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Modification of protein hydrogen bonds influences the efficiency of picosecond electron transfer in bacterial photosynthetic reaction centers

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Picosecond absorption spectroscopy was used to monitor laser-induced oxidation-reductions of reaction center (RC) bacteriochlorophyll (P) and bacteriopheophytin (I) in *Rhodopseudomonas sphaeroides* RC preparations on exposure to different chemicals. The D_2O isotope substitution of H_2O or partial substitution of water by organic solvents (ethylene glycol, glycerol, propylene glycol, dimethyl sulfoxide) causes the appearance of a fast, nanosecond component of P^+ reduction, the result of an increased probability of recombination of the primary ion-radical products $P^+I^- \rightarrow PI$. The effect is accompanied by a noticeable slowing down of electron transfer from photoreduced bacteriopheophytin to the primary quinone acceptor Q_A. The effect of the organic solvents, known as cryoprotectors, is correlated with their degree of hydrophobicity, i.e. the ability to penetrate the RC protein and interact with bound water and protein hydrogen bonds. The conclusion drawn from the data is that the dielectric relaxation processes through which the intermediate energy levels of the carriers in the PIQ_A system are lowered to levels necessary for the stabilization of the photochemically separated charges proceed with the involvement of protons of the nearest water-protein surrounding of the RC pigments and electron transport cofactors.

Photosynthesis; Reaction center; Picosecond charge separation; Cryoprotection; Isotopic substitution effect; (*Rps. sphaeroides*)

1. INTRODUCTION

In purple bacteria, during photosynthesis, the bacteriochlorophyll special pair P (primary donor), excited to the first singlet state, donates an electron, for a time of 4–7 ps, to bacteriopheophytin I (intermediate acceptor) from which the electron is transferred to quinone Q_A (relatively stable primary acceptor) [1–6]. These fast reactions (scheme 1)

$$PIQ_A \xrightarrow{h\nu} P^*IQ_A \longrightarrow P^+I^-Q_A \longrightarrow P^+IQ_A^- \quad (1)$$

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proceed with a high quantum and energetic efficiency in the so-called reaction center (RC), a protein complex to which pigments and electron transport cofactors are specifically linked.

The thermal effects in the primary photosynthetic reactions are not large [3,4,6–8]. At physiological and low temperatures (down to liquid helium temperature) electron transfer from P to Q_A takes about 100 ps. The time of recombination of ion radicals in the reactions $P^+I^-Q_A \longrightarrow PIQ_A$ and $P^+IQ_A^- \longrightarrow PIQ_A$ which occur when they are blocked for straightforward utilization, is on the nano- and millisecond time scale [9]. This means that the photochemically separated changes can be stabilized, in the absence of conformational degrees of freedom in the system, for a time about

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8 orders of magnitude longer than that of the primary events (picsecond–milliseconds). The relaxation processes following electron transfers within the RC, whereby some energy of the photoproducts may be dissipated, and which prevent reverse electron transfer reactions, seem to be limited in scope. The high efficiency of electron transfer is presumably provided by dielectric polarization processes within the pigment-protein complex in close proximity to the primary reactants [8,10–15,17]. These processes can neutralize the interactions of separated charges and stabilize them for further utilization [15].

The elucidation of the nature of the particles involved in polarization rearrangements of the RC is important for developing physical models of electron transfer in biological systems. The present investigation is concerned with this problem. As found, the dehydration of RCs and photosynthetic membranes (chromatophores) alters the protein hydrogen bonds and affects the energetics of the separated charges; the quantum efficiency is lowered more than 10-fold on dehydration [16,17]. It seems natural to continue investigating the kinetics of the basic elementary electron transfer events by substituting D_2O for H_2O, and also by partially substituting water by organic solvents. Of interest is the effect of multi-atomic alcohols and aprotonic compounds, the cryoprotectors: extrusion of water from biological structures and incorporation into hydrogen bonds, the effect enhancing the rigidity of the respective segments of the macromolecule.

