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Effect of End Group Blockage on the Properties of a Class A Amphipathic Helical Peptide

Y.V. Venkatachalapathi,[1] Michael C. Phillips,[2] Richard M. Epand,[3] Raquel F. Epand,[3] Ewan M. Tytler,[1] Jere P. Segrest,[1] and G.M. Anantharamaiah[1]

[1]Departments of Medicine, Biochemistry and The Atherosclerosis Research Unit, The University of Alabama School of Medicine, Birmingham, Alabama 35294, [2]Department of Physiology and Biochemistry, Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129, and [3]Department of Biochemistry, Mc Master University Health Sciences Centre, Hamilton, Ontario, Canada L8N 3Z5

ABSTRACT **In a recent classification of biologically active amphipathic α-helixes, the lipid-associating domains in exchangeable plasma apolipoproteins have been classified as class A amphipathic helixes (Segrest, J.P., De Loof, H., Dohlman, J.G., Brouillette, C.G., Anantharamaiah, G.M. *Proteins* 8:103–117, 1990). A model peptide analog with the sequence, Asp Trp Leu Lys Ala Phe Tyr Asp Lys Val Ala Glu Lys Leu Lys Glu Ala Phe (18A), possesses the characteristics of a class A amphipathic helix. The addition of an acetyl group at the α-amino terminus and an amide at the α-carboxyl terminus, to obtain Ac-18A-NH_2, produces large increases in helicity for the peptide both in solution and when associated with lipid (for 18A vs Ac-18A-NH_2, from 6 to 38% helix in buffer and from 49 to 92% helix when bound to dimyristoyl phosphatidylcholine in discoidal complexes). Blocking of the end-groups of 18A stabilizes the α-helix in the presence of lipid by approximately 1.3 kcal/mol. There is also an increase in the self-association of the blocked peptide in aqueous solution. The free energy of binding to the PC–water interface is increased only by about 3% (from −8.0 kcal/mol for 18A to −8.3 kcal/mol for Ac-18A-NH_2). The Ac-18A-NH_2 has a much greater potency in raising the bilayer to hexagonal phase transition temperature of dipalmitoleoyl phosphatidylethanolamine than does 18A. In this regard Ac-18A-NH_2 more closely resembles the behavior of the apolipoprotein A-I, which is the major protein component of high-density lipoprotein and a potent inhibitor of lipid hexagonal phase formation. The activation of the plasma enzyme lecithin: cholesterol acyltransferase by the Ac-18A-NH_2 peptide is greater than the 18A analog and comparable to that observed with the apo A-I. In the case of Ac-18A-NH_2, the higher activating potency may be due, at least in part, to the ability of the peptide to micellize egg PC vesicles.**

Key words: apolipoprotein A-I, α-helix stabilization, hexagonal phase, LCAT activation, peptide–lipid interactions, synthetic peptides

INTRODUCTION

The exchangeable plasma apolipoproteins possess lipid associating domains which have the characteristics of an amphipathic α-helix, a unique secondary structural and functional motif involved in lipid interaction.[1] Amphipathic helixes have been identified in several biologically active polypeptides and proteins.[2–5] An amphipathic helix is defined as an α-helix with opposing polar and nonpolar faces oriented along the long axis of the helix.[1]

Based on the position of the charged residues along the helix, the biologically active amphipathic helixes have been classified into seven different classes.[2] Helical segments of several exchangeable apolipoproteins correspond to class A amphipathic helixes. Class A amphipathic helixes are characterized by a unique secondary structural feature with positively charged residues clustered at the polar–nonpolar interface and negatively charged residues at the center of the polar face.[2] Studies of the model peptide analogs mimicking the charge distribution

Abbreviations: 18A, a model 18 amino acid peptide having a class A amphipathic helix; Ac-, N-acetyl group; Ac-18A-NH_2, N-acetyl-18A-amide; apo A-I, apolipoprotein A-I; Boc, *t*-butyloxycarbonyl; CD, circular dichroism; DCC, dicyclohexylcarbodiimide; DMPC, dimyristoyl phosphatidylcholine; DPoPE, dipalmitoleoyl phosphatidylethanolamine; egg PC, egg phosphatidylcholine; Gdn·HCl, guanidine hydrochloride; HDL, high-density lipoprotein; HOBt, hydroxy-benzotriazol; HPLC, high-performance liquid chromatography; H_{II} phase, inverted hexagonal phase; LCAT, lecithin:cholesterol acyltransferase; PBS, phosphate-buffered saline; T_H, bilayer to hexagonal phase transition temperature; TFA, trifluoroacetic acid.

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Address reprint requests to Dr. G.M. Anantharamaiah, Department of Medicine, U.A.B. Medical Center, Birmingham, AL 35294.

in the amphipathic helical domains of exchangeable apolipoproteins have revealed that this property is important for their lipid affinity.[2,5–11]

We have used an 18-residue model peptide, 18A, to explore the properties of class A amphipathic helixes. The sequence of this peptide is Asp Trp Leu Lys Ala Phe Tyr Asp Lys Val Ala Glu Lys Leu Lys Glu Ala Phe. The lipid binding characteristics of this peptide and the lipid packing in the discoidal complexes with dimyristoyl phosphatidylcholine (DMPC) have been elucidated.[5,9,12] In addition, this peptide has been shown to mimic apolipoprotein (Apo) A-I, the major protein component of high-density lipoprotein (HDL), exhibiting properties such as inhibiting HIV- and HSV-induced cell fusion, stimulating the release of human placental lactogen from trophoblasts,[13–17] and activating the plasma enzyme lecithin:cholesterol acyltransferase (LCAT).[6] It also competes for the surface of HDL with apo A-I.[6]

A number of modifications of this peptide have been studied.[5,6,9–11] A peptide with two 18A domains connected by a Pro residue, 37pA, was found to possess enhanced lipid binding and biological activities compared with 18A, mimicking more closely the properties of apo A-I.[5,6,13–16] In an attempt to increase the potency of 18A and to design shorter peptides mimicking apo A-I properties, we replaced the amino acids having less helix forming potential such as Asp and Ala in 18A with Glu and Leu, respectively. The resulting peptides were found to possess increased helicity, lipid affinity, and LCAT-activating ability.[5,6] Although there were increases in the α-helicity in aqueous buffer and in the presence of the lipid for the modified peptide analogs, these increases were only marginal. We therefore considered other possible methods for increasing the helicity of the model amphipathic peptide 18A with a view to design a closer mimic of apo A-I.

