Motions and Interactions of Phospholipid Head Groups at the Membrane Surface. 3. Dynamic Properties of Amine-Containing Head Groups†

Jeffrey L. Browning*

ABSTRACT: The dynamic properties of the amine-containing head groups of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and a new phospholipid, phosphatidylserine methyl ester, were studied. Deuterium NMR spin–lattice (T_1) relaxation times of deuterium labels specifically incorporated in the head groups were measured in multilamellar dispersions of these phospholipids. As a reference point, the data were compared with T_1 values from a phospholipid with a simple phosphopropanol head group. In the liquid-crystalline state at both the α (P–O–CD_2–CH) and β (P–O–CH_2–CD) head-group segments, the values of the T_1 relaxation times decreased in the order propyl > choline > ethanolamine > serine = serine methyl ester. In the propyl and choline head groups, the β-segment T_1 values were longer than those of the α segment, indicating increasing flexibility as one progressed toward the free end of the head group. The ethanolamine and serine head groups had essentially identical T_1 values at all positions. Phosphorus-31 NMR spin–lattice relaxation times were found to parallel the deuterium results for the neighboring α segment. These data indicate that the phosphatidylserine head group is less flexible than that of phosphatidylethanolamine which in turn was more rigid than the phosphatidylcholine head group. The phosphatidylserine head-group T_1 values were as short as those of the glycerol backbone moiety of phosphatidylcholine which is known to be a relatively rigid section of the phospholipid molecule. The relaxation time data for liquid-crystalline phase phosphatidylserine and gel phase phosphatidylglycerol were quantitatively similar, indicating that for those motions which contribute to T_1 relaxation, the two head groups are in similar states. These differences in head-group rigidity are discussed in terms of the capacity of the various head groups to bind noncovalently to their neighbors in the plane of the membrane surface.

In the first two papers of this series (Browning, 1981a,b), interactions at the membrane surface between phospholipid head groups were studied in relatively simple systems. First, alkyl head groups were investigated, resulting in a set of reference data for the case where no noncovalent interactions (ignoring van der Waals interactions) were possible with the esterified portion of the head group. Next, hydroxyl groups were introduced onto the alkyl "skelton", and the experiments indicated the presence of hydrogen bonds between the hydroxyl groups and other phosphates. In this paper, another class of phospholipids whose head groups contain amine functions has been examined. This group encompasses most of the abundant naturally occurring phospholipids including phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine.

Interactions at the surfaces of phospholipid bilayers whose head groups contain amine groups have long been postulated [see reviews by Boggs (1981) and Hauser & Phillips (1979)]. A hydrogen-bonded ion pair of the type $-PO_2^-\cdots H-{}^+NH_2-$ is called a *proton transfer complex*, a terminology which includes both the hydrogen-bonding and electrostatic components of this type of bond (Vinogradov & Linnell, 1971). There is a fair amount of indirect experimental evidence supporting the existence of this bond in membranes of phosphatidylethanolamine. Increased phase transition temperatures (Jacobson & Papahadjopoulos, 1975; Michaelson et al., 1974; Phillips et al., 1972) and decreased surface areas relative to those for phosphatidylcholine (Phillips & Chapman, 1968; Hayashi et al., 1972) attest to the presence of this type bond. Measurements of surface viscosity (Hayashi et al., 1975) while being difficult to interpret quantitatively (Adamson, 1976) have revealed that the interface region of phosphatidylethanolamine monolayers is considerably more viscous than the surface of a phosphatidylcholine layer. These authors suggested that the surface of the phosphatidylethanolamine monolayer may be almost "polymeric" in character. More directly, a hydrogen-bonded proton observed with proton NMR (Seimiya et al., 1978), bond shortening in a number of crystal structures [cf. Sundaralingam (1972) and Elder et al. (1977)], and infrared spectroscopy measurements (Akutsu et al., 1975) have supported the concept of this noncovalent interaction in the phosphatidylethanolamine head group. These infrared studies have shown that such a bond not only is present in dehydrated lipid films but also is retained in the hydrated state (Akutsu et al., 1981). The decreased affinity of water for the phosphatidylethanolamine head group relative to phosphatidylcholine was attributed by these authors to strong noncovalent bonding in the phosphatidylethanolamine head group. NMR studies of proton line-width changes (Michaelson et al., 1974) and $^{31}P\{^1H\}$ nuclear Overhauser effects (Yeagle et al., 1975, 1976, 1977) and anisotropy in the motion of the water bound to lipid head groups [cf. Hauser & Phillips (1979) and Finer & Darke (1974)] have led to similar conclusions.

Structural aspects of this amine-containing set of phospholipids have been studied by using a variety of techniques including ^2H NMR [see reviews by Seelig & Seelig (1980), Hauser & Phillips (1979), Büldt & Wohlgemuth (1981), Yeagle (1978), and Hauser et al. (1980)]. However, information on the dynamic properties of these head groups exists only for phosphatidylcholine (Lee et al., 1976; Gally et al., 1975; Burns & Roberts, 1980). The NMR relaxation behavior of a deuterium nucleus provides a direct approach for the study of the dynamic properties of a C–D segment. With a simple analysis, the relative rates of head-group motion in nonsonicated lipid bilayers are obtained. From the relative rates of motion and a consideration of the head-group chemistry, it is hoped that the existence or nonexistence of noncovalent interactions in the plane of the membrane surface may be

† From the Biocenter, Department of Biophysical Chemistry, University of Basel, CH-4056 Basel, Switzerland. *Received January 7, 1981.* This work was supported by Swiss National Science Foundation Grant 3.409.78.

* Address correspondence to the Department of Physiology, School of Medicine, University of California, San Francisco, CA 94143.

0006-2960/81/0420-7144$01.25/0

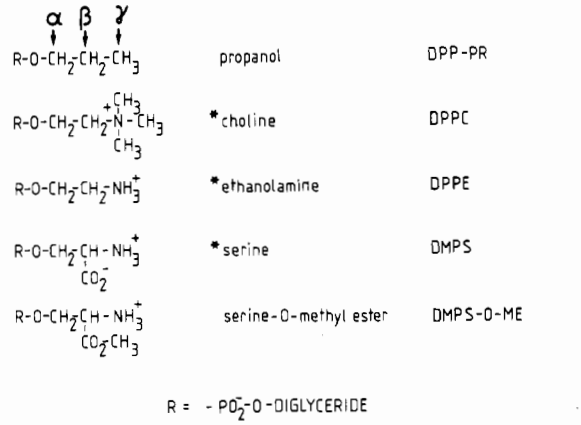

FIGURE 1: Head-group structures, names, and abbreviations of the phospholipids used in this study. Charged forms refer to the states found at pH 7.0 under these conditions. In this system of abbreviations, DPP, DMP, and DOP refer to 1,2-dipalmitoyl-*sn*-glycero-3-phospho-, 1,2-dimyristoyl-*sn*-glycero-3-phospho-, and 1,2-dioleoyl-*sn*-glycero-3-phospho-, respectively. Compounds marked with asterisks are found in nature. Phase transition temperatures (°C) of these lipids under these conditions are DMPC 23, DPPC 41, DMPE 49, DPPE 63, DMPS 36, DOPS −11, DPP-PR 42, and DPPG 41.

deduced. These dynamic properties were examined both in membranes of the pure lipid and in mixtures with either phosphatidylcholine or cholesterol. As a premise, phosphatidylcholine addition should not affect the spacing of the proton transfer acceptors, whereas cholesterol will increase the separation between all of the groups bonding in an intermolecular manner. The study of these mixtures represents an attempt to separate inter- from intramolecular bonding.

