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Proton Dissociation Dynamics in the Aqueous Layer of Multilamellar Phospholipid Vesicles

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Summary. The water layers interspacing between the phospholipid membranes of a multilamellar vesicle are 3–10 water layers across and their width is adjusted by osmotic pressure (Parsegian, V.A., et al., 1986. *Methods Enzymol.* **127**:400–416).

In these thin water layers we dissolved pyranine (8 hydroxypyrene 1,3,6 trisulfonate), a compound which, upon photo excitation, ejects it hydroxy proton with time constant of 100 psec. (Gutman, M. 1986. *Methods Enzymol.* **127**:522–538).

In the present study we investigated how the width of the aqueous layer, the density of phosphomoieties on the membrane's surface and the activity of water in the layer affect the capacity of protons to diffuse out from the electrostatic cage of the excited anion before it decays to the ground state.

Using a combination of steady-state and subnanosecond time-resolved fluorescence measurements we determined the average number of proton excited-anion recombinations before the proton escapes from the Coulomb cage.

The probability of recombination in thin water layer is significantly higher than in bulk. The factor contributing most to enhancement of recombination is the diminished water activity of the thin aqueous layer.

The time frame for proton escape from an electrostatic trap as big as a membrane-bound protein is 3 orders of magnitude shorter than turnover time of membrane-bound enzymes. Thus the effects of local forces on proton diffusion, at the time scale of physiological processes, is negligible.

Key Words hydration layer · water activity · multilamellar vesicles · pH jump · proton diffusion · dissociation dynamics

Introduction

Reversible proton dissociation is a reaction common to all proton-coupled energy transformation in bioenergetic organelles (chloroplasts, mitochondria) where it proceeds in the thin water layers confined between the thylakoids membranes or the mitochondrial cristea. The kinetic implications emanating from the shape of the water body where protons diffuse in these organelles are the subject of this research, using multilamellar vesicles as a model system.

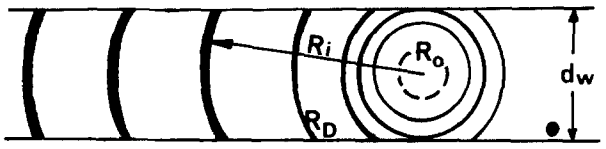
The reactions of protons with a site located in inter lipid membrane space are affected by the following factors:

Inhomogenecity of the Aqueous Matrix

The width of the aqueous layer between the membranes is not more than a few water layers across. Under such conditions the homogeneity of the water body is lost. Water molecules which are in contact with the phospholipid headgroup (i.e., 3–4 molecules/lipid (Rand & Parsegian, 1989)) interact strongly with it, forming the first hydration shell. Water molecules in the first hydration shell exhibit a lower rotational and translational mobility in comparison with those in the second hydration shell, or those in the bulk. This ordering of water, decreasing from the interface towards the middle of the aqueous layer, has an immediate effect on the diffusion coefficient of protons (Gutman & Nachliel, 1990).

Geometrical Restriction on the Diffusion Process

The shape of the reaction space approximates a two-dimensional space. This approximation is acceptable for diffusion experiments lasting some microseconds. Within this time frame the diffusing length ($l = \sqrt{2Dt}$) exceeds by orders of magnitude the width of the aqueous layer. Under such conditions the two-dimensional approximation led to consistent accurate evaluation of the proton diffusion coefficient (Gutman, Nachliel & Moshiach, 1989). Very fast reactions, lasting a few nanoseconds or less,



Scheme I. Schematic presentation of the reaction space for proton-excited pyranine anion recombination in the thin water layer between phospholipid membranes of multilamellar vesicle. The proton release is depicted at the center of the layer, and it diffuses in concentric shells. When the diffusion radius (R_i) exceeds the distance to the membrane ($dw/2$), the shape of the diffusion space deviates from spheric symmetry, approaching at large values of R_i a cylindrical one. R_o is the reaction radius, R_d is the unscreened Debye radius of pyranine (R_d = 28.3 Å). $d_{(w)}$ in this scheme is 30 Å and the size of water molecule is drawn to scale.

cannot be subjected to such approximation as the diffusion length is comparable with the width of the aqueous layer. As seen in Scheme I, after a few diffusion steps, when the diffusion sphere is coming into contact with the membrane, the shape of the reaction space is changing and wall effects must be considered.

Distorted Electric Fields

The electrostatic cage surrounding an ion within a thin water layer deviates from spherical symmetry (Kjellander & Marcelja, 1988). Image charges and fixed charges on the membrane or proteins reshape the electric potential well. As a result more than one minimum in the ionic potential space may be found.

