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Conformational change of poly(L-lysine) induced by lipid vesicles of dilauroylphosphatidic acid

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The effect of negatively charged dilauroylphosphatidic acid (DLPA) vesicles on the conformation of poly(L-lysine) was investigated by circular dichroism measurements. DLPA vesicles induced a conformational change of poly(L-lysine) from the random coil to β-structure in 5 mM Tes, pH 7.0. The fraction of induced β-structure (F_β) was determined via a procedure of curve fitting of the observed spectra to the reference spectra. F_β increased linearly with the molar ratio, r, of DLPA to lysine residues up to $r = 0.7$, and reached a saturation value of 1 at $r > 1$. Within the range $0.7 \lesssim r \lesssim 1$, precipitation occurred. The effect of dilution of the negative charge on vesicle membranes was examined by mixing DLPA with dilauroylphosphatidylcholine (DLPC). Although the β-structure of poly(L-lysine) was also induced by mixed vesicles, the saturation value of F_β decreased with decreasing DLPA content in mixed vesicles. The variation in saturation value of F_β with the composition of mixed vesicles was interpreted in terms of the change in average distance between DLPA head groups in mixed vesicles.

1. Introduction

Some bioactive polypeptides undergo changes in conformation on incorporation into or onto biological membranes [1,2]. Investigation of interactions of polypeptides with phospholipid vesicles should provide insights into the functions of biological membranes or the characteristics of the interaction between proteins and biological membrane. So far polypeptide-phospholipid interactions have been extensively studied by many workers [3–9].

Circular dichroism (CD) is a powerful tool for monitoring the conformational changes of polypeptides, and has been frequently utilized in studying the conformation of polypeptide-lipid systems. In an early work in this field, Hammes and Shullery [3] reported that the α-helix was induced when random coils of poly(L-lysine) interacted with phosphatidylserine vesicles. Bach et al. [7] also observed conformational changes in random copolymers containing L-lysine in the presence of phosphatidylserine vesicles. However, these studies appear to be rather qualitative. Conformational analysis on a more quantitative basis may be required to elucidate the details of vesicle-induced conformational changes of polypeptides.

In the present work, conformational changes of poly(L-lysine) induced by dilauroylphosphatidic acid (DLPA) vesicles and DLPA-dilauroylphosphatidylcholine (DLPC) mixed vesicles were studied using CD measurements. In contrast to the case of phosphatidylserine [3], the CD spectra indicated a conformational change from the random coil to β-structure. The observed spectra were analyzed with the aid of the respective reference

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spectra, and the degree of induced structure was estimated as a function of lipid concentration by a least-squares curve-fitting method.

2. Experimental

2.1. Materials

Synthetic DLPA and DLPC were purchased from Sigma and used without further purification. Poly(L-lysine) ($M_r = 5000$–15000) was obtained from Wako as the hydrobromide salt. The poly(L-lysine) was converted to the hydrochloride by dialysis against 0.1 M HCl for 36 h, and then against 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 was determined using a modified ninhydrin method [10] after hydrolysis in 6 M HCl for 24 h.

The buffer solution employed in sample preparation was 5 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (Tes) at pH 7.0. At this pH, DLPA possesses a single negative charge [11].

A stock suspension of DLPA vesicles or DLPA-DLPC mixed vesicles in Tes solution was prepared by sonication in the cup-horn of a Branson model 185 sonifier at 35°C for about 30 min. The sizes of the vesicles thus prepared were estimated to be about 110 nm diameter for both DLPA vesicles and DLPA-DLPC (1:1) mixed vesicles by means of quasielastic light scattering.

A sample suspension was prepared by mixing the lipid suspension and poly(L-lysine) solution to give the desired lipid concentration, and sonicated again for about 5 min. The concentration of lysine residues was maintained at about 5×10^{-4} M throughout unless otherwise noted.