Experimental Procedures

The structures, names, and abbreviations of the lipids used in this study are presented in Figure 1. The head-group carbon segments are labeled, starting from the phosphate as α, β, and γ as shown in this figure. Head-group-deuterated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholines (DPPC's)[1] and DPPE's were the generous gifts of Dr. J. Seelig (Biocenter, Basel). DMPS was prepared as previously described (Browning & Seelig, 1979).

*N-tert-Butoxycarbonyl-*DL*-serine Methyl Ester (I).* *N-tert*-Butoxycarbonyl-DL-serine dicyclohexylamine salt (4 g, 10 mM), synthesized after Moroder et al. (1976), was reacted with 25 g (177 mM) of iodomethane in 40 mL of dry dimethylformamide at 30 °C. After 1 h, the precipitated dicyclohexylamine iodide was removed by filtration and the solvent was removed by distillation (bath at 60 °C, 12 mmHg). The product was taken up in carbon tetrachloride, and any insoluble salts were removed. The solvent was removed and replaced with dichloromethane. The product was chromatographed over silica gel and could be eluted with 50/1 dichloromethane/methanol. The resultant preparation contained only the product and some dicyclohexylamine salts. The salts were removed by treatment with Dowex 50 as described (Browning & Seelig, 1979). The product in dichloromethane was washed with aqueous sodium acetate, pH 5.5. The organic phase was dried over sodium sulfate, the solvent was removed, and the resultant oil was dried under high vacuum. The product was deemed pure enough for coupling to phosphatidic acid. The product (yield 30–40%) was ninhydrin positive on silica gel thin-layer plates with an R_f of 0.74 with a chloroform/methanol/H_2O (65/25/4) solvent system. Selectively deuterated I was prepared from 2-deuterated (–CH–CD_2–OH) and 1-deuterated (–CD–CH_2–OH) *N-tert*-butoxycarbonyl-DL-serine dicyclohexylamine salt whose syntheses have been described (Browning & Seelig, 1979). Proton NMR ($CDCl_3$, 60 mHz) showed that the serine resonances of the product corresponded exactly to those of the similar compound *N-tert*-butoxycarbonyl-DL-serine phthalimidomethyl ester (Browning & Seelig, 1979) and that the deuterium labels were retained in the desired positions δ 1.45 (singlet, 9.7 H, *tert*-butyl), 3.0 (singlet, 1.2 H, –OH), 3.9 (singlet, 3.4 H, –O–CH_3), 3.9 (singlet in the 1-deuterated compound, 2 H, –CH_2–OH), 4.35 (doublet in the 2-deuterated compound, 1.1 H, –CH–CH_2OH), and 5.57 (doublet, 1 H, –CO–NH–, this signal collapsed to a singlet in the 1-deuterated compound).

*1,2-Dimyristoyl-sn-glycero-3-phospho-*DL*-serine Methyl Ester.* This compound was prepared by coupling 1,2-dimyristoyl-*sn*-glycero-3-phosphoric acid to I with the triisopropylsulfonyl chloride method as described for DMPS (Browning & Seelig, 1979; Aneja et al., 1970). The *tert*-butoxycarbonyl amine protective group was cleaved with trifluoroacetic acid as described by Browning & Seelig (1980). The resultant product was purified by silica gel chromatography. The column was prepared with dichloromethane/methanol (50/1), and the product was eluted with a 20/1 solution. DMPS-OME was obtained as a white powder after precipitation with acetone, R_f = 0.6 for TLC on silica gel in chloroform/methanol/H_2O (65/25/4), ninhydrin positive. Anal. Calcd for $C_{35}H_{68}O_{10}NP{\cdot}H_2O$ (M 711): C, 59.12; H, 9.84; N, 1.97. Found: C, 58.06; H, 9.89; N, 2.12. The identity of the product was confirmed by comparison with the product obtained by transesterification of DMPC with serine methyl ester hydrochloride salt by phospholipase D. In 70, 25, 12, and 6% (w/v) solutions of serine methyl ester hydrochloride [synthesis of Guttmann & Boissonnas (1958)], DMPC was reacted with cabbage phospholipase D after the procedure of Comfurius & Zwaal (1977) at 40 °C. High concentrations of serine methyl ester were apparently inhibitory for the enzyme. After 15 h, thin-layer chromatography of the 12 and 6% solutions showed in addition to the expected hydrolysis product, phosphatidic acid, a small amount of a ninhydrin- and phosphate-positive lipid, i.e., the transesterification product with serine methyl ester, DMPS-OME. This product comigrated on thin-layer plates with the totally synthetically prepared DMPS-OME.

Sample Preparation. Multilamellar lipid dispersions were formed in 50 mM Pipes–Tris, pH 7.0, with 0.1 M NaCl and 1.0 mM EDTA as described (Browning, 1981a,b). Lipid mixtures were first dissolved in chloroform/methanol (2/1), and the solvent was removed. This process was repeated with pure chloroform followed by extensive drying under high vacuum. This sample was then dispersed as described above.

Measurements. ^2H NMR experiments were carried out as described previously (Browning, 1981a). ^{31}P NMR spin–lattice relaxation times were determined with nondeuterated, multilamellar dispersions of the lipids in the buffer system described above. Measurements were performed at 36.4 mHz with a Bruker HX-90 spectrometer with an inversion–recovery pulse sequence. The 90° pulse was 9–11 μs. No broad-band proton decoupling power was applied.

[1] Abbreviations used: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DMPE, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DMPS, 1,2-dimyristoyl-*sn*-glycero-3-phospho-DL-serine; DOPS, 1,2-dioleoyl-*sn*-glycero-3-phospho-DL-serine; DMPS-OME, 1,2-dimyristoyl-*sn*-glycerophospho-DL-serine methyl ester; DPP-PR, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-1-propanol; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol; Pipes, 1,4-piperazinediethanesulfonic acid.

