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Interactions of Synthetic Peptide Analogs of the Class A Amphipathic Helix with Lipids

EVIDENCE FOR THE SNORKEL HYPOTHESIS*

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Class A amphipathic helixes present in exchangeable plasma apolipoproteins are characterized by the location of positively charged amino acid residues at the non-polar-polar interface and negatively charged amino acid residues at the center of the polar face. The objectives of the present study were: (i) to investigate the role of hydrocarbon side chain length of the interfacial positively charged amino acid residues in the lipid affinity of class A amphipathic helixes, and (ii) to investigate the importance of the nature of interfacial charge in the lipid affinity of class A amphipathic helixes. Toward this end, lipid interactions of the following two analogs of the class A amphipathic helix, Ac-18A-NH_2 (acetyl-Asp-Trp-Leu-Lys-Ala-Phe-Tyr-Asp-Lys-Val-Ala-Glu-Lys-Leu-Lys-Glu-Ala-Phe-NH_2), and Ac-18A(Lys>Haa)-NH_2 (acetyl-Asp-Trp-Leu-Haa-Ala-Phe-Tyr-Asp-Haa-Val-Ala-Glu-Haa-Leu-Haa-Glu-Ala-Phe-NH_2) (Haa = homoaminoalanine), were studied. The side chain of Haa has two CH_2 groups less than that of lysine. The lipid affinities of these two peptide analogs were compared with that of Ac-18R-NH_2, an analog of Ac-18A-NH_2 with positions of the charged amino acid residues reversed. The techniques used in these studies were circular dichroism, fluorescence spectroscopy, right-angle light scattering measurements, and differential scanning calorimetry. The results of these studies indicated the following rank order of lipid affinity: Ac-18A-NH_2 > Ac-18A(Lys>Haa)-NH_2 > Ac-18R-NH_2. These results are in agreement with the "snorkel" model proposed earlier to explain the higher lipid affinity of class A amphipathic helixes (Segrest, J. P., Loof, H. D., Dohlman, J. G., Brouillette, C. G., and Anantharamaiah, G. M. (1990) *Proteins Struct. Funct. Genetics* 8, 103–117). In addition, it was observed from the differential scanning calorimetry studies that Ac-18A-NH_2 and Ac-18A(Lys>Haa)-NH_2 interact more strongly than Ac-18R-NH_2 with negatively charged dimyristoyl phosphatidylglycerol. The weaker interaction of Ac-18R-NH_2 with dimyristoyl phosphatidylglycerol is suggested to be due to electrostatic repulsion between the negatively charged lipid and the interfacial negative charges of the peptide.

Exchangeable plasma apolipoproteins are protein detergents and are responsible for carrying lipids in circulation in the form of lipoproteins. There is now considerable evidence that the structural motif that is responsible for their detergent property is the amphipathic α-helix (1). While many other proteins and biologically active peptides have been found to possess this secondary structural motif, the exchangeable apolipoproteins are unique in that they possess multiple copies of amphipathic α-helixes with positively charged amino acid residues at the non-polar-polar interface and negatively charged amino acid residues at the center of the polar face (2). The amphipathic α-helixes with this kind of distribution of charged amino acid residues in the polar face have been classified as class A (3–5). The lipid-associating properties of exchangeable apolipoproteins are believed to reside predominantly in the amphipathic helical domains possessing the class A motif (3).

On the basis of the key structural features predicted for the lipid-associating properties of the amphipathic helix (1, 2), many laboratories, including ours, have studied structural and functional properties of *de novo* designed peptide analogs of the amphipathic helix (6–16). We have addressed the question whether or not the location of charged amino acid residues on the polar face of the class A amphipathic helical peptide play a role in determining its lipid affinity (17–19). A model peptide with 18 amino acid residues was designed to mimic the general features of the amphipathic helix described in the original model (2, 17). The model peptide 18A, with primary sequence Asp-Trp-Leu-Lys-Ala-Phe-Tyr-Asp-Lys-Val-Ala-Glu-Lys-Leu-Lys-Glu-Ala-Phe, in the helical wheel representation has positively charged Lys residues at the non-polar-polar interface and negatively charged Asp and Glu at the center of the polar face (17). To address the question of contribution of interfacial Lys residues to the lipid affinity of 18A, a peptide with the same amino acid composition, but the positions of charged amino acid residues reversed, was synthesized. The peptide reverse-18A (18R) had the following amino acid sequence: Lys-Trp-Leu-Asp-Ala-Phe-Tyr-Lys-Asp-Val-Ala-Lys-Glu-Leu-Glu-Lys-Ala-Phe. A helical wheel representation of this sequence shows negatively charged amino acid residues Asp and Glu at the non-polar-polar interface and positively charged Lys residues at the center of the polar face (17). Studies on these two peptides and their analogs showed that class A amphipathic peptides with positively charged residues at the non-polar-polar interface and negatively charged residues at the center of the polar face have higher lipid affinity compared with the corresponding charge-reversed peptide analogs (17–20).

