2D ^{1}H-NMR Conformational Study of Phosphatidylserine Diluted in Perdeuterated Dodecylphosphocholine Micelles. Evidence for a pH-Induced Conformational Transition

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ABSTRACT: The conformation of phosphatidylserine (DMPS) diluted in perdeuterated dodecylphosphocholine micelles (DPC) has been investigated by 1D and 2D proton NMR spectroscopy. Chemical shift pH dependence showed that the p*K* relative to the serine carboxyl titration (3.4 ± 0.05) was nearly identical to that measured in bilayers. Chemical shift and NOE data revealed that the phosphatidylserine molecule undergoes a conformational transition upon titration of the serine carboxyl group. The NOE network observed between the different parts of the molecule was sufficiently abundant to allow, in combination with molecular modeling methods, an assessment of the conformational changes. The conformational changes mainly involve the glycerol backbone, which is parallel to the whole molecule, that is, to the layer normal, at low pH and becomes perpendicular to the whole molecule at neutral pH. In both cases, the conformations are remarkably close to those observed for the crystal forms of zwitterionic and negatively charged phospholipids. Two-dimensional proton NMR study of phospholipids, diluted in perdeuterated DPC micelles, appears to be a simple and relevant method to obtain complete and direct information on their conformations in a model membrane−solution interface.

The role of phosphatidylserine as regulator of membrane protein functions is now well established. Phosphatidylserine, associated with calcium ions, was found to modulate enzyme activity as well as anchoring of peripheral proteins to the membrane. There are many examples of association between protein, calcium, and phosphatidylserine such as membrane recognition of proteins involved in blood coagulation (Esmon, 1993), protein kinase C activity, which displays an absolute specificity for phosphatidylserine (Lee & Bell, 1989; Newton, 1993), and binding of Annexins to membranes (Moss, 1992). Understanding these mechanisms at the molecular level may require knowledge of the conformational properties of phosphatidylserine. Indeed, numerous studies have been carried out to obtain structural information on phosphatidylserine in various conditions. A large number of these works were devoted to pure phosphatidylserine bilayers using different spectroscopic techniques to detect conformational changes upon binding of various ions and biological molecules [^{31}P-NMR:[1] Hope and Cullis (1980) and Lopez-Garcia et al. (1993, 1994). ^{2}H-NMR: Browning and Seelig (1980) and de Kroon et al. (1991). IR: Casal et al. (1987) and Hübner et al. (1994)]. In parallel, ^{1}H-NMR experiments were performed on short-chain diacyl phosphatidylserine in aqueous solution as well as on dimyristoylphosphatidylserine solubilized in mixed organic solvents that allowed estimation of the rotamer proportions around the glycerol C2−C1 bond from *J* coupling measurements (Hauser et al., 1988). In addition, proton chemical shift analysis of spectra of phosphatidylserine−Triton mixed micelles and pure phosphatidylserine sonicated vesicles (De Bony & Dennis, 1981) showed that, as for other phospholipids, one can discriminate CH_2 signals of the *sn*-1 and *sn*-2 chains (the two first methylene protons). However, since the proportion of phosphatidylserine in biological membranes is always low (less than 20%), it is important to get information on phosphatidylserine diluted in membranes. Such studies were performed using ^{2}H-NMR experiments with specifically deuterated phosphatidylserine and monitored conformational changes induced by the binding of various ions (Roux & Bloom, 1990, 1991), calcium and diacylglycerols (Goldberg et al., 1994), or peripheral and intrinsic membrane peptides or proteins (Roux et al., 1988, 1989; Bitbol et al., 1989; Dempsey et al., 1989) through the order parameter variations. Nevertheless, at the present time, no complete description of phosphatidylserine using direct information is available.

In a recent work (Macquaire et al., 1992), we showed that, by using the now standard 2D experiments combined with molecular modeling, one can obtain a precise description of the average conformation of a diacyl lipopeptide diluted in perdeuterated dodecylphosphocholine micelles mimicking a membrane interface. Since a continuous NOE network was

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[1] Abbreviations: DG, distance geometry; DQF COSY, double quantum filtered correlated spectroscopy; DLPA, 1,2-dilauroyl-*sn*-glycero-3-phosphate; DLPE, 1,2-dilauroyl-D,L-glycero-3-phosphoethanolamine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPE, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol; DMPS, 1,2-dimyristoyl-*sn*-glycero-3-phosphoserine; DPC, dodecylphosphocholine; EDTA, ethylenediaminetetraacetic acid; IR, infrared; JR, "jump and return"; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPS, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoserine; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser enhancement spectroscopy; SA, simulated annealing.

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observed from the discriminated first methylene protons of both *sn*-1 and *sn*-2 chains up to the C-terminal amino acid, our study provided not only the conformation of the peptide but also that of the diacylglyceric fragment and the average orientation of the peptide segment with respect to the micelle surface. Therefore, we decided to study the conformation of dimyristoylphosphatidylserine using this method. The phospholipid was diluted in perdeuterated dodecylphosphocholine micelles at different pH values. Our aim was also to study whether conformational changes occur between the charged phosphatidylserine (at neutral pH) and zwitterionic phosphatidylserine when the serine carboxyl group is protonated (at acidic pH).

