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Lipid-Protein Interactions between Human Apolipoprotein A-I and Defined Sphingomyelin Species

A ^{13}C-NMR Spectroscopic Study

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Summary: Chromatographically and immunologically homogeneous apolipoprotein A-I (apoLp A-I) from human serum has been recombined in separate experiments with three species of sphingomyelin. They differed in the degree of saturation of their fatty acyl residues, stearoyl (18:0), oleoyl (18:1) and linoleoyl (18:2). The lipoprotein complexes formed were purified by CsCl density gradient centrifugation between 1.07 - 1.09 g/cm^3 and by gel filtration. Stearoyl-sphingomyelin does not recombine with the apoprotein A-I below its phase transition temperature (t_c = 41.5 °C)*.

The lipoproteins eluted with the following apparent molecular weights: 18:0-sphingomyelin apoLp A-I, 8.0 x 10^5; 18:1-sphingomyelin apoLp A-I, 4.0 x 10^5; and 18:2-sphingomyelin apoLp A-I, 4.0 x 10^5.

In electron microscopy the particles appear as discs of 160 - 170 Å diameter and 50 - 60 Å thickness. Their tendency to form stacked aggregates of discs decreases with the degree of their unsaturation.

CD measurements underline the considerable increase in α-helicity of the secondary structure of apo A-I after recombination with the phospholipids. This increase in order is equal for the three sphingomyelin species (α-helicity of apoLP A-I = 0.46, after recombination 0.89).

If the three sphingomyelin species are used in equal molar amounts in the recombination experiment, no preference for any one sphingomyelin species is observed.

Recombination of apoLp A-I with sphingomyelin, labelled with the isotope ^{13}C in the choline group, C-14 of stearic or linoleic, or C-11 of oleic acid, were performed for spin lattice relaxation time (T_1) experiments. Compared with sphingomyelin liposomes, the polar head groups of these lipids in the lipoprotein particles possess a considerably higher mobility, whereas the changes in T_1-times of the C-atoms in the centre of the fatty acid chains of the lipids refer to their interactions with the polypeptide side chains.

A model of the lipoprotein complexes formed is proposed on the basis of the experimental data.

Enzyme:
Phospholipase A_2, phosphatide 2-acylhydrolase (EC 3.1.1.4).

Abbreviations:
apoLp A-I = Apolipoprotein A-I of human high density lipoprotein (HDL); lecithin = phosphatidylcholine; prefixes (18:0, 18:1, 18:2) indicate substituting fatty acyl residues (stearoyl, oleoyl, linoleoyl) in sphingomyelins.

* For long chain base composition see Results.

Lipid-Protein-Wechselwirkung zwischen menschlichem Apolipoprotein A-I und spezifischen Spezies von Sphingomyelin: Eine ^{13}C-NMR-Untersuchung

Zusammenfassung: Chromatographisch und immunologisch einheitliches Apolipoprotein A-I aus menschlichem Serum wurde in getrennten Experimenten mit je drei Spezies von Sphingomyelinen, die sich durch die steigende Zahl der Doppelbindungen der Fettsäuresubstituenten unterschieden, rekombiniert. Die gebildeten Lipoproteinkomplexe wurden durch Caesiumchlorid-Dichtegradientenzentrifugation bei einer Dichte von 1.07 - 1.09 g/cm^3 und durch Gelfiltration gereinigt. Die Lipoproteine wurden mit folgenden apparenten Molekulargewichten eluiert: 18:0-Sphingomyelin-apoLp A-I 8.0×10^5, 18:1-Sphingomyelin-apoLp A-I 4.0×10^5 und 18:2-Sphingomyelin-apoLp A-I 4.0×10^5.

Unterhalb des Phasenumwandlungspunktes des 18:0-Sphingomyelins (t_c = 41.5 °C)* findet keine Lipid-Apoprotein-Assoziation statt.

Elektronenmikroskopisch besitzen die Partikeln Scheibenform von 160 - 170 Å Durchmesser und 50 - 60 Å Dicke. Ihre Aggregationsneigung nimmt mit dem Grad der Ungesättigtheit ab.

CD-Messungen unterstreichen die erhebliche Zunahme des α-helicalen Gehaltes der Sekundärstruktur nach Assoziation mit diesen Phospholipiden. Ein Unterschied zwischen den Spezies besteht nicht (α-Helicität des apoLP A-I = 0.46, nach Rekombination 0.89).

Werden äquimolare Mengen der drei verschiedenen Sphingomyelinspezies gleichzeitig mit apoLp A-I rekombiniert, so wird keine Spezies bevorzugt gebunden.

