be better studied in a chemically defined media which does not contain serum proteins. We hope to investigate this in future studies.

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pH-Dependent Interaction of Amphiphilic Polypeptide Poly(Lys-Aib-Leu-Aib) with Lipid Bilayer Membrane

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ABSTRACT: A sequential polypeptide, poly(Lys-Aib-Leu-Aib) (Aib represents 2-aminoisobutyric acid), was synthesized, and the interaction with lipid membrane was studied. Poly(Lys-Aib-Leu-Aib) was designed to take an amphiphilic structure upon the formation of α-helix. Circular dichroism of poly(Lys-Aib-Leu-Aib) in an aqueous solution showed a negative Cotton effect due to α-helix. The content of α-helix increased when the pH was raised above 7.5 or in the presence of small unilamellar vesicles composed of egg yolk lecithin. On the other hand, α-helical conformation was broken by increasing the ionic strength of solution. Carboxyfluorescein leakage from dipalmitoylphosphatidylcholine (DPPC) vesicles induced by binding of poly(Lys-Aib-Leu-Aib) to the lipid membrane was facilitated in an alkaline solution and/or in a solution of low ionic strength. These phenomena can be related to the α-helix content of the polypeptide. It was shown that poly(Lys-Aib-Leu-Aib) induced fusion of DPPC vesicles in an alkaline solution below the phase-transition temperature of the membrane. It was further shown that the aggregation and fusion of the neutral lipid membrane was regulated by changing the pH of solution.

An amphiphilic structure found in polypeptide molecules is considered to be one of the most important structural units for biological activities (Massey et al., 1981). Kaiser and Kezdy (1984) have pointed out that apolipoproteins and peptide toxins potentially form an amphiphilic α-helix, which is supposed to be favorable for the interaction with phospholipid vesicles and other amphiphilic surfaces (Segrest et al., 1973; Mao et al., 1981). It has been shown that many peptide hormones likewise take amphiphilic secondary structure, which is essential in interactions with receptor. Schwyzer (1986) has

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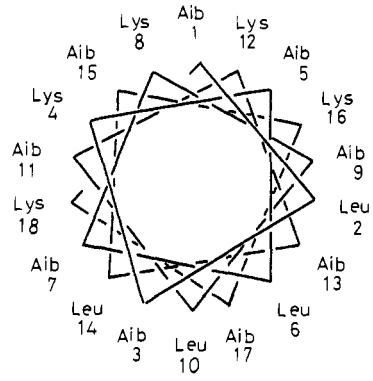

FIGURE 1: Top view of part of α-helical conformation of poly(Lys-Aib-Leu-Aib).

reported, however, that peptide hormones having an amphiphilic property bind to lipid membrane by taking a specific conformation and orientation, which affects their binding to receptor in the membrane. For example, glucagon was shown to take an amphiphilic α-helical conformation and bind to the membrane with an orientation of the helix axis parallel to the surface of the membrane (Epand et al., 1977).

Amphiphilic peptide sequence was also found in the N-terminal region of hemaglutinin, taking part in the fusogenic activity in low pH regions (White et al., 1983). A 30-residue amphiphilic peptide consisting of repeating units, Glu-Ala-Leu-Ala, was synthesized and studied on pH-dependent interactions with lipid membrane (Subbarao et al., 1987). It was shown that the binding was related with the helical content of peptide, but this peptide did not show the fusogenic activity. On the other hand, basic amphiphilic peptides, such as poly(Lys) (Gad, 1983), albumin fragment (Garcia et al., 1984), melittin (Morgan et al., 1983), polymixin B (Gad & Eytan, 1983), and gramicidin S (Eytan et al., 1988), are known as fusogens. It was postulated that the peptide molecules with an appropriate arrangement of hydrophobic units and cationic charges possess the fusogenic activity. Recently, Suenaga et al. (1989) synthesized Ac-(Leu-Ala-X-Leu)$_3$-NHCH$_3$ (X = Arg or Lys), which took an amphiphilic helical structure, and observed the fusogenic activity. It should be noted that these sequential polypeptides induce the fusion of neutral as well as acidic lipid membranes.

Despite extensive studies that have been made on the membrane fusion, the molecular mechanism is still unknown. It is therefore worthwhile to study the interaction of synthetic amphiphilic peptides with lipid membrane. However, since synthetic α-helical polypeptides are usually insoluble and aggregative, the investigations have not gone into detail. In the present investigation, an amphiphilic sequential polypeptide, poly(Lys-Aib-Leu-Aib) (Aib[1] represents 2-aminoisobutyric acid), was synthesized (Figure 1). When poly(Lys-Aib-Leu-Aib) takes an α-helical conformation in an alkaline solution, the hydrophilic Lys residues will be located on one face of the helical cylinder and the hydrophobic Leu residues on the other. The Aib residue was used because it prevents the formation of β-sheet structure (Narita et al., 1985a,b), which makes peptides insoluble. The conformational

