992). As was shown by theoretical analysis of the X-ray structural data (Churg et al. 1983; Zeng et al. 1991) and by resonance Raman scattering (Schomacker et al. 1984), the reorganization energy of the cytochrome-c redox transition is ca. 0.100 eV. The reorganization energy of P^+ formation in RC from Rps. viridis was calculated to be about 0.130 eV (Parson 1990). Therefore, the sum of the heme reorganization energy (estimated to be close to λ for cytochrome-c) and P^+ formation in RC is expected to be between 0.200 and 0.300 eV. This agrees well with the λ values calculated according to Eq. (6). The Marcus relation Eq. (5) predicts that at the initial stage of the increase of G the ET rate will increase (normal regime).

The maximum of the ET rate will be reached when the free energy is equaly to the reorganization energy (optimized, activationless regime), since according to Markus (Markus and Sutin 1985), the activation energy of the reaction is $E_a = 0.25\,\lambda^{-1}\,(G-\lambda)^2$. When the free energy exceeds the reorganization energy, the ET rate is expected to decrease (inverted regime).

Since the activation energies of C_{380}–P^+ transfer are relatively low – about 0.050 eV (Ortega and Mathis 1993) and also G_3 is about λ, one can speculate that the rate of P^+ reduction by C_{380} is close to its maximal value, i.e. it is optimized. It can be considered as an evolutional bioengineering solution of the problem of rapid regeneration of P^+ to the initial state preparing the RC for a next cycle of charge photoseparation.

The peculiarities of the C_{380}–P^+ electron transfer at low temperature

At all three redox states the temperature dependence of the ET between C_{380} heme and P^+ is characterised by a common feature: the efficiency, Φ, of P^+ reduction abruptly decreases with decreasing temperature (Ortega and Mathis 1993). Φ can be defined as $\Phi = 100 \cdot A/A_0$, where A_0 is the difference in the optical density between P^+ and P immediately after the flash and A is the difference in the optical density between P^+ and P measured after 10 μs i.e. practically under saturation conditions when compared to the ET time at room temperature. The temperature at which Φ equals 0.5 ($\Phi_{1/2}$) depends on the redox state. If before the flash only the C_{380} heme is reduced, then $\Phi_{1/2}$ is about 250 K (Fig. 2). Since at a temperature below 250 K the C_{380} heme cannot be photooxidized, the P^+ reduction takes place owing to the back ET from the reduced Q_A with a relatively slow rate of about $1.4 \cdot 10^2\ s^{-1}$ (Ortega and Mathis 1993). The rate of the C_{380}–P^+ reaction decreases slightly with temperature and shows a weak activation energy of about 0.070 eV. $\Phi_{1/2}$ is shifted to 210 K if in addition the C_{310} heme is reduced before the flash (see Fig. 2).

At the redox state where all three hemes are reduced before the flash the $\Phi_{1/2} = 80$ K (see Fig. 2). From all reduced hemes only the C_{20} heme oxidation is observed experimentally. There are two paths to oxidize C_{20}. At 150 K the efficiency of the fast path for C_{20} heme oxidation starts continuously to decrease and simultaneously the amplitude of another slower path for the C_{20} oxidation starts to increase with a rate of $6.3 \cdot 10^2\ s^{-1}$ (not shown in Fig. 2). It is assumed that at low temperature the neighbouring C_{380} heme is unable to participate in the reduction of P^+, but the more distant C_{20} heme remains fully photooxidizable. This paradoxical fact has been observed in many bacterial species – see Ortega and Mathis (1993) and references cited therein.

What can be the reason for the inhibition of the C_{380}–P^+ ET reaction at low temperature (Fig. 2). It is difficult to explain in terms of an increased C_{380}–P^+ distance resulting from a low temperature conformation change as already proposed (Ortega and Mathis 1993). Let us assume that the C_{380}–P^+ ET is slower than the Q_A–P^+ recombination, so that $k_1 < 1.4 \cdot 10^2\ s^{-1}$. In this case according to Eq. (5) the C_{380}–P^+ distance should be increased by more than 7Å which seems to be unlikely.

