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Study of the Effect of Poly(L-lysine) on Phosphatidic Acid and Phosphatidylcholine/Phosphatidic Acid Bilayers by Raman Spectroscopy†

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ABSTRACT: The effect of polylysine (PLL) on dimyristoylphosphatidic acid (DMPA), on dimyristoylphosphatidylcholine (DMPC), and on mixtures of these lipids was investigated by Raman spectroscopy. These results show that long polylysine ($M_r \approx 200\,000$) increases the stability of the acyl chain matrix of DMPA to form a more closely packed structure with a stoichiometry of one lysine residue per PA molecule. On the other hand, short PLL (M_r 4000) destabilizes the PA bilayer, and the complex formed undergoes a gel to liquid-crystalline transition at a lower temperature than of the pure lipid. For both cases, we have observed that bound polylysine adopts a β-sheet conformation as opposed to the α-helical structure previously found for dipalmitoylphosphatidylglycerol/long PLL complexes [Carrier, D., & Pézolet, M. (1984) *Biophys. J. 46*, 497-506]. The difference in the thermal behavior of complexes of DMPA with long and short polylysines is believed to be associated with the fact that in the complex the long polypeptide adopts the β-sheet conformation over the whole range of temperatures investigated while the short one undergoes a change of conformation from β-sheet of random coil upon heating. Therefore, the conformation of the lipid-bound polypeptides depends on the nature of the polar head group of the lipid, not only on its net charge, and it affects considerably the thermotropism of the lipid. On the other hand, both long and short polylysines show no affinity for phosphatidylcholine since the temperature profiles of DMPC and of DMPC/PLL complexes exhibit exactly the same behavior. When mixed with PA/PC mixtures, long PLL induces a partial lateral phase separation. One phase consists mainly of DMPC but contains some DMPA, and the second phase is richer in the PA component and is perturbed by PLL. On the contrary, short polylysine does not induce phase separation as both lipids of the mixture exhibit the same thermotropic behavior.

The miscibility of membrane lipids and the occurrence of lateral phase separation are of prime interest in the study of membrane structure and function. The organization of membrane lipids in domains has been detected in almost all bacteria and cells of complex organisms, with the exception of erythrocytes (Karnovsky et al., 1982). It has even been observed in the viral membrane of the influenza mixovirus (Bukrinskaya et al., 1987). This heterogeneity seems to have a functional significance. For example, one of the first requirements for the fusion between membranes is believed to be the presence of specific local domains (Portis et al., 1979; Scheule, 1987).

Lateral phase separation in a membrane can be induced by external agents like multivalent cations, among which calcium has been extensively studied (Onishi & Ito, 1974; Jacobson & Papahadjopoulos, 1975; Galla & Sackmann, 1975a,b; Van Dijck et al., 1978; Hui et al., 1983; Silvius & Gagné, 1984a,b; Kouaouci et al., 1985). Polycationic species can also be very effective, and one of the most studied is poly(L-lysine) (PLL),[1] a polypeptide often taken as a model of extrinsic protein. PLL-induced phase separation was first observed in dipalmitoylphosphatidic acid bilayers, by electron spin resonance and fluorescence spectroscopy (Galla & Sackmann, 1975a,b; Hartmann & Galla, 1978; Hartmann et al., 1977). The formation of domains of bound phosphatidic acid within the pure phospholipid, or in mixtures with dioleoylphosphatidylcholine, has also been proved by electron microscopy (Hartmann et al., 1977). Later, PLL was shown to induce a very peculiar behavior in dipalmitoylphosphatidylglycerol bilayers, where three types of domains were observed for a lipid to lysyl residue ratio greater than 1 (Carrier & Pézolet, 1985). A fundamental finding of the same study is that the degree of polymerization of the peptide is very important when dealing with the structure and thermotropism of phosphatidylglycerol/PLL complexes. In fact, long polylysine (M_r >150 000) causes a shift of the gel to liquid-crystalline tran-

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[1] Abbreviations: DMPA, dimyristoylphosphatidic acid; DMPC, dimyristoylphosphatidylcholine; DMPC-d_{54}, DMPC with perdeuteriated acyl chains; DMPG, dimyristoylphosphatidylglycerol; DPPG, dipalmitoylphosphatidylglycerol; EDTA, ethylenediaminetetraacetic acid; PLL, poly(L-lysine); R_i, phospholipid to lysine residue incubation molar ratio.

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sition from 40 °C for pure DPPG to 45 °C for a lipid to peptide molar ratio of 1, while for short polylysine (M_r 4000), the transition of the complex occurs at 37 °C. This difference between the stability of the two complexes was attributed to the fact that bound long PLL adopts the α-helical conformation in the complex while short polylysine remains unordered.

In this study, our aim is to determine if short and long poly(L-lysines) induce phase separation in binary mixtures of a negatively charged lipid, dimyristoylphosphatidic acid (DMPA), with a zwitterionic lipid, dimyristoylphosphatidylcholine (DMPC). We will then focus on the characterization of the structure of these complexes and on the conformation of the bound peptide.

Vibrational spectroscopy is well-suited to study biological membranes since it provides direct information on the conformation of the membrane components (Mushayakarara & Levin, 1984; Knoll, 1986; Pézolet et al., 1982). Full deuteriation of acyl chains allows an elegant and efficient investigation of multicomponent systems. For binary mixtures of phospholipids with normal and deuteriated acyl chains, it is possible to monitor simultaneously and independently the conformation of each lipid by looking at the C–H and C–D stretching mode regions. The usefulness of this technique has been put forward in several studies (Mendelsohn & Tarashi, 1978; Mendelsohn & Maïsano, 1978; Kouaouci & Pézolet, 1985).

Materials and Methods

Materials. The disodium salt of dimyristoylphosphatidic acid (DMPA) and dimyristoylphosphatidylcholine with perdeuteriated chains (DMPC-d_{54}) were obtained from Avanti Polar Lipids. The bromide salts of PLL of molecular weight 4000 (short PLL) and 180 000 or 260 000 (long PLL) were purchased from Sigma (St. Louis, MO). All materials were used without further purification.

Raman Measurements. Binary phospholipid mixtures were prepared by dissolving appropriate amounts of each component in chloroform/methanol (87:3 v/v) at 50–60 °C to ensure complete dissolution of the solids. Afterward, the solvent was evaporated under a stream of nitrogen, and final traces of solvent were removed by pumping at least 12 h over the samples in a vacuum desiccator.

Dispersions of pure lipids or of DMPA and DMPC-d_{54} mixtures were prepared by mixing appropriate amounts of solids in a solution 150 mM NaCl/10 mM EDTA, pH 6.5. Samples (10% weight in total lipid) were then heated to approximately 65 °C for 10 min, agitated with a vortex mixer, and cooled down below the gel to liquid-crystalline phase transition. This cycle was repeated at least 3 times. The pH of the dispersions was measured with a microelectrode (Microelectrodes Inc.) and adjusted to pH 6.5, if necessary. Samples were then transferred in glass capillary tubes and centrifuged in a hematocrit centrifuge. Spectra were obtained from the white precipitate. Samples containing polylysine were prepared similarly except that the lipid concentration was 1%. Appropriate amounts of 3% PLL were added to the dispersions, and the incubation cycle was repeated once again. The PA to lysine residue incubation molar ratio (R_i) was 1 for pure phospholipids and 0.33 for lipid mixtures in order to have an excess of PLL.

