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Pressure Dependence of 1,6-Diphenyl-1,3,5-hexatriene Fluorescence in Single-Component Phosphatidylcholine Liposomes†

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ABSTRACT: Small unilamellar vesicles (SUV) and multilamellar vesicles (MLV) of dimyristoyl-L-α-phosphatidylcholine (DMPC) and dipalmitoyl-L-α-phosphatidylcholine (DPPC) and the MLV of dioleoyl-L-α-phosphatidylcholine (DOPC) were examined by steady-state polarization fluorometry under pressure in the range 10^{-3}–2 kbar. Isothermal pressure induced phase transitions were observed in DPPC and DMPC vesicles incorporated with 1,6-diphenyl-1,3,5-hexatriene. The temperature to pressure equivalences, dT/dP, estimated from the transition point, $P_{1/2}$, are 29.5 °C kbar^{-1} for DPPC(SUV), 22.7 °C kbar^{-1} for DPPC(MLV), 22.2 °C kbar^{-1} for DMPC(SUV), and 25.9 °C kbar^{-1} for DMPC(MLV) in an aqueous phase containing 0.1 M KCl and 0.01 M tris(hydroxymethyl)-aminomethane at pH 8.2. Even though there is no phase transition, we are still able to estimate a dT/dP of about 21 °C kbar^{-1} for DOPC(MLV). All the values of dT/dP obtained from this study are within the range typical for lipid-involving processes. The fluidity changes in the pretransition in DMPC(MLV) and in DPPC(MLV) observed in isobaric temperature studies are not detected in the isothermal pressure study, suggesting that the thermal pretransition may not involve an appreciable volume change in the hydrocarbon regions.

The temperature-induced gel to liquid-crystalline phase transition has been observed in many phospholipid vesicles by different physical techniques such as electron spin resonance (ESR)[1] (Hubble & McConnell, 1971), light scattering (Abramson, 1971), nuclear magnetic resonance (Sheetz &

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[1] Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; PRODAN, 6-propionyl-2-(dimethylamino)naphthalene; DPPC, dipalmitoyl-L-α-phosphatidylcholine; DMPC, dimyristoyl-L-α-phosphatidylcholine; DOPC, dioleoyl-L-α-phosphatidylcholine; SUV, small unilamellar vesicles; MLV, multilamellar vesicles; ESR, electron spin resonance; Tris, tris(hydroxymethyl)aminomethane.

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Chan, 1972), dilatometry (Nagle, 1973), fluorescence polarization (Lentz et al., 1976; Suurkuusk et al., 1976; Andrich & Vanderkooi, 1976), ultrasonic absorption (Mitaku et al., 1978), and calorimetry (Albon & Sturtevant, 1978). The general conclusions of these studies are the following: the temperature transition point is dependent upon the chain length and type of fatty acid chain; below the phase transition temperatures, the lipid bilayer is an "ordered" gel; above the phase transition temperatures, the lipid bilayer exists as a fluid liquid state. This fluid liquid state has been proposed as a requirement for "optimum" biological functions of membranes. To fully describe the physical state of membranes, one should include all the important thermodynamic variables. Although pressure is one, equal in importance to temperature and membrane composition, it has not been applied to an extent comparable to the studies of temperature and lipid composition.

Previously, pressure effects on the phase transition in synthetic phospholipids have been studied by the methods of spin-label ESR (Trudell et al., 1974), volumetry (Srinivasan et al., 1974; Liu & Kay, 1977), light scattering (DeSmedt et al., 1975; Ceuterick et al., 1978), and calorimetry (Mountcastle et al., 1978), and it has been shown that the phase transition temperature, T_m (or pressure, P_m), increases linearly with the increase of pressure (or temperature), giving a dT_m/dP_m value between 17 and 24 °C kbar^{-1}.

In our study, steady-state fluorescence polarization measurements of 1,6-diphenyl-1,3,5-hexatriene (DPH) incorporated in phospholipid vesicles were employed to examine the pressure effect on the membrane fluidity. Since phosphatidylcholine is the most prominent phospholipid in nearly all the mammalian membranes, and the physical study of phosphatidylcholine vesicles has been well documented, we chose it as the model system for initial pressure studies.

Experimental Procedures

Materials. Dipalmitoyl-L-α-phosphatidylcholine (DPPC), dimyristoyl-L-α-phosphatidylcholine (DMPC), and dioleoyl-L-α-phosphatidylcholine (DOPC) purchased from Sigma Chemical Co. were used without further purification. DPH and perylene were obtained from Aldrich Chemical Co. 6-Propionyl-2-(dimethylamino)naphthalene (PRODAN) was synthesized by the method of Weber & Farris (1979). Fluorescein was purchased from Baker Chemical Co. Millipore water (10^{18} Ω cm^{-2}), reagent-grade chemicals, and glass-distilled organic solvents were used in all experiments.

