Phospholipid Head-Group Conformations; Intermolecular Interactions and Cholesterol Effects†

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ABSTRACT: The predominant orientation of the phosphorylcholine polar head group in phosphatidylcholine and sphingomyelin bilayers and cholesterol perturbations of that orientation have been identified by exploiting the $^{31}P\{^1H\}$ nuclear Overhauser effect (NOE) in the ^{31}P NMR spectra of phospholipid bilayers. In pure egg phosphatidylcholine bilayers, a NOE of 40% is observed. The magnitude of the NOE has been measured as a function of continuous-wave proton-decoupler frequency in order to identify the proton source of the NOE. In pure egg phosphatidylcholine bilayers, the maximum NOE occurs at the *N*-methyl proton resonance position of the choline moiety. In a modified phosphatidylcholine in which all the *N*-methyl protons were replaced by deuterium, the NOE arose from methylene protons next to the phosphate. In mixed systems of phosphatidylcholine and phosphatidylethanolamine, and phosphatidylcholine and diphosphatidylglycerol, both phospholipid resonances attained maximum NOE at the position of the *N*-methyl proton resonance of phosphatidylcholine. An analogous result was obtained with pure sphingomyelin. These results are explained by orienting the phosphorylcholine portion of the molecule parallel to the surface of the bilayer so that the positively charged *N*-methyl moiety is located close to the negatively charged phosphate on a neighboring phospholipid in an intermolecular interaction. Addition of cholesterol is shown to disrupt the intermolecular interaction in phosphatidylcholine bilayers.

Phospholipid polar head-group conformation is an important component of the structure of phospholipid bilayers, both in pure lipid systems and in cell membranes. The head group constitutes the surface of the bilayer and bears the charges of the phospholipid. These charges will affect the membrane surface potential, and provide binding sites for ions from solution. Further, they are the first part of a bilayer with which a molecule must contend in order to cross the membrane. Certain membrane-bound proteins require particular phospholipid for activity, and some membranes have been shown to be asymmetric with respect to phospholipids of different head-group structures (Rothman and Lenard, 1977). Information on the behavior of phospholipid head groups is thus of considerable interest. In addition, any affects of the ubiquitous membrane component, cholesterol, on the phospholipid head groups might provide clues to that molecule's role in membranes.

Phospholipid polar head-group behavior has only recently been explored in any detail due to the lack of adequate probing methods. Because the properties of the various phospholipids are dependent on specific structures, perturbations of the surface with probes had to be avoided, resulting in more stringent conditions on the investigator than studies of the interior of the bilayer. ^{31}P NMR has proven to be a useful nonperturbing probe of the head-group region. In particular, the $^{31}P\{^1H\}$ nuclear Overhauser effect (NOE)[1] has been explored in phospholipid bilayers to determine if phospholipid head-group conformation information could be obtained. Early work suggested that in egg phosphatidylcholine bilayers the phosphorylcholine moiety was oriented parallel to the bilayer surface (Yeagle et al., 1975), and engaged in intermolecular interactions (Yeagle et al., 1976). This proposition is examined in greater depth in this report by developing further the $^{31}P\{^1H\}$ NOE probing method and by examining the behavior of several new phospholipid systems. The conclusion reached is that the zwitterionic dipole in phosphatidylcholine and sphingomyelin, along with what was already known for phosphatidylethanolamine, is oriented parallel to the bilayer surface and that cholesterol (at a 2:1 phospholipid/cholesterol mole ratio) is capable of completely disrupting the intermolecular association in bilayers.

Materials and Methods

Egg phosphatidylcholine and phosphatidylethanolamine were purified by silicic acid chromatography (Huang, 1969; Litman, 1973). Egg phosphatidylcholine fully deuterated in the *N*-methyl groups was obtained from Lipid Specialties, Inc. (Boston). Dipalmitoylphosphatidylcholine was purchased from Calbiochem, diphosphatidylglycerol from Sigma, and sphingomyelin from Analabs. Cholesterol was purchased from General Biochemical and purity was checked by thin-layer chromatography.

Vesicles were prepared by sonication until clear with a Heat Systems W-350 sonifier in an ice bath (except for dipalmitoylphosphatidylcholine and sphingomyelin which were sonicated at 45 and 25 °C, respectively) until clear in a 100 mM NaCl, 10 mM EDTA, D_2O solution. Mixed lipid systems, including those with cholesterol, were colyophilized from benzene before sonication. The vesicles were used for NMR experiments within 48 h and were stable during that time. Phosphate concentration was determined by the method of Bartlett (1958).

