Microenvironments of Basic Amino Acids in Amphipathic α-Helices Bound to Phospholipid: ^{13}C NMR Studies Using Selectively Labeled Peptides†

Sissel Lund-Katz,*,‡ Michael C. Phillips,‡ Vinod K. Mishra,§ Jere P. Segrest,§ and G. M. Anantharamaiah§

Department of Biochemistry, Medical College of Pennsylvania and Hahnemann University, Philadelphia, Pennsylvania 19129, and Departments of Medicine, Biochemistry and Molecular Genetics and the Atherosclerosis Research Unit, University of Alabama Medical Center, Birmingham, Alabama 35294

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ABSTRACT: The lipid-binding properties of serum apolipoproteins are mediated by class A amphipathic α-helices in which basic and acidic amino acid residues tend to be localized at the helix polar−nonpolar boundary and the center of the polar face, respectively. To better understand the role of the interfacial basic residues, the microenvironments and titration characteristics of lysine (Lys) residues have been examined using two 18-residue peptides. The parent 18A molecule (AspTrpLeuLysAlaPheTyrAspLysValAlaGluLysLeuLysGluAlaPhe) binds phospholipid relatively well because of its class A amphipathic helix, whereas 18R, which has the positions of basic and acidic residues reversed, binds to phospholipid relatively weakly. Lys residues were ^{13}C-dimethylated, and NMR spectra were obtained of the peptides complexed with dimyristoylphosphatidylcholine (1/1 (w/w) DMPC/peptide). The four [^{13}C]-ε-dimethyl-Lys in 18A gave four resonances at chemical shifts (δ) of 42−43 ppm, whereas only a single resonance at δ 42.70 ppm was observed from the four Lys in 18R. Measurements of δ as a function of pH gave pK_a values. The four Lys in 18R had a common pK_a of 10.3, indicating that the Lys in the polar face of the amphipathic helix have the pK_a expected for noninteracting, fully hydrated groups. In contrast, the four Lys in Ac-18A-NH_2 had pK_a's in the range 9.4−11.0, indicating that they exist in various microenvironments at the polar−nonpolar boundary of the 18A helix. To examine individual Lys, 18A was synthesized with various Lys selectively labeled. Lys-4, -9, -13, and -15 in Ac-18A-NH_2 associated with DMPC had pK_a values of 11.0, 9.4, 9.4, and 10.3, respectively. The various Lys pK_a values in 18A or Ac-18A-NH_2 are determined primarily by interhelix interactions between antiparallel peptide molecules. In this configuration, the pK_a's of Lys-4 and Lys-15 are increased by the formation of attractive amino-aromatic and ion-pair interactions, respectively. Lys-9 and Lys-13, which are positioned on the opposite side of the amphipathic helix, experience a basic microenvironment that decreases their pK_a's. Novel insights into interactions between class A amphipathic helices in lipoprotein particles can be gained by the approach used in this study.

The structures of serum lipoprotein particles are stabilized in part by the interaction of apolipoproteins with the microemulsified phospholipid, triglyceride, cholesteryl ester, and unesterified cholesterol. It has been known for some 20 years that the lipid-binding domains in the exchangeable apolipoproteins contain amphipathic α-helices (Segrest et al., 1974). To understand the details of the lipid−protein interactions, it is necessary to define the conformations of apoproteins at the lipoprotein particle surface. This is a difficult task because lipoprotein particles cannot be crystallized in a form suitable for X-ray diffraction, and the particles are too large for study by current multidimensional NMR methods. Another complication is that the conformations of apolipoprotein molecules are dependent upon the size of the lipoprotein particle with which they are associated. For these reasons, a variety of physical−biochemical methods have been applied to the study of apolipoprotein conformation and interaction with lipids [for a review, see Atkinson and Small (1986)]. The data from these experiments are broadly consistent with lipid binding being mediated by amphipathic helices, as originally proposed by Segrest and colleagues (Segrest et al., 1974). More recently, the biologically active amphipathic helices have been classified into seven different groups on the basis of the position of charged residues of the polar face of the helix (Segrest et al., 1990, 1992). Class A amphipathic helices have positively charged residues clustered at the polar−nonpolar interface and negatively charged residues at the center of the polar face. The helical segments of several exchangeable apolipoproteins correspond to class A amphipathic helices.

The structural basis for variations in the lipid-binding properties of different amphipathic helices is being investigated in these laboratories. An 18-residue model peptide designated 18A has been used to explore the properties of class A amphipathic helices. This peptide, which has the sequence AspTrpLeuLysAlaPheTyrAspLysValAlaGluLysLeuLysGluAlaPhe, binds to dimyristoylphosphatidylcholine (DMPC)[1] in an fashion analogous to apolipoprotein (apo) A-I to form diskoidal complexes (Anantharamaiah et al., 1985). The four lysine (Lys) residues in 18A are situated

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* Author to whom correspondence should be addressed.
‡ Medical College of Pennsylvania and Hahnemann University.
§ University of Alabama Medical Center.

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[1] Abbreviations: apo-A-I, apolipoprotein A-I; δ, chemical shift; DMPC, dimyristoylphosphatidylcholine; HDL, high-density lipoprotein.

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close to the polar−nonpolar interface of the amphipathic helix, whereas the peptide designated reverse 18A (or 18R), in which the positions of the acidic and basic residues are exchanged, has lower lipid binding affinity than 18A (Anantharamaiah et al., 1985; Spuhler et al., 1994). The amino acid sequence of 18R is LysTrpLeuAspAlaPheTyr-LysAspValAlaLysGluLeuGluLysAlaPhe. To explain the higher lipid binding affinity of 18A, it has been suggested (Segrest et al., 1992; Mishra et al., 1994) that the Lys residues extend ("snorkel") toward the polar phase, so that the charged amino group is located in the aqueous phase while the hydrophobic methylene groups in the side chain remain buried in the lipid milieu. By adopting this conformation, the lipid affinity of class A amphipathic helices is enhanced. The organization of the amphipathic helices in a molecule such as apo-A-I is dependent upon the size and shape of the lipoprotein particle with which it is associated. Helix−helix interactions between class A amphipathic helices in human apo-A-I are thought to be important in both diskoidal and spherical HDL particles (Brasseur et al., 1990; Rosseneu et al., 1992). However, information about the helix−helix interactions is not available at this time. As the results presented here show, the microenvironments, as reflected in the pK_a values, of Lys residues in class A amphipathic helices are sensitive to helix−helix interactions. It is possible to examine the helix organization in a lipoprotein particle in some detail from knowledge of the pK_a values of specific Lys residues.

