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Fast electron transfers in photosynthetic reaction centre: effect of the time-evolution of dielectric response

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Abstract

The total dielectric response of a protein in due to different kinds of motion each of them having its own, and often very different characteristic time. This situation has been described phenomenologically in terms of a set of effective dielectric constants ϵ_τ operative in the corresponding time intervals. The effects of this time dependence on the reaction free energy ΔG and on the medium reorganization energy E_s were considered employing the models of an infinite homogeneous dielectric and of a three-layer membrane. For the primary charge separation, the effects of the variable ϵ_τ on both these energies, ΔG and E_s, are practically equal and opposite in sign, and hence compensate each other. As a result, the primary charge separation proves to be activationless irrelevant to the value of ϵ_τ. On the basis of the existing experimental data, the semiquantitative estimates of ϵ_τ at different times were given. With these values of ϵ_τ, the activation energy close to zero was calculated for the fast reactions of electron transfer from bacteriopheophytin to quinone and of recombination of the primary radical-ion pair with formation of a neutral triplet.

Keywords: Photosynthesis; Electron transfer; Charge separation; Dielectric relaxation; Protein relaxation; Protein as a dielectric

1. Introduction

The principal electron transfer processes in photosynthetic reaction centre (RC) are very fast reactions developing in a picosecond time intervals. These reactions (primary charge separation, pheophytin–quinone electron transfer) are practically activationless (for review see, e.g., [1–4]).

There are some approximations and not fully clarified points in the quantitative theoretical description of the kinetics of these processes in a very wide range of temperatures [5–8]; however, the activationless character of these reactions, at least under physiological conditions, seems to be well established.

For an activationless reaction, the (negative) energy gap between the initial and final states coincides with the reorganization energy of this process. This is clear from the well known expression for activation energy of the elementary act of reaction (E^*)

$$E^* = \frac{(E_r + \Delta G)^2}{4E_r} \tag{1}$$

where E_r is the reorganization energy (which can be represented as a sum of the inner-sphere E_i and medium E_s contributions), ΔG is the reaction free energy (at a given partners disposition).

The medium reorganization energy characterizes the process of the medium repolarization due to the charge transfer, it is determined by the inertial part of the dielectric response of the reactants surroundings [3,9]. In the case that the dielectric relaxation time is much shorter than the time of reaction, the rate of dielectric response does not affect the reaction kinetics. However, when these times are comparable (the situation possible, e.g., for adiabatic reactions in solutions), the relaxation parameters influence directly the rate of the charge transfer; in particular, the pre-exponential factor in the rate equation may become inversely proportional to the so-called 'longitudinal' (or 'constant-charge') relaxation time. The problem of the dielectric relaxation effects in charge-transfer kinetics is

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Abbreviations: *Rb., Rhodobacter*; *Rps., Rhodopseudomonas*; RC, reaction centre; P, special pair (bacteriochlorophyll dimer); Bph, bacteriopheophytin; Q_A, Q_B, quinones in branches A and B

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now in focus of many investigations, including those related to electron transfer in the photosynthetic reaction centre (see, e.g., [10–15] and the literature cited therein).

The protein inertial polarization is made up of several contributions: changes of covalent bonds lengths and angles, libration of side chains, turns and bends of backbone resulting in the shifts of protein fragments of various size. It is known that, in proteins, a hierarchy of different motions exists, their characteristic relaxation time varying by several orders of magnitude [16–19]. The polarization processes which are many orders of magnitude slower than the reaction rate cannot manifest themselves in the course of the charge transfer. As regards photosynthetic reaction centres, the experiments with substantially different measurement times give markedly different values of the energy gap between the initial (excited P) and final (P^+Bph) states (these data will be discussed in more detail in Section 3.2). The observed increase in the energy gap was explained in [5,20] as a result of stabilization of the charged state due to a gradual development in time of the protein dielectric polarization.

The purpose of this paper is to explore the consequences of the set of strongly different dielectric relaxation times for the fast electron transfers, especially in RC.

2. The model

In the papers quoted above [10–15], a dielectric relaxation process was considered which influenced directly the reaction rate. In the present communication, we will not touch the problem of an immediate effect of dielectric relaxation parameters on the charge transfer kinetics. We will consider the protein as a dielectric medium having several relaxation times differing so strongly that, for some time interval elapsed from the start of the process, a part of the possible polarization changes is already completed while the polarization of the other kinds has not yet entered the scenario. This can be expressed as a set of effective dielectric constants ϵ_τ characteristic of definite time intervals. This time dependence is due to the temporal development of the components of inertial polarization, the inertialess contribution (ϵ_0) being practically constant.

