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Relationship between Fluidity and Ionic Permeability of Bilayers from Natural Mixtures of Phospholipids

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Summary. Proton and calcium permeability coefficients of large unilamellar vesicles made from natural complex mixtures of phospholipids were measured in various conditions and related to membrane fluidity. Permeability coefficients at neutral pH and 25°C were in the range of 10^{-4} cm sec^{-1} and 2.5×10^{-11} cm sec^{-1} for protons and calcium, respectively. With the exception of two cases, $(H^+) > 10^{-4}$ M and $(Ca^{2+}) > 10^{-3}$ M, fluidity increases correspond to permeability increases. Theoretical analysis shows that, for both ions, the measured values of permeability coefficients imply that the permeation process is controlled by the product D_1D_2 of the diffusion coefficient from the medium into the membrane (D_1) by the diffusion coefficient in the membrane (D_2). Further analysis of D_1 values deduced from combined use of permeability and fluidity data shows that the solubilization should occur in a medium of dielectric constant of about 12, suggesting the involvement of the hydration water of membranes. High proton concentrations, although having virtually no effect on fluidity, trigger the appearance of (i) lateral heterogeneity in membranes, as seen by ^{31}P NMR, and (ii) large permeability increases. It is proposed that the main effect of fluidity and/or lateral heterogeneity on permeability may be *via* the membrane hydration control. We conclude that the current assumption that permeability is controlled by fluidity should be regarded with caution, at least in the case of ions and natural mixtures of phospholipids.

Key Words proton permeability · calcium permeability · fluidity · membrane heterogeneity · natural phospholipid mixtures

Introduction

A primary function attributed to the lipid layer of biological membranes is to act as a permeability barrier. In current formalism, permeability properties are evaluated by permeability coefficients. As defined operationally, these coefficients represent the ratio of the diffusion coefficient in the lipid layer to its thickness. Two basic assumptions are made to define such coefficients: (i) the phospholipid layer is homogeneous, and (ii) diffusion across the lipid layer is the limiting step of the permeation process. A consequence of the latter hypothesis is that permeability coefficients should be proportional to the lipid layer fluidity (via the Einstein-Stokes relation; *see*, for example, Rojas, 1981). This supports the current idea that the permeability barrier is sensitive to fluidity effectors. The former assumption is usually implicit. However, it is well known that at temperatures around the gel-fluid transition temperature, phospholipid bilayers have anomalous permeabilities (Papahadjopoulos et al., 1973; Blok et al., 1975). Furthermore, pure phospholipid membranes are known to exhibit complex polymorphism which should be accompanied by the development of lateral and transverse heterogeneity in bilayers. In fact, some reports describe permeability increase in response to polymorphism (Gerritsen et al., 1980; Noordam et al., 1980).

The overall picture is therefore that phospholipid bilayers act as fluidity-controlled permeability barriers with the exception of cases where some bilayer components quit the fluid lamellar organization. Direct evaluation of this conclusion has not been attempted, with the exception of water permeability which has been recently related to the physical state of bilayers (Carruthers & Melchior, 1983). However, no structural or dynamic parameter determinations were made in this study, and no quantitative relation may be proposed between membrane structure or dynamics and water permeability.

In the case of ions, with the exception of protons (Nichols et al., 1980; Rossignol et al., 1982*a*; Deamer & Nichols, 1983), published data for permeability coefficients are available only in early studies of small unilamellar vesicles. These results have not been re-examined in recent years despite the availability of methods for obtaining large unilamellar vesicles. These allow the preparation of stable and homogeneous membranes which offer a convenient system for permeability studies. In addition, all available results were obtained with pure

phospholipids or simple mixtures. Measurements with natural phospholipidic mixtures are rare, although recent studies show that these mixtures may exhibit polymorphism dependent on ionic conditions (Gounaris et al., 1983; Albert et al., 1984).

During the past decade, fluidity parameters have been measured by several different techniques, the most popular one being steady-state fluorescence anisotropy (FA). In principle, a true fluidity may be derived from FA determinations (Shinitzky & Yuli, 1982), provided that care is taken to separate dynamic and structural information (Heyn, 1979; Jähnig, 1979; Van Blitterswijck et al., 1981; Pottel et al., 1983). Polymorphism can also be monitored by methods such as ^{31}P NMR or freeze-fracture microscopy (Verkleij & de Gier, 1981).

