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A Comparative Study of Diffusive and Osmotic Water Permeation Across Bilayers Composed of Phospholipids with Different Head Groups and Fatty Acyl Chains

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ABSTRACT Osmotic and diffusive water permeability coefficients P_f and P_d were measured for lipid vesicles of 100–250 nm diameter composed of a variety of phospholipids with different head groups and fatty acyl chains. Two different methods were applied: the H_2O/D_2O exchange technique for diffusive water flow, and the osmotic technique for water flux driven by an osmotic gradient. For phosphatidylcholines in the liquid-crystalline state at 70°C, permeability constants P_d between 3.0 and $5.2 \cdot 10^{-4}$ cm/s and ratios P_f/P_d 7 and 23 were observed. The observation of a permeability maximum in the phase transition region and the fact that osmotically driven water flux is higher than diffusive water exchange suggest that water is diffusing through small transient pores arising from density fluctuations in the bilayers. The P_d values depend on the nature of the head group, on the chemical structure of the chains, and on the type of chain linkage. In the case of charged lipids, the ionic strength of the solution has a strong influence. For phosphatidylethanolamines, phosphatidic acids, and ether phosphatidylcholines, permeability constants P_d were considerably lower ($2–4 \cdot 10^{-6}$ cm/s at 70°C). For liquid-crystalline phosphatidylcholines, a strong reduction of P_d after addition of ethanol was observed ($2–4 \cdot 10^{-6}$ cm/s at 70°C). The experimental values are discussed in connection with different permeation models.

INTRODUCTION

The permeation of small molecules through lipid bilayers is a phenomenon that is still not completely understood but of significant importance for understanding the permeability of biological membranes (Sháafi, 1981; Finkelstein, 1987). Nonpolar molecules more or less obey Overton's rule (Overton, 1895), i.e., the permeability coefficient of these molecules is proportional to their partition coefficient between a nonpolar solvent, such as a hydrocarbon, and water (Finkelstein, 1987; Overton, 1895). The permeation mechanism for polar molecules seems less clear, but models have been proposed where molecules diffuse particularly effectively through domain boundaries when the lipids are in the phase transition region (Tsong et al., 1977; Kanehisa and Tsong, 1978). It was shown that for many polar molecules the permeability coefficient P_d is proportional to the product of the partition coefficient K_w and the diffusion coefficient D_w. Although for H_2O, significant deviations are observed. Apparently, water permeation does not proceed according to the simple solubility diffusion mechanism (Hanai and Haydon, 1966; Finkelstein and Cass, 1968; Lieb and Stein, 1969; Reeves and Dowben, 1970). At least two different independent pathways for water transport were suggested, the permeation through fluctuating defects playing the major role (Deamer and Bramhall, 1986; Lawaczeck, 1988).

Additional information on the permeation mechanism can be obtained from a comparison of the permeability coefficient P_f for osmotic water flow with the permeability coefficient P_d for diffusional water exchange (Sháafi, 1981; Finkelstein, 1987). The osmotic transport of water induced by an impermeable solute is a net volume flow of water through the membrane. In the case of diffusional exchange of water, no net water flux occurs. This diffusional exchange can be followed by tracer methods using deuterated or tritiated water (Finkelstein, 1987; Lawaczeck, 1979, 1984, 1988; Engelbert and Lawaczeck, 1984a, b).

Only few reports on measurements on artificial bilayers exist, where osmotic permeability coefficients P_f were compared with diffusional permeability coefficients P_d. For pure lipid bilayers, the ratio P_f/P_d was reported to be 1, whereas introduction of pore-forming molecules, such as nystatin, amphotericin, or gramicidin increased this ratio to 3–5 (Finkelstein, 1987). In many biological membranes, much larger P_f/P_d ratios have been observed so that the existence of macroscopic pores or specific water channels in these

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Abbreviations used: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; PS, phosphatidylserine; PG, phosphatidylglycerol; cyPC, ω-cyclohexyl phosphatidylcholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; 11cyPC, 1,2-di-ω-cyclohexyl-undecanoyl-*sn*-glycero-3-phosphocholine; 13cyPC, 1,2-di-ω-cyclohexyl-tridecanoyl-*sn*-glycero-3-phosphocholine; 14cyPC, 1,2-di-ω-cyclohexyl-tetradecanoyl-*sn*-glycero-3-phosphocholine, DMPE,1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine;DPPE,1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DLPA, 1,2-dilauroyl-*sn*-glycero-3-phosphoric acid; DMPA, 1,2-dimyristoyl-*sn*-glycero-3-phosphoric acid; DPPA, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoric acid; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol; DPPS, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine; DHPC, 1,2-dihexadecyl-*rac*-glycero-3-phosphocholine; EtOH, ethanol; DLS, dynamic light scattering; T_m, transition temperature.

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membranes has been proposed (Sidel and Solomon, 1957; Pagnelli and Solomon, 1957, Stein, 1990).

We have undertaken a systematic study of the osmotic and diffusional water permeability of lipid bilayer vesicles. We studied water permeability as a function of the chemical nature of the phospholipid head group and the length of the acyl chains. We will show that varying the thickness of the hydrophobic region has only a minor influence on water permeability. Major effects on water permeability are observed when the size and charge of the lipid head group, the type of chain linkage, and the chemical structure of the chains are altered. Finally, the comparison of osmotic and diffusional water flux measured on unilamellar vesicles gives additional information on the permeability pathways. We found that permeation through transient pores created by density fluctuations is the most likely mechanism by which water passes through liquid-crystalline bilayers.

MATERIALS AND METHODS

DMPC, DPPC, DSPC, DLPA, DMPA, DPPA, and DPPS were purchased from Sigma-Chemie (Deisenhofen, Germany). DMPE, DPPE, DHPC, and DPPG were products of Fluka AG (Neu-Ulm, Germany). All lipids were pure as detected by TLC and used without further purification. 11cyPC, 13cyPC, and 14cyPC were synthesized using established procedures as described previously (DasGupta et al., 1982; Blume et al., 1987, 1988; Habel, 1989). Deuterium oxide (99%) was obtained from Campro Scientific (Emmerich, Germany).

Vesicle preparation

Lipid vesicles for stopped-flow experiments were prepared by sonication of the crude lipid suspension in water or buffer in a bath type sonicator (Branson 1200) at temperatures 10°C above the temperature of the main transition of the lipid. The lipid concentration was 1 mg/ml. The sonication time varied from 3 min for PAs to 45 min for PEs. The resulting vesicle preparation was annealed for another 60–120 min at the sonication temperature and then immediately cooled to room temperature. With exception of phosphatidic acids, all lipid dispersions were extruded in a LiposoFast extrusion apparatus (Avestin, Inc., Ottawa, Canada) using filters with a pore size of 200 nm to obtain unilamellar vesicles and to remove vesicles with larger diameters. The samples were degassed for 15 min under water aspirator vacuum before starting the stopped-flow experiment.

For the H_2O/D_2O exchange experiments, the vesicles were prepared in bidistilled H_2O, 20 mM aqueous phosphate buffer (pH = 7), or 20 mM aqueous NaCl solution. They were mixed with D_2O containing the equivalent salt or buffer concentrations. For osmotic experiments, the lipid vesicles were prepared in a solution containing 100 mM D(+)-glucose and 20 mM KCl. They were mixed with a hypotonic medium containing only 20 mM KCl. The resulting osmotic gradient was 50 mOsm.