As ϵ_τ is time-dependent, all the energies (e.g. of solvation, interaction, reorganization) are also functions of time.

For spherical particles, the charging energy of an ion of radius a is

$$E_{ch} = \frac{e^2}{2\epsilon_\tau a} \tag{2}$$

where e is the electronic charge (we will consider, if not specified, only neutral and single-charged reactants and a one-electron transfer). The Coulombic interaction energy

$$E_c = \pm\frac{e^2}{\epsilon_\tau R} \tag{3}$$

signs + and − correspond to two ions of similar (+) or opposite (−) polarity, R the distance between their centres. The medium reorganization energy, E_s, is

$$E_s = e^2\left(\frac{1}{\epsilon_0} - \frac{1}{\epsilon_\tau}\right)\left(\frac{1}{2a_1} + \frac{1}{2a_2} - \frac{1}{R}\right) \tag{4}$$

All these formulae relate to a homogeneous infinite medium. Strictly speaking, this condition is not met for RC because the reactants are situated inside a membrane of a rather complex structure surrounded by an aqueous electrolyte solution. This question was analyzed in the first approximation in [21]. In the case of the reactant being not too close to membrane boundaries, the corrections in E_s and ΔG due to heterogeneity of the medium are of subordinate importance. At the moment, we will not consider them explicitly because our first purpose is to elicit the main physical effects connected with the temporal dependence of ϵ. Later, we will discuss the heterogeneity effects more quantitatively.

The molecules of chlorophylls, quinones etc. are far from spherical: much closer to some oblate spheroids. The formulae for a spheroidal reactant were derived in [22]; practically, Eqs. (2) and (4) can be used, substituting for radii some effective values. Their estimates were given in [23].

Eqs. (2)–(4) presuppose that the electric field of an ion is a field of a point charge, undistorted by the presence of the second particle which introduces, in principle, some inhomogeneity in the surroundings of the first ion. In our case, we deal with the reactants of fairly high polarizability; hence, their effective dielectric constants should be somewhat higher than the average ϵ_0 of protein. At the same time, they should be lower than protein's static constant ϵ_s. As ϵ_0 and ϵ_s are quantities of a similar order of magnitude (e.g., 2.5 and 4), and $\epsilon_\tau \leq \epsilon_s$, we can conclude that the effective ϵ of reactants is rather close to ϵ of surroundings and hence the field distortion can play only a minor role.

As stressed above, the time-evolution of ϵ results in time-evolution of both E_s and ΔG. Let us consider now from this point of view specific cases of electron transfer in RC.

3. The primary charge separation

3.1. The model of an infinite medium

In the process of primary charge separation two ions arise from the originally neutral particles. Let us designate ΔG^0_{cs} the free energy of the reaction under conditions of an equilibrium polarization, i.e., polarization corresponding to ϵ_s. Then ΔG_{cs} under nonequilibrium conditions, i.e., at some moment τ (dielectric constant ϵ_τ) is

$$\Delta G_{cs} = \Delta G^0_{cs} + e^2\left(\frac{1}{\epsilon_\tau} - \frac{1}{\epsilon_s}\right)\left(\frac{1}{2a_1} + \frac{1}{2a_2} - \frac{1}{R}\right) \tag{5}$$

Here it is taken into account that charging energies and Coulombic attraction energy at ϵ_τ differ from the equilibrium ones (i.e., at ϵ_s)[1]. The term in parentheses in Eq. (5) describing these energies is similar in form to the last term of Marcus equation (4). Substituting (4) and (5) in (1) we obtain

$$E^* = \frac{\left[E_i + e^2\left(\frac{1}{\epsilon_o} - \frac{1}{\epsilon_s}\right)\left(\frac{1}{2a_1} + \frac{1}{2a_2} - \frac{1}{R}\right) + \Delta G^o_{cs}\right]}{4\left[E_i + e^2\left(\frac{1}{\epsilon_o} - \frac{1}{\epsilon_\tau}\right)\left(\frac{1}{2a_1} + \frac{1}{2a_2} - \frac{1}{R}\right)\right]} = \frac{(E^o_r + \Delta G^o_{cs})^2}{4E_r} \tag{6}$$

Here, the inner-sphere reorganization energy, E_i, is independent of τ. The numerator of this equation does not depend on the value of ϵ_τ: the effects of changing E_s and ΔG_{cs} with changing ϵ are opposite and strictly compensate each other. Both remaining parts of the numerator correspond to equilibrium (at ϵ_s) values of E^o_r and ΔG^o_{cs}. That means that if this numerator equals zero at equilibrium values of ϵ_s, it will remain zero at any moment of the temporal evolution of dielectric response.

