RAMAN SPECTROSCOPIC STUDY OF THE INTERACTION OF POLY-L-LYSINE WITH DIPALMITOYLPHOSPHATIDYLGLYCEROL BILAYERS

DANIELLE CARRIER AND MICHEL PÉZOLET
Départment de Chimie, Université Laval, Québec, Canada G1K 7P4

ABSTRACT The interaction of the basic polypeptide poly-L-lysine with the negatively charged phospholipid dipalmitoylphosphatidylglycerol was studied using Raman spectroscopy. The nature of the interaction appeared to depend on the molar ratio of the constituents. At up to one lysine group per lipid molecule, the bilayer was stabilized by the polypeptide that underwent a conformational transition toward an ordered α-helical structure, in which the electrostatic interactions were probably maximal. The stabilization of the bilayer was detected by an increase in both the temperature of the thermotropic transition of the lipid and the interchain vibrational coupling of the methylene C-H vibrations. At higher poly-L-lysine concentration, hydrophobic interactions must have been involved to explain the binding of excess polypeptide. There seemed to be a penetration of poly-L-lysine in the bilayer that increased with the polypeptide concentration. Under these conditions, the chain-packing lattice gradually changed from hexagonal to either orthorhombic or monoclinic symmetry. We believe that this change of structure is associated with the interdigitation of the acyl chains.

INTRODUCTION

Natural membranes are known to play an important role in several biological processes. Therefore, a considerable amount of work has been done to unravel the molecular organization of lipid bilayers and to understand the relationship between the properties of biological mambranes and their rather heterogeneous compositions. Proteins account for ~60% of the dry weight of biological membranes. Depending on their nature, proteins are either embedded in the hydrophobic core of the bilayer, in which case they are referred to as intrinsic or integral proteins, or they are simply attached to the outside of the bilayer, in which case they are referred to as extrinsic or peripheral proteins.

Poly-L-lysine is one of the most interesting models of extrinsic proteins because of its basic character. Each lysine group bears a positive charge at physiological pH and can thus bind to negatively charged phospholipids. Important modifications of the structure of both constituents are expected because of these attractive electrostatic forces. The purpose of the present study was thus to examine the interaction of poly-L-lysine (PLL) with the negatively charged phospholipid, dipalmitoylphosphatidylglycerol (DPPG).

Several techniques have been used to investigate the effect of PLL on the molecular organization of phospholipids. Using differential scanning calorimetry, Papahadjopoulos et al. (1975) have shown that the binding of PLL to DPPG bilayers causes an increase in the enthalpy and in the temperature of the gel-to-liquid crystalline phase transition. These results were interpreted to be caused by a stabilization of the bilayer structure after the negative charge of the polar head groups was neutralized, and it was concluded that PLL is simply attached to the bilayer surface without penetrating into the hydrocarbon region. PLL binding also leads to an increase in the transition temperature (T_c) of dipalmitoylphosphatidic acid (Galla and Sackmann, 1975*a*, *b*; Hartmann et al., 1977; Hartmann and Galla, 1978) and of dimyristoylphosphatidylcholine, which has a zwitterionic rather than an anionic polar head group (Susi et al., 1979). Electron microscopy has provided direct evidence for the formation of crystalline patches of bound phosphatidic acid within the pure phospholipid or in mixtures with dioleoylphosphatidylcholine (Hartmann et al., 1977). The presence of charge-induced domains has also been postulated from electron spin resonance and fluorescence data (Galla and Sackmann, 1975*a*, *b*; Hartmann and Galla, 1978; Hartmann et al., 1977).

There is little experimental data on the conformation of phospholipid-bound PLL. Hammes and Schullery (1970) first proposed from their circular dichroism results that PLL adopts the α-helical conformation when it is bound to phosphatidylserine bilayers. More recently Hartmann and Galla (1978) concluded from their spin-labeling study on phosphatidic acid vesicles that the conformation of bound PLL is partially ordered, but not α-helical. Therefore, there is a discrepancy between the findings of these investigations. However, this structural transformation of

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PLL appears to be too intricate to account for all analogous systems made of a basic polypeptide interacting with an acidic phospholipid. For example, Bach et al. (1975) have found that upon binding to phosphatidylserine vesicles, random copolymers of L-lysine and either L-phenylalanine or L-tyrosine undergo a conformational transition to the α-helical or β-sheet structures, respectively. On the other hand, the copolymer of L-lysine and of L-serine always remains in the random coil structure under the same conditions.

In a recent investigation on cardiotoxins, Pézolet et al. (1982) have shown that Raman spectroscopy is a particularly effective tool to study the conformation of phospholipid-bound proteins. In addition, several studies have shown that the Raman spectra of lipid bilayer systems are remarkably sensitive to the conformation of the hydrocarbon chains of the phospholipid molecules (Tu, 1982; Carey, 1982). We have thus used this technique to investigate the conformation of DPPG-bound poly-L-lysine, as well as the effects of this polypeptide on the thermotropic behavior of aqueous dispersions of DPPG.

MATERIALS AND METHODS

The ammonium salt of DPPG and the bromide salt of PLL (molecular weight of 150,000) were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. The purity of the DPPG sample was ascertained by thin-layer chromatography using a mixture of chloroform/methanol/acetic acid/water (65:25:8:4 by volume) as the eluant.

