Conformation of Phosphatidylserine in Bilayers as Studied by Fourier Transform Infrared Spectroscopy†

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ABSTRACT: The ^{13}C labeled lipid 1[1′-^{13}C]DPPS–NH_4^+ and its metal salts were used to unambiguously assign all carbonyl vibrations in the infrared spectrum of phosphatidylserines. It is shown that the C=O stretching band at 1741 cm^{-1} of phosphatidylserines previously assigned to the *sn*-1 C=O vibration contains contributions from both the *sn*-1 and the *sn*-2 carbonyls. The C=O stretching band at frequencies between 1715 and 1730 cm^{-1} previously assigned to the *sn*-2 C=O vibration also contains contributions from both carbonyl groups. The frequency dependence observed with the ester carbonyls primarily reflects hydrogen bonding and the polarity of the immediate vicinity. Conformational changes are accounted for in terms of frequency shifts if the conformational change involves the disposition of the C=O groups and in turn the hydrogen bonding properties. The infrared spectra of phospholipids dispersed in aqueous medium in the liquid crystalline state are inconsistent with a simple phospholipid conformation, e.g., with a conformation as found in the single-crystal structure of 1,2-dimyristoyl-*sn*-phosphatidylcholine and 1,2-dilauroyl-*rac*-phosphatidylethanolamine. The spectra support the hypothesis proposed earlier (Hauser et al., *Biochemistry*, 1988) on the basis of existing single-crystal phospholipid structures and NMR evidence. The hypothesis states that several conformations are present in liquid crystalline phospholipid dispersions. The interconversion between these conformers takes place at rates that are fast on the NMR time scale but slow on the infrared time scale giving rise to motionally averaged NMR spectra but clearly discernible component infrared spectra. Changes in the infrared spectra observed for aqueous dispersions of phosphatidylserines induced at the order–disorder phase transition and upon adding cations such as Li^+, Mg^{2+}, and Ca^{2+} are interpreted in terms of changes in hydrogen bonding and in turn as conformational changes.

Phospholipids are integral constituents of biological membranes, and for this reason their physicochemical properties have been the subject of extensive studies. The knowledge of the conformation and dynamics of phospholipids present in bilayers is essential for an understanding of their functional role. The glycerol backbone of the phospholipid molecule is regarded as the central part; two fatty acyl chains are linked through ester bonds to carbon atoms *sn*-1 and *sn*-2 of the glycerol group, and the polar group is esterified to carbon atom *sn*-3. The parallel stacking of the two fatty acyl chains appears to be a fundamental principle of the conformation of phospholipids. It not only prevails in phospholipid bilayers and phospholipid aggregates in general but was also shown to exist in phospholipid monomers (Hauser et al., 1980a,b; Hauser et al., 1988). A question of central interest is whether or not the parallel chain stacking is associated with a single, unique conformation of the glycerol backbone. Information pertinent to this question has been provided by X-ray crystallography (Hauser et al., 1981; Hauser & Poupart, 1991), NMR studies including high-resolution and broad-line NMR using different nuclei (Seelig, 1977, 1978; Browning, 1981 and references cited therein; Fuson & Prestegard, 1983) and more recently by Fourier transform infrared spectroscopy (Dluhy et al., 1983; O'Leary & Levin, 1984; Casal et al., 1987a–c; Blume et al., 1988; Mantsch & McElhaney, 1991).

Here we tackle this question by studying ^{13}C-labeled phosphatidylserine bilayers by Fourier transform infrared spectroscopy. Phosphatidylserine is the major anionic phospholipid of many cell membranes, and its negative charge and interactions with mono- and divalent cations have been implicated in important membrane processes such as membrane fusion, enzyme regulation, and signal transduction. The C=O stretching vibration arising from the carbonyl groups of the fatty acyl chains was shown to respond sensitively to hydrogen bonding (Blume et al., 1988). The C=O stretching band is therefore expected to be a good probe of the conformation of the glycerol backbone and related to it of the mode of chain stacking in an indirect way. Changes in the glycerol conformation and hence in the mode of chain stacking are assumed to affect the precise location of the C=O groups at the apolar (hydrophobic)–polar (hydrophilic) interface of the phospholipid bilayer which determines the hydration, i.e.,

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[1] Abbreviations: DSC, differential scanning calorimetry; Tes, 2{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}ethanesulfonic acid; ap, antiperiplanar, sc, synclinal or gauche; DPPS–NH_4^+, ammonium salt of 1,2-dipalmitoyl-*sn*-glycero-3-phospho-L-serine or 1,2-dipalmitoyl-3-*sn*-phosphatidylserine; DMPS–NH_4^+, ammonium salt of 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine or 1,2-dimyristoyl-3-*sn*-phosphatidylserine; 1[1′-^{13}C]DPPS,1[1′-^{13}C]palmitoyl-2-palmitoyl-*sn*-glycero-3-phospho-L-serine or 1[1′-^{13}C]palmitoyl-2-palmitoyl-3-*sn*-phosphatidylserine; POPS, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine.

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the degree of hydrogen bonding. A complication in the analysis of the C=O vibrational modes is the assignment of the complex pattern of C=O bands to the *sn*-1 and *sn*-2 fatty acyl chains. In order to facilitate this assignment we have synthesized 1,2-dipalmitoyl-3-*sn*-phosphatidylserine (DPPS) specifically ^{13}C-labeled in the carbonyl group of the *sn*-1 chain. As a result of the isotopic substitution of ^{12}C=O by ^{13}C=O the frequency of the ^{13}C=O vibration is shifted to lower values by 40–42 cm^{-1} compared to the frequency of the ^{12}C=O vibration (Blume et al., 1988). With this in mind the infrared absorption bands of 1[1′-^{13}C]DPPS between 1670 and 1700 cm^{-1} can be assigned to ^{13}C=O vibrations of the *sn*-1 chain, while the ^{12}C=O vibrations of the *sn*-2 chain are in the normal frequency range of 1710–1742 cm^{-1}. As a rule of thumb, the frequencies of the *sn*-1 carbonyl stretching vibration in unlabeled DMPS or DPPS are obtained by adding ~41 cm^{-1} to the frequencies of the *sn*-1 ^{13}C=O vibrations of 1[1′-^{13}C]-DPPS. As pointed out before (Blume et al., 1988), a further significant advantage of using 1[1′-^{13}C]DPPS labeled phospholipids is that the C=O bands of both fatty acyl chains are observed separately, and the information content is increased.

