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Human High Density Apolipoprotein A-I-Lysolecithin-Lecithin and Sphingomyelin Complexes

A Method for High Yield Recombinations to Lipoprotein Complexes of Reproducible Stoichiometry

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Summary: High density apolipoprotein A-I (apoLp A-I) has been prepared in a chromatographically and immunochemically homogeneous form. This apoprotein forms trimeric and tetrameric aggregates in aqueous solution at higher concentrations. ApoLp A-I has been recombined in almost quantitative yield in the presence of lysolecithin with phosphatidylcholine and sphingomyelin to particles of reproducible stoichiometry. *Lysolecithin is not required for* the interactions of lecithin and sphingomyelin with the apoprotein A-I or for the stability of these complexes.

Dialysis removes most of the lysolecithin without the loss of lecithin and sphingomyelin.

ApoLp A-I-lecithin particles have a molecular weight of 200000 and contain 50 molecules lecithin and 25 of lysolecithin. ApoLp A-I-sphingomyelin complexes contain 50 sphingomyelin and 13 lysolecithin molecules. The former particles show up as discs of 100 Å diameter, and the latter particles are 250 Å in diameter. Their thickness was estimated as 25 Å in the apoLp A-I lecithin and 60 Å in the apoLp A-I-sphingomyelin particles.

ApoLp A-I and lysolecithin form complexes whose densities depend on the lysolecithin concentration. Lysolecithin enhances the binding of phosphatidylcholine to apoLP A-I, yielding lipoprotein complexes with decreasing density.

The yield of apoLp A-I-sphingomyelin-lysolecithin complexes is proportional to the lysolecithin concentration. The ratio of apoLp A-I to sphingomyelin in all these complexes remains constant.

Komplexe von menschlichem High-Density-Apolipoprotein A-I mit Lysolecithin, Lecithin und Sphingomyelin. Ein Verfahren zur Rekombinierung mit hoher Ausbeute und in reproduzierbaren stöchiometrischen Verhältnissen.

Zusammenfassung: Das High-Density-Apolipoprotein A-I (apoLp A-I) wurde in einheitlicher und immunchemisch reiner Form dargestellt. Dieses „hydrophobe" Protein neigt in wäßriger Lösung dazu, *in höherer Konzentration trimere* oder tetramere Aggregate zu bilden.

Es wurde Apolipoprotein A-I in fast quantitativer Ausbeute in Gegenwart von Lysolecithin alleine und von Lysolecithin mit Phosphatidylcholin und Sphingomyelin in reproduzierbaren stöchiometrischen Verhältnissen rekombiniert.

Abbreviations: PC = phosphatidylcholine = lecithin; SPM = sphingomyelin; lyso-PC = 1-acyl-3-*sn*-glycerophosphocholine; apoLp A-I = high density apolipoprotein A-I; HDL = high density lipoproteins.

Lysolecithin ist weder für die Bindung des Phosphatidylcholins und Sphingomyelins im Komplex, noch für seine Stabilität erforderlich. Es kann durch Dialyse weitgehend aus dem Komplex ohne Verlust des gebundenen Phosphatidylcholins oder Sphingomyelins entfernt werden.

Der Lecithin-apoLp A-I-Komplex enthält 50 Moleküle Phospholipid pro Molekül apoLp A-I, hat ein Molekulargewicht von 200000 und zeigt eine einheitliche, scheibenförmige Struktur in der Elektronenmikroskopie (100 Å Durchmesser, 25 Å Dicke).

Das Molekulargewicht des Sphingomyelin-apoLp A-II Komplexes wurde mit 400000 bestimmt. Der Komplex enthält rund 50 Moleküle Sphingomyelin und weist gleichfalls Scheibenform auf (250 Å Durchmesser und 50 Å Dicke).

ApoLp A-I und Lysolecithin bilden Komplexe, deren Dichte von der Lysolecithin-Konzentration abhängt.

Die Bindung von Phosphatidylcholin an das apoLp A-I wird durch steigende Lysolecithin-Konzentrationen erhöht, wodurch Lipoproteinkomplexe abnehmender Dichte entstehen.

Proportional der Lysolecithin-Konzentration steigt die Ausbeute von apoLp A-I-Sphingomyelin-Lysolecithin-Komplexen, die jedoch alle durch das gleiche apoLp A-I/Sphingomyelin-Verhältnis charakterisiert sind.

Key words: Apolipoprotein A-I-lecithin-lysolecithin complex; apolipoprotein A-I sphingomyelin-lysolecithin complex; stoichiometry; electron microscopy.

