Membrane Packing Geometry of Diphytanoylphosphatidylcholine Is Highly Sensitive to Hydration: Phospholipid Polymorphism Induced by Molecular Rearrangement in the Headgroup Region

Chang-Huain Hsieh, Shih-Che Sue, Ping-Chiang Lyu, and Wen-guey Wu
Department of Life Sciences, National Tsing Hua University, Hsinchu, Taiwan 30043

ABSTRACT Diphytanoylphosphatidylcholine (DPhPC) has often been used in the study of protein-lipid interaction and membrane channel activity, because of the general belief that it has high bilayer stability, low ion leakage, and fatty acyl packing comparable to that of phospholipid bilayers in the liquid-crystalline state. In this solid-state ^{31}P and ^{2}H NMR study, we find that the membrane packing geometry and headgroup orientation of DPhPC are highly sensitive to the temperature studied and its water content. The phosphocholine headgroup of DPhPC starts to change its orientation at a water content as high as ~16 water molecules per lipid, as evidenced by hydration-dependent ^{2}H NMR study at room temperature. In addition, a temperature-induced structural transition in the headgroup orientation is detected in the temperature range of ~20–60°C for lipids with ~8–11 water molecules per DPhPC. Dehydration of the lipid by one more water molecule leads to a nonlamellar, presumably cubic, phase formation. The lipid packing becomes a hexagonal phase at ~6 water molecules per lipid. A phase diagram of DPhPC in the temperature range of −40°C to 80°C is thus constructed on the basis of NMR results. The newly observed hydration-dependent DPhPC lipid polymorphism emphasizes the importance of molecular packing in the headgroup region in modulating membrane structure and protein-induced pore formation of the DPhPC bilayer.

INTRODUCTION

Synthetic diphytanoyl (3,7,11,15-tetramethylhexadecanoic) phosphatidylcholine (DPhPC) has been a good choice of phospholipid in the reconstitution study of channel-forming proteins such as cyanobacterial toxin (Spassova et al., 1995), *Clostridium botulinum* toxin (Schmid et al., 1994), tetanus toxin (Gambale and Montal, 1988), anthrax toxin (Blaustein et al., 1989), rhodopsin (Mollevanger et al., 1987; Baldwin and Hubbell, 1985), alamethicin (Taylor and de Levie, 1991), and gramicidin (Koeppe and Anderson, 1996; Durkin et al., 1990). It has also been successfully applied in the crystallization of proton channel peptides (Lovejoy et al., 1992). Apparently, the high resistance to proton as well as other ion flows of the isoprenoid chain array of DPhPC (Gutknecht, 1987) and its analog (Yamauchi et al., 1992, 1993) account for the popularity in choosing it in ion-conducting measurements. The protein- or polypeptide-induced formation of a new ion conducting pathway was usually attributed to the putative pore formation, because DPhPC was thought to form stable liquid-crystalline bilayers over a wide temperature range from −120°C to 120°C (Lindsey et al., 1979).

There has also been recent interest in characterizing the binding mode of polypeptides with DPhPC bilayers (Huang and Wu, 1991; Ludtke et al., 1996). Membrane thinning followed by insertion of polypeptides of magainin 2 and alamethicin into DPhPC bilayers has been demonstrated by Huang and co-workers by oriented circular dichroism (OCD), x-ray diffraction, and neutron scattering studies (Ludtke et al., 1995; Wu et al., 1995; He et al., 1996). The membrane pores induced by polypeptide penetration account for its cytolytic property, but the long-range nature of bilayer deformation under the influence of increasing polypeptide concentration is still poorly understood. Both the structure of polypeptides and the packing of PC membrane are believed to play a role, because the capacity for polypeptide penetration has been shown to be hydration- as well as lipid-dependent (Huang and Wu, 1991; He et al., 1996).

In this communication, we show, by ^{31}P and ^{2}H NMR study of DPhPC selectively deuterated at both C_α and C_β methylene segments (d_4-DPhPC) and D_2O in DPhPC, that DPhPC exhibits a wide spectrum of lipid polymorphism at relatively high water content. Not only a novel dehydration-induced lamellar-to-lamellar transition of DPhPC, identified recently by an x-ray diffraction study (Wu et al., 1995), is shown here to be related to headgroup reorientation, but also several nonlamellar structures, including hexagonal and complex lipid packing arrangements, presumably cubic phase, are detected in the hydration state of ~5–11 water molecules per lipid. The result suggests that polymorphism of lecithin (Luzzati et al., 1968) is also related to the hydration-dependent molecular rearrangement in the headgroup region. In addition, the polymorphic DPhPC, in contrast to the presumed bilayer stability, may play a significant

Received for publication 21 February 1997 and in final form 28 April 1997.

Address reprint requests to Dr. Ping-Chiang Lyu or Dr. Wen-guey Wu, Department of Life Sciences, National Tsing Hua Unniversity, Hsin Chu, 30043 Taiwan. Tel.: 886-3-5742752; Fax: 886-3-5715934; E-mail: lswwg@life.nthu.edu.tw.

0006-3495/97/08/870/08 $2.00

role in affecting the binding mode of polypeptides with DPhPC membranes.

MATERIALS AND METHODS

Spectra were obtained on a 7.05-Tesla Bruker MSL-300 spectrometer, using a broadband probe with a 5-mm insert as described previously (Hsieh and Wu, 1995a–c, 1996). Briefly, the ^{31}P-NMR spectra were recorded with a Hahn echo pulse sequence, using a 90° pulse of 4 μs in the presence of ^{1}H decoupling, and ^{2}H NMR spectra were recorded with a quadrupolar echo pulse sequence, using a 90° pulse length of 2.2–2.5 μs. The recycle delays, longer than five times the respective spin-lattice relaxation times (T_1), were in the range of 7 s and 200 ms, respecitvely, for ^{31}P and ^{2}H NMR measurements. When necessary, depakeing of the ^{31}P and ^{2}H NMR spectra (Sternin et al., 1983) was performed to quantitate the chemical shift anisotropy (CSA) and quadrupolar splitting ($\Delta\upsilon_q$) of the overlapping signals. The temperature of the samples was controlled by evaporation N_2 gas from a liquid nitrogen dewar for low-temperature measurements or by passing heated dry air for higher than ambient temperature measurement, and monitored with a Bruker VT-1000 thermal system.