The 1[1′-^{13}C]-labeled DPPS is used here to unambiguously assign the C=O stretching bands and to derive conformational information. Since cations such as Li^+, Mg^{2+}, and Ca^{2+} are known to interact strongly with saturated phosphatidylserines (Hauser & Shipley, 1983, 1984; Casal et al., 1987a–c, 1989), 1[1′-^{13}C]DPPS was also used to study the effect of these metal ions on the glycerol conformation and hydrocarbon chain packing.

MATERIAL AND METHODS

DMPS and DPPS were synthesized and purified as described by Hermetter et al. (1982). The acid form was converted to the monoammonium salt according to Bligh and Dyer (1959). All phospholipids used in this work were pure by TLC standards and by chemical microanalysis. Alkali metal chlorides, $MgCl_2$ and $CaCl_2$ (Puriss grade), were purchased from Merck (Rahway, NJ).

Preparation of 1[1′-^{13}C]Palmitoyl-2-palmitoyl-sn-glycero-3-phospho-L-serine. 1[1′-^{13}C]Palmitoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine was prepared by reacting 1-*O*-trityl-2-palmitoyl-*sn*-glycero-3-phosphocholine with [1-^{13}C]palmitic acid anhydride in the presence of borontrifluoride etherate in anhydrous methylene chloride (Hermetter et al., 1989). The ^{13}C-labeled dipalmitoylphosphatidylcholine was converted to the corresponding phosphatidic acid by treatment with a crude preparation of phospholipase D from cabbage as described by Eibl and Kovatchev (1981). The ^{13}C-labeled dipalmitoylphosphatidic acid was then converted to 1[1′-^{13}C]palmitoyl-2-palmitoyl-*sn*-glycero-3-phosphoserine by condensation with *N*-((*tert*-butyloxy)carbonyl)serine benzhydryl ester, followed by removal of the protective groups in the presence of HCl in anhydrous chloroform (Hermetter et al., 1982). The product was purified by precipitation from a chloroform solution with acetone. The purity of the product was checked by thin-layer chromatography using as the solvent chloroform/methanol/25% ammonia (65:35:5, by volume) and chloroform/acetone/methanol/acetic acid/water (50:20:10:10:5, by volume). The yield of pure product was 60% based on phosphatidic acid. The isotopic purity of the phospholipid with the ^{13}C=O group being present in the *sn*-1 fatty acyl chain was better than 96% (Blume et al., 1988).

Preparation of Phospholipid–Metal Ion Complexes. 1[1′-^{13}C]DPPS suspended in 2 mM Tes, 2 mM His pH 7.0 containing 0.1 M NaCl and 0.1 mM EDTA in D_2O was heated to 60 °C and vortexed, and the appropriate amount of a solution of LiCl, $MgCl_2$, or $CaCl_2$ in D_2O (60 °C) was added to a final phospholipid/cation mole ratio of 0.5. For infrared spectroscopic measurements the precipitate of the phospholipid–metal ion complex thus formed was deposited on CaF_2 or BaF_2 windows and assembled into cells of 12 μm path lengths. For the ATR experiment a preheated and vortexed suspension of 1[1′-^{13}C]DDPS–NH^{4+} in H_2O (10% w/v) was spread on a zinc selenide crystal and dried continuously under a stream of nitrogen inside the spectrometer chamber. Loss of water from the lipid film was monitored by the disappearance of the ν_{OH} stretching mode of the water molecule at 3600 cm^{-1}.

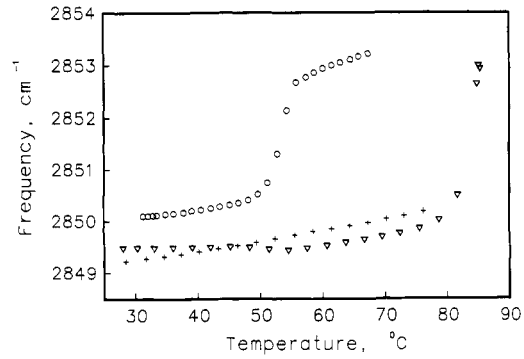

FIGURE 1: Frequency versus temperature relation of the symmetric CH_2 stretching vibration of 1[1′-^{13}C]DPPS–NH_4^+ (circles), 1[1′-^{13}C]DPPS–Li^+ (triangles), and 1[1′-^{13}C]DPPS–Ca^{2+} (+).

Infrared Spectra. Infrared spectra at 2 cm^{-1} resolution were collected with a Digilab FTS-60 spectrometer, equipped with a DTGS detector, using procedures for data collection and analysis described elsewhere (Casal et al., 1987b,c). The temperature of the sample was controlled by pumping a thermostated C_2H_5OH–H_2O mixture through the hollow cell mount. Temperatures were measured by a copper-constantan thermocouple placed against the edge of the cell window.