Rekombinate von apoLp A-I mit Sphingomyelinen, die in der Cholingruppe bzw. an C-14 der Stearin- und Linolsäure bzw. C-11 der Ölsäure mit ^{13}C markiert waren, wurden Spin-Gitter-Relaxationszeitmessungen unterzogen. Verglichen mit den aus den Sphingomyelinen hergestellten Liposomen zeigte sich eine deutliche Zunahme der Beweglichkeiten der polaren Köpfe in den Lipoproteinkomplexen, während die T_1-Zeiten der Alkanketten-C-Atome der Lipide auf eine Wechselwirkung mit den Polypeptidketten hinweisen.

Es wird ein Modell für die gebildeten Lipoproteinkomplexe auf der Basis der erhaltenen experimentellen Daten vorgeschlagen.

Key words: Apolipoprotein A-I-^{13}C-labelled sphingomyelin recombinations; sphingomyelin species; lipoprotein particles; spin-lattice relaxation times; structure of particles.

Human high density serum lipoproteins (HDL) are spherical particles of 80 - 100 Å diameter suitable for the study of lipid-protein interactions. They are easily accessible and of limited complexity with regard to their lipid and protein composition. Due to the known amino acid sequence of their two main apoproteins apoLp A-I[1] and apoLp A-II[2], the apoproteins present themselves as promising models for the study of the mode and site of the binding of phospholipids to polypeptide chains in recombination experiments. The understanding of the spatial arrangement of the components in recombined lipoprotein complexes might give insight into the native structure of these lipid-transporting particles; moreover, general aspects of the nature of lipid binding to apoproteins, also governing membrane architecture, may evolve. In addition, our understanding of the physiology of high density lipoproteins could be promoted.

Our experimental approach to these problems is based mainly on two techniques, namely NMR spectroscopy[3,4] and photoaffinity labelling techniques*. NMR spectroscopy, particularly ^{13}C and ^{31}P NMR, allows insight into the dynamic properties of the lipid components in a lipoprotein complex, provided the ^{13}C nucleus has been

* Sphingosinbasenzusammensetzung siehe Results.

* Stoffel, W. and Därr, W., manuscript in preparation.

enriched in particular positions, e.g. the polar head groups or the alkyl chain of the fatty acid residues of phospholipids[5]. Our previous ^{13}C NMR studies have shown that the polar head groups of the lecithin and sphingomyelin molecules are freely mobile, whereas their fatty acyl chains have restricted mobilities when bound to the apoHDL[3,4].

The main apolipoprotein of HDL is apoLp A-I. In this paper the results of experiments are reported, in which we have investigated the properties of lipoprotein complexes recombined under standardized conditions[6], and consisting of apoLp A-I and sphingomyelin, with regard to its stoichiometry by radiochemical analysis, molecular weight of the particle formed, electron microscopic appearance of the lipoprotein complex, changes in α-helicity induced by lipid binding by CD measurements and the dynamics of the lipid mobility in the lipoprotein complex by spin-lattice relaxation time experiments in partially relaxed Fourier transform NMR-spectroscopy.

Methods and Materials

Apolipoprotein A-I was prepared from human high density lipoprotein isolated by the sedimentation-flotation method[7] and purified by DEAE-cellulose[8,9] and Sephadex G-200 chromatography[10]. The apoprotein proved to be homogeneous in disc[11] and slab gel polyacrylamide gel electrophoresis[12] and in the immunodiffusion test[13]. *N*-Stearoyl-, *N*-oleoyl- and *N*-linoleoylsphingosylphosphocholines labelled either with the corresponding radioactive or ^{13}C-labelled fatty acids were synthesized in this laboratory[14]. Lysolecithin prepared from soy lecithin by phospholipase A_2 *(Crotalus adamanteus)* hydrolysis was catalytically hydrogenated.

The recombination procedure described before[6] has been slightly modified: vesicles from sphingomyelin (35 μmol) were prepared by sonication (30 min, 70 W, nitrogen atmosphere) above the phase transition temperature, i.e. at 50 °C in the case of *N*-stearoylsphingomyelin (t_c = 41.5 °C), but at 4 °C with the other sphingomyelins: 3.0 m*l* 0.01M Tris buffer, pH 8.2 (1.17 g NaCl, 7.44 g EDTA, 0.2 g NaN_3 per liter) was used as suspension medium. 1-Stearoyl-3-glycerophosphocholine (14.5 μmol) and apoLp A-I (0.27 μmol) were dissolved in 50 m*l* recombination buffer, pH 8.2, containing 8M urea and stirred for 3 h. The temperature was raised to 50 °C in the three recombinations, so the conditions would be uniform, and the apoLp A-I lysolecithin complex added to the vesicle preparation over a period of 10 min with stirring, which was continued in an N_2 atmosphere at room temperature for 2 h more. The recombination mixture was then dialysed against a solution containing 0.01% NaN_3 and 0.01% EDTA (pH 8.0) for 24 h at 4 °C. The retentate was concentrated to a volume of 1 - 2 m*l* by ultrafiltration using an Amicon UM2 ultrafilter. The lipoprotein complex separated well on a Bio-Gel A-5m column (90 x 1.6 cm) from excess lipid vesicles, which eluted with the void volume, and traces of unrecombined apoLp A-I.

