Coupled Changes between Lipid Order and Polypeptide Conformation at the Membrane Surface. A ^2H NMR and Raman Study of Polylysine–Phosphatidic Acid Systems†

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ABSTRACT: Thermotropism and segmental chain order parameters of *sn*-2-perdeuteriated dimyristoylphosphatidic acid (DMPA)–water dispersions, with and without poly(L-lysine) (PLL) of different molecular weights, have been investigated by solid-state deuterium NMR spectroscopy. The segmental chain order parameter profile of this negatively charged lipid is similar to that already found for other lipids. Addition of long PLL (MW = 200 000) increases the temperature, T_c, of the lipid gel-to-fluid phase transition, whereas short PLL (MW = 4000) has practically no effect on T_c. In the fluid phase both varieties of PLL increase the "plateau" character of segmental order parameters up to carbon position 10. At the same reduced temperature, long PLL more significantly increases the segmental ordering, especially at the methyl terminal position. This leads to the conclusion that polar head-group capping and charge neutralization by PLL induce severe changes in lipid chain ordering, even down to the bilayer core. The structure of PLL bound to the lipid bilayer surface was monitored by Raman spectroscopy, following the amide I bands. Results show that the lipid gel-to-fluid phase transition triggers a conformational transition from ordered β-sheet to random structure of short PLL, while it does not affect the strongly stabilized β-sheet structure of long PLL. It is concluded that both short and long PLL can efficiently cap and neutralize lipid head groups, whatever their structure, and that peptide length is a key parameter in whether lipids or peptides are the driving force in conformationally coupled changes of both partners in the membrane.

The results of a recent investigation by Raman spectroscopy of the interaction between dimyristoylphosphatidic acid (DMPA)[1] bilayers and poly(L-lysine) (PLL) of low (4000) and high (200 000) molecular weight have clearly shown that when both types of PLL are bound to DMPA bilayers, they adopt the β-sheet conformation (Laroche et al., 1988) as opposed to the α-helical structure previously found for long PLL bound to dipalmitoylphosphatidylglycerol (Carrier & Pézolet, 1984). For long PLL, the conformation of the polypeptide bound to DMPA remains stable over the whole range of temperatures investigated, whereas short PLL undergoes a conformational change from β-sheet to random coil structure at nearly the same temperature as that of the gel-to-fluid phase transition of the lipid (Laroche et al., 1988).

In the same study, it has been concluded that, in contrast to short PLL, long PLL induces a 20 °C increase in T_c and a lateral phase separation in mixtures of DMPA with dimyristoylphosphatidylcholine (DMPC). We believe that this phenomenon might be associated with the fact that long PLL adopts an ordered structure (β-sheet) while short PLL is in the random coil conformation in this mixture.

Even though Raman spectroscopy has provided valuable information on the structure of DMPA/PLL complexes, it is not very sensitive for monitoring the order of the acyl chains in the fluid phase, and thus, important questions are still unanswered: (i) What is the effect of the polypeptide on the DMPA acyl chain dynamics, and does the conformation adopted by the polypeptide in the fluid phase influence the dynamical properties of the membrane? (ii) Is the phase transition of the DMPA/short PLL system driven by the conformational change of the polypeptide or is the latter change triggered by the physical state of the lipid? This last question is particularly important regarding the possible influence of the physical state of phospholipids on the conformation of proteins in biological membranes.

In this paper deuterium nuclear magnetic resonance spectroscopy (^2H NMR) has been used to document lipid structure and dynamics. This technique is a powerful tool for investigating membrane dynamics (Davis, 1983; Dufourc et al., 1984; Dufourc & Smith, 1985; Huschilt et al., 1985; Devaux et al., 1986). Up to now, ^2H NMR studies on lipid chain dynamics have almost exclusively been performed on zwitterionic lipids. This is the first time, to our knowledge, that ^2H NMR is successfully applied to follow perturbations of the chains of negatively charged lipids as induced by polypeptides acting like peripheral proteins, which cap only the lipid head groups. One must mention that several studies using the same technique have dealt with modifications of lipid head-group structure and dynamics induced by cations and polypeptides (Altenbach & Seelig, 1984; Sixl et al., 1984; Sixl & Watts,

