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Effect of Isotope Substitution and Controlled Dehydration on the Photoinduced Electron Transport Reactions of Quinone Acceptors and Multiheme Cytochrome *c* in Bacterial Photosynthetic Reaction Center

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Abstract—Isotope substitution of H_2O by ^2H_2O causes an increase in the rate of dark recombination between photooxidized bacteriochlorophyll (P^+) and reduced primary quinone acceptor in *Rhodobacter sphaeroides* reaction centers (RC) at room temperature. The isotopic effect declines upon decreasing the temperature. Dehydration of RC complexes of *Ectothiorhodospira shaposhnikovii* chromatophores containing multiheme cytochrome *c* causes a decrease in the efficiency of transfer of a photomobilized electron between the primary and secondary quinone acceptors and from cytochrome to P^+. In the case of H_2O medium these effects are observed at a lower hydration than in ^2H_2O-containing medium. In the *E. shaposhnikovii* chromatophores subjected to dehydration in H_2O, the rate of electron transfer from the nearest high-potential cytochrome heme to P^+ is virtually independent of hydration within the P/P_0 range from 0.1 to 0.5. In samples hydrated in ^2H_2O this rate is approximately 1.5 times lower than in H_2O. However, the isotopic effect of this reaction disappears upon dehydration. The intramolecular electron transfer between two high-potential hemes of cytochrome *c* in samples with ^2H_2O is inhibited within this range of P/P_0, whereas in RC samples with H_2O there is a trend toward gradual inhibition of the interheme electron transfer with dehydration. The experimental results are discussed in terms of the effects of isotope substitution and dehydration on relaxation processes and charge state of RC on implementation of the reactive states of RC providing electron transfer control.

Key words: purple bacteria, photosynthetic reaction center, cytochromes, electron transport, deuteration, dehydration

It is now beyond doubt that dynamic processes in proteins and protein-bound water play a very active role in energy transformation by the pigment–protein complexes of photosynthetic reaction centers (RC) of purple bacteria. These processes contribute to various reactions of energy transformation in RC including the initial stages of photoinduced charge separation, intraprotein electron transfer to quinone acceptors, interprotein electron transfer from cytochrome *c* to oxidized bacteriochlorophyll dimer (P^+), and recombination between P^+ (recombination is observed if electron transfer from cytochrome or other external electron donor to P^+ is inhibited) [1-7]. Dehydration and deuteration of the pigment–protein complexes of interest provide an informative experimental approach to studies of the role of dynamics of low molecular weight proton-containing groups of RC in electron transport reactions. For example, it was found that effects of dehydration and isotope substitution (H_2O by ^2H_2O) of hydrated water on the efficiency of the initial stages of light energy transformation in *Rhodobacter sphaeroides* RC are most probably mediated by modification of relaxation processes. This modification is accompanied by stabilization of the photomobilized electron in pigment cofactors of RC—bacteriopheophytin and primary quinone acceptor (Q_A) [1, 2, 6]. The deuteration-induced effects in this case were attributed not only to trivial mass changes associated with isotope substitution of H_2O by ^2H_2O but also (and mostly) to modification of the network of hydrogen bonds in RC protein. The deuteration-induced modification of the network of hydrogen bonds of RC protein itself was found to be accompanied by a decrease in the mobility of the macromolecule and an increase in its rigidity. However, the resulting changes in molecular dynamics may also have a substantial effect on slower stages of electron transport. In this case, the modifying agent may also induce

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structural changes in macromolecules, provided that the structural transitions are associated with certain stages of electron transport.

The goal of this work was to study the role of conformational dynamics in the intraprotein process of charge recombination between photooxidized bacteriochlorophyll and reduced primary quinone acceptor and in the interprotein electron transfer from cytochrome *c* to bacteriochlorophyll using the experimental approach based on dehydration and isotope substitution of H_2O by ^2H_2O. The objects of study were RC preparations from *Rb. sphaeroides* and RC cytochrome complexes of *Ectothiorhodospira shaposhnikovii* chromatophores.

MATERIALS AND METHODS

Cells of the sulfur purple bacterium *E. shaposhnikovii* were grown in a modified Larsen medium as described in [8]. Cells were disrupted by sonication at 4°C using an UZDN-1 ultrasonic disintegrator. Chromatophores were isolated by differential centrifugation.

Cells of the nonsulfur purple bacterium *Rhodobacter sphaeroides* were grown in Ormerod liquid culture medium [8] under anaerobic conditions in a luminostat at a temperature of 30°C for 3-5 days. Cells were disrupted by sonication. Complexes of RC were isolated from chromatophores treated with detergent lauryl dimethylamine oxide (LDAO) by ultracentrifugation and chromatography on a column with hydroxyapatite as described in more detail in [9]. The resulting RC preparations were suspended to the final RC concentration of 10 μM in 0.01 M sodium phosphate buffer (pH 7.0) containing 0.05% LDAO.