To explain the higher lipid affinity of class A amphipathic helixes, the "snorkel" model was proposed (3). The bulk of the van der Waals surface area of the positively charged lysine and arginine residues is hydrophobic. In the snorkel model, it was proposed that the interfacial positively charged amino acid residues of the peptide, when associated with lipid, extend toward the polar face of the helix to insert their charged moieties into the aqueous environment for solvation. Thus, in the snorkel orientation, the entire uncharged van der Waals surface of the class A amphipathic helix is buried within the hy-

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drophobic interior of a lipid bilayer.

Apolipoprotein A-I (apoA-I),[1] the major protein constituent of plasma high density lipoproteins, and class A amphipathic helical peptide analogs have been shown to stabilize the bilayer structure of phospholipids, and these properties were correlated to their ability to protect against lytic peptide-induced erythrocyte lysis (21). These molecules also exhibit anti-viral and anti-inflammatory properties and protect fatty acid containing dye-entrapped phospholipid vesicles from albumin-induced leakage (22–24). These properties were attributed to the snorkeling effect of interfacial Lys residues in these molecules. This snorkeling effect was thought to create a "wedge" cross-sectional shape which is responsible for the stabilization of phospholipid vesicles (21, 24). It was, therefore, important to determine if indeed the longer hydrocarbon side chain of interfacial Lys residues increases the lipid-associating ability, which is a key determinant of the properties of apoA-I and the corresponding peptide analogs.

Earlier, we reported that N- and C-terminal protection of 18A drastically increased its helicity as well as lipid affinity and the resulting peptide, Ac-18A-NH_2 (acetyl-Asp-Trp-Leu-Lys-Ala-Phe-Tyr-Asp-Lys-Val-Ala-Glu-Lys-Leu-Lys-Glu-Ala-Phe-NH_2), closely mimicked the properties of apoA-I (25). In the present report, we compare the lipid affinity of Ac-18A(Lys>Haa)-NH_2 (acetyl-Asp-Trp-Leu-Haa-Ala-Phe-Tyr-Asp-Haa-Val-Ala-Glu-Haa-Leu-Haa-Glu-Ala-Phe-NH_2) with that of Ac-18A-NH_2 and Ac-18R-NH_2 (acetyl-Lys-Trp-Leu-Asp-Ala-Phe-Tyr-Lys-Asp-Val-Ala-Lys-Glu-Leu-Glu-Lys-Ala-Phe-NH_2). The side chain of Haa residue has two methylene groups less than that of Lys. The peptide Ac-18A(Lys>Haa)-NH_2 was designed to investigate the role of longer hydrocarbon side chain of the interfacial lysine residues in the lipid affinity of Ac-18A-NH_2. The three peptides were studied for their ability to interact with dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), and egg yolk phosphatidylcholine (EYPC) multilamellar vesicles. The following criteria were used for the lipid affinity of these peptides (26–28): (i) increase in the α-helical structure in the presence of the lipid as detected by far-UV CD spectra; (ii) blue-shift in the tryptophan emission maximum and shielding from the aqueous-phase quenchers, iodide and acrylamide, in the presence of the lipid as monitored by steady-state fluorescence spectroscopy; (iii) clarification of the turbidity due to EYPC multilamellar vesicles as measured by right-angle light scattering; and (iv) reduction in the transition enthalpy and broadening in the gel to liquid-crystalline phase transition of the DMPC and DMPG multilamellar vesicles as measured by differential scanning calorimetry (DSC).

EXPERIMENTAL PROCEDURES

Materials—Synthesis and purification of the peptide Ac-18A-NH_2 has been described earlier (25). Peptide Ac-18R-NH_2 was synthesized and purified following similar methodology (20). For the synthesis of the peptide Ac-18A(Lys>Haa)-NH_2, Boc-Haa(Z), instead of Boc-Lys(2-ClZ), was used. The amino acid derivative was prepared using the procedure described elsewhere (29). The other steps in the synthesis and purification were similar to those described earlier for Ac-18A-NH_2 (25).

DMPC, DMPG, and EYPC, purity > 99%, were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL) and used without further purification. Potassium iodide (KI) was obtained from Fisher Scientific Company, and acrylamide (>99.9%) was purchased from Bio-Rad. (1*S*)-(+)-10-Camphorsulfonic acid (99%), 2,2,2-trifluoroethanol (TFE, 99.5+%, NMR grade), and guanidine hydrochloride (99%) were obtained from Aldrich. All other chemicals were of highest purity commercially available.