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[1] Abbreviations: PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; DPPG, dipalmitoylphosphatidylglycerol; DMPA, dimyristoylphosphatidic acid; DPPA, dipalmitoylphosphatidic acid; ^2H NMR, deuterium nuclear magnetic resonance; PLL, poly(L-lysine); T_c, temperature of the gel- ($L_{\beta'}$) to-fluid ($L_{\alpha'}$) phase transition; DMPC, dimyristoylphosphatidylcholine; [*sn*-2-$^2H_{27}$]DMPA, 1-myristoyl-2-perdeuteriomyristoyl-*sn*-glycero-3-phosphoric acid.

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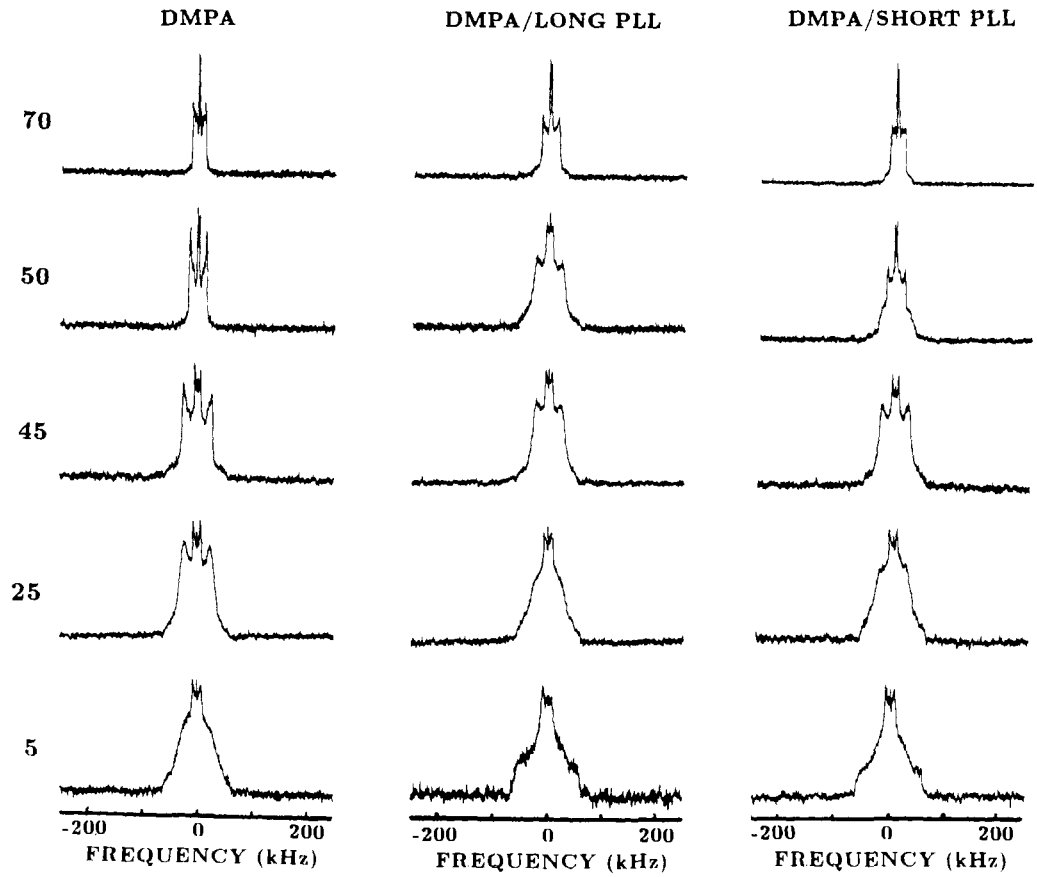

FIGURE 1: Temperature dependence of ^2H NMR powder spectra of [*sn*-2-$^2H_{27}$]DMPA in the absence (left) and the presence of long (center) and short (right) PLL. Temperature in °C is indicated on the left-hand side. Experimental parameters: $\pi/2$ pulse length 5.3 μs, pulse spacing in the quadrupolar echo sequence 25 μs, spectral window 500 kHz, recycling time 1.5–2 s, 3000 acquisitions.