MATERIALS AND METHODS

1,2-Dimyristoyl-*sn*-glycero-3-phosphoserine (DMPS, from Avanti) (1.2 mg) was solubilized in 0.5 mL of a 20 mM phosphate buffer containing 16 mg of perdeuterated dodecylphosphocholine (DPC, from MSD), giving a DMPS:DPC molar ratio of 2:50, and 0.1 mM EDTA. The solution was sonicated for 10 min with a Branson bath sonicator at 20 °C. The study of the DMPS proton chemical shift pH dependence was achieved with a sample in a 9:1 H_2O:D_2O buffer at 10 °C using a "jump and return" sequence (Plateau & Guéron, 1982) on a Bruker AMX 600 spectrometer. The pH was adjusted by adding small amounts of NaOD or DCl solution and was measured before and after the NMR experiment to obtain the most precise average value. A standard two-dimensional phase-sensitive DQF-COSY experiment was performed on a Bruker AMX 500 with a sample in D_2O buffer at 20 °C at pH 7.5 and then at pH 2.6. The pH value was checked from the chemical shift calibration curves preliminarily obtained. A total of 64 transients were acquired with a recycling delay of 1 s. Four hundred increments of 4K data points for a 2500-Hz spectral width were collected, leading after Fourier transformation and zero filling to a 4K × 1K matrix with a digital resolution of 0.6 Hz/point in the F_2 dimension, allowing measurement of J values. Gaussian−Lorentzian and sine functions were used for apodization in the F_2 and F_1 domains, respectively. For both pH values, phase-sensitive NOESY spectra with mixing times of 80 and 160 ms were recorded on a Bruker AMX 600 spectrometer. NOESY experiments containing a jump and return sequence with a 160-ms mixing time were also performed on a sample in a 9:1 H_2O:D_2O buffer at 10 °C. In this case, the high pH value had to be decreased from 7.5 to 6.3 since the signal of the NH_3 protons vanishes above pH 7. A total of 64 transients were acquired with a recycling delay of 1 s. Four hundred increments of 2K data points for a 3000-Hz (D_2O buffer) or a 6000-Hz (9:1 H_2O:D_2O buffer) spectral width were collected. Shifted squared sine-bell functions were used for apodization in both dimensions. NOE data were converted into interproton constraints, using for calibration the intensities of the cross peaks connecting the magnetically inequivalent methylene protons. Distance geometry/simulated annealing (DG/SA) calculations were performed using Sybyl 6.1 software (Tripos Associates) containing an efficient simulated annealing facility. The molecules were first embedded using additional chain constraints as described in the text, roughly minimized, and then submitted to several 5-ps annealing cycles from 700 to 0 K and finally thoroughly minimized. A Tripos force field was used without any electrostatic term.

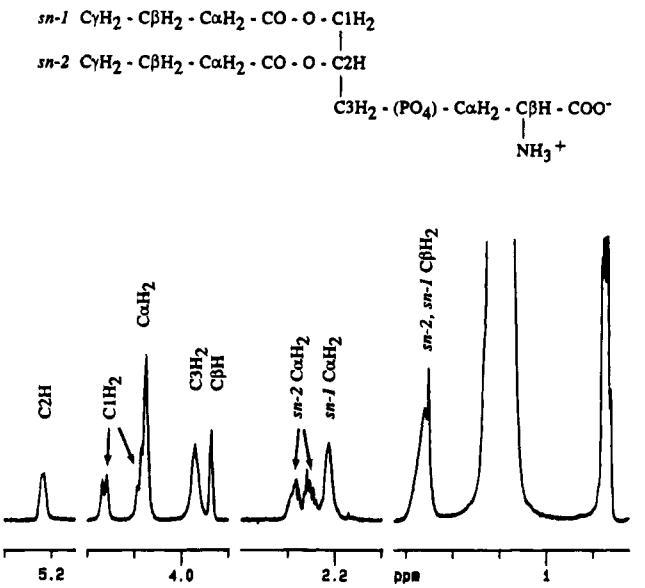

FIGURE 1: 600-MHz proton NMR spectrum of DMPS solubilized in perdeuterated DPC micelles (D_2O phosphate buffer, 10 °C, pH 7.5).

RESULTS AND DISCUSSION

Chemical Shift and J Coupling Analysis of DMPS in DPC Micelles at pH 7.5. Figure 1 shows the proton numbering and the ^{1}H-NMR spectrum of DMPS solubilized in a D_2O buffer (pH 7.5, 10 °C) containing perdeuterated DPC micelles. The resonances of the glycerol $C1H_2$−C2H−$C3H_2$ and serine $C\alpha H_2$−$C\beta H$ protons are close to those previously observed for different PS lipids in various conditions [sonicated vesicles, Neumann et al. (1985); mixed organic solvents, Hauser et al. (1988); short-chain diacyl lipids in aqueous solution, Hauser et al. (1988)]. Measurement of vicinal J coupling constants gave 3/7.5 Hz for the glycerol $C1H_2$−C2H segment and 4.5/4.5 Hz for the serine $C\alpha H_2$−$C\beta H$ segment, in agreement with the values measured in the other conditions previously mentioned (except for the sonicated PS vesicles because of two large line widths). The J values relative to the glycerol $C1H_2$−C2H bond indicate that the two gauche± rotamers are highly predominant and roughly equally populated (Hauser et al., 1988). In the following, we used the notations A and B of Hauser et al. to identify these two families of glycerol rotamers. The overlap between the $C3H_2$ signals precluded the measurement of the other glycerol J values. With a sample in 9:1 H_2O:D_2O buffer and by using the jump and return (JR) sequence for water signal suppression, a broad signal corresponding to the serine NH_3 protons was detected only when the pH became less than 7 (δ = 8.14 ppm at pH 6.3).

