Effect of Double Bonds on the Dynamic Properties of the Hydrocarbon Region of Lecithin Bilayers†

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ABSTRACT: The motional properties of the hydrophobic fluorescent probe 1,6-diphenyl-1,3,5-hexatriene have been assessed in liposomes of various lecithins: dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylcholine, 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), 1-palmitoyl-2-linoleoylphosphatidylcholine (PLPC), and 1-palmitoyl-2-arachidonoylphosphatidylcholine (PAPC). The temperature dependence of the steady-state fluorescence anisotropy was determined within the range of 10–50 °C. Nanosecond measurements were also made and the results analyzed by a model of wobbling diffusion confined within a cone. In this treatment the cone angle (θ_c) obtained relates to the degree of order of the fatty acyl chains, and the wobbling diffusion constant (D_w) relates to the rate of motion of the hydrocarbon chains. θ_c was increased (order decreased) and D_w increased (rate increased) for the first double bond (POPC) as compared to the fully saturated DPPC. In all four unsaturated lecithins the temperature dependences of θ_c and D_w were similar: θ_c increased by about 4–5°, and D_w increased by a factor of 1.5 per 10 °C. DPPC in the liquid-crystalline phase also showed a similar trend. The absolute values, on the other hand, showed some differences: for θ_c, DOPC (78° at 37 °C) > PAPC (75°) > POPC (70°) ≃ PLPC (69°) > DPPC (20°), and for D_w, PAPC (0.28 ns^{-1}) > DOPC (0.22 ns^{-1}) ≃ PLPC (0.24 ns^{-1}) > POPC (0.21 ns^{-1}) > DPPC (0.05 ns^{-1}). The differences among the unsaturated lecithins are relatively small suggesting that the first double bond introduced into a lecithin plays the most important role at physiological temperatures. In 1:1 mixtures of the disaturated lecithin, DPPC, with the unsaturated lecithins, the temperature of the phase transition of the former was lowered and broadened, by approximately the same amount, irrespective of the differences in unsaturation.

Cell membrane phospholipids may potentially undergo considerable variation in chemical composition including a variation of the fatty acid carbon chain length and of the number and position of double bonds per chain. Together with variation in head group substitution, this gives rise to a large number of possible molecular species of phospholipids. An understanding of the relationship between composition and physical properties of the phospholipids in membranes, as a basis for the control of membrane mediated functions, has been gained partly from model membrane studies and partly from studies of the effects of specific modifications on intact membranes. Both model membranes and intact cell membranes have been used to examine the effect of modifying phospholipid fatty acyl saturation by employing a variety of techniques. These include the use of ^2H NMR[1] (Seelig & Seelig, 1977; Seelig & Waespe-Šarčevič, 1978; Gally et al., 1979; Rance et al., 1980), ESR (King & Spector, 1978; Hatton et al., 1978), and fluorescent probe techniques (Martin & Thompson, 1978; Martin & Foyt, 1978; Rintoul et al., 1978, 1979; Gilmore et al., 1979a,b; Sklar et al., 1979; Cossins et al., 1980; Stubbs et al., 1980; McVey et al., 1981). One problem in using cell systems, however, is the great complexity of the lipid composition and also the fact that changes may often occur in other lipid components which may compensate for the other alterations made.

The effects of unsaturation in a model system were first examined in monolayer studies, and the extent and number of double bonds were shown to be of importance in interactions of phospholipids with cholesterol and to correlate with liposome permeabilities (Demel et al., 1972; Ghosh & Tinoco, 1972; Evans & Tinoco, 1978). A number of physical techniques have been used which yield specific physical parameters, in order to quantify the nature of the interactions and structural roles of cholesterol, phospholipids, and proteins, in membranes. These parameters have often been interpreted in terms of "membrane fluidity", which is now recognized as a general term referring to the overall physical state of the membrane lipids. More specifically the physical state of the membrane can be considered from the point of view of (a) the order of the fatty acyl chains, often determined as the "order parameter", together with (b) the rate of motion of the fatty acyl chains, as discussed by Seelig & Seelig (1977). By use of this approach, the effect of a single cis double bond on lecithin bilayers was found to be an increase in the rate of motion and a decrease in the order (Seelig & Seelig, 1977). Previously, POPC had been investigated in a comprehensive study of various lipids by Lentz et al. (1976), and fluorescence measurements of DPH, embedded in the lipid bilayer, revealed lower microviscosity values, which relate both to the order and to the rate for POPC as compared to other saturated lecithins. Previous attempts to explain the effect of double bonds simply in terms of the percentage of phospholipids containing double bonds, i.e., the percentage unsaturation, led to the observation that changes brought about in the unsaturation of animal cell membrane phospholipids had little affect on the steady-state fluorescence polarization of DPH (Stubbs et al., 1980; McVey et al., 1981).