1985; Watts & Poile, 1986; Roux & Neumann, 1986; MacDonald & Seelig, 1987, 1988; Roux et al., 1989).

The last question about the relationship between the conformational change of short PLL and the physical state of the lipid was addressed by Raman spectroscopy, a technique particularly efficient for monitoring the conformation of both the lipid and the polypeptide (Pézolet et al., 1982; Carrier & Pézolet, 1984; Laroche et al., 1988). In order to define which of the two components imposes the constraint for conformational change, the binding of identical peptides on DMPA and DPPA bilayers, which have gel-to-fluid phase transition temperatures differing by about 15 °C, has been studied.

Materials and Methods

Materials. The disodium salts of dipalmitoylphosphatidic acid and of dimyristoylphosphatidic acid with a perdeuteriated *sn*-2 chain were obtained from Avanti Polar Lipids (Birmingham, AL). The hydrobromide salts of PLL of molecular weights 3300–4000 (short PLL, i.e., about 26 amino acids long) and 180000–260000 (long PLL, i.e., 1200–1700 amino acids long) were purchased from Sigma (St. Louis, MO). All materials were used without further purification.

Deuterium NMR Experiments. Aqueous dispersions of [*sn*-2-$^2H_{27}$]DMPA were prepared by mixing appropriate amounts of lipid in deuterium-depleted water containing 150 mM NaCl and 10 mM EDTA at pH 6.5. Samples were then heated to 65 °C for 10 min, vortexed, and cooled down below the gel-to-fluid phase transition temperature. This cycle was repeated at least three times. The pH of the dispersion was measured and adjusted to 6.5, if necessary, and samples were transferred to 10-mm-diameter NMR tubes. Samples containing polylysine were prepared by adding appropriate amounts of a 3% PLL solution to the lipid dispersion, and the incubation cycle was repeated once again. For all experiments, the lipid-to-lysine residue molar ratio was 1, which corresponds to the stoichiometry of the complex (Laroche et al., 1988). In all cases, the final phospholipid concentration was about 70 mM (5% by weight). Spectra were recorded on a Bruker MSL 200 spectrometer operating at 30.7 MHz by means of the quadrupolar echo sequence (Davis, 1979). Quadrature detection was utilized and the temperature was regulated to ±1 °C. Samples were allowed to equilibrate at a given temperature for at least 30 min prior to recording the NMR signal. Typical experimental parameters were $\pi/2$ pulse length of 5.3 μs, spacing of the two $\pi/2$ pulses in the quadrupolar echo sequence of 25 μs, spectral window of 500 kHz, and recycle time of 1.5–2 s. Data treatment was performed on a VAX/VMS 8600 computer.

Raman Experiments. Aqueous dispersions of DPPA were prepared in the same solvent as for NMR experiments in order to obtain a final lipid concentration of 10% by weight. Complexes of DPPA and short PLL were prepared by mixing appropriate volumes of a 1% DPPA dispersion with a 3% PLL solution. Samples were then transferred to glass capillary tubes and centrifuged in a hematocrit centrifuge. Raman spectra were obtained from the white pellet. The Raman spectrophotometer used in this work is described elsewhere (Savoie et al., 1979; Pézolet et al., 1983).

Results

Thermotropic Behavior of DMPA Alone and in the Presence of PLL. Figure 1 (left) shows ^2H NMR spectra of pure [*sn*-2-$^2H_{27}$]DMPA dispersions. As the temperature is raised, spectra decrease in width and become more structured. The most important change occurs between 45 and 50 °C, i.e., at