2.2. Methods

CD spectra were recorded with a Jasco J-40A spectropolarimeter using a 1 mm cell with water jacket, thermostatted at 35°C. The spectra from 240 to 190 nm, averaging over eight accumulations, were recorded on a pen recorder and digitized with a resolution of 1 nm using a Hewlett Packard 9111A graphics tablet connected to a Hewlett Packard model 310 microcomputer. Molecular ellipticity was expressed in terms of the mean residue ellipticity.

Observed spectra were analyzed as a linear combination of those for the three conformations: α-helix, β-structure, and random coil. Reference spectra for the three conformations were obtained under the following conditions: α-helix, NaOH solution (pH 11.5) at 4°C [12]; β-structure, SDS solution (3.5×10^{-2} M) at 35°C [13]; random coil, Tes solution (pH 7.0) at 35°C [12].

Let F_i be the fraction of the i-th conformation, where $i = \alpha$ (α-helix), β (β-structure) or r (random coil). Then, the ellipticity, $[\theta]_\lambda$, at wavelength λ 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 $[\theta]_\lambda^i$ represents the ellipticity of the i-th conformation, and is taken from the respective reference spectra. The values of F_i 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 spectra, and the summation was taken from $\lambda = 190$ to 240 at 1-nm intervals.

3. Results

Fig. 1 shows the CD spectra for poly(L-lysine) obtained in the presence of DLPA at various concentrations. The reference spectra for the β-structure and random coil are also shown in the inset to fig. 1. It is seen that with increasing DLPA concentration, the spectral pattern is transformed continuously from the random coil to β-structure. The spectra observed at high DLPA concentrations exhibit an appreciable red shift probably due to the turbidity of the sample. The red shifts of CD spectra in turbid samples have been frequently observed in biological systems [14,15], and were attributed to the multiple scattering in turbid suspensions [16]. The suspensions of DLPA vesicles themselves are slightly turbid at the concentrations employed. Mixing of

