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Charge recombination kinetics of photosynthetic reaction centres in phospholipid organized systems

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Abstract. Liposomes, *n*-hexane phospholipids reverse micelles and organogel have been used as model membrane systems in which the photosynthetic reaction centres (RCs) from *Rb. Sphaeroides* have been incorporated. The influence of the liposomial composition on the RC photochemical properties have been tested, using liposomes made of different phospholipids. The charge recombination of flash generated states of the RC solubilized in reverse micelles have been followed as a function of the quinone concentration at two different temperatures. In this mimetic membrane system the quinone molecules are in fast exchange between the Q_B site of the protein and the bulk organic phase. The thermodynamic parameters relative to the electron transfer from Q_A^- to Q_B and to the binding processes of the quinone to the Q_B site have been determined. Complex multiphasic kinetics for RCs in organogel have been observed. In this system, both the quinone binding to the Q_B site and the $P^+Q_A^-$ state stabilization are markedly influenced by the W_0. The role played by the water activity in the RC binding affinity for the ubiquinone molecule has been considered.

Keywords. Photosynthetic reaction centres; liposomes; *n*-hexane phospholipid reverse micelles; organogel.

1. Introduction

The primary process of photosynthesis involves the light-induced transfer of an electron from a primary electron donor to a sequence of electron acceptors. In photosynthetic bacteria, this is accomplished in a pigment-protein complex called Reaction Centre (RC).

The RC is an integral protein complex that spans the bacterial lipid membrane [1], in which the absorption of a photon promotes the primary electron donor, a bacteriochlorophyll dimer (P), to its excited state. An electron is consequently transferred through a molecule of bacteriopheophytin (I) to the first quinone electron acceptor (Q_A, which is located in a hydrophobic pocket of the protein) and subsequently to a secondary quinone molecule (Q_B which binds a relatively polar protein domain). The replacement of the electron on P^+ is performed by a secondary electron donor (a reduced cytochrome (cyt)c_2). In the absence of cyt c_2 the charge recombination between P^+ and Q_B^- is observed. Otherwise, if the Q_B binding site is empty, or in the presence of an inhibitor of the electron transfer between Q_A^- and Q_B, the light-induced charge separation is limited to $P^+Q_A^-$ and, obviously, so is the observed charge recombination process.

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Comparative EPR and ENDOR studies of the radical cation electronic structure of the primary electron donor carried out on four different species of purple bacteria, have clearly shown that the surfactant environment surrounding the reaction centres strongly affects the electronic structure of the cofactors as well as the electron transfer rate [2]. These experimental evidences are a further confirmation of the relevant role played by the membranes in regulating the activity of a large number of membrane-associated proteins [3-6].

In order to clarify any detail in the photosynthetic process involving the RC, the influence of the membrane should be taken into account. To obtain this information however, the study of the intact bacterial cells is not the best approach. In fact, scattering problems together with the complexity of dealing with an intact organism, do not allow the determination of the kinetic and thermodynamic parameters with the needed accuracy. One possibility could be the use of the chromatophores (the native bacterial membrane mechanically broken and then reconstituted). This approach, although extremely useful, is again limited by the intrinsic complexity of the native membranes. For this reason, the use of phospholipid-based membrane models (such as liposomes) is recommended. These systems, in fact, offer to the guest species an environment of defined chemical composition and structure. The use of liposomes incorporating the RC therefore represents a main step towards better comprehension of the energy stabilization of the excited states in photooxidised RCs.

Despite widespread interest on the RC, little is known about RC-phospholipid interactions. However, there are experimental evidences showing that in the case of RC reconstituted in liposomes, the lipid phase transition influences the thermodynamic parameters associated with the electron transfer processes [7,8].

Several papers have been published in the last ten years dealing with the existence *in vivo* of isotropic aggregation forms of the lipids into the membrane. For example, both freeze fracture electron microscopy and ^{31}P-*NMR* reveal the presence of reverse micelles in coexistence with the predominant phospholipid bilayer phase, which are, probably, precursors of hexagonal phase formation [9-11]. Although the existence and the possible role of such structures in the native membranes remain a topic of research and discussion, the reverse micelles provide a powerful tool to investigate *in vitro* the interactions of integral proteins with hydrophobic cofactors such as ubiquinone. Since some of the biological membrane peculiarities are preserved in these structures (e.g. anisotropy and amphiphatic nature), phospholipid based reverse micelles have been extensively used as host-systems for RC from *Rb. sphaeroides*, also because of the retained photochemical activity in these systems [12,13]. As already mentioned, the fluidity of the bilayer affects the charge transfer process taking place within the RC. Such an effect could be conveniently investigated in reverse micelles, as long as a way to modulate the local mobility of the surfactant molecule is available. Some years ago it was found that lecithin reverse micellar solution, in a number of apolar solvents, can be transformed into transparent, highly viscous, and thermodynamically stable viscoelastic systems (organogel) by the addition of tiny (somewhat critical) amounts of water [14]. Such systems can thus provide a tool to investigate the influence of the local viscosity on the photochemical properties of RC, which can be solubilized, without denaturation in these systems [15,16]. Moreover, the structure of the organogels is quite well known (at least for some particular cases), thanks to extensive ^{31}P-NMR [17-19], ^{1}H Self Diffusion NMR [20,21], SANS and light-scattering [22] measurements.

