Sonderdruck aus
Hoppe-Seyler's Zeitschrift für Physiologische Chemie
Walter de Gruyter & Co., Berlin 30

Hoppe-Seyler's Z. Physiol. Chem.
Bd. 355, S. 1381–1390, November 1974

^{13}C Nuclear Magnetic Resonance Spectroscopic Studies on Lipid-Protein Interactions in Human High-Density Lipoprotein (HDL)

A Model of the HDL Particle

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(Received 2 July 1974)

Dedicated to Professor Dr. Hj. Staudinger on the occasion of his 60th birthday

Summary: Phosphatidylcholines and sphingomyelins enriched with ^{13}C in the choline moiety, in different carbon atoms of oleic and linoleic acid chains of lecithins, sphingomyelin and cholesterol esters, and in C-26, 27 of cholesterol were recombined with total apoproteins of human high-density lipoproteins and the separated apolipoproteins apoA-I (apoLpGln-I) and apoA-II (apoLpGln-II). The stoichiometry of the reassembled lipoprotein particles was determined by the respective radioactive lipids. ApoA-I preferentially binds phosphatidylcholine, although its lipid-binding capacity is smaller than that of apoA-II. The latter avidly reassembles with sphingomyelin by hydrophobic interactions. Due to the enrichment of ^{13}C, the spin-lattice relaxation times (T_1) of particular segments of the different lipid molecules could be accurately measured. The ^{13}C NMR data established that in reassembled HDL, the phospholipid molecules bind to the apoprotein moieties with their hydrophobic fatty acid chains and not with their hydrophilic zwitterionic groups. The integration of cholesterol esters into the particle also is discussed on the basis of ^{13}C NMR data.

A model of the molecular organization of the HDL particle has been derived on the basis of the chemical analysis, the molecular dimensions of its lipids, other well known properties of the lipoprotein, and our ^{13}C NMR data.

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^{13}C-Kernmagnetische Resonanz-Untersuchungen über Lipid-Protein-Wechselwirkungen im menschlichen High-Density-Lipoprotein (HDL). Ein Modell der HDL-Partikel

Zusammenfassung: Phosphatidylcholine und Sphingomyeline, die mit Kohlenstoff 13 als innere Sonde in der Cholingruppe des hydrophilen Teils und in verschiedenen Kohlenstoffatomen der Öl- und Linolsäure als Acylgruppen der Lecithine, Sphingomyeline und Cholesterinester und im Kohlenstoffatom 26 bzw. 27 des Cholesterins markiert wurden, wurden mit den Gesamtproteinen und den getrennten Apolipoproteinen A-I und A-II des menschlichen „high-density" Lipoproteins, d = 1.06–1.21, rekombiniert. Die Stöchiometrie der Lipid-Protein-Bindung der rekonstituierten Lipoproteinpartikeln wurde mit den entsprechenden radioaktiven Lipidmolekülen bestimmt. Auf Grund der ^{13}C-Anreicherung konnten die Spin-Gitter-Relaxationszeiten (T_1) von bestimmten Segmenten der verschiedenen Lipidmoleküle exakt gemessen werden. Die ^{13}C-NMR-Daten beweisen, daß in den

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Abbreviations: HDL = high density serum lipoprotein; apoHDL = delipidated proteins of HDL; apoA-I (apoLp Gln-I) and apoA-II (apoLp Gln-II) = main two protein components of HDL; PC = phosphatidylcholine; SPM = sphingomyelin; C = cholesterol; CE = cholesterol ester.

rekombinierten HDL-Partikeln die Phospholipidmoleküle mit ihren hydrophoben Fettsäureketten und nicht mit ihren hydrophilen zwitterionischen Gruppen Bindungen mit den Apoproteinen eingehen. ApoA-I bindet bevorzugt Phosphatidylcholin, obwohl seine Lipidbindungskapazität kleiner ist als die des ApoA-II. ApoA-II assoziiert begierig mit Sphingomyelin durch hydrophobe Wechselwirkungen. Auch die Integration der Cholesterinester in die HDL-Partikel wird aufgrund der ^{13}C-NMR-Daten diskutiert.

Ein Modell der molekularen Organisation der HDL-Partikel wird auf der Basis der chemischen Komponentenanalyse, der molekularen Dimensionen seiner Lipide, anderer schon bekannter Eigenschaften des Lipoproteins und unserer ^{13}C-NMR-Daten entwickelt.

