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REASSEMBLY OF HUMAN APOPROTEINS A-I AND A-II WITH UNILAMELLAR PHOSPHATIDYLCHOLINE-CHOLESTEROL LIPOSOMES

ASSOCIATION KINETICS AND CHARACTERIZATION OF THE COMPLEXES

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Summary

The kinetics of association between the human apoprotein A-I and apoprotein A-II and cholesterol dimyristoyl phosphatidylcholine (DMPC) vesicles are compared in this study and the lipid-apoprotein complexes are characterized.

The association kinetics are followed by turbidity measurements monitoring the decrease of the vesicular size and by fluorescence polarization measurements monitoring the decrease in the mobility of the phospholipid acyl chains during complex formation.

The influence of the incubation temperature and of the cholesterol/DMPC ratio has been studied by both techniques. Under all incubation conditions the apoprotein A-II associates more readily with cholesterol-DMPC vesicles than apoprotein A-I, as the kinetics are faster and the complex yield larger. With both apoproteins optimal complex formation takes place around the phospholipid transition temperature and around 10 mol% cholesterol. The apoprotein A-I/lipid association seems restricted to this narrow range for the temperature and the cholesterol/DMPC ratio, while the apoprotein A-II still associates with vesicles containing 20 mol% cholesterol and at temperatures up to 32°C.

The lipid-apoprotein complexes were isolated by gradient ultracentrifugation and by gel chromatography. According to these data the apoprotein A-II associates more readily than apoprotein A-I with cholesterol-DMPC vesicles to form protein-rich complexes, whilst the optimal apoprotein A-I-lipid association requires a more disordered lipid structure.

Abbreviation: DMPC, dimyristoyl phosphatidylcholine.

Introduction

The distribution of the apoproteins A-I and A-II at the surface of the high-density lipoproteins (HDL) and their preferential interaction with cholesterol and phospholipids is not yet completely elucidated. In view of the role attributed to the HDL in the exchange and transport of cholesterol between lipoproteins and membranes [1,2], the relative contribution of its major apoprotein components to this process is of major importance.

The major HDL proteins, apoprotein A-I and apoprotein A-II, from various species have been shown to recombine with phospholipids to yield discrete complexes [3,4]. The yield and composition of the complexes are dependent upon the incubation conditions. The two apoproteins behave distinctly, as the apoprotein A-I recombines more readily with DMPC in a more exothermal reaction [5]. The reassembly of the apoprotein A-I with mixed multilamellar liposomes consisting of DMPC and cholesterol has been investigated by Pownall et al. [6], by Tall and Lange [7] and by Jonas and Krajnovich [8], who isolated lipid-protein complexes. The association kinetics between the apoprotein A-I and the DMPC-cholesterol liposomes are dependent upon the liposomal composition and upon the incubation temperature [6].

In this paper we compare the kinetics of association between cholesterol-DMPC vesicles and the human apoproteins A-I and A-II, in order to define optimal conditions for the coexistence of lipid and apoproteins within stable structures. The complexes generated under optimal reassembly conditions are subsequently isolated and their composition is analyzed.

A comparative evaluation of the reassembly kinetics and of the compositional data should provide more information about the specific function of apoprotein A-I and apoprotein A-II in the HDL molecule.

Methods

Apoprotein isolation

The human apoproteins A-I and A-II were isolated from HDL originated from normal fasting donors after ultracentrifugation at $d = 1.063—1.21$ g/ml, delipidation by diethyl ether/ethanol (2 : 1) and separation on DEAE-cellulose column in 7 M urea [9].

The purity of the fractions was checked by polyacrylamide gel electrophoresis and by immunodiffusion with specific anti-sera against albumin, apoprotein B, apoprotein A-I and apoprotein A-II.

Preparation of unilamellar liposomes

Mixed liposomes were prepared from cholesterol (Schuchardt, Austria) and dimyristoyl phosphatidylcholine (DMPC) (Sigma Chemical Co.) at ratios between 0 and 20 mol% cholesterol. The lipids were dissolved in ethanol, evaporated and cosonicated at 37°C for 30 min, in 0.01 M Tris-HCl (pH 8.0)/0.1 M NaCl, using a Branson sonifier equipped with a microtip probe. They were subsequently centrifuged at 100 000 × g for 30 min [10]. The size of the vesicles was determined by chromatography on a Sepharose 4B column, from which the phospholipids eluted within the column volume.