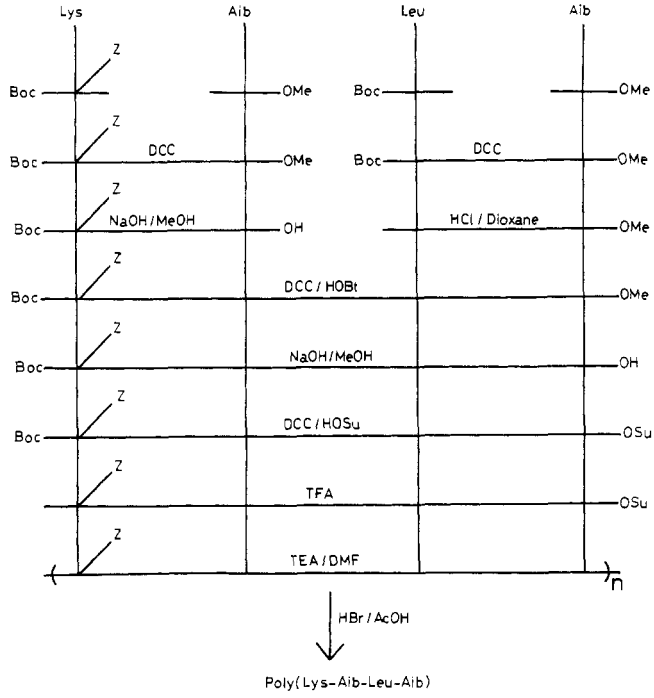

FIGURE 2: Synthetic route to poly(Lys-Aib-Leu-Aib).

change of poly(Lys-Aib-Leu-Aib) was studied by circular dichroism (CD) measurements by changing pH and ionic strength. It was shown that the aggregation and fusion of dipalmitoylphosphatidylcholine vesicles induced by poly(Lys-Aib-Leu-Aib) was pH dependent.

Experimental Procedures

Materials. Poly(Lys-Aib-Leu-Aib) was synthesized by the conventional liquid-phase method according to the synthetic scheme shown in Figure 2. Gel permeation chromatography of the protected product [poly(Lys(Z)-Aib-Leu-Aib)] on a Sephadex LH-60 column using *N*,*N*-dimethylformamide as eluant showed that the most frequent molecular weight was 5000 [taking poly(oxyethylene) as standard], indicating 3800 for poly(Lys-Aib-Leu-Aib).

Dipalmitoylphosphatidylcholine (DPPC), egg yolk phosphatidylcholine (EYPC), phosphatidylethanolamine (PE), dioleoylphosphatidylethanolamine (DOPE), lissamine Rhodamine B sulfonyl chloride (Rho-Cl), and 5(6)-carboxyfluorescein (CF) were purchased from Sigma and used without further purification. *N*-(7-Nitrobenz-2-oxa-1,3-diazo-4-yl)-dipalmitoylphosphatidylethanolamine (NBD-DPPC) was obtained from Molecular Probes Inc. and used without further purification. *N*-(Lissamine Rhodamine B sulfonyl)dioleoylphosphatidylethanolamine (Rho-DOPE) was synthesized from Rho-Cl and DOPE according to the method reported by Struck et al. (1981).

Measurements. CD spectra were measured on a JASCO J-600 spectropolarimeter using a cell with 1-cm optical path length. The concentration of polypeptide was 8.0×10^{-5} M (residue unit), and pH was adjusted by aliquots of 1 N NaOH or 1 N HCl. CD spectra of poly(Lys-Aib-Leu-Aib) in the presence of EYPC vesicles were measured after incubation for 15 min.

The α-helix content (f_H) was calculated from CD spectra according to the equation reported by Chen et al. (1972).

$$f_H = -(\theta_{222} + 2340)/30300$$

UV and fluorescence measurements were carried on a JASCO Ubest-50 and a Hitachi MPF-4 fluorescence spec-

[1] Abbreviations: Aib, 2-aminoisobutyric acid; CD, circular dichroism; CF, 5(6)-carboxyfluorescein; DOPE, dioleoylphosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; EYPC, egg yolk lecithin; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid; NBD-DPPC, *N*-(7-nitrobenz-2-oxa-1,3-diazo-4-yl)dipalmitoylphosphatidylethanolamine; Rho-DOPE, *N*-(lissamine Rhodamine B sulfonyl)dioleoylphosphatidylethanolamine; SUV, small unilamellar vesicle.

trophotometer, respectively. The stock solution of polypeptide was prepared in a trifluoroethanol (TFE) solution, and the fraction of TFE added to the vesicle suspension was within 1% of the total volume of the suspension.

