Association of a Model Class A (Apolipoprotein) Amphipathic 
α Helical Peptide with Lipid

HIGH RESOLUTION NMR STUDIES OF PEPTIDE-LIPID DISCOIDAL COMPLEXES

Received for publication, October 21, 2005, and in revised form, December 29, 2005

Published, JBC Papers in Press, January 9, 2006, DOI 10.1074/jbc.M511475200

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Class A amphipathic helical peptides have been shown to mimic apolipoprotein A-I, the major protein component of high density lipoproteins and have been shown to inhibit atherosclerosis in several dyslipidemic mouse models. Previously we reported the NMR structure of Ac-18A-NH2, the base-line model class A amphipathic helical peptide in a 50% (v/v) trifluoroethanol-d6/water mixture, a membrane-mimic environment (Mishra, V. K., Palgunachari, M. N., Anantharamaiah, G. M., Jones, M. K., Segrest, J. P., and Krishna, N. R. (2001) Peptides 22, 567–573). The peptide Ac-18A-NH2 forms discoidal nascent high density lipoprotein-like particles with 1,2-dimyristoyl-sn-glycero-3-phosphocholine. Because subtle structural changes in the peptide-lipid complexes have been shown to be responsible for their antiatherogenic properties, we undertook high resolution NMR studies to deduce detailed structure of recombinant peptide 1,2-dimyristoyl-sn-glycero-3-phosphocholine complexes. The peptide adopts a well defined amphipathic α helical structure in association with the lipid at a 1:1 peptide-lipid weight ratio. Nuclear Overhauser effect spectroscopy revealed a number of intermolecular close contacts between the aromatic residues in the hydrophobic face of the helix and the lipid acyl chain protons. The pattern of observed peptide-lipid nuclear Overhauser effects is consistent with a parallel orientation of the amphipathic α helix, with respect to the plane of the lipid bilayer, on the edge of the disc (the belt model). Based on the results of chemical cross-linking and molecular modeling, we propose that peptide helices are arranged in a head to tail fashion to cover the edge of the disc. This arrangement of peptides is also consistent with the pKα values of the Lys residues determined previously. Taken together, these results provide for the first time a high resolution structural view of the peptide-lipid discoidal complexes formed by a class A amphipathic α helical peptide.

Lipoproteins are macromolecular complexes of lipids and proteins (apolipoproteins) that serve to transport lipids and lipid-soluble and water-insoluble molecules in blood throughout the body. Epidemiological studies have established an inverse correlation between the plasma levels of high density lipoprotein (HDL) cholesterol and the risk of coronary artery disease. In support of a protective role of human apolipoprotein A-I (apoA-I), the major protein accounting for 70% of the total protein present in HDL, it has been shown that expression of human apoA-I in atherosclerosis-sensitive mouse models leads to inhibition of atherosclerosis (1–4). In addition, recently it has been shown that infusion of recombinant HDL particles consisting of apoA-I Milano regressed already existing lesions in humans (5). Therefore, there is a great deal of interest in understanding structure-function relationships of HDLs.

The common lipid-associating motif identified in apoA-I and other exchangeable apolipoproteins is the amphipathic α helix with opposing polar and apolar faces (6). Most of the amphipathic α helices present in exchangeable apolipoproteins possess positively charged amino acid residues at the polar and nonpolar interface and negatively charged amino acid residues at the center of the polar face (7). Such amphipathic helices were grouped into class A (apolipoprotein class) motif (8). Over the past several years, our laboratory has been involved in the de novo design and studies of relatively small synthetic apolipoprotein-mimic peptides. An 18-residue peptide, namely 18A, was designed to mimic the class A amphipathic helical motif (9, 10). The peptide 18A has the following amino acid sequence: Asp-Trp-Leu-Lys-Ala-Phe-Tyr-Asp-Lys-Val-Ala-Glu-Glu-Leu-Asp-Glu-Ala-Phe. It has been shown that acetylation of the N-terminal end and amidating the C-terminal end of 18A to produce Ac-18A-NH2 results in helix stabilization and increased lipid affinity of the peptide (11). The peptide Ac-18A-NH2 has been shown to mimic certain properties of human apoA-I. For example, it has been shown that Ac-18A-NH2 is able to disrupt multilamellar vesicles of DMPC to form discoidal peptide-lipid complexes analogous to apoA-I (11). The global secondary structure of Ac-18A-NH2 under different environments has been studied using CD (11, 12) and oriented CD (13) spectroscopy. In addition, we have determined the solution NMR structure of Ac-18A-NH2 in 50% (v/v) trifluoroethanol-d6/H2O mixture, a membrane-mimic environment (14). However, these studies did not distinguish subtle variations in lipid-associated structures of peptides that may distinguish them from atheroprotective and inactive complexes (15). For example, the orientation of the peptide with respect to lipid in discoidal peptide-lipid complexes has to be understood before we can understand the subtle changes in the structure of peptide-lipid complexes.