Both the reverse micelles and the organogels allow to carry out a detailed study of the dynamic processes taking place at the interface water/organic solvent, between the RC and liposoluble species (mainly ubiquinone).

In this paper, the kinetic behaviour of the RC charge recombination in the liposomes, in the *n*-hexane phospholipid reverse micelles and in the organogels is discussed.

2. Materials and methods

2.1 *Chemicals*

Ubiquinone- 10 (UQ_{10}), phosphatidylserine (brain extract, type III: Folch fraction III from bovine brain; PS) and phosphatidylethanolamine (type II-S: from sheep brain; PE) from Sigma Chem. Co. were used without further purification. Soybean lecithin (Epikuron 200, 98% pure phosphatidylcholine; PC) was a generous gift from Lucas Meyer Ltd. Sephadex G-50 was purchased from Pharmacia, whilst terbutryne was from Chem Service. All the other chemicals were from Fluka and were of the highest purity available.

2.2 *Samples preparation*

RC was isolated and purified from *Rhodobacter sphaeroides* (R-26 strain) as already described [23], and in each preparation its concentration was spectrophotometrically determined at 802 nm ($\varepsilon_{802} = 288\,mM^{-1}\,cm^{-1}$). For liposomes preparation the lipid(s) were first dissolved in $CHCl_3$ (at this stage, it was possible to add the ubiquinone as a chloroform solution, to obtain the desired RC/UQ_{10} ratio), dried under nitrogen stream, then resuspended in a 10 mM imidazole/ 100 mM KCl and 3% Na-cholate buffer at *p*H = 7, and sonicated on ice for 3 min. The RC was added to the lipidic dispersion at approximately 30 μM of the final concentration, so as to accomplish a final lipid/RC ratio of 4500/1. Typically, 0·5 ml of the final mixture was sonicated twice for 3s, applied onto a Sephadex G-50 column (1 × 10 cm) and then eluted with a 10 mM imidazole/100 mM KCl buffer at *p*H = 7. The fractions containing the proteoliposomes were diluted to a final volume of 2 ml. About the liposomes preparation, a further clarification must be made in order to avoid any misunderstanding. Both PE and PS used in this work were not pure phospholipids; the former purity being, in fact, 70% while in the case of the latter, it was 80%. In both the commercial preparations the contaminants were mostly other phospholipids, among which the phosphatidylcholine was the most abundant impurity (checked by means of quantitative HPTLC). In the discussion of the experimental data we will refer to 'pure PE' and/or to 'pure PS' made liposomes to those preparations obtained using just one commercial lipid at a time. This is particularly a tricky point for the 'pure PE' liposomes. In fact, due to the unfavourable packing parameter [24], pure PE doesn't form lamellar phases at all. Only preparations containing PE and PC can form lamellar phases in general, and liposomes in particular. So, even in those liposomes prepared starting with only PE, it is clear that there is enough phosphatidylcholine to allow the bilayer formation [25].

For the *n*-hexane extract preparation, typically 200 μL of a 1 M $MgCl_2$ solution were added to a 2 ml liposome sample. 3 ml of *n*-hexane were then stratified onto this solution and the whole system stirred for 3 min at room temperature (25 ± 2°C). The two phases were separated by centrifugation for 5 min in a desk centrifuge and the

organic one retained. UQ_{10} was added to the samples at the desired concentration as a *n*-hexane solution.

For the organogels preparation, the water present in the *n*-hexane extracts was removed by means of anhydrous Na_2SO_4 (50 mg/ml) and the suitable amount of lecithin and phosphatidylserine (as concentrated PC/PS 1:1 (w/w) *n*-hexane solution) was then added in order to achieve a final total lipid concentration of 160 mg/ml. To obtain the desired Wo values (W_0 = [water]/[lipids]), water was added to the samples by means of a Hamilton microsyringe.

In the experiments performed at increasing total lipid concentration and fixed W_0 ($W_0 = 4$), the lipids, as concentrated PC/PS 1:1 (w/w) *n*-hexane solution, were gradually added to the *n*-hexane extracts together with the water required to keep constant the W_0.

2.3 *Instrumentals*

The kinetic competence of RCs in all the systems investigated has been checked by means of a single beam spectrophotometer of local design, whose technical details have been described elsewhere [15].

The NIR-VIS spectra have been recorded by means of a Varian Cary 5E spectrophotometer. The actinic light, provided by a 75W tungsten lamp, was directed to the samples by an optical fibreglass guide, shielded with a 10 cm water filter in order to avoid any heating of the sample due to the infrared component of the incident light. Spectra resolution was set at 0·5 nm, with a spectral bandwidth of 2 nm. In all the experiments, the temperature was set at 21 ± 0·1°C by a circulating water bath kept at the right temperature by means of a Haake F3-K thermocryostat.

