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Study of Effect of Molecular Mobility in Chromatophore Membranes of the Bacterium *E. shaposhnikovii* on Processes of Photoinduced Electron Transport Using the NMR-Spin-Echo Method with Isotope Substitution and Dehydration

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Abstract—The effect of dehydration and $^2H_2O/H_2O$ isotope substitution on electron transport reactions and relaxation of proton-containing groups was studied in chromatophore membranes of *Ectothiorhodospira shaposhnikovii*. During dehydration (including isotope substitution of hydrate water) of preliminarily dehydrated isolated photosynthetic membranes there was a partial correlation between hydration intervals within which activation of electron transport from high-potential cytochrome *c* to photoactive bacteriochlorophyll dimer P890 of photosynthetic reaction center and variation of spin–lattice and spin–spin proton relaxation time was observed. Partial correlation between hydration intervals can be considered as evidence of correlation between mobility of non-water proton-containing groups with proton relaxation frequency $\sim 10^8$ sec^{-1} with efficiency of electron transfer at the donor side of the chain.

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Interaction of membrane-bound enzymes with the membrane environment plays a crucial role in the functioning of the enzyme. Lipid bilayer prevents denaturation of membrane protein and provides interaction between enzymes involved in catalytic cycles. In many cases, lipids also regulate catalytic cycles themselves [1, 2]. Interaction of a membrane protein with its lipid environment is a prerequisite for the necessary level of enzyme conformational mobility allowing its functional activity [3].

This is also valid in the case of photosynthetic proteins providing effective transformation of light energy, including membrane-bound protein complex of photosynthetic reaction center (RC) of purple bacteria. RC of purple bacteria implements photoinduced separation of primary opposite charges. The lipid composition of bacterial photosynthetic membranes depends on growth conditions (e.g. oxygen concentration in the atmosphere). The main lipid components of photosynthetic membrane are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, and cardiolipin [4-6]. It was shown that lipid mobility in chromatophores was limited as a result of interaction with membrane proteins [7]. Some lipids remain bound to RC proteins even after a procedure of intense purification. For instance, RC protein of *Rhodobacter sphaeroides* was found to be strongly bound to three lipid molecules (cardiolipin, phosphatidylcholine, and glucosylgalactosyldiacylglycerol) [8]. This implies specific interaction between phospholipids and RC protein. It was shown that thermodynamic and kinetic parameters of electron transfer in the quinone acceptor site of photosynthetic electron transport chain of purple bacteria RC depended on the presence of physiologically important lipids (phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, and cardiolipin) in the protein environment [9, 10]. It is presently obscure how lipids modify processes of photosynthetic energy conversion. However, it was suggested in [9] that anion phospholipids exerted an electrostatic effect on the primary quinone acceptor Q_A in photosynthetic RC. This effect was manifested as an increase in the lifetime of the state with photoseparated charges. It was demonstrated in our earlier works that electron transport processes in pho-

Abbreviations: NMR, nuclear magnetic resonance; RC, reaction center.

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tosynthetic RC were associated with structure–dynamic states of the RC macromolecular complexes. Studies of temperature dependence, dependence on hydration extent, and isotope substitution of hydrate water revealed a correlation between molecular mobility of photosynthetic membrane domains and electron transport activity of specific sites of the photosynthetic electron transport chain [11-13].