^{31}P NMR (40.48 MHz) spectra were obtained with a JEOL PS-100/EC-100 Fourier transform spectrometer at 23.5 kG and 23 °C with a JEOL 5 kHz crystal filter. T_1 measurements were made using the $180°-\tau-90°$ pulse sequence with proton decoupling. NOE measurements were made with the JEOL OA-1M/SD-HC gated decoupler as previously described (Yeagle et al., 1976). The magnitude of the NOE in the figures

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[1] Abbreviations used are: NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; PC, phosphatidylcholine; EDTA, (ethylenedinitrilo)tetraacetic acid.

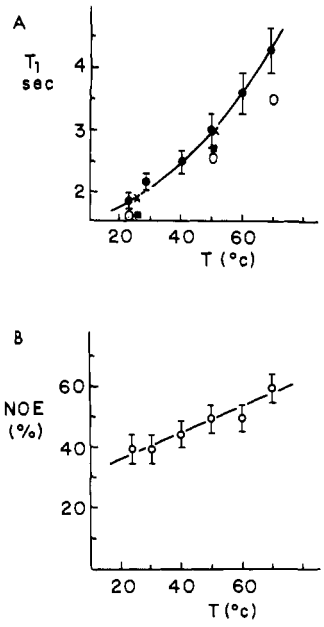

FIGURE 1: Temperature dependence of ^{31}P T_1 and NOE for phosphatidylcholine vesicles. (A) (●) Sonicated egg PC; (x) unsonicated egg PC; (○) sonicated dipalmitoyl-PC; (■) unsonicated dipalmitoyl-PC. (B) sonicated egg PC.

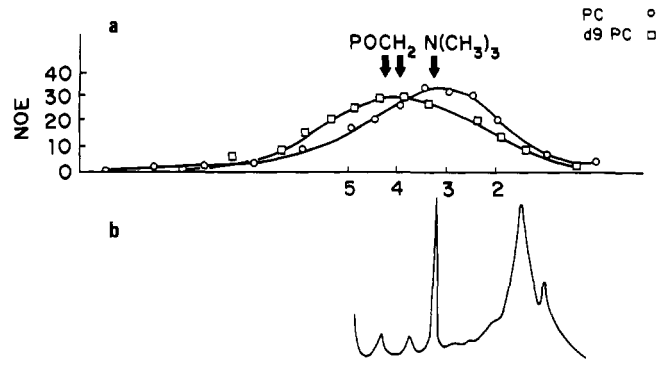

FIGURE 2: Frequency dependence of NOE. (a) $^{31}P\{^1H\}$ NOE of egg PC (○) and d-9 PC (□) plotted as a function of the cw proton decoupler frequency which is labeled in ppm from Me_4Si. $POCH_2$ refers to methylene groups adjacent to the phosphate and $N(CH_3)_3$ refers to *N*-methyl groups (see Levine et al. (1976) for assignments). (b) ^1H NMR spectrum of egg PC vesicles on same scale as cw proton decoupler frequency above. All data were obtained at 23 °C.

is reported as percentage enhancement which can vary from 0 to 124%.

In the following frequency-dependence measurements, the instrumental parameters are different than those described above, and while the position of maximum NOE (which is of most importance in this paper) is accurately determined, the absolute magnitude is not, and should not be used for comparisons. In order to identify which protons are the source of the NOE in the ^{31}P NMR resonance of a phospholipid, one can selectively decouple portions of the proton spectrum with weak continuous-wave proton decoupling rather than strong broad-band decoupling. If the magnitude of the $^{31}P\{^1H\}$ NOE is plotted as a function of the portion of the proton spectrum being decoupled, a maximum should be observed in the region in which resonate the protons responsible for the NOE. In the following experiments, the continuous-wave proton decoupler was gated off during accumulation of the free-induction decay and on the remainder of the time to retain proton coupling while exhibiting the NOE. Delays of 4 to 5 T_1 of both phosphorus and proton were employed between pulses. In this way, the peak shape remained the same throughout the experiment, whether or not the proton irradiation was of the correct frequency to decouple the methylene protons next to the phosphate (which would have narrowed the line). Intensities could then be compared with the computer-calculated peak heights which greatly facilitated repeated measurements, and three to eight determinations were collected for each decoupler frequency, depending on whether the proton decoupler was set far from, or close to, the region of maximum NOE, respectively. The magnitude of the NOE was calculated by comparing the intensity of the ^{31}P resonance when the proton decoupler was in the region of interest to the intensity when the decoupler was placed far from the region of maximum NOE. The power level of the proton decoupler was adjusted for the greatest differentiation in the NOE as a function of frequency: too low a power level would lead to little or no NOE being observed at any decoupler frequency, and too high a power level would increase the bandwidth of the decoupler so that NOE would be observed across too wide a portion of the spectrum.