In the DQF-COSY network of the acyl chain protons, the discernible signals, on a 2D spectrum and with a decreasing chemical shift, are the α (CH_2CO), β (CH_2CH_2CO), and γ ($CH_2CH_2CH_2CO$) methylene protons and the terminal CH_3 group. The other methylene signals are not distinguishable. The *sn*-1 and *sn*-2 chains give well-separated α methylene resonances. A small difference between the chemical shifts of the *sn*-1 and *sn*-2 resonances is also observed for the β and γ protons. In addition, the α and α′ proton resonances

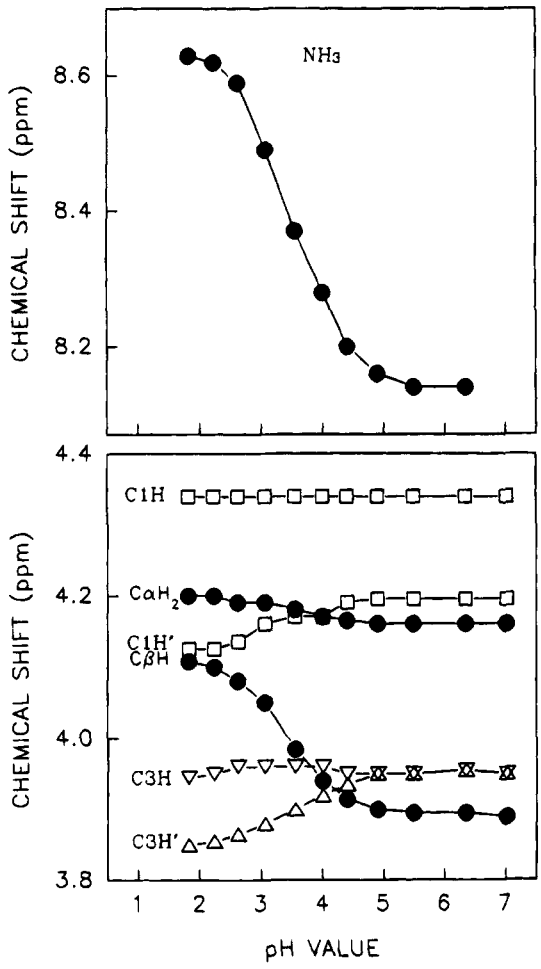

FIGURE 2: Chemical shift pH dependence of serine protons (●) and methylene glycerol protons (□, △, ▽) of DMPS solubilized in perdeuterated DPC micelles (9:1 H_2O:D_2O phosphate buffer, 10 °C).

are discernible for one chain (the low-field signal). Chain discrimination in proton NMR spectra of diacyl phospholipids solubilized in Triton micelles as well as organized in small unilamellar bilayer vesicles has been reported previously (De Bony & Dennis, 1981, and references therein). More recently, we showed that diacyl lipopeptides exhibit the same spectral feature (Macquaire et al., 1992). Assignment of *sn*-1 and *sn*-2 signals was achieved by Roberts and Dennis using partially deuterated lipids (Roberts & Dennis, 1977), and in all the cases, the *sn*-1 α methylene signal shifted upfield from the *sn*-2 one. These features were related to the typical phospholipid conformation in which the *sn*-1 chain starts perpendicular to the layer surface, while the *sn*-2 chain starts parallel and then bends sharply at the α carbon atom (Hitchcock et al., 1974; Seelig & Seelig, 1975; Haberkorn et al., 1977; Pearson & Pascher, 1979).

pH Dependence of DMPS Resonances. Figure 2 shows the pH dependence—between pH 7.02 and 1.82—of the serine and glycerol proton chemical shifts of DMPS solubilized with DPC micelles in a 9:1 H_2O:D_2O buffer at 10 °C. Both serine NH_3 and Hβ chemical shift variations fit well-defined sigmoid curves reflecting the serine carboxyl titration. The corresponding pK value is 3.4 ± 0.05. In contrast to the serine CαH_2 signal, which is hardly sensitive to the pH variation, one of the glycerol $C3H_2$ protons is significantly affected. Magnetically equivalent at pH 7, the H3 and H3′ protons give well-separated signals ($\Delta\delta$ = 0.1 ppm) at pH 1.8. The J_{HH} values of the C2H−$C3H_2$ segment and the J_{PH} values of the $C3H_2$−O−P segment are 5/5.5 and 4/4.5 Hz, respectively. Furthermore, whereas the glycerol H1 and H1′ protons remain magnetically nonequivalent between pH 1.8 and 7, one of these signals undergoes a significant shift reflecting the serine carboxyl titration. Lastly, the glycerol H2 signal is almost insensitive to the pH variation ($\Delta\delta$ < 0.01 ppm; data not shown), and the $J(C1H_2$−C2H) values are not modified. As regards the chain protons, no variation was observed upon pH titration except for a slight decrease of the chemical shift difference between the *sn*-2 α and α′ resonances.