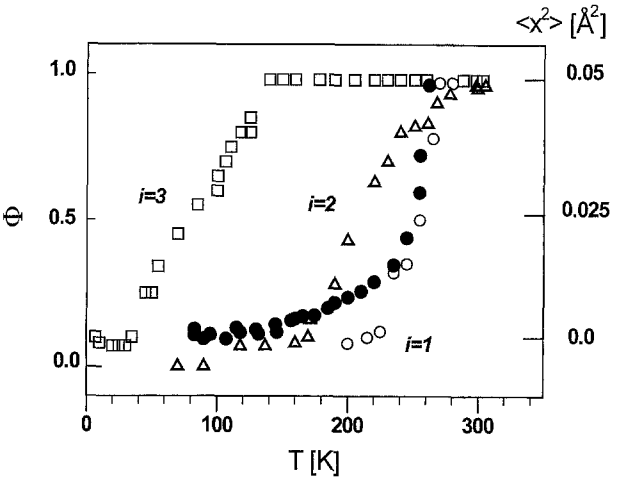

Fig. 2 The temperature dependence of the efficiency, Φ, of the P^+–P regeneration due to C_{380}–P^+ ET at different redox conditions when only the C_{380} heme is reduced (○), C_{380} and C_{310} are reduced (△), or C_{380}, C_{20}, and C_{310} are reduced (□) (taken from Ortega and Mathis [1993]). The temperature dependence of the mean square displacement of iron atoms (●) measured by Mössbauer spectroscopy

In the following we want to extend our room temperature model in order to get a better understanding of the low temperature behaviour.

Considering the low temperature peculiarities of ET in RC one should take into account that the Marcus equation (5) is adequate only at high temperatures, where the Boltzmann thermal energy, k_BT, is large compared to the energy of the characteristic vibrational frequency, $\hbar\omega$, or nuclear motion involved in the reorganization process. At high temperatures the vibrations are fully excited and the ET reaction proceeds as a thermally – activated passage over a potential energy barrier, separating the initial and final states of the system. At low temperature, when $k_BT < \hbar\omega$ the quantum correction should be applied for description of the population of the characteristic vibration mode (Markus and Sutin 1985; DeVault 1980; Levich and Dogonadze 1959; Jortner 1976; Hopfield 1974). Such a correction led to the conclusion that at very low temperatures the displacements of the nuclei involved in the reorganization process comes from tunneling. Compared to Eq. (5) three additional parameters should be taken into consideration at low temperatures: the characteristic frequency, ω, the relative displacement, d, and the reduced mass, M, of the involved nuclei. The reorganization energy in this case is determined by: $\lambda = 0.5\ d^2\omega^2 M$ (DeVault 1980).

As shown by Goldanskii (1979) the relative nuclear displacement, d, determining the rate, k, of ET can be connected with the mean square displacement, $\langle x^2 \rangle$, by

$$k = k_0 \exp\left(-d^2/\langle x^2 \rangle\right) \qquad (7)$$

The mean square displacement in proteins can be measured by methods which allow the determination of the Debye-Waller factor, e.g. X-ray diffraction, neutron scattering or Mössbauer spectroscopy (for a review see Frauenfelder et al. (1988)). Mössbauer spectroscopy yields information at the iron position only, and is sensitive only to true dynamic contributions, while X-ray data also include static contributions (Frauenfelder et al. 1988). The Mössbauer time threshold is of the order of the lifetime of the excited nuclear level which is equal to 140 ns. This fits to the time of the $C_{380}-P^+$ reaction (on the average 100 ns.).

It can be seen from Fig. 2 that by lowering the temperature both the mean square displacement and the efficiency of the $C_{380}-P^+$ reaction decrease simultaneously in the temperature region 250 K when only the C_{380}–heme is reduced. Such a coupling between protein dynamics and ET efficiency has been already seen in the case of the electron transition from the primary to the secondary quinone in the photosynthetic membrane from Rsp. rubrum (Parak et al. 1980). Initial Mössbauer experiments on the RC from Rps. viridis were described by Frolov et al. (1991) and Parak et al. (1990) and here we have extended these data. The effect of freezing the intraprotein mobility on the ET rate and the mean square displacement has been also proposed by Knapp and Fischer (1987).