The purpose of this paper is to study the relationship between fluidity and ionic permeability coefficients in order to determine to which extent ionic permeability may be assumed to be controlled by fluidity. The study was performed with large unilamellar vesicles made from complex natural phospholipid mixtures. Protons and calcium were chosen since cells must maintain internal concentration of these ions as low as 10^{-7} M, even though the external abundance may be several orders of magnitude higher.

Materials and Methods

Phospholipid Obtention

Phospholipids were extracted and purified from roots of six-day-old seedlings of horse bean and converted to their potassium salts as described earlier (Rossignol et al., 1982*b*). Phosphatidic acid and phosphatidylglycerol were obtained, respectively, by phospholipase D-catalyzed degradation or transphosphatidylation of horse bean phosphatidylcholine (Bergelson, 1980). In all cases, fatty acid auto-oxidation was less than 0.5%, as judged by absorbance at 231 nm (Kates, 1972). Phosphorus assay after thin-layer chromatography indicated the presence of phosphatidylinositol (7.9%), phosphatidylcholine (44.5%), phosphatidylglycerol (6.7%), phosphatidylethanolamine (30.7%), phosphatidylserine (4.0%), diphosphatidylglycerol (2.5%) and phosphatidic acid (3.7%). Gas chromatography of fatty acid methyl esters showed the presence of palmitic (18.2%), stearic (5.9%), oleic (3.5%), linoleic (66.3%) and linolenic (6.1%) acids.

Proton Permeability Determinations

Measurements were carried out using the pyranine method (Rossignol et al., 1982*a*). Liposomes were prepared by a modified ether injection method (Deamer & Bangham, 1976) in 50 mM PIPES buffer, pH 5.6, containing 100 mM KCl, 1 mM pyranine and 1 mM EDTA unless otherwise stated. The internal volume of the vesicles, calculated from their pyranine content, was repeatedly of 8 to 10 liters/mole, a high value typical of large and primarily unilamellar liposomes (Szoka & Papahadjopoulos, 1978). After removal of untrapped probe by gel filtration through Sephadex G50, 0.2 ml of the liposome fraction was mixed with 2 ml of 50 mM PIPES buffer, pH 7.4, containing 100 mM KCl and 1 mM EDTA. The ratio I_{463}/I_{405} of the pyranine fluorescence intensities at 511 nm upon excitation at 405 and 463 nm allowed accurate determination of internal pH (Rossignol et al., 1982*a*). Experiments were performed in a Jobin-Yvon JY3D spectrofluorometer equipped with a thermostated cell holder. External pH was continuously monitored by a combined glass microelectrode over a period of one hour.

For each time interval, the net proton flux J can be described by the relation $J = P_{eqH} \cdot \Delta H$, where ΔH is the proton activity difference across the liposome membrane. P_{eqH} is the permeability coefficient of all species equivalent to protons which contribute to decay of pH gradient. The flux J can be otherwise directly calculated from the relation $J = B_i \cdot V_i \cdot \Delta pH_i / A \cdot \Delta t$, where ΔpH_i is the change in internal pH during the time interval Δ_t, V_i the internal volume and B_i the buffer capacity of the internal volume determined from a separate acid-base titration of the buffer. A is the total membrane area estimated assuming a mean packing area of 75 Å^2.

Liposomes were used within 6 hr after the gel filtration. Aging experiments over a 24-hr period after the gel filtration do not provide evidence of any significant difference for P_{eqH}. As, on the other hand the proton fluxes decreased exponentially with time (Rossignol et al., 1982*a*), the diffusion may be assumed to be actually limited by a surface barrier. No correction was made for an eventual diffusion potential slowing the fluxes. This eventually may lead to an overestimation of P_{eqH} of a factor 2 in the conditions used here (Rossignol et al., 1982*a*; Rossignol, 1984). Also P_{eqH} were not corrected for surface potential effects which, although identical at both sides of the membranes in the pH range used, locally lead to overconcentration of protons and underconcentration of hydroxide ions. In the case of our liposomes (surface potential of −40 mV, as determined by microelectrophoresis, Gibrat and Grignon, 1982) this leads to an underestimation of P_{eqH} by a factor of 2 (Rossignol, 1984). For all presented data the standard deviations amounted to 10 to 30% of the corresponding mean values.