CF Release from DPPC Vesicles. Carboxyfluorescein-containing DPPC vesicles were prepared according to the method reported by Barbet et al. (1984). A dry thin membrane of DPPC was dispersed in water containing CF (0.1 M) and was sonicated and centrifuged to obtain small unilamellar vesicle (SUV). Free CF was removed by gel chromatography on a Sephadex G-50 column. CF-encapsulated vesicles were dispersed in HEPES buffer (0.2 or 10 mM) with preadjusted pH and ionic strength. An aliquot of poly(Lys-Aib-Leu-Aib) in a TFE solution was added, and the fluorescence change was monitored. One hundred percent release of CF was obtained by disruption of vesicles by Triton X-100 (final concentration 0.12%) except at pH 4.3, where the 100% value was determined from the fluorescence intensity after the dispersion stood overnight at 50 °C. CF leakage during 5 min in the absence of poly(Lys-Aib-Leu-Aib) was dependent on pH and ionic strength of medium as follows: in the presence of 0.1 M NaCl, 8.9% at pH 4.3, 1.9% at pH 7.5, and 1.0% at pH 8.6; at pH 7.3, 3.7% at 0.4 M NaCl, 1.1% at 0.1 M NaCl, 2.3% at 0.01 M NaCl, and 6.8% at 0.002 M NaCl. However, these CF leakages in the absence of the polypeptide did not influence the evaluation of CF leakage induced by the polypeptide, because the latter was dominant over the former. Excitation and monitoring wavelengths of CF were 470 and 520 nm, respectively.

Aggregation of SUV. Poly(Lys-Aib-Leu-Aib) (30 μM in residue unit) was added to a dispersion of DPPC SUV (1.36 mM) at room temperature, and pH was adjusted with 1 N NaOH or 1 N HCl. Aggregation of SUV was monitored by following the absorbance at 300 nm.

Membrane Fusion. Peptide-induced fusion of lipid membrane was monitored by measuring the excitation energy transfer between NBD-DPPE and Rho-DOPE (Struck et al., 1981). Intermixing of lipid molecules due to the fusion of membranes was evaluated by the "probe dilution" assay (Duzgunes et al., 1987). DPPC SUV (0.57 mM) containing 1 mol % of NBD-DPPE and Rh-DOPE was incubated at pH 6.0 with different amounts of DPPC SUV followed by the addition of poly(Lys-Aib-Leu-Aib) (1.5×10^{-4} M in residue unit). pH was adjusted with 1 N NaOH or 1 N HCl, and the ratio of fluorescence intensities of NBD-DPPE at 530 nm and of Rh-DOPE at 593 nm were measured. Excitation wavelength was 450 nm.

Results

Conformation. Conformation of poly(Lys-Aib-Leu-Aib) in aqueous solution of varied pH was studied by CD measurement (Figure 3). In these spectra, the doubled minima at 208 and 222 nm were commonly observed, which are assigned to α-helical conformation. The intensity of Cotton effect at 222 nm was plotted against pH (Figure 4). The α-helix content remained constant below pH 5.8 and above pH 7.6 with a sharp conformational transition in the pH region between 5.8 and 7.6.

The ionic strength of solution also affected the stability of α-helix conformation of the peptide. Figure 5 shows the change in CD spectra of poly(Lys-Aib-Leu-Aib) with the addition of NaCl. The intensity of Cotton effect at 222 nm decreased as the NaCl concentration was increased at pH 8.8. On the other hand, the Cotton effect increased the intensity with the addition of a small amount of NaCl and then decreased with further additions of NaCl at pH 6.3.

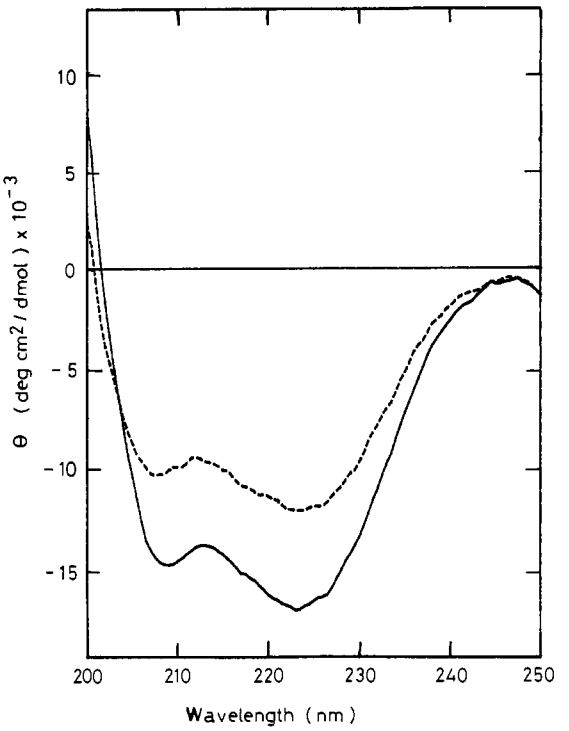

FIGURE 3: CD spectra of poly(Lys-Aib-Leu-Aib) in aqueous solution at pH 6.4 (---) and pH 7.6 (—).