Each day these kinds of measurements were taken the position of the proton decoupler was calibrated to compensate for any change in the lock frequency. A standard sample of trimethyl phosphate (in D_2O) was subjected to low-power single-frequency proton decoupling, changing the proton-decoupler frequency and power until a narrow frequency range ($\leq$5 Hz) of effective proton decoupling of the methyl protons from the phosphorus was obtained. The known proton frequency of those methyl protons (3.75 ppm) could then be used to calibrate the position of the proton decoupler.

Results

Temperature Dependence of T_1 and NOE. Previous work led to the conclusion that the spin-lattice relaxation mechanism of phosphorus in phosphatidylcholine was dominated by dipolar interactions with nearby protons (Yeagle et al., 1975). This conclusion was tested further by measuring the temperature dependence of the spin-lattice relaxation time, T_1, and the $^{31}P\{^1H\}$ NOE in egg phosphatidylcholine and dipalmitoylphosphatidylcholine dispersions. The results for both sonicated and unsonicated dispersions are shown in Figure 1. All the systems behave in a qualitatively similar fashion, reflecting in the increased T_1 and NOE the increase in motion brought on by increased temperature. This result adds to the argument presented previously that the phosphorus T_1 is dominated by dipolar interactions with the protons because spin-rotation relaxation would be expected to have the opposite temperature dependence. The only other likely mechanism, chemical shift anisotropy, was ruled out previously by the independence of T_1 from magnetic field strength (Yeagle et al., 1975). Therefore, the NOE observed is motionally limited, and not reduced due to other competing relaxation mechanisms.

Frequency Dependence of the $^{31}P\{^1H\}$ NOE. The frequency dependence of the $^{31}P\{^1H\}$ NOE was measured in the above manner for several phospholipids. The results for single phospholipid vesicle systems consisting of egg phosphatidylcholine, d-9 egg phosphatidylcholine, dipalmitoylphosphatidylcholine, and bovine brain sphingomyelin, all in D_2O, 100 mM NaCl, 10 mM EDTA, are presented in Figures 2 and 3. In d-9 egg PC the *N*-methyl groups were replaced with fully deuterated methyl groups. The proton spectrum of the deuterated phospholipid proved to be the same as for the native phospholipid, except for the total lack of an *N*-methyl proton

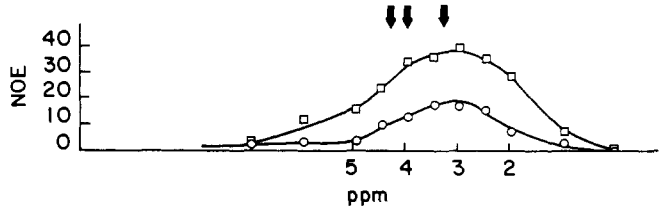

FIGURE 3: As in Figure 2, with dipalmitoylphosphatidylcholine (□) and sphingomyelin (○). Arrows represent the same proton resonances as in Figure 2. Data obtained at 57 and 52 °C, respectively.

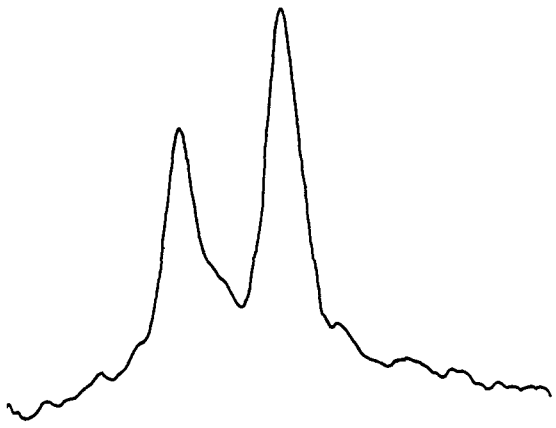

FIGURE 4: ^{31}P NMR spectra of 3.6:1 egg PC/diphosphatidylglycerol vesicles. The upfield peak is PC and the downfield peak (leftmost) is diphosphatidylglycerol. Separation between the peaks is 1 ppm. Data were obtained at 23 °C.

