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Electron transfer kinetics in photosynthetic reaction centers embedded in polyvinyl alcohol films

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Abstract

The coupling between electron transfer and protein dynamics has been studied at room temperature in isolated reaction centers (RCs) from the photosynthetic bacterium *Rhodobacter sphaeroides* by incorporating the protein in polyvinyl alcohol (PVA) films of different water/RC ratios. The kinetic analysis of charge recombination shows that dehydration of RC-containing PVA films causes reversible, inhomogeneous inhibition of electron transfer from the reduced primary quinone acceptor (Q_A^-) to the secondary quinone Q_B. A more extensive dehydration of solid PVA matrices accelerates electron transfer from Q_A^- to the primary photooxidized electron donor P^+. These effects indicate that incorporation of RCs into dehydrated PVA films hinders the conformational dynamics gating Q_A^- to Q_B electron transfer at room temperature and slows down protein relaxation which stabilizes the primary charge-separated state $P^+Q_A^-$. A comparison with analogous effects observed in trehalose-coated RCs suggests that protein motions are less severely reduced in PVA films than in trehalose matrices at comparable water/RC ratios.

Keywords: Photosynthetic reaction center; Polyvinyl alcohol film; Protein dynamics; Conformational relaxation

1. Introduction

The photosynthetic reaction center (RC) from purple bacteria provides an ideal system for exploring the relationship between electron transfer and internal protein motions. This integral pigment–protein complex promotes the primary event of photosynthetic energy conversion, i.e., a light-induced charge separation across the membrane dielectric. Within the RC of *Rhodobacter sphaeroides*, following absorption of a photon, the primary electron donor P, a bacteriochlorophyll dimer, delivers an electron (via a bacteriopheophytin molecule) to the primary ubiquinone acceptor, Q_A, forming the primary charge-separated state $P^+Q_A^-$ in less than 200 ps. The photoreduced Q_A^-, in turn, reduces a ubiquinone-10 molecule, bound at the secondary acceptor Q_B site, generating the $P^+Q_AQ_B^-$ state in the hundreds-of-microsecond timescale. When no electron donor to P^+ is available, the electron on Q_B^- recombines with the hole on P^+ in a back reaction which proceeds essentially by thermal repopulation of the $P^+Q_A^-Q_B$ state [1].

The interplay between internal protein motions and electron transfer has been studied in bacterial RCs mainly by hampering conformational relaxations and protein-substate interconversion at cryogenic temperatures and by comparing the kinetics of specific electron transfer processes in RCs frozen in the dark and under illumination. The RC can be trapped at cryogenic temperatures in a *dark-adapted* and a *light-adapted* conformation, strongly differing in the stability of the $P^+Q_A^-$ state. Both conformations consist of distributions of substates which are reflected in a large, continuous spectrum of rate constant for charge recombination of the $P^+Q_A^-$ state at cryogenic temperatures [2,3]. The occurrence of structural changes accompanying light-in-

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duced charge separation is confirmed by temperature dependence studies of Q_A^--to-Q_B electron transfer: the reaction is blocked in RCs frozen in the dark below 200 K, but persists even at 50 K in RC frozen under illumination [2,4]. This electron transfer process has been proposed to be conformationally gated and appears to be governed by a complex energy landscape [5,6].

We have recently shown that the relationship between electron transfer and RC dynamics can be studied *at room temperature* by exploiting a different approach, originally developed in the analysis of function-dynamics coupling in heme proteins [7]. Protein conformational dynamics coupled to electron transfer could be severely conditioned by embedding the RC within a dehydrated trehalose matrix [8]. Trehalose was chosen among other saccharides in view of its high glass transition temperature and of its peculiar properties in the preservation of biostructures [9]. Spectroscopic studies and molecular dynamics simulations performed on myoglobin/trehalose/water systems have shown that the protein motional freedom is strongly reduced, approaching the one of a harmonic solid in extremely dry matrices [10,11]. A progressive dehydration of *trehalose-coated* RCs blocks Q_A^--to-Q_B electron transfer in a progressively increasing fraction of the RC population [12]. Further reducing the amount of residual water in the amorphous matrix hinders at room temperature RC relaxation from the dark-adapted to the light-adapted conformation as well as interconversion between conformational substates. This impairment is reflected in strongly distributed $P^+Q_A^-$ recombination kinetics. The average rate constant and distribution width increase in parallel with dehydration reaching, at room temperature, in extremely dry matrices, values comparable to those measured in glycerol–water mixtures at cryogenic temperature [8]. Interestingly, extensive dehydration in the absence of the disaccharide has limited effects on Q_A^--to-Q_B electron transfer and negligibly affects $P^+Q_A^-$ recombination kinetics, suggesting that the conformational dynamics controlling electron transfer is strongly slaved to the structure and dynamics of the surrounding medium [8,12]. In the present work, to further assess the role of the embedding matrix, we have studied the kinetics of charge recombination in RCs incorporated into polyvinyl alcohol (PVA) films characterized by a variable content of residual water. Incorporation into PVA matrices is a widely used practice in spectroscopic studies to attain high RC concentrations, to increase RC stability and/or to prevent diffusion of RC complexes at room temperature (see, e.g., Refs. [13,14]). We show that decreasing the water/RC ratio has remarkably different effects on charge recombination kinetics in RC embedded in PVA films and in trehalose amorphous matrices.