Preparation of Peptide Solutions—Peptide solutions were prepared by dissolving the peptide in 4 M guanidine hydrochloride and dialyzing against phosphate-buffered saline (PBS, pH 7.4; KH_2PO_4 1.47 mM, $Na_2HPO_4.7H_2O$ 6.45 mM, NaCl 136.89 mM, KCl 2.68 mM) extensively (overnight, with at least three buffer changes). All the studies described below were done in the same buffer. Peptide concentrations were determined in 4 M guanidine hydrochloride by measuring absorbance at 280 nm (ϵ_{280} = 7300 M^{-1} cm^{-1}).

Circular Dichroism—The CD spectra were recorded with an AVIV 62DS spectropolarimeter interfaced to a personal computer. The instrument was calibrated with (1*S*)-(+)-10-camphorsulfonic acid (30, 31). The CD spectra were measured from 260 to 190 nm every nm with 1 s averaging per point, and a 2-nm bandwidth. An 0.01-cm path length cell was used for obtaining the spectra. All CD spectra were signal averaged by adding four scans, base-line corrected, and smoothed. All the CD spectra were recorded at 25 °C. Temperature was regulated with a Lauda RS2 circulating water bath. Final peptide concentrations of 100 μM or less were used for obtaining the CD spectra. DMPC multilamellar vesicles suspension was prepared by dissolving a known amount of lipid (~10 mg) in ethanol in a test tube and evaporating the solvent slowly under a stream of dry nitrogen. The residual solvent was removed by storing the tube under high vacuum overnight in a vacuum oven at 25 °C. To the dried lipid film appropriate volume of buffer was added to give a final lipid concentration of 14.7 mM. The lipid film was hydrated by vortexing the mixture for 30 min at room temperature. Peptide-DMPC complexes for CD studies were prepared as follows. Appropriate volume of peptide solution in buffer was added to the DMPC multilamellar vesicles suspension to give lipid to peptide molar ratio of 20:1. The mixture was incubated overnight at room temperature. This yielded a clear solution of the peptide-DMPC complex.

The mean residue ellipticity, $[\Theta]_{MRE}$(deg·cm^2·$dmol^{-1}$), was calculated using the following equation:

$$[\Theta]_{MRE} = MRW \cdot \Theta / 10.c.l \qquad \text{(Eq. 1)}$$

where, MRW is mean residue weight of the peptide, Θ is the observed ellipticity in degrees, c is the concentration of the peptide in g/ml, and l is the path length of the cell in centimeters. The percent helicity of the peptide was estimated from the following equation:

$$\%\ \alpha\ \text{helix} = ([\Theta]_{222} + 3{,}000)/(36{,}000 + 3{,}000) \qquad \text{(Eq. 2)}$$

where, $[\Theta]_{222}$ is the mean residue ellipticity at 222 nm (32).

Fluorescence Measurements—Steady-state fluorescence emission spectra were recorded in the ratiometric mode on an SLM 8000C photon counting spectrofluorometer (SLM Instruments, Inc., Urbana, IL) at 25 °C. Excitation and emission slit widths were both 4 nm. An excitation wavelength of 280 nm was used for recording emission spectra. Wavelengths at which maximum emission occurred (λ_{max}) were determined by positioning the cursor manually at the peak maximum and reading the corresponding wavelength. The final peptide concentration used was 14 μM (A_{280} = 0.1).

Fluorescence quenching experiments were performed using 295-nm excitation wavelength in order to minimize absorptive screening by the quenchers used. Aliquots (10 μl) of freshly prepared potassium iodide (4 M) or acrylamide (4 M) stock solutions were added to a constantly stirred and thermostated (25 °C) 2-ml peptide solution. After every addition of the quencher, the emission spectra were recorded from 310 to 450 nm, and the emission intensity at λ_{max} were determined as described above. Stock solution of potassium iodide contained 1 mM sodium thiosulfate ($Na_2S_2O_3$) in order to prevent I_3^- formation (33). For acrylamide quenching, corrections for inner filter effects (ϵ_{295} = 0.25 M^{-1} cm^{-1}, for acrylamide) were made (34). The fluorescence quenching data were analyzed according to the Stern-Volmer equation for the collisional quenching (35):

$$F_0/F = 1 + K_{SV}[Q] = \tau_0/\tau = 1 + k_q\tau_0[Q] \qquad \text{(Eq. 3)}$$

where, F_0 and F are fluorescence intensities in the absence and presence of quencher, respectively, K_{sv} is Stern-Volmer constant for the collisional quenching process, [Q] is quencher concentration, τ_0 and τ are fluorescence lifetimes of the flurophore in the absence and presence of the quencher, respectively, and k_q is the rate constant for the bimolecular quenching process. The above equation predicts a linear plot of F_0/F (or τ_0/τ) *versus* [Q] for a homogeneously emitting solution. The slope of this plot yields the value of K_{sv}.