The amino acid residues at the center of the polar face (7). Such amphipathic helices were grouped into class A (apolipoprotein class) motif (8). Over the past several years, our laboratory has been involved in the de novo design and studies of relatively small synthetic apolipoprotein-mimic peptides. An 18-residue peptide, namely 18A, was designed to mimic the class A amphipathic helical motif (9, 10). The peptide 18A has the following amino acid sequence: Asp-Trp-Leu-Lys-Ala-Phe-Tyr-Asp-Lys-Val-Ala-Glu-Glu-Leu-Asp-Glu-Ala-Phe. It has been shown that acetylation of the N-terminal end and amidating the C-terminal end of 18A to produce Ac-18A-NH2 results in helix stabilization and increased lipid affinity of the peptide (11). The peptide Ac-18A-NH2 has been shown to mimic certain properties of human apoA-I. For example, it has been shown that Ac-18A-NH2 is able to disrupt multilamellar vesicles of DMPC to form discoidal peptide-lipid complexes analogous to apoA-I (11). The global secondary structure of Ac-18A-NH2 under different environments has been studied using CD (11, 12) and oriented CD (13) spectroscopy. In addition, we have determined the solution NMR structure of Ac-18A-NH2 in 50% (v/v) trifluoroethanol-d6/H2O mixture, a membrane-mimic environment (14). However, these studies did not distinguish subtle variations in lipid-associated structures of peptides that may distinguish them from atheroprotective and inactive complexes (15). For example, the orientation of the peptide with respect to lipid in discoidal peptide-lipid complexes has to be understood before we can understand the subtle changes in the structure of peptide-lipid complexes.
NMR Studies of Discoidal Complexes

complexes that lead to atheroprotective or inactive complexes. With a view to establish a sensitive and high resolution method to understand subtle differences in peptide-lipid complexes, we used two-dimensional 1H NMR spectroscopy to investigate the structure of Ac-18A-NH₂ bound to DMPC discs. The present studies for the first time show that the peptide helix axis is oriented parallel to the bilayer plane on the edge of the disc. The results also reveal several intermolecular interactions of the hydrophobic amino acid side chains with lipid acyl chains. Based on the results of chemical cross-linking of the Lys residues in the peptides on the disc and molecular modeling, we propose that two amphipathic α helices arranged in an antiparallel fashion cover the lipid acyl chains on the edge of the disc. This is in agreement with our earlier 13C NMR studies of the discoidal complexes of Ac-18A-NH₂ and DMPC that indicated that Lys⁹ and Lys¹³ residues located on the same side (right side, viewing through the axis of the helix from the N terminus) of the amphipathic α helix have reduced pKₐ values because of their exposure to a basic microenvironment (16).