Incubation of the apoproteins and lipids

For the kinetics experiments the apoproteins and lipids were mixed in the cuvette of the microviscosimeter and of the spectrophotometer and the recording was started immediately. In these experiments the lipid/apoprotein (w/w) ratio was 2 : 1 and the lipid concentrations were around 0.07 mg/ml for the microviscosity measurements and 0.6 mg/ml for the absorbance measurements.

For the complex isolation by gradient ultracentrifugation and by gel filtration a lipid/protein ratio of 2 : 1 (w/w) was selected. 1.5 mg lipid was incubated with 0.75 mg protein for 3 h at 25°C before ultracentrifugation, while 4 mg lipid were incubated with 2 mg protein for 3 h at 25°C before fractionation on a Sepharose 6B column.

Complex isolation and characterization

1. Complex isolation. The complexes generated between the mixed phosphatidylcholine-cholesterol vesicles and the apoproteins A-I and A-II were isolated by gradient ultracentrifugation [10] and gel filtration [11].

Gradient ultracentrifugation was carried out in an NaBr gradient spanning the densities 1.05—1.15 g/ml. The gradient was prepared by use of an Auto-Densiflow (Büchler Instr.) and spun in a SW 50.1 rotor in a Beckman L5-65 preparative ultracentrifuge for 60 h. The gradient was eluted by means of the same system and collected in 0.2 ml fractions in a Gilson fraction collector. The fractions' densities were derived from refractive index measurements on an Abbe refractometer.

Gel filtration was performed on a Sepharose 6 B column equilibrated in a Tris-HCl buffer 0.01 M, pH 8.0, 0.1 NaCl. For column calibration, HDL, ferritin, catalase, albumin, chymotrypsinogen and cytochrome *c* were used as standards and 2,4-dinitrophenylasparagine was used as marker for the total column volume.

2. Complex characterization. The composition of the isolated complexes was derived from protein measurements with the Folin's reagent [13]. This assay can be carried out in the presence of NaBr, whilst KBr causes precipitation. Phosphorus quantitation was carried out according to Eibl and Lands [14], and cholesterol quantitation using a fluorimetric technique according to Gamble et al. [15].

The dimension of the complexes and the Stoke's radius were derived both from gel chromatography and from electron microscopy after negative staining with phosphotungstate on a JEOL electron microscope.

Kinetics of association

The kinetics of association between the apoproteins and the mixed liposomes were followed by two techniques: (a) turbidity measurements based on the decrease of the size of the liposomes [6]; and (b) fluorescence polarization measurements monitoring the changes in the fluidity of the lipid phase as a consequence of the apoprotein-lipid binding [16]. The association kinetics were investigated at the various cholesterol/phosphatidylcholine ratios stated above and as a function of temperature in the range 22—38°C.

1. Turbidity measurements. The absorbance at 325 nm was measured during incubation of the liposome dispersion with apoprotein A-I and apoprotein A-II

on a Pye-Unicam spectrophotometer equipped with thermostatically controlled cuvettes. The temperature of the mixture was measured by means of a thermistor placed in one of the cuvettes. The measurements were carried out at a lipid concentration of 0.6 mg/ml and the extent of reaction was expressed as $\alpha = (E - E_0)/(E_\infty - E_0)$, where E_∞ is the absorption of the isolated complex, E_0 that of the pure lipid and E that of the mixture at time t. This parameter does not express the percentage of apoprotein-lipid complex but provides only an estimate of the decrease of the size of the original liposomes.