The sedimentation of the recombinants was studied by CsCl gradient centrifugation (11 - 20.5%). The lipoprotein complexes had a density of 1.07 - 1.09 g/cm^3. The determination of the radioactivity of the sphingomyelins and phosphorous[15] in lysolecithin allowed the calculation of the stoichiometry of the lipoprotein complexes. Protein was determined with the Lowry method[16] and corrected for lipoproteins[17].

Lipoprotein complexes were delipidated with chloroform/methanol 2:1 (v/v). Lipids were separated by preparative thin-layer chromatography (solvent system: chloroform/methanol/10% ammonia 60:30:8). Fatty acids were released from sphingomyelins by acid hydrolysis[18] and analysed by gas-liquid chromatography (2.5% EGS on Chromosorb, 2-m column at 180 °C).

The phase transition temperature t_c of *N*-stearoylsphingomyelin vesicles was determined by 90° light scattering in a Perkin Elmer MPF III fluorimeter (d = 1 cm; 400 nm excitation, 400 nm emission). Electron microscopy was performed with a Philips EM 300 of samples negatively stained with buffered 2% phosphotungstic acid.

Circular dichroism was measured in a Jobin-Yvon dichrometer III at 25 °C. The conditions were as follows: lipoprotein complexes were dialysed against a buffer containing 0.15mM KF, 1mM Tris, 1mM EDTA, pH 8.0. The clear suspension was diluted to a protein concentration of 0.025 - 0.030 mg/m*l*, which corresponds to an absorbance of 1.9 at 195 nm in a 0.1-cm cuvette. Molar ellipticity [Θ] [deg x cm^2 x dmol^{-1}] was calculated according to

$$[\Theta] = \frac{(\epsilon_L - \epsilon_R) \times MRW}{c \times l} \times 3300$$

where ϵ_L and ϵ_R are extinction coefficients, MRW is the average molecular residue weight, c is the protein concentration (g/l), and l is the light path (cm). The α-helicity (f_H) of the free apoLp A-I and the lipoprotein complexes was calculated according to the approximation of Chen and Yang[19].

$$f_H = \frac{[\Theta]_{222} - 2340}{30300}$$

Protein decoupled ^{13}C-NMR spectra of the lipid vesicles and the purified lipoprotein complexes were recorded with a Bruker WH 90 pulse-spectrometer at 22.63 MHz at 37 °C. Samples containing 6 to 12 mg apoLp A-I were thoroughly flushed with argon. Spin-lattice relaxation times and standard deviations were calculated with a homemade computer program. Other conditions of the T_1-experiments have been described before[3-5,14].

Results

Recombination of sphingomyelin species with apoLp A-I

Three species of sphingomyelins substituted with stearic, oleic or linoleic acid were synthesized. The fatty acids were either radioactively labelled and/or enriched with ^{13}C in position C-14 of stearic and linoleic and C-11 of oleic acid. Fig. 1 visualizes the chemical and radiochemical purity of the substrates *N*-stearoyl-, *N*-oleoyl- and *N*-linoleoylsphingosylphosphocholine.

N-Stearoylsphingomyelin did not recombine at room temperature, in contrast to the unsaturated species. Therefore it was essential to know the phase transition temperature of *N*-stearoylsphingosylphosphocholine. This was determined to be 41.5 °C. (The phase transition temperature of *N*-linoleoylsphingosylphosphocholine was 12 °C, that of the corresponding oleoyl compound could not be determined because of the limited amount available.) The sphingomyelins contained 39.8% sphinganine and 60.2% 4 *t*-sphingenine. A vesicle suspension obtained by ultrasonication (30 min, 70 W, N_2 atmosphere) was submitted to a reversible heating-cooling cycle and simultaneous light scattering measurement of the absorption emitted at 400 nm and excitation at 400 nm (Fig. 2) and by the monolayer technique.