[1] The abbreviations used are: apoA-I, apolipoprotein A-I; Boc, tertiary-butyloxycarbonyl; Z, benzyloxycarbonyl; (2Cl)Z, 2-chlorobenzyloxycarbo-nyl; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)](sodium salt); EYPC, egg yolk L-α-phosphatidylcholine; NATA, *N*-acetyltryptophanamide; PBS, phosphate-buffered saline; TFE, 2,2,2-trifluoroethanol; DSC, differential scanning calorimetry; Haa, homoaminoalanine; Ac, acetyl; NH_2, carboxamide.

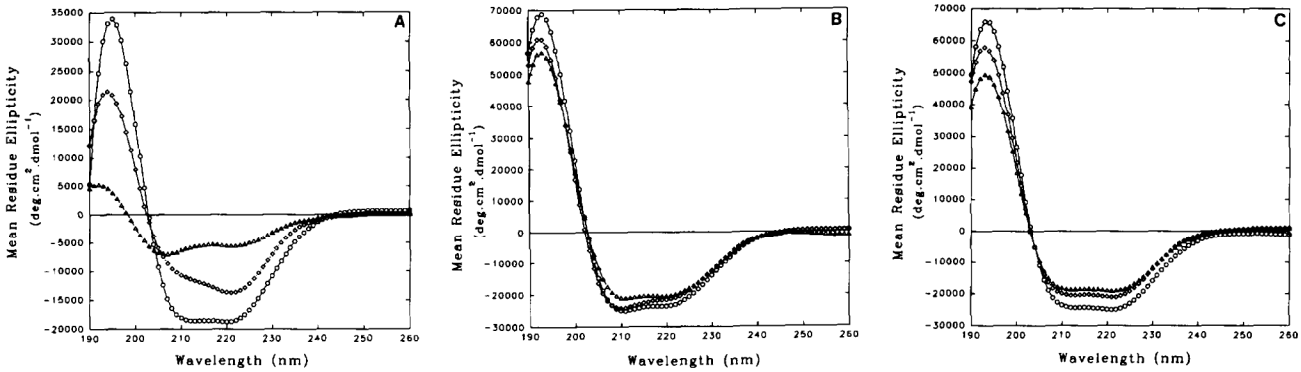

FIG. 1. **Far-UV CD spectra of peptides (~100 μM) in buffer (PBS, pH 7.4) (*A*), 50% (v/v) TFE/PBS (*B*), and DMPC complex (lipid/peptide molar ratio 20:1) (*C*).** Ac-18A-NH_2 (○), Ac-18A(Lys>Haa)-NH_2 (△), Ac-18R-NH_2 (◇).

Right-angle Light Scattering Measurements—The association of the peptides with EYPC multilamellar vesicles was followed by monitoring the rate of clarification of the turbidity due to the vesicles (36). The rate of turbidity clarification was measured by right-angle light scattering using SLM 8000C photon counting spectrofluorometer with both excitation and emission monochromators set at 400 nm. Data were acquired using slow time based acquisition. EYPC multilamellar vesicles suspension was prepared as described above for the DMPC. The sample containing 105 μM EYPC and an equimolar amount of the peptide was maintained at 25 °C and was continuously stirred. Complete dissolution of the EYPC vesicles was achieved by the addition of Triton X-100 to the vesicles suspension at a final concentration of 1 mM.

Differential Scanning Calorimetry—The high sensitivity DSC studies were performed in Microcal MC-2 scanning calorimeter (MicroCal, Inc., Amherst, MA) at the scan rate of 20°h^{-1} at an instrumental sensitivity of 1 and with a filtering constant of 10 s. The lipid multilamellar vesicles and the peptide-lipid mixtures for DSC were prepared as follows. DMPC or DMPG (~2 mg) was dissolved in chloroform in a test tube and dried by slow evaporation under a stream of dry nitrogen. The residual solvent was removed under high vacuum in the vacuum oven as described above. To the dried lipid film either buffer alone or buffer containing the peptide was added to obtain lipid/peptide molar ratio 100:1. The lipid was hydrated by vortexing at room temperature for 30 min. The suspension was degassed for 30 min, and an aliquot of 1.2 ml of suspension was used and run against buffer in the reference cell. Four consecutive scans with 60 min equilibration time between each scan were run for the same sample. No significant changes in the thermograms were observed between the first scan and the fourth scan. The observed transitions were analyzed using software provided by MicroCal, Inc., Amherst, MA.