Calcium Permeability Determinations

Liposomes were prepared by the procedure described above but in the presence of either 5×10^{-5} or 10^{-3} M $CaCl_2$ labeled with ^{45}Ca, and 1 mM pyranine. External ^{45}Ca and pyranine were removed by gel filtration and liposomes were dialyzed against tracer and pyranine-free buffer in an apparatus allowing fast equilibration (Dianorm). At 1-hr intervals external medium was changed and assayed for radioactivity (Packard scintillation spectrometer). Due to the low permeability of calcium, experiment duration was one day. As the ionic conditions used in these experiments may lead to some destabilization of the vesicles for this duration, pyranine was also assayed in the external medium and correction was made in the calculations, at each time interval, for internal volume released in the medium. Standard deviations amounted to 5 to 20% of the corresponding mean values.

Fluidity Determinations

Fluidity coefficients were derived from DPH-FA measurements. Liposomes were prepared by taking to dryness under argon flow chloroform solutions of phospholipids (1 μmol) and DPH (1 nmol). After addition of 3 ml of aqueous buffer containing 100

mM KCl and 1 mM EDTA (or 1 mM $CaCl_2$), phospholipids were dispersed by Vortex agitation (10 min) and sonication (15 min) in a cooled bath-type apparatus. Measurements were performed with a Jobin-Yvon JY3D spectrofluorometer and correction was made for the grating polarization (Azumi & Mc Glynn, 1962; Rossignol et al., 1982*b*). The fluorescence lifetime was calculated from total fluorescence intensity assuming that the lifetime at 25°C is that of DPH in dicetylphosphate-egg lecithin bilayers (9.4 nsec, Shinitzky & Barenholz, 1974). The apparent microviscosity was calculated by the relations given by Shinitzky and Barenholz (1978). The true microviscosity was calculated according to Shinitzky and Yuli (1982). The relation between the steady-state FA, r, and the asymptotic value of the time-resolved anisotropy, r_∞, is $r_\infty = 2.29\ r^2 + 0.44\ r - 0.02$. This relation was obtained, in a way analogous to that used by Van Blitterswijck et al. (1981), by fitting a plot of available simultaneous determinations of r and r_∞ for DPH embedded in unsaturated phospholipid membranes (Rossignol, 1984). This equation holds for $0.04 < r < 0.28$. Fluidity was defined as the reciprocal of the true microviscosity. Standard deviations amount to 3 to 20% of the corresponding mean values.

^{31}P NMR Experiments

Broad-band proton-decoupled ^{31}P NMR spectra were recorded on a Brucker WP 200 pulse Fourier transform spectrometer operating in inverse-gated mode. The pulse width was 32.5 μsec (*ca.* 90°) and 6000 scans were recorded at 30°C. 10 mm sample tubes were used. Sample (50 μmol) was dispersed in buffered solution containing 20% D_2O for the lock.

Results

Relation between Proton Permeability and Fluidity

Several parameters were varied to modify the fluidity of horse bean phospholipid bilayers: temperature, calcium, cholesterol, modification of the polar head spectrum (addition of phosphatidic acid or phosphatidylglycerol). Their effects on fluidity and on proton permeability are summarized in Fig. 1. In all situations fluidity was in the range of 1.5 to 4 P^{-1}. This result was expected from the high unsaturation of horse bean phospholipids. The proton permeability coefficients (P_{eqH}) averaged 1.03×10^{-4} cm sec^{-1} at 25°C, in agreement with previous determinations on the same lipids (Rossignol et al., 1982*a*) or on other lipids (Deamer & Nichols, 1983; Gutknecht, 1984).