Vesicle sizing

The diameters and the size distribution of the vesicles were determined by dynamic laser light scattering (DLS) using a Malvern Zetasizer 3 (Malvern, Ottobrunn, Germany). The autocorrelation function was analyzed using the Malvern software, applying the cumulant method and the multi-exponential fit method. The average diameter of the vesicles varied between 100 and 250 nm, depending on the phospholipid used.

Stopped-flow technique

The stopped-flow experiments were performed using a HighTech Scientific SF 51 instrument (HiTech Scientific, Salisbury, U.K.). Intensity versus time curves were stored using a Thurlby DSA524 digital storage adaptor. The data were then transferred to a Atari 1040 STF computer and analyzed using linear regression and nonlinear fit methods. The time resolution of the stopped-flow apparatus was determined by using the method by Tonomara et al. (1978). The shortest relaxation times that could be detected were 2 ms. The experiments were performed in a temperature range between 10 and 70°C. Osmotic and diffusive water permeation were measured by recording the light scattering signal at a wavelength of 450 nm. The samples were equilibrated for ~5 min at each temperature before starting the experiment.

RESULTS

Methodology

For water permeability measurements, it is advantageous to use large unilamellar vesicles for the following reasons. a) In unilamellar vesicles, permeation through only one bilayer has to be considered. b) Large vesicles allow the determination of higher permeability constants because the time constant for the permeation is inversely proportional to the vesicle radius r. c) The signal-to-noise ratio improves because the intensity of the scattered light increases with the radius. On the other hand, vesicles with diameter larger than 250 nm are difficult to prepare, have a higher probability of being oligolamellar, and are more sensitive to rupture or leakiness under shear when rapidly mixed in the stopped-flow apparatus. Vesicles with diameters of 100–250 nm are a good compromise. The size of the vesicles was determined by DLS before and after mixing to ensure that rapid mixing did not lead to vesicle rupture and/or consecutive fusion.

H_2O/D_2O exchange experiments

Diffusive water permeation of was measured using the method of Lawaczeck (Lawaczeck, 1979, 1984, 1988; Engelbert and Lawaczeck, 1985a, b). Because H_2O and D_2O have different indices of refraction, a time-dependent change of the light scattering signal is observed after mixing vesicles prepared in H_2O with D_2O. The intravesicular index of refraction, n_i, as function of time, t, follows first-order kinetics

$$n_i(t) = n_o + \{n_i(0) - n_o\}\exp(-kt), \quad (1)$$

with $n_i(0) = n_i$ at the time $t = 0$, n_o = extravesicular index of refraction, and k = rate constant.

Because no further factors influence the permeation process, the permeability coefficient P_d can be calculated easily when the vesicle size is known

$$P_d = k \cdot \frac{V}{A} = \frac{1}{3} \cdot r \cdot k, \quad (2)$$

with V = vesicle volume, A = vesicle surface, r = vesicle radius, and k = rate constant.

Osmotic experiments

If vesicles prepared in 100 mM glucose solution are mixed with water, essentially only water permeates into the vesicles because of the concentration gradient, because the vesicles are relatively impermeable to glucose. The vesicles swell,

and this leads to a change in light scattering that can be followed as a function of time. It was shown that a linear correlation of the signal intensity and the relative vesicle volume exists (Sidel and Solomon, 1957; Sháafi, 1967) and that large unilamellar vesicles behave like ideal osmometers (Bangham et al., 1967; Bittmann and Blau, 1972). The osmotic permeability coefficient P_f is calculated using the following expression:

$$P_f = \frac{dV}{dt} \cdot \frac{RT}{\pi \cdot A \cdot V_w}, \quad (3)$$

with dV/dt = time-dependent volume change, V_w = partial molar volume of H_2O, A = surface area of vesicles, T = absolute temperature, R = gas constant, $\pi = RT \cdot \Delta c_i$ (osmotic coefficient) with Δc_i = applied glucose concentration gradient.

The major problem in determining P_f values is to find a relation between the change in light scattering and the relative volume change. With the assumption that the vesicles are spherical, a volume change can only occur when the membrane expands under tension. Needham and Evans (1988) determined the elastic area compressibility modulus K of liquid-crystalline DMPC membranes to ~150 dyn/cm. Using this value and a concentration difference of 50 mM glucose, a membrane tension T of ~5 dyn/cm is calculated for a vesicle with a radius r of 100 nm, because $T = \pi \cdot r/2$ (Sun et al., 1986). This leads to a relative increase in surface area $\Delta A/A = T/K$ of approximately 4% and an increase in volume of ~6%. Carruthers and Melchior (1983) determined a volume change of 6.8% for DMPC vesicles for the same osmotic gradient we used. Because no values for the elastic area compressibility modulus are known for all other lipids used in our study, we had to estimate the volume change occurring during the osmotic swelling. As a compromise, we used for all our lipid samples a relative volume change dV/V of 5% for $dt = t_{1/2}$, the experimental half-time. The total volume change is therefore somewhat larger than the value measured by Carruthers and Melchior. This was done to account for nonspherical shapes and lower elastic moduli (Mui et al., 1993). That this assumption is reasonable can be shown when an ellipsoidal shape with an axial ratio of 1.5 is assumed as the initial vesicle form. The shape change from an oblate ellipsoid to a sphere with 100-nm radius with constant surface area would lead to an additional ~5% volume increase. Starting with a prolate ellipsoid, one calculates an additional ~2.5% volume increase during swelling. The values for P_f, therefore, may be even higher, depending on the values for the corrections for K and the deviations from spherical symmetry. The P_f values were calculated using the following expression derived from Eq. 3 with $dt = t_{1/2} = \ln 2/k$, $A = 3V/r$, and $dV/V = 0.05$

$$P_f = \frac{0.05 \cdot r \cdot k}{3 \cdot \ln 2} \cdot \frac{RT}{V_w \cdot \pi}, \quad (4)$$

with r = radius of vesicles and k = rate constant determined from the experimental curves.

Estimations of the volume changes for gel state vesicles are even more problematic. The elastic area compressibility increases by a factor of approximately 2–5 (Needham and Evans, 1988). This would lead to a lower relative volume change for the same osmotic gradient. On the other hand, surface corrugations, particularly in the P_β-phase of PCs, may be present and be eliminated under stress, compensating for the increase in K. Therefore, we have used the same relative volume change of 5% at $t = t_{1/2}$ to be able to compare our different lipid samples.

EXPERIMENTAL RESULTS

Phosphatidylcholines: H_2O/D_2O-exchange

Fig. 1 shows an experimental light scattering curve obtained after mixing DPPC vesicles in H_2O with D_2O. The decrease of the scattering intensity can be described by a single exponential. In a temperature range of approximately 2–4°C around T_m, it is not possible to obtain reproducible light scattering curves with significant amplitude changes. Fig. 2 shows the light scattering amplitude for DMPC as a function of temperature with drastic decrease in the transition region. Obviously, water exchange around T_m is so fast that it is complete within the dead time of our instrument (~2 ms). The P_d-value in the phase transition region, therefore, must