This study provides two important pieces of information. First, the pK relative to the serine carboxyl group of DMPS solubilized in DPC micelles is significantly higher than that of the isolated serine (pK = 2.9). Moreover, its value is close to that measured in mixed phosphatidylserine/phosphatidylcholine bilayers (pK = 3.6; Tsui et al., 1986) using potentiometric titrations and surface potential measurements. Therefore, one can conclude that specific physicochemical properties of the membrane interface are conserved in the DPC micellar environment. Second, the pH dependence of the $C1H_2$ and $C3H_2$ chemical shifts suggests that at least the glycerol backbone undergoes a conformational transition upon the serine carboxyl titration. In the following section, the conformation of DMPS in DPC micelles at pH 7.5 and 2.6 is analyzed using the NOE data.

Comparative Analysis of NOE Data at pH 7.5 and 2.6: Evidence for a Conformational Transition. For both pH values, two NOESY experiments of a sample in D_2O buffer at 10 °C were recorded with mixing times of 80 and 160 ms under the same experimental conditions. NOESY experiments containing the JR sequence (9:1 H_2O:D_2O buffer, at pH 6.3 and 2.6) were also performed, but no NOE arising from the NH_3 signal was detected, probably because of its large line width.

Figure 3a shows the region of the NOESY spectrum (mixing time of 160 ms) corresponding in the F_2 dimension to the methylene protons (α, β) of the *sn*-1 and *sn*-2 chains and in the F_1 dimension to the glycerol $C1H_2$ and $C3H_2$ and the serine CαH_2 protons, at both pH values. This figure readily reveals that, as previously suggested by the chemical shift analysis, a conformational transition occurs upon pH titration. First, the NOE pattern relative to the H3 and H3′ signals strongly differs according to the pH value: these protons exhibit NOE cross peaks with both *sn*-1 (α, β) and *sn*-2 (α) chain protons at pH 7.5 but only a weak NOE with one of the *sn*-2 α protons at pH 2.6. In contrast, the glycerol H1 and H1′ signals are only connected to the *sn*-1 α and β signals at both pH values. Second, whereas well-resolved NOEs between the serine α protons and the *sn*-2 chain α and α′ protons are observed at both pH values, the NOE intensities measured at pH 2.6 are 2 times greater than those measured at pH 7.5, and a weak serine α−sn-2 β NOE is observed at pH 2.6 and absent at pH 7.5. Furthermore, NOEs between the serine α protons and the glycerol H2 and H3 protons only appear at pH 2.6 (boxed cross peaks in Figure 3b). In contrast, the serine β proton only exhibits a sequential NOE cross peak with the α protons at both pH values. As regards the other NOE patterns relative to the glycerol protons as well as the NOEs between the discernible α, β, and γ chain protons within a given chain, only slight differences are observed. Lastly, no interchain NOE can be