A number of physical methods has been utilized for the study of lipid-lipid and lipid-protein interactions in lipoproteins and membranes, e. g. electron spin resonance (ESR), fluorescence, proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectroscopy and mono- and bimolecular films. ^{13}C NMR has the properties of a good probe: it is an internal probe, causes no perturbation of the structure to be analyzed, does not interfere with the function, can be exactly localized by its sharp resonance lines with well separated chemical shifts and reliable assignment.

Spin-lattice relaxation rates T_1 of the individual C atoms give insight into the mobility of the C atoms as a function of the molecular geometry, segmental motion, viscosity of the surrounding medium, and noncovalent interactions.

The enrichment of ^{13}C in the hydrophilic and hydrophobic moiety of the predominant lipid components of lipoproteins and membranes, namely phosphatidylcholines, sphingomyelins, cholesterol and cholesterol esters, may substantially overcome the drawback of CMR spectroscopy due to the low natural abundance of ^{13}C. We have described the chemical synthesis of these lipids enriched in C atoms[1,2] which were required in order to determine T_1 times of special regions of the lipid molecules in reasonable periods of time, and which served for the study of hydrophilic and hydrophobic interactions of lipid-protein complexes.

A readily accessible system for the study of lipid-lipid and lipid-protein interactions is the high-density lipoprotein (HDL) fraction of human serum. Detailed information is available on the lipid and protein composition of these particles[3–16]. The HDL particle consists of approximately equal amounts of lipids and proteins. The lipid components are mainly phosphatidylcholine, sphingomyelin, cholesterol esters and cholesterol. The acyl residues of the ester lipids are predominantly unsaturated fatty acids (oleic 18:1[9] and linoleic 18:2[9,12] acids). Two apoproteins (apoA-I [apoLpGln-I] and apoA-II [apoLpGln-II]) comprise almost 90% of the total and are present in a ratio of 3:1. Circular dichroism measurements indicate that the two proteins possess a high degree (60–70%) of order, either in α-helical or pleated sheet structures[5,6]. The amino acid sequence of apoA-II has been determined[10].

The molecular organization of the molecules in the HDL particle relates closely to other important questions concerning the structure and interactions of lipids and proteins in more complex lipoprotein aggregates such as biological membranes. The objective of our ^{13}C NMR studies was to provide experimental evidence that would help us to understand the interactions of lipids and proteins in general, and of the molecular organization of a complex structure like HDL in particular. Furthermore, we would like to demonstrate by these studies that ^{13}C NMR spectroscopy is a powerful tool for the study of lipid-protein interactions, provided the ^{13}C probe is enriched at sites of the molecules involved in the assembling process of lipids and proteins, which then yield ^{13}C NMR spectra of good quality in a reasonable period of time.

Experimental

The chemical synthesis of [*N-methyl*-^{13}C]choline-labelled phosphatidylcholines and sphingomyelins has been described previously[1, 2]. [26,27-^{13}C]cholesterol has been synthesized according to established procedures[17] starting from 3β-hydroxy-nor-5-cholestene-25-one. Cholesterol esters (cholesteryl[^{13}C]oleate and [^{13}C]linoleate) were synthesized by acylation using the respective acyl chlorides and *N*-dimethylaminopyridine as catalyst. They were purified by silicic acid chromatography. [*N-methyl*-^{13}C]Choline-labelled phosphatidylcholine and sphingomyelin were treated with phospholipase A_2 (*Crotalus adamanteus*), and the resulting lyso derivatives were catalytically reduced and then acylated with the acyl chlorides of the following fatty acids:

[1-^{13}C], [3-^{13}C], [8-^{13}C] and [11-^{13}C]oleic acid, and [1-^{13}C], [3-^{13}C] and [14-^{13}C]linoleic acid as described before[2]. Sphingomyelin was deacylated by treatment with aqueous methanolic HCl to sphingosylphosphorylcholine[18], which was purified by silicic acid chromatography and then reacylated with the following fatty acids: [1-^{13}C]palmitic acid, [3-^{13}C] and [11-^{13}C]oleic acid. Phospholipase A_2 hydrolysis of portions of phosphatidylcholines with subsequent gas chromatographic analysis of the methyl esters of the released fatty acid proved that less than 5% of positional isomerization of fatty acids had occurred. All lipid samples were carefully stored in the dark under purified argon at −24°C in benzene solution.