Raman spectra were recorded with a computerized Spex Model 1400 double monochromator (Savoie et al., 1979) with spectral resolution of 5 cm^{-1}. The monochromator was calibrated periodically with a neon discharge lamp, and the frequencies cited below are believed to be accurate to ±2 cm^{-1} for sharp peaks. Samples were excited with the 514.5-nm line of a Spectra Physics Model 2020 argon ion laser; the laser power at the sample was approximately 150 mW. Capillaries containing the samples were placed in a thermoelectrically regulated sample holder (Pézolet et al., 1983) whose temperature was monitored at ±0.2 °C with a copper–constantan thermocouple. Spectra were recorded digitally with an integrating period of 2 s. For the C–D stretching mode region (1950–2350 cm^{-1}), the increment was 1 cm^{-1}, and spectra were seven-point-smoothed by using the algorithm of Savitsky and Golay (1964). For the C–H stretching mode region, the increment was 2 cm^{-1}, and, if needed, spectra were corrected for the choline contribution at 2962 cm^{-1} (choline CH_3 symmetric stretching vibration) using the 3037 cm^{-1} band (choline CH_3 asymmetric stretching vibration) as an internal intensity standard.

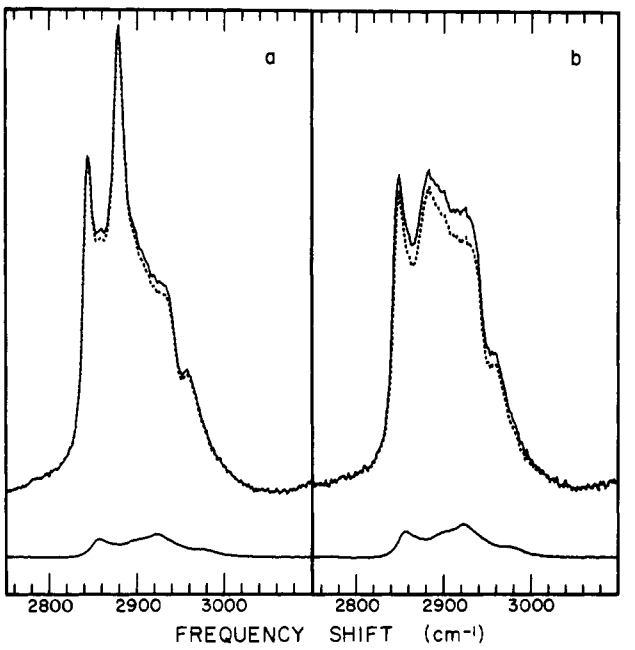

FIGURE 1: Spectral contribution of PLL in the C–H stretching mode region. Raman spectra of DMPA/long PLL complexes at R_i = 1 (one scan of 2 s/2 cm^{-1}) before (solid line) and after (dotted line) correction for the PLL spectral contribution (shown below the lipid spectra) in (a) the gel and (b) the liquid-crystalline states.

For the DMPA/PLL complexes, the polypeptide contribution in the C–H region was subtracted by using the amide I band of PLL as an internal intensity standard. For complexes of DMPA with long polylysine, the spectrum of PLL in the β-sheet conformation was used to correct the spectra of the complexes since the conformation of the bound polypeptide is β-sheet and is not temperature dependent (Figure 4a). An example of such a correction is given in Figure 1 for the lipid in the gel and in the liquid-crystalline states. As can be seen, the spectral contribution of polylysine is approximately 6% of the total integrated intensity of the C–H stretching mode region, the strongest band of PLL being at 2930 cm^{-1}. For complexes of DMPA with short polylysine, the correction was not as straightforward since the conformation of the bound polypeptide is temperature dependent (see Figure 4b,c). The following correction procedure was used. Since the conformation of bound short PLL is mainly β-sheet below 55 °C (Figure 8), the spectrum of β-sheet polylysine was used to correct the spectra of the complexes, while above 55 °C, spectra were corrected with the spectrum of PLL in the random-coil conformation. This procedure, although quite adequate, introduces some error between 50 and 60 °C since the conformation of the bound polypeptide is partly β-sheet and

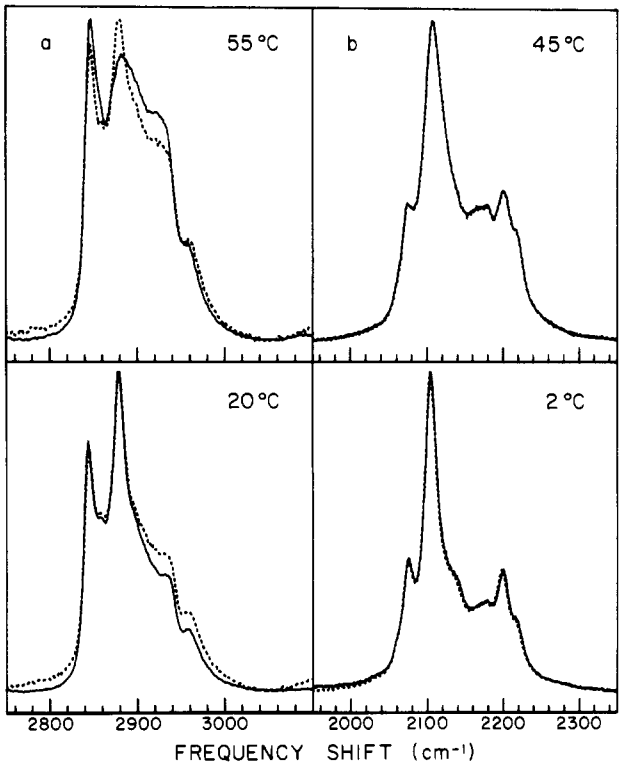

FIGURE 2: Raman spectra of dispersions of (a) DMPA (one scan at 2 s/2 cm^{-1}) and (b) DMPC-d_{54} (one scan at 2 s/1 cm^{-1}) in the absence (solid line) and in the presence (dotted line) of long polylysine (R_i = 1).

random coil in this temperature range. Subtractions done on the spectrum of the complex at 55 °C using PLL in either the β-sheet or the random-coil conformation show that the error introduced on the h_{2880}/h_{2850} and h_{2930}/h_{2880} ratios is less than 4%. Therefore, we believe that none of the effects observed for long or short polylysine is due to the spectral contribution of the polypeptides. For binary mixtures, this correction was not done because of the poor signal to noise ratio in the amide I region. Spectra were corrected for the water contribution by subtracting an appropriate polynomial function.