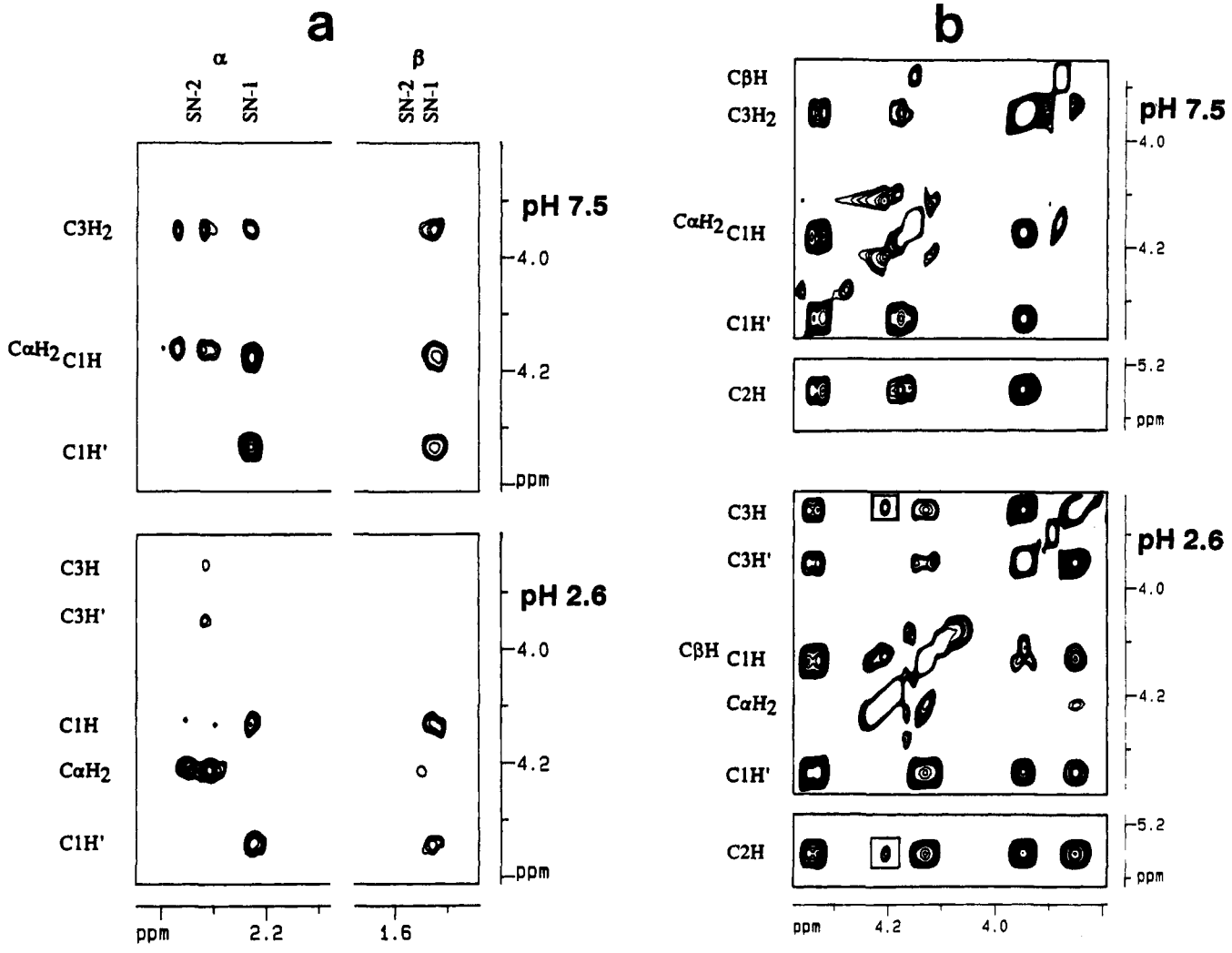

FIGURE 3: (a) Part of the 600-MHz NOESY spectra (mixing time of 160 ms) of DMPS solubilized in perdeuterated DPC micelles (D_2O phosphate buffer, 10 °C) corresponding in the F_2 dimension to the methylene protons (α, β) of the *sn*-1 and *sn*-2 chains and in the F_1 dimension to the glycerol $C1H_2$ and $C3H_2$ and serine $C\alpha H_2$ protons, at pH 7.5 and 2.6. (b) Part of the same spectra corresponding in the F_2 dimension to the glycerol $C1H_2$ and $C3H_2$ protons and the serine $C\alpha H_2$ and $C\beta H$ protons, and in the F_1 dimension to the same protons plus the glycerol C2H proton, at pH 7.5 and 2.6; the boxed cross peaks in the pH 2.6 spectrum correspond to NOEs between the serine α protons and the glycerol H2 and H3 protons.

observed, probably because of chemical shift degeneracy from the γ protons on one hand and the relative conformation of the *sn*-1 and *sn*-2 chains (see the following section) on the other hand. Figure 4 shows several NOE intensity plots versus the two mixing time values, representing the three classes of cross-peak intensity (upper trace, strong NOEs; middle trace, medium NOEs, lower trace, weak NOEs) for both pH values. For the pH 7.5 plots, the values of NOEs involving the magnetically equivalent H3 and H3′ protons [referred to as H(3,3′) in Figure 4] correspond to *half of the measured cross-peak intensity* in order to facilitate the comparison between the plots relative to both pH values. These plots indicate that the cross-peak intensities measured with a mixing time of 80 ms are approximately in the NOE linear limit.

The *main* differences between the NOE data at pH 7.5 and that at pH 2.6 are schematically summarized in Figure 5. In a simple way, this scheme shows that the protonation of the serine carboxyl group induces a drastic change of the average orientation of both the glycerol and the serine moieties.

A possible difficulty for quantitatively analyzing the NOE values arises from the flexibility of DMPS and the variations of the apparent correlation time along the molecule. In the region including the glycerol backbone and the two first carbons of the chains, we have experimental evidence that the dispersion of the order parameters and apparent correlation times is weak: (i) the protons exhibit similar line widths, and (ii) the three NOEs between the nonequivalent protons of glycerol C1 and C3 and the *sn*-2 chain Cα methylene groups, corresponding to the same shortest distance (1.8 A), exhibit similar intensities. In the case of the head group protons, Browning and Seelig (Browning & Seelig, 1980) showed that the dilution of PS in PC bilayers significantly increased the head group flexibility in comparison with pure PS bilayers (this point will be elaborated below). In our case, the fact that the serine $C\beta H$ proton exhibits no other NOE than that with the adjacent $C\alpha H_2$ protons can probably be explained not only by the conformation of the head group relative to the glycerol backbone and the chains but also by an increased flexibility of the head group, leading, for a given interproton distance, to a decreased NOE intensity compared to that obtained for the other distinguishable protons. This means that the distance ranges associated with the NOEs between the serine $C\alpha H_2$ protons and the glycerol or chain protons are probably underestimated, but this is of course

