Blackburn, M. N., Smith, R. L., Carson, J., & Sibley, C. C. (1984) *J. Biol. Chem. 259*, 939–941.
Busby, T. F., Atha, D. H., & Ingham, K. C. (1981) *J. Biol. Chem. 256*, 12140–12147.
Chandra, T., Stackhouse, R., Kidd, V. J., & Woo, S. L. C. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1845–1848.
Chang, J. Y., & Tran, T. H. (1986) *J. Biol. Chem. 261*, 1174–1176.
Fish, W. W., Danielsson, Å., Nordling, K., Miller, S. H., Lam, C. F., & Björk, I. (1985) *Biochemistry 24*, 1510–1517.
Gettins, P. (1987) *Biochemistry 26*, 1391–1398.
Jorgensen, A. M., Borders, C. L., & Fish, W. W. (1985) *Biochem. J. 231*, 59–63.
Koide, T., Odani, S., Takahashi, K., Ono, T., & Sakuragawa, N. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 289–293.
Kress, L. F., & Catanese, J. J. (1981) *Biochemistry 20*, 7432–7438.
Kurachi, K., Schmer, G., Hermodson, M. A., Teller, D. C., & Davie, E. W. (1976) *Biochemistry 15*, 368–372.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.
Mitra, G., Schneider, P. M., & Lundblad, J. L. (1982) *Biotechnol. Bioeng. 24*, 97–107.
Nordenstrom, B., Nystrom, C., & Björk, I. (1977) *Eur. J. Biochem. 78*, 195–201.
Pecan, J. M., & Blackburn, M. N. (1984) *J. Biol. Chem. 259*, 935–938.
Petersen, T. E., Dudek-Wojciechowska, G., Sottrup-Jensen, L., & Magnusson, S. (1978) in *The Physiological Inhibitors of Blood Coagulation and Fibrinolysis* (Collen, D., Wirman, B., & Verstraete, M., Eds.) pp 43–54, Elsevier/North-Holland, Amsterdam.
Privalov, P. L. (1979) *Adv. Protein Chem. 33*, 182–186.
Rosenberg, R. D., & Damus, P. S. (1973) *J. Biol. Chem. 248*, 6490–6505.
Rosenfeld, L., & Danishefsky, I. (1984) *Arch. Biochem. Biophys. 229*, 359–367.
Rosenfeld, L., & Danishefsky, I. (1986) *Biochem. J. 237*, 639–646.
Thaler, E., & Schmer, G. (1975) *Br. J. Haematol. 31*, 233–243.
Villanueva, G. B. (1984) *J. Biol. Chem. 259*, 2531–2536.
Villanueva, G. B., & Allen, N. (1983a) *J. Biol. Chem. 258*, 11010–11013.
Villanueva, G. B., & Allen, N. (1983b) *J. Biol. Chem. 258*, 14048–14053.
Zavyalov, V. P., Troitsky, V. V., Khechinashvili, N. N., & Privalov, P. L. (1977) *Biochim. Biophys. Acta 492*, 102–111.

Infrared Studies of Fully Hydrated Saturated Phosphatidylserine Bilayers. Effect of Li^+ and Ca^{2+} †

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ABSTRACT: The thermotropic phase behavior of fully hydrated Na^+ and/or NH_4^+ salts of 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (DMPS) was determined by temperature-dependent infrared spectra. The molecular level properties and thermal phase behavior of DMPS–Li^+ complexes were also characterized by infrared spectroscopy. With increasing concentrations of Li^+, the infrared spectra reveal the appearance of a second, more ordered, lipid phase which shows a gel to liquid-crystal transition at significantly higher temperatures (75–95 °C) than the Na^+ or NH_4^+ salts of DMPS (39 °C). Li^+ binds to the phosphate and carboxylate groups of DMPS, resulting in the following changes: (1) water of hydration is lost from both the carboxylate and phosphate groups; (2) there are changes in the conformation of the glycerol backbone but not in the P–O ester bonds of the phosphate group which remain in the gauche–gauche conformation; and (3) the packing of the fatty acyl chains becomes more ordered. In addition, the properties of the DMPS–Ca^{2+} complex were studied by infrared spectroscopy. While the DMPS–Ca^{2+} complex is also characterized by rigidly packed, well-ordered fatty acyl chains, the mode of Ca^{2+} binding to the DMPS head groups differs significantly from that of Li^+ binding. By comparison, with dry DMPS–Ca^{2+} [Casal, H. L., Mantsch, H. H., Paltauf, F., & Hauser, H. (1987) *Biochim. Biophys. Acta* (in press)], the phosphate group undergoes a conformational change, probably to the antiplanar–antiplanar conformation, and loses its water of hydration. In contrast to the DMPS–Li^+ complex, the carboxylate group remains hydrated in the DMPS–Ca^{2+} complex, indicating that Ca^{2+} is chelated by phosphate groups only. Furthermore, in the DMPS–Ca^{2+} complex, one of the ester carbonyl groups is engaged in hydrogen bonding; such a hydrogen bond is not found in DMPS–Na^+, DMPS–NH_4^+, and DMPS–Li^+.

The interaction of metal ions with membrane lipids is of great importance in the control of the structure and function of biological membranes. The interaction of metal ions with anionic phospholipids such as phosphatidylserine (PS)[1] seems to be related to many of the roles which these lipids play in

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[1] Abbreviations: DMPS, 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine; PS, phosphatidylserine; DSC, differential scanning calorimetry; Tes, *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid or 2-[[tris(hydroxymethyl)methyl]amino]ethanesulfonic acid; His, histidine; EDTA, ethylenediaminetetraacetic acid.

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membrane function. In particular, lithium and calcium ions interact with PS, inducing specific changes in the lipid structure which may be relevant to membrane function. Ca^{2+}, on the one hand, induces membrane fusion, and Li^+, on the other, has therapeutic effects, presumably interacting at the membrane level in nerve cells (Williams, 1973; Schou, 1976; Papahadjopoulos, 1978).

The interaction of Li^+ and Ca^{2+} ions with DMPS has been studied in some detail by DSC and X-ray diffraction and compared to that of other mono- and divalent cations (Hauser et al., 1982; Hauser & Shipley, 1981, 1983, 1984). In general, Li^+ and Ca^{2+} form crystalline complexes with DMPS, in which the bilayer structure is preserved, but their gel to liquid-crystal phase transitions occur at much higher temperatures than those of other salts of DMPS. Infrared spectroscopy has shown that Ca^{2+} binds directly to the phosphate group of ox brain PS, forming a bidentate complex inducing dehydration of this functional group (Dluhy et al., 1983). Other spectroscopic techniques have reached the same conclusions, Ca^{2+} causing dehydration of the head group while inducing an isothermal crystallization of the PS acyl chains (Hauser et al., 1977; Kurland et al., 1979; Holwerda et al., 1981).

In many aspects, the interaction of Li^+ with DMPS resembles that of Ca^{2+}; however, in a recent study of anhydrous complexes of PS with Li^+ and Ca^{2+}, we found differences in the modes of binding of Li^+ and Ca^{2+} (Casal et al., 1987). While in complexes of Li^+ with PS the PO_4 group is in the same conformation as in the Na^+ or NH_4^+ salts of DMPs, in the case of Ca^{2+} complexes the PO_4 group is in a drastrically different conformation. At the same time, complexation with Ca^{2+} induces formation of hydrogen bonds to the ester carbonyl functional groups.

In this report, we present a detailed investigation by infrared spectroscopy of the temperature-induced phase behavior of aqueous DMPS–NH_4^+ and DMPS–Li^+. In this manner, the mode of interaction of Li^+ with DMPS is characterized in detail. The infrared spectra of the DMPS–Li^+ complexes indicate that the carboxylate and phosphate groups lose their water of hydration. In the gel phase of DMPS–Li^+, the spectra are compatible with orthorhombic-like packing of the fatty acyl chains. The infrared spectra of the DMPS–Ca^{2+} complex in aqueous medium are also studied, and the properties of these complexes are compared with those of DMPS–Li^+.