Proton Reactivity of the Surface

The surface of the membrane is not proton inert; it contains numerous proton binding sites. Each phosphomoiety of a phospholipid is a rather strong acid (pK = 2.25 (Nachliel & Gutman, 1988)) which delays a proton for a period of 1–2 nsec. The effect of these fixed buffer moieties on the propagation of proton has been analyzed by Junge and McLaughlin (1987). According to them the reduction of the apparent diffusion coefficient can be in orders of magnitudes. A detailed mechanistic analysis of proton-site interaction which accounts simultaneously for all these factors calls for a model system which allows one parameter to vary selectively while others are kept constant. The model we selected is the thin aqueous layers spacing between lipid membrane of large multilamellar vesicles (MLV) containing pyranine in their outermost layers (Gutman et al., 1989). This preparation has the following advantages:

(*i*) The activity of water in the aqueous lamella is experimentally adjustable through the activity of water in the suspending medium.

(*ii*) The width of the aqueous layer ($d_{(w)}$) and its dependence on the osmotic pressure is well known (for review, *see* Rand & Parsegian, 1989). The width of the layer and the surface packing density were extensively measured by X-ray diffraction over a wide range of external pressures for many phospholipids (McIntosh & Simon, 1986; Parsegian et al., 1986; Rand & Parsegian, 1989; Ito, Yamazaki & Ohnishi, 1989). Thus we can select conditions where $d_{(w)}$ is an experimentally adjustable parameter.

The $d_{(w)}$ values given in this report are based on the data compiled by Rand, Parsegian and their colleagues (1980). These values differ from those determined by McIntosh and Simon (1986) using electron density maps. Yet, the conclusions we draw are not affected by the method used for quantitating $d_{(w)}$.

(*iii*) The width of the water layer is modulated by addition of cholesterol (Rand et al., 1980; Lis et al., 1982; McIntosh, Magid & Simon, 1987; McIntosh, Magid & Simon, 1989). The separation ($d_{(w)}$) between DPPC membranes or DPPC + cholesterol (1 : 1) membranes, under the same external pressure, is not equal. Thus the effect of a_{H_2O} *and* $d_{(w)}$ can be isolated from each other and their effect evaluated quantitatively.

(*iv*) The density of the phosphohead groups on the surface is modulated by addition of cholesterol which acts as an inert spacer (with respect to H^+).

The probing reaction we selected is the binding of proton to excited pyranin anion (8 hydroxypyrene 1,3,6 trisulfonate) dissolved in the aqueous lamellas (Gutman et al., 1989). Due to the short time frame of our observation we monitor the excited dye during its initial acid dissociation and subsequent reprotonation by a proton which is still confined to the Coulomb cage of the excited anion. Such a process is called geminate recombination.

Geminate recombination is a reaction common to all reversible dissociation processes. It takes place when the two products are formed at an ultimate proximity to each other; thus, there is a certain probability that the pair will recombine to reform the parent molecule. In a case where the two products attract each other electrostatically or are slowly diffusing, the probability of recombination increases. Through this instrument we gain an inside look at diffusional processes and electric forces as they operate in the ultrathin water layers between phospholipid membranes.

The mechanism of geminate recombination in bulk water has been analyzed recently by Agmon (1988), Agmon, Pines and Huppert (1988) and Pines,

Huppert and Agmon (1988). This analysis is not directly applicable to the nonspheric reaction space defined by the phospholipid membranes, yet it is capable of determining the average number of recombinations exercised by a proton before it escapes from the Coulomb cage. Using this analysis we demonstrate that the probability of proton to react with a very close anion is enhanced almost 10-fold when the pair is sandwiched between phospholipid membranes.

Materials and Methods

Lipids: Dipalmitoyl phosphatidyl choline (synthetic) and cholesterol 99+% were obtained from Sigma, pyranine (8 hydroxypyrene 1,3,6 trisulfonate) was from Kodak.

Large multilamellar vesicles containing pyranine in the aqueous lamella were prepared as in Gutman et al. (1989). Ten mg of lipids (either DPPC or 1 : 1 ratio with cholesterol) were dry evaporated from chloroform in a rotating 100-ml round bottom flask. Ten ml of 2 mM pyranine in water (pH = 5.5) were added, and the lipids were swelled overnight in a shaker bath with very slow (15 rpm) stirring at 45°C. The suspension was diluted to 40 ml with water and the vesicles were spun down. The precipitate was gently broken by double passage in a glass Teflon homogenizer, diluted to 40 ml and spun again. This washing procedure was repeated four times. In the last one the vesicles were spun down in solution containing 5 mM MES buffer, pH 5.5. The external concentration of the dye is reduced by this procedure to less than 1 nM.

The vesicles were finally suspended in 0.5 ml of 5 mM MES buffer, pH = 5.5, and kept at +4°C. Samples were used within two days of preparation.

Fluorescence spectrum was measured by a Shimadzu FR 540 spectrofluorometer using baseline correction.