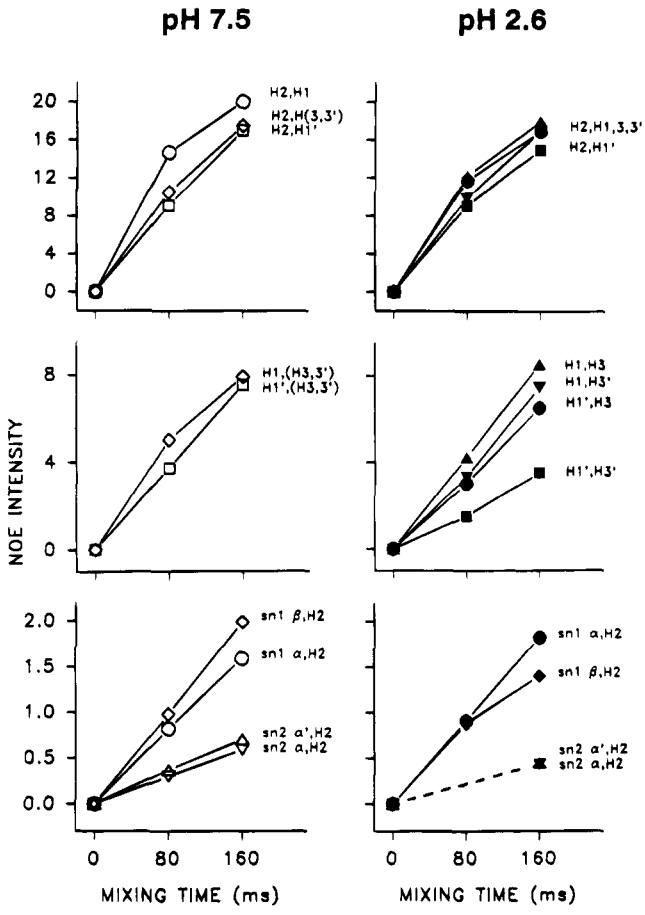

FIGURE 4: Plots of NOE intensity (arbitrary units) versus two mixing time values (80 and 160 ms) measured from the NOESY spectrum of DMPS solubilized in perdeuterated DPC micelles (D_2O phosphate buffer, 10 °C) at pH 7.5 and 2.6. The upper trace corresponds to the strong NOEs connecting the glycerol H2 resonance to the glycerol $C1H_2$ and $C3H_2$ methylene protons; the middle trace corresponds to the medium NOEs between the glycerol $C1H_2$ and $C3H_2$ methylene protons; the lower trace corresponds to the weak NOEs connecting the glycerol H2 proton to the *sn*-1 and *sn*-2 α and β chain protons.

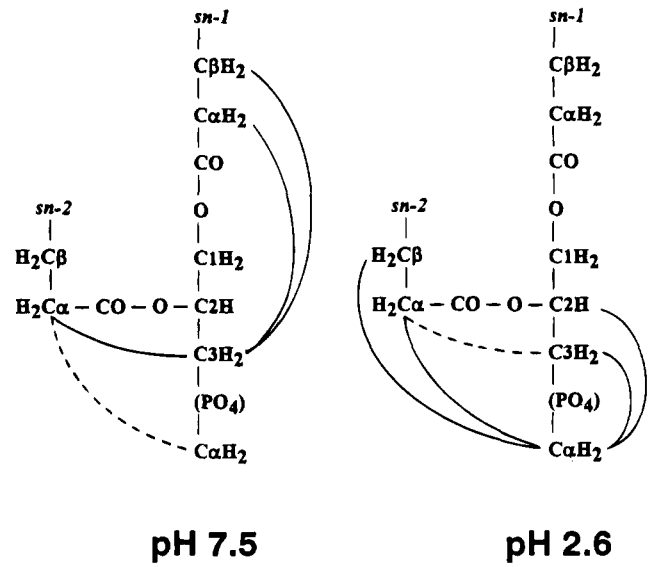

FIGURE 5: Schematic picture of the main differences observed between the NOE network of DMPS at pH 7.5 and that of DMPS at pH 2.6. For NOEs observed in both networks but whose intensities significantly differ according to the pH value, the dotted line symbolizes a reduced intensity.

not critical for the polar head. The following section presents a molecular modeling study using the NOE data as constraints to give the possible conformations of DMPS at pH 7.5 and 2.6.

Different Conformers Explored by the DMPS Molecules Diluted in Perdeuterated DPC Micelles and Deduced from NOE Data by Molecular Modeling. Phospholipids are flexible molecules and *experience different conformations*. The NOE data thus reflect a *set of the most populated conformers* rather than a unique average conformation. A consequence of such an intrinsic flexibility is that not all of the NOE derived constraints can be satisfied simultaneously. Our aim is thus to obtain the limiting conformations—those of lowest energy—which are explored by the DMPS molecule in the two different pH conditions. Therefore, we were led to select mutually exclusive subsets of NMR constraints which are supposed to represent different conformers in equilibrium. Our strategy to transform the NOE data into meaningful conformational information, using distance geometry/simulated annealing calculations (DG/SA), was as follows:

(a) In order to obtain final calculated energies that can be compared, extra constraints were introduced to maintain the aliphatic chains in a full trans configuration (a Cα—methyl constraint for *sn*-1 and a Cβ—methyl constraint for *sn*-2). These constraints were removed at the final minimization step. An additional constraint for bringing the chains in contact was also transiently introduced (at the embedding step) to simulate the intermolecular lipidic pseudopotential absent in the calculation but whose contribution is very important for the conformation of the lipid molecules. This interaction constraint was always removed after the rough minimization following the embedding step and was of course absent during the annealing cycles.