Materials and Methods

1,2-Dimyristoyl-*sn*-glycero-3-phospho-L-serine (DMPS) was synthesized and purified as described elsewhere (Hermetter et al., 1982); methods for conversion from the acid form to the various salts have also been described (Hauser et al., 1982; Casal et al., 1987). For comparison, the sodium salt of DMPS was purchased from Avanti Polar Lipids (Birmingham, AL), and its purity was checked by thin-layer chromatography using the solvent mixture $CHCl_3/CH_3OH/NH_3$ (65:35:4 by volume).

Precipitates of DMPS with Li^+ and Ca^{2+} were prepared by adding LiCl or $CaCl_2$ in buffer (100 mM NaCl, 2 mM Tes, 2 mM His, and 0.1 mM EDTA, pH 7, prepared in either D_2O or H_2O) to ready-made unsonicated DMPS dispersions in the same buffer. In all cases, the LiCl or $CaCl_2$ solutions and DMPS were mixed at 45 °C. In general, the samples were ~0.13 M in DMPS and contained different amounts of Li^+ or Ca^{2+} to yield samples of various compositions. The precipitate formed in this way is referred to as aqueous precipitate. The aqueous precipitates were deposited on CaF_2 or BaF_2 windows and assembled into cells of 12-μm path length for infrared measurement. A comment on the equilibration of the PS dispersion with the metal ion is appropriate here. As found previously (Hauser & Shipley, 1981, 1983) and confirmed here by infrared spectroscopy, the order–disorder transition of the DMPS–Li^+ complex occurs at significantly higher temperatures (75–95 °C) than that of DMPS–NH_4^+ or DMPS–Na^+ (39 °C). The order–disorder transition temperature of the DMPS–Ca^{2+} complex was determined by DSC to be at 155 °C (Hauser & Shipley, 1984). Information as to the degree of equilibration of the PS dispersion with Li^+ or Ca^{2+} can be obtained from the temperature dependence of the $\nu_s(CH_2)$ mode at 2850 cm^{-1}. The order–disorder transition of DMPS–NH_4^+ or DMPS–Na^+, which has not interacted with either Li^+ or Ca^{2+}, is readily detected by the increase in $\nu_s(CH_2)$ wavenumber at 39 °C (cf. Figure 1). In the presence of Ca^{2+}, as used in this study, no transition occurred at 39 °C, indicating that the DMPS–NH_4^+ was converted to the Ca^{2+} salt. This would require that Ca^{2+} ions were equilibrated across the PS bilayers. In contrast to Ca^{2+}, in the presence of Li^+ under comparable experimental conditions, the transition at 39 °C is still observable (cf. Figure 4). This is interpreted to mean either that Li^+ is not equilibrated across the DMPS bilayers or, alternatively, if it is equilibrated, that it must reflect the equilibrium of Li^+ binding to DMPS.

Infrared spectra at 2 cm^{-1} resolution were collected with a Digilab FTS-11 or a Digilab FTS-15 spectrometer using procedures for data collection and analysis described elsewhere (Cameron & Jones, 1981; Mantsch et al., 1986).

Results and Discussion

Thermotropic Phase Behavior of DMPS–NH_4^+. The thermotropic phase behavior of DMPS–NH_4^+ has been well characterized by DSC and X-ray diffraction (Hauser et al., 1982); there is a gel to liquid-crystal phase transition at 39.0 °C with a transition enthalpy of 7.4 kcal/mol. X-ray diffraction studies have demonstrated that DMPS–NH_4^+ forms bilayers with the same general characteristics as those of other membrane lipids. The order–disorder transition involves primarily the conformational melting of the fatty acyl chains which in the gel phase are packed in a hexagonal lattice.

In this first section, we present the relevant features of the infrared spectrum of hydrated DMPS–NH_4^+ bilayers to serve as a basis for the comparison with the DMPS–Li^+ and DMPS–Ca^{2+} complexes. As is commonly done, we followed the temperature-induced changes in the infrared spectrum (Casal & Mantsch, 1984). The gel to liquid-crystal transition temperature is conveniently determined from the temperature dependence of the wavenumber of the CH_2 symmetric stretching mode as shown in Figure 1. The transition is found at 41 °C and involves a discrete increase of the wavenumber of 2.8 cm^{-1} (from 2850.4 to 2853.2 cm^{-1}), a change typical of this type of transition and consistent with the known ΔH of the transition.

Other vibrational modes due to the DMPS–NH_4^+ acyl chains are also consistent with the known properties of these bilayers. For example, the CH_2 scissoring mode [$\delta(CH_2)$] gives rise to a single band at 1468 cm^{-1} in the spectra of gel phase DMPS–NH_4^+ (vide infra), which is typical of systems with acyl or alkyl chains packed in a hexagonal lattice. In the spectra of the liquid-crystalline phase, this band decreases markedly in intensity.

However, the spectrum of hydrated DMPS–NH_4^+ at temperatures below −30 °C shows that the $\delta(CH_2)$ mode gives rise to two bands at 1473 and 1465 cm^{-1}. This splitting of the $\delta(CH_2)$ mode is consistent with orthorhombic-like packing of the DMPS–NH_4^+ acyl chains (Casal & Mantsch, 1984).

Among the vibrational modes originating from the lipid

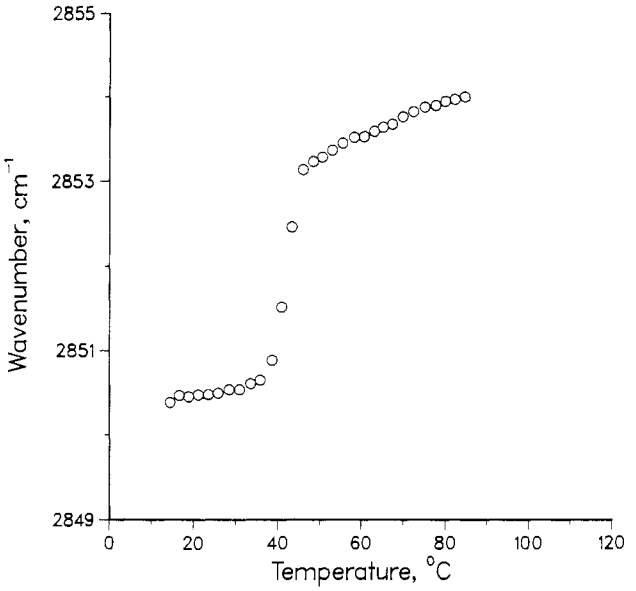

FIGURE 1: Temperature dependence of the wavenumber of the $\nu_s(CH_2)$ mode in the infrared spectra of aqueous dispersions of DMPS–NH_4^+.

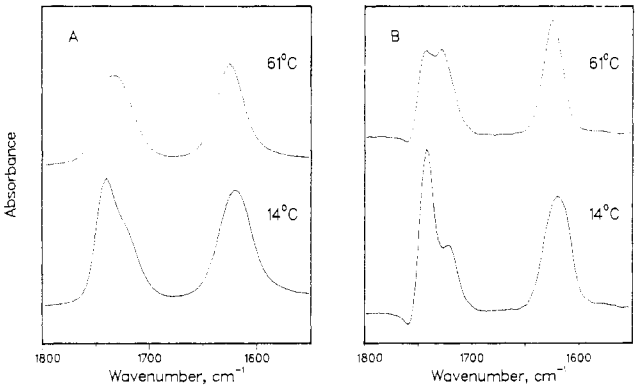

FIGURE 2: 1800–1550 cm^{-1} region of the infrared spectrum of hydrated (D_2O buffer) DMPS–NH_4^+ at 14 and 61 °C. The traces in (B) represent the result of deconvolution with Lorentzian lines of 15 cm^{-1} full width at half-height and a resolution enhancement factor of 2, using the methods described by Kauppinen et al. (1981).

polar group, the stretching of the ester carbonyl groups is a convenient monitor of membrane structure (Levin, 1984). These vibrational modes are sensitive to interactions at the head-group level. The two ester carbonyl groups in diacyl lipid assemblies are nonequivalent due to the different conformation of the C_2–C_3 segments of the two acyl chains and give rise to two bands which have been assigned (Mushayakarara & Levin, 1982).