The aqueous solutions (3% by weight) of PLL used to obtain the Raman spectra were prepared by dissolving the proper amount of the dry polypeptide in water, and adding small volumes of HCl or NaOH (1 M) to adjust their pHs to the desired values. Lipid dispersions (4% by weight) were prepared by weighing out the solid, adding the appropriate volume of 0.1 M phosphate buffer (pH 7.0), and shaking mechanically at 50°C for 10–15 min. Phospholipid-polypeptide complexes were formed by mixing the appropriate amount of a 4% DPPG dispersion and of a PLL solution in phosphate buffer. The concentration of this solution was 10, 1, or 0.1% (wt/wt), depending on the lipid-peptide molar ratio. Mixtures were then vortexed, incubated at 50°C for 10–15 min, transferred into a capillary cell (1.5 mm diam), and centrifuged to yield white pellets that were used to obtain the spectra.

Raman spectra were recorded with a computerized spectrometer (model 1400; Spex Industries, Inc., Edison, NJ) described elsewhere (Savoie et al., 1979), with a spectral resolution of 5 cm^{-1}. The monochromator was calibrated with a neon discharge lamp, and the frequencies that are cited later are believed to be accurate to ± 2 cm^{-1} for sharp peaks. Exictation radiation was provided by an argon ion laser (model 165; Sepctra-Physics Inc., Mountain View, CA) operating at 514.5 nm with ~200 mW laser power at the sample.

Raman spectra of aqueous solutions of poly-L-lysine were computer-corrected for the water contribution by using the band associated with the HOH bending mode at ~1,635 cm^{-1}. The water spectrum was subtracted from that of a solution at the same pH and temperature to obtain a symmetrical amide I band and a straight baseline between 1,720 and 1,900 cm^{-1}. For the concentration range used to obtain spectra of aqueous solutions of PLL, this method gave results similar to the method described by Williams and Dunker (1981), which corrects for the water contribution using the 3,000–3,200-cm^{-1} region. For pure DPPG dispersions, the subtraction criterion was simply to cancel the water band at 1,635 cm^{-1} with the phosphate buffer spectrum obtained under identical conditions. For the spectra of DPPG-PLL complexes, the method of Williams and Dunker (1981) was used in the C-H stretching region, whereas the method based on the 1,635-cm^{-1} water band was preferred for the rest of the spectrum because the corrections gave different results at high-protein concentration (Cavatorta et al., 1976). When dealing with the lipid behavior, the polypeptide contribution was canceled taking the amide I band as an internal intensity standard. When needed, spectra were corrected for fluorescence backgrounds by subtracting appropriate polynominal functions (Savoie et al., 1979).

RESULTS

Poly-L-Lysine in Aqueous Solution

Fig. 1 displays the Raman spectra of PLL in the three possible conformations that this polypeptide can adopt in aqueous solution. Several features of these spectra allow one to distinguish between these conformers. Because the detailed vibrational assignments can be found in the literature (Yu et al., 1973; Painter and Koenig, 1976; Sugawara et al., 1978), we will restrict the discussion to the most characteristic bands.

Below pH 9, PLL side chains are positively charged so that the repulsion between them usually prevents the polypeptide from adopting an ordered structure. In the absence of specific counterions like SCN^- (Conio et al., 1974) or $C10_4^-$ (Painter and Koenig, 1976) and at low

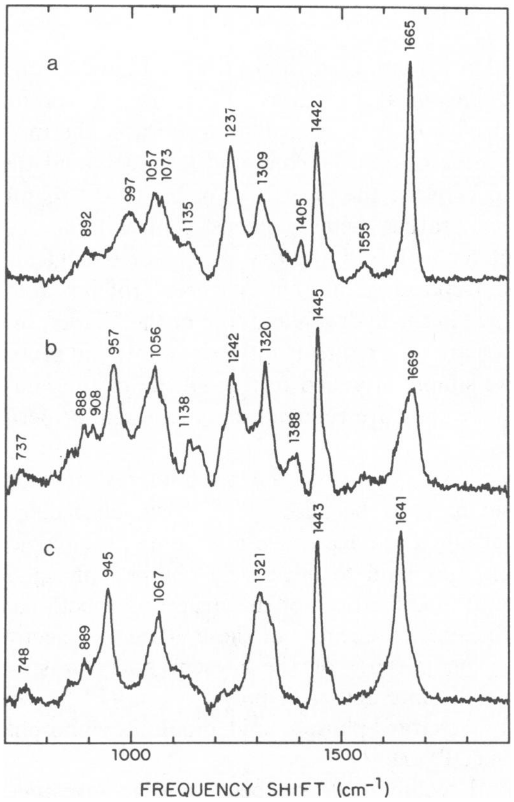

FIGURE 1 Raman spectra of 3% (wt/wt) poly-L-lysine solutions (four scans at 2 s/2 cm^{-1}). The spectra represent (*a*) β-sheet structure, pH 11.4, 52°C; (*b*) random-coil form, pH 5.6, 20°C; and (*c*) α-helical conformation, pH 11.4, 4°C.