It appears on Fig. 1 that a decrease of the fluidity generally corresponds to a decrease of the proton permeability. This effect may also be deduced from data of other workers (Nichols & Deamer, 1980; Nichols et al., 1980; Deamer & Nichols, 1983) on egg phosphatidylcholine/phosphatidic acid vesicles (Fig. 1, panel PC + PA). The largest changes were obtained by modifying the temperature (Fig. 1, panel T). Decreasing the temperature from 50 to 10°C promotes a fluidity decrease from 3.88 to 1.54 P^{-1} and a proton permeability decrease from 7.49×10^{-4} cm sec^{-1} to 1.07×10^{-5} cm sec^{-1}. Other well-known effectors, such as cholesterol, promote similar changes: the addition of 10% cholesterol (mol/mol) induces, at 25°C, a fluidity decrease from 2.16 to 2.07 P^{-1} and a proton permeability decrease from 1.03×10^{-4} to 3.54×10^{-5} cm sec^{-1} (Fig. 1, panel cholesterol). One notable exception is the case of liposomes prepared in the presence of 10 mM $CaCl_2$:

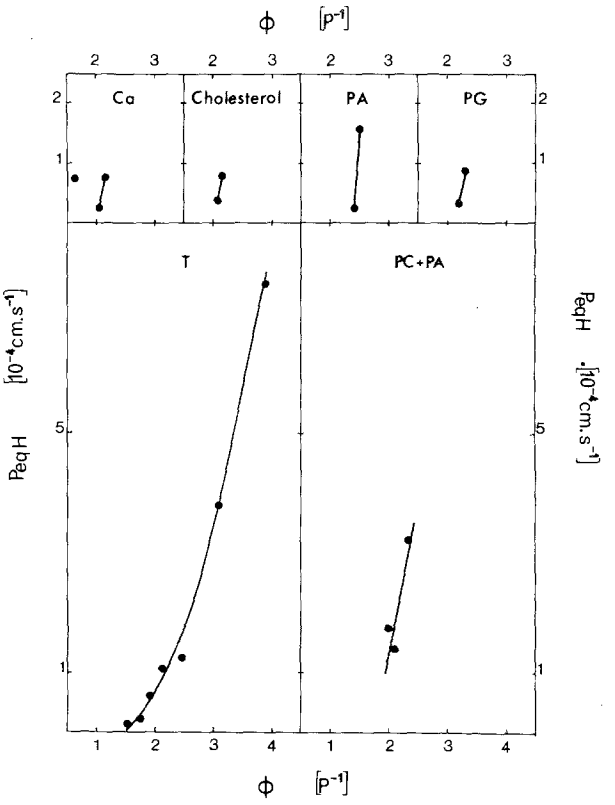

Fig. 1. Relation between proton permeability and fluidity. Ca: measurements at 25°C in the presence of calcium; from the left to the right calcium concentrations are 10, 1 and 0 mM. Cholesterol: measurements at 25°C with bilayers containing 10% cholesterol; the point to the right is the reference without cholesterol. PA: measurement at 25°C on bilayers supplemented with phosphatidic acid, in presence (lower point), or in absence (upper point) of 1 mM calcium. PG: measurements at 25°C on bilayers supplemented with phosphatidylglycerol, in presence (lower point) or in absence (upper point) of 1 mM calcium. T: measurements at various temperatures, from the left to the right: 10, 15, 20, 25, 30, 40 and 50°C. PC + PA: results from the literature concerning egg phosphatidylcholine liposomes containing, from the bottom to the top: 2, 0 and 10% of egg phosphatidic acid; measurements are from Deamer and Nichols (1983, point 0%), Nichols et al. (1980, point 2%) and Nichols and Deamer (1980, point 10%); corresponding fluidities are calculated, according to the procedure described in Materials and Methods, from data of Shinitzky and Barenholz (1974)

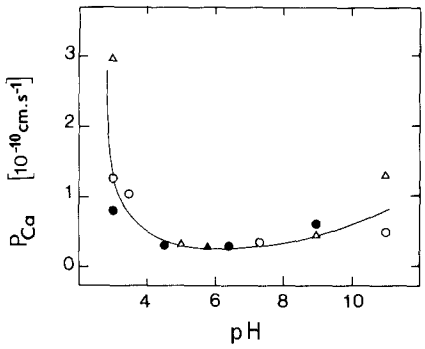

Fig. 2. Effects of pH on calcium permeability coefficients, P_{Ca}. Calcium permeability coefficients were measured at 25°C. Each point corresponds to the mean value of five to eight determinations with variation coefficients ranging from 5 to 20%. Open symbols: measurements in presence of 5×10^{-5} M $CaCl_2$; full symbols: measurements in presence of 10^{-3} M $CaCl_2$; circles: horse bean phospholipid vesicles; triangles: yellow lupine phospholipid vesicles

whereas the presence of 1 mM $CaCl_2$ reduces the fluidity by 5% and the proton permeability by 70%, that of 10 mM $CaCl_2$ reduces the fluidity by 25% with essentially no effect on the proton permeability (Fig. 1, panel Ca).