Preparation of Liposomes. Multilamellar vesicles (MLV) were prepared by the method of Bangham et al. (1967) using an aqueous phase either containing 137 mM NaCl, 2.7 mM KCl, 12.2 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.2, or containing 0.1 M KCl and 0.01 M Tris, pH 8.2 (measured at 19 °C). Small unilamellar vesicles (SUV) were prepared following a modification of the method of Huang (1969) by first evaporating chloroform from a phospholipid/chloroform solution. Then the dry residues in an aqueous solution of 0.1 M KCl, 0.15 mM EDTA, and 0.01 M Tris, pH 8.2, were sonicated under nitrogen by a Heat Systems 375-W sonifier. Finally, the dispersions were spun at 40 000 rpm for 1 h. The clear supernatant was used for the experiments. During the preparation of DPPC and DMPC vesicles, either MLV or SUV, the samples were kept above the phase transition temperature except for the period of ultracentrifugation. The vesicles were stored under nitrogen or argon above their phase transition temperatures. DPH dispersions in buffer were made by first heating the buffer solution followed by the injection of concentrated DPH/tetrahydrofuran into the solution while stirring. The stirring and heating helped the evaporation of tetrahydrofuran. The DPH dispersions were then mixed with phospholipid vesicles, making the mole ratio of DPH to phospholipid between 1/1600 and 1/500. The phospholipid concentration was measured as inorganic phosphate by the method of Ames (1966). Phosphatidylcholine concentrations in dispersions used for fluorescence measurements were about 1.0–0.1 mM.

Fluorescence Polarization Measurements under Pressure. DPH fluorescence polarization measurements under pressure were made with a high-pressure bomb mounted in a photon-counting polarization fluorometer. The high-pressure apparatus, including the pressure bomb, inner cuvette, and pressure-generating system, and the polarization instrument have been described in detail elsewhere (Paladini & Weber, 1981). Briefly, the phospholipid dispersions incorporating DPH were loaded into an inner quartz cuvette capped with a small polyethylene tube sealed at one end, forming a closed system. The sample system is surrounded with alcohol which served as the pressure-transmitting fluid. All polarization measurements were made by employing the L-format method; with vertically polarized excitation light, the parallel ($I_\parallel$) and perpendicular ($I_\perp$) components of the fluorescence emission were observed through a polarizer, and the polarization, p, was calculated by the equation

$$p = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp} \quad (1)$$

Excitation with horizontally polarized light should ideally give $p = 0$. In our system, it was 0.01. Therefore, a correction for residual polarization was judged unnecessary. However, a correction is necessary for the depolarization due to pressure-induced birefringence from the bomb windows and can be carried out as described by Paladini & Weber (1981). We determined the correction factors by using four different probes: fluorescein, perylene, DPH, and PRODAN. Since birefringence is due to pressure effects on the windows, the obtained correction factors must not include direct effects of the high pressure upon the fluorophores. These direct effects, if present to a significant extent, ought to affect the dependence of polarization upon pressure to a varying extent in the four fluorophores. Four sets of correction factors without appreciable differences between them should be conclusive with regard to the origin of the pressure depolarization. Experimentally, the testing fluorescent probe dissolved in glycerol was cooled down to −10 to −12 °C. Under these conditions, the observed fluorescence polarizaiton at 1 atm is essentially the limiting polarization, p_0, of that probe and should remain unchanged at all higher pressures. The scrambling coefficient, α, was determined as described by Paladini & Weber (1981), and the polarization values observed at high pressure were corrected through the equation

$$P_{cor} = \frac{P_{obsd}}{1 - \alpha(3 - P_{obsd})} \quad (2)$$

Excitation at 355 nm was employed for all DPH polarization measurements, with a Corning 7-54 band-pass excitation filter and a 2-mm layer of 2 M $NaNO_2$ plus a Corning 3-73 as emission filters. At the concentration of phospholipid vesicles and with the optical filters employed, a correction for the scattering light was not necessary.

The temperature of the sample and pressure bomb was controlled by a circulating bath, which was connected to the circulating line firmly attached on the high-pressure bomb.

Temperature was read as a digital output of a thermocouple placed in the bomb.

The method employed in this study of the pressure effects on membranes has some features of its own, distinct from other methods previously used. First, we could register membrane fluidity in a direct way as it is generally accepted that the changes in fluorescence polarization of DPH reflect fluidity changes (Andrich & Vanderkooi, 1976; Shinitzky & Barenholz, 1978). Second, the sample was in a closed system completely isolated, thus avoiding possible perturbations of the membrane by the contacting pressure-transmitting fluid. As an example, MacDonald (1975) has shown that helium pressure gave different results from pure hydrostatic pressure in the study of the rate of cell division of *Tetrahymena*. Third, the range of 10^{-3}–1.5 kbar was employed so that the results directly refer to the environmental pressure range ($\sim$1 kbar) and beyond, and need not be presumed to apply to it by extrapolation from observations over a smaller pressure range.