Serum high density lipoproteins (HDL) form a group of lipoproteins, which are not only of biological importance, but also suitable models for studies on the interaction of complex lipids and apoproteins. HDL is composed mainly of two proteins, apolipoprotein A-I and A-II (apoLp A-I and apoLp A-II) with traces of C-apoproteins and the lipid classes phosphatidylcholine, sphingomyelin, cholesterol and cholesterol esters.

The amino acid sequences of apoLp A-I and apoLp A-II have been elucidated[1,2]. Studies in our laboratory are concerned with the mode of interaction between these main apolipoproteins and the main lipids of the native HDL particles, namely phosphatidylcholines and sphingomyelin, cholesterol and unsaturated cholesterol esters. The ultimate aim is the elucidation of the structure of the HDL particle. ^{13}C-NMR spectroscopic relaxation studies of phosphatidylcholines and sphingomyelins enriched with C-13 in the polar head groups or in different carbon atoms of the acyl chains [3- or 14-^{13}C]linoleic and [3- or 11-^{13}C]-oleic acid indicated that the binding of the lipids to the apoLp A-I and A-II is due to the interaction of the aliphatic chains and not the zwitterionic polar head groups[3,4]. We have developed computer calculated models of the secondary structures of the apoLp A-I and A-II with the empirical rules elaborated by Chou and Fasman[5,6]. Corey-Pauling-Koltun (CPK) models indicate that not only α-helices, but also extensive sequences in β-sheet configuration of both apolipoproteins possess a polar and an apolar side*. Amphipathic helices have been suggested for apoLp A-II[7,8].

All the evidence available suggests that the two major apolipoproteins are arranged at the surface of the HDL particle with their hydrophilic side directed towards the aqueous medium and their hydrophobic side towards the interior of the spherical particle. In the model of the HDL particle proposed by us previously[3], the lipids are oriented perpendicular to the surface, their polar head groups in the plane of the hydrophilic side of the apoproteins and their fatty acids interacting with the hydrophobic side chains of the amino acid residues. In order to further study the validity and details of this model, more information about these interactions and the topography are required. A prerequisite is reproducible methods and conditions for the recombination of apoHDL or apoLp A-I and A-II with given phospholipids to yield lipoprotein complexes of constant stoichiometry.

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In this study we describe lysolecithin as a suitable natural mediator for the reconstitution of lipoprotein complexes with apoLp A-I and the two main phospholipids of native HDL, phosphatidylcholine and sphingomyelin. We report on the analysis of the lipoprotein complexes formed between apoLp A-I and lysolecithin alone and in association with lecithin and sphingomyelin.

Materials and Methods

[*Me*-^{14}C]choline-labelled phosphatidylcholine and sphingomyelin were prepared according to the procedure described before[9]. Lysolecithin was obtained by phospholipase A_2 hydrolysis *(Crotalus adamanteus)* of dilinoloyllecithin in borate buffer (0.1M, pH 7.4) containing 0.04M calcium acetate. It was purified by column chromatography.

Human high density lipoprotein (HDL) was prepared by the sedimentation-flotation technique, following established procedures[10] and delipidated with chloroform/methanol 2:1 and ether. 120 mg apoHDL was separated into the apoprotein A-I and apoprotein A-II fractions by DEAE-cellulose chromatography in 6M urea and an *increasing Tris/HCl buffer gradient (0.04M to 0.09M)*, pH 8.4, column dimensions 2.6 × 50 cm[11]. The apoprotein fractions were pooled and rechromatographed on a 2.6 × 95 cm Sephadex G-200 column[12]. Apo-Lp A-I was homogeneous, as proven by disc electrophoresis[13,14] and the immunodiffusion technique [15,16]. The amino acid composition was very close to that given by Baker et al.[1] and devoid of cysteine. The molecular weight was determined by sodium dodecylsulfate polyacrylamide gel electrophoresis to be between 28000 and 29000. Rabbit anti-apoLp A-I serum was prepared by repeated apoLp A-I boosters as follows.

35 - 40 mg apoLp A-I was dissolved in 1.0 ml 0.9% NaCl and *emulsified with an equal volume of complete* Freund's adjuvans. 0.5 ml of the emulsion was injected i.m. into rabbits (approx. 3 kg weight). Four boosters were given at 10-day intervals. One week after the last injection, the rabbits were bled by cardial puncture. The antiserum was stored at − 20 °C after the addition of NaN_3 in a final concentration of 0.01%.

After a 60 h diffusion at 4 °C, nonprecipitated proteins were washed out with water and the gels stained with 1% amido black in a mixture of water/methanol/acetic acid 40:50:10. The gels were destained in water/methanol/acetic acid 70:20:10.