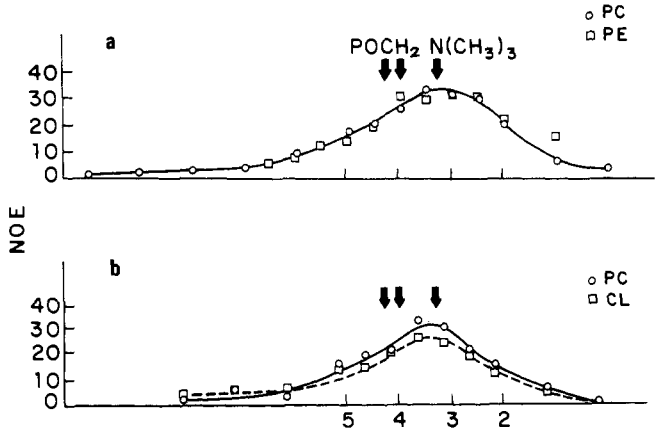

FIGURE 5: Frequency dependence of ^{31}P $\{^1H\}$ NOE for mixed vesicle systems, at 23 °C. (a) (○) Egg PC and (□) egg PE, 1.2:1; (b) (○) egg PC and (□) diphosphatidylglycerol, 3.6:1.

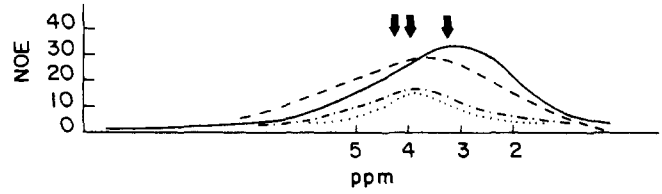

FIGURE 6: Frequency dependence of ^{31}P $\{^1H\}$ NOE for PC-cholesterol systems. (—) Pure egg PC; (- - -) PC/cholesterol, 3:1; (- • -) PC/cholesterol 3:2; (• • •) PC-cholesterol, 1:1. Arrows represent the same proton resonances as in Figure 5. Data were obtained at 23 °C.

resonance. Two binary mixtures of phospholipids were studied, egg PC with phosphatidylethanolamine (1.2:1) and egg phosphatidylcholine with diphosphatidylglycerol (3.6:1). In both vesicle systems, separate resonances corresponding to each of the phospholipids were observed. A ^{31}P NMR spectrum of phosphatidylcholine with diphosphatidylglycerol is given in Figure 4 (for the other mixture, see Yeagle et al., 1976). The frequency dependence of the NOE appears in Figure 5 for the mixed systems.

Cholesterol Effects. The effect of cholesterol on the frequency dependence of the NOE was measured to see whether cholesterol could exert any influence on phosphatidylcholine head-group conformation. Cholesterol was colyophilized with egg PC in differing mole ratios and sonicated, with the results presented in Figure 6. Data in the absence of cholesterol are shown for comparison.

In all the above data, the breadth of the maximum of the NOE vs. decoupler frequency is most likely due to broad proton lines and a broad decoupler bandwidth from the power level necessary to saturate the protons with short spin-lattice relaxation times. It was not possible to perform similar experiments with unsonicated phospholipid dispersions because the very broad, overlapping proton resonances characteristic of such systems prevent selective proton decoupling.

In addition to the frequency dependence of the ^{31}P $\{^1H\}$ NOE in d-9 egg PC, the magnitude of the NOE and T_1 was measured. As reported before, the NOE was less in the deuterated phospholipid than in the native phospholipid, though the decrease was from 40 to 30% enhancement in the recent experiments, rather than 40 to 10% previously reported (Yeagle et al., 1975). That much more material, and therefore much greater signal to noise, was available in the more recent experiments may explain the difference, since these NOE measurements require very good signal to noise ratios. This problem is unique to the deuterated results. The measurement has been repeated several times on two independent synthetic preparations for this work, with consistency. T_1 of the deuterated phospholipid was 2.4 s, compared to 1.5 s reported for native egg PC (Yeagle et al., 1975). If the *N*-methyl protons are contributing to the relaxation mechanism of the phosphorus, as is argued under Discussion, then replacing those protons with deuterium should lengthen the T_1, as is observed.