This paper describes such a study using the model 18A and 18R peptides. This investigation represents an extension of our prior collaborative work (Lund-Katz et al., 1990; Venkatachalapathi et al., 1993) on the lipid-binding properties of amphipathic peptides. It is apparent that the titration behavior of Lys residues is dependent on their location with respect to the polar−nonpolar interface of the amphipathic helix. In diskoidal complexes with DMPC, the microenvironments of the Lys residues in the 18A α-helix are determined by helix−helix interactions between peptide molecules arranged in an antiparallel fashion.

EXPERIMENTAL PROCEDURES

Peptide Synthesis. Samples of unlabeled 18A and 18R peptides were prepared by the solid phase synthetic procedure described previously (Anantharamaiah et al., 1985; Anantharamaiah, 1986). The peptides were purified by reversed phase HPLC and characterized by amino acid and sequence analyses. The final peptides were >99% pure. The Lys residues and the α-amino group were labeled with $^{13}CH_3$ by conversion to dimethylamino derivatives using a reductive methylation procedure (Jentoft & Dearborn, 1983), as described previously (Sparks et al., 1992). The peptides were labeled as diskoidal complexes with DMPC, and essentially complete dimethylation was insured by adding a 20/1 molar excess of [^{13}C]formaldehyde to amino groups and using a final sodium cyanoborohydride concentration of 10 mM. The degree of methylation was determined both by ^{13}C NMR and also by mixing the [^{13}C]formaldehyde with [^{14}C]-formaldehyde and determining the level of radioactivity incorporated into the peptide.

The preparation of selectively labeled [^{13}C]dimethyl-18A analogs involved a selective protection and preferential deprotection scheme (Figure 1), using a solid phase peptide

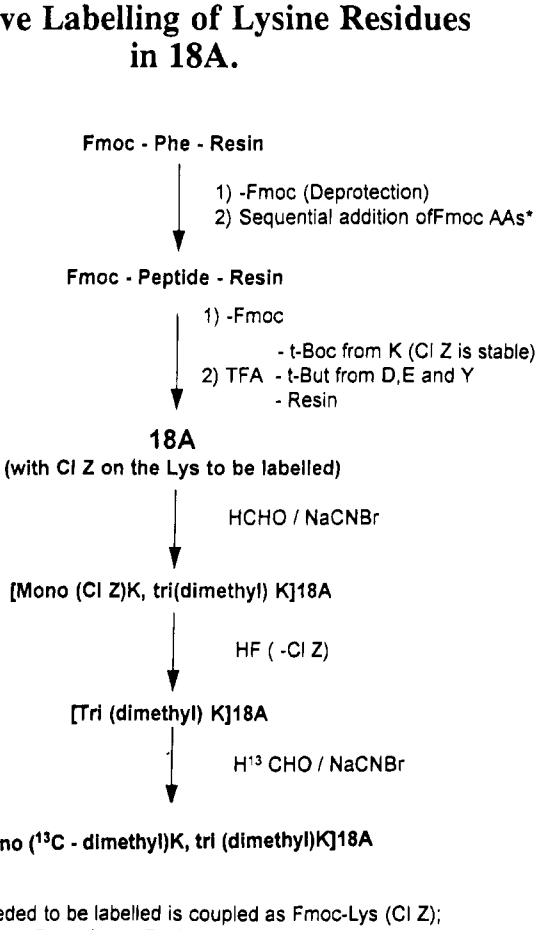

FIGURE 1: Outline of the scheme used to synthesize peptide 18A with selectively ^{13}C-dimethylated lysine residues.

synthesis method on a Wang resin (Peptide International, Louisville, KY) (Anantharamaiah, 1986; Atherton & Sheppard, 1989). The first C-terminal amino acid FMOC-Phe was coupled to the resin using dicyclohexylcarbodiimide in the presence of catalytic amounts of 4-(dimethylamino)-pyridine. The ε-NH_2 groups of all Lys except for the Lys residue to be labeled were protected with BOC groups that are cleavable with trifluoroacetic acid (TFA). The residue to be labeled was protected with the carboxybenzoxy (Z) group, which that is stable under the conditions of BOC group cleavage. The other protecting groups that are also cleavable by TFA are *tert*-butyl ester for Asp and Glu and *tert*-butyl ether for Tyr. Thus, at the end of the synthesis, cleavage of the peptide along with the cleavage of all side chain protecting groups, except for that of the ε-NH_2 of the Lys to be labeled, was accomplished with TFA treatment. The intermediates in the synthesis were characterized by proton NMR; for instance, the presence of an ε-Z group on a particular Lys after the cleavage of the peptide from the resin with TFA and the removal of this group using anhydrous HF was ascertained by the presence or absence of characteristic benzyl CH_2 resonances at around 5−5.5 ppm. The selectively protected peptide was treated with formaldehyde and sodium cyanoborohydride to convert the Lys free ε-NH_2 to dimethyl amino groups (Jentoft & Dearborn, 1983). The only ε-Z group present on the side chain of the Lys to be labeled was then cleaved with anhydrous HF. Dimethylation of this with ^{13}C-labeled formaldehyde/sodium cyanoborohydride gave the selectively