Recombinations

Increasing amounts of lysolecithin (0.0, 0.4, 0.8, 1.2 and 1.6 μmol) were stirred under nitrogen together with 0.84 mg (0.0269 μmol) apoLp A-I in 5 ml recombination buffer (1.17 g NaCl, 1.21 g Tris, 0.2 g NaN_3, 7.44 g EDTA per *l*), pH 8.2, 8M in urea for 2 h. Phosphatidylcholine vesicle suspensions (3 μmol, spec. act. 1.9×10^5 dpm/μmol PC) in 1.0 ml of the urea-free buffer were added and stirring continued for 2 h. The mixture was centrifuged in a CsCl gradient and dialyzed against a 0.01% NaN_3 and 0.01% EDTA solution adjusted to pH 8.2 with NH_4OH. The distribution of protein and radioactivity in the gradient fractions was measured before and after dialysis of radioactive fractions. The concentration of each fraction was determined by ultrafiltration, using Amicon filters (UM 2). CsCl gradients were built up between *11* and *20.5%* CsCl solutions and centrifuged at 38000 rpm for 72 h at 10 °C in an SW 41 rotor. Total phosphorus was determined in the fractions according to Rouser[17]. The lecithin bound was calculated from the radioactivity measured. The difference between total phosphorus and lecithin corresponds to the amount of residual lysolecithin in the lipoprotein complex.

Sphingomyelin was recombined with apoLp A-I in the same way. Its sedimentation behaviour was also studied by CsCl gradient centrifugation. Apparent molecular weights of the lipoprotein recombinants were determined by chromatography on calibrated Bio-Gel A-5m *columns with 0.1M Tris, pH 8.2, 0.15M NaCl, 0.001M* EDTA and 0.02% NaN_3 as elution buffer.

Disc electrophoresis of lipoproteins was performed on 3.5% polyacrylamide gel according to Masket et al.[18]. A Philips electron microscope model 300 was used for visualizing the lipoprotein complexes, which were negatively stained with 2% phosphotungstic acid.

Results

Serum high density apolipoprotein A-I and A-II (*apoLP A-I and apoLp A-II*) were separated by DEAE-cellulose chromatography in 6M urea and a Tris/HCl buffer gradient (0.04 to 0.09M)[11], Fig. 1a. The apoproteins were rechromatographed on Sephadex G-200[12] (6M urea, 0.1M NH_4HCO_3 buffer, pH 8.6), Fig. 1b. They were obtained in a homogeneous form as shown by disc electrophoresis in systems containing 6M urea and sodium dodecylsulfate. Fig. 2 shows highly loaded disc electrophoresis gels of apoLp A-I. No contaminants could be observed. The apoprotein also proved to be immunologically pure and free from HDL protein components as shown in Fig. 3. The monospecific antiserum of rabbits immunized with the purified apoLp A-I formed no precipita-

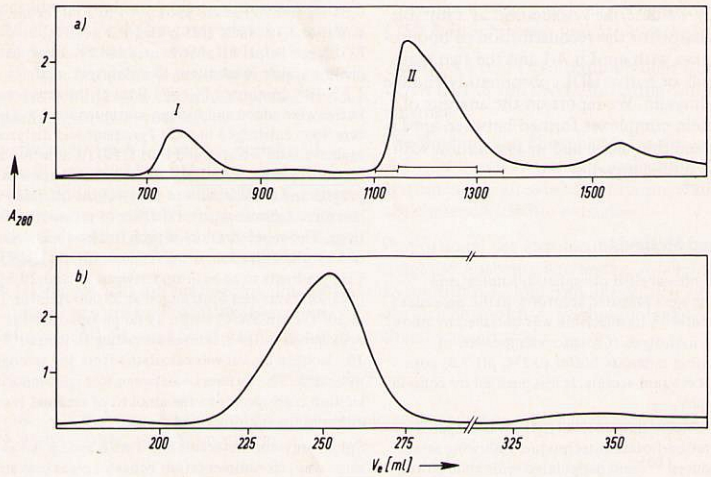


Fig. 1. a) Elution profile of high density apolipoproteins from DEAE-cellulose column (2.5 x 50 cm). A Tris-HCl gradient of 1000 ml of 0.04M and 1000 ml of 0.09M Tris in 6M urea eluted first apoLp A-II (peak I) followed by apoLp A-I (peak II).
b) Sephadex G-200 chromatography (2.5 x 95 cm) of 35 mg apoLp A-I (peak II) of Fig. 1a.
Elution buffer: 0.01M Tris HCl, pH 8.6, 0.01% NaN_3, 0.1% mercaptoethanol, 0.001M EDTA.

tion lines with purified apoLp A-II in the double immunodiffusion test[15,16] but only against apoLp A-I and apoHDL. Molecular weight determination by sodium dodecylsulfate gel electrophoresis[14] indicated a molecular weight of 28500, which agreed well with that given by Baker et al.[1]. Amino acid analysis also agreed well with that elucidated by these authors. No isoleucine, cystine or cysteine was present in this polypeptide. ApoLp A-I dissolved in water or low ionic strength buffers tends to form trimers or tetramers. Chromatography on Agarose columns calibrated with Dextran Blue 2000 and protein standards (glutamate dehydrogenase, aldolase and trypsin) indicated a molecular weight of about 100000 (Fig. 4).