3. Results and discussion

3.1 *Liposomes*

As already mentioned in the introduction, the interactions among phospholipids and membrane proteins could play a relevant role in modulating the biochemical and/or biophysical properties of the intrinsic proteins. Regarding the bacterial reaction centre, in order to test the influence of the bilayer composition on its photochemical properties, several experiments of flash-photolysis have been performed. The RCs have been incorporated into liposomes made of three different phospholipids (namely PC, PE and PS) in the presence of an excess of ubiquinone ($[UQ_{10}]/[RC] = 50$). In these conditions, the proteins lack any secondary electron donor to P^+, while the functionality of the secondary electron acceptor site, Q_B, is almost completely reconstituted. The photo-oxidation of the bacteriochlorophyll dimer and its dark re-reduction by charge recombination from Q_B can thus be followed by monitoring the absorption change at 600 nm (a minimum in the RC light minus dark spectrum) by means of a single turn-over flash experiment. These experiments should give a hint on the stabilization of the RC charge separated state upon changing the lipidic composition of the bilayer, taking as a reference the RC charge recombination kinetics in LDAO, where its behaviour is quite well known: a 800 ms half-time kinetics characterizes the back reaction in the detergent stabilized protein [1].

The results of such an experimental approach are summarized in table 1, where the charge recombination half-times between P^+ *and* Q_B^- are reported, together with the

Table 1. Charge recombination half-times between P^+ and Q_B^- and ΔG^0 values relative to Q_A to Q_B charge transfer calculated for RC reconstituted in liposomes.

Liposomes	Half-times (ms)	ΔG^0 (meV × mol^{-1})
pure PC	1580	− 75
pure PE	2790	− 90
pure PS	3440	− 96
PC/PE/PS (1:1:2)	1580	− 75
	2460	− 89

$\Delta G^0 s$ relative to the Q_A to Q_B charge transfer. When the Q_B sites are completely occupied or in the presence of a slow exchange (with respect to the $P^+Q_A^-$ recombination) of the quinone bound to this site with those dissolved in the bilayer, the observed half-times of the charge recombination from Q_B^- to P^+ ($t_{1/2P}$) is related to the equilibrium constant relative to the electron transfer from Q_A^- to Q_B (L_{AB}) by the equation [26],

$$\frac{t_{1/2P}}{t_{1/2AP}} - 1 = L_{AB} \tag{1}$$

where, $t_{1/2AP}$ is the half-time of the charge recombination from Q_A^-, experimentally determined in the presence of terbutryne an inhibitor of the electron transfer from Q_A to Q_B. Strictly, (1) defines an apparent equilibrium constant which accounts for both, the electron transfer and quinone binding processes (see § 3.2). However, when the Q_B site is fully occupied, as in the present case, the apparent equilibrium constant and the L_{AB} coincide. From the experimentally determined half-times for the fast and slow components of the P^+ dark relaxation, one can estimate the value of the L_{AB}, and then the corresponding ΔG according to the equation,

$$\Delta G = -RT\ln L_{AB}. \tag{2}$$

The P^+ decay can be accounted for a single exponential in all the 'pure' systems examined. Actually, a small contribution (always less than 10%) of a second exponential component is always found. This second component is characteristic of a charge recombination process involving the first electron acceptor (Q_A) and, due to the aquisition parameter used, it doesn't interfere with the monoexponential fit of the traces. As can be clearly seen in table 1, a generalized slowing down of the charge recombination event, if compared to the LDAO system, is always coupled with the RCs reconstitution in liposomes. In particular, it should be noted that extreme half-time value is obtained with 'pure PS' liposomes. A particularly high stabilization of the charge separated state of the protein should correspond to this particular composition of the bilayer, as confirmed by the very negative value of the ΔG_{AB}.

When the liposomes are made of a mixture of lipids, an interesting feature arises. The experimental kinetic trace cannot be fitted by a single exponential and two exponential components are needed to fit the data. The life-times values obtained are consistent with a charge recombination from Q_B, since they are of the same order of magnitude as

those obtained by the deconvolution of the pure lipid traces. Morover, the addition of terbutryne leads to a monoexponential decay with a time constant of $10\,s^{-1}$ (data not shown), typical of a charge recombination from Q_A. The biexponential decay could arise from the presence of two different populations of RCs, each one sharing a different lipidic environment. Such an interpretation is further supported by the observation that the mixed liposomes show a component, which in terms of both life-times and ΔGs, is equal to the 'pure PC' bilayer, suggesting the presence of PC domains in the mixed synthetic membrane. Moreover, the presence of PE can affect the disymmetry in the lipid distribution in the membrane. In fact, due to geometrical characteristics, PE tends to form structures characterized by a negative curvature [25]. This feature could lead one to predict a greater concentration of PE in the inner leaflet of the bilayer, contributing to the total asymmetry of the membrane (as it has indeed been found in many biological membranes). These conclusions are not peculiar since it has been demonstrated, both experimentally and theoretically, the presence of a not uniform distribution of lipids in both natural and synthetic membranes. In particular, in the case of PC and PS mixed bilayer, the presence of domains of lipids in certain membrane areas has been shown by several authors [26,27]. It has also been shown that a domain formation can be induced by the presence of a transmembrane protein such as bacteriorhodopsin [28]. It is clear, however, that at this stage of our research, it is premature to draw any conclusion, because of the limited experimental data available. It is however clear that a detailed comparison of the kinetic behaviour of the RCs incorporated into liposomes and in the native membrane can give a lot of information about the lipid-protein interactions.

