The synthetic lipid-associating peptide, LAP-20 (VSSL LLSSLKEYWSSLKEFS), activates lecithin-cholesterol acyltransferase (LCAT) despite its lack of sequence homology to apolipoprotein A-I, the primary in vivo activator of LCAT. Using SDS and dodecylphosphocholine (DPC) to model the lipoprotein environment, the structural features responsible for LAP-20’s ability to activate LCAT were studied by optical and two-dimensional $^1$H NMR spectroscopy. A large blue shift in the intrinsic fluorescence of LAP-20 with the addition of detergent suggested that the peptide formed a complex with the micelles. Analysis of the CD data shows that LAP-20 lacks well defined structure in aqueous solution but adopts helical, ordered conformations upon the addition of SDS or DPC. The helical nature of the peptides in the presence of both lipids was confirmed by upfield H$_3$N MR secondary shifts relative to random coil values. Average structures for both peptides in aqueous solutions containing SDS and DPC were generated using distance geometry methods from 329 (SDS) and 309 (DPC) nuclear Overhauser effect-based distance restraints. The backbone (N, C$,^\alpha$, C=O) RMSD from the average structure of an ensemble of 17 out of 20 calculated structures was 0.41 ± 0.15 Å for LAP-20 in SDS and 0.41 ± 0.12 Å for an ensemble of 20 out of 20 calculated structures for LAP-20 in DPC. In the presence of SDS, the distance geometry and simulated annealing calculations show that LAP-20 adopts a well defined class A amphipathic helix with distinct hydrophobic and hydrophilic faces. A similar structure was obtained for LAP-20 in the presence of DPC, suggesting that both detergents may be used interchangeably to model the lipoprotein environment. Conformational features of the calculated structures for LAP-20 are discussed relative to models for apolipoprotein A-I activation of LCAT.

In human plasma, free and esterified cholesterol circulate as constituents of lipoproteins. The primary enzyme responsible for modulating plasma levels of cholesterol is lecithin-cholesterol acyltransferase (LCAT; EC 2.3.1.43)$^1$ (1, 2), a 60-kDa glycoprotein that catalyzes transesterification of a fatty acid from the sn-2 position of lecithin (phosphatidylcholine) to cholesterol. The key in vivo metabolic activator of LCAT is apoA-I (3), a protein also believed to play an important role in the biogenesis of high density lipoprotein particles, which serve as acceptors of peripheral cell-associated free cholesterol in the reverse cholesterol transport pathway (4, 5). High plasma high density lipoprotein-cholesterol levels have been inversely correlated with the risk of developing coronary artery disease (6).

A prominent feature of apoA-I and the other exchangeable apolipoproteins is repeating amino acid motifs of 11 or 22 residues, which, based upon predictions from primary sequences, may adopt amphipathic helical structures when associated with lipid (7). Such a motif is characterized by polar and nonpolar amino acid residues aligned on opposing faces of the long axis of an $\alpha$-helix. The positively and negatively charged amino acid residues are distributed in the polar-nonpolar interface and along the center of the polar face, respectively. The lipid-associating properties of the apolipoproteins are believed to result from hydrophobic interactions between the nonpolar amino acid side chains and the phospholipid acyl chains (8). The model also allows for ionic interactions between (i) the positively charged protein side chains in the interface with the negatively charged phosphate groups of the phospholipid and (ii) the negatively charged protein side chains in the hydrophobic face with the positively charged quaternary amines of the phospholipid.

