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Mechanisms of hydration effects on the structural-dynamic and functional characteristics of photosynthetic membranes in various purple bacteria

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Abstract NMR spectra and T_1, T_2 relaxation times for ^1H, ^{13}C and ^{31}P nuclei in membranes of *R. rubrum* and *Rb. sphaeroides* recorded at different relative humidity, as well as hydration curves and electron transfer efficiency of these membranes and membranes of *E. shaposhnikovii*, reveal complicated relations between structural-dynamic and functional characteristics. A number of sites of the electron transfer chain are shown to be under the control of structural-dynamic mechanisms. Different parameters characterizing these membranes at low humidity and during hydration have been established. These findings and analysis of the data from model systems reveal four different stages of hydration. Each of them is associated with specific changes in structure, dynamics, and function of photosynthetic membranes and their components. In the first stage the hydration of some polar groups leads to local changes in the dynamics of the protein component and this influences the recombination between photoactive pigment P and intermediate acceptor Q_A. The second stage is induced by incorporation of water molecules into the hydrogen bonds between the polar head groups of the lipids and within macromolecules. This results in changes of the dynamics of the membranes, the efficiency of the electron transfer between the quinones and the efficiency of photooxidation of cytochrome c. In the third stage all polar groups are hydrated owing to the appearance of free water with a high dielectric constant. This makes possible lateral mobility of membrane components and changes in distances between the interacting macromolecular components. Therefore, the regulation of photosynthetic processes can be mediated with the participation of mobile carriers. Finally, in the fourth stage, complete humidification provides conditions for regulation of photosynthesis at the cell level. The mechanisms influencing these processes and the efficiency and regulation of electron transfer in various parts of the photosynthetic chain are discussed.

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Key words Protein and lipid dynamics · ^1H, ^{13}C, ^{31}P NMR · Electron transfer efficiency and regulation

Abbreviations *BChl* Bacteriochlorophyll · *ET* Electron transfer · *LDAO* Lauryldimethylamineoxide · *PE* Phosphatidylethanolamine · *RC* Reaction center · *RH* Relative humidity

Introduction

Interaction with water has a major effect on the dynamics of various biological structures and their functional activity, as has been demonstrated by a variety of methods (Likhtenstein 1976; Frauenfelder et al. 1979; Beece et al. 1980; Hauslay and Stanley 1982; Welch et al. 1982; Lakowicz 1986; McCammon and Harvey 1987; Suzdalev 1988; Wüthrich 1988; Rupley and Carreri 1991; etc.). In particular, the study of the dynamics of chromatophores of purple bacteria by methods of spin-echo of protein resonance, spin label and γ-resonance spectroscopy revealed the direct relationship between dynamics and efficiency of electron transfer (ET) in the photosynthetic chain between primary (Q_A) and secondary (Q_B) quinones (Berg et al. 1979; Nikolaev et al. 1980; Aksyonov et al. 1985; Kononenko et al. 1986). However, for different ET stages and for chromatophores of various purple bacteria such a relationship is sometimes uncertain and may even be virtually absent. This discrepancy can be attributed to the specific organization of different chromatophore structures and to the complicated character of water effects. Effects of hydration of polar groups, incorporation of water molecules into hydrogen bonds of proteins or membranes, as well hydrophobic interaction must be taken into account. Thus the dynamics and functional characteristics of biological structures depend upon a great number of factors which can be elucidated and investigated only using a comprehensive approach. To solve a multiparametric problem, it is necessary to have a corresponding number of structural-dynamic pa-

rameters which in turn must be compared with certain functional characteristics of the systems in question.

Photosynthetic membranes are suitable objects for this purpose, because in the photosynthetic chain separate stages differ dramatically in their kinetic constants (Clayton 1980). There is a variety of data concerning functional and structural-dynamic characteristics of different purple bacteria species and their membranes. It is also essential that some of the primary ET stages do not depend on diffusive restrictions since this allows one to correlate directly the observed changes in the dynamics of the membrane structures with their biological function. Hydration effects may also provide for important additional information on properties of such membranes. Finally, the use of different methods for their investigation, in addition to the data obtained in model systems, allows one to increase considerably the reliability of the interpretation of the results obtained.

To solve such a problem, photosynthetic membranes of three species of purple bacteria were studied: *Rhodospirillum rubrum, Rhodobacter sphaeroides* and *Ectothiorhodospira shaposhnikovii.* These species differ from each other in a number of functional and structural-dynamic characteristics. Measuring relaxation times T_1 and T_2 for protons, isotope effects, ^{13}C and ^{31}P NMR spectra were studied during the process of hydration. In these membranes we studied the dynamics of the protein and the polar and non-polar lipid phases. The results obtained were analyzed together with the functional characteristics of ET reactions and compared with data obtained for the model systems. The effects studied on these membranes revealed 4 stages of hydration which are characterized by different water content and parameters of dynamics and ET efficiency in the photosynthetic chain.