ApoLp A-I was recombined with lysolecithin (1-stearoyl-3-*sn*-glycerophosphocholine) in increasing concentrations (0.4 to 2.0 μmol).

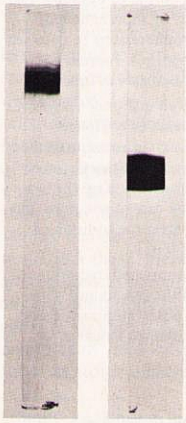


Fig. 2. Polyacrylamide gel electrophoresis (7.5% acrylamide) of purified apoLp A-I.
I) ApoLp A-I in 6M urea[13];
II) apoLp A-I in sodium dodecylsulfate[14].

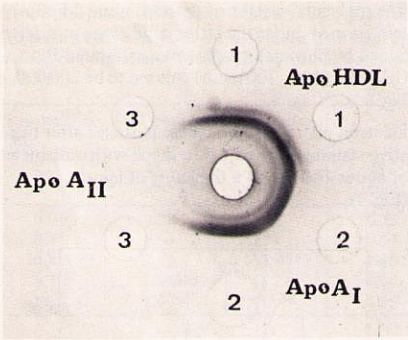


Fig. 3. Immunodiffusion of apoHDL (1.1), apoLp A-I (2.2) and apoLp A-II (3.3).
5 µg each against anti apoLp A-I serum (10 µl) (central well). Staining with amido black.

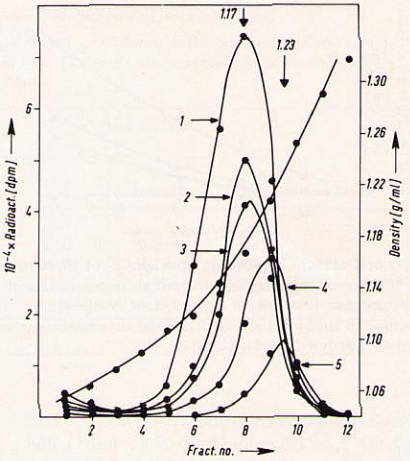


Fig. 5. Distribution of radioactivity in CsCl gradients of apoLp A-I [*N-methyl*-^{14}C]lysolecithin recombinants.
Lysolecithin concentrations decreased from 2.0 µmol to 0.4 µmol. Lysolecithin concentrations: 1) 2.0 µmol; 2) 1.6 µmol; 3) 1.2 µmol; 4) 0.8 µmol; 5) 0.4 µmol. 72 72 h run at 38000 rpm and 10 °C, SW 41 rotor.

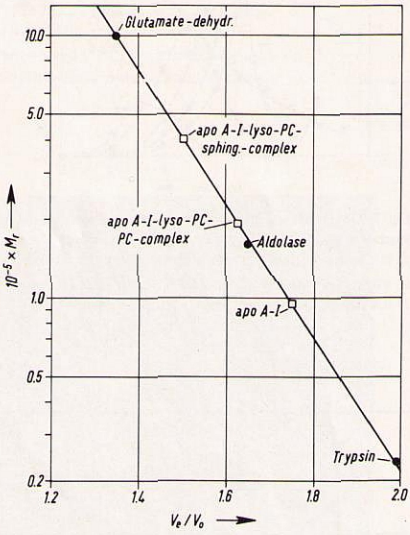


Fig. 4. Determination of molecular weights of lipoprotein complexes by Agarose gel chromatography (Bio-Gel A-5m, 200 - 400 mesh) (1.6 × 90 cm).
Elution buffer: 0.1M Tris HCl, pH 8.2, 0.15M NaCl, 0.01% NaN_3, 0.01% EDTA.

Complexes with decreasing densities between 1.23 to 1.17 g × ml^{-1} were formed (Fig. 5). The lysolecithin-apoLp A-I complexes proved to be easily dissociable. Dialysis led to the loss of 50 to 90% of the lysolecithin with increasing time. This release of lysolecithin from the complex was measured with [*N-methyl*-^{14}C]lysolecithin in the dialysate. The ratio lysolecithin : apoLp A-I changed from initially 67 : 1 to 27 : 1 after 48 h (Fig. 6 and Table 1).