The effect of isotope substitution and controlled hydration on photoinduced reactions of quinone acceptors and multiheme cytochrome *c* of photosynthetic RC of sulfur bacteria *Ectothiorhodospira shaposhnikovii* or nonsulfur bacteria *Rhodobacter sphaeroides* was studied in our preceding work [14]. It was demonstrated that $H_2O/^2H_2O$ isotope substitution caused an increase in the rate of dark recombination between photo-oxidized RC bacteriochlorophyll (P^+) and reduced primary quinone acceptor in RC of *Rb. sphaeroides* at room temperature. In RC complexes with active cytochrome *c*, dehydration of membrane preparations of *E. shaposhnikovii* chromatophores induces inhibition of electron transfer from primary to secondary quinone acceptors and from high-potential cytochrome *c* to P^+. This effect in the presence of H_2O is observed at lower hydration than in the presence of ^2H_2O. Dehydration of *E. shaposhnikovii* chromatophores in the presence of H_2O had virtually no effect on the rate of electron transfer from the cytochrome *c* heme closest to RC bacteriochlorophyll to P^+. This independence was observed within hydration range P/P_0 ~ 0.5-0.1. In chromatophore preparations hydrated in ^2H_2O this rate was ~1.5 times lower than in such preparations hydrated in H_2O. However, the isotope effect disappears upon deep dehydration. The intracytochrome electron transfer between two high-potential hemes was blocked in the presence of ^2H_2O within this hydration range. In the presence of H_2O within this P/P_0 range there was a gradual decrease in the rate of the intracytochrome electron transfer between the two high-potential hemes. These results were discussed in terms of the effect of isotope substitution and dehydration on relaxation processes and changes in charge environment in RC. Realization of isolated reaction-competent states regulating functional activity of RC was also discussed.

The goal of this work was to continue this research using the method of NMR-spin-echo to study molecular dynamics of bacterial photosynthetic RC membrane complexes under conditions of dehydration and isotope substitution. Dependence of spin–lattice time on hydration degree of samples allows not only variation of molecular mobility but also frequency of internal mobility to be determined if this frequency within a certain hydration range coincides with resonance frequency. Additional information about the role of hydrate water in the primary processes of photosynthesis can be obtained using simultaneous isotope substitution and controlled dehydration of photosynthetic preparations. We developed an original technique of preparation of photochemically active cytochrome-containing membrane preparations of bacterial RC with simultaneous control of humidity and isotope substitution.

MATERIALS AND METHODS

The purple photosynthetic bacterium *E. shaposhnikovii* was grown and chromatophore preparations were isolated as described earlier [15, 16]. Chromatophore films capable of being studied spectrally at fixed humidity were prepared as described in [17]. Photoinduced reactions of cytochromes were measured using methods of optical differential spectrophotometry with pulse laser or continuous laser excitation [18]. Efficiency of electron transport from high-potential cytochrome c_h to RC bacteriochlorophyll dimer P890 was assessed using absorption changes in the gamma-band at 424-425 nm. Efficiency of electron transport from the primary to the secondary quinone acceptors was assessed using the ratio of fast and slow kinetic components of recombination of quinone acceptors with P890. These methods were described in more detail in [19, 20]. NMR experiments were carried out using an NMR-spin-echo spectrometer with digital detection of proton spin-echo signal amplitude (working frequency, 17 MHz) and signal averaging system [21]. Curves of spin–lattice relaxation saturation were measured using a Bruker PC-20 (Germany) NMR-spectrometer at frequency 20 MHz. Samples for NMR experiments were prepared using lyophilization of chromatophore membranes at 10^{-3} atm for 6-8 h. Lyophilized preparations were further incubated in a desiccator in the presence of saturated salt solution for 2-3 days. Water sorption was implemented using transition from low to high humidity. Isotope substitution was implemented by double exchange of H_2O by ^2H_2O in lyophilized preparations. In special experiments it was demonstrated that hydration degree of chromatophore powder used in NMR experiments was the same as in chromatophore films used in optical experiments (the difference was 1-3%). Water content was measured gravimetrically using a VLR-20 analytical scales (Russia). The conventional method of drying to constant weight at 110-130°C was used.

RESULTS AND DISCUSSION

Curves of dependence of spin–lattice (T_1) and spin–spin (T_2) proton relaxation times in *E. shaposhnikovii* chromatophores on hydration in atmosphere of vapors of H_2O and ^2H_2O are shown in Fig. 1 (a and b). A wide minimum of T_1 (Fig. 1a) is evidence of either a wide distribution of mobility frequency or a relatively small increase in the frequency of rotation of presumably lipid molecules within a certain humidity range [13, 22].