Small-Angle X-ray Measurements. Samples were prepared as for Raman measurements and were transferred into X-ray thin-wall glass capillary tubes; capillaries were then centrifuged and sealed. X-ray measurements were performed with a Warhus–Statton camera, using the nickel-filtered Cu Kα line (λ = 0.154 nm). The photographic plates were placed at 32 cm from the sample in an evacuated enclosure. Exposure times were 5–7 days.

RESULTS

Raman Spectra of Pure Lipid Components. For the study of the lipid conformation and thermotropism, the signals arising in the C–H (2750–3100 cm^{-1}) and C–D (1950–2350 cm^{-1}) stretching mode regions are the most interesting. Figure 2a shows the C–H stretching mode region of the Raman spectrum of a DMPA multilamellar dispersion (continuous line). One can see that below the phase transition of the lipid (49 °C), two bands clearly emerge from the broad background at 2850 and 2880 cm^{-1}, corresponding to the symmetric and antisymmetric C–H stretching vibrations of the methylenes of the acyl chains, respectively (Gaber & Peticolas, 1977). The two weaker bands at 2930 and 2954 cm^{-1} are due to the symmetric and asymmetric C–H stretching modes of the terminal methyl groups (Spiker & Levin, 1975).

As can be seen from Figure 2, heating the sample above the phase transition temperature causes an increase of the intensity of the 2930 cm^{-1} band. This change results in part from an underlying infrared-active methylene antisymmetric stretching mode that becomes Raman-active when the chain symmetry is lowered (Bunow & Levin, 1977) and also from a change in the Fermi resonance interaction between the symmetric C–H stretching mode of the terminal methyl groups and the first overtone of the asymmetric CH_2 bending mode (Hill & Levin, 1979). Molecular disordering also affects the 2880 cm^{-1} feature considerably. When the temperature is increased, this band shifts to higher frequency, broadens, and decreases in intensity. This latter change is partially due to the lowering of the intensity of the broad underlying background, which arises from inter- and intramolecular Fermi resonance interactions between the methylene symmetric C–H stretching mode and the different binary combinations of the methylene bending fundamental (Snyder et al., 1978; Snyder & Scherer, 1979).

It has previously been shown that the h_{2880}/h_{2850} and h_{2930}/h_{2880} intensity ratios are well-suited to study the thermotropic behavior of phospholipid bilayers (Faucon et al., 1983; Taraschi & Mendelsohn, 1980; Gaber & Peticolas, 1977; O'Leary & Levin, 1984). These two ratios monitor essentially the overall disorder of the lipid acyl chain matrix, but the h_{2880}/h_{2850} ratio is also sensitive to the intermolecular vibrational coupling and the lateral packing of the acyl chains (Carrier & Pézolet, 1984).

The C–D stretching mode region of a DMPC-d_{54} multilamellar dispersion (continuous line) is shown in Figure 2b. The main feature of this region is the 2103 cm^{-1} band assigned to the CD_2 symmetric stretching mode (Mendelsohn et al., 1976). Weaker bands due to the CD_2 antisymmetric stretch at 2177 cm^{-1} and to CD_3 vibrations, at 2076, 2125, and 2213 cm^{-1}, are also observed (Bryant et al., 1982).

When a dispersion of phospholipids with fully deuteriated acyl chains undergoes the gel to liquid-crystalline phase transition, the width of the 2103 cm^{-1} band increases markedly. It has been shown by Mendelsohn and Koch (1980) that the half-width, measured from a straight base line between 2060 and 2240 cm^{-1}, monitors essentially the formation of gauche bonds along the acyl chains. Later, Kouaouci et al. (1985) have measured the bandwidth of the 2103 cm^{-1} feature from a straight base line between 1950 and 2350 cm^{-1}. This width was determined at different heights according to the algorithm of Cameron et al. (1982), and it was then found that the bandwidth at 65% of the peak height intensity ($\Delta\nu_{0.65}$) gives very reproducible results. On the other hand, their experiments on perdeuteriated hexadecane dissolved in normal hexadecane have demonstrated that $\Delta\nu_{0.65}$ is insensitive to intermolecular chain interactions throughout the whole range of concentrations and monitors mainly the gauche rotamer population.

Effect of Long Polylysine on Pure DMPA and DMPC-d_{54}. As seen in Figure 2a, the spectral changes induced by long PLL (dotted line) are quite different whether DMPA is in the gel or liquid-crystalline state. For the gel phase, the h_{2880}/h_{2850} and h_{2930}/h_{2880} ratios are approximately the same for the pure lipid and the complex, which indicates that the order within the bilayer is not affected by PLL. On the other hand, the binding of long PLL to the polar head group of DMPA increases the stability of the acyl chain matrix of the lipid. In fact, both intensity ratios indicate that at 55 °C pure DMPA is in the liquid-crystalline phase, while the acyl chains of the lipid in the complex with long PLL are still quite ordered.

As opposed to DMPA, DMPC-d_{54} does not show any affinity toward polylysine (Figure 2b, dotted line). For both the gel and liquid-crystalline states, the spectra of pure DMPC-d_{54}

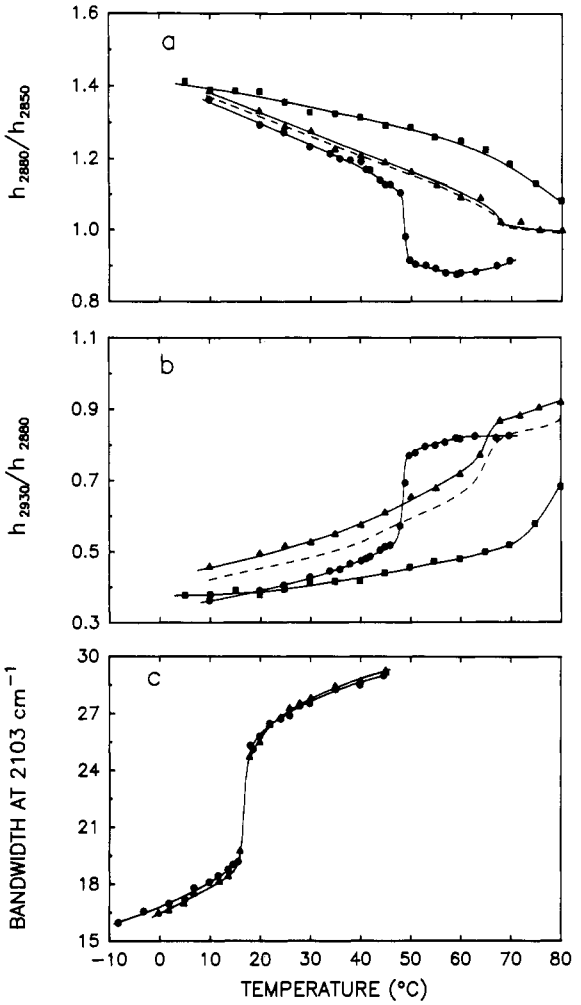

FIGURE 3: (a and b) Temperature profiles of DMPA in the absence (●) and in the presence (▲) of long PLL (R_i = 1) or of calcium ions (■) [reproduced from Kouaouci et al. (1985)] derived from the Raman h_{2930}/h_{2880} and h_{2880}/h_{2850} peak height intensity ratios. The dashed curve is after correction for the PLL spectral contribution. (c) Temperature profiles of DMPC-d_{54} in the absence (●) and in the presence (▲) of long PLL (R_i = 1) derived from the bandwidth at 65% of the height of the 2103 cm^{-1} Raman band.

and of a complex of DMPC-d_{54} and long PLL at R_i = 1 (Figure 2b) are identical, suggesting that the polypeptide does not interact with the lipid.