Table I: ^2H Spin-Lattice (T_1) Relaxation Times (ms) for a Series of Phospholipids with Amine-Containing Head Groups (47 °C)

deuterated position in head group	lipid	head group	DMPC (mol %) 0	DMPC (mol %) 50	cholesterol (mol %) 50
α	DPP-PR	propyl	32 ± 1	27 ± 1	33 ± 1
	DPPC	choline	26 ± 1		33 ± 1
	DPPE	ethanolamine	20 ± 2[a]	22 ± 1	34 ± 1
	DMPE	ethanolamine	20 ± 1		
	DMPS[b]	serine	8.5 ± 0.2	10.6 ± 0.2	11.7 ± 0.4
	DOPS[b]	serine	10.8 ± 0.3		
	DMPS-OME	serine methyl ester		7.3 ± 0.5	10.9 ± 0.4
β	DPP-PR	propyl	56 ± 10	53 ± 3	
	DPPC	choline	39 ± 1		42 ± 1
	DPPE	ethanolamine	22 ± 1[a]	23 ± 1	36 ± 1
	DMPS	serine	9.3 ± 0.3	11.9 ± 0.3	10.6 ± 0.2
	DOPS	serine	11.8 ± 0.2		
	DMPS-OME	serine methyl ester		7.5 ± 0.2	11.0 ± 0.4
γ	DPP-PR	propyl	195 ± 20	173 ± 20	
	DPPC	choline	91 ± 2		

[a] Extrapolated from an Arrhenius plot of data obtained from fluid state DPPE. [b] Both deuterons had the same T_1.

Results

^2H Spin–Lattice (T_1) Relaxation Times. ^2H spin–lattice (T_1) relaxation times of multilamellar dispersions of headgroup-deuterated phospholipids were measured with an inversion–recovery experiment. The recovery of magnetization could be characterized by one decay time as was indicated by the linearity of a ln ($M_0 - M_\tau$) vs. τ plot, where τ is the interpulse delay in an 180°–τ–90° experiment. Data are presented for the α and β positions of DPPC, DPPE, and DMPS in Table I and in Figure 2 in the form of an Arrhenius plot of ln T_1 vs. reciprocal temperature (K). ^2H T_1 values for the α and γ segments of DPPC have been previously reported (Gally et al., 1975). Quantitatively the older data agree roughly with those values reported here, the differences reflecting the vast superiority of the present generation of spectrometers. Qualitatively, these ^2H T_1 data are in excellent agreement with ^{13}C T_1 data from sonicated DPPC preparations [Lee et al., 1976; cf. also Seelig & Seelig (1980)]. The ratios of ^{13}C NT_1 values for the α, β, and γ segments to the 3-glycerol position almost exactly parallel the same ratios obtained with ^2H T_1 relaxation times. The two deuterons of the α position of phosphatidylserine have different quadrupole splittings (at 47 °C, DMPS 3.6 and 12.1 kHz, DOPS 0.7 and 9.8 kHz; Browning & Seelig, 1980), and the T_1 of each deuteron could be determined separately. Both deuterons of this segment for both DMPS and DOPS relaxed at the same rate. Conditions were found where both signals disappeared with the same interpulse delay (τ) in the inversion–recovery experiment; i.e., the signal disappears at the point where $\tau = 2 \ln T_1$. The equivalence of the T_1 values for these two deuterons indicates that the orientational contribution to relaxation is very small. With DOPS, the standard plot of ln ($M_0 - M_\tau$) vs. τ gave a T_1 value for the deuteron with the smaller quadrupole splitting consistently 1–2 ms longer than that of the other deuteron. This discrepancy is probably due to an overlap of a small amount of an isotropic DOPS phase complicating an exact analysis of this T_1.

The ^2H T_1 relaxation times were found to vary over a wide range. At both the α and β segments, the T_1 values decreased in the order propyl > choline > ethanolamine > serine ≃ serine methyl ester. The α-segment T_1 values for DMPE and DPPE were similar, and likewise only small differences were observed at the same segment between DMPS and DOPS. Thus, the effect of the fatty acyl chain length on the headgroup relaxation times was not large. The T_1 values of the α segment of DMPS and DOPS in the liquid-crystalline phase were in the same range as data from the same segment in gel phase DPPG. A comparison of T_1 data from DMPS, DOPS, and DPPG is presented in Figure 3.

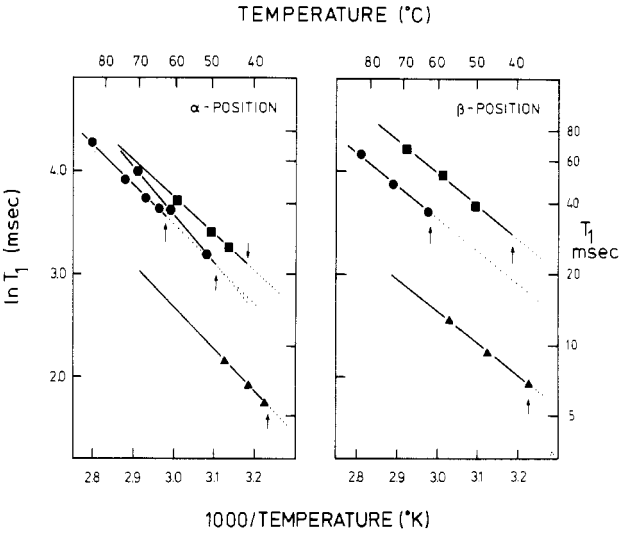

FIGURE 2: Deuterium spin–lattice (T_1) relaxation times for the α- and β-deuterated segments of dispersed multilamellar preparations of DPPC (■), DMPE (●), DPPE (●), and DMPS (▲). Data are presented in the form of an Arrhenius plot where the slopes of the lines were used to calculate apparent activation energies. Arrows indicate phase transition temperatures. Standard deviations determined from a regression analysis of the experimental data were generally smaller than the symbols and were within the ranges given in Table I.

The T_1 relaxation times for all positions decreased with decreasing temperature, indicating that relaxation was in the short correlation time regime ($\omega_0\tau_c < 1$, $\omega_0 = 2.9 \times 10^8$ rad s^{-1}, where ω_0 is the NMR resonance frequency for deuterium and τ_c is the molecular correlation time). An attempt was made to extend the DOPS T_1 data to the point where $\omega_0\tau_c > 1$, that is, into the long correlation time regime. Such data have been obtained for the phosphate group of dioleoylglycero-3-phosphocholine (Seelig et al., 1981). Arrhenius plots of the DOPS and DPPG data did not extend into the long correlation time regime. However, the data did not appear to be linear in the low-temperature region (Figure 3).