In our previous paper [23] we have concluded that $E^o_r \simeq -\Delta G^o_{cs}$, i.e., $E^* \simeq 0$, using experimental estimates of ΔG^o_{cs} and calculating E^o_s with the aid of an equilibrium ϵ_s (for a more detailed discussion, see the next subsection). According to Eq. (6) this result holds true also under conditions of nonequilibrium polarization.

Speaking of the activation energy being equal to zero, we should keep in mind that, to agree with the experimental data, it is enough to have $E^* \ll kT$, the behaviour of the process will be indiscernible from a strict activationless reaction.

As was noted above, Eqs. (2)–(4) imply several approximations. It should be stressed, however, that some of them (effectively spherical shape, the field not distorted by the second ion's body) bring about practically equal corrections both in E_s and ΔG_{cs}, and they compensate each other, not affecting our main conclusion. The problem of a nonuniform dielectric will be considered in the next subsection.

A lowering of temperature will bring about considerable changes in dielectric constant of the medium: a drastic change in the dynamic properties of an RC at low temperatures is a well-known fact [24]. That will result in a corresponding change in the process energetics (the necessity to account for the temperature dependence of E_s was stressed recently by Franzen and Boxer [25]). In discussing this problem, we can use the considerations similar to the given above: decrease in ϵ_s due to 'freezing-out' of some polarization modes will affect both E_s and ΔG_{cs} in opposite directions, and the sum of them will remain the same as the steady-state value at ambient temperature, i.e., close to zero. This explains why, in spite of a self-evident effect of the temperature on the reaction energetics, the (classical) contribution into the activation energy remains practically temperature-independent.

Strictly speaking, with the temperature lowering, some increase in optical dielectric constant will take place because of an increase in protein's density. However, the change of ϵ_o is much smaller than that of ϵ_s. For instance, the volume of myoglobin decreases at 80 K only by 3% as compared to 300 K [26]: at constant molar refraction, this will increase ϵ_o from 2.5 to 2.57 causing a decrease in the reorganization energy by 0.015 eV, a rather low decrement. At the same time, the main expected effect of low temperature, viz. a drastic decrease in ϵ_s, is fully compensated.

For a true activationless reaction, decrease in E_r with decreasing temperature will result in some additional acceleration because the pre-exponential factor is inversely proportional to $(E_r T)^{1/2}$ (see, e.g., [2,3,5,9]). This effect may contribute into an unexpectedly fast increase in the rate of the activationless electron transfer at low temperature. It is more important, however, that the 'freezing-out' of some polarization modes means that their behaviour becomes a quantum one (instead of a classical one at room temperatures), and hence the process cannot be described in a quasiclassical approximation. More appropriate here is the quantum multiphonon theory taking into account the distribution of the frequencies of polarization modes. In some cases, the experimental data could be successfully simulated with only one effective mode [5,7], in other cases the introduction of at least three modes is shown to be necessary [6].

Eq. (6) can be applied, at least semiquantitatively, to any charge separation reaction in arbitrary homogeneous media. For instance, it can explain why for an optical electron transfer ($DA + h\nu \Rightarrow D^+A^-$) in different media the transition energy depends rather on ϵ_o than on ϵ_s [27]. This energy is the sum of E_r and ΔG_{cs}, and this sum is, as we have seen, independent of ϵ_s. However, this is strictly true only in the case when the charge transferred and the final charge of each of reactants are the same, i.e., if the values of e in Eqs. (2)–(4) are identical. In the case of the partial charge transfer in the initial state (e.g., $D^{+\delta}A^{-\delta} \Rightarrow D^{+\delta+\gamma}A^{-\delta-\gamma}$) this identity is violated and the independence of λ_{max} on ϵ_s will be observed only approximately, the better the smaller is δ in comparison to γ.

In our analysis, we considered the reaction medium as an homogeneous structureless dielectric. At the same time, one of the main characteristic features of proteins is the presence of some 'preorganization', i.e., of some coordinates-dependent permanent electric field due to a definite and fixed arrangement of numerous polar groups, the field

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[1] We do not consider in principle a possible small effect of the variable ϵ_τ on the energy of the excited state. This energy was found to be practically time-independent [20].

existing before the introduction of a free charge into the medium [28–30]. This electric field can influence substantially the energetics of the charge transfers including those in RC. The magnitude of the field depends, naturally, on the dielectric constant of the medium. However, the distinction of this dielectric constant from the value ϵ_τ lies in the fact that the preexisting dipole field is independent of the appearance of the newly-formed charge, and hence it corresponds to the static value ϵ_s. This is one of the typical cases when the value of ϵ to be used in calculations depends on the problem under consideration [31]. Time-dependent dielectric response to the creation of new charges described formally by ϵ_τ superimposes on the permanent preexisting dipole field. The effect of the latter forms a part of ΔG°_{cs} and is time-independent.