In order to more precisely define secondary structural features responsible for LCAT activation, synthetic model peptides have been studied. Synthetic fragments of apoA-I designed to localize the position of the major LCAT activating region indicate that residues 143–185 and 121–164, which both contain two predicted amphipathic regions (9, 10), activate LCAT 24 and 30%, respectively, of the rate of native apoA-I. De novo peptides have shown that charge distribution plays a role in the peptides’ affinity for lipid and ability to activate LCAT (11, 12). For example, one acidic 30-residue peptide, GALA, was observed to activate LCAT almost as effectively as apoA-I (13). Studies using de novo amphipathic peptides of various lengths suggest that 10–12 residues are enough for lipid association (14) but that longer sequences are necessary for LCAT activation (15). The critical role of the primary sequence is illustrated by the inability of a 16-residue peptide, LAP-16, to activate with DMPC or activate LCAT, while the addition of four residues to the amino terminus of LAP-16 (LAP-20) results in a dramatic increase in lipid association and LCAT activation (16). ApoA-I mimetic peptides have also illustrated that proline punctuation between contiguous pairs of predicted amphipathic $\alpha$-helices increases lipid affinity and LCAT activation (17), although the absence of such a residue in LAP-20 and

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*COSY, double-quantum-filtered correlation spectroscopy; RMSD, root mean square deviation; DMPC, dimyristoylphosphatidylcholine.

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‡ The abbreviations used are: LCAT, lecithin-cholesterol acyltransferase; DPC, dodecylphosphocholine; NOESY, nuclear Overhauser enhancement spectroscopy; TOCSY, total correlation spectroscopy; DQF-

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GALA suggests that intramolecular interaction between proline and LCAT is likely not involved in LCAT activation.

LAP-20 is unique in that, while it has no sequence homology with apoA-I, it activates transacylation by LCAT to 65% of that of apoA-I in the presence of DMPC and cholesterol (16). Because the latter value is greater than twice the value obtained with the most potent synthetic peptide from apoA-I, we chose to study in greater detail the conformational changes that occur upon the association of LAP-20 with lipid.

Sodium dodecyl sulfate and dodecylphosphocholine, agents commonly used to model membranes (18, 19), were used to model the lipoprotein environment (20, 21). The amount of increasing concentrations of SDS and DPC was estimated by circular dichroism spectroscopy and H NMR secondary shift analysis. Two-dimensional 1H NMR studies were performed in the presence of perdeuterated SDS (SDS-d25) and perdeuterated DPC (DPC-d25) at a molar ratio of peptide to detergent of 1:40. Using distance restraints obtained from NOE data, average structures for LAP-20 in SDS and DPC were obtained using distance geometry/simulated annealing methods. A comparison was made between the structures calculated for LAP-20 in the presence of SDS and DPC to determine if the different lipid head groups have any significant structural effects. The biological significance of the major structural features observed for LAP-20 in the model lipid environments is discussed in relation to models of LCAT activation.

EXPERIMENTAL PROCEDURES

Materials—LAP-20, VSSLSSLSKEYWSSLKESFS, was synthesized by Dr. Ian Clark-Lewis (University of British Columbia) using solid-phase methods (22). SDS was purchased from BDH Chemicals Ltd.; SDS-d25 and DPC-d25 were purchased from Cambridge Isotope Laboratories Inc.; D2O was purchased from STOHLEK/R STOR Isotopes Inc. Insight II (version 2.3.0), MM2/3, FELIX, and FLEX (version 2.1, Hare Research Inc.) software was purchased from Biosym Technologies Inc. (San Diego, CA).

Fluorescence Spectroscopy—Fluorescence measurements were obtained at 20 °C on a Jasco J710 spectropolarimeter calibrated using ammonium d(+)-camphorsulfonate. The measurements were performed with 0.53 (SDS) and 0.64 (DPC) mg peptide (pH 5.0) and various concentrations of lipid (0–25 μmol) in a quartz cell of 0.02-cm path length at 25 °C. Spectra were the average of two consecutive scans from 260 to 190 nm recorded with a bandwidth of 0.5 nm, a time constant of 0.25 s, and a scan rate of 10 nm/min. Following base-line correction and noise reduction, the observed ellipticities were converted to mean residue ellipticities, [θ], in units of degrees cm2/dmol.

NMR Spectroscopy—Samples (5 mM) for 1H NMR spectroscopy were prepared in either 90% H2O, 10% D2O or in 99.9% D2O by dissolving LAP-20 in 500 μl of 200 mM SDS-d25 or DPC-d25 in a quartz cell of 0.02-cm path length at 25 °C. Spectra were the average of two consecutive scans from 260 to 190 nm recorded with a bandwidth of 0.5 nm, a time constant of 0.25 s, and a scan rate of 10 mHz. Following base-line correction and noise reduction, the observed ellipticities were converted to mean residue ellipticities, [θ], in units of degrees cm2/dmol.