ApoLp A-I-phosphatidylcholine-lysolecithin complexes

Increasing amounts of lysolecithin (0.0, 0.4, 1.2 and 1.6 µmol) were first combined with a constant amount of apoLP A-I (0.03 µmol) and of lecithin (3 µmol) in liposome form. The recombination mixture was dialyzed and then centrifuged to equilibrium in a CsCl gradient. The radioactivity and density profile of the gradient were determined. Fig. 7 indicates that the density of the complexes formed decreased with increasing

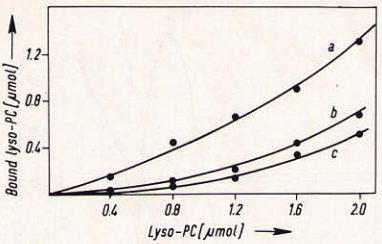


Fig. 6. Release of lysolecithin from apoLp A-I [*N*-methyl-^{14}C]lysolecithin complexes formed at concentrations of lysolecithin between 0.4 and 2.0 μmol, lysolecithin bound to apoLp A-I after a) CsCl gradient centrifugation, b) 48 h dialysis, c) 84 h dialysis.

lysolecithin concentrations (0.4 μmol, d = 1.19 g × ml^{-1}, to 1.6 μmol, d = 1.07 g × ml^{-1}), due to an increased binding of lecithin, Table 2.

These particles were dialyzed up to 48 h and again analyzed for bound phospholipids. Lysolecithin had passed out of the complex, whereas lecithin remained firmly bound to apoLp A-I (see Table 2, column 2 - 4).

The reaggregated lipoproteins formed at lysolecithin concentrations of 1.2 and 1.6 μmol, densities 1.086 and 1.074 g × l^{-1}, respectively, were completely freed from contaminating liposomes by Agarose 4B chromatography. The particles have the following molecular stoichiometry: apoLp A-I/PC/lysoPC = 1:48:13 and 1:51:23, respectively, with 1.2 and 1.6 μmol of lecithin in the starting recombination mixture.

The molecular weight of the recombined lipoprotein formed under the latter conditions was determined by chromatography on a calibrated Bio-Gel A 5m (100 - 200 mesh) column to be 200000, Fig. 8.

Electron micrographs of these particles after negative staining showed a disc shape with a diameter of about 100 Å and a thickness of approx. 25 Å (Fig. 9).

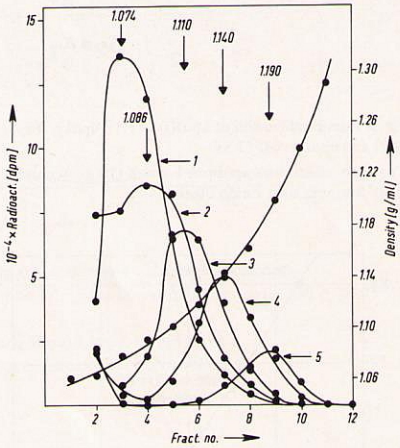


Fig. 7. Densities and distribution of radioactivity in CsCl gradients of apoLp A-I-lysolecithin-lecithin complexes at increasing concentrations of lysolecithin.
Lysolecithin concentration: 1) 1.6 μmol, 2) 1.2 μmol, 3) 0.8 μmol, 4) 0.4 μmol and 5) without lysolecithin.

Table 1. Yields of lysolecithin binding to apoLp A-I.

Lysolecithin		Yield of recombined lysolecithin after CsCl gradient centrifugation		48 h dialysis		
[μmol]	lyso-PC / apoLp A-I	[μmol]	[%]	[μmol]	[%]	lyso-PC / apoLp A-I
0.4	13	0.15	36	0.02	5.2	0.8
0.8	26	0.43	53	0.10	12.4	4.0
1.2	40	0.64	53	0.20	17.0	8.0
1.6	53	0.89	56	0.43	27.0	17.0
2.0	67	1.30	65	0.67	33.3	27.0

Table 2. Binding of phosphatidylcholine to apoLp A-I; dependence on lysolecithin concentrations.
The particles were dialyzed. 3 μmol PC and 0.03 μmol apoLp A-I were recombined in the presence of lyso-PC at increasing concentrations. The lipoprotein complex was purified by CsCl density gradient centrifugation and dialysis for 48 h.