The data of table 1 can be tentatively interpreted in terms of an environment similar to the native membrane than the situation in detergent. It has to be kept in mind, that the more stability there is in the charge separated state, the higher is the probability of an electron transfer to the bc_1 complex, making the reaching of the goal of the whole photosynthetic process easier, i.e. the production of chemical energy.

3.2 *Reverse micelles*

By means of the extractive procedure described in detail § 2 of this paper, the RC solubilization in *n*-hexane phospholipid reverse micelles (RM) is achieved, leaving a protein still photoactive. As already mentioned, the liposome composition strongly affects the yield of the whole extractive process. In the following, all the data refer to a liposome composition already described in the literature to give the best result in terms of both yield and photochemical activity [15,16]. If in the liposomes preparation any UQ_{10} is added, during the RC extraction with *n*-hexane a deprivation of the Q_A site is observed [12], leading to a protein which is no more photoactive. In figure 1 a titration of the Q_A site with UQ_{10} *n*-hexane solution is reported. Each point represents the value of the bleaching at 860 nm normalized for RC concentration, *R*,

$$R = \frac{Abs^{D}_{860} - Abs^{L}_{860}}{Abs_{802}}, \tag{3}$$

plotted as function of the UQ_{10} concentration, where the *D* and *L* supercripts refer to the dark and light absorbance. The experimental titration data have been fitted according to the quadratic treatment of a ligand binding to a population of identical noninteracting sites, obtaining a binding constant value of $9{\cdot}7 \times 10^{-4}\,M^{-1}$.

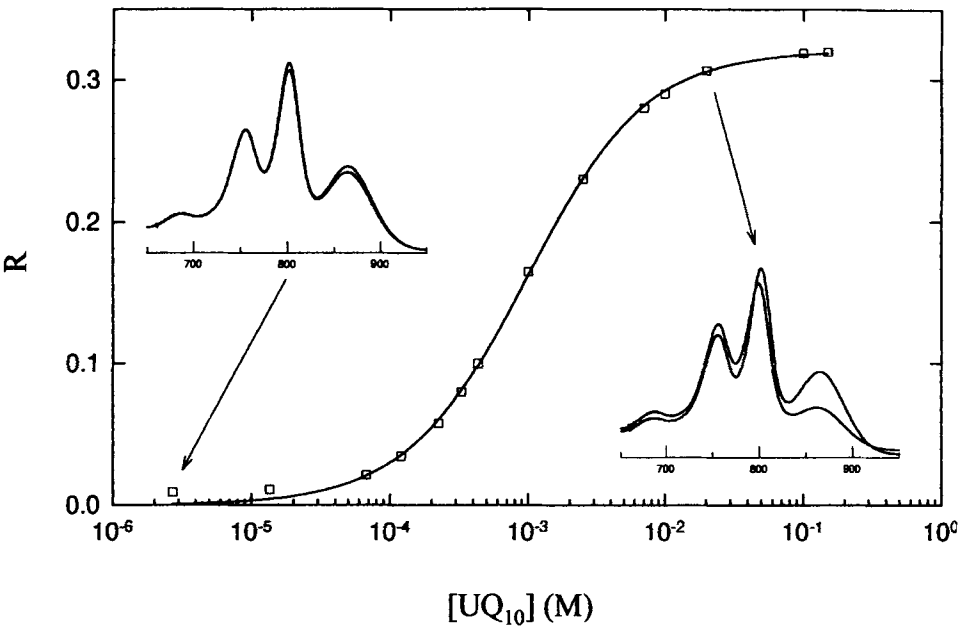

Figure 1. Semilog plot of the Q_A site titration with UQ_{10}. The insets show the electronic absorption spectra before and after the partial reconstitution of the QA site, recorded in the dark (D) and under subsaturing actinic light (L). The R value is defined by equation 3.

The charge recombination of flash generated states of the RC solubilized in reverse micelles, has been followed as a function of the quinone concentration at two different temperatures. Under all the conditions tested, the decay of P^+ is described by two exponential phases: a slow component, always accounting for 90% of the recovery and a fast minor phase, characterized by kinetic parameters which are unaffected by temperature and UQ_{10} concentration (see figure 2). In view of this independence, we attribute the latter component to $P^+Q_A^-$ recombination taking place in a fraction (approximately 10%) of the RCs in which the Q_B site functionality has been lost (this is presumably related to the high degree of organization of the water molecles, see § 3.3). The half-times of the slow phase, originating from RCs in which the photoinduced charge separation involves Q_B, increase at increasing UQ_{10} concentrations. This predicted behaviour is when the binding equilibrium of the quinone to the Q_B site is rapidly established as compared to the charge recombination process from $P^+Q_A^-$ [29]. Under this assumption, the overall recombination from $P^+Q_B^-$ is described by a single component, with an apparent rate constant k_p given by,

$$k_P = \frac{k_{AP}}{1 + L_{AB}^{app}}, \tag{4}$$

where k_{AP} is the rate constant of $P^+Q_A^-$ recombination and L_{AB}^{app} can be derived by the equation,