Fluorescence Lifetime Measurements under Pressure. Fluorescence lifetime measurements were employed on the cross-correlation phase/modulation fluorometer (Spencer & Weber, 1969). The pressure bomb on a specially made adapter was placed at the turret position used during normal lifetime measurements. Modulation frequency was 18 MHz, and the excitation wavelength and excitation and emission filters were as described for the polarization measurements. A suspension of glycogen in Millipore water (10^{18} Ω cm^{-2}) was used to provide a scattered reference signal. To obviate frequent removal of the bomb, a signal of zero phase delay was generated by diverting a fraction of the exciting light through an alternate pathway of equivalent length to the one leading to the pressure bomb.

Emission and Excitation Measurements under Pressure. Fluorescence intensity measurements were done by using an analog spectrofluorometer (Wehrly, 1979). The pressure bomb was placed at the cuvette holder position used during normal intensity measurements.

Results

Correction Factors for Window Birefringence. The correction factors for the window birefringence obtained from fluorescein, DPH, perylene, and PRODAN in glycerol are presented in Figure 1, showing that the scrambling coefficient, α, which is used as the correction factor, is independent of probe. At 2 kbar, the scrambling coefficient from the two specific windows which were used in this study resulted in a 58.5% decrease of the polarization values observed at 1 atm. This is somewhat larger than that reported previously by Paladini & Weber (1981) for the same bomb. Redetermination of α was required when a new window was installed, and the observed difference in α is not unreasonable.

The fluorescence excitation polarization spectra of each of the above probes, at 1 atm and 2.04 kbar ($\approx$30 kpsi), are shown in the inset of Figure 1. The uncorrected polarization spectrum at 2.04 kbar is parallel, generally speaking, to the spectrum at 1 atm in the wavelength range corresponding to the last absorption band for each probe. This parallelism shows the correction factors to be wavelength independent, in the range covered in the graph, so that the same correction factor can be used to bring the uncorrected polarization values to the true value regardless of the excitation wavelength. Figure 1 also demonstrates that the excitation polarization spectrum remains somewhat flat even at a pressure as high as 2.04 kbar. The flatness assures that the observed polarization change at high pressure is not influenced by pressure-induced shifts in the absorption maximum in the range below 2.04 kbar.

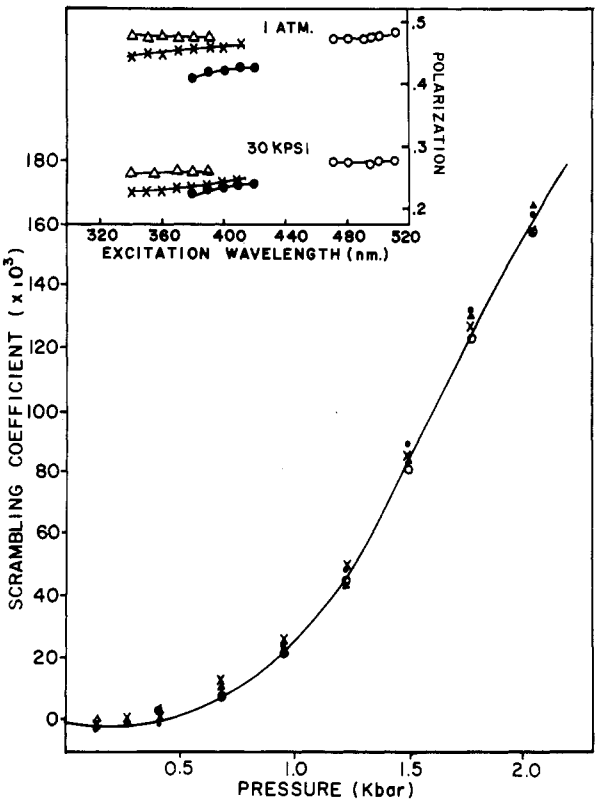

FIGURE 1: Scrambling coefficient (α) as a function of hydrostatic pressure. (Δ) Data obtained from DPH in glycerol, λ_{ex} = 355 nm; ($\bullet$) obtained from perylene in glycerol, λ_{ex} = 410 nm; (O) obtained from fluorescein in glycerol, λ_{ex} = 495 nm; ($\times$) obtained from PRODAN in glycerol, λ_{ex} = 360 nm; measured at −10 to −20 °C. Inset: Excitation polarization spectra of DPH, perylene, fluorescein, and PRODAN at 1 atm and 30 kpsi ($\approx$2 kbar). Polarization values were not corrected for scrambling of the windows.