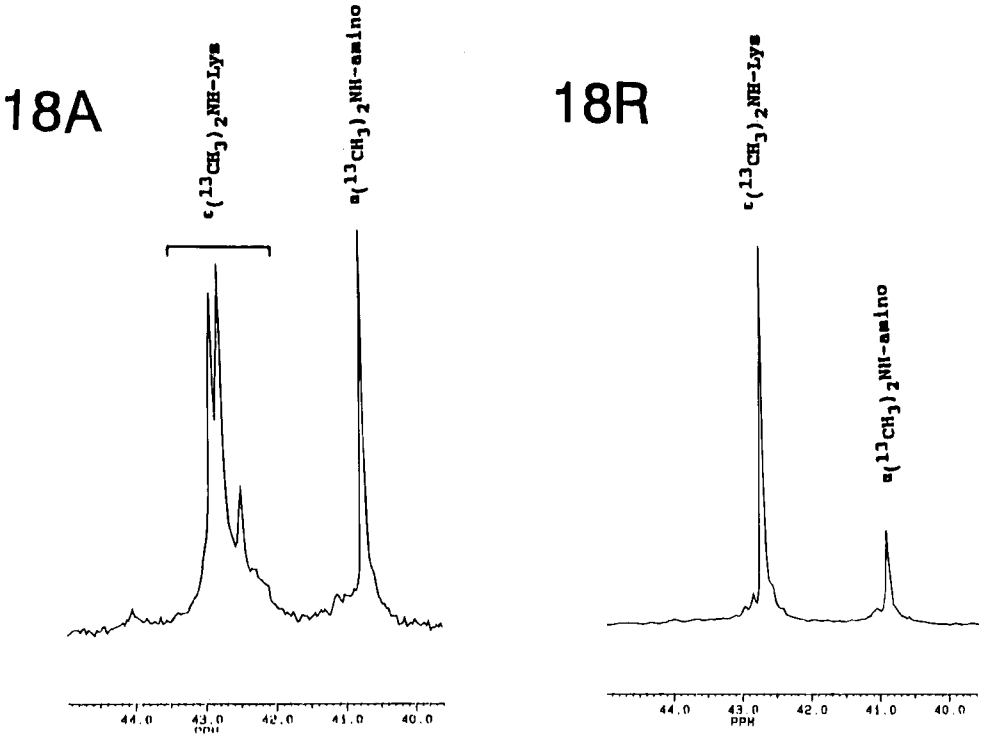

FIGURE 2: Proton-decoupled ^{13}C NMR spectra (126 MHz) of [^{13}C]dimethyl-Lys 18A (1.7 mg/mL, 37 900 acquisitions) and 18R (2.1 mg/ml, 33 631 acquisitions) dissolved in aqueous solution (0.02 M sodium borate, 0.15 M NaCl, 1 mM EDTA, and 0.05% NaN_3, pH 8.5) at 37 °C. The spectra were accumulated using 90° pulses of 8 μs duration and a recycling time of 1.08 s; the nuclear Overhauser effect was not suppressed. Chemical shifts were measured relative to external 1,4-dioxane (66.55 ppm). The spectra were processed with 2.0 Hz exponential filtering.

^{13}C-dimethylated peptide. By using this procedure, 18A peptides were synthesized in which the four different Lys side chains were selectively labeled.

^{13}C NMR of Peptide/DMPC Complexes. Diskoidal 1/1 (w/w) [^{13}C]dimethyl peptide/DMPC complexes were prepared by a bath sonication procedure, as described previously (Lund-Katz et al., 1990). Samples of these complexes in 1.5 mL of 0.02 M sodium borate, 0.15 M NaCl, 1 mM EDTA, and 0.02% NaN_3 (pH 8.5) were combined with D_2O (as the NMR lock compound) to a final volume of 2.5 mL. Proton-decoupled ^{13}C NMR spectra at 126 MHz were obtained on a Bruker AM500 spectrometer using procedures described previously (Sparks et al., 1992). Chemical shifts were measured by using external aqueous 1,4-dioxane (66.55 ppm) as a reference and are accurate to ±0.05 ppm. Titration curves were obtained over the pH range 6−12, and pK_a (±0.1 pH unit) values were derived by fitting to the Henderson−Hasselbalch equation (Sparks et al., 1992).

Analytical Procedures. The stoichiometries of the peptide/lipid complexes were determined by analyzing for inorganic phosphorus and using the absorbance at 280 nm to assay for peptide (Lund-Katz et al., 1990). The α-helix contents of the peptides and the sizes of the diskoidal complexes were monitored by using circular dichroism spectroscopy and negative stain electron microscopy, respectively (Sparks et al., 1992).

RESULTS

The abilities of the amphipathic helical peptides 18A and 18R, which have opposite distributions of acidic and basic residues to interact with DMPC have been compared in detail (Anantharamaiah et al., 1985; Mishra et al., 1994; Spuhler et al., 1994). Manipulation of the DMPC/peptide stoichiometry allows the formation of diskoidal complexes of different sizes (Anantharamaiah et al., 1985). For this study, a 1/1 (w/w) DMPC/peptide ratio was selected so that the diskoidal particles are small, which optimizes the resolution in the ^{13}C NMR spectra. In addition, essentially all of the peptide residues are located at the edge rather than in the faces of the small disks, so that the amphipathic helices are interacting primarily with the phospholipid acyl chains (cf. Brasseur et al., 1990; Lund-Katz et al., 1990; Wald et al., 1990). The ionization characteristics of the Lys residues of 18A and 18R in such disks can be determined from NMR studies of the peptides in which the Lys residues are ^{13}C-dimethylated. The pK_a's of Lys and dimethyl-Lys are quite similar, with the latter being slightly less basic (Jentoft & Dearborn, 1983; Huque & Vogel, 1993). Importantly, dimethylation does not significantly affect the ability of the peptides to interact with DMPC. The latter point is substantiated by the following experimental data. (1) The major diameters of the 1/1 (w/w) (3.2/1 mol/mol) DMPC/18A and DMPC/dimethyl-18A disks are 85 ± 9 (Anantharamaiah et al., 1985) and 97 ± 12 Å, respectively, as determined by negative stain electron microscopy. The equivalent dimensions for 18R and dimethyl-18R/DMPC disks are 198 ± 25 (Anantharamaiah et al., 1985) and 159 ± 19 Å, respectively; these disks are longer than the equivalent 18A particles because of the lower lipid affinity of the 18R peptide (Anantharamaiah et al., 1985; Spuhler et al., 1994). (2) The α-helix contents of either the unmodified peptide or the dimethyl derivative in the disks are 35−40% and 25−30% for the 18A and 18R peptides, respectively. (3) Particles that are indistinguishable in the NMR experiments described in the following are obtained if dimethylation is performed before or after complexation with DMPC.

