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PHOTO-INDUCED ELECTRON TRANSPORT AND WATER STATE IN *RHODOSPIRILLUM RUBRUM* CHROMATOPHORES

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Summary

It is shown that in bacterial chromatophores the pronounced changes in the free water content with a proton spin-spin relaxation time (T_2) of 10^{-3}—10^{-2} s does not influence the efficiency of electron transfer from the photosynthetic reaction centre to the membrane pool of secondary acceptors. An abrupt inhibition of this process occurs only after the loss of the water with faster proton spin-spin relaxation time (T_2 of 10^{-4} s). The process is reversible. The water fraction in question is obviously bound to the chromatophore proteins and forms the primary hydration layer.

Introduction

As far back as in 1962 Clayton has attempted to investigate the effects of hydration on the functional activity of the photosynthetic apparatus as an approach to studies of the physical mechanisms of the primary act of solar energy conversion [1]. Using photosynthetic membranes of purple bacteria, he studied the general effects of hydration on absorption and photochemical characteristics of the photoactive pigment and its adjacent components. Investigations have been continued in recent years using preparations of isolated photosynthetic reaction centres [2,3]. However, estimation of water content and its state was not made in those works, though the necessity of such studies was emphasized.

Abbreviations: BChl, bacteriochlorophyll; X_1 and X_2, primary and secondary acceptor respectively; NMR, nuclear magnetic resonance; ESR, electron spin resonance.

In some of our previous works [4—6] we have studied spectral, electron transfer and other characteristics of various preprations from purple bacteria at a wide range of hydration of the preparations. It has been found in experiments with whole cells [4], isolated chromatophores [5] and photosynthetic reaction centres [6], that dehydration of the preparations causes considerable, but reversible changes in the structure and functional activity of the photosynthetic apparatus. A sharp decelleration of electron transfer from photoexcited reaction centres to secondary quinoid acceptors has been observed in samples (chromatophore and isolated reaction centre films) incubated at a relative humidity (P/P_s) of 0.3 to 0.1 This effect is correlated to the reversible breaking of local conformational mobility of macromolecular components of reaction centres that has been observed in experiments with spin- and ^{57}Fe-labeled preparations [7]. The shift of the absorption band of reaction centre bacteriochlorophyll $(BChl)_2$ to longer wavelengths was also seen in the same P/P_s region. Obviously, the spectral position of this band depends, in large measure, on the relative position of the conjugated π-π systems of porphyrin macrocycles forming dimer $(BChl)_2$ [8—10]. In maintenance of a specific mutual orientation of components of the special pair, some role is obviously played by interactions with the side chains of some amino acids of the protein carrier. It is possible that the latter may be involved in the stabilization of the structure of dimer $(BChl)_2$ in vivo. If so, structural changes of the protein of the reaction center, and therefore its conformational dynamics, must influence the spectral properties of dimer $(BChl)_2$.

It seemed reasonable to continue investigations along these lines, monitoring in parallel the water state in chromatophores by direct methods. The NMR pulsed spin-echo technique is quite a suitable tool for such studies. The results of our experiments are reported here. We found that pronounced changes in the free water content with a proton spin-spin relaxation time (T_2) of 10^{-3}—10^{-2} s do not influence the efficiency of an electron transfer from the reaction centre to the membrane pool of secondary acceptors. However a marked but reversible inhibition of this process was seen upon removal of the water with $T_2 = 10^{-4}$ s, which is obviously bound to the chromatophore proteins forming the primary hydration layer.

Materials and Methods

Experiments were carried out on *Rhodospirillum rubrum* chromatophores isolated by the method described elsewhere [11]. The incubation mixture contained 0.25 M sucrose, 50 mM Tris-HCl buffer, pH 7.6 and 6 mM $MgCl_2$. Without addition of reductants, a rather high redox potential is built up in the mixture, sufficient for the oxidation of cytochromes. Meanwhile, in *R. rubrum* chromatophores isolated from sonicated cells, most of cytochromes is missing [12]. The chromatophores obtained were freeze-dried for 20 h at approx. 1.3 Pa. They were frozen first in a thin layer at —30°C. Then dehydration was carried out at room temperature. NMR measurements were done on powdered chromatophores and absorbance changes measurements on thin films. The films were prepared by evaporation of a concentrated aqueous suspension of freeze-dried chromatophores on a glass plate. The procedure was carried out at

room temperature, atmospheric pressure and humidity in the dark. Variable hydration levels of the chromatophore powders and films were obtained by equilibrating the sample with the desired relative humidities provided by saturated solutions of different salts [13]; 24 h were normally allowed for the equilibrium to be attained. The preparations were kept at a given humidity for 48—72 h before being transferred into sealed test cuvettes. Water content was measured by a 'standard weight' method, that is by drying the preparations at 105° C and estimating the weight of water lost.