RESULTS

CD Studies—Secondary structures of the peptides were determined by recording their far-UV CD spectra in buffer (PBS, pH 7.4), in 50% (v/v) TFE/PBS, and in the presence of DMPC (lipid/peptide molar ratio 20:1). The CD spectra are shown in Fig. 1. The CD spectrum of an α-helix is characterized by two negative bands, one at 222 nm and another at 208 nm, and a positive band at 192 nm (37). The CD spectrum of Ac-18A-NH_2 in buffer (Fig. 1*A*) indicates that the peptide is largely helical. As estimated from mean residue ellipticity at 222 nm $[\Theta]_{222}$, the peptide is 55% α-helical. The CD spectrum of Ac-18R-NH_2 indicates that it is less helical (43%) than Ac-18A-NH_2. The CD spectrum of Ac-18A(Lys>Haa)-NH_2 in buffer is distinct from the CD spectra of the other two peptides because of its greatly reduced mean residue ellipticity values as well as a large shift in the zero cross-over point (wavelength at which mean residue ellipticity is zero). This suggests a largely non-helical structure of the peptide in buffer. The peptide was estimated to have 22% α-helical structure.

TFE is a most commonly used structure-inducing, hydrophobic, cosolvent (38). CD spectra of the three peptides in 50% (v/v) TFE/PBS were recorded to estimate their α-helical contents in a relatively hydrophobic environment. As is evident from Fig. 1*B*, among the three peptides, Ac-18A-NH_2 has the maximum α-helical structure (67%). The α-helical contents of Ac-18R-NH_2 and Ac-18A(Lys>Haa)-NH_2 are less than that of Ac-18A-NH_2 (61 and 59%, respectively). However, a closer inspection of the CD spectra of the peptides in TFE reveals that while Ac-18A(Lys>Haa)-NH_2 has a zero cross-over point (202.7 nm) nearly identical with that of Ac-18A-NH_2 (202.6 nm), the zero cross-over point of Ac-18R-NH_2 is shifted toward a shorter wavelength (202 nm). This indicates that Ac-18A(Lys>Haa)-NH_2 has a higher α-helical content than Ac-18R-NH_2 in this solvent system (39).

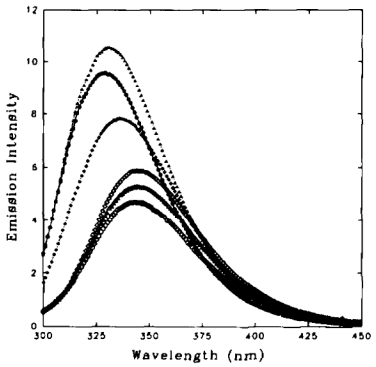

FIG. 2. **Fluorescence emission spectra of peptides in buffer (PBS, pH 7.4) and peptide-DMPC complexes (lipid/peptide molar ratio 20:1).** Peptide concentration = 14 μM, excitation wavelength = 280 nm. Ac-18A-NH_2 in buffer (○), Ac-18A-NH_2-DMPC complex (●), Ac-18A(Lys>Haa)-NH_2 in buffer (△), Ac-18A(Lys>Haa)-NH_2-DMPC complex (▲), Ac-18R-NH_2 in buffer (◇), Ac-18R-NH_2-DMPC complex (◆).

The CD spectra of peptide-DMPC complexes (lipid/peptide molar ratio 20:1) are shown in Fig. 1*C*. When complexed with the lipid, all the three peptides are largely α-helical. The three peptides were estimated to have the following α-helical contents: Ac-18A-NH_2 72%, Ac-18R-NH_2 62%, and Ac-18A(Lys>Haa)-NH_2 56%. We would like to point out that the α-helical content of Ac-18A-NH_2 in DMPC complex was earlier reported to be 92% (25). This value was recently found to be an overestimation because of the improper calibration of the spectropolarimeter.

Fluorescence Measurements—The fluorescence emission maximum of tryptophan is highly sensitive to its microenvironment (40). The microenvironments of tryptophan residues in the peptides were probed by recording their emission spectra in buffer and in the presence of DMPC (lipid/peptide molar ratio 20:1). The emission spectra are shown in Fig. 2. All three peptides disrupt DMPC vesicles completely at lipid/peptide molar ratio 20:1 resulting in an optically clear solution. In buffer

TABLE I

Tryptophan emission maxima and fluorescence quenching parameters of the peptides and the peptide-DMPC complexes

Peptide concentration was 14 μM; lipid/peptide molar ratio was 20:1. Fluorescence emission maxima were determined using excitation wavelength of 280 nm; quenching studies were done using excitation wavelength of 295 nm. K_{SV} is Stern-Volmer quenching constant determined from the slopes of the lines for the plots of F_0/F-1 *versus* [*Q*]; slopes were determined by linear regression analysis of the fluorescence quenching data using the least squares method.