TABLE I
SPECTRAL CHARACTERISTICS OF THE AMIDE I AND AMIDE III RAMAN BANDS OF POLY-L-LYSINE IN AQUEOUS SOLUTIONS

Conformation	Amide I			Amide III	
	Frequency	Intensity*	Half-width	Frequency	Intensity*
	cm^{-1}		cm^{-1}	cm^{-1}	
α-Helix	1,641	1.05	30	~1,305	—
β-Sheet	1,665	1.60	18	1,237	1.00
Random	1,669	0.60	52	1,242	0.70

*Peak-height intensity relative to the ~1,442-cm^{-1} methylene band.

ionic strength (Yu et al., 1973), poly-L-lysine remains in the random-coil conformation. Fig. 1 *b* shows its Raman spectrum under such conditions. As seen, the amide I band appears at 1,669 cm^{-1}; it is broad (Table I) and much weaker than the methylene bending mode at 1,445 cm^{-1}, which is often used as an internal intensity standard in the Raman spectra of proteins (Pézolet et al., 1976). The amide III vibration gives a peak of medium intensity at 1,242 cm^{-1}.

At high pH and temperature, PLL changed to the β-sheet structure, which is characterized in the Raman spectrum (Fig. 1 *a*) by strong and narrow amide I and amide III bands centered at 1,665 cm^{-1} and 1,237 cm^{-1}, respectively. Finally, the α-helical conformation could be obtained at low temperature and high pH. In this case, the Raman spectrum (Fig. 1*c*) has an intense and rather sharp amide I band whose frequency, 1,641 cm^{-1}, is much lower than those in the other two spectra. The band associated with the amide III vibration seems to be absent, at least in the usual 1,220–1,280-cm^{-1} range for this mode. However, recent results on the thermal denaturation of the highly helical protein tropomyosin (Pézolet, M., M. Pigeon-Gosselin, and J. P. Caillé, unpublished data) indicate that the amide III band for long α-helices appears around 1,305 cm^{-1}. Close examination of the α-helical PLL spectrum indicates that the methylene twisting band at ~1,320 cm^{-1} is quite broad, most likely because an overlapping amide III band is present at ~1,300 cm^{-1}. The main spectral characteristics of the amide I and III vibrations of PLL are summarized in Table I.

The C-C skeletal stretching region is also valuable for conformational identification. For example, the strong and sharp C-C stretching band at 945 cm^{-1} is associated with the α-helix. The corresponding feature in the random-coil spectrum is also fairly strong, but it arises at 957 cm^{-1}. In the β-structure spectrum, the C-C band is rather weak and broad and is located at 997 cm^{-1} in a bulky region.

DPPG-bound Poly-L-Lysine

Because the Raman spectra of supernatants taken from above the DPPG-PLL complexes (results not shown) indicate that some uncomplexed polypeptide was present, we attempted to determine the real molar ratios of the precipitates. The colorimetric method of Itzhaki (1972) was used to assay the peptide concentration in the supernatants. For incubation molar ratios (R_i) that were greater than one lipid molecule per lysine residue, the accuracy of this method was satisfactory, and we found that at up to one lysine group per lipid molecule, most of the peptide was bound to DPPG. For example, at $R_i = 1$, the real molar ratio in the complex was only 10% higher than the incubation ratio. However, at lower R_i ratios (between 1 and 0.01), the real molar ratios were always higher than the incubation ratios and the discrepancy increased with

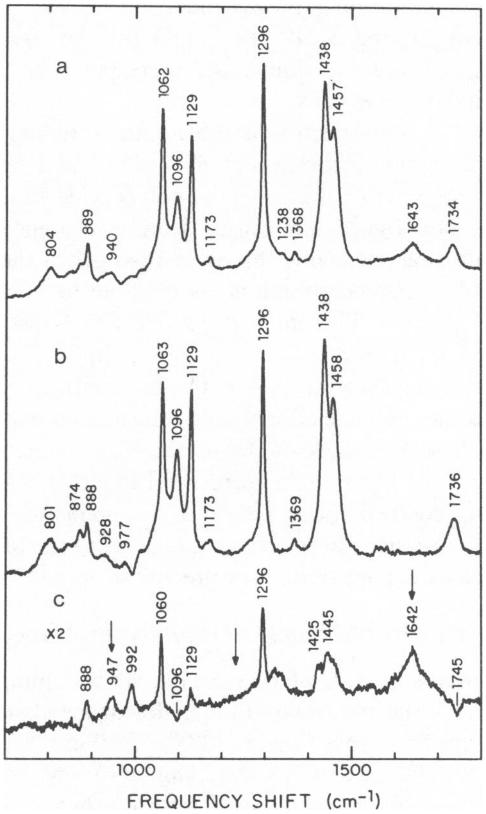

FIGURE 2 (*a*) Raman spectrum of DPPG-PLL complex with $R_i = 1$ (incubation lipid/protein molar ratio) at 20°C, taken 5 d after preparation (10 scans at 2 s/cm^{-1}); (*b*) Raman spectrum of a 4% DPPG dispersion at 20°C, which was corrected for the buffer contribution (eight scans at 2 s/0.5 cm^{-1}); and (*c*) spectrum obtained from spectrum *a* after subtraction of the pure lipid contribution.

decreasing R_i. Because small volumes of solution were used to prepare the Raman samples, very inaccurate results were obtained when the colorimetric method was used for complexes with low incubation ratios. We thus decided to use the relative integrated intensity of the PLL amide I band and of the DPPG carbonyl band in the spectra of precipitated complexes to evaluate their real molar ratios. From this method, and knowing from the colorimetric assay that essentially all the PLL was bound at $R_i = 1$, we found that the real molar ratio of complexes prepared with an incubation ratio of 0.01 is ~0.3. Therefore, one has to be careful when dealing with mixing-molar ratios to propose models of phospholipid-Pll complexes.