NMR spectroscopy—Samples (5 mM) for 1H NMR spectroscopy were prepared in either 90% H2O, 10% D2O or in 99.9% D2O by dissolving LAP-20 in 500 μl of 200 mM SDS-d25 or DPC-d25. The pH was adjusted to obtain the best dispersion of the amide resonances with minimal loss of signal intensity (5.0 ± 0.1 (SDS) and 4.0 ± 0.1 (DPC)) with the addition of 0.1 M NaOH and 0.1 M HCl (pH meter readings uncorrected for the deuterium isotope effect).

NMR experiments were run on a Bruker AMX spectrometer operating at a proton resonance frequency of 600.13 MHz as reported earlier (20). Standard phase-sensitive (TPPI) two-dimensional NOESY (23), TOCSY (24), and DQF-COSY (26) spectra were recorded at 25 °C. Water suppression in the TOCSY and NOESY experiments was by WATERGATE (27) employing a 3-9-19 pulse sequence (28). NOESY data were recorded using mixing times of 75, 100, 150, and 225 ms. A 75-ms mixing time and 2.5-ms trim pulse were used in the MLEV-17 spinning sequence of the TOCSY experiments. Prior to Fourier transformation, the data were zero-filled to generate a 2K × 2K matrix and apodized by a cosine function in D2 and a sine function in D1. A fifth-order polynomial function was applied to base-line correct all processed spectra in both dimensions.

Fig. 1. Circular dichroism spectra of LAP-20 at 25 °C, pH 5.0, in aqueous solution and at various molar ratios of peptidedetergent. a. no detergent; b, 1:2.4 DPC (dashed line); c, 1:1 SDS; d, 1:40 DPC (dashed line); e, 1:40 SDS.

Structure Calculations—Three-dimensional structures were calculated from the NOE distance data (FELIX) using the distance geometry program (DGII) as described by Rozek et al. (29), except the initial distance restraints used to calculate structures for LAP-20 in DPC were obtained by classifying the peak volumes into strong (1.80–3.0 Å), medium (2.80–4.0 Å), and weak (3.80–5.50 Å) ranges as opposed to ranges of 1.80–2.50, 2.51–3.50 and 3.51–5.00 Å used to classify NOEY volumes for LAP-20 in SDS.

RESULTS

Optical Spectroscopy—The addition of 40 μM excess SDS and DPC to an aqueous solution of LAP-20 produced a 21-nm blue shift in the intrinsic tryptophan fluorescence. A blue shift of 16 nm was reported for LAP-20 upon the addition of 20 μM excess DMPC (29). Such a shift in the maximum emission wavelength of the fluorescence of tryptophan suggests an association of the peptide with the lipid accompanied by a transfer of the tryptophan residue from a polar to nonpolar environment (16, 29–31).