Lysolecithin conc.	CsCl gradient centrifugation		48 h dialysis				
[μmol]	PC [μmol]	[%]	PC [μmol]	[%]	lyso-PC [μmol]	[%]	PC / lyso-PC
0.0	0.28	9.3	0.25	9.3	–	–	–
0.4	0.66	22	0.57	19.0	0.034	8.5	17
0.8	1.03	34	0.89	30.0	0.22	27.5	4
1.2	1.38	46	1.14	38.0	0.35	29.0	3.25
1.6	1.53	51	1.35	45.0	0.60	40.0	2.25

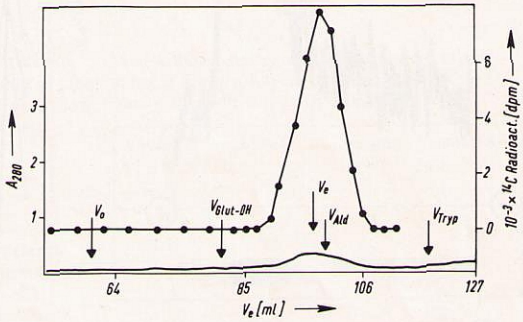


Fig. 8. Molecular weight determination of lipoprotein complex consisting of apoLp A-I, PC and lyso-PC (ratio 1:51:23) by Bio-Gel A-5m chromatography calibrated with test proteins. Protein and [^{14}C]PC radioactivity appeared with identical elution volumes.

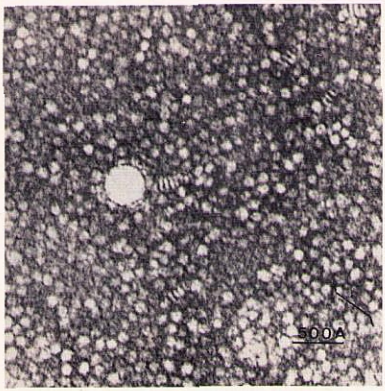


Fig. 9. Electron micrograph of lipoprotein recombinants consisting of apoLp A-I, PC and lyso-PC (1:53:13), molecular weight 200000, after Agarose chromatography.

The lipoprotein complex thus formed from apoLp A-I and radioactive phosphatidylcholine and lysolecithin migrates as a single band in 3.5% acrylamide gels according to Masket et al.[18], as shown in Fig. 10.

ApoLp A-I-sphingomyelin-lysolecithin complexes

ApoLp A-I formed complexes with sphingomyelin in the presence of lysolecithin which had densities of 1.08 to 1.10, almost independently of the lysolecithin concentration in the recombination mixture (Fig. 11). Due to this density range, the particles had to be separated from the sphingomyelin liposomes on Agarose (Bio-Gel A-5m). An example of the elution diagram of the Agarose (Bio-Gel A-5m) chromatography is given in Fig. 12. The sphingomyelin liposomes elute sharply separated from the lipoprotein complex. Table 3 lists the ratios of sphingomyelin to lysolecithin bound in these purified complexes. The

Fig. 11. CsCl gradient centrifugation of apoLp A-I-sphingomyelin-lyso-PC complexes.

Distribution of radioactivity of [*N-methyl-*^{14}C]sphingomyelin and protein coincided. Lipo-PC concentrations were as follows: 1) 2.0 μmol, 2) 1.6 μmol, 3) 1.2 μmol, 4) 0.8 μmol, 5) 0.4 μmol.

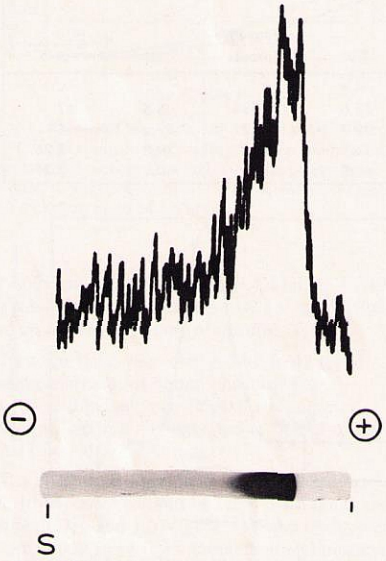
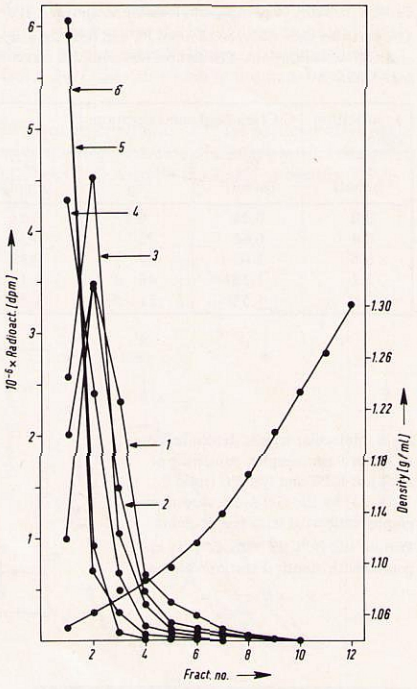


Fig. 10. Polyacrylamide gel electrophoresis of lipoprotein complex recombined from apoLp A-I, [*N-methyl-*^{14}C]phosphatidylcholine and lysolecithin.