Fig. 2 *a* displays the Raman spectrum of a precipitated DPPG-PLL complex prepared with an incubation molar ratio of 1. This spectrum is quite similar to the spectrum of the pure lipid dispersion (Fig. 2 *b*). The main differences are in the carbonyl region, where a new peak is present at 1,643 cm^{-1}, and on the high-frequency wing of the 1,296-cm^{-1} peak. To emphasize these features, we subtracted the lipid spectrum from the spectrum of the complex in order to minimize the lipid contribution (Fig. 2 *c*) Comparing this difference spectrum with those of Fig. 1 unambiguously reveals that bound-PLL is in the α-helical conformation because (*a*) the 1,642-cm^{-1} band can be assigned to the amide I vibration of the α-helical polypeptide, (*b*) there is no peak around 1,240 cm^{-1}, and (*c*) the small peak appearing at 947 cm^{-1} obviously corresponds to the 945 cm^{-1} band of the α-helix.

In Fig. 3, the spectra in the amide I region of the DPPG-polylysine complexes at $R_i = 0.01$ and 1 are compared with those of the α-helical polypeptide in aqueous solution. This figure demonstrates the coincidence of the peaks' maxima and shows that the half-width of the amide I band of bound polylysine is comparable to that of the aqueous polymer. Therefore, polylysine was essentially all converted from random coil to α-helix upon binding to phosphatidylglycerol at $R_i = 1$. On the contrary, Fig. 3 *c* indicates the presence of another conformation in addition to the helical one: the shoulder on the high-frequency side of the amide I band could correspond to either β-sheet or disordered conformations. However, this nonhelical polylysine must also have been some way bound to the phospholipid because it appeared in the precipitate spectrum.

Dipalmitoylphosphatidylglycerol Spectrum

The Raman spectrum of DPPG (Fig. 4) is dominated by bands due to the vibrations of the hydrocarbon chains. The skeletal optical modes region (1,000–1,200 cm^{-1}) consists mainly of delocalized C-C stretching vibrations, involving long portions of the acyl chains. There are three characteristic bands in the gel state (Fig. 4 *a*). The 1,063- and 1,129-cm^{-1} features have been respectively assigned to the out-of-phase and in-phase skeletal C-C stretching modes of the all-*trans* segments (Lippert and Peticolas, 1971; Spiker and Levin, 1975). The peak at 1,096 cm^{-1} originates from the superposition of the symmetric PO_2^- stretching mode with C-C vibrations of *gauche* conformers in the fatty acyl chains and C-C skeletal motion of all-*trans* portions with a phase angle different from 0 or π (Spiker and Levin, 1975). As the temperature was raised, the frequency of this complex band shifted to 1,085 cm^{-1} whereas its intensity increased, which reflects that the number of *gauche* bonds increases when going to the liquid crystalline state (Fig. 4 *c*). At the same time, the intensity of the *trans* features decreased and the 1,129 cm^{-1} peak shifted to lower frequency. Levin and Bush (1981) have attributed this displacement to the appearance, on the low frequency side, of a C-C stretching band due to *gauche* bonds involving the acyl terminal methyl group. The skeletal stretching mode region is therefore particularly sensitive to the intrachain disorder. Intensity ratios of the 1,085 cm^{-1}-band to the all-*trans* conformer peaks are currently used as a probe of the *gauche*/*trans* population.

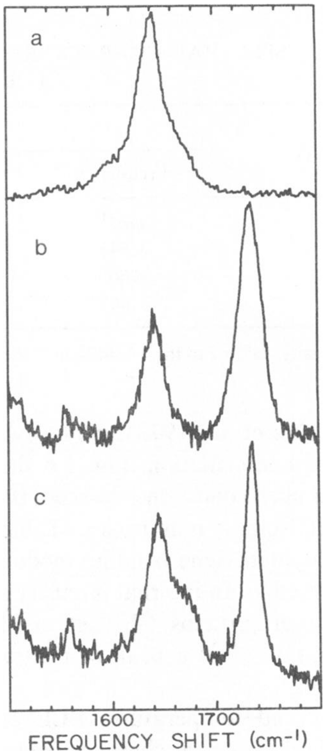

FIGURE 3 Raman spectrum of the amide I region for (*a*) α-helical polylysine in aqueous solution at pH 11.4 and 4°C (four scans at 2 s/cm^{-1}); (*b*) DPPG-PLL at $R_i = 1$ and 20°C (10 scans at 2 s/cm^{-1}); and (*c*) DPPG-PLL at $R_i = 0.01$ (real molar ratio of the complex = 0.3) and 20°C (three scans at 2 s/cm^{-1}). All spectra were corrected for the water spectral contribution.

The methylene twisting mode appeared at 1,296 cm^{-1}. It broadened and shifted to higher frequency when the lipid went through the gel-to-liquid crystalline phase transition. The strong doublet at 1,450 cm^{-1} arose from the methylene-bending vibration. In the fluid state, the high-