The effect of PLL on the thermotropism of DMPA and DMPC-d_{54} was also examined through the temperature dependence of the h_{2880}/h_{2850} and h_{2930}/h_{2880} ratios for DMPA, and from the $\Delta\nu_{0.65}$ of the 2103 cm^{-1} band for DMPC-d_{54}. These results are shown in Figure 3.

For pure DMPA at pH 6.5, the highly cooperative gel to liquid-crystalline phase transition occurs at 49 °C which is in very good agreement with the values of 49 and 51 °C determined by calorimetry (Graham et al., 1985) and Raman spectroscopy (Mushayakarara & Levin, 1984), respectively. When long PLL is bound to DMPA (R_i = 1), the transition is shifted toward higher temperatures and is considerably broader.

Below the transition, the h_{2930}/h_{2880} ratio is slightly higher for the complex than for the pure lipid. We can thus conclude that long PLL increases somewhat the disorder of the acyl chains of DMPA below its normal transition temperature. On the other hand, above the phase transition of pure DMPA, both the h_{2880}/h_{2850} and h_{2930}/h_{2880} ratios show that long polylysine affects considerably the packing as well as the disorder of the lipid acyl chains.

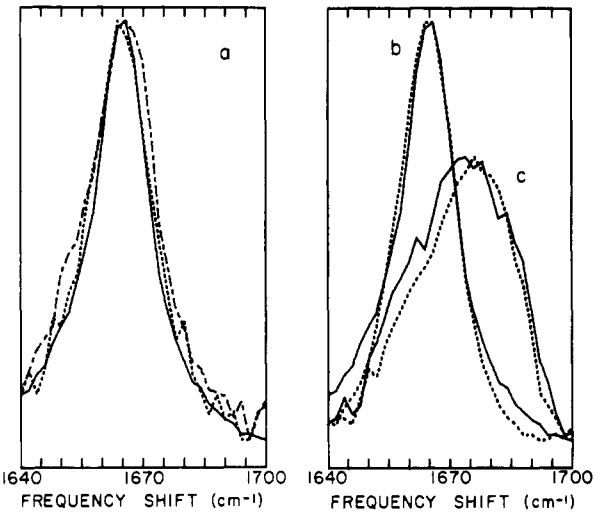

FIGURE 4: (a) Raman spectra of the amide I region of polylysine in the β-sheet conformation (solid line) at pH 11.5 and 60 °C (four scans at 2 s/2 cm^{-1}); DMPA/long PLL complex at R_i = 1 at pH 6.5 and 20 °C (dotted line) and at 70 °C (dashed dotted line) (10 scans at 2 s/2 cm^{-1}). (b and c) Raman spectra of the amide I region of polylysine in the β-sheet (b, solid line) and in the random-coil (c, solid line) conformation (4 scans at 2 s/2 cm^{-1}); DMPA/short PLL complex with R_i = 1 at 20 °C (b, doted line) and at 70 °C (c, dotted line) (25 scans at 2 s/2 cm^{-1}). These spectra were corrected for the water spectral contribution.

The results of Kouaouci et al. (1985) for DMPA/Ca^{2+} complexes are also reproduced on Figure 3. On the basis of their Raman results and the calorimetric data of Graham et al. (1985), they concluded that DMPA/Ca^{2+} forms cochleates (Liao & Prestegard, 1981; Van Dijck et al., 1978) that do not exhibit any cooperative thermotropic transition between 5 and 100 °C. As the profile for the DMPA/PLL complex is very smooth, the possibility of a cochleate phase cannot be ruled out. However, the complexes formed between long polylysine and DMPA seem to be less stable than those of Ca^{2+} and DMPA because for the former the acyl chains of the lipid are completely melted at 68 °C. Moreover, the h_{2880}/h_{2850} ratio indicates that, over the entire range of investigated temperatures, the acyl chains in DMPA/Ca^{2+} complexes are more closely packed than for the DMPA/long PLL system.

Small-angle X-ray measurements also reveal significant differences between complexes of DMPA with long PLL and Ca^{2+}. The lamellar repeat distance is 5.95 nm for an equimolar DMPA/long PLL complex while it is only 4.85 nm for the cochleate phase formed by DMPA and calcium ions.

In order to obtain more information on the structure of DMPA/long PLL complexes, we have also studied the conformation adopted by the bound polypeptide using the amide I spectral region. Carrier and Pézolet (1984) have demonstrated that long polylysine adopts the α-helical conformation when it is bound to DPPG bilayers. Because the polar head groups of DPPG and DMPA are both negatively charged, one would expect PLL to adopt the same conformation in the DMPA/PLL system. Figure 4a illustrates the amide I region of the DMPA/long PLL R_i = 1 complex. Surprisingly, this spectrum clearly reveals that bound polylysine adopts the β-sheet structure instead of the α-helical conformation over the whole range of temperatures investigated. These results provide strong evidence that the structure of polar head groups can affect considerably the conformation of bound polypeptides when they are bound to phospholipids.

In order to further characterize the system, we have determined the stoichiometry of DMPA/long PLL complexes by recording their spectra in the C–H stretching region at 55

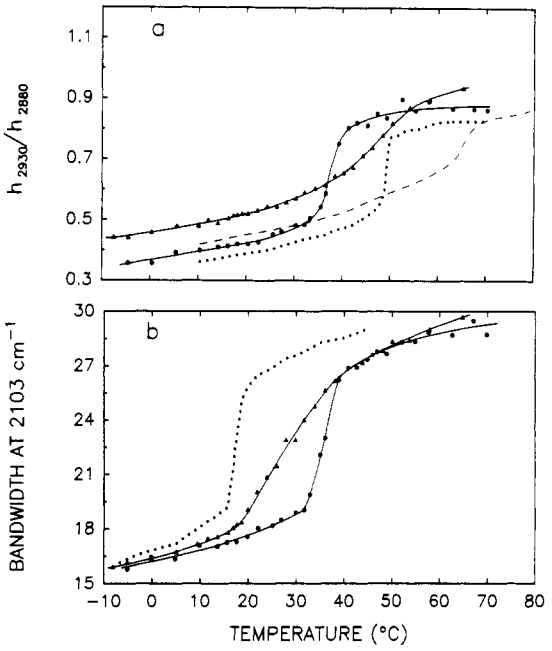

FIGURE 5: (a) Temperature profiles of DMPA derived from the Raman spectral h_{2930}/h_{2880} peak height intensity ratio. Equimolar mixture of DMPA/DMPC-d_{54} in the absence (●) and in the presence of (▲) of an excess of long PLL. Temperature profiles of pure DMPA (dotted line) and of DMPA/long PLL complex at $R_i = 1$ (dashed line) are also shown. (b) Temperature profiles of DMPC-d_{54} derived from the bandwidth at 65% of the height of the 2103 cm^{-1} Raman band. Equimolar mixture of DMPA/DMPC-d_{54} in the absence (●) and in the presence (▲) of an excess of long PLL. The temperature profile of pure DMPC-d_{54} (dotted line) is also shown.

