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Conformational study of poly(L-lysine) interacting with acidic phospholipid vesicles

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Poly(L-lysine); Conformational change; Acidic phospholipid; Phospholipid vesicle; Circular dichroism

Circular dichroism measurements were carried out on poly(L-lysine) in the presence of vesicles of the negatively charged phospholipids, phosphatidylserine (PS; from bovine brain), phosphatidic acid (PA; prepared from egg yolk lecithin) and dimyristoylphosphatidylglycerol (DMPG). PS vesicles induced a conformational change in poly(L-lysine) from random coil to α-helix structure in 5 mM Tes (pH 7.0), whereas PA vesicles gave rise to β-structure in the same buffer. The fraction of α-helix, F_α (or β-structure, F_β), increased with increasing PS (or PA) concentration, reaching a saturation value of about 0.7 (or about 1). Mixed vesicles comprising PS and dilauroylphosphatidylcholine (DLPC) also induced α-helix conformation, however, the saturation value of F_α diminished with decreasing PS content in mixed vesicles. On the other hand, the spectral patterns for poly(L-lysine) in DMPG vesicle suspensions exhibited the coexistence of α-helix and β-structure. Both F_α and F_β increased with DMPG concentration and reached saturation values of about 0.5. Mixed vesicles composed of DMPG and dimyristoylphosphatidylcholine (DMPC) led to a reduction in F_β, while F_α remained almost constant. The diversity in ordered structure induced by different phospholipid vesicles suggests the participation of lipid head groups in determining the secondary structure of poly(L-lysine) adsorbed on the vesicular surface.

1. Introduction

Phospholipid-polypeptide interactions have been widely investigated in relation to those occurring between proteins and biomembranes [1–9]. The conformational behavior of polypeptides adsorbed on a membrane/water interface is an interesting objective for study, since some bioactive polypeptides are known to undergo conformational change on incorporation into or onto a biomembrane [10,11].

In a previous paper [12], we reported that dilauroylphosphatidic acid (DLPA) vesicles induced a conformational change in poly(L-lysine) from the random coil to β-structure. In contrast, Hammes and Shullery [1] reported that the random coil conformation of poly(L-lysine) was transformed to the α-helix on interaction with phosphatidylserine (PS) vesicles. The primary factor responsible for inducing an ordered structure of vesicle-bound poly(L-lysine) must be charge neutralization of the positive charges on the side chain of the polypeptide by negative charges on the vesicular surface; in fact, no ordered structure of poly(L-lysine) is induced by the vesicles composed of neutral phospholipid. These observations, however, suggest that the type of ordered structure induced is not only determined by simple charge neutralization, but also governed by other factors.

Our interest was focused on elucidation of the dependence of the type of ordered structure in-

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duced on the head group structure of acidic phospholipids. In the present work, we performed a conformational analysis of the interactions between poly(L-lysine) and vesicles of two types of acidic phospholipid, bovine brain PS and dimyristoylphosphatidylglycerol (DMPG), by means of CD measurements. Furthermore, the contribution of lipid acyl chain composition to the type of ordered structure induced was determined by comparing results obtained on phosphatidic acid (PA) prepared from egg yolk lecithin with those of DLPA.

2. Experimental

2.1. Materials

All phospholipids were purchased from Sigma and used without further purification. Poly(L-lysine) ($M_r = 15\,000$–$30\,000$) was obtained from Wako as the hydrobromide salt, and was converted to the hydrochloride by dialysis vs. 0.1 M HCl for 36 h, and then vs. water for 24 h, using cellulose dialysis tubing with an M_r cut-off of 3500. The concentration of lysine residues in the poly(L-lysine) stock solution (about 5×10^{-3} M) was determined via a modified ninhydrin method [13] after hydrolysis in 6 M HCl for 24 h.

The buffer solution used for sample preparation was 5 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (Tes, pH 7.0). At this pH value, PA, PS and DMPG possess a net, singly negative charge.