The lipids used in this work, DPhPC and d_4-DPhPC, were obtained commercially (Avanti Polar Lipids, Alabaster, AL). Spectroscopic grade D_2O and deuterium-depleted H_2O were from Cambridge Isotope Laboratory (Woburn, MA). Lipid lyophilized overnight in chloroform under high vacuum was assumed to contain about two water molecules per lipid (Ulrich and Watts, 1994; Hsieh and Wu, 1996). All of the water/lipid ratios were estimated by including these two tightly bound water molecules. In general, the error in the reported hydration value was plus or minus one water molecule per lipid.

Lipids with different hydration states were prepared gravimetrically in preweighed NMR tubes from a known amount of lyophilized lipid. These hydration samples were freeze-thawed between 50°C and −20°C at least five times to ensure the homogenization of the sample. The spectra obtained from homogenized samples are reversible upon hydration and dehydration as long as there is no detectable degradation of the lipid studied. It is found that incubation of the sample at high temperature (>60°C) for a long time during NMR spectra accumulation may induce the hydrolization of a DPhPC molecule (<5% as detected on thin-layer chromatography plate) and have an irreversible effect on the obtained spectra.

RESULTS AND DISCUSSIONS

Polymorphic DPhPC as revealed by ^{31}P NMR

Fig. 1 shows representative ^{31}P NMR spectra obtained from DPhPC at indicated hydration states and temperatures. Previous x-ray and ^{1}H NMR studies of DPhPC have established that DPhPC forms liquid-crystalline bilayers (L_α) in an excess of water and at ambient temperature (Wu et al., 1995; Lindsey et al., 1979). As indicated by the axially symmetrical ^{31}P NMR spectra, with CSA values of about −40 ppm, obtained at a water/lipid ratio (n_w) higher than 11, the lipid molecules undergo free axial rotational motions along the normal of the membrane bilayer surface (Seelig, 1978). Surprisingly, dehydration of the sample by one or two water molecules per DPhPC induces the formation of isotropic signals centered at 0 ppm, an indication of the formation of complex lipid structures with either small lipid particles or cubic phase membranes (Cullis and Hope, 1991). Further dehydration causes the complete disappearance of bilayer structure at a water content of ~7–8 water molecules per lipid and the appearance of an inverted hexagonal (H_{II}) phase assembly at 4–6 water molecules per

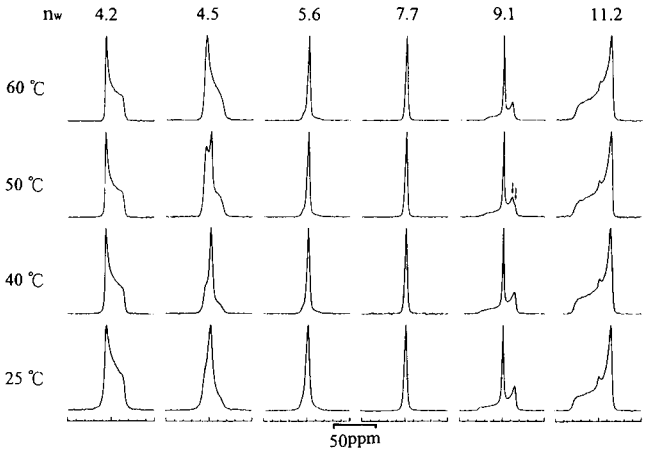

FIGURE 1 Representative ^{31}P NMR spectra obtained at indicated temperatures and water/DPhPC molar ratios (n_w).

DPhPC, as indicated by the obtained ^{31}P NMR spectra with a CSA of ~17 ppm. The described lipid polymorphisms are evident at all of the temperatures studied (shown in Fig. 1).

Many synthetic phospholipids, such as unsaturated phosphatidylethanolamine (PE), e.g., dioleoyl PE (DOPE), or their mixtures with unsaturated PC, e.g., dioleoyl PC (DOPC) or 1-palmitoyl-2-oleoyl PC (POPC), e.g., have been shown to exhibit liquid crystalline lamellar-to-nonlamellar (i.e., L_α-H_{II}) transition (Gruner et al., 1988; Koynova and Caffrey, 1994; Lafleur et al., 1996). Polymorphism of lecithin has also been reported at the extreme high temperature of ~100°C and the low water content of <4% (or $n_w < 4$) H_2O (Luzzati et al., 1968). In contrast, DPhPC was thought to form a stable L_α phase bilayer over a wide temperature range (Lindsey et al., 1979) and was used extensively in many ion conductance investigations because of its high resistance to ion leakage. However, in view of the significantly large cross-sectional area ratio between the fatty acyl chain region and headgroup region of DPhPC, the intrinsic curvature $1/R_0$ (Gruner, 1985; Hui and Sen, 1989) or the molecular packing parameter (Marsh, 1996) of DPhPC may be considered to be large and are expected to undergo a L_α-H_{II} transition from the theoretical point of view. It is somewhat surprising, however, that the L_α-to-nonlamellar transition of DPhPC occurs at relatively high water content.

A novel lamellar-to-lamellar transition of DPhPC

A first indication of a phase transition when DPhPC was dehydrated was reported in an x-ray diffraction study of oriented bilayer membranes interacting with amphiphilic helical polypeptides (Wu et al., 1995). A lamellar-to-lamellar transition was detected by the presence of two coexisting phases, as evidenced by the double peaks in every Bragg order. The structural difference between the two phases was not studied at that time. The transition, however, is interesting because the plot of bilayer thickness versus lamellar

spacing obtained for pure DPhPC at ambient temperature indicates that dehydration decreases the peak-to-peak bilayer thickness. This observation is in sharp contrast to x-ray diffraction studies on other saturated PCs, which show an increase in bilayer thickness during the process of dehydration-induced gel phase, with fatty acid chains in the all-*trans* state formation.

We observe in Fig. 1 that the CSA of the ^{31}P NMR spectra obtained for samples with the hydration of 9–10 water molecules per lipid seems to vary significantly as a function of temperature. For instance, the spectrum obtained at 60°C is much narrower than that obtained at 25°C, an indication of smaller CSA for DPhPC at high temperature. The reduction of CSA as a function of increasing temperature, however, is not continuous. As emphasized by the arrows shown in the figure, there are two overlapping signals with distinct CSA values for spectra obtained at 50°C.