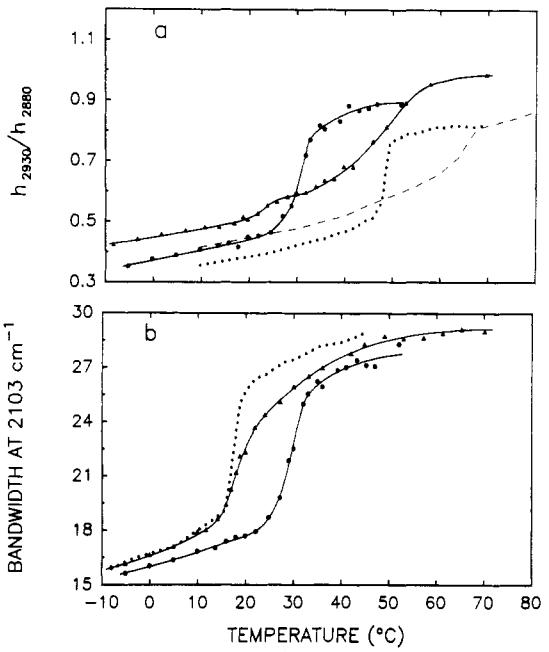

FIGURE 6: (a) Temperature profiles of DMPA derived from the Raman spectral h_{2930}/h_{2880} peak height intensity ratio. DMPA/DMPC-d_{54} 1:2 in the absence (●) and in the presence (▲) of an excess of long PLL. Temperature profiles of pure DMPA (dotted line) and of DMPA/long PLL complex with $R_i = 1$ (dashed line) are also shown. (b) Temperature profiles of DMPC-d_{54} derived from the bandwidth at 65% of the height of the 2103 cm^{-1} Raman band. DMPA/DMPC-d_{54} in the absence (●) and in the presence (▲) of an excess of long PLL. The temperature profile of pure DMPC-d_{54} (dotted line) is also shown.

°C (e.g., between the transition temperature of pure DMPA and that of the complex) for several R_i values. The curve of the h_{2880}/h_{2850} peak height intensity ratio as a function of $1/R_i$ (data unshown) reaches a plateau at $1/R_i = 1$, indicating that the stoichiometry of the complex is one lysine residue for each phosphatidic acid molecule.

For DMPC-d_{54}, the thermotropic behavior presented in Figure 3c confirms the conclusion obtained from the spectra of Figure 2. The temperature profiles for pure DMPC-d_{54} and the DMPC-d_{54}/long PLL complex at $R_i = 1$ are identical, and no Raman signal in the amide I region has been obtained for the polypeptide (results not shown), showing that long PLL does not interact with DMPC-d_{54} and that the presence of charges is necessary for the binding of the polypeptide.

Effect of Long Polylysine on a 1:1 DMPA/DMPC-d_{54} Mixture. Figure 5 shows the temperature profiles of an equimolar mixture of DMPA/DMPC-d_{54} in the absence and in the presence of an excess of polylysine (three lysine residues per phosphatidic acid molecules). On the basis of these results, we can conclude that the two lipids are completely miscible at equimolar concentration since only one transition is observed on the temperature profiles derived from either the DMPC-d_{54} or the DMPA component of the mixture; moreover, the transition midpoint temperature is close to the average between the transition temperatures of pure DMPA and DMPC-d_{54}. When an excess of long PLL is added to the mixture, the profile of the DMPA transition (Figure 5a) looks like that of the DMPA/PLL complex (Figure 3b) except that the transition appears at a lower temperature. Yet, the transition temperature is higher than that of lipid mixture without polylysine. We can thus conclude that the C–H stretching region monitors the presence of a phase containing a mixture of DMPA and DMPC, this mixture being bound to long PLL. Spectra of the amide I region (results not shown) of polylysine bound to the equimolar PA/PC mixture also indicate that the polypeptide adopts the β-sheet conformation as for pure DMPA/PLL complexes.

The thermotropic behavior of the DMPC-d_{54} component of the mixture (Figure 5b) differs from that of the DMPA component in the presence of PLL. The transition also becomes broader, but it shifts to lower temperature, that is, toward the transition temperature of pure DMPC-d_{54}. However, it is higher than the transition temperature of the pure lipid. Therefore, these results suggest that only a partial lateral phase separation has occurred with a phase rich in DMPC-d_{54} and a phase rich in DMPA to which PLL is bound.

If there is a partial phase separation of the lipids, one might ask why the profiles derived from both the C–H and the C–D regions do not exhibit two transitions. In fact, it is possible that two transitions are present on the temperature profiles but their midpoint temperatures are too close to be well resolved. In addition, the fact that the transition of the DMPA-rich phase is very broad makes biphasic transitions hard to distinguish.

Effect of Long Polylysine on a 1:2 DMPA/DMPC-d_{54} Mixture. In order to support the conclusions obtained with the equimolar DMPA/DMPC-d_{54} mixture, we have studied a 1:2 DMPA/DMPC-d_{54} mixture. The results obtained are shown in Figure 6.

Even at this molar ratio, the lipids appear to be completely miscible. The transition temperature determined from both the DMPA and the DMPC-d_{54} component is 29 °C, which is closer to the transition temperature of pure DMPC-d_{54} since the mixture is richer in this lipid.