Peptide	λ_{max} (nm)		K_{SV} (M^{-1})			
	Buffer	DMPC complex	Buffer, iodide quenching	DMPC complex, iodide quenching	Buffer, acrylamide quenching	DMPC complex, acrylamide quenching
Ac-18A-NH_2	343	329	5.7	1.4	13.5	2.6
Ac-18A(Lys>Haa)-NH_2	344	331	7.2	3.7	15.5	6.2
Ac-18R-NH_2	344	336	8.0	5.4	17.4	10.3
NATA	350	ND[a]	11.0	ND	27.7	ND

[a] Not determined.

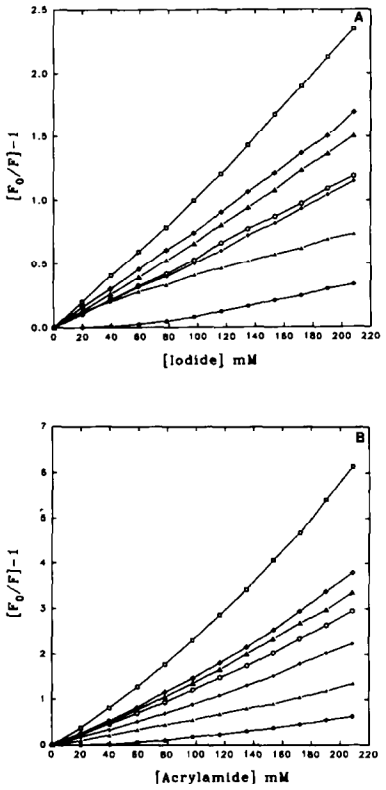

FIG. 3. **Stern-Volmer plots of fluorescence quenching of the peptides in buffer (PBS, pH 7.4), and peptide-DMPC complexes (lipid/peptide molar ratio 20:1) by iodide (*A*) and acrylamide (*B*).** Peptide concentration = 14 μM, excitation wavelength = 295 nm. Ac-18A-NH_2 in buffer (○), Ac-18A-NH_2-DMPC complex (●), Ac-18A(Lys>Haa)-NH_2 in buffer (△), Ac-18A(Lys>Haa)-NH_2-DMPC complex (▲), Ac-18R-NH_2 in buffer (◇), Ac-18R-NH_2-DMPC complex(◆), NATA in buffer (□).

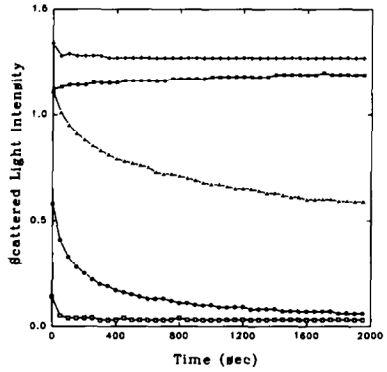

FIG. 4. **Rate of decrease of scattered-light intensity measured at 90° from a 105 μM suspension of EYPC vesicles.** Equimolar amounts of the peptides were added to the lipid suspension. Complete dissolution was achieved by adding Triton X-100 solution to the lipid suspension at a final concentration of 1 mM. Excitation and emission wavelengths both were 400 nm. EYPC alone (■), Ac-18A-NH_2 (●), Ac-18A(Lys>Haa)-NH_2 (▲), Ac-18R-NH_2 (◆), Triton X-100 (□).

alone, while Ac-18A-NH_2 has its emission maximum (λ_{max}) at 343 nm, Ac-18R-NH_2 and Ac-18A(Lys>Haa)-NH_2 both have their emission maxima at 344 nm. When complexed with DMPC, all the three peptides show an increase in the emission intensity as well as a blue-shift in their emission maxima compared with those in buffer, indicating partitioning of tryptophan into a more hydrophobic environment. However, the extent of the blue-shift in the tryptophan emission maximum, compared with that in buffer, is different for the three peptides. In the presence of DMPC, while Ac-18A-NH_2 emission maximum shows a blue-shift of 14 nm, Ac-18A(Lys>Haa)-NH_2 and Ac-18R-NH_2 show blue-shifts of 13 and 8 nm, respectively (Table I). This indicates that the tryptophan residue in Ac-18R-NH_2 is in a less hydrophobic environment than the tryptophan residues in Ac-18A-NH_2 and Ac-18A(Lys>Haa)-NH_2.