In this study, in order to investigate further these effects of unsaturation on the physical properties of membrane phospholipids, we have made liposomes of pure phospholipids,

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[1] Abbreviations used: DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPC, dipalmitoyl-3-*sn*-phosphatidylcholine; DOPC, dioleoyl-3-*sn*-phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl-3-*sn*-phosphatidylcholine; PLPC, 1-palmitoyl-2-oleoyl-3-*sn*-phosphatidylcholine; PLPC, 1-palmitoyl-2-linoleoyl-3-*sn*-phosphatidylcholine; PAPC, 1-palmitoyl-2-arachidonoyl-3-*sn*-phosphatidylcholine; 16:0, palmitic acid; 18:0, stearic acid; 18:1, Δ^9-oleic acid; 18:2, $\Delta^{9,12}$-linoleic acid; 20:4, $\Delta^{5,8,11,14}$-arachidonic acid; ^2H NMR, deuterium magnetic resonance; ESR, electron spin resonance.

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in which the precise position of the acyl chain(s) containing double bonds and the number and position of the bonds within the acyl chains were known (DPPC, DOPC, POPC, PLPC, and PAPC). Data are presented relating to both the order and rate motion of the fatty acyl chains, over the temperature range 10–50 °C. PLPC and PAPC, containing two and four double bonds, respectively, have not previously been examined in such a manner. We have investigated multilayer liposomes made from these lipids, on the basis of the motional properties of DPH in terms of the "static" orientational constraint (the cone angle θ_c, relating to the order) and the wobbling diffusion constant (D_w, relating to the rate of motion), using the "wobbling-in-cone" model (Kawato et al., 1977; Kinosita et al., 1977).

Materials and Methods

DPPC and DOPC were obtained from Sigma Chemical Co. POPC was extracted from egg yolk lecithin (Sigma), according to the method of Arvidson (1965). The fatty acid composition, determined by gas–liquid chromatography, revealed 9.3% of 18:0 and 2.0% of 18:2 as impurities. Positional analysis was not carried out since it is known that egg lecithin contains essentially no saturated fatty acid at position 2 and no unsaturated fatty acid at position 1 (Renkonen, 1965; Kuksis & Marai, 1967). PLPC and PAPC, obtained from Nihon Shoji (Japan), were prepared by the reaction of glycerophosphorylcholine with the fatty acid imidazole, subsequent removal of the position 2 fatty acid by phospholipase A2, and further reaction with the required fatty acid imidazole (U.S. Patent 4 130 571). The purity (data specially supplied by Nihon Shoji) for PLPC was as follows: position 1, 16:0, 92.5%, 18:2, 7.5%; position 2, 16:0, 9.1%, 18:2, 90.9%. The purity for PAPC was as follows: position 1, 16:0, 95.9%, 20:4, 4.1%; position 2, 16:0, 7.8%, 20:4, 92.2%. The content of other fatty acids was <1%. DPH was obtained from the Aldrich Chemical Co. All other chemicals used were of analar grade. Lipids were stored in chloroform or benzene (PLPC and PAPC) at −20 °C. Lipids susceptible to oxidation were stored under nitrogen.

Liposomes. Multilayer liposomes were prepared in 0.2 M phosphate-buffered saline (pH 7.4, 0.15 M sodium chloride). From the required amounts of lipid and DPH (in tetrahydrofuran), the solvents were removed by evaporation under reduced pressure. The molar ratio of lipid to DPH used was 500:1. Buffer was then immediately added and the contents of the tube vortexed thoroughly at not less than 5 °C above the phase transition temperature of the lipid (the final concentration of lipid in the liposomes was 0.2–1.0 mg/mL). The operations were performed in the dark, and the preparations were used immediately. Lipids containing two or more double bonds in a single fatty acyl chain are susceptible to oxidation, and tests were therefore performed on liposome preparations treated in the same manner as those used for fluorescence measurements. PLPC was tested by the thin-layer chromatographic method of Oette (1965) and also by the diene conjugation method of Klein (1970), but oxidation products were not found. For PAPC, oxidation products of the order of 1–2% were detectable by the thiobarbituric acid method of Dahle et al. (1962), after treatment of liposomes for 1 h at 50 °C. These may have had some effects on the fluorescence measurements, although heating a sample from 7 to 50 °C and recooling, over a period of over 3 h, had no detectable effect on the fluorescence anisotropy. With respect to nanosecond measurements, we made a series of measurements on the same sample, in the order 10, 25, 37, 50, and 10 °C, taking ~1.5 h for each measurement and the results of the first and last agreed within experimental error for all the parameters determined.