A close examination of the DMPA temperature profile for the lipid mixture with long PLL allows the detection of two transitions: the first one, shallow, around 20 °C, most likely pertains to the DMPC-d_{54}-rich phase, and its occurrence in

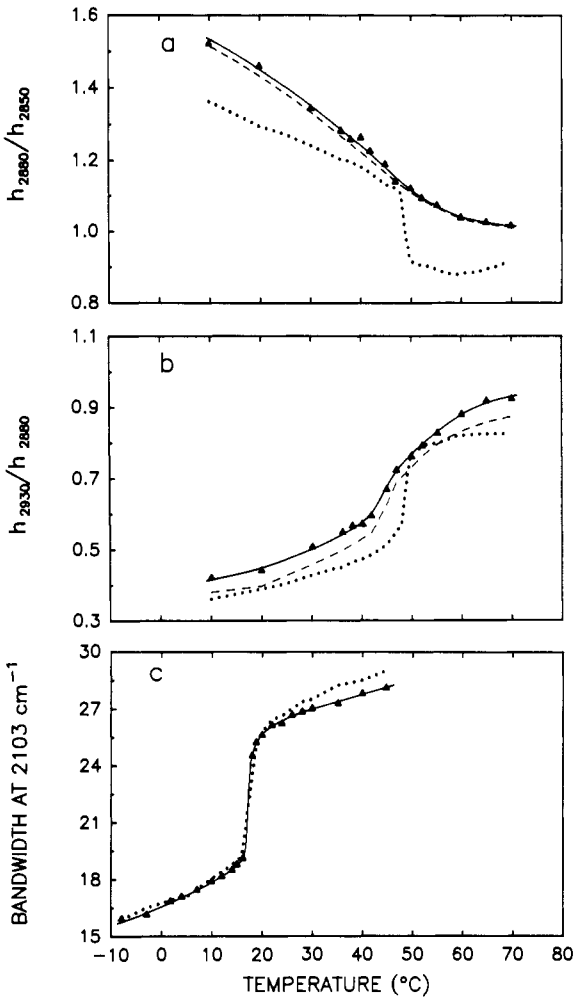

FIGURE 7: (a and b) Temperature profiles of DMPA derived from the Raman spectral h_{2930}/h_{2880} and h_{2880}/h_{2850} peak height intensity ratios. DMPA in the absence (dotted line) and in the presence (▲) of short PLL (R_i = 1). The dashed curve is after correction for the PLL contribution. (c) Temperature profiles of DMPC-d_{54} derived from the bandwidth at 65% of the height of the 2103 cm^{-1} Raman band. DMPC-d_{54} in the absence (dotted line) and in the presence (▲) of short PLL (R_i = 1).

the C–H stretching region profile demonstrates that this phase contains some DMPA. The main transition, appearing at higher temperature, is broad and occurs at lower temperature than for the equimolar mixture (Figure 5a), indicating that it is richer in DMPC-d_{54}. The melting curve obtained from the deuteriated lipid also shows two transitions, at approximately the same temperatures as those obtained from the spectra of the DMPA component. In this case, the two transitions have about the same amplitude. The first one should correspond to domains composed mainly of pure DMPC-d_{54} since its midpoint is very close to the pure DMPC-d_{54} transition temperature. The broader transition observed at higher temperature must be due to a phase containing DMPA and DMPC in more comparable concentrations. Clearly, this latter phase is more strongly perturbed by polylysine.

Effect of Short Polylysine on Pure DMPA and DMPC-d_{54}. Figure 7 illustrates the thermotropic behavior of DMPA and DMPC-d_{54} in the absence and in the presence of short polylysine. While long PLL shifts the PA transition toward higher temperatures, short PLL displaces the transition in the opposite direction. In view of this dramatic difference in the effect of short and long PLL, we have also examined the amide I band for short polylysine bound to DMPA; spectra are presented

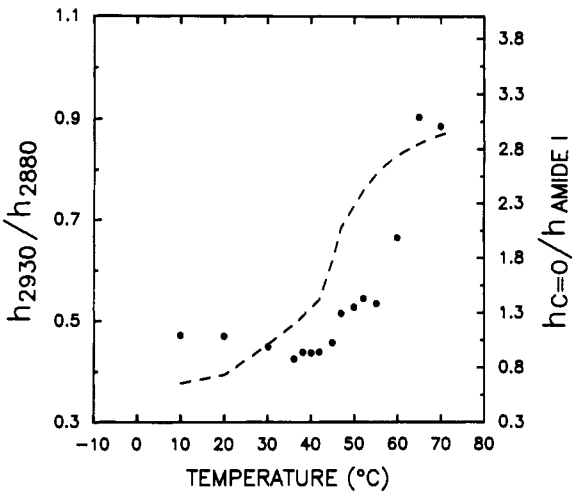

FIGURE 8: Temperature profiles of DMPA (dashed line) and of short PLL (●) in the DMPA/short PLL complexes (R_i = 1) derived from the h_{2930}/h_{2880} and $h_{C=O}/h_{amide\ I}$ peak height intensity ratios, respectively.

in Figure 4b,c. One can see that at 20 °C the amide I band for the complex is almost superimposable with the amide I band of polylysine in the β-sheet conformation but at 70 °C PLL adopts the random-coil conformation. In order to follow this change of conformation, we have measured the ratio of the peak height intensity of the lipid carbonyl band at 1740 cm^{-1} relative to that of the amide I band of PLL. The former band is not very sensitive to temperature while the height of the amide I band decreases as the polypeptide undergoes the β-sheet to random-coil transition since the band is much sharper for the β-sheet conformation (Carrier & Pézolet, 1984). The effect of temperature on the $h_{C=O}/h_{amide\ I}$ and h_{2930}/h_{2880} ratios is shown in Figure 8. Although there is a fair amount of scattering in the data points for the $h_{C=O}/h_{amide\ I}$ ratio, due to the lower signal-to-noise ratio in the amide I region, results of Figure 8 show that the break around 45 °C on the temperature profile of DMPA/short PLL complex nearly coincides with the onset of the conformational transition of PLL. Therefore, the contrasting thermotropic behavior of complexes of DMPA with long and short PLL most likely results from the change of conformation of short PLL in the complex when the temperature is increased. These results support the conclusion of Carrier and Pézolet (1986) that the thermotropic behavior of complexes of PLL with charged lipids is highly dependent on the conformation of the polypeptide.

Effect of Short Polylysine on a 1:1 DMPA/DMPC-d_{54} Mixture. For the complex of an equimolar mixture of DMPA and DMPC-d_{54} with short polylysine, the temperature profiles derived both from the C–D and from the C–H stretching regions (Figure 9) are very similar, and their midpoint temperature is 30 °C, which corresponds to a lowering of approximately 7 °C by comparison with the 1:1 mixture in absence of short PLL. This shift is similar to that observed for the DMPA/short PLL complex at R_i = 1 and suggests that, as opposed to long PLL, short PLL, when bound to DMPA/DMPC-d_{54} bilayers, does not induce phase separation within the system. The increase of the h_{2930}/h_{2880} ratio observed for this mixture is most likely due to the spectral contribution of PLL in the C–H stretching region, and the peptide probably does not affect substantially the overall disorder of the lipid acyl chain matrix.