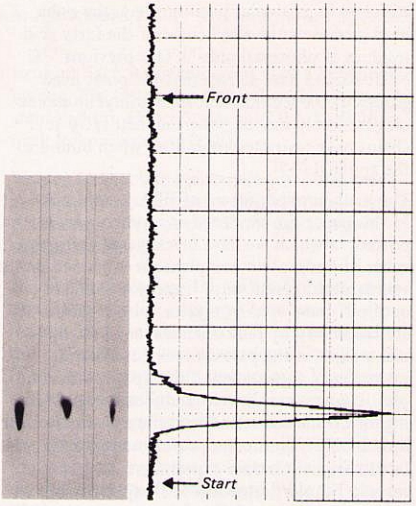

Fig. 1. Radio thin-layer chromatogram of *N*-[^{3}H]stearoyl-, *N*-[^{3}H]oleoyl- and *N*-[^{3}H]linoleoylsphingosylphosphocholine.
Solvent system: chloroform/methanol/water 65 : 25 : 4.

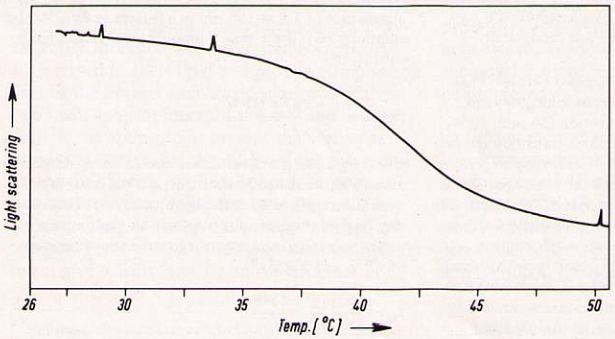

Fig. 2. Determination of phase transition temperature (t_c) of *N*-stearoylsphingosylphosphocholine by light scattering.
A temperature cycle between 27 °C and 50 °C was applied, excitation and emission wavelength of the Perkin Elmer MPF III fluorescence spectrometer was set at 400 nm.

The principle of the recombination procedure described before[6] was further elaborated: the apoLp was unfolded in 8M urea and complexed with saturated lysolecithin, mainly 1-stearoyl-3-glycerophosphocholine. Then a suspension of sphingomyelin vesicles, which after negative staining appeared as unilammelar liposomes of 500 to 1000 Å diameter in electron microscopy, was added and the exchange of lysolecithin by sphingomyelin molecules allowed to proceed, first at room temperature and afterwards under dialysis conditions at 4 °C. The reaction mixture then consisted of the lipoprotein complex, trace amounts of non-recombined apoLp A-I and dissociated lysolecithin, and traces of excess sphingomyelin. In general this recombination procedure was carried out at 50 °C in all association experiments because *N*-stearoylsphingosylphosphocholine, with a phase transition of 41.5 °C, did not bind below this temperature.

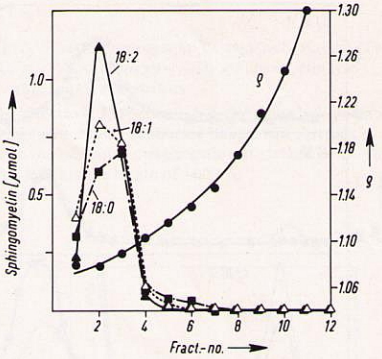

Fig. 3. CsCl density gradient centrifugation of sphingomyelin-lysolecithin-apoLp A-I complexes.
The fatty acids in the sphingomyelin component are indicated at the curves.

Purification of sphingomyelin-apoLp A-I complex

The lipoprotein complexes were purified in two ways. The dialysed preparations were concentrated by ultrafiltration to a small volume and then submitted to a continous CsCl density gradient centrifugation (11 - 20.5% CsCl). The three sphingomyelin-lysolecithin-apoLp A-I complexes (Fig. 3) concentrated at a density of 1.07 - 1.09 g/cm^3. The 18:0-sphingomyelin-apoLp A-I complex aggregates at this density but completely disaggregates during the 24 h dialysis at pH 8.0. Component analysis of the purified lipoprotein complexes resulted in the molar ratios given in Table 1.

The lipoprotein complexes can also be separated from non-recombined apoLp A-I and excess lipids by agarose chromatography (Bio-Gel A-5m 1.60 x 90 cm), Fig. 4.

The lipoprotein complex separates well from excess lipid, which elutes in the void volume, and traces of non-recombined apoLp A-I. The analyses of the lipoprotein complexes corresponded to those of the respective recombinants purified by CsCl gradient centrifugation, given in Table 1.

Agarose chromatography of the CsCl gradient preparation, or CsCl gradient centrifugation of the lipoprotein purified by agarose chromatography, did not alter the stoichiometry of the complexes. ApoLp A-I and sphingomyelin were recovered in the lipoprotein particles in high yields. They are summarized in Table 2.

Electron microscopy of lipoprotein particles

Negatively stained lipoprotein particles of the aforementioned composition have a disc shape, 160 - 170 Å in diameter and 50 - 60 Å high. The tendency to form stacked discs decreases with increasing degree of saturation of the fatty acyl residue of the sphingomyelin in the lipoprotein complexes (Fig. 5).