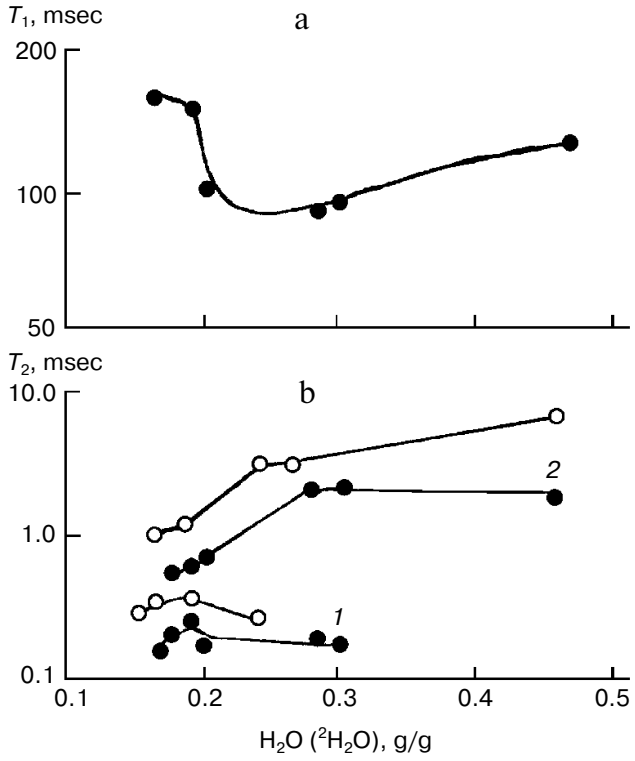

Fig. 1. Curves of dependence of spin–lattice (a) and spin–spin (b) proton relaxation times in *E. shaposhnikovii* chromatophores on hydration. H_2O, open symbols; ^2H_2O, closed symbols. Curves: *1*) fast component of spin–spin relaxation; *2*) slow component of spin–spin relaxation.

Proton spin–spin relaxation within this hydration range (0.15-0.5 g/g) contains both rapid and slow components (Fig. 1b). Within hydration range 0.15-0.25 g H_2O per gram of dry weight in H_2O and 0.15-0.3 g ^2H_2O per gram of dry weight in ^2H_2O there was a substantial increase in the time of the slow component of proton spin–spin relaxation ($T_2 > 1$ msec). This component was observed in addition to the fast component (T_2 ~ hundreds of microseconds) (Fig. 1b, curve *1*). There was a significant decrease in the T_1 value of non-water protons. This value was minimal within hydration range 0.2-0.3 g H_2O per gram of dry weight. Therefore, mobility frequency of non-water molecules (perhaps rotation of phospholipid molecules around their long axis) at this humidity is ~10^8 sec^{-1}. This conclusion about anisotropic mobility of molecules with averaging of local fields over certain directions is consistent with relative invariability of T_2 at high humidity. It should also be noted that anisotropic mobility of molecules with averaging of local fields over certain directions is responsible for decrease in T_1 of non-water protons at low humidity and its increase at higher humidity. Relative invariability of T_2 at high humidity is observed simultaneously with increase in T_1 indicating increasing molecular mobility. In this case mobility increase is accompanied by a trend toward T_2 change to the value non-averaged as a result of motion between neighboring moments of nuclei rather than to zero. This suggestion is consistent with rotation of lipid molecules around their long axes. This rotation is accompanied by averaging of interaction of proton magnetic moments in CH_2-groups, whereas proton interaction in neighboring groups oriented along the long axis of the molecule remains invariable. In contrast to proton spin–spin relaxation time in non-water molecules, spin–lattice relaxation time depends on frequency of motion.

An increase in the hydration degree from 0.3 to 0.5 is accompanied by a significant increase in the contribution of slow component of dark reduction of photo-oxidized RC bacteriochlorophyll dimer P890. This contribution characterizes efficiency of electron transfer from the primary to the secondary quinone acceptors in bacterial photosynthetic RC in *E. shaposhnikovii* chromatophores (Fig. 2a).