Gel electrophoresis was performed according to Masket et al.[18]. The radioscan of the gel (upper tracing) is included.

molecular weight of the apoLP A-I-sphingomyelin-lysolecithin complex has been estimated as 400 000 from its chromatographic behaviour on an Agarose (Bio-Gel A 5m) column calibrated with test proteins.

The stoichiometry of the complexes formed at different concentrations of lysophosphatidylcholine is summarized in Table 4. It demonstrates that the amount of sphingomyelin bound to apoLp A-I was independent of the lysolecithin concentration. The high molecular weight of the apoLp A-I-



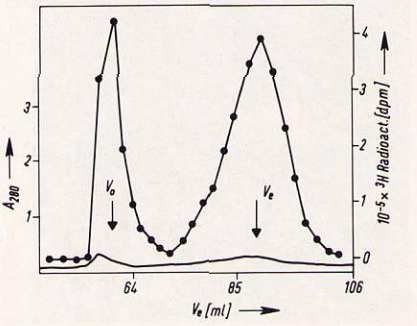


Fig. 12. Chromatographic separation of lipoprotein complexes consisting of apoLp A-I-sphingomyelin and lyso-PC on Bio-Gel A-5m (see Experimental).

Table 3. [*N-methyl-*^{14}C]sphingomyelin bound to apoLp A-I in the presence of increasing concentrations of lyso-PC.

Each recombination mixture contained 0.030 μmol apoLp A-I, 3.0 μmol [*N-methyl-*^{14}C]sphingomyelin and lysolecithin in the concentration indicated below. Analytical data refer to composition of complexes purified by Bio-Gel A 5m chromatography.

Conc. of lyso-PC [μmol]	Sphingomyelin bound		Lyso-PC		sphing./lyso-PC
	[μmol]	[%]	[μmol]	[%]	
0.4	0.3	10	0.048	12	6.25
0.8	0.44	15	0.056	7	7.8
1.2	0.78	26	0.11	9	7.1
1.6	0.72	24	0.155	9.6	4.6
2.0	0.80	26	0.225	11	3.6

sphingomyelin complexes (4×10^5) is consistent with the particle size. Electron micrographs of these lipoprotein particles (negatively stained) revealed discs of 250 Å diameter and 50 Å thickness (Fig. 13 a, b).

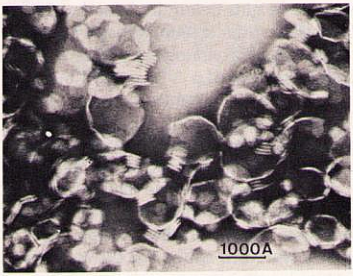
Discussion

The chemical composition of HDL particles is well known, and so are the primary structures of the two major apolipoproteins A-I and A-II,

Table 4. Stoichiometry of lipoprotein complexes consisting of apoLp A-I, sphingomyelin and lyso-PC and formed at lyso-PC concentration indicated below.

Conc. of lyso-PC [μmol]	Lyso-PC	Sphingomyelin	apoLp A-I
0.8	6.5	52	1
1.2	9.0	65	1
1.6	10.5	50	1
2.0	13.5	48	1

a)



b)

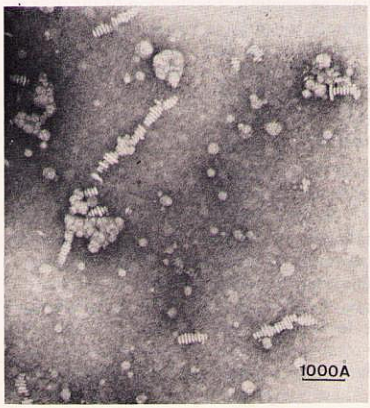


Fig. 13. Electron micrographs of negatively stained lipoprotein complexes consisting of apoLp A-I, sphingomyelin and lysolecithin a) before, b) after Agarose chromatography (note that particles are then free of liposomes).

which are present in the particle in a molar ratio of 3:1. About 100 to 120 molecules of phosphatidylcholine, sphingomyelin and cholesterol are associated with the two apolipoproteins. The additional cholesterol esters are supposedly located in the interior of the spherical particle, as indicated by the electron-poor nucleus seen in low-angle X-ray diffraction studies. ^{13}C-NMR[3,4] and ^{31}P-NMR studies[8,19] prove that the polar head groups of the phospholipids are arranged in the plane of the particle surface, whereas fatty acyl chains interact with the hydrophobic side chains of the apolar interior surface of the apoprotein arranged in the shell of HDL. ApoLp A-I not only is the main apolipoprotein component but also functionally important. Its essential role in the lecithin-cholesterol acyltransferase (LCAT) reaction has been recognized[20]. Recombinations between defined phospholipids and single apolipoproteins may be useful models to study the molecular basis of the interaction between phospholipids and apoproteins.