2. Experimental

The RC were purified as in Ref. [15]. The Q_B activity, close to 60%, was not reconstituted. RC containing PVA films were prepared as follows. RCs were suspended in 10 mM Tris–HCl, pH 7.5, 0.025% lauryl dimethylamine-*N*-oxide, 2.5% w/v PVA (Fluka, $M_w \approx 130000$) to a final concentration of ≈ 9 μM. To form the film, the suspension was dehydrated under dry N_2 flow for approximately 5 h. Further dehydration was obtained under vacuum. PVA films could be partially rehydrated by exposure to water vapor.

The water content of the PVA films was evaluated by NIR spectroscopy as described in Ref. [8] (see also Section 3.1). To limit water exchange with the environment, the PVA film was sandwiched between two optical glass plates, sealed with silicon grease. Charge recombination kinetics were monitored at 422 and 605 nm by flash absorption spectroscopy as described in Ref. [8]. RC photochemistry was elicited by a 20-ns pulse from a dye laser (RDP-1 Radiant Dyes, Wermelskirchen, Germany) pumped by a frequency-doubled Q-switched Nd-YAG laser (Surelite 10, Continuum, Santa Clara, CA). Stryryl 9 was used as a dye (λ_{max} at 810 nm). Nonlinear fitting was performed as in Ref. [8].

3. Results and discussion

3.1. Water/RC ratio and RC spectral features in PVA films

In parallel with measurements of charge recombination kinetics (see Section 3.2), spectra of RC-containing PVA films, characterized by a variable water content, were collected in the visible–NIR region in the range from 700 to 2300 nm. Three representative spectra are shown in Fig. 1A. The combination band of water centered at $\lambda \approx 1930$ nm [16] is progressively reduced when the PVA film is exposed to dry N_2 or dehydrated under vacuum for a longer time (see inset). The water content was estimated by the same method used in trehalose matrices [8], i.e., on the basis of the area S of the combination band, assuming a proportionality constant $k = 100$ absorbance units nm M^{-1} cm^{-1} [17]. Since the effective optical path of the film is not easily accessible and could change with dehydration, the RC absorption at 802 nm (A_{802}) was used as an internal standard (extinction coefficient $\varepsilon_{802} = 288 \times 10^3$ M^{-1} cm^{-1}). Under these assumptions, the water/RC molar ratio is determined as $(H_2O/RC)=(\varepsilon_{802}S)/(kA_{802})$. Interestingly, the water band in PVA films is blue-shifted by ≈ 10 nm as compared to that observable in trehalose matrices. Moreover, the progressive red shift of the band occurring in trehalose under conditions of severe drought (characteristic of water involved in strong H-bonds [8]) is not detected in PVA films.