Relation between Calcium Permeability and Membrane Organization

Calcium permeability coefficients were measured (Fig. 2) at different pH values and at two calcium concentrations: 5×10^{-5} M (open symbols) and 10^{-3} M (full symbols). In addition to determinations on horse bean phospholipids, results on yellow lupine root phospholipids are also presented. This latter mixture differed from the former by the presence of slightly higher contents of saturated fatty acids and acidic phospholipids (Rossignol et al., 1982*b*). The measured P_{Ca} at neutral pH was in the range of 2.5×10^{-11} cm sec^{-1}. This value may not be directly compared to early determinations for two reasons: (i) these studies were done with either small unilamellar (Vanderkooi & Martonosi, 1971) or multilamellar (Hyono et al., 1975) vesicles; (ii) the results from these investigations are not expressed in terms of permeability coefficients. However, the earlier studies also suggested low calcium permeabilities.

Decreasing the pH triggered an increase of calcium permeability. A similar but less pronounced behavior was observed at high pH values. The extent of these pH effects depended upon the phospholipid mixture and the calcium concentration.

In the same pH range, fluidity remained practically constant (Fig. 3*A*) so that no conclusion could

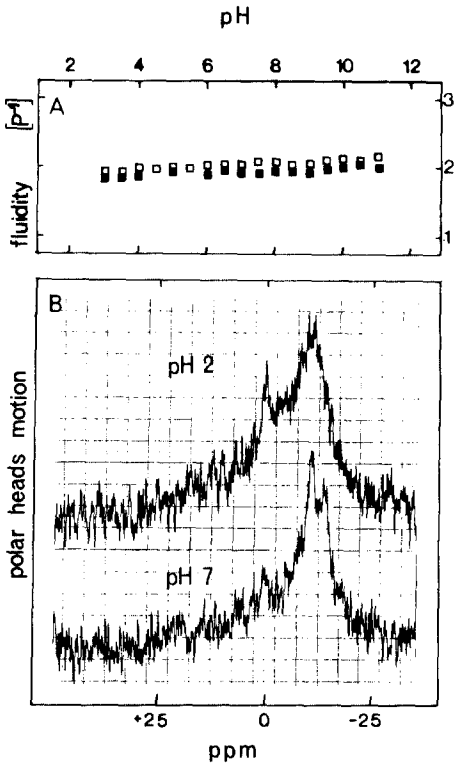

Fig. 3. Effects of pH on physical properties of horse bean phospholipid bilayers. All measurements were performed at 25°C with vesicles dispersed in 0.1 M KCl. (*A*) Fluidity values derived from fluorescence polarization measurements. The medium contained 1 mM EDTA (open symbols) or 1 mM $CaCl_2$ (full symbols). (*B*) 81 MHz ^{31}P NMR spectra recorded at two pH values in a medium containing 1 mM EDTA

be drawn concerning the relation between P_{Ca} and ϕ. However, polar head motion (Fig. 3*B*) was sensitive to pH: the ^{31}P NMR spectrum of horse bean phospholipid membranes at pH 7 was broad and asymmetric with a low field shoulder and a high field peak separated by approximately 40 ppm, whereas, at pH 2 an additional peak at 0 ppm was observed indicating that a growing proportion of phospholipids was allowed to move more isotropically.

Discussion

Permeability coefficients, as they are measured, correspond to two separate events: the entry into the membrane, and the diffusion across the membrane. According to Zwolinsky et al. (1949), P may be described as:

$$\frac{l}{P} = \frac{2\lambda}{D_1} + \frac{l}{KD_2} \qquad (1)$$

where λ is the length of a jump from one potential minimum to the neighboring, l the thickness of the apolar layer and K the partition coefficient between the two phases. D_1 and D_2 are the diffusion coefficients, respectively, from the medium into the membrane, and in the membrane interior. The current concept that P is controlled by the fluidity lies on the assumption that D_1 is largely higher than D_2, so that $P \cong KD_2/l$, or, after developing D_2 according to the Einstein-Stokes relation, $P \cong kTK\phi/6\pi Rl$. The partition coefficient K is introduced in order to deduce the intramembranous concentrations, which are those which determine the fluxes, from the external concentrations, which are the only known. However, K is related to D_1. According to the absolute rate theory, D_1 may be expressed as:

$$D_1 = \frac{\lambda^2 kT}{h} \exp(-\Delta G_0/RT) \tag{2}$$

where k and h are the Boltzman and Planck constants, respectively, and ΔG_0 the free energy difference for an ion between the two phases. Substituting $-RT \ln K$ for ΔG_0 leads to:

$$K = \frac{hD_1}{\lambda^2 kT} \tag{3}$$

and the general equation for P may be rewritten as:

$$\frac{1}{P} = \frac{2\lambda}{D_1} + \frac{l\lambda^2 kT}{hD_1D_2}. \tag{4}$$

This relation shows that in no case, the diffusion in the membrane, and therefore the fluidity, may control alone the measured permeability coefficients. Calculations made for P_{eqH} and P_{Ca} at 25°C, by introducing arbitrary values of D_1 (or D_2) lead to the following conclusions (Fig. 4): (i) the product D_1D_2 remains constant, except when D_1 falls below 10^{-10} cm^2 sec^{-1} in the case of proton, and below 10^{-15} cm^2 sec^{-1} in the case of calcium; (ii) these later situations are obtained for values of D_2 greater than 10^{-2} cm^2 sec^{-1} in the case of proton, and 10^{-3} cm^2 sec^{-1} in the case of calcium, *i.e.* values which are orders of magnitude higher than those of the corresponding diffusion coefficients in water; (iii) in all other cases, the term $l\lambda^2 kT/hD_1D_2$ contributes to nearly 100% of $1/P$. The most likely conclusion is therefore that for both cations the permeability is controlled by the product D_1D_2.

In all results summarized in Fig. 1, changes of P_{eqH} are always larger than the corresponding ϕ changes. This implies that the used effectors induce large variations of D_1. For example, increasing the temperature from 10 to 50°C promotes a 70-fold increase of P and a threefold increase of ϕ. Deducing D_2 from ϕ, one can calculate that the corresponding D_1 change is about 10-fold larger than that of D_2. On the other hand, as ionic radius differences between ions are limited, the values of D_2 deduced from ϕ should be relatively close for different ions: for example, if R = 2Å for H^+ and 3Å for Ca^{2+}, D_2 amounts to 2.36×10^{-7} cm^2 sec^{-1} for H^+ and to 1.57×10^{-7} cm^2 sec^{-1} for Ca^{2+} at 25°C. The large difference between the permeability coefficients of the two ions implies that the main difference concerns the solubilization of ions measured by D_1. These remarks suggest that D_1 is more sensitive to fluidity effectors than D_2 (or fluidity itself).

It is however noteworthy that values of D_1 deduced from P and ϕ determinations are rather high