RESULTS

Thermotropic Phase Behavior of DPPS Multilayers. The gel-to-liquid crystal phase transition of 1[1′-^{13}C]DPPS–NH_4^+ and the Li^+ complex of this phospholipid (phospholipid/Li^+ mole ratio 0.5) can be detected in the temperature dependence of the infrared spectra as shown in Figure 1. The melting of the fatty acyl chains is indicated by the increase in frequency of the symmetric CH_2 stretching band of the fatty acyl chains observed at 53 and 86 °C for 1[1′-^{13}C]DPPS–NH_4^+ and 1[1′-^{13}C]DPPS–Li^+, respectively. In contrast, both 1[1′-^{13}C]-DPPS–Ca^{2+} and 1[1′-^{13}C]DPPS–Mg^{2+} at phospholipid/divalent cation mole ratios of 0.5 show no order–disorder transition in the temperature range of 30–90 °C. The lower values of the frequency of the symmetric CH_2 stretching band in the lithium and calcium precipitates compared to 1[1′-^{13}C]DPPS–NH_4^+ in the gel phase (see Figure 1) are consistent with a more ordered and rigid packing arrangement of the fatty acyl chains in the former.

These results are in good agreement with published DSC measurements. Fully hydrated DPPS–NH_4^+ bilayers reveal a phase transition at 53 °C (Hauser & Shipley, 1983), while precipitates of DPPS formed in excess of Li^+, Mg^{2+}, and Ca^{2+} exhibit phase transitions at 90, 95, and 150 °C, respectively (Hauser & Shipley, 1983; Hauser & Shipley, 1984).

In 1[1′-^{13}C]DPPS–NH_4^+ and 1[1′-^{13}C]DPPS–Ca^{2+} we observed a gradual temperature-induced linear increase in frequency of the symmetric CH_2 stretching vibration between 30 and 50 °C. In 1[1′-^{13}C]DPPS–Li^+ this frequency remains

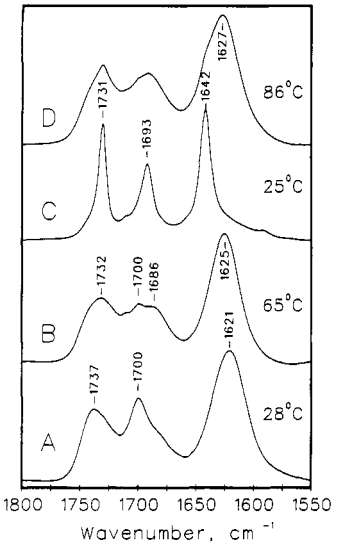

FIGURE 2: Infrared spectra of the region of the carbonyl and carboxylate stretching bands of 1[1′-^{13}C]DPPS-NH_4^+ in the gel phase (A) and in the liquid crystalline phase (B) and of 1[1′-^{13}C]DPPS-Li^+ in the crystalline phase (C) and in the liquid crystalline phase (D).

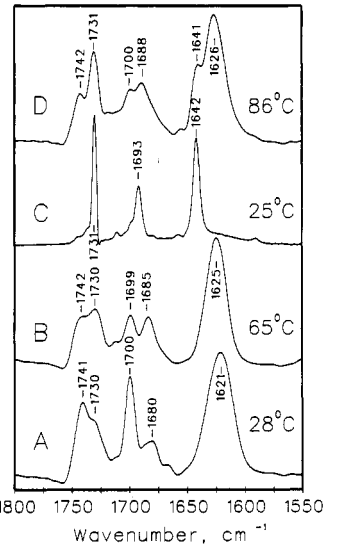

FIGURE 3: Same spectra as in Figure 2 after resolution enhancement by Fourier self-deconvolution. Spectra A, B, and D were deconvolved with a Lorentzian line L = 20 cm^{-1} full width at half-height and a resolution enhancement factor K = 2 using the method described by Kauppinen et al. (1981). The deconvolution parameters for spectrum C were L = 7 cm^{-1} and K = 2.

constant up to ~50 °C and then shows a minimum at ~53 °C. Between 55 and 80 °C one finds the usual temperature induced increase comparable to that observed in 1[1′-^{13}C]-DPPS–Ca^{2+} (Figure 1). We conclude that after sample preparation of 1[1′-^{13}C]DPPS–NH_4^+ with Li^+ (mole ratio 1:2) a small fraction of uncomplexed 1[1′-^{13}C]DPPS–NH_4^+ is still present, but on heating the sample in the IR-cell to 53 °C the binding of lithium at the lipid/water interface, possibly achieved by lateral diffusion of the cation at the bilayer surface, is complete. This behavior differs from the results obtained with DMPS–Li^+ (mole ratio 1:1; Casal et al., 1987b) which undergoes a pretransition due to the melting of uncomplexed DMPS–NH_4^+.

Infrared Spectra of the Carbonyl Stretching Region. Figures 2 (parts A and B) shows infrared spectra of the region of the carbonyl and carboxylate stretching vibrations of hydrated 1[1′-^{13}C]DPPS–NH_4^+ at 28 °C (gel phase) and at 65 °C (liquid crystalline phase). As mentioned above we now can assign the *sn*-1 and *sn*-2 carbonyl vibrations without ambiguity. In the gel phase the two C=O bands of 1[1′-^{13}C]DPPS–NH_4^+ having maxima at 1737 and 1700 cm^{-1} are asymmetric in shape and exhibit a shoulder on the low frequency side (Figure 2A). Band narrowing by deconvolution reveals that the contour of the *sn*-2 C=O band consists of two underlying component bands at 1741 and 1730 cm^{-1} (Figure 3A). Furthermore, the composite band due to the *sn*-1 ^{13}C=O vibration consists of two major bands, at 1700 and 1680 cm^{-1}. At the phase transition temperature T_m one observes a frequency shift of the overall maximum of the *sn*-2 C=O band from 1737 to 1732 cm^{-1} (Figure 2). This apparent shift in frequency is caused by an increase in intensity of the component at 1730 cm^{-1} relative to that at 1741 cm^{-1}. Simultaneously the *sn*-1 ^{13}C=O band component at 1680 cm^{-1} shifts to 1685 cm^{-1} and increases in intensity relative to the *sn*-1 ^{13}C=O component at 1700 cm^{-1} (cf. spectra B in Figures 2 and 3). The additional strong band at 1621 or 1625 cm^{-1} in Figure 2 (parts A and B) is due to the antisymmetric COO^- vibration of the serine moiety. It is well-known that this vibration occurs in the spectral range 1620–1626 cm^{-1} in the hydrated state and at around 1640 cm^{-1} in the anhydrous state (Casal et al., 1987b). It follows that the serine carboxylate group in 1[1′-^{13}C]DPPS–NH_4^+ is fully hydrated over the whole temperature range studied.