MATERIALS AND METHODS

Peptide Synthesis, Purification, and NMR Sample Preparation—The peptide Ac-18A-NH₂ was synthesized and purified as described earlier (11). Three separate sets of samples were prepared for the NMR studies: (a) perdeuterated DMPC ([1,2-dimyristoyl-sn-glycero-3-phosphocholine-1,2,2,2-d₂,N,N,N-trimethyl-d₁₄, Avanti Polar-Lipids, Inc., Alabaster, AL) and peptide in 90% H₂O, 10% D₂O (100% deuterium oxide, ratio of 1:1 (w/w). Methanol was evaporated under a stream of nitrogen separately in methanol. Appropriate amounts of peptide and DMPC were incubated at room temperature, the cross-linked peptide was purified using reverse-phase HPLC. An analytical C₁₈ reverse-phase column (4.6 mm × 25 cm; particle size, 5 μm, Vydac), with a flow rate of 1.2 ml/min using a linear gradient of 25–90% acetonitrile (containing 0.1% trifluoroacetic acid) during 65 min, was used for separation. Individual fractions collected from HPLC were digested with trypsin (sequencing grade, Roche Applied Science, 12.5 ng/μl) at 37 °C for 16 h. The peptide fragments were analyzed by matrix-assisted laser desorption ionization time-of-flight (Perspective Biosciences, Model Voyager-DE2 PRO) and electrospray ionization-tandem mass spectrometry sequencing (MicroMass Q TOF-2). These analyses were performed at the Comprehensive Cancer Center Mass Spectrometric Shared Facility of the University of Alabama at Birmingham.

NMR Measurements and Structure Calculations—All one-dimensional and two-dimensional NMR experiments were performed on Bruker Avance-600 and -500 NMR spectrometers at 37 °C. One-dimensional ^31P experiments were run at 202.45 MHz on Avance-500 with sodium phosphate buffer as an external reference set at 0.0 ppm. 50-, 100 – and 200-ms mixing times were used in NOESY, and a 100-ms spin-lock time was used in total correlation spectroscopy measurements. Total correlation spectroscopy artifacts in rotating frame Overhauser enhancement spectroscopy with 200-ms spin-lock time were suppressed by using a train of 180° pulses (17). All two-dimensional data sets were collected with 2048 complex t₁ points with 512 t₂ increments. Time proportional phase incrementation as described by Stales et al. (18) was used for frequency discrimination in the indirect dimension. Chemical shifts were referenced with respect to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (0.0 ppm) used as an internal standard.

The NMR data were transferred to a Silicon Graphics IRIS Indigo work station and processed using the program FELIX (version 2004) (Accelrys Software Inc., San Diego, CA). NMR structures were calculated on a Silicon Graphics IRIS Indigo work station using X-PLOR (on-line) version 3.851. The structure calculation protocol (19) involved (a) generation of a “template” coordinate set; (b) bound smoothing, full structure embedding, and regularization to produce a family of 100 distance geometry structures (20, 21); (c) simulated annealing regularization and refinement for embedded distance geometry structures (20–22); and (d) simulated annealing refinement (20). Average coordinates of the accepted structures were energy-minimized using 200 cycles of conjugate gradient energy minimization. All the NMR constraints were enforced during energy minimization.

Distance and Dihedral Angle Constraints—On the basis of cross-peak intensities in the NOESY spectra, recorded with mixing times of 100 and 200 ms, the NOE distance constraints were classified as follows (23). For NOEs involving intraresidue and sequential NH, dH, and dβ protons, a distance constraint of 2.0–2.5 Å for strong, 2.0–4.0 Å for medium, and 2.0–4.0 Å for weak NOEs was used; for medium or long range NOEs involving NH and dH protons, a distance constraint of 2.0–4.0 Å was used; for NOEs involving medium or long range side chain protons, a distance constraint of 2.0–5.0 Å was used. Pseudoatom corrections, where appropriate, were added to the upper distance bounds as described previously (23, 24). Based on the difference (Δδ) in the observed and “coil” NH proton chemical shifts, H-bond (H–O) distances were calculated as described earlier (23, 25). These H-bond distances were included as additional constraints (26) during structure calculation. Dihedral angles Φ and Ψ were based on the difference in the observed and coil C₄–H proton chemical shifts (23, 25); a minimum deviation of 40° was allowed in the dihedral constraints. Molecular modeling was performed on a Silicon Graphics IRIS Indigo work station using the program SYBYL (version 7.0) (Tripos, Inc., St. Louis, MO).