Fluorescence Measurements. Measurements of steady-state fluorescence anisotropy were made at 1 °C intervals for continuously increasing temperatures (15 or 30 °C/h) from below 10 to 50 °C. Once the temperature reached 50 °C, the procedure was reversed and the sample gradually cooled again. In all experiments the heating and cooling curves coincided. For the nanosecond measurements samples were measured at 10, 25, 37, and 50 °C.

Instrumentation. Decays of fluorescence anisotropy and total fluorescence intensity were measured by a single photon counting fluorometer. The present instrumentation (details to be described elsewhere) incorporates a number of modifications over the previous design (Kinosita et al., 1976). The light source consisted of a free-running discharge lamp, filled with hydrogen at 11–14 atm, providing light pulses of duration of <0.8 ns with a frequency of 6–10 kHz. The excitation light was first passed through a 8-nm band-pass monochromator (360 nm), a Glan prism polarizer and then a Hoya U-340 filter. The sample was contained in a temperature-controlled water jacket, with a tip thermister directly immersed into the sample. The emission light was passed through Hoya L-39 and L-42 filters (transmission above 420 nm) and split into vertical and horizontal components by a pair of Polaroid HNP′B sheet polarizers, and each component was passed into separate Hamamatsu R943-02 photomultiplier tubes, operated at −20 °C. By the use of two photomultiplier tubes, the vertical and horizontal components could be measured simultaneously. With this system it was possible to use DPH concentrations as low as 0.2 μM for nanosecond measurements taking ~1.5 h, an improvement over the previous system. The response function of the apparatus was measured by using dilute Ludox (Du Pont) in place of the sample, at 455 nm (Kawato et al., 1977). The apparatus was run by a DEC LSI-11 microcomputer.

Analysis of Fluorescence Data. The nanosecond decay data were analyzed by assuming exponential decays of the forms

$$I_T^{\delta}(t) = \alpha_1 \exp(-t/\tau_1) + \alpha_2 \exp(-t/\tau_2) \quad (1)$$

$$r^{\delta}(t) = (r_0 - r_\infty) \exp(-t/\phi) + r_\infty \quad (2)$$

where $I_T^{\delta}(t)$ is the decay of the total fluorescence with time t, τ_1, τ_2 and α_1, α_2 are the fluorescence lifetimes under double-exponential approximation and their respective decay amplitudes, $r^{\delta}(t)$ is the decay of fluorescence anisotropy with time, r_0 is the anisotropy in the absence of motion, taken to be 0.395 (Kawato et al., 1977), ϕ is the apparent relaxation time, and r_∞ is the residual equilibrium anisotropy. The superscript δ indicates that these quantities are responses to truly impulsive (δ function like) excitation (Kinosita et al., 1976, 1977; Kawato et al., 1977). The decay of the total fluorescence intensity was routinely analyzed as a double-expontential decay, although generally the results indicated that to a good approximation the decay could be described as a single exponential (see Figure 1).

In this paper we analyzed the anisotropy data in terms of the wobbling-in-cone model (Kawato et al., 1977; Kinosita et al., 1977), where the major axis of DPH wobbles uniformly within a cone of half-angle θ_c around a normal to the membrane and is related to r_∞