Table 1. Molar ratios of components in sphingomyelin-lysolecithin-apoLp A-I complexes purified by a) CsCl gradient centrifugation and b) Bio-Gel A-5m chromatography.

	a) CsCl gradient	b) Bio-Gel A-5m
N-Stearoylsphingosylphosphocholine:lysolecithin:apoLp A-I	110 : 20 : 1	103 : 21 : 1
N-Oleoylsphingosylphosphocholine:lysolecithin:apoLp A-I	80 : 17 : 1	95 : 21 : 1
N-Linoleoylsphingosylphosphocholine:lysolecithin:apoLp A-I	70 : 17 : 1	85 : 19 : 1

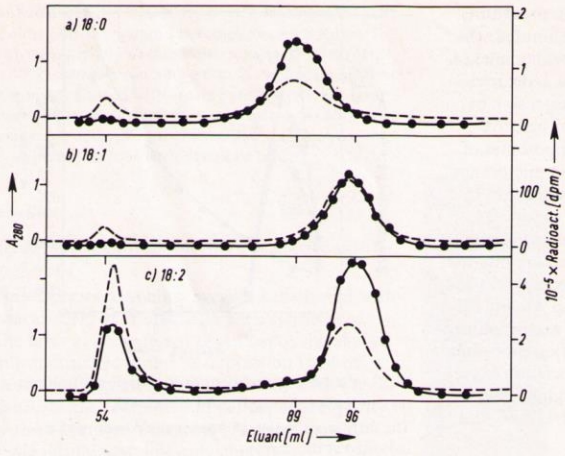

Fig. 4. Agarose chromatography (Bio-Gel A-5m, 100 - 200 mesh) of a) *N*-[^{3}H]stearoyl-, b) *N*-[^{3}H]-oleoyl- and c) *N*-[^{3}H]linoleoyl-sphingomyelin-lysolecithin-apoLp A-I complexes. – – –, A_{280}; •——•, radioactivity.

Table 2. Recovery of apoLp A-I and sphingomyelin in purified lipoproteins.

Sphingomyelin	Recovery [%]	
	ApoLp A-I	Sphingomyelin
18 : 0	77	68
18 : 1	87	69
18 : 2	75	54

Molecular weight determination of lipoprotein particles by gel filtration

Gel filtration of the three purified lipoprotein complexes on a Bio-Gel A-5m column (1.6 x 90 cm) calibrated with the test proteins glutamate dehydrogenase (M_r = 1.0 x 10^6), aldolase (M_r = 1.6 x 10^5) and trypsin (M_r = 1.8 x 10^4) revealed apparent molecular weights of 8.0 x 10^5 when *N*-18 : 0-sphingomyelin and 4.0 x 10^5 when the *N*-18 : 1 and *N*-18 : 2-sphingomyelin species were reassembled with the apoLp A-I.

Circular dichroism of reassembled sphingomyelin-apoLp A-I particles

ApoLp A-I in aqueous solution preserves part of its secondary structure, Fig. 6. Its ellipticity Θ is still 1.6 x 10^4 deg x cm^2 x dmol^{-1}, which corresponds to a degree of α-helicity of f_H = 0.46. Binding of any of the three model sphingomyelins enhances the helix content considerably, as shown in Table 3. This is independent of the structure of the sphingomyelin species (Table 3).

Table 3. Ellipticity (Θ) and α-helix content (f_H) of apoLp A-I (first line), free and recombined with the three sphingomyelin species.
The stoichiometry is that given in Table 1.

Sphingomyelin species	$10^{-4} \times \Theta$ [deg x cm^2 x dmol^{-1}]	f_H
–	– 1.6	0.46
18:0	– 3.1	0.94
18:1	– 2.9	0.89
18:2	– 3.1	0.94

The CD spectrum of the *N*-stearoylsphingomyelin containing lipoprotein complex is characterized by a pronounced negativity in the far UV region, which is caused by the light scattering of the highly aggregated lipoprotein particles.