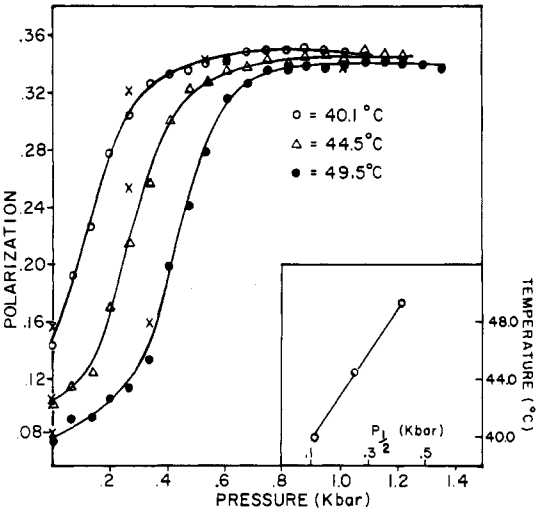

FIGURE 2: Pressure dependence of DPH polarization in DPPC(SUV) in a solution of 0.1 M KCl and 0.01 M Tris, pH 8.2 (measured at 19 °C), at different temperatures. ($\times$) Data were obtained after the release of pressure. Inset: Phase transition pressure, $P_{1/2}$, as a function of temperature.

Phase Transition in DPPC and DMPC Vesicles. The effect of hydrostatic pressure on DPH fluorescence polarization in DPPC(SUV) is shown in Figure 2, indicating that DPH polarization values increase with the increase of pressure. No matter what rationalization is employed to clarify the meaning of DPH polarization in bilayers, either by the concept of "microviscosity" (Shinitzky & Barenholz, 1978) or by the theory of "viscosity in the cone" (Kawato et al., 1977), the

Table I: Parameters in Thermal and Pressure Studies of Single-Component Phosphatidylcholine Vesicles[a]

	T_m[b] at 1 atm (°C)	ΔT_t[b] (°C)	$P_{1/2}$ at 42 °C (kbar)	ΔP_t (kbar)	dT/dP (°C kbar^{-1})
DPPC(MLV)	41.1	5.1	0.06[c]	0.23[c]	24.3[c]
			0.02[d]	0.25[d]	22.7[d]
DPPC(SUV)	36.4	10.9	0.15[d]	0.54[d]	29.5[d]
DMPC(MLV)	24.4	7.1	0.80[c]	0.44[c]	22.4[c]
			0.80[d]	0.49[d]	25.9[d]
DMPC(SUV)	20.9	13.1	0.84[d]	1.44[d]	22.2[d]
DOPC(MLV)					21.0[c]

[a] Abbreviations: T_m, transition point in the thermal-induced phase transition; ΔT_t, transition range in the thermal-induced phase transition; $P_{1/2}$, transition point in the pressure-induced phase transition; ΔP_t, transition range in the pressure-induced phase transition; dT/dP, temperature to pressure equivalence. [b] Data taken from Lentz et al. (1976). [c] In 137 mM NaCl, 2.7 mM KCl, 12.2 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.2. [d] In 0.1 M KCl and 0.01 M Tris, pH 8.2 (at 19 °C).

results refer to a general phenomenon: the decrease in lipid bilayer fluidity under pressure. On each isothermal curve, the DPH polarization value shows an initial monotonic increase with pressure followed by a dramatic change which corresponds to the well-recognized liquid-crystalline–gel phase transition. Finally, the DPH polarization levels off at pressures which bring on the gel state of the vesicle. The DPH polarization values for the liquid-crystalline state can be approximately extrapolated from the curve. A phase transition pressure, $P_{1/2}$, is defined as the pressure at which the polarization change is 50%. The span of the main transition, ΔP_t, is defined as the pressure change between 10% and 90% of the total change in polarization. The main transition can be observed only at temperatures higher than the phase transition temperature, T_m; below T_m, the packing in the lipid bilayer is already tight, and a higher pressure will just make the packing even tighter. As the temperature increases, the isothermal transition curves move in the direction of higher pressures. For each different type of vesicle, a plot of temperature against $P_{1/2}$ gives a straight line (see the inset in Figure 2). Its positive slope yields $dT/dP_{1/2}$, the "temperature to pressure equivalence". The significance of dT/dP is that, on maintaining a constant DPH polarization value or inferentially constant fluidity in the vesicles, changes in dT and dP have the same effect in terms of their sign and magnitude. It implies that high temperature and high pressure (or low temperature and low pressure) can compensate each other to keep the membrane fluidity the same. The pressure effects on DPH polarization in DMPC(MLV), DMPC(SUV), DPPC(MLV), and DPPC(SUV) are summarized in Table I. The dT/dP values cover a small range, between 21 and 30 °C kbar^{-1}. However, each type of vesicle bears different characteristics (Table I). For example, MLV have a sharp transition indicated by a small ΔP_t while SUV have a rather broad one.