$$r_\infty/r_0 = [(1/2) \cos \theta_c(1 + \cos \theta_c)]^2 \quad (3)$$

and the wobbling diffusion constant in the cone, D_w, is approximately given by

$$1/\phi = D_w/\langle\sigma\rangle \quad (4)$$

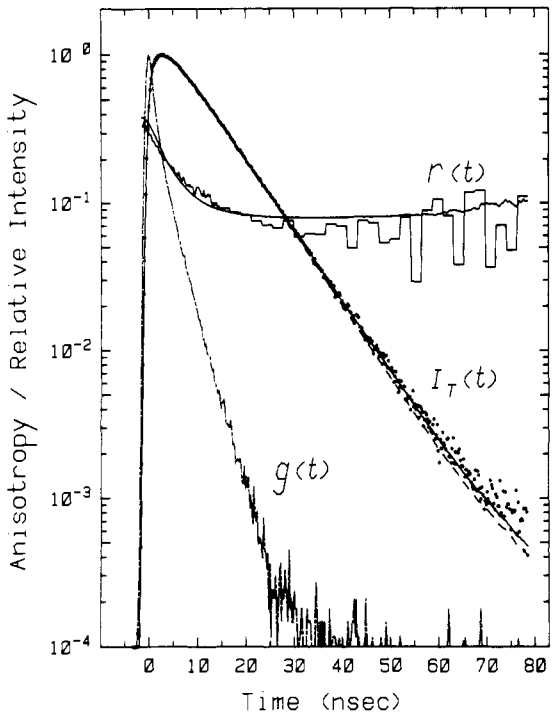

FIGURE 1: A typical fluorescence decay of DPH in multibilayer phospholipid liposomes (in this example: PLPC at 10 °C). Chain line, the instrumental response function, g(t); dots, the total fluorescence intensity, $I_T(t)$; zigzag solid curves, fluorescence anisotropy, $r(t)$. The broken and solid lines superimposed on $I_T(t)$ are calculated best-fit curves for single- and double-exponential approximations, respectively. The smooth line superimposed on the $r(t)$ curve is the calculated best-fit curve according to eq 2. Experimental conditions were as described under Materials and Methods.

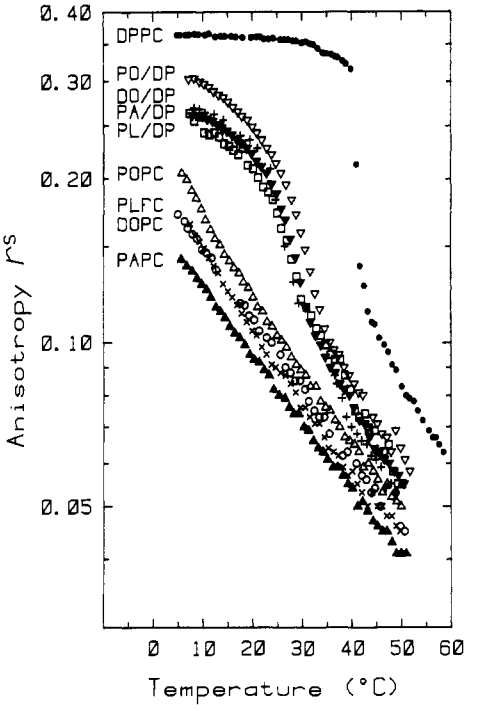

FIGURE 2: Temperature dependence of the steady-state fluorescence anisotropy (r^s). Single-component multibilayer liposomes: DPPC (●); POPC (△); PLPC (○); DOPC (×); PAPC (▲). Two-component liposomes (1:1 mixtures): POPC/DPPC = PO/DP (▽), DOPC/DPPC = DO/DP (+), PAPC/DPPC = PA/DP (▼), PLPC/DPPC = PL/DP (□).

where σ is a constant that depends only on θ_c.

The steady-state anisotropy r^s contains contributions from both the order and rate of motion of the fatty acyl chains. From eq 1 and 2 we have

$$r^s = (r_0 - r_\infty)\frac{\phi}{\langle\tau\rangle}\left(\frac{\alpha_1}{1+\frac{\phi}{\tau_1}} + \frac{\alpha_2}{1+\frac{\phi}{\tau_2}}\right) + r_\infty \quad (5)$$

where $\langle\tau\rangle \equiv \alpha_1\tau_1 + \alpha_2\tau_2$ (average fluorescence lifetime).

The first term can be considered to represent the dynamic contribution (it should be noted that the term is dependent on both D_w and θ_c), and the second term represents the static contribution to the steady-state anisotropy [see also Hildebrand & Nicolau (1979)]. The order parameter used in NMR and ESR is related to r_∞ (Kawato et al., 1977), and this relationship has recently been discussed in detail (Heyn, 1979; Jähnig, 1979; Lipari & Szabo, 1980).