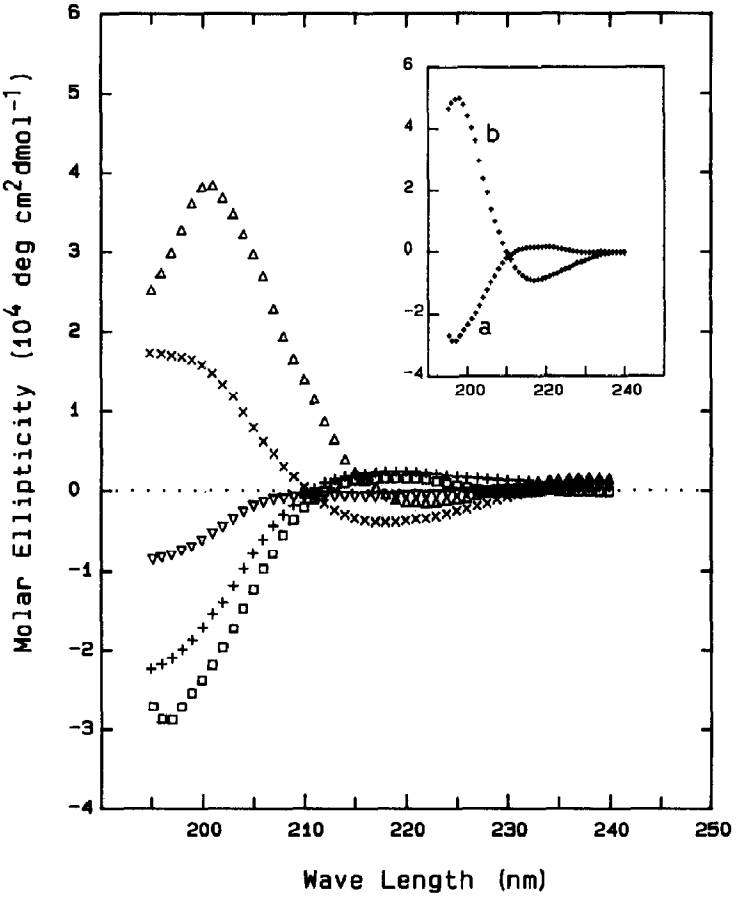

Fig. 1. CD spectra of poly(L-lysine) interacting with DLPA vesicles in 5 mM Tes buffer (pH 7.0) at 35°C. Concentration of poly(L-lysine): 5.47×10^{-4} M as lysine residues. DLPA concentrations: 0 (□), 3.87×10^{-5} M (+), 1.03×10^{-4} M (▽), 2.58×10^{-4} M (×), and 1.16×10^{-3} M (△). (Inset) CD spectra of poly(L-lysine) for random coil (a) and β-structure (b), obtained under the conditions described in the text.

DLPA suspension with poly(L-lysine) solution leads to further increase in turbidity especially for [DLPA]/[lysine residues] > 1.

The observed CD spectra were analyzed as a linear combination of reference spectra, and the fractions of the respective reference conformations were determined by the least-squares curve-fitting procedure described in section 2. It was found that the fraction of α-helix was essentially zero for all spectra obtained in this study. Thus, the reference spectra for the random coil and β-structure were used for the spectral analysis. The observed and computed spectra are compared in fig. 2 for some typical cases. The agreement of both spectra is satisfactory. As mentioned above, the red shift of the spectra was appreciable at high DLPA concentrations. In this case, the reference spectra were used for curve fitting after appropriate correction of the wavelength.

Fig. 3 plots the fraction of β-structure of poly(L-lysine), F_β, as a function of DLPA concentration. The concentration is expressed in terms of the molar ratio of DLPA to lysine residues of poly(L-lysine), i.e., r = [DLPA]/[lysine residues]. F_β increases almost linearly with increasing r up to $r \simeq 0.7$. In the range $0.7 \lesssim r \lesssim 1$, precipitation occurs, which prevents measurements of the CD spectra. At $r > 1$, F_β reaches 1. This profile of F_β