The surface charge of DPPC and DMPC vesicles at neutral pH is zero so that we do not expect an appreciable effect of buffers, with a pH around the neutral value, on the structure of vesicles. We have examined the pressure effect on the phase transition of DPPC(MLV) and DMPC(MLV) in two different buffers, phosphate and Tris. The former has the larger pressure dependence of pH values, +0.3 kbar^{-1} as compared to −0.002 kbar^{-1} (Neuman et al., 1973), while Tris has the larger temperature dependence (−0.03 °C^{-1}) (Ceuterick et al., 1978). The pressure data for 0.1 M KCl/0.01 M Tris at pH 8.2 and for phosphate/saline buffer containing 137 mM NaCl, 2.7 mM KCl, 12.2 mM Na_2HPO_4, and 1.5 mM KH_2PO_4 at pH 7.2 show very close values of $P_{1/2}$ and no appreciable difference in ΔP_t between them (Table I). Furthermore, dT/dP values are also independent of the buffer (Table I). Thus, small differences in ionic strength, or in pH induced by either temperature or pressure, have no significant effects on the physical state of the vesicles.

Table II: DPH Fluorescence Lifetimes and Rotational Relaxation Times in DMPC(MLV) under Various Pressures at 32.1 °C

pressure (kbar)	lifetime (ns) (18 MHz) modulation	lifetime (ns) (18 MHz) phase	polarization	rotational relaxation time[a] (ns)
1×10^{-3}	8.21	8.25	0.144	8.9
0.1	8.20	8.12	0.155	9.9
0.2	8.24	8.19	0.171	11.5
0.3	8.27	8.21	0.195	14.3
0.4	9.05	8.64	0.338	54.3
0.5	9.03	8.49	0.371	77.4
0.6	8.91	8.30	0.379	84.2
0.8	8.63	8.03	0.388	91.7

[a] Values were calculated through the Perrin equation (Perrin, 1929) by using the measured modulation lifetimes and polarization values.

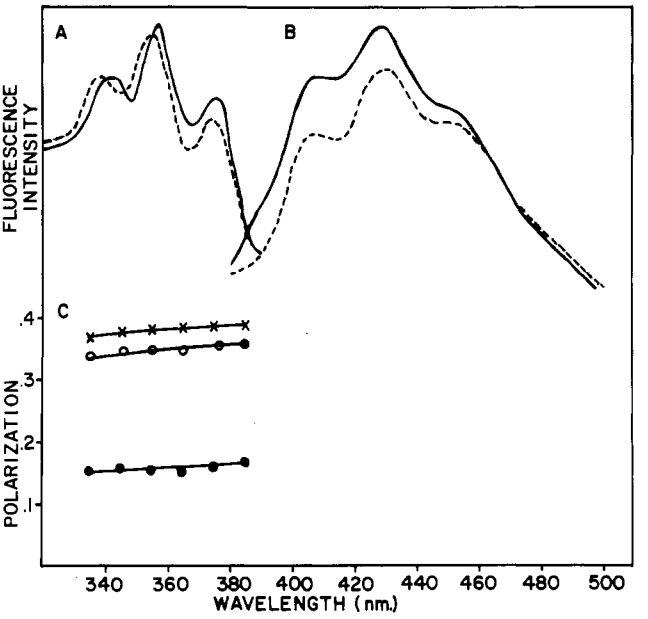

FIGURE 3: Fluorescence excitation (A) (λ_{em} = 430 nm) and emission (B) (λ_{ex} = 355 nm) spectra of DPH in DMPC(MLV) in 0.1 M KCl and 0.01 M Tris, pH 8.2 (at 19 °C), at 1 atm (---) and at 1.1 kbar (—). The temperatures were 35.5 and 34.7 °C for (A) and (B), respectively. In (C) are the excitation polarization spectra of the same sample at 1 atm (●) and 1.1 kbar [(○) uncorrected; (×) corrected] at 34.7 °C.

Control Experiments. The reversibilty of the pressure effect was checked during and after all experiments. As shown in Figures 2 and 5, the reversibility of pressure effects on DPH polarization in phospholipid vesicles is excellent.

The lifetime of DPH fluorescence in DMPC (MLV) under pressure is shown in Table II. With the measured lifetimes and polarization values, we calculated the apparent rotational relaxation times which gave a phase transition with the midpoint pressure at 0.40 kbar, in agreement with the $P_{1/2}$ determined directly by a 50% polarization change of 0.38 kbar. Thus, the apparent polarization change itself reflects quite closely the lipid fluidity change.

The fluorescence intensity of DPH in vesicles under pressure does not show significant change. For example, the total