Results and Discussion

In this study we have examined the physical properties of lecithins with an increasing number of double bonds in the hydrocarbon chain esterified at position 2. The results have been analyzed in terms of the motional properties of DPH embedded in the membrane according to the wobbling-in-cone model (Kawato et al., 1977; Kinosita et al., 1977). The basic parameters measured were the static orientational constraint, given by the cone angle θ_c (which relates to the "average order" of the fatty acyl chains), and the wobbling diffusion constant, D_w (which relates to the rate of motion of the fatty acyl chains), measured at 10, 25, 37, and 50 °C. The steady-state anisotropy, r^s, which relates both to the order and to the rate, was determined at 1 °C intervals over the range 10–50 °C.

Single-Component Liposomes. The DPH technique has been widely applied in the study of disaturated lecithins. Studies include those based on steady-state measurements (Lentz et al., 1976; Andrich & Vanderkooi, 1976) and time-resolved measurements (Chen et al., 1977; Kawato et al., 1977; Lakowitz et al., 1979a,b). Although the disaturated lecithin DPPC has a phase transition which is above mammalian physiological temperatures (41 °C), it is nevertheless found in cell membranes, for instance as 18% of the lecithins of lymphocytes (Morimoto & Kanoh, 1980). In this study, in order to determine the effect of the first double bond, we have examined the motional properties of DPH in POPC, which is the most commonly occurring lecithin, and compared it with DPPC. The results for the r^s vs. temperature plots (Figure 2) show a large decrease on introduction of the first double bond, even at a temperature of 10 °C or more above the phase transition of the fully saturated lecithin. This result is in very close agreement with that of Lentz et al. (1976), who used a synthetic POPC in contrast to the naturally occurring POPC used here. This shows that the low levels of linoleate and stearate in our POPC had little effect on the results. Previously, Seelig & Seelig (1977) compared in detail POPC and DPPC by using ^{2}H NMR, which yields information on the order of specific carbons of the hydrocarbon chains. The order was found to substantially decrease on the introduction of the first double bond, and this was also shown in the present work for θ_c (Figure 3a), which relates to the average order across the bilayer. ESR measurements, in the same study by Seelig & Seelig (1977), indicated that the effect of a single first double bond alone was to increase the rate of motion, and this was confirmed in the present study where an increase in D_w was found (Figure 3b).

Another common unsaturated fatty acyl lecithin of natural membranes is PLPC. The difference between POPC and PLPC lies in the extra double bond in the hydrocarbon chain esterified at position 2. Generally the results for POPC and

Table I: Analysis of the Fluorescence Data for Lipids Labeled with DPH[a]

	temp (°C)	r^s	r_∞	ϕ (ns)	D_w (ns^{-1})	θ_c (deg)	α_1	τ_1 (ns)	α_2	τ_2 (ns)	$\langle\tau\rangle$ (ns)	τ (ns)
DPPC	10	0.363	0.358 (0.001)	1.37	0.014 (0.002)	14.6 (0.1)	0.16	3.4	0.84	9.6	8.7	9.3
	25	0.357	0.353 (0.001)	0.86	0.029 (0.013)	15.6 (0.3)	0.14	2.9	0.86	9.8	8.8	9.5
	37	0.331	0.326 (0.001)	0.78	0.049 (0.014)	20.4 (0.2)	0.17	2.6	0.83	9.9	8.7	9.6
	50	0.091	0.054 (0.003)	0.75	0.290 (0.012)	60.9 (1.1)	0.12	1.8	0.88	8.2	7.4	8.0
DOPC	10	0.149	0.031 (0.001)	3.51	0.069 (0.001)	66.4 (0.3)	0.25	2.6	0.75	8.9	7.3	8.3
	25	0.089	0.013 (0.002)	1.84	0.136 (0.003)	73.5 (0.9)	0.25	2.2	0.75	8.2	6.7	7.7
	37	0.063	0.007 (0.004)	1.19	0.215 (0.012)	77.6 (2.7)	0.20	2.5	0.80	7.5	6.5	7.1
	50	0.045	0.001 (0.000)	0.76	0.340 (0.020)	84.8 (1.0)	0.21	2.2	0.79	6.7	5.7	6.3
POPC	10	0.171	0.096 (0.006)	2.93	0.062 (0.001)	52.3 (1.0)	0.22	2.8	0.79	9.8	8.3	9.3
	25	0.104	0.040 (0.003)	1.80	0.127 (0.007)	63.8 (0.8)	0.20	2.4	0.80	9.1	7.7	8.7
	37	0.071	0.021 (0.005)	1.17	0.209 (0.006)	69.8 (1.8)	0.23	1.6	0.77	8.4	6.9	8.1
	50	0.050	0.013 (0.006)	0.72	0.348 (0.022)	73.7 (3.2)	0.21	1.7	0.79	7.5	6.3	7.2
PLPC	10	0.157	0.083 (0.003)	2.70	0.072 (0.003)	54.6 (0.6)	0.14	4.2	0.86	8.9	8.3	8.6
	25	0.102	0.042 (0.002)	1.62	0.139 (0.008)	63.3 (0.6)	0.10	3.3	0.90	8.4	7.8	8.2
	37	0.072	0.025 (0.007)	1.01	0.241 (0.010)	68.6 (2.5)	0.14	2.8	0.87	7.8	7.1	7.5
	50	0.049	0.015 (0.005)	0.65	0.384 (0.014)	72.9 (2.3)	0.17	2.5	0.83	7.1	6.3	6.8
PAPC	10	0.128	0.051 (0.006)	2.22	0.098 (0.002)	61.1 (1.5)	0.19	3.1	0.81	8.7	7.6	8.3
	25	0.080	0.023 (0.004)	1.32	0.184 (0.014)	69.2 (1.5)	0.18	2.2	0.82	7.9	6.9	7.7
	37	0.059	0.012 (0.004)	0.89	0.283 (0.014)	74.5 (2.4)	0.16	1.9	0.84	7.3	6.4	7.1
	50	0.041	0.008 (0.001)	0.56	0.453 (0.016)	76.8 (0.9)	0.17	1.7	0.84	6.6	5.8	6.3