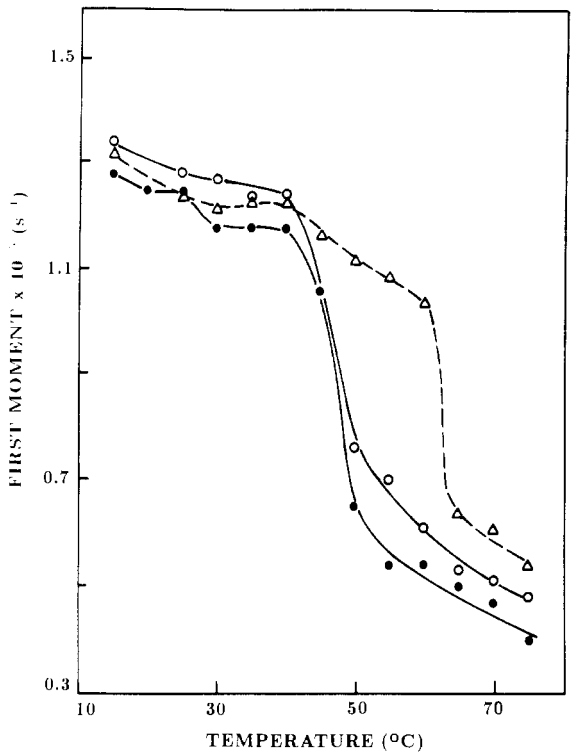

FIGURE 2: Temperature variation of the first moment of [*sn*-2-$^2H_{27}$]DMPA spectra in the absence (●) and the presence of long (△) and short (○) PLL.

the temperature of the lamellar gel ($L_{\beta'}$) to lamellar fluid ($L_{\alpha'}$) phase transition of the phospholipid. In order to quantitate these observations, ^2H NMR spectral moments were calculated (Bloom et al., 1978; Davis, 1979). The first spectral moment, M_1, calculated with respect to the middle of the spectrum (zero frequency) and using absolute values of frequencies, allows an estimation of the orientational order parameter for a given deuterium-labeled carbon position. Since M_1 is calculated on spectra originating from perdeuteriated acyl chains, it provides a measure of the overall chain order parameter. Figure 2 displays the thermal dependence of M_1 and indicates that the first spectral moment of pure [*sn*-2-$^2H_{27}$]DMPA dispersions undergoes a sharp decrease on increasing the temperature above 50 °C. This order–disorder transition occurs at $T_c \simeq$ 48 °C. Interestingly, the spectrum at 45 °C shows axially symmetric character, indicating the presence of chain disorder even at the beginning of the phase transition.

In Figure 1 are also reported spectra of [*sn*-2-$^2H_{27}$]DMPA in the presence of short (right) and long (center) PLL. Spectra at low temperatures (5 °C) are wider in the presence of short and long PLL, as compared to spectra of the pure lipid. As the temperature is increased, spectra decrease in width at ca. 50 °C for short PLL/DMPA complexes and between 50 and 70 °C for long PLL/DMPA complexes. It is interesting to note that the spectrum of DMPA at 50 °C in the presence of short PLL results from the superimposition of two spectra, one of gel phase and the other of fluid phase. The thermal dependence of M_1 calculated from the above results is shown in Figure 2. As can be seen, the first spectral moments in the gel phase and in the presence of both long and short PLL are markedly higher than those of pure DMPA. As the temperature is increased, M_1 values for complexes of DMPA with long and short PLL remain slightly higher than those of pure lipid, and a sudden decrease in M_1 occurs at ca. 48 °C and ca. 62 °C in the presence of short and long PLL, respectively. In addition, the width of the transition appears broader (ca. 15 °C) in the presence of short PLL, whereas that of