From the slopes of the Arrhenius plots, apparent activation energies for the motions involved in T_1 relaxation are obtained (Table II). For the α segment of DPPC, DPPE, and DMPS, the activation energies were in the range of 30–33 kJ/mol. The

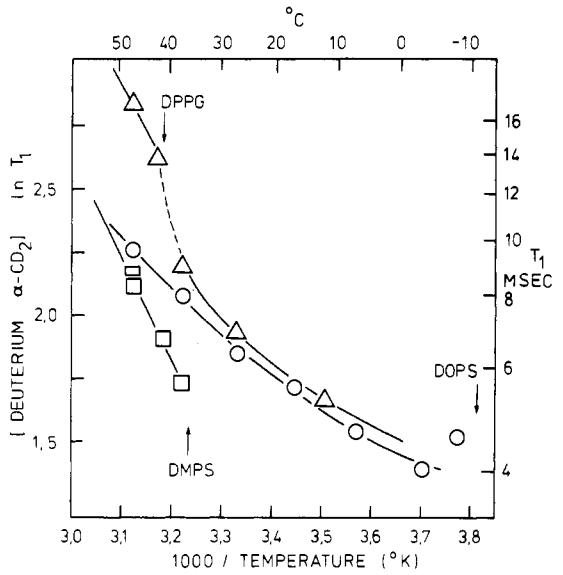

FIGURE 3: Comparison of the deuterium spin–lattice (T_1) relaxation times of the α segments of DMPS (□), DOPS (○), and DPPG (△). Arrows indicate phase transition temperatures.

Table II: Apparent Activation Energies

lipid	head group	activation energy (kJ/mol)[a]
	^{2}H T_1 Relaxation	
3-glycerol		
DPPC	choline	14.5[b]
α position		
DMP-PR	propyl	11.8
DPP-PR	propyl	11.2
DPPC	choline	30.1
DMPE	ethanolamine	38.0
DPPE	ethanolamine	31.4
DMPS	serine	33.5
DOPS	serine	15.6
β position		
DPPC	choline	27.1
DPPE	ethanolamine	27.2
DMPS	serine	25.9
γ position		
DPPC	choline	16.7
	Other Measurements	
choline dipole		
DPPC	choline	16.6[c]
^{31}P T_1 relaxation		
DOPC	choline	16.7[d]

[a] From data on the liquid-crystalline state. [b] Brown et al. (1979). [c] Activation enthalpy from dielectric relaxation measurements of Shepherd & Büldt (1978). [d] DOPC = dioleoyl-*sn*-glycero-3-phosphocholine; Seelig et al., 1981.

activation energy of the α-CD_2 position of DMPE was slightly higher (38 kJ/mol). These α-position data are larger than those values for the same position in the alkyl head groups presented in the first paper of this series (Browning, 1981a). The β-position activation energies for these compounds varied between 26 and 27 kJ/mol. No data from the β position of a simple alkyl head group are available for comparison. The activation energy of the γ position of DPPC was 16 kJ/mol [the same value was found earlier by Gally et al. (1975)], apparently reflecting a lower energy barrier to rotation of this group. The α-segment DMPS and DOPS activation energies varied dramatically. It is possible that relaxation in this DOPS segment is approaching the long correlation time regime in which case the measured activation energy would not necessarily reflect an average energy barrier. Alternatively, there

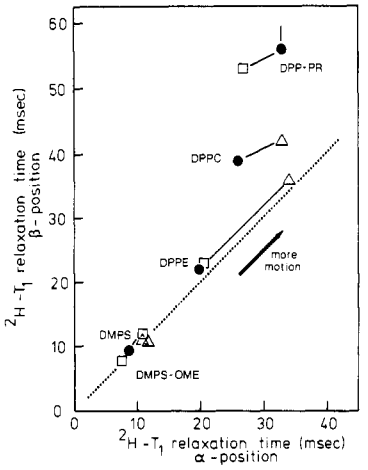

FIGURE 4: Plot relating the α- and β-segment deuterium spin–lattice (T_1) relaxation times of various phospholipids with amine groups in their head groups. Data were obtained at 47 °C except in the case of DPPE and DMPE where T_1 values at higher temperatures were extrapolated to 47 °C. Points are for the pure lipid (●) and in 1/1 (molar ratio) mixtures with DMPC (□) and cholesterol (△). The points directly on the α-T_1 = β-T_1 dotted line are for DMPS-OME. No data are given for pure DMPS-OME.

are probably large differences in the surface area available to the head group between these two lipids which may affect these activation energies.

The ^{2}H T_1 relaxation times have been collected in Table I for all the positions investigated. Examination of the α-CD_2 T_1 data for DMPE and DPPE indicates that an absolute temperature scale is more appropriate for the comparison of T_1 data rather than a reduced temperature scale (Seelig & Browning, 1978). The same conclusion was reached on the basis of ^{2}H T_1 data from the alkyl head groups (Browning, 1981a) and from ^{31}P T_1 relaxation experiments (see below). In Figure 4, these data have been compared in an α-position vs. β-position T_1 plot at a common absolute temperature. This figure illustrates the progression of decreasing T_1 values from the propyl to the serine head groups. The phosphatidylethanolamine head group is found to lie intermediate between the serine and choline head groups. The phosphatidylethanolamine head-group T_1 values are almost identical with the values found previously for the phosphatidylglycerol head group (Browning, 1981b).

The equivalence of the α- and β-T_1 values represents another differentiating parameter in addition to the abolute magnitude of the data [cf. Browning (1981b)]. The dotted line in Figure 4 indicates equal α- and β-T_1 values. Only the choline (DPPC) and propyl (DPP-PR) head groups deviate significantly from this condition. In all the head groups investigated to date, no points below the α-T_1 = β-T_1 line have been found.

Perturbations in the form of cholesterol addition or dilution with phosphatidylcholine were introduced to probe the question of intra- vs. intermolecular interactions. These data are presented in Table I and Figure 4. Generally, the phase transition temperature of a mixture of two phospholipids can be approximated as $T_{c\,\text{mixture}} = X_1 T_{c1} + X_2 T_{c1}$, where X_i is the mole fraction of component i. Good miscibility is expected in mixtures of DMPC with either DPPE or DMPS at temperatures above $T_{c\,\text{mixture}}$ [cf. van Dijck et al. (1978), Luna & McConnell (1977), Stewart et al. (1979), and Lee (1977)]. ^{2}H NMR spectra show little change in the size of the quadrupole splitting of the DPPE head-group labels in mixtures with DMPC or DPPC at temperatures above $T_{c\,\text{mixture}}$ (J. L. Browning, unpublished data). Likewise the dynamic behavior of the DPPE head group was not appreciably affected by

Table III: Comparison of ^{31}P and ^{2}H Spin-Lattice Relaxation Data

compd	relaxation times[a] at 47 °C: ^{31}P (s)	relaxation times[a] at 47 °C: α-segment ^{2}H (ms)	relative values[b]: ^{31}P	relative values[b]: α-segment ^{2}H
DPPC	3.05	26	1.00	1.00
DMPC	3.01		0.99	
DPPE		20		0.77
DMPE	2.13	20	0.70	0.77
DLPE	2.20		0.72	
DMPS	0.53	8.5	0.17	0.33

[a] Data were obtained from a least-squares fit to an Arrhenius plot such as in Figure 2. For DMPE and DPPE, the data were extrapolated to temperatures below the phase transition. [b] The value for DPPC was taken as 1.00.

phosphatidylcholine addition. With DMPS, only small changes were observed.