Figure 2 shows infrared spectra of hydrated DMPS–NH_4^+ at 14 °C (gel phase) and 61 °C (liquid-crystalline phase) where the two C=O stretching bands are clearly distinguished, particularly after resolution enhancement as shown in Figure 2B. In the spectrum of the DMPS–NH_4^+ gel phase, the two ester carbonyl stretching bands are quite dissimilar in intensity; the band due to the *sn*-1 C=O group (1742 cm^{-1}) is much more intense than the one due to the *sn*-2 C=O group (1721 cm^{-1}). This type of different relative intensities of the two C=O stretching bands has been observed in the spectra of many other lipids in the gel phase. The difference in intensity could indeed be due to the different conformations of the two acyl chains in the vicinity of the C=O groups. In the liquid-crystalline phase, the conformational differences of the two chains are somewhat averaged, and in the spectrum of DMPS–NH_4^+ at 61 °C (Figure 2B), the two C=O stretching bands are of comparable intensity.

In terms of comparison with the spectra of DMPS–Li^+ and DMPS–Ca^{2+} complexes, we emphasize that in the case of DMPS–NH_4^+ there are *two* bands due to C=O stretching. We also note that, as currently accepted (Levin, 1984), the values of the wavenumbers of these two vibrations indicate that neither of the ester carbonyl groups is engaged in hydrogen bonding. When a sample of hydrated DMPS–NH_4^+ is cooled to −75 °C, the corresponding infrared spectrum reveals that even at this low temperature there are *only two* bands due to ester carbonyl stretching, one at 1740 cm^{-1} (*sn*-1 chain) and the second at 1716 cm^{-1} (*sn*-2 chain). Furthermore, the relative intensity of the two bands at −75 °C is the same as in the 14 °C spectrum. Thus, when the DMPS–NH_4^+ aqueous dispersion is immobilized (at −75 °C), the conformations of the ester carbonyl groups are the same as in the gel phase at temperatures close to T_1 (the gel to liquid-crystal transition temperature of DMPS–NH_4^+).

The spectra displayed in Figure 2 also show a band at 1622 cm^{-1} due to the antisymmetric stretching vibration of the carboxylate group of the serine moiety in the DMPS head group $\nu_{as}(CO_2)$. In the case of DMPS–NH_4^+, the $\nu_{as}(CO_2)$ mode is at 1622 cm^{-1} in gel phase spectra and at 1626 cm^{-1} in liquid-crystal phase spectra (Figure 2). The values of the $\nu_{as}(CO_2)$ wavenumber demonstrate that the carboxylate group is well hydrated in both gel and liquid-crystalline phases. In contrast, in the spectra of anhydrous DMPS–NH_4^+ the $\nu_{as}(CO_2)$ mode is at 1640 cm^{-1} (Casal et al., 1987). The finding that the carboxylate group is hydrated above and below the transition temperature is consistent with the swelling behavior of DMPS–NH_4^+ as determined by X-ray diffraction. Both the gel and liquid-crystalline phases show infinite swelling (Hauser et al., 1982). The spectra of Figure 2 also demonstrate that there is only one band for $\nu_{as}(CO_2)$. Moreover, in the spectra of DMPS–NH_4^+ at −75 °C, the wavenumber of the $\nu_{as}(CO_2)$ mode is 1618 cm^{-1}, compatible with this group being hydrated.

The phosphate group in the DMPS–NH_4^+ head group gives rise to several vibrational modes which are useful for membrane studies. In the case of anhydrous samples, examination of the single-bond P–O stretching and the PO_2 wagging modes allowed the determination of the local conformation of this group (Casal et al., 1987). In the case of hydrated samples, the spectral region below 850 cm^{-1}, where these two types of vibrations occur, is not amenable to study. Thus, we concentrate on the double-bond O=P=O stretching modes. The antisymmetric PO_2^- stretching, $\nu_{as}(PO_2)$, is between 1240 and 1220 cm^{-1} while the corresponding symmetric stretching, $\nu_s(PO_2)$, is at 1090 cm^{-1}.

Figure 3A shows spectra of hydrated DMPS–NH_4^+ (in H_2O buffer) in the 1300–1150 cm^{-1} region at 24 and 51 °C, corresponding to the gel and liquid-crystalline phases, respectively. In the gel phase spectrum, the $\nu_{as}(PO_2)$ mode is at 1218 cm^{-1} while in the liquid-crystalline phase spectrum it is at 1220 cm^{-1}. These values of the $\nu_{as}(PO_2)$ wavenumbers are consistent with a hydrated phosphate group. The wavenumber of this vibrational mode is sensitive to the hydration of the phosphate group with values around 1220 cm^{-1}, typical of hydrated samples, and values of 1235–1250 cm^{-1}, characteristic of dehydrated phosphate groups. The very small change observed for the $\nu_{as}(PO_2)$ wavenumber between the gel and liquid-crystalline phase is in keeping with previous observations on other membrane lipids (Casal & Mantsch, 1984).

The $\nu_s(PO_2)$ mode is in the 1150–1000 cm^{-1} region crowded with other vibrational modes. Figure 3B shows spectra in this

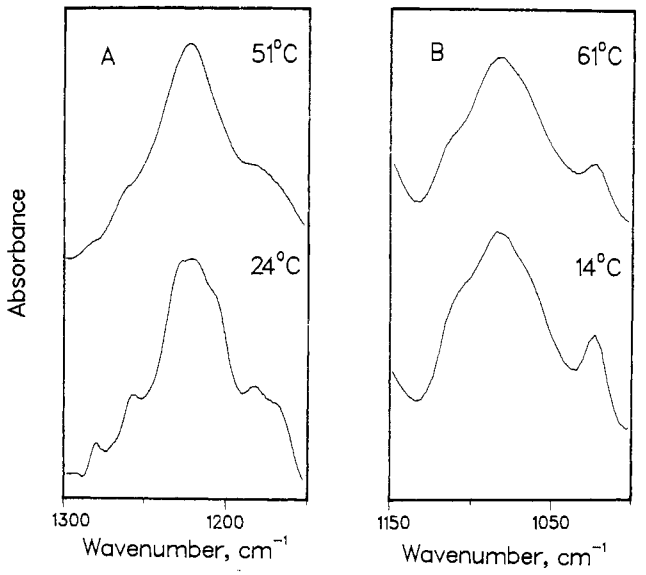

FIGURE 3: (A) 1300–1150 cm^{-1} region of the infrared spectrum of aqueous (H_2O) dispersions of DMPS–NH_4^+ at 24 and 51 °C. (B) 1150–1000 cm^{-1} region of the infrared spectrum of aqueous (D_2O) dispersions of DMPS–NH_4^+ at 14 and 61 °C.

Table I: Transition Temperatures of Aqueous Samples of DMPS–NH_4^+ Containing Increasing Concentrations of LiCl[a]

[DMPS] (M)	[LiCl] (M)	DMPS:Li molar ratio	pH	T_1 (°C)	T_2 (°C)
0.14	0		6.9	41	
0.14	0.05	2.8	7.1	42	65
0.14	0.1	1.6	7.3	43	74
0.14	0.14	1.0	7.1	43	73
0.14	0.25	0.56	6.9		75–92
0.14	0.31	0.44	6.9		70–72
0.16	0.5	0.32	7.0		70–94

[a] Determined from the temperature dependence of the wavenumber of the $\nu_s(CH_2)$ mode in the corresponding infrared spectra of these samples.

region, of DMPS–NH_4^+ (in D_2O buffer), at 14 and 61 °C. There are three main bands, at 1100, 1085, and 1068 cm^{-1}; the pattern is similar to that observed in the spectra of other phospholipids in aqueous media (Casal & Mantsch, 1984). Transition from the gel to the liquid-crystalline phase induces practically no change in these modes; nevertheless, as shown in the case of anhydrous samples (Casal et al., 1987), this spectral region is sensitive to metal ion interactions.

Infrared Spectra of DMPS–Li^+ Complexes. We have studied the temperature dependence of the infrared spectra of DMPS–Li^+ complexes obtained by mixing DMPS–NH_4^+ with increasing amounts of LiCl; samples ranging in DMPS:Li molar ratios from 2.8 to 0.32 were studied.