There are many classic examples of using model amphipathic peptides to understand peptide/protein structure-function.[2–6,18–25] In these studies, it has been established that end group modification of an α-helical peptide increases the ordered structure and an improvement in the functional properties. In our earlier designs the interaction of the charged residues with the helical dipole was not considered. A negatively charged residue near the amino terminal end would stabilize the helix, while a positive charge at this position would be destabilizing.[24] The opposite is the case for the carboxy terminal end. Furthermore, the α-helix can be stabilized by the removal of the charges from the amino and carboxy-terminal end.[25] While it has been shown that the α-helix in general can be stabilized by modification of the end groups, the effects of these modifications on the properties of a lipid-associating, apolipoprotein mimicking (class A), amphipathic helical peptide have not been systematically investigated.

The present studies focus on the effect of the end-group modification of 18A on the solution properties, lipid-affinity, effect on membrane properties, and on LCAT-activating properties. The results from these studies show that the end group modifications have a profound effect on the properties of 18A. Both of the peptides mimic apo A-I in raising the bilayer to hexagonal transition temperature (T_H), with Ac-18A-NH_2 exerting a larger effect, approaching that of apo A-I. Furthermore, the two peptides activate the plasma enzyme LCAT to different extents with the protected peptide analog, at higher concentrations, surpassing the natural apolipoprotein activator, apo A-I.

MATERIALS AND METHODS

Materials

Dipalmitoleoyl phosphatidylethanolamine (DPoPE), dimyristoyl phosphatidylcholine (DMPC), and egg PC were purchased from Avanti Polar Lipids Inc., (Birmingham, AL). Human apo A-I was isolated from normal subjects using the procedures described previously.[26] The protected amino acids for the synthesis of the peptides were purchased from either Advanced Chemtech (Louisville, KY) or Bachem California (Torrance, CA).

Peptide Synthesis

The synthesis of peptide 18A has been described elsewhere.[5,27] Peptide Ac-18A-NH_2 was synthesized by the solid phase method on a benzhydrylamine resin (0.575 meq of NH_2/g of resin, Bachem Inc., Torrance, CA), essentially using the same protocol that was used for the synthesis of 18A except the first amino acid Boc-Phe, which was directly coupled to the benzhydrylamine resin support using the DCC-HOBt condensation procedure. After the addition of the last amino acid, the N-terminal Boc-protecting group was removed and the protected peptide-resin was reacted with 10 equivalents of acetic acid in dichloromethane using DCC as the condensing reagent, to incorporate the acetyl (Ac-) group to the N-terminus of the peptide. The peptide resin was then subjected to the modified HF procedure.[28] Because of the functionality (benzhydrylamine) on the starting resin, HF treatment of the finished peptide resin resulted in a peptide amide. The released peptide was extracted with 50% acetonitrile/water containing 0.1% TFA, the organic solvent was evaporated and the remaining water was removed by lyophilization. The crude peptide was dissolved in 6 M guanidine hydrochloride (Gdn·HCl), dialyzed against water using Spectrapor dialysis membrane (1000 MW cutoff), and lyophilized. Further purification was achieved by preparative HPLC on a C_4 reverse-phase preparative column (2.5 × 25 cm, VYDAC) using water/acetonitrile (0.1% TFA) gradient (5 to 50% in 60 min; flow rate of 4.8 ml/min; and monitored at 280 nm). Purity of the peptide was as-

certained by amino acid analysis and analytical HPLC.

Preparation of Peptide Solutions for Studies

Peptides were dissolved in Gdn·HCl (6 M) to obtain a concentration of about 5 mg/ml. These solutions were dialyzed against Tris buffer (10 mM Tris and 150 mM sodium chloride adjusted to pH 7.4 with HCl) using 1000 MW cutoff dialysis bags to ensure the proper pH of the peptides solutions. The concentrations of the solutions were determined either by quantitative amino acid analysis or by using molar extinction coefficients of Trp and Tyr.[12]

Circular Dichroism

The CD spectra were obtained using either a JASCO-500 spectropolarimeter coupled to a DPN-500 integrator or an AVIV 62 DS circular dichroism spectrometer linked to a 80386 microprocessor for data acquisition and reduction. The CD was measured in a 1 mm path length cell. Eight or 16 scans were averaged and the reported spectra was corrected for the appropriate baselines. The corrected data were used to calculate the mean residue ellipticities; the percent helix was estimated by the procedure of Chang et al. as described.[29] Complexes of the peptides with DMPC were prepared by mixing the peptide with a suspension of DMPC multilamellar vesicles at the different lipid:peptide ratios. The solutions were incubated overnight at room temperature and diluted to 50 μg/ml of peptide. The concentration-dependent CD spectra was measured using a 1 mm pathlength cell for peptide concentrations of 25 μg/ml and above and a 1 cm pathlength cell for more dilute solutions. pH-dependent CD was measured for both the peptides at a peptide concentration of 50 μg/ml. The solutions at approximately 50 μg/ml were adjusted to various pH values and then the exact concentrations of the peptides were determined. For the denaturation studies the lipid–peptide complexes were prepared at a molar ratio of 20, incubated with various concentrations of Gdn·HCl for 72 hr at room temperatures before CD spectra were recorded.

Intrinsic Tryptophan Fluorescence

Fluorescence spectra of both peptide and peptide–DMPC complexes were recorded at room temperature with excitation at 287 nm using a SPF-500 spectrofluorimeter. The relative fluorescence intensity was measured in ratio mode which corrects for time-dependent fluctuations in lamp intensity.

Right Angle Light Scattering

The rates of dissolution of multilamellar suspensions of DMPC and egg PC were measured by right angle light scattering using a SPF-500 spectrofluorimeter. Excitation and emission wavelengths were both set at 400 nm. Solutions of the peptide (50 μg/ml) in Tris buffer with various amounts of lipid were used for measurements. At zero time the lipid was mixed with the peptide and the intensity of the light scattered was measured for 30 min. A similar experiment in the absence of the peptide was carried out which served as control.