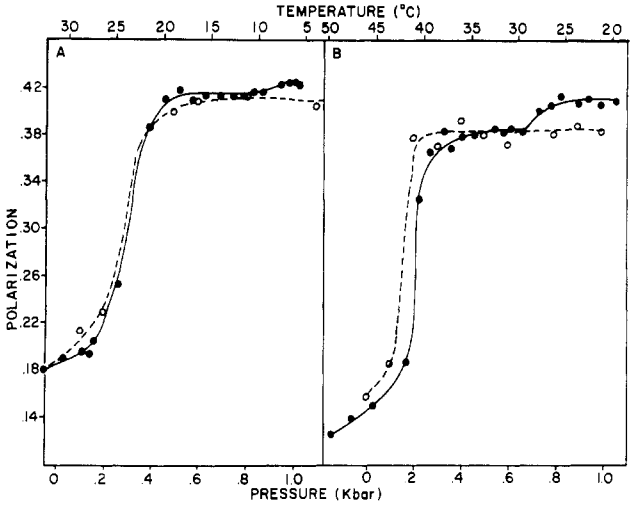

FIGURE 4: (A) DPH polarization as a function of temperature (at 1 atm) (●) and as a function of pressure (at 30.5 °C) (○) in DMPC(MLV) in 0.1 M KCl and 0.01 M Tris, pH 8.2. (B) DPH polarization as a function of temperature (at 1 atm) (●) and as a function of pressure (at 44.8 °C) (○) in DPPC(MLV) in 0.1 M KCl and 0.01 M Tris, pH 8.2.

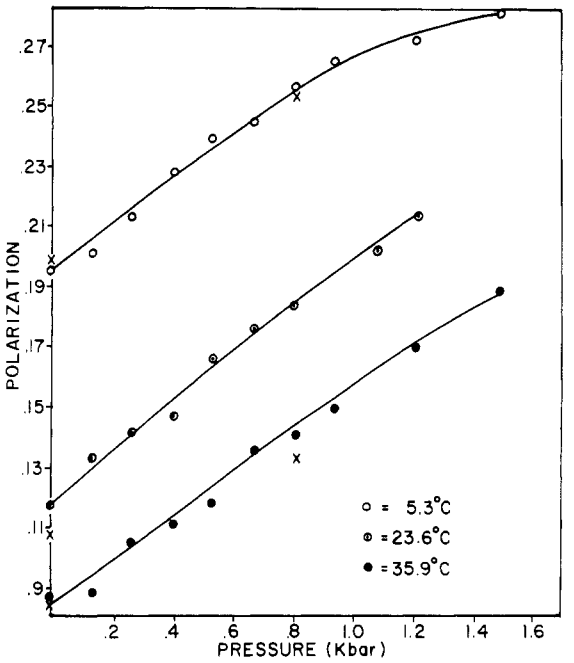

FIGURE 5: Pressure dependence of DPH polarization on DOPC(MLV) in 137 mM NaCl, 2.7 mM KCl, 12.2 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.2, at three different temperatures. (×) Data were obtained after the release of pressure.

fluorescence intensity of DPH in DMPC(MLV) increases only about 5.2% at 1.1 kbar over that at 1 atm (Figure 3B). A significant drop in fluorescence intensity should follow if the probes leave the membrane under pressure, since the quantum yield of DPH fluorescence in water is about 1000 times lower than that in phospholipid bilayers (Shinitzky & Barenholz, 1974). The small emission maximum shift (about 2 nm) from 1 atm to 1.1 kbar is probably due to the increased dipole interaction in the vesicles as the volume decreases (Macgregor & Weber, 1981) and has no effect on the interpretation of polarization results. The release of pressure from 1.1 kbar back to 1 atm gave an emission spectrum virtually identical with the original one, showing good reversibility of the fluorescence intensity and the spectrum as well as polarization. Figure 3C shows that uncorrected and corrected excitation polarization spectra at 1.1 kbar and 1 atm are quite flat and virtually parallel in the range of 335–385 nm, which corresponds to the last absorption band in DPH/phospholipid dispersions. The fluorescence excitation spectra of the same sample at 1 atm and at 1.1 kbar show a shift of excitation maxima of about 2–3 nm (Figure 3A). These data demonstrate that the observed polarization change in synthetic phospholipid membranes has no apparent error resulting from the shift of the excitation maximum under pressure.

Pretransition. Besides the main transition described above, we also examined the pressure effect on pretransitons in DPPC(MLV) and DMPC(MLV). The thermal pretransition in phospholipid vesicles has been studied by many groups (Hinz & Sturtevant, 1972; Nagle, 1973; Mitaku et al., 1978), but its molecular mechanism remains unclear. The current belief is that the pretransition results from a structural change somewhere between the hydrophobic region and the lipid bilayer surface, not as a result of the melting of the hydrocarbon chains (Janiak et al., 1976). In Figure 4, an isobaric temperature study done at 1 atm with the sample in the high-pressure bomb shows that a pretransition is evident in each plot of DPH polarization against temperature. The pretransition temperatures shown in Figure 4 are located at 26–31 °C for MLV of DPPC and at 7–11 °C for MLV of DMPC, close to values reported by Lentz et al. (1976), who also used DPH polarization to observe the thermal phase transitions in DMPC(MLV) and DPPC(MLV). However, no such pretransition appears in the plot of DPH polarization against pressure (Figure 4). Instead, the DPH polarization starts leveling off with pressures at the region where the thermal pretransition occurs in the isobaric temperature study. This indicates that the pretransition observed in the isobaric temperature studies does not involve an appreciable volume change or more correctly that it does not involve a conspicuous fluidity change in the region that we assume to harbor the probe, the hydrocarbon region of the vesicles. Thus, our data match well with the current thoughts about the thermal pretransition in phosphatidylcholine vesicles.