2. Fluorescence polarization. The fluorescence polarization ratio of the lipid vesicles and of the apoprotein-lipid complexes was measured after labeling with diphenyl hexatriene [17], on an Elscint microviscosimeter. The cholesterol-DMPC vesicles (0.2 mM) were incubated with diphenylhexatriene for 30 min at 25°C prior to mixing with the apoproteins. The temperature of the apoprotein solution and the lipid dispersion were thermostatically controlled separately at the reaction temperature, and subsequently mixed inside the cuvette of the microviscosimeter. The fluorescence polarization ratio of the mixture was recorded for 2 h and the incubation temperature was measured by means of a thermistor placed inside the cuvette. The fluorescence polarization ratio of the mixture is larger than the value measured for the pure liposomes, p_0, and smaller than that of the isolated complex, p_∞. To compare the time course of the reaction at various temperatures, the parameter $\alpha' = (p - p_0)/(p_\infty - p_0)$ was used, as it expresses the degree of advancement of the lipid-protein association.

Results

Kinetics of association monitored by fluorescence polarization measurements

The kinetics of association between apoproteins A-I and A-II and the cholesterol-DMPC vesicles are dependent both on temperature and on vesicle composition, as illustrated in Figs. 1 and 2. At a cholesterol/DMPC content of 10 mol% the optimal reaction rate with both apoproteins was observed around the transition temperature of the phospholipid (24.5°C) (Fig. 1). At this particular temperature the effect of cholesterol was specially pronounced for the apoprotein A-I, in the case of which an optimal association was observed at approx. 10 mol% (Fig. 2). These figures show that under the conditions tested the association kinetics are slower with apoprotein A-I than with apoprotein A-II, as confirmed by Tables I and II.

Table I summarizes the reaction rates for the two apoproteins as a function of temperature and of the vesicles' composition. The reaction rates are expressed as $k = 1/t_{1/2}$, as the order of the reaction could not be determined [6]. With both apoproteins, the reaction rates (Table I) as well as the increase of p, expressed by α, are optimal around the transition temperature of the vesicles, whilst the effect of the temperature is especially pronounced upon the A-I-lipid association. The value of α, measured after 15 min incubation of the apoproteins and cholesterol-DMPC vesicles containing 10 mol% cholesterol, decreases from 0.9 at 24°C to 0.2 at 28°C for the apoprotein A-I-lipid association and from 0.8 at 24°C to 0.7 at 28°C for the apoprotein A-II-lipid complex. Under these conditions, the rate constant, k_1, decreases by a factor of 20 for