To provide isotope substitution of H_2O by ^2H_2O, a fraction of RC preparation was lyophilized for 4-5 h at 10^{-3} mm Hg and resuspended in heavy water (the degree of isotope substitution was 99.8%). After another fraction of RC preparations had been subjected to similar lyophilization, it was resuspended in distilled water. This fraction of RC preparation served as control.

Dehydrated preparations of photosynthetic membranes and RC preparations containing photoactive cytochromes *c* were obtained as described earlier [10].

Samples for NMR spectroscopy were prepared by lyophilization for 6-8 h at 10^{-3} mm Hg and further incubation in desiccators above saturated solutions of salts in H_2O or ^2H_2O for 2-3 days.

Light-induced photoreactions in RC of *Rb. sphaeroides* were studied spectrophotometrically (by measuring absorption changes in the Q_y absorption band of bacteriochlorophyll P at 870 nm) using a locally made computer-assisted single-beam differential spectrophotometer with pulse light photoactivation with an ISSh-100 3M stroboscopic lamp (pulse duration, 10 μsec; spectral range, 400-600 nm; pulse energy, 9 mJ). To measure kinetic curves of the process of dark reduction of photooxidized bacteriochlorophyll from the primary quinone acceptor, photoreactions were measured in the presence of 10^{-2} M *o*-phenanthroline. As noted above, *o*-phenanthroline is the inhibitor of direct electron transfer from Q_A to Q_B, which displaces the secondary quinone from its binding site in the RC structure. Low-temperature measurements were performed using RC suspension containing 70 vol. % glycerol.

Kinetics of photoinduced electron transport reactions of P890 induced in the *E. shaposhnikovii* chromatophores by steady-state light ($\lambda > 640$ nm; 180 W/m^2) were measured in the spectral band at 890 nm using a differential single-beam spectrophotometer equipped with a cryostat as described in [3].

Kinetics of electron transfer from the multiheme cytochrome *c* to photooxidized bacteriochlorophyll RC *E. shaposhnikovii* was measured at 424 nm using the automated pulsed laser spectrophotometer described in [11].

NMR measurements were performed using a Bruker PC-20 spectrometer (Germany) equipped with an NMR spin-echo unit and a signal averaging system with working frequency of 17 MHz.

Water content in samples was measured gravimetrically using a VLR-20 analytical balance. The samples were dried at 110-130°C to constant weight.

Mathematical simulation of kinetic curves and identification of model parameters by experimental data was performed using the Nedler–Meed method as described in [12].

Lauryl dimethylamine oxide (LDAO) was from Onyx Chem. Corp. (USA). The other reagents were chemical or analytical purity grade products of domestic manufacturers.

RESULTS AND DISCUSSION

Effect of isotope substitution of H_2O by ^2H_2O on the process of charge recombination between photooxidized bacteriochlorophyll and reduced primary quinone acceptor in *Rb. sphaeroides* RC. Isotope substitution of H_2O by ^2H_2O in *Rb. sphaeroides* RC causes an increase in the rate of dark transfer of an electron from the reduced primary quinone acceptor Q_A to the photooxidized bacteriochlorophyll dimer P870. In this particular series of experiments, the characteristic time of the process at 22°C in control and deuterated RC preparations was 101 ± 2 and 90 ± 2 msec, respectively. It has been already reported in [13, 14] that isotope substitution of H_2O by ^2H_2O induces acceleration of the reaction of recombination between P^+ and Q_A^-. It was suggested in [14] that the effect of acceleration is due to substitution of certain protons in the vicinity of Q_A. According to the viewpoint suggested in [14], the vibronic modes of these protons could be coupled with electron transfer. Studies of temperature-

induced effects in control and deuterated RC preparations provide important information about mechanisms of influence of isotope substitution on electron transport. Although the temperature-induced effects were not studied in [14], the temperature dependence of the rate of recombination between P^+ and Q_A^- in nondeuterated RC was measured by other authors. It was experimentally observed in [15] that temperature decrease is accompanied by an atypical increase in the rate of this reaction. This pattern of temperature dependence of the reaction rate was attributed to possible low-temperature compression of the protein globule of RC [15] or to switching between high- and low-temperature conformational states of RC protein that are characterized by different rates of electron transfer from Q_A^- to P^+ [16].

The temperature dependencies of the rate of recombination between P^+ and Q_A^- in control and deuterated RC samples studied in our experiments within the range from room temperature to −180°C are shown in Fig. 1. Comparative analysis of these curves revealed that the difference between control and deuterated samples decreased upon decreasing the temperature. This difference was virtually absent at cryogenic temperatures (i.e., isotopic effect virtually disappeared within this temperature range). This finding provides a new insight into mechanisms of regulation of the rate of electron transfer from Q_A^- to P^+ in RC. In our opinion, relaxation processes involving light proton-containing molecular groups control this process.