Water proton spin-spin relaxation time (T_2) was measured by the Hahn two-pulse method [14], using the 'Minispec p 20' spectrometer which operates at a resonant frequency of 19.8 MHz ('Bruker', F.R.G.).

Photo-induced electron transport reactions in chromatophores were followed by the method of differential absorption spectroscopy.

Results

a. Reaction centre activity

As is known after light excitation of chromatophores of photosynthesizing bacteria the separation of charges occurs rapidly in the reaction centre between $(BChl)_2$ and the acceptor of quinone nature (X_1). The photomobilized electron passes then into the pool of secondary acceptors, also quinones (X_2). In the absence of the constitutive electron donors for $(BChl)_2^+$ (lost in the course of chromatophore preparations or oxidized chemically at a high redox potential) the reversal of the photoinitiated process occurs in the dark. As for *R. rubrum* in particular, the reduction of $(BChl)_2^+$ was observed to be rather slow at normal humidity and room temperature ($t_{1/2}$ from 2 to 15 s). It was shown by Slooten [15] and some other investigators (see the review by Parson and Cogdell [16]) that the interaction between $(BChl)_2^+$ and photo-reduced X_2 is responsible for the slow recovery. This is in good agreement with our previous findings [17]. On lowering the hydration of chromatophores a fast millisecond component appears in the kinetics of $(BChl)_2^+$ dark recovery, due to the backward reaction in the $(BChl)_2^+ - X_1^-$ pair. A similar behavior was also seen under cryogenic temperatures as well as in the presence of *o*-phenanthroline, the inhibitor of electron transfer between X_1 and X_2 [15—18].

To elucidate in greater detail the characteristics of the observed effects in relation to the amount and state of water bound to the photosynthetic membrane we conducted an investigation of *R. rubrum* chromatophores by the NMR spin-echo technique together with electron transport studies.

Electron transport efficiency at the $X_1 - X_2$ site was measured in terms of the contribution of the slow component to the dark reduction of $(BChl)_2^+$ by photoreduced X_2 after the steady-state redox equilibrium has been attained in the saturating actinic light. The kinetics of $(BChl)_2^+$ dark recovery were monitored by absorbance changes at 870 nm. Contributions of the individual slow and fast components were determined to a high degree of accuracy since the rates of the corresponding reactions differ by at least an order of magnitude [18].

As is seen from Fig. 1A, the efficiency of the $X_1^- - X_2$ electron transfer (N) remains at the same level wherever P/P_s varies from 0.9 to 0.3. Further

dehydration of chromatophores over a small interval of P/P_s from 0.3 to 0.1 leads to a sharp decrease in N, which occurs simultaneously with the red band-shift of $(BChl)_2$. At $P/P_s \geqslant 0.3$ the maximum of the absorption band of $(BChl)_2$ is centred at 870 nm, but at low humidity it is abruptly shifted toward 880 nm.

The curve that is presented in Fig. 1B illustrates the effect of hydration on intramolecular mobility in chromatophore membranes. This curve is taken from our previous work [7]. The parameter τ' that is given along the ordinate axis is proportional to the correlation time of rotational diffusion of nitroxyle radicals, covalently bound to the SH groups of the membrane proteins. The τ' value greatly depends on the mobility in the local surroundings of the spin label and therefore may be used as a characteristic for the dynamic state of the medium. In analysing the curves of Fig. 1, A and B, it should be remembered that curve 1B refers to powder preparations of photosynthetic membranes which, it was experimentally found, absorb by 20—50% less than films at P/P_s from 0.1 to 0.5. For this reason, the sorption curve for powder preparations is shifted by 0.1—0.15 units of P/P_s toward greater humidity, as compared with that for film preparations. It is clear, taking into account the above considerations, that there is rather close correlation between the curves of Fig. 1A and 1B.