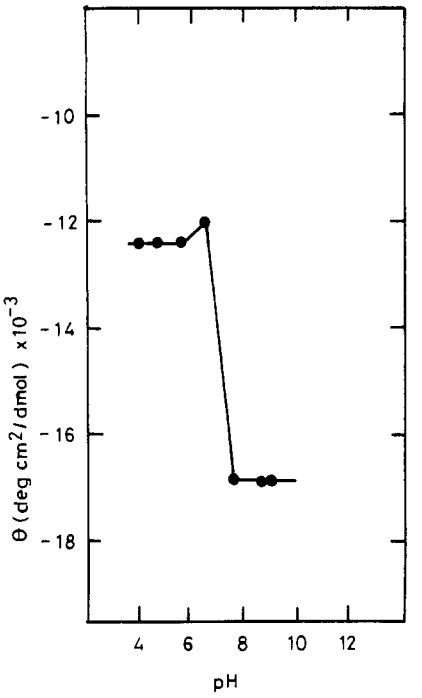

FIGURE 4: pH dependence of molar ellipticity at 222 nm of poly(Lys-Aib-Leu-Aib) in water.

CD spectra of poly(Lys-Aib-Leu-Aib) were measured in the presence of various concentrations of EYPC vesicles (Figure 6). The Cotton effects of poly(Lys-Aib-Leu-Aib) became more negative with increasing amounts of EYPC vesicles at both pH 6.8 and 8.8 (Table I). These results indicate that α-helical conformation of poly(Lys-Aib-Leu-Aib) is stabilized by binding to the lipid bilayer membrane.

CF Leakage. The interaction of poly(Lys-Aib-Leu-Aib) with the lipid bilayer membrane was investigated by measuring CF leakage from DPPC vesicles. The addition of poly(Lys-Aib-Leu-Aib) to CF-containing DPPC vesicles increased CF leakage in a pH-dependent manner. Figure 7 shows CF

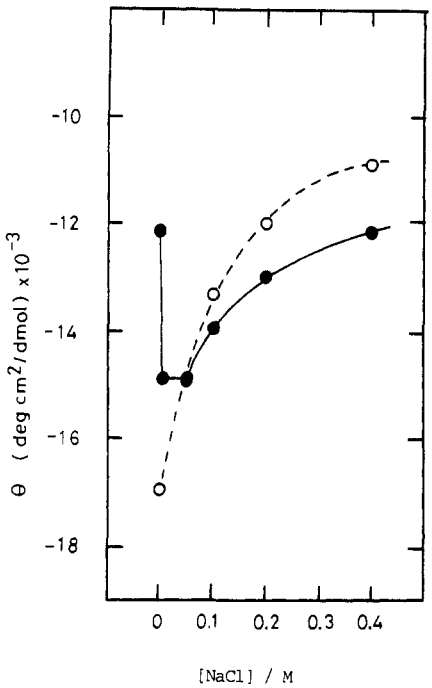

FIGURE 5: Change of molar ellipticity at 222 nm of poly(Lys-Aib-Leu-Aib) in water with varying NaCl concentration at pH 6.3 (●) and at pH 8.8 (○).

Table I: Molar Ellipticity n–π* Transition of Poly(Lys-Aib-Leu-Aib) in the Presence of Various Amounts of EYPC SUV

[EYPC] (M)	[EYPC]/[amino acid residue]	θ (deg cm² cmol⁻¹) pH 6.3	pH 8.6
0	0	12000	17000
8.2×10^{-5}	1	15500	20000
2.1×10^{-4}	2.5	19000	21400

leakage in the presence of polypeptide added at various pH regions. An abrupt increase of CF leakage was observed immediately after the addition of polypeptide. This initial rapid leakage became more significant as pH of the solution increased.

On the other hand, CF leakage was suppressed with increasing NaCl concentrations at pH 7.3 (Figure 8).

Aggregation of Vesicles. The turbidity of the suspension of DPPC vesicles was monitored in the presence of poly(Lys-Aib-Leu-Aib) in varying pH regions. Only a little change in optical absorbance at 300 nm was detected in acidic regions, whereas the absorbance increased strongly when the pH was raised to 7.5, which is indicative of aggregation of vesicles (Figure 9). The aggregate formed at pH 7.5 was partly dissociated by lowering the pH to 6.2. It is noteworthy that the aggregation of vesicles was observed only below the phase-transition temperature of the membrane and at low ionic strengths.