The situation is quite different for the 1[1′-^{13}C]DPPS–Li^+ complex (see spectra in Figure 2 (parts C and D)). At a mole ratio phospholipid/Li^+ of 0.5 the carboxylate group is completely dehydrated up to 80 °C. At 86 °C the COO^- band at 1627 cm^{-1} indicates hydration of the carboxyl group. However, as seen in the deconvolved spectrum (Figure 3D), even at 86 °C there still remains a fraction of anhydrous COO^- groups indicating residual binding of Li^+ to the carboxylate group. The carbonyl bands in 1[1′-^{13}C]DPPS–Li^+ show an interesting and somewhat unusual behavior. First of all only two sharp bands are present at 1731 (*sn*-2 C=O) and 1693 cm^{-1} (*sn*-1 ^{13}C=O). The extremely narrow width of the carbonyl and carboxylate bands (half-bandwidth of 4–8 cm^{-1}) indicates that the polar group is completely immobilized. At 86 °C the infrared spectrum of 1[1′-^{13}C]DPPS–Li^+ exhibits four bands in the carbonyl stretching region (see Figure 3D). The frequencies of the four components are comparable though not identical with those found in the infrared spectrum of 1[1′-^{13}C]DPPS–NH_4^+ in the liquid crystalline state.

Of special interest was the assignment of the narrow C=O bands in the crystalline precipitates of 1[1′-^{13}C]DPPS–Ca^{2+} and 1[1′-^{13}C]DPPS–Mg^{2+} (Figure 4). At 30 °C the carbonyl frequencies of 1[1′-^{13}C]DPPS–Ca^{2+} are 1730 and 1711 cm^{-1} for the *sn*-2 chain and 1699 and 1678 cm^{-1} for the *sn*-1 chain (Figure 4A). By adding 41 cm^{-1} to compensate for the isotope effect we obtain frequencies of 1740 and 1719 cm^{-1} for the *sn*-1 C=O bands in unlabeled DPPS–Ca^{2+}. These values are in very good agreement with the experimentally observed frequencies of unlabeled DMPS–Ca^{2+}, exhibiting carbonyl bands at 1740, 1728, 1716, and 1710 cm^{-1} (Casal et al., 1987b). Now we can unequivocally assign the unusually low frequency component at 1711 cm^{-1} to a *sn*-2 C=O vibration, which is also found in 1[1′-^{13}C]DPPS–Mg^{2+} (see spectra C and D of Figure 4), in unlabeled DMPS–Mg^{2+} (Casal et al., 1989), and in ox brain PS–Ca^{2+} complexes (Dluhy et al., 1983). The bands observed at 1716 cm^{-1} in DMPS–Ca^{2+} (Casal et al., 1987) and at 1718 cm^{-1} in ox brain PS–Ca^{2+} at lower temperature (Dluhy et al., 1983) are due to a *sn*-1 carbonyl

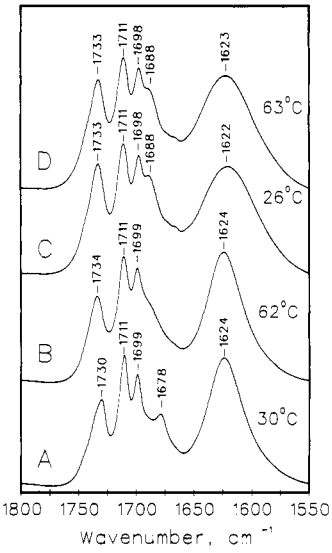

FIGURE 4: Infrared spectra of the carbonyl stretching region of 1[1′-^{13}C]DPPA-Ca^{2+} (A and B) and of 1[1′-^{13}C]DPPS-Mg^{2+} (C and D) at the indicated temperatures.

vibration and not due to a *sn*-2 C=O vibration as was suggested in previous publications. We note that the *sn*-1 ^{13}C=O band observed at 1678 cm^{-1} in 1[1′-^{13}C]DPPS-Ca^{2+} (at 30 °C) is shifted in 1[1′-^{13}C]DPPS-Mg^{2+} (at 26 °C) by 10 wavenumbers to 1688 cm^{-1} (Figure 4). Thus in the unlabeled DPPS-Mg^{2+} the *sn*-1 C=O band should occur at about 1729 (1688 + 41) cm^{-1} and would be expected to overlap with the 1733-cm^{-1} band of the *sn*-2 C=O vibration. This explains why in a previous publication only three carbonyl bands at 1741, 1728, and 1711 cm^{-1} were observed in DMPS-Mg^{2+} (Casal et al., 1989). In 1[1′-^{13}C]DPPS-Ca^{2+} the *sn*-1 ^{13}C=O band at 1678 cm^{-1} broadens and shifts to 1686 cm^{-1} over the temperature range 40–60 °C (Figure 4). This process is reversible on cooling. In contrast, the *sn*-1 ^{13}C=O band at 1699 cm^{-1} does not change in frequency, intensity, and width over the temperature range 30–80 °C. Also whereas one component of the *sn*-2 carbonyl vibration shifts gradually from 1730 to 1734 cm^{-} as the temperature increases from 40 to 60 °C, the second *sn*-2 component at 1711 cm^{-1} remains constant in frequency, intensity, and bandwidth. For this reason we exclude correlation field (site symmetry) splitting as an explanation for the appearance of two *sn*-1 and two *sn*-2 C=O stretching bands, because in the latter case the two *sn*-1 C=O and the two *sn*-2 C=O bands would be expected to change simultaneously.