Apolipoproteins: Human high-density lipoprotein was obtained by ultracentrifugation sedimentation flotation cycles in potassium bromide between densities 1.063 and 1.210[19]. The lipoprotein fraction was delipidated completely with chloroform/methanol 2:1 (v/v), followed by ether extraction. Portions of 300 mg apoHDL were separated by chromatography on DEAE-cellulose columns (2.5 × 45 cm) in 8M urea[4] and a Tris buffer gradient between 0.04 and 0.10M. The two main fractions apoA-I and apoA-II were isolated in a homogeneous form as shown by polyacrylamide gel electrophoresis[20] and by immunoprecipitation (antisera were kindly provided by *Dr. H. Greten*, Medizinische Klinik, Heidelberg).

The amino acid composition of the two proteins compared well with those reported in the literature[19, 21].

Recombination of lipoprotein complexes: apoHDL, apoA-I and apoA-II were dissolved in 0.05M NH_4HCO_3, pH 7.4, 0.1% NaN_3. Phosphatidylcholine and sphingomyelin were sonicated with or without cholesterol and cholesterol esters in proportions resembling the composition of the native HDL particles (PC:SPM:C:CE 5:1:2:5) in 0.05M NH_4HCO_3, pH 7.4 in the presence of 0.1% NaN_3 for 60 min under a stream of purified nitrogen at 75 W with a Branson sonifier in a temperature-controlled vessel. Temperatures were chosen above the highest transition temperature of the components, which had been determined by isotherms of monomolecular films at the air-water interphase by the monolayer technique[22].

Phosphatidylcholine and sphingomyelin liposomes were prepared as described in the preceding paper[23]. They were homogeneous in size as shown by Sepharose 4B chromatography[24] and by electron microscopy after negative staining. Phospholipid dispersions containing cholesterol and cholesterol esters were opalescent. These lipid dispersions were combined with the apoprotein solutions with magnetic stirring at 37°C in an argon atmosphere over 12 h. The density of the reassembled mixture was adjusted to 1.063 g/ml with solid KBr, and the recombined lipoproteins were freed from unbound lipid by sedimentation at 60000 rpm for 24 h. The slightly yellow infranatant was obtained by slicing the tube above this layer and adjusted to a density of 1.210 with solid KBr and spun for 48 h at 60000 rpm. The lipid apoprotein complex was freed from any excess apoprotein by this flotation process. The lipid composition was analyzed by quantitative radio thin-layer chromatography of portions in the following solvent systems: chloroform/methanol/acetic acid/water 25:15:4:2[25] for phospholipids and sphingomyelin; diisopropylether/acetic acid 96:4 and hexane/ether/acetic acid 70:30:1 for cholesterol and cholesterol esters[26]. The radioactive bands were recovered from the plates and their radioactivity determined after elution from silica gel. No decomposition of any of the lipids had occurred during the sonication and reassembling procedures.

^{13}C NMR spectroscopy: The conditions of ^{13}C NMR spectroscopy under proton-noise decoupling were the same as described in the preceding paper. Portions of the lipids and apoproteins were analyzed in order to exclude any alterations which might occur during the spectroscopy, lipids by thin-layer chromatography and apoproteins by disc-electrophoresis in 6M urea[20]. No structural changes could be observed in these analyses. Force area isotherms of purified individual lipid classes (phosphatidylcholine, sphingomyelin and cholesterol esters) of HDL were recorded by an automatical Langmuir balance as described before[22].

Protein was determined according to Lowry et al.[27].

Results

In order to get insight into the lipid-lipid and lipid-protein interactions in human high-density lipoproteins, the spin-lattice relaxation times (T_1) of the main lipids participating in their molecular organization of this lipid transport particle, namely phosphatidylcholine, sphingomyelin, cholesterol and cholesterol esters, were determined after reassembling with a) total apoHDL and b) the separated main components apoA-I and apoA-II (Fig. 1). Sonicated lipid dispersions were used for the recombination process and for comparison, which is described in detail under Experimental. In order to determine the stoichiometry of the lipid composition integrated into the HDL particle, radioactive phosphatidylcholine, sphingomyelin, cholesterol and cholesteryl linoleate identical in structure with the ^{13}C-labelled lipid were used in the reassembling procedures. Table 1 summarizes the stoichiometry of the lipid molecules reassembled with apoHDL, apoA-I and apoA-II.