A stock suspension of phospholipid vesicles was prepared as follows. The lipid (about 30 mg) was dissolved in a small amount of ultrapure chloroform (Wako), the solvent being then evaporated under a stream of N_2. After drying in vacuo, Tes solution (25 ml) was added, and lipid thereafter was dispersed by sonication in the cuphorn of a Branson model 185 sonifier at 25° C (for PS and PA) or 30° C (DMPG) for approx. 30 min. Phospholipid concentrations in stock suspensions (about 2×10^{-3} M) were monitored by weighing lipid of defined molecular weight, except for PA and PS. The PA and PS concentrations were determined by inorganic phosphate assay after hydrolysis. PS/DLPC and DMPG/DMPC mixed vesicles were prepared by dissolving both lipids in chloroform followed by the same procedure as that employed for pure phospholipid. A sample suspension was prepared by mixing the lipid stock suspension and poly(L-lysine) stock solution to give the desired lipid concentration. After mixing, the sample suspension was again sonicated for about 5 min. This procedure was necessary in order to resuspend the material precipitated on increasing the concentration of phospholipid above that of the lysine residues. Turbidity of sample suspensions was reduced to such an extent via this procedure that CD measurements are possible; however, the precipitate formed in the region of phospholipid concentrations comparable to that of lysine residues was not resuspended in this way. The concentration of lysine residues was fixed at about 5×10^{-4} M for PA, DMPG and mixed DMPG/DMPC vesicles, and at about 2.5×10^{-4} M for PS and mixed PS/DLPC vesicles.

2.2. Methods

CD spectra were recorded on a Jasco J-600 spectropolarimeter using a 1 mm cell with a water-jacket for temperature control. Spectral data were acquired over the range 240–190 nm at intervals of 0.1 nm, averaged over 8 accumulations, and stored on a floppy disk for analysis according to the procedure described below.

Poly(L-lysine) in solution can assume three conformations, α-helix, β-structure, and random coil, depending on the solvent conditions. It is reasonable to regard the observed spectra as the composite of the weighted linear combination of the spectra corresponding to the three conformations. Hence, the ellipticity at wavelength λ, $[\theta]_\lambda$, is expressed as

$$[\theta]_\lambda = F_\alpha [\theta]_\lambda^\alpha + F_\beta [\theta]_\lambda^\beta + F_r [\theta]_\lambda^r$$

$$F_\alpha + F_\beta + F_r = 1$$

where F_i represents the fraction of the i-th conformation where $i = \alpha$ (α-helix), β (β-structure) or r (random coil), and $[\theta]_\lambda^i$ the ellipticity of the i-th conformation at wavelength λ, which is known from the respective reference spectra. The refer-

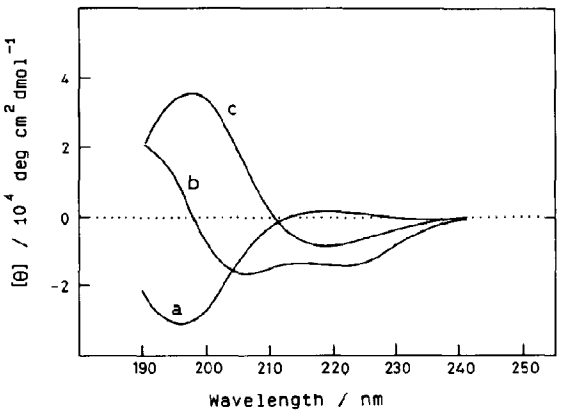

Fig. 1. CD spectra of poly(L-lysine) for random coil (a), α-helix (b) and β-structure (c) obtained under the conditions described in the text. [θ] denotes molar residue ellipticity.

ence spectra for the three conformations are shown in fig. 1, being obtained under the following conditions: α-helix, NaOH solution (pH 11.5) at 4°C [14]; β-structure, SDS solution (3.5×10^{-2} M) at 35°C [15]; random coil, Tes solution (pH 7.0) at 35°C [14]. The values of F_i, the respective fractions of the conformations observed in the CD spectra, were determined so as to minimize the quantity $\Sigma_\lambda([\theta]_\lambda^{obs} - [\theta]_\lambda)^2$, where $[\theta]_\lambda^{obs}$ is the ellipticity of the observed spectrum at wavelength λ.

CD measurements were carried out at 25°C unless otherwise stated. Ellipticity is expressed in terms of molar residue ellipticity.