To more clearly define the two different states of DPhPC as revealed by the two overlapping signals, the obtained ^{31}P powder spectra were depaked. As shown in Fig. 2 *A*, the CSA obtained at low temperature is about −40 ppm, which is almost 10 ppm different from those obtained at high temperature. The variation in the CSA as a function of temperature clearly indicates that a structural transition in the PC headgroup region occurs in the studied temperature range. The structural transition can be due either to the disordering effect at high temperature or to a more specific conformational change, such as reorientation of the phosphocholine headgroup. The reorientation of the phosphocholine headgroup either away or toward the membrane surface has been shown to occur in response to a variation in electrical potential (Seelig et al., 1987; Roux et al., 1989; Scherer and Seelig, 1989; Macdonald et al., 1991; Pinheiro et al., 1994) and hydration states (Bechinger and Seelig, 1991; Ulrich and Watts, 1994).

We therefore study d_4-DPhPC with deuterium selectively labeled at both C_α and C_β methylene segments of the phosphocholine headgroup, because it has been demonstrated before that phosphocholine headgroup reorientation will result in an opposite change (i.e., increase and decrease, respectively) in the ^{2}H NMR quadrupolar splitting, $\Delta\nu_q$, for -CD_2- labeled at the C_α and C_β methylene positions. As shown in Fig. 2 *B*, the $\Delta\nu_q$ of -CD_2- at both C_α and C_β positions is also found to be sensitive to temperature. Similar to the case of ^{31}P NMR, there are also overlapping signals in the temperature range of ~40–50°C. More importantly, although both α and β splittings are decreased when the temperature is increased, the β splitting of the second component detected at high temperatures is larger than that of the first component detected at low temperatures. It suggests strongly that the two distinct CSA values of ^{31}P NMR are due to the different orientation of the phosphocholine headgroup, and a structural transition in the phosphocholine headgroup region has occurred as a function of temperature. The exact conformational change of the phosphocholine headgroup is unknown at present. According to the "choline-tilt" model (Seelig et al., 1987; Roux et al., 1989; Macdonald et al., 1991), one of the possible scenarios is that the choline group moves toward the water phase.

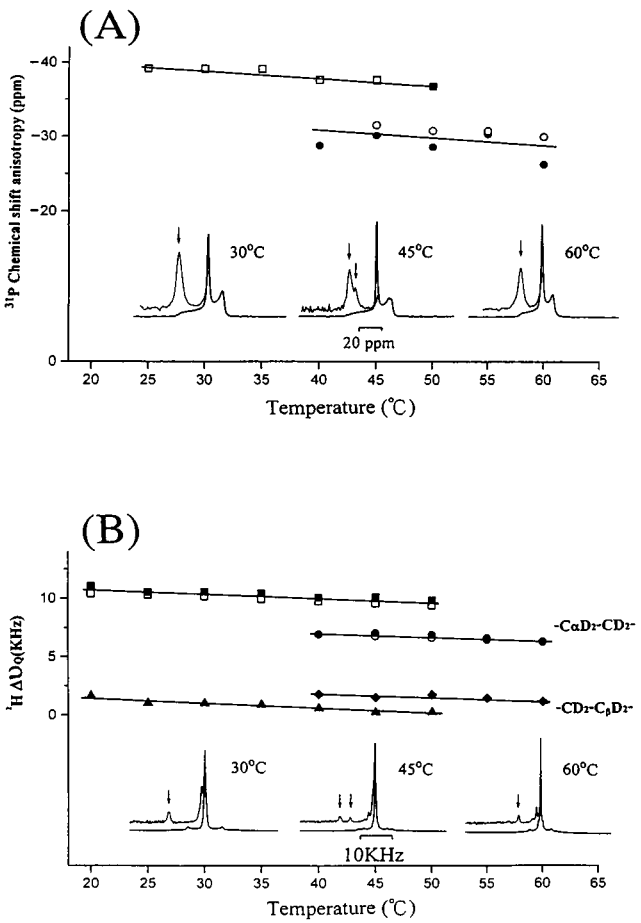

FIGURE 2 Plot of chemical shift anisotropy of ^{31}P NMR (*A*) and quadrupolar splitting, $\Delta\nu_q$, of ^{2}H NMR (*B*) of d_4-DPhPC as a function of temperature at a water/DPhPC molar ratio of 9.8. The open and filled symbols represent data extrapolated from powder and depaked spectra, respectively. The arrows point out the depaked peak for these powder spectra. Two arrows in the same spectrum at 45°C indicate that there are two lamellar phases in coexistence, as detected by -C_αD2-. The unmarked sharp peaks in the depaked spectra are from -C_βD2-.

The newly revealed lamellar-to-lamellar transition of DPhPC as a function of temperature has not been reported before, but, as we mentioned earlier, a novel lamellar-to-lamellar transition during the dehydration process of DPhPC has been detected by x-ray diffraction studies on the oriented lipid sandwiched between glass plates. It is interesting, therefore, to see whether a similar hydration dependent structural transition can also be detected by NMR study of powder samples. Shown in Fig. 3, *A* and *B*, are ^{31}P NMR spectra obtained at two hydration states. Despite the presence of isotropic signals, the CSA of ^{31}P NMR spectra for a sample with a n_w of 8.2 water molecules per DPhPC is clearly smaller than that with a n_w of 10.3 at 25°C. In fact, it corresponds to the ^{31}P NMR signal with a low CSA value obtained for the high hydration sample at high temperature.

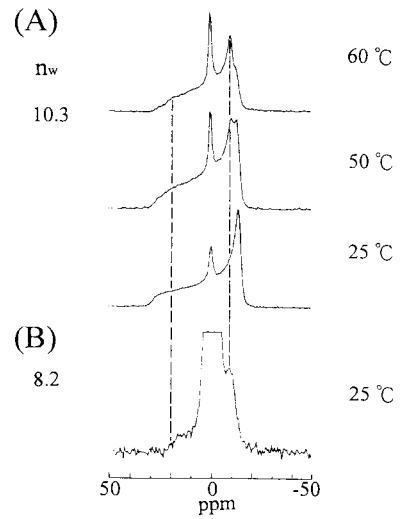

FIGURE 3 ^{31}P NMR spectra of DPhPC, indicating the existence of two distinct conformational states of phosphocholine at different hydration states and temperatures.

Therefore, the phosphocholine orientation of DPhPC at low hydration and ambient temperature is similar to that of DPhPC with high hydration at higher temperature.