Previous DSC studies (Hauser & Shipley, 1981; Hauser et al., 1982) have shown that the addition of LiCl to DMPS–NH_4^+ dispersions produces complexes which, depending on the LiCl concentration, show two endothermic events. The first, at about 40 °C, corresponds to the gel to liquid-crystal transition of DMPS–NH_4^+ (referred to as T_1) while the second, at temperatures varying from 70 to 90 °C, corresponds to the conformational melting of the DMPS–Li^+ complex (referred to as T_2). The transition temperatures, as determined from the temperature dependence of the $\nu_s(CH_2)$ wavenumber, are summarized in Table I. There are some discrepancies between these temperatures and those determined by DSC at comparable molar ratios which could be due to differences in the sample preparation and in the measurement of temperature.

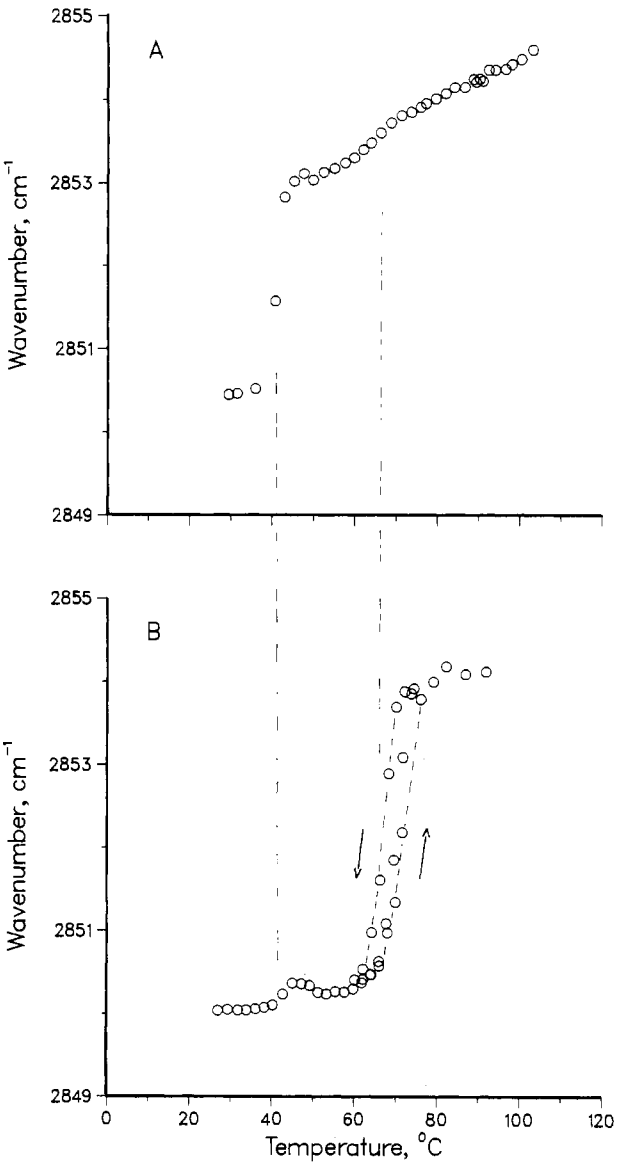

FIGURE 4: Temperature dependence of the wavenumber of the $\nu_s(CH_2)$ band in the infrared spectra of DMPS–NH_4^+ in samples containing (A) 0.05 M LiCl and 0.14 M DMPS–NH_4^+ (DMPS:Li ratio = 2.8) and (B) 0.14 M LiCl and 0.14 M DMPS–NH_4^+ (DMPS:Li ratio = 1.0).

Figure 4 shows the temperature dependence of the $\nu_s(CH_2)$ wavenumber of samples with DMPS:Li molar ratios of 2.8 and 1.0. In the first case (molar ratio = 2.8; Figure 4A), there are two transitions evident as discrete increases in the $\nu_s(CH_2)$ wavenumber; they are at 42 and 65 °C. The first one (larger change) corresponds to T_1, the conformational melting of uncomplexed DMPS–NH_4^+; the second (smaller change) corresponds to T_2, the conformational melting of the DMPS–Li^+ complex. These data show that even at this low concentration of Li^+ there is phase separation, the two phases being DMPS–NH_4^+ and DMPS–Li. The present data cannot distuinguish between two-dimensional and three-dimensional phase separation.

In the case of the sample with a DMPS:Li molar ratio of 1.0, there are also two transitions at 43 and 73 °C (Figure 4B). The first one (smaller change) corresponds to T_1 of the uncomplexed DMPS–NH_4^+ while the second one (larger change) is the conformational melting of the DMPS–Li^+ complex (T_2). In Figure 4B, we also include data points obtained by cooling the sample immediately after heating through T_2. The transition at T_2 is reversible, with hysteresis of about 4 °C, and

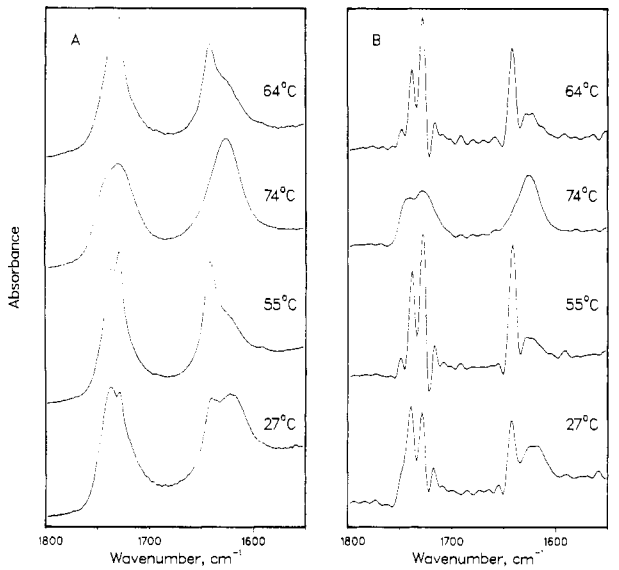

FIGURE 5: 1800–1550 cm^{-1} region of the infrared spectrum of hydrated (D_2O buffer) DMPS–NH_4^+ containing equimolar quantities of LiCl at 27, 55, and 74 °C; the spectrum at 64 °C was recorded after recording that at 74 °C; see text. The traces in (B) are the result of deconvolution with Lorentzian lines of 15 cm^{-1} full width at half-height (see caption to Figure 2).

it is interesting to note that the values of the $\nu_s(CH_2)$ wavenumbers are the same after cooling from temperatures above T_2. Furthermore, the final values of the $\nu_s(CH_2)$ wavenumber attained in the liquid-crystalline phase are the same (2854 cm^{-1}) for DMPS–NH_4^+ (Figure 1) and DMPS–Li^+ (Figure 4) at different molar ratios. A priori, this indicates that the final liquid-crystalline phases obtained are very similar. The same conclusion is obtained by examination of other spectral features (see below).

Since the shift to higher wavenumbers of the $\nu_s(CH_2)$ mode reflects primarily the introduction of gauche bonds in the DMPS acyl chains, the higher temperature required to "melt" the DMPS–Li^+ complexes indicates that complexation with Li^+ induces crystallization of the fatty acyl chains, which is in accord with previous reports (Hauser & Shipley, 1981, 1983).

The mode of interaction of Li^+ with DMPS at the molecular level can be studied by analysis of vibrational modes originating from other functional groups in DMPS. As a representative example, we present data obtained with DMPS–Li^+ complexes (of molar ratio 1.0). The effects of increasing the concentration of Li^+ are cumulative.

Figure 5 shows the 1800–1550 cm^{-1} region of the infrared spectrum of aqueous precipitates of DMPS–Li^+ (1:1 molar ratio) at 27, 55, 74, and 64 °C. The spectrum at 64 °C was recorded after the sample had been heated through the T_2 transition of the DMPS–Li^+ complex at 73 °C (see Figure 4B). As already discussed for DMPS–NH_4^+ (Figure 2), the bands in this spectral region are due to ester carbonyl stretching and CO_2^- antisymmetric stretching. The spectral features due to the ester C=O groups are quite different from those of the DMPS–NH_4^+ spectrum. In the spectrum of DMPS–Li^+ at 27 °C, there are three main bands at 1740, 1728, and 1716 cm^{-1} which are clearly separated in the deconvolved spectrum (Figure 5B) and also a shoulder at 1745 cm^{-1}. The same pattern of bands was found in the spectrum of anhydrous DMPS–Li^+. The situation is more complicated in the case of the aqueous precipitates of DMPS–Li^+, since in the spectrum recorded at 27 °C there is coexistence of two gel phases, that of DMPS–NH_4^+ and that of DMPS–Li^+. In the spectrum recorded at 55 °C, the DMPS–Li^+ gel phase and the DMPS–NH_4^+ liquid-crystalline phases now coexist since the event at 43 °C was ascribed to the main transition of uncomplexed DMPS–NH_4^+ (see above). The separation of the spectra into the different contributions is not feasible; however, the general features can be understood in terms of the complexation of Li^+ to the DMPS head group.