RESULTS

The size exclusion profile of the peptide:DMPC complex is shown in Fig. 1. The peptide elutes predominantly as a DMPC complex at the peptide:DMPC ratio used (1:1, w/w or 1:3.3, mol/mol) (Fig. 1). The apparent Stokes diameter of the complex, estimated using high and low
molecular weight calibration kits (Amersham Biosciences), is 69 Å (Fig. 1). The lipid to peptide ratio in the peak fraction was determined to be 1:2:1 (w/w). Trypsin digestion of the cross-linked Ac-18A-NH2 on the DMPC discoidal complexes yielded two major products: one in which Lys9 and Lys13 (corresponding to one cross-linker/molecule of the peptide) and the other in which Lys8 and Lys13 of two neighboring peptides were intermolecularly cross-linked by two bis(sulfosuccinimidy) suberate molecules as shown in the supplemental material.

A combination of total correlation spectroscopy (spectrum not shown) and NOESY spectrum was used to make sequence-specific resonance assignments of the individual amino acids in Ac-18A-NH2 (27). The chemical shifts of the observed 1H signals, referenced with respect to internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate at 0.0 ppm, are shown in Table 1.

Fig. 2, A and B, show the amide/aromatic proton region and the fingerprint region, respectively, of the NOESY spectrum of Ac-18A-NH2 on the DMPC discoidal complexes yielded two major products: one in which Lys8 and Lys13 were intramolecularly cross-linked (mass spectral value corresponded to the fragment AFYDKVAKELK of Ac-18A-NH2 with the addition of 138, corresponding to one cross-linker/molecule of the peptide) and the other in which Lys8 and Lys13 of two neighboring peptides were intermolecularly cross-linked by two bis(sulfosuccinimidyl) suberate molecules as shown in the supplemental material.

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Fig. 2, A and B, show the amide/aromatic proton region and the fingerprint region, respectively, of the NOESY spectrum of Ac-18A-NH2 obtained using a mixing time of 200 ms. Sequential dNN(i, i + 1), NOESY cross-peaks for all the residues in Ac-18A-NH2 are identified in Fig. 2A. In addition, dNN(i, i + 2) cross-peaks were observed for Leu7/Ala8 and Lys13/Lys15 pairs and are indicated in Fig. 2A. In Fig. 2B intra-residue HN/CαH NOEs for all the residues in Ac-18A-NH2 are labeled. A number of other long range (i, i + 3 and i, i + 4) NOEs involving side chain protons of the nonpolar amino acid residues (Trp7/Ala8, Trp7/Phe6, Leu3/Tyr5, Phe6/Val10, Tyr7/Val10, Tyr7/Ala8, and Leu14/Phe18) were observed (Fig. 2, A and B). The methyl protons of the N-terminal acetyl protecting group showed NOEs to Asp1 and Trp7 backbone NH protons as well as to the Trp7 aromatic ring proton (2H) (Fig. 2B). In addition, a NOE was observed between the aromatic ring protons of Phe6/Tyr7 (Fig. 2A). The observed (i, i + 1), (i, i + 2), (i, i + 3), and (i, i + 4) NOEs are schematically shown in Fig. 3. For the sequential NOEs, the thickness of the line is in proportion to their intensity (Fig. 3). The NOEs that could not be assigned unambiguously because of the resonance overlap are shown with empty rectangles (Fig. 3).

Proton chemical shifts are related to the secondary structures of peptides and proteins (25, 28). The plots of the difference between the observed chemical shift and the coil chemical shift (23, 25) ($\Delta\delta = \delta_{\text{observed}} - \delta_{\text{coil}}$) for the CαH and the NH protons in Ac-18A-NH2 are shown in Fig. 4, A and B, respectively. A periodic (3–4-residue) variation is evident in the case of NH proton chemical shifts ($\Delta\delta$) (Fig. 4B).