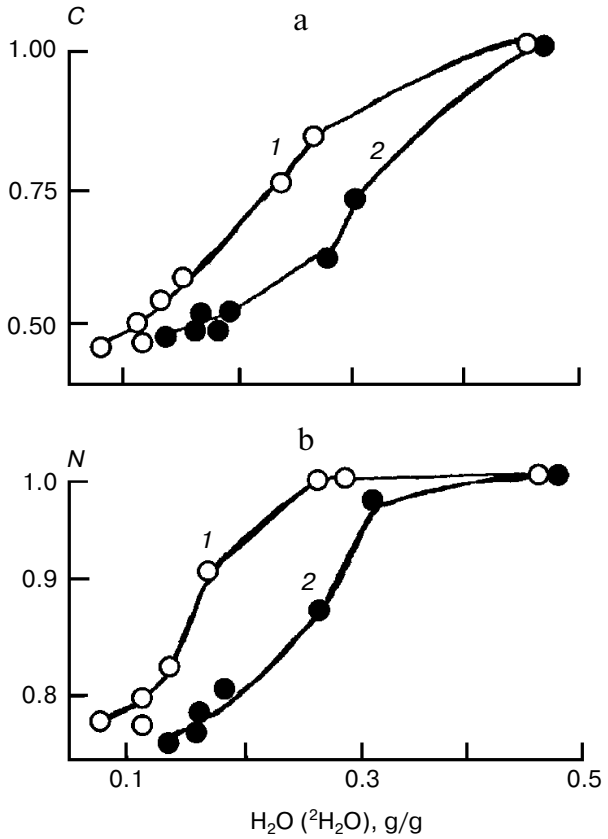

Fig. 2. Effect of hydration degree on efficiency of electron transfer at the acceptor (a) and donor (b) sides of photosynthetic electron transport chain in *E. shaposhnikovii* chromatophores. *C* value is proportional to concentration of cytochrome *c* hemes subjected to photoinduced oxidation; *N* value is proportional to fraction of photoreduced secondary quinone RC acceptors. *C* and *N* values were calculated from experimental results of photoinduced kinetics of cytochromes *c* and RC bacteriochlorophyll (P890) at different hydration degree (for more detail about methods of calculation see [17]): *1*) H_2O; *2*) ^2H_2O.