From the data of Figure 4B, it is justified to conclude that in the temperature range below T_2 (73 °C) the concentration of DMPS–Li^+ is larger than that of DMPS–NH_4^+. Thus, the spectra at 27 and 55 °C (Figure 5) are mostly due to DMPS–Li^+ with minor contributions from DMPS–NH_4^+. The main difference between the C=O stretching bands at 27 and 55 °C of Figure 5 is a reduction of the intensity of the band at 1740 cm^{-1}; this is compatible with the effects observed upon "melting" of DMPS–NH_4^+ (see Figure 2). As a consequence of the reduction of the 1740 cm^{-1} band intensity, the shoulder at 1745 cm^{-1} becomes evident. Thus, there are four C=O stretching bands in the gel phase spectrum of the DMPS–Li^+ complex at 1745, 1740, 1728, and 1716 cm^{-1}, as opposed to the gel phase of DMPS–NH_4^+ where there are only two bands at 1740 and 1728 cm^{-1}. The other important difference between the spectra of DMPS–NH_4^+ and DMPS–Li^+ (compare Figures 2 and 5) is in the relative intensities of the 1740 and 1728 cm^{-1} C=O stretching bands. The 1740 cm^{-1} band is more intense in the DMPS–Li^+ complex. Thus, there are two main effects due to Li^+ binding, i.e., a doubling of the C=O stretching bands and a reversal of the relative band intensities.

The appearance of two main groups of C=O stretching bands suggests that the conformational nonequivalence of the two acyl chain persists in the DMPS–Li^+ complex, since it has been shown that C=O stretching bands between 1747 and 1730 cm^{-1} are due to chains which do not contain gauche bonds in the C_2–C_3 segment of the chain while bands between 1728 and 1716 cm^{-1} are due to chains containing gauche bonds in the C_2–C_3 segment of the chain [see Levin (1984) and references cited therein]. In this manner, we associate the bands at 1745 and 1740 cm^{-1} with the ester carbonyl group in the *sn*-1 chain and the bands at 1728 and 1716 cm^{-1} with the ester carbonyl group at the *sn*-2 chain.

The values of the wavenumbers of the C=O stretching bands indicate that these two functional groups are not engaged in hydrogen bonding. When ester carbonyl groups such as those of lipid acyl chains form strong hydrogen bonds, the wavenumber of the C=O stretching mode is below 1715 cm^{-1}; in the DMPS–Li^+ spectra, no bands are observed below 1716 cm^{-1} (Levin, 1984).

The observed doubling of the two C=O stretching bands may be due to crystal effects or to motional effects, and the two are naturally related. Site-symmetry splitting could be responsible for the doubling of the C=O stretching bands. This type of splitting occurs when a molecular unit has its conformational freedom reduced, e.g., by crystallization or conversion to another crystal form with lower symmetry. The reduction in symmetry may result in the splitting of degenerate vibrations and the appearance of new bands (Decius & Hexter, 1977). In the case of lipids, "new" C=O stretching bands that appear at low temperature have been attributed to individual rotational isomers of the acyl chains (Bush et al., 1980). In the present comparison of DMPS–Li^+ and DMPS–NH_4^+, besides splitting of bands we observe changes in relative intensities Also, the spectrum of hydrated DMPS–NH_4^+ at −75 °C showed *only* two bands at 1740 and 1716 cm^{-1}, with the same relative intensities as in the spectrum

recorded at 20 °C. Thus, the interaction of Li^+ with DMPS has more profound effects than simply restricting the motional freedom of the acyl chains. If the effects of Li^+ binding were related only to restricting the motional freedom of the ester C=O groups, the spectra would indicate that this restriction is more pronounced than cooling to −75 °C. Therefore, we believe that the effect of Li^+ at the level of the ester C=O groups involves immobilization, leading to changes in the local symmetry (inducing site-symmetry splitting). This change could be brought about by changes in the conformation of the glycerol backbone.

We now return to analyzing the efects of temperature on the C=O stretching bands of the DMPS–Li^+ complex. The spectrum at 74 °C in Figure 5 is practically identical with that of DMPS–NH_4^+ in the liquid-crystalline phase (see Figure 2). At 74 °C, the DMPS–Li^+ complex is in the liquid-crystalline phase, and the fact that this spectrum is the same as that of liquid-crystalline DMPS–NH_4^+ demonstrates that the liquid-crystalline phases are the same, as already indicated by the same values of the $\nu_s(CH_2)$ wavenumber (vide supra). When the sample is cooled below T_2, the spectra are the same as those before heating through T_2. Thus, the conformational melting of the fatty acyl chains in the DMPS–Li^+ complex at T_2 is entirely reversible.

The bands due to the CO_2^- antisymmetric stretching are also shown in Figure 5. In the spectrum at 27 °C, there are two bands, at 1640 and 1622 cm^{-1}, which are associated with the DMPS–Li^+ complex and with DMPS–NH_4^+, respectively. In the spectrum of the DMPS–NH_4^+ gel, there is only one band, at 1622 cm^{-1} (Figure 2). The band at 1640 cm^{-1} can be assigned unequivocally to the DMPS–Li^+ complex since this band, absent in the spectra of DMPS–NH_4^+ or DMPS–Na^+, appears only when Li^+ is present and grows in intensity as the concentration of Li^+ increases (data not shown). The band is observed even in spectra of samples in which the DMPS:Li^+ molar ratio is 2.8 and [LiCl] = 0.05 M. The wavenumber of 1640 cm^{-1} for the $\nu_{as}(CO_2)$ mode is characteristic of dehydrated PS samples. The band at 1640 cm^{-1} undergoes practically no change when passing through T_1 as seen by comparing the spectrum at 55 °C with that at 27 °C; this is also confirmation that the band is due to the DMPS–Li^+ complex. The band due to DMPS–NH_4^+, at 1622 cm^{-1}, moves to 1625 cm^{-1} as a consequence of the melting of DMPS–NH_4^+, as already described (Figure 2).

Upon heating through T_2, the band at 1640 cm^{-1} disappears by coalescing with the 1620 cm^{-1} band, indicating that upon formation of the liquid-crystalline phase the carboxylate group becomes hydrated. Thus, at T_2, the melting of the fatty acyl chains of the DMPS–Li^+ complex is accompanied by hydration of the carboxylate group. These two phenomena, i.e., conformational chain melting and hydration of the carboxylate group, occur simultaneously, and when the sample is cooled below T_2, the chains become well ordered conformationally and the carboxylate group loses its water of hydration concomitantly. The spectrum recorded at 64 °C after cooling from above T_2 (Figure 5, top) is practically the same as that at 55 °C, recorded before heating through T_2.

In the spectrum of the DMPS–Li^+ complex, the $\nu_{as}(CO_2)$ mode is at 1640 cm^{-1}, i.e., at the same wavenumber as in dry PS samples (Dluhy et al., 1983; Casal et al., 1987); thus, Li^+ binds to the carboxylate group of DMPS, replacing the water of hydration from the DMPS head group. It also implies that Li^+ bound to PS loses at least part of its own hydration shell.