The CD spectra from a titration of an aqueous solution of LAP-20 with lipid are shown in Fig. 1. In the absence of detergent the LAP-20 spectrum shows a negative band around 200 nm and a very weak band near 220 nm, which characterize peptides that lack a well defined secondary structure (32). Spectra obtained without lipid did not change over the pH range 3–11 and, as observed by Pownall et al. (16), over the temperature range of 10–50 °C. The addition of SDS or DPC to LAP-20 effected changes in the CD spectra, which suggested an increase in ordered secondary structure. Above a peptide: detergent ratio of 1:4 (SDS) and 1:6 (DPC), no further changes were observed in the CD spectra, suggesting that LAP-20 was completely associated with lipid in a micelle-bound state (20, 33). The lower molar ratio of SDS required to obtain such a condition may reflect a marginally greater affinity of LAP-20 for the negatively charged SDS than the zwitterionic DPC (31). In both lipid complexes the CD spectra of LAP-20 possess a double minimum at 222 and 208–210 nm and a substantial maximum at 191–193 nm. Such features are indicative of a helical conformation (34) and were observed for LAP-20 in complexes with DMPC (16, 29). The helical content of LAP-20 in the absence and presence of SDS and DPC was estimated by deconvoluting the CD spectra using convex constraint analysis (35), and the results are pre-
in both lipids is less than 0.04 ppm, suggesting a similar conformation (44, 45). The results, tabulated in Table I, agree with earlier Fourier transform infrared spectroscopy data, which suggested that, in the absence of lipid, LAP-20 self-aggregated (43). On the other hand, in the presence of either SDS or DPC, the solubility of LAP-20 increases severalfold and the 1H NMR spectrum contains well resolved resonances with linewidths of approximately 10 Hz, and there is now a dispersion of the downfield amide resonances between 9 and 7.5 ppm and the side chain resonances between 4.5 and 0.5 ppm. Such observations suggest that LAP-20 forms a complex with the detergent micelles and that the conformation adopted by LAP-20 in the presence of lipid differs significantly from its structure in water alone.

FIG. 2. H−H' regions of the 600-MHz NOESY spectra (t_m = 150 ms) of LAP-20 (5 mM) in the presence of 200 mM SDS-d_{25} (pH 5.0) and 200 mM DPC-d_{28} (pH 4.0) at 25 °C. The constructs follow the sequential connectivities, with the H−H', cross-peaks labeled.

Proton Resonance Assignments and Secondary Shifts—In a non-lipid aqueous environment, the 1H NMR spectrum of a saturated LAP-20 solution (approximately 1 mM) contains poorly resolved resonances characteristic of self-aggregation (41, 42). Such observations agree with earlier Fourier transform infrared spectroscopy data, which suggested that, in the absence of lipid, LAP-20 self-aggregated (43). On the other hand, in the presence of either SDS or DPC, the solubility of LAP-20 increases severalfold and the 1H NMR spectrum contains well resolved resonances with linewidths of approximately 10 Hz, and there is now a dispersion of the downfield amide resonances between 9 and 7.5 ppm and the side chain resonances between 4.5 and 0.5 ppm. Such observations suggest that LAP-20 forms a complex with the detergent micelles and that the conformation adopted by LAP-20 in the presence of lipid differs significantly from its structure in water alone.

Table I summarizes the percentage of secondary structures and helical parameters R1 and R2 for LAP-20 in the presence of various ratios of SDS and DPC. The CD data were collected in H_2O, pH 5.0, at 25 °C. Variation of the pH over the range 3.0–11.0 had no effect on the spectra. The constructs follow the sequential connectivities, with the H−H', cross-peaks labeled.

Using random coil proton chemical shifts determined for peptides in unstructured conformations (44), it is possible to calculate chemical shift changes that occur when a peptide or protein adopts an ordered conformation. With respect to random coil chemical shift values, the H−N resonances move upfield in an α-helical conformation and downfield in a β-sheet conformation (44, 45). H−N secondary shifts (ΔH−N) were calculated by subtracting the measured H−N chemical shifts of LAP-20 in detergent complexes (Tables II and III) from the corresponding random coil values obtained by Wüthrich (44). Most of the H−N secondary shifts are positive, which suggests that LAP-20 adopts a helical conformation in the presence of lipid. Furthermore, except for Trp^{32}, Lys^{16}, and two residues at the N-terminal, the difference in ΔH−N for LAP-20 in the presence of both lipids is less than 0.04 ppm, suggesting a similar conformation in both lipid environments. A semiquantitative estimation of this helical content is obtained by dividing the average H−N secondary shift by 0.35, the average upfield H−N shift observed in the amino acid residues of proteins in an α-helical conformation (46). The results, tabulated in Table I, agree with the CD data and show that LAP-20 is highly helical in the presence of both detergents. A more detailed examination shows that the H−N secondary shifts of residues toward the N- and C termini approach 0 in both lipids, suggesting some fraying at both ends.