Additional experiments were carried out in order to shed light on the question of the assignment of the C=O bands. Infrared attenuated total reflectance spectroscopy applied to a dry film of 1[1′-^{13}C]DPPS-NH_4^+ gave only two narrow, symmetric carbonyl stretching bands at 1741 and 1700 cm^{-1} for the *sn*-2 and *sn*-1 chain, respectively (data not shown). Correcting for the isotope effect it is clear that a single C=O band would be obtained for unlabeled DPPS-NH_4^+ under these conditions. The frequency observed is characteristic of an anhydrous C=O group. If D_2O is added to the film of 1[1′-^{13}C]DPPS-NH_4^+ two asymmetric C=O bands are observed very similar to those shown in Figure 2A for the gel phase of 1[1′-^{13}C]DPPS-NH_4^+. Moreover, the infrared spectra of the carbonyl stretching region consist of a single C=O band at a frequency of about 1740 cm^{-1} for DPPS-NH_4^+ dissolved in chloroform and of two bands for 1[1′-^{13}C]DPPS-NH_4^+ dissolved in the same solvent.

DISCUSSION

Assignment of Carbonyl Vibrations. Based on the data presented we now are able to unambiguously assign the various carbonyl bands observed with unlabeled aqueous dispersions of phosphatidylserines. As summarized in Table I synthetic phosphatidylserines usually exhibit two C=O bands at ~1741 and 1721 cm^{-1} in the gel phase and at ~1741 and 1728 cm^{-1} in the liquid crystalline phase (Casal et al., 1987a–c, 1989). Table I illustrates that the data obtained with 1[1′-^{13}C]DPPS-NH_4^+ and corrected for the isotope effect are in very good agreement with those obtained with unlabeled phosphatidylserines. The most important result of Table I, however, is that both the *sn*-1 C=O and the *sn*-2 C=O band consist of two components; furthermore, the previous tentative assignment of the C=O band at ~1741 cm^{-1} to the *sn*-1 carbonyl vibration and that of the C=O bands between 1720 and 1730 cm^{-1} to the *sn*-2 carbonyl vibration cannot be sustained in light of the results presented here (cf. Table I). It is clear from the data in this table that both the C=O band at 1741 cm^{-1} and those between 1720 and 1730 cm^{-1} consist of contributions from both the *sn*-1 and the *sn*-2 carbonyls.

The data summarized in Table I also provide an answer to the question of the origin of the frequency differences observed for the C=O bands. A dehydrated film of a multilamellar arrangement of 1[1′-^{13}C]DPPS gives rise to a single C=O band at 1741 cm^{-1} after correction for the isotope effect. This frequency is apparently characteristic of the anhydrous C=O group, a conclusion which is corroborated by the infrared spectrum of DMPS-NH_4^+ or 1[1′-^{13}C]DPPS-NH_4^+ dissolved in $CHCl_3$. A single C=O band at 1741 cm^{-1} is obtained after correction for the isotope effect (Table I). As was reported earlier diacyl phospholipids form small inverted micelles in $CHCl_3$ (Dervichian, 1964). It is highly unlikely that under extremely different conditions as in the dehydrated, crystalline lamellar phase and in small inverted micelles the DPPS-NH_4^+ molecules have exactly the same conformation, yet in both phases a single C=O band at the same frequency is observed (Table I). This result enforces the conclusion that the frequency differences observed in the C=O stretching bands are not due to different conformations in the glycerol backbone and the two hydrocarbon chains. Our data (cf. Table I) clearly indicate that the frequency of the C=O stretching vibration is not a sensitive probe of phospholipid conformation in the sense that changes in torsion angles affecting the position and orientation of the C=O groups are directly expressed as shifts in the C=O stretching bands. Blume et al. (1988) arrived at the same conclusion from a Fourier transform infrared study of aqueous dispersions of dimyristoyl phospholipids including phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidic acid all ^{13}C-labeled in the ester–carbonyl group. Blume et al. (1988) also proposed that the immediate cause of the differences in frequency of C=O bands is hydrogen bonding. Besides hydrogen bonding the polarity and/or the dielectric properties of the medium in which the C=O group is embedded is thought to affect the frequency of the ester carbonyl vibration. Increasing polarity of the immediate surroundings is supposed to have a lowering effect on the frequency of the ester carbonyl vibration. Our results indicate that the frequency of the anhydrous or water-free ^{12}C=O (^{13}C=O) group is at 1741 cm^{-1} (1700 cm^{-1}) (cf. Table I), and the frequencies of "hydrated" ^{13}C=O (^{12}C=O) groups, i.e., groups involved in hydrogen bonding, are at lower frequencies (cf. Figures 2–4 and Table I). We conclude that the frequency of the C=O stretching vibration depends primarily on hydrogen bonding and the observed shift to lower

Table I: Assignment of the Ester Carbonyl Stretching Bands of Aqueous Phosphatidylserine Dispersions

phospholipid	temp (°C)	lipid phase	total C=O bands (cm^{-1})	*sn*-1 C=O band (cm^{-1})	*sn*-2 C=O band (cm^{-1})
DMPS-NH_4^+	14	gel	1742, 1721		
	61	liquid crystalline	1742, 1727		
POPS-NH_4^+	3	gel	1740, 1720		
	30	liquid crystalline	1740, 1728		
1[1′-^{13}C]DPPS-NH_4^+ [a]	28	gel	1741, 1730, 1741, 1721	1700, 1680	1741, 1730
	65	liquid crystalline	1742, 1730, 1740, 1726	1699, 1685	1742, 1730
1[1′-^{13}C]DPPS-NH_4^+	room temp	dehydrated crystalline lamellar	1741	1700	1741
1[1′-^{13}C]DPPS-NH_4^+	room temp	inverted micelles in $CHCl_3$	1741	1700	1740