Headgroup reorientation of DPhPC

A better picture of the hydration dependent structural transition in the headgroup region can be seen in the hydration-dependent study of the $\Delta\upsilon_q$ of -CD_2- at both C_α and C_β positions. Fig. 4 *A* shows the representative ^{31}P and ^{2}H NMR spectra at the indicated hydration, and Fig. 4 *B* summarizes and compares the hydration dependence of $\Delta\upsilon_q$ of -CD_2- at both C_α and C_β positions between DOPC and DPhPC. In general, the hydration-dependent change in $\Delta\upsilon_q$ of -CD_2- at both C_α and C_β positions for DPhPC is similar to the previous report on DOPC (Ulrich and Watts, 1994) and POPC (Bechinger and Seelig, 1991), suggesting that the choline end of the DPhPC headgroup also moves closer to the hydrocarbon layer upon initial dehydration.

There are, however, three new features emerging from the result of this DPhPC study. First, DPhPC is more sensitive to the water content than DOPC and POPC, because the NMR parameters start to change at relatively higher hydration states (Fig. 4 *B*). Second, an isotropic ^{2}H signal can be detected for DPhPC at the rather high water content of ~10–11 water molecules per lipid (Fig. 4 *A*), but it is absent for both DOPC and POPC, even at the lowest studied water content of ~4 water molecules per lipid. Third, there is an additional dehydration-induced lamellar-to-lamellar transition for DPhPC at 25°C (Fig. 3).

The absence of isotropic components with DOPC and POPC membranes is probably correlated with the observation that these two lipids do not form the HII phase. The chemical structures of the phosphocholine headgroup of these PC molecules are essentially identical; therefore, the

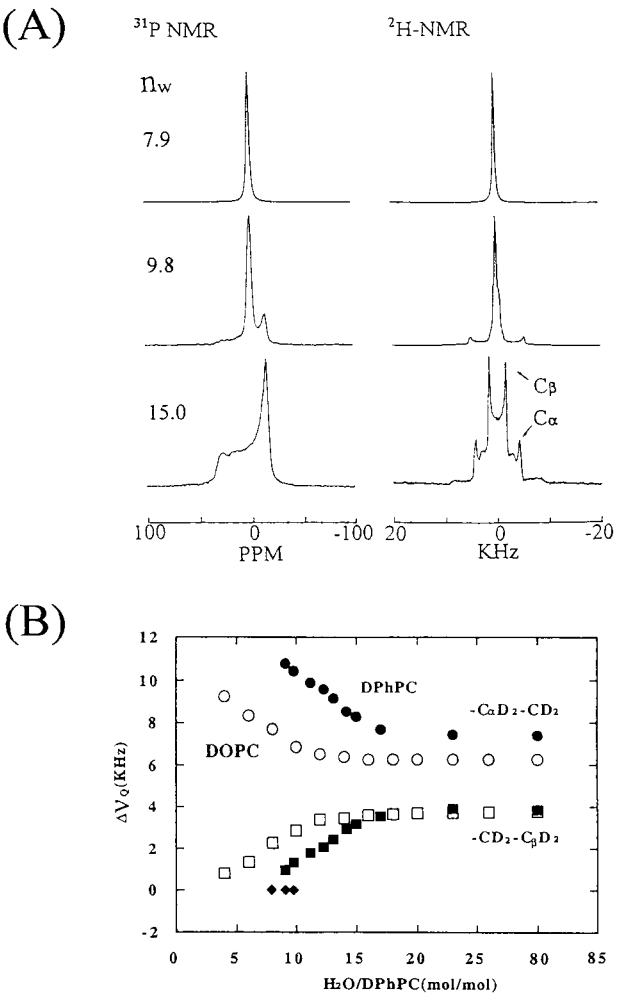

FIGURE 4 Representative ^{31}P and ^{2}H NMR spectra of DPhPC at 25°C and indicated water/lipid molar ratio n_w (*A*) and ^{2}H NMR quadrupolar splitting, $\Delta\upsilon_q$, recorded as a function of the water/lipid molar ratio of DPhPC multilamellar at 25°C (*B*). The quadrupolar splitting of DOPC is taken from Ulrich and Watts (1994). It should be noted that the amount of isotropic component in the ^{31}P spectrum is similar to the related ^{2}H spectrum for n_w = 9.8, if one considers that there are actually α and β splitting of the ^{2}H signal. The filled diamonds represent the presence of isotropic signals at the studied water content.

quantitatively different behavior of the PC headgroup can only be attributed to the distinct physical packing property of DPhPC bilayers. For instance, one of the major differences in a physicochemical property is that the molecular surface of DPhPC determined by the monolayer method is 17 Å^2 greater than that of POPC at all surface pressures measured (Pownall et al., 1987). The designated extra surface area for DPhPC, if the depth of the phosphocholine group is taken into further consideration, can easily accommodate four or five water molecules per lipid. It suggests that the packing geometry near the headgroup region plays a crucial role in the dehydration-induced headgroup reorientation and membrane structure of DPhPC.

The equilibrium coexistence of two phases of DPhPC has also been observed by x-ray diffraction with relative hu-

midity near 85–90% (Wu et al., 1995). Their interbilayer space, estimated from the difference between the bilayer thickness and lamellar spacing (see figure 6 in Wu et al., 1995) of DPhPC, is ~9–10 Å, the water content of which can be estimated (~10–12 water molecules) to be similar to the hydration content of DPhPC used in this study. Therefore, the lamellar-to-lamellar transition detected by this NMR study is indeed associated with the transition detected by the x-ray diffraction method. The discrepancy in not detecting nonlamellar structure in previous x-ray studies on oriented multilamellar bilayers may be attributed to the boundary condition imposed by the glass plates. In fact, recent x-ray diffraction studies on powdered DPhPC samples have also detected nonlamellar structures under similar experimental conditions (Harroun et al., 1996; H. W. Huang, personal communication).

To explain the decreased hydrophobic thickness detected by x-ray diffraction (Wu et al., 1995) and the altered headgroup orientation detected by NMR, a schematic model of DPhPC transition with special emphasis on the headgroup region is proposed (Fig. 5). Under excess water, or with a water content higher than 16 water molecules per DPhPC, the PC headgroup adopts a "normal" orientation, with the P-N dipole approximately parallel to the membrane surface. As in other PC membranes, initial dehydration of the sample moves the N-end of the choline group toward the hydrocarbon region (Bechinger and Seelig, 1991; Ulrich and Watts, 1994). However, as the water content reaches ~8–10 water molecules per DPhPC, a novel lamellar-to-lamellar transition occurs. The cross-sectional area of the fatty acyl chain expands (He et al., 1996), and the orientation of the phosphocholine headgroup tilts to a different direction. Specifically, we propose that the choline group may protrude back to the water layer to account for the opposite effect on the quadrupolar splitting $\Delta\nu_q$ of -CD_2- at both C_α and C_β positions (Fig. 2), and the sudden increase in the interbilayer space detected by x-ray diffraction (Fig. 5).