In PVA films, the RC retains its spectral features, which remain unaffected for months when the film is stored at 5 °C and changes in the hydration state are prevented (see Section 2). The only relevant spectral changes induced progressively upon decreasing the H_2O/RC ratio are a decrease in amplitude and a blue shift of the Q_y band of P ($\lambda_{max} \approx 860$ nm in solution) (Fig. 1B). Both effects revert upon rehydration. Analogous spectral changes have been

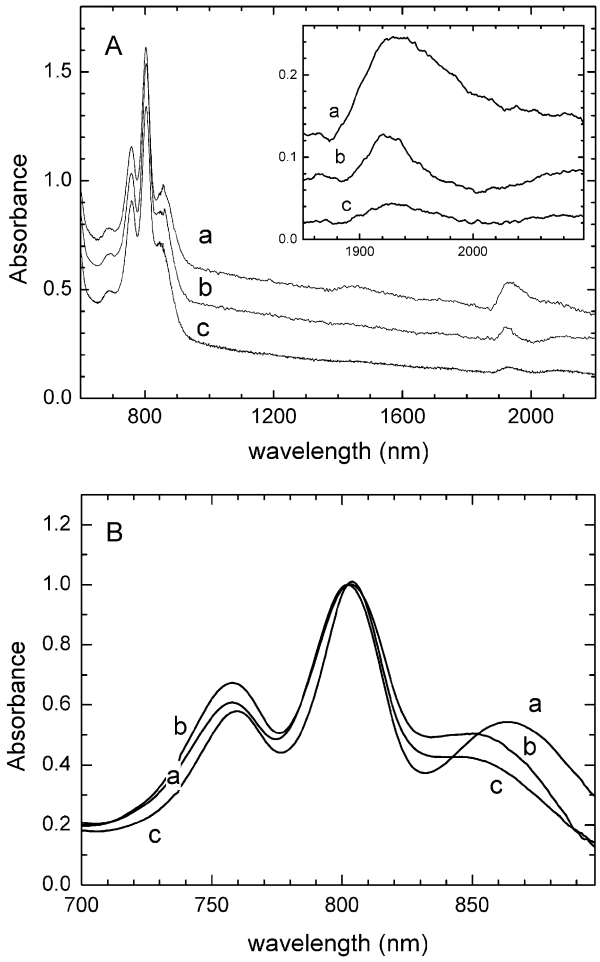

Fig. 1. Visible–NIR absorbance spectra of RC containing PVA films at the following different H_2O/RC ratios: (a) 3.3×10^4 (sample 1 rehydrated); (b) 5.9×10^3 (sample 1 dehydrated); (c) 3.1×10^3 (sample 2 dehydrated). Samples are identified as in Table 1. Panel A shows Q_y absorption bands of the RC and the water combination band around 1930 nm, magnified in the inset. In panel B, Q_y spectral features are compared, normalizing the spectra to the bacteriochlorophyll peak at 802 nm.

reported in response to: dehydration both in the presence and in the absence of trehalose [8]; changes in detergent type and concentration [18]; phase segregation of RC at acidic pH values [19]; and increase of the applied hydrostatic pressure up to 0.6 GPa [20]. In general, these effects are not coupled with changes in the rate of $P^+Q_A^-$ recombination and have been attributed to very slight reorganizations in the dimeric structure of P which can induce important variations in the electronic states of the bacteriochlorophyll dimer [20].

3.2. Kinetic analysis of charge recombination

Charge separation induced by a short light pulse and the subsequent charge recombination is described by:

$$PQ_AQ_B \underset{k_{AP}}{\overset{h\nu}{\rightleftarrows}} P^+Q_A^-Q_B \underset{k_{BA}}{\overset{k_{AB}}{\rightleftarrows}} P^+Q_AQ_B^- \quad (1)$$

where, under physiological conditions, $k_{AP} \approx 10\ s^{-1}$, $k_{AB} \approx 6 \times 10^3\ s^{-1}$ and $k_{BA} \approx 4.5 \times 10^2\ s^{-1}$ [21]. Charge recombination of the $P^+Q_AQ_B^-$ state occurs slowly with an observed rate constant $k_s \approx 1\ s^{-1}$. In the absence of the secondary acceptor Q_B, or when Q_A^- to Q_B electron transfer is blocked, recombination of the $P^+Q_A^-$ state occurs and P^+ decays with k_{AP}. In general, P^+ decay includes two kinetic components, a fast and a slow one, ascribed to RC subpopulations which undergo $P^+Q_A^-$ and $P^+Q_B^-$ recombination, respectively. The relative contributions of the two components depend on the quantum yield of flash-induced Q_B formation, with the rate of the slow kinetic components being determined by k_{AP}, k_{AB} and k_{BA}. In principle, therefore, kinetic analysis of charge recombination yields information on two electron transfer reactions simultaneously: primary charge recombination and electron transfer from Q_A^- to Q_B.