To further probe the location of the tryptophan residues of the three peptides in the lipid bilayer, fluorescence quenching experiments were carried out. Iodide and acrylamide were used as aqueous-phase quenchers of the tryptophan fluorescence (35). While iodide is an anionic quencher, acrylamide is a neutral but polar quencher. Iodide is considered to have access only to surface tryptophans, whereas acrylamide has good access to all but the most highly buried tryptophan residues (41). The Stern-Volmer plots of the quenching of tryptophan fluorescence by iodide and acrylamide are shown in Fig. 3. For comparison, Stern-Volmer plots of fluorescence quenching of NATA (*N*-acetyltryptophanamide), a model compound for free tryptophan, in buffer by iodide and acrylamide are also included. In buffer, compared with NATA, tryptophans in all the three peptides are less exposed to the quenchers. In the presence of DMPC, compared with buffer, tryptophans in all the three peptides become less accessible to the quenchers, suggesting shielding by the lipid bilayer. However, the extent of shielding from the quenchers is different for the three peptides. In the presence of DMPC, compared with buffer, the following order was observed in the shielding of the tryptophan residue of the three peptides from the quenchers: Ac-18A-NH_2 > Ac-18A(Lys>Haa)-NH_2 > Ac-18R-NH_2. Thus, among the three peptides, tryptophan in Ac-18R-NH_2 experiences least shielding in going from buffer to the lipid bilayer. The Stern-Volmer quenching constants for a bimolecular collisional quenching process were calculated from the apparent slopes of the plots of F_0/F-1 *versus* [*Q*] (slopes were calculated by linear regression analysis using the "least squares" method; $0.989 < r < 0.999$) and are given in Table I. The Stern-Volmer plots of the fluorescence quenching by acrylamide for the three peptides as well as that of NATA in solution

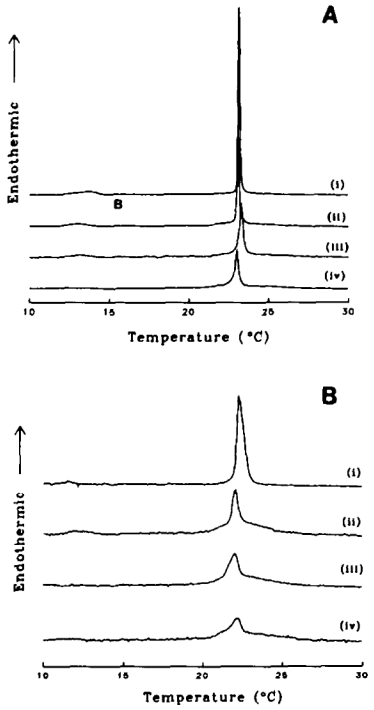

FIG. 5. **DSC heating thermograms of lipids and lipid-peptide mixtures (lipid/peptide molar ratio 100:1).** *A*, DMPC alone (1.5 mM) (*i*), DMPC + Ac-18R-NH_2 (*ii*), DMPC + Ac-18A(Lys>Haa)-NH_2 (*iii*), DMPC + Ac-18A-NH_2 (*iv*). *B*, DMPG alone (1.4 mM) (*i*), DMPG + Ac-18R-NH_2 (*ii*), DMPG + Ac-18A(Lys>Haa)-NH_2 (*iii*), DMPG + Ac-18A-NH_2 (*iv*).

TABLE II

Phase transition parameters for lipids and peptide-lipid mixtures as determined by DSC

DMPC concentration 1.5 mM, DMPG concentration 1.4 mM, lipid/peptide molar ratio 100:1. T_m is the temperature at which excess heat capacity is maximum; ΔH_0 is calorimetric enthalpy; ΔH_{VH} is Van't Hoff enthalpy; $\Delta T_{1/2}$ is width of the transition at half of the peak heat capacity.

Sample	T_m	ΔH_0	ΔH_{VH}	$\Delta T_{1/2}$
	°C	kcal/mol		°C
DMPC	23.1	5.4	4284.4	0.2
DMPC/Ac-18A-NH_2	23.0	3.3	1389.6	0.6
DMPC/Ac-18A(Lys>Haa)-NH_2	23.3	4.1	1566.8	0.6
DMPC/Ac-18R-NH_2	23.1	4.2	2537.5	0.3
DMPG	22.3	5.5	1171.1	0.6
DMPG/Ac-18A-NH_2	22.2	3.0	480.4	0.8
DMPG/Ac-18A(Lys>Haa)-NH_2	22.0	3.8	568.4	0.7
DMPG/Ac-18R-NH_2	22.1	4.8	674.3	0.4

(Fig. 3*B*) curve upward, indicating the occurrence of static quenching (42).