Interresidue NOEs and Secondary Structure—An unambiguous indication of protein secondary structure is provided by the magnitude and pattern of the interresidue nuclear Overhauser effects (44). NOESY spectra highlighting the H−H' regions of LAP-20 (Fig. 2) show strong and medium, and in most cases well resolved, sequential H−H', cross-peaks. As
summarized in Fig. 3, many strong and medium H$_i^N$-H$_{i+1}^N$ and medium and weak H$_i^N$-H$_{i+1}$, H$_i^N$-H$_{i+2}$, H$_i^N$-H$_{i+3}$, H$_i^N$-H$_{i+4}$, and H$_i^N$-H$_{i+5}$ NOE cross-peaks are also observed. The presence of strong to medium (i) – (i+1) and medium to weak (i) – (i+2), (i) – (i+3), and (i) – (i+4) NOE contacts throughout both structures suggests that they both adopt highly helical conformations when bound to detergent (44, 47) in accord with the CD and secondary shift analyses.

Three-dimensional Structure Calculations—Based on the CD data, H$_i^N$ secondary shifts, and NOE connectivity patterns for LAP-20, a right-handed $\alpha$-helix was chosen as the starting structure for the distance geometry and simulated annealing calculations. A total of 329 (132 intraresidue and 197 interresidue, SDS) and 309 (144 intraresidue and 165 interresidue, DPC) distance restraints were used in the final calculation of 20 energy-minimized structures for LAP-20 in SDS and DPC, respectively. Fig. 4A shows 17 out of 20 structures calculated for LAP-20 in the presence of SDS, and Fig. 4B shows 20 out of 20 structures calculated for LAP-20 in the presence of DPC. In the figure the N-C$^\alpha$-C=O backbone atoms are drawn superimposed on residues 3-18 of the average structure. In Fig. 4C the backbone of the average structure of the ensembles in Fig. 4, A and B, has been replaced by a ribbon and superimposed on residues 3-18. The quality of the final structures is apparent in Fig. 5, plots of the pairwise RMSDs to the mean structure for each residue. In general, the pairwise RMSD per residue is below 0.2 Å for the backbone atoms and never rises above 1.4 Å for all atoms. For instance, the backbone (N, C$^\alpha$, C=O) RMSD for residues 3-18 is 0.29 ± 0.14 Å in SDS and 0.27 ± 0.09 Å in DPC. If all atoms are included the RMSD for residues 3-18 is 1.13 ± 0.14 Å in SDS and 1.02 ± 0.13 Å in DPC.

DISCUSSION

LAP-20 was shown to associate with both SDS and DPC by optical spectroscopy and displayed optical properties similar to those observed for LAP-20 in the presence of DMPC (16, 29), a lipid in which LAP-20 activates LCAT, suggesting that the peptide’s conformation is similar in all three lipid environments. Previously, Pownall et al. (16) confirmed a physical association of LAP-20 with DMPC discoidal complexes using a variety of methods, including ultracentrifugation in a density gradient and size exclusion chromatography. Our $^1$H NMR
data indicate that LAP-20 associates with SDS and DPC, and all the evidence suggests it associates as a monomer. While the unique set of proton resonance linewidths for LAP-20 in the presence of detergent are sharp enough to allow unambiguous assignments, they are still too broad to obtain \( H^N \text{--} H^N \) coupling constants, indicating the peptide is part of a large molecular weight micellar complex (19, 20, 46).

The CD data, \( H^N \text{--} H^N \) secondary shifts (Table I), and the pattern of the interresidue NOEs (Figs. 2 and 3) (44, 47) suggest that LAP-20 adopts a helical conformation when associated with SDS or DPC. Detailed three-dimensional structures generated for LAP-20 in the presence of SDS and DPC, using distance geometry calculations, verify such structures as illustrated by the superimposed backbone atoms of the calculated structures in Fig. 4, A (SDS) and B (DPC). Fig. 4C, which overlays ribbons drawn through the backbone atoms of the mean LAP-20 structure of the ensembles in Fig. 4, A and B, illustrates the formation of a similar, well defined helical structure over the length of the molecule in the presence of both detergents, apart from some dynamic fraying at the termini. Because the different headgroups on SDS and DPC have little effect on the structure adopted by LAP-20 in the micelle, we suggest that both detergents may be equally suited to model the lipoprotein environment.