During recombination studies with the purified apoLp A-I and A-II we observed inconsistencies in the stoichiometry but particularly in the recovery of the apolipoproteins in the recombined lipoprotein complexes. ApoLp A-I forms aggregates at higher concentrations in aqueous solution, apparently with trimeric structure. We dissociate these aggregates and unfold the apoLp A-I molecule in 8M urea, and by the addition of lysolecithin (1-stearoyl-3-glycerophosphocholine) in increasing amounts. Elimination of the urea by dialysis leads to apoLp A-I-lysolecithin complexes with refolding of the polypeptide chain (unpublished results). ApoLp A-I-lysolecithin complexes have also been described by Verdery and Nichols[21], Gwynne et al.[22] and Haberland and Reynolds[23].

We found that a characterization of the apoLp A-I-lysolecithin complexes as reported previously[21,22] by the sedimentation-flotation method is insufficient. Lysolecithin binds only weakly to the apoprotein. It dissociates easily on dialysis from the complexes, which are formed in the presence of increasing lysolecithin concentration with a density range from 1.17 to 1.21. Essentially the total apoLp A-I was complexed with the micellar lyso-compound, lysolecithin/apoLp A-I 40:1. These complexes extracted phospholipid molecules either from unsaturated (dilinoloyl-phosphatidylcholine) liposomes (250 Å diameter) or sphingomyelin liposomes (800 to 1000 Å diameter) with the formation of distinct particles. The newly formed apoLp A-I-lysolecithin-lecithin (A) and apoLp A-I-lysolecithin-sphingomyelin (B) complexes differed not only in their densities but also in their size. The density of A decreased with increasing lysolecithin concentrations as determined by CsCl gradient centrifugation. The ratio of lecithin bound to apoprotein A-I in the aforementioned dialyzed lipoprotein complexes (A) was rather constant (50:1). These complexes have been purified by Agarose chromatography to ensure the absence of any liposomes and easily exchangeable lipids. The 100 × 25 Å disc-shaped particles with density 1.086 contained 50 lecithin molecules per apoLp A-I molecule and 13 molecules of lysolecithin, while the particles of density 1.074 contained 23 molecules of lysolecithin. The apoLp A-I-lysolecithin-sphingomyelin complexes (B) also yielded particles of disc shape and size 250 × 50 Å. The stoichiometry of the lipoproteins was like that of the much smaller apoLp A-I-lysolecithin-lecithin particles. 50 molecules of sphingomyelin were bound per apoLp A-I and 10 molecules of lysolecithin (d = 1.08).

Unlike the lecithin-containing lipoproteins (A), the apoLp A-I-sphingomyelin aggregates (d = 1.10) did not become denser with increasing lysolecithin concentration; instead, the yield of complexed apoLp A-I increased.

The molecular weights determined for the smaller apoLp A-I-lysolecithin-lecithin particles by calibrated Agarose gel chromatography were 200000 and 400000 for the apoLp A-I-lysolecithin-sphingomyelin particles. These values must be taken with all necessary reservations. Along with the size and shape revealed by negatively stained electron micrographs of these particles, the data suggest the following structures and mechanisms by which lysolecithin favours the formation of the two lipoprotein complexes: the hydrophobic regions of the unfolded apoLp A-I surface tend to interact with each other in aqueous solution. The binding of amphiphiles such as the detergent lysolecithin prohibits this process by the interaction of the fatty acyl chain and the hydrophobic segments of the apolipoprotein, inducing conformational changes, and also supplies this sur-

face area with zwitterionic hydrophilic groups. Lysolecithin furthermore "opens" the lecithin liposome bilayer, as suggested in lysolecithin-induced fusion processes. It enables phosphatidylcholine acyl chains to combine with the hydrophobic side of the apoLp A-I, partially displacing lysolecithin molecules. The lecithin used for the recombination is highly unsaturated. The height of the disc (23 - 25 Å) suggests a structure which cannot consist of a phospholipid bilayer (60 - 80 Å) with apoLp A-I embedded in it. We suggest that the helices and β-sheet segments of apoLp A-I are spread in a plane with its hydrophilic polypeptide side chains forming one face of the disc. The other side is comprised of the hydrophobic areas, now rendered hydrophilic by the zwitterionic polar head groups of lysolecithin and lecithin molecules bound with their acyl chains to the hydrophobic sites of the protein. The shape and dimensions,of the apoLp A-I-lysolecithin-sphingomyelin complex (250 × 50 Å), on the other hand, could result from a partial bilayer arrangement of the sphingomyelin and lysolecithin molecules with the apoLp A-I molecules (4 to 6 per particle) extended in the two planes of the bilayer. Further studies are in progress to define the structures of these two types of lipoproteins.

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