Right-angle Light Scattering Measurements—Interactions of the three peptides with EYPC were studied by monitoring the rate of clarification of the turbidity due to the lipid vesicles after addition of the peptide. The results of this study are shown in Fig. 4. As has been noted earlier (25), at equimolar lipid/peptide ratio, Ac-18A-NH_2 clarifies the lipid turbidity within 30 min. At an identical lipid/peptide molar ratio, while the rate of turbidity clarification was slower with Ac-18A(Lys>Haa)-NH_2, Ac-18R-NH_2 failed to clarify the lipid turbidity within the duration of the experiment (33 min). A slight increase in the turbidity of the lipid suspension was observed after the addition of Ac-18R-NH_2. The reason for this is not clear at present.

DSC Studies—The thermotropic phase transition properties of DMPC and DMPG multilamellar vesicles were studied in the absence and in the presence of the peptides by DSC. The heating endotherms of the pure lipid vesicles and the peptide-lipid mixtures (lipid/peptide molar ratio 100:1) are shown in Fig. 5. At neutral pH, while DMPC is zwitterionic, DMPG is negatively charged. Interactions of the peptides with DMPG vesicles were studied to investigate possible electrostatic interactions between the positively charged amino acid residues of the peptides and the negative charges of the lipid. DMPC vesicles alone exhibited endothermic transitions at 13.7 and 23.1 °C, which correspond to the pretransition (lamellar to periodic gel) and the gel to liquid-crystalline (order-disorder) phase transition of the lipid, respectively (43). Addition of peptides to the DMPC vesicles resulted in lowering of the transition enthalpy and broadening of the gel to liquid-crystalline phase transition of DMPC vesicles (Table II). Among the three peptides, Ac-18A-NH_2 caused the maximal reduction in the enthalpy of the main phase transition, while Ac-18A(Lys>Haa)-NH_2 is intermediate to Ac-18A-NH_2 and Ac-18R-NH_2 (Table II). None of the peptides changed the chain-melting temperature (T_m) of DMPC vesicles by more than 0.2 °C. While the pretransition in the DMPC vesicles is observed with Ac-18A(Lys>Haa)-NH_2 and Ac-18R-NH_2, it is not detected in the presence of Ac-18A-NH_2. Similar to DMPC vesicles, DMPG vesicles exhibited endothermic transitions at 11.5 and 22.7 °C, corresponding to the pretransition and the main phase transition of the lipid, respectively (44). The effect of the three peptides on the phase transition properties of the DMPG vesicles is similar to that observed with DMPC (Table II). The maximum change in T_m of the lipid vesicles observed in the presence of the peptides is less than 0.3 °C. The width at half of the peak height of the main phase transition in DMPG is more than that in DMPC (Table II). This is presumably due to the poor packing of the lipid molecules in the bilayer because of electrostatic repulsion between the negative charges in the head group of the phospholipid molecules (36).

DISCUSSION

A comparison of the CD spectra of the three peptides in the DMPC complex (Fig. 1*C*) shows the following rank order of helicity: Ac-18A-NH_2 > Ac-18R-NH_2 > Ac-18A(Lys>Haa)-NH_2. While the same order is observed in the helicity of the three peptides in buffer (Fig. 1*A*), the helicity of Ac-18A(Lys>Haa)-NH_2 in the presence of TFE is more than that of Ac-18R-NH_2 (Fig. 1*B*). In the presence of TFE, Ac-18A(Lys>Haa)-NH_2 was estimated to have less helical structure than Ac-18R-NH_2 based on the mean residue ellipticity at 222 nm of the two peptides. However, comparison of the zero cross-over point of the two peptides revealed that Ac-18A(Lys>Haa)-NH_2 has more helical structure than Ac-18R-NH_2 (39). It has been pointed out that since the shape and zero cross-over of a CD spectrum directly reflect the proportional contribution of different secondary structures, any genuine change in secondary structure would lead to a change in either the shape or the zero cross-over, or both, of that spectrum (39). Therefore, it is important to note that a comparison of helicity should not be based on ellipticity values alone. The results of the CD studies indicate that the lipid affinities of Ac-18R-NH_2 and Ac-18A(Lys>Haa)-NH_2 are less than the lipid affinity of Ac-18A-NH_2. The lipid affinity of Ac-18A(Lys>Haa)-NH_2, based on the results of the CD studies alone, is lower than that of Ac-18R-NH_2.

A comparison of the fluorescence emission maxima of the peptides, however, reveals that in the DMPC complex, while tryptophan in Ac-18A(Lys>Haa)-NH_2 is in a less hydrophobic environment than the tryptophan in Ac-18A-NH_2, it is in a more hydrophobic environment than the tryptophan in Ac-18R-NH_2 (Table I). These conclusions are further supported by the results from the fluorescence quenching studies (Fig. 3). The

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