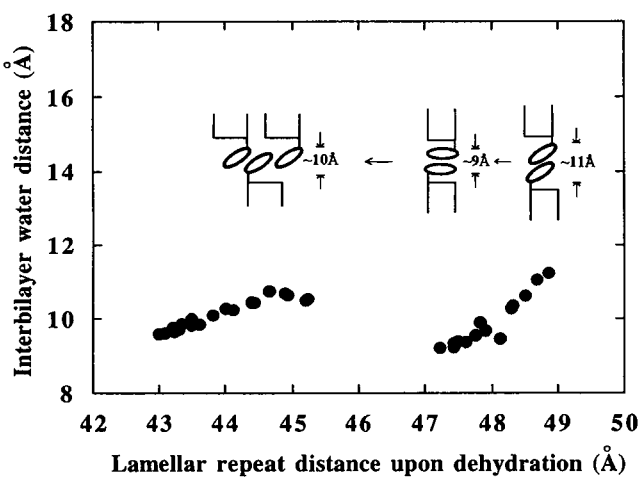

FIGURE 5 A schematic model to explain the respective effect of dehydration on the headgroup orientation and interbilayer water space detected by NMR and x-ray study. The data points of interbilayer water distance are calculated from the difference between the lamellar repeat distance and the bilayer peak-to-peak distance reported by Wu et al. (1995).

One of the possible factors responsible for the proposed rearrangement of the phosphocholine headgroup of DPhPC as compared with other DOPC or POPC molecules is the relatively large cross-sectional area in DPhPC, which is determined to be ~76–80 $Å^2$ and is at least 10 $Å^2$ larger than the 68 $Å^2$ determined for most of the PC molecules in the L_α phase (Beschiaschvili and Seelig, 1990). Upon dehydration, the relatively large empty space of DPhPC in the polar headgroup region would allow interdigitation of the phosphocholine headgroup, as depicted in Fig. 5. Interdigitation of the phospholipid headgroup has at least been detected in the crystal structures of phosphatidic acid (PA) and PE (Pascher et al., 1992). These molecules also contain smaller cross-sectional areas of headgroup than that of the fatty acid chain region. Future study of the headgroup orientation, using oriented DPhPC bilayers, may allow detailed characterization of the proposed conformational change of the PC headgroup.

Phase diagram of DPhPC

In light of the temperature-dependent behavior of DPhPC, we also attempted to explore the possibility that there are other phase transitions of DPhPC at subzero temperature ranges, as detected by NMR, because it was claimed that there was no detectable gel-to-liquid crystalline phase transition for DPhPC over a wide temperature range from −120°C to 120°C (Lindsey et al., 1979). The absence of structural transition of phospholipid at subzero temperatures as low as −120°C appears unlikely, because we have recently observed headgroup freezing events in all of the studied phosphocholine-containing lipids near the homogeneous nucleation temperature, T_H, of ice formation, i.e., −40°C (Wu et al., 1991; Hsieh and Wu, 1996). It has been known for some time that the axial rotation of the entire lipid molecule is associated with the pretransition in phosphatidylcholine bilayers (Marsh, 1980). The observed freezing event of the phospholipid headgroup near −40°C is expected to impose a lower limit for the main phase transition of water containing phospholipid dispersion.

As shown in Fig. 6, the lamellar structures of DPhPC remain in the L_α phase at subzero temperatures higher than T_H for hydration states higher than ~11 water molecules per lipid. Not only is an axial symmetrical pattern maintained; the CSA value also remains at about −40 ppm. The ^{31}P NMR lineshapes start to change into an asymmetrical pattern and represent a rigid lattice-like structure when the temperature is reaches T_H. At a water/lipid ratio of 8.2, the broadening effect on the ^{31}P NMR spectra is clearly visible at −30°C, suggesting that a phase transition occurs above this temperature. In general, dehydration will elevate the transition temperature of DPhPC, as detected by the freezing event of the phosphocholine headgroup (Fig. 6 *A*).

As a further check on the phase transition point at subzero temperatures, we also obtained ^2H NMR spectra of D_2O in

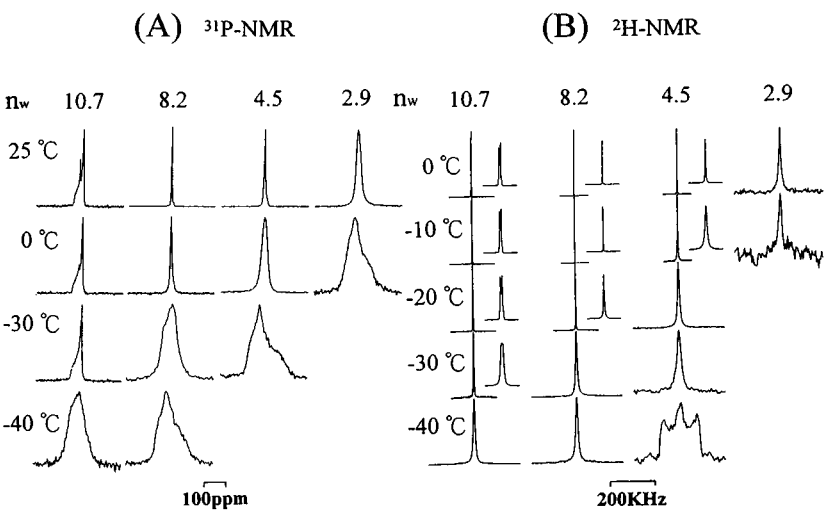

FIGURE 6 ^{31}P and ^{2}H NMR spectra obtained from D_2O/DPhPC at indicated temperatures and water/lipid molar ratios during the heating process. The second set of ^{2}H NMR spectra were 10× expended for the details of the linewidth and lineshape of the associated spectra. The D_2O ^{2}H spectra were reversible during the cooling/heating process at the studied water content, suggesting that there was no excess water removed from the interbilayer space during the cooling process.

DPhPC dispersion. The detection of ^{2}H NMR signal of D_2O in the interbilayer space has previously been shown as a sensitive method for determining the phase diagram of dipalmitoyl PC (Ulmius et al., 1977). A similar approach may then be used to check the phase behavior based on the ^{2}H NMR result at subzero temperatures. For instance, the small residual splitting of the interbilayer D_2O signal is also evident for DPhPC in the lamellar phase, which is consistent with similar studies on DMPC bilayers (Hsieh and Wu, 1996). By examining the linewidth and lineshape of the obtained ^{2}H NMR spectra, we are able to extend the phase diagram established at high temperatures into the subzero temperature range. Fig. 7 summarizes the phase diagram of DPhPC based on ^{31}P and ^{2}H NMR results obtained from −40°C to 80°C.