Effects of dehydration upon kinetics of the pigment-acceptor interactions were studied also on reaction centre preparations, isolated with lauryldimethylaminoxide, from the carotenoidless mutant of *Rhodopseudomonas spharoides* [2,3]. In this work the increase of the recombination rate between oxidized $(BChl)_2$ and photoreduced acceptor X_1 was observed after drying. However, the quantitative relation of the effect to sample water content was not determined. We have demonstrated, in experiments with reaction centre preparations, made

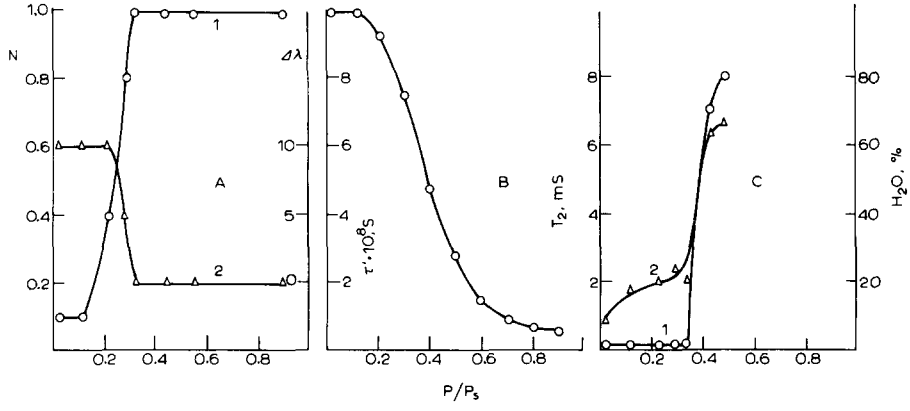

Fig. 1. Reaction centre characteristics and water state in *R. rubrum* chromatophores equilibrated with different relative humidities (P/P_s). (A) N, effectivity of the photoinduced electron transfer from primary (X_1) to secondary (X_2) acceptor in the reaction centres (curve 1); $\Delta\lambda$, shift of the main near infrared absorption band of $(BChl)_2$ in the reaction centres (curve 2). (B) τ', intramolecular mobility in the chromatophore membranes as determined from the correlation time measurements of rotational diffusion of ESR-spin labels covalently bounded to SH-groups of membrane proteins. (C) T_2, spin-spin relaxation time for protons in the chromatophore preparations (curve 1); H_2O, water content in the chromatophore preparations as measured by the NMR spin-echo method (curve 2).

with sodium dodecylsulfate from the wild type of *Rps. sphaeroides*, that the above acceleration of recombination occurs in samples whose humidity falls within the P/P_s range from 0.3 to 0.1 [6].

b. Water state

In freeze-dried chromatophores the proton spin-echo relaxation signal shows a single exponential decay in the time domain of measurements (Fig. 2A). From comparison with NMR spin-echo signals of the control it appears that the protons responsible for the resonance in lyophilized chromatophores constitute approx. 9% of the sample weight.

The spin-echo signal from protons was observed earlier for whole freeze-dried cells of photosynthesizing purple bacteria. In *Chromatium vinosum* the amplitude of such signal corresponds to 2.9% of proton contribution [4]. In both cases spin-spin relaxation times (T_2) for these fractions of protons were about 10^{-4} s. Drying in vacuo (approx. 1.3 mPa. 10 h) of *C. vinosum* cells [4], as well as *R. rubrum* chromatophores does not affect the residual spin-echo signals. There is a possibility, of course, that in addition to the contribution from the protons of strongly bound water with restricted motions, the residual NMR signal may have the contributions from protein and lipid protons too. Because of the low mobility of protons in lyophilized samples, strong dipole-dipole interactions can take place in them thus leading to a respective shortening of T_2 (T_2 approaches 10^{-4} s) [19]. After equilibration with low humidities the number of water molecules with $T_2 \approx 10^{-4}$ s increases somewhat, perhaps as a result of the filling of vacant binding sites. A similar effect was observed after moistening freeze-dried preparations of globular proteins.

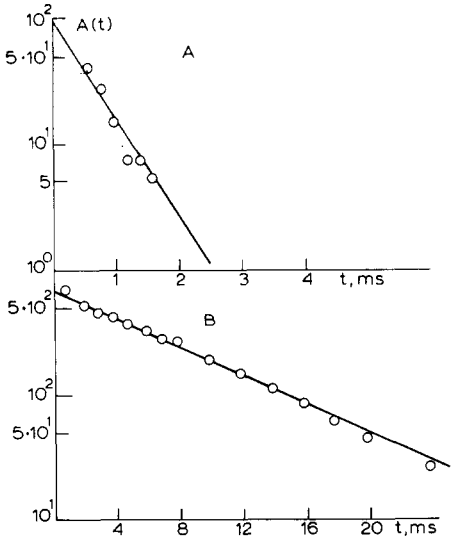

Fig. 2. The decay of transverse magnetization from protons in *R. rubrum* chromatophores. (A) Freeze-dried preparations (weight 1 g). (B) Preparations equilibrated with P/P_S = 0.43 (weight 1 g). The ordinate axis: amplitude of NMR signal (arbitrary units). The abscissa axis: time spacing between radio frequency pulses (ms).