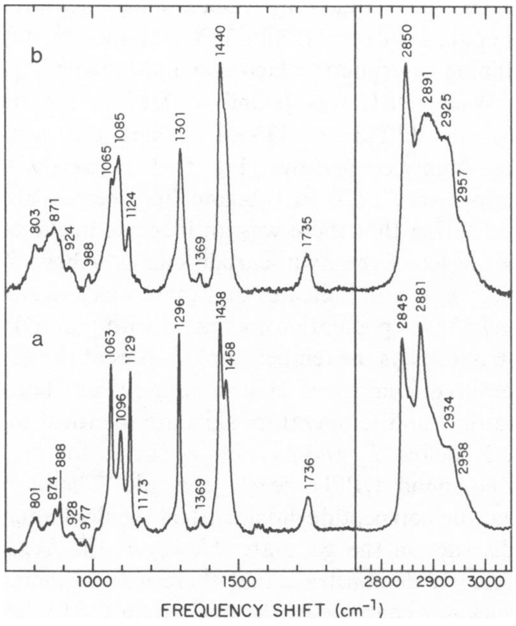

FIGURE 4 Raman spectrum of a 4% DPPG dispersion: (*a*) in the gel state at 20°C (eight scans at 2 s/0.5 cm^{-1} for the 700–1,900-cm^{-1} region and one scan at 2 s/cm^{-1} for the C-H stretching region) and (*b*) in the liquid crystalline phase at 50°C (two scans at 2 s/2 cm^{-1} for the first part and one scan at 2 s/cm^{-1} for the second part).

frequency component tended to disappear. Bush et al. (1980) have shown that this scissoring mode is sensitive to small change in lattice disorder.

The carbonyl-stretching mode shifted from 1,736 to 1,733 cm^{-1} when the bilayer melted. Several papers dealing with the conformation dependence of this feature have been published (Brown et al., 1980; Mushayakarara et al., 1982; Bunlow and Levin, 1977), but this subject will not be discussed here.

The carbon-hydrogen stretching region extends from 2,800 to 3,000 cm^{-1} and contains a series of overlapping vibrational bands associated with the acyl chains and complicated by Fermi resonance with overtones of the methylene deformation modes. The peaks at 2,844 and 2,880 cm^{-1} are respectively assigned to the methylene symmetric and antisymmetric C-H stretching vibrations. The shoulders at ~2,930 and 2,954 cm^{-1} are a result of the symmetric and asymmetric C-H stretching mode of terminal methyl groups, respectively (Spiker and Levin, 1975).

The intensity of the 2,880-cm^{-1} feature is enhanced by an underlying broad band attributed to the Fermi resonance interaction between the methylene symmetric C-H stretching mode and the first overtone of the methylene-bending fundamental at 1,450 cm^{-1} (Snyder et al., 1978; Snyder and Scherer, 1979). Upon melting, the 2,880-cm^{-1} band seemed to decrease in intensity relative to the 2,844-cm^{-1} peak, and it broadened and shifted to higher frequency (Fig. 4). The intensity lowering has been related to the reduction of the interchain Fermi interaction (Verma and Wallach, 1977). By comparing the spectra of pure solid hexadecane and hexadecane dissolved in a solid perdeuterohexadecane matrix, Gaber and Peticolas (1977) have shown that the interchain Fermi interaction accounts for 50% of the total intensity change upon melting. The additional intensity reduction results from the broadening

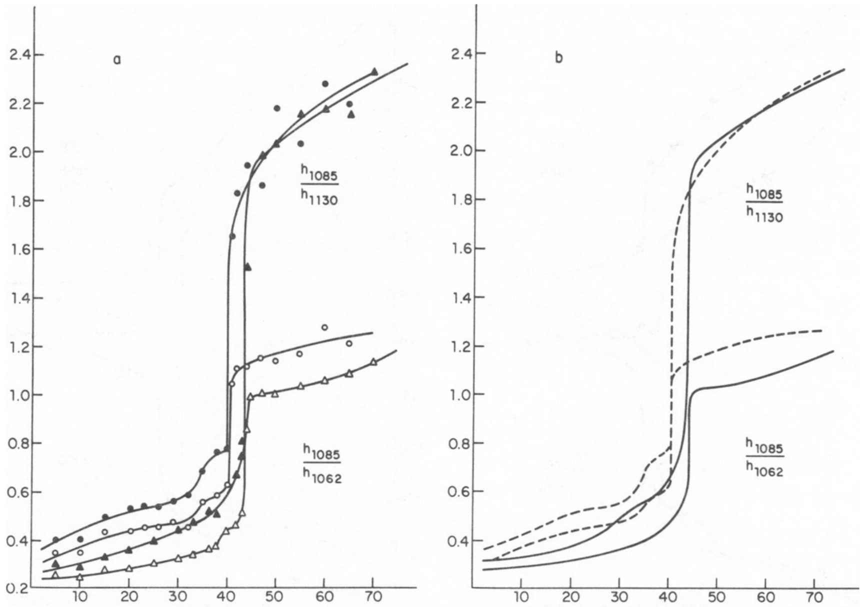

FIGURE 5 (*a*) Temperature profiles of pure DPPG (circles) and DPPG-PLL (triangles) at $R_i = 1$ derived from the Raman spectral $h_{1,085}/h_{1,130}$ (solid symbols) and $h_{1,085}/h_{1,062}$ (open symbols) peak height intensity ratios. (*b*) Corresponding curves after correction for phosphate buffer and polylysine spectral contribution. Dashed lines are used for the pure lipid.

that occurs when several conformers in the liquid are present, and to the increase of the acyl chain rotational mobility (Snyder et al., 1980). The ratio $h_{2,880}/h_{2,845}$ is thus a sensitive probe for the intermolecular vibrational coupling and the lateral packing of the acyl chains.

The spectra of Fig. 4 also show an increase of the 2,930-cm^{-1} band with temperature. This change is attributed to an underlying infrared active methylene asymmetric stretching mode that becomes Raman because the chain symmetry is lowered (Bunow and Levin, 1977). Therefore, the $h_{2,930}/h_{2,880}$ ratio is a convenient measure to monitor the interchain disorder of lipids in bilayers.

