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In Vitro Binding of Synthetic Acylated Lipid-Associating Peptides to High-Density Lipoproteins: Effect of Hydrophobicity†

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ABSTRACT: To measure the effect of hydrophobicity on the binding of model apoproteins to lipoproteins, we synthesized a 15 amino acid lipid-associating peptide (LAP) with acyl chains of various lengths (0–18 carbons) bound to the N-terminal amino acid through a peptide bond. The acylated LAPs preferentially bound to high-density lipoprotein (HDL) and were activators of lecithin:cholesterol acyltransferase. Circular dichroic spectra indicated that the LAP association with phospholipid was accompanied by increased α-helical structure. The LAPs self-associated in solution as judged from tryptophan fluorescence analysis. These characteristics, which are comparable to those of apolipoprotein A-I, were strongly dependent upon the acyl chain length of the LAPs. The equilibrium constants (K_{eq}) for the association of LAPs to reassembled HDL were measured by equilibrium dialysis at several temperatures. At 37 °C, K_{eq} increased by 3 orders of magnitude as the number of carbon units was increased from 0 to 16; there was a log-linear relationship between K_{eq} and the acyl chain length. The free energy of association (ΔG_a) decreased by a constant value for each methylene unit added to the acyl chain (0.35 kcal mol^{-1}), clearly demonstrating a strict hydrophobic effect. This change of ΔG_a was enthalpy rather than entropy driven. Our data show that, with all other parameters including putative α-helicity, sequence, and molecular weight being constant, the binding of a lipid-associating peptide to lipoprotein is governed by its hydrophobicity.

The plasma lipoproteins are water-soluble lipid–protein complexes that transport lipids in the circulation. They are operationally defined according to their densities as the high, low, intermediate, and very low density lipoproteins (HDL, LDL, IDL, and VLDL, respectively)[1] and chylomicrons. The lipoproteins are composed of a central core of nonpolar lipids, cholesteryl esters, and triglycerides surrounded by a monomolecular layer of polar lipids, unesterified cholesterol, phospholipids, and specific apoproteins (Schaefer et al., 1978; Smith et al., 1978). Most of the polar components spontaneously exchange among lipoproteins. The mechanism of transfer of apoproteins probably involves transport of monomers through the aqueous phase (Imaizume et al., 1978; Patsch et al., 1978; Pownall et al., 1978a, 1981). Analysis of the amino acid sequence of apolipoproteins led to the amphipathic helical theory of the lipid binding of apoproteins (Segrest et al., 1974). Hypothetically, when an apoprotein assumes a helical structure, the polar residues lie on one face of the helix, and the hydrophobic residues appear on the opposite side. Presumably, the nonpolar face of the helix penetrates the lipid matrix, and the polar face interacts with the aqueous phase. This theory has now been supported by numerous studies from many laboratories employing a variety of physicochemical techniques. When combined with a lipid matrix, apolipoproteins undergo changes in secondary structure consistent with formation of an amphipathic helix (Morrisett et al., 1973, 1977; Fukushima et al., 1980). Moreover, self-association of apoproteins in solution is one manifestation of their relatively high hydrophobicity (Stone & Reynolds, 1975; Mantulin et al., 1980; Massey et al., 1981a). Finally, the amphipathic helical theory has been tested with a number of model lipid binding peptides and with fragments of native apolipoproteins corresponding to lipid binding regions (Sparrow et al., 1973; Sigler et al., 1976; Mao et al., 1977; Chen et al., 1979; Fukushima et al., 1980; Kanellis et al., 1980; Pownall et al., 1980). These studies permitted the formulation of a number of properties of native or synthetic lipid-associating apoproteins: (i) the polypeptide must have the potential to form an am-

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[1] Abbreviations: HDL, high-density lipoprotein(s); LDL, low-density lipoprotein(s); IDL, intermediate-density lipoprotein(s); VLDL, very low density lipoprotein(s); LCAT, lecithin:cholesterol acyltransferase; POPC, 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; apoA-I, apoprotein A-I; apoA-II, apoprotein A-II; C_n-LAP, lipid-associating peptide bearing an acyl chain of n carbons; R-HDL, reassembled HDL consisting of POPC and apoA-I (100:1); HPLC, high-performance liquid chromatography; EDTA, ethylenediaminetetraacetic acid; Tris·HCl, tris(hydroxymethyl)aminomethane hydrochloride; CD, circular dichroism.