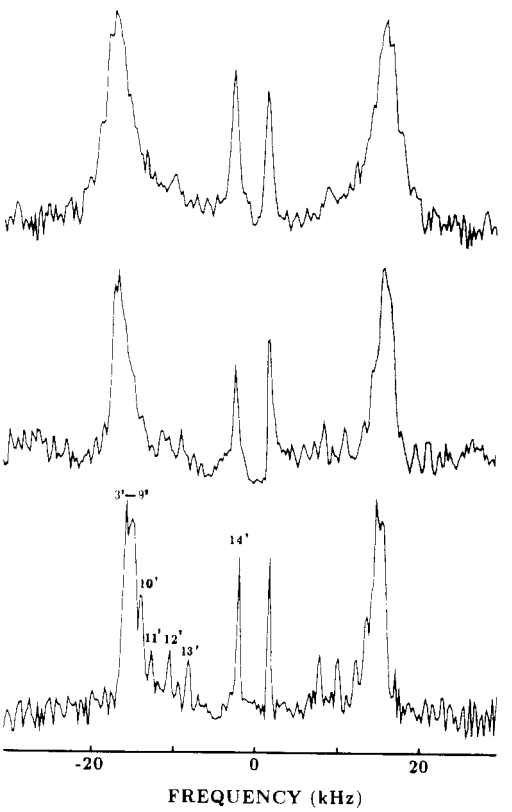

FIGURE 3: De-Paked [*sn*-2-$^2H_{27}$]DMPA spectra (90° orientations are shown) in the absence (bottom) and the presence of short (center) and long (top) PLL. Spectra are shown at T_c + 7 °C. Deconvolution is performed on ca. 1000 experimental points with five iterations.

DMPA/long PLL is as cooperative as that of pure DMPA. It is also interesting to note that M_1 in the fluid phase, in the presence of both types of PLL, remains higher than M_1 of DMPA spectra. This clearly indicates an ordering effect of poly(L-lysine) on the phospholipid acyl chains.

Orientational Order Parameters for Chain CH_2 and CH_3 Groups of DMPA with and without PLL. In the fluid phase, one can "de-Pake" the powder patterns, i.e., obtain oriented-like spectra on which the quadrupolar splittings can easily be measured (Bloom et al., 1981; Sternin et al., 1983). A de-Paked spectrum corresponding to bilayer normals oriented at 90° with respect to the magnetic field direction is shown in Figure 3 (bottom) for pure DMPA. One observes several doublets, which correspond to the different deuterium-labeled CH_2 and CH_3 groups of the DMPA *sn*-2 chain. Although no selectively labeled DMPA was available to assign unambiguously each splitting, a tentative attribution has been made on the basis of previously published results on DMPC and DPPC (Seelig, 1977; Davis, 1983; Dufourc et al., 1986). For instance, the central doublet, which corresponds to the area of three deuterons, can be assigned to the terminal C^2H_3. The larger splitting corresponds to the area of about 14 deuterons and may be assigned to labeled positions 3′ to 9′, i.e., the "plateau" positions. The de-Paked spectrum of [*sn*-2-$^2H_{27}$]-DMPA is almost superimposable on that of [*sn*-2-$^2H_{27}$]DMPC (Dufourc et al., 1984), thus affording the assignment of other quadrupolar splittings shown by numbers in Figure 3 (bottom) for the labeled carbon positions on the *sn*-2 chain. Labeled position 2′ on DMPC or DPPC exhibits two quadrupolar splittings (Seelig, 1977); herein, the corresponding splittings for DMPA cannot be reasonably assigned due to the relatively low signal-to-noise ratio of the de-Paked spectrum.

Figure 4 shows the variation of the quadrupolar splitting as a function of labeled carbon position, for DMPA at about