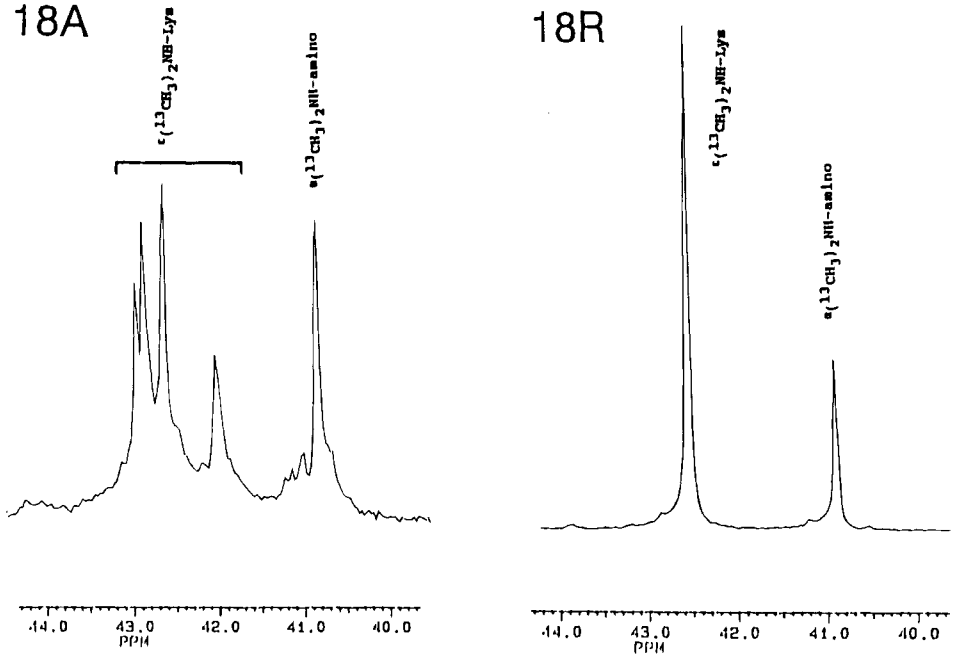

FIGURE 3: Expansions of the proton-decoupled ^{13}C NMR spectra of 1/1 (w/w) complexes of DMPC/[^{13}C]dimethyl-18A (2000 acquisitions, 4.1 mg of peptide/mL) and DMPC/[^{13}C]dimethyl-18R (38 300 acquisitions, 1.0 mg of peptide/mL) at 37 °C and pH 8.5. The spectral conditions were the same as those described in Figure 2. The resonances originate from the [^{13}C]-ϵ-dimethyl-Lys and [^{13}C]-α-dimethylamino groups in the peptides.

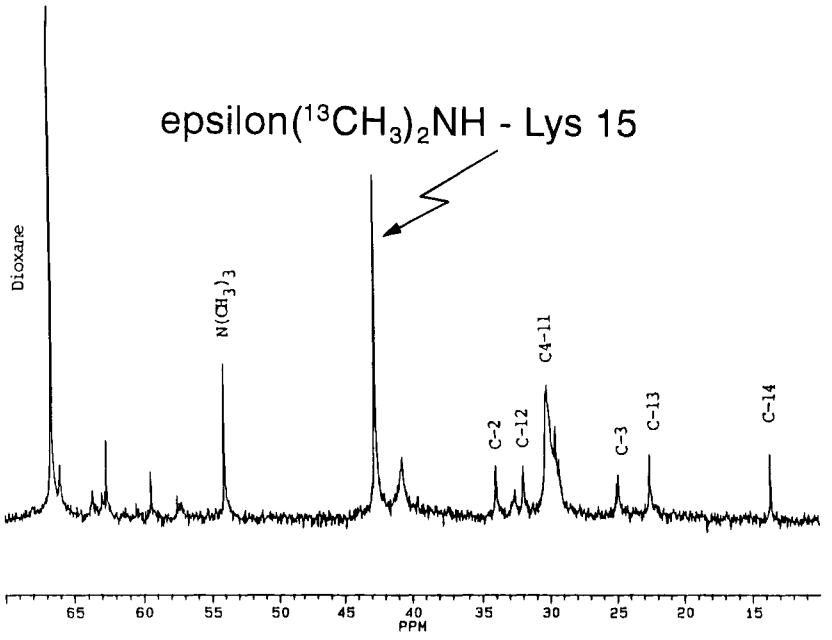

FIGURE 4: Proton-decoupled ^{13}C NMR spectrum of a 1/1 (w/w) DMPC/[^{13}C]dimethyl-Lys-15−dimethyl-18A complex (4.7 mg of dimethyl-18A/mL) obtained at 37 °C and pH 8.5 (14 620 acquisitions). The remaining spectral conditions were the same as those described in Figure 2.

The 18A and 18R molecules that each contain a single amphipathic α-helix exist in reversible equilibrium between the free and bound states when present with equal weights of DMPC (Venkatachalapathi et al., 1993). Free 18A and 18R can self-associate at concentrations of ~1 mg/mL, presumably by hydrophobic interaction between the nonpolar faces of the amphipathic helix, to form oligomers with molecular weight <14 000 (Venkatachalapathi et al., 1993), and the dimethyl derivatives are presumed to behave similarly. Consistent with this, dimethylation only slightly reduces the ability of 18A to penetrate PC monolayers (data not shown) and has no detectable effect on the interaction with DMPC to form diskoidal complexes. The purpose of the following NMR experiments is to obtain sequence-specific assignments and thereby determine the pK_a values for the various Lys residues in 18A and 18R molecules associated with DMPC. Since free and bound molecules are present in the NMR samples (cf. Lund-Katz et al., 1990), we initially examined the peptides free in aqueous solution. Figure 2 shows the ^{13}C NMR spectra of the two peptides at pH 8.55, where dimethyl-Lys resonances are known to be well resolved (Lund-Katz et al., 1988; Sparks et al., 1992). It is apparent that, for fully dimethylated 18A, three [^{13}C]-ϵ-dimethyl-Lys resonances are visible at chemical shift (δ)