2. MATERIALS AND METHODS

Preparations used in the experiments were RCs isolated from chromatophores of cells of the non-sulphur bacterium *Rhodopseudomonas sphaeroides*, wild-type 1760-1, and the carotenoidless strain R-26 (cells of *Rps. sphaeroides* R-26 were kindly provided by Professor R. Cogdell, University of Glasgow, Scotland). The isolation procedure was that described in [18]. In dealing with the R-26 strain, the preparation procedure (precipitation with ammonium sulphate, dialysis and chromatography) was performed as rapidly as possible in the dark and cold. The characteristics of the RC preparations obtained meet the generally accepted criteria for structural and functional integrity and purity. The preparation used in the experiment contained about 10^{-3} M BChl (P). The suspension mixture contained 0.03 M sodium phosphate buffer, pH 7.1–7.2, and 0.05% (v/v) LDAO.

For H_2O substitution by D_2O, the RC suspension (0.5 ml) was dried under low pressure (10^0–10^{-1} mmHg). Air was evacuated from the desiccator containing the dried sample, D_2O vapor was sprayed in, and 0.5 ml D_2O added (extent of D_2O isotope substitution: 99.8%). The preparation was resuspended in the added amount of D_2O. The procedure was repeated twice. The reverse substitution of D_2O by H_2O was performed similarly.

Ethylene glycol, glycerol, propylene glycol and dimethyl sulfoxide (DMSO), at concentrations of 25–90% (v/v) were added to the RC suspension. Reversible aggregation was observed after adding more than 70% propylene glycol or more than 50% DMSO, but not denaturation of the pigment-protein complex.

N,N,N',N' - Tetramethyl - *p* - phenylenediamine (to 10^{-4} M) and sodium ascorbate (10^{-3} M) were added immediately before measurements to increase the regeneration rate of initially oxidized bacteriochlorophyll P.

Absorption changes, resulting from electron transfer within the PIQ_A system, were recorded as in [3,4] on a computer-based picosecond spectrometer. A pulse selectivity technique was used for excitation (λ_{exc} = 532 nm) and probe (λ = 870 and 750 nm) pulses to maintain the pulse energy and duration within the specified limits. The duration of the pulses was ≈30 ps. The energy of the excitation pulses was 10^{14} quanta/cm^2 and the PRF was 2 Hz.

3. RESULTS AND DISCUSSION

Fig.1 shows the effect of H_2O-D_2O isotope substitution on the kinetic behavior of the laser-induced 870-nm absorption changes of photochemically active bacteriochlorophyll P in *Rps. sphaeroides* (strain R-26) RC preparations. The fast changes, which are due to P oxidation (decrease in 870-nm absorbance), cannot be resolved in time. As seen in the control, the reduction of P^+ proceeds slowly, since it results from reverse electron transfer from the quinone acceptors which oc-

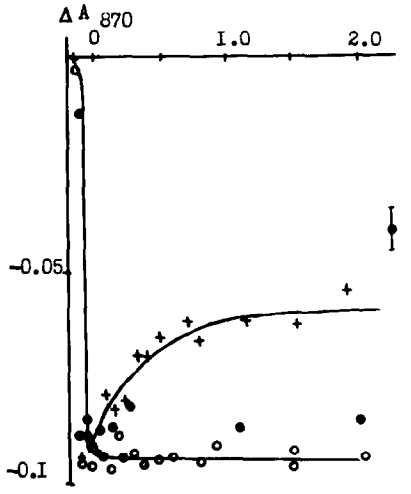

Fig.1. Effect of substituting H_2O by D_2O in RC preparations from *Rps. sphaeroides*, strain R-26, on the kinetic behavior of laser-induced absorption changes (A) of bacteriochlorophyll P at 870 nm. (○—○) Control, (+ — +) after substituting H_2O by D_2O, (●—●) reversal of the effect after substituting D_2O by H_2O (each point, average of 20 measurements; the maximum measurement error shown by vertical bars is less than 6×10^{-3} A units). Solid line, theoretical least-squares approximation.

curs for a time (about tens of milliseconds) which falls beyond the time scale of measurement. After substitution by D_2O, the kinetic behavior of the $P^+ \longrightarrow P$ process changes significantly. A fast component, whose lifetime (τ) is less than 1 ns, appears in addition to the slow reduction. Its contribution (A) (the percentage of its amplitude relative to the total signal at 870 nm) is 0.4 (table 1).

As seen from fig.1, the observed isotope effect is completely reversible.