Thermotropic Behavior of Free and Bound DPPG

To analyze the thermotropic properties of DPPG, we used the intensity ratios mentioned in the last section. The $h_{1,080}/h_{1,130}$ and $h_{1,080}/h_{1,062}$ ratios were taken as spectral indices of intrachain disorder, whereas lateral interactions and intermolecular chain disorder were monitored using the ratios $h_{2,880}/h_{2,845}$ and $h_{2,930}/h_{2,880}$.

Fig. 5 *a* shows the temperature dependence of the ratios $h_{1,080}/h_{1,130}$ and $h_{1,080}/h_{1,062}$ for a pure DPPG dispersion and for a DPPG-polylysine complex with $R_i = 1$. These curves are characterized by two transitions: a sharp one at 40°C for the pure lipid corresponding to the gel-to-liquid crystalline phase transition, and a small pretransition at ~33°C. Both temperatures agree well with the values of 40.2°C and 32.8°C determined by fluorescence measurements (Watts et al., 1978) or 41° and 35°C obtained by differential scanning calorimetry (Jacobson and Papahadjopoulos, 1975). When PLL was bound to DPPG, the melting temperature shifted to 43°–44°C, but the transition remained highly cooperative (Fig. 5 *a*). Below the transition temperature (T_c), PLL-bound lipid curves are lowered, indicating that there was an increase in the conformational order of the hydrocarbon chains. Above T_c, the ratio $h_{1,080}/h_{1,062}$ still indicates that there was a decrease of the *gauche*/*trans* population compared with pure DPPG.

Fig. 6 *a* displays the temperature profiles of the parameters measured from the C-H stretching region. Transition temperatures and temperature shift are identical to those of Fig. 5. Below T_c, the $h_{2,930}/h_{2,880}$ curves for pure and polylysine-bound DPPG nearly coincide. Therefore, we infer that the polypeptide did not affect the lipid intermolecular disorder in the gel state. However, the $h_{2,880}/h_{2,845}$ curves clearly demonstrate that there was an increase of intermolecular coupling of the acyl chain C-H vibrations when polylysine was bound to DPPG, for the whole temperature range. For a DPPG-PLL complex at $R_i = 0.01$ (results not shown), the effect on the $h_{2,880}/h_{2,845}$ ratio was much more pronounced below T_c, whereas in the fluid state the ratio was almost the same as the one obtained at $R_i = 1$.

The methylene HCH deformation region also reflects changes in lateral packing (Snyder et al., 1978; Verma and Wallach, 1977). Fig. 7 *a* reveals the appearance of a novel

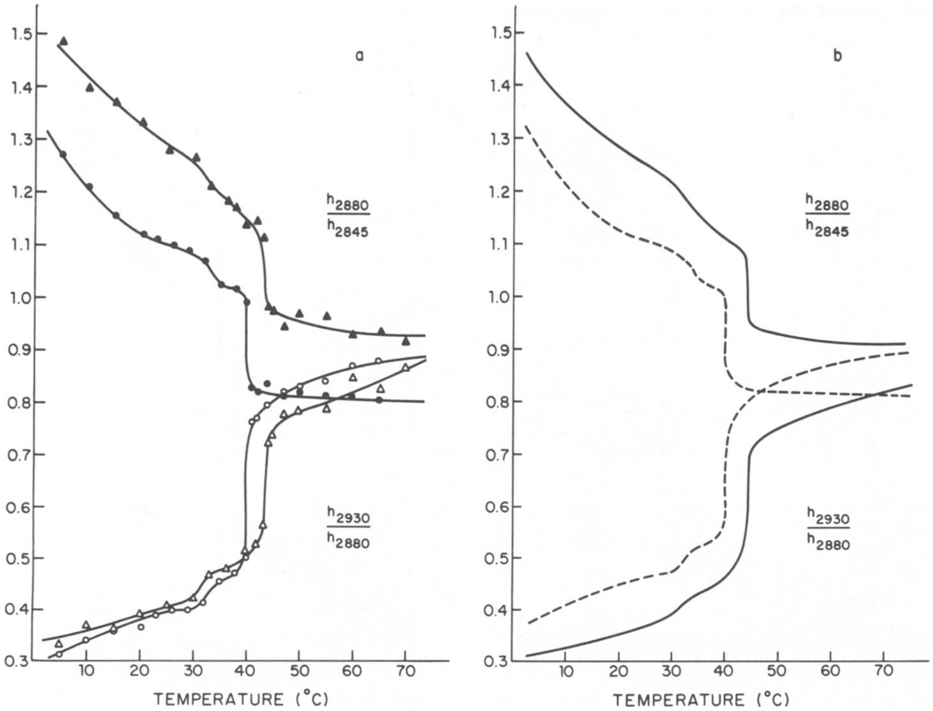

FIGURE 6 (*a*) Temperature profiles of pure DPPG (circles) and DPPG-PLL (triangles) at $R_i = 1$ derived from the Raman spectral $h_{2,880}/h_{2,845}$ (solid symbols) and $h_{2,930}/h_{2,880}$ (open symbols) peak height intensity ratios. (*b*) Corresponding curves after correction for phosphate buffer and polylysine spectral contribution. Dashed lines are used for the pure lipid.

feature at 1,422 cm^{-1} in the presence of polylysine. The intensity of this peak increased with PLL concentration: it existed at $R_i = 1$ as a weak shoulder that was unveiled in the difference spectrum of Fig. 2, or as an overlapping band at lower R_i (Fig. 7 *a*). We also found that this peak continuously grew during the first 3–4 wk after the sample was prepared when it was stored below the pretransition temperature. In addition, it should be mentioned that the band at ~1,422 cm^{-1} disappeared above the pretransition temperature and did not recur immediately after cooling down to the initial temperature.