[a] The four listed frequencies consist of those of the two ^{12}C=O bands of the *sn*-2 carbonyl group plus those of the two C=O bands of the *sn*-1 carbonyl group obtained by adding 41 cm^{-1} to the frequency of the *sn*-1 ^{13}C=O bands to compensate for the isotope effect.

frequencies appears to be directly proportional to the strength of the hydrogen bonding. The data presented herein are interpreted in this sense. For example, the ^{13}C=O group of 1[1′-^{13}C]DPPS-NH_4^+ is less mobile in the gel than in the liquid crystalline phase as indicated by the widths of the C=O bands in spectra A and B of Figure 3; this decreased mobility of the ^{13}C=O group in the gel phase may lead to the formation of stronger hydrogen bonds to the *sn*-1 C=O group. As a result the frequency of this group is lowered by 5 cm^{-1} from 1685 cm^{-1} in the liquid crystalline phase to 1680 cm^{-1} in the gel (cf. Figure 3 (parts A and B) and Table I).

At the order–disorder phase transition of 1[1′-^{13}C]DPPS-NH_4^+ the intensity of the two low-frequency components at 1730 and 1685 cm^{-1} increases relative to the intensity of the C=O bands at 1741 and 1700 cm^{-1}, assigned to the two anhydrous C=O groups (cf. spectra A and B of Figure 3). This finding is interpreted to indicate a loosening of the polar group packing at the phase transition and in turn an increase in the extent of hydration. Furthermore, we note from the intensity ratio $I(1730)/I(1680)$ that in the gel phase the *sn*-2 carbonyl group is qualitatively more hydrated than the *sn*-1 carbonyl group. In the gel phase the *sn*-1 ester group appears therefore to be deeper buried in the hydrophobic hydrocarbon chain matrix and thus less accessible to water molecules for hydrogen bonding than the *sn*-2 ester group. The same explanation was given by Blume et al. (1988) for similar changes in intensity of the C=O stretching band observed at the phase transition of dimyristoylphosphatidylcholine ^{13}C-labeled at the *sn*-2 carbonyl group.

Conformational Implications. Considering that the frequency of the C=O stretching vibration responds sensitively to interactions of the C=O group with water molecules or to changes in the immediate polarity, we predict that conformational changes of the phospholipid molecule affecting these two parameters will be expressed in shift changes. The C=O stretching vibration will response as sensitively to conformational changes as these conformational changes lead to changes in the hydrogen bonding properties of the ester carbonyl groups.

Figure 5 illustrates four single-crystal structures of phospholipids which differ in the mode of chain stacking. Rotamers A (left-hand side of Figure 5) differ from the rotamers B in torsion angles Θ_3/Θ_4 about the C(*sn*-1)–C(*sn*-2) glycerol bond. For the atom numbering and notation for torsion angles see Hauser et al. (1988). Rotamers A are characterized by Θ_3/Θ_4 = ap/sc, rotamers B by Θ_3/Θ_4 = sc/–sc. Representative phospholipid crystal structures for rotamers A are 1,2-dimyristoyl-*sn*-phosphatidylcholine (Pearson & Pascher, 1979), 1,2-dilauroyl-*rac*-phosphatidylethanolamine (Hitchcock et al., 1974) and, the monosodium salt of 1,2-dilauroyl-*sn*-glycero-3-phosphate (Pascher and Sundell, personal communication), while the crystal structures of 1,2-dilauroyl-*rac*-glycero-3-phospho-*N*,*N*-dimethylethanolamine (Pascher & Sundell, 1986) and 1,2-dimyristoyl-*sn*-phosphatidylglycerol (Pascher et al., 1987) are representative for rotamers B. For the interpretation of our infrared data it is important to note that the two rotamers A depicted in Figure 5 differ in the chain stacking: in rotamer A_γ the *sn*-1 or γ fatty acyl chain forms a continuous zigzag with the glycerol group and the β-chain is initially oriented layer-parallel and twists at carbon atom C(22) such that parallel chain stacking is accomplished. This is contrasted by rotamer A_β in which the *sn*-2 or β chain extends straight upward from the glycerol atom C(2) whereas the *sn*-1 or γ-chain is initially oriented layer-parallel with an about 90 °C twist at carbon atom C(32). Rotamers B_γ and B_β shown on the right-hand side of Figure 5 differ by the same features as rotamers A_γ and A_β, respectively. Further details regarding the differences between the four single-crystal structures shown in Figure 5 are discussed by Hauser et al. (1988).