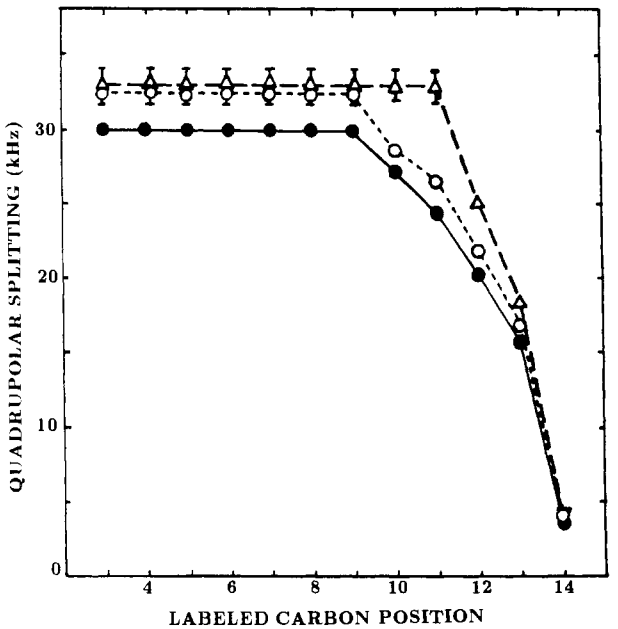

FIGURE 4: Variation of the quadrupolar splitting as a function of labeled carbon position on the *sn*-2-DMPA chain, in the absence (●) and the presence of short (○) and long (△) PLL. Quadrupolar splittings are reported at T_c + 7 °C for each system. Bars and symbols give an estimate of the error.

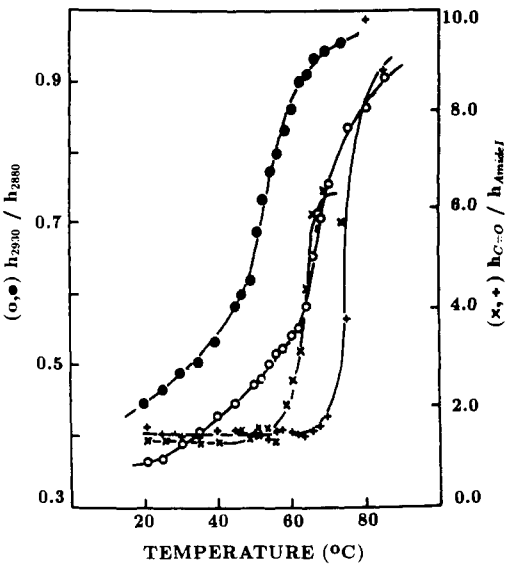

FIGURE 5: Temperature profiles of the h_{2930}/h_{2880} and $h_{C=O}/h_{amideI}$ peak intensity ratios derived from Raman spectra of short PLL bound to DMPA and DMPA. DMPA (●)–short PLL (×) mixture; DPPA (○)–short PLL (+) mixture.

T_c + 7 °C (55 °C). DMPA spectra in the presence of short (Figure 3, center) and long (Figure 3, top) PLL were also de-Paked. In order to compare systems in the same physical state, "de-Pake-ings" were performed at about the same temperature compared to T_c (i.e., T_c + 7 °C). From Figure 3 one can notice that spectra in the presence of both types of PLL are wider than the spectrum in their absence and that individual line widths are larger in the presence of polypeptides. As shown on Figure 4, this results in a marked increase of the quadrupolar splittings, especially for the plateau positions, due to the presence of both types of PLL. In addition, and only in the case of long PLL, one notices that there is an increase in the length of the plateau region, indicating that positions 10′ and 11′, which could be resolved as doublets for pure DMPA, merge into the unresolved doublet due to positions 3′–9′. This is reflected in Figure 4 by an extension of the plateau character in the presence of long PLL. Similar effects are also induced by short PLL but to a much lesser extent.

Correlation between Lipid Phase Transition and Peptide Conformational Change As Followed by Raman Spectroscopy. The thermotropic behavior of the DPPA/short PLL system was followed by measuring the Raman $h_{C=O}/h_{amideI}$ and h_{2930}/h_{2880} intensity ratios, which probe the peptide secondary structure (Laroche et al., 1988) and the lipid chain disorder, respectively (Gaber & Peticolas, 1977; O'Leary & Levin, 1984; Lafleur et al., 1987). As seen in Figure 5, the gel-to-fluid phase transition temperature of DPPA, which is ca. 62 °C in pure systems, is not markedly modified by the presence of short PLL, the transition being, however, slightly wider. On the other hand, the $h_{C=O}/h_{amideI}$ peak height intensity ratio indicates that short PLL drastically changes its conformation around 70 °C, that is, when the DPPA transition is completed. A similar effect occurs at approximately a 20 °C lower temperature in the case of DMPA/short PLL, as shown in the same figure and in agreement with an earlier study (Laroche et al., 1988); that is, short PLL undergoes a conformational transition when the DMPA transition is completed. Therefore, data of Figure 5 lead to the conclusion that the change in lipid physical state (i.e., gel to fluid) triggers the peptide conformational transition from β-sheet to random coil.