3. Results and discussion

Fig. 2 shows typical CD spectra for poly(L-lysine) obtained in the presence of PS vesicles at various concentrations. One observes that with increasing PS concentration, the spectral pattern indicates transformation from a random coil to α-helix characterized by diminution of the negative peak at around 198 nm and negative peaks appearing at around 208 and 222 nm. The observed spectra were analyzed via the procedure described in section 2; since the fraction of β-structure was found to be essentially zero for all spectra of PS-poly(L-lysine) systems, the reference spectra for the random coil and α-helix were used for spectral analysis. The CD spectra observed at high lipid concentration exhibited an appreciable red shift due to turbidity of samples [16–18]. In such cases, reference spectra were used for curve fitting after carrying out appropriate correction of wavelengths. The observed and computed spectra are compared in the inset to fig. 2. Satisfactory agreement is seen between the observed and computed spectra, although a somewhat poorer level of agreement occurred at high PS concentration.

The fraction of α-helix structure for poly(L-lysine), F_α, is plotted in fig. 3 as a function of PS concentration, the concentration being expressed in terms of the molar ratio of PS to lysine residues of poly(L-lysine), i.e., $r = [PS]/[\text{lysine residue}]$. The interval between the dashed lines indicates the concentration range within which precipitation and interruption of CD measurements occur; poly(L-lysine)-induced aggregation, which takes place around $r = 1$, appears to be a general feature of negatively charged lipid vesicles, as observed previously for DLPA [12], and in the current article for PA, PS, and DMPG.

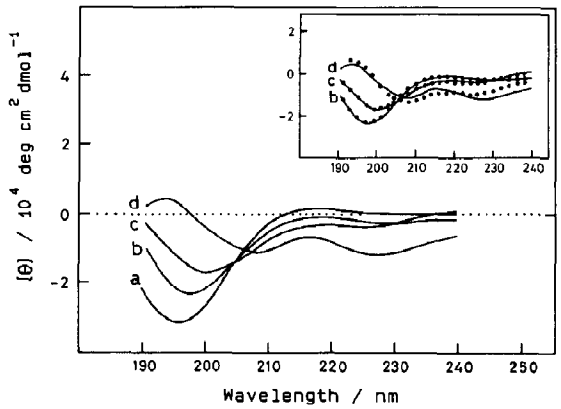

Fig. 2. CD spectra of poly(L-lysine) interacting with PS vesicles in 5 mM Tes buffer (pH 7.0) at 25°C: [poly(L-lysine)] = 2.27×10^{-4} M as lysine residues; [PS] = 0 (a), 9.16×10^{-5} M (b), 1.60×10^{-4} M (c), 3.21×10^{-4} M (d). (Inset) Comparison of observed and computed CD spectra of poly(L-lysine). Dots indicate the spectra computed using the fractions of random coil, F_r, and α-helix, F_α, determined via least-squares analysis. Values of F_r and F_α: 0.78, 0.22 (b); 0.59, 0.41 (c); 0.29, 0.71 (d); respectively.

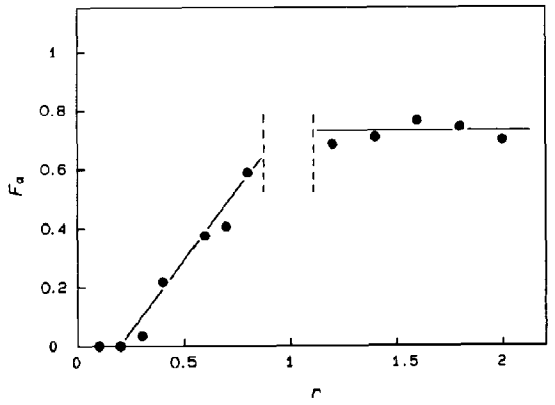

Fig. 3. Plot of fraction of α-helix of poly(L-lysine), F_α, vs. molar ratio r = [PS]/[lysine residue]. Dashed lines indicate the concentration range in which precipitation takes place.

