Hoppe-Seyler's Z. Physiol. Chem.
Bd. 359, S. 465 - 472, April 1978

The Binding of Lysolecithin to Human Serum High Density Apoprotein A-I
A ^{13}C-NMR Study

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(Received 16 January 1978)

Summary: 1-[14,15-$^{3}H_2$;16-^{13}C]Palmitoyl-, 1-[9,10-$^{3}H_2$;11-^{13}C]- and 1-[9,10,12,13-$^{3}H_4$;-14-^{13}C]stearoyl-, 1-[9,10-$^{3}H_2$;11-^{13}C]oleoyl-, 1-[9,10,12,13-$^{3}H_4$;14-^{13}C]linoleoyl-*sn*-glycero-3-phosphocholine and 1-palmitoyl-*sn*-glycero-3-phospho-[*N*-$^{13}CH_3$,$^{14}CH_3$]choline have been prepared by chemical synthesis. They recombine with apolipoprotein A-I of human serum high density lipoproteins. This binding leads only to minor changes in circular dichroism with an increase of α-helicity (f_H) from 0.44 to 0.54 on the average. The lipid-apoprotein A-I-complexes remain stable during agarose chromatography. 60 - 70 lysolecithin molecules were bound to the apolipoprotein, approximately the number associating with the apolipoprotein A-I during equilibrium dialysis (70 lysolecithins per apolipoprotein A-I molecule).

Spin-lattice relaxation time (T_1) experiments indicate that only the hydrophobic fatty acyl chains of the saturated as well as the unsaturated lysolecithins bind to the polypeptide chains, whereas the mobility of the choline moiety of the hydrophilic, polar group remains completely unaltered. The T_1-times were compared with those of the respective lysolecithin in aqueous micellar solution. The function of lysolecithin in the synthesis of the high density lipoprotein particle during biosynthesis is discussed.

Über die Bindung von Lysolecithinen an Apoprotein A-I des menschlichen Serums: Eine ^{13}C-NMR-Untersuchung

Zusammenfassung: 1-[14,15-$^{3}H_2$;16-^{13}C]Palmitoyl-, 1-[9,10-$^{3}H_2$;11-^{13}C]- und 1-[9,10,12,13-$^{3}H_4$;14-^{13}C]Stearoyl-, 1-[9,10-$^{3}H_2$;11-^{13}C]-Oleoyl-, 1-[9,10,12,13-$^{3}H_4$;14-^{13}C]Linoleoyl-*sn*-glycero-3-phosphocholin und Palmitoyl-*sn*-glycero-3-phospho-[*N*-$^{13}CH_3$,$^{14}CH_3$]cholin wurden synthetisiert. Sie rekombinieren mit Apolipoprotein A-I des menschlichen Serum-High-Density-Lipoproteins. Diese Bindung bewirkt nur geringe Änderungen des Circulardichroismus mit einem Anstieg des α-Helix-Gehaltes (f_H) von 0.44 auf 0.54 im Mittel. Die Lysolecithin-Apolipoprotein-Komplexe sind während der Agarose-Chromatographie stabil und binden ungefähr 60 - 70 Lysolecithinmoleküle. Ähnlich viele Lysolecithinmoleküle assoziieren mit Apolipoprotein A-I in der Gleichgewichtsdialyse (70 Lysolecithine pro Apolipoprotein A-I Molekül).

Spin-Gitter-Relaxationszeit-Messungen zeigen, daß nur die hydrophoben Fettsäureketten der gesättigten und ungesättigten Lysolecithine an die Polypeptidketten binden. Die Beweglichkeit der Cholingruppe in der polaren hydrophilen Gruppe ändert sich hingegen nicht. Die T_1-Zeiten wurden jeweils mit denen der entsprechenden Lysolecithine in wäßrigen micellaren Lösungen verglichen. Die Funktion des Lysolecithins in der Synthese des High-Density-Lipoproteins wird diskutiert.

Key words: ^{13}C-labelled lysolecithin species, binding to apolipoprotein A-I, spin-lattice relaxation time studies.