$$L_{AB}^{app} = \frac{L_{AB}K_{bind}[Q]}{1 + K_{bind}[Q]}, \tag{5}$$

in which L_{AB} is the equilibrium constant for electron transfer from Q_A^- to Q_B^-, and K_{bind} is the equilibrium constant for the UQ_{10} binding to the *RC* Q_B site. In terms of half-times, (4) can be rewritten as,

$$\frac{t_{1/2P}}{t_{1/2AP}} - 1 = L_{AB}^{app}, \tag{6}$$

which is formally identical to equation 1. From the data reported in figure 2, the apparent equilibrium constant L_{AB}^{app} can be derived by means of (6) and of a $t_{1/2AP}$ value of 80 ms (obtained by measuring the charge recombination process in the presence of terbutryne). The values of L_{AB}^{app} as a function of UQ_{10} concentration at 298 K and 279 K are reported in figure 3, where the data have been fitted according to (5), yielding the L_{AB} and K_{bind} parameters listed in table 2. From the data of table 2, ΔH^0 and ΔS^0 for the electron transfer and binding processes can be determined according to the Van't Hoff relationship (table 3). Due to the not negligible uncertainties on k_P and k_{AP} and the strong correlation between K_{bind} and L_{AB} in (5), the relative errors on the equilibrium constants of table 3 are quite high. As a consequence, the thermodynamic parameters derived by the Van't Hoff relationship, are greatly affected by the uncertainties (table 3).

However, although with the due cautiousness, the data of table 3 outline an enthalpically driven binding of the quinone to the Q_B site, as reported in the case of Q_0 binding in aqueous systems [30]. It should be mentioned that both the Q_A and Q_B binding constant measured by us are lower than that reported for the corresponding UQ_{10} binding processes in aqueous systems [30]. This discrepancy is probably due to the dramatic contribution of the hydrophobic effect always present in water surfactant

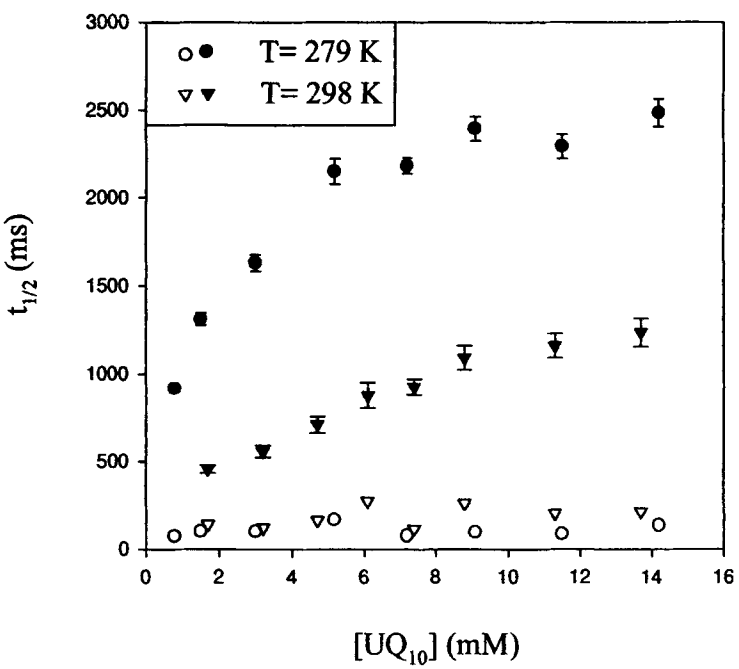

Figure 2. Quinone dependence of P^+ decay in RM at two different temperatures.

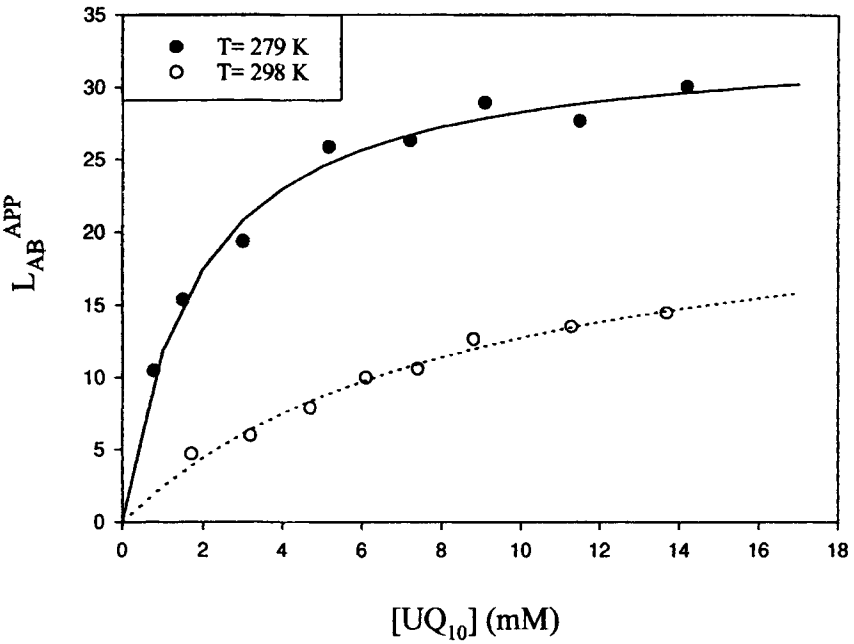

Figure 3. L_{AB}^{app} as a function of quinone concentration at two different temperatures. The lines are the best fit according to equation 5.