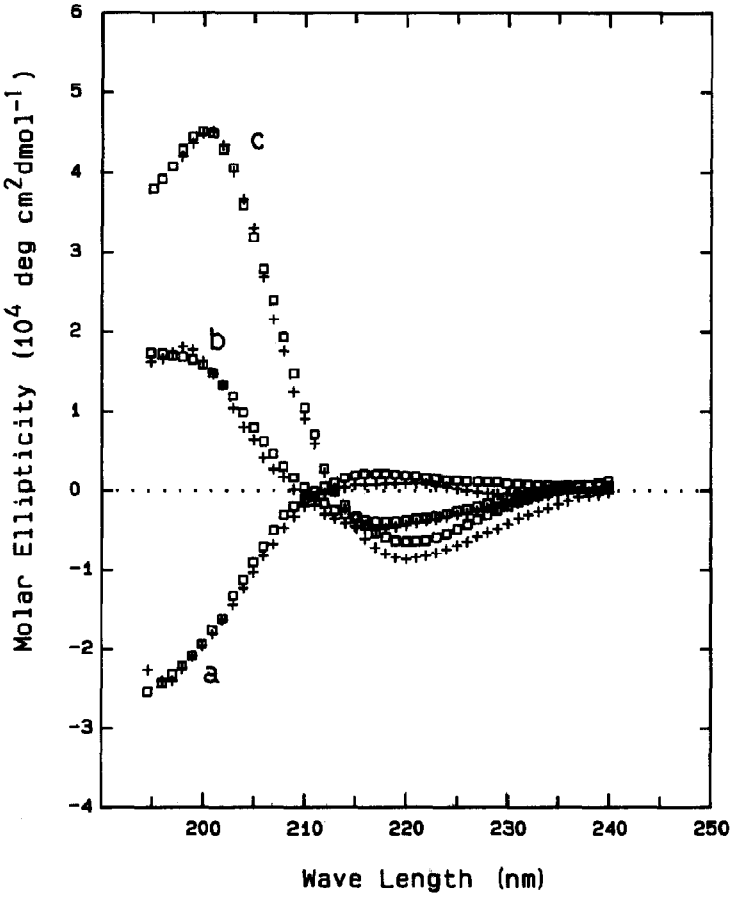

Fig. 2. Comparison of observed and computed CD spectra of poly(L-lysine). (□) Observed spectra and (+) computed spectra using the fractions of random coil, F_r, and β-structure, F_β, determined by least-squares analysis. Concentration of poly(L-lysine): 5.47×10^{-4} M as lysine residues. DLPA concentrations and values of F_r and F_β: (a) 2.58×10^{-5} M, $F_r = 0.93$, $F_\beta = 0.07$; (b) 2.58×10^{-4} M, $F_r = 0.41$, $F_\beta = 0.59$; and (c) 6.45×10^{-4} M, $F_r = 0.06$, $F_\beta = 0.94$.

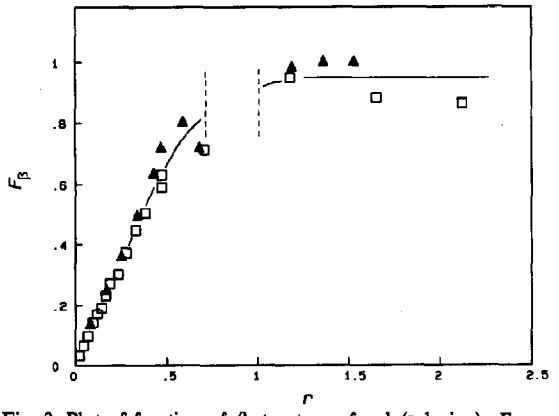

Fig. 3. Plot of fraction of β-structure of poly(L-lysine), F_β, vs. molar ratio r = [DLPA]/[lysine residues]. Concentrations of poly(L-lysine) as lysine residues: 5.47×10^{-4} M (□) and 2.95×10^{-4} M (▲). Dashed lines indicate the concentration range where precipitation takes place.

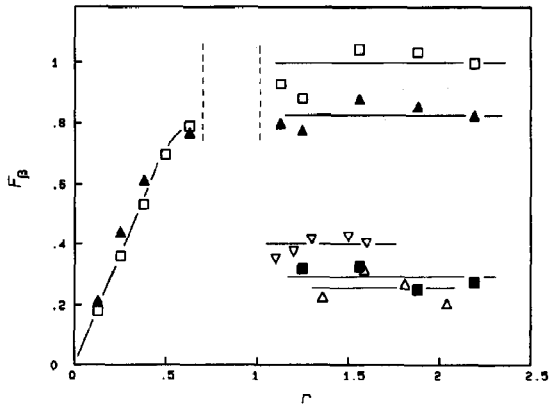

Fig. 4. Plot of F_β vs. r for DLPA-DLPC mixed vesicles of various compositions. r, molar ratio of DLPA in mixed vesicles to lysine residues. Compositions of mixed vesicles (molar ratio of DLPA to DLPC): 9:1 (□), 7:3 (▲), 5:5 (▽), 3:7 (■), and 2:8 (△). Dashed lines indicate the precipitation region.

with respect to r is independent of the poly(L-lysine) concentration; two different poly(L-lysine) concentrations give almost the same result, as shown in fig. 3.