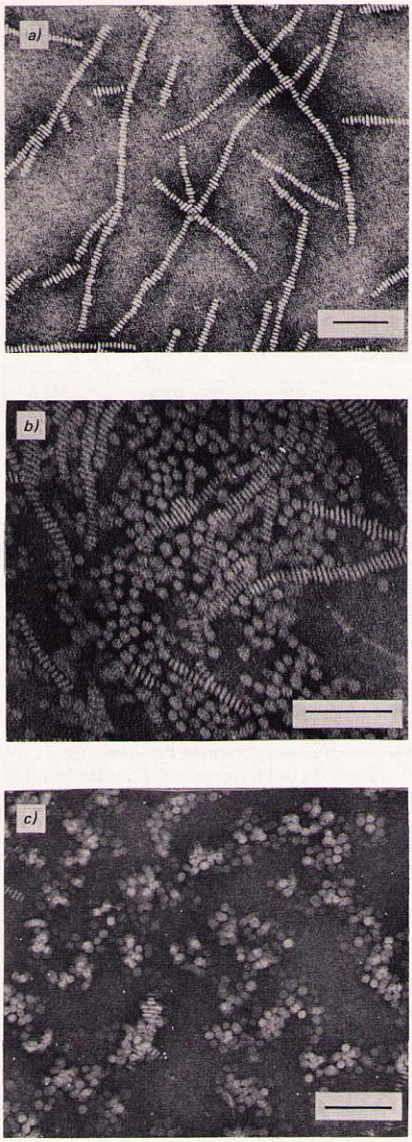

Fig. 5. Electron micrographs of negatively stained (2% buffered phosphotungstic acid) sphingomyelin-lysolecithin-apoLp A-I particles.

a) *N*-Stearoyl-, b) *N*-oleoyl- and c) *N*-linoleoyl-sphingosylphosphocholine containing lipoprotein particles. Note the increasing disaggregation of stacked discs. The bars indicate the length of 1000 Å.

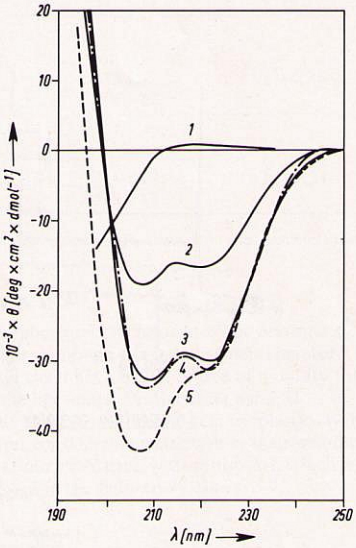

Fig. 6. Circular dichroism measurements.

1) Sphingomyelin vesicles (for conditions see Material and Methods)
2) apoLp A-I
3) *N*-linoleoylsphingomyelin-apoLp A-I complex
4) *N*-oleoylsphingomyelin-apoLp A-I complex
5) *N*-stearoylsphingomyelin-apoLp A-I complex.

Role of the double bonds of the sphingomyelin species for the affinity to apoLp A-I

Although the stoichiometry, molecular weight, particle size and CD strongly indicated a lack of preference of the apoLp A-I for any one of the

three sphingomyelin species, the crucial experiment consisted of the reassembly of sphingomyelin liposomes containing the three species in equimolar amounts with apoLp A-I. The composition of the fatty acid methyl esters isolated from the equimolar sphingomyelin mixture of the liposomes by acidic hydrolysis corresponds to that of the sphingomyelins bound in the purified lipoprotein complexes and recovered by chloroform/methanol extraction.

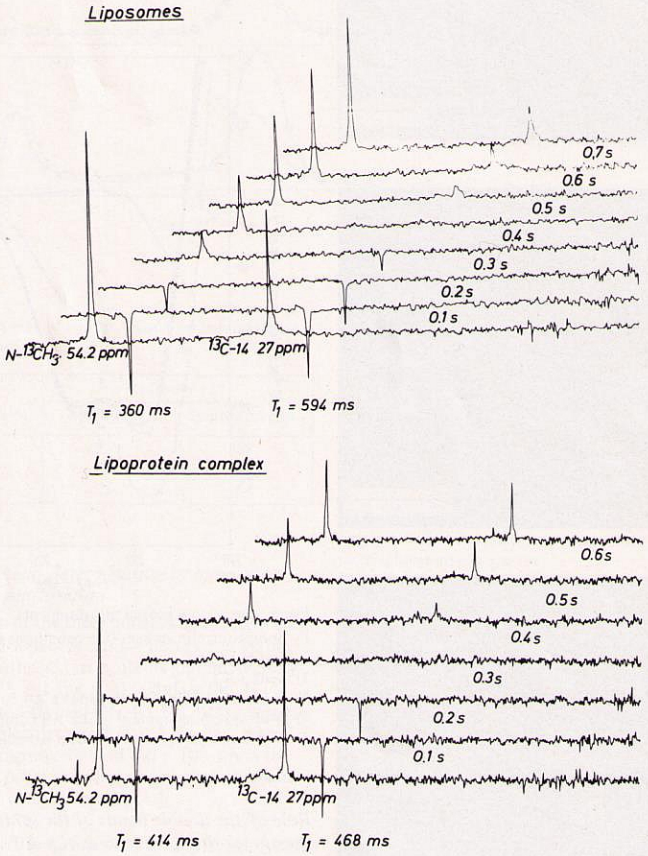

Fig. 7. Spin-lattice relaxation time determination of *N*-[14-^{13}C]linoleoylsphingosylphospho-[*N*-$^{13}CH_3$]choline in liposomes and in the sphingomyelin-lysolecithin-apoLp A-I complex.