Fig. 2 shows normalized kinetics of P^+ decay measured in a PVA solution and in two PVA films characterized by a decreasing H_2O/RC ratio. Incorporation of the RC into a sufficiently hardened matrix is expected to slow down fluctuations of the RC protein among different conformational substates (characterized by individual rate constants), eventually revealing at room temperature the structural heterogeneity of the RC protein, as observed for solid trehalose samples. When substate interconversions become

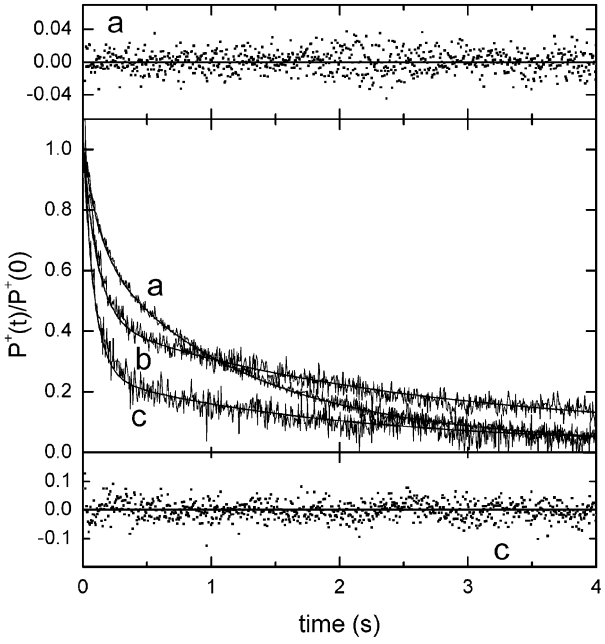

Fig. 2. Kinetics of charge recombination following flash excitation of RCs in a PVA solution (trace a, sample 1 resuspended) and in a PVA film (sample 1) progressively dehydrated: trace b, H_2O/RC = 5.9×10^3; trace c, H_2O/RC = 3.6×10^3. P^+ decay has been normalized to the maximal amplitude at the time of the laser pulse ($t = 0$). Traces have been fitted to the sum of an exponential decay (fast phase) and a power law (slow phase) as described in detail in the text (see also Table 1). Residues for traces a and c are shown in the upper and lower panel, respectively.

slower than the observed electron transfer process, the kinetics of electron transfer exhibit a nonexponential character and are described by a continuous rate distribution [8]. We have previously shown that in trehalose matrices, each kinetic component of charge recombination can be adequately described by a power law [8,12], so that normalized P^+ decay following a flash is, in general, fitted to:

$$P^+(t)/P^+(0) = (1 - A_s)/(1 + \lambda_f t)^{n_f} + A_s/(1 + \lambda_s t)^{n_s} \quad (2)$$

where the subscripts f and s identify the fast and slow components, respectively. Parameters λ and n are related to the average rate constant $\langle k \rangle$ and to the width σ of the rate distribution function by $\langle k \rangle = n\lambda$, $\sigma^2 = n\lambda^2$. In PVA solution and dehydrated films, except for the driest ones (see below), the fast rate distribution was very narrow ($\sigma_f \approx 0$); in these cases, the first power law in Eq. (2) was replaced by a simple exponential decay. The kinetic parameters obtained by such an analysis are summarized in Table 1 for different samples at different degree of dehydration.

In liquid PVA solution, the slow component accounts for approximately 60% of P^+ decay and is characterized by a narrow distribution ($\sigma_s = 0.3\ s^{-1}$) around an average rate constant $\langle k_s \rangle = 0.8\ s^{-1}$. The fast kinetic component, attributed to RCs which lack the secondary acceptor Q_B, is reasonably described by a simple exponential decay ($\sigma_f \approx 0$) with a rate constant $k_f = 9\ s^{-1}$, close to values measured for $P^+Q_A^-$ recombination in the absence of PVA [8,21]. Formation of the PVA film following dehydration causes a sizable decrease in the amplitude A_s and in the average rate constant $\langle k_s \rangle$ of the slow component (Fig. 2). The relative contribution of the slow component, A_s, further decreases when the H_2O/RC ratio of PVA films is decreased and practically vanishes in the driest samples characterized by approximately 3×10^3 water molecules per RC (see Table 1 and Fig. 2). The (average) rate constant of the residual fast phase increases systematically upon decreasing the amount of residual water in the film, reaching a value of approximately 14 s^{-1} in the driest matrices. The nonexponential character of this phase becomes evident only under conditions of extreme drought (H_2O/RC $\approx 3 \times 10^3$). The described effects revert upon rehydration of the film; charge recombination kinetics observed in the liquid sample are fully restored upon redissolving the matrix in water (see Table 1 and Fig. 2).