Materials und methods

The nonsulphur purple bacteria *Rhodospirillum rubrum*, *Rhodobacter sphaeroides* and sulphuric *Ectothiorhodospira shaposhnikovii* were grown for 3–5 days under anaerobic conditions in a luminostat at a temperature of about 30 °C. Cells of *R. rubrum* and *Rb. sphaeroides* were grown on Ormerod's culture medium and *E. shaposhnikovii* on Larsen's medium (Kondratyeva 1963). Photosynthetic membranes (chromatophores) were isolated from bacterial cells using routine methods (Samuilov and Kondratyeva 1969). Cells separated from culture medium were suspended in 0.05 M Tris-HCl buffer, pH 6.8–7.0 containing 0.05 M sucrose (pH 8.0 and 0.25 M sucrose for *E. shaposhnikovii*) and 0.005 M $MgSO_4$. The cells were disrupted by ultrasonic treatment (20–22 kHz, 0.5 A) for 3–5 min. Debris was discanted by centrifugation and the fraction of the chromatophores was sedimented from the supernatant by ultracentrifugation at 100,000–150,000 *g* for 1–2 h at 0–4 °C. Chromatophores of *E. shaposhnikovii* with active cytochromes were prepared as described (Pottosin et al. 1984; Chamorovsky et al. 1986).

The chromatophores for the NMR measurements were lyophilized and then wetted in dessicators over saturated salt solutions to attain the necessary hydration degree according to Winston and Bates (1960). ET activity of chromatophores was measured by optical differential spectroscopy in film preparations (Chamorovsky et al. 1976) obtained after drying on a glass support of either the suspension of the chromatophores or wetted lyophilized powder used for NMR spectroscopy. The hydration of the preparations was determined gravimetrically and by direct titration with Fisher's reagent using electrometric detection of the equivalent point (Klimova 1976).

The NMR spectra for the nuclei ^{13}C and ^{31}P and relaxation times T_1 and T_2 for them were measured on a "Bruker" CXP-300 spectrometer at frequencies of 75 and 121 MHz, respectively. To measure the spin-lattice relaxation time T_1, a standard "saturation-recovery" sequence from a series of 90° pulses was used. The curves of recovery of the longitudinal magnetization for the nuclei ^{13}C were approximated in the form of one exponent and the curves for the nuclei ^{31}P – in the form of the sum of two exponents ("fast" and "slow"). The T_1 values and the relative proportions of these exponents were determined using the EXPO Bruker program. A similar procedure was performed to determine the T_1 from amplitude values of the curves as well as from the integral values of the curves using not less than 200 measurements. The standard deviations of T_1 values were not more than 10%.

The T_2 relaxation times (more strictly $T_2^* = 1/\Delta\omega$) (Abragam 1961) for ^{31}P were measured directly by half-width of resonance and were used only for relative estimates. To determine T_2 each curve was averaged for not less than 1000 measurements.

The relaxation times T_1 and T_2 for protons were measured on a home-made NMR spin-echo spectrometer at a frequency of 20 MHz with the aid of pulse sequences of $180° - \tau - 90°$ and $90° - \tau - 180°$, respectively (Farrar and Becker 1971). Intensities of the spin-echo signals were standardized.

Results

It is evident from Fig. 1 that hydration curves for photosynthetic membranes of the studied species of purple bacteria (*R. rubrum*, *Rb. sphaeroides* and *E. shaposhnikovii*) differ greatly from each other. In the membranes of *R. rubrum,* phosphatidylethanolamine (PE) occupies about 65% of the lipid phase (Snozzi and Bachofen 1979). Hydrogen bonds are formed between the polar heads of PE lipid molecules, resulting in a sharp increase in water adsorption within the interval of relative humidity (RH) $P/P_0 = 0.3–0.5$. A further RH increase up to 0.8 has virtually no effect on the hydration level of the membranes (Fig. 1 A). These effects are even more clear in the protein-pigment complexes of the reaction centres (RC) extracted from membranes using lauryldimethylamineoxide (LDAO). Like PE, the detergent forms hydrogen bonds between its

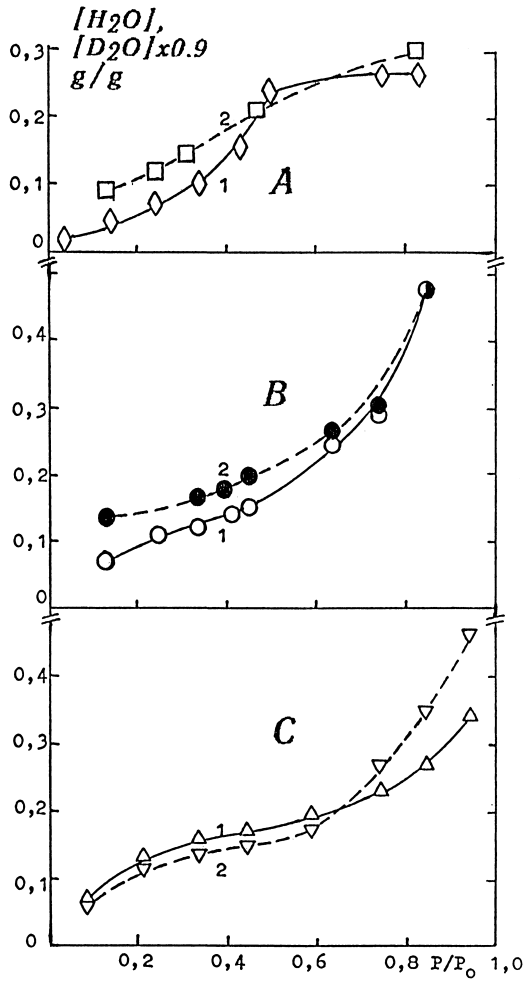

Fig. 1 **A** Hydration isotherms of bacterial membranes of *R. rubrum* (◇) (Aksyonov et al. 1985) and *Rb. sphaeroides* (□) (g H_2O per g of dry weight of membranes); P/P_0 – relative humidity. **B** Same for membranes of *E. shaposhnikovii* humidified in H_2O (○) and D_2O (●) vapours (Aksyonov et al. 1987). Mass values for D_2O multiplayed by 0.9 (proportionality ratio between the mass of H_2O and D_2O); **C** Same for bovine serum albumin (△) and 4% low-molecular ligands and BSA (▽) (Aksyonov et al. 1990 a)