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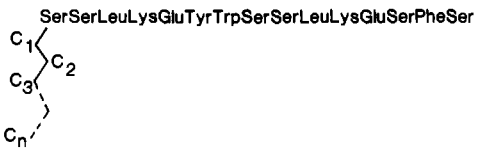

FIGURE 1: Structure of the acylated lipid-associating peptide. Saturated fatty acids of various lengths (0–18 carbons) were covalently bound to the N-terminal serine of the peptide through a peptide bond. This was achieved by reacting a given fatty acid with the resin-bound peptide in the presence of dicyclohexylcarbodiimide and the catalyst *N,N*-dimethylaminopyridine.

phipathic α-helix in the presence of a lipid matrix, although the α-helical structure in solution is not necessary; (ii) the peptide must have a critical amphipathic length of approximately 20 residues; (iii) the peptide must have a high hydrophobicity.

The relative importance of each of these criteria is not easily determined since they are not independent. For example, both the potential to form an α-helix and the hydrophobic content of a peptide obviously are partially dependent upon its length. Therefore, the use of purely peptidic molecules precludes a clear distinction between these criteria. To study the quantitative effect of hydrophobicity on the lipid–protein interaction, independent of both the α-helix potential and the length of the peptide, we synthesized a family of acylated lipid-associating peptides which have a hydrophobic content that increases with the acyl chain length. The family consists of a series of peptides bearing saturated fatty acyl chains of various lengths. Herein we report the binding characteristics of these acyl peptides to native and reassembled lipoproteins.

Experimental Procedures

Materials

The amino acid sequence of the acylated lipid-associating peptides (LAPs) is that of the 15 amino acid C-terminal fragment of the previously studied peptide, LAP-20 (Pownall et al., 1980). A saturated acyl chain of various numbers of carbons ($0 < n < 18$) was covalently bound to the N-terminal serine through a peptide bond (Figure 1). The peptides were prepared by the solid-phase method of Merrifield (1969) using the resin of Sparrow (1975). The acylation of the LAP was achieved by reacting a given fatty acid with the resin-bound peptide in the presence of dicyclohexylcarbodiimide and the catalyst *N,N*-dimethylaminopyridine. Following cleavage and deblocking of protecting groups, the acyl LAPs were purified by reversed-phase high-performance liquid chromatography (HPLC) using a linear gradient of 2-propanol in a 1% aqueous solution of triethylamine phosphate (Hancock & Sparrow, 1981). Following desalting, the purity of each peptide was verified by amino acid analysis, analytical HPLC, ultraviolet spectroscopy ($\epsilon = 6850\ M^{-1}\ cm^{-1}$ at 280 nm), and isoelectric focusing. The nomenclature adopted here is of the form C_n-LAP where n is the number of carbons in the acyl chain of the LAP.

DMPC and POPC were obtained from Calbiochem, La Jolla, CA, and from Avanti Polar-Lipids, Birmingham, Al, respectively. Human plasma lipoproteins were isolated by the density flotation method of Scanu (1967), and human apoA-I and apoA-II were purified as previously described (Pownall et al., 1978b). LCAT was purified to homogeneity by the method of Albers et al. (1979).

Methods

All the experiments were carried out by using a buffer containing 150 mM NaCl, 0.01 M EDTA, 0.01 M sodium azide, and 10 mM Tris·HCl, pH 7.4.

Spectral Analysis of the LAPs. The intrinsic fluorescence of tryptophan was monitored by using an SLM photon counting fluorometer equipped with an externally controlled temperature regulator. The excitation wavelength was 280 nm, and the emission spectra were recorded between 300 and 450 nm. Circular dichroic spectra were recorded on a Cary 61 spectropolarimeter.

Preparation of Reassembled HDL (R-HDL). POPC and apoA-I (100:1 molar ratio) were mixed in the presence of sodium cholate at room temperature. The detergent was then separated from the lipid–protein complex by dialysis (Matz & Jonas, 1982). When LAPs, which pass through dialysis membranes, were incorporated into R-HDL, a desalting column (Bio-rad P4) was used instead of dialysis (Pownall et al., 1982).