Let the time of electron transition from Q_A to P be denoted as τ_e. If this value satisfies the inequality $\tau_e >> \tau_r$ (where τ_r is the time of relaxation of surrounding medium), the process of electron transfer is regarded as slow and taking place under steady-state conditions of relaxed medium. In this case, the effect of such factors as deuteration or temperature on electron transfer is mediated by mechanisms other than relaxation processes. However, it should be emphasized that temperature may have an effect not only on characteristic time of relaxation but also on the established value of relaxed parameter. The temperature of the characteristic time of relaxation is described by the Arrhenius equation:

$$\tau_r = \tau_r^0 \exp(E_A/k_B T), \quad (1)$$

where E_A is the activation energy; k_B is the Boltzmann constant; T is temperature; τ_r^0 is the relaxation time at $T \to \infty$. Any relaxation process is associated with displacement of atoms from initial positions. The amplitude of the displacement is determined by the rigidity k of chemical bonds. Parameter k itself is a function of temperature. Therefore, the amplitudes of established value of relaxing parameter at different temperatures differ from each other. Because the rigidity of chemical bonds usually decreases upon increasing the temperature, the amplitude of relaxing parameter should increase with increasing T.

Within the framework of a simple approximation by a curve with single relaxation time, the process of relaxation (e.g., polarization W) can be described by the following equation:

$$W(t,T) = W_0(T)[1 - \exp(-t/\tau_r)], \quad (2)$$

where $W_0(T)$ is the established value of W, i.e., $W(T, t \to \infty) \to W_0(T)$. The values of $W_0(T)$ at different T differ from each other. For example, if $T_2 > T_1$, then $W_0(T_2) > W_0(T_1)$.

It should be noted that parameter W_0 is included in the well-known expression for medium reorganization energy, which determines the activation character of the rate constant of electron transfer [17]. Changes in reorganization energy lead to changes in the rate constant. On the other hand, changes in the value of W_0 cause a shift in the energy level position of donor and acceptor. This may have a substantial effect on the probability of tunneling, changing thereby the rate of electron transfer. Therefore, although the time of electron transfer τ_e in this case is much larger than the relaxation time τ_r, the rate of electron transfer may be in principle modulated by the process of relaxation (through the value of $W_0(T)$). For example, let the energy level difference between electron transfer carriers be

$$\Delta = \Delta_0 + W(t, T), \quad (3)$$

where Δ_0 is the initial value of the difference; $W(t, T)$ is described by Eq. (2). If $\tau_e >> \tau_r$, then $W(\tau_e, T) \approx W_0(T)$, and Eq. (3) can be recast as:

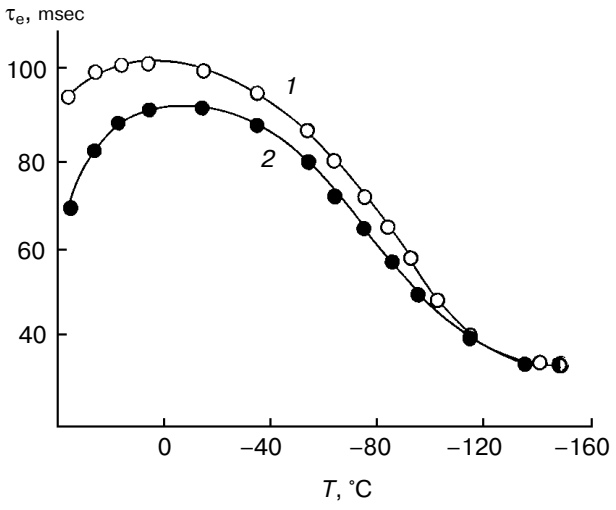

Fig. 1. Temperature dependence of the rate of dark reduction of photooxidized bacteriochlorophyll from reduced primary quinone acceptor in RC of *Rb. sphaeroides*: *1*) H_2O; *2*) ^2H_2O.

$$\Delta(T) = \Delta_0 + W_0(T). \quad (4)$$

If $T_2 > T_1$, $W_0(T_2) > W_0(T_1)$, it follows from Eq. (4) that $\Delta(T_2) > \Delta(T_1)$. Therefore, different temperatures correspond to different values of $\Delta(T)$. The probability of tunneling (therefore, the time of electron transfer) depends on the value of Δ. Therefore, in the case considered above: $\tau_e(T_2) > \tau_e(T_1)$.