3.2. *The model of a three-layer membrane*

The approximation of an infinite homogeneous dielectric should work well in the case of reactants immersed deep enough inside the membrane [21], and it was concluded in [23] that the geometry of RC corresponds to this condition. In our subsequent work [32], a more sophisticated model was developed which describes better the membrane electric responses. In this model, we took into account the different dielectric properties of the membrane's low-polar inner core (hydrocarbon chains of lipids, α-helical segments of the hydrophobic intramembrane proteins) and of the membrane's outer layers of intermediate polarity (lipid polar heads, hydrophilic segments of proteins). The different dielectric constants of inner and outer parts of a photosynthetic membrane reveal themselves in the magnitudes of electrogenic effects in these membranes [33].

With an account of this more complex structure of membrane, we see that the redox reactants of RC are really far from aqueous phase but they are close to the intermediate layer which ϵ_s is estimated usually as lying between, e.g., 10 and 20 (summarized in [32]). The coupling constant of this layer ($C = 1/\epsilon_o - 1/\epsilon_s$) is substantially larger than for inner core, and hence a correction in the reorganization energy calculations is necessary.

The formulae for reorganization energy in such a multilayer membrane were derived in [34]. Making use of them, we have performed calculations describing the membrane RC as an inner layer 3.3 nm thick with $\epsilon_s = 4$ and two layers at each side of membrane 1.0 nm thick with $\epsilon_s = 16$. Variations of the latter ϵ_s in reasonable limits as well as of thicknesses of layers does not change the results substantially (in the range of a few millielectronvolts).

Among the geometric parameters, that most influencing the calculations is the shortest distance between the centre of the charged particle (the special pair in our case) and the boundary separating the inner core and the outer layer of membrane. This boundary has a conditional character because one should expect more probably a smooth change of dielectric constant along the normal to the surface rather than a sharp drop at some boundary. Nevertheless, in absence of any experimental data on the spatial distribution of ϵ_s, we prefer to use a stepwise model function to obtain a reasonable estimate of the effects due to the medium inhomogeneity. For the position of the boundary we have chosen the plane separating hydrophobic and hydrophilic side-chains of the outer segments of L and M subunits, more precisely, the middle of the α-helices Lcd and Mcd (and Lc, Mc) covering the special pair. The distance from the special pair centre and this boundary equals 0.5–0.6 nm (all the geometric parameters were calculated from the data on homologous structures of *Rps. viridis* and *Rb. sphaeroides*: see, e.g., [35,36]).

The value of ϵ_o for both layers was accepted to be 2.5 (optical dielectric constant of amides equals to 2.1–2.2 but we have to include in the effective value of ϵ_o not only electronic polarization but also the polarization components due to fast, high-frequency quantum modes like, for example, N-H vibrations [9,37]). With $\epsilon_o = 2.5$ and $\epsilon_s = 4$ we get $C = 0.15$: precisely this value of the coupling parameter was used in our previous calculations [23] giving for E_s of the primary charge separation value of 0.22 eV. The correction to this value calculated in the three-layer model equals to 0.08–0.10 eV increasing the total reorganization energy to 0.31 ± 0.01 eV.

Earlier [23], the value $E_s = 0.22$ eV was compared to the experimental $\Delta G_{cs} \simeq -0.2$ eV, and their agreement was considered to be quite good. The new corrected value of E_s is rather far from $-\Delta G_{cs}$ given above. However, one should use the value $\Delta G_{cs} \simeq -0.2$ eV with a great reservation because it corresponds definitely to a non-relaxed, nonequilibrium state while E_s° was calculated using a steady-state static ϵ_s.