Time-resolved fluorometry was measured with 10-psec resolution as described by Pines et al. (1988).

Osmotic pressure was applied by addition of sucrose to the desired concentration, followed by 15-min equilibration. The osmotic pressure of sucrose solutions, and their corresponding $a_{(H_2O)}$ values were taken from Robinson and Stokes (1954).

Results

Time-Resolved Fluorescence of Pyranine in Thin Water Layers

Figure 1 depicts the time-resolved fluorescence of ΦOH* and the steady-state (time-integrated) emission spectrum of pyranine.

The lower curve was recorded with pyranine in bulk water. Following excitation, there is a rapid exponential decay of the emission of ΦOH* (τ = 100 psec) to a level of 5–10% of the initial amplitude. After that the relaxation proceeds nonexponentially in a shape of a very low shallow tail.

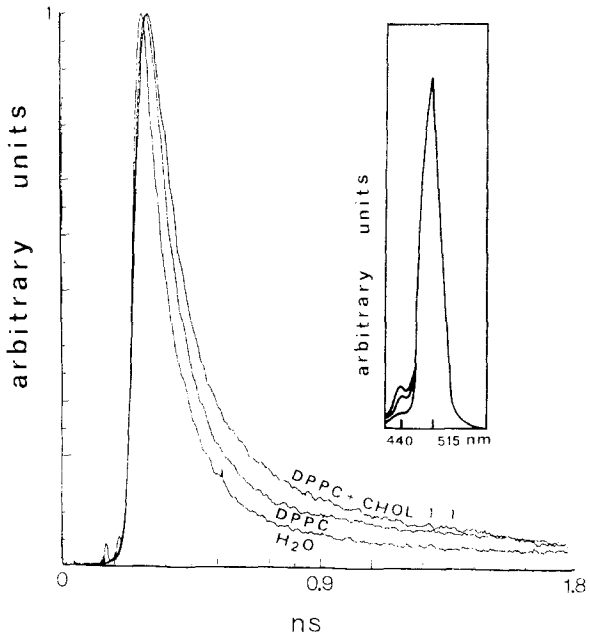


Fig. 1. Time-resolved fluorescence of pyranine at the wavelength of maximum ΦOH* emission. The dye was excited by a 10-psec laser pulse (λ = 335 nm) and the fluorescence recorded with a streak camera and multichannel analyzer as detailed by Pines et al. (1988). The traces correspond with fluorescence decay dynamics measured for pyranine in water, entrapped in the aqueous layers of multilamellar vesicles made of DPPC or those made of DPPC + cholesterol (1 : 1). *Inset:* Steady-state fluorescence spectra of the samples shown in the main frame. The spectra were normalized to have the same value at 515 nm, where emission of ΦO^{*-} is maximal. This presentation emphasizes the incremental emission of the membranal preparation at 440 nm. The three curves correspond with dye dissolved in water (lowermost curve), entrapped in DPPC vesicles (middle curve), or in DPPC + cholesterol vesicles (uppermost curve)

According to the analysis of Pines et al. (1988) this two-phase dynamics is typical for the geminate recombination process. The initial fast decay is due to the dissociation of a proton from ΦOH*. The long tail reflects repeated reformation of ΦOH* by the encounter of a proton with the ΦO^{*-} anion before it relaxes to the ground state.

The other two curves were measured with pyranine entrapped in the thin water layer of the multilamellar structures. Both curves show a remarkably enhanced geminate recombination. The fluorescence tail is significantly larger than that of pyranine in bulk water. The decay of ΦO^{*-} (the excited pyranine anion) measured at 515 nm exhibits no pecularities and was the same as in bulk water (*not shown*), with a time constant of 5.5 nsec.

The enhanced emission of ΦOH*, as detected in the tail of the decay, is readily observed in steady-state fluorescence spectrum (*see* inset to Fig. 1) where the emission intensity of ΦO^{*-} (λ = 515 nm)