The effects of ^2H_2O can be described similarly. Isotope substitution may change the value of $W_0(T)$ by modifying the chemical bond rigidity. Deuteration causes an increase in the rigidity of a hydrogen bond [18]. Therefore, the following inequality should be observed at any temperature: $W_0(T)$ in H_2O > $W_0(T)$ in ^2H_2O. Therefore, isotope substitution of H_2O by ^2H_2O should cause an increase in the rate of the process. At low temperature all possible activation processes are strongly inhibited and relaxation is mainly determined by nuclear tunneling. In the case considered in this work, both protons and deuterons are involved in formation of hydrogen bonds. The time of tunneling of protons or deuterons (10^{-10}-10^{-11} sec) in this case is much shorter than the time of electron transfer. Therefore, during the time interval of electron localization in the carrier at low temperature the tunneling-mediated relaxation of nuclei certainly occurs and effects observed in H_2O and ^2H_2O should be identical to one another, because the electrical charges transferred in H_2O and ^2H_2O are equal to one another.

Thus, studies of temperature dependence of the effects of deuteration supports the suggestion put forward above in this work that the relaxation processes mediated by light proton-containing groups may indeed modify the rate of relatively slow electron transfer (e.g., reaction of charge recombination between P^+ and Q_A^-).

Effect of dehydration and deuteration on structural and dynamic characteristics and processes of electron transfer from the primary to the secondary quinone acceptors and from the multiheme cytochrome *c* to bacteriochlorophyll dimer in *Ectothiorhodospira shaposhnikovii* chromatophores. It was shown in our earlier works that dehydration of membrane preparations of chromatophores and RC of *Rhodospirillum rubrum* within the range of relative ambient humidity from 0.5 to 0.3 P/P_0 is accompanied by desorption of a considerable amount of water, a sharp decrease in the mobility of non-water components, and inhibition of electron transfer from the primary (Q_A) to the secondary (Q_B) quinone acceptors [19]. Similar experiments in *E. shaposhnikovii* chromatophores revealed that the efficiency of electron transfer from the multiheme cytochrome *c* to RC bacteriochlorophyll dimer is changed at a higher threshold relative humidity (~0.7 P/P_0) [10]. The goal of this work was to study the effects induced by simultaneous dehydration and isotope substitution H_2O by ^2H_2O.

It follows from Fig. 2 that the main increment of water adsorption in *E. shaposhnikovii* chromatophores

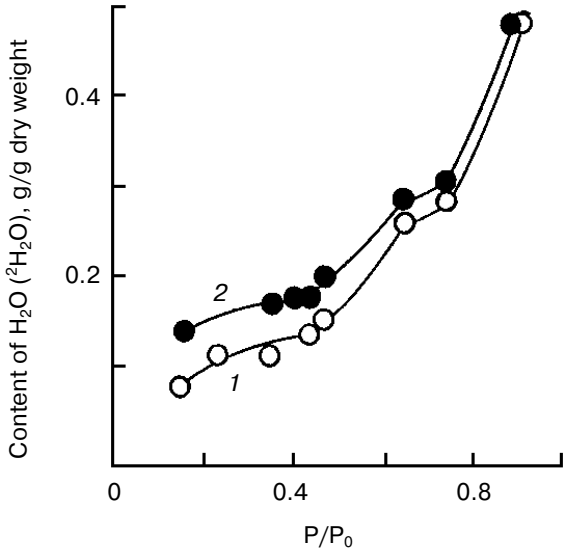

Fig. 2. Isotherms of water sorption by dry powder of chromatophores *E. shaposhnikovii*. The weight values in ^2H_2O were multiplied by 0.9 (ratio of molecular weights of H_2O (*1*) and ^2H_2O (*2*)).

was observed within the ranges of relative humidity from 0.47 to 0.66 and from 0.75 to 0.88 P/P_0. At low humidity the amount of adsorbed ^2H_2O is higher than the amount of adsorbed H_2O. At high humidity this difference disappears. According to the results obtained at high humidity, the number of adsorption centers for ^2H_2O in these preparations was virtually equal to the number of adsorption centers for H_2O. The isotope effect at low humidity is thought to be caused by different accessibility of absorption centers. Another explanation (which does not exclude the first one) is based on different values of adsorption heat in H_2O and ^2H_2O. It should be noted that in our experiments relative humidity was poised using exposure of experimental samples to vapors of saturated solutions of various salts. Because the levels of solubility in H_2O and ^2H_2O slightly differ from one another, only qualitative estimates of the isotope effect can be obtained from sorption curves. Hydration numbers rather than relative humidity values are used below in this work to obtain more accurate quantitative description of hydration curves and isotope effect.