Figure 6A shows the region of the antisymmetric PO_2^- stretching mode (1300–1150 cm^{-1}) in the spectrum of

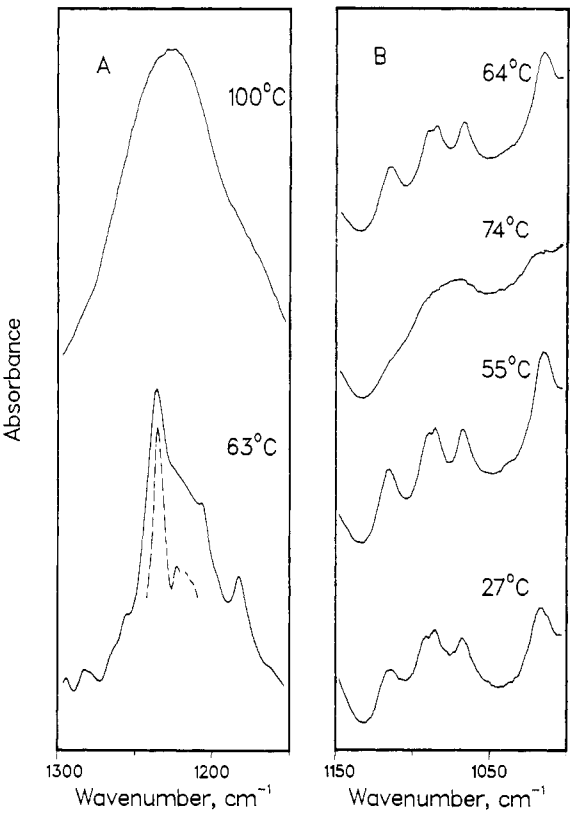

FIGURE 6: (A) 1300–1150 cm^{-1} region of the infrared spectrum of hydrated (H_2O buffer) DMPS–NH_4^+ containing 0.14 M DMPS–NH_4^+ and 0.25 M LiCl (i.e., DMPS:Li ratio = 0.56) at 63 and 100 °C. The broken line in the 63 °C spectrum is the result of deconvolution. (B) 1150–1000 cm^{-1} region of the infrared spectrum of hydrated (D_2O buffer) DMPS–NH_4^+ containing 0.14 M DMPS–NH_4^+ and 0.14 M LiCl (i.e., DMPS:Li ratio = 1.0).

DMPS–Li^+ of molar ratio 0.56 (in H_2O buffer). In the 63 °C spectrum, corresponding to the gel phase of DMPS–Li^+, the $\nu_{as}(PO_2)$ mode appears as two bands at 1230 and 1220 cm^{-1} (see broken curve, deconvolved spectrum). In the spectrum at 100 °C, corresponding to the liquid-crystalline phase of DMPS–Li^+, the $\nu_{as}(PO_2)$ mode gives a single broad band at 1220 cm^{-1}. Thus, the spectra in Figure 6 indicate that at 63 °C there is coexistence of two species; one, characterized by a $\nu_{as}(PO_2)$ band at 1230 cm^{-1}, due to DMPS–NH_4^+ and a second, characterized by a $\nu_{as}(PO_2)$ band at 1220 cm^{-1}, due to DMPS–NH_4^+. The shift in wavenumber of the $\nu_{as}(PO_2)$ mode from 1220 to 1230 cm^{-1} is consistent with loss of bound water from the DMPS phosphate group. As in the case of the carboxylate group, it can be proposed that Li^+ binds to the phosphate group, replacing the water of hydration. The ^{31}P NMR spectra of aqueous precipitates of DMPS–Li^+ demonstrate that the phosphate group is rigid and the spectral shape is identical with that of ^{31}P NMR spectra of a dry solid sample of DMPS–Li^+ (Casal et al., 1987). From the infrared and ^{31}P NMR results obtained with different synthetic phosphatidylserines, a correlation evolves between the state of hydration of the phosphate group and its mobility: whenever water of hydration is lost, the phosphate group becomes immobilized.

Figure 6B shows the region of the symmetric PO_2^- stretching mode in the infrared spectrum of the DMPS–Li^+ complex (1:1 molar ratio, D_2O buffer) at 27, 55, 74, and 64 °C after cooling from 80 °C. As already mentioned, this mode is in a crowded spectral region, and detailed band assignments are not possible. There are three main bands, at 1115, 1085, and 1068 cm^{-1}, and comparison with the spectra of the DMPS–NH_4^+ gel

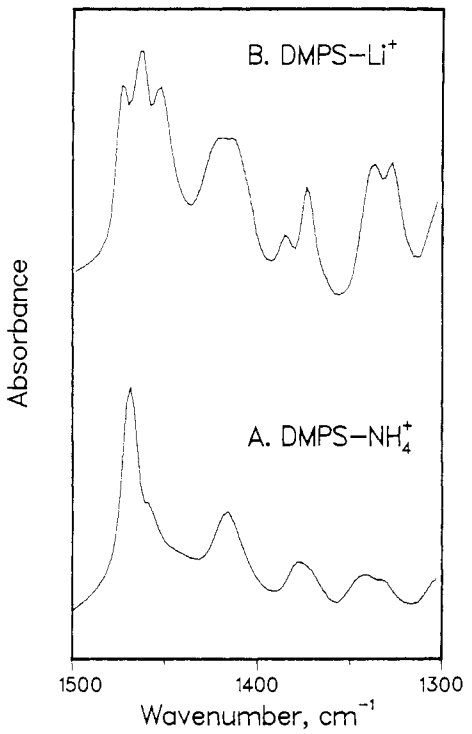

FIGURE 7: 1500–1300 cm^{-1} region of the infrared spectrum of hydrated DMPS–NH_4^+ at 14 °C (A) and of DMPS–Li^+ at 29 °C (B).

(Figure 3B) reveals that complexation with Li^+ does not lead to the appearance of new bands or to wavenumber shifts. The main effect is a pronounced band narrowing, dependent on Li^+ concentration; the bands narrow progressively as the Li^+ concentration increases. The spectra at 27 and 55 °C are practically the same; i.e., T_1 has no effect on $\nu_s(PO_2)$, as expected, given the data on DMPS–NH_4^+ (Figure 3B) and previous studies (Casal & Mantsch, 1984). The spectrum at 74 °C is characteristic of the liquid-crystalline phase and is the same as that of liquid-crystalline DMPS–NH_4^+. Again, this shows that the final liquid-crystalline phases reached by DMPS–NH_4^+ and DMPS–Li^+ are the same. The top spectrum shown in Figure 6B, recorded at 64 °C after cooling from 80 °C, demonstrates the reversibility of the events at T_2.

The pronounced band narrowing observed for the $\nu_s(PO_2)$ bands upon Li^+ binding indicates a reduction of the motional freedom of the phosphate group. In fact, the band narrowing observed in the DMPS–Li^+ complex is more pronounced than that observed by cooling DMPS–NH_4^+ to −75 °C. Thus, the results on DMPS–Li^+ indicate that binding of Li^+ induces not only restrictions to the motional freedom (as is the case when the samples are cooled) but also other changes probably due to direct binding to the phosphate group The overall picture emerging for the interaction of Li^+ with DMPS is consistent with crystallization of the fatty acyl chains, removal of water of hydration from the carboxylate and phosphate groups, and immobilization of the head group of DMPS. Since the ester carbonyl stretching bands are compatible with the two acyl chains being conformationally nonequivalent at the segments next to the ester groups, it is interesting to analyze whether these changes in the polar groups induce changes in the fatty acyl chain packing. The CH_2 scissoring mode, $\delta(CH_2)$, can be used to characterize this packing [see Casal and Mantsch (1984) and references cited therein]. Figure 7A shows the 1500–1300 cm^{-1} region of the spectrum of aqueous precipitates of DMPS–NH_4^+ in the gel phase (14 °C) with the $\delta(CH_2)$ band at 1467 cm^{-1}. The wavenumber observed, and the fact that the $\delta(CH_2)$ mode gives only one band, indicates that the

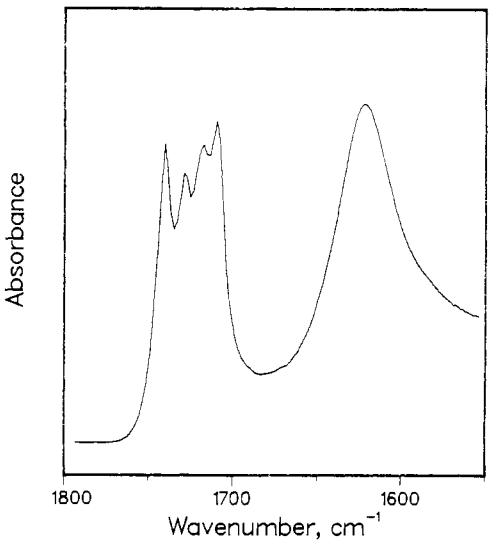

FIGURE 8: 1800–1550 cm^{-1} region of the infrared spectrum of aqueous precipitates of DMPS–Ca^{2+} at 30 °C (DMPS:Ca^{2+} ratio = 0.5).