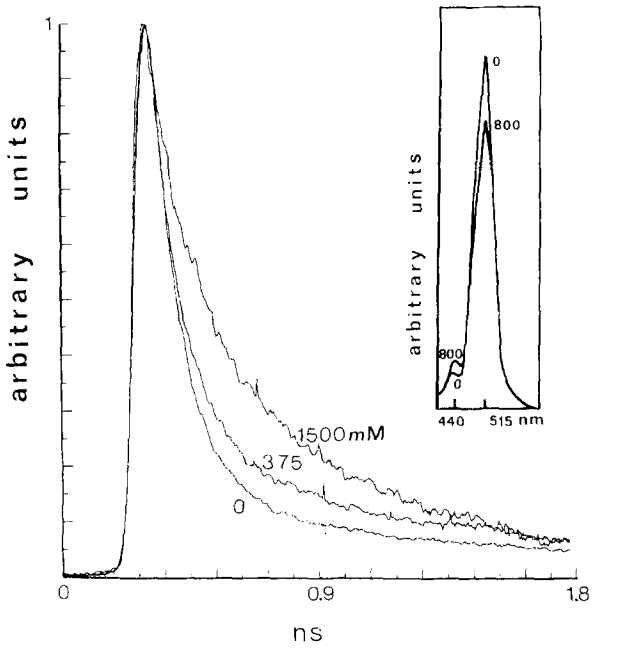


Fig. 2. Time-resolved fluorescence of pyranine trapped in the water layers of DPPC multilamellar vesicles under applied osmotic pressure. The measurements were carried out as in Fig. 1, except that the vesicles were suspended in sucrose solution at the concentration indicated in the figure. Inset: Steady-state emission spectrum of the entrapped dye, in the absence and presence of osmotic stress applied by 800 mM sucrose

was normalized as 100%. In accord with the kinetics measurements, the emission of ΦOH* (at 440 nm) was weakest in bulk water and strongest in the DPPC + cholesterol preparation.

The Effect of Osmotic Pressure on Fluorescence of Pyranine in Thin Water Layers

Under applied osmotic pressure the fluorescence dynamics undergoes a gradual transition corresponding with enhanced geminate recombination. As seen in Fig. 2 the tail of the decay became more prominent and the transition between the initial fast decay and the slower process is less distinguished.

The steady-state fluorescence (*see* inset) indicates that the enhanced emission of ΦOH* is at the expense of ΦO^{*-} emission. We noted no change in the lifetime of ΦO^{*-} under osmotic pressure. Thus the enhanced emission ratio corresponds with enrichment of ΦOH* population by geminate recombination which consumes the ΦO^{*-} population.

Quantitation of Geminate Recombination

The enhanced steady-state emission of ΦOH* originates from two mechanisms.

(*i*) The rate of proton dissociation is controlled by the availability of water molecules needed to stabilize, by hydration, the ejected proton. For these reasons the lifetime of ΦOH* is prolonged in solution of low a_{H_2O}, and the steady-state emission of this form is intensified (Huppert et al., 1982; Bardez et al., 1984; Politi & Chaimovich, 1986; Gutman & Nachliel, 1990).

(*ii*) The second mechanism leading to the enhanced emission of ΦOH* is the geminate recombination. Whenever proton and ΦO^{*-} recombine to form ΦOH*, there will be a delay period of τ_d (the intrinsic dissociation time of ΦOH* in the pertinent system) before the ion pair is formed again. Thus the apparent lifetime of ΦOH* in solution where geminate recombination takes place is given by

$$\tau_{app} = \tau_d + \tau_d \cdot NR \tag{1}$$

where NR is the average number of successive recombination-dissociation events characterizing the excited population of ΦOH.

τ_d, the time constant of proton dissociation, is measured directly from the most initial phase of ΦOH* emission decay curve (*see* Figs. 1 and 2).

The apparent time constant is calculated as a product of the effective dissociation yield (given by $I\Phi OH^*/I\Phi O^{-*}$) times the fluorescence lifetime of ΦOH* (6000 psec) as measured under conditions where no dissociation takes place (for more comprehensive discussion, *see* Pines & Huppert, 1989).

$$\tau_{app} = \tau_{\Phi OH^*}(I_{\Phi OH^*}/I_{\Phi O^{*-}}). \tag{2}$$

The combination of Eqs. (1) and (2) yields the expression for the average number of recombinations.

$$NR = \frac{\tau_{\Phi OH^*}(I_{\Phi OH^*}/I_{\Phi O^{*-}})}{\tau_d} - 1. \tag{3}$$

NR is a pure number denoting the average recombination events within the excited geminate pair population before the proton escapes from the Coulomb cage. A more intuitively accepted expression is τ_{gr}.

$$\tau_{gr} = \tau\Phi O^{*-}/NR. \tag{4}$$

τ_{gr} is the average lapse time between successive geminate recombinations. In bulk water $NR = 1.27$ and $\tau_{gr} = 4.3$ nsec (Pines et al., 1988; Pines & Huppert, 1989).