Electron Microscopy of Peptide:Dimyristoyl Phosphatidylcholine Complexes

Peptides mixed with multilamellar vesicles of DMPC at 2.5 and 5 (DMPC/peptide) weight ratio (or 8 and 16 molar ratio) were stained with 2% potassium phosphotungstate, pH 5.9 and were examined with a Philips EM400 microscope on carbon-coated Formvar grids.

Nondenaturing Gradient Polyacrylamide Gel Electrophoresis

A 2–16% linear gradient gel was formed. Electrophoresis was performed at 4°C at a constant voltage of 125 V for 20 hr. The running buffer was Tris-glycine with no SDS. The gels were fixed with 10% trichloroacetic acid and then stained with 0.125% Coomassie Blue G-250 (Eastman Kodak Co., Rochester, NY) in 30% methanol and 10% acetic acid.

Interaction of Peptides With Phospholipid Monolayers

The relative affinities of the peptides 18A and Ac-18A-NH_2 for the lipid–water interface were investigated using a surface balance technique. Employing former procedures,[30,31] an insoluble monolayer of egg PC was spread at the air–water interface (85 cm^2) in a circular Teflon dish containing 80 ml of phosphate-buffered saline (pH 7.0) at room temperature. The surface pressure (π) was monitored by the Wilhelmy plate technique using a mica plate connected to a Cahn RTL recording electrobalance.[32] Sufficient egg PC was spread from a 9:1 (v:v) hexane:ethanol solution to give an initial surface pressure of 10 dyn/cm. Peptides dissolved in the above buffer containing 1.5 M Gdn·HCl were injected into the subphase to give the desired initial concentration; a small Teflon tube which projected through the monolayer into aqueous subphase was used for this injection so that the PC monolayer was not disrupted. The presence of Gdn·HCl ensured that the peptide molecules were initially present as random coil monomers. The peptide molecules renatured in the subphase as the Gdn·HCl was diluted to a final concentration of $\leq$ 1 mM. The solution was stirred continuously with a magnetic stirrer and the increase in surface pressure ($\Delta\pi$) with time was recorded until a steady-state value of $\Delta\pi$ was obtained. The complete adsorption isotherms characterizing the binding of the peptides to the egg PC monolayer were obtained by measuring $\Delta\pi$ as a function of the subphase concentrations of each peptide.

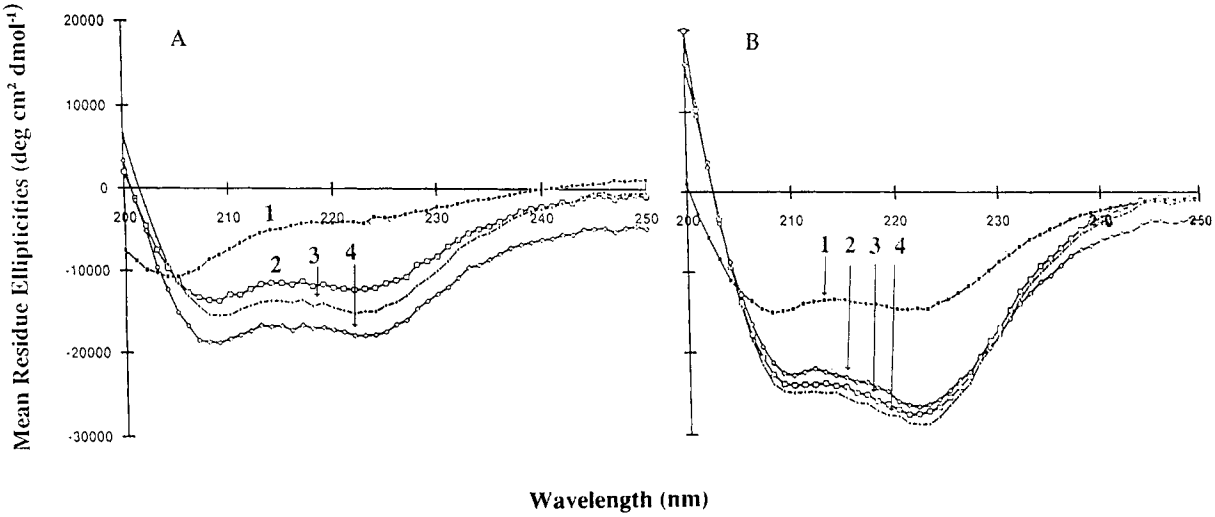

Fig. 1. Circular dichroism spectra of **(A)** 18A and **(B)** Ac-18A-NH_2 in phosphate buffered saline (PBS) (10 mM Na_2HPO_4/NaH_2PO_4 and 150 mM NaCl; pH 7.4) with increasing amounts of DMPC. The molar ratios of DMPC/peptide are 1, without lipid; 2, 10; 3, 20, and 4, 50.

Transition Temperatures of Peptide–Lipid Mixtures

The dipalmitoleoyl phosphatidylethanolamine (DPoPE) was dissolved in chloroform/methanol (2/1, v/v) and the solvent evaporated with a stream of nitrogen so as to deposit the lipid as a film on the wall of a glass tube. Final traces of solvent were removed in a vacuum desiccator at 40°C for 1 hr. The lipid films were suspended in a solution of peptide in 20 mM Pipes, 1 mM EDTA, 150 mM NaCl with 0.002% NaN_3 at pH 7.4 by vortexing at 45° for 30 sec. The final lipid concentration was 20 mg/ml. The lipid concentration was verified by phosphate determination after perchloric acid washing of the sample. The lipid suspensions were degassed under vacuum before loading into an MC-2 high sensitivity scanning calorimeter (Microcal Co., Amherst, MA). A scan rate of 39 K/hr was employed. Duplicate heating runs and duplicate samples were often run to ensure reproducibility of the observations. The fact that phosphatidylethanolamines at neutral pH do not form well sealed vesicles makes it likely that the peptides and protein have access to all the lipid.[33] This is confirmed by the similarity of the heating scans performed after removing the bilayer phase from the H_{II} phase by cooling. In addition, peptides were also dissolved or suspended in methanol, mixed with the chloroform/methanol solution of lipid and dried to a film which was then hydrated. These preparations gave similar results to those in which the peptide was added to the lipid. The enthalpy of the transitions show a variability of ±25% because these lipids do not form well-dispersed homogeneous suspensions. No systematic change in the transition enthalpy was observed in the presence of the peptides or the apo A-I. The bilayer to hexagonal phase transition was fitted to a single Van't Hoff component and the T_H reported as that for the fitted curve.