From the data on delayed fluorescence in RC of *Rb. sphaeroides*, Woodbury and Parson [20] have found the initial (in the time range of picoseconds) ΔG_{cs} for primary charge separation equal to -0.16 eV, after ~ 10 ns it increased by absolute value to -0.21 eV. However, these data were obtained in presence of Q_A^-, and hence the value of $-\Delta G$ is somewhat diminished by the difference of electric potentials set up by this negative ion at the centres of the special pair and Bph. Woodbury et al. [38] have obtained the initial $\Delta G_{cs} \simeq -0.18$ to -0.19 eV in absence of Q_A; the corresponding value at 10 ns can be estimated as -0.23 to -0.24 eV (a similar value of -0.26 eV was found in [39]; some intermediate state with $\Delta G_{cs} = -0.20$ to -0.21 eV was proposed in [40]). Goldstein et al. [41] have found $\Delta G = -0.26$ eV in their experiments on the triplet decay rate; the time scale of these measurements was about 100 μs. One can suppose that at longer time intervals the $-\Delta G_{cs}$ values will be larger. For the RC of *Rps. viridis*, the recombination kinetics determined by the equilibrium $Q_A^- \rightleftarrows \mathrm{Bph}^-$ gives $\Delta G_{cs} = -0.30$ eV, the characteristic time being few milliseconds [42]. A slightly larger value −0.34 eV was deduced from the data on equilibrium titration [38]. These data are probably somewhat influenced by additional relaxation processes which cannot be purely dielectric in nature (see below, Section 4). However, taking into account the total set of energies discussed above and the general trend of their time evolution we can accept that $\Delta G^{\circ}_{\mathrm{cs}}$ is close to −0.3 eV, and this value agrees quite well with the calculated reorganization energy of 0.31 ± 0.01 eV.

Let us consider now to what extent the heterogeneous structure of membrane will affect the principal conclusion of the previous subsection, namely, that the activation energy of the charge separation process is practically insensitive to the value of ϵ_{s} (and of ϵ_{τ}), in particular that the process will remain activationless in spite of the temporal evolution of the medium dielectric constant.

As is described in detail in [34], the solution of the electrostatic problem for a charge in the multilayer membrane can be presented in the form of superposition of electric fields set up by the charge and several infinite series of its images. In calculation of E_{s}, one should integrate the flux of the vector $\varphi\nabla\varphi$ (φ is potential, $\nabla\varphi$ its gradient) over the surfaces of ions and over the planar boundaries of dielectric layers. The first type of integral depends markedly on the ϵ_{τ} value. However, the same integrals are involved in calculations of the charging and interaction energies of ions, i.e., in ΔG_{cs} calculations. Therefore, just as in the case of E_{s} and ΔG_{cs} calculations for an infinite medium, we have here again a strict compensation of these contributions.

The integrals over planar boundaries have no counterpart in the formulae for ΔG_{cs}, and hence they remain uncompensated. However, their dependence on the ϵ_{τ} value is rather weak. The larger of these integrals equates to

$$e^2\left(\frac{\epsilon_m}{\epsilon_{\mathrm{p}}+\epsilon_{\mathrm{m}}}\right)^2\left[\frac{1}{\epsilon_{\mathrm{om}}}-\frac{1}{\epsilon_{\mathrm{m}}}-\left(\frac{1}{\epsilon_{\mathrm{op}}}-\frac{1}{\epsilon_{\mathrm{p}}}\right)\frac{\epsilon_{\mathrm{p}}}{\epsilon_{\mathrm{m}}}\right]S \qquad (7)$$

where ϵ_{p} and ϵ_{m} are ϵ_{s} for the membrane core and interlayer, respectively, ϵ_{op} and ϵ_{om} the corresponding optical constants, S is the function of geometric parameters which is only slightly dependent on ϵ_{s} values. At the constant ratio $\epsilon_{\mathrm{m}}/\epsilon_{\mathrm{p}}$ (see below) and $\epsilon_{\mathrm{op}}=\epsilon_{\mathrm{om}}$, the coefficient before S is constant, and the variations of S and of the other integrals should be very small. This conclusion has been supported by direct calculations carried out with various sets of parameters. Let us give here as an example the results obtained for a set which seems to be one of the most reasonable.

Due to very fast relaxation of water (the longitudinal dielectric relaxation time lies in the picosecond range) we have accepted ϵ_{s} for aqueous surroundings to be equal to the stationary value, viz., 78. For the intermediate layer which, in the vicinity of the special pair, is proteinaceous, it is reasonable to suppose approximately the same relaxation kinetics as for the intramembrane protein. Hence we accepted its ϵ_{τ} permanently 4-times larger than ϵ_{τ} of the inner core (i.e., it is changing from 12 to 16 during the evolution of the inner ϵ_{τ} from 3 to 4; the ratio of 4 or somewhat larger was deduced in [32] on the basis of the data on electrogenic effects). The other parameters were as listed before. The calculated contribution of all planar-boundary integrals is 0.127 eV but its change with increase of inner ϵ_{τ} from 3 to 4 is only 0.0004 eV. Similar results, viz., effects of order of less than 1 meV, were obtained with the other choice of parameters, e.g., with a constant ϵ_{s} of the intermediate layer. Hence we can conclude that this contribution is practically independent of ϵ_{τ}; that means, in concordance with conclusion of the simplified homogeneous model, that if an analysis based on an experimental equilibrium $\Delta G^{\circ}_{\mathrm{cs}}$ and a steady-state ϵ_{s} predicts the activation energy close to zero, this result will be valid at lower ϵ_{τ} too.