Mixtures of cholesterol with DPPC and DPPE have been well examined [Blume, 1980; reviews by Lee (1977) and Demel & de Kruijff (1976)], but the properties of cholesterol/DMPS mixtures have not been well investigated (van Dijck, 1979). On the basis of ^{2}H NMR spectra of DPPC and DPPE in mixtures with cholesterol, good mixing occurred on the ^{2}H NMR time scale (Brown & Seelig, 1978). Unusual ^{2}H NMR spectra of DMPS deuterated at either the α or the β position were observed in an equimolar mixture with cholesterol. An intense peak arises which cannot be attributed to isotropic or hexagonal phases (J. L. Browning, unpublished experiments). Axial asymmetry is tentatively proposed to explain the unusual spectral shape. The T_1 data for the DMPS/cholesterol mixture were determined from the outer quadrupole splittings and not from the central peak. The T_1 relaxation times of the DPPC and DPPE head groups increased with cholesterol addition. An increase in the rates of motion of the DPPC head-group dipole has been reported (Shepherd & Büldt, 1979). The dynamic behavior of the DMPS probed by the T_1 experiment was relatively unaffected by cholesterol.

The dynamic properties of a new lipid, DMPS-OME, are reported here. Bilayers composed of this lipid alone appear to have somewhat atypical phase transition behavior, and for this reason, data for the pure compound have not been included. From the phosphorus NMR powder spectra of mixtures of DMPS-OME with either DMPC or cholesterol, one sees that normal lamellar phases were formed. The dynamic properties of the DMPS-OME head group in these mixtures are very similar to those of the parent compound DMPS. It can be concluded that the removal of the net negative charge from DMPS does not change the dynamic properties of this head group at least for those motions contributing to T_1 relaxation.

^{31}P Spin–Lattice (T_1) Relaxation Times. ^{31}P T_1 relaxation times of dispersed multilamellar preparations of these phospholipids were measured at 36.4 mHz with an inversion–recovery experiment. The recovery of phosphorus magnetization could be described by one decay time. Because of the differing mechanisms for phosphorus and deuterium relaxation, the ^{31}P T_1 values are much longer than those for deuterium. Data are presented in Figure 5 in the form of an Arrhenius plot for the phosphates of DMPC, DMPE, and DMPS. Table III contrasts some of the ^{31}P T_1 data at a single temperature with those of the neighboring α-CD_2 segment deuterium data. The results agree well with measurements on egg yolk phosphatidylcholine (Yeagle et al., 1977) and DMPC and DPPC (Vita & Minetti, 1981).

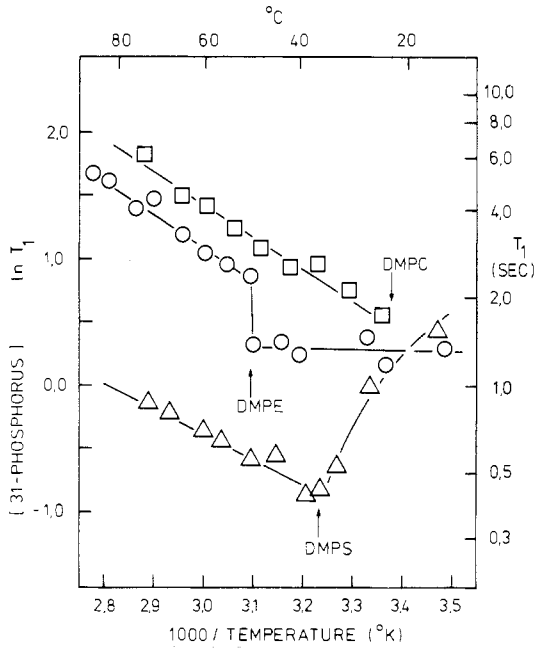

FIGURE 5: Arrhenius plot of phosphorus-31 NMR spin–lattice (T_1) relaxation times for dispersed multilamellar preparations of DMPC (□), DMPE (○), and DMPS (△). Measurements were performed at 36.4 mHz in the absence of any proton decoupling. Arrows indicate phase transition temperatures. Standard deviations in the ln T_1 values were in the range of 0.1–0.2 in the liquid-crystalline phase. Errors in the gel phase data were somewhat larger.

From Figure 5, it can be seen that the phosphorus T_1 values for all three lipids, DMPC, DMPE, and DMPS, decreased linearly in the liquid-crystalline phase. This indicates that relaxation is in the short correlation time regime ($\omega_0\tau_c < 1$, $\omega_0 = 2.3 \times 10^8$ rad s^{-1}). The slopes of these lines are roughly similar and the overall appearance of this plot is strikingly similar to Figure 2 (left panel). Activation energies for the motions involved in ^{31}P T_1 relaxation were 16–24 kJ/mol in the liquid-crystalline phase for all the lipids listed in Table III. This value is intermediate between those of the glycerol backbone (Brown et al., 1979) and the neighboring α segment. The length of the fatty acyl chains has little affect on the ^{31}P T_1 values provided only lipids with saturated chains were compared. DMPE and DLPE as well as DPPC and DMPC had very similar ^{31}P T_1 values in accord with previous data on the neighboring α segment of both the alkyl (Browning, 1981a) and phosphatidylethanolamine head groups. This result reinforces the concept of using an absolute temperature scale for the comparison of relaxation data.

At the phase transition, there was a dramatic break in the Arrhenius plot, reflecting large changes in the dynamic properties of the head groups. In the gel phase, the ^{31}P T_1 values of DMPE, DLPE, and DPPC were only slightly temperature dependent (data not shown for DLPE or DPPC). Most of the data fell in the range indicated in Figure 5 for DMPE. The DMPS ^{31}P T_1 values began to increase in the gel phase, indicating that in this region relaxation had shifted into the long correlation time regime ($\omega_0\tau_c > 1$). Calcium had a similar effect on the ^{31}P T_1 relaxation times of DOPS (J. L. Browning, unpublished experiments).

Discussion

The measurement of deuterium spin–lattice (T_1) relaxation times is an excellent tool for analyzing the relative and to some extent the absolute rates of motion in phospholipid head

groups. Deuterium relaxation is dominated by the quadrupole interaction, and only intramolecular motions need be considered. Here, in a relatively simple treatment of the data, an isotropic model will be employed to describe the motions involved in T_1 relaxation. This approach and its validity for these studies have been discussed in the second paper of this series (Browning, 1981b). With this treatment, the apparent or average correlation time, τ_c, is inversely proportional to the relaxation time in the short correlation time regime ($\omega_0\tau_c < 1$), which is the case for these data. For a more complete discussion of deuterium relaxation in membranes, see Brown (1979), Brown et al. (1979), and Seelig (1977).