We note that in structures A_γ and B_γ the *sn*-2 C=O group is closer to the lipid–water interface while the *sn*-1 C=O group is further away from this interface and more deeply buried in the hydrocarbon layer. In contrast, in the structures designated A_β and B_β the situation is reversed: the *sn*-1 carbonyl is closer to the lipid water interface, while the *sn*-2 carbonyl appears to be buried in the hydrocarbon chains. In the former structures (A_γ and B_γ) the *sn*-2 carbonyls are expected to be more accessible to water molecules and thus involved in hydrogen bonding, while in structures A_β and B_β the *sn*-1 carbonyls are more likely to form hydrogen bonds. The infrared spectra of aqueous phosphatidylserine dispersions consist of two components each for the *sn*-1 carbonyl and the *sn*-2 carbonyl. This is true in the gel as well as in the liquid crystalline phase (cf. Figures 2 and 3 and Table I). However, as to the intensity of the four C=O component bands, in the gel state the intensities of the anhydrous components of both *sn*-1 and *sn*-2 carbonyls are dominant while in the liquid crystalline state the intensities are more evenly distributed among all four components (cf. spectra A and B of Figure 3). By comparison with the four possible single-crystal structures depicted in Figure 5 we can conclude that the infrared spectra are clearly inconsistent with the presence of a single conformation in the liquid crystalline phase of phosphatidylserine or phospholipids in general. Such a generalization appears to be justified by comparing the present study with previous infrared (Blume et al., 1988) and NMR studies (Hauser et al., 1988). Both these studies provide good evidence that the main feature of the conformation of different phospholipids are very similar, at least in the liquid crystalline phase. For instance, Strenk et al. (1985) interpreted deuterium NMR results obtained with aqueous dispersions of dimyristoylphosphatidylcholine in the liquid crystalline phase in terms of a single phospholipid conformation. The authors proposed the NMR data to be consistent with rotamer A_γ. The infrared data are, however, consistent with a hypothesis put forward on the basis of existing single-crystal structures and proton

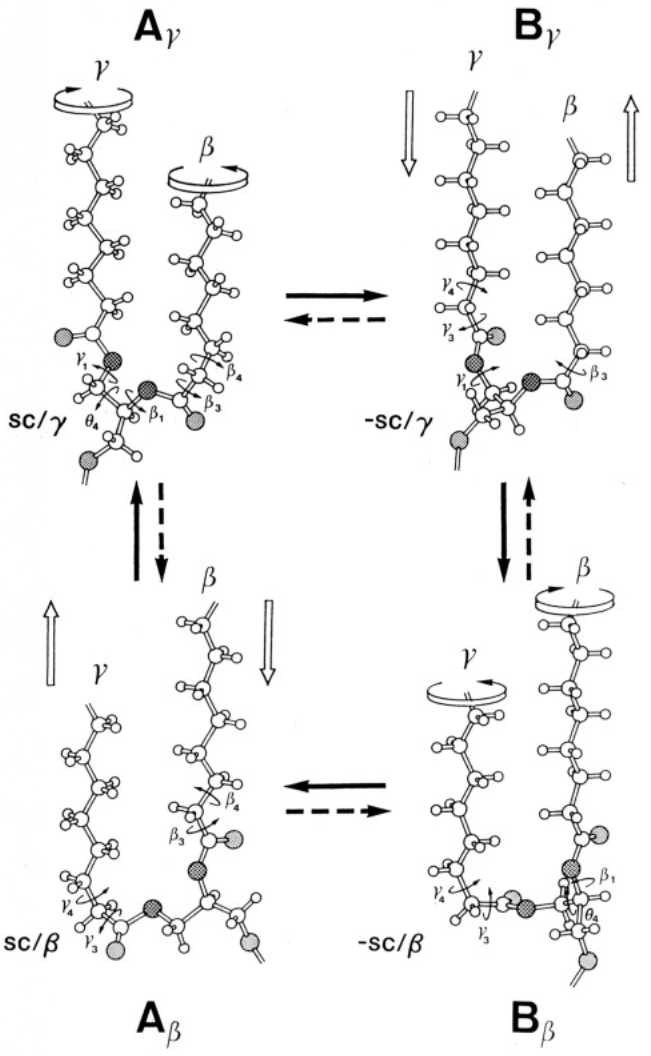

FIGURE 5: Four single-crystal structures of phospholipids are shown: 1,2-dimyristoyl-*sn*-phosphatidylcholine (Aγ), 1,2-dilauroyl-*sn*-glycero-3-phosphate (Aβ), 1,2-dilauroyl-*rac*-glycero-3-phospho-*N*,*N*-dimethylethanolamine (Bγ), and 1,2-dimyristoyl-*sn*-phosphatidylglycerol (Bβ). Rotamers A and B differ in the conformation of the C(*sn*-1)–C(*sn*-2) glycerol bond: rotamer A, θ_3 = ap, θ_4 = sc; rotamer B, θ_3 = sc, θ_4 = –sc. The subscripts β and γ refer to the chain stacking, β indicating that the β-chain (*sn*-2 chain) extends as a straight zigzag chain from glycerol (C(*sn*-2) and γ indicating that this is true for the γ-chain (or *sn*-1 chain) attached to the glycerol C(sn-1). The ester oxygens attached to the glycerol C(*sn*-1)–C(*sn*-2) bond are emphasized by heavy shading; all other oxygens are shaded. Note that structure Aγ and Aβ also differ in the orientation of the glycerol group with respect to the bilayer plane. The glycerol group is oriented approximately perpendicular and parallel in structures Aγ and Aβ, respectively. In contrast, the orientation of the glycerol study is about 45° in both structures Bγ and Bβ. The double arrows indicate the reversible nature of the conformational transitions between the four rotamers shown. The small arrows associated with torsion angles β_1, β_3, β_4, and γ_1 in rotamer Aγ indicate that these torsion angles undergo conformational changes during the transition from structure Aγ to Bγ. The conformational changes ensure that the parallel chain stacking is maintained (adapted from Hauser et al., 1988).