packing of the fatty acyl chains is hexagonal in accord with previous X-ray diffraction results (Hauser et al., 1982). Figure 7B shows the 1500–1300 cm^{-1} region of the spectrum of an aqueous precipitate of DMPS–Li^+ in the gel phase (29 °C). In this case, the $\delta(CH_2)$ mode gives rise to three bands at 1474, 1467, and 1462 cm^{-1}. The appearance of these three bands can be correlated with the coexistence of two gel phases, i.e., one that gives rise to the band at 1467 cm^{-1} corresponding to hexagonal packing and a second gel phase giving rise to the bands at 1474 and 1462 cm^{-1} compatible with orthorhombic or monoclinic packing of the fatty acyl chains (Snyder, 1979). These data indicate that the hexagonal packing originates from the uncomplexed DMPS–NH_4^+ while the fatty acyl chains in the DMPS–Li^+ complex are packed in an orthorhombic-like subcell. This type of packing for the DMPS–Li^+ complex is entirely compatible with the concept of crystallized chains (Hauser & Shipley, 1981, 1983) and gives rise to interchain coupling of the $\delta(CH_2)$ vibrational mode, and hence, two bands at 1472 and 1462 cm^{-1} are observed.

Infrared Spectra of DMPS–Ca^{2+} Complexes. The thermal phase behavior of DMPS–Ca^{2+} complexes has been investigated by DSC (Hauser & Shipley, 1984). In the presence of excess Ca^{2+}, DMPS crystallizes and undergoes a phase transition at 155 °C. This transition temperature is very high, and at this temperature, samples are not amenable to infrared study, while ensuring their integrity. Thus, in this section, we discuss the room temperature spectra of aqueous precipitates of DMPS–Ca^{2+} in comparison with gel phase spectra of DMPS–NH_4^+ and DMPS–Li^+. The infrared spectra of DMPS–Ca^{2+} do not change with temperature in the range 5–80 °C.

Figure 8 shows the 1800–1550 cm^{-1} region of the infrared spectrum of an aqueous precipitate of DMPS–Ca^{2+}, the region that contains the ester carbonyl and the carboxylate antisymmetric stretching bands. The bands due to the ester C=O groups are at 1740, 1728, 1716, and 1710 cm^{-1}. There are several striking features of these bands when compared with their counterparts in the spectra of DMPS–NH_4^+ (Figure 2) and DMPS–Li^+ (Figure 5). First, they are very narrow when compared to the corresponding bands in the spectra of DMPS–NH_4^+ and DMPS–Li^+. Since (as a first approximation) band narrowing can be associated with reduction in motional freedom, the pronounced narrowing observed for DMPS–Ca^{2+} demonstrates that Ca^{2+} binds tightly to DMPS, leading to the immobilization of the entire phospholipid polar

group. The mode of Ca^{2+} binding to PS appears to be different from that of Li^+ and other monovalent cations. This can already be inferred from the high transition temperature of the DMPS–Ca^{2+} complex as compared to that of DMPS–Li^+ and DMPS–NH_4^+. The same conclusions were reached in our previous study of dry PS–metal ion complexes (Casal et al., 1987). Second, in the spectrum of DMPS–Ca^{2+}, there is a band at 1710 cm^{-1} when this complex is prepared in D_2O buffer and at 1706 cm^{-1} when prepared in H_2O buffer. Ester carbonyl stretching bands of lipids are normally observed in the 1745–1716 cm^{-1} range. On the basis of Raman spectra of lipids and model compounds, Levin and co-workers [see Levin (1984) and references cited therein] have shown that bands at wavenumbers below 1716 cm^{-1} are characteristic of carbonyl groups engaged in hydrogen bonding. According to this, the spectrum of the aqueous precipitate of DMPS–Ca^{2+} indicates that chelation of Ca^{2+} to DMPS involves the formation of hydrogen bond(s) to the ester carbonyl group, in marked contrast to Li^+ binding where no new hydrogen bonds are formed.

The other important difference between ester carbonyl stretching bands in the spectra of DMPS–Ca^{2+} and DMPS–Li^+ relates to the band at 1740 cm^{-1}. In the case of DMPS–Li^+ in the gel phase, this band is split, giving rise to two components at 1745 and 1740 cm^{-1} (Figure 5). In the case of DMPS–Ca^{2+}, only one band, at 1740 cm^{-1}, appears which would correspond to the C=O group at the *sn*-1 chain. The lack of band doubling in the case of the DMPS–Ca^{2+} complex could be taken to indicate either that the conformation of this group is such that no site-symmetry splitting occurs or that only one rotational isomer of the entire *sn*-1 chain is found. Thus, the *sn*-1 chain would be locked in a given position, a situation compatible with the pronounced immobilization of the DMPS molecule brought about by Ca^{2+} chelation. The two bands at 1728 and 1716 cm^{-1} are at the same position as those of DMPS–Li^+, suggesting that the *sn*-2 chain is in a similar environment in the two complexes of DMPS–Li^+ and DMPS–Ca^{2+}.

The band due to the CO_2^- antisymmetric stretching mode is at 1620 cm^{-1} in the spectrum of DMPS–Ca^{2+} (Figure 8), a value characteristic of hydrated carboxylate groups. Thus, even though Ca^{2+} interacts strongly with the DMPS head group, it does not induce dehydration of the carboxylate group. In the case of DMPS–Li^+, however, dehydration of the carboxylate group occurs readily even at low concentrations of Li^+ (see above, Figure 5).

The effect of chelation of Ca^{2+} by DMPS on the phosphate group is seen by examination of the $\nu_{as}(PO_2)$ and $\nu_s(PO_2)$ vibrational modes. The 1500–1150 cm^{-1} region of the spectrum of DMPS–Ca^{2+} prepared in H_2O buffer is shown in Figure 9A while Figure 9B shows the 1150–1000 cm^{-1} region of the spectrum of DMPS–Ca^{2+} prepared in D_2O buffer. The four bands at 1110, 1090, 1080, and 1065 cm^{-1} in Figure 9B are in a pattern almost identical with that found in the spectrum of anhydrous DMPS–Ca^{2+} (Casal et al., 1987). In the case of anhydrous DMPS–Ca^{2+}, we found that Ca^{2+} induces a conformational change in the phosphate group of the phospholipid. Upon Ca^{2+} binding, the torsion angles α_2 and α_3 [for notation of torsion angles, see Hauser et al. (1981)] of the two P–O ester bonds change from gauche–gauche, usually found in phosphate esters, to antiplanar–antiplanar. In contrast to DMPS–Ca^{2+}, the predominant conformation of the P–O ester bonds (α_2, α_3) in anhydrous DMPS–Na^+, DMPS–NH_4^+, and DMPS–Li^+ is gauche–gauche. Since the spectral details observed in the region of the $\nu_s(PO_2)$ mode are

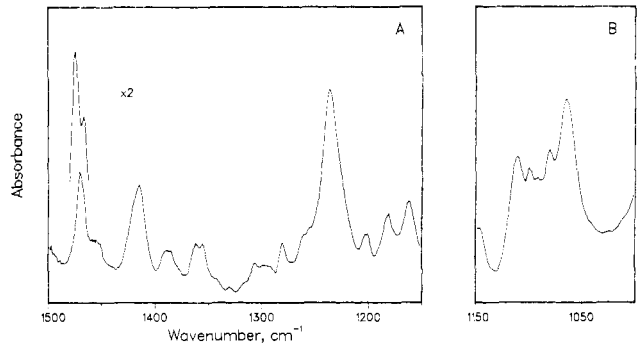

FIGURE 9: 1500–1150 cm^{-1} region of the infrared spectrum of DMPS–Ca^{2+} as aqueous precipitates in H_2O buffer (A) and the 1150–1000 cm^{-1} region of the infrared spectrum of DMPS–Ca^{2+} as aqueous precipitates in D_2O buffer (B). In both cases, the DMPS:Ca^{2+} ratio is 0.5 and the temperature 30 °C. The inset in part A is the result of deconvolution with a Lorentzian line of 6 cm^{-1} full width at half-height.

the same in anhydrous DMPS–Ca^{2+} and in the aqueous precipitate of DMPS–Ca^{2+}, it is reasonable to conclude that the antiplanar–antiplanar conformation is also present in the aqueous precipitate of DMPS–Ca^{2+}. Furthermore, the phosphate group is dehydrated in the presence of Ca^{2+} as demonstrated by the wavenumber of the $\nu_{as}(PO_2)$ mode. Figure 9A shows that the $\nu_{as}(PO_2)$ band is at 1240 cm^{-1} in the spectrum of DMPS–Ca^{2+}, a value indicative of a dehydrated phosphate group.