From the size of the T_1 relaxation times, the relative rates of motion for these various head groups are obtained. At both the α and β segments, the rates of head-group motion decreased in the order propyl > choline > ethanolamine > serine = serine methyl ester. The T_1 values varied 3–4-fold between the serine and propyl head groups at the α position and 5–6-fold at the β position. The short T_1 values for the serine and serine methyl ester head group could result from purely steric effects due to the large size of these head groups; however, this is unlikely since both the phosphatidylserine and phosphatidylcholine head groups have similar sizes[2] but very different T_1 relaxation times.

A second criterion for judging the relative dynamic properties of these head groups is the equivalence of the α- and β-segment relaxation times. Equal α- and β-segment relaxation times were found for all the head groups except propyl and choline. In the case of the propyl group, it is clear that no interactions of the alkyl portion of the head group are possible with neighboring phosphates. As a result, the T_1 values reflect the motions of an anchored chain; i.e., segments nearest the anchoring point will have the slowest rates of motion followed by increasing rates toward the free end. This is in direct analogy to the data found for the hydrocarbon chains in a bilayer (Lee et al., 1976; Brown et al., 1979; Davis, 1979). In contrast, in the head groups with identical α- and β-segment T_1 values, the motions of both segments must be governed by similar constraints. This trend suggests that the overall head group behaves more as a rigid entity with rapid internal oscillations about the segmental bonds. The phosphatidylcholine head group lies somewhere between these two extremes of a flexible chain (propyl head group) and a chain anchored at both ends (DPPE and DMPS). The introduction of an amine group which can participate as a donor in a proton transfer complex results in equivalent α- and β-position T_1 values, as was observed for the addition of a hydroxyl group to the head group (Browning, 1981b).

A third aspect of the properties of these head groups is the apparent activation energy for ^2H T_1 relaxation. The addition of hydroxyl groups to an alkyl "skeleton" increased the activation energy from 10–15 to 25–30 kJ/mol (Browning, 1981a,b). Here again, the addition of amine groups increased this value to 30–35 kJ/mol. One interpretation of these data is that the introduction of noncovalent interactions into the membrane surface increases the energy barriers for the motions involved in T_1 relaxation. On the other hand, the large activation energy for the α segment of the phosphatidylcholine head group indicates that steric factors are clearly important and cannot be separated in these experiments from other contributions.

The analysis of phosphorus-31 spin–lattice (T_1) relaxation is more complicated than that of deuterium. There are two predominant mechanisms for T_1 relaxation in phospholipids; dipole–dipole relaxation and relaxation via chemical shift anisotropy. A previous study on the magnetic field strength dependence of ^{31}P T_1 relaxation showed that at field strengths around 2 T (36-MHz phosphorus NMR resonance frequency), dipole–dipole relaxation is the dominant mechanism [Yeagle et al., 1977; cf. Seelig et al. (1981)]. With both mechanisms, the T_1 relaxation time in the short correlation time regime is inversely proportional to the molecular correlation time as with ^2H T_1 relaxtion. For comparison of ^{31}P T_1 data from several different classes of phospholipids, two conditions should be fulfilled: the arrangement of protons in the immediate environment of the phosphate should be similar, and the relative intra- and intermolecular contributions to relaxation should be the same in each case. Since the segment $-CH_2-O-PO_2^--O-CH_2-$ has almost the same conformation in both the phosphatidylcholine and phosphatidylethanolamine head groups (Seelig & Seelig, 1980; Büldt & Wohlgemuth, 1981; Büldt & Seelig, 1980) and most likely also in the phosphatidylserine head group, the intramolecular proton arrangements around the phosphates of these various lipids should be similar. The intermolecular contribution to ^{31}P T_1 relaxation is likely to differ between these head groups. The $N(CH_3)_3$ portion of the choline head group is known to influence ^{31}P T_1 relaxation in an intermolecular manner (Yeagle et al., 1977). The extent of hydrogen bonding and the hydration shells around the phosphates may also differ between these head groups. Despite the complications of the intermolecular factor, the relative trends indicated by the ^{31}P and ^2H T_1 data agree well. Thus, measurement of the ^{31}P T_1 relaxation behavior may serve in some cases as an additional tool for the determination of the dynamic properties of these head groups.

In the gel phase, the ^{31}P T_1 values for DMPS shifted into the long correlation time regime ($\omega_0\tau_c > 1$), indicating a dramatic reduction in the rates of those motions whose frequency components contribute to relaxation. Deuterium results have indicated previously that the phosphatidylethanolamine head group may be more rigid in the gel phase than the phosphatidylcholine head group (Seelig & Gally, 1976). Thus, the order of decreasing rates of head-group motion observed in the liquid-crystalline phase appears to be retained in the gel phase. The same result was found for the hydroxyl group containing series of head groups (Browning, 1981b).

Given that there are these differences in rates of motion or the relative rigidity of these various head groups, an important question is the following: How large are these differences on a molecular scale? This is a common problem in the interpretation of NMR relaxation data and is often not addressed. To develop a "context" for viewing these data, it is useful to examine other systems where a molecular picture can be more easily visualized.

First, these data can be compared with T_1 values from other parts of a phospholipid molecule [cf. Browning (1981a) and Seelig & Seelig (1980)]. The phosphatidylserine head-group values are shorter than those of the 3 segment of the glycerol backbone (Brown et al., 1979) and in the same range as the 2 and 1 segments of this backbone (R. Ghosh, unpublished data). The glycerol backbone is considered to be quite rigid since its motions are constrained by the geometry of the fatty acyl chains in a bilayer and the necessity for a hydrophilic interaction of the head group with the solvent.

[2] Surface areas of these head groups including the phosphate were estimated from the projection of space-filling models (CPK) onto the membrane surface to be (Å^2) choline 32, ethanolamine 24, serine 31, and serine methyl ester 35. Parallel head-group conformations similar to those found in the phosphatidylcholine (Pearson & Pascher, 1979) and phosphatidylethanolamine (Hitchcock et al., 1974) crystal structures were assumed.

A second framework is provided by comparison with ^{13}C and ^{2}H NMR studies on small cyclic hormonal peptides which contain a rigid cyclic peptide section and a short flexible tripeptide "tail" extending out from the ring portion. The ^{13}C T_1 relaxation times are inversely proportional to an apparent correlation time; however, this correlation time contains terms from both internal motions and diffusion of the entire molecule. The ^{13}C T_1 values for the rigid ring portion of these peptides were 3–5-fold shorter (slower rates of motion) than the values for the freer tripeptide "tail" (Deslaurier et al., 1974; Meraldi et al., 1977; Allerhand & Komoroski, 1973). In a ^{2}H spin–spin (T_2) study, similar conclusions were reached (Glasel et al., 1973). Likewise, a comparison of tightly and loosely bound enzyme inhibitors with proton NMR relaxation times showed an order-of-magnitude difference in the rotational correlation times between the "tight" and "loose" states (London & Schmidt, 1974).