Lecithin:Cholesterol Acyltransferase Activation

The enzyme LCAT was purified by the procedure of Matz and Jonas.[34] Peptides were studied for their ability to activate the enzyme LCAT using cholesterol-containing egg PC liposomes containing a trace of [7α-^{3}H]cholesterol according to the procedure described previously.[9]

RESULTS

Circular Dichroism and Tryptophan Fluorescence Studies

CD spectra of 23 μM peptide solutions (Fig. 1) indicate that the peptide Ac-18A-NH_2 has considerably higher α-helicity (38%) than 18A (6%). The helix content in the presence of lipid is also higher for the end group-modified peptide (92%) compared to 18A (49%). Figure 1 shows that there is an increase in the magnitude of the ellipticity at 222 nm indicating increased helicity of 18A with increasing lipid:peptide ratio; with Ac-18A-NH_2, the magnitude of the ellipticity reached a maximal value at a lower DMPC:peptide ratio (<10/1 lipid/peptide). At higher lipid:peptide molar ratios (>20), the solutions are turbid due to incomplete transformation of all of the DMPC into discoidal complexes. Intrinsic tryptophan fluorescence studies are consistent with the CD data. As can be seen in Figure 2, there is a larger blue shift in the presence of lipid for Ac-18A-NH_2 (from a maximum at 362 nm in buffer to 338 nm in the presence of DMPC compared with 18A which has maxima at 362 and 350 nm in buffer and

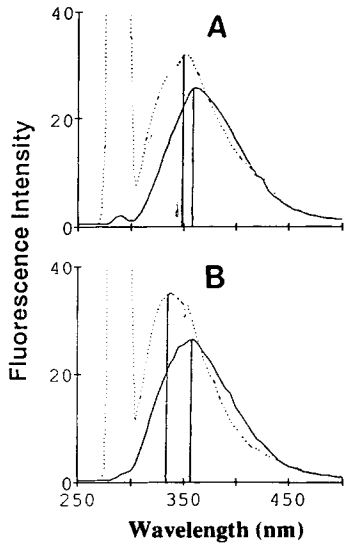

Fig. 2. Intrinsic tryptophan fluorescence maxima of 22.5 μM of peptides **(A)** 18A and **(B)** Ac-18A-Nh_2 solutions in PBS with (dotted line) or without (solid line) 450 μM of DMPC.

in the presence of DMPC, respectively). There are probably two contributing factors. First, there may be a larger shift of Trp from a polar aqueous environment to a nonpolar lipid environment with the end-group modification of the 18A molecule. Second, the ratio of the bound to free peptide is greater for Ac-18A-NH_2. This explanation is supported by analysis of the band widths at half height of the four spectra in Figure 2. The spectra of both of the peptides free in aqueous solution are identical with the band widths being 80 nm. However, when bound to lipid, the spectra show that the band width for 18A did not change, while that for Ac-18A-NH_2 becomes narrower (70 nm). Furthermore, the spectrum of lipid-associated 18A appears to be asymmetric, indicating that this spectra is a combination of two spectra, one for the free peptide and another for the lipid-associated peptide. This explanation is further supported by nondenaturing gradient gel electrophoresis of peptide–lipid complexes. Thus the Trp of 18A appears to be in a less hydrophobic environment because a portion of the peptide partitions into water.

Figure 3A shows that the change in ellipticity at 222 nm is minimal up to about 25 μM peptide for both of the peptides. At higher concentrations, there is a gradual increase in the magnitude of the ellipticity for Ac-18A-NH_2 reaching a maximum at 250 μM. This increase in the magnitude of the ellipticity of Ac-18A-NH_2 at higher concentrations of peptide is probably due to self-association of the peptide. With dilution, the magnitude of the ellipticity decreased, indicating the reversible nature of this self-association.

The results of the effect of pH on the ellipticities at 222 nm of both peptides are shown in Figure 3B.

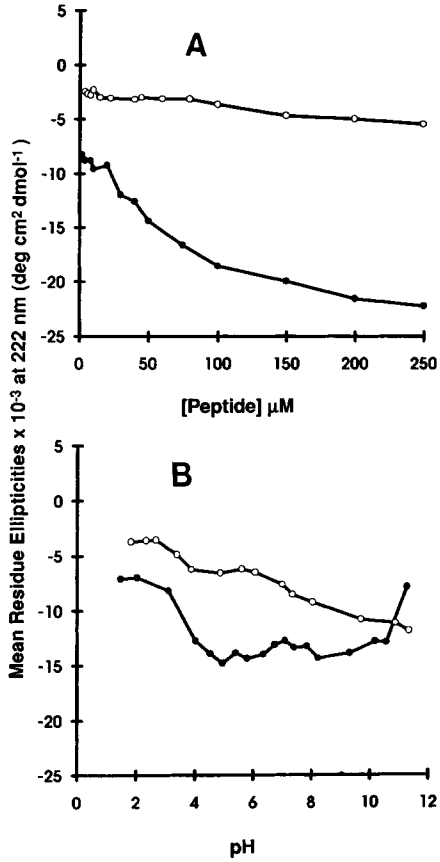

Fig. 3. Mean residue ellipticities at 222 nm in PBS. **(A)** Different peptide concentrations, and **(B)** different pH. ○, 18A and ●, Ac-18A-NH_2.