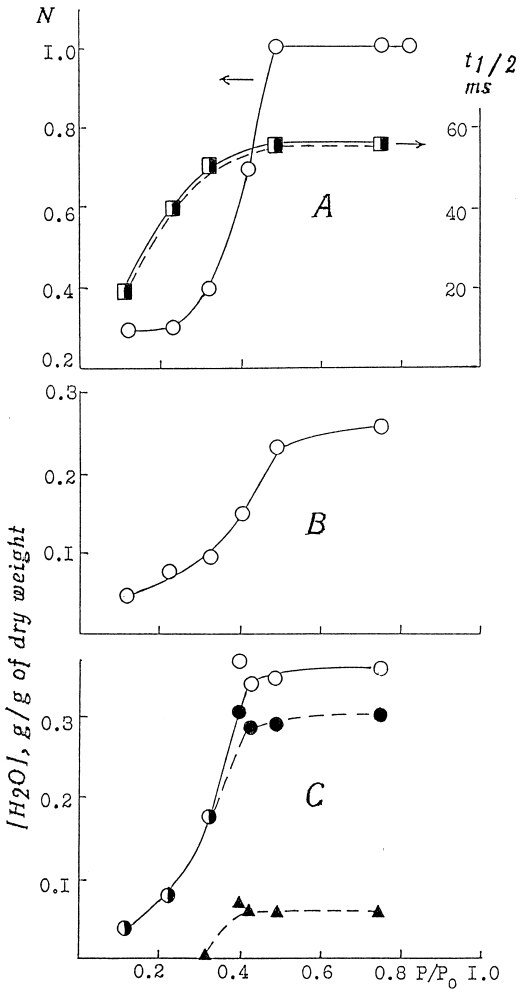

Fig. 2 **A** Efficiency (N) of photoinduced electron transfer from the primary (Q_A) to the secondary (Q_B) quinone acceptors in chromatophores of *R. rubrum* (○) and time $t_{1/2}$ of recombination between photoactive pigment P^+ and primary acceptors Q_A in RC preparations isolated from *R. rubrum* membranes by LDAO (–□–) or Triton X-100 (–■–); **B** water adsorption isoterms in *R. rubrum* membranes; **C** total amplitude of NMR spin-echo signals of mobile protons in *R. rubrum* chromatophores (○) (as calculated per water weight) and its components: fast ($T_2 = 0.1 – 0.4$ ms) (●) and slow ($T_2 = 2.5 – 3.2$ ms) (▲) (Aksyonov et al. 1985)

molecules. However, if LDAO is replaced by Triton X-100, the area of humidity-dependent hydration increase is extended markedly (Aksyonov et al. 1985).

The hydration curves for membranes of *Rb. sphaeroides* are more smooth than those for membranes of *R. rubrum* (Fig. 1 A). In membranes of *E. shaposhnikovii* (Fig. 1 B) the main increase in water adsorption is shifted to higher RH values of 0.5 – 0.7 and more as compared with 0.3 – 0.5 in *R. rubrum*. There is a noticeable difference in the adsorption of H_2O and D_2O at intermediate and low RH values which virtually disappears at high humidity (Fig. 1 B) (Aksyonov et al. 1987; Chamorovsky et al. 1988). The hydration curve for this type of membrane in H_2O is quite close to the hydration curve for an equimolar mixture of bovine serum albumin (BSA) with ligands (average molecular weight of about 3 kD) (Aksyonov et al. 1990 a) (Fig. 1 C). However, the hydration curve for BSA without ligands differs markedly from both of these curves (Fig. 1 B, 1 C).

The increase in the amount of adsorbed water in membranes of *R. rubrum* and *E. shaposhnikovii* at intermediate humidity leads to a considerable increase in mobility of the molecules and their individual groups, other than water. This can be observed in the excess of the number of mobile groups over the water content in the system (Fig. 2 B and 2 C) and in the change in the T_1 and T_2 values for protons. The same effects are observed under conditions of humidification in heavy water vapours (Fig. 3 B, 3 C).

Such changes are more pronounced in the membranes of *R. rubrum* and are also accompanied by a sharp increase