Similar results were obtained in Tris buffer at pH 8.2 (Figure 4) and in our phosphate/saline buffer (not shown), indicating that the pH changes brought about by variation of temperature or pressure do not produce artifacts in the pretransition study. For example, Tris buffer changes its pH from 8.2 to 7.6 when the temperature increases from 19 to 39 °C. One might argue that this 0.6 unit pH change could bring some artifacts which could then lead to the appearance of the thermal pretransition shown in our isobaric temperature study. Since phosphate buffer is much less temperature sensitive, the pretransition we observed in pH 7.2 phosphate buffer should clear up the doubts in this respect.

The inability to observe the pretransition at elevated pressures does not imply that the pretransition shows no pressure sensitivity; rather, it belongs to a region away from the fluorescent probe. A recent study of pressure effects on Raman spectra of DMPC vesicles by Wong et al. (1982) has shown that a thermal pretransition is detectable at 1 kbar. We do not observe the pretransition at 1 kbar or even at 1.5 kbar (data not shown). Such differences are easy to reconcile. The fluorescence probe technique reflects only membrane fluidity changes while the Raman transitions may be modified by changes in molecular properties unconnected to fluidity changes. Hence, our conclusion that the pretransition does not involve an appreciable volume change in the hydrocarbon region is appropriate.

Pressure Effect on DOPC Multilamellar Vesicles. The results of a pressure study on DOPC are presented in Figure

5. In phosphate/saline buffer, the DPH polarization increases with the increase of pressure. This increase is smooth through the whole pressure range we examined. At 35.9 °C, DPH polarization increases 50% at a pressure of 0.7 kbar. Even though no sharp transition allows one to exactly determine the temperature to pressure equivalence, its value may be estimated from the three different isothermal curves shown in Figure 5. In the case of DOPC vesicles at 23.5 °C and 1 atm, for example, the DPH polarization increases from 0.118 to 0.152 when the pressure is raised to 0.4 kbar. In order to bring the DPH polarization back to 0.118 at 0.4 kbar pressure, the temperature must be raised to 35.9 °C. In this way, we estimate the dT/dP for DOPC(MLV) to be about 21 °C kbar^{-1}.

As shown in Figure 5, at 5.3 °C, the DPH polarization tends to level off at about 1.5 kbar with a DPH polarization value of about 0.28, which is fairly low when compared to the DPH polarization value at the gel state of DMPC and DPPC vesicles (between 0.35 and 0.41). The fact that the polarization change with pressure, $d(pol)/dP$, becomes smaller at low temperatures (5.3 °C) and high pressures (>1.5 kbar) suggests a very small compressibility under those conditions. However, during the phase transition of DPPC and DMPC vesicles, the DPH polarization levels off [$d(pol)/dP \approx 0$] at the gel state. The difference in $d(pol)/dP$ values, at low temperature and high pressure, suggests that the free volume change or the compressibility is larger in DOPC vesicles, rather than in DPPC and DMPC vesicles. The slight variation of the dT/dP value with pressure in DOPC vesicles, as estimated from Figure 5, may be a result of experimental error. As most biological membranes do not exhibit clear phase transitions, the pressure dependence of DPH polarization in DOPC vesicles probably comes closer to that of actual biological membranes.

Discussion

The fluidity of a homogeneous medium may be assessed by measuring the rotational rate of a molecular probe immersed in it. An assumption necessary for the computation of the fluidity by this method is that the probe behavior is determined solely by its size and shape (Stokes–Einstein assumptions). It is known that this is not the case in practice and that the rotational rate is greatly influenced by the type of bonding interactions between probe and medium [see Mantulin & Weber (1977) and references cited therein]. Moreover, there is the additional complication of possible partition of the probe among regions endowed with different properties. However, these considerations are of secondary importance in assessing the transition point in a phase transition. What is required from the molecular probe is the capacity to generate signals that are quite distinct for the two cases so that the transition from one to the other is measured with good precision. Thus, although for a physical analysis one might prefer to use real-time depolarization methods (Chong & Cossins, 1983), the greater precision of measurement of stationary fluorescence polarization should be able to better reveal individual differences, one example being the detection of the pretransition when the temperature is changed at constant pressure and its absence when the pressure is changed isothermally. Differences between lipids with regard to their phase transitions revealed by the temperature studies are also shown in the pressure behavior. As an example, the pressure-induced phase transition in multilamellar vesicles is much sharper than in small, unilamellar vesicles, a phenomenon already observed in thermal studies in DPPC and DMPC vesicles by Lentz et al. (1976). The phase transition data from both thermal and pressure studies by DPH fluorescence polarization are listed in Table I. Generally speaking, temperature and pressure exert opposite effects; that is, high pressure has an effect equivalent to low temperature. From the thermodynamic point of view, temperature and pressure effects depend respectively on entropy and volume:

$$\left(\frac{\partial \Delta G^\circ}{\partial T}\right)_P = -\Delta S^\circ \qquad \left(\frac{\partial \Delta G^\circ}{\partial P}\right)_T = \Delta V^0 \qquad (3)$$