Taking into consideration the contribution of heme-heme electrostatic interactions and dynamic aspects revealed by Mössbauer experiments one can propose the following picture for the low temperature ET from the cytochrome subunit to the P^+.

i) Only the C_{380} heme is reduced. Equilibrium redox titration or room temperature photoinitiation of the P^+ cation-radical formation involves the movement of the axial histidine residue consuming an energy of about 0.130 eV (Parson 1990). At temperatures were the intraprotein mobility is frozen, the photoinitiation of the P^+ generates an "out-of-equilibrium" state of an induced dipole. The energy level is increased relative to the equilibrium by the reorganization energy of 0.130 eV. In this way it becomes practically the same as the C_{380} energy level. The $C_{380}-P^+$ ET reaction is blocked because $G \approx 0$. At the same time the freezing of intraprotein dynamics also prevents the thermal fluctuations responsible for transitions from the reduced to the oxidized heme configuration which could also diminish G. The picture given explains the correlation between freezing of protein dynamics measured by Mössbauer spectroscopy and the restriction of the $C_{380}-P^+$ ET. For the rearrangement of the axial histidines the dynamics of all parts of the protein are necessary.

ii) The high potential C_{380} and C_{310} hemes are reduced. The energy level of the C_{380} heme is displaced upwards by 0.040 eV owing to the interheme electrostatic interaction, as noted in the first part of this paper. This destroys the energetic inhibition at 250 K existing in the state where only the C_{380} heme is reduced. The $C_{380}-P^+$ ET process is still possible at 250 K. A further decrease of the temperature freezes the protein dynamics completely as indicated by the Mössbauer spectroscopy. As mentioned above, the total protein reorganization energy including P^+ formation and C_{380} redox transition is about 0.240 eV. That means, that a total freezing of protein dynamics makes the ET reaction endothermic again and the $C_{380}-P^+$ ET is again suppressed at 210 K.

iii) The C_{380}, C_{310} and C_{20} hemes are reduced. The energy level of the C_{380} heme is electrostatically shifted up by a further 0.110 eV. The energetic inhibition at 210 K is destroyed and the $C_{380}-P^+$ ET process is again possible even at very low temperature. However, we have at present no explanation for why the reduction of P^+ decreases again at about 120 K. Nevertheless, we want to draw attention to the following facts: the recent refined X-ray structure localized about 20 internal water molecules bound to the cytochrome subunit as well as two water molecules which form H-bridges with the dimer of bacteriochlorophyll (Deisenhofer et al. 1995). The reorganization energy and thermal properties of such intraprotein bound water have to be taken into account. Specific heat measurements indicate that the temperature of freezing of amorphous water spreads to about 100 K (Czybulka et al. 1992). Therefore, the restriction of $C_{380}-P^+$ transition at 80 K could be associated with a disperse freezing of internally bound water. The fast thermally activated processes of the $C_{380}-P^+$ and following $C_{20}-C_{380}$ ET is replaced by slow activationless direct $C_{20}-P^+$ transition.

Concluding remarks

The contribution of heme-heme electrostatic interactions together with the protein dynamics revealed by Mössbauer experiments resolves some of the paradoxical peculiarities of the ET from the cytochrome subunit to P^+ and provides a more consistent picture.

The interheme electrostatic interaction plays a double role: it is responsible for the acceleration of the ET rate from C_{380} to P^+ and it compensates for the lack of free energy of reaction arising from freezing of the reorganization process at low temperatures. A reorganization can only take place if there are enough degrees of motional freedom. The freezing of protein dynamics is displayed by the Mössbauer effect as glass-like transitions at about 240 K and 180 K. The behaviour of the water molecules strongly bound in the protein interior is still not well understood at low temperatures and may be connected with the ET below 120 K.

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