Equilibrium Dialysis. The LAPs were radioiodinated by using the chloramine T method (Greenwood et al., 1963). The equilibrium constants, K_{eq}'s, of the binding of ^{125}I-LAPs to R-HDL were calculated after equilibrium dialysis at several temperatures. In a typical experiment, 1 mL of R-HDL, at various concentrations, was placed inside the dialysis tubing (Spectrapor 2; molecular weight cutoff 14 000–16 000). The outside compartment contained 50 mL of buffer in a sealed Nalgene test tube. To avoid self-association, only traces of each ^{125}I-LAP (approximately 10^6 cpm) were added to the dialysis system in a small volume (20 μL of 50% 2-propanol). Even under these conditions, the high degree of self-association of C_{16}- and C_{18}-LAPs precluded their use in the equilibrium dialysis experiments. Although preliminary experiments showed that the time necessary to reach equilibrium depended upon both the temperature and the acyl chain length of LAP, it never exceeded 3 days. Therefore, we elected to conduct all subsequent equilibrium measurements with gentle shaking for 4 days in a temperature-controlled water bath. At the end of the experiment, the concentration of the lipid phase of R-HDL in the dialysis tubing was measured by using the phosphorus assay of Bartlett (1959). The amount of ^{125}I-LAP bound to R-HDL was obtained from the difference between the radioactivities of the inside and outside compartments.

Zonal Ultracentrifugation. Traces of ^{125}I-C_{16}-LAP and ^{131}I-apoA-II were equilibrated with rat serum overnight at 4 °C. Samples were then analyzed by zonal ultracentrifugation in a Ti-14 zonal rotor using two different salt gradients (Patsch et al., 1973). In the first, a nonlinear NaBr density gradient in the density range of 1.0–1.4 g/mL was used, and centrifugation was performed at 41 000 rpm for 22 h at 10 °C. This separated HDL from VLDL and LDL. Another gradient centrifugation in the density range of 1.0–1.3 g/mL was performed at 42 000 rpm for 140 min at 15 °C to separate VLDL from LDL. At the end of each run, 20-mL fractions were collected while the absorbance at 280 nm was continuously recorded with an ISCO monitor. ^{125}I and ^{131}I radioactivities of 1-mL aliquots of each fraction were measured in a two-channel γ counter. ^{125}I radioactivity was corrected for ^{131}I spillover.

LCAT Assay. Enzyme activity was assayed by using a modification of the method of Glomset (1962) in which minicolumns of silica gel (1 mL) constructed from Pasteur pipets were substituted for thin-layer plates. Cholesteryl esters were extracted with 1 mL of hexane, transferred to the minicolumn, and eluted directly into scintillation vials with ether–hexane (1:6).

Results

Spectral Properties of the LAPs. We measured the circular dichroism and tryptophan fluorescence maxima of the acylated

Table I: Fluorescence and Circular Dichroic Properties of LAPs

	fluorescence				circular dichroism			
	-DMPC		+DMPC[a]		[θ_{222}][c]		helical content (%)[d]	
no. of carbons	λ_{max}	$\Delta\lambda_{max}$[b]	λ_{max}	$\Delta\lambda_{max}$[b]	-DMPC	+DMPC[a]	-DMPC	+DMPC
0	356		356	0	nil	nil	nil	nil
4	356	0	356	0	nil	-1000	nil	10
8	356	0	343	-13	nil	-10900	nil	35
12	350	-6	341	-15	-600	-10900	9	35
16	345	-11	341	-15	-6000	-11000	23	36

[a] Concentrations of DMPC and LAPs were 2 and 0.1 mM, respectively. [b] $\Delta\lambda_{max}$ values are calculated by reference to the λ_{max} of C_0-LAP. [c] The molar ellipticity at 222 nm was calculated from $[\theta_{222}] = MRW\theta_{222}/(10lc)$ where C is the LAP concentration, l is the cuvette path length, MRW is the mean residue weight of LAP, and θ_{222} is the measured ellipticity angle at 222 nm. [d] Percent helical content was calculated from % helix = $([\theta_{222}] + 3000/39000) \times 100$.