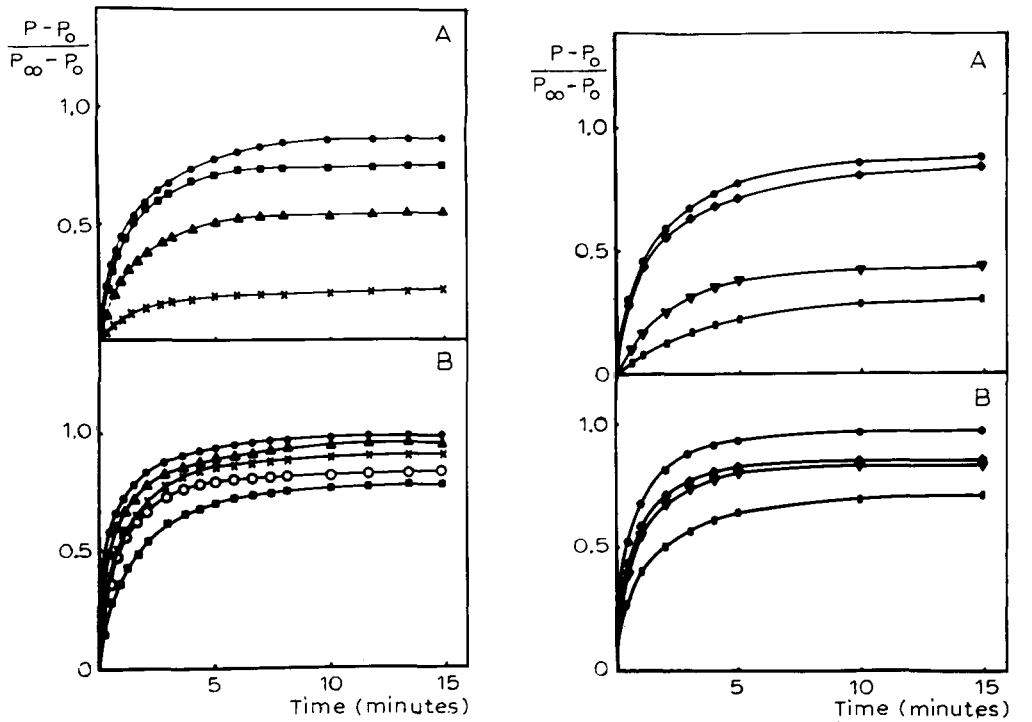

Fig. 1. Association kinetics of apoprotein A-I (A) and apoprotein A-II (B) with DMPC-cholesterol vesicles containing 10 mol% cholesterol. The relative increase of the fluorescence polarization ratio $(p - p_0)/(p_\infty - p_0)$ is plotted vs. time at various incubation temperatures (°C): 23 (■———■); 24.5 (●———●); 28 (▲———▲); 32 (X———X) and 37 (○———○).

Fig. 2. Association kinetics of apoprotein A-I (A) and apoprotein A-II (B) with DMPC-cholesterol vesicles, measured at 24.5°C as a function of the liposomal composition. The relative increase of the fluorescence polarization ratio $(p - p_0)/(p_\infty - p_0)$ as plotted vs. time at various cholesterol contents (mol%): 0 (▼———▼); 5 (◆———◆); 10 (●———●) and 20 (■———■).

the apoprotein A-I-lipid complex compared to a factor of 2 for the apoprotein A-II-lipid association.

The association kinetics are also sensitive to the composition of the cholesterol-DMPC vesicles, as the rate and extent of association between apoprotein

TABLE I

RATE CONSTANTS FOR APOPROTEIN-LIPID ASSOCIATION k_1 (min^{-1}), DEDUCED FROM FLUORESCENCE POLARIZATION

T (°C)	mol% cholesterol					
	0		5		10	
	A-I	A-II	A-I	A-II	A-I	A-II
23	0.05	5	0.11	4	0.07	0.6
24.5	0.03	4	0.8	6	0.7	3.5
28	0.015	3	0.25	3	0.04	1.7
32	0.01	0.12	0.04	1.7	0.03	1.2

TABLE II

RATE CONSTANTS FOR APOPROTEIN-LIPID ASSOCIATION k_2 (min^{-1}), DEDUCED FROM ABSORBANCE DECREASE (325 nm)

T (°C)	mol% cholesterol					
	0		5		10	
	A-I	A-II	A-I	A-II	A-I	A-II
23	1.0	3.0	0.6	1.0	3	10
24.5	0.5	10	2	1.8	4	10
28	0.03	1	0.1	1	0.11	2.0
32	0.01	0.05		0.4		0.7

A-I and mixed DMPC-cholesterol vesicles are optimal at a cholesterol content around 10 mol%. After incubating apoprotein A-I with the mixed lipid vesicles at 24.5°C for 15 min, the fluorescence polarization ratio increases up to 90% of the value of the isolated complex at 10 mol% cholesterol, compared to a 40% increase with pure DMPC and a 30% increase at 20 mol% cholesterol. The rate constant, k_1, decreases by a factor of 20 between 10 and 0 mol% cholesterol and could not be evaluated at 20 mol%. The presence of cholesterol affected the kinetics of apoprotein A-II-lipid association to a lesser extent. At 24.5°C after 15 min incubation, 80% of the apoprotein A-II is associated with DMPC vesicles, whilst 70% of the apoprotein A-II associates with vesicles containing 20 mol% cholesterol.

These data indicate that each apoprotein has a specific association behaviour with cholesterol-DMPC vesicles. The apoprotein A-II-lipid association seems less sensitive to the physical state of the phospholipid within the vesicles which is modulated by both the cholesterol content and the temperature (Fig. 1).

Kinetics of association monitored by turbidity measurements

The turbidity of the apoprotein-liposomes mixtures was monitored at 325 nm for about 2 h at the same temperatures and liposomal composition as used for the fluorescence polarization measurements.