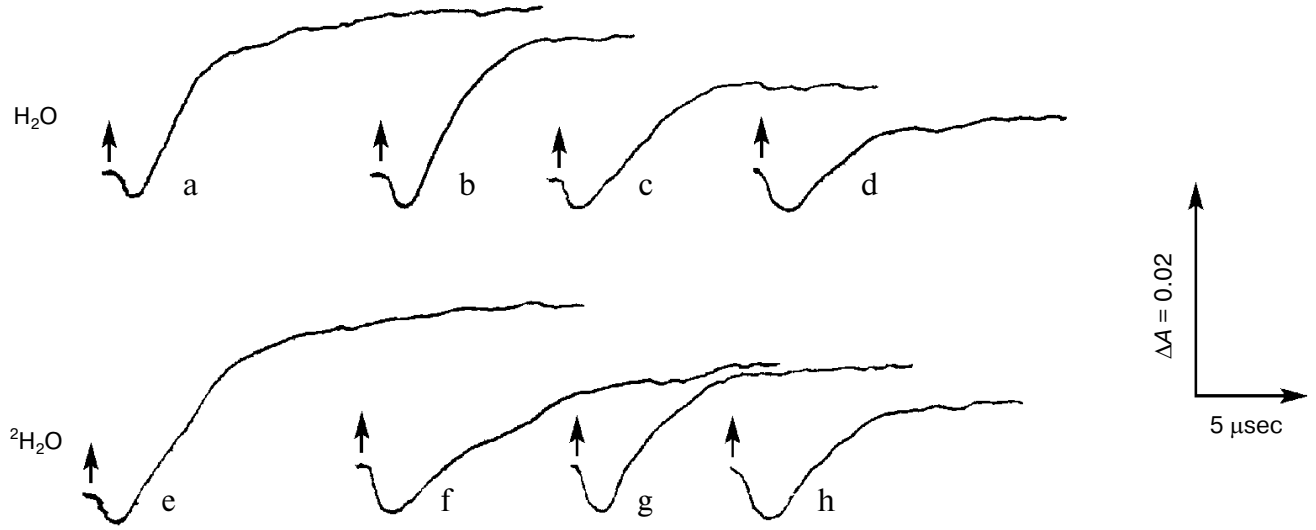

Fig. 3. Kinetics of laser-induced oxidation of cytochrome *c* in *E. shaposhnikovii* chromatophore films at different hydration degree in H_2O (a-d) or ^2H_2O (e-h). Hydration degree (g H_2O per g dry weight): 0.47 (a), 0.25 (b), 0.15 (c), 0.08 (d), 0.47 (e), 0.28 (f), 0.18 (g), 0.14 (h). Spectral measurements were performed at λ_{mean} = 424 nm. Chromatophore films were prepared by drying in the presence of 50 μM tetramethylphenylenediamine and 5 mM sodium ascorbate. Arrows indicate moments of laser-induced photoactivation.

Within this range of hydration degree (0.3-0.5) the efficiency of electron transport at the RC donor side in *E. shaposhnikovii* chromatophores, as assessed by the amount of photoactive cytochrome, remains virtually unchanged (Fig. 2b). Efficiency of this process was also measured at higher hydration degree (up to 0.88) (not shown in Fig. 2).

Pulse laser spectrophotometry allowed the effect of dehydration and isotope substitution H_2O for ^2H_2O on cytochrome *c*–P890 electron transfer rate constant to be measured. The results of this study are summarized in Fig. 3 and the table. It follows from Fig. 3 and the table that in *E. shaposhnikovii* chromatophore films at hydration degree 0.47 g/g the rate constant of this reaction in H_2O is 1.7 times that in ^2H_2O. At low hydration degree the isotope effect decreases. Because at low water vapor pressure the water exchange in samples is slower than at high humidity, it is probable that isotope substitution at low hydration degree was incomplete. To exclude this possibility, we carried out control experiments in samples prepared by dissolution of lyophilized material in H_2O and ^2H_2O. Although the photochemical activity of cytochromes *c* was partially inhibited, the results of kinetic measurements in such preparations at fixed hydration in H_2O and ^2H_2O coincided accurate to ±0.3 μsec with similar results obtained in preparations prepared using the conventional method.

Time of laser-induced half-oxidation of cytochrome *c* in films of *E. shaposhnikovii* chromatophores at different hydration degree in H_2O and ^2H_2O

Hydration degree, g/g	Time of half-oxidation in H_2O, μsec	Time of half-oxidation in ^2H_2O, μsec	Isotope effect
0.14-0.15	2.7	2.7	1
0.25-0.28	2.3	4.2	1.8
0.47	2.3	3.8	1.7

Note: Error in time of half-oxidation was 0.3 μsec.

The effect of dehydration and $^2H_2O/H_2O$ isotope substitution on electron transport reactions and relaxation of proton-containing groups was studied in *E. shaposhnikovii* chromatophore membranes. Proton relaxation was studied by the method of spin-echo of proton magnetic resonance (PMR). It was suggested that the PMR signal in deuterated preparations was due to protons of fatty acids of membrane lipids rather than water protons. The position of the extremum in the curve of the dependence of signal of spin–lattice relaxation on hydration provides information about proton mobility. During variation of hydration degree there was a partial coincidence between hydration ranges within which electron transport from high-potential cytochrome *c* to photoactive bacteriochlorophyll dimer P890 of photosynthetic RC was activated and spin–lattice relaxation and spin–spin relaxation time was changed. Partial coincidence between hydration ranges can be regarded as evidence of correlation between mobility of non-water protons with relaxation frequency ~10^8 sec^{-1} and electron transfer activity at the donor side.