While the ellipticity of the protected peptide remains constant between pH 4.5 and 9.5, the ellipticity for 18A decreases with increasing pH over this range. Since the α-carboxyl group is expected to be fully ionized, the change in conformation is presumably due to titration of the α-amino group. Deprotonation of this group stabilizes the α-helical structure in 18A, by removing the positive charge that interacts unfavorably with the helix dipole.[24]

Right Angle Light Scattering Studies With DMPC and Egg PC Vesicles

The rates of micellization (conversion to discoidal complexes) of DMPC and egg PC liposomes by the peptides are compared in Figure 4. Similar to exchangeable apolipoproteins, both of the peptides disrupted DMPC liposomes with the micellization rate being faster for Ac-18A-NH_2 than for 18A. As can be seen in Figure 4, addition of intermediate concentrations of 18A caused an initial clarification of the solution which was followed by increased turbidity. This indicates that the discoidal complexes which are originally formed are metastable. The results also show that 18A was not as effective as the pro-

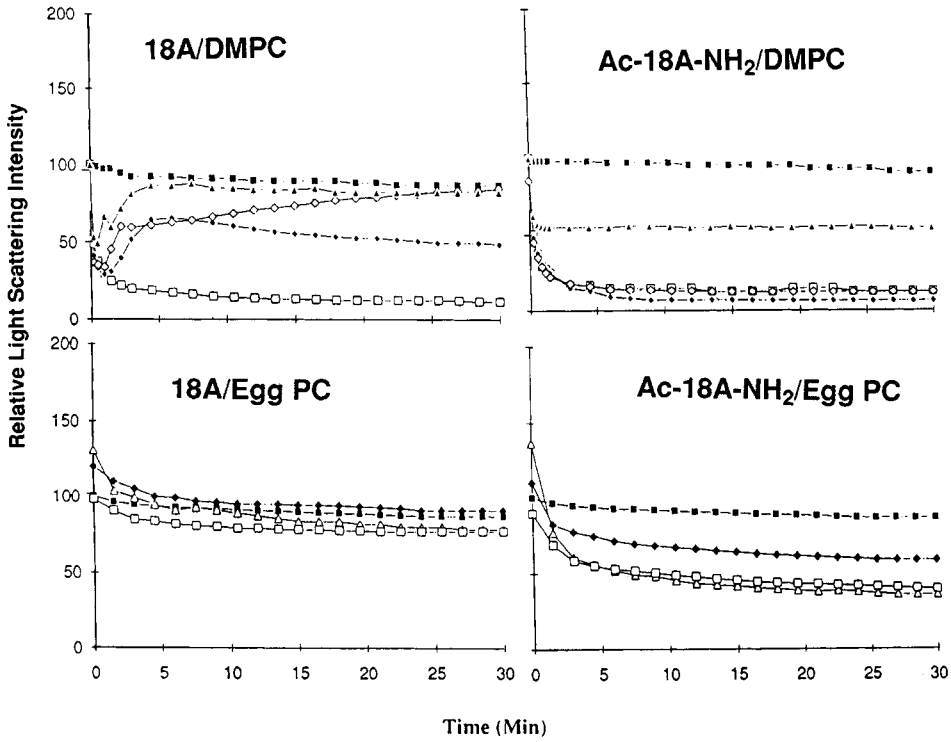

Fig. 4. Rate of decrease of scattered light at 90° from a suspension of DMPC and egg PC. Peptide concentrations were 25 μM in PBS buffer. Scattering intensity at 400 nm was expressed relative to the intensity measured in the absence of peptides for different amounts of lipid. ■, lipid alone, △, □, ◆, ◇, and ▲ correspond to lipid:peptide molar ratios of 1, 5, 10, 20, and 40, respectively.

tected peptide in converting egg PC multilamellar vesicles into discoidal complexes.

Denaturation of Peptide–Lipid Complexes With Guanidine Hydrochloride

Peptide/DMPC (1/20 mol/mol) complexes were prepared by the cholate dialysis procedure.[6] Analysis of the complexes indicated no loss of peptide or lipid as determined by quantitative amino acid analysis for the peptide and phosphate estimation for the phospholipid[35] (results not shown). These complexes at 23 μM peptide concentrations were subjected to denaturation by incubating with increasing concentrations of Gdn·HCl for 72 hr at room temperature. The denaturation was followed by a decrease in the magnitude of the ellipticity at 222 nm (Fig. 5); in these experiments it is assumed that the denatured peptide has zero ellipticity at 222 nm. Gdn·HCl, 2.5 M, was required for 50% denaturation of Ac-18A-NH_2; the equivalent figure for 18A is 1.3 M. The concentration of Gdn·HCl for the denaturation of similar discoidal complexes of apo A-I with DMPC is 2.8 M.[36] Thus, the Ac-18A-NH_2–lipid complexes are approximately as stable as apo A-I:lipid complexes. The Gdn·HCl denaturation data (Fig. 5) provide an estimate of the conformational stabilities of the two peptides when associated with lipid. Thus, the free

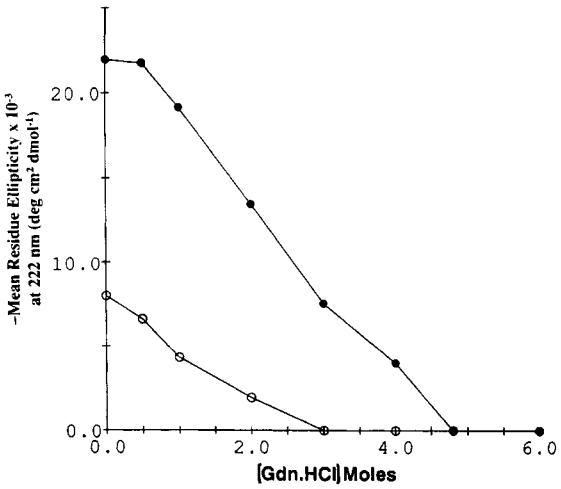

Fig. 5. Mean residue ellipticity at 222 nm of ○, 18A:DMPC complexes (1:20 mol/mol), and ●, Ac-18A-NH_2:DMPC complexes (1:20 mol/mol), with increasing guanidine hydrochloride concentrations.

energy of denaturation, ΔG°_{D}, gives a measure of the stability of the folded α-helica form of the peptide relative to the unfolded conformation. The data in Figure 5 were analyzed as described previously[36]