Effect of Short Polylysine on a 1:2 DMPA/DMPC-d_{54} Mixture. In order to confirm the inability of short polylysine to induce phase separation in a DMPA/DMPC mixture, we have also investigated a 1:2 DMPA/DMPC-d_{54} mixture

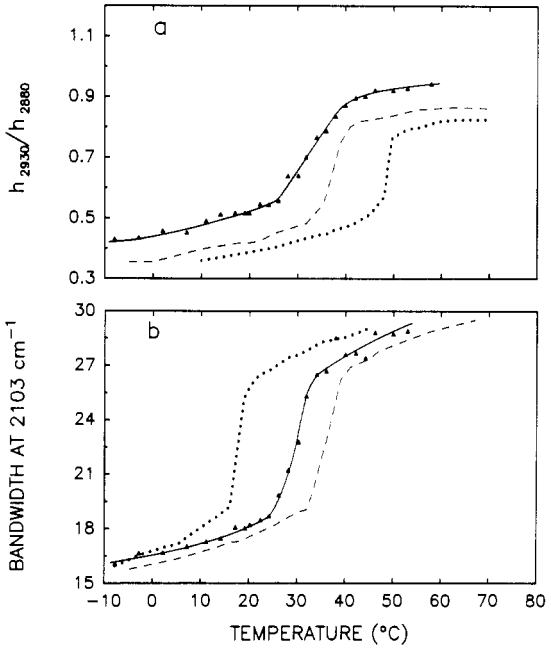

FIGURE 9: (a) Temperature profiles of DMPA derived from the Raman spectral h_{2930}/h_{2880} peak height intensity ratio. DMPA/DMPC-d_{54} 1:1 in the absence (dashed line) and in the presence (▲) of an excess of short PLL. The temperature profile of pure DMPA (dotted line) is also shown. (b) Temperature profiles of DMPC-d_{54} derived from the bandwidth at 65% of the height of the 2103 cm^{-1} Raman band. DMPA/DMPC-d_{54} 1:1 in the absence (dashed line) and in the presence (▲) of an excess of short PLL. The temperature profile of pure DMPC-d_{54} (dotted line) is also shown.

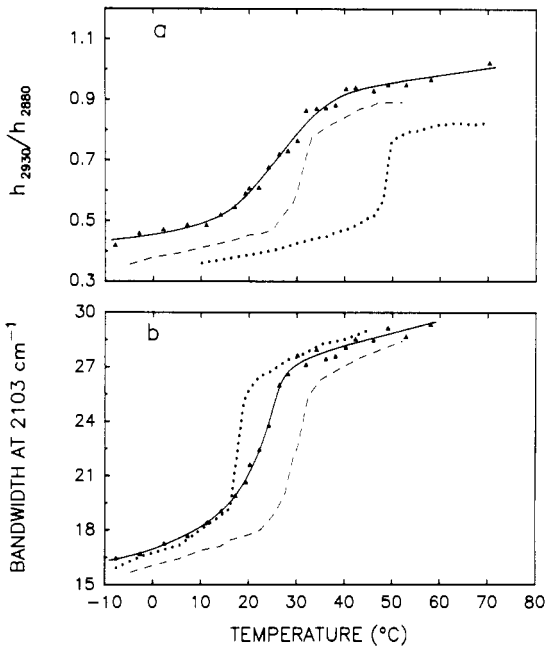

FIGURE 10: (a) Temperature profiles of DMPA derived from the Raman spectral h_{2930}/h_{2880} peak height intensity ratio. DMPA/DMPC-d_{54} 1:2 in the absence (dashed line) and in the presence (▲) of an excess of short PLL. The temperature profile of pure DMPA (dotted line) is also shown. (b) Temperature profiles of DMPC-d_{54} derived from the bandwidth at 65% of the height of the 2103 cm^{-1} Raman band. DMPA/DMPC-d_{54} 1:2 in the absence (dashed line) and in the presence (▲) of an excess short PLL. The temperature profile of pure DMPC-d_{54} (dotted line) is also shown.

(Figure 10). The transition temperatures observed from the C–H and C–D stretching regions for the complex are essentially the same (approximately 22 °C), and the two phospholipids present the same thermotropic behavior, indicating the presence of a single phase. Moreover, the transition temperature of the complex is shifted down once again as for complexes of DMPA with short polylysine (from 30 to 22 °C). Therefore, according to our results, there is no evidence that short polylysine induces phase separation in the DMPA/DMPC-d_{54} mixture.

DISCUSSION

The results presented here enlighten the subtleties of the interaction of polylysine with membrane lipids. The degree of polymerization of PLL has revealed to be a key factor in the determination of effects on DMPA and DMPA/DMPC mixtures, as expected from the previous work of Carrier and Pézolet (1986) on DPPG. We showed that under appropriate conditions, PLL can lead to lateral phase separation within homogeneous bilayers of DMPA and DMPC. However, before discussing this phenomenon, it is certainly worth reviewing the behavior of the complexes formed with each pure lipid.

As seen in Figure 3, the gel to liquid-crystalline phase transition of DMPA becomes less cooperative and shifts toward much higher temperatures when long PLL is present. A similar shift has been observed for the DPPA/PLL (M_r 30000) system, using electron spin resonance (Galla & Sackmann, 1975a) and fluorescence spectroscopy (Hartmann & Galla, 1978). The Raman intensity ratios h_{2930}/h_{2880} and h_{2880}/h_{2850} both indicate that below the phase transition of the pure lipid, the packing and the conformation of the acyl chains of DMPA are almost unmodified by the polypeptide. However, PLL makes the gel phase more "temperature-resistant" since the fluid phase is reached slowly and at a much higher temperature. In that way, one can say that polylysine stabilizes DMPA bilayers. This system thus behaves quite differently from the DPPG/PLL system where the transition cooperativity is preserved. The very large temperature shift observed upon binding of long PLL to DMPA (which bears a single negative charge at pH 6.5) is not surprising since a simple acidic neutralization of the PA charges leads to a comparable 20 °C increase (Trauble & Eibl, 1974). In the case of DPPG, a mere 4 °C positive shift can be observed upon binding to PLL (Carrier et al., 1985) while acidification gives a 15 °C rise of the transition temperature (Watts et al., 1978). Therefore, one may conclude that long PLL neutralizes more efficiently DMPA than DPPG head groups, the extra glycerol of this latter probably impeding the access of the lysyl groups to the phosphate charges. The lack of cooperativity of the DMPA/long PLL transition indicates that the molecular organization of this complex is different from that of DPPG/PLL and also, on the lipid side, from the acid-neutralized DMPA. Actually, the DMPA/long polylysine thermotropic behavior is somewhat similar to the DMPA/Ca^{2+} one (Kouaouci et al., 1985).

Under the conditions used in this work, long as well as short PLL did not induce any change in the Raman spectra and of the thermotropic behavior of the zwitterionic lipid DMPC-d_{54}. We may thus infer that long polylysine does not interact with DMPC. These latter results are somewhat in contradiction with those obtained by Susi et al. (1979), who observed that PLL (M_r 17000) causes a shift of 4 °C to higher temperature of the gel to liquid-crystalline phase transition of DMPC. This apparent disagreement may be due to a difference of ionic strength of the solution used to prepare the dispersions.

The effect of short polylysine on DMPA differs markedly from that of the peptide with longer chains. The lipid transition temperature is lower for the DMPA/short PLL complexes than for the pure lipid. This parallels the behavior of DPPG/short PLL complexes (Carrier et al., 1985). For both

lipids, however, the h_{2880}/h_{2850} intensity ratio indicates a better packing of the acyl chains, showing that there is indeed an interaction between the charged head group and the polypeptide.