The distance and dihedral constraints were used to calculate NMR structures of the peptide associated with DMPC disc. Starting from a set of initial 100 extended structures of Ac-18A-NH2, 62 structures were accepted based on the following criteria: (a) no NOE violations >0.5 Å, (b) in dihedral angle violations >5°, (c) r.m.s. difference for bond deviations from ideality <0.01 Å, and (d) r.m.s. difference for angle deviations from ideality <2°. To obtain the average NMR structure, average coordinates of the 62 accepted structures were energy-minimized. All the distance and the dihedral angle constraints were enforced during the energy minimization of the average coordinates. The structural statistics for the 62 accepted NMR structures and the energy-minimized average structure of Ac-18A-NH2 are summarized in Table 2. The 62 accepted structures were superimposed onto the energy-minimized average structure using MATCH (SYBYL, version 7.0). The program MATCH performs an automatic least squares fit of two molecules that differ only in the coordinates of their atoms. Fig. 5 shows superposition of the Cα atoms of all the 62 accepted structures on the energy-minimized average NMR structure of Ac-18A-NH2.

Fig. 6 shows a DMPC molecule with different protons labeled. To
observe intermolecular NOEs between peptide and lipid protons, NOESY and rotating frame Overhauser enhancement spectroscopy spectra using three different mixing times of 50, 100, and 200 ms were obtained with the peptide and protonated lipid in 100% D\textsubscript{2}O. The NOESY spectra were compared with those obtained using perdeuterated DMPC in H\textsubscript{2}O. Fig. 7 shows an overlay view of the two NOESY spectra obtained using a mixing time of 200 ms. The spectrum obtained with protonated DMPC is shown in black, and that with perdeuterated DMPC is in red. The NOEs that are seen only with protonated DMPC are enclosed in rectangles. The assignments of the observed lipid protons are marked next to the observed NOESY cross-peaks (Fig. 7). Fig. 8A shows the one-dimensional horizontal slices of the NOESY spectrum obtained in the presence of protonated DMPC using a mixing time of 100 ms. The corresponding lipid protons are indicated next to the horizontal slices (Fig. 8A). It was shown previously that peptides homologous to Ac-18A-NH\textsubscript{2} also give NOESY cross-peaks between aliphatic protons of the lipid and aromatic protons from the peptide side chains (29). However, with one such analog, such cross-peaks were observed only in the absence of cholesterol (30). Fig. 8B shows the one-dimensional horizontal slice of the 100-ms NOESY spectrum corresponding to the $(\text{CH}_2)_n$ protons of DMPC alkyl chain and the observed peptide aromatic proton signals. The aromatic proton signals as well as the lipid [2,CH\textsubscript{2}] proton signals are labeled in Fig. 8B.

DISCUSSION

An amphipathic $\alpha$ helix (henceforth referred to as amphipathic helix) is an often encountered secondary structural motif in lipid-associating peptides and proteins (6–8). A number of studies, including the x-ray crystal structure of an N terminus deletion mutant of human apolipoprotein A-I, apoA-I(1–43)A-I (31), have provided strong experimental evidence for the involvement of amphipathic helices in the lipid association of apolipoproteins (32).

The peptide Ac-18A-NH\textsubscript{2} is a model class A amphipathic $\alpha$ helix that
has been shown previously to mimic certain physical-chemical and biological properties of human apoA-I (9–11). One of the properties this peptide shares with apoA-I is its ability to solubilize phospholipids to form discoidal particles (11).

We have shown that in a set of class A amphipathic helical peptides, despite similar physical-chemical properties, only some of the peptide/lipid complexes are antiatherogenic, and they are antiatherogenic by sequestering oxidized lipids (15). Such complexes are also involved in improving the antiatherogenic properties of HDL in atherosclerosis-sensitive animal models (15). To understand subtle differences in the peptide/lipid complex properties that lead to differences in the biological properties, it is important to gain a detailed structural understanding of these complexes. Because Ac-18A-NH₂ is a prototype peptide from which several other active analogs were designed (15), we have studied the discoidal DMPC complex of this peptide in detail using high resolution NMR spectroscopy.