An additional viewpoint is provided by comparison of the DMPS and DPPG data. From these data, one can conclude that the phosphatidylserine head group in the liquid-crystalline state is as rigid as the phosphatidylglycerol head group in the gel phase. This conclusion is valid only for those motions whose frequency components contribute to relaxation, and in this case, only relatively high-frequency motions are involved. An examination of the deuterium spectra of the head-group labels in gel phase DPPG and liquid-crystalline phase DMPS reveals differences in the motional properties of the two states. Well-resolved powder spectra are obtained with liquid-crystalline phase DMPS, whereas broader more featureless spectra are found for gel state DPPG. Within certain frequency ranges, the dynamics of these two head groups in these states cannot be the same. Most likely, rates of motion of gel phase head groups in the range 10^6–10^3 s^{-1} are slowed, resulting in broadened ^{2}H spectra. Changes in this frequency range would not appreciably affect ^{2}H T_1 relaxation.

Typically, differences of 2–4-fold in the ^{2}H T_1 relaxation times were found in the hydroxyl group containing series of lipids, and between the propyl and serine head groups described here, a range of 4–6-fold was found. In view of the similar size of the effects found in the systems described above and these phospholipid data, it is felt that the differences between the phospholipid head groups are significant and reflect variations in the extent and strength of the noncovalent interactions at the membrane surface.

Phosphatidylcholine cannot form a proton transfer complex with neighboring phosphates and can only interact electrostatically. This lack of a hydrogen-bonded proton appears to account for the more flexible nature of the choline head group compared with that of phosphatidylethanolamine. The electrostatic interaction alone appears to be quite weak since the flexibility of the choline head group is approaching that of the simple propyl chain. The proton transfer complex described for the phosphatidylethanolamine head group is also possible at the surface of phosphatidylserine membranes. Moreover, there are additional possibilities for hydrogen bond formation with the carboxylate group of phosphatidylserine or the carbonyl of phosphatidylserine methyl ester. These additional noncovalent interactions appear to render the phosphatidylserine head group more rigid than the other head groups. Extensive interaction within the plane of the membrane surface would demand a head-group conformation parallel to this plane. Such a parallel conformation has been established for the head groups of phosphatidylcholine and phosphatidylethanolamine (Seelig & Seelig, 1980; Büldt & Seelig, 1980; Büldt & Wohlgemuth, 1981), and on this basis, such an orientation appears likely for the phosphatidylserine head group.

When the distance between head groups is increased, interactions between these groups should be weakened. Cholesterol addition increases this spacing (Demel & de Kruijff, 1976) and was effective in reducing the effect of the of the $N(CH_3)_3$ group on the surrounding phosphates (Yeagle et al., 1977). Similar reasoning can be applied to these ^{2}H T_1 data. Cholesterol addition increased the rates of motion in both the phosphatidylcholine and phosphatidylethanolamine head groups. Furthermore, dilution of the deuterated lipid with phosphatidylcholine should not change the arrangement of proton transfer accepting groups; i.e., the phosphate spacing remains the same, and indeed, the phosphatidylethanolamine head group was unaffected by dilution with DMPC. These two points clearly suggest that the interactions of the phosphatidylethanolamine head group are to some extent intermolecular. For phosphatidylserine and its methyl ester analogue, the head-group dynamic states were relatively unperturbed by either cholesterol or DMPC. It is possible that the interactions of these head groups are mostly intramolecular, but it is felt either that the cholesterol-induced expansion was insufficient to affect the noncovalent bonding or that cholesterol itself participates in the bonding network. A similar result was obtained with the phosphatidylglycerol head group.

It is useful in forming an image of these packed head groups to consider the available crystal data. The interphosphate distance in crystals of phospho-L-serine monohydrate is 5.3–7.7 Å along the *b* and *c* axes, respectively (Sundaralingam & Putkey, 1970). In the crystal structure of dilauroylglycero-3-phosphoethanolamine–acetic acid, the same spacing is 6.8–7.7 Å in the *b*–*c* plane (Hitchcock et al., 1974). Within one layer of the phosphoserine crystal, there is a two-dimensional network of hydrogen bonds with five to six noncovalent intermolecular bonds per molecule. Likewise, each amine in the phosphatidylethanolamine structure is hydrogen bonded to two neighboring phosphates. In the liquid-crystalline bilayer phase, the interphosphate distance is about 8–9 Å [cf. Shepherd & Büldt (1978)]. Even with a 1–2-Å expansion over the values found in the crystal, noncovalent bonding would appear to be geometrically feasible in a membrane.

The existence of a network of noncovalent bonds at the membrane surface may have several implications for biological systems. The retention of these properties in mixtures with other lipids indicates that these interactions will be present to varying extents in the bulk lipid phase of natural systems. Some of the present confusion regarding the requirements for specific lipids for the optimal functioning of membrane enzymes (Sandermann, 1978) or membrane-oriented systems such as blood clot formation (Zwaal, 1978) may have arisen from a lack of consideration of the capacity of the various head groups to interact noncovalently or, more broadly expressed, their ability to form weak short-lived aggregates. Many of the net negatively charged phospholipids, e.g., phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, and most likely phosphatidic acid (Blume & Eibl, 1979), are also capable of interacting through hydrogen bonding. It would be difficult comparing only phosphatidylcholine and phosphatidylserine to establish a requirement for negative charge for a specific function. Likewise, disrupting effects on these noncovalent bonds should be considered when investigating the interactions of monovalent and divalent cations and anesthetics and the binding of extrinsic membrane proteins to membranes.

Acknowledgments

I thank Dr. J. Seelig for many enlightening discussions and

helpful advice and R. Jenni for expert technical assistence.

References

Adamson, A. W. (1976) *Physical Chemistry of Surfaces*, 3rd ed., Wiley, New York.

Akutsu, H., Kyogoku, Y., Nakahara, H., & Fukuda, F. (1975) *Chem. Phys. Lipids 15*, 222–242.

Akutsu, H., Ikematsu, M., Kyogoku, K. (1981) *Chem. Phys. Lipids 28*, 149–158.

Allerhand, A., & Komoroski, R. (1973) *J. Am. Chem. Soc. 95*, 8228–8231.

Aneja, R., Chadha, J. S., & Davies, A. P. (1970) *Biochim. Biophys. Acta 218*, 102–111.