Table 1: NMR and Titration Characteristics of Peptide [^{13}C]Dimethyllysine Residues in 1/1 (w/w) Complexes with DMPC

resonance	chemical shift (ppm)[a]	pK_a[b]
	Peptide 18R	
Lys-1, -8, -12, -16	42.70	10.3
	Peptide Ac-18R-NH_2	
Lys-1, -8, -12, -16	42.70	10.1
	Peptide 18A	
Lys-4	42.00	11.5
Lys-9	42.90	9.5
Lys-13	42.95	9.5
Lys-15	42.65	10.6
	Peptide Ac-18A-NH_2	
Lys-4	42.00	11.0
Lys-9	42.90	9.4
Lys-13	43.05	9.4
Lys-15	42.65	10.3

[a] From spectra of the type shown in Figure 3 obtained at pH 8.55. [b] Obtained from titration curves of the type shown in Figure 5 (±0.1 pH unit).

values of 42.45, 42.75, and 42.85 ppm. In contrast, a single ϵ-dimethyl-Lys resonance is apparent for 18R at 42.65 ppm. In the spectra of both peptides, the α-dimethylamino group resonates at 40.7 ppm; the chemical shift of the latter resonance from both peptides is shifted 0.1−0.2 ppm downfield upon addition of DMPC (cf. Figures 2 and 3). The resonances from the DMPC molecules in the diskoidal complexes are not shown in Figure 3, but they have been described in detail in a prior paper (Lund-Katz et al., 1990). The [^{13}C]-ϵ-dimethyl-Lys groups in the 18R/DMPC disk give a single resonance at δ 42.70, which is the same as that observed for the peptide in aqueous solution (Figure 2). In contrast, the spectrum of 18A in the DMPC disk shows four ϵ-dimethyl-Lys peaks with δ values in the range 42−43 ppm (Figure 3).

To assign the four peaks in the spectrum of the dimethyl-18A/DMPC complex, spectra were obtained from samples in which the Lys at positions 4, 9, 13, and 15 in the 18A molecule were specifically ^{13}C-dimethylated, as summarized in Figure 1. As an example, Figure 4 depicts the spectrum of 18A in which Lys-15 is selectively ^{13}C-dimethylated. The resonance at 42.65 ppm originates from Lys-15, while that at 40.80 ppm is due to the α-dimethylamino group. The only other resonance from the peptide is that for ϵ-monomethyl-Lys at ~33 ppm. Comparison of the intensity of this peak to that of the dimethyl-Lys peak at ~43 ppm confirms that the reductive methylation procedure leads to essentially complete formation of the dimethyl derivative. Analogous spectra were obtained for 18A with Lys-4, -9, and -13 selectively labeled. The chemical shift assignments for the four Lys residues are presented in Table 1. The titration curves in Figure 5 were derived by obtaining spectra at pH values across the range indicated. The data points were fitted to the Henderson−Hasselbalch equation (cf. Sparks et al., 1992) to obtain the pK_a values for the various Lys residues in 18A (Table 1). The titration curve for the single resonance from 18R was used to derive the common pK_a value for the four Lys residues in this molecule.

The effects of increasing helix length on the titration properties of the Lys residues were investigated by making parallel measurements on acetyl-18A-amide. Blocking the ends of the 18A molecule by adding an acetyl group at the amino terminus and an amide at the carboxyl terminus to

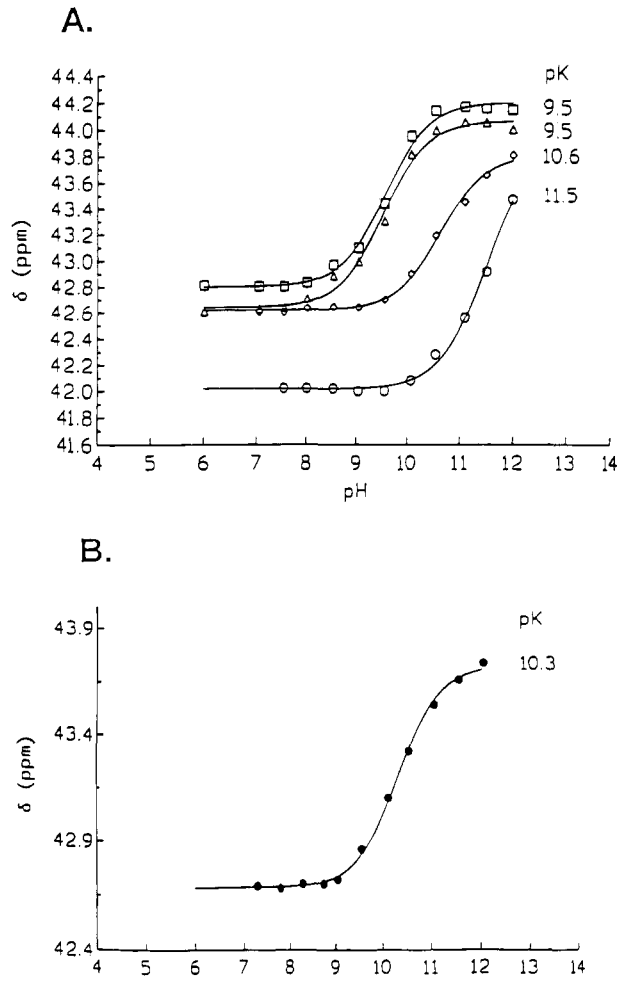

FIGURE 5: ^{13}C NMR chemical shifts (ppm) as a function of pH for the [^{13}C] dimethyl-Lys residues of 1/1 (w/w) peptide/DMPC diskoidal complexes. The titration curves and pK_a values were derived by fitting to the Henderson−Hasselbalch equation. (A) [^{13}C]-Dimethyl-18A/DMPC complexes (4.1 mg of peptide/mL). (B) [^{13}C]-Dimethyl-18R/DMPC complexes (1.0 mg of peptide/mL).