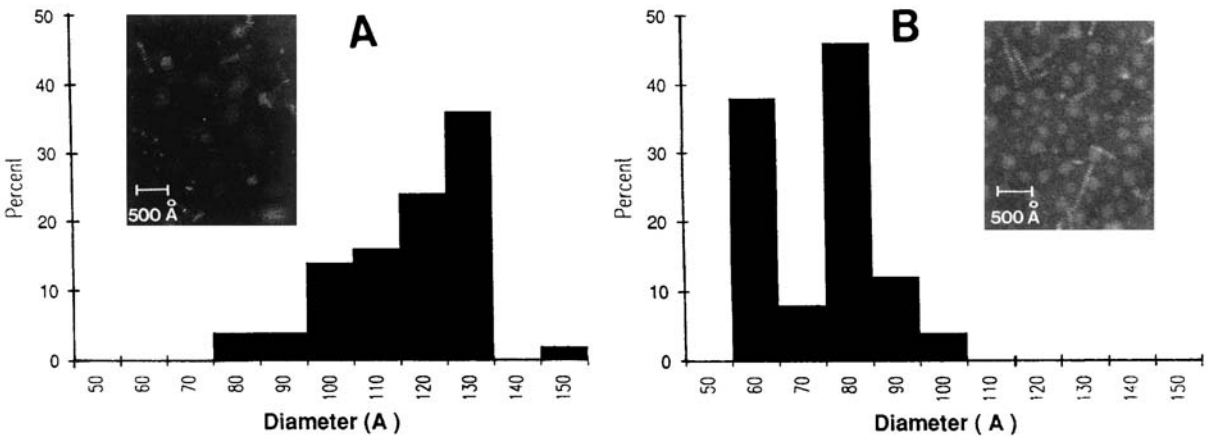

Fig. 6. Histograms of discoidal peptide:DMPC (1:20 mol/mol) complexes in PBS as measured by negative stain electron microscopy (inset). **(A)** 18A; and **(B)** Ac-18A-NH_2.

using a model in which the denaturation of peptide is driven by the enhancement of the binding of Gdn·HCl to the peptide due to the exposure of binding sites upon unfolding.[37,38] Linear regression analysis of the denaturation data yields ΔG°_{D}, = 1.7 ± 0.2 and 3.0 ± 0.6 kcal/mol peptide for 18A and Ac-18A-NH_2, respectively. Thus, blocking the endgroups of 18A stabilizes the α helix in the presence of lipid by about 1.3 kcal/mol. The model for the denaturation process indicates that about 9 mol of Gdn·HCl bind per mol peptide to induce the unfolding. In this regard Ac-18A-NH_2 more closely resembles apo A-I than does 18A.

Sizing of Peptide/Lipid Complexes

At the same peptide:lipid ratio, Ac-18A-NH_2 formed smaller peptide–DMPC complexes than 18A (Fig. 6). Since smaller complexes are more stable,[5] this result is consistent with the rates of micellization by the peptides in which the protected peptide was more effective in interacting with DMPC to form stable complexes. Peptides alone were subjected to nondenaturing gradient gel electrophoresis (Fig. 7A). In both cases, the bands observed indicate that peptides self-associate. The band due to Ac-18A-NH_2 has an apparent molecular weight of 200,000 while 18A migrates to a region of lower molecular weight. As shown in Figure 7B, at higher peptide:lipid ratios no free Ac-18A-NH_2 was detected in the gel. In contrast, with 18A, free peptide was observed at higher peptide:lipid ratios. With decreasing peptide:lipid ratios, the relative intensity of the lipid-unassociated peptide band decreased. These results can be explained by the higher lipid affinity of Ac-18A-NH_2 compared to 18A. The blocked peptide also seems to form more homogeneous discoidal complexes with DMPC than 18A.

Surface Chemistry

Figure 8 compares the adsorption isotherms for the two peptides interacting with an egg PC monolayer. Measurements of the dependence of the increase in surface pressure ($\Delta\pi$) on the subphase concentration of peptide provides a convenient method for determining the relative lipid affinities of the peptides.[32] Unlike the situation in bulk solution where the interaction of lipid and protein can involve a rearrangement of the phospholipid vesicle to some micellar structure, the PC monolayer provides a stable lipid surface to which the peptide binds. The binding of peptide can be analyzed in terms of a Langmuir adsorption isotherm which is equivalent to a Scatchard analysis. The peptide is presumed to bind reversibly to independent sites in the PC monolayer. The lines in Figure 9 show fits to this model using a nonlinear regression analysis (Program Enzfitter, Elsevier Biosoft, Cambridge, UK) on an IBM PC-compatible computer. The dissociation constants K_d for 18A and Ac-18A-NH_2 are 6.5×10^{-8} and 4.4×10^{-8} M peptide, respectively. From the relationship for the free energy of association $\Delta G_a = -RT \ln K_d$, the binding free energies for the 18A and Ac-18A-NH_2 peptides are −8.0 and −8.3 kcal/mol residue. Consistent with the higher lipid affinity of the blocked peptide, Ac-18A-NH_2 also exerts a maximal increase in surface pressure of 19 dyn/cm compared to the equivalent value of 16 dyn/cm obtained with the 18A molecule.

Effects on Lipid Polymorphism

Peptides and proteins that partition into the polar group region of a lipid bilayer are expected to raise the temperature of the transition from bilayer to hexagonal phase (T_H) by increasing the intrinsic radius of curvature of the membrane.[39,40] It is appar-