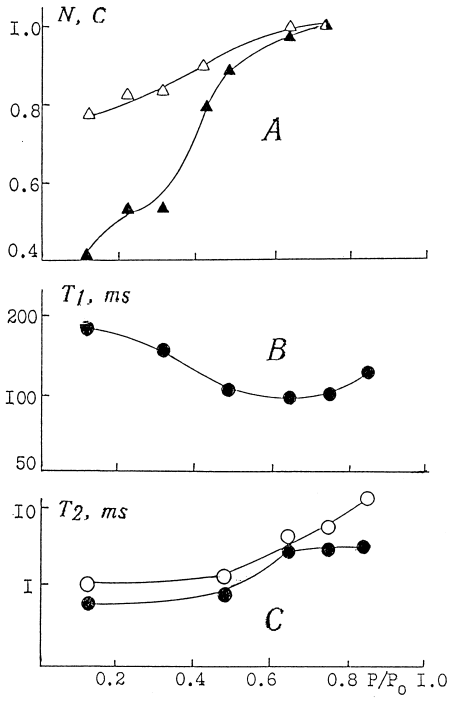

Fig. 3 **A** Efficiency (N) of photoinduced electron transfer from the primary (Q_A) to the secondary (Q_B) quinone acceptors (△) and efficiency (C) of photoinduced oxidation of cytochrome C (▲) in chromatophores of *E. shaposhnikovii* at different humidity (Pottosin et al. 1984); **B** T_1 values for protons in these chromatophores under humidification in D_2O vapours; **C** T_2 values for membrane protons under humidification in H_2O (○) and D_2O (●) vapours (Aksyonov et al. 1987)

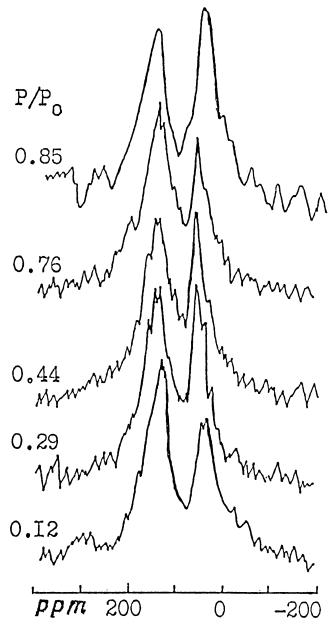

Fig. 4 NMR spectra of carbon ^{13}C nuclei as measured at 75.476 MHz in *R. rubrum* chromatophores poised at different relative humidity P/P_0 (Aksyonov et al. 1991)

in the ET efficiency between primary (Q_A) and secondary (Q_B) quinones within the RH range $P/P_0 = 0.3-0.5$ (Fig. 2 A). Such effects are even more pronounced in the system RC-LDAO (Aksyonov et al. 1985). However, in chromatophores of *E. shaposhnikovii* the ET between quinones remains effective even in the dry state, and it increases only slightly with RH increase (Fig. 3 A). A similar pattern is also observed in membranes of *Rb. sphaeroides.*

No direct relationship was found in the isolated RC of *R. rubrum* between the changes in dynamics and the recombination of photoactive pigment P and intermediate acceptor Q_A (Fig. 2 A). Unlike ET between quinones, recombination does not depend upon the type of detergent used. However, this changes markedly at low RH levels where noticeable changes in the hydration and dynamics of the membranes were not yet observed (Fig. 2 A and 2 B).

There is only partial overlap between the area of significant growth of water adsorption and the area of increase in the dynamics and growth of efficiency of another ET process, which is observed in membranes of *E. shaposhnikovii* – the photoinduced oxidation of cytochrome c (Fig. 3 A) (Pottosin et al. 1984, 1987; Kononenko and Aksenov 1987). The area of rapid growth of its efficiency is much closer to the area of the sharp change in the dynamics in the membranes of *R. rubrum* (Fig. 2 C). However, the ET efficiency from cytochrome c to bacteriochlorophyll (BChl) of RC increases markedly only at $P/P_0 = 0.5-0.88$ (Chamorovsky et al. 1988).

Thus, the relationship between the dynamics and function during hydration of the photosynthetic membranes has a complicated character. To elucidate this problem the details of the hydration process were studied by the ^{13}C and ^{31}P NMR spectroscopy.

The NMR spectra of carbon 13 at the frequency of 75 MHz for the membranes of *R. rubrum* in the solid phase contain 2 quite well resolved lines (Fig. 4) (Aksyonov et al. 1990 b, 1991). High resolution NMR spectra for the membranes of *R. rubrum* and *Rb. sphaeroides* were recorded with samples rotating at the "magic" angle (Aksyonov et al. 1991). Using these data it was shown that the left line of the spectrum (Fig. 4) belongs mainly to the molecular groups of proteins and the right spectral line mainly to the groups of non-polar lipid regions (Aksyonov et al. 1991), the whole spectrum being weakly dependent on RH.

Additional important information was obtained by measuring the T_1 values for two ^{13}C spectral lines in the membranes of *R. rubrum* under various RH. The NMR data on T_1 for protein dynamics in the solid state (Andrew et al. 1978; Aksyonov 1983) showed that the partial contribution of protein groups to the line related to lipids hardly affects the dependence of the lipid T_1 values on RH (Aksyonov et al. 1991). This is also true for the contribution of lipid groups to the protein line. T_1 values of relevant lipid and protein groups in the membranes of *R. rubrum* have opposite dependences upon RH (Fig. 5, 6 A).