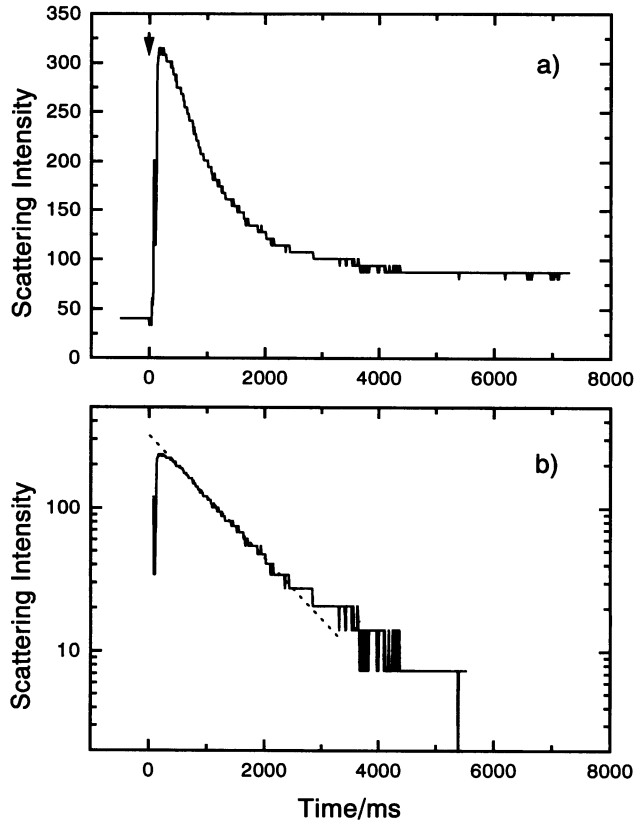

FIGURE 1 (*a*) Experimental light scattering intensity curve as a function of time for H_2O/D_2O exchange across bilayers of DPPC vesicles (T = 21°C.) (*b*) Logarithmic plot of scattering intensity after subtraction of baseline.

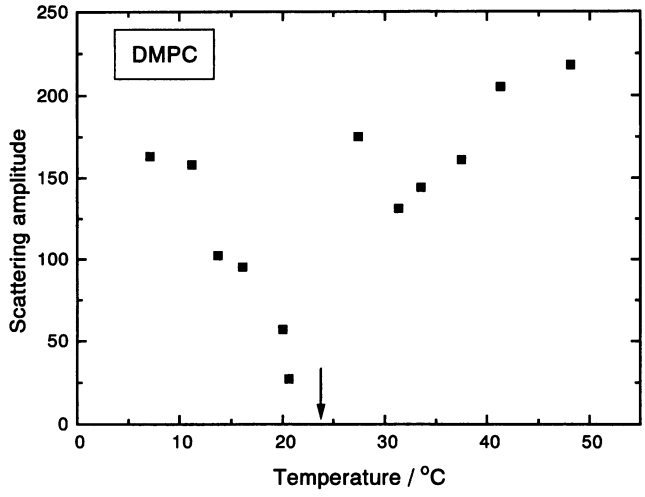

FIGURE 2 Light scattering amplitude for H_2O/D_2O exchange of DMPC vesicles as function of temperature. Arrow indicates transition temperature.

be higher than 10^{-2} cm/s (see below). This phenomenon was not only observed for DMPC but also for all other lipids.

Fig. 3 *a* shows a plot of log P_d as a function of $1/T$ for three PCs with saturated fatty acids. A marked decrease in water permeability by a factor of 50–100 is observed at the main-phase transition temperature T_m. For the liquid-crystalline phase, the P_d-values are in the range of $1.9–3.3 \cdot 10^{-4}$ cm/s and, thus, are almost the same within the error margins of our P_d determination. The length of the fatty acid chain of the PCs does not have a measurable influence on the diffusive water permeability. In the gel phase, the water permeability is lower with P_d values between $4.1 \cdot 10^{-7}$ and $2.5 \cdot 10^{-6}$ cm/s. The activation energies calculated by linear regression in the temperature ranges above and below T_m are between 16 and 33 kJ/mol. In the transition region, no reliable data could be obtained, although in some cases an increase in permeability was observed when the transition region was approached from the low temperature side. In the case of a permeability maximum at T_m, one should also observe an increase approaching T_m from the high temperature side. It was not possible to check this because of the low scattering amplitudes in the transition region and the short relaxation times (see Fig. 2).

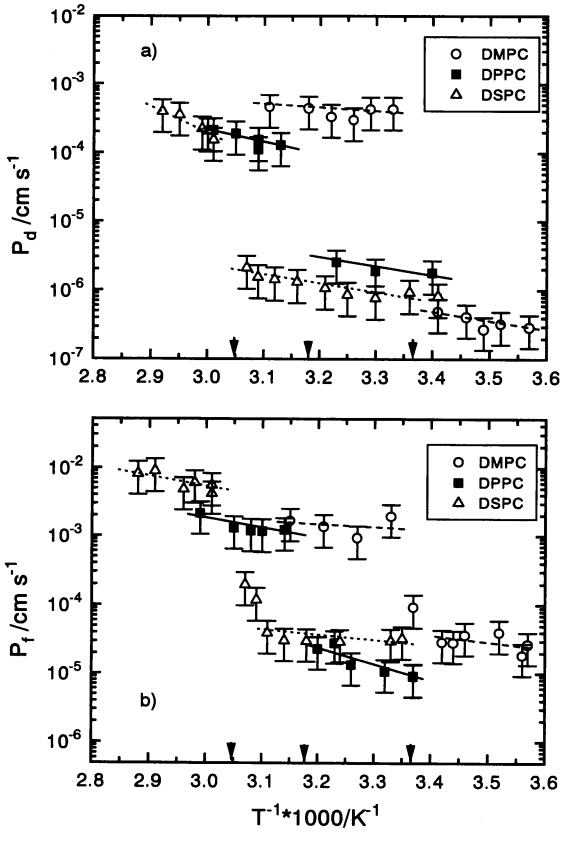

FIGURE 3 (*a*) Permeability coefficient P_d for diffusive water permeation through vesicles of phosphatidylcholines with different chain lengths as function of temperature. (*b*) Permeability coefficient P_f for osmotic water permeation through vesicles of phosphatidylcholines with different chain lengths as function of temperature. Arrows indicate transition temperatures.

Phosphatidylcholines: osmotic water permeability

Plots of log P_f for osmotic water permeation vs. $1/T$ for DMPC, DPPC, and DSPC vesicles are shown in Fig. 3 *b*. Again, a large change in permeability is observed at T_m, the P_f value decreasing for the gel phase by a factor of 50–100. Ten degrees above their respective T_m, permeability constants between $1 \cdot 10^{-3}$ and $8 \cdot 10^{-3}$ cm/s are measured. The activation energies are similar to those determined in the H_2O/D_2O exchange experiments. Again, we observed a strong reduction and then disappearance of the light scattering amplitude in the vicinity of T_m. The vesicles seem to be very permeable in the phase transition region.

The ratio P_f/P_d has values between 7 and 23 for the liquid-crystalline phase. The values for the gel state show considerably more scatter, probably due to leakiness of the gel state vesicles under osmotic stress.

To check whether the size of the vesicles had any influence on the osmotic water permeability, we also measured P_f of DMPC and DSPC vesicles with diameters between 90 and 400 nm. No size dependence was observed, and all P_f-values were within the same range (not shown).

Phosphatidic acid, phosphatidylethanolamine, phosphatidylserine, and phosphatidylglycerol: H_2O/D_2O exchange

Phosphatidic acids

We studied the permeability of vesicles of DLPA, DMPA, and DPPA. Representative results for DMPA are shown in Fig. 4 *a*. At pH 7 PAs in pure water are in the singly charged form (Eibl and Blume, 1979; Blume and Eibl, 1979). However, the surface potential and the amount of counter ion condensation can be influenced by changing the ionic strength of the solution by addition of salt or buffer (Träuble and Eibl, 1974; Träuble et al., 1976, Jähnig, 1976). For this reason, we studied the permeability of PA vesicles in 20 mM phosphate buffer and also in 20 mM NaCl solution.