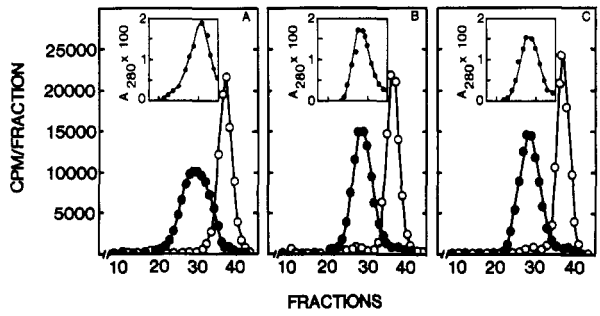

FIGURE 2: Chromatography of ^{125}I-labeled C_0-LAP (○) and C_{16}-LAP (●) on Sepharose CL-4B after incubation with rat serum (A), purified human HDL (B), and R-HDL (C). The samples in 1 mL were applied to the column (1.5 × 30 cm) at room temperature and eluted with buffer. The insets show the absorbances at 280 nm. The total elution volume appeared at fractions 36–37.

peptides in the presence or absence of DMPC (Table I). The fluorescence maxima of C_0-, C_4-, and C_8-LAP, in the absence of DMPC, corresponded to that of tryptophan in an aqueous environment (Longworth, 1971). The blue shift of C_{12}- and C_{16}-LAP relative to the others was assigned to self-association since dilution shifted it back to 356 nm (data not shown). As seen from the circular dichroic data, this self-association was accompanied by the formation of an α-helical structure. Addition of DMPC induced a blue shift of fluorescence, corresponding to the transfer of the tryptophan to the hydrocarbon region of the lipid. This shift increased with acyl chain length and was accompanied by a progressive increase of the peptide ellipticity.

Binding of LAPs to Lipoprotein. In early experiments, the affinity of LAPs for R-HDL was qualitatively assessed by reassembling HDL in the presence of traces of ^{125}I-labeled LAPs. The C_n-LAP bound to R-HDL was separated from the free peptide by filtration on a Bio-Rad P4 column (data not shown). The association of the LAPs to R-HDL was a sensitive function of the acyl chain length, going from virtually no binding with C_0-LAP to almost total binding with C_{16}-LAP. The spontaneous binding properties of the LAPs were then examined by mixing traces of ^{125}I-LAPs with either previously prepared R-HDL, purified human HDL, or rat serum. After overnight incubation at 4 °C, the mixtures were separated on a Sepharose CL-4B column. Figure 2 shows as examples the results obtained with C_0- and C_{16}-LAP. In all cases, C_0-LAP was essentially recovered as free peptide. In contrast, C_{16}-LAP was almost entirely associated with either R-HDL or native HDL. In the presence of rat serum, C_{16}-LAP eluted in the HDL zone. The preferential association of C_{16}-LAP with HDL was confirmed by zonal ultracentrifugation. Samples consisting of rat serum equilibrated with traces of ^{125}I-C_{16}-LAP were subjected to zonal ultracentrifugation under conditions where HDL separate from VLDL–LDL (Figure 3, panel A)