Discussion

The ^{31}P $\{^1H\}$ nuclear Overhauser effect arises from protons that are dipolar coupled to the phosphorus nucleus being observed. It is for this reason that the relaxation mechanism of the phosphorus nucleus in phospholipids must be understood. The experiments described previously and in the present work establish that the dominant mode of phosphorus spin-lattice relaxation is a dipole-dipole mechanism involving nearby protons, so that some protons in the sample are dipolar coupled to the phosphorus of the phospholipid phosphate. As a result, there exists a possible relaxation pathway which involves coupled transitions of both the phosphorus and a proton. Saturation of the protons, which perturbs the equilibrium population of the proton energy levels by equalizing them, will perturb the population of the phosphorus energy levels via the coupled relaxation pathway. Saturation of the protons can thus lead to an increase in the intensity of the phosphorus resonance, up to a maximum of 124% (Noggle and Schirmer, 1971).

In order for a proton to be effective in relaxing the phosphorus and thus be capable of causing a NOE, criteria must be met which can be described by the following equation for the spin-lattice relaxation of the phosphorus:

$$\frac{1}{T_1} = \sum_i (Kn_i/r_i^6)(f_i(\tau_c)) \quad (1)$$

K is a collection of constants, r_i is the internuclear, through-space distance between the proton and the phosphorus nucleus, n_i is the number of protons a given distance r_i away, and $f_i(\tau_c)$ is a function describing the motion of the phosphorus–proton internuclear vector. The relative effectiveness of a particular proton a given distance, r_i, away at causing a NOE, which is proportional to its contribution to the dipolar spin–lattice relaxation rate, is then highly dependent on the distance between that proton and the phosphorus, compared to other protons, and to a lesser extent on the number of protons of that type. In addition, motion is important, and if slow enough may essentially eliminate the $^{31}P\{^1H\}$ NOE (Yeagle et al., 1975). While not simply related to distance, it is this distance dependence which provides the structural information to be discussed below.

Since a $^{31}P\{^1H\}$ NOE is observed in the phosphorus resonance of several phospholipids, one can ask which protons of the phospholipid are causing the NOE and then inquire whether structural information concerning the polar head group of the phospholipid can be derived. Previous work (Yeagle et al., 1975, 1976) suggested that the N-methyl protons of the positively charged quaternary ammonium ion interacted with the negatively charged phosphate and that the polar head group was therefore preferentially oriented parallel to the surface of the phospholipid bilayer. This suggestion is tested in depth in this discussion.

The first system to consider is egg phosphatidylcholine vesicles. The methylene hydrogens next to the phosphate are the protons closest in chemical bonds to the phosphate, but the N-methyl protons can also be brought close to the phosphate, particularly one on a neighboring molecule, with a suitable set of bond rotations in the polar head group. The other methylene protons, next to the nitrogen, cannot be brought as close to the phosphate as the previously mentioned protons. In Figure 2a, a maximum in the NOE occurs when the proton decoupler is situated on the N-methyl proton resonance, indicating that the N-methyl protons make a substantial contribution to the relaxation rate of the phosphorus. Since they must compete with the methylene protons next to the phosphate to relax the phosphorus, the N-methyl protons must be close to the phosphate, because of the r^{-6} dependence of the dipolar interactions (eq 1). As a check on this conclusion, the frequency dependence of the NOE was measured for a modified egg phosphatidylcholine in which the N-methyl protons were replaced with deuterium. As expected, the maximum in the NOE shifted to the position of the methylene next to the phosphate, once the N-methyl protons were chemically removed (deuterium cannot cause an NOE under the conditions of these experiments). The conclusion remains that the N-methyl moiety must be close to the phosphate. The same result was obtained for dipalmitoylphosphatidylcholine so the interaction is not dependent on the nature of the fatty acyl chains of the phospholipid. At this point the experimental results do not permit a distinction between an inter- or intramolecular interaction, though the former seems more likely from model building. But it is clear that the positively and negatively charged moieties must be close to each other, and therefore that the polar head group cannot be oriented perpendicular to the bilayer surface.