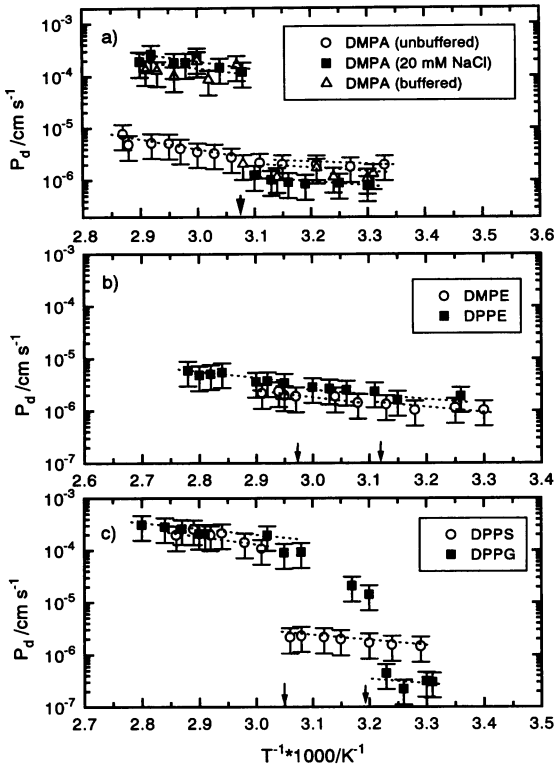

FIGURE 4 Permeability coefficient P_d for diffusive water permeation as a function of temperature. (*a*) DMPA vesicles prepared in H_2O_{bidest} (pH 6–6.5), 20 mM NaCl (pH 6–6.5), and 20 mM phosphate buffer (pH 7). (*b*) DMPE and DPPE vesicles prepared in H_2O_{bidest}. (*c*) DPPS and DPPG vesicles in 20 mM phosphate buffer (pH 7). Arrows indicate transition temperatures.

Surprisingly, no large increase in permeability is observed at the phase transition when PAs are dispersed in pure water. Only in buffer or 20 mM NaCl solution do we observe the characteristic increase of P_d by a factor of 50–100 at T_m. Typical P_d values above T_m at 70°C in buffer are $1–2 \cdot 10^{-4}$ cm/s and in H_2O $2–4 \cdot 10^{-6}$ cm/s. For PA vesicles dispersed in water, the permeability above T_m is thus lower by a factor of 50–100 compared with PAs in buffer solutions and also compared with the corresponding PCs.

Phosphatidylethanolamines

Phosphatidylethanolamines do not readily form vesicles that are stable over longer periods of time. Only vesicle suspensions of DMPE and DPPE in pure water could be prepared. For vesicle formation, the crude lipid suspension (0.5 mg/ml) had to be sonicated in a bath-type sonicator above T_m of the respective lipid for at least 45 min and then immediately filled into the syringes of the stopped-flow apparatus. DLS measurements indicated normal vesicle sizes with diameters between 150 and 250 nm. Fig. 4 *b* shows that PE vesicles seem to behave very similarly to PA vesicles suspended in pure water. Again, the values for P_d do not change at T_m; they are in the range of $1–4 \cdot 10^{-6}$ cm/s at high temperature.

Phosphatidylserine, phosphatidylglycerol

Fig. 4 *c* shows the results obtained for DPPS and DPPG. Both lipids were only be prepared in a 20 mM phosphate buffer (pH = 7), because preparations in pure water gave no stable vesicle dispersions that could be investigated over the whole temperature range. For both lipids, an increase of P_d by a factor of 100 was found at T_m. The permeability coefficients of DPPS vesicles are similar to those of DPPC, whereas the P_d-values for DPPG are slightly lower.

Di-ω-cyclohexyl-phosphatidylcholines: H_2O/D_2O exchange and osmotic water permeability

The phase transition properties of PCs with ω-cyclohexyl fatty acids show a strong odd-even effect (Lewis and McElhaney, 1985; Blume et al., 1987, 1988; Hübner et al., 1990), the lipids with an odd number of carbon atoms having higher T_m values and forming a highly ordered, partially dehydrated lamellar phase. It is still not clear, however, whether interdigitation or strong tilting of the molecules occurs. In the liquid-crystalline phase, it was shown by ^{2}H-NMR that the chains are slightly more ordered because of the cyclohexyl rings at the chains ends (Blume et al., 1988). Measurements of H^+/OH^--permeation through bilayers of these lipids showed reduced proton permeability values (Habel, 1989). This is of importance because lipids of this type occur in the thermoacidophilic *Bacillus acidocaldarius*, an organism that lives at low pH and high temperatures (Blume et al., 1978; Poralla et al., 1980; Kannenberg et al., 1985).

Fig. 5, *a* and b show representative results for cyPCs. A strong odd-even effect of the permeability is observed, 11cyPC and 13cyPC having two orders of magnitude lower P_d-values than 14cyPC. High P_f/P_d ratios are observed for 11cyPC and 13cyPC. Bilayers of 14cyPC behave more like DPPC bilayers, the osmotic water permeability being only slightly lower.

Dihexadecyl-phosphatidylcholine: H_2O/D_2O exchange and osmotic water permeability

The thermotropic behavior of phospholipids also depends on the type of linkage of the chains to the glycerol back bone that can modify the intermolecular interactions in the lipid-water interface. To study this influence, we measured the water permeability of vesicles of the ether lipid DHPC, which forms an interdigitated gel phase but a normal liquid-crystalline bilayer (Ruocco et al., 1985). The results for DHPC are shown in Fig. 6 *a*. A significantly lower permeability in comparison with DPPC is observed. P_d drops to values to $3 \cdot 10^{-6}$ cm/s above T_m, whereas P_f-values are much higher ($1 \cdot 10^{-4}$ cm/s). Again, a permeability maximum is observed in the phase transition region.

DPPC in water/ethanol: H_2O/D_2O exchange

An interdigitated gel phase can also be induced by the addition of ethanol to ester PCs (Simon and McIntosh, 1984;

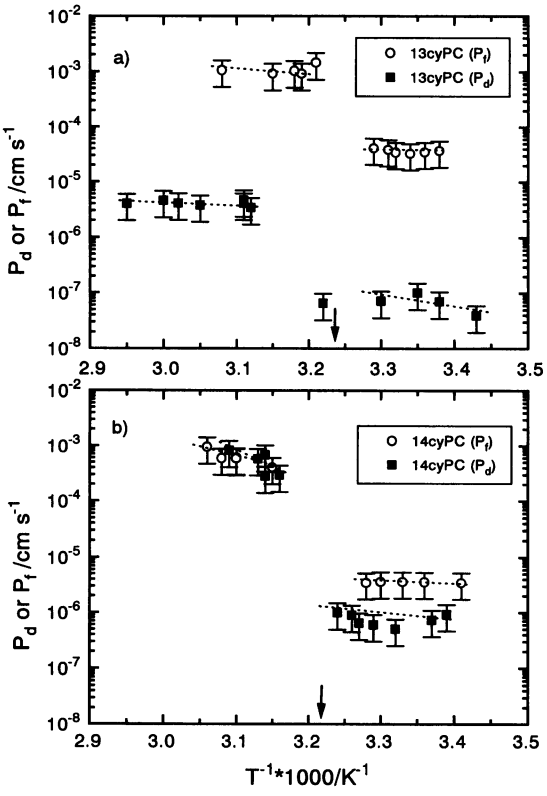

FIGURE 5 Permeability coefficient P_d for diffusive and P_f for osmotic water permeability as a function of temperature for vesicles composed of (*a*) 13cyPC in H_2O_{bidest} (pH 6–6.5) and (*b*) 14cyPC in H_2O_{bidest} (pH 6–6.5). Arrows indicate transition temperatures.