Serum high density lipoproteins (HDL) represent a group of lipoproteins very suitable to study the interactions of their apoproteins A-I and A-II and lipids by physical and chemical methods and finally to construct from these data a model of these particles. This should explain the lipid binding, the lipid transport function and the substrate function of the lipids and the activator function of the apolipoprotein A-I for lecithin-cholesterol acyltransferase[1].

Phosphatidylcholine, cholesterolester and cholesterol are the main classes among the lipid components of HDL, but about 2% lysolecithin is consistently found, which corresponds to about 4 mol lysolecithin per mol apoprotein[2].

In previous studies we have analysed the influence of lysolecithin on the reaggregation of HDL-apolipoproteins, particularly apolipoprotein A-I, with phosphatidylcholine and sphingomyelin species[3,4]. In this recombination procedure, lysolecithin apparently occupies sites of the partially unfolded apoprotein from which it is substituted by the diacylphospholipid or sphingomyelin with two long alkyl chains, one from the fatty acid and the other from the long chain base.

This paper reports experiments which on physical grounds prove that lysolecithin molecules bind to the apoprotein not by interactions with polar side chains of the polypeptide, but by hydrophobic interactions of the alkane chains with hydrophobic sites of the apolipoprotein. This information was derived from ^{13}C-NMR spin-lattice relaxation time experiments with lysolecithins substituted with palmitic, stearic, oleic and linoleic acid, enriched with carbon-13 in specific positions along the fatty acyl chain and in the choline moiety of the polar head groups.

The secondary structure of these lysolecithin-apolipoprotein A-I complexes was scarcely different from the apolipoprotein, as shown by circular dichroism spectra.

The stable complexes had densities between 1.11 - 1.19 g/cm^3.

Methods and Materials

^{3}H-labelled lysolecithin substituted with ^{13}C-labelled fatty acids ([16-^{13}C]palmitic acid, spec. radioact. 5.8 mCi/mmol, [11-^{13}C]oleic acid, spec. radioact. 22.6 mCi/mmol, [14-^{13}C]linoleic acid, spec. radioact. 3.75 mCi/mmol, [11- and 14-^{13}C]stearic acids) and 1-acylglycerophospho-[*N*-$^{13}CH_3$,$^{14}CH_3$]choline, spec. radioact. 3.52 mCi/mmol, were synthesized by acylation of *sn*-glycero-3-phosphocholine (W. Stoffel and K.P. Salm, to be published). The syntheses of the radioactive ^{13}C-enriched fatty acids have been described before[5]. The fatty acid residue in the 2-position of the diacylglycerophosphocholine molecules was removed by phospholipase A_2 *(Crotalus adamanteus)* hydrolysis and separated from the lysolecithin by silicic acid column chromatography. The fatty acid was eluted with chloroform and lysolecithins with methanol/chloroform 4 : 1 and methanol. All compounds were chemically and radiochemically pure.

Preparation of high density lipoprotein and its purified apolipoproteins: Human serum high density lipoproteins (density 1.063 - 1.21 g/cm^3) were isolated by the sedimentation-flotation ultracentrifugation method[6]. They were delipidated with chloroform/methanol[7] and the apoproteins separated by the combination of DEAE-cellulose[8] and Sephadex G-150 or G-200 chromatography in 8M urea[7]. The main apolipoproteins A-I and A-II were obtained in homogenous form as demonstrated by sodium dodecylsulfate polyacrylamide, disc and slab gel electrophoresis[9-12], in the immunodiffusion assay[13] and amino acid stoichiometry.

Purification of apolipoprotein A-I-lysolecithin complexes: Apolipoprotein A-I was stirred together with different molar amounts of the lysolecithins in aqueous solution. The complexes were separated from unbound lysolecithin

a) by ultracentrifugation in a caesium chloride gradient 10 to 20.5% in a SW 41 Beckman rotor at 38000 rpm for 72 h,

b) by agarose chromatography on a Bio-Gel A-5 m 100 - 200 mesh 90 × 2.5 cm column. The stoichiometry was calculated from the bound radioactive lysolecithin per apolipoprotein.

The radioactivity was assayed in a Tricarb liquid scintillation spectrometer, Packard, model 3380.