The question of inter- or intramolecular interaction was investigated using two binary mixed lipid systems: phosphatidylcholine and phosphatidylethanolamine, and phosphatidylcholine and diphosphatidylglycerol. In the ^{31}P NMR spectra of these systems, separate resonances appear for each lipid so that the two phospholipids can be studied independently yet simultaneously within the same vesicle. In both systems the phosphatidylcholine and the second lipid each received significant contributions to the NOE from the N-methyl protons of the choline, so that the N-methyl moiety must be close to the phosphatidylcholine phosphates and to the phosphates of the second phospholipid. Therefore, the interaction must be intermolecular, with the phosphorylcholine dipole parallel to the surface of the bilayer so the N-methyl moiety can get close to the phosphate on a neighboring phospholipid. Later discussion of cholesterol effects provides further support for this conclusion.

This head-group orientation is not unique to phosphatidylcholine. Sphingomyelin, whose polar head group also consists of phosphorylcholine, exhibits the same frequency dependence of the NOE as phosphatidylcholine and therefore possesses the same orientation in the head-group region. The differences in the properties of the two phospholipids most likely originate in the interface region which contains, among other differences, an amide bond in the case of sphingomyelin.

The orientation of the head group described should be considered a preferred conformation in a dynamic state. It has been shown that the head group of phosphatidylcholine is capable of considerable motion (Gally et al., 1975), and the conclusion drawn here that the head group is predominantly parallel to the bilayer surface is compatible with a head group undergoing motion. The head group would be expected to be constantly changing partners as the N-methyl group jumps from one neighboring phosphate to another. The likelihood of this kind of motion is encouraged by the observation of intermolecular interactions in the binary mixtures regardless of whether the mole ratio of phosphatidylcholine to the other phospholipid is 3.6:1 or 1.2:1, and further suggests that the bilayer surface in the binary mixture does not contain large pools of the second lipid.

Since the head group is in motion, it would be interesting to know at any instant of time how many head groups are in the orientation described above, or how preferred is this preferred orientation. The starting point for an answer to that question must be with an estimation of the proportion of the NOE observed that arises from the N-methyl protons. Three independent lines of reasoning suggest that about one-half of the NOE is contributed by the N-methyl protons.

From reconstruction of frequency dependence of NOE plots a contribution as low as 33% from the N-methyl protons would lead to a maximum at the methylene protons, clearly different from the maximum observed. The frequency-dependence plots therefore require a minimum 40% contribution from the N-methyl protons to the NOE. A value of about 40% can be obtained combining the T_1 of the deuterated lipid and the native lipid, but that is approximate at best, because one should compare two synthetic lipids, one deuterated and one protonated, rather than one synthetic and one native, for sensitive T_1 measurements. The value obtained is encouraging, nonetheless. Finally, from the crystal structure of glycerophosphorylcholine (Abrahamsson and Pascher, 1966) proton–phosphorus distances for the four closest methylene protons (with which the N-methyl protons must compete to cause NOE) of 2.63, 2.87, 3.18, and 2.59 Å are obtained. Since some of these distances approach the sum of the van der Waals radii of phosphorus and hydrogen atoms, the N-methyl protons cannot be significantly nearer the phosphorus than the methylene hydrogens. Since the NOE is motionally limited in both native and deuterated egg phosphatidylcholines, a similar correlation function, $f(\tau_c)$, may be assumed for both kinds of protons. Therefore, the N-methyl protons cannot contribute much more to the NOE

than the methylene protons, and the three arguments converge to an estimate of 50% contribution of the *N*-methyl protons to the NOE.

Because the *N*-methyl protons must compete with methylene protons held close to the phosphate by chemical bonds, and because of the $1/r^6$ dependence of T_1, if the *N*-methyl moiety is not in its position next to the phosphate then it is effectively an infinite distance away. One can conceive of *N*-methyl proton sites, *r* Å away from the phosphate, with a variable occupancy, less than one for each site, which would allow for the proportion of time the head group is not in the preferred orientation discussed above. To the extent the head group is not in the preferred orientation, the protons must approach the phosphorus more closely to continue to contribute 50% of the relaxation rate. Since the *N*-methyl protons cannot get much closer to the phosphorus than some of the methylene hydrogens, the *N*-methyl protons must interact with the phosphate nearly all the time. The head-group orientation required for this intermolecular interaction is one where the phosphorylcholine dipole is predominantly oriented approximately parallel to the bilayer surface.