ApoHDL binds phosphatidylcholine and sphingomyelin, very similarly to the native HDL, in a ratio of approximately 5:1, but only one half of the

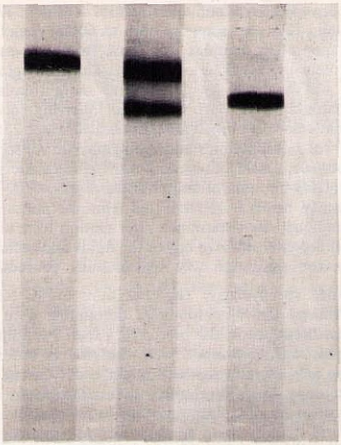

Fig. 1. Disc-electrophoresis of delipidated a) apoA-I, b) total apoHDL and c) apoA-II.

cholesterol and one third of the cholesterol esters are incorporated into the particles. ApoA-I reassembles preferentially with phosphatidylcholine (phosphatidylcholine:sphingomyelin 12:1), whereas apoA-II avidly associates with sphingomyelin (phosphatidylcholine:sphingomyelin 1:4). Phosphatidylcholines and sphingomyelins were used then, which were labelled a) in the *N*-methyl group of choline, representing the probe for the hydrophilic group, and b) in carbon atoms 1, 3, 8, 11 and 14 of oleic and linoleic acid respectively, which allow the observation of the mobility of the alkyl chain of the fatty acid in the 2-position of the lecithins or the *N*-acyl group of sphingomyelin, thus providing a probe of different segments of the hydrophobic moiety of the lipids. In Fig. 2 1-stearoyl-2-[14-^{13}C]linoleoyl-3-glycerophosphorylcholine, in Fig. 3, *N*-[11-^{13}C]oleoylsphingosyl-phos-

Table 1. Number of individual lipid molecules bound in reassembled lipoproteins.

Lipids	Native HDL[a]	Protein component apoA-I[b]	apoA-II[c]	apoHDL[d]
PC	46	12	6	58 (42)
SPM	8	1	24	8 (10)
C	21	–	1	9 (17)
CE	46	1	3	15 (17)

[a] Contains 3 molecules of apoA-I and 1 molecule apoA-II.

[b] 5.2 mg apoA-I bound 2.7 mg of lipids (2.4 mg PC, 0.2 mg SPM, 0.1 mg CE).

[c] 11 mg apoA-II bound 16.3 mg of lipids (3.4 mg PC, 11.5 mg SPM, 1.3 mg CE and 0.1 mg cholesterol).

[d] 15.2 (51.0) mg apoHDL were associated with 9.5 (28.0) mg of lipid, 6.8 (16) mg PC, 0.8 (3.4) mg SPM, 1.4 (5.5) mg CE and 0.5 (3.1) mg cholesterol.

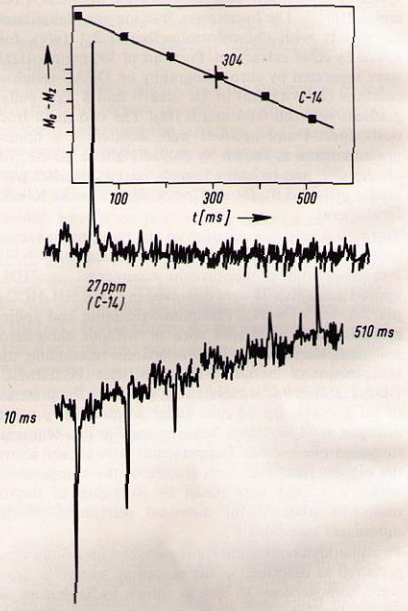

Fig. 2. Spin-lattice relaxation (T_1) measurements of human high-density lipoprotein (HDL) after reassembling total apoHDL with a lipid mixture resembling the native composition (see Experimental).

Phosphatidylcholine was substituted with 1-stearoyl-2-[14-^{13}C]linoleoyl-3-glycerophosphorylcholine.

Chemical shifts [ppm] are related to $Si(CH_3)_4$. $M_0 - M_z$ is expressed in arbitrary units; t = delay time in (180^0-t-90^0) pulse sequence.