Peptide Forms a Well Defined Class A Amphipathic α-Helix—It has been shown previously that the end group blockage increases the helical content of the unblocked peptide 18A by removing the destabilizing interactions of the helix macrodipole with the charged termini (11). It is worthy of note that in an α helix the N-terminal acetyl and the C-terminal amide blocking groups also provide for the formation of two additional H-bonds (one at each end) between residues i (acceptor) and i + 4 (donor) compared with the unblocked peptide. The N-terminal acetyl group shows NOEs to Asp₁ (strong) and Trp₂ (weak) backbone NH protons (Fig. 2B), suggesting its involvement in helix stabilization.
The observation of NOEs between hydrophobic side chains spaced at (i, i + 3) and (i, i + 4) positions in the linear sequence (Trp/Pro, Trp/Val, Ala/Leu, Leu/Trp, Phe/Val, Tyr/Val, Ala/Leu, and Leu/Pro) is also consistent with an α helical structure of the peptide. In addition, the aromatic rings of Phe and Tyr are sufficiently close in space to give rise to the observed NOEs between their ring protons (Fig. 2A).

The pattern of stronger dNN(i, i + 1) and weaker dNN(i, i + 4) NOEs as well as the observation of several well resolved dNN(i, i + 3) NOEs (Fig. 3) confirms that Ac-18A-NH₂ adopts an α helical structure in DMPC discs. A few of the dNN(i, i + 4) and dNN(i, i + 3) NOEs could not be confirmed due to resonance overlap but are likely to be present as well (Fig. 3).

The 1H NMR chemical shifts in peptides and proteins are dependent on the secondary structure (25, 28), e.g. CαH protons move upfield (relative to their random coil value) on helix formation. An inspection of Fig. 4A reveals that all of the CαH protons in Ac-18A-NH₂ experience an upfield chemical shift relative to their coil values (23, 25). Thus, the chemical shifts of CαH protons are consistent with an α helical structure of Ac-18A-NH₂.

The NH protons in Ac-18A-NH₂ show a periodic 3–4-residue variation in the (Δδ) chemical shifts (Fig. 4B). The periodicity in the NH proton chemical shifts has been related to the amphipathicity of the helix (28, 33–36). The periodicity in the NH proton chemical shift is thought to result from a shortening (strengthening) of the H-bonds on the hydrophobic side (a medium of low dielectric) and a lengthening (weakening) of the H-bonds on the hydrophilic side (a medium of high dielectric) of the helix. The NMR structures obtained based on the distance and dihedral angle constraints show that the peptide Ac-18A-NH₂ forms a well defined class A amphipathic α helix with hydrophobic residues clustered on one side and hydrophilic residues clustered on the opposite side of the long axis of the helix (Fig. 5).

**Peptide Interacts with the Lipid Acyl Chains and Is Oriented Parallel to the Plane of the Bilayer—**To determine genuine intermolecular peptide/lipid NOEs, NOESY spectra of the peptide obtained with protonated DMPC in D₂O and deuterated DMPC in H₂O were compared (Fig. 7). This comparison of the two spectra should rule out any incorrect assignment of intramolecular peptide NOE as intermolecular peptide/lipid NOE. A number of intermolecular NOEs were present in the case of protonated DMPC that were absent in the case of deuterated DMPC (Fig. 7). These NOEs were further identified and compared by extracting one-dimensional horizontal slices of the NOESY spectrum obtained with protonated DMPC (Fig. 8A). A maximum number of NOEs were seen between lipid alkyl chain methylene protons, (CH₂),
and the peptide aromatic protons (Fig. 8, A and B). It is important to note that these intermolecular NOEs (Fig. 8B) were seen with aromatic ring protons of the residues that are far apart in the linear sequence (e.g., Trp<sup>2</sup>, Tyr<sup>7</sup>, and Phe<sup>18</sup>) and are not expected to come close in space in an α-helical conformation. This observation strongly suggests that the peptide is oriented parallel to the plane of the membrane (and, therefore,
perpendicular to the lipid acyl chains) in the DMPC discoidal particles (Fig. 9A). To support this conclusion, it is noted that if the peptide adopted an orientation that is perpendicular to the plane of the membrane (and, therefore, parallel to the lipid acyl chains, as shown in Fig. 9B), we should have observed intermolecular NOEs, e.g. between Trp and Phe and lipid head group protons. We did not observe such intermolecular NOEs.