Their opposing actions may be explained because an increase in pressure at constant temperature results in increased molecular interactions that oppose the thermal disorder and lead therefore to a decreased entropy. Conversely, an increase in temperature at constant pressure leads to a new equilibrium in which the average intermolecular distance, and therefore the volume, is increased. Simultaneous changes in temperature and pressure that keep the volume constant bring the system to a state that differs from the original only in the mean thermal energy. We thus except that over a relatively large range, temperature and pressure effects can compensate each other. The relation between "free volume", or simply volume, and fluidity is well documented (e.g., that of oleic acid; Bridgman, 1925). Although it is not necessary to give a precise physical significance to the change in polarization of fluorescence in order to describe the lipid phase transition, it appears that the changes in fluidity calculated from the simple formula (Shinitzky & Barenholz, 1978)

$$\bar{n} = \frac{2p}{p_0 - p} \qquad (4)$$

where $\bar{n}$ is the microviscosity and p_0 is the limiting polarization of DPH can give a good description of the change in various lipidlike phases and, therefore, of the changes in viscosity during the phase transition.

Our dT/dP values for DPPC and DMPC vesicles are close to those reported by other groups (Liu & Kay, 1977; Trudell et al., 1974; Ceuterick et al., 1978; Mountcastle et al., 1978) and within the range typical for lipid-involving processes, 17–30 °C kbar^{-1} (DeSmedt et al., 1979; Wann & MacDonald, 1980). Qualitatively, we interpret the value of dT/dP as the temperature to pressure equivalence in terms of constant membrane fluidity, as inferred from DPH fluorescence polarization values. The thermodynamic significance of dT/dP makes an explanation of the range of values observed quite straightforward. At constant volume, the variation of pressure with temperature is the ratio of isothermal compressibility, β, to thermal expansivity, ϵ:

$$\left(\frac{\partial T}{\partial P}\right)_v = \frac{\beta}{\epsilon} \qquad (5)$$

From the known ϵ and β values of phospholipid vesicles, the β/ϵ values are just about 17–30 °C kbar^{-1}. For example, in the sonicated DPPC vesicles,[2] $\epsilon \approx 1.6 \times 10^{-3}$ K^{-1} and $\beta \approx 3.6 \times 10^{-5}$ bar^{-1}, giving $\beta/\epsilon = 23$ °C kbar^{-1}. We can therefore rationalize the effects of pressure as simple volume effects that produce increased molecular interactions and order in the lipid layers.

Figure 3 shows that the three vibrational bands observed in the DPH emission do not uniformly change in intensity

[2] ϵ was estimated from Figure 8 of Sheetz & Chan (1972) for the sonicated DPPC vesicles at 30 °C. β was taken from Table I of Mitaku et al. (1978) for the sonicated DPPC vesicles at 30 °C in the case of 0.5 g of water/1 g of the DPPC lipid bilayer, and with the assumption that the ratio of isothermal compressibility to adiabatic compressibility for DPPC(SUV) comes close to 1.0–1.1.

under pressure, and there are small shifts in peak maxima when the pressure changes from 1 atm to 1.1 kbar. A recent study by Knutson et al. (1982) shows that rotational heterogeneity of DPH exists in the mixture of DMPC and DPPC vesicles. They have observed a small but significant difference between the emission spectrum of DPH in pure DMPC vesicles and that in DPPC vesicles, with the spectrum of the mixture (DMPC + DPPC) between those of the component spectra. By employing time-resolved emission spectroscopy, they found that the spectrum of DPH in the mixture was time dependent. The late spectrum, assigned to the "immobilized" (better "less mobile") probes (Knutson et al., 1982) in the mixture, resembled the DPPC spectrum. The emission spectra of DPH in DMPC(MLV) at 1 atm and 1.1 kbar shown in Figure 3 in this paper are respectively similar to the early and late spectra of DPH in the mixture of DPPC and DMPC observed by Knutson et al. (1982). This implies that DPH in vesicles at high pressure is in a less mobile environment than at 1 atm.

In conclusion, we point out that besides the determination of dT/dP in various cases, studies of membrane functions under different pressures or a combination of pressures and temperatures should be sufficient to demonstrate which functions are dependent upon the fluidity, that is, the volume, of the system and which are independent of it. The former are connected with transport phenomena in one way or another, while the relative insensitivity to pressure (or volume) must be expected from ordinary chemical reactions in which covalent bond exchanges take place exclusively (Weber & Drickamer, 1983).

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