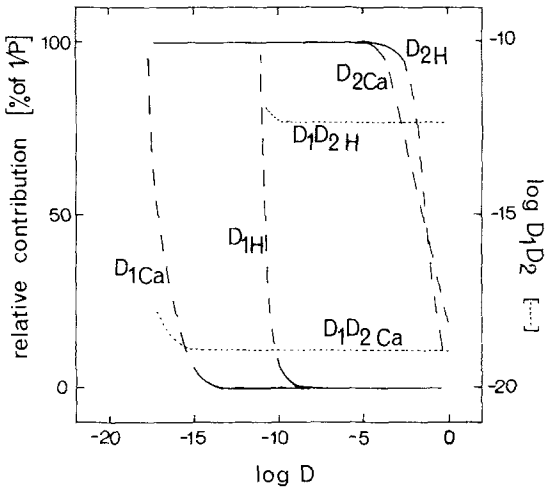

Fig. 4. Theoretical diffusion coefficients deduced from H^+ and Ca^{2+} permeability coefficients measured at 25°C. D_1: diffusion coefficient from the aqueous medium into the membrane; D_2: diffusion coefficient in the membrane; D_1D_2: product of the two coefficients D_1 and D_2. Subscripts H and Ca refer to diffusion coefficients for protons and calcium. D_1 (or D_2) are calculated by introducing in the general permeability equation ($1/P = 2\lambda/D_1 + l\lambda^2 kT/hD_1D_2$, *see* the Discussion section): (i) arbitrary values of D_2 (or D_1), (ii) $P = 1.03 \times 10^{-4}$ cm sec^{-1} for protons and $P = 2.5 \times 10^{-11}$ cm sec^{-1} for calcium (*see* the Results section), and (iii) 5 Å for the length of a jump λ, and 30 Å for the thickness of the apolar layer l. The curves D_{1H} and D_{1Ca} (left scale) show the contribution of $D_1/2\lambda$ to the measured P, as a function of D_1. The curves D_{2H} and D_{2Ca} (left scale) show the contribution of $hD_1D_2/l\lambda^2 kT$ to the measured P, as a function of D_2. The curves D_1D_2 (right scale) show the values of the product D_1D_2, as a function of D_1 (or D_2), necessary to satisfy the general permeability equation after introduction of the measured P values. Dashed portions on D_2 curves correspond to unlikely values of D_2, *i.e.* values greater than the corresponding diffusion coefficients in water. Dashed portions on D_1 curves refer to corresponding unlikely values of D_1 deduced from the D_1D_2 curves

in view of the currently admitted dielectric constant difference between the two phases: 78.5 at 25°C for the aqueous medium and about 2.5 for the hydrocarbon layer. The free energy change of an ion between the aqueous phase and the membrane phase should at least correspond to the Born charging energy. According to Parsegian (1969) the related work is:

$$W = \frac{z^2 \cdot e^2 \cdot (E_1 - E_2)}{8\pi \cdot E_0 \cdot E_1 \cdot E_2 \cdot R} - \frac{z^2 \cdot e^2}{4\pi \cdot E_0 \cdot E_2 \cdot l} \ln(2E_1/E_1 + E_2) \quad (5)$$

where z is the valency; e the electronic charge; E_0, the permittivity of the free space and E_1 and E_2 the dielectric constants of aqueous and membrane phase, respectively. Using relations (2) and (5), one can calculate tha the D_1 values derived from P and ϕ determinations should correspond to a dielectric constant of the hydrocarbon layer of about 12. A tentative explanation of this situation is that solubilization occurs in the hydration water of membranes. Several lines of evidence indicate that this hydration may affect permanently up to the 5th or 6th first methylene groups of acyl chains (Ashcroft et al., 1981; Simon et al., 1982; Blume, 1983). Recently Gutknecht (1984), measuring the proton conductance changes as a function of pH on black lipid membranes, suggested that the limiting step may be the hydration of membranes. Whatever the precise significance of this E_2 value concerning the membrane organization, it may be stressed that, for such a value, the frictions experienced by an ion crossing the membrane are different from those experienced by DPH, from which ϕ is deduced. In the absence of direct determinations of K or D_1 for ions in membranes, this ambiguity may not be resolved and further questions the use of the current fluidity parameters in reasoning permeability properties.

Role of the Membrane Organization

Unexpectedly high permeabilities are observed in the case of protons when calcium concentration rises to 10^{-2} M (Fig. 1, panel Ca) and, in the case of calcium, when proton (or hydroxyl) concentration reaches 10^{-3} M (Figs. 2 and 3). These ionic conditions are known to trigger structural changes in pure phospholipid membranes (Hope & Cullis, 1980; Van Venetie & Verkleij, 1981; Farren et al., 1983; Seddon et al., 1983*a*,*b*). In some cases permeability increases have been reported to be associated with such changes (Gerritsen et al., 1980; Noordam et al., 1980). The anomalous permeability increases shown in Fig. 2 may be associated with the departure of some of the membrane components from the fluid lamellar organization, in agreement with ^{31}P NMR spectra (Fig. 3*B*) which suggest that high proton concentration induces a lateral heterogeneity in membranes. In view of the preceding discussion it may be supposed that this heterogeneity allows a higher membrane hydration which facilitates solubilization and/or diffusion.

In conclusion, this work shows that the usual assumption that permeability is controlled by fluidity should be regarded with caution in the case of ions. The main effect of fluidity is perhaps to be searched concerning membrane hydration. Additional work is needed to get quantitative data on this point. The question of the relevance of these conclusions for natural membranes is still open. In this view it is noteworthy that roots, from which the phospholipids used here are extracted, lose the control of their permeability for ions in the same ionic conditions as observed here for phospholipid bilayers (Ghorbal et al., 1978).

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