form Ac-18A-NH_2 stabilizes the α-helix and enhances the lipid affinity of the peptide molecule (Venkatachalapathi et al., 1993). The α-helix content of Ac-18A-NH_2 bound to DMPC is about 75% (cf. Mishra et al., 1994), which is about double the helicity of 18A. Figure 6 depicts the NMR spectrum of fully ^{13}C-dimethylated Ac-18A-NH_2 in a 1/1 (w/w) complex with DMPC; the δ values of the four dimethyl-Lys resonances are the same as those for the 18A peptide (Table 1). The pK_a values of Lys-9 and Lys-13 are the same in 18A and Ac-18A-NH_2, while Lys-4 and Lys-15 have pK_a values that are, respectively, 0.5 and 0.3 unit lower in Ac-18A-NH_2. This result is consistent with the idea that the stabilization of the α-helix and prevention of the fraying of the ends that occurs in Ac-18A-NH_2 affect the microenvironments of Lys-4 and Lys-15 that are near the ends of the molecule. When these residues are located in a class A α-helix at the edge of a DMPC disk their affinity for protons is reduced. In contrast, stabilization of the non-class A helix in 18R by blocking the ends to form Ac-18R-NH_2 has relatively little effect on the Lys microenvironments because their pK_a values in complexes with DMPC are 10.1, compared to the value of 10.3 for 18R/DMPC complexes. The N-terminal α-amino groups in the 18A and 18R molecules associated with DMPC exhibit pK_a values of 7.3 and 7.2, which are close to the range of 7.5−8.0 normally

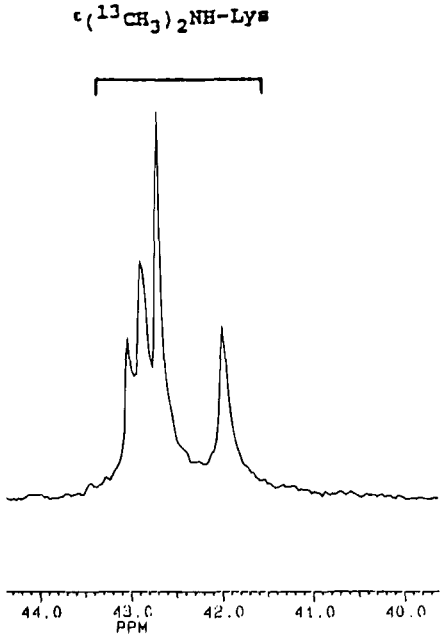

FIGURE 6: Expansion of the proton-decoupled ^{13}C NMR spectrum of a 1/1 (w/w) complex of DMPC with [^{13}C]dimethyl-Ac-18A-NH_2 at 37 °C and pH 8.5 (30 000 acquisitions, 2.7 mg of peptide/mL). The spectral conditions were the same as those described in Figure 2. The resonances originate from the [^{13}C]-ϵ-dimethyl-Lys groups in Ac-18A-NH_2.

observed for the α-amino terminus of water-soluble proteins (Tanford, 1962) and the value of 7.0 observed for apo-A-I in diskoidal complexes (Sparks et al., 1992). This implies that the α-dimethyl-amino groups in the 18A and 18R molecules are not involved in any unusual interactions.

DISCUSSION

The structural characteristics of the diskoidal complexes formed by the interaction of peptides 18A and 18R with DMPC have been described in detail (Anantharamaiah et al., 1985; Mishra et al., 1994). The numbers of lipid and peptide molecules per particle can be estimated from the disk volume and the partial specific volumes of the constituents. Such a calculation indicates that the 1/1 (w/w) 18A/DMPC complex contains about 170 lipid molecules and 53 peptide molecules. The equivalent numbers for the 18R/DMPC disk are 454 lipid molecules and 142 peptide molecules. The α-helical peptide molecules are located mostly around the edge of the disk where they cover the PC acyl chains (Lund-Katz et al., 1990). Studies with similar synthetic model peptides have shown that the helices are oriented roughly parallel to the acyl chains of the phospholipid bilayer; interactions between contiguous amphipathic helices that are arranged in an antiparallel fashion can contribute to the overall stability of the lipid/protein complex (Brasseur et al., 1993; Corijn et al., 1993). This packing arrangement is analogous to that of apo-A-I molecules in diskoidal complexes (Brasseur et al., 1990; Nolte & Atkinson, 1992; Rosseneu et al., 1992; Sparks et al., 1992; Wald et al., 1990). The present NMR results demonstrate that the titration behavior of Lys residues can be affected by the location of the Lys side chain with respect to the polar and nonpolar faces of the amphipathic helix and by interhelix interactions.

In the case of 18R, where Lys-1, -8, -12, and -16 are located toward the center of the polar face of the amphipathic helix (Figure 7), the ionization behavior of the Lys residues is relatively straightforward to understand. The fact that all four Lys have a common pK_a value of 10.3 (10.1 in Ac-18R-NH_2) when the helix is associated with DMPC indicates that the side chains of these residues extend into the aqueous phase, where they are fully hydrated as expected. Since all four residues have a pK_a similar to that of an isolated Lys group (pK_a = 10−10.5), it follows that, regardless of their position along the α-helix, the microenvironments of these Lys residues in the polar face of the helix are essentially unaffected by either intrahelix interactions or the presence of lipid molecules. For example, Lys-8 could perhaps form an intramolecular salt bridge with either adjacent Asp-9 or Asp-4, which are one turn removed along the α-helix (Figure 7). Such participation in a salt-bridge with a carboxyl group would elevate the pK_a of the ϵ-amino group relative to a free Lys because the affinity for protons is enhanced. Since all the Lys in 18R have the same pK_a value, it follows that intrahelix ionic interactions are not significant. Intermolecular interactions also do not affect the Lys microenvironments in 18R. Because the Lys are located on the polar face of the amphipathic helix facing away from the DMPC molecules, the interaction of protons with the ϵ-amino groups is not altered. The phosphate−phosphate distance across a liquid-crystalline DMPC bilayer at 37 °C is about 30 Å (Janiak et al., 1979), which is similar in length to the 18A helix (an 18-residue, 5-turn α-helix is 27 Å long). However, since the Lys residues are removed by at least one turn from the ends of the helix, interactions between the DMPC phosphate groups and the Lys residues in the 18A helices arrayed parallel to the phospholipid acyl chains around the edge of the diskoidal particle do not occur. Finally, because the Lys in 18R are located in the center of the polar face of the amphipathic helix (Figure 7), they do not interact significantly with any amino acid side chains of adjacent 18R helices.