The patterns observed with the two apoproteins closely resemble those of the fluorescence polarization data. For the apoprotein A-I and apoprotein A-II the turbidity decrease is optimal around the transition temperature of DMPC and is dependent upon the vesicle composition especially for the apoprotein A-I-liposomes association (Fig. 3). The apoprotein A-II induces a breakdown of the vesicles at a faster rate and a higher extent than apoprotein A-I under all conditions investigated.

The reaction rates between lipid vesicles and the two apoproteins are summarized in Table II for the turbidity measurements. These data show that the apoprotein A-II-lipid association occurs more readily than with apoprotein A-I. The rate constants differ by a factor of about 10, in the absence of cholesterol or at temperatures far from the phospholipid transition. At the optimal incubation conditions (24.5°C, 10 mol% cholesterol), the figures become closer.

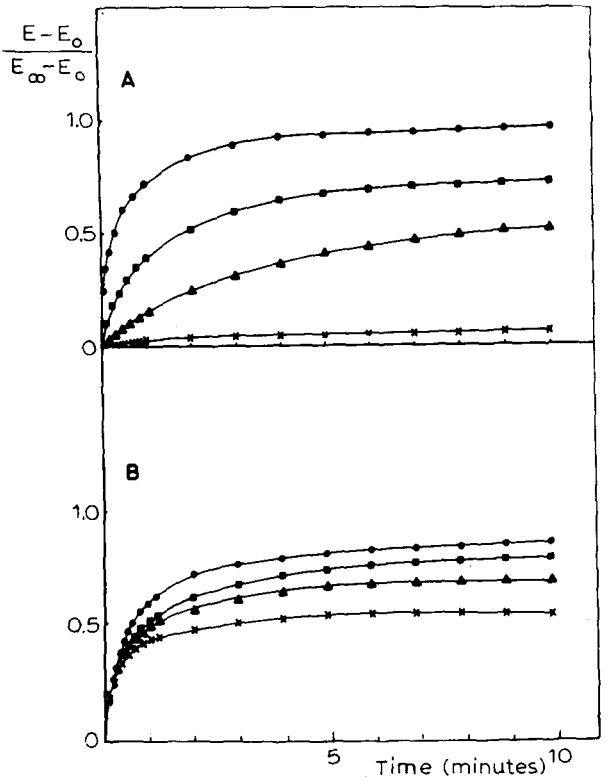

Fig. 3. Association kinetics for apoprotein A-I (A) and apoprotein A-II (B) with DMPC-cholesterol containing 10 mol% cholesterol. The relative turbidity changes $(E - E_0)/(E_\infty - E_0)$ are plotted as function of time at various temperatures (°C); 23 (■———■); 24.5 (●———●); 28 (▲———▲); and 32 (X———X).

Ultracentrifugal isolation and characterization of the apoprotein-lipid complexes

As shown by the kinetics experiments, the optimal complex yield between apoprotein A-I and apoprotein A-II and the cholesterol-DMPC liposomes is obtained around 24°C after 2 h incubation. These conditions were selected for the preparation of the complexes, which were subsequently isolated and characterized.

Figs. 4 and 5 (A—D) represent the ultracentrifugation patterns for the isolation of the complexes between the apoproteins A-I and A-II and the cholesterol-DMPC vesicles containing 0, 5, 10 and 20 mol% cholesterol. As all original mixtures contained an excess of protein compared to the complex stoicheiometry, no free lipid was detected after gradient elution, except for the apoprotein/DMPC-cholesterol mixture containing 20 mol% cholesterol (Figs. 4D and 5D). At 5 and 10 mol% cholesterol, the complex yield is comparable for apoprotein A-I and apoprotein A-II, as previously observed with pure DMPC [10].