As seen from Fig. 5, the T_1 value for the non-polar lipid regions decreases with humidification and then, having attained its minimum at an RH of about 0.3, increases again. The existence of such a minimum provides unequivocal evidence that the motion frequencies (related to the lipid

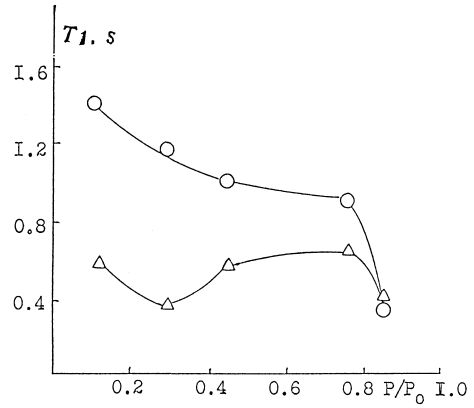

Fig. 5 T_1 values for carbon ^{13}C nuclei in protein (○) and lipid (△) components of *R. rubrum* membranes (Aksyonov et al. 1990 b)

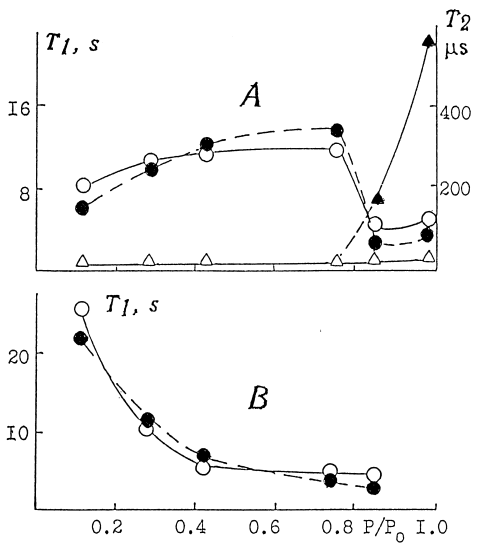

Fig. 6 **A** T_1 values for phosphorus ^{31}P nuclei for the amplitude (○) and integral (●) values of slow relaxation components of longitudinal magnetization and T_2 values for broad (△) and narrow (▲) spectral lines of these nuclei in cromatophores of *R. rubrum* poised at different relative humidity (Aksyonov et al. 1990 b); **B** Same for T_1 in membranes of *R. sphaeroides*

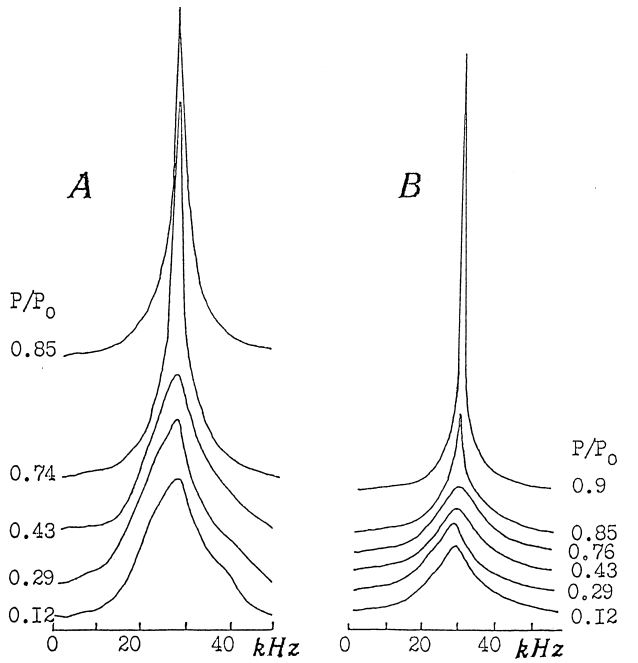

Fig. 7 NMR spectra of ^{31}P nuclei at 121 MHz in *Rb. sphaeroides* (**A**) and *R. rubrum* (**B**) membranes poised at different relative humidity

tails) are close to the resonance frequency (Abragam 1961). The T_1 minimum is also observed for the lipid protons in the membranes of *E. shaposhnikovii* when they are humidified in heavy water vapours (Fig. 3 B).

At the onset of the next stage of humidification the T_1 value for the ^{13}C nuclei in the lipids of the *R. rubrum* membranes increases, showing that there is a further increase in lipid mobility. Within the interval RH 0.5–0.76 the T_1 value has an approximately constant value. However, for P/P_0 >0.8 a new and pronounced decrease in T_1 values occurs again (Fig. 5). Such a new drop in the T_1 value of the lipid line at high RH above 0.8 testifies to the appearance of a new type of motion in the lipids. The latter is likely to be not only related to lipid tails but also to the whole lipid molecules (Aksyonov et al. 1991).

In the case of the ^{13}C NMR line related to the protein, the drop in the T_1 values occurs at the initial stage of humidification (Fig. 5). This fact indicates an activation of the internal dynamics of the protein. Subsequent increases of the RH up to about 0.5 results in an additional drop of the T_1 value from the carbon nuclei of the protein component. A new drop of the T_1 value at an RH of about 0.8 points to a considerable increase in internal protein dynamics. One cannot exclude the possibility that there is some contribution of the motions of the whole protein globules.

In addition, in the polar part of the lipids, as seen in the rise of the T_1 values for ^{31}P with humidification of the membranes of *R. rubrum*, a quite unexpected dependence on RH is observed. As follows from NMR theory (Abragam 1961), the increase of T_1 values at the first stages (RH <0.5) indicates a decrease rather than an increase in mobility for the main slow component of ^{31}P NMR (Fig. 6 A). The same dependence was also found for the minor fast component of T_1 (Aksyonov et al. 1991). A dependence of this kind is unlikely to be seen in the case of lipids in a hexagonal phase with low humidity where the polar groups must directly interact with each other. This conclusion does not conflict with the shape of the NMR spectra for ^{31}P (Fig. 7 B), but it can be explained by the complex composition of this membrane.