Protein was determined according to Lowry et al.[14] with serum albumin as standard.

Proton noise decoupled ^{13}C-nuclear magnetic resonance spectra of lipids (^{13}C-enriched fatty acids and lysoleci-

thins) dissolved in deuterochloroform and apolipoprotein A-I-lysolecithin complexes dissolved in 3 ml water-20% D_2O were recorded in the Fourier transform mode in a Bruker WH 90 NMR spectrometer at 22.63 MHz as described before[4]. A temperature control unit Bruker ST 100/700 controlled the temperature within 1 °C.

For T_1-measurements, partially relaxed Fourier transform (PRFT) spectra were recorded, applying the inversion-recovery method[15]. T_1 was calculated from the slope of the computerized semilog plot of ln $(M_0 - M_t)$ against τ, the delay time. The magnetization *(M)* for the $(180° - \tau - 90°)$ pulse sequence is $M_{(t)} = M_0 (1 - 2 \exp - \tau/T_1)$[15]. The experimental error ranges between 5 and 10%.

Circular dichroism in the UV-range was measured with a Jasco J-41A spectropolarimeter. The molar ellipticity ($[\Theta]$) was calculated and corrected according to Chen and Yang[16]. A molecular residue weight (MRW) of 116.5 for apolipoprotein A-I was used.

Results

The NMR spectroscopic properties of six lysolecithins have been studied in 1) organic, 2) aqueous micellar solutions and 3) associated with apolipoprotein A-I. These were 1-[16-^{13}C]-palmitoyl-, 1-[11-^{13}C]- and 1-[14-^{13}C]stearoyl-, 1-[11-^{13}C]oleoyl-, 1-[14-^{13}C]linoleoylglycerophosphocholine and a hydrogenated [*N*-$^{13}CH_3$]-lysolecithin from soy lecithin*. Palmitoyl- and stearoylglycerophosphocholine have phase transition temperatures of 18.5 °C and 31 °C, respectively, as determined by light scattering at 400 nm; the others are liquid in the temperature range of the experiments (4 - 37 °C). All recombinations and spin-lattice relaxation time studies were carried out at 37 °C.

1) Spin-lattice relaxation times in organic solvents. Phospholipid molecules and fatty acids form inverted micelles in apolar solvents with their hydrophilic ionic groups closely packed due to electrostatic interactions. Their fatty acyl chains are directed into the organic solvent. The T_1-times of the aforementioned lysolecithins and fatty acids in $CDCl_3$ underline this suggestion (Table 1).

The choline methyl groups of lysolecithins in $CDCl_3$ are highly immobilized, as indicated by the very short T_1-time. The T_1-times of the

* Fatty acid composition in the 1-position: 16:0 24%; 18:0 76%.

Table 1. ^{13}C-NMR spin-lattice relaxation times T_1 of ^{13}C-labelled lysolecithins in $CDCl_3$ and water.

Lipid	T_1-times [ms]	
	in $CDCl_3$	in H_2O
Fatty acid		
[16-^{13}C]16 : 0	4479 ± 181	
[11-^{13}C]18 : 1	2060 ± 42	
[14-^{13}C]18 : 2	3560 ± 184	
Lysolecithin containing		
[16-^{13}C]16 : 0	4319 ± 143	1942 ± 15
[14-^{13}C]18 : 0	1536 ± 17	292 ± 10
[11-^{13}C]18 : 0	921 ± 39	221 ± 6
[11-^{13}C]18 : 1	1457 ± 68	344 ± 13
[14-^{13}C]18 : 2	2446 ± 131	728 ± 42
[*N*-$^{13}CH_3$]Choline	95 ± 4	633 ± 32

^{13}C-enriched fatty acyl chains in both the free fatty acids and the lysolecithins increased towards the terminal CH_3-group. This is due to the fixation of the carboxy terminal end at the glycerophosphate-backbone, but also due to reduced van der Waals' forces toward the terminal CH_3-group. *Cis*-double bonds in oleic acid and linoleic acid lead to a considerable elevation of the mobility of the carbon segment beyond the double bond most distant from the carboxy group. The *cis*-double bonds cause a disordered arrangement of the acyl chains, with facilitated *trans-gauche* isomerisations and kink formations thereby reducing the rotational correlation time, but prolonging the T_1-time.