With the apoprotein A-II, two complexes were observed at 0, 5 and 10 mol% cholesterol with a distinct density and composition (Table III). The lighter complex contains about 100 mol lipid/mol protein, whereas the heavier complex consists of 75 mol lipid/mol protein. In both complexes, the cholesterol/

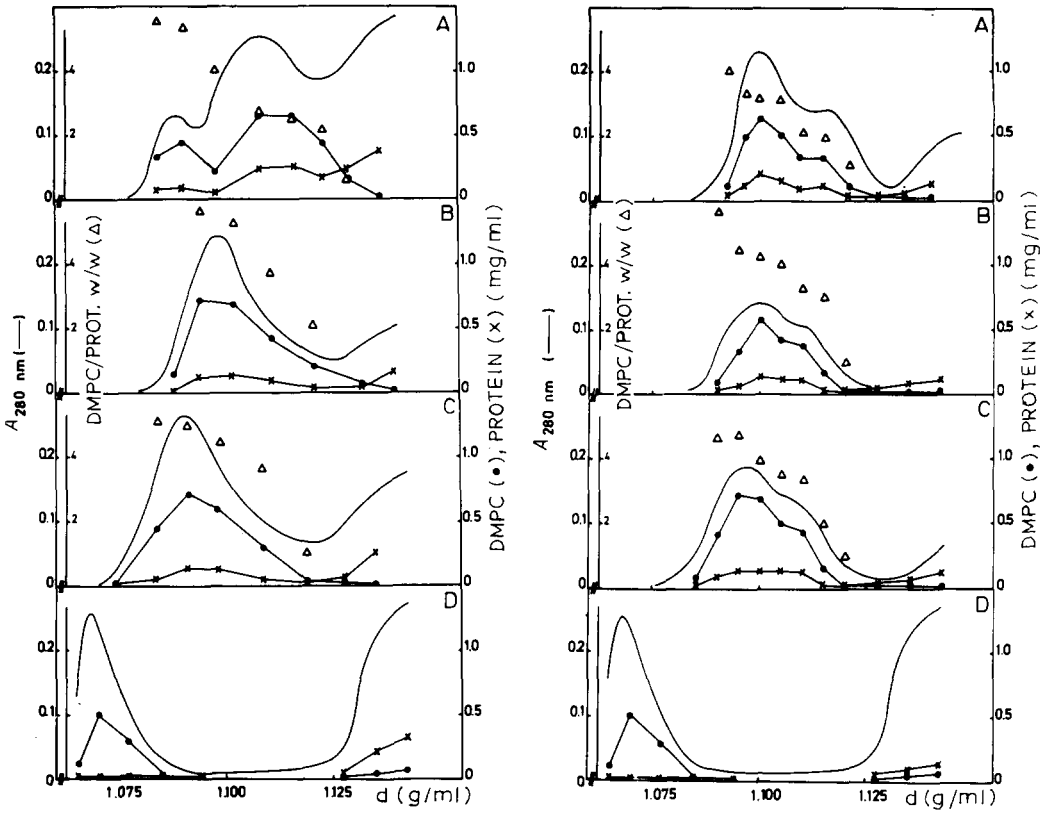

Fig. 4. Gradient ultracentrifugation of DMPC/cholesterol/apo A-I mixtures. DMPC-cholesterol vesicles (1.5 mg in DMPC) were incubated with apoprotein (0.75 mg) at 25° C for 3 h. The absorbance elution pattern (———), the DMPC (•———•) and apoprotein (X———X) concentrations and the lipid/apoprotein (w/w) ratio (△) are plotted against density. Elution patterns are from: A. apoprotein A-I/DMPC mixture; B. apoprotein A-I/DMPC/cholesterol mixture containing 5 mol% cholesterol; C. apoprotein A-I/DMPC/cholesterol mixture containing 10 mol% cholesterol; D. apoprotein A-I/DMPC/cholesterol mixture containing 20 mol% cholesterol.

Fig. 5. Gradient ultracentrifugation of DMPC/cholesterol/apo A-II mixtures. DMPC-cholesterol vesicles (1.5 mg in DMPC) were incubated with apoprotein (0.75 mg) at 25° C for 3 h. The absorbance elution pattern (———), the DMPC (•———•) and apoprotein (X———X) concentrations and the lipid/apoprotein, (w/w) ratio (△) are plotted against density. Elution patterns are from: A. apoprotein A-II/DMPC mixture; B. apoprotein A-II/DMPC/cholesterol mixture containing 5 mol% cholesterol; C. apoprotein A-II/DMPC/cholesterol mixture containing 10 mol% cholesterol; D. apoprotein A-II/DMPC/cholesterol mixture containing 20 mol% cholesterol.