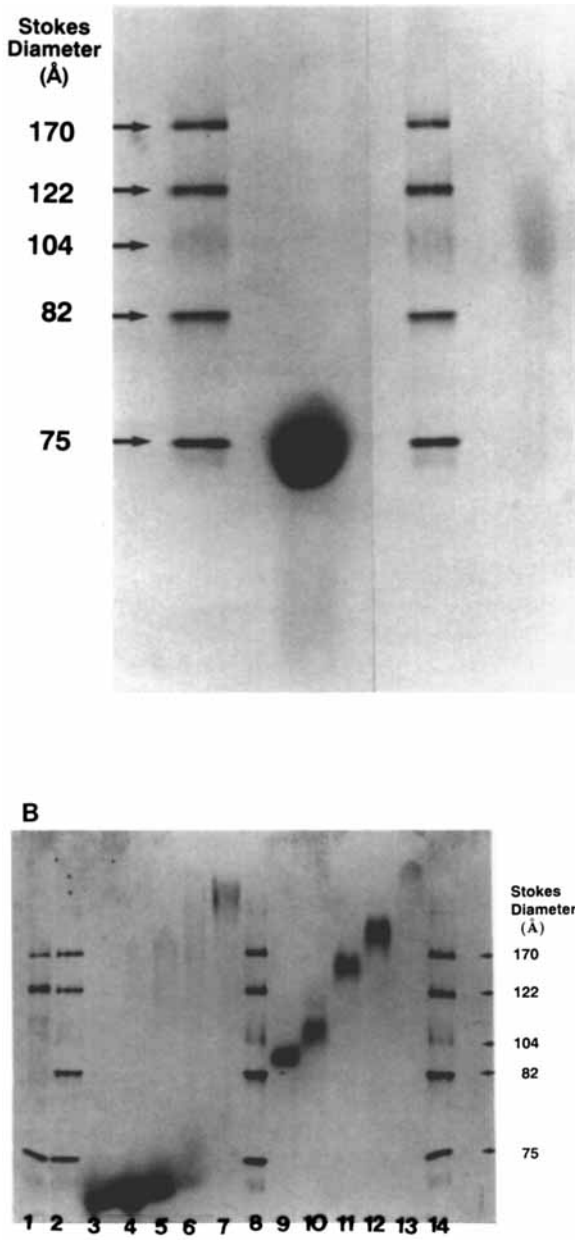

Fig. 7. Nondenaturing gradient gel electrophoresis of peptides **(A)** lanes 1 and 3, molecular weight standards; 2, 18A and 4, Ac-18A-NH_2 and peptide:DMPC complexes **(B)** at varying peptide:lipid ratios. Lanes 1, 2, 8, and 14, molecular weight standards; Lanes 3 to 7, DMPC:18A complexes at 1, 2.5, 5, 10, and 20 (mol/mol) ratios, respectively; and Lanes 9 to 13, DMPC:Ac-18A-NH_2 complexes at the above ratios, respectively.

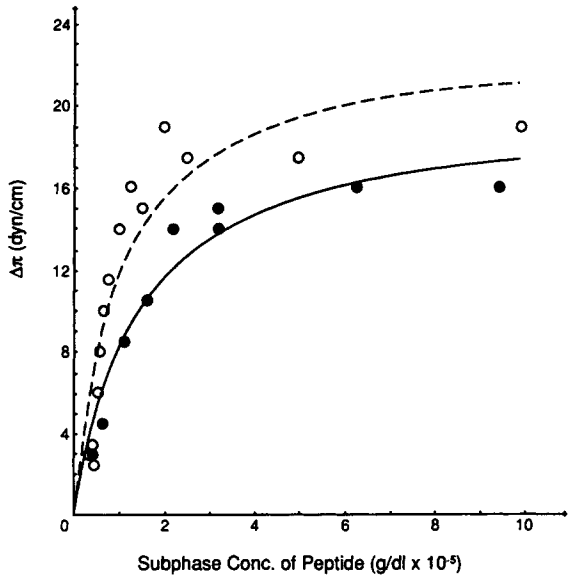

Fig. 8. Adsorption isotherms for peptides 18A and Ac-18A-NH_2 binding to monolayers of egg PC spread at an initial surface ressure of 10 dyn/cm. The increases in surface pressure ($\Delta\pi \pm$ dyn/cm) at various subphase concentrations of peptide in PBS, H 7.0 at room temperature are shown by the data points ●, 18A, nd ○, Ac-18A-NH_2. The continuous and dashed lines show the angmuir adsorption isotherms determined by nonlinear regression analysis for 18A and Ac-18A-NH_2. The K_d values given in the ext are derived from these lines.

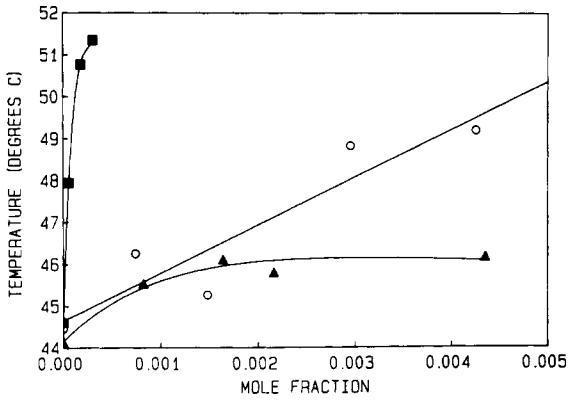

Fig. 9. Dependence of the bilayer to hexagonal phase transition temperature (T_H) of DPoPE on the mole fraction of added ▲, 18A, ○, Ac-18A-NH_2, and ■, apo A-I.

ent from Figure 9 that apo A-I is extremely potent in raising T_H of DPoPE and that Ac-18A-NH_2 is more effective than in 18A raising T_H. Furthermore, Ac-18A-NH_2 has an effect on T_H which is proportional to peptide concentration over a larger range of mole fractions of peptide suggesting that Ac-18A-NH_2 is more soluble than 18A in DPoPE membranes. The effect of apo A-I is about 28 times greater than that of Ac-18A-NH_2 on a molar basis but only 2-fold greater on a weight basis. Thus, Ac-18A-NH_2, on a weight basis, approaches the effectiveness of apo A-I in increasing T_H and in this regard, is much more potent than 18A.

Lecithin:Cholesterol Acyltransferase-Activating Ability

Peptides were studied for their ability to activate the enzyme LCAT. The results of these experiments

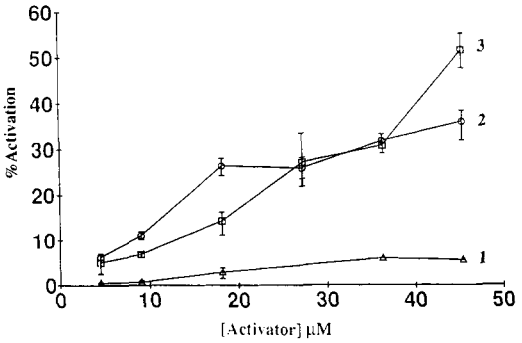

Fig. 10. Lecithin:cholesterol acyltransferase activation by apo A-I and synthetic peptide analogs of the amphipathic helix as measured using small unilamellar vesicles of egg PC as a substrate 1, 18A; 2, apo A-I; and 3, Ac-18A-NH_2. The data in the figure represent the mean of three experiments.

are shown in Figure 10. In the assay system studied, end group-blocked 18A had a greater LCAT-activating ability than unprotected 18A at all concentrations studied. At higher activator concentrations, the activation by Ac-18A-NH_2 was significantly greater than that induced by apo A-I.