2) Lysolecithins form micelles in water with an average molecular weight of 92000[17], which corresponds to about 170 to 190 molecules. The lysolecithin concentrations used in these experiments were about 10mM. This is far above the critical micellar concentration, which has been suggested to be below 120μM[17,18] and above 7μM[19]. The T_1-values of the ^{13}C-enriched lysolecithins in aqueous solution (Table 1) therefore stand for their micellar arrangement. The fatty acyl chains are drastically immobilized due to strong hydrophobic inter-chain interactions, whereas the choline group reaches a high T_1-time, which excluded electrostatic interactions between polar head groups of lipid molecules.

3a) Apolipoprotein A-I-lysolecithin complexes. Lysolecithin easily associates with apolipoprotein A-I at temperatures above their phase transition point. The aforementioned six lysolecithins formed stable complexes in a ratio of 1 apolipoprotein A-I and 55 - 60 lysolecithin molecules. They eluted from an agarose column (2.5 x 90 cm) calibrated with test proteins as single bands with no lysolecithin in the void volume. Lysolecithin produced from highly unsaturated soy lecithin had a retention volume identical with that of the apolipoprotein A-I-lysolecithin complex. The apparent molecular weight determined by this agarose gel chromatography was around 90000 to 100000, very close to that of unsaturated lysolecithin micelles, Fig. 1. Therefore the complexes were centrifuged in a CsCl gradient, Fig. 2. The analysis of the gradients clearly proved that lysolecithin was completely bound to the apolipoprotein with no free apolipoprotein left. Whereas 1-palmitoyl-, 1-oleoyl- and 1-linoleoylglycerophosphocholine-A-I complexes banded at ρ = 1.16 - 1.19 g/cm^3, 1-stearoylglycerophosphocholine had a density of 1.11 g/cm^3. These results are in good agreement with earlier studies on apolipoprotein A-I-lysolecithin complexes of molar ratios 1 : 73 to 1 : 15, which had densities between 1.17 and 1.23 g/cm^3[3].

b) Circular dichroism. We measured the molar ellipticity ([Θ]) of the different apolipoprotein A-I-lysolecithin complexes in order to follow structural perturbation, particularly in α-helicity (f_H) by the binding of the lipid. Fig. 3 and Ta-

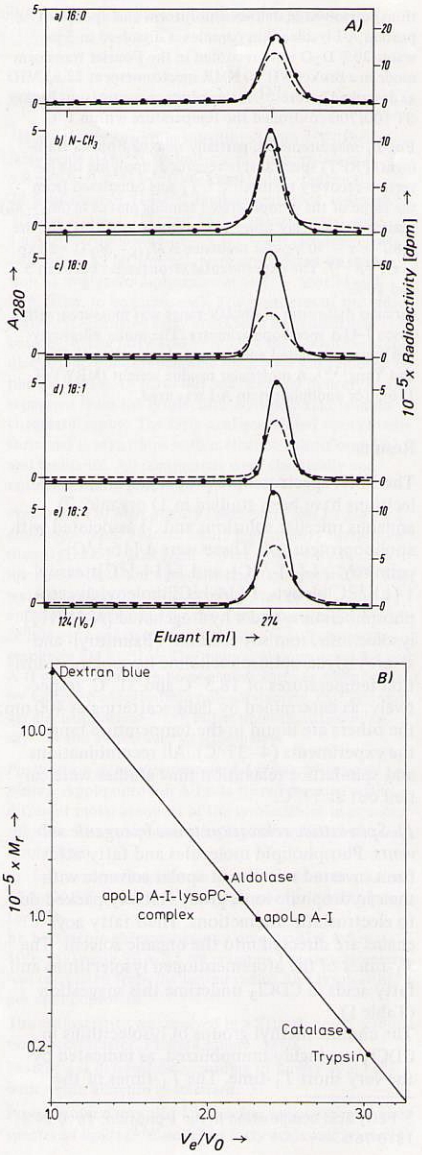

Fig. 1. Determination of molecular weight of apolipoprotein-A-I-lysolecithin complexes by agarose gel chromatography (Bio-Gel A-5 m 200 - 400 mesh (2.5 x 90 cm)) on a calibrated column.