Recent work utilizing independent approaches provide further evidence for this conclusion. X-ray and neutron-diffraction data from egg PC bilayers containing cholesterol are consistent with a model of the polar head-group orientation as described here (Worcester and Franks, 1976). Analysis of the ^{31}P NMR spectra of oriented dipalmitoylphosphatidylcholine monohydrate bilayers, using the ^{31}P chemical shift tensor, demonstrates that the O-P-O plane, where the O atoms are not esterified, is tilted at 50° to the bilayer plane, placing the head group approximately parallel to the membrane surface (Griffin et al., 1977). A similar conclusion is arrived at by Seelig and Gally (1976) from ^{31}P chemical-shift anisotropy data. They describe a picture in which the polar head group rotates flat on the bilayer surface. These approaches measure an average orientation and thus confirm the estimation given above that the head-group orientation deduced from the ^{31}P $\{^1H\}$ NOE data is the preferred orientation.

Not only is the polar head group parallel to the surface in phosphatidylcholine and sphingomyelin bilayers as described above, but a similar orientation has been found for phosphatidylethanolamine in a single crystal study (Hitchcock et al., 1974), in which the quaternary ammonium group is hydrogen bonded to the phosphate on a neighboring molecule. Thus, for three of the most common zwitterionic phospholipids, the polar head group is found to lie in the surface of the bilayer, rather than to extend out from it, and also to engage in intermolecular interactions.

Some evidence suggests that the polar head-group conformation may be different when ions are bound to the phospholipid. In the presence of both cationic and anionic shift reagents, Hauser et al. (1976) came to the conclusion that the polar head group was oriented perpendicular to the bilayer surface in phosphatidylcholine bilayers. It is not surprising that an ion would break up the electrostatic interaction between phospholipids. In the case of phosphatidylethanolamine, neutralization of the positive charge in the head group at high pH introduces considerable motion evidently resulting from just such a disruption of intermolecular electrostatic interactions (Seelig and Gally, 1976; Michaelson et al., 1974). Furthermore, Hauser et al. (1976) incorrectly assumed a single rigid conformer of the glycerol portion of phosphatidylcholine, as the nonequivalence of glycerol CH_2 protons is consistent with the two major conformers that occur in the liquid crystalline state. This and other assumptions of their treatment result in an unusual geometry for Ln^{3+} binding.

The association of cholesterol with phosphatidylcholine has been the subject of considerable study (for a review, see Jain, 1975). The present discussion will focus on what effects cholesterol may have on the polar head group of phospholipids in bilayers. On the basis of ^{31}P chemical shift and T_1 data, the possibility of a hydrogen bond between the cholesterol hydroxyl and the phosphate on the phospholipid was rendered unlikely (Yeagle et al., 1975). The present data show that as the mole fraction of cholesterol in the egg phosphatidylcholine bilayer increases, the source of the NOE shifts from the *N*-methyl protons to the methylene protons. The *N*-methyl protons of phosphatidylcholine must be sufficiently removed from the phosphate in bilayers of high-cholesterol content that the methylene protons next to the phosphate can dominate the phosphorus relaxation and consequently the NOE. Addition of cholesterol to egg PC bilayers increases the separation between phospholipids until intermolecular interactions between phospholipid head groups can no longer occur. X-ray diffraction data also indicate that addition of cholesterol to the bilayer increases the separation of the phospholipid head groups (Levine, 1972). This frees the head groups, increasing the freedom of motion of the phosphorylcholine moiety, which can be seen in the phosphorus order parameters (McLaughlin et al., 1975), and increasing the hydration of the bilayer surface (Newman and Huang, 1975). Changes have been observed before in bilayer properties that reach an end point when the mole fraction of cholesterol exceeds about $\frac{1}{3}$, but here the nature of the probe allows a specific molecular interpretation. That the *N*-methyl protons do not contribute to the ^{31}P $\{^1H\}$ NOE in the presence of high cholesterol provides additional evidence that an intramolecular *N*-methyl–phosphate interaction does not occur.