Fig. 5 illustrates the superposition of the side chains for the ensemble of calculated structures shown for the backbone atoms of LAP-20 in SDS from Fig. 4A, while Fig. 7 illustrates a stereo view of the mean orientation of the side chains for the ensemble of calculated structures for the backbone atoms of LAP-20 in DPC from Fig. 4B. In both lipid environments, the side chains are clustered in three distinct regions: hydrophobic,
hydrophilic, and interfacial. Trp$^{12}$ is fixed in the interfacial region with the hydrophobic six-membered ring oriented toward the hydrophobic face and the polarizable imino group intruding into the hydrophilic face. Such an orientation (which generated a 21-nm blue shift in the maximum Trp fluorescence) is predicted to be energetically favored (48–51) and is similar to the orientation observed for the Trp residue in a proposed lipid binding domain of apoC-I (35–53) (20). Glu$^{10}$ and Glu$^{17}$, which are negatively charged in the SDS solution at pH 5.0, lie along the center of the hydrophilic face. The two positively charged residues, Lys$^{9}$ and Lys$^{16}$ (dark gray), are located in the polar-nonpolar interface. Such an overall orientation of the peptide side chains for LAP-20 in the presence of SDS and DPC fits the definition of a class A amphipathic helix (7, 51) and was predicted for LAP-20 from the primary structure (16).

The nonpolar side chains that extend from the hydrophobic face of LAP-20 presumably interact with the hydrophobic acyl chains of the lipid. On the other hand, the polar and charged side chains, located at the polar-nonpolar interface or the hydrophilic face, presumably interact with the aqueous milieu that includes the negatively charged head groups of SDS or the zwitterionic head groups of DPC. Because the LAP-20 structures are similar regardless of the head group of the detergent, hydrophobic interactions between the nonpolar face of the peptide and the hydrophobic interior of the micelle are likely the primary force stabilizing the helix (50, 52). The surface occupied by the hydrophobic face, as estimated by looking down the long axis of the helix, is a pie-shaped wedge that occupies ~30% of the total area. This proportion of peptide, which presumably penetrates into the micelle surface, is considered optimal for enzyme activation (9).

In addition to hydrophobic interactions, $\alpha$-helical structuress are stabilized by intramolecular hydrogen bond formation between backbone amide and backbone carbonyl groups four residues apart (5–7 kcal/bond). LAP-20, in a perfect $\alpha$-helix, is predicted to form 16 backbone hydrogen bonds with a $N^\text{H}$$^\text{N}^\text{--O}$ distance of $2.06 \pm 0.16$ Å and a $N^\text{H}$–$N^\text{H}$–$O$ bond angle of $155 \pm 11^\circ$ (53). The calculated structures for LAP-20 in SDS and DPC show that the $N^\text{H}$–$N^\text{H}$–$O$ atoms are in a position to form 11 and 14 hydrogen bonds, respectively, that meet the following conditions: (i) $\angle (i)$ (i + 4), $N^\text{H}$–$N^\text{H}$–$O$ bond angle between 120 and 180°, $N^\text{H}$–$O$ distance < 3.0 Å. Therefore, it is likely that the helical structure of LAP-20, in both detergents, is stabilized by the formation of intramolecular hydrogen bonds.

Segrest (7, 51) has proposed a stabilization of amphipathic structures by a “snorkeling” of basic amino acid side chains located at the polar-nonpolar interface. In such a model the Lys and Arg residues are oriented with the side chains aligned along the edge of the hydrophobic face, and the remaining side chains are shown in light gray. Except for the N-terminal valine, the side chains are segregated into distinct hydrophobic and hydrophilic domains. One-letter amino acid codes are used.