Table 2. Equilibrium constants relative to the electron transfer from Q_A^- to Q_B (L_{AB}) and to the quinone binding to the RC Q_B site (K_{bind}) determined for RC in reverse micelles.

T(°C)	L_{AB}	K_{bind} (M^{-1})
25 ± 0·21	23·9 ± 2·0	114 ± 19
6 ± 0·2	33·4 ± 1·0	550 ± 65

Table 3. Thermodynamic parameters relative to the electron transfer from Q_A^- to Q_B and to the binding process of the quinone to the Q_B site for RC in reverse micelles. The ΔG^0 values have been calculated at 298 K.

ΔG^0_{AB}(meV)	− 82 ± 2	ΔG^0_{bind}(meV)	− 122 ± 4
ΔH^0_{AB}(meV)	− 130 ± 40	ΔH^0_{bind}(meV)	− 590 ± 110
ΔS^0_{AB}(meV K^{-1})	− 0·15 ± 0·14	ΔS^0_{bind}(meV K^{-1})	− 1·60 ± 0·4

solution. Further measurements are in progress in order to obtain reliable values of the thermodynamic parameters of both the electron transfer and binding processes.

3.3 *Organogel*

In order to evaluate the influence of the phospholipid local mobility on the RC's charge recombination process, a study of this protein in the organogels has been undertaken.

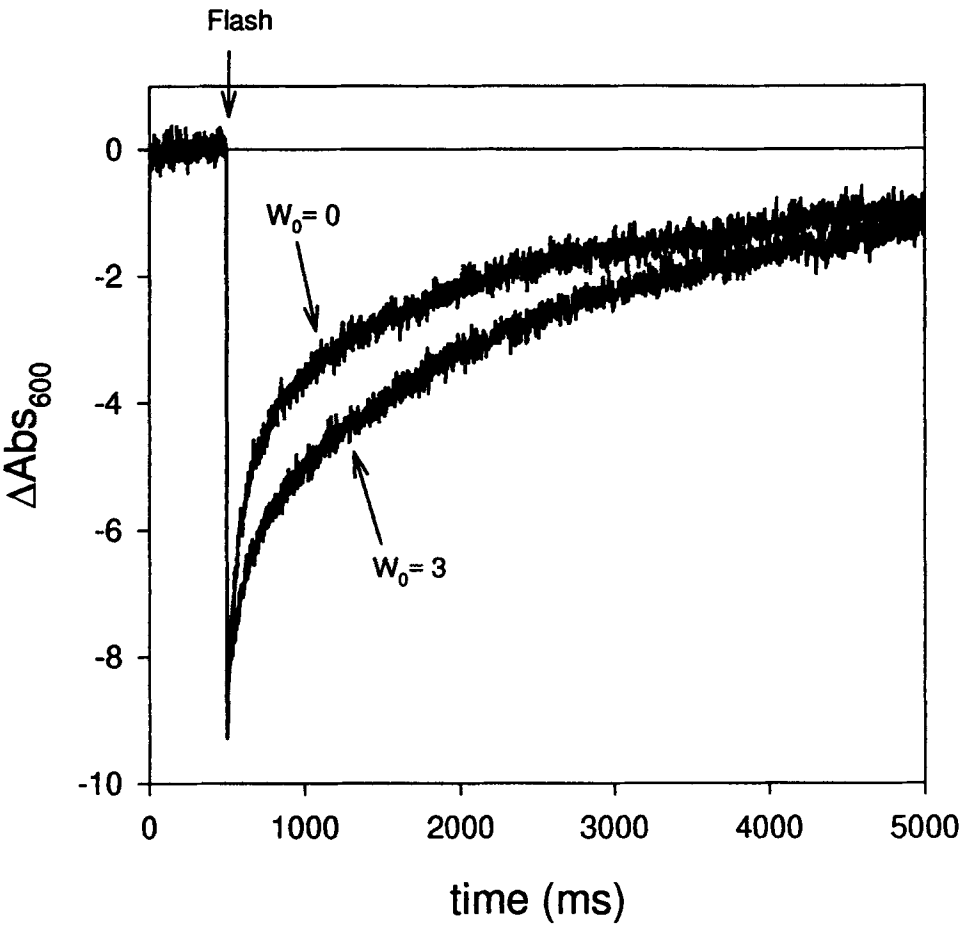

Figure 4. Charge recombination kinetics of RC in PC/PS *n*-hexane gels ([PL] = 160 mg/ml) at two different W_0.