To explain these differences of behavior between long and short PLL, Carrier and Pézolet (1986) relied on the fact that the long polymer adopts the α-helical conformation when it is bound to DPPG bilayers, whereas the short one remains unordered. Hartmann and Galla (1978) also suggested a change of conformation from the random coil to a partially ordered structure when long PLL is bound to DPPA. For the DMPA/polylysine systems, Figure 4 shows unambiguously that both short and long PLL adopt the β-sheet conformation. However, the conformation of short PLL bound to DMPA is readily converted to the unordered conformation when the temperature is raised as opposed to long PLL. The difference in thermal stability of the conformation adopted by bound PLL is most likely at the origin of the drastically different thermotropic behavior of the complexes formed with DMPA. This hypothesis is supported by the fact that the break around 45 °C on the temperature profile of DMPA/short PLL complexes (Figure 8) nearly coincides with the onset of the conformational transition of PLL.

A few examples exist in the literature showing that polypeptides bound to phospholipids can adopt the β-sheet structure. Bach et al. (1975) have found by circular dichroism that upon binding to either phosphatidylserine or phosphatidylcholine vesicles, copolymers of lysine and tyrosine undergo a conformational transition from a random-coil to a β-sheet structure. Pézolet et al. (1982) have shown by Raman spectroscopy that reduced cardiotoxins adopt a β-sheet conformation when bound to DMPA. More recently, Surewicz et al. (1987) have reported that myelin basic protein forms a β-sheet (53%) upon binding to DMPG bilayers while Bazzi et al. (1987) have found, by IR spectroscopy, that the random copolymer poly(Leu^{50}, Lys^{50}) undergoes a partly unordered structure to a β-sheet transition in the presence of lysolecithin.

The X-ray data are in good agreement with PLL conformational results, as identical lamellar repeat distances ($\approx$6.0 nm) were found for both short and long PLL/DMPA complexes. For DPPG/PLL systems (Carrier & Pézolet, 1986), the lamellar repeat distances were markedly different for short and long polypeptides, and the failure of short PLL to order in an α-helix was accounted for by end group effects. These effects appear to be less important for the β-sheet formation.

At this point, a question that arises from the above results is the following: Why do DPPG and DMPA lead to different PLL ordered structures? We believe that this behavior of PLL is associated with the degree of neutralization of its ionized amino group when it is bound to charged lipids. As discussed above, the thermotropism of complexes of DMPA and DPPG with long PLL leads to the conclusion that PLL neutralizes more effectively the charged head group of DMPA than that of DPPG because of the presence of the bulky glycerol group for the latter lipid. According to Davidson and Fasman (1967), any residual charges on the lysyl residues are more disruptive for the β-sheet structure than for the helical conformation since in the β-structure, the amino side chains are closer. The formation of β-sheet structure would then be favored for PLL bound to DMPA. This conclusion is further supported by the fact that short polylysine, which remains unordered when bound to DPPG (Carrier et al., 1984), also adopts the β-sheet structure in complexes with DMPA. However, it is less stable and is readily destabilized when the temperature is increased.

Raman results as a function of the incubation molar ratio show that the stoichiometry of the DMPA/PLL complexes is 1. This value is in very good agreement with geometrical considerations based on the surface areas of the β-sheet structure and of DMPA. For the β-sheet structure, the distance between two residues is 0.7 nm along the chains and 0.5 nm between adjacent chains. This give a surface area of 0.35 nm^2 for each peptide group which agrees well with the value of 0.40 nm^2 determined by Blume (1979) from measurements on DMPA monolayers.

The second question that arises is the following: What is the structure of the long PLL/DMPA complexes? The analogy of the noncooperative transition of the DMPA/long PLL complexes with that of the DMPA/Ca^{2+} system suggests that the planar bilayer structure may even not be present. Small-angle X-ray diffraction measurements reveal that the lamellar repeat distance is 5.95 nm for DMPA/long PLL complexes which corresponds essentially to the thickness of a bilayer of DMPA separated by polylysine in the β-sheet conformation. Therefore, the X-ray results suggest that polylysine may form bridges between neighboring bilayers. The same conclusion was reached for the DPPG/PLL system (Carrier & Pézolet, 1986).

The temperature profiles shown above demonstrate that 1:1 and 1:2 mixtures of DMPA and DMPC-d_{54} are perfectly miscible. Long polylysine gives rise to a lateral phase separation within these mixtures. However, the segregation is not as complete as with calcium (Kouaouci et al., 1985): the two phases formed are in fact enriched in one component. For the 1:1 mixture, the temperature profile of the PA-rich phase is similar to that of the pure DMPA/long PLL complex, but the transition occurs at a slightly lower temperature, suggesting the presence of some DMPC-d_{54} molecules. The results on the amide I band of bound polylysine also indicate that the peptide is in the β-sheet conformation, as for the pure DMPA/long PLL system. The profile derived from the PC component consists of a single, cooperative transition, at a temperature lower than that of the pure lipidic mixture. This suggest that a phase rich in PC is formed in the presence of long PLL.

The results obtained for the DMPA/DMPC-d_{54} 1:2 mixture also provide very convincing evidence of phase separation induced by long PLL. The transition temperature of both PA and PC profiles is lowered relative to the corresponding curves for the 1:1 mixture. This indicates that both phases have incorporated a fraction of the additional PC. Since the transition temperature of the PC-rich phase is about the same as that of pure DMPC-d_{54}, this phase does not contain a lot of DMPA.

Short PLL, by contrast to the long one, does not induce phase separation in the investigated mixtures. For the two PA/PC molar ratios studied, both the PA and PC components display the same thermal behavior, with a single cooperative transition. This indicates that DMPA and DMPC-d_{54} are in the same phase. The transition temperature is lower than in the absence of the polypeptide; the lipid/short PLL interaction is thus of the same type as for DMPA/short PLL. The larger shift obtained in this case, 8 °C instead of 3 °C for the DMPA/short PLL system, is due to the fact that the β-sheet structure in this case is less stable than for pure DMPA since the charges on the bilayer surface are further apart.

In summary, we have shown that long polylysine interacts strongly with DMPA to form a more stable and closely packed structure. This polypeptide induces a lateral phase separation in DMPA/DMPC-d_{54} mixtures. Short polylysine produces

very different effects. When bound to DMPA, it induces a small decrease of the transition temperature. In addition, short PLL does not lead to phase separation in DMPA/DMPC-d_{54} mixtures. In all cases, we have observed that the bound polypeptide has a β-sheet conformation as opposed to the α-helical structure previously found for DPPG/long PLL complexes (Carrier & Pézolet, 1984). However, DMPA-bound short polylysine forms a β-sheet structure only at low temperature and undergoes a conformational transition toward the disordered conformation when the temperature is increased. Therefore, our results demonstrate that the conformation of the lipid-bound polypeptides depends on the nature of the polar head group of the lipid, not only on its net charge, and that it affects considerably the thermotropism of the lipid.

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