Implication in polypeptide-membrane interactions and the intrinsic curvature of membranes

DPhPC has been used extensively in the electrophysiological and physicochemical interaction of polypeptides with membranes, but its membrane packing geometry has not been investigated before. We show in this ^{31}P and ^{2}H NMR study that the headgroup orientation of DPhPC is highly sensitive to hydration, and there is a sequel of phase transformation from lamellar to nonlamellar packing geometry upon dehydration from 12 to 4 water molecules per lipid.

The constructed phase diagrams of DPhPC are expected to aid our understanding of the mechanism of insertion of polypeptides into lipid membranes and pore formation. For instance, the orientation of alamethicin in the DPhPC bilayer has been shown to be hydration dependent; dehydration in the DPhPC bilayer inhibits the insertion of alamethicin (Huang and Wu, 1991; He et al., 1996). Because alamethicin binds peripherally to DPhPC membranes in such a way as to expand the membrane surface area by 280 $Å^2$ for each adsorbed peptide, alamethicin may simply fill the void volume created by the dehydration-dependent headgroup reorientation and thus stabilize the peripheral binding state.

The hydration-dependent binding of amphiphilic polypeptides as a result of the molecular rearrangement in the headgroup region may provide an alternative explanation for other protein-lipid interactions. For instance, hydration may also play important role in gramicidin-induced H_{II} phase formation in DOPC model membranes; dehydration in DOPC inhibits the gramicidin-induced H_{II} phase formation (Killian and de Kruijff, 1985). If one assumes that the inserted mode of gramicidin in DOPC may behave simply to increase the cross-sectional area of packing geometry in the hydrophobic region, and thus mimic the packing of

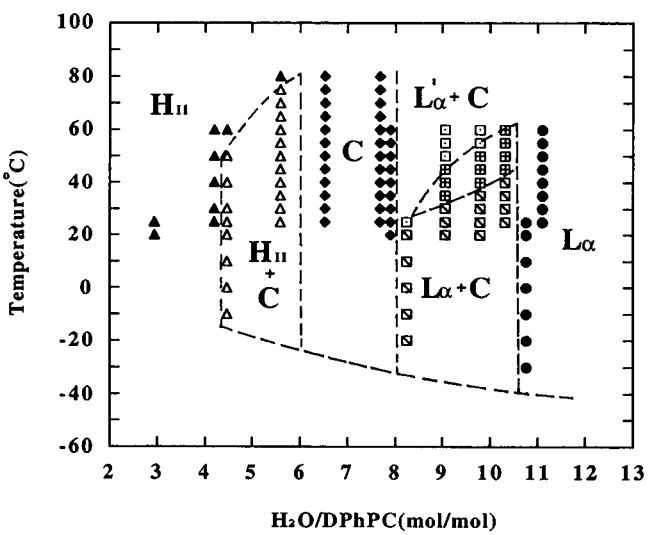

FIGURE 7 Phase diagram of DPhPC in water. The approximate phase boundaries were constructed based on the NMR data points representing the designated phase. ⊞, Coexistence of two lamellar phases, L_α and L'_α, with the presumably cubic phase, C. The filled symbols represent lipid phases with pure bilayer (L_α), cubic (C), and inverted hexagonal (H_{II}) phases; the open symbols represent lipid polymorphism with the indicated phase mixtures.

DPhPC, dehydration should enhance H_{II} phase formation. To reconcile the apparent contradiction, domains of specific interaction or energetical accessibility to the gramicidin/lipid system at low water content were proposed (Chupin et al., 1987; Watnick et al., 1990). It may be possible that there is also a hydration-dependent change in the binding mode of gramicidin in DOPC membranes. Hydration-dependent binding, such as in the case of alamethicin in DPhPC, may therefore provide an alternative explanation on gramicidin-lipid interaction suggested before.

Other lines of evidence are consistent with the interpretation that the peripheral binding mode of amphiphilic polypeptides may be stabilized by the void volume created by dehydration or molecular rearrangement in the headgroup region. It has been shown by OCD measurement that there is an apparent increase in the alamethicin/lipid stoichiometry for the same amount of alamethicin insertion into DPhPC as in DOPC (Huang and Wu, 1991). The insertion of alamethicin into the DPhPC membrane is, therefore, more difficult than that into DOPC because of the strong tendency of alamethicin to remain in the peripheral binding mode of DPhPC. Recent study of the interaction of the PE/PC mixture with alamethicin also indicates that the effect of PE may simply create a space for alamethicin to fill in the water layer and stabilize the peripheral binding state of alamethicin (H. W. Huang, personal communication). Therefore, the molecular packing of molecules in the headgroup region should also play a role, in addition to the hydrophobic match, in determining the exact binding mode of amphiphilic polypeptides with membrane bilayers.

The hydration-dependent curvature of DPhPC membrane will also help in establishing the role of molecular packing in the determination of intrinsic curvature in membranes. Most of the previous studies on the effect of curvature on the phospholipid packing in membranes have mainly focused on the fatty acyl chain region (see, for instance, Lafleur et al., 1996), but it is known that the structure and dynamics of polar headgroup are also significantly perturbed, as evidenced by the differential linewidth of the NMR signal from the inner and outer monolayers of sonicated membrane vesicles in the headgroup region (Wu, 1995). Despite the advances in relating the elastic property (Helfrich, 1978; Gruner, 1985) of the monolayer to the molecular shape (Israelachvili, 1992; Marsh, 1996), the packing parameter can only be operationally considered to be a general quantity that effectively includes interactions between the lipid polar headgroups and between the lipid hydrocarbon chains. We show in this study that "dehydration" of a DPhPC molecule at a water/lipid ratio higher than the primary hydration shell (Hsieh and Wu, 1996), thus without dramatic perturbation of their chemical interaction, may induce change in the membrane packing geometry in an interesting manner. Therefore, molecular rearrangement in the headgroup region also plays a role in modulating the intrinsic curvature of membranes. Future study to provide a detailed molecular information and elastic property of DPhPC may allow quantitative estimates of important packing parameter useful for our understanding of the polymorphic membrane structures.