A definite result was obtained for the membranes of *Rb. sphaeroides*. Here at low humidity the T_1 values for ^{31}P decrease with increasing RH (Fig. 6 B) and the resonance line is asymmetric with an additional component in the low frequency wing (Fig. 7 A). This is in full agree-

ment with the assumed behavior of the hexagonal phase (Cullis and De Krujiff 1979; Yeagle 1990). With the increase in RH the asymmetry of the resonance from the ^{31}P nuclei is less pronounced, which indicates that at least part of the assumed hexagonal phase has disappeared. According to the T_1 data for the ^{31}P nuclei, this process is also accompanied by the growth of mobility in the polar area of the membranes. Further increase in humidity gives rise to a noticeable narrowing of the resonance line with a virtual disappearance of its asymmetry (Fig. 7 A). Moreover, the fraction of more mobile groups in the polar area of the membranes increases markedly and together with the broad line from the ^{31}P nuclei with $T_2 = 25$ μs there appears a narrow line with $T_2 = 200$ μs. These effects are also observed in the NMR spectra of the *Rb. sphaeroides* membranes as well as *R. rubrum* (Aksyonov et al. 1991). This points to the influence of rotational averaging of the interaction between magnetic moments of the phosphorus nuclei and protons which is associated with an increased lateral mobility of the lipid molecules within the membranes (Cullis and De Krujiff 1978, 1979; Yeagle 1990). The RH range where these effects appear coincides with the range where a substantial drop of the T_1 values for the ^{13}C nuclei which belong both to the non-polar area of the lipids and the protein component can be observed (Fig. 5).

Discussion

The results obtained should be discussed with respect to the characteristics of the hydration processes and the role of water in the internal dynamics of biological macromolecules and membranes and their functioning.

Hydration processes in the photosynthetic membranes

In the process of the membrane hydration, one can distinguish a few stages which differ with respect to structural changes and dynamics caused by water molecules, as well changes in functional characteristics of the membranes. We shall now discuss these separate hydration stages in more detail.

It is suggested in the literature that in the initial stages the polar groups of the proteins and lipids must be hydrated. However, with a real biological membrane, one should take into account its multicomponent character. A model system, BSA-ligand, can serve as a good subject for the study of the role of different polar groups in hydration processes. The number of polar groups available for hydration in the BSA-ligand system is higher than in the protein BSA alone. However, the amount of adsorbed water in the low RH region turns out to be markedly lower in the former case (Fig. 1 C). Hence, not all the polar groups in the BSA-ligand system can be hydrated at low RH. This is obviously due to the interaction of oppositely charged groups of the ligands and the protein BSA (Aksyonov et al. 1990 a). This interaction is virtually undisturbed in the first stage of hydration when only bound water with a low dielectric constant value is present. This effect may be important for the hydration of biological membranes where the interaction of opposite charges of the lipid molecules with each other and with the charged protein groups play a marked role.

The first stage of hydration influences only a part of the polar groups in the complex protein-lipid systems. It is likely to give rise to local changes in their structure and dynamics only. The decrease in the T_1 values for the protein component at this level of hydration points to faster dynamics.

The second stage of hydration within the RH interval 0.3–0.5 is connected with the involvement of water molecules in hydrogen bonds between the lipid molecules and within protein globules. This effect is observed more distinctly in the membranes of *R. rubrum*, where PE forms 65% of the lipid phase, especially in the system RC-LDAO (Aksyonov et al. 1985) and to a lesser extent in the membranes of *Rb. sphaeroides* (Fig. 1 A) with a PE content of about 37% (Wood et al. 1965). Direct evidence on the presence of hydrogen-bonded interlamellar water interacting strongly with head groups of PE was obtained by one- and two-dimentional NMR spectroscopy, whereas in other lipids studied such effects were not observed (Chen et al. 1996).

The peculiarity of the above processes is that they appear only when some minimum amount of water is available. In particular, this is the case for the data from the model system polyethylenimine $(CH_2–CH_2–NH)_n–H_2O$ a linear polymer which contains about 2% branchings. Here, in the first stages of hydration there emerges relatively mobile water. However, in the subsequent process of humidification, the water mobility decreases down to a minimum level which corresponds to the hydration with 1 M H_2O per 1 M NH-groups. If the water content increases further the water mobility starts to increase again (Aksyonov et al. 1977). This effect is most likely to be due to the incorporation of water molecules into interchain bonds of the polymer, which may also lead to the breaking of the neighbouring direct hydrogen bonds. As a result, this process becomes possible only with the participation of at least a few molecules of water in the given structural domain. This process is also influenced by the local density of the protein and lipid packing. Data concerning the pronounced difference between the hydration curves for pure PE and for an equimolar mixture of PE with phosphatidylcholine (Jendrasiak and Hastly 1974) support this conclusion. According to these authors, pure PE, in contrast to the mixture of PE with phosphatidylcholine, adsorbs virtually no water at low and intermediate humidity values.

Taking such effects into account, one may explain the data on the small growth of water content in the membranes of *R. rubrum* at the initial stage of hydration with a subsequent sharp growth of water absorption in the interval of $P/P_0 = 0.3–0.5$ (Fig. 1 A). A stronger adsorption of D_2O as compared to H_2O in the membranes of *E. shaposhnikovii* (Fig. 1 B) at low and intermediate humidity may be due, at least in part, to the stronger hydrogen bonds of heavy water.