Peptide Helices Are Arranged in a Head to Tail Fashion to Cover the Edge of the Disc—The apparent Stokes diameter of the peptide-lipid disc is 69 Å (Fig. 1). For this disc size, the number of DMPC molecules per disc (assuming a helix diameter of 10 Å and a surface area/DMPC of 70 Å²) are 54 (number of DMPC per disc = 2π(d − 20)/2)/70) (37). Because the peptide-lipid molar ratio is 1:3:3, there are ~16 peptide molecules per disc. Molecular modeling studies indicate that at least two Ac-18A-NH₂ molecules, with axes of two helices separated by 10 Å, are required to shield the hydrophobic lipid acyl chains of the DMPC on the edge of the discoidal particles in the aqueous environment. It is interesting to note that 16 helical peptide molecules per disc arranged in a double belt fashion will result in a ~79-Å-diameter disc (38). Our earlier ¹³C NMR studies showed that Lys residues in Ac-18A-NH₂ are in different microenvironments (16). Interestingly these studies indicated that Lys⁹ and Lys¹³ possess identical pKa values of 9.4, whereas pKa values of Lys⁷ and Lys¹⁵ were 11.0 and 10.3, respectively (16). The pKa value for the free Lys residue is 10.1 (16). As shown in Fig. 9A, residues Lys⁷ and Lys¹³ and residues Lys⁹ and Lys¹⁵ are on the opposite sides of the helix. Earlier we proposed that the reason for lower pKa values for residues Lys⁹ and Lys¹³ is because they are present in a basic microenvironment (16). An antiparallel orientation of two Ac-18A-NH₂ molecules on the edge of the DMPC disc will create such a basic microenvironment for Lys⁹ and Lys¹³ residues (Fig. 9A). As shown in Fig. 9A, the other Lys residues (Lys⁴ and Lys¹⁵) are in a polar environment with a possible proximity to the lipid head group. It is also noted that in this orientation the hydroxyl group of the Tyr residue will be placed closer to the aqueous environment. The spatial proximity of Lys⁷ and Lys¹³ residues of the two Ac-18A-NH₂ molecules was further supported by chemical cross-linking using bis(sulfosuccinimidy) suberate (spacer arm length, 11.4 Å). It is important to note that none of the alternative arrangements of two Ac-18A-NH₂ molecules on the edge of the DMPC disc (supplemental Fig. 10) will be consistent with the results of either the ¹³C NMR studies (16) or the cross-linking studies.

In conclusion, the present NMR studies support the arrangement of the peptide helices on the edge of the peptide-lipid discoidal particles in which peptide helices are arranged in an antiparallel manner with the helix axes perpendicular to the lipid acyl chains. This structure satisfies not only the observed pattern of intermolecular NOEs but also the different pKa values that have sidedness for four Lys residues in Ac-18A-NH₂. In addition, these studies provide us with a possible unique methodology for understanding subtle differences in peptide-lipid discoidal complexes that result in atheroprotective HDL-like particles.

Acknowledgments—We thank Professor Axel T. Brünger for access to the online version 3.851 of X-PLOR. We thank Martin K. Jones for help during the computational work. Marion Kirk is acknowledged for the mass spectral analysis of the products of the chemical cross-linking of the peptide on the DMPC discs.

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FIGURE 9. A, molecular model showing two average energy-minimized molecules of Ac-18A-NH₂ oriented parallel to the plane of the membrane in a head to tail fashion. B, molecular model showing average energy-minimized molecule of Ac-18A-NH₂ oriented perpendicular to the plane of the membrane. Molecular models were generated using the program SYBYL. DMPC molecules were extracted from a lipid bilayer containing 120 DMPC molecules after a molecular dynamic simulation of 200 ns (39).