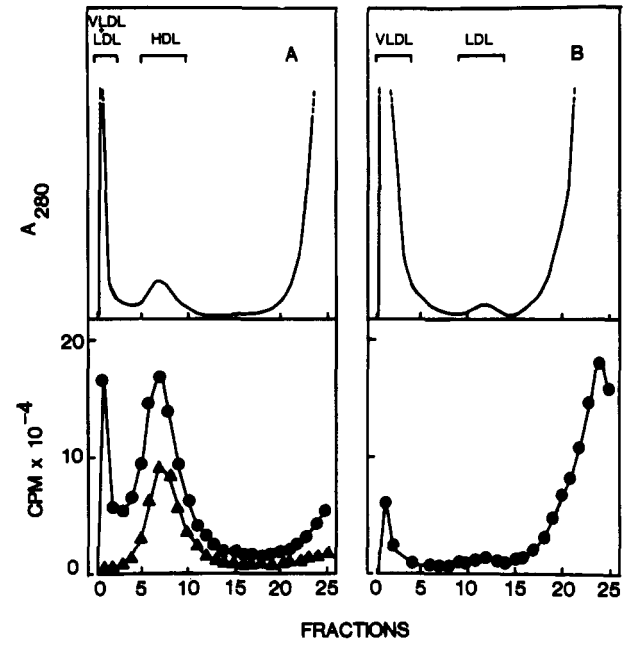

FIGURE 3: Zonal ultracentrifugation of ^{125}I-C_{16}-LAP (●) after incubation with rat serum. The sample was submitted to zonal ultracentrifugation under two different conditions as described under Experimental Procedures. Panel A shows the separation of HDL from VLDL–LDL. ^{131}I-ApoA-II (▲) was used together with the absorbance at 280 nm to identify the HDL-containing fractions. Panel B shows the separation of VLDL from LDL.

or under conditions where VLDL separate from LDL (Figure 3, panel B). Approximately 80% of the lipoprotein-bound radioactivity was associated with HDL. The residual part was recovered in the VLDL rather than LDL fractions. To ensure that these results were not simply due to the large concentration of HDL in rat serum relative to those of LDL and VLDL, the peptide was incubated overnight with a mixture of purified HDL, LDL, and VLDL, having the same phospholipid concentration. The sample was then chromatographed on a Sepharose 4B column. Approximately 74% of the peptide coeluted with HDL whereas only 8% and 5% were associated with VLDL and LDL, respectively (data not shown).

LCAT Activation. LCAT activation in the presence of LAPs was compared to that obtained with apoA-I (Table II). C_0-LAP did not activate LCAT for any of the tested concentrations. In contrast, C_8- and C_{18}-LAP activated the enzyme in a concentration-related manner. When compared on a mass concentration basis, apoA-I was 2 and 5 times more potent than C_{18}- and C_8-LAP, respectively.

Quantitative Aspects of R-HDL–LAP Association. Since the spectral and binding properties of LAP appeared to correlate with the acyl chain length, we studied the quantitative aspects of the binding of LAPs to R-HDL by equilibrium

Table II: Comparison of the Effects of LAPs and ApoA-I on LCAT Activity[a]

C_n-LAPs				apoA-I	
	LCAT activity ($\times 10^{-3}$ cpm/h)				
concn (μg/mL)	C_0-LAP	C_8-LAP	C_{18}-LAP	concn (μg/mL)	LCAT activity ($\times 10^{-3}$ cpm/h)
0	13	27	25	0	17
19	10	24	54	6	95
38	8	183	563	12	224
76	6	239	407	24	479
152	7	450	1137	57	1217
228	12	595	1403	114	1536

[a] LCAT activity was assayed by measuring the formation of [^{3}H]-cholesteryl ester from POPC vesicles containing [^{3}H]cholesterol. C_n-LAPs or apoA-I was incubated with 20 μL of 3.3 mM POPC for 1 day at 37 °C. Then 10 μL of LCAT (80 μg/mL) was added to the samples, and the amount of [^{3}H]cholesteryl ester formed was determined after 15 min. The results are expressed as the amount of [^{3}H]-cholesteryl ester formed per hour.

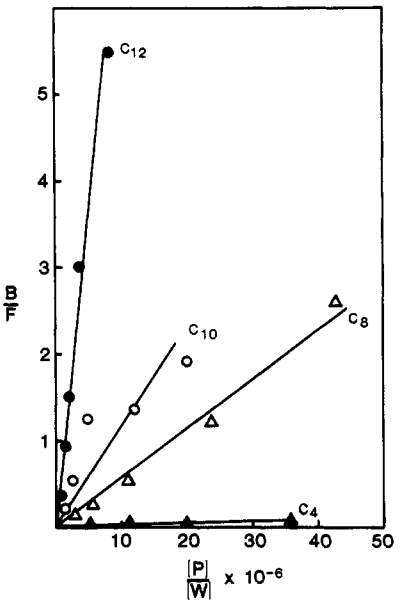

FIGURE 4: Determination of the equilibrium constant (K_{eq}) for the binding of C_4- (▲), C_8- (△), C_{10}- (○), and C_{12}-LAP (●) to R-HDL at 37 °C. Measurements of the ratio of R-HDL-bound LAP to free LAP (B/F) were done after 4 days of dialysis as described under Experimental Procedures. Several concentrations of R-HDL were used with each LAP. The molar ratios of phospholipid, [P], in water, [W], were determined by phosphorus assay. The K_{eq} values are given by the slopes of the regression lines. The values for C_0-LAP almost coincided with the x axis and are not shown for clarity of the figure. The high degree of self-association of C_{16}- and C_{18}-LAP precluded their use in equilibrium dialysis experiments.