A similar effect of H_2O-D_2O substitution on oxidation-reductions of P was observed in wild-type *Rps. sphaeroides* RC preparations (fig.2, table 1). In these preparations, laser-induced absorption changes of bacteriopheophytin I at 750 nm were monitored simultaneously. One can see from fig.2 that the appearance of the fast component of P^+ reduction after isotope substitution is accompanied by a marked slowing down of the decay of the intermediate state $P^+I^-Q_A$ (see scheme 1): an indication of this is the increase in recovery time (τ_0) of the 750-nm absorption changes (compared with the control).

It is evident from the data that the nearly irreversible electron transfer within the PIQ_A system (scheme 1) is violated by the D_2O substitution. The replacement of the slow P^+ reduction by the fast nanosecond process presumably reflects the lower efficiency of electron transfer and an increase in probability of P^+I^- recombination. With the appearance of the fast recombination process and the direct I^- to Q_A transfer rate remaining unchanged, one may expect a decrease in decay time τ_0 of the intermediate state $P^+I^-Q_A$, not an increase as observed in the experiment (fig.2). An implication is that in this pathway direct electron transfer is hindered in the presence of D_2O.

We also investigated the kinetics of laser-induced oxidation-reductions of RC bacteriochlorophyll after partially substituting H_2O by organic solvents and found that the compounds used, which are known to exhibit cryoprotective properties, influence electron transport in the PIQ_A system in a similar way. The results obtained are listed in tables 1 and 2. The addition of a multiatomic alcohol (ethylene glycol, glycerol, propylene glycol) or an aprotonic solvent (DMSO), at certain concentrations, causes the appearance of a fast component of P^+ reduction ($\tau \cong 1$ ns) and increases the probability of P^+I^- recombination. This is accompanied by a marked fall in the rate of electron transfer from I^- to Q_A.

A fact that deserves attention is that the characteristic time τ of the P^+I^- recombination observed in our substitution experiments is nearly the same as the lifetime of the fastest component of delayed luminescence emitted by the RC ($\tau \cong$ 700 ps) when the recombination of the ion radicals P^+ and I^- takes place, i.e. under conditions where the quinone acceptor Q_A is held reduced [14].

It should be noted that the extent of the effect of the organic solvents appeared to differ in RC preparations from the two strains (wild type, R-26) under study whereas the greatest isotope effect was observed to be the same and reversible (table 1; figs 1,2).

The changes in kinetic parameters induced by the organic solvents (propylene glycol and DMSO) were also observed to differ noticeably in wild-type RC preparations isolated from different samples of *Rps. sphaeroides* cells (cf. parameter A in samples 1–3, table 1). It can be seen that with 50% content of propylene glycol, parameter A shows

Table 1

Relative contribution (A) and characteristic time (τ) of the fast kinetic component of RC bacteriochlorophyll reduction (as monitored by 870-nm absorption changes) after substituting H_2O by D_2O and in the presence of organic solvents

Bacterial strain	Sample	Modification procedure	A (arbitrary units)	τ (ps)
Wild type	1	H_2O by D_2O	0.4 ± 0.04	600 ± 50
R-26	1	H_2O by D_2O	0.4 ± 0.04	600 ± 50
Wild type	1	propylene glycol (50%)	0.64 ± 0.05	600 ± 80
Wild type	2	propylene glycol (50%)	0.4 ± 0.04	720 ± 50
Wild type	3	propylene glycol (50%)	0	–
R-26	1	propylene glycol (50%)	0	–
Wild type	3	propylene glycol (70%)	0.3 ± 0.03	–
R-26	1	propylene glycol (70%)	0	–
R-26	2	propylene glycol (70%)	0	–
R-26	2+DDS (1%)	propylene glycol (70%)	0.4 ± 0.04	650 ± 50
Wild type	1	DMSO (40%)	0.51 ± 0.03	730 ± 50
Wild type	2	DMSO (40%)	0.25 ± 0.07	600 ± 80
R-26	1	DMSO (40%)	0	-
R-26	1+DDS (1%)	DMSO (40%)	0.38 ± 0.03	660 ± 50

Preparations used were made from different cell samples (1–3) by a common isolation method, from *Rps. sphaeroides*, wild type and carotenoidless strain R-26