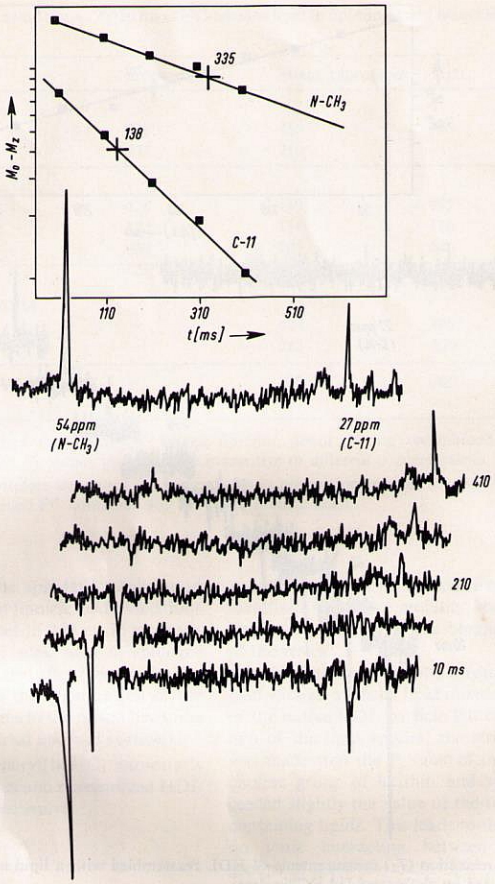

Fig. 3. Spin-lattice relaxation (T_1) measurements of HDL after reassembling total apoHDL with a lipid mixture of native stoichiometry, in which sphingomyelin was substituted by *N*-[11-^{13}C]oleoylspingosyl-phosphoryl-[*N-methyl*-^{13}C]choline.
The signal of 54 ppm corresponds to the *N*-methyl group of choline and the one at 27 ppm to C-11 of oleic acid. Chemical shifts (ppm) are related to $Si(CH_3)_4$. $M_0 - M_z$ is expressed in arbitrary units; t = delay time in (180^0-t-90^0) pulse sequence.

phoryl-[*N-methyl*-^{13}C]choline and in Fig. 4, cholesteryl [14-^{13}C]linoleate were the labelled lipid components in the artificial lipid mixture, which resembled that of the native HDL.

The results of the spin-lattice relaxation (T_1) studies of all other recombined lipoproteins in comparison with those of the same lipids in aqueous dispersion are summarized in Table 2. The reconsti-

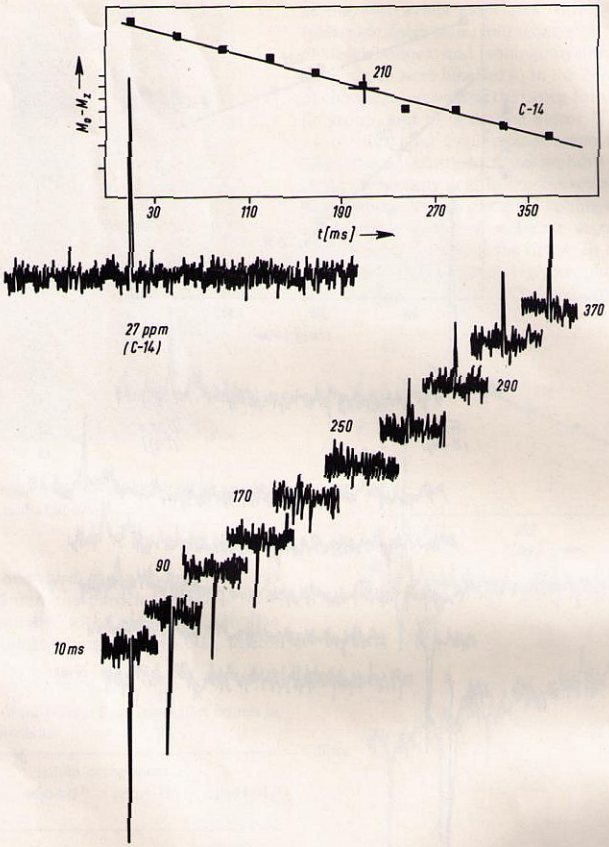

Fig. 4. Spin-lattice relaxation (T_1) measurements of HDL reassembled with a lipid mixture in which cholesterol esters were substituted by cholesteryl [14-^{13}C]linoleate.
Chemical shifts (ppm) are related to $Si(CH_3)_4$. $M_0 - M_z$ is expressed in arbitrary units; t = delay time in (180^0-t-90^0) pulse sequence.