Table 4. Spin-lattice relaxation times (T_1) of ^{13}C-labelled sphingomyelin liposomes and the respective sphingomyelin-lysolecithin-apoLp A-I complexes.

^{13}C-labelled sphingomyelin	T_1 [ms] of nuclei*		
	Liposomes	Lipoprotein	Molar ratio of apoLp A-I : sphingomyelin lysolecithin
N-[11-^{13}C]- and *N*-[14-^{13}C]stearoyl-sphingosylphospho-[*N*-$^{13}CH_3$]choline			
N-CH_3	274 ± 8*	389 ± 31	1:75:27
C-11	50	183 ± 28	
C-14	50	234 ± 11	1:121:16
N-[11-^{13}C]oleoyl-sphingosylphospho-[*N*-$^{13}CH_3$]choline			
N-CH_3	341 ± 6	435 ± 7	1:84:44
C-11	227 ± 12	206 ± 12	
N-[14-^{13}C]linoleoyl-sphingosylphospho-[*N*-$^{13}CH_3$]choline			
N-CH_3	360 ± 12	414 ± 13	1:72:24
C-14	594 ± 13	468 ± 17	

* Figures indicate the maximal error possible in the graphical evaluation of the T_1-times. The experimental error does not exceed 5%.

^{13}C NMR spectroscopic studies on the sphingomyelin-lysolecithin-apoLp A-I complexes

The ^{13}C NMR spectroscopic studies required recombinations with 5 to 10 mg apoLp A-I. They were reassembled with the three sphingomyelin species now labelled at C-14 (stearic), C-11 (oleic) and C-14 (linoleic acid) and in addition, in the *N*-CH_3 group of the choline moiety in the way outlined before. These preparations gave excellent signals of the ^{13}C enriched positions in the fatty acyl chains or the polar head group. Spin-lattice relaxation times (T_1) of these nuclei could be accurately determined in the lipid molecule associated with the apoLp A-I and in liposomes prepared from the lipids after extraction of the lipoprotein particles. An example is given in Fig. 7. T_1-times are summarized in Table 4.

The T_1-times of choline methyl groups of all sphingomyelins increase considerably after binding to the apoLp A-I. The T_1-time of the ^{13}C nuclei in positions 11 and 14 of the *N*-stearoyl group in sphingomyelin is not accurately measurable in the liposomes, because they form rigid, crystalline bilayers. However, associated with the apolipoprotein, its resonance line becomes clearly visible, although as a broad signal. Therefore, the alkyl chain has a higher degree of mobility than in the liposomes. On the other hand, C-11 of oleic and C-14 of linoleic acid in sphingomyelin suffer considerable restriction in their mobility after recombination with apoLp A-I, which is expressed in the reduced T_1-times.

Discussion

ApoLp A-I is the major apoprotein of the human high density lipoprotein particle which, together with apoLp A-II and the phospholipid classes phosphatidylcholine and sphingomyelin and cholesterol, forms the skeleton of this spherical lipoprotein, the main function of which is the transport of the cholesterol esters to the liver for further catabolism. We have proposed a model of the HDL particle[3,4], in which the polar head groups of the phospholipid molecules, together with the hydrophilic face of the amphiphilic apoproteins, are turned to the surface of the spherical particle, whereas the fatty acyl chains of lecithins and sphingomyelin interact with the

hydrophobic side of the apoproteins, which we believe to border the hydrophobic nucleus consisting mainly of cholesterol esters.

Systematic investigations of the lipid-binding properties of the individual apolipoproteins have now been initiated, which not only should facilitate our understanding of the HDL particle structure, but more generally of the principles of lipid to polypeptide binding.

The line of our approach reported in this paper is to reconstitute lipoprotein particles consisting of apoLp A-I and single sphingomyelin species. By changing the degree of unsaturation of the fatty acid substituting the sphingosylphosphocholine moiety, we should be able to elucidate the influence of double bond systems of the acyl residue in the association to the polypeptide chains. The stoichiometry of the lipoprotein particles formed, their electron microscopic shape and size, their apparent molecular weight, together with the dynamic changes in the secondary structure of the apoprotein induced by the binding of the lipids, and the mobility properties of their hydrophobic chains and their hydrophilic polar head groups should suffice to derive a proposal for the molecular arrangement of the components in these lipoproteins.