Mixed vesicles of DLPA and DLPC also gave CD spectra of poly(L-lysine) superimposed with those of the random coil and β-structure. Fig. 4 shows plots of F_β vs. lipid concentration for mixed vesicles of various compositions. The expression of lipid concentration is the same as that used for the pure DLPA-poly(L-lysine) system; i.e., $r =$ [DLPA in mixed vesicles]/[poly(L-lysine)]. The general behavior of F_β with respect to r is similar to that observed with pure DLPA vesicles; F_β increases linearly with r up to $r \simeq 0.7$, precipitation occurs within the range $0.7 \lesssim r \lesssim 1$, and then F_β reaches the saturation value at $r > 1$. The saturation value of F_β depends strongly on the composition of the mixed vesicles. It should be noted here that a dependence of the CD spectra on time was observed under the conditions of high DLPC content (DLPA/DLPC = 5 : 5) and low r ($r < 0.7$). The CD spectra changed over about 30 h, and during the time course, a spectral pattern irreproducible by the combination of the three reference spectra was observed. However, even with mixed vesicles of high DLPC content, no time dependence was seen for the CD spectra of poly(L-lysine) when $r > 1$.

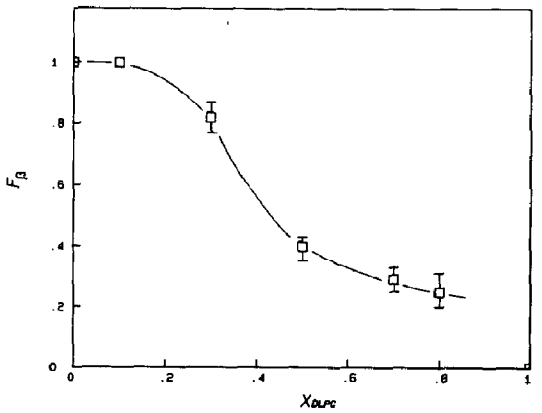

Fig. 5. Variation in saturation value of F_β with composition of mixed vesicles. X_{DLPC}, mole fraction of DLPC in DLPA-DLPC mixed vesicles.

Fig. 5 shows the dependence of the saturation value of F_β on composition of the mixed vesicles, where F_β is plotted vs. the mole fraction of DLPC in the mixed vesicles, X_{DLPC}. F_β obtained with $X_{DLPC} = 0.1$ is the same as that of pure DLPA vesicles. Further increase in X_{DLPC} leads to a decrease in F_β. Since no conformational change of poly(L-lysine) was induced by DLPC alone, the β-structure observed in the presence of mixed vesicles is attributed entirely to the interaction of poly(L-lysine) with DLPA in mixed vesicles. The results in fig. 5 may be interpreted as that the effectiveness of DLPA for inducing the β-structure of poly(L-lysine) is reduced by the dilution of DLPA with DLPC in mixed vesicles.

4. Discussion

Hammes and Shullery [3] have reported that poly(L-lysine) is transformed from the random coil to α-helix by phosphatidylserine vesicles. On the other hand, Hartmann and Galla [9] have concluded, based on a spin label study, that dipalmitoylphosphatidic acid transforms the conformation of poly(L-lysine) from the random coil to some ordered structure other than the α-helix. The present study, based on the CD spectra, revealed that poly(L-lysine) is transformed from the random coil to β-structure interacting with phosphatidic acid.

It is well known that charged surfactants induce conformational changes in charged polypeptides [17,18], the main driving force of which is charge neutralization resulting from the binding of singly dispersed surfactants to the polypeptides. For the case of a phospholipid-induced conformational change in poly(L-lysine), there remained the possibility that the lipid molecule acts in a manner similar to a surfactant molecule, i.e., singly dispersed lipid interacts with poly(L-lysine). In order to confirm the participation of vesicles instead of the singly dispersed species in conformational changes of poly(L-lysine), quasielastic light-scattering measurements were carried out with DLPA-poly(L-lysine) mixtures. The results are shown in fig. 6, where the hydrodynamic radius of a DLPA vesicle, R_h, is plotted vs. r. At low r

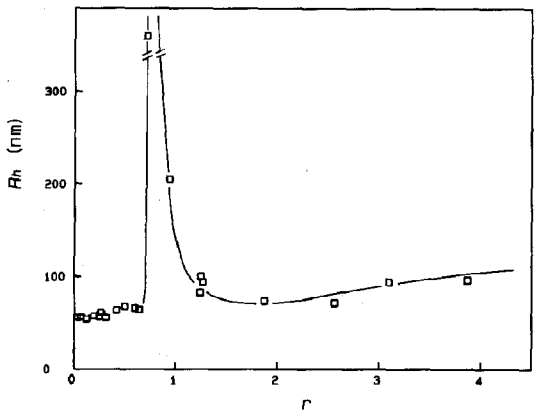

Fig. 6. Plot of hydrodynamic radius of DLPA vesicles, R_h, vs. r. R_h was determined by quasielastic light-scattering measurements. Concentration of poly(L-lysine): 5.0×10^{-4} M.