Also shown in Figure 9A is the region of the $\delta(CH_2)$ mode. In this case, there are two bands, at 1471 and 1464 cm^{-1} (see deconvolved spectrum, inset). As discussed above, this pattern of bands is compatible with the fatty acyl chains in the DMPS–Ca^{2+} complex packed in an orthorhombic-like subcell. This is common with DMPS–Li^+ (Figure 7) and also with DMPS–NH_4^+ at temperatures below −30 °C. We note, however, that in DMPS–Li^+ the separation of the $\delta(CH_2)$ bands is larger, suggesting "tighter" packing in DMPS–Li^+ than in DMPS–Ca^{2+}; this is in accord with X-ray studies (Hauser & Shipley, 1983, 1984).

CONCLUSIONS

The results of the infrared spectroscopic measurements reported here give detailed information regarding the structure and dynamics of the DMPS–Li^+ and DMPS–Ca^{2+} complexes. Formation of the DMPS–Li^+ complex is equivalent to an isothermal crystallization of the fatty acyl chains as already demonstrated by DSC and X-ray diffraction studies (Hauser & Shipley, 1981, 1983). When the Li^+ complex is formed from DMPS–NH_4^+ in the liquid-crystalline phase, the fatty acyl chains crystallize. In the gel phase, the infrared spectra of DMPS–Li^+ are compatible with the fatty acyl chains being packed in an orthorhombic-like lattice, in which there is interchain vibrational coupling. In contrast, the gel phase of DMPS–NH_4^+ is characterized by the fatty acyl chains being packed in a hexagonal lattice consistent with a previous study (Hauser et al., 1982). The data presented here indicate that the lithium ion binds directly to the phosphate group of DMPS, thereby removing the water of hydration of this group. However, there are no changes in the conformation of the phosphate group induced by binding of Li^+. Li^+ binds to the carboxylate group of DMPS also by removing its water of hydration. In the liquid-crystalline phase of DMPS–Li^+, both the phosphate and carboxylate groups become reversibly hydrated. In preparations of DMPS–NH_4^+ containing LiCl at molar ratios of 1.0 or less, the final liquid-crystalline phase obtained by these mixtures is the same as the liquid-crystalline

phase of DMPS–NH_4^+ (or DMPS–Na^+); however, when there is a 2-fold molar excess of LiCl, the infrared spectra of the liquid-crystalline phase show some characteristics of the DMPS–Li^+ complexes, such as a dehydrated carboxylate group. The changes brought about by Li^+ binding to the DMPS head group are transmitted to the interfacial region. The bands due to the ester carbonyl stretching modes indicate that the local conformation of these groups is different from that of DMPS–NH_4^+ (or DMPS–Na^+). The effects observed for the C=O stretching bands are different from those obtained by cooling DMPS to −75 °C; this indicates that the interaction of Li^+ with DMPS involves conformational changes probably in the glycerol backbone and is not simply due to immobilization.

The interaction of calcium ions with DMPS is in many respects similar to that of Li^+ (Hauser & Shipley, 1983, 1984). The present study, however, demonstrates that there are significant differences in the mode of interaction of these two metal ions. Ca^{2+} binds to the phosphate group of DMPS, replacing water of hydration. At the same time, the conformation of the phosphate group changes: the two P–O bonds change from the usual gauche–gauche conformation to antiplanar–antiplanar. In contrast to the phosphate group, Ca^{2+} binding to DMPS does not affect the hydration of the carboxylate group. This group remains hydrated even if Ca^{2+} is present in a 2-fold molar excess with respect to DMPS. Ca^{2+} binding also immobilizes the ester carbonyl groups and induces the formation of a hydrogen bond to one of the ester carbonyl groups. This is a unique feature of the Ca^{2+} binding to PS and had been first observed with ox brain PS (Dluhy et al., 1983). Our data do not allow us to assign the C=O group involved in hydrogen bonding; experiments with specifically labeled compounds would be needed to answer this question.

Further experiments are in progress in order to investigate the effect of unsaturation in the fatty acyl chains of PS on metal ion binding. At the same time, studies are carried out to shed light on the question whether the PS–metal ion complexes are unique or whether similar complexes are formed with other anionic phospholipids.

Registry No. DMPS, 64023-32-1; DMPS (NH_4^+ salt), 80581-65-3; DMPS (Na^+ salt), 105405-50-3; Li, 7439-93-2; Ca, 7440-70-2.

REFERENCES

Bush, S. F., Levin, H., & Levin, I. W. (1980) *Chem. Phys. Lipids 27*, 101–111.

Cameron, D. G., & Jones, R. N. (1981) *Appl. Spectrosc. 35*, 448.

Casal, H. L., & Mantsch, H. H. (1984) *Biochim. Biophys. Acta 779*, 381–401.

Casal, H. L., Mantsch, H. H., Paltauf, F., & Hauser, H. (1987) *Biochim. Biophys. Acta* (in press).

Decius, J. C., & Hexter, R. M. (1977) *Molecular Vibrations in Crystals*, McGraw-Hill, New York.

Dluhy, R. A., Cameron, D. G., Mantsch, H. H., & Mendelsohn, R. (1983) *Biochemistry 22*, 6318–6325.

Hauser, H., & Shipley, G. G. (1981) *J. Biol. Chem. 256*, 11377–11380.

Hauser, H., & Shipley, G. G. (1983) *Biochemistry 22*, 2171–2178.

Hauser, H., & Shipley, G. G. (1984) *Biochemistry 23*, 34–41.

Hauser, H., Finer, E. G., & Darke, A. (1977) *Biochem. Biophys. Res. Commun. 76*, 267–274.

Hauser, H., Pascher, I., Pearson, R. H., & Sundell, S. (1981) *Biochim. Biophys. Acta 650*, 21–51.

Hauser, H., Paltauf, F., & Shipley, G. G. (1982) *Biochemistry 21*, 1061–1067.

Hermetter, A., Paltauf, F., & Hauser, H. (1982) *Chem. Phys. Lipids 30*, 35–45.

Holwerda, D. L., Ellis, P. D., & Wuthier, R. E. (1981) *Biochemistry 20*, 418–428.

Kauppinen, J. K., Moffatt, D. J., Mantsch, H. H., & Cameron, D. G. (1981) *Appl. Spectrosc. 35*, 271–276.

Kurland, R. J., Hammoudah, M., Nir, S., & Papahadjopoulos, D. (1979) *Biochem. Biophys. Res. Commun. 88*, 927–932.

Levin, I. W. (1984) *Adv. Infrared Raman Spectrosc. 11*, 1–48.

Mantsch, H. H., Casal, H. L., & Jones, R. N. (1986) *Adv. Spectrosc. 13*, 1–46.

Mushayakarara, E., & Levin, I. W. (1982) *J. Phys. Chem. 86*, 2324–2327.

Papahadjopoulos, D. (1978) *Cell Surf. Sci. 5*, 760–790.

Schou, M. (1976) *Annu. Rev. Pharmacol. Toxicol. 16*, 231–242.

Snyder, R. G. (1979) *J. Chem. Phys. 71*, 3229–3235.

Williams, R. J. P. (1973) in *Lithium: Its Role in Psychiatric Research and Treatment* (Gershon, S., & Shipsin, B., Eds.) pp 15–31, Plenum Press, New York.