(b) An all-trans configuration of the aliphatic chains being assumed, we have now to consider more attentively the constraints relative to the *sn*-1 α and β protons. The effect of segmental motion of the chains is to shorten the average distance between these protons and the glycerol protons. Indeed, NOE derived distances are systematically shorter or slightly shorter than the "crystallographic distances". We were thus led to relax some or all the *sn*-1-glycerol constraints depending on the particular limiting configuration being observed. Indeed, the number of configurations of the glycerol backbone is small, and our objective is precisely to determine these limiting configurations, which are sequentially explored by the whole molecule rather than an average configuration. As a consequence, a set of simulated molecules was first generated using *loose constraints* for each set of NOEs (pH 2.6 or 7.5). Then, stereospecific attribution and constraint selections were made for each characteristic configuration sorted after this first step.

(c) For modeling the conformation of DMPS at pH 2.6, the constraints between the glycerol $C1H_2$ and C2H protons on one hand and the *sn*-1 α and β protons on the other hand were relaxed. Two populations of molecules emerged according to the configuration, A or B, of the glycerol $C1H_2$—C2H atoms (Figure 6). This result is in agreement with our *J* coupling data giving approximately equal populations for the A and B configurations. In either case, we had to make stereospecific attribution in order to use realistic constraints between the glycerol protons and the *sn*-2 or serine protons.

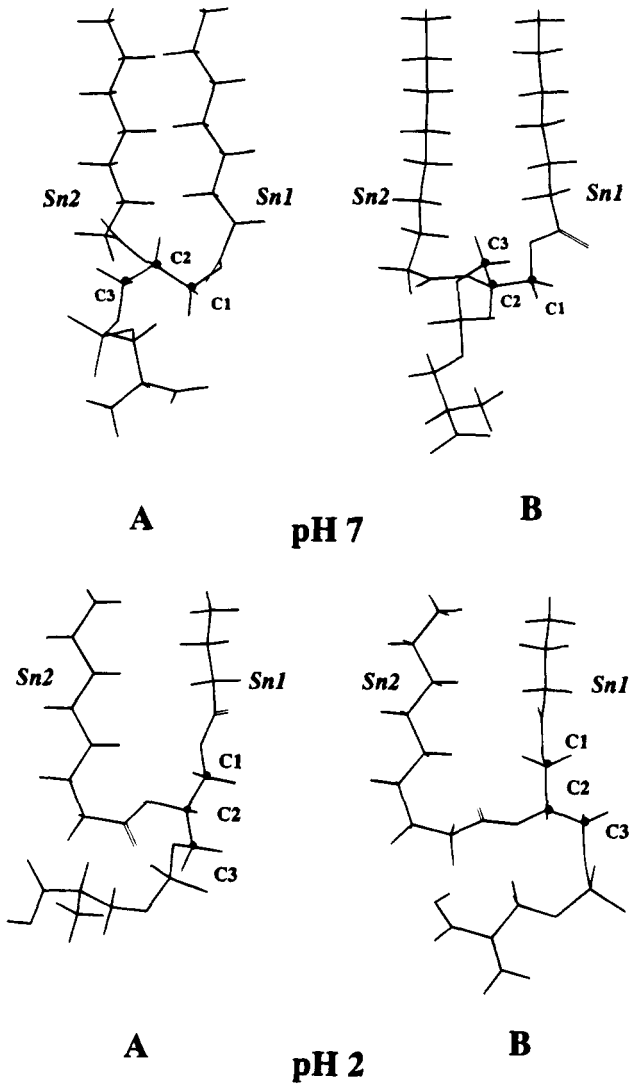

FIGURE 6: Two DMPS molecules exhibiting the lowest energy obtained by DG/SA calculations and representative of the two A and B rotamer families, at pH 7.5 and 2.6. The A and B rotamers correspond to the gauche+ and gauche− configurations of the C1 and C2 ester groups.

(d) For the simulation of the pH 7.5 conformation, we simply introduced the important $C3H_2$−*sn*-1 constraints and relaxed the other unobserved constraints. Loose constraints involving $C3H_2$ and *sn*-1 protons (d_{max} = 5.5 Å) were introduced first and immediately gave a bend for the relative glycerol−*sn*-1 configuration. This also allowed a separation of the simulated molecules into A and B families. Then the constraints were specifically adjusted and strengthened to their NMR values, taking into account the NMR equivalence of protons and stereospecificity. As an example (see Figure 6), the C2H−*sn*-1 α or β constraint was either relaxed (conformation A) or strengthened (conformation B). Furthermore, *either* the $C1H_2$−*sn*-1 α *or* the $C1H_2$−*sn*-1 β constraint was naturally satisfied, in contrast with the pH 2.6 conformation, without the need of invoking the segmental motion in the *sn*-1 chain. Finally, for each set of molecules obtained, an *intensive* minimization was performed, providing low-energy conformations within the two A and B families (Figure 6).