The three-layer model of membrane presents, of course, some approximation, and one could suppose the possibility of a more complex geometry of the regions of different dielectric constants. However, in any case the general structure of the formulae for E_{s} will be the same: integrals over the surfaces of ions and integrals over the other boundaries. As was explained before, there will be an automatic exact compensation of the ϵ_{τ}-dependent contributions in the integrals of the first type and in the corresponding integrals in the expressions for solvation and interaction energies. The integrals of the second type will have the form analogous to Eq. (7), and hence they can hardly lead to a substantial dependence upon ϵ_{τ}. Therefore, the conclusion derived above seems to be rather general.

The calculations in the framework of the multilayer model of membrane have given the E°_{s} value practically equal to $-\Delta G^{\circ}_{\mathrm{cs}}$, the result being almost insensitive to the variation within the reasonable limits of the model parameters. Further, we have shown that the final result, viz. the activation energy close to zero, holds true independently of the time evolution of the medium dielectric response. This conclusion obtained without resorting to any adjustable parameter reveals the physical reason why the primary charge separation in RC is activationless.

We have shown above that the activationless character of the primary charge separation does not depend on the temporal value of ϵ: it is important only that both E_{s} and ΔG_{cs} were related to the same time, i.e., to the same ϵ_{τ}. Let us use this result to get, on the basis of experimental data, some information on the temporal evolution of ϵ. We will start with a steady-state $\epsilon_{\mathrm{s}}=4$ and $E^{\circ}_{\mathrm{s}}\simeq-\Delta G^{\circ}_{\mathrm{cs}}=$ 0.31 eV. From this value, 0.13 eV is the practically ϵ_{τ}-independent contribution of the integrals over planar boundaries, and the rest, i.e., 0.18 eV, involves two contributions: 0.22 eV corresponding to E_{s} in an infinite medium, and a correction of −0.04 eV originating from the integrals over ions surfaces due to the field of images. E°_{s} is proportional to coupling constant $C = 1/\epsilon_0 - 1/\epsilon_T$ (see Eq. (4)). The (negative) correction for the field of images is also proportional to C, but it involves also some dependence on ϵ_T due to dependence of image charges on dielectric constants of all media. However, the latter dependence is rather weak, and the effect of C is predominant. So, with $\epsilon_0 = 2.5$, the values of C at $\epsilon_T = 3$ and 4 differ by 2.5 times while the correction terms calculated by an exact formula differ, depending on parameters, by 2.22–2.23 times. Hence we can state with a sufficient accuracy that the difference ($E_s - 0.13$) eV is proportional to C.

As described above, in the picosecond time span the probable value of $\Delta G_{cs} = -0.18$ to -0.19 eV, after approx. 10 ns $\Delta G_{cs} = -0.23$ to -0.24 eV, and after 100 μs $\Delta G_{cs} = -0.26$ eV. Using the equality $E_s = -\Delta G_{cs}$ and proportionality between ($E_s - 0.13$) and C we can find C, and hence ϵ_T. The three characteristic ϵ_T for these time intervals are, correspondingly,: 2.79 to 2.85; 3.16 to 3.24; 3.42. We see that in the picosecond time interval the inertial polarization almost has not developed, and the dielectric constant remains rather close to its optical (better to say quantum) value ($\epsilon_0 = 2.5$), and even after 10 ns it is far from the static one.

In comparing these continuum calculations with a molecular dynamic analysis, we have to keep in mind that the latter relates to the short time spans, namely picoseconds. Molecular dynamic simulations have shown a very fast protein relaxation with the characteristic time of about 100 fs [43] or 200 fs [44]. No further relaxation was found in the course of 20 ps simulation. So, these data revealed a distinct region of protein dielectric response with a single relaxation time [15] corresponding to the interval several orders of magnitude shorter than the time of many experiments described above (10 ns and longer). The simulations carried out in [43–46] have given reorganization energies of about 0.19 eV–0.22 eV. These quantities match quite well the energy $\Delta G_{cs} = -0.18$ to -0.19 eV characteristic of this time interval. The same result, viz., practically coinciding $-\Delta G_{cs}$ and λ_s was obtained by the longest in time (60 ps) simulation in [47].

4. Pheophytin–quinone electron transfer

In this case, ΔG_{tr} depends not on the sum, as for charge separation, but on the difference of charging energies. Further, it does not involve the direct Coulombic interaction between reaction partners. Hence, the expressions for ΔG_{tr} and E_s for this reaction have a quite different form, and we cannot expect a strict compensation of effects of ϵ_T on these two quantities.