Measurements of water adsorption isotherms of several bilayer systems have been interpreted as providing evidence for increasing hydration in the series phosphatidylethanolamine, phosphatidylcholine, and phosphatidylcholine with cholesterol bilayers. These results were used to argue that the head-group conformation was different in phosphatidylethanolamine than in phosphatidylcholine (Jendrasiak and Mendible, 1976). Another way to interpret these results, however, is that the extent of hydration is inversely related to the strength of the intermolecular interactions between phospholipid head groups, and thus to the extent to which the charged moieties are accessible to the solvent. The data in this report demonstrate that the head groups in pure phospholipid bilayers are more tightly associated than in phospholipid/cholesterol bilayers, and it is reasonable that phosphatidylethanolamine head groups are more tightly associated yet. The ammonium ion forms stronger hydrogen bonds with its protons to a phosphate than can methyl groups. ^{31}P and ^2H NMR data (Seelig and Gally, 1976) show much less motional freedom in phosphatidylethanolamine than in phosphatidylcholine head groups. That the ammonium proton resonance is observable (Lange et al., 1975) and yet does not contribute to the NOE also suggests slow motion, so the phosphatidylethanolamine head groups appear to be more tightly associated than phosphatidylcholine head groups. Thus, the extent of hydration of the bilayer surface can be related to the strength of the interaction between the charged moieties of phospholipid head groups, rather than to a difference in conformation.

Acknowledgment

We thank Dr. R. Miller and Dr. R. Bryan for calculating the proton–phosphorus distances.

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A Theory of Phase Transitions and Phase Diagrams for One- and Two-Component Phospholipid Bilayers†

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ABSTRACT: A statistical mechanical partition function for phospholipid bilayers is constructed to obtain a theoretical description of the chain melting phase transition in lipid bilayer membranes and of the phase diagrams for two-component bilayers. In addition to providing an accurate representation of the transition temperatures and enthalpies of one-component bilayers composed of 1,2-diacylphosphatidylcholines, the theory can also account for the shapes of the phase diagrams observed for bilayers which are binary mixtures of these compounds with two different hydrocarbon chain lengths.

Aqueous dispersions of phospholipid bilayers can undergo a phase transition which involves a disordering of the hydrocarbon chains in the interior of the bilayer (Ladbrooke and Chapman, 1969; Engleman, 1970; Hubbell and McConnell, 1971; Lippert and Peticolas, 1971; Hinz and Sturtevant, 1972; Nagle, 1973a,b; Scott, 1975; Marčelja, 1974; Marsh, 1974; Ranck et al., 1974; Träuble and Eibl, 1974; Sklar et al., 1975, 1976, 1977; Jacobs et al., 1975; McCammon and Deutch, 1975; Mabrey and Sturtevant, 1976). The same type of transition occurs in biological membranes (Steim et al., 1969; Reinert and Steim, 1970; Melchior et al., 1970; Ashe and Steim, 1971; Schechter et al., 1972; Overath et al., 1975; Linden and Fox, 1975; Morrisett et al., 1975; Cronan and Gelmann, 1975; Melchior and Steim, 1976; Thilo and Overath, 1976; Tecoma et al., 1977), and many properties of membranes, such as transport (Overath et al., 1967; Wilson et al., 1970), enzyme function (Chapman and Urbina, 1970; Papahadjopoulos et al., 1975a), drug susceptibility (Jain et al., 1975), and interaction with anesthetics (Papahadjopoulos et al., 1975b; Trudell et al., 1975), are affected by the transition. In this paper, we develop a statistical thermodynamic model for a bilayer which accounts for many of the phase-transition properties observed in a homologous series of 1,2-diacylphosphatidylcholines (DLPC,[1] DMPC, DPPC, DSPC, DBPC) as well as for the qualitative features of the phase diagrams observed for binary mixtures of these lipids.

In the following section, the experimental observations on one- and two-component phosphatidylcholine bilayers are reviewed. This is followed by a discussion of the motivation for the present three-dimensional model in terms of an earlier two-dimensional model (Jacobs et al., 1975). The present model is developed in detail in the next section and the theoretical results are then compared with the experiment.

Experimental Data

There is a wealth of experimental data for the 1,2-diacylphosphatidylcholines with two saturated acyl chains. The

† From the Department of Chemistry, Stanford University, Stanford, California 94305. *Received May 2, 1977.* This work was partially supported by the National Science Foundation (Grant CHE75-06634 to H.C.A.) and the National Institutes of Health (Grants GM21149 to B.S.H. and GM23085 to H.C.A. and B.S.H.).

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[1] Abbreviations used are: DLPC, dilauroylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; DBPC, dibehenoylphosphatidylcholine. The fatty acids in these esters have the formula $C_nH_{2n}O_2$, where n is 12, 14, 16, 18, and 22, respectively.