Blume, A. (1980) *Biochemistry 19*, 4908–4913.

Blume, A., & Eibl, H. (1979) *Biochim. Biophys. Acta 558*, 13–21.

Boggs, J. M. (1981) *Can. J. Biochem. 58*, 755–770.

Brown, M. F. (1979) *J. Magn. Reson. 35*, 203–215.

Brown, M. F., & Seelig, J. (1978) *Biochemistry 17*, 381–384.

Brown, M. F., Seelig, J., & Häberlen, U. (1979) *J. Chem. Phys. 70*, 5045–5053.

Browning, J. L. (1981a) *Biochemistry* (first of three papers in this issue).

Browning, J. L. (1981b) *Biochemistry* (second of three papers in this issue).

Browning, J. L., & Seelig, J. (1979) *Chem. Phys. Lipids 24*, 103–118.

Browning, J. L., & Seelig, J. (1980) *Biochemistry 19*, 1262–1270.

Büldt, G., & Seelig, J. (1980) *Biochemistry 19*, 6170–6175.

Büldt, G., & Wohlgemuth, R. (1981) *J. Membr. Biol. 58*, 81–100.

Burns, R. A., & Roberts, M. F. (1980) *Biochemistry 19*, 3100–3106.

Comfurius, P., & Zwaal, R. (1977) *Biochim. Biophys. Acta 488*, 36–42.

Davis, J. H. (1979) *Biophys. J. 27*, 339–358.

Demel, R. A., & de Kruijff, B. (1976) *Biochim. Biophys. Acta 457*, 109–132.

Deslaurier, R., Smith, I. C. P., & Walter, R. (1974) *J. Am. Chem. Soc. 96*, 2289–2291.

Elder, M., Hitchcock, P., Mason, R., & Shipley, G. G. (1977) *Proc. R. Soc. London, Ser. A 354*, 157–170.

Finer, E. G., & Darke, A. (1974) *Chem. Phys. Lipids 12*, 1–16.

Gally, H.-U., Niederberger, W., & Seelig, J. (1975) *Biochemistry 14*, 3647–3652.

Glasel, J. A., Hruby, V. J., Mckelvy, J. F., & Spatola, A. F. (1973) *J. Mol. Biol. 79*, 555–575.

Guttmann, S., & Boissonnas, R. A. (1958) *Helv. Chim. Acta 41*, 1852–1867.

Hauser, H., & Phillips, M. C. (1979) *Prog. Surf. Membr. Sci. 13*, 297–413.

Hauser, H., Guyer, W., Pascher, I., Skrabal, P., & Sundell, S. (1980) *Biochemistry 19*, 366–373.

Hayashi, M., Muramatsu, T., & Hara, I. (1972) *Biochim. Biophys. Acta 255*, 98–106.

Hayashi, M., Muramatsu, T., Hara, I., & Seimiya, T. (1975) *Chem. Phys. Lipids 15*, 209–215.

Hitchcock, P. B., Mason, R., Thomas, K. M., & Shipley, G. G. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3036–3040.

Jacobson, K., & Papahadjopoulos, D. (1975) *Biochemistry 14*, 152–161.

Lee, A. G. (1977) *Biochim. Biophys. Acta 472*, 285–344.

Lee, A. G., Birdsall, N. J., Metcalfe, J. C., Warren, G. B., & Roberts, G. C. K. (1976) *Proc. R. Soc. London, Ser. B 193*, 253–274.

London, R. E., & Schmidt, P. G. (1974) *Biochemistry 13*, 1170–1179.

Luna, E. J., & McConnell, H. M. (1977) *Biochim. Biophys. Acta 470*, 303–316.

Meraldi, J.-*P.*, Hruby, V. J., & Brewster, A. I. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1373–1377.

Michaelson, D. M., Horwitz, A. F., & Klein, M. P. (1974) *Biochemistry 13*, 2605–2615.

Moroder, L., Hallet, A., Wünsch, E., Keller, D., & Wersin, G. (1976) *Hoppe-Seyler's Z. Physiol. Chem. 357*, 1651–1653.

Pearson, R., & Pascher, I. (1979) *Nature (London) 281*, 499–501.

Phillips, M. C., & Chapman, D. (1968) *Biochim. Biophys. Acta 163*, 301–313.

Phillips, M. C., Finer, E. G., & Hauser, H. (1972) *Biochim. Biophys. Acta 290*, 397–402.

Sandermann, H. (1978) *Biochim. Biopys. Acta 515*, 209–238.

Seelig, J. (1977) *Q. Rev. Biophys. 10*, 353–418.

Seelig, J., & Gally, H. U. (1976) *Biochemistry 15*, 5199–5204.

Seelig, J., & Browning, J. L. (1978) *FEBS Lett. 92*, 41–44.

Seelig, J., & Seelig, A. (1980) *Q. Rev. Biophys. 13*, 19–61.

Seelig, J., Tamm, L., Hymel, L., & Fleischer, S. (1981) *Biochemistry 20*, 3922–3932.

Seimiya, T., Ashida, M., Hayashi, M., Muramatsu, T., & Hara, I. (1978) *Chem. Phys. Lipids 21*, 69–76.

Shepherd, J. C. W., & Büldt, G. (1978) *Biochim. Biophys. Acta 514*, 83–94.

Shepherd, J. C. W., & Büldt, G. (1979) *Biochim. Biophys. Acta 558*, 41–47.

Stewart, T. P., Hui, S. W., Portis, A. R., & Papahadjopoulos, D. (1979) *Biochim. Biophys. Acta 556*, 1–16.

Sundaralingam, M. (1972) *Ann. N.Y. Acad. Sci. 195*, 324–355.

Sundaralingam, M., & Putkey, E. (1970) *Acta Crystallogr., Sect. B B26*, 790–799.

van Dijck, P. W. M. (1979) *Biochim. Biophys. Acta 555*, 89–101.

van Dijck, P. W. M., de Kruijff, B., Verkleij, A., van Deenan, L., & de Gier, J. (1978) *Biochim. Biophys. Acta 512*, 84–96.

Vinogradov, S. N., & Linnell, R. H. (1971) *Hydrogen Bonding*, p 163, Van Nostrand-Reinhold, New York.

Vita, V., & Minetti, M. (1981) *Chem. Phys. Lipids 28*, 215–225.

Yeagle, P. (1978) *Acc. Chem. Res. 11*, 321–327.

Yeagle, P., Hutton, W., Huang, C.-H., & Martin, R. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3477–3481.

Yeagle, P., Hutton, W., Huang, C.-H., & Martin, R. (1976) *Biochemistry 15*, 2121–2124.

Yeagle, P., Hutton, W., Huang, C.-H., & Martin, R. (1977) *Biochemistry 16*, 4344–4349.

Zwaal, R. F. A. (1978) *Biochim. Biophys. Acta 515*, 163–206.