CONCLUSION

Diphytanoyl phosphatidylcholine, DPhPC, is shown to exhibit a diverse membrane packing geometry in response to variations in temperature and water content, in contrast to the general belief that it forms a stable liquid-crystalline bilayer. A lamellar-to-lamellar transition of DPhPC associated with the headgroup reorientation is also demonstrated by ^{31}P and ^{2}H NMR spectroscopic investigation. The transition is hydration- as well as temperature-dependent and exhibits a novel property in expanding the lipid surface area and in changing the phosphocholine headgroup orientation upon dehydration. This is in sharp contrast to the dehydration-induced reduction of the surface area and movement of the choline group toward the hydrocarbon layer in most of the other PC dispersions. A schematic model is proposed to explain the existing data of DPhPC membranes. The observation is interesting because it provides an explanation of the hydration-dependent binding of polypeptides with phospholipid membrane. It is suggested that DPhPC may serve as an interesting system for investigating the relationship between molecular packing geometry and the intrinsic curvature of membranes.

We thank Professor H. W. Huang for the initiation of the project and many helpful discussions during the course of this research project.

This work was supported by the National Science Council, Taiwan (grants 85-2113-M007-035Y and 85-2311-B007-025).

REFERENCES

Baldwin, P. A., and W. L. Hubbell. 1985. Effects of lipid environment on the light-induced conformational changes of rhodopsin. 2. Roles of lipid chain length, unsaturation, and phase state. *Biochemistry.* 24: 2633–2639.

Bechinger, B., and J. Seelig. 1991. Conformational changes of the phosphatidylcholine headgroup due to membrane dehydration: a ^{2}H-NMR study. *Chem. Phys. Lipids.* 58:1–5.

Beschiaschvili, G., and J. Seelig. 1990. Melittin binding to mixed phosphatidylglycerol/phosphatidylcholine membrane. *Biochemistry.* 29: 52–58.

Blaustein, R. O., T. M. Koehler, R. J. Collier, and A. Finkelstein. 1989. Anthrax toxin: channel-forming activity of protective antigen in planar phospholipid bilayers. *Proc. Natl. Acad. Sci. USA.* 86:2209–2213.

Chupin, V., J. A. Killian, and B. de Kruijff. 1987. ^{2}H-Nuclear magnetic resonance investigations on phospholipid acyl chain order and dynamics in the gramicidin-induced hexagonal HII phase. *Biophys. J.* 51:395–405.

Cullis, P. R., and M. J. Hope. 1991. Physical properties and functional roles of lipids in membranes. *In* Biochemistry of Lipids, Lipoproteins and Membranes. D. E. Vance and J. Vance, editors. Elsevier Scientific Publishers, Amsterdam. 1–41.

Durkin, J. T., R. E. Koeppe, II, and O. S. Andersen. 1990. Energetics of gramicidin hybrid channel formation as a test for structural equivalence. Side-chain substitutions in the native sequence. *J. Mol. Biol.* 211: 221–234.

Gambale, F., and M. Montal. 1988. Characterization of the channel properties of tetanus toxin in planar lipid bilayers. *Biophys. J.* 53:771–783.

Gruner, S. L. 1985. Intrinsic curvature hypothesis for biomembrane lipid composition: a role for non-bilayer lipids. *Proc. Natl. Acad. Sci. USA.* 82:3665–3669.

Gruner, S. L., M. W. Tate, G. L. Kirk, P. T. C. So, D. C. Turner, D. T. Keane, C. P. S. Tilcock, and P. R. Cullis. 1988. X-ray diffraction study of the polymorphic phase behaviour of *N*-methylated dioleoylphosphatidylethanolamine. *Biochemistry.* 27:2853–2866.

Gutknecht, J. 1987. Proton/hydroxide conductance and permeability through phospholipid bilayer membranes. *Proc. Natl. Acad. Sci. USA.* 84:6443–6446.

Harroun, T., K. He, W. T. Heller, S. J. Ludtke, H. W. Huang, C.-H. Hsieh, and W. Wu. 1996. Lyotropic phases of diphytanoyl phosphatidylcholine. *Biophys. J.* 70:A88.

He, K., S. J. Ludtke, W. T. Heller, and H. W. Huang. 1996. Mechanism of alamethicin insertion into lipid bilayers. *Biophys. J.* 71:2669–2679.

Helfrich, W. 1978. Steric interaction of fluid membranes in multilayer systems. *Z. Naturforsch.* 33a:305–315.

Hsieh, C.-H., and W. Wu. 1995a. Solvent effect on phosphatidylcholine headgroup dynamics as revealed by the energetics and dynamics of two gel-state bilayer headgroup structures at subzero temperatures. *Biophys. J.* 69:4–12.

Hsieh, C.-H., and W. Wu. 1995b. Molecular order and hydration property of amine group in phosphatidylethanolamine and its *N*-methyl derivatives at subzero temperatures. *Biophys. J.* 69:2521–2530.

Hsieh, C.-H., and W. Wu. 1995c. Three distinct types of unfrozen water in fully hydrated phospholipid bilayers: a combined ^{2}H and ^{31}P NMR study. *Chem. Phys. Lipids.* 78:37–45.

Hsieh, C.-H., and W. Wu. 1996. Structure and dynamics of primary hydration shell of phosphatidylcholine bilayers at subzero temperatures. *Biophys. J.* 71:3278–3287.

Huang, H. W., and Y. Wu. 1991. Lipid-alamethicin interactions influence alamethicin orientation. *Biophys. J.* 60:1079–1087.

Hui, S.-W., and A. Sen. 1989. Effects of lipid packing on polymorphic phase behavior and membrane properties. *Proc. Natl. Acad. Sci. USA.* 86:5825–5829.

Israelachvili, J. N. 1992. Intermolecular and Surface Forces, 2nd Ed. Academic Press, San Diego.

Killian, J. A., and B. de Kruijff. 1985. Importance of hydration for gramicidin-induced hexagonal HII phase formation in dioleoylphosphatidylcholine model membrane. *Biochemistry.* 24:7890–7898.

Koeppe, R. E., II, and O. S. Andersen. 1996. Engineering the gramicidin channel. *Annu. Rev. Biophys. Biomol. Struct.* 25:231–258.

Koynova, R., and M. Caffrey. 1994. Phase and phase-transition of the hydrated phosphatidylethanolamines. *Chem. Phys. Lipids.* 69:1–34.