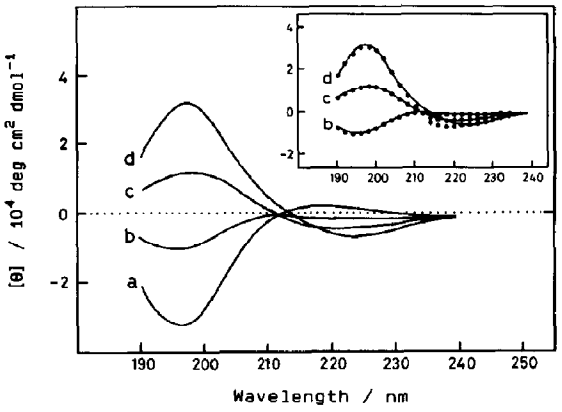

Fig. 4. CD spectra for the interaction of poly(L-lysine) with PA vesicles in 5 mM Tes buffer (pH 7.0) at 35 °C: [poly(L-lysine)] = 5.60×10^{-4} M as lysine residues; [PA] = 0 (a), 1.04×10^{-4} M (b), 2.08×10^{-4} M (c), 7.80×10^{-4} M (d). (Inset) Comparison of observed and computed CD spectra of poly(L-lysine). Dots indicate the spectra computed using the fractions of random coil, F_r, and β-structure, F_β, determined through least-squares analysis. Values of F_r and F_β: 0.68, 0.32 (b); 0.35, 0.65 (c); 0.07, 0.93 (d); respectively.

It has been reported that poly(L-lysine) assumes a β-structure when adsorbed on DLPA vesicles [12]. In contrast to DLPA, PS vesicles induce a conformational change in poly(L-lysine) from the random coil to α-helix. This is the most striking difference between DLPA and PS. It may be possible that the lipid acyl chain composition influences the ordered structure of poly(L-lysine) adsorbed on the vesicular surface; bovine brain PS is not homogeneous but, in fact, a mixture with different acyl chains containing unsaturated fatty acids, while synthetic DLPA has a uniform acyl chain. In order to ascertain the possibility of this occurring, CD spectra were recorded for the interaction of poly(L-lysine) with vesicles of PA prepared from egg yolk lecithin, which also has differing acyl chains including unsaturated fatty acids. The CD spectra observed at various PA concentrations are demonstrated in fig. 4. One observes that with increasing PA concentration, the pattern of spectra changes from the random coil to β-structure (cf. reference spectra in fig. 1). The inset to fig. 4 depicts a comparison of the observed spectra with those computed, the former being analyzed as a superposition of random coil and β-structure. Fig. 5 depicts plots for the fraction of β-structure, F_β, with respect to PA concentration. The concentration profile of F_β in fig. 5 is essentially the same as that of the poly(L-lysine)-DLPA system [12]. This demonstrates that the ordered structure of poly(L-lysine) adsorbed on the vesicular surface is rather insensitive to the acyl chain composition of the lipids. Moreover, it was observed in a previous study [12] that the F_β values obtained for DLPA vesicles were not affected significantly by temperatures below and above the gel-to-liquid-crystalline phase-transition temperature of the vesicle membrane. These facts

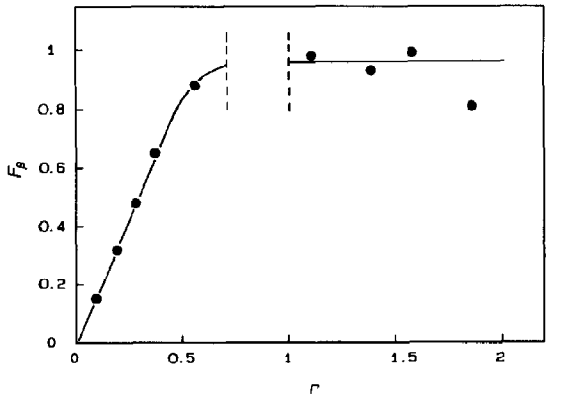

Fig. 5. Plot of the fraction of β-structure for poly(L-lysine), F_β, vs. molar ratio r = [PA]/[lysine residue]. Dashed lines indicate the precipitation region.

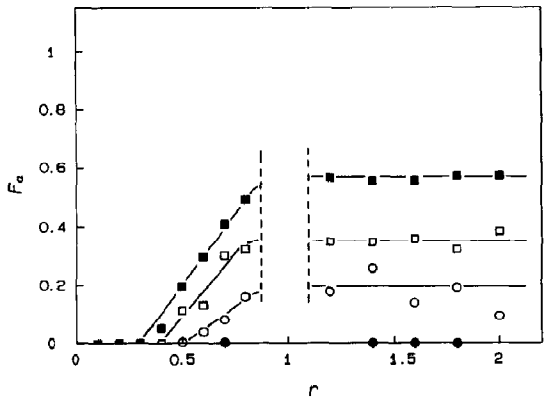

Fig. 6. Plot of F_α vs. r (molar ratio [PS]/[lysine residue]) for PS/DLPC mixed vesicles of various compositions. Composition of mixed vesicles (PS/DLPC molar ratio): 8:2 (■), 7:3 (□), 5:5 (○), and 4:6 (●). Dashed lines indicate the precipitation region.

appear to be quite reasonable, since the site of action of vesicles regarding poly(L-lysine) is considered to reside at the vesicular surface rather than with the lipid hydrocarbon interior.