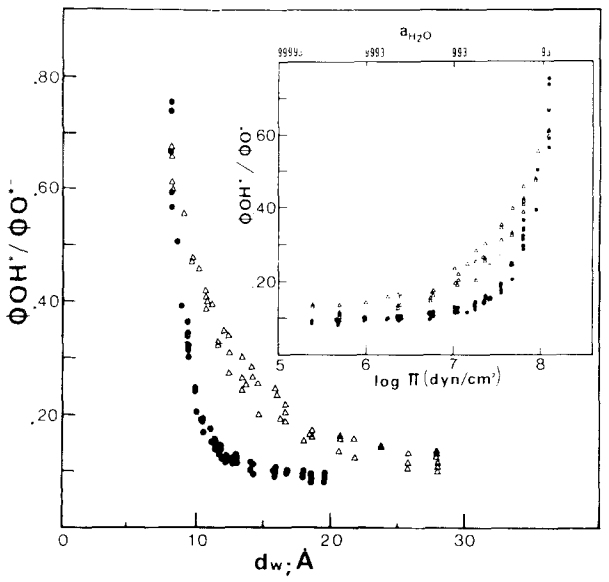


Fig. 3. The correlation between the ratio of excited pyranine population (ΦOH*/ΦO*⁻) with the width of the aqueous layer. The fluorescence emission spectrum of pyranine trapped in multilamellar vesicles was measured from vesicles suspended in a sucrose solution of known osmotic pressure. As the fluorescence quantum yield of the two forms is ~1 (Pines & Huppert, 1989) the ratio of emission intensity is expressed as the molar ratio of the excited species. The width of the aqueous layers was calculated from the data published by Lis et al. (1982) and Rand et al. (1980). The activity of water of the sucrose solutions was calculated from data published by Robinson and Stokes (1954). Inset: The dependence of the excited population ratio on the osmotic pressure. △, pyranine in thin water layer of MLV made of DPPC + cholesterol; ●, pyranine in MLV made of DPPC

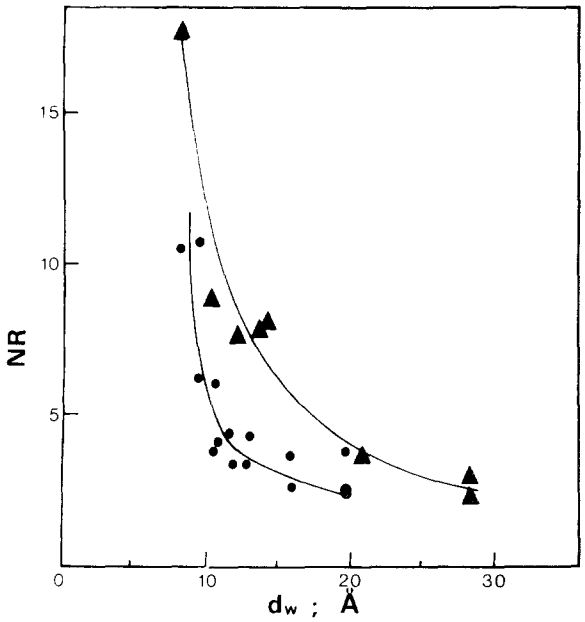


Fig. 4. The dependence of the average number of sequential recombinations of proton with their excited geminate anion on the width of the aqueous layer where the reaction takes place. The average number of recombinations was calculated according to Eq. (3) using steady-state and time-resolved fluorescence measurements as described in Figs. 1–3. The width of the aqueous layer was determined as explained in the legend to Fig. 3. ▲, pyranine in thin water layer of MLV made of DPPC + cholesterol; ●, pyranine in MLV made of DPPC

Using NR and τ_{gr} as quantitable parameters we shall evaluate our experimental results, looking in particular at the distinction between the effect of the geometrical constraint (expressed by $d_{(w)}$) and the chemical potential of the solvent (a_{H_2O}) as determinants of the geminate recombination probability.

The Effect of Geometric Constraints on Geminate Recombination

Figure 3 depicts the effect of diminishing $d_{(w)}$ on the steady-state ratio of the two excited species (ΦOH*/ΦO*⁻). According to expectations, as the width of the aqueous lamella decreases the fraction of ΦOH* increases, yet when we compare the results obtained with two vesicular preparations, i.e., DPPC and DPPC + cholesterol, we note that at the same $d_{(w)}$ each preparation is characterized by its own ratio. The distinction between the two populations indicates that $d_{(w)}$ is not the major determinant affecting the overall process.

The inset of Fig. 3 relates the excited population ratio with the force applied on the vesicles, quantitated by its osmotic pressure and the (a_{H_2O}) of the suspending medium (Robinson & Stokes, 1954).

At very high pressure $\Pi > 5 \times 10^7$ dyn/cm² the results obtained with the two preparations merge. Yet at lower pressure (or $a_{H_2O} > 0.93$) the two preparations are distinguishable from each other.