As the reaction takes place before neutralization of the special pair we have to take into account the interaction of the negative charge on Bph or Q_A with the positive charge of P^+. The final equation for an infinite dielectric medium is

$$E^* = \frac{\left[E_r^o + \Delta G_{tr}^o + e^2\left(\frac{1}{\epsilon_s} - \frac{1}{\epsilon_T}\right)\left(\frac{1}{a_1} - \frac{1}{R} + \frac{1}{R_2} - \frac{1}{R_1}\right)\right]^2}{4E_r} \qquad (8)$$

Here subscript 1 refers to pheophytin, 2 to quinone, R_1 and R_2 are distances of the centres of corresponding ions from the centre of the positive charge on the special pair. The last term in the nominator gives a negative correction to the value characteristic of the static ϵ_s(~ -0.11 eV at $\epsilon_T = 3$). Without discussing Eq. (8) in more detail we will proceed to results of calculations according to the three-layer model.

The first problem to consider is the value of ΔG_0. The data on delayed fluorescence from the state P^+Q (in RC of *Rb. sphaeroides*, the characteristic time 0.1 s) give this state lying by 0.86 eV lower by free energy than P^* [38,48]. Subtracting the estimated above $\Delta G_{cs}^o = -0.3$ eV we obtain $\Delta G_{cs}^o = -0.56$ eV. However, this value cannot be considered as corresponding to the energy level stabilized by the dielectric relaxation only. As a matter of fact, a relaxation process with a characteristic time of hundreds of microseconds was observed [49,50]. The nature of this relaxation is not clear, but one can hypothesize, with a rather good probability, that it is due to protonation of some side-chain groups of the protein [2]. In the fast electron transfer, the protonation does not take place, and hence its effect should not be included in the standard value of ΔG_{tr}^o appropriate for the analysis of the process. Protonation affects the energies of both Q_A^- and Bph^-, and hence its influence on the energy difference should be rather weak [42]. According to data of [53], $-\Delta G$ for $P^+Q_A^-$ recombination increases from pH 8 (to this pH relates $\Delta G_{tr}^o = -0.56$ eV) to pH 10 by 0.03 eV. At pH 10, the ΔG-pH dependence shows some trend to a plateau, and we believe that it approaches the maximal value corresponding to the process almost undistorted by the protonation effect. The Bph^-–Q_A transfer should be less affected than $P^+Q_A^-$ recombination because Bph is closer to Q_A and to a hypothetical protonated group than P. Hence without protonation ΔG_{cs}^o should be more positive, probably by ≤ 0.03 eV, and we can accept as a reasonable estimate $\Delta G_{cs}^o = -0.54$ eV.

The second point is the localization of the boundary between the membrane core and the layer of the intermedi-

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[2] The proton uptake upon Q_A reduction is a well documented phenomenon (see, e.g., [42,51–53]. Probably, this effect is the cause of an unexpectedly low influence of the quinone charging on the energy of the primary charge transfer, namely about 0.03 eV [20,38,39], while our electrostatic calculations employing the three-layer model of membrane give a value of 0.11 eV. At longer times (tens of milliseconds), biphasic recombination processes were noticed [54,55]; most probably, they should be ascribed not to some relaxation process but rather to pre-existence of different conformational states [55].

ate polarity. According to structural data [56], Q_A contacts mainly the hydrophobic residues but the nearby Q_B has many hydrophilic residues in its environment, and, moreover, one can discern near it a void filled, probably, with mobile water molecules [57]. Hence we draw the boundary not far from the Q_A centre but at the distance of 0.3 nm, i.e., practically at the van-der-Waals contact with this molecule.

The effective dielectric constant at the times of a few picoseconds was estimated in the previous section as $\simeq 2.8$, at nanoseconds $\epsilon \simeq 3.2$; hence for the process under consideration having a characteristic time of a hundred picosecond the value of $\epsilon_\tau \simeq 3.0$ could be accepted.

With all parameters given above we have calculated a correction to ΔG_{tr} due to the difference of solvation energies of ions in the multilayer membrane at $\epsilon_s = 4$ and $\epsilon_\tau = 3$. The corrected $\Delta G_{\text{tr}} = -0.35$ eV. In the same model and with the same parameters the reorganization energy was calculated to be equal to 0.43 eV, the corresponding activation energy $E^* = 3.7$ meV, i.e., the value low enough to describe the process as practically activationless one. It should be noted that this result is almost insensitive to the choice of ϵ_τ (e.g., for $\epsilon_\tau = \epsilon_s = 4$, $E^* = 3.9$ meV) but at an attempt to choose a boundary closer to Q_A the situation changes substantially: e.g., with this distance equal to 0.2 nm (what seems to be unrealistically low), and at $\epsilon_\tau = 3$ a low but still marked barrier has been calculated $E^* = 33.6$ meV. However, at a farther boundary's position, the activation energy decreases; e.g., at 0.4 nm it falls to less than 0.1 meV. Summarizing, we can conclude that the model predicts, in full concordance with the experiment, the activationless character of this electron transfer.