Incorporation of water molecules into the polar phase of the membranes of *R. rubrum* leads to an increase in the volume which, in turn, induces a loosening of the packing of the non-polar lipid tails. As a result, one can observe a sharp increase in the dynamics in the lipid non-polar phase (Fig. 2 C).

Participation of water molecules in hydrogen bonds also takes place within the protein component of the membranes. However it is difficult to detect quantitatively its contribution to the total hydration effect on the basis of the typical smooth hydration curves for the proteins (example Fig. 1 C). On the other hand, incorporation of water molecules into the hydrogen bond network of the protein is likely to promote the formation of its native conformation. From Fig. 3 B one can see the increase in internal mobility of membranes of *E. shaposhnikovii* in the RH interval 0.3 – 0.5, which may correspond to the effect of water incorporation. This is also accompanied by a rapid growth of the efficiency of the photoinduced cytochrome oxidation (Fig. 3 A) within the same RH interval (0.3 – 0.5). In the given RH interval the change in the internal dynamics of the proteins also follows from the additional drop of the T_1 values for the ^{13}C nuclei from the protein component of the *R. rubrum* membranes (Fig. 5). It is interesting that at RH values higher than 0.3 other biopolymers reveal similar behavior. Thus the enzymatic reaction may proceed in solid samples (Khurguin 1976). The rate of irreversible changes in DNA secondary structure also increases considerably at RH >0.3 (Novikov and Sukhorukov 1977). All these cases are characterised by a sharp dependence upon the RH.

The third stage of the hydration is observed at RH values exceeding 0.7 (Fig. 1 C) where free water with a high dielectric constant appears in the sample and weakens any interactions between oppositely charged groups. As a result, a visible excess of the amount of adsorbed water in the BSA-ligand system compared to the protein sample is observed at RH higher than 0.7 (Fig. 1 C). Considerable growth of water adsorption in the membranes of *E. shaposhnikovii* at these RH values is also observed (Fig. 1 B). Evidently, the hydration of practically all of the charged groups in the membrane occurs here, and this is similar for the membranes and for the protein-ligand model system.

The presence of free water in such a system induces lateral mobility of lipid molecules. This mobility is manifested by the appearance of a narrow line of resonance for a part of the ^{31}P nuclei in the studied membranes of *R. rubrum* and *Rb. sphaeroides* for which the T_2 values increase by an order of magnitude. The other indication for lipid mobility is a drop of the T_1 values for both ^{31}P and ^{13}C nuclei of the lipid component (Fig. 5, 6). The sharp drop of T_1 is also observed for proteins (Fig. 5).

The considerable increase in the stability of DNA secondary structure at RH >0.7 (Novikov and Sukhorukov 1977), suggests that free water induces an increase in hydrophobic interaction.

Finally, at high water content the transfer of macromolecules into water medium and changes in the association of proteins with each other occur. This provides various possibilities for the regulation of membrane processes in cells. An increase in the number of mobile protein groups and lipid molecules on their release from the bound state corresponds to an increase of entropy in such processes and must lead to a small change in free energy and high sensitivity of this system of regulation to different kinds of effects (Aksyonov 1985 a, 1990). Protein transfers from the bound to the released state of proteins may be the result of a small loss of water accompanied by the small change of its contribution to the hydration energy of macromolecules (up to a few per cent) and this may be insensitive to the physical measurements used to investigate the state of water (Aksyonov 1985 b, 1990).

There is a series of direct and indirect data confirming these ideas. Thus, it is sufficient to increase the ionic strength up to 0.15 M NaCl in order to release some peripheral proteins from the state in which they are bound to the membranes (Friedrich 1984). Peripheral protein-lipid interactions are sensitive to many other factors (Sancaram and Marsh 1993). In terms of transitions of the proteins from the bound state into water and vice versa the peculiarities of the data could be explained by considering the changes in cytoplasm viscosity. Analogous conclusions were drawn from light scattering and the distribution of dyes in cells (Aksyonov 1990) which accompany the activation of any metabolic processes (Heilbrunn 1956; Alexandrov 1985).

The relationship between the dynamics and function of photosynthetic membranes

For three types of the studied photosynthetic membranes the above mentioned hydration effects result in different relations between the structural-dynamic and functional characteristics. Their examination allows one to identify the role of various types of motion in providing the high ET efficiency in different parts of the photosynthetic chain and in regulating these processes.

The existence of the assumed hexagonal phase with loose packing of the lipid tails at low RH in the membranes of *Rb. sphaeroides* and possibly in the *E. shaposhnikovii* agrees with the fact that they preserve the mobility and high ET efficiency between the quinones. In the membranes of *R. rubrum*, where the ET efficiency at low RH is smaller than in these types of membranes, the lipid tails are packed more densely. This becomes obvious from a comparison of the T_2 values of protons at low RH in the membranes of *R. rubrum* which are markedly lower (Aksyonov et al. 1985) than the T_2 values in the membranes of *E. shaposhnikovii* (Fig. 3 C).

In the first stage only some of the polar groups are hydrated but the decrease in the T_1 values for the protein component shows that the hydration leads to changes in the dynamics of some protein groups. This results in an increase in the recombination time of the photoactive pigment P and the intermediate acceptor Q_A. This effect is only related to the dynamics of protein since it is identical with different types of detergents (Fig. 2 A).