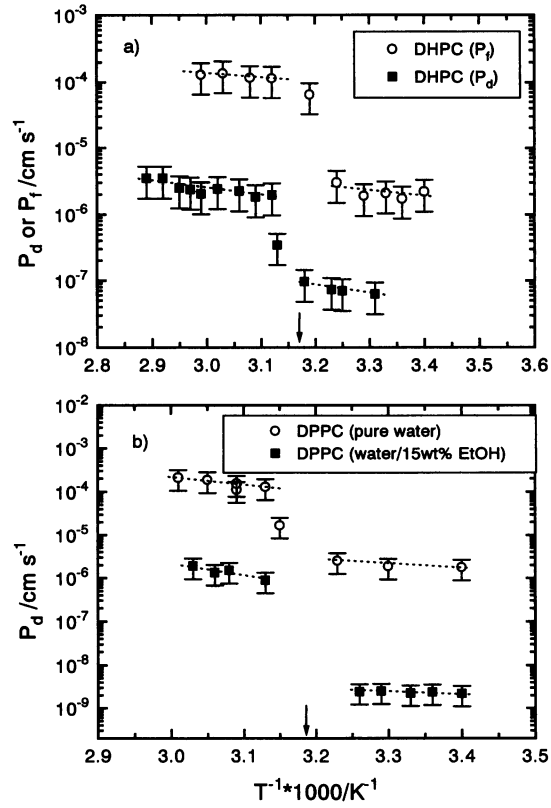

FIGURE 6 (*a*) Permeability coefficient P_d for diffusive P_f for osmotic water permeation through DHPC vesicles in water as a function of temperature. (*b*) Permeability coefficient P_d for diffusive water permeation through DPPC vesicles in water and in water/15 wt% ethanol as a function of temperature. Arrows indicate transition temperatures.

Nagel et al., 1992). Apparently, this is caused by modifications of hydrophilic interactions at the lipid-bilayer water interface. An interesting question, therefore, was whether alcohols had any effect on the water permeability. Fig. 6 *b* shows a comparison of P_d for DPPC vesicles in water and in water with 15 wt% ethanol. Above T_m, P_d-values in water/ethanol are lower by a factor of ~100. Systematic measurements of P_d as a function of ethanol concentration showed that for liquid-crystalline DPPC bilayers 5 wt% of ethanol in water is sufficient to reduce the water permeability by a factor of 100. Further addition does not lead to any further reduction of P_d (not shown).

Table 1 shows a summary for P_d- and P_f-values for all lipid systems at two different temperatures.

DISCUSSION

Theories

Solubility-diffusion model

On the basis of Overton's rule for the permeation of unpolar molecules through biological membranes, the permeation of H_2O through a lipid bilayer can be calculated using the solubility-diffusion mechanism. In this model, the hydrophobic part of the bilayer has the characteristics of an oil layer in which H_2O is only sparingly soluble, so that they do not collide during the permeation process. The solubility of H_2O in the bilayer is estimated from the solubility of water in long chain aliphatic hydrocarbons such as *n*-hexadecan (partition coefficient $K_w = 0.42 \cdot 10^{-4}$ (Schatzberg, 1965; Finkelstein, 1976)). According to the solubility-diffusion model, both osmotic and diffusive water permeation can be considered to be purely diffusive processes. Based on the Fick's law for diffusion, one obtains the same result for P_f and P_d

$$P_f = P_d = \frac{D_w \cdot K_w \cdot V_w}{d \cdot V_{lipid}}, \quad (5)$$

with d = thickness of the bilayer, D_w = diffusion coefficient of H_2O in the bilayer, K_w = partition coefficient of H_2O between bilayer and water, V_w = molar volume of H_2O, and V_{lipid} = molar volume of lipid.

For a given thickness of the bilayer, P_d and P_f are directly proportional to the product $D_w \cdot K_w$. Using the further assumption that the diffusion coefficient of water is the same in all liquid-crystalline bilayers, differences in permeability are then only related to changes in the partition coefficient K_w. If $P_f/P_d = 1$, the permeability of water cannot be increased by an additional osmotic concentration gradient across the membrane. Such a permeation behavior would only be observed for permeation of water monomers, because a cooperative movement of the water molecules would lead to an increase in the P_f/P_d ratio. The ratio P_f/P_d,

TABLE 1 Permeability coefficient P_d for diffusive and P_f for osmotic water permeation and P_f/P_d ratios for phospholipids with different chain lengths and head groups

		$T = 20°C$			$T = 70°C$		
Lipid	Solution	P_d	P_f	P_f/P_d	P_d	P_f	P_f/P_d
DMPC	H_2O	$5.0 \cdot 10^{-7}$	$3.0 \cdot 10^{-5}$	60	$5.2 \cdot 10^{-4}$	$3.5 \cdot 10^{-3}$	7
DPPC	H_2O	$1.7 \cdot 10^{-6}$	$8.5 \cdot 10^{-6}$	5	$3.0 \cdot 10^{-4}$	$2.1 \cdot 10^{-3}$	7
DSPC	H_2O	$8.1 \cdot 10^{-7}$	$2.8 \cdot 10^{-5}$	35	$3.9 \cdot 10^{-4}$	$8.9 \cdot 10^{-3}$	23
DLPA	buffer*	$3.1 \cdot 10^{-6}$			$1.7 \cdot 10^{-4}$		
	H_2O	$6.7 \cdot 10^{-6}$			$1.3 \cdot 10^{-6}$		
DMPA	buffer*	$1.2 \cdot 10^{-6}$			$1.3 \cdot 10^{-4}$		
	H_2O	$2.1 \cdot 10^{-6}$			$3.8 \cdot 10^{-6}$		
	20 mM NaCl	$7.5 \cdot 10^{-7}$			$1.8 \cdot 10^{-4}$		
DPPA	buffer*	$1.9 \cdot 10^{-6}$			$1.4 \cdot 10^{-4}$		
	H_2O	$1.0 \cdot 10^{-6}$			$3.6 \cdot 10^{-6}$		
DPPG	buffer*	$1.2 \cdot 10^{-7}$			$2.1 \cdot 10^{-4}$		
DPPS	buffer*	$7 \cdot 10^{-7}$			$1.9 \cdot 10^{-4}$		
DMPE	H_2O	$9.0 \cdot 10^{-7}$			$2.3 \cdot 10^{-6}$		
DPPE	H_2O	$1.3 \cdot 10^{-6}$			$3.7 \cdot 10^{-6}$		
DHPC	H_2O	$3.7 \cdot 10^{-8}$	$2.0 \cdot 10^{-8}$	54	$3.4 \cdot 10^{-6}$	$1.4 \cdot 10^{-4}$	41
11cyPC	H_2O (14°C)	$1.4 \cdot 10^{-7}$	$2.0 \cdot 10^{-5}$	142	$2.8 \cdot 10^{-6}$	$1.5 \cdot 10^{-3}$	536
13cyPC	H_2O	$6.5 \cdot 10^{-8}$	$3.3 \cdot 10^{-5}$	507	$4.5 \cdot 10^{-6}$	$1.0 \cdot 10^{-3}$	222
14cyPC	H_2O	$5.0 \cdot 10^{-7}$	$3.4 \cdot 10^{-6}$	8	$5.3 \cdot 10^{-4}$	$5.8 \cdot 10^{-4}$	1
DPPC	H_2O/EtOH	$2.5 \cdot 10^{-9}$			$1.5 \cdot 10^{-6}$		

*20 mM phosphate buffer, pH 7.0.
Values for P_f and P_d in cm/s.

therefore, can be used to check the validity of the solubility-diffusion model.