dialysis. The experiments were carried out with a wide range of R-HDL concentrations. For each peptide, K_{eq} was calculated from

$$K_{eq} = (B/F)([W]/[P])$$

where B and F represent the concentrations of R-HDL-bound LAP and free LAP, respectively. [W] and [P] represent the molar concentrations of water and of the R-HDL phospholipid, respectively. For all the peptides, we found a linear relationship between [P]/[W] and B/F, demonstrating that the K_{eq}'s were independent of lipid concentration (Figure 4). The K_{eq}'s calculated from the slopes of these lines exhibited a log-linear relationship with respect to the acyl chain length (Figure 5). From the K_{eq}'s, we calculated the free energy of association of the LAPs (ΔG_a) according to

$$\Delta G_a = -RT \ln K_{eq}$$

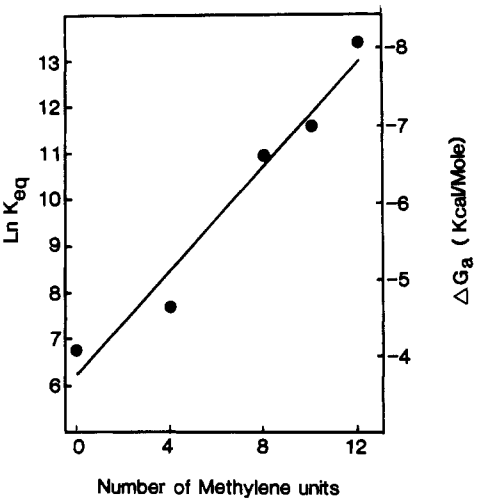

FIGURE 5: Effect of the acyl chain length on the binding of acylated LAPs to R-HDL. The logarithms of the equilibrium constants (ln K_{eq}) were calculated from the data of Figure 4 and plotted vs. the number of methylene units. The slope of the straight line corresponded to a variation of the free energy of association of −0.35 kcal mol^{-1} per methylene unit.

Table III: Effect of Temperature on the Binding of LAPs to Reassembled HDL[a]

	C_8-LAP			C_{12}-LAP		
temp (°C)	POPC[b] (mM)	K_{eq}[c] ($\times 10^{-4}$)	ΔG_a[d] (kcal mol^{-1})	POPC[b] (mM)	K_{eq}[c] ($\times 10^{-5}$)	ΔG_a[d] (kcal mol^{-1})
4	5.48	0.15	−4.02	0.63	0.63	−6.08
	3.13	0.26	−4.33	0.26	0.90	6.28
	1.34	0.14	−3.95	0.15	0.90	−6.28
				0.09	0.34	−5.74
				0.06	1.10	−6.39
				0.04	1.14	−6.41
		0.18[e]	−4.10[e]		0.84[e]	−6.20[e]
25	3.14	0.42	−4.94	0.21	3.39	−7.54
	1.35	0.71	−5.25	0.10	4.59	−7.72
	0.73	0.88	−5.38	0.05	5.44	−7.82
	0.39	0.64	−5.19	0.02	5.43	−7.82
		0.66[e]	−5.19[e]		4.71[e]	−7.72[e]
37	5.42	4.22	−6.56	0.46	6.66	−8.26
	2.38	6.43	−6.82	0.21	8.23	−8.39
	1.33	4.96	−6.66	0.12	7.11	−8.30
	0.61	4.88	−6.65	0.08	6.77	−8.27
	0.31	4.57	−6.61	0.05	4.10	−7.96
	0.17	4.36	−6.58	0.04	3.48	−7.86
		4.90[e]	−6.66[e]		6.06[e]	−8.17[e]
50	0.63	1.21	−6.03	0.13	5.22	−8.45
	0.45	0.70	−5.68	0.06	6.50	−8.59
	0.18	1.50	−6.17	0.03	4.83	−8.40
	0.09	5.12	−6.96	0.02	8.47	−8.76
		2.13[e]	−6.21[e]		6.26[e]	−8.55[e]