DISCUSSION

The results presented in this communication elucidate the nature of the polylysine-DPPG association. First, our data provide direct evidence that for up to at least one lysine group per DPPG molecule, the conformation adopted by the bound polypeptide at pH 7.0 was α-helical. This agrees well with the circular dichroic results of Hammes and Schullery (1970) on the binding of PLL to phosphatidylserine at pH 7.0. In addition, we found that when there was a molar excess of polylysine ($R_i < 1$), part of the bound peptide did not undergo the transition to the α-helical structure.

We may then question the binding stoichiometry of the complex. For a molar ratio of one lysine group per phosphatidic acid molecule, Hartmann and Galla (1978) have proposed a 1:1 stoichiometry. However, according to their model, only half of the lipids and lysine groups are bound. Their electron microscopy and electron spin resonance data indicate that crystalline patches of bound lipids are formed. Raman spectroscopy can also be used to detect domain formation when two transitions occur. Knoll (1978) has obtained such a biphasic Raman transition curve with DPPA-PLL mixtures. Our results for $R_i = 1$ show there was a single transition temperature (Figs. 5 and 6). Therefore, either there was no domain formation with DPPG or all the lipid molecules were bound to PLL. The calorimetric data of Papahadjopoulos et al. (1975) agree that two phases form at high DPPG/PLL ratios, one for free and unperturbed lipids and another for bound lipids. To detect the presence of two transitions, we investigated the thermotropic behavior of a complex at $R_i = 2$. The observed profiles shown in Fig. 8 demonstrate the lipid phase separation. As our Raman transition curves do not provide evidence for a similar domain formation at $R_i = 1$, we must conclude that all the lipid molecules were involved in the interaction at this molar ratio. The phase separation observed by Hartmann and Galla (1978) for PLL-DPPA at $R_i = 1$ could be explained by a different binding stoichiometry resulting from the double charge of phosphatidic acid at pH 9.

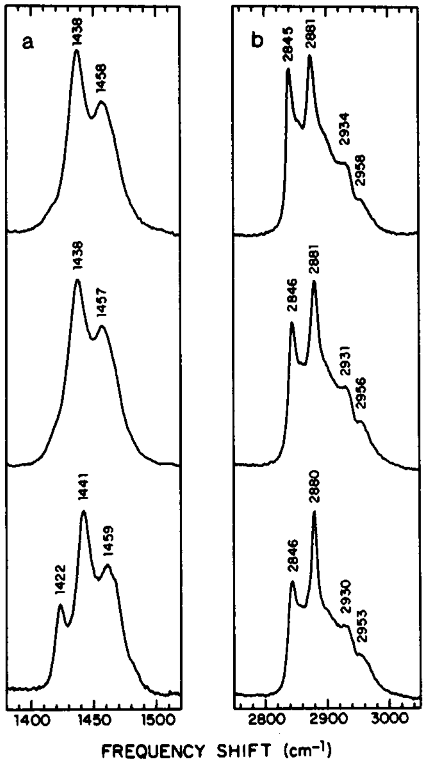

FIGURE 7 Raman spectra of the (*a*) methylene deformation and (*b*) C-H stretching regions for a DPPG dispersion (top spectra), a DPPG-PLL complex at $R_i = 1$ (middle spectra), and a DPPG-PLL complex at $R_i = 0.3$ (bottom spectra).

The binding of polylysine to DPPG also led to structural modifications of the lipid. There was a small increase in the conformational order of the lipid hydrocarbon chains (Fig. 5) as well as a shift of ~4°C in the gel-to-liquid crystalline

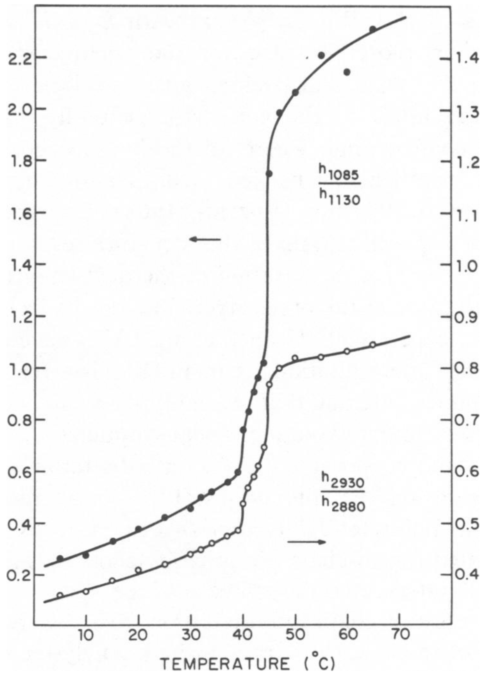

FIGURE 8 Temperature profiles of DPPG-PLL at $R_i = 2$, derived from the Raman spectral $h_{1,085}/h_{1,130}$ (solid circles) and $h_{2,930}/h_{2,880}$ (open circles) peak height intensity ratios.

phase transition towards higher temperatures. This stabilization of the DPPG bilayers by PLL was interpreted by Papahadjopoulos et al. (1975) to be a result of the neutralization of the negative charge of the lipid head groups. Moreover, our Raman data show an increase of the intermolecular vibrational coupling between adjacent acyl chains below and above T_c. To explain this effect in the liquid crystalline phase, we may infer that there was a tighter packing of PLL-bound DPPG. The same explanation holds for the gel state, but the effect could have also resulted from a modification of the molecular arrangement.