Discussion

Prior to the discussion of the results on DMPA/PLL systems, it is interesting to comment on the ^2H NMR data on pure DMPA, since this is the first time that this technique has been used to follow chain ordering of phosphatidic acid bilayers. The quadrupolar splitting dependence of labeled carbon positions is very similar to what has been found for other phospholipids studied (PC, PE, and PS; Seelig & Browning, 1978), i.e., a plateau of order parameters up to carbon 9 followed by a gradual decrease down to the terminal methyl group (Seelig, 1977). Therefore, it appears that this peculiar order parameter profile is independent of the nature of the lipid head group and reflects a characteristic of the bilayer core in saturated-chain phospholipid systems.

The binding of PLL to DMPA bilayers results in a significant increase of the lipid chain orientational order parameter in the fluid phase. When this parameter is compared at the same temperature relative to T_c, here T_c + 7 °C, both long and short PLL increase the quadrupolar splitting of the plateau positions to the same extent. This phenomenon can be accounted for by neutralization of negative charges of DMPA, which leads to a better packing of the lipid chains, i.e., to a reduction of conformational space for reorientation of methylene and methyl groups. Interestingly, labeled positions near the bilayer center are less affected by the presence of the polypeptide, indicating that the driving force comes from the head-group capping. As already mentioned, long PLL appears to be more efficient in capping than short PLL. This is felt for CH_2 positions near the bilayer center, which are markedly more ordered in the presence of long PLL than the corresponding ones in the presence of the short polypeptide. Therefore, the secondary structure of the bound polypeptide, β-sheet or random coil, seems to induce in the fluid phase small differences compared to the main organizing effect due to charge neutralization.

Such an increase in order has also been recently found for the very short polypeptide pentalysine on the head group of phosphatidylserine, where significant increases in quadrupolar splittings were also observed by Roux et al. (1989). Although

more qualitative, the conclusion of a general decrease of the chain motion was also proposed by de Kruijff et al. (1985), from ^{13}C NMR line broadening induced by PLL on cardiolipin.

One must also recall that the accurate conclusions proposed here from NMR agree with the results obtained by fluorescence anisotropy of the diphenylhexatriene probe with the same PLL in DPPG, which showed that both long and short PLL decreased the mobility of the probe in the lipid fluid phase, with, however, a weaker effect promoted by the short ones (Carrier et al., 1985; Houbre et al., 1988).

At this stage of the discussion it is also interesting to compare the effects of PLL with those of other polypeptides or proteins. One must recall that generally no severe effect on lipid order was detected with intrinsic membrane proteins; on the other hand, basic and amphipathic peptides change the order parameter of lecithins in their fluid phase. For example, melittin (Dufourc et al., 1986) and δ-hemolysin from *Staphylococcus aureus* (Dufourc et al., 1989) lower the chain quadrupolar splittings at high temperature relative to T_c. Near T_c, δ-hemolysin increases the ordering of the entire chain, while melittin affects only the chain segments near the bilayer center. This was interpreted as a penetration of both natural peptides into the bilayer core at high temperatures, while at $T \simeq T_c$, melittin is internalized in the membrane and δ-hemolysin is expelled but remains capping the polar head groups.

The present observations for PLL support the idea that peripheral proteins increase lipid ordering by head-group capping, whereas a hydrophobic contact, implying some penetration of proteins, disorders the lipid matrix, at least at high temperatures.

Although the above results demonstrate that short and long PLL induce rather similar changes in the DMPA fluid phase, the temperature of the gel-to-fluid phase transition of the complexes is quite different. This has to be paralleled by the different conformation of PLL bound to DMPA. The structure of short PLL changes from β-sheet to random coil when the system is heated, while the long polypeptide chain remains in the β-sheet structure (Laroche et al., 1988). Thus T_c of DMPA is not significantly affected when the bound peptide has no strongly preferred structure. On the other hand, the stable β-sheet structure adopted by long PLL bound to DMPA is able to induce a constraint on the lipids and to increase the stability of their regular packing, resulting in an increase of T_c and the ability to induce phase separation.