According to the results of NMR spin-echo, within the hydration range 0.15-0.25 g H_2O per g dry weight and 0.15-0.3 g ^2H_2O per g dry weight there was a sharp increase in the mobility of proton-containing groups. According to the results of ^{1}H-NMR, the total amount of mobile protons in sample as calculated per water weight is 0.35 and 0.65 g/g H_2O at hydration numbers of 0.15-0.2 and 0.3 g H_2O per g dry weight, respectively. The difference between the total number of mobile protons and number of water protons corresponds to the contribution

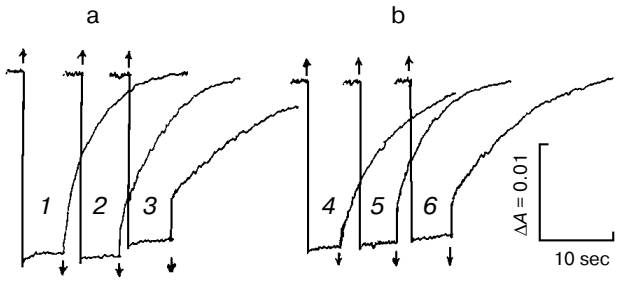

Fig. 3. Kinetic curves of photoinduced absorption changes of P890 in films of *E. shaposhnikovii* chromatophores poised at different levels of hydration in H_2O (a) or ^2H_2O (b). Degree of hydration (g H_2O per g dry weight): *1*) 0.27; *2*) 0.15; *3*) 0.12; *4*) 0.30; *5*) 0.28; *6*) 0.18. Chromatophores were dried without addition of exogenous donor. Upward and downward arrows indicate the moments of actinic light on and off, respectively.

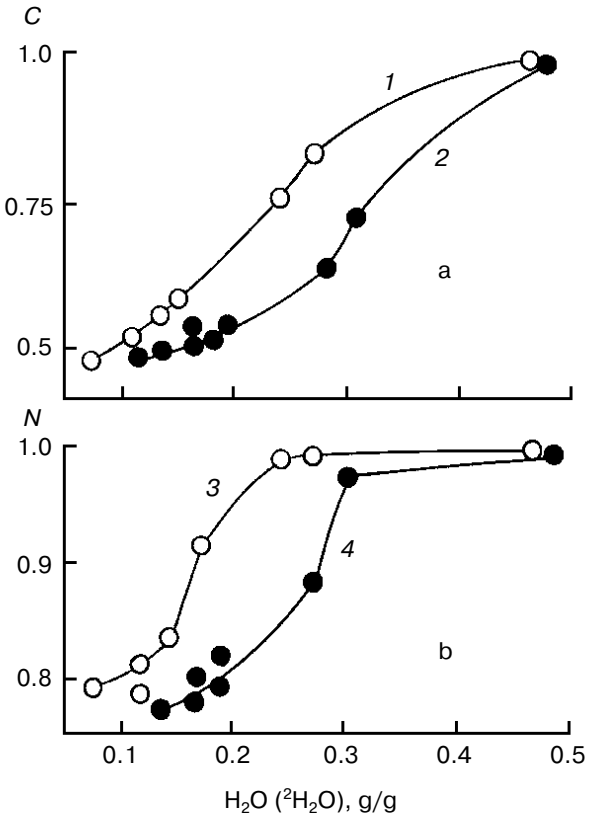

Fig. 4. Effect of the degree of hydration on the efficiency of electron transfer at cytochrome (a) and acceptor (b) sides of RC in *E. shaposhnikovii* chromatophores. The value of *C* was determined from the amplitude of laser-induced absorption changes of cytochrome C_h at 424 nm, whereas the value of *N* was calculated from the magnitude of fast component of dark reduction of bacteriochlorophyll P890 under oxidative conditions: *1*, *3*) H_2O; *2*, *4*) ^2H_2O.

of protons of proton-containing molecules other than water. Therefore, in addition to water content increase, the preparation humidity increase within the hydration range 0.15-0.3 g H_2O per g dry weight was accompanied by an increase in the number of mobile proton-containing groups.

Typical kinetic curves of the photoinduced absorption changes associated with redox reactions of RC bacteriochlorophyll (P890) in films of *E. shaposhnikovii* chromatophores poised at different levels of hydration in H_2O and ^2H_2O are shown in Fig. 3.

The results of kinetic analysis showed that changes in NMR characteristics corresponding to a sharp increase in the mobility of non-water components were observed within the same range of hydration that corresponded to a sharp increase in the efficiency of electron transfer from Q_A to Q_B. The efficiency of this reaction was assessed by changes in the contribution of fast component ($\tau \sim 0.1$ sec) of dark reduction of bacteriochlorophyll P890 (this component represents the reaction of transfer of photomobilized electron from Q_A^- to P^+) in films of chromatophores containing no exogenous donors. This method of calculation of the efficiency of electron transfer from the primary (Q_A) to the secondary (Q_B) quinone acceptors was substantiated and described in more detail in [3].

In films of *E. shaposhnikovii* chromatophores prepared in the presence of exogenous donor of electrons as described in [10] there were photoinduced absorption changes of the high-potential cytochrome C_h rather than bacteriochlorophyll P890. The dependencies of the efficiency of electron transfer at the acceptor (from Q_A to Q_B) and donor (from high-potential cytochrome C_h to bacteriochlorophyll P890) sides of RC on the degree of hydration in H_2O and ^2H_2O are shown in Fig. 4. It follows from Fig. 4 that efficiency of electron transfer both at the acceptor and the donor sides of RC under otherwise identical level of hydration in ^2H_2O is higher than in H_2O. Perhaps the isotope effect in this case is due to the fact that packing density of photosynthetic membranes in ^2H_2O is higher than in H_2O [20]. This fact also supports the suggestion of correlation of the functional reactions considered above with processes of structural dynamics.