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REFERENCES

1. Sandermann, H., Jr. (1978) *Biochim. Biophys. Acta*, **515**, 209-237.
2. Latruffe, N., Berrez, J. M., and El Kebbaj, M. S. (1986) *Biochimie*, **68**, 481-491.
3. Lee, S. Y., Lee, A., Chen, J. Y., and MacKinnon, R. (2005) *PNAS USA*, **102**, 15441-15446.
4. Wood, B. J. B., Nichols, B. W., and James, A. T. (1965) *Biochim. Biophys. Acta*, **106**, 261-273.
5. Onishi, J. C., and Niederman, P. (1982) *J. Bacteriol.*, **149**, 831-839.
6. Benning, C. (1998) in *Lipids in Photosynthesis: Structure, Function and Genetics* (Siegenhaler, P. A., and Murata, N., eds.) Kluwer, Dordrecht, pp. 83-101.
7. Birrell, G. B., Sistrom, W. R., and Griffith, O. H. (1978) *Biochemistry*, **17**, 3768-3773.
8. Camara-Artigas, A., Brune, D., and Allen, J. P. (2002) *PNAS USA*, **99**, 11055-11060.
9. Agostiano, A., Milano, F., and Trotta, M. (2005) *Photosynth. Res.*, **83**, 53-61.
10. Milano, F., Dorogi, M., Szebenyi, K., Nagy, L., Maroti, P., Varo, G., Giotta, L., Agostiano, A., and Trotta, M. (2007) *Bioelectrochemistry*, **70**, 18-22.
11. Kononenko, A. A., Knox, P. P., Chamorovsky, S. K., Rubin, A. B., Likhtenshtein, G. I., Krupyansky, Yu. F., Suzdalev, I. P., and Goldansky, V. I. (1986) *Chem. Phys. (Moscow)*, **5**, 795-804.
12. Rubin, A. B., Kononenko, A. A., Pachshenko, V. Z., Gulyaev, B. A., and Chamorovsky, S. K. (1987) *Advances in Science and Technology. Biophysics* [in Russian], Vol. 20, VINITI Press, Moscow.
13. Aksyonov, S. I., Knox, P. P., Kononenko, A. A., Chamorovskii, S. K., and Rubin, A. B. (1997) *Eur. Biophys. J.*, **26**, 461-470.
14. Chamorovsky, S. K., Krasilnikov, P. M., and Knox, P. P. (2002) *Biochemistry (Moscow)*, **67**, 1298-1306.
15. Samuilov, V. D., and Kondrat'eva, E. N. (1969) *Nauch. Dokl. Vyssh. Shkoly. Biol. Nauki*, **5**, 97-100.
16. Chamorovsky, S. K., Pyt'eva, N. F., and Rubin, A. B. (1977) *Stud. Biophys.*, **66**, 129-136.
17. Pottosin, I. I., Chamorovsky, S. K., Kononenko, A. A., and Uspenskaya, N. Ya. (1984) *Mol. Biol. (Moscow)*, **18**, 821-826.
18. Rubin, A. B. (ed.) (1974) *Modern Methods in Studies of Photobiological Processes* [in Russian], MGU Publishers, Moscow.
19. Chamorovsky, S. K., Remennikov, S. M., Kononenko, A. A., Venediktov, P. S., and Rubin, A. B. (1976) *Biochim. Biophys. Acta*, **430**, 62-70.
20. Chamorovsky, S. K., Remennikov, S. M., Kononenko, A. A., and Rubin A. B. (1980) *Biochim. Biophys. Acta*, **589**, 151-155.
21. Aksyonov, S. I., Voronkov, A. E., Nikolaev, G. M., and Gelfand, V. E. (1971) *Nauch. Dokl. Vyssh. Shkoly. Biol. Nauki*, **12**, 109-113.
22. Aksyonov, S. I., Bobrova, M. N., Bocharov, E. A., Gippius, A. A., Knox, P. P., Kononenko, A. A., Revokatov, O. P., Rubin, A. B., and Shmeleva, E. L. (1997) *Biol. Membr. (Moscow)*, **14**, 5-17.