Elution buffer: 0.1M Tris/HCl, pH 8.2, 0.15M NaCl, 0.01% NaN_3, 0.01% EDTA in water.

A) Elution pattern of complexes consisting of apolipoprotein A-I and ^{3}H-labelled a) 1-[16-^{13}C]palmitoylglycerophosphocholine; b) 1-stearoylglycerophospho-[*N*-$^{13}CH_3$]choline; c) 1-[11-^{13}C]stearoylglycerophosphocholine; d) 1-[11-^{13}C]oleoylglycerophosphocholine; e) 1-[14-^{13}C]linoleoylglycerophosphocholine. --- A_{280}; —— radioactivity.

B) Semilogarithmic plot of molecular weight against the relative elution volume V_e/V_0 (V_e = elution volume, V_0 = void volume).

ble 2 summarize these results. α-Helicity increases by 14 - 27% due to the binding of lysolecithins. This change in the secondary structure of apolipoprotein A-I is small in comparison to that after recombination of apolipoprotein A-I with lecithin or sphingomyelin. These particles had an f_H of approximately 0.8 - 0.9. An increase in the lysolecithin content of the complexes did not further elevate the α-helical structure.

c) Spin-lattice relaxation studies. The ^{13}C-enriched lysolecithins bound to the apoprotein A-I made spin-lattice relaxation time studies feasible.

Table 2. Molar ellipticity ([Θ]) and α-helicity (f_H) of apolipoprotein A-I free and associated with different lysolecithin species.

Lysolecithin containing	$10^{-3} \times [\Theta]$ [degree × cm^2 × $dmol^{-1}$]		f_H
	uncorrected	corrected[16]	
16 : 0	− 14.7	− 18.7	0.54
18 : 0	− 14.3	− 18.2	0.52
18 : 1	− 15.5	− 19.2	0.56
18 : 2	− 13.7	− 17.4	0.50
apoA-I	− 12.3	− 15.6	0.44

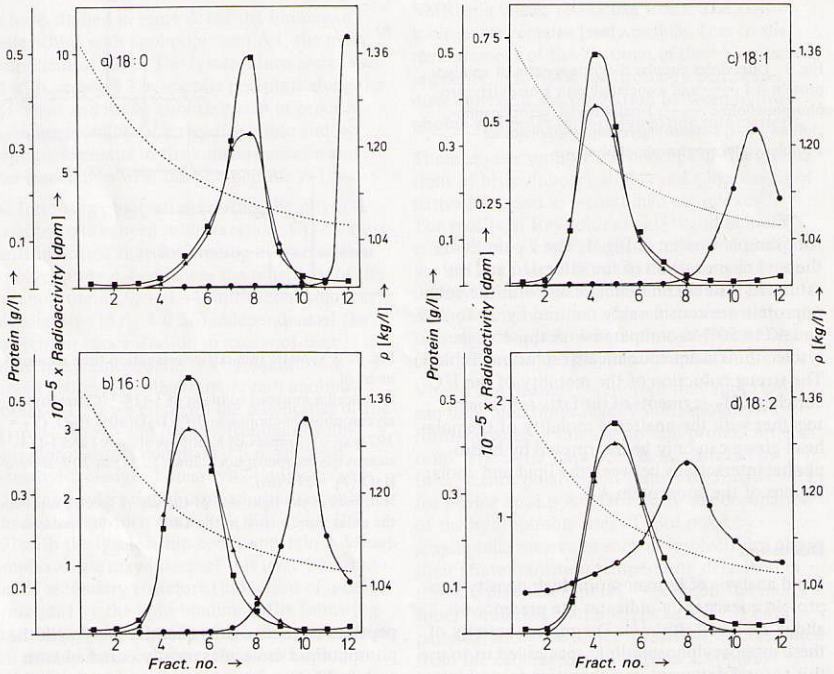

Fig. 2. Patterns of CsCl gradient centrifugation of apolipoprotein A-I complexes with a) 1-stearoyl (18 : 0)-; b) 1-palmitoyl (16 : 0)-; c) 1-oleoyl (18 : 1)-; d) 1-linoleoyl (18 : 2) glycerophosphocholine.
■——■ protein; ▲——▲ radioactivity in complex; ●——● lysolecithin micelles; ····· density.