The first observation of such an approach is the observation that in organogels a slowing down of the whole dark re-reduction of P^+ is found decreasing the local lipid mobility of the RM solutions (figure 4). Indeed, a quite complex multiphasic kinetics is found wherein three exponentials are required to accurately fit the P^+ decay [20]. A fast phase, with a half-time shorter than 100 ms, could account for the $P^+Q_A^-$ recombination. Also in the gel, in fact, the addition of terbutryne leads to a monoexponential P^+ decay with this characteristic time constant. Accordingly, the slower phases, (intermediate and slow) whose half-time values range from 150 to 700 ms and from 1 to 2·7 s respectively, can be ascribed to the $P^+Q_B^-$ recombination.

The multiexponential behaviour for the P^+ dark recovery is not a surprise since the presence of a two exponential time course is also expected in the case of homogeneous population of RCs when the rate of quinone exchange is of the same order of magnitude as that of the $P^+Q_A^-$ recombination [29]. Another explanation could be the heterogeneity of both the protein and/or its surrounding which can lead to the same phenomenological description. These considerations, together with the difficult-to-obtain reliable

deconvolution of the multi-exponential decays [31] and lack of information on the influence of the quinone concentration, induced us to simplify the discussion of the organogel results by referring to a 'fast phase' (relative to the $P^+Q_A^-$ recombination) and to a 'slow phase' (obtained by the sum of the slower ones).

The elevated half-time values of the slow phase suggest, that as already seen in the liposomes, the charge separated state, $P^+Q_B^-$, is more stabilized in a system characterized by a reduced lipid mobility like the organogel. This interpretation is further supported by investigations carried out on cubic phases made of palmitoyl–phosphocholine where a dramatic increase in the half-time for the $P^+Q_B^-$ recombination has been reported [32]. Moreover, at constant quinone concentration, the water to phospholipid concentration ratio (W_0) plays a relevant role in stabilizing the charge separated states. The comparison of the kinetic behaviour of the RC in gel at different values of W_0 and fixed PL concentration and also at fixed W_0 and different lipid concentration, permits one to resolve the influence of both parameters [20].

A direct proportionality between the slow phase relative amplitude and the W_0 is shown in figure 5A where it is also possible to observe the fast phase amplitude decrease with W_0, suggesting an increase in the number of the Q_B sites accessible to the quinone dissolved in the organic bulk. On the contrary, the relative amplitude of the two phases are unaffected by any variation in the overall water amount as long as the W_0 remains constant (figure 5B). It is interesting to note that at maximum W_0 value, before the phase separation, the fraction of Q_B sites not accessible to the quinone binding is about 10% which is the same value observed in RM (see § 3.2). Both, the amount of water solubilized during the extraction of proteoliposomes in hexane and the phase separation boundary in organogels, reflect the maximum water chemical potential compatible with a three-component isotropic single phase. In both cases we found the same fraction of RCs lacking the Q_B site functionality. In addition, the degree of organization of the water molecules in RM is usually believed to be a function of W_0 (and not of the overall water amount) [33]. As a whole, this information indicates that the activity of water may seem to play a role in the RC binding affinity for the ubiquinone molecules. This conclusion is in agreement with the X-ray structure of the RC from *Rhodobacter sphaeroides* which reveals several water molecules buried in the core of the protein, some of them being well positioned to play a role in the binding process of the secondary quinone molecule, Q_B [34]. It should be mentioned that similar conclusions were recently proposed on the basis of investigations in aqueous systems at high osmolality [35].

Furthermore, figure 6A shows how the half-time of the $P^+Q_A^-$ recombination is more sensibly shortened at low W_0 values. A similar influence of the water content on the charge recombination rate of RC films has been reported several years ago [36], and it has been recently reported that the rate constant for the charge recombination between P^+ and Q_A^- is increased by a factor of two or three in dehydrated phospholipid reverse micelles in *n*-hexane [37]. An independence of the fast phase half-times upon any changes in the total lipid concentration is clearly deducible from figure 6B. A comparison of figures 6A and B allow us to conclude that the decrease in the stability of the $P^+Q_A^-$ state is related to the W_0 and not merely due to the total amount of water. Once again, this agrees with the high resolution X-ray structure of the RC recently reported which suggests a direct role for the water in the binding process of the ubiquinone at the Q_A site and in the stabilization of the corresponding semiquinone radical [38].