($r \lesssim 0.7$), R_h is almost constant and comparable to that for pure DLPA vesicle. An abrupt increase in R_h in the range $0.7 \leq r \leq 1$ corresponds to the occurrence of precipitation. At $r > 1$, R_h increases gradually.

Fig. 6 demonstrates that the vesicles exist throughout the concentration range of DLPA employed for CD measurements, and hence, it is suggested that the conformational change of poly(L-lysine) is induced by the interaction with DPLA vesicles. One can envisage a situation in which poly(L-lysine) is adsorbed on the vesicular surface where negative charges are distributed on a two-dimensional lattice. It is generally accepted [19,20] that lipid molecules in bilayers are packed in a hexagonal lattice below the main phase transition temperature. (The packing symmetry is considered to be disturbed above the main transition temperature, nevertheless short-range order may still exist.) Then, it can be considered that the positive charges on the side chains of the lysine residues are neutralized by negative charges distributed on the DLPA vesicle membrane, and that charge neutralization plays a dominant role in the conformational change of poly(L-lysine) from the random coil to an ordered structure. Thus, it is reasonable to assume that the two-state model is valid for the conformational state of poly(L-lysine) in a DLPA vesicle suspension, i.e., poly(L-lysine) has a random coil structure in the bulk phase and a β-structure on the surface of DLPA vesicles. According to this assumption, the linear increase in F_β with r in the range $r \lesssim 0.7$ (fig. 3) is explained as being the result of stoichiometric poly(L-lysine) binding to the DLPA vesicle and the bound lysine residue assuming the β-structure conformation. Furthermore, $F_\beta = 1$ under conditions of excess DLPA ($r > 1$) indicates that all poly(L-lysine) molecules are bound on the vesicle surface.

The saturation value of F_β obtained with DLPA-DLPC mixed vesicles decreased with increasing DLPC content in mixed vesicles (see fig. 5). If the neutralization of the positive charges on the side chains of lysine residues by the negative charges of DLPA head groups is a dominant factor in inducing the β-structure of poly(L-lysine), the distance between DLPA head groups could be the main factor in determining the saturation value of F_β, at least to a first approximation, since negative charges separated by a large distance may be less effective in the formation of a continuous β-structure for poly(L-lysine). It is interesting to compare the pitch of the β-structure with the distance between DLPA head groups in the vesicle. The established values for the pitch of β-structure are 6.5 Å (parallel β-sheet) and 7.0 Å (anti-parallel β-sheet). Based on an X-ray diffraction study, Harlos [19] has reported that the area occupied by phosphatidylethanolamine at the bilayer surface is 39 Å^2 per lipid molecule. It has also been suggested from the pressure-area curves obtained with phospholipid monolayers that the close packed area per head group is about 40 Å^2 irrespective of the type of head group [21]. According to these data on the surface area of lipid molecules at bilayer or monolayer surfaces, the distance between neighboring DLPA head groups is estimated to be about 7 Å, which is comparable to the pitch of the β-structure of polypeptides.