The next stage of the hydration is determined by the effects of incorporating the water molecules into the hydrogen bonds within the membranes. In the membranes of *R. rubrum* the incorporation of the water molecules between the polar heads of PE molecules leads to a sharp increase in mobility of the lipid tails that results in a sharp increase in the ET efficiency between the quinones. To a smaller extent, the same effects are also present in the membranes of *Rb. sphaeroides* and *E. shaposhnikovii* where the mobility of the lipid tails was already rather high in the dry state.

The influence of lipid mobility on the ET between the quinones is likely to be due to electrostatic stabilization of the reduced quinones. Changes in the $Q_A - Q_B$ distance and between the charged groups of Q_B and protein in the vicinity of the quinone Q_B (Lankaster et al. 1996) make their contribution. As a result, the probability of the back reaction decreases sharply without a marked loss of energy which may be the case in the static situation. This effect may be also accompanied by a change in Q_B benzene ring orientation which promotes the second electron transfer from Q_A. According to Clayton (1980) the second electron transfer to the quinone Q_B is not observed in the rigid membrane structure.

To reduce the probability of back reaction to less then 1% it is necessary that the characteristic times of such motions should be at least 2 orders of magnitude less than the times of the backward electron transition from Q_B to Q_A. Mobile groups must also be displaced at some minimal distance within this time interval. To meet these requirements, corresponding motions of molecular groups in the non-polar phase of the membranes of *R. rubrum* at RH values from 0.5 and higher must have characteristics times within the nanosecond time range. This follows directly from the data in Fig. 5 where the transition through the minimum T_1 for the ^{13}C nuclei in the non-polar lipid areas with RH growth is presented. The minimum T_1 is also observed for protons in the membranes of *E. shaposhnikovii* (Fig. 3 B). Characteristic times of molecular motions can be compared with the time of Q_B^- reduction in the microsecond time range (Clayton 1980).

Participation of the water molecules in the hydrogen bonds within protein is likely to influence the formation of the native protein conformation, the efficiency of photooxidation of cytochrome c (Fig. 2 A) being due to the latter. Thus, only in the native "contact" state is the rapid ET from cytochrome c to the special pair (P) possible through a tunneling mechanism (Chang and Austin 1982; Petrov et al. 1985; Chamorovsky et al. 1986, 1990).

It should be noted that even for the RC protein there are data demonstrating the effects of conformational changes on the ET. This also follows from the difference of the effective values of the ET rates under the light and dark conditions in RC components (Chamorovsky et al. 1990; Riznichenko et al. 1990, 1993).

The next stage of hydration is connected with the apperance of free water with a high dielectric constant. As a result, this allows the lateral diffusion of the lipid molecules and apparently also of the proteins in the membranes. At this stage of hydration changes in the distance between the interacting macromolecules is likely to influence the additional change of the ET efficiency from the cytochrome c to the BChl of RC in the *E. shaposhnikovii* membranes (Aksyonov et al. 1987). Thus the data for the model system cytochrome c – hexacyanide Fe show that the increase in the distance between the Fe atoms from 1 to 1.5 nm completely blocks the ET process (Chang and Austin 1982).

The diffusion will also influence the regulation of the ET owing to mobile carriers (Riznichenko et al. 1990, 1993).

In two other species of purple bacteria the cytochromes c have a weaker bond with the membrane as compared to the membranes of *E. shaposhnikovii*. For these cytochromes it was shown that an increase in pH (Vanderkooi et al. 1972; Vanderkooi and Eresinska 1973), lipid saturation and the tightness of the carbon chain packing (Mateu et al. 1978), by adding cholesterin (Papahajopulos et al. 1973, 1975) and decreasing the concentration of bivalent ions result in the cytochrome c leaving the hydrophobic part of the membrane. At the same time, weakening the polar interactions with an increase in pH and ionic strength results in the cytochrome c being transfered into the solution. A similar effect takes place when a voltage difference of corresponding polarity is applied to membranes (Kszenzhek et al. 1977). It is also known that the cytochromes c are easily separated from the membranes of a number of purple bacteria species in the process of extracting chromatophores with increasing ionic strength.

The above cited data suggest that the degree of binding of cytochromes to the membrane can change even in the course of the photosynthetic membranes functioning under the conditions of high water content. A similar system can be used for regulating the activity of the processes of photosynthesis.

Conclusion

Thus the comparative study of the hydration effects in three types of photosynthetic membranes of purple bacteria allowed us to reveal a number of the structural peculiarities of such membranes and mechanisms of its change during the hydration process. In the course of the hydration process, it is possible to determine 4 stages of the hydration influence. In the first stage, there take place local changes in the protein structure and dynamics with the possibility of displacing certain groups. This influences the recombination of photoactive pigment P and intermediate acceptor Q_A. In the next stage of hydration the incorporation of water molecules into the hydrogen bonds results in structural reorganization of protein macromolecules and a considerable part of the lipid texture. The displacement of each of the separate groups has rather local character. But as a result of the motion of many groups the protein conformation and the state of a number of the lipid phase areas change markedly. This results in changes in the dynamics

of the membranes, the ET efficiency between the quinones and efficiency of photooxidation of cytochrome c. Only in the third stage with the appearance of free water having a high dielectric constant, there arises the possibility for mutual displacements of the membrane component at distances much greater than the interatomic ones.

Finally, in the fourth stage with total humidification, the ET become sensitive to regulation, when there must be some influence of the state of the cells. This points to the close interrelation of the hydration processes with the character of the internal motion in the membrane structures, which is one of the decisive factors in reaching the high ET efficiency in the photosynthetic chain.

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