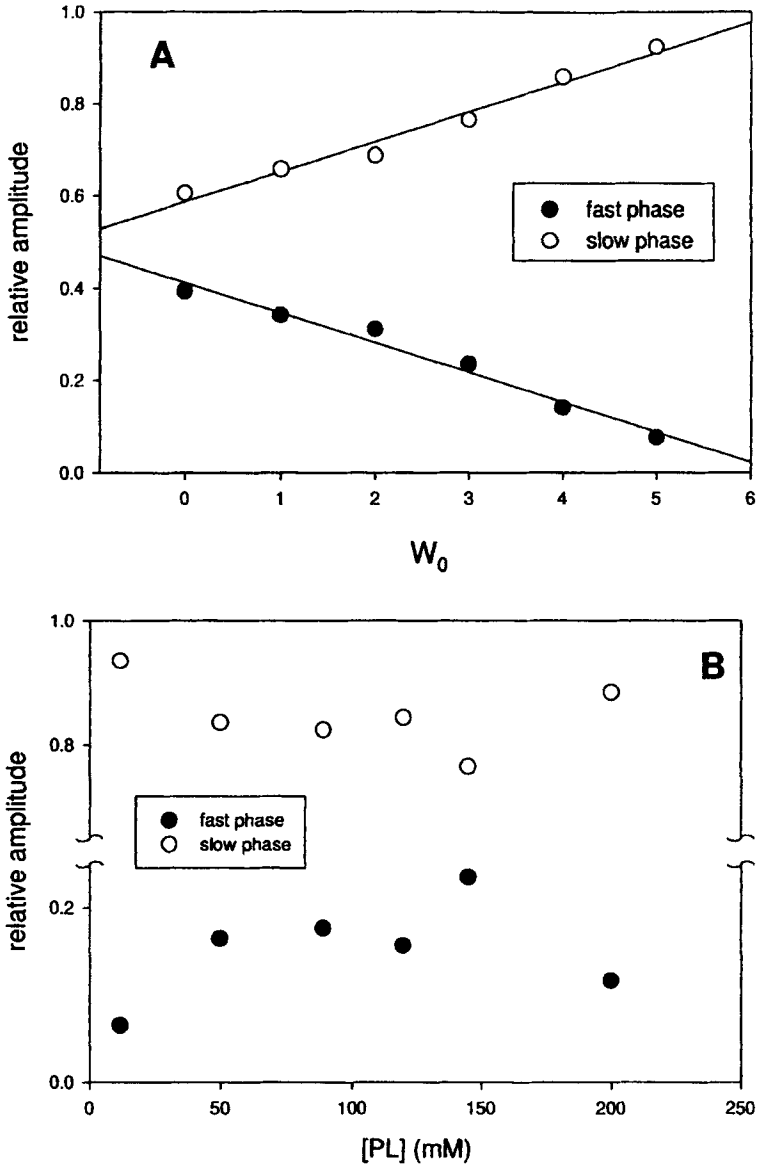

Figure 5. Relative amplitudes of the exponential phases obtained by the deconvolution of the experimental traces recorded in RC contained in PC/PS *n*-hexane gels at (**A**) different W_0 ([PL] = 160 mg//ml) and (**B**) at different PL concentration ($W_0 = 4$).

Conclusions

The data reported in this paper point out a marked dependence of the photochemical properties of the RC on the lipid environment. In fact, the results, have shown how the different molecular organization of the lipid forming the hosting systems, influences the charge recombination kinetics in the RC. It has been found particularly that biomimetic systems characterized by a reduced lipid mobility, stabilize the charge

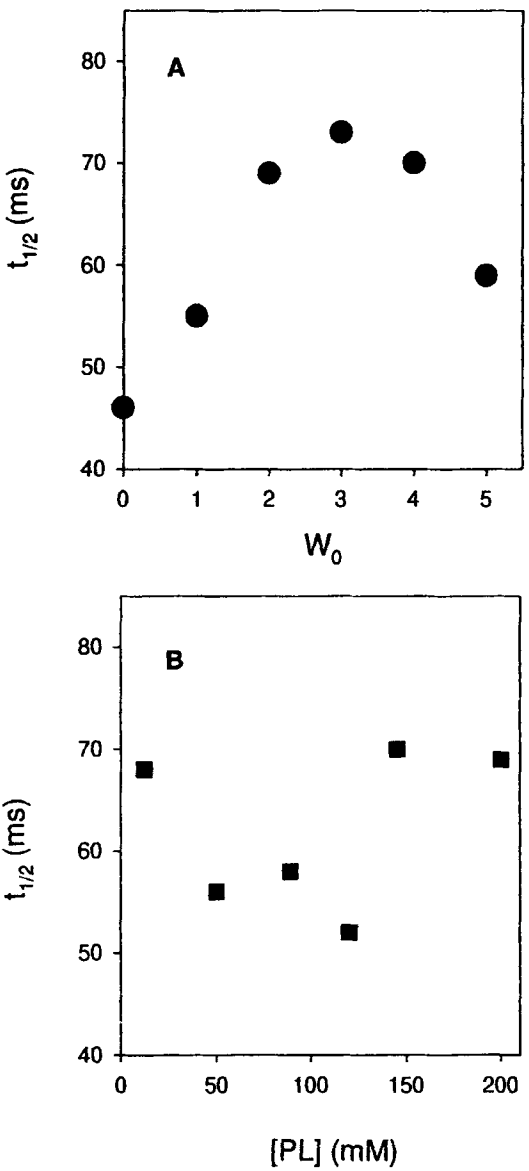

Figure 6. Half-times of the fast phases obtained by the deconvolution of the experimental traces recorded in RC contained in PC/PS *n*-hexane gels at (**A**) different W_0 ([PL] = 160 mg/ml) and (**B**) at different PL concentration ($W_0 = 4$).

separate states in the protein. Moreover, the binding of the quinone to the RC and the microheterogenity of the environment surrounding the protein seem to account for the multiphasic kinetic behaviour of the charge recombination observed in the lipidic systems examined. Finally, this study has also highlighted the relevant role played by the water activity on the RC binding affinity for the ubiquinone molecules, supporting the recent literature data that suggest a role of water on both, the ubiquinone binding process and the stabilization of the semiquinone radical.

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