The difference between PS and PA is also evident from the dependence of the fraction of ordered structure on lipid concentration. For DLPA(or PA)-poly(L-lysine) systems, F_β increased on raising the lipid concentration without a concentration delay and reached a saturation value of

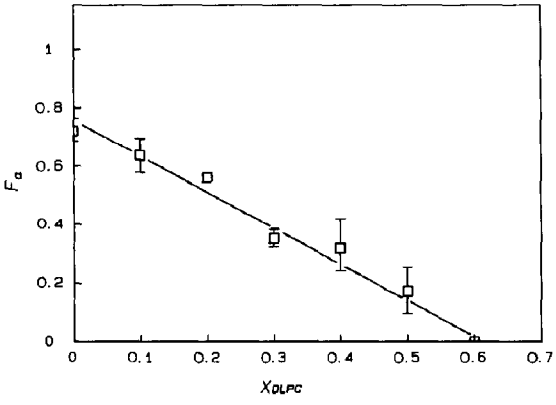

Fig. 7. Variation in saturation value of F_α with composition of PS/DLPA mixed vesicles. X_{DLPC}, mole fraction of DLPC in mixed vesicles. Vertical bars indicate scattering range of F_α for $r > 1$.

$F_\beta = 1$ (fig. 5 and ref. 12). On the other hand, for the PS-poly(L-lysine) system, a concentration delay occurs for increasing F_α, the saturation value of F_α being about 0.7. The concentration profile of F_β for DLPA-poly(L-lysine) suggested that poly(L-lysine) bound to DLPA stoichiometrically for the region $r < 1$, and that all of the polypeptides bound at the vesicular surface for $r > 1$ [12]. This may also be valid for PS-poly(L-lysine) systems due to the strong electrostatic attraction caused by the polypeptide-lipid interaction. Thus, the concentration delay for the increase in F_α and the saturation value below unity observed for PS vesicles may suggest the existence of random coiled poly(L-lysine) even at the vesicular surface of PS.

Poly(L-lysine) in suspensions of mixed vesicles comprising PS and DLPC gave rise to CD spectra indicative of random coil and α-helix, as well as the case for pure PS vesicles. As shown in fig. 6, the decrease in PS content of mixed vesicles led to enhanced concentration delay for increase in F_α and a decrease in the saturation value of F_α. These observations indicate that the effectiveness of PS at inducing poly(L-lysine) to adopt the α-helix

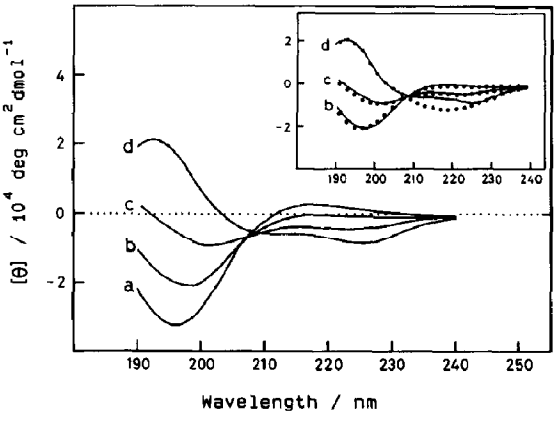

Fig. 8. CD spectra of poly(L-lysine) interacting with DMPG vesicles in 5 mM Tes buffer (pH 7.0) at 25 °C: [poly(L-lysine)] = 5.11×10^{-4} M as lysine residues; [DMPG] = 0 (a), 1.03×10^{-4} M (b), 2.07×10^{-4} M (c), 7.15×10^{-4} M (d). (Inset) Comparison of observed and computed CD spectra of poly(L-lysine). Dots indicate the spectra computed using the fractions of random coil (F_r), α-helix (F_α) and β-structure (F_β) determined from least-squares analysis. Values of F_r, F_α and F_β: 0.78, 0.10, 0.12 (b); 0.52, 0.24, 0.24 (c); 0, 0.57, 0.43 (d); respectively.