[a] Reassembled HDL were prepared by the cholate method. [b] POPC content of R-HDL was determined by the phosphorus assay. [c] Calculated from equilibrium dialysis experiments as described under Experimental Procedures. [d] Calculated from $\Delta G_a = -RT \ln K_{eq}$. [e] Mean values.

where R is the gas constant and T the absolute temperature. ΔG_a decreased linearly with respect to the number of methylene units in the acyl chain of the LAP. The change of ΔG_a was −0.35 kcal mol^{-1} per methylene unit.

To further examine the thermodynamics of association of the LAPs with R-HDL, we measured the K_{eq}'s by equilibrium dialysis at four different temperatures: these were 4, 25, 37, and 50 °C. The K_{eq}'s of C_0- and C_4-LAPs were too low to permit an accurate determination of their change with temperature. Therefore, only C_8- and C_{12}-LAPs were used in this group of experiments. K_{eq}'s increased with temperature (Table III). This increase was larger for C_8-LAP than for C_{12}-LAP,

25 and 5 times, respectively, between 4 and 50 °C. The variations in the corresponding ΔG_a with temperature were used to calculate the enthalpies (ΔH_a) and the entropies (ΔS_a) of association. ΔH_a was estimated from the regression line of $\Delta G_a/T$ vs. $1/T$. ΔH_a decreased when the acyl chain length of LAPs increased (10.7 ± 2.5 vs. 8.3 ± 1 kcal mol^{-1} for C_8- and C_{12}-LAP, respectively). These estimates were relatively uncertain due to some deviation from linearity. However, ΔS_a, calculated from $\Delta G_a = \Delta H_a - T\Delta S_a$, appeared to be constant over the range of temperatures studied and independent of the acyl chain length. Average values for C_8- and C_{12}-LAP were 53.5 and 53 cal mol^{-1} deg^{-1}, respectively. This suggested that the variation of ΔG_a with respect to the acyl chain length reflected a change of ΔH_a rather than ΔS_a.

Discussion

It has been suggested that the hydrophobic content of apolipoproteins is one of the critical factors responsible for their binding to lipoproteins. To test this hypothesis quantitatively, we synthesized a family of model apolipopeptides whose hydrophobicity increased in a predictable way.

Structure of the LAPs. The peptide structure of the LAPs is that of acylated peptides. The peptidic core consists of a 15 amino acid sequence derived from that of a previously studied 20-residue lipid-associating peptide (Pownall et al., 1980). The acyl chain length of the LAPs varies from 0 to 18 carbons. In a homologous family of amphiphiles, the addition of each methylene unit increases the hydrophobicity to the same extent (Tanford, 1981; Pownall et al., 1983). Therefore, the free energy of association (ΔG_a) of an amphiphile with a lipid surface can be fitted to an equation of the form

$$\Delta G_a = A - Bn$$

where A (−3.8 kcal mol^{-1}) is a constant characteristic of the amphiphile family, B is the contribution of each methylene unit to ΔG_a, and n is the number of methylene units. This rule implies that the variation of the acyl chain length of the LAPs induces large changes of hydrophobicity without greatly altering their molecular weight (approximately 10% difference between the molecular weights of C_0- and C_{18}-LAP) and without altering their amino acid number or sequence. Therefore, any change in the binding characteristics of the acyl peptides with respect to the acyl chain length can be considered as resulting from the change of hydrophobicity inherent to the acyl chain.

Lipid Binding Properties of the LAPs. As judged from circular dichroic data, the α-helical content of the free LAPs was low, but their binding to lipid surfaces was clearly accompanied by a transition from random-coil to α-helical structure. The blue shift of the intrinsic fluorescence of tryptophan of the LAPs upon forming a complex with DMPC is comparable to that of native apoproteins (Morrisett et al., 1977). This shift is due to the transfer of the tryptophan from a polar to a nonpolar environment (Pownall et al., 1980). The more hydrophobic LAPs (C_{12}- and C_{16}-LAPs) exhibited a partial blue shift of fluorescence in the absence of lipid, indicating a marked tendency to self-associate.