The reorganization energy for this electron transfer is substantially higher than for the charge separation step: this is necessary to adjust to a larger energy gap. We believe that this adjustment has been achieved by Nature using a proper geometry of the system, primarily a smaller effective radius of the electron acceptor [23].

For the Bph–Q_A reaction, there are in the literature some microscopic simulations [43,58]. Both of them, in concordance with semi-continuum results, give the reorganization energy practically 2-times higher than for the charge separation.

5. Charge recombination

In the charge recombination reaction, contrary to charge separation, the effects of variable ϵ_τ on ΔG and E_s have the same sign, and hence do not compensate each other. The recombination of the P^+Q^- pair is rather slow (~ 0.1 s [6,38,42,48]), and hence the problem of the time-evolution of dielectric constant is practically irrelevant to this reaction. However, another recombination process, namely P^+Bph^- recombination to form the triplet special pair ^3P is fast, the characteristic time being about 2 ns [59].

The energy difference obtained from the experiments of 100 μs time span equals -0.17 eV [41,60] (in this difference, the correction for multiplicity of spin states should not be included because spin rephasing takes place in the radical pair, prior to electron transfer). The reorganization energy for charge recombination is the same as for charge separation. If we tried to combine $E_s = 0.31$ eV calculated for the static value of $\epsilon_s = 4$ with the experimental $G = -0.17$ eV we would obtain a low but non-zero activation energy $E^* \simeq 16$ meV. This does not seem to be in very good agreement with the activationless character of the process [59]. However, as was described in the previous sections, one should use the quantities corresponding to the characteristic time of the reaction. From the data on ϵ_τ obtained in Section 3 we can estimate that at 2 ns $\epsilon_\tau \approx 3.1$–$3.2$. Using this parameter we were able to calculate both E_s and a small correction to ΔG due to transition from $\epsilon_\tau = 3.4$ (~ 100 μs) to $\epsilon_\tau = 3.1$–3.2. The corrected $\Delta G \simeq -0.18$ eV, $E_s = 0.22$ eV, and hence $E^* = 1.8$ meV, i.e., a quite realistic result. We see that the estimates of the temporal variation of the effective dielectric constant based on the data for one reaction provide a reasonable description of the other.

6. Conclusions

The dielectric response of any polar medium evolves in time; for proteins this evolution is much more extended than for usual low-molecular solvents, and it involves motions of substantially different types with characteristic times differing by several orders of magnitude. We describe this situation in terms of a set of effective dielectric constants ϵ_τ corresponding to various time intervals. In the framework of this phenomenological approach, the fast electron-transfer processes in the photosynthetic bacterial reaction centre were considered. The temporal evolution of dielectric constant affects both the free energy of reaction and the medium reorganization energy. The quantitative estimates of the reaction energetics were carried out using two models, namely the model of an infinite homogeneous dielectric, and the three-layer membrane model.

For the primary charge separation, the effects of the variable ϵ_τ on both the reaction free energy and the reorganization energy are practically equal and opposite in sign, the result being true as well for the infinite dielectric as for the three-layer membrane. Therefore, these two effects are mutually compensating, and the process remains activationless irrelevant to the varying value of ϵ_τ. The same relates to the temperature influence on the static dielectric constant.

On the basis of the experimental data on the energy gaps corresponding to different time spans, the effective dielectric constants could be evaluated. They vary substantially in time starting with the value of about 2.8 at a few picoseconds, through $\epsilon_T \approx 3.2$ at 10 ns and ≈ 3.4 at 100 μs, to static value of 4. With these parameters, the activation energies close to zero were calculated for two other fast reactions in RC, viz., for electron transfer from Bph^- to Q_A and for charge recombination in the radical pair P^+Bph^- with formation of the triplet special pair ^3P. All the results described above are only slightly sensitive to variation, within reasonable bounds, of the model parameters.

We are quite aware that the model employed involves some approximations, and that the parameters used are not strictly quantitative. Our task here was to obtain a general picture of the dielectric response in different intervals of time and to outline its influence on the kinetics of the electron transfer in photosynthetic RC. From this point of view, the results presented here can give us a semiquantitative characterization of this phenomenon.

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