For pure lipid membranes, the occurrence of fluctuating defects acting as pores has been discussed (Deamer and Bramhall, 1986; Lawaczeck, 1988). These pores are assumed to be so small ($r < 0.3$ nm) that water in the pores loses its colligative properties and that water molecules cannot pass each other during permeation. This behavior can be described by the so-called single-file model for water transport through small pores and leads to $P_f/P_d > 1$.

Single-file model (Finkelstein, 1987)

Osmotic water transport: The osmotic permeability coefficient P_f can be described as the product of the osmotic permeability coefficient per pore p_f and the number of pores n per unit area A:

$$P_f = p_f \cdot \frac{n}{A}. \tag{6}$$

The osmotic permeability coefficient per pore p_f is under steady-state conditions given by

$$p_f = \frac{v_w \cdot k \cdot T \cdot N}{\gamma \cdot L^2} = \frac{v_w \cdot D_w \cdot N}{L^2}, \tag{7}$$

with v_w = volume of a water molecule, k = Boltzmann constant, T = temperature, γ = friction coefficient of a water molecule in a pore, D_w = diffusion coefficient of water in pore, L = length of the pore, and N = number of water molecules per pore.

If P_f is known from experimental data, the number of pores per unit area can be calculated using Eq. 6, on the basis of the estimated p_f values from Eq. 7. The number of water molecules in the hydrophobic part of the bilayer is then obtained by multiplying the calculated number of pores per unit area with the average number of water molecules per pore. Because the number of lipid molecules per unit area is known, it is also possible to calculate a formal partition coefficient K_w of water:

$$K_w = \frac{x_w(\text{bilayer})}{x_w(\text{water})} \tag{8}$$

$$x_w(\text{bilayer}) = \frac{n_w}{n_{lip} + n_w}, \tag{9}$$

with x_w(bilayer) = mol fraction of water in the bilayer, x_w(water) = mol fraction of water in the water phase, n_w = number of water molecules in the bilayer, and n_{lip} = number of lipid molecules in the bilayer. Diffusive water transport: The diffusive permeability coefficient P_d can be expressed in a similar way using the permeability coefficient per pore p_d and the number of pores per unit area n:

$$P_d = p_d \cdot \frac{n}{A}. \tag{10}$$

The diffusive permeability coefficient per pore p_d is given by

$$p_d = \frac{v_w \cdot k \cdot T}{\gamma \cdot L^2} = \frac{v_w \cdot D_w}{L^2}. \tag{11}$$

The ratio of P_f/P_d thus corresponds to the mean number of the water molecules per pore as seen by division of Eq. 8 by Eq. 11:

$$\frac{P_f}{P_d} = \frac{p_f}{p_d} = N. \tag{12}$$

The P_f/P_d ratio for the single-file model thus differs from $P_f/P_d = 1$ for the solubility diffusion mechanism. In the

single-file model, N water molecules cooperatively cross the membrane in the presence of an osmotic gradient. Diffusive water permeation is lower because the water molecules are now independent but cannot pass each other because of the limited size of the pore.

The experimentally determined diffusive and osmotic permeability coefficients can be analyzed using the single-file model. The P_f/P_d ratio yields the number of water molecules per transient pore. Estimation of the permeability coefficients per pore p_d or p_f (Eqs. 8 and 11) can be made using the assumption that the diffusion coefficient of water in the pore is approximately equal to the diffusion coefficient of free water (Finkelstein, 1987; Levitt, 1973, 1974a, b). Using this assumption, the number of pores per cm^2 and the partition coefficient of water between the lipid bilayer and the water phase can be calculated (see below).

Error analysis

In the case of diffusive water permeation, the main sources of error are possible leakiness of the vesicles during the mixing process in the stopped-flow spectrometer and the uncertainty in the value of the average radius of the vesicles as measured by dynamic light scattering.

For osmotic water permeability, a major uncertainty is the unknown volume increase of the vesicles induced by the water influx. We assumed a 5% volume increase at $t = t_{1/2}$ for all temperatures below and above T_m. This is a conservative estimate; the real volume changes in the liquid-crystalline phase are probably larger. This would increase the P_f values and thus also the P_f/P_d ratios (see Results, Methodology).

Excluding this latter error, the maximal estimated error margins were assumed to be ±50% of the averaged values obtained from at least three different experimental curves, including the errors in the determination of the time constants by linear least-square fitting of the logarithm of the scattering intensity curves. These error margins are indicated in all figures. Differences in permeability values that are less than a factor of three are therefore within the precision of our method and will be regarded as not significant.

Phosphatidylcholines

The goal of the comparison of DMPC, DPPC, and DSPC vesicles was to show whether the thickness of the hydrophobic part of a lipid bilayer influences P_f and P_d. The results show no systematic correlation between water permeability and bilayer thickness (see Fig. 3). All data are within our estimated error margins. This was unexpected, because the variation in chain length was only four CH_2 groups, resulting in a thickness difference of 1 nm (~20%). Our results for P_f agree with those reported by Carruthers and Melchior (typical values above T_m: $P_f = 6 \cdot 10^{-4}$ cm/s, and below T_m: $P_f = 1 \cdot 10^{-5}$ cm/s (Carruthers and Melchior, 1983). Carruthers and Melchior also found no systematic correlation between P_f and the length of the fatty acid chain of the lipid. P_d-values were first measured by Lawaczeck et al. in his original papers describing the H_2O/D_2O exchange method (Lawaczeck, 1979, 1984, 1988; Engelbert and Lawaczeck, 1985a, b). Lawaczeck found P_d-values for PCs in the range of 10^{-4}-10^{-3} cm/s above and 10^{-6}-10^{-5} cm/s below T_m, which again is in good agreement with our data.

Previously, no systematic comparison of diffusive and osmotic water permeability was performed using a consistent experimental approach. In this study, we used the same lipid preparations prepared in the same consistent way, the vesicle sizes checked by dynamic light scattering. Despite the unknown volume increase during the osmotic water influx, we feel confident that considerable differences for diffusive and osmotically driven water permeation exist. The ratio P_f/P_d in the liquid-crystalline phase is between 7 and 23 for all three PCs with saturated fatty acyl chains. Few other data exist with which our values can be compared. For biological membranes, P_f/P_d was determined to be larger than 3. For pure lipid vesicle membranes without protein content, no data have been reported so far. For black lipid membranes, values for P_f/P_d of approximately unity were reported (Sháafi, 1981; Finkelstein, 1987). This is in contrast to our results.

The small osmotic pressure of 50 mOsm used in our studies is not sufficient to induce vesicle lysis. This has been shown by an analysis of the osmotic properties of egg-PC/cholesterol (Mui et al., 1993) and DOPG vesicles (Ertel et al., 1993; Hallet et al., 1993). For egg-PC/cholesterol vesicles, lysis occurs at a differential of 400 mOsm and for DOPG at 300 mOsm. We therefore believe that the differences between P_f and P_d are not induced by our experimental approach. The large scattering of our P_f/P_d ratios is probably due to the assumption of a fixed 5% volume change at $t = t_{1/2}$. As discussed above, vesicles prepared by the extrusion technique are not necessarily spherical in isotonic solution (Mui et al., 1993), but become so only after swelling. The real change of internal volume can thus be larger than estimated, increasing the P_f/P_d values (see Materials and Methods). The variations in vesicle shapes are probably responsible for the scattering of our P_f/P_d values.