The last point to be addressed was to better know how the coupled structural changes of both short PLL and DMPA occur at T_c. The answer is provided herein by the Raman data shown in Figure 5, which demonstrate unambiguously that the conformational change of short PLL bound to DMPA and DPPA occurred when these lipids reached their fluid phase and therefore was triggered by the gel-to-fluid phase transition of the phospholipid chains. Thus it appears that the conformational disorder of the lipid molecules is able to destablize the ordered conformation of short PLL. In the gel phase the ordered network of negative charges stabilizes the ordered β-sheet structure of low molecular weight poly(L-lysine), which is poorly structured in solution (G. Laroche and M. Pézolet, unpublished data).

Going further to speculate about the structure of PLL bound to PA, one can follow the already proposed models (de Kruijff et al., 1985; Carrier & Pézolet, 1986; Laroche et al., 1988). If PLL in β-sheet is extended with the charged groups connecting alternatively two adjacent bilayers through interactions with phosphate groups, then one must also take into account the effect of the polypeptide length on the stability of the sheet structure. A 25 amino acid long sheet is not very stable and can be formed either by folding a single chain, which indeed results in a rather short intramolecular sheet, or, alternatively, by pairing different short PLL molecules together, which will allow longer and thus more stable β-sheet structure. In the case of this second hypothesis, the bound polypeptide structure would be better fitted on a regular interface of fixed lipid molecules in their gel phase, while in an L_α phase, lateral diffusion would probably decrease the stability of contacts between different PLL molecules. Indeed, for very long PLL, the β-sheets can be intramolecular and are stronger, and they remain stable even in the presence of fluctuating lipid charges.

This important result showing that the conformation of a phospholipid-bound peptide is modulated by the physical state of the lipid is one of the first direct observations and strengthens the conclusions of several studies demonstrating that membrane lipids are necessary to maintain the structural stability and functionality of a number of membrane-associated enzymes and transport proteins (Sandermann, 1978; McElhaney, 1982). The fact that the conformation of a phospholipid-bound peptide is modulated by the physical state of the lipid, as it is clearly demonstrated in this report, was also observed very recently by Cornell et al. (1989) for the insertion of signal peptides in phospholipids. They have shown that the conformation of the signal peptide of LamB protein from *E. coli* bound to phospholipid monolayers is modulated by the lateral pressure of the film. At low initial pressure, part of the peptide penetrates the lipid film and adopts an α-helical conformation while the rest of the peptide is in the β-sheet structure, flat on the film. At high pressure, the peptide does not penetrate the film and is coplanar with the lipid film, adopting almost entirely the β-sheet conformation.

More results have, however, been published on the stabilization of proteins by phospholipids. For example, Pézolet et al. (1982) have shown by Raman spectroscopy that the lytic protein cardiotoxin from snake venom, which undergoes a thermal unfolding at 70 °C in aqueous solution, remains in an ordered conformation well over this temperature when it is bound to DMPA. On the other hand, Yu et al. (1985) and Rigell et al. (1985) have shown by calorimetry that phospholipids stabilize cytochrome *c* oxidase in its native and functional configuration. Moreover, Akrem et al. (1982) have determined that the presence of sufficient quantities of phospholipids increases the temperature of the thermal unfolding of cytochrome P-450. Therefore, it appears that the conformational thermal stability of proteins is higher when they are bound to phospholipids.

In summary, we have shown that the structure of complexes of DMPA and polylysine is governed by electrostatic interactions between the charged polypeptide and the anionic phospholipid. Both long and short PLL increase the order of the lipid acyl chains by neutralizing the lipid negative charges. However, in the fluid phase, this ordering effect is more important for DMPA/long PLL complexes, where the polypeptide adopts a stable β-sheet conformation over the whole range of temperatures investigated, constraining the lipids to fit the charged network of the polypeptide. Conversely, short PLL undergoes a change of conformation from β-sheet to random coil at the gel-to-fluid transition of the lipids, showing that the lipid constraints modulate the state of the bound peptide.

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