The enhanced fraction of ΦOH* reflects both slower dissociation of the proton from the excited molecule and higher frequency of geminate recombination. To eliminate the contribution of the dissociation dynamics we calculated the average number of geminate recombinations (NR) which quantitates only the post dissociation trajectory of the proton. The results of these calculations are given in Fig. 4. The average number of recombinations, as measured for excited pyranine-proton pair trapped in a thin water layer, is about twice that measured in bulk water, yet we cannot ascribe it exclusively to the width of the layer. In the absence of external pressure $d_{(w)}$ of the two preparations varies by 40%, while the average number of recombinations is essentially the same. The distinction between the two populations is maintained over the whole $d_{(w)}$ range

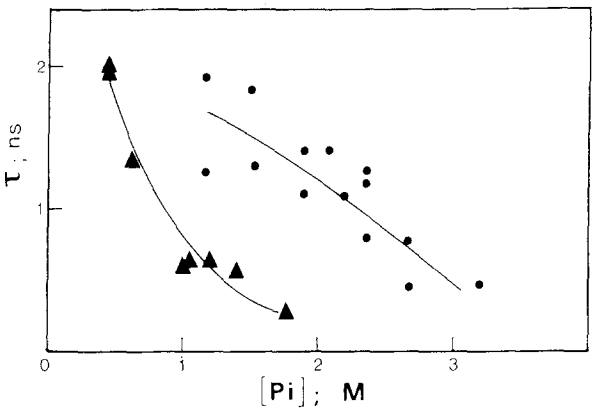


Fig. 5. The dependence of geminate recombination time on the density of phosphocholin headgroups facing the aqueous phase between the membranes. The geminate recombination time, calculated according to Eq. (4), is the average time interval between recombination events. The phosphomoieties concentration was calculated from data published by Rand et al. (1980) and converted to molar units. ▲, pyranine in thin water layer of MLV made of DPPC + cholesterol; ●, pyranine in MLV made of DPPC

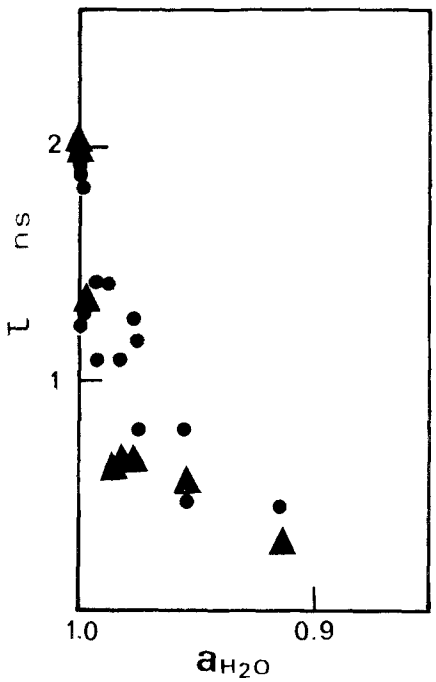


Fig. 6. The dependence of geminate recombination time on the activity of water in the aqueous layer. The activity of the water in equilibrium with the MLV was calculated from data given by Robinson and Stokes (1954). ▲, pyranine in MLV made of DPPC + cholesterol; ●, pyranine in MLV made of DPPC

we investigated. This is an indication that besides the geometrical factor there is another term, specific to the membrane-water systems, which affects the probability of geminate recombination.

Reactivity of Surface Groups

In the absence of osmotic pressure the number of phosphohead groups confined within the volume of the Coulomb cage is high (≈80 in DPPC vesicles and ≈40 in DPPC + cholesterol vesicles). Applying osmotic pressure increases the spatial density of the sites by two mechanisms: lateral compression of the lipids (Lis et al., 1982; McIntosh et al., 1987; Ito et al., 1989; McIntosh et al., 1989) and smaller distance between the membranes. As a result, under osmotic pressure, the number of reversible proton binding sites within the boundaries of the electrostatic cage of pyranine will increase. The outcome will be a better confinement of proton to the vicinity of the excited anion (Junge & McLaughlin, 1987). In parallel the excited anion may also regain its protonated state by collisions with a protonated phosphomoiety (Gutman et al., 1989). Thus the high density of temporary binding sites within the electrostatic cage will shorten the average time interval between recombination events.

The results depicted in Fig. 5 illustrate this point. As the membranes are compressed the density of phosphomoieties increases (expressed in equivalent molar units) while the average delay between successive recombinations decreases from 2000 to ~ 300 psec.

While the observations are in accord with the predictions, the two populations still maintain their identity, indicating that the basic mechanism underlying the process is not directly coupled with the density of reacting sites within the volume of the reaction space.

The Effect of the Activity of Water on Geminate Recombination

The dependence of the recombination time (τ_{gr}) on the activity of the water in the aqueous layer, taken as that of the external medium, is shown in Fig. 6. This figure relates the average time between successive recombination events with the activity of the solvent in the reaction space. In this refined analysis we find that data points of the two preparations merge to a single curve, indicating that the major factor affecting the observation is the solvent in the reaction space, not the vessel.