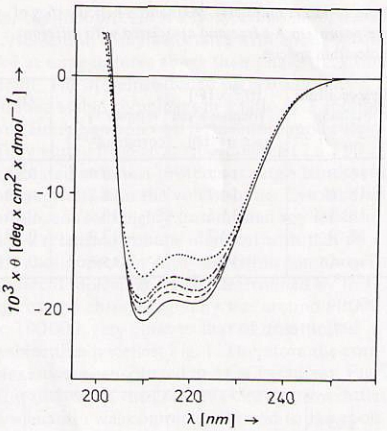

Fig. 3. Ultraviolet circular dichroic spectra of apolipoprotein A-I ····· and associated with 1-oleoylglycerophosphocholine ——; 1-palmitoylglycerophosphocholine – – –; 1-stearoylglycerophosphocholine –.–.–; 1-linoleoylglycerophosphocholine –..–..–.

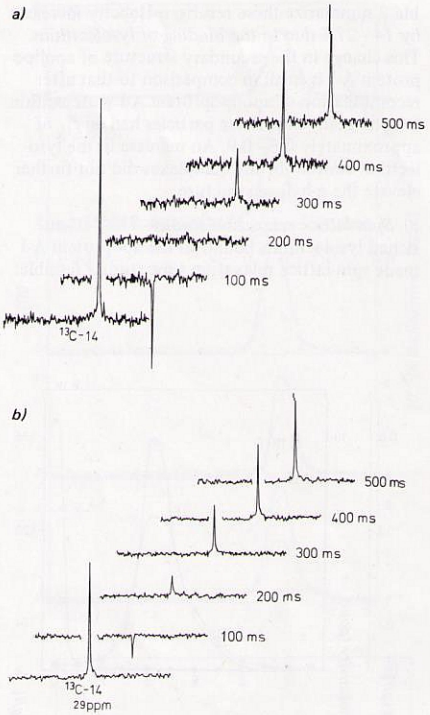

Fig. 4. ^{13}C-NMR spin-lattice relaxation time measurement of
a) micellar aqueous solution of 1-[14-^{13}C]stearoylglycerophosphocholine in 20% D_2O/80% H_2O, (T_1 = 292 ms); b) complex of apolipoprotein A-I and 1-[14-^{13}C]-stearoylglycerophosphocholine (1 : 57) in 20% D_2O/80% H_2O (T_1 = 216 ms).
Numbers at the right side of the set of spectra represent the delay time τ (ms) in the 180° – τ – 90° pulse sequence.

An example is given in Fig. 4. The T_1-times of the acyl chain carbon of the saturated and unsaturated lysolecithins complexed with the apolipoprotein are considerably reduced by 20 to 25% and 40 to 50% as compared with those of the lysolecithins in aqueous micellar solution (Table 3). The strong reduction of the mobility of the ^{13}C-enriched CH_2-segments of the fatty acyl chains together with the unaltered mobility of the polar head group can only be interpreted by hydrophobic interactions between the lipid and apolar regions of the apoprotein A-I.

Discussion

Lipid analysis of human serum high density lipoprotein consistently indicates the presence of about 2% lysolecithin[2]. The surface activity of these monoacylphospholipid species led us to use this natural detergent in elaborating recombination methods for HDL apolipoproteins with bisacylphospholipids or sphingomyelin with two hydrophobic alkyl chains[3,4]. In fact the polypeptide A-I reassociates quantitatively with the phospholipid molecules mostly enriched with carbon 13. Our interpretation is that the acyl chains of lysolecithin molecules occupy the hydrophobic sites of apoLp A-I, but then they are substituted by lecithin or sphingomyelin mole-

Table 3. ^{13}C-spin-lattice relaxation times T_1 of apolipoprotein A-I complexed with ^{13}C-labelled lysolecithin after agarose chromatography.