The appearance of the band at ~1,422 cm^{-1} is direct evidence that a change occurred in the packing symmetry of the acyl chains. Such a splitting of the methylene scissoring mode characterizes monoclinic and orthorhombic crystal lattices whose unit cells contain two molecules instead of one for the hexagonal structure (Boerio and Koenig, 1970). The 1,422-cm^{-1} peak was very weak at $R_i = 1$ (Figs. 2 and 7), but became more important at higher concentration of PLL. In addition, this band increased in intensity with time. Therefore, when the concentration of polylysine was high enough, part of the lipid matrix underwent a slow rearrangement from an initial, already stabilized state to a more stable structure where the hydrocarbon chains formed an orthorhombic or a monoclinic lattice. This process took place below the pretransition temperature and was reserved by simple heating above the temperature.

It has been shown that the $h_{2,880}/h_{2,845}$ ratio reflects the chain-packing characteristics in the gel state (Snyder et al., 1978, 1980). Orthorhombic and monoclinic crystalline *n*-alkanes display Raman spectra with $h_{2,880}/h_{2,845}$ ratios higher than those obtained for the triclinic structure, whereas, the hexagonal packing gives the lowest values. Diacylated lipids in the gel phase generally adopt an $L_{\beta'}$-type conformation where all the hydrocarbon chains are stiff, parallel, and packed according to a hexagonal two-dimensional lattice (Luzzati, 1968). Charged lipids may give a special variant of this structure resulting from interdigitation, i.e., penetration of the acyl chains of one layer into those of the other layer. The results from x-ray scattering experiments (Ranck et al., 1977) have demonstrated that interdigitation occurs in DPPG-water systems at low temperature and that several types of chain packing exist with different two-dimensional symmetry. According to Boggs and co-workers (1982), interdigitation explains their results on the interaction of the basic protein of myelin and phosphatidylglycerol. In a recent Raman study of saturated mixed-chain phosphatidylcholines, Huang et al. (1983) interpreted the increase of the $h_{2,880}/h_{2,845}$ ratio to be a result of chain interdigitation. Fig. 7 *b* shows the increase of the $h_{2,880}/h_{2,845}$ ratio at high polylysine concentration, for which the appearance of the ~1,422-cm^{-1} band has been related to the modification of the chain-packing system. Interdigitation could explain the increased chain interactions and could yield this crystal lattice modification. However, interdigitation first requires the spacing out of the lipid head groups.

Shah (1969) has reported that stearic acid monolayers expand when polylysine is added. This effect, not induced by calcium ions, was related to the penetration of PLL side chains in the monolayers. Electron paramagnetic resonance data (Hartmann and Galla, 1978) indicate that the polylysine side groups are located in a less polar environment when the polypeptide binds to phosphatidic acid. Hydrophobic interactions were also shown to be involved in the binding of different lysine copolymers to phosphatidylserine monolayers (Miller and Bach, 1974), because the most hydrophobic polypeptide investigated was the most strongly adsorbed. Therefore, the binding of PLL to DPPG bilayers should not be considered as a simple electrostatic interaction. This latter effect, however, could initiate a more complex mechanism of binding involving hydrophobic forces, which would lead to a penetration of the polypeptide into the bilayer. The lipid lattice would thus be loosened, and this could possibly lead to interdigitation. Polymyxin B is known to interact with DPPG similarly (Theretz et al., 1983), although the extent of the penetration of this antibiotic is probably more important because it has a hydrophobic segment that is longer than PLL.

Polymyxin is different from polylysine because it destabilizes the bilayer. It lowers the DPPG phase transition by 10°C (Sixl and Galla, 1981), whereas PLL had an opposite and smaller effect. However, the temperature shift of 4°C obtained when PLL was at $R_i = 1$ was reduced to ~1°C when there was a large excess of polypeptide (unshown results). Differential scanning calorimetry (Papahadjopoulos et al., 1975) shows the same reduced effect under similar conditions. A perturbing effect seems to compete with the usual stabilizing effect of PLL, and it is consistent with the concept that a more extensive penetration of the bilayer occurs similar to that of polymyxin or intrinsic proteins. Hammes and Schullery (1970) have suggested that polylysine molecules form channels, which explains the osmotic behavior of phosphatidylserine vesicles in the presence of excess polypeptide.

In summary, the nature of the interaction of polylysine and DPPG bilayers depends on their molar ratio. At up to one lysine group per lipid molecule, the bilayer was stabilized and the polypeptide took an ordered, α-helical conformation in which the electrostatic interactions were probably maximized. At higher PLL concentration, hydrophobic interactions must have been involved to explain the binding of excess polypeptide. A new chain-packing lattice gradually formed. There seemed to be a penetration of PLL inside the bilayer that increased with the polypeptide concentration. A medium penetration could produce the spacing out of the polar head groups and led to the interdigitation of the acyl chains. A strong penetration induced a perturbation of the bilayer core similar to the one observed with polymyxin or integral proteins.

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