[a] r_∞, residual equilibrium anisotropy; ϕ, apparent relaxation time; r^s, steady-state fluorescence anisotropy; D_w, wobbling diffusion constant; θ_c, cone angle; τ_1, τ_2 and α_1, α_2, fluorescence lifetimes under double-exponential approximation and their respective relative decay amplitudes; $\langle\tau\rangle \equiv \alpha_1\tau_1 + \alpha_2\tau_2$; τ, fluorescence lifetimes under single-exponential approximation. Results are expressed as the mean of three or more experiments; standard deviation is in parentheses.

PLPC were very similar, over the temperature range examined, although definite differences were found. The results of the r^s vs. temperature plot (Figure 2) show that at the lower temperature the values for POPC and PLPC diverge. The results of the nanosecond experiments (Table I) reveal that the difference in r^s is due to small differences in both D_w and θ_c.

The lecithin PAPC was examined, since arachidonic acid has been implicated in many cell processes and since it contains four double bonds. The results for the r^s vs. temperature plot (Figure 2) show lower values for r^s compared to those for POPC and PLPC especially at lower temperatures. The differences are, however, rather small. Comparisons are better made for θ_c and D_w, and the results given in parts a and b of Figure 3 reveal that the values for θ_c and D_w for PAPC are only slightly greater than those of POPC and PLPC. It should be noted here that parameters relating to the order and rate may change in a somewhat independent manner as discussed by Seelig & Seelig (1974).

The lecithin DOPC was investigated to compare the situation where there are two double bonds on a single hydrocarbon chain of lecithin, the other being saturated (PLPC), with one double bond on each chain (DOPC). The r^s vs. temperature plot (Figure 2) for PLPC and DOPC revealed no differences in the temperature range 10–50 °C. However, in terms of the cone angle θ_c (Figure 3a) DOPC gave a value greater than PLPC and even greater than PAPC. In terms of D_w DOPC and PLPC were essentially identical. The lack of a difference in r^s, in spite of the difference in θ_c, can be explained by the relatively small contribution of r_∞ to r^s at the temperatures measured (see below) and the fact that the differences between τ, ϕ, and r_∞ for the two lipids tend to compensate for each other leaving r^s unchanged (see eq 5 and Table I).

If the values of r_∞ and r^s are compared (Table I), the relative contributions of the dynamic component to the steady-state anisotropy can be determined by referring to eq 5. It is clear from the results of the present work that below the phase transition, the main contribution to r^s is from the static component and that the opposite is true above the phase transition. On increasing the temperature even further above the transition, the contribution of the static component to r^s eventually becomes very small and can be virtually neglected (e.g., DOPC at 50 °C, see Table I).