tuted and ^{13}C-labelled HDL and lipoprotein A-I and A-II complexes showed the following striking changes:

a) The spin-relaxation times of the polar head groups [*N-methyl*-^{13}C]choline of phosphatidylcholine and sphingomyelin increase compared to those of the liposomal bilayer arrangement (430 ms in liposomes to 480 ms and 560 ms respectively in reconstituted lipoproteins).

b) The T_1 values for C-3 of linoleic acid present in the 2-position of phosphatidylcholine are very short but not altered (120 ms).

Table 2. ^{13}C spin-lattice relaxation times (T_1) in ms of ^{13}C-labelled lipid in liposomes and reassembled with apoHDL and apoA-II [a].

Lipid	Liposomes	Mixed Liposomes [b]	HDL	apoA-II
[*N*-^{13}C]18:0; [^{13}C]18:1 PC				
N-CH_3	428	430	485	–
C-11	278	210	–	–
[*N*-^{13}C]18:0; [^{13}C]18:2 PC				
N-CH_3	428	430	485	586
C-3	122	134	116	120
C-14	638	705	440	–
[1-^{13}C]16:0 SPM		660	675	
[11-^{13}C]18:1 [*N-methyl*-^{13}C]SPM				
N-CH_3	–	424	485	560
C-11	–	242	200	144
Cholesteryl[14-^{13}C]18:2		250	305	–
Cholesteryl[14-^{13}C]18:1		177	–	–

[a] Concentration of liposomes was about 30–40 mg lipid/ml, about 30 mg recombined HDL and 7 mg recombined apoA-II/ml. The T_1 values proved to be insensitive to different concentrations.

[b] PC, SPM, C and CE dispersions contained the components in proportions present in native HDL. Liposomes with no ^{13}C-labelled PC contained soy PC (70% linoleic acid).

c) On the other hand, the spin-lattice relaxation times of carbon atom 14 of linoleic acid of lecithins (705 ms in mixed liposomes to 440 ms in reconstituted HDL) and C-11 of oleic acid in sphingomyelin are drastically reduced after these molecules have been integrated into the organization of the lipoprotein complex (242 ms in the mixed liposome to 144 ms in the reconstituted apoA-II particle).

d) The T_1 value for cholesteryl[14-^{13}C] linoleate is also very short in liposomes and reassembled HDL (250 ms and 305 ms, respectively).

Discussion

As shown in the preceding paper, the T_1 values of the methyl groups of the choline residue as it stands for the zwitterionic group in lecithins and sphingomyelins are quite dependent on the interactions with their surroundings. The choline T_1 values observed for the micellar forms in chloroform solutions are very short, which indicates that the ionic interaction of the zwitterions immobilizes them, and this supports the idea that inversed micelles with the hydrophilic group in the interior are formed in chloroform, whereas in the liposomes in an aqueous medium, the choline groups are characterized by a large mobility, since they are pointing to the exterior of the bimolecular lipid layer of the vesicle.

When high-density apolipoproteins are reassembled with an artificial lipid mixture resembling that of the native HDL particle but enriched in one or two of the lipid species, the striking observation was made that the T_1 value of the [*N-methyl*-^{13}C]-choline group of lecithin and sphingomyelin exceeded slightly the value of the liposomal choline-containing lipids. This leads to the conclusion that no ionic interaction between the hydrophilic groups of these two main lipid components of the HDL particle with the apolipoproteins A-I and A-II occurs. The diameter of the reassembled HDL particle approximates closely that of the native particle (80 to 90 Å), whereas the diameter of the vesicles is about 250 Å as demonstrated by negative staining in electronmicroscopy, a value which is identical with that given by Huang[24]. Due to the curvature effect, the space available for the choline head group should be higher in the smaller particle, which allows a greater mobility and therefore an increased T_1 value[28]. Following the carbon chain of the acyl residue from the carboxy to the