Some similarities between the kinetics measured in extensively dehydrated PVA films and the ones observed in moderately dehydrated trehalose matrices lead to a straightforward interpretation of the present results. Incorporation in PVA films and progressive dehydration of the matrix appears to block Q_A^- to Q_B electron transfer in a progressively larger fraction of the RC population. Charge recombination in the residual subpopulation, which still experience Q_B photoreduction, is slowed down, possibly reflecting a stabilization of the $P^+Q_B^-$ state relative to $P^+Q_A^-$. This behavior is quantitatively similar to that observed in trehalose-coated RCs. However, comparable effects on the slow kinetic component of charge recombination are observed in the two matrices at markedly different values of H_2O/RC. The block of Q_A^- to Q_B electron transfer over the whole RC population occurs in PVA films at approximately 3×10^3 water molecules per RC, while the same effect is observed in trehalose at five times larger H_2O/RC ratio [12]. In the driest PVA film, the average rate constant of the fast phase $\langle k_f \rangle$ ($P^+Q_A^-$ recombination) is 1.5 times larger than in solution, suggesting that the relaxation of the RC protein from the dark-adapted to the light-adapted conformation is slowed down on the timescale of $P^+Q_A^-$ recombination, leading to an appreciable destabilization of the primary charge-separated state. However, this acceleration effect is two times smaller than the analogous one observed in solid trehalose matrices [8]. Again, a comparable $\langle k_f \rangle$ value is observed in trehalose-coated RCs at more than 10^4 water molecules per RC [8] vs. approximately 3×10^3 in the PVA film. Moreover, the width of the fast rate distribution never exceeds 10 s^{-1} in PVA, as compared to 16 s^{-1} in trehalose. It appears that incorporation into PVA films, even at very low contents of residual water, does not block completely the interconversion between protein substates on the timescale of tens of milliseconds.

Table 1
Kinetic parameters of charge recombination in PVA solutions and films at different H_2O/RC ratios

Sample	H_2O/RC	A_s (%)	$\langle k_f \rangle$ (s^{-1})	σ_f (s^{-1})	$\langle k_s \rangle$ (s^{-1})	σ_s (s^{-1})
1, solution	$\approx 6\ 10^6$	58	9.0	≈ 0	0.82	0.30
1, dehydrated	$(5.9 \pm 0.9)\ 10^3$	41	9.1	≈ 0	0.37	0.19
1, dehydrated	$(3.6 \pm 0.4)\ 10^3$	24	11.8	≈ 0	0.50	0.22
1, rehydrated	$(3.3 \pm 0.6)\ 10^4$	62	6.2	≈ 0	0.59	0.21
1, resuspended	$\approx\ 2\ 10^6$	65	7.9	≈ 0	0.82	0.30
2, dehydrated	$(3.1 \pm 0.4)\ 10^3$	4	13.8	5.0	0.40	0.20
2, dehydrated	$(2.9 \pm 0.5)\ 10^3$	0	15.3	10.0	–	–

4. Conclusions

Analysis of charge recombination suggests that embedding the RC in progressively dehydrated PVA films reversibly slows at room temperature the RC protein dynamics coupled to $P^+Q_A^-$ and blocks the conformational transitions which gates Q_A^- to Q_B electron transfer. As already observed in trehalose-coated RCs, the latter effect occurs at larger H_2O/RC ratios than the former one. The conditioning of RC protein dynamics exerted by PVA films appears to be milder than that induced by incorporation into trehalose amorphous matrices. Solid trehalose matrices are more effective particularly in blocking interconversion between conformational substates over the timescale of $P^+Q_A^-$ recombination. Moreover, when comparable effects are observed in the kinetics of charge recombination, the water-to-RC ratio in PVA films is almost 1 order-of-magnitude lower

than in trehalose matrices. As a whole, these results point to a selective correlation between the dynamics of the external medium and protein motions governing specific electron transfer processes, suggesting that slaving of the protein dynamics to the solvent can be modulated at room temperature by the structure of the embedding matrix.

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