To the first approximation, the fact that sharp changes in the efficiency of electron transfer at the acceptor (from Q_A to Q_B) and donor (from high-potential cytochrome C_h to bacteriochlorophyll P890) sides of RC are observed in different ranges of dehydration can be attributed to specific features of protein–lipid interaction, which has a significant effect on the structural dynamic state of certain domains of macromolecular complexes. Interaction of integral proteins with lipids is much stronger than interaction between lipids and peripheral proteins. Therefore, changes in the efficiency of functional contact of cytochrome complex with membrane and RC protein can be observed at relatively milder

conditions than changes in the efficiency of processes of electron transfer between quinone acceptors inside the protein globule of RC. More severe structural rearrangement of lipid–protein complexes is required to change the efficiency of these processes. This suggestion agrees well with the results of our studies of structural dynamic properties of *E. shaposhnikovii* membranes obtained in collaboration with Yu. F. Krupyanskii et al. using the method of Rayleigh scattering of Mössbauer radiation (RSMR) [21]. According to the RSMR data, there are smooth changes in the membrane microviscosity within the hydration range corresponding to significant changes in the efficiency of cytochrome reaction. In contrast to that, a sharp decrease in the membrane microviscosity was observed within the hydration range 0.15-0.25 g/g. It should be noted that changes in the RSMR parameters correspond to mobility inhibition of molecular groups with frequencies of 10^7-10^8 sec^{-1}. Therefore, parameters of molecular dynamics of RC domains of location of electron transport components (quinone acceptors and multiheme cytochrome *c*) correlate with the efficiency of both interquinone electron transfer (*N*) and electron transfer from cytochrome to P^+. A similar conclusion of possible involvement of certain conformational states of a macromolecular system in the control of processes mediated by the system were drawn earlier from detailed studies of temperature dependence of functional reactions or RC and molecular mobility of various physical labels (spin, luminescence, Mössbauer, etc.) [3, 22, 23]. As noted above, the deuteration-induced shift in the curves of the dependence of the efficiency of inter-quinone electron transfer and efficiency of electron transfer from cytochrome to P^+ on the level of hydration toward a larger value of P/P_0 can be attributed to a certain increase in the rigidity of the macromolecular system induced by isotope substitution.

The effect of isotope substitution of H_2O by ^2H_2O on the elementary rate constant of electron transfer from high-potential cytochrome C_h to bacteriochlorophyll P890 was studied using pulse laser spectrophotometry. In suspension of cells or chromatophores of *E. shaposhnikovii* or in dry films of chromatophores at hydration level above 0.5 g/g the elementary rate constant of this reaction in H_2O was found to be approximately 1.5 times higher than in ^2H_2O. At lower levels of hydration there is a trend toward a decrease in the isotope effect of the reaction. The kinetic curves of the laser-induced absorption changes of cytochrome *c* in dry films of the *E. shaposhnikovii* chromatophores at different levels of hydration are shown in Fig. 5. Because at low levels of water vapor pressure the rate of water exchange in the sample is lower than at high humidity, it was conceivable that isotope substitution at low humidity was incomplete. To test such a possibility, we performed control measurements in samples prepared by dissolving lyophilized chromatophores in H_2O or ^2H_2O. Although the photochemical activity of

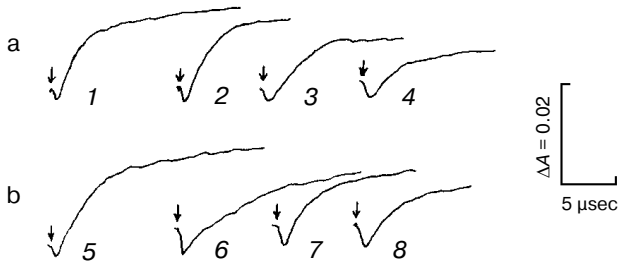

Fig. 5. Kinetic curves of laser-induced absorption changes of cytochrome C_h in dry films of *E. shaposhnikovii* chromatophores at different levels of hydration in H_2O (a) or ^2H_2O (b). Degree of hydration (g H_2O per g dry weight): *1*) 0.47; *2*) 0.25; *3*) 0.15; *4*) 0.08; *5*) 0.47; *6*) 0.28; *7*) 0.18; *8*) 0.14. Monitoring light wavelength, λ_{mon} = 424 nm. Chromatophores were dried in the presence of 50 μM N,N,N′,N′-tetramethyl-*p*-phenylenediamine and 5 mM sodium ascorbate. Arrows indicate the moments of activation of samples with single laser pulse.

cytochrome C_h in such preparations was reduced, the values of characteristic time of photoinduced oxidation of cytochrome C_h at given levels of hydration in H_2O or ^2H_2O coincided accurate to ±0.3 μsec with the values of characteristic time of this reaction in preparations prepared by the conventional method described above.