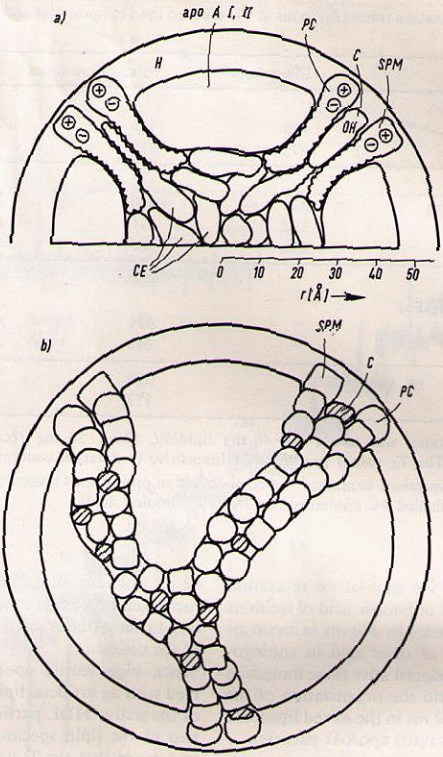

Fig. 5. Model proposed for the molecular arrangement of lipids and apolipoproteins in human high-density lipoprotein (HDL).

a) Cross-section; b) top view.

Hatched circles, cholesterol; □, sphingomyelin; ○, phosphatidylcholine; H, hydration shell.

terminal methyl group, we observed no changes of the spin-lattice relaxation times of C-3, which is close to the ester linkage and in a region of tight packing. On the other hand, the significant decrease of T_1 of C-14 of linoleic acid in phosphatidylcholine and C-11 of oleic acid in sphingomyelin when reassembled with apoHDL and apoA-II, respectively, give direct evidence for the hydrophobic interaction between the fatty acid chains of these lipids and the apoproteins. The terminal segments of the two fatty acids used here are involved in the hydrophobic lipid-protein interaction.

Our reassembling studies also give strong evidence for the nonrandom distribution of phosphatidylcholine and sphingomeylin in HDL. The rather selective interaction of sphingomyelin molecules with apoA-II and of phosphatidylcholine with apoA-I and the lower lipid binding capacity of apoA-I compared with apoA-II require further studies.

Although the tertiary structure of apoA-II is still unknown, clusters of aliphatic amino acid residues might be organized into hydrophobic sites in these highly ordered apoproteins (more than 70% α-helical form) by the sidedness of pitches with aliphatic and aromatic amino acid side chains. These areas have yet to be defined.

Component analyses[8], succinylation[29], fluorescence studies[30], small-angle X-ray diffraction[12,13], electron microscopy[11] and the results of these studies which prove the hydrophobic binding of the fatty acyl residues of lecithin and sphingomyelin to apoproteins of HDL, but exclude ionic interactions between these components, favor a model of the HDL particle which is proposed in Fig. 5.

The hydrated head groups of the phospholipids and cholesterol and the hydrophilic areas of the apolipoproteins form the surface of the spherical HDL particle, the latter covering about half of the surface. The polar group of dipalmitoyl-lecithin has been determined by X-ray[31] analysis to be 11 Å, which corresponds to the dimension of the electron-dense shell in small-angle X-ray studies[12,13]. The phospholipids must be organized as a monolayer in which the polar head groups of approximately 100–120 phospholipid molecules and cholesterol are freely mobile in the hydration shell of the HDL particle. This has been proven also by complex formation with the shift reagent praseodymium nitrate. There was only one visible signal for the choline *N*-methyl carbon[13] atom. We suggest further, from our ^{13}C NMR studies with [26, 27-^{13}C] cholesterol and cholesteryl [14-^{13}C]linoleate, that free cholesterol intercalates with the phospholipid monolayer because its influence on the carboxy proximal part of the acyl chain and its mobility in the aliphatic side chain is barely altered. The terminal segment of [14-^{13}C] linoleate residue of the cholesterol ester exhibits a rather low mobility (T_1 = 350 ms, τ_c = 0.45×10^{-9} s). We are inclined to interpret this as indicated in the model given in Fig. 5. The terminal acyl chain either intercalates with the acyl residues of the phospholipids or interacts with the hydrophobic region of the apoproteins, and the cholesterol nucleus of the esters resides in the interior of the HDL sphere.

The model proposed here also reflects the observation that the lysolecithin-cholesterol acyl transferase which transfers the fatty acid from position 2 of lecithin to cholesterol is stimulated by apoA-I[32]. This reaction could easily occur at the surface of the particle. The cholesterol ester which is formed, and which is much more hydrophobic, could then move into the interior of the HDL particle and stored there until being metabolized in the liver.