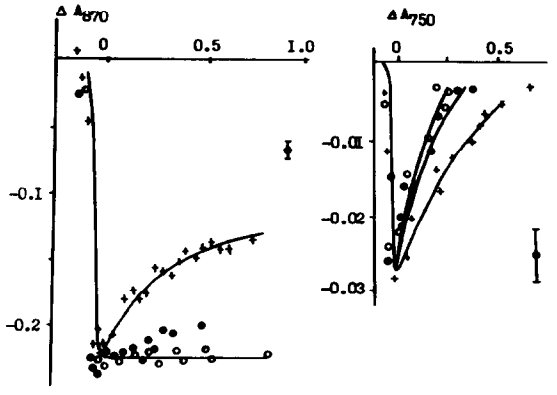

Fig.2. Effect of substituting H_2O by D_2O in RC preparations from wild-type *Rps. sphaeroides* on the kinetic behavior of laser-induced absorption changes of bacteriochlorophyll P at 870 nm and bacteriopheophytin at 750 nm. (○—○) Control, (+ — +) after substituting H_2O by D_2O, (●—●) reversal of the effect after substituting D_2O by H_2O (each point, average of 20 measurements; the maximum measurement error shown by a vertical bar is less than 6×10^{-3} A units). Solid line, theoretical least-squares approximation.

Table 2

Relative contribution (A, arbitrary units) and characteristic time (τ, ps) of the fast component of RC bacteriochlorophyll reduction (as monitored by 870-nm absorption changes) and decay time (τ_0) of the intermediate ion-radical state $P^+I^-Q_A$ (as monitored by 750-nm absorption changes) in wild-type *Rps. sphaeroides* RC preparations in the presence of different organic solvents/cryoprotectors

Solvent	Parameter	Ethylene glycol	Glycerol	Propylene glycol	DMSO
0	A	0	0	0	0
	τ_0	150 ± 25	150 ± 25	150 ± 25	150 ± 25
25	A				0.29 ± 0.07
	τ				450 ± 70
	τ_0				310 ± 80
40	A				0.52 ± 0.03
	τ				700 ± 50
	τ_0				650 ± 100
50	A		0	0.64 ± 0.05	[a]
	τ		–	600 ± 80	
	τ_0		160 ± 30		
75	A		0.23 ± 0.04	[a]	
	τ		320 ± 60		
	τ_0		220 ± 80		
80	A		0.37 ± 0.06		
	τ		400 ± 60		
	τ_0		300 ± 80		
85	A	0.27 ± 0.04			
	τ	330 ± 60			
90	A	0.33 ± 0.03	0.65 ± 0.02		
	τ	400 ± 60	650 ± 50		
	τ_0		500 ± 100		

[a] Onset of aggregation

wide variability: from 0.6 in sample 1 to 0 in sample 3. With the propylene glycol content increased to 70%, the effect also becomes noticeable in sample 3, with A amounting to as much as 0.3. Interestingly, the 70% content of propylene glycol and 40% of DMSO (maximal concentrations) have virtually no effect on R-26 preparations that have not been pretreated with SDS. After adding 1% SDS, in the presence of 70% propylene glycol or 40% DMSO, a fast, nanosecond component appears in such preparations, as in the wild-type, with the contribution amounting to 0.4. The observed differences in interaction between the organic solvent and RC pigment-protein complex in preparations from different cell samples of a single strain are probably the consequence of some variability in the structural and dynamic state of the RCs in the isolated state as it is difficult to reproduce strictly identical conditions at preparation. The difference seen in the two strain types suggests that the globular structure of the wild-type RC is somewhat unfolded. In the compact folded conformation, it is difficult for the molecules to diffuse into the hydrophobic nucleus of the RC protein – the locality of the pigments and electron transport cofactors – unless the surface of the globule is loosened by added detergent.

As follows from table 2, the effect of cryoprotectors on the kinetics of electron transfer in the PIQ_A system increases in the order: ethylene

glycol, glycerol, propylene glycol, DMSO. The effectiveness of these substances is correlated with their hydrophobicity, i.e. the capability of penetrating the RC pigment-protein complex (cf. the distribution coefficients in an octane/water system [19]).