Comparison with theory

Previously, the solubility diffusion mechanism was assumed for the transport of water through pure lipid membranes (Hanai and Haydon, 1966; Finkelstein and Cass, 1968; Lieb and Stein, 1969; Reeves and Dowben, 1970; Träuble, 1971; Lawaczeck, 1979; Stein, 1990). An alternative transport mechanism, diffusion through water filled pores and fluctuating defects or transient pores was also discussed (Pagnelli and Solomon, 1957; Huang and Thomspon, 1966; Carruthers and Melchior, 1983; Lawaczeck, 1988).

On the basis of our permeability values and the P_f/P_d ratios, we believe that water permeation occurs through fluctuating defects or transient pores because of the following reasons: a) The calculated P_f/P_d ratios are larger than unity. Such a large difference between osmotic and diffusive water permeation is not compatible with a solubility diffusion

mechanism (see above).b) The P_f and P_d values have a small temperature dependence. The activation energies E_a are within a range of 8–35 kJ/mol. Our E_a-values agree with those reported by Carruthers and Melchior (1983) for osmotic water permeation (E_a = 10–27 kJ/mol). This low activation energy E_a is similar to the activation energy for self-diffusion of bulk water (Landolt-Börnstein, 1961; Sháafi, 1981) and suggests that water molecules can pass the lipid membrane with hardly any additional resistance. This finding supports the assumed diffusion through transient pores. For a solubility diffusion process, higher activation energies would be expected because the temperature dependence of the partition coefficient of water would have an additional influence.c) No reproducible light scattering curves with sufficient amplitudes are observed in the temperature range of the gel-to-liquid-crystalline phase transition because diffusion obviously occurs within the dead time of our instrument. This result can only be explained by assuming a strong increase of water permeability in the phase transition region. Our experimental results are at variance with the data of Lawaczeck (1979) and Carruthers and Melchior (1983). At present, we have no satisfactory explanation for this difference. In this temperature range, vesicles might become sensitive to the mechanical stress during the mixing process. To test this hypothesis, we reduced the driving pressure for the syringes or pushed them by hand. This had no effect on the experimental values. We also determined the vesicle size before and after the mixing, but no size changes were observed. This excludes the possibility that the vesicles are mechanically so stressed that they become leaky or that they fuse with concomitant loss of vesicle contents.

In view of the proposed permeation through transient pores, it is reasonable to expect a permeability maximum in the transition range. This was indeed found for other molecules, for ions, and dye molecules and is also predicted by theories on the basis of a cluster model for the lipid phase transition. (Papahadjopoulos et al., 1973; Tsong et al., 1977; Kanehisa and Tsong, 1978; Cruzeiro-Hansson and Mouritsen, 1988). In the transition region, lateral density fluctuations are particularly pronounced and line defects between gel and liquid-crystalline domains occur, increasing the number of transient pores and, therefore, the permeability.

Calculation of pore numbers

From the experimental P_d-values and the estimated permeability coefficients for a single pore p_d, we can by use of Eqs. 10 and 11 estimate the number of pores per vesicle and, using the experimental P_f/P_d ratios, calculate the number of water molecules N per single-file pore. From N and the number of pores, the distribution coefficient K_w of water between water and bilayer interior can be estimated (Eq. 8). If we assume for water the same diffusion coefficient in the bilayer as in bulk water ($D_w = 2.3 \cdot 10^{-5}$ cm^2/s at 25°C), a length L for the pore of $4 \cdot 10^{-7}$ cm (thickness of the bilayer), and for the volume of a water molecule $16–30 \cdot 10^{-24}$ cm^3 (lower value from van der Waals surface of water, upper value from density of bulk water), we calculate for the single-file pore p_d values between 2.3 and $4.3 \cdot 10^{-15}$ cm^3/s.

A vesicle with a radius of 100 nm has a surface area of $1.25 \cdot 10^{-9}$ cm^2 and $\sim 1.9 \cdot 10^5$ molecules in the outer leaflet (calculated with a value of ~ 65 Å^2 for the molecular area for PC in the liquid-crystalline phase). With a typical P_d value of $5 \cdot 10^{-4}$ cm/s (lower temperatures in the liquid-crystalline phase), we calculate from Eq. 10 for the number of pores n per vesicle values of 145–270. This corresponds to approximately 1 pore per 700–1300 lipid molecules in the outer leaflet or 1400–2600 lipids in both monolayers. This calculation is quite instructive because it shows that the total number of transient pores or defects per vesicle is not very large.

Taking for the sake of simplicity $N = 10 = P_f/P_d = p_f/p_d$ for the number of water molecules per single-file pore (experimental P_f/P_d-values are 7–23), we have then 200 pores per vesicle and 2000 water molecules in the bilayer of a vesicle with $3.8 \cdot 10^5$ lipid molecules. The partition coefficient K_w (in mole fraction units) is then to $5.2 \cdot 10^{-3}$. This is two orders of magnitude higher than the partition coefficient for water between hexadecane and water (Finkelstein, 1976), which is $4.2 \cdot 10^{-5}$.

Density fluctuations leading to transient hydrophobic and hydrophilic pores are also thought to be necessary to understand the phenomenon of electroporation, the formation of large hydrophilic pores after application of large transmembrane voltages leading to electrical break down of the bilayer (Abidor et al., 1979; Benz et al., 1979; Benz and Zimmermann, 1981; Sugar et al., 1987; Glaser et al., 1988; Neumann et al., 1989; Tsong, 1991; Freeman et al., 1994). Glaser et al. (1988) presented estimations for the free energy of formation of hydrophobic pores as a function of pore radius assuming a hydrophobic free energy $\sigma(R)$ of $50 \cdot 10^{-3}$ N/m in the limit of large pores. For pores with smaller radius, the free energy decreases strongly because of van der Waals interactions across the pore walls. For a pore with radius R of 0.2 nm and a length L of 4 nm, the free energy $E = 2\pi \cdot L \cdot R \cdot \sigma(R)$ per pore is $3.8 \cdot 10^{-20}$ J using a value of 5 mN/m for $\sigma(R)$. Similar results for the pore energy were calculated by Freeman et al. (1994) using a value for the pore edge energy density of $2 \cdot 10^{-11}$ J/m. The pore density can then be estimated from the Boltzmann factor $N_{pore}/N_{lipid} = \exp(-E/kT)$. For T = 300 K, one calculates one pore per 1000 lipid molecules in both leaflets of a vesicle, a value well within the range of our estimates considering the large uncertainties for the value of the pore energies.

Recently, a 120-ps molecular dynamics simulations of a DPPC bilayer composed of 64 lipid and 736 water molecules has been reported by Marrink and Berendsen (1994). They proposed an inhomogeneous solubility diffusion model for water, in which the inhomogeneous diffusion constant and the inhomogeneous free energy for water partitioning as determined from the MD simulation is used to calculate the permeability constant P_d. For 350 K (77°C), they find $P_d = 7 \cdot 10^{-2}$ cm/s. Our values at 70°C are $4–5 \cdot 10^{-4}$ cm/s and, thus, are considerably lower. Molecular dynamics simulations of

120 ps are certainly not long enough to decide whether transient pores of the estimated size can exist. As on the average, only one pore per 2000 lipid molecules is present, an ensemble of 64 lipids and a 120-ps simulation time are definitely not sufficient to decide for or against a diffusion through transient pores.