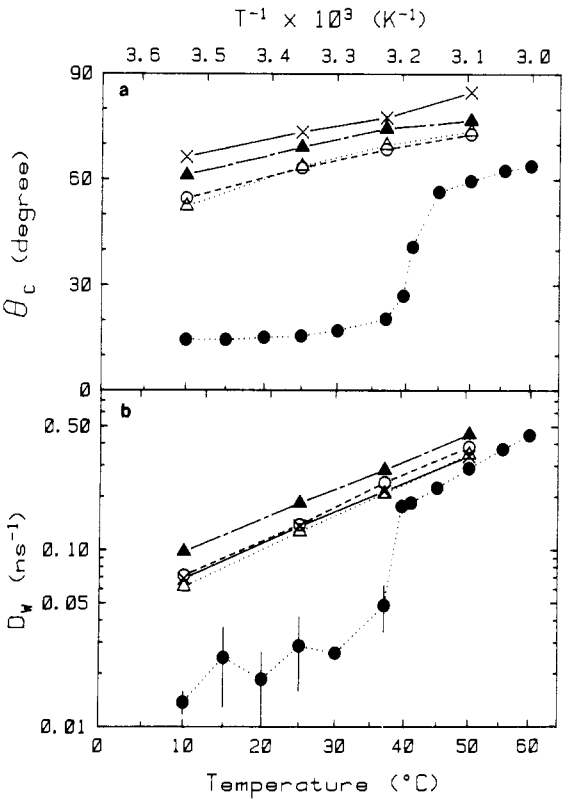

FIGURE 3: (a) Temperature dependence of the cone angle (θ_c). (b) Temperature dependence of the wobbling diffusion constant (D_w). Except where shown, the size of the errors in the measurements are approximately equal to the size of the symbols. The data points at 10, 25, 37, and 50 °C represent the mean of at least three separate experimental values. For an explanation of the symbols, see the legend to Figure 2.

It has been pointed out by Seelig & Seelig (1977) that, according to theories describing phase transitions, at equal temperatures above the phase transition different systems are subject to the same average molecular forces. When they

compared DPPC and POPC in this manner, at 19 °C above the respective phase transitions, the ^2H NMR derived order profiles for various labeled carbon atoms along the chain were accordingly found to be parallel except for the region between carbon atoms 4 and 10 (near the double bond); also under this condition of comparison the unsaturated system now was the more ordered. In the present study comparison can be made of DOPC, POPC, and DPPC, also at 19 °C above the phase transition [the phase transition of DOPC is −22 °C (Phillips & Hauser, 1972), POPC, −5 °C (de Kruijff et al., 1973), and DPPC, 41 °C (from present data)]. This comparison reveals a cone angle (from Figure 3a) of close to 60° for each of the lipids, in agreement with the theory. Whereas the structural parameter θ_c represents an equilibrium property, the kinetic parameter D_w involves collision frequency. The magnitude of D_w therefore cannot be simply related to the transition temperature. Of course, it is not entirely appropriate to compare the results of the ^2H NMR and DPH studies since they are both concerned with very different types of motion. Over the range of temperature examined, for the unsaturated lipids, the curves obtained for D_w (Figure 2b) were almost parallel, and from the slope of the curves the apparent activation energies were calculated to be 7–8 kcal/mol.

The findings of the present work can be usefully compared with the monolayer studies of Ghosh & Tinoco (1972), who examined a series of synthetic lecithins. It was shown that at a pressure of 40 dyn/cm, for example, the area per molecule (Å^2) is 55.3 for DPPC, 65.8 for POPC, 67.7 for PLPC, 76.3 for 1-palmitoyl-2-($\Delta^{9,12,15}$-linolenoyl)phosphatidylcholine, and 70.0 for PAPC. Thus as found in the present study the first double bond produced a much more marked effect compared to the second and subsequent double bonds. This was also shown in the study of Evans & Tinoco (1978) for an increasing number of double bonds in a chain consisting of 20 carbons esterified at the position 2 of phosphatidylcholine. Here the importance of the position as well as the number of bonds along the chain was emphasized. Barton & Gunstone (1975) previously demonstrated the importance of the position of the first double bond in POPC and isomers with the bond in other positions.