Fusion of Vesicles. The excitation energy transfer from NBD-DPPE to Rho-DOPE in lipid membrane was used to estimate the extent of fusion of vesicles. The energy transfer can be expressed by the ratio of fluorescence intensities, R ([fluroescence intensity of NBD-DPPE]/[fluorescence intensity of Rho-DOPE]) (Conner et al., 1984). When the vesicles containing both probes are fused with probe-free DPPC vesicles, the R value will increase due to the dilution of probes in membrane. The R value increased in the presence of poly(Lys-Aib-Leu-Aib) in an alkaline solution, whereas it did not change so much at pH 6.0. As shown in Figure 10, the R value increased with increasing amounts of DPPC vesicles

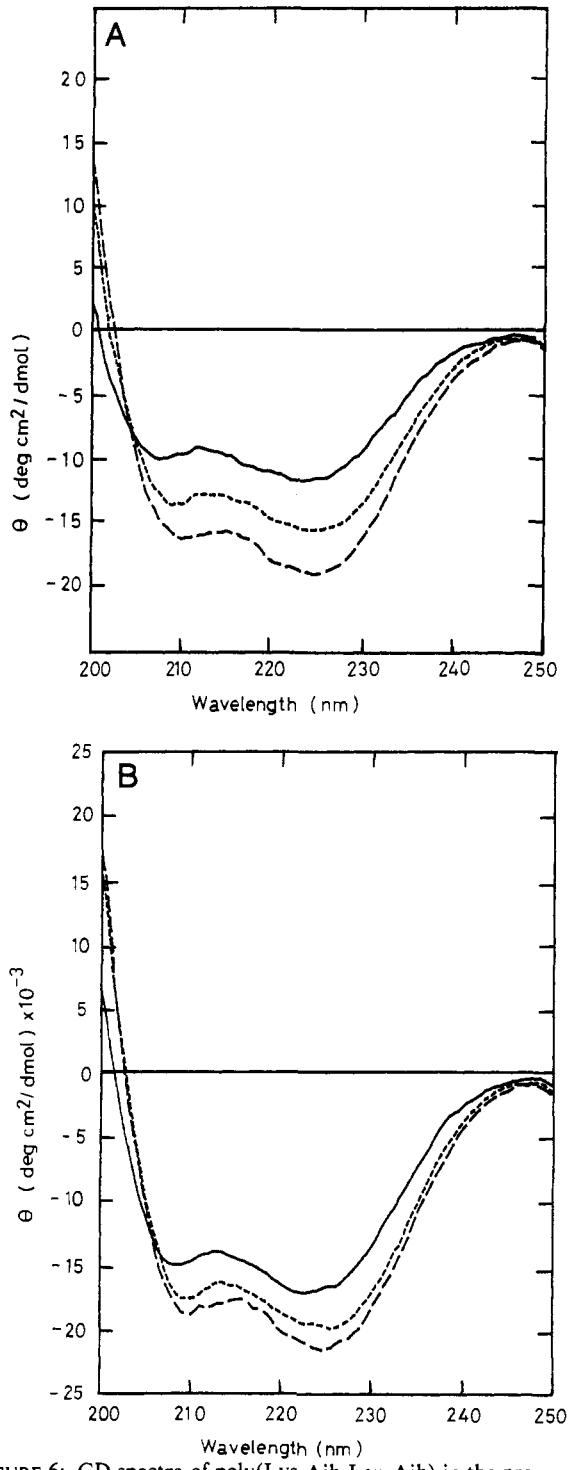

FIGURE 6: CD spectra of poly(Lys-Aib-Leu-Aib) in the presence of EYPC vesicles: (···) 2.1×10^{-4} M; (- - -) 4.1×10^{-4} M. (—) In the absence of EYPC vesicles. (A) At pH 6.3; (B) at pH 8.8.

added to the vesicles containing the probes. It was confirmed that the R value did not change under the same conditions without the addition of the probe-free DPPC vesicles.

Discussion

The Aib residue was introduced into the present polypeptide, because it suppresses the formation of β-sheet structure. The CD spectrum of poly(Lys-Aib-Leu-Aib) showed double minima in an aqueous solution, which is a pattern typical of α-helix. On the other hand, the CD spectrum of a comparative polypeptide poly(Lys-Ala-Leu-Ala), which was synthesized in the present investigation, consisted of a strong and a weak negative Cotton effect at 208 ($\theta = 3.8 \times 10^4$) and 222 nm (θ

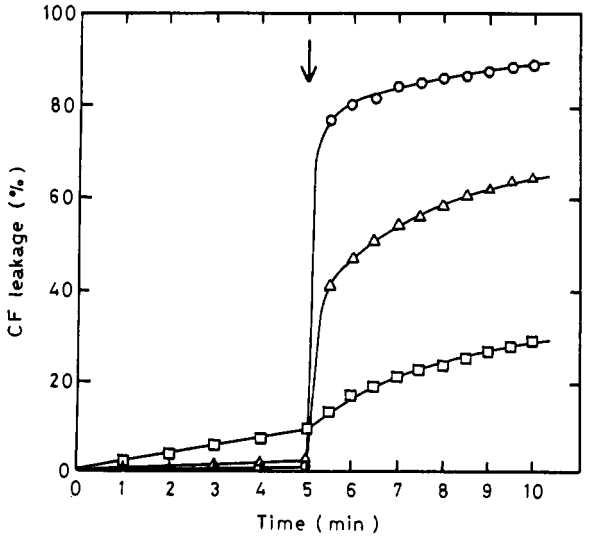

FIGURE 7: CF leakage from DPPC SUV induced by poly(Lys-Aib-Leu-Aib) in aqueous solution containing HEPES (10 mM) and NaCl (0.1 M) at pH 8.6 (○), pH 7.5 (△), and pH 4.3 (□). [Poly(Lys-Aib-Leu-Aib)] = 6×10^{-6} M (in residue unit). [DPPC] = 1.7×10^{-5} M. 35 °C. The addition of the polypeptide is indicated by an arrow.