Phosphatidic acids, phosphatidylethanolamines, phosphatidylserine, and phosphatidylglycerol

Phosphatidic acids

When PAs were dispersed in pure water (pH 6–6.5), we observed the peculiar effect of only a small change in permeability at the phase transition temperature. The P_d-values in the liquid-crystalline phase were then a factor of 50–100 lower than those for PCs (see Fig. 4 *a* and Table 1). For vesicle suspensions in 20 mM phosphate buffer (pH 7) or 20 mM NaCl (pH 6–6.5), we observed the normal permeability increase at T_m. However, P_d-values in the liquid-crystalline phase are still a factor of 2–3 lower than for the corresponding PCs.

Addition of salt or buffer changes the apparent pK_a of the head group (Träuble and Eibl, 1974; Blume and Eibl, 1979; Blume and Tuchtenhagen, 1992). This is caused by counter ion condensation and the concomitant screening of the negative head group charges. These screening effects can be described using the Gouy-Chapman theory for the diffuse electrical double layer (Träuble et al., 1976; for reviews, see Cevc and Marsh, 1987; Cevc, 1990). However, specific binding effects and changes in hydration upon ion binding cannot be described by this theory (Cevc, 1988; 1990). Our experimental data indicate that head group interactions play an important role in the permeability properties. PAs have higher transition temperatures than PCs because of intermolecular hydrogen bonding and decreased hydration (Eibl and Woolley, 1979; for review, see Boggs, 1987). This can then lead to lower permeabilities. However, it remains surprising that this effect can be modulated to such an extent by changes in ionic strength (see Fig. 4 *a*).

Phosphatidylethanolamines

PEs have similar transition temperatures as PAs because of intermolecular H-bonds between positively charged ammonium and the negatively charged phosphate groups, and the head groups are less hydrated than those of PC (Jendrasiak and Hasty, 1974; Simon and McIntosh, 1986; Boggs, 1987; Cevc, 1988; Damodaran and Merz, 1994). The P_d-values for PEs in the liquid-crystalline phase are similar to those of PAs. Again, intermolecular head group interactions and lower hydration of the interface reduces water permeability (see Fig. 4 *b*).

Phosphatidylglycerol, phosphatidylserine

The permeability of PG and PS vesicles in aqueous salt solution is very similar to the permeability of PC vesicles, indicating that the intermolecular interactions between the head groups are comparable (see Figs. 3 *a* and 4 *c*).

Di-ω-cyclohexylphosphatidylcholines

The permeability values of cyPCs in the liquid-crystalline phase show a strong odd-even effect. This is surprising because odd-even effects in the thermotropic behavior are a consequence of packing problems in the gel phase. Large packing differences in the liquid-crystalline phase are not expected. For the cyPCs one can assume that the cyclohexyl residues fill out the region in between the two monolayers where normally free volume exists. This should reduce the probability that defects serving as pathways for water permeation traverse both monolayers. The difference between 11cyPC and 13cyPC on one hand and 14cyPC on the other, however, is difficult to conceive. One can speculate that the conformation of the cyclohexyl ring at the chain ends must be such that the free volume is filled more effectively in the case of odd-numbered cyPCs.

The P_f-values for all cyPCs are much higher and similar to the values of PCs with straight chains. The P_f/P_d ratios for 11cyPC and 13cyPC are between 200 and 500, much too high to be explainable by diffusion through single-file pores. These vesicles are probably particularly sensitive to osmotic stress and become leaky even at low membrane tensions of 5 dyn/cm. The other assumption, namely, that the volume increase under osmotic stress is much less than 5% at $t = t_{1/2}$, leads to a decrease of P_f but not to such a large extent that the P_f/P_d ratio is reduced to values found for straight chain PCs.

Dihexadecyl-phosphatidylcholine

The assumption that interfacial hydration is one major factor governing the permeability properties can be tested by the investigation of ether lipids. From x-ray- and neutron scattering, FT-IR spectroscopy, and molecular dynamics simulation, the extent of hydration of the lipid-water interface can be determined (Simon and McIntosh, 1986; Knott and Schoenborn; 1986; Blume et al., 1988; Damodaran and Merz, 1994; Marrink and Berendsen, 1994). It was found that water can easily reach both carbonyl groups of ester PCs. In DHPC bilayers, the hydration site of the carbonyl oxygen is no longer present. As a consequence, DHPC forms an interdigitated gel phase below T_m (Ruocco et al., 1985; Laggner et al., 1987). In the liquid-crystalline phase, the reduced hydration of the interface is obviously the reason for the much lower water permeability compared with DPPC.

DPPC in water/ethanol mixtures

The drastically reduced water permeability of DPPC vesicles in water-ethanol mixtures is also a strong support for the assumption that water penetration occurs through transient defects or pores. Not only is water permeation in the liquid-crystalline phase strongly reduced, but it is also reduced in the gel phase. In the gel phase, water permeates probably

through defects at domain boundaries that are stable over longer periods of time. In the liquid-crystalline phase, the system is highly dynamic and the defects or pores are of transient nature. The defects in the gel phase and the transient pores in the liquid-crystalline bilayer are probably predominantly occupied by ethanol molecules. From FT-IR spectroscopy on reversed PC micelles, it was concluded that ethanol displaces water from the phosphate and the two carbonyl groups. The partition coefficient of ethanol between bilayer and water was determined to 16–17 (Diamond and Katz, 1974; Chiou et al., 1992). At 5 wt% ethanol in water (~1 M) and lipid concentrations of 1–2 mM, there is one molecule of ethanol per lipid molecule in the bilayer. With the assumption of 1 pore per 2000 lipid molecules (see above), there are therefore enough ethanol molecules available that not only the hydrations sites can be occupied but also that the defects can be filled with ethanol molecules. Thus, hydrophilic and "hydrophobic sites" are blocked by ethanol molecules, and this leads to the strongly reduced water permeability.

SUMMARY AND CONCLUSIONS

We have measured the water permeability of lipid vesicles composed of a variety of different phospholipids with different head groups and fatty acyl chains using the H_2O/D_2O exchange method for diffusional water flow and the osmotic method to study osmotically driven water flux. Our main results are that osmotically driven water flow is higher than the diffusional flow. We suggest that this can arise when water permeation proceeds via transient defects or pores that are filled by several water molecules that then cooperatively traverse the bilayer. In contrast to previous results, we suggest a permeability maximum at the phase transition temperature as would be expected when permeation occurs via defects. The permeability is strongly influenced by intermolecular interactions in the head group region via hydrogen bonds between the lipids and the water molecules in the hydration sphere. In the case of charged phospholipids, these can be modified by pH or changes in ionic strength. For PCs an exchange of ester versus ether linkage of the chains reduces the permeability strongly. In the framework of the permeation model, this means that the probability for the formation of transient defects does not only depend on the hydrophobic energy for pore formation but also on the energy necessary to break up polar intermolecular interactions in the head group region. Addition of ethanol leads to a marked decrease of water permeability. In accordance with the suggested permeation mechanism, this reduction can be explained by the fact that ethanol molecules not only replace water in the hydration shells of the head groups but also accumulate in transient defects in the hydrophobic part of the bilayer, excluding water molecules from these sites and, therefore, reducing the water permeability.

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