conformation is reduced on diluting PS with DLPC; note that the lipid concentration in fig. 6 is expressed as the molar ratio of PS in the mixed vesicles to lysine residues. The relation between the saturation value of F_α and composition of mixed vesicles is shown in fig. 7, where F_α is plotted vs. mole fraction of DLPC in mixed vesicles, X_{DLPC}. F_α decreases almost linearly with mole fraction of DLPC, and reaches 0 at $X_{DLPC} = 0.6$. This behavior is somewhat different from that observed for DLPA/DLPC mixed vesicles, where F_β decreased rapidly up to $X_{DLPC} = 0.5$, thereafter continuing to decrease more slowly; β-structure was retained to an appreciable extent even at $X_{DLPC} = 0.8$ [12]. The reduction in the rate of decrease and the residual F_β at high X_{DLPC} were interpreted as the result of phase separation in mixed vesicles; i.e., DLPA-rich domains are produced in mixed vesicles as a result of poly(L-lysine) binding. On the other hand, for PS/DLPC mixed vesicles, F_α decreases continuously with X_{DLPC} and finally vanishes. It may be considered from this result that poly(L-lysine) does not induce phase separation in PS/DLPC mixed vesicles to such an extent that the α-helix conformation is maintained in poly(L-lysine).

Fig. 8 shows typical CD spectra for poly(L-lysine), recorded in the presence of DMPG vesicles at various concentrations. With increasing DMPG concentration, the spectral pattern undergoes continuous transformation from a random coil to an ordered structure. The CD spectra observed at high DMPG concentrations (e.g., curve d in fig. 8) exhibit neither the typical α-helix nor β-structure (cf. reference spectra in fig. 1). The spectra observed for $r < 1$ were reproduced well as a linear combination of the three reference spectra. The spectra at high DMPG concentrations ($r > 1$) were approximated by a superposition of the α-helix and β-structure; the fraction of random coil was less than 0.05. The observed and computed spectra are compared in the inset to fig. 8. Agreement is excellent for the spectra obtained at DMPG concentrations of $r < 1$ (curves b and c, fig. 8). For $r > 1$ (curve d, fig. 8), agreement is also satisfactory, although appreciable deviation is observed within the region 225–210 nm. Based on Raman spectroscopy, Carrier and Pézolet [19] have reported that the conformation of poly(L-lysine) of M_r 150 000 is transformed into α-helix by interaction with dipalmitoylphosphatidylglycerol (DPPG) pellets. However, the CD spectra observed in DMPG vesicle suspensions cannot be reproduced satisfactorily without the introduction of β-structure together with α-helix. The discrepancy between these results may be attributable in part to the difference in the M_r values of poly(L-lysine).

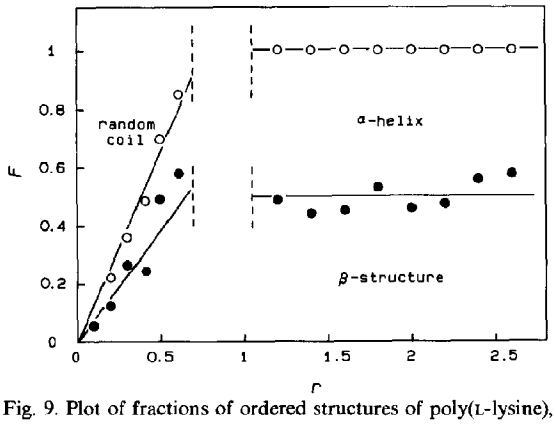

Fig. 9. Plot of fractions of ordered structures of poly(L-lysine), F, vs. molar ratio r = [DMPG]/[lysine residue]. (●) F_β, (○) $F_\alpha + F_\beta$. Dashed lines indicate the precipitation region.