Furthermore, our results emphasize that ^{13}C NMR spectroscopy can serve as powerful tool for the study of lipid-lipid and lipid-protein interactions.

We gratefully acknowledge the support of the *Deutsche Forschungsgemeinschaft* (SFB 74).

Literature

[1] Stoffel, W., LeKim, D. & Tschung, T. S. (1971) *this J.* **352**, 1058–1064.

[2] Stoffel, W., Zierenberg, O. & Tunggal, B. D. (1973) *this J.* **354**, 1962–1969.

[3] Shore, B. & Shore, V. (1968) *Biochemistry* **7**, 2773–2777.

[4] Shore, B. & Shore, V. (1969) *Biochemistry* **8**, 4510–4516.

[5] Scanu, A. M., Toth, J., Edelstein, C., Koga, S. & Stiller, E. (1969) *Biochemistry* **8**, 3309–3316.

[6] Jackson, R. L. & Gotto, A. M. (1972) *Biochim. Biophys. Acta* **285**, 36–47.

[7] Camejo, G. (1969) *Biochim.Biophys.Acta* **175**, 290–300.

[8] Scanu, A. M. (1972) *Biochim. Biophys. Acta* **265**, 471–508.

[9] Lux, S. E., Hirz, R., Shrager, R. I. & Gotto, A. M. (1972) *J. Biol. Chem.* **247**, 2598–2606.

[10] Brewer, H. B., Jr., Lux, S. E., Rowan, R. & John, K. M. (1972) *Proc. Nat. Acad. Sci. U.S.A.* **69**, 1304–1308.

[11] Forte, T. & Nichols, A. (1972) *Advan. Lipid Res.* **10**, 1–41.

[12] Shipley, G. C., Atkinson, D. & Scanu, A. M. (1972) *J. Supramol. Structure* **1**, 98–103.

[13] Laggnar, P., Müller, K., Kratky, O., Kostner, G. & Holasek, A. (1973) *FEBS Lett.* **33**, 77–80.

[14] Nichols, A. (1969) *Proc. Nat. Acad. Sci. U.S.A.* **64**, 1128–1137.

[15] Gotto, A. M. Jr. (1969) *Proc. Nat. Acad. Sci. U.S.A.* **64**, 1119–1127.

[16] Scanu, A. M. (1967) *J. Biol. Chem.* **242**, 711–719.

[17] Murray, A. III. & Williams, D. L. (1958) Organic Syntheses with Isotopes, part I, pp. 1056–1059, Interscience Publ., New York.

[18] Stoffel, W. & Assmann, G. (1972) *this J.* **353**, 65–74.

[19] Scanu, A. M. (1966) *J. Lipid Res.* **7**, 295–306.

[20] Reisfeld, R. A. & Small, P. A. (1966) *Science* **152**, 1253–1255.

[21] Edelstein, C., Lim, C. T. & Scanu, A. M. (1972) *J. Biol. Chem.* **247**, 5842–5849.

[22] Stoffel, W. & Pruss, H. D. (1969) *this J.* **350**, 1385–1393.

[23] Stoffel, W., Tunggal, B. D., Zierenberg, O., Schreiber, E. & Binczek, E. (1974) *this J.* **355**, 1367–1380, preceding paper.

[24] Huang, C. (1969) *Biochemistry* **8**, 344–352.

[25] Skipsky, V. P., Peterson, R. F. & Barclay, M. (1964) *Biochem. J.* **90**, 374–378.

[26] Skipsky, V. P., Smolowe, A. F., Sullivan, R. C. & Barclay, M. (1965) *Biochim. Biophys. Acta* **106**, 386–396.

[27] Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.

[28] Sheetz, M. P. & Chan, S. I. (1972) *Biochemistry* **11**, 4573–4581.

[29] Scanu, A. M. (1967) *J. Biol. Chem.* **242**, 711–719.

[30] Jonas, A. (1973) *Biochemistry* **12**, 4503–4507.

[31] Phillips, M. C., Finer, E. G. & Hauser, H. (1972) *Biochim. Biophys. Acta* **290**, 397–402.

[32] Fielding, C. J., Shore, V. G. & Fielding, P. E. (1972) *Biochem. Biophys. Res. Commun.* **46**, 1493–1498.