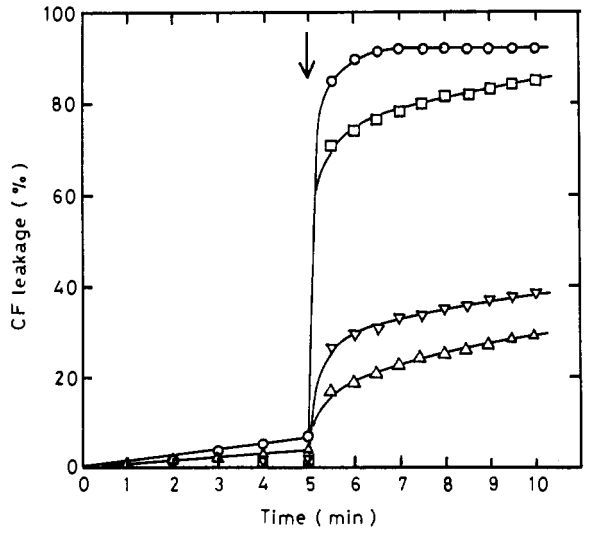

FIGURE 8: CF leakage from DPPC SUV induced by poly(Lys-Aib-Leu-Aib) in HEPES (0.2 mM) buffer solution at pH 7.3 in the presence of varying concentrations of NaCl: (○) 2 mM; (□) 10 mM; (▽) 0.1 M; (△) 0.4 M. [Poly(Lys-Aib-Leu-Aib)] = 3×10^{-6} M (in residue unit). [DPPC] = 1.7×10^{-5} M. 35 °C. The addition of the polypeptide is indicated by an arrow. The CF leakage induced by the polypeptide at 0.1 M was lower than that at 0.1 M in Figure 7. The difference is ascribed to the change in the helix content between pH 7.5 and 7.3, because the stability of helix changed markedly in this region.

= 1.5×10^4), respectively. This spectral behavior is supposedly due to the distortion of α-helix of the latter. Therefore, Aib-containing peptide should facilitate the formation of α-helical conformation in solution as compared with Ala-containing peptide. Although shorter peptides containing Aib residues have been reported to take 3_{10} helix conformation (Benedetti et al., 1982; Francis et al., 1983), longer peptides tend to take an α-helical structure (Vijayakumar et al., 1984; Bosch et al., 1985). The molecular weight of the polypeptide should be high enough for taking an α-helical conformation.

The α-helix content of poly(Lys-Aib-Leu-Aib) changed in the pH region between 6.4 and 7.6 and increased in an alkaline solution. The increase of α-helix content could be ascribed to deprotonation of side-chain ammonium groups of Lys

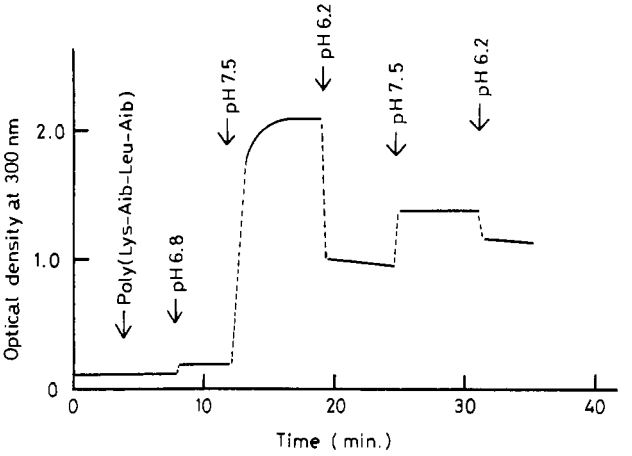

FIGURE 9: pH-dependent aggregation and dissociation of DPPC SUV in the presence of poly(Lys-Aib-Leu-Aib) at room temperature. [Poly(Lys-Aib-Leu-Aib)] = 3.0×10^{-5} M (in residue unit). [DPPC] = 1.4×10^{-4} M. The initial pH was 6.0.