The data are in good agreement with the substitution effects of heavy water of different isotopic composition (^2H_2O and $H_2^{18}O$), mono- and multi-atomic alcohols and proton-free solvents on the dynamics of charge separation in *Rps. sphaeroides* chromatophores observed in experiments in which recombination-induced nanosecond luminescence was monitored by phase fluorometry [20]. It has also been shown that the functional changes induced by the addition of a cryoprotector are correlated with the extent of its penetration into the interior of the photosynthetic membrane. Such physical properties as viscosity and dielectric constant have much less effect than the hydrophobic properties of the solvent. Of course, organic solvents produce more than one effect on the structure and dynamic behavior of biological macromolecules. However, stabilization of the structure of biopolymers is generally accepted to be their major effect. The similarity in the effects of D_2O substitution and cryoprotectors suggests one possible interpretation of the effect of the latter – modification of intra-protein hydrogen bonds.

An indication of the specific effect of substances being studied comes from results of a special experiment in which we used a water/glycerol suspension of wild-type *Rps. sphaeroides* RCs. The parameters A and τ of laser-induced oxidation-reductions of P and I were measured in the presence of glycerol (60–80%, v/v) at different temperatures. The parameters remained unchanged over temperatures from 20 to 0°C. The viscosity of glycerol is known [21] to increase 5–7-fold at low temperatures such as these. Presumably, the effects we observed cannot be explained in terms of solvent viscosity, although in principle solvent viscosity can influence the microviscosity of biological structures and change their functional activity [22].

We also investigated electron transfer in the P-I-Q_A system as a function of pH. A change in pH toward the acidic (pH 5.5–5.8) or alkaline region (pH 10–10.5) retards forward electron transfer between I and Q_A (the characteristic time increasing more than 2-fold) which is accompanied by the appearance of a kinetic component due to P^+-I^- recombination (characteristic time ~1 ns). The relative contribution of the latter (A) is about 0.2 at pH 5.8 and 10.5 in an RC preparation from the *Rps. sphaeroides* wild type and 0.1–0.15 in an RC preparation from the carotenoidless strain R-26. Interesting results are obtained on comparison with fluorescence from the RC tryptophan which has been studied in our earlier investigation using RC preparations from *Rps. sphaeroides* wild type [23]. The position of the fluorescence peak was observed to be unchanged in the pH range 6.5–9.5 but showed a shift toward longer or shorter wavelengths at higher pH (>9.5) or smaller pH (below pH 6.5), respectively. As known [24], such shifts may be a response to a change in orientational mobility of the dipoles in closest proximity to the excited chromophores. The shifts of the fluorescence maxima that were observed in [23] are associated with the fact that the structure in the vicinity of the tryptophan residues becomes tighter at acidic pH and loosens at alkaline pH. Hence, the observed changes in kinetic parameters of electron transfer in the P-I-Q_A system in an RC whose structure was stabilized by D_2O or by added cryoprotector molecules may be a general phenomenon, a change in the electron transfer rates as a result of the deviation of the structural and dynamic state of the RC from some optimal physiological state.

The observed effects of modification of RCs, affecting protein hydrogen bonds, confirm the concept that the relaxation processes through which the energy levels of the electron carriers are lowered to levels necessary for the stabilization of separated charges proceed with the involvement of mobile protons of the protein and bound water in the nearest surrounding of the donor-acceptor system. A change in polarization of the hydrogen bonds in response to the charge of the electron carriers (i.e. the intramolecular process involving conversions of the type AH-B $\longrightarrow$ A-HB) can occur, with high efficiency, even at extremely low temperature. However, removal of water from the RC by drying that disrupts the hydrogen bonds, by solvent or by substituting protons for deuterons, i.e. by exposure that reduces the internal mobility and increases the rigidity of the system [21,25],

may hinder normal relaxation. This violates the conditions of formation of the energetic barrier by which the recombination of P and I is prevented. The mismatch in the energetic levels in the PIQ_A system may well be responsible for the electron-carrying capacity of the direct pathway between P and I and may reduce it.

The conclusion drawn from these findings is that the primary, fast events of photosynthesis and, presumably, of other biological processes, which are associated with the strong redistribution of charge density in pigment-protein complexes, cannot be considered without due regard for the molecular dynamics of light, charged particles (groups). This is essential for constructing adequate physical models of electron transfer in biological systems.

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