The dilution of DLPA by DLPC may increase the distance between neighboring negative charges, and hence may reduce the effectiveness of DLPA in inducing conformational changes in poly(L-lysine). The average distance between DLPA head groups in mixed vesicles can be roughly estimated as a function of the composition of mixed vesicles based on the simple statistical model described

below. We employ the following assumptions: (i) the β-structure extends linearly. Thus, it is sufficient to consider only the one-dimensional average distance between DLPA head groups on the surface of the mixed vesicle. (ii) DLPA and DLPC are distributed randomly over the one-dimensional lattice at an interval of a. According to these assumptions, the probability of finding the neighboring DLPA in the n-th site far from the particular DLPA is expressed by $X_{\mathrm{DLPC}}^{n-1}(1 - X_{\mathrm{DLPC}})$, where X_{DLPC} is the mole fraction of DLPC in the mixed vesicle. Then, the average distance between DLPA head groups, $\langle d \rangle$, is related to the composition of the mixed vesicle as

$$\langle d \rangle = \sum_{n=1}^{\infty} naX_{\mathrm{DLPC}}^{n-1}(1 - X_{\mathrm{DLPC}}) = \frac{a}{1 - X_{\mathrm{DLPC}}}$$

The saturation value of F_β obtained with the mixed vesicle (fig. 5) is re-plotted vs. $\langle d \rangle / a$ in fig. 7, where $\langle d \rangle / a$ represents the relative increase in average distance between DLPA head groups resulting from dilution of DLPA by DLPC in mixed vesicles. F_β is unaltered up to $\langle d \rangle \simeq 1.1a$; i.e., the separation of negative charges up to $1.1a$ retains full effectiveness in formation of the β-structure for poly(L-lysine). Further increase in $\langle d \rangle$ leads to a rapid decrease in F_β. Extrapolation of the rapidly decreasing portion (dashed line in fig. 7) predicts that the effect of DLPA on the conformational change of poly(L-lysine) diminishes at $\langle d \rangle \simeq 2.5a$.

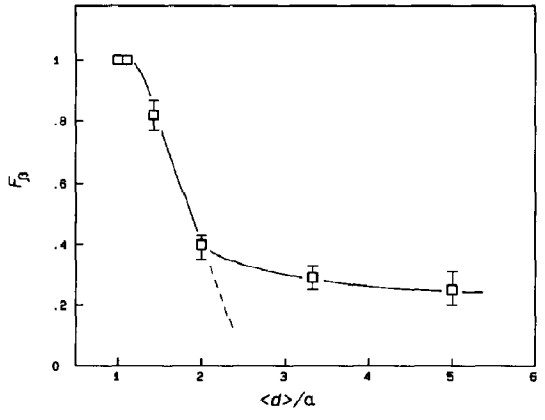

Fig. 7. Plot of saturation value of F_β vs. $\langle d \rangle / a$, the relative average distance between neighboring DLPA head groups in mixed vesicles.

However, residual effectiveness is apparently observed even at $\langle d \rangle \simeq 5a$. This is probably due to phase separation in the mixed vesicle induced by bound poly(L-lysine); i.e., DLPA is not distributed randomly but DLPA-rich domains exist in the mixed vesicles. Phase separation induced by addition of external charges has been reported for mixed vesicles of negatively charged phospholipids and phosphatidylcholine [9,22,23].

It has been shown in the present work that DLPA vesicles transform the conformation of poly(L-lysine) from the random coil to β-structure. On the other hand, the α-helix of poly(L-lysine) is induced by interaction with phosphatidylserine vesicles [3]. Furthermore, it has been demonstrated that phosphatidylserine vesicles induce the α-helix for random copolymers of L-lysine and L-phenylalanine, whereas the β-structure is found for random copolymers of L-lysine and L-tyrosine [7]. These observations indicate that the conformations of positively charged polypeptides bound on the surface of negatively charged phospholipid vesicles are not unique but depend on several factors, such as charge distribution and hydrophobicity of polypeptides, and the type of head group of the phospholipids. The results obtained with mixed vesicles of DLPA and DLPC suggest that matching of the pitch of the ordered structure of polypeptides with the distance between negative charges of lipid head groups in the bilayer is an important factor in the process of lipids inducing conformational changes in polypeptides.

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