A set of about 40 molecules was obtained by this procedure. All of the energies, calculated without electrostatic terms, were negative. In each class of configuration (A or B), the conformations of the glycerol backbone exhibit small fluctuations which mainly concern the rotation around the C2−C3 bond. The molecules naturally differ mainly by the disposition of the phosphoserine polar head with respect to the glycerol backbone. This of course has to be related to the intrinsic mobility of the polar head. Only slight variations of the *sn*-1 and *sn*-2 chain directions, up to about 10°, with respect to the glycerol backbone were observed. Only the lowest energy molecule for each class and each pH value is shown in Figure 6. We stress again that the conformations obtained by molecular modeling from NOE data represent the most populated conformers or alternatively the longest lived conformers which are experienced by the molecule at pH 7.5 and 2.6. Indeed, at intermediate pH values, a fast exchange, on the chemical shift time scale (Figure 2), is observed between the low- and high-pH conformations.

Comparison between the Two Conformations of DMPS. At pH 2.6, the polar head of the DMPS molecule is zwitterionic following the carboxylate protonation, and the conformations of lowest energy are remarkably similar to that of DLPE (Hitchcock et al., 1974) and also of DMPC (Pearson & Pascher, 1979). The glycerol backbone, whatever the A or B configuration, is predominantly parallel to the aliphatic chains, that is, to the layer normal (or micelle radius). Conversely, the polar head is mostly elongated perpendicular to this direction. Of course, other less probable conformations of the polar head which would not give rise to NOE contacts are not excluded. However, the number of contacts observed seem to indicate a strong persistence for the conformation obtained by molecular modeling, which brings the polar head under the *sn*-2 chain, that is, within the cylinder roughly determined by the whole molecule. At pH 7.5, the polar head is negatively charged and the average conformations of the DMPS molecule are strongly different, with the glycerol backbone rather perpendicular to the chain direction. This is exactly the average conformation observed for the negatively charged phospholipids like DLPA (Hauser et al., 1988) and DMPG (Pascher et al., 1987). In addition, the polar head is found to be more parallel to the layer normal at pH 7.5. This probably results from electrostatic interactions which tend to increase the distance between the serine carboxylate and the layer of negatively charged phosphate groups.

As already mentioned, both A and B configurations of the glycerol backbone are compatible with the NMR data whatever the pH value. However, whereas the A ↔ B exchange is very easily obtained at pH 2.6 with a minimum of independent bonds involved (essentially C1−C2 and *sn*-1), this is not the case at pH 7.5. Indeed, at pH 7.5, the A ↔ B exchange involves correlated rotations around the C1−O−(*sn*-1) and C2−O−(*sn*-2) bonds. Besides, we cannot exclude the existence of a "pH 2.6 like" conformation as a possible low populated conformer at pH 7.5 during this exchange. To end, it is worth noting that, for *all* the conformations of the DMPS molecule, the polar head is linked to the glycerol backbone by at least one bond parallel to the long axis of the whole molecule which is entropically favorable. These are the O−P (A family) or the C3−O (B family) bond at pH 2.6 and C3−O (A family) or the O−P (B family) bond at pH 7.5.

Comparison with Previous NMR Studies. Comparing our results to those obtained by [2]H-NMR studies and a

previous one-dimensional ^{1}H-NMR study, several features can be pointed out. The pioneering ^{2}H-NMR study of Browning and Seelig indicated an unusual rigid head group for pure PS bilayers compared to pure PC or PE bilayers (Browning & Seelig, 1980). However, dilution of charged DMPS or POPS with neutral DMPC or POPC (Browning & Seelig, 1980; Roux & Neumann, 1986; Roux & Bloom, 1991) up to PS:PC molar ratios of 1:5 dramatically increases the head group flexibility, mainly monitored by the variation of the magnetic nonequivalence of the two deuterons of the serine $C\alpha D_2$ group. The dilution effect is enhanced when DMPS is diluted in DMPE bilayers since, for a PS:PE molar ratio of 1:5, one of two quadrupolar splittings becomes nearly zero (Roux & Neumann, 1986). These results strongly suggested that intermolecular interactions between PS head groups occur in pure PS bilayers (Browning & Seelig, 1980). In the present study, the DMPS:DPC molar ratio is 1:25, and it is thus not surprising that our data, i.e., the magnetic equivalence of the two serine α protons and the structures obtained by NOE derived modeling, indicate a large flexibility of the head group. As regards the glycerol backbone, our results confirm the existence of an equilibrium between two roughly equally populated A and B conformations previously observed by one-dimensional ^{1}H-NMR spectroscopy (Hauser et al., 1988).

CONCLUSION

We showed that 2D NMR and NOE spectroscopies are able to give a direct conformational description of DMPS diluted in perdeuterated DPC micelles, that is, the average orientation of both glycerol backbone and polar head group with respect to the layer normal. Our results confirm that the conformation of the phospholipidic molecules depends on the polar head charge.

We have now undertaken an extensive molecular dynamic calculation to describe in more detail the conformational equilibrium as detected by NMR. Our experiments were performed with DMPS diluted in DPC micelles. The overall consistency with the DMPS conformations obtained with the bilayer and crystal studies confirms that the DPC micelles are a suitable model for lipidic environment allowing more extended studies using high-resolution NMR. We are now extending the use of this model membrane to study the conformation of other lipids as well as their interactions with cations, especially calcium.

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