The studies described in this paper demonstrate that the recombination method developed before[6] yields lipoprotein complexes of reproducible stoichiometry not significantly dependent on the sphingomyelin species. Each apoLp A-I molecule binds 80 - 100 sphingomyelin and 20 lysolecithin molecules. An association of the sphingomyelin molecules occurs only above their phase transition temperature (t_c), which was clearly proven with *N*-stearoylsphingosylphosphocholine. This species did not form a lipoprotein complex at all below $t_c = 41.5$ °C. The molecular weight determination by agarose chromatography gave values of 800000 for the 18:0-sphingomyelin-apoLp A-I complex but 400000 for the two unsaturated species 18:1- and 18:2-sphingomyelin apoLp A-I particles.

We have no explanation for the high apparent molecular weight of the former particle, but that it elutes as a dimer. It does not differ in size and shape from the other two particle preparations in electron microscopy.

This explanation is supported by the great tendency of these disc-shape particles to aggregate to stacked discs.

The three lipoproteins have the same appearance in electron microscopy. They form discs of 160 to 170 Å diameter and 50 to 60 Å height. Higher resolution reveals a sandwich structure of these particles. α-Helicity of the apoLp A-I is increased considerably by lipid binding (80%), which indicates the high degree of order induced. It allows the helices to arrange in the two dimensions of the surface. We have calculated, according to Chou and Fasman[20], that apoLp A-I potentially can form up to 70% α-helix and 10% β-structures. These areas possess opposite, bilateral properties, amphiphilicity, with a concentration of hydrophilic residues on one side and hydrophobic on the other. This can be demonstrated clearly in Corey-Pauling-Koltun models of the polypeptide chains. Induction of α-helicity by lipid association therefore neccessarily increases this amphiphilicity.

The ^{13}C NMR spin-lattice relaxation time experiments indicate that the polar head groups in the apoLp A-I sphingomyelin-lysolecithin lipoprotein complexes gain considerably in mobility as compared to the well-ordered arrangement in the liposome, with its limited space available for the polar head group. The increase in the T_1-time of the choline group expresses the reduction of the packing, and therefore the surface density on the lipoprotein. On the other hand, the reduction of the T_1 time of C-11 and C-14 of oleic and linoleic acid in sphingomyelin after binding to the polypeptide chains of apoLp A-I refers to the restricted mobility of the hydrophobic chains due to their interactions with the hydrophobic side chains of the apoLp A-I. The reversed observation is made with *N*-stearoylsphingosylphosphocholine. Liposomes of this sphingomyelin species labelled at C-14 of the stearoyl residue exhibit only a very broad signal, even beyond the t_c. An accurate evaluation is not possible, due to the small T_1 (< 50 ms). In association with apoLp A-I however the resonance line sharpens and the T_1 experiment gave a value of 234 ms.

This increase in motional freedom can be explained either by the increased space available per molecule lipid in the lipoprotein, as compared to the quasi crystalline state in the liposome with the limited possibility of *trans-gauche* isomerizations

and kink formations, which are the main mechanisms of the spin-lattice relaxation or the interactions ("solution") of the hydrocarbon chain of the stearoyl residues in the hydrocarbon milieu of the hydrophobic side of the apoLp A-I. This would lead to the same effect on the relaxation mechanism. Taking all the stoichiometric, physical, electron microscopic and NMR data together, we suggest the molecular arrangement of these lipoprotein particles shown in Fig. 8. The hydrophobic sides of two apoLp A-I molecules on each side of the sandwich structure are opposite each other, and likewise the fatty acid tails of the sphingomyelin and lysolecithin molecules interact with their "sticky ends". This would mean that only hydrophobic forces between apoLp A-I molecules and the sphingomyelins stabilize the lipoprotein complex. The polar head groups, on the other hand, are oriented together with the hydrophilic side chains of the apoLp A-I molecules towards the outside of the disc-shaped particle.

Present studies are concerned with a chemical approach to localizing the polypeptide neighbourhood of the sphingomyelin and lysolecithin molecules in the reassembled lipoprotein complexes.

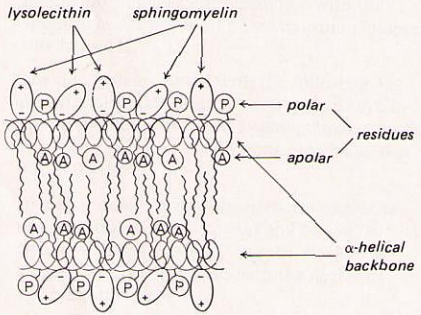

Fig. 8. Proposed model of the lipid-protein arrangement in the apoLp-A-I sphingomyelin-lysolecithin particles.

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