Two-Component Liposomes. When DPPC and DOPC are mixed in a 1:1 ratio, the average number of double bonds per lecithin molecule is the same for the mixture as is found in POPC (i.e., for an average of one double bond per lecithin molecule). However, the phase transition of POPC is −5 °C (de Kruijff et al., 1973) while according to the heating curve obtained for DPPC/DOPC, the transition is much higher (Lentz et al., 1976; S. M. Johnson, C. D. Stubbs, and A. D. Smith, unpublished results and Figure 2). In the present work we have extended the investigation to include mixtures of the mixed acid lecithins with DPPC (Figure 2). The results show that the phase transition for these mixtures occurs at a lower temperature and over a much broader temperature range than is the case for DPPC alone. Interestingly, the transition temperatures for all the mixtures of DPPC with unsaturated phospholipids fall within a narrow range (25–30 °C) whereas the transitions of the unsaturated phospholipids alone, as single component liposomes, lie within a much broader range.

In a number of previous studies the effect on the phase transition of free fatty acids, added in various amounts to specified lecithins, has been investigated (Eliasz et al., 1976; Usher et al., 1978; Verma et al., 1980). A useful comparison can be made here of the effect of a free fatty acid added to DPPC with the effect of the same acid added as a position 2 component of the phospholipid in liposomes. The effect of adding saturated acids to disaturated lecithins is to raise the transition temperature (Eliasz et al., 1976), while with the unsaturated acids the effect is to lower and broaden the transition temperature (Usher et al., 1978; Verma et al., 1980). Thus, with DPPC Verma et al. (1980) showed that the addition of oleic acid causes the transition midpoint to be lowered to 35 °C, while with linoleic acid it was 28 °C (lecithin/fatty acid was 1:0.6). These results are very similar to the results with the two-component liposomes of the present study. Thus, a much lower phase transition occurs when the first single double bond occurs in the fatty acid chain of the lecithin molecule itself, rather than by adding to the lecithin bilayer a further free fatty acid containing the double bond. This may have important implications in cells where fatty acids are released into the membrane from the position 2 of membrane phospholipids by phospholipases (as in the synthesis of prostaglandins), to be replaced by other fatty acids from exogenous sources.

General Discussion. The order of the hydrocarbon chains as sensed by DPH may be thought of as an average order for the membrane bilayer. It is important to distinguish between terms relating to this average order and the order parameter of specifically deuterated carbons of the hydrocarbon chains, which give information pertaining to specific areas of the bilayer. Thus Seelig & Waespe-Šarčevič (1978) have pointed out that the results of the ^2H NMR studies indicate that the area around the double bond may be more ordered than areas nearer the methyl end of the hydrocarbon chain. Further, they concluded that this may lead to the existence of distinct structural zones, parallel to the bilayer surface, and that therefore these lipids, which are in the liquid-crystalline state, cannot be described as being in a fully disordered state as might be expected. This conclusion is in full accord with the results of the present work where even at 50 °C lipids in the liquid-crystalline state show definite differences in the average order (as well as in the rate). It is possible then that phospholipids with multiple double bonds may possibly contain a complex of structurally different zones within the bilayer at physiological temperatures. Only ^2H NMR type studies, yielding order parameters of specific sections along the chain length, could verify this point. Also ESR studies such as those of King & Spector (1978), sensitive to specific regions within the bilayer, are relevant here. However, the extent to which processes such as enzyme activities, transport functions, and receptor functions might be sensitive to such differences remains to be determined. The possibility that membrane proteins may be sensitive not only to changes in the average order but also may be subject to fine control from specific areas within the bilayer with differing degrees of order merits further investigation.

In summary, the main conclusion arising from this work is that in contrast to the large decrease in the average order of the bilayer and the increase in the rate of motion within the bilayer, brought about by the first double bond in a phospholipid, as demonstrated previously and also in the present work, the second and subsequent double bonds produce changes of a much smaller magnitude. Although there is a large increase in D_w and θ_c on the introduction of the first double bond and only relatively small changes for the second and fourth double bonds, this should not be taken as a reflection of the unimportance of such small differences with regard to cell membrane functions which may well be sensitive to small differences in the physical state of the membrane lipids. Indeed the wobbling diffusion constant, at 37 °C, increases in proportion to the number of double bonds, at least

from the first to the fourth bond. The relationship between changes in the absolute magnitude of these parameters, as affected by unsaturation, with changes in cell function such as membrane enzyme activities would be a worthwhile subject of further study.

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