DMPC ratio is the same as in the original mixture, in agreement with the data of Pownall et al. [6]. The proportion of the two species can be altered by varying the composition of the incubation mixture. An excess of lipid would increase the amount of the lipid-rich complex, whilst an excess of apoprotein would have the opposite effect. The lipid/protein ratio of the two isolated species remains independent of the composition of the incubation mixture. These complexes have a lipid-to-protein ratio comparable to that of the DMPC-apoprotein A-II complexes (Table III). With the apoprotein A-I and DMPC, two complexes were isolated, with molar ratios of, respectively, 250 and 122 mol DMPC/mol apoprotein A-I, whilst only one complex could be detected after

TABLE III

CHARACTERIZATION AND COMPOSITION OF THE COMPLEXES RECONSTITUTED FROM THE HUMAN APOLIPOPROTEIN A-I AND APOLIPOPROTEIN A-II AND DMPC-CHOLESTEROL VESICLES

Chol., cholesterol; apopr., apoprotein.

Original mixture		Complex isolated by ultracentrifugation				Complex isolated by gel filtration	
Apo-protein	Chol./DMPC (mol%)	*d* g/ml	mol DMPC/mol apopr.	Chol./DMPC (mol%)	Diameter (E.M.) (Å)	mol DMPC/mol apopr.	Stokes radius (Å)
Apo A-I	0	1.090	254	0	120	102	58.5
		1.120	122	0			
	5	1.096	200	5.6	—	—	—
	10	1.090	228	10	120	200	56
Apo A-II	0	1.100	90	0	100	60	59
		1.120	70	0			
	5	1.095	100	5.2	250	—	—
		1.105	75	4.9	130		
	10	1.096	110	10	250	124	59
		1.106	80	10	130	100	63

incubation with cholesterol/DMPC mixtures. This single cholesterol-DMPC-apoprotein A-I complex contains 210 mol lipid/mol protein and its composition is close to that of the lipid-rich species generated after DMPC-apoprotein A-I incubation. These data suggest that for the apoprotein A-I, the presence of cholesterol is a limiting factor for the number of apoprotein molecules incorporated into the lipid-protein complex, as the cholesterol-rich complexes contain half as much apoprotein as that generated from pure phospholipids. At 20 mol% cholesterol, the kinetic data suggested that apoprotein association takes place at 24.5°C with apoprotein A-I and between 22 and 32°C with apoprotein A-II. However, with both apoproteins, no discrete complex could be isolated by ultracentrifugation, suggesting that some dissociation must have occurred during centrifugation in a high-salt medium (Figs. 4D and 5D).

Gel filtration of the apoprotein-lipid complexes

The complexes generated with the apoproteins A-I and A-II and the phospholipid-cholesterol vesicles were isolated by gel filtration on a Sepharose 6B column (Figs. 6 and 7) and their composition followed by chemical analysis. With pure DMPC and at cholesterol contents up to 20 mol%, the apoproteins A-I and A-II are incorporated into particles with a size intermediate between that of the original vesicles and that of human HDL (Figs. 6 and 7). The Stoke's radii, calculated by the calibration of the Sepharose 6B column with globular proteins lie around 59 ± 3 Å for the various apoprotein-lipid complexes. The size of the complexes does not vary significantly with the cholesterol content of the vesicles between 0 and 20 mol%. The radii of the human apoprotein A-I-lipid complex are higher by about 10 Å than the values reported by Jonas and Krajnovich [8] for bovine apoprotein A-I-DMPC-cholesterol complexes separated on a Sepharose 4B column. With the apoprotein A-II-lipid