Discussion

In this study we used geminate recombination as a probing reaction, looking for the effect of the shape of the reaction space on the dynamics of proton

transfer. The short observation period inherent to the reaction ensures that the space is small. Furthermore the large Coulomb cage of the fluorophore (28.3 Å) (Agmon, 1988; Agmon et al., 1988; Pines et al., 1988) ensures that the whole width of the thin water layer is under the influence of electrostatic potential which is stronger than the thermal energy. Thus the conclusions drawn from the study are directly applicable for evaluating conditions encountered in closed packed membranal structures like thylakoids or mitochondria where the water layers are of comparable width. The proton dissociated in our study is analogous to the initial events following a proton discharge by a protogenic enzyme on these membranes.

The model structure we selected is suitable for investigating the role of three major variables which dominate the reaction space: the width of the aqueous layer, the reactivity of the membrane boundaries and the properties of the water molecules in the thin lamella.

The membranes used (DPPC and DPPC + cholesterol) differ in their hydration energy, the amount of tightly held water per lipid moiety, the lateral compressibility, surface density of zwitterionic sites and most probably their orientation with respect to the plane (McIntosh et al., 1987; Rand & Parsegian, 1989). These differences suffice to endow the preparations with unique properties expressed by the nonidentical kinetics of proton-anion recombination (*see* Fig. 1) and the response of the indices we used (Figs. 3–5) bear the mark of the phospholipid composition.

The nonidentity of the dynamics as measured in the two preparations allowed us to investigate which of the characterizing parameters is the dominating force controlling the probability of the test reaction. The algorithm underlying this search was to isolate experimentally the effect of each term and examine its effect on the test reaction. The one that unifies the data points of the two preparations to a single curve is the major factor controlling the reaction.

The first index for comparison is the population ratio $\Phi OH^*/\Phi O^{*-}$ shown in Fig. 3. It reflects two processes which may vary independently:

(*i*) The rate of proton dissociation, which is function of the capacity of water to rapidly hydrate the proton (Huppert et al., 1982; Bardez et al., 1984; Politi & Chaimovich, 1986). This process dominates at the most initial phase and thus contributes substantially to the steady-state emission of ΦOH^*.

(*ii*) The probability of the proton to escape from the electrostatic cage. This process produces the long "tail" and thus contributes less to the steady-state emission of ΦOH^*.

To quantitate the latter process, we employed two parameters which are free of the initial dissociation step, the average number of recombinations (*NR*) or the average time interval between successive recombinations. Using these refined parameters we could exclude geometrical constraints and reactivity of surface as the major determinants of geminate recombination in the system under study. Within the present experimental and analytic resolution it seems that the solvent properties are those governing most (but not exclusively) the geminate recombination.

The exit of proton from the Coulomb cage is a diffusion process. It must execute within the lifetime of the excited anion many random walk steps, up and down the electric potential field. As the electric field is relatively constant in all preparations, the probability of escape becomes, to a large extent, a function of the matrix capacity to serve as an efficient proton carrier. The proton transfer in water is an ensemble property of the environment, associated with dipole orientation of the adjacent water molecule. If the freedom of these molecules to rotate is diminished, such as by strong interaction with the phospholipid membrane, the response of the environment to proton translocation will be delayed, producing an electrodynamic drag (Hubbard & Onsager, 1977). This projection is confirmed by our results. When the membranes are pressed together and the residual aqueous matrix has diminished activity, the proton mobility is reduced. This observation based on subnanosecond studies is in accord with our previous measurement at the microseconds time scale (Gutman et al., 1989) where reduced diffusion coefficient of proton was measured in thin water layers.

There is another aspect to proton diffusion in "partially organized" water. The water-surface interactions tend to form sequences of hydrogen-bonded molecules with a higher stability (time averaged) than in bulk water. These ordered structures form a multipotential surface where the protons exhibit a continuum infrared absorption, as for example on water-glass interface (Zundel & Roberts, 1980). These structures are inadequate for rapid proton diffusion (Gutman & Nachliel, 1990). Because of their temporal and spatial stability they localize the proton in the hydrogen-bonded structure (Brzezinski, Zundel & Kramer, 1987). Diffusion *per definition* is a random process. Thus ordering of the diffusion matrix will lower the probability of the proton to escape from the electrostatic cage.

Biomembranes, in contrast to our simple model, are heterogenous, so the extent of surface-water interaction will vary between different loci. Yet our studies demonstrated that within a few nanoseconds the protons can escape from a large trap of $\simeq$60 Å

diameter. A trap of that size is already comparable with the dimensions of a membrane-bound protein. We therefore conclude that local forces in a biomembrane can affect the dynamics of a proton transfer only for a short period of a few nanoseconds. Since catalytic events on biomembranes are characterized by the millisecond time constant, we can disregard any local effect with regard to proton mobility.