TABLE I

NMR QUANTITATIVE ESTIMATIONS FOR PROTON MOBILITY AND WATER CONTENT IN *R. RUBRUM* CHROMATOPHORES EQUILIBRATED WITH DIFFERENT RELATIVE HUMIDITIES (*P/Ps*)

P/Ps	Spin-spin relaxation time for protons (ms)	Water content per 1 g sample wt. (%)
Freeze-dried samples	0.05	9
0.12	0.05	18
0.23	0.08	20
0.3	0.10	23
0.33	0.10	21
0.43	7.0	64
0.49	8.0	66

In chromatophores equilibrated at $P/P_s = 0.33$, 23% of protons contribute to the NMR spin-echo signal (as estimated by an equivalent amount of water).

Estimation of the total water contents by the 'standard weight' method in samples equilibrated at P/P_s from 0.12 to 0.33 yields values by 5—9% lesser than by NMR measurements. This may be the result of some contribution to the spin-echo signals from protons of membrane proteins and lipids.

After equilibration at $P/P_s = 0.43$ the chromatophores have a much greater amount of water, as detected by the NMR method. The proton spin-spin relaxation in this case occurs on a longer time scale. The spin-echo decay curve remains single exponential, but the T_2 value increases (Fig. 2B). That is an obvious indication of the appearance of some water with greater mobility. Molecules of the water fraction are exchanging rapidly with the bound water fraction thus resulting in the observed increase of the proton spin-spin relaxation time ($T_2 \simeq 10^{-3}$—10^{-2} s). Table I represent T_2 values and water contents, measured by the NMR spin-echo method in chromatophores equilibrated at different P/P_s from 0.12 to 0.49. In samples incubated at $P/P_s > 0.5$ both the amount of water and T_2 parameter do not show appreciable changes. For comparison with the reaction centre activity data, the NMR results are also given in Fig. 1C. As is seen, considerable changes in T_2, $[H_2O]$ and N, $\Delta\lambda$ refer to the same critical region of relative humidities. In fact the correlation is even more pronounced, keeping in mind, as it is mentioned above, that water sorbing capacity of chromatophore powders is lower that of dry films.

Discussion

It has already been inferred that structural dynamics of the reaction centre pigment-protein complex in the photosynthetic membrane are necessary to provide for its functional activity [6,7,17]. In particular, the electron transfer from X_1^- to X_2 is associated with dielectric polarization and conformational changes in the reaction centre protein [6,7,17,20]. The conformational hypothesis to explain the stabilization of primary redox products $(BChl)_2^+$ and X_1^-, tightly bounded to the reaction centre protein, refers to the relaxation energy losses during the electron tunneling in macromolecular systems. This is

analysed in current theories of electron transfer mechanisms in photosynthesis [21—24]. Such relaxation losses are believed to proceed in a stepwise manner through several non-equilibrated states to the equilibrium comformation of the reaction centre complex with reduced X_2 [25,26]. The structural rearrengements as a tool to prevent the back ward electron tunneling from X_1^- to $(BChl)_2^+$ are accompanied by shifts in energy levels of carries involved. During these processes the electron energy level lowers in the reduced acceptor so that the difference between electron levels of donor and acceptor molecules increases. Therefore the backward electron tunneling becomes impossible and electron passes from X_1^- to X_2.

The intramolecular mobility is essential to provide the above mentioned transitions between different conformational states in the reaction centre complex. On the other hand it is known that water critically influences the steric configuration of biopolymers and in some cases is incorporated in the structure of active centres of functional macromolecules [27].

The results reported here indicate that water directly bound to polypeptide chains of the reaction centre complex is necessary to provide for conformational changes associated with the photoinduced electron transfer. This water obviously forms the primary hydration coating over the protein components of the reaction centre. In fact when the content of relatively free water ($T_2 = 10^{-3}$—10^{-2} s) is varied over a large range of $P/P_s \geqslant 0.3$ the efficiency of the electron transfer from the reaction centre to the secondary acceptors ($X_1^- \rightarrow X_2$) remains constant. An abrupt, but reversible, inhibitor of the electron removal from the reaction centre and concomitant red bandshift of $(BChl)_2$ occur just at humidity range over which no relatively free water is observed. These effects clearly manifest that strongly bound water with the proton spin-spin relaxation time of about 10^{-4} s, that is water fraction incorporated into the structure of the reaction centre pigment-protein complex, controls its functional activity.

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