Mathematical simulation of the effects of dehydration and deuteration on the processes of electron transfer from the multiheme cytochrome *c* to bacteriochlorophyll dimer in *Ectothiorhodospira shaposhnikovii* chromatophores. To study the effects of dehydration and deuteration on the processes of electron transfer from the multiheme cytochrome *c* to bacteriochlorophyll dimer in more detail, we constructed a mathematical model of these processes.

It is well known that the cytochrome subunit of bacterial photosynthetic RC contains two high-potential hemes C_h, which differ from one another by the distance to the RC bacteriochlorophyll and by the value of midpoint redox potential [24]. Therefore, the aggregate of electron transport reactions in this system can be described by the following scheme:

$$C_{h1} \overset{k_1}{\rightarrow} C_{h2} \overset{k_2}{\rightarrow} P890\ , \qquad (5)$$

where C_{h1} and C_{h2} are the first and the second hemes of the high-potential cytochrome C_h, respectively; P890 is the RC bacteriochlorophyll dimer; k_1 and k_2 are the rate constants of corresponding reactions of electron transfer.

The levels of population of hemes C_{h1} and C_{h2} are independent parameters. Therefore, the system may have four initial states, which differ from one another by the redox state of hemes: (I) C_{h1}^{red} C_{h2}^{red}; (II) C_{h1}^{red} C_{h2}^{ox}; (III) C_{h1}^{ox} C_{h2}^{red}; (IV) C_{h1}^{ox} C_{h2}^{ox}, where C_i^{ox} and C_i^{red} are oxidized and

reduced states of the *i*-th heme, respectively. Because in state (IV) the two hemes are oxidized, this state does not contribute to the photoinduced absorption changes. It was shown in [10, 12] that solution of corresponding set of simultaneous differential equations can be described as:

$$P^+(t) = P_1(t) + P_2(t) + P_3(t) =$$

$$= (C_1 + C_2)e^{-k_2 t} + C_2k_2/(k_2 - k_1)\, e^{-k_1 t}, \qquad (6)$$

where P_j and C_j are the probability and concentration of the *j*-th state, respectively; *t* is time.

The kinetic curves calculated from the model were compared with experimental data shown in Fig. 5. Model parameters were identified by fitting experimental curves using the Nedler–Meed method as described in [12]. Rate constants k_1 and k_2 and contributions of states (I)-(III) to the degree of initial reduction of cytochrome pool were used as the search parameters of the model. The results of computer simulation are given in table.

It follows from the table that kinetic curves recorded at high hydration in vapors of H_2O (0.47 g H_2O per g dry weight) are approximated by a bi-exponential function with rate constants $k_1 = 0.09$ and $k_2 = 0.33$. As the hydration level decreases, the kinetic curves of the reaction are gradually transformed into monoexponential with dominant fast component ($k_2 = 0.3$). It can be suggested that dehydration primarily inhibited the reaction of electron transfer from heme C_{h1} to heme C_{h2} and only after that, from heme C_{h2} to P890. When the threshold level of 0.15 g H_2O per g dry weight had been attained, further dehydration had no effect on reaction parameters.

As noted above, in suspension of chromatophores of *E. shaposhnikovii* or in dry films of chromatophores at high level of hydration, the rate of reaction of electron transfer from cytochrome C_h to P890 in H_2O was found to be approximately 1.5 times higher than in ^2H_2O. It follows from the table that the efficiency of interheme electron transfer under these conditions is negligible. At the hydration level below 0.18 g ^2H_2O per g dry weight, the rate constant of this reaction increases and becomes comparable with the rate constant in H_2O at the same level of hydration.

The rate of electron tunneling from donor to acceptor center significantly depends on the electron energy level difference Δ between these centers. Local environment has a strong effect on the energy level position of electron localization center. Displacement of atoms from initial positions modifies the potential field shape, which is accompanied by local changes in electronic states. It is conceivable that the isotope substitution H_2O by ^2H_2O may also cause displacement of atoms or atomic groups and corresponding changes in the initial value of Δ.

Consider the rates of electron transfer in the chain of cofactors (5). Because the processes of dehydration/rehydration of samples in H_2O to a first approximation had virtually no effect on the rate constant k_2, it might be suggested that under these conditions the conformation of the protein component of the system is conserved in the state providing effective electron transfer from heme C_{h2} to P890. The increase in the rate constant k_2 induced by dehydration and/or deuteration of samples can be interpreted as follows. Let the system in general be in the same conformational state as protein in H_2O. Because the hydrogen bond rigidity in ^2H_2O is slightly (an average of 5%) higher than in H_2O [18], the position of deuteron in protein in sample hydrated in ^2H_2O under otherwise identical conditions is slightly shifted relative to the position of proton in sample hydrated in H_2O. Such a shift accounts for about 2% of the bond length (i.e., ~10^{-2} Å).