The experiments involving rat serum, purified human lipoproteins, or R-HDL showed that the LAPs bound predominantly to HDL. The binding to VLDL was low, and that to LDL was almost nil. These characteristics are comparable to those of HDL apoproteins and especially to those of apoA-I since the LAPs and apoA-I are activators of LCAT (Fielding et al., 1972). The properties of the LAPs, namely, binding to HDL, α-helicity, self-association, and activation of LCAT, are strongly dependent upon the acyl chain length of the LAPs. Indeed, we found a log-linear relationship between the equilibrium constants of the binding of LAPs to R-HDL and the number of methylene units in their acyl group. Since $\Delta G_a = -RT \ln K_{eq}$, this implied that the contribution of each methylene unit of the LAP to ΔG_a was the same. This is characteristic of a pure hydrophobic effect as discussed above. Therefore, with respect to the properties of native or synthetic apoproteins as defined in the introduction, we have shown the influence of the hydrophobicity per se on the binding of model apolipopeptides to HDL without altering their potential to form an α-helix and without altering the putative amphipathic length of the peptide. It is notable that we obtained a very strong binding to HDL with a peptide of only 15 amino acids. This means that the so-called critical amphipathic length of 20 residues for a purely peptidic LAP results from the combination of two different parameters, the α-helicity and the hydrophobicity. Provided that the hydrophobicity is maintained (by an acyl chain in our case), a high binding of LAP to HDL can be reached with an α-helix of less than 20 amino acids.

Thermodynamics of LAP Binding to HDL. We found that the entropy of association (ΔS_a) of the LAPs with R-HDL was independent of the acyl chain length of the peptide. Therefore, the variation of ΔG_a with respect to the hydrophobicity of the LAP reflected the corresponding change of enthalpy (ΔH_a). The variation of ΔG_a per methylene unit reported here (0.35 kcal mol^{-1}) is smaller than that of approximately 0.7 kcal mol^{-1} observed by others (Davis et al., 1974; Tanford, 1981). This difference likely results from the particular structure of the acyl peptides. The peptide moiety of the LAPs might cause a significant change in the depth and/or angle of penetration of the acyl group into the lipid matrix. Moreover, this peptidic moiety has its own thermodynamic properties (Jahnig, 1983), and it has been clearly demonstrated that when a peptide binds to an acceptor surface, ΔH_a is proportional to the percent of α-helicity (Massey et al., 1979, 1981b). Thus, the variation of ΔG_a that we observed is probably the sum of the combined effects of the peptide and fatty acyl moiety of the LAP. Alteration of the lipid surface by the peptide or apolipoproteins may change the structure of the microenvironment that surrounds the acyl chain. This might be achieved if the peptide or apoprotein increases the water content of the hydrophobic region of the phospholipid (Pownall et al., 1981). Alternatively, the lowered hydrophobic effect may be due to some association of the peptide moiety with its own acyl chain even when it is an aqueous monomer. If this is the case, the full hydrophobic effect would not be expressed when the acyl peptide is transferred from water to phospholipid.

Biological Implications. Following our demonstration of the large effect of acyl chain length on the lipid binding properties of the acylated LAPs, one can consider several biological implications: (i) acylation of a peptide is a way of increasing its affinity for lipid without altering its biological activity; (ii) use of a highly hydrophobic acyl chain provides a means for identifying the minimal peptide sequence for a given biological activity when binding to a lipid surface is a necessary but not sufficient condition for that activity; (iii) since there is a precise relationship between the acyl chain length of the LAPs and their lipid binding properties, the acylated LAPs can be used in vivo to study directly the effect of hydrophobicity on the metabolism of model apopeptides.

Registry No. C_0-LAP, 91879-73-1; C_4-LAP, 91879-74-2; C_8-LAP, 91879-75-3; C_{12}-LAP, 91879-76-4; C_{16}-LAP, 91879-77-5; C_{18}-LAP,

91879-78-6; LCAT, 9031-14-5; POPC, 26853-31-6; DMPC, 18194-24-6.

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