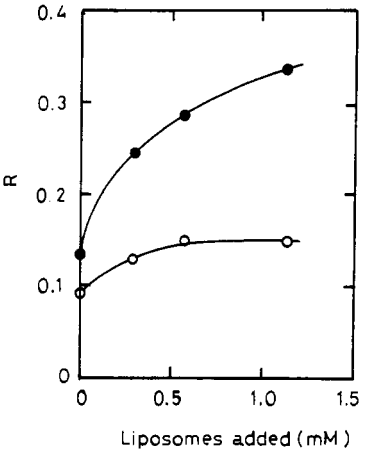

FIGURE 10: Dilution of NBD-DPPE and Rho-DOPE by intermixing of lipid molecules between probe-containing DPPC SUV and probe-free DPPC SUV as monitored by the ratio of emission intensities of NBD-DPPE and Rho-DOPE, R. (○) At pH 6.3; (●) at pH 9.0 ± 0.5. [Poly(Lys-Aib-Leu-Aib)] = 1.5×10^{-4} M (in residue unit).

residues arrayed on one side of the helical cylinder to reduce electrostatic repulsions. However, the change in the stability of α-helix occurred at a much lower pH region (around pH 7) than that of poly(lysine) (pH 9.8) (Kakiuchi & Akutsu, 1981). It occurred at the pH region much closer to the pK_a of the N-terminal α-amino group. The positive charge at the N-terminal of a polypeptide chain has been shown to destabilize α-helix due to unfavorable interactions with the macrodipole of α-helix (Shoemaker et al., 1987). It was therefore concluded that deprotonation of the N-terminal ammonium group should be the major reason for the increase of α-helical conformation.

The α-helix content was estimated to be around 32% under acidic conditions, which is higher than that of poly(Lys) in acidic regions. The higher helix content of poly(Lys-Aib-Leu-Aib) in an acidic solution than that of poly(Lys) should be due to the low density of positive charges along the polypeptide chain and to the presence of α-helix-forming Leu and Aib residues (Narita et al., 1985). The molar ellipticity at 222 nm of poly(Lys-Aib-Leu-Aib) was −13 900 deg cm^2 dmol^{-1} at pH 6.3 in the presence of 0.1 M NaCl, which is more negative than the reported values of a sequential polypeptide composed of Glu-Ala-Leu-Ala (−11 500 deg cm^2 dmol^{-1} at pH 5.1 in the presence of 0.1 M KCl) (Subbarao et al., 1987) and of Ac-(Leu-Ala-Arg-Leu)$_4$-NHCH$_3$ (about −10 000 deg cm^2

$dmol^{-1}$ in water) (Suenaga et al., 1989), indicating that poly(Lys-Aib-Leu-Aib) has a higher tendency for taking α-helical conformation.

The effect of ionic strength on the stability of α-helix is complicated. At pH 6.4, a small amount of NaCl (1.4 mM) increased the α-helix content, but the latter decreased with further increase of the NaCl concentration. On the other hand, at pH 8.6 the α-helix content decreased with increasing concentrations of NaCl. It is considered that the electrostatic repulsion between the positive charges should be reduced by the addition of NaCl, and hence the α-helix content increases. However, a large amount of NaCl should destroy the intramolecular hydrogen bonds, resulting in the decreased α-helix content. The α-helix content at high salt concentrations was lower in an alkaline solution than that in an acidic solution. The intermolecular electrostatic repulsions should be reduced in an alkaline solution with high salt concentrations. This change should promote aggregation of polypeptides, resulting in decreased α-helix content. In practice, the analysis using gel chromatography indicated the formation of aggregates at high ionic strengths at pH 7.2, but no aggregates were formed at low ionic strengths. (The polypeptide was eluted faster through a gel chromatography column in the presence of 0.5 M NaCl at pH 7.2 than without NaCl. The apparent molecular weight at 0.5 M NaCl was 4-fold larger than that without NaCl.)

When poly(Lys-Aib-Leu-Aib) was incubated for 15 min with EYPC vesicles either at pH 6.5 or at pH 8.8, the α-helix content increased. This observation is consistent with other basic amphiphilic peptides, which increased α-helix content when they were bound to the lipid membrane (Kanellis et al., 1980; Lee et al., 1986). The α-helix conformation should be facilitated in the lipid membrane, because the polar amide groups become shielded by intramolecular hydrogen bondings and energetically stable (Von Heijne & Blomberg, 1979).

The profile of peptide-induced leakage of CF is composed of two stages, i.e., an abrupt increase immediately after the addition of polypeptide and a subsequent moderate increase. The initial leakage is ascribed to the transient disturbance in membrane structure upon the binding of polypeptide (Yagi et al., 1988). Therefore, the extent of leakage could be correlated with the binding strength of polypeptide to the membrane. Since the peptide-induced leakage was significant under the conditions of high pH and low ionic strength, which are favorable for the polypeptide to take amphiphilic α-helical conformation, binding strength of the polypeptide to the lipid membrane should be enhanced under these conditions.

Peptide-induced aggregation of DPPC vesicles was observed in an alkaline solution, where the α-helix content of the polypeptide is high. Since the change of turbidity was not completely reversible with changing pH, membrane fusion should have occurred to some extent. In fact, the mixture of probe-free and probe-containing vesicles showed the membrane fusion induced by poly(Lys-Aib-Leu-Aib) in an alkaline solution.