high-resolution as well as deuterium NMR results (Hauser et al., 1988 and references cited therein). The hypothesis states that all four structures shown in Figure 5 are present in the liquid crystalline state of phospholipids though to a different extent and that there is interconversion between these structures which is fast on the NMR time scale. Since the infrared time scale is on the order of 10^{-12} s and hence much shorter than that of NMR measurements, infrared spectroscopy is capable of discerning the different conformers present in the liquid crystalline phase. Unfortunately, absolute values for band intensities cannot be derived from our spectra (Figures 2 and 3), and hence we refrained from a quantitative interpretation of the data by curve-fitting. Nevertheless, the infrared data allow us to draw some important conclusions. The spectra obtained with phosphatidylserine in the liquid crystalline state are consistent with approximately equal contributions of conformers ($A_\gamma + B_\gamma$) and (A_β and B_β). The spectra clearly indicate that under these conditions conformers A_β and/or B_β are present and contribute significantly to the total populations of conformers. Only in conformers A_β and B_β the *sn*-1 carbonyl can readily form hydrogen bonds and only these conformers can account for the C=O band intensity observed at frequencies $\leq$1685 cm^{-1}. Qualitatively the intensity ratio $I(1730)/I(1680)$ is greater in the gel phase than in the liquid crystalline phase (cf. spectra A and B of Figure 3). Such a change in the intensity ratio is attributed to a conformational change upon going through the order–disorder transition: the weighting of the different conformers must change at this phase transition. In the gel phase, conformers A_γ and B_γ are probably dominant accounting for the preferential hydrogen bonding to the *sn*-2 carbonyl. The infrared data support the conclusions derived from both proton high-resolution NMR on phospholipid micelles (Hauser et al., 1988) and deuterium NMR measurements on phospholipid bilayers (Gally et al., 1981; Blume et al., 1982). We want to stress that the infrared data, though consistent with the hypothesis of four different conformers contributing to the observed infrared spectra, do not rule out other conformers (not illustrated in Figure 4).

Phosphatidylserine–Cation Complexes. Binding of Li^+ to 1[1′-^{13}C]DPPS–NH_4^+ is consistent with dehydration of the entire phospholipid polar group confirming earlier results (Casal et al., 1987b,c). Adding the isotope effect of 41 cm^{-1} to the *sn*-1 ^{13}C=O band in the spectra of the 1[1′-^{13}C]DPPS–Li^+ complex the values of 1734 cm^{-1} for the *sn*-1 C=O and 1731 cm^{-1} for the *sn*-2 C=O are very close to those observed with "dry" DMPS–Li^+ (Casal et al., 1987a). Compared to 1[1′-^{13}C[DPPS–NH_4^+ exhibiting anhydrous C=O bands at 1741 and 1700 cm^{-1}, the dry carbonyl bands in 1[1′-^{13}C]-DPPS–Li^+ at 1731 and 1693 cm^{-1} have a relatively low frequency. Assuming that water is absent in the polar group of 1[1′-^{13}C]DPPS–Li^+, Li^+ (with or without its hydration shell) may increase the polarity in the vicinity of the ester groups thus accounting for the low frequency. Both carbonyl groups are probably oriented toward the Li^+ and thus participate in chelating the Li^+. At the phase transition of 1[1′-^{13}C]DPPS–Li^+ at 86 °C the –COO^- group and the *sn*-1 carbonyl group are freed from the chelate and become hydrated, whereas the *sn*-2 carbonyl remains anhydrous and probably chelated to Li^+. Besides Li^+, Ca^{2+}, and Mg^{2+} also induce the crystallization of phosphatidylserine bilayers (Casal et al., 1987a–c, 1989). It was shown earlier that the phosphodiester moiety of the polar group becomes dehydrated on binding of these cations (Casal et al., 1987b,c, 1989). However, while Li^+ induces the complete dehydration of the polar headgroup, including the carbonyl and carboxyl groups, Ca^{2+} and Mg^{2+} are chelated mainly by neighboring phosphate groups with the serine carboxylate group remaining hydrated. Water is apparently still present in the vicinity of both carbonyl groups in the Ca^{2+} and Mg^{2+} complexes. This "trapped" water is immobilized near the *sn*-1 C=O group in 1[1′-^{13}C]DPPS–Ca^{2+} giving rise to a narrow carbonyl absorption at 1678 cm^{-1} indicative of a strong hydrogen bond. On heating the Ca^{2+} complex to higher temperatures the hydrocarbon chain lattice

widens slightly resulting in a higher degree of rotational/translational freedom of the "trapped" water molecules near the *sn*-1 C=O group and in a loss of hydrogen bonding strength. Consequently the *sn*-1 ^{13}C=O band broadens and shifts from 1678 to 1688 cm^{-1}. It is known that Mg^{2+} has a weaker binding affinity to phospholipids (Dawson & Hauser, 1970; Casal et al., 1989), and as a result its effect of promoting the crystallization of the hydrocarbon chains is also weaker. The water molecules near the *sn*-1 C=O region appear to be more mobile and consequently the frequency of the hydrogen-bonded *sn*-1 carbonyl absorption at 26 °C is higher (1688 cm^{-1}) than in the corresponding Ca^{2+} complex. The narrow bands and the extremely low frequency of the *sn*-2 C=O absorption observed at 1711 cm^{-1} at all temperatures in both 1[1′-^{13}C]DPPS–Ca^{2+} and 1[1′-^{13}C]DPPS–Mg^{2+} is indicative of immobilized carbonyl groups and strong hydrogen bonding. We infer that the *sn*-2 carbonyl group is partially bound to the immobilized water of the hydration shell of the Ca^{2+} or Mg^{2+} ions. In the Ca^{2+} and Mg^{2+} complexes of 1[1′-^{13}C]-DPPS the frequency of the non-hydrogen-bonded *sn*-2 C=O vibration (1730–1734 cm^{-1}) is lower by 6–10 wavenumbers than the frequency of the non-hydrogen bonded *sn*-1 C=O group (1740 = 1699 + 41 cm^{-1}). This may again be attributed to a polarity effect assuming that the *sn*-2 carbonyl group is closer to the phosphate-divalent cation group and the *sn*-1 carbonyl group is more deeply buried in the hydrocarbon chain matrix. The infrared data are consistent with the Ca^{2+} or Mg^{2+} complexes favoring conformations with a more dominant contribution from conformers A_γ and B_γ.

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