**FIG. 6.** Superposition of the side chains of the ensemble of calculated structures for LAP-20 in SDS shown in Fig. 4A. The hydrophilic amino acids (Ser$^3$, Ser$^4$, Ser$^6$, Ser$^7$, Glu$^{10}$, Tyr$^{11}$, Ser$^{13}$, Ser$^{14}$, Glu$^{15}$, Ser$^{18}$, and Ser$^{20}$) are shown in boldface, the positively charged amino acids (Lys$^9$ and Lys$^{16}$) are shown in dark gray, and the remaining side chains are shown in light gray. One-letter amino acid codes are used.

**FIG. 7.** Stereo view of the side chains of the calculated structure for LAP-20 in DPC that most closely superimposes on the average structure of the ensemble in Fig. 4B. Ser$^3$, Ser$^4$, Ser$^6$, Ser$^7$, Glu$^{10}$, Tyr$^{11}$, Ser$^{13}$, Ser$^{14}$, Glu$^{15}$, Ser$^{18}$, and Ser$^{20}$ are shown in boldface, the positively charged amino acids (Lys$^9$ and Lys$^{16}$) are shown in dark gray, and the remaining side chains are shown in light gray. If the lysine side chains truly snorkeled, their positively charged termini should have been observed to extend fully into the hydrophilic face.
sive in apoC-I fragments bound to SDS, and consequently, this study reinforces our contention that snorkeling is likely not a general characteristic of amphipathic helices (20).

Intraresidue charge interactions, in the form of salt bridges between oppositely charged side chains, may stabilize α-helical structures by up to 6 kcal/mol/salt bridge (54–56). While LAP-20 contains two positively and two negatively charged side chains at pH 5.0, they are either too close (1 residue) or too distant (>5 residues) to stabilize the α-helix through intramolecular salt bridge formation. It is therefore not surprising to find that the structure of LAP-20 at pH 4.0 in DPC, where the Glu residues are protonated, does not differ significantly from the structure obtained at pH 5.0 in SDS, where the Glu residues are negatively charged. Furthermore, detailed structural studies of the proposed lipid binding domains of apoC-I, which showed helical structures in the presence of SDS, showed that salt bridges did not form (20).

Features associated with peptides that activate LCAT are affinity for lipid (57) and helical secondary structure (58). Our NOE-derived structures for LAP-20 confirm a helical conformation and show that ionic interactions (intermolecular salt bridges and snorkeling) play a minor role in stabilizing the lipid-bound state. We find, instead, that hydrophobic interactions between the nonpolar amino acid side chains and the phospholipid acyl chains (8) play the most important role in stabilizing the complex, followed by intermolecular backbone hydrogen bonding. The hydrophobic interactions are likely crucial for LCAT activation because LCAT is surface-active, i.e. it binds to the lipid/water interface (3). Indeed, it has been suggested that the primary role of the amphipathic helix in activating LCAT is to disrupt the water-phospholipid interface and expose buried substrate to LAP-20 (15, 59). Support for this hypothesis is the observation that LCAT does not require a co-factor to hydrolyze water soluble substrates such as the p-nitrophenyl esters of fatty acid (59). However, while lipid binding is necessary for LCAT activation, many amphipathic peptides that bind to lipid do not activate LAP-20 (51). Consequently, there must be one or more key topological features of these bound peptides responsible for their ability to effectively activate LCAT.

Segrest et al. (51) have proposed that the unique position of two negatively charged Glu residues, located in the nonpolar face of apo-A-I regions 66–87 and 99–120, play a major role in the apoA-I activation of LCAT. In each of these two apoA-I 22-residue regions, the Glu is located at the 13th position. While a consensus 22-residue sequence containing a Glu at the 13th position is a poor activator of LCAT, a 44-residue dimer, obtained by linking two 22-mers together head-to-tail, activates LAP-20 as well as apoA-I (57). Because the two Glu residues of LAP-20, a potent activator of LAP-20, are located in the center of the hydrophilic face when bound to lipid, it is unlikely that negatively charged Glu residues in the nonpolar face are directly involved in the intermolecular activation of LCAT.

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