DISCUSSION

The original observation on the amphipathic helix motif as present in exchangeable serum apolipoproteins[1] noted the presence of positively charged residues at the polar–nonpolar interface and negatively charged residues at the center of the polar face. The model peptide 18A was designed to mimic the amphipathic helical domains of apolipoproteins.[5] Thus, positively charged lysines were placed at the polar–nonpolar interface and negatively charged aspartic and glutamic acids at the center of the polar face. This peptide has been shown to mimic many of the properties of exchangeable apolipoproteins, such as association with phospholipids to form stable discoidal complexes and competing with apo A-I for binding to HDL.[9,10]

Apo A-I possesses tandem 22-mer amphipathic helical domains punctuated by Pro. Although the model peptide 18A mimics many of the properties of apo A-I, the helical content when bound to lipid is approximately only 50%. The helix is thus too short to be an ideal model for apo A-I. For this reason, we have attempted to increase the helical content by end group modification of 18A to make it a better mimic of apo A-I. Earlier, we have shown that substituting glutamic acid and leucine for aspartic acid and alanine, respectively, in a lipid-associating amphipathic helical peptide, increases both the helicity and lipid-associating ability.[9,10] The results presented here demonstrate that larger increases in α-helicity of 18A result from the end-group modifications. Such an increase in α-helicity with end-group modification is in agreement with published data on model α-helical peptides by Baldwin and coworkers.[24,25] In a model peptide such as 18A, five complete α-helical turns are possible. It is known that charged residues near the ends affect the stability of an isolated α helix.[24] It is also known that the charge on the α-NH_3^+ group destabilizes the helix through a repulsive electrostatic interaction with the peptide dipole. Similarly, the α-COO^- can also have a helix-destabilizing effect. An acetyl group at the α-NH_3^+ end and amidation of the carboxyl end stabilizes the α-helix of 18A by 1.3 kcal/mol. This change in free energy originates from the removal of repulsive electrostatic interactions between the terminal charged groups and the helix macro dipole which has its positive and negative poles located at the N- and C-terminal ends, respectively.[25] The end group modification stabilizes the entire 18A molecule as an amphipathic α-helix thereby enhancing the lipid affinity relative to the unblocked peptide molecule. End group modification of peptides has been used in other systems to improve the functional properties.[23]

Increased ability to interact with the lipid is evident from the intrinsic tryptophan fluorescence experiments which show a larger blue shift for Ac-18A-NH_2 compared to 18A. This increased ability of Ac-18A-NH_2 to interact with the lipid is also reflected in the right angle light scattering studies (Fig. 4) showing that complete micellization occurs at lipid:peptide ratios of 5, 10, and 20, whereas with 18A complete micellization occurs only at a lipid:peptide ratio of 5. The K_d values derived from the data in Figure 8 give approximate measurements of the high affinities of the amphipathic α helical 18A and Ac-18A-NH_2 molecules for lipid–water interfaces. The free energy of binding of about −8 kcal/mol residue reflects the various changes in molecular interactions and conformation involved in inserting the nonpolar faces of the amphipathic helix into the lipid environment. The burial of nonpolar residues on folding of a water-soluble globular protein is typically associated with a free energy change of about −2 kcal/mol residue.[41] This smaller free energy change is presumably due to a less effective removal of hydrophobic residues from contact with water relative to the interaction of the amphipathic helix with the lipid–water interfaces. It is interesting that the 43% higher helix content of Ac-18A-NH_2 when associated with lipids only increases the free energy of binding to the egg PC monolayer by about 3% (i.e., from −8.0 kcal/mol for 18A to −8.3 kcal/mol for Ac-18A-NH_2). The reasons for this are not clear at this time but may reflect a lower free energy for the lipid-free Ac-18A-NH_2 in aqueous solution due to enhanced self-association as noted for Ac-18A-NH_2 in the results section. These results are supported by the sedimentation coefficient values obtained for 18A (1.43 S) and Ac-18A-NH_2 (2.11 S) (data not shown).

We have shown that the blocked Ac-18A-NH_2 molecule, but not 18A, can convert egg PC vesicles into micellar structures at lipid/peptide molar ratios greater than 10. These results are analogous to the results observed by us for a 37-residue peptide that represents two 18A peptides linked through a Pro (37pA). The peptide was also capable of forming micellar structures with egg PC vesicles.[6] Since the discoidal particles are known to be better substrates for LCAT than vesicular substrates,[42] the peptide 37pA produced greater LCAT activation than apo A-I. As can be seen in Figure 10, the LCAT activation with the blocked Ac-18A-NH_2 peptide is bimodal; at activator concentrations below 18 μM, the LCAT activity is less than that induced by apo A-I (but more than that induced by 18A). The rank order of LCAT activity below 18 μM of the activators, which is apo A-I > Ac-18A-NH_2 > 18A, probably reflects the relative lipid affinities. Apo A-I and 18A were unable to disrupt egg PC vesicles; this was confirmed in the light scattering studies (Fig. 4 for 18A) and also in the LCAT-activation studies where the turbid vesicular substrates were not affected by the addition of increasing amounts of apo A-I and 18A. In contrast, with Ac-18A-NH_2, the egg PC liposomes were converted to micelles at higher concentrations of peptide (Fig. 4). It follows that the enhanced activation of the plasma enzyme LCAT with high concentrations of Ac-18A-NH_2 is due, at least in part, to the conversion of vesicular substrates into discoidal structures.

The blocked peptide increases the T_H of DPoPE, thus stabilizing the bilayer structure of this lipid. This is probably because of the increased length of the helix, which has an effect on the potential curvature of the interface stabilizing the bilayer and preventing the hexagonal phase formation. Consistent with this is the larger Trp blue shift indicating higher interaction of the N-terminal end of the blocked peptide (trptophan is the second amino acid from the N-terminus) with the acyl chains of the lipid than that occurs with 18A. Since membrane disruption and fusion have been implicated to involve hexagonal phase formation,[39,40] the potent antiviral and neutrophil inhibitory properties of apo A-I and model amphipathic peptides[13–16,43] could in part be explained by their ability to inhibit hexagonal phase formation.

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