Fig. 9 depicts the calculated fractions of α-helix, β-structure, and random coil as a function of DMPG concentration. As observed for the concentration profiles of F_α and F_β, no concentration delay is found and the saturation value of the fraction of ordered structure is unity ($F_\alpha + F_\beta = 1$). This indicates that all of the poly(L-lysine) bound on DMPG vesicles has an ordered structure, and is quite similar to those noted for DLPA/poly(L-lysine) and PA/poly(L-lysine) systems. The coexistence of α-helix and β-structure is quite characteristic as one of the features of DMPG; DLPA, PA and PS induced either one of the two ordered structures of poly(L-lysine). One may consider that the DMPG head group has the potential ability to induce α-helix as well as β-structure when interacting with poly(L-lysine), whereas the effects of the PA and PS head groups have a preferential effect toward the adoption of either of the two ordered structures compared to the others.

The effect of poly(L-lysine) on the bilayer phase-transition behavior of DPPG has been investigated by Carrier et al. [20]. These authors found that poly(L-lysine) of M_r 17000, comparable to the value of the poly(L-lysine) used in the present study, resulted in a multiple-step phase transition for DPPG vesicle membranes, and they suggested that the formation of distinct domains in the DPPG vesicle membrane is due to binding of poly(L-lysine). If this is also valid for DMPG, it is likely that the poly(L-lysine) bound on such domains assumes a different ordered structure. The mode of packing of the lipid molecules or their spacing, and hence the distance separating the negative charges may differ between the two distinct domains. The distance between negative charges on the vesicular surface is probably a determining factor in the process of adopting one of the two possible ordered structures, since the distance between positive charges in poly(L-lysine) may differ between the two conformations.

The form of the spectra observed for mixed vesicles comprising DMPG and DMPC also showed the coexistence of two ordered structures. The general behavior of F_α and F_β with respect to lipid concentration is similar to that observed for pure DMPG vesicles; both F_α and F_β increase linearly with r followed by saturation at $r > 1$. In fig. 10, the saturation values of F_α and F_β are plotted as a function of the mole fraction of DMPC in the mixed vesicles, X_{DMPC}.

As demonstrated in fig. 10, the saturation values of F_α and F_β remain nearly constant up to $X_{DMPC} \simeq 0.2$, and further increase in X_{DMPC} leads to the appearance of the random coil in parallel with a decrease in F_β; F_α is not altered significantly over the measured mixing range. This indicates that the β-structure is converted to random coil by dilution of DMPG with DMPC in the vesicles. In other words, the effectiveness of DMPG at inducing the β-structure of poly(L-lysine) is lowered on mixing with DMPC, whereas that for induction of α-helix is much less affected.

The present study has revealed that PS vesicles induce conformational change in poly(L-lysine) from the random coil to α-helix, DMPG vesicles leading to β-structure in parallel with the α-helix. On the other hand, poly(L-lysine) assumes the

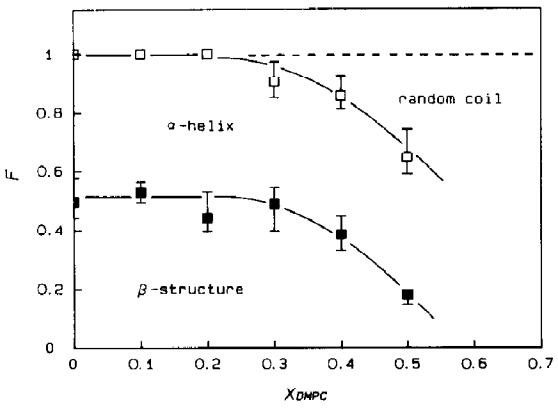

Fig. 10. Variation in saturation value of F with composition of DMPG/DMPC mixed vesicles. X_{DMPC}, mole fraction of DMPC in mixed vesicles. (■) F_β, (□) $F_\alpha + F_\beta$. Vertical bars indicate scattering range of F obtained at $r > 1$.

β-structure when adsorbed onto vesicles of DLPA [12] or PA. All of the phospholipids possess a single net negative charge, although the head group structure differs according to the phospholipid. The main driving force for inducing conformational change in poly(L-lysine) from a random coil to an ordered structure should be the charge neutralization occurring with the positive charges on lysyl side chains being counterbalanced by the negative charges on the phospholipid head groups. However, the type of ordered structure induced depends on the lipid head group structure. This suggests that the head group of a lipid participates in determining the secondary structure of poly(L-lysine) adsorbed on negatively charged phospholipid vesicles, although the details are not clear at the present stage.

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