Lysolecithin containing	Apo A-I/lysoPC	T_1-times [ms]	
		ApoA-I-lysoPC complex	Lysolecithin micellar solution
[16-^{13}C]16 : 0	1 : 57	1580 ± 115	1942 ± 15
[14-^{13}C]18 : 0	1 : 57	216 ± 14	292 ± 10
[11-^{13}C]18 : 0	1 : 57	176 ± 6	221 ± 6
[11-^{13}C]18 : 1	1 : 57	190 ± 12	344 ± 13
[14-^{13}C]18 : 2	1 : 57	360 ± 27	728 ± 42
[*N*-$^{13}CH_3$]choline	1 : 57	650 ± 25	633 ± 30

cules monomerized by lysolecithins from the liposomal arrangement.

We have studied in more detail the binding of lysolecithins with apolipoprotein A-I, the main component of HDL. The lysolecithins were labelled with carbon 13 in specific positions along the acyl chain and in the choline group in order to probe the mobility of its hydrophobic and hydrophilic segments in the aqueous micelle and after association with the polypeptide A-I.

The following observations during the physical characterization need interpretation: CD-measurements indicated that the binding of lysolecithin to polypeptide A-I enhances the α-helicity of the free apoprotein (f_H = 0.44) in aqueous solution only slightly (to f_H = 0.56) independent of the lysolecithin concentration in excess of that bound to apolipoprotein A-I by equilibrium dialysis (70 mol lysolecithin per mol apolipoprotein A-I). If we compare the α-helicities of the stearoyl-, oleoyl- and linoleoylsphingomyelin-apolipoprotein A-I complexes[4] or those of 1-stearoyl-2-oleoyl-, 1-stearoyl-2-linoleoyl- and 1,2-dilinoleoylglycerophosphocholine (unpublished results) which reach 80 - 90% (f_H = 0.8 - 0.9) with the lysolecithin-apolipoprotein A-I complexes, we may interpret this large perturbation of secondary structure (induction of α-helix formation) by the lipid binding in the following way: the polypeptide A-I, which in aqueous solution already has a considerable α-helix content, loads all its hydrophobic sites with monoacyl-phospholipids and changes its surface with the dipolar phosphocholine groups. The lyso-compounds are exclusively bound with their fatty acyl tails to the apoprotein, the immobilisation of which is apparent from the strongly reduced NMR-spin-lattice relaxation times. The zwitterionic group remains freely mobile. Due to the measurement of the T_1-times of the ^{13}C-enriched segments, ^{13}C-NMR spectroscopy permits a direct physical discrimination between hydrophobic and polar lipid-polypeptide interactions.

These studies support our concept of the interactions of bisacylphospholipids and apoproteins of native HDL and in recombined complexes[4,20]. The results of Reynolds et al.[21] and Makino et al.[22] on comparable associations of apolipoprotein A-I from HDL with the detergent anion dodecylsulfate and the cationic tetradecyltrimethylammonium ion allow the same interpretation regarding polar interactions between these detergents and the polypeptide chains.

Vitello and Scanu[23] reported that apoLp A-I can only bind lipids readily in its monomeric form, because of missing protein-protein interactions. The latter have been demonstrated by fluorescence polarisation studies by Jonas[24,25] for bovine apoLp A-I. Obviously the occupation of the hydrophobic sites of apoLp A-I by amphiphilic molecules such as lysolecithins above their phase transition temperature described in this and previous papers[3,4] prohibit the multimer formation (tetramer, octamer[23]) but apparently allow the dimer formation, as concluded from the calibrated gel filtration chromatography.

The in-vitro binding process of lysolecithin described in this paper combined with the displace-

ment of the monoacyl- by the diacylphospholipids of the previous publication[4] could be transposed into the situation in vivo. Lysolecithin present in most subcellular membranes and also in those of the endoplasmic reticulum could well prepare the nascent polypeptide chain for the recombination with the diacylphospholipids which induce the high α-helicity of the final particle structure. Further studies will be concerned with the degree of lipid-polypeptide and lipid-lipid interactions of the diacylphospholipids within the HDL particle.

This study was supported by the *Deutsche Forschungsgemeinschaft*.

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