Lafleur, M., M. Bloom, E. F. Eikenberry, S. M. Gruner, Y. Han, and P. R. Cullis. 1996. Correlation between lipid plane curvature and lipid chain order. *Biophys. J.* 70:2747–2757.

Lindsey, H., N. O. Petersen, and S. I. Chan. 1979. Physicochemical characterization of 1,2-diphytanoyl-sn-glycerol-3-phosphocholine in model membrane systems. *Biochim. Biophys. Acta.* 555:147–167.

Lovejoy, B., K. S. Akerfeldt, W. F. DeGrado, and D. Eisenberg. 1992. Crystallization of proton channel peptides. *Protein Sci.* 1:1073–1077.

Ludtke, S. J., K. He, W. T. Heller, T. A. Harroun, L. Yang, and H. W. Huang. 1996. Membrane pores induced by magainin. *Biochemistry.* 35:13723–13728.

Ludtke, S. J., K. He, and H. W. Huang. 1995. Membrane thinning caused by magainin 2. *Biochemistry.* 34:16764–16769.

Luzzati, V., T. Gulik-Krzywicki, and A. Tardieu. 1968. Polymorphism of lecithins. *Nature.* 218:1031–1034.

Macdonald, P. M., J. Leisen, and F. M. Marassi. 1991. Response of phosphatidylcholine in the gel and liquid-crystalline states to membrane surface charges. *Biochemistry.* 30:3558–3566.

Marsh, D. 1980. Molecular motion in phospholipid bilayers in the gel phase: long axis rotation. *Biochemistry.* 19:1632–1637.

Marsh, D. 1996. Intrinsic curvature in normal and inverted lipid structures and in membranes. *Biophys. J.* 70:2248–2255.

Mollevanger, L. C., A. P. Kentgens, J. A. Pardoen, J. M. Courtin, W. S. Veeman, J. Lugtenburg, and W. J. de Grip. 1987. High-resolution solid-state ^{13}C-NMR study of carbons C-5 and C-12 of the chromophore of bovine rhodopsin. Evidence for a 6-S-*cis* conformation with negative-charge perturbation near C-12. *Eur. J. Biochem.* 163:9–14.

Pascher, I., M. Lundmark, P.-G. Nyholm, and S. Sundell. 1992. Crystal structures of membrane lipids. *Biochim. Biophys. Acta.* 113:339–373.

Pinheiro, T. J. T., A. A. Duralski, and A. Watts. 1994. Phospholipid headgroup-headgroup electrostatic interactions in mixed bilayers of cardiolipin with phosphatidylcholines studied by ^{2}H NMR. *Biochemistry.* 33:4896–4902.

Pownall, H. J., Q. Pao, H. L. Brockman, and J. B. Massey. 1987. Inhibition of lecithin-cholesterol acyltransferase by diphytanoly phosphatidylcholine. *J. Biol. Chem.* 262:9033–9036.

Roux, M., J. M. Neumann, R. S. Hodges, P. F. Devaux, and M. Bloom. 1989. Conformational changes of phospholipid headgroup induced by a cationic integral membrane peptides as seen by deuterium magnetic resonance. *Biochemistry.* 28:2313–2321.

Scherer, P. G., and J. Seelig. 1989. Electric charge effects on phospholipid headgroups: phosphatidylcholine in mixtures with cationic and anionic amphiphiles. *Biochemistry.* 28:7720–7728.

Schmid, A., R. Benz, I. Just, and K. Aktories. 1994. Interaction of Clostridium botulinum C2 toxin with lipid bilayer membranes. Formation of cation-selective channels and inhibition of channel function by chloroquine. *J. Biol. Chem.* 269:16706–16711.

Seelig, J. 1978. ^{31}P nuclear magnetic resonance and the head group structure of phospholipid in membrane. *Biochim. Biophys. Acta.* 515: 105–140.

Seelig, J., P. M. Macdonald, and P. G. Scherer. 1987. Phospholipid head groups as sensors of electric charge in membranes. *Biochemistry.* 26: 7535–7541.

Spassova, M., I. R. Mellor, A. G. Petrov, K. A. Beattie, G. A. Codd, H. Vais, and P. N. Usherwood. 1995. Pores formed in lipid bilayers and in native membranes by nodularin, a cyanobacterial toxin. *Eur. Biophys. J.* 24:69–76.

Sternin, E., M. Bloom, and A. L. Mackay. 1983. De-pake-ing of NMR spectra. *J. Magn. Res.* 55:274–282.

Taylor, R. J., and R. de Levie. 1991. "Reversed" alamethicin conductance in lipid bilayers. *Biophys. J.* 59:873–879.

Ulmius, J., H. Wennerstrom, G. Lindblom, and G. Arvidson. 1977. Deuteron nuclear magnetic resonance studies of phase equilibria in a lecithin-water system. *Biochemistry.* 16:5742–5745.

Ulrich, A. S., and A. Watts. 1994. Molecular response of the lipid headgroup to bilayer hydration monitored by ^{2}H-NMR. *Biophys. J.* 66: 1441–1449.

Watnick, P. I., S. I. Chan, and P. Dea. 1990. Hydrophobic mismatch in gramicidin A'/lecithin system. *Biochemistry.* 29:6215–6221.

Wu, W. 1995. Sonicated membrane vesicles. *In* Encyclopedia of NMR. D. M Grant and R. K. Harris, editors. John Wiley and Sons, Sussex, England. 4485–4492.

Wu, W., L.-M. Chi, T.-S. Yang, and S.-Y. Fang. 1991. Freezing of phosphocholine headgroup in fully hydrated sphingomyelin bilayers and its effect on the dynamics of nonfreezable water at subzero temperatures. *J. Biol. Chem.* 266:13602–13606.

Wu, Y., K. He, S. J. Ludtke, and H. W. Huang. 1995. X-ray diffraction study of lipid bilayer membranes interacting with amphiphilic helical peptides: diphytanoyl phosphatidylcholine with alamethicin at low concentrations. *Biophys. J.* 68:2361–2369.

Yamauchi, K., K. Doi, M. Kinoshita, F. Kii, and H. Fukuda. 1992. Archaebacterial lipid models: highly salt-tolerant membranes from 1,2-diphytanylglycero-3-phosphocholine. *Biochim. Biophys. Acta.* 1110: 171–177.

Yamauchi, K., K. Doi, Y. Yoshida, and M. Kinoshita. 1993. Archaebacterial lipids: highly proton-impermeable membranes from 1,2-diphytanyl-sn-glycero-3-phosphocholine. *Biochim. Biophys. Acta.* 1146: 178–182.