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References

Agmon, N. 1988. Geminate recombination in proton-transfer reaction. III. Kinetics and equilibrium inside a finite sphere. *J. Chem. Phys.* **88:**5639–5642

Agmon, N., Pines, E., Huppert, D. 1988. Geminate recombination in proton-transfer reaction. II. Comparison with diffusional and kinetic schemes. *J. Chem. Phys.* **88:**5631–5638

Bardez, E., Goguillon, B.T., Keh, E., Valeur, B. 1984. Dynamics of excited-state reactions in reversed micelles. I. Proton transfer involving a hydrophilic fluorescent probe. *J. Phys. Chem.* **88:**1909–1913

Brzezinski, B., Zundel, G., Kramer, R. 1987. Proton polarizability caused by collective proton motion in intramolecular chains formed by two and three H bonds. Implications for charge conduction in bacterial rhodopsin. *J. Phys. Chem.* **91:**3077–3080

Gutman, M., Nachliel, E. 1990. The dynamic aspects of proton transfer processes. *Biochim. Biophys. Acta* **1015:**391–414

Gutman, M., Nachliel, E., Moshiach, S. 1989. Dynamics of proton diffusion within the hydration layer of phospholipid membranes. *Biochemistry* **28:**2936–2940

Hubbard, J., Onsager, L. 1977. Dielectric dispersion and dielectric friction of electrolytic solution. *J. Chem. Phys.* **67:**4850–4857

Huppert, D., Kolodney, E., Gutman, M., Nachliel, E. 1982. Effect of water activity on the rate of proton dissociation. *J. Am. Chem. Soc.* **104:**6949–6953

Ito, T., Yamazaki, M., Ohnishi, S.I. 1989. Osmoelastic coupling in biological structure: A comprehensive thermodynamic analysis of the osmotic response of phospholipid vesicles and a reevaluation of the hydration force theory. *Biochemistry* **28:**5626–5630

Junge, W., McLaughlin, S. 1987. The role of fixed and mobile buffers in the kinetics of proton movement. *Biochim. Biophys. Acta* **890:**1–5

Kjellander, R., Marcelja, S. 1988. Inhomogeneous Coulomb fluids with image interaction between planar surfaces. III. Distribution functions. *J. Chem. Phys.* **88:**7138–7146

Lis, L.J., McAllister, M., Fuller, N.L., Rand, R.P., Parsegian, V.A. 1982. Measurement of lateral compressibility of several phospholipid bilayers. *Biophys. J.* **37:**667–672

McIntosh, T.J., Simon, S.A. 1986. Hydration forces and bilayer deformation, a reevaluation. *Biochemistry* **25:**4058–4066

McIntosh, T.J., Magid, A.D., Simon, S.A. 1987. Steric repulsion between phosphatidylcholine membranes. *Biochemistry* **26:**7323–7325

McIntosh, T.J., Magid, A.D., Simon, S.A. 1989. Cholesterol modifies the short range repulsive interactions between phosphatidyl choline membranes. *Biochemistry* **28:**17–25

Nachliel, E., Gutman, M. 1988. Time resolved proton-phospholipid interaction methodology and kinetic analysis. *J. Am. Chem.Soc.* **110:**2629–2635

Parsegian, V.A., Rand, R.P., Fuller, N.L., Race, D.C. 1986. Osmotic stress for the direct measurement of intramolecular forces. *Methods Enzymol.* **127:**400–416

Pines, E., Huppert, D. 1989. Salt effect in photo-acids quantum yields measurements: A demonstration of the geminate recombination role in deprotonation reactions. *J. Am. Chem.Soc.* **111:**4096–4097

Pines, E., Huppert, D., Agmon, N. 1988. Geminate recombination in excited state proton transfer reactions: I. Numerical solution of the Debye-Smoluchowski equation with back reaction and comparison with experimental results. *J. Chem. Phys.* **88:**5620–5630

Politi, M.J., Chaimovich, H. 1986. Water activity in reversed sodium *Bis* (2-ethylhexyl)sulfosuccinate micelles. *J. Phys. Chem.* **90:**282–287

Rand, R.P., Parsegian, V.A. 1989. Hydration layer between phospholipid bilayers. *Biochim. Biophys. Acta* **988:**351–376

Rand, R.P., Parsegian, V.A., Henry, J.A.C., Lis, L.J., McAlister, M. 1980. The effect of cholesterol on measured interactions and compressibility of dipalmitoyl phosphotidyl vesicles. *Can. J. Biochem.* **59:**959–968

Robinson, R.A., Stokes, R.H. 1954. Electrolyte Solutions Appendix 8. G. Butterworth Scientific, London

Zundel, G., Roberts, N.K. 1980. Long range structuring of water by quartz and glass surface as indicated by infrared continuum and diffusion coefficient of excess protons. *J. Phys. Chem.* **84:**3655–3660

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