Initial degree of reduction of cytochrome pool and rate constants of electron transfer calculated as a result of model identification

Hydration, g/g dry weight	$C_{h1}^{red}C_{h2}^{red} + C_{h1}^{ox}C_{h2}^{red}$	$C_{h1}^{red}C_{h2}^{ox}$	$k_1 \times 10^6$, sec^{-1}	$k_2 \times 10^6$, sec^{-1}
		^2H_2O		
0.47	0.95	$3 \cdot 10^{-4}$	< 0.01	0.22
0.28	0.82	$2 \cdot 10^{-4}$	< 0.01	0.18
0.18	0.62	$3 \cdot 10^{-4}$	< 0.01	0.33
0.14	0.5	10^{-4}	< 0.01	0.4
		H_2O		
0.47	0.8	0.13	0.09	0.33
0.25	0.8	10^{-5}	0.01	0.4
0.15	0.58	10^{-4}	< 0.01	0.34
0.08	0.53	10^{-4}	< 0.01	0.32

Similar estimates of the mean-square amplitude of thermal oscillations gave the values of 0.246 and 0.236 Å in H_2O and ^2H_2O, respectively. Let an individual water molecule be at a distance $R = 10$ Å from the electron location. Then the shift of the charge $+e$ at a distance of $\delta R \sim 10^{-2}$ Å in the radial direction causes the following changes in the electron potential energy:

$$\Delta U = e^2 [1/R - 1/(R + \delta R)] \approx e^2/R \cdot \delta R/R = U_0 \cdot \delta R/R,$$

$$U_0 = 23 \cdot 10^{-20}/10^{-7} = 2.3 \cdot 10^{-12} \text{ erg},$$

$$\Delta U = 2.3 \cdot 10^{-12} \cdot 10^{-3} = 0.23 \cdot 10^{-14} \text{ erg}.$$

Theoretical estimates of changes in $\delta\Delta$ (i.e., difference between energy levels of donor and acceptor) showed that a 1.5-fold decrease in the reaction rate corresponded to the value of $\delta\Delta \approx 0.5 \cdot 10^{-14}$ erg or $\delta\Delta \approx 0.8 \cdot 10^{-14}$ erg if electron transition was accompanied by phonon absorption or emission, respectively. Because energy is an additive parameter, the presence of N molecules of ^2H_2O in the vicinity of an electron causes a $N \cdot \Delta U$ decrease in the electron energy. To meet the condition $\delta\Delta = N \cdot \Delta U$, it is sufficient to place 3-5 molecules ^2H_2O at a distance of 10 Å from the electron. This situation is fairly realistic, because, according to X-ray diffraction analysis of the RC cytochrome complexes from *Rhodopseudomonas viridis*, about 40 molecules of water are bound to the cytochrome subunit of the complex [25]. Dehydration causes a decrease in the number N of ^2H_2O molecules in the electron vicinity, thereby decreasing the value of Δ and accelerating the process of electron transfer.

The dehydration-induced changes in the rate constant k_1 can also be qualitatively explained by an increase in the hydrogen bond rigidity in ^2H_2O. However, the effect of isotope substitution in this case is manifested quite differently. Indeed, dehydration of protein hydrated in H_2O causes at least a tenfold decrease in the value of k_1. This corresponds to the minimum value $\delta\Delta \approx 2.3 \cdot 10^{-14}$ erg. The extent of sample humidification in ^2H_2O had no effect on the rate constant k_1 (table). These effects can be explained as follows. The protein hydrated in H_2O attains the conformation required for electron transfer, whereas dehydration eliminates this conformation. Because of enhanced hydrogen bond rigidity, this conformation in protein hydrated in ^2H_2O is not attained at all. Perhaps these effects can be explained only in terms of electrostatic interaction rather than conformational changes. Because the position of deuteron at the binding site in protein hydrated in ^2H_2O under otherwise identical conditions is slightly shifted relative to the position of proton at the binding site in sample hydrated in H_2O (see discussion above), the resulting value of Δ is too large, and electron transfer is blocked. Theoretical estimates showed that in protein hydrated in H_2O effective electron transfer is observed if about ten water molecules are at a distance of ~10 Å from the electron.

The results of this work showed that molecular dynamics of the pigment–protein complexes mediating energy transformation in photosynthesis exerts a significant effect both on the intraprotein electron transfer between pigment cofactors and on the interprotein electron transfer from cytochrome *c* to RC bacteriochlorophyll. Studies of the effects of deuteration and dehydration provide valuable information about mechanisms of functional activity correlation with structural dynamics of the system at individual segments of photosynthetic electron transport chain.

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