Compared to the situation with the 18R molecule, the location of the Lys residues near the boundary between the polar and nonpolar faces of the class A amphipathic helix in 18A or Ac-18A-NH_2 leads to significant differences in their ionization behavior. Because the chemical shifts of the Lys resonances are similar for lipid-free and lipid-bound 18A (cf. Figures 2 and 3), it seems that similar helix−helix interactions arise from either self-association or interaction with lipid. The four Lys residues situated at the polar−nonpolar boundary of the Ac-18A-NH_2 amphipathic helix have pK_a values in the range 9.4−11.0 (Figure 7). The fact that there is no obvious correlation of pK_a value with position relative to the polar−nonpolar interface of the amphipathic helix indicates that hydrophobic effects do not influence the ionization behavior of the Lys residues. The pK_a values are determined primarily by polar interactions which, as discussed earlier for the 18R case, must arise from interactions of neighboring 18A molecules around the edge of the DMPC diskoidal particle.

As has been reported by Brasseur and colleagues (Brasseur et al., 1993) for peptides similar to 18A, energy minimization indicates that an antiparallel arrangement of the α-helical molecules is preferred. Ionic interactions between residues along the edges of the adjacent helices contribute to the stability of this configuration; interaction between the helix dipoles also favors an antiparallel arrangement. Figure 8 shows this structure for two pairs of 18A molecules, and it

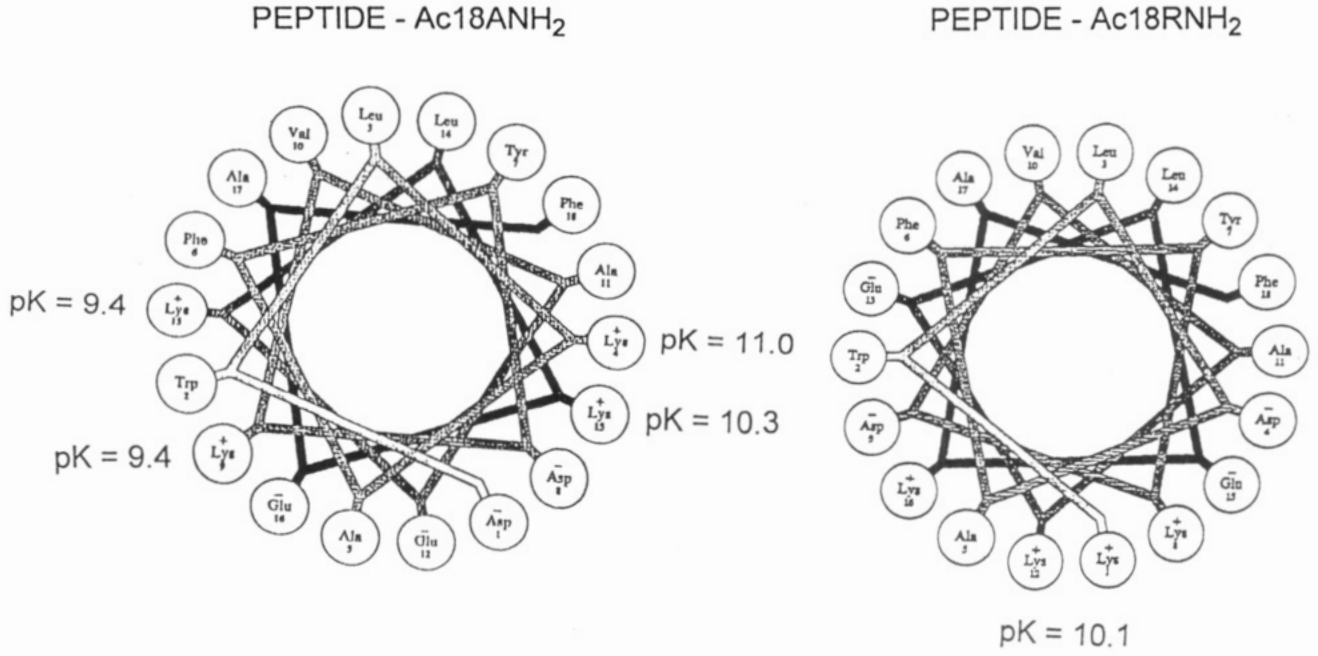

FIGURE 7: Helical wheel projections of peptides Ac-18A-NH_2 and Ac-18R-NH_2. The amphipathic nature of the α-helices is evident, and the positions of the Lys residues and their pK_a values are shown.