It should be noted, however, that the aggregation and fusion of vesicles does not seem to occur simply by binding of the polypeptide to the lipid membrane. It occurred only in an alkaline solution below the phase-transition temperature of the membrane, but did not occur above the phase-transition temperature, although the polypeptide increased the α-helix content in the presence of liquid-crystalline membrane under acidic condition and induced CF leakage from DPPC vesicles above the phase-transition temperature. This discrepancy indicates that the binding of poly(Lys-Aib-Leu-Aib) to the lipid membrane does not necessarily induce aggregation or fusion of the lipid membrane. It is therefore considered that poly(Lys-Aib-Leu-Aib) with low α-helix content is bound so loosely to the lipid membrane that it is not sufficient to induce aggregation and fusion of vesicles. On the other hand, those polypeptides that have high α-helix content cross-link several vesicles, thereby disturbing the membrane structure. The defect formed in the lipid membrane upon binding of the polypeptide in this case should be more significant below the phase-transition temperature than above the phase-transition temperature, resulting in fusion of neutral lipid membranes (Boni et al., 1984).

The α-helix content increased from 55% at pH 6.3 to 63% at pH 8.8 in the presence of EYPC vesicles ([EYPC]/[Lys-Aib-Leu-Aib] = 2.5). Therefore, about a 10% change of α-helix content significantly affects the mode of interaction of the polypeptide with lipid membrane. It is of interest to note that ionization and deionization at the NH_2 terminus of the poly(Lys-Aib-Leu-Aib) chain according to pH change should be responsible for the change of α-helix content and different degrees of interactions with lipid membrane, leading to aggregation and fusion of DPPC vesicles.

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Interactions of Serum Proteins with Small Unilamellar Liposomes Composed of Dioleoylphosphatidylethanolamine and Oleic Acid: High-Density Lipoprotein, Apolipoprotein A1, and Amphipathic Peptides Stabilize Liposomes†

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ABSTRACT: Small unilamellar liposomes composed to dioleoylphosphatidylethanolamine (DOPE) and oleic acid (OA) are stabilized by incubation with normal human serum or plasma [Liu, D., & Huang, L. (1989) *Biochemistry 28*, 7700-7707]. The present report describes a systematic study of interactions of purified serum proteins and lipoproteins with these liposomes. Albumin destabilized liposomes by extracting OA from the liposomes, whereas immunoglobulins and lipoproteins (HDL, LDL, and VLDL) had no effect. However, HDL and, to some extent, VLDL showed a rapid stabilization activity against the lytic effect of albumin. HDL added together with or shortly after the addition of albumin completely abolished the liposome leakage and aggregation effects induced by albumin. SDS-PAGE analysis of the HDL-stabilized liposomes revealed that apolipoprotein A1 was associated with liposomes. Purified apolipoprotein A1, but not a lipid mixture resembling the lipid composition of HDL, showed comparable liposome stabilization activity as HDL. Furthermore, synthetic peptides resembling the amphipathic helices found in apolipoprotein A1 also showed strong liposome stabilization activity. Peptides which were able to form amphipathic helixes of a wedge shape were more effective stabilizers than those which could not. These data indicate that HDL plays a major role in human serum or plasma for the liposome stabilization activity. HDL exerts its activity probably by the interactions of the amphipathic helices of apolipoprotein A1 with the hydrophobic voids found on the outer surface of the highly curved, small liposomes.

One of the crucial aspects of using liposomes as a drug delivery vehicle is to understand their interactions with plasma or serum components. Previous work has revealed that a number of serum proteins become associated with liposomes [for reviews, see Juliano and Lin (1980) and Senior (1987)]. For example, albumin, immunoglobulins, fibronectin, and other serum proteins are found to coat the surfaces of multilamellar liposomes composed of phosphatidylcholine (PC)[1] and cholesterol, and additionally containing phosphatidylserine or stearylamine (Juliano & Lin, 1980). Interaction of HDL with multilamellar liposomes composed of PC causes the lysis of liposomes and transfer of liposomal PC to HDL (Tall & Green, 1981; Kirby et al., 1980; Senior et al., 1983). Insertion of apolipoprotein(s) into the membrane of small unilamellar liposomes at or near the chain-melting temperature causes a rapid leakage of the entrapped contents (Weinstein et al., 1981; Scherphof et al., 1984). Activated complement components also induce liposome leakage by forming pores in the liposome membrane (Hexby et al., 1969). Some yet unidentified serum protein(s) is (are) likely to be opsonin(s), which promote(s) the uptake of liposome by macrophage, primarily those in liver and spleen (Ivanov et al., 1985).

It is well-known that liposomes composed of unsaturated PC are not stable in serum or plasma, unless cholesterol is

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[1] Abbreviations: DOPE, dioleoylphosphatidylethanolamine; OA, oleic acid; PC, phosphatidylcholine; BSA, bovine serum albumin; HDL, high-density lipoprotein(s); LDL, low-density lipoprotein(s); VLDL, very low density lipoprotein(s); PBS, phosphate-buffered saline; Apo A1, apolipoprotein A1; Igs, human immunoglobulins.

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