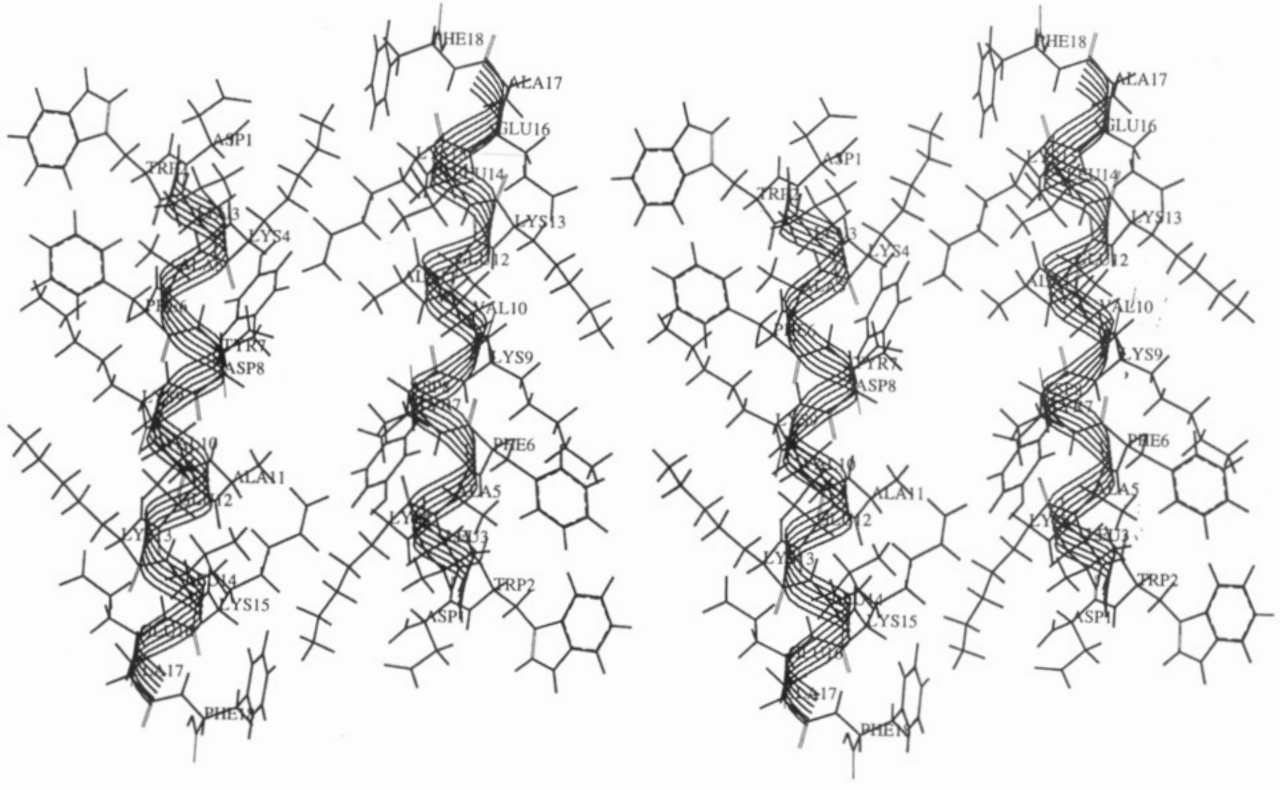

FIGURE 8: Packing of four α-helical 18A molecules arranged in an antiparallel orientation. Two fully helical 18A molecules arranged in an antiparallel orientation were docked and energy-minimized. To elucidate the interactions with other neighboring 18A molecules, another identical pair of 18A molecules was docked onto the first pair and energy-minimized. The molecular modeling was done using SYBYL (Tripos Associates, St. Louis, MO) running on a Silicon Graphics workstation. 1000 cycles of conjugate gradient were employed with a dielectric constant of 16.5 and a repulsive cutoff value of 10 Å during energy minimization. Within each pair of antiparallel helices, the juxtaposition of Asp-8 and Lys-15 that creates two salt bridges and the two amino−aromatic interactions between Lys-4 and Phe-18 are apparent. The protrusion of Lys-9 and Lys-13 to the outside of each pair of antiparallel helices is also clear; the basic microenvironment formed by the four ϵ-amino groups of these Lys residues is apparent between the two central helices. The configuration shown with the pairs of helices on the left and right in the same orientation has enhanced van der Waals attraction and a lower free energy than the configuration where the two pairs of helices have opposite orientations.

is apparent that two salt bridges between Asp-8 and Lys-15 help stabilize the helix−helix interaction. Relative to the situation in Ac-18R-NH_2, the salt bridge raises the pK_a of Lys-15 by 0.2 unit which means that the free energy of the salt bridge ($\Delta G = 2.3RT\Delta pK$) is about 0.3 kcal/mol; this is a weak salt bridge (cf. Fersht, 1972). The reason for the elevated pK_a of 11.0 for Lys-4 [the value for Ac-18A-NH_2 rather than that for 18A is used (Table 1) because this

molecule is more nearly 100% helical when complexed with DMPC] is also apparent from Figure 8. The ϵ-amino group is located close to and above the aromatic ring of Phe-18 of the paired, antiparallel helix. Such an amino−aromatic interaction (Burley & Petsko, 1986) can be about half as strong as a normal hydrogen bond (Levitt & Perutz, 1988). In the case of two interacting Ac-18A-NH_2 helices (Figure 8), the ΔpK of 0.9 unit for Lys-4 indicates that the amino−aromatic interaction free energy in this case is about 1.3 kcal/mol. Analysis of the amino−aromatic interactions in proteins of known structure indicates that the positively charged amino group locates preferentially over the center of the aromatic ring (Burley & Petsko, 1986). Such an interaction for the [^{13}C]dimethylamino group of Lys-4 would be expected to give rise to a ring current shift so that the resonance is shifted upfield (Wuthrich, 1986). Inspection of the chemical shift values in Table 1 reveals that the [^{13}C]-dimethylamino group of Lys-4 does indeed resonate at the highest field relative to the other Lys in either 18A or 18R.

In addition to explaining the increases in pK_a for Lys-4 and Lys-15, the model depicted in Figure 8 also shows why the pK_a values for Lys-9 and Lys-13 are decreased. When 18A or Ac-18A-NH_2 molecules are arranged in this antiparallel fashion around the edge of a diskoidal particle, Lys-9 and Lys-13 on adjacent helix pairs become juxtaposed. This creates a basic microenvironment, which tends to suppress ionization of the ϵ-amino groups and decrease the pK_a values. As an example of such an effect, an interresidue electrostatic interaction between two Lys residues that are close in the active site of mandelate racemase decreases the first pK_a of one of the ϵ-amino groups to 6.4 (Landro et al., 1991). The electrostatic repulsions of Lys-9 and Lys-13 in Ac-18A-NH_2 decrease this pK_a to 9.4 from the reference value of 10.1 for the Lys in Ac-18R-NH_2 (Table 1). Consequently, the electrostatic free energy of repulsion experienced by these residues is about 1 kcal/mol.

In conclusion, the present data show that knowledge of Lys pK_a values gives information about the interhelix interactions of class A amphipathic helices. This effect arises because the Lys (and other basic residues) are located at the edges of juxtaposed helices, so that they interact with amino acid side chains on the next helix. The resultant ionic interactions probably contribute to the tertiary structure of exchangeable apolipoprotein molecules such as apo-A-I when they are associated with lipoprotein particles (cf. Nolte & Atkinson, 1992; Rosseneu et al., 1992; Brasseur et al., 1993). The results observed with the 18A peptides suggest that the antiparallel arrangement of class A amphipathic helices is preferred even when they are not covalently linked.

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