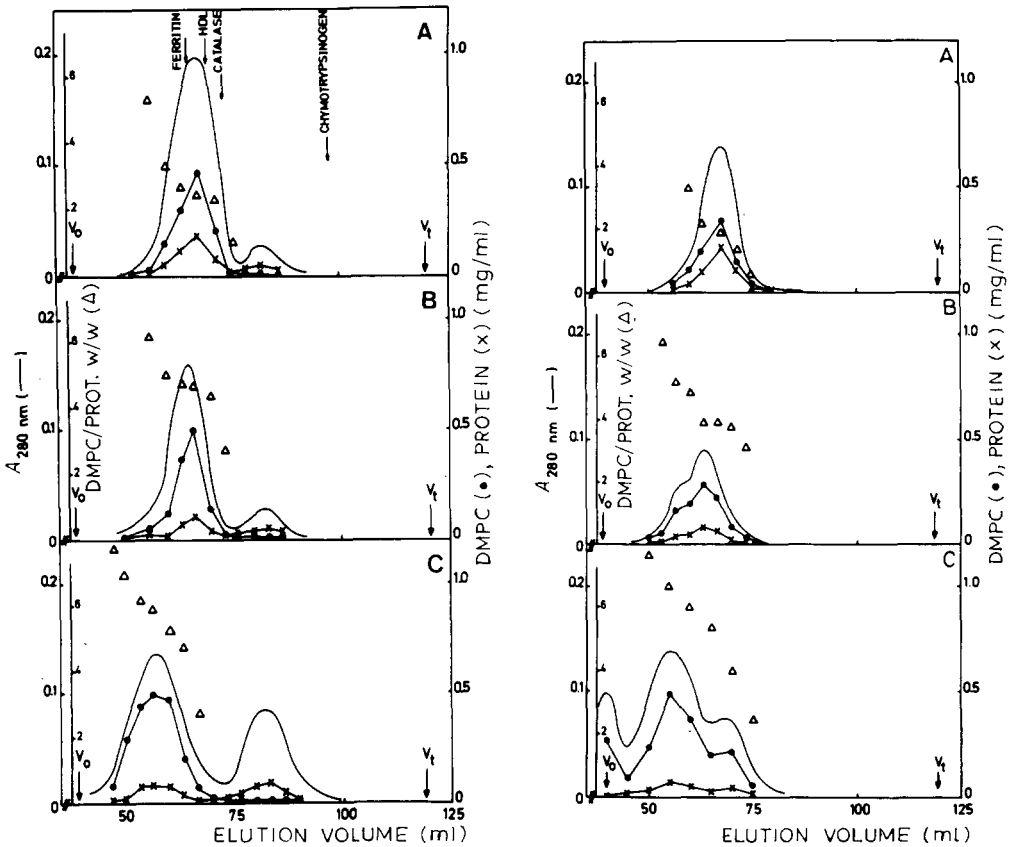

Fig. 6. Gel chromatographic elution patterns of cholesterol/DMPC/apo A-I mixtures after incubation for 3 h at 25°C. The absorbance elution pattern (———), the DMPC (•———•) and apo A-I (X———X) concentrations and the lipid/apoprotein (w/w) ratio (△) are plotted against elution volume. Elution patterns are from: A. apoprotein A-I/DMPC mixture; B. apoprotein A-I/DMPC/cholesterol mixture containing 10 mol% cholesterol; C. apoprotein A-I/DMPC/cholesterol mixture containing 20 mol% cholesterol.

Fig. 7. Gel chromatographic elution patterns of cholesterol/DMPC/apo A-II mixtures after incubation for 3 h at 25°C. The absorbance elution pattern (———), the DMPC (•———•) and apo A-II (X———X) concentrations and the lipid/apoprotein (w/w) ratio (△) are plotted against elution volume. Elution patterns are from: A. apoprotein A-II/DMPC mixture; B. apoprotein A-II/DMPC/cholesterol mixture containing 10 mol% cholesterol; C. apoprotein A-II/DMPC/cholesterol mixture containing 20 mol% cholesterol.

mixtures, two complexes were detected, corresponding to the species isolated by gradient ultracentrifugation.

At a higher cholesterol/DMPC ratio (20 mol%) the lipid/apoprotein ratio throughout the main peak kept decreasing with increasing elution volume, in the case of apoprotein A-I (Fig. 6C) as well as apoprotein A-II (Fig. 7C). The apoprotein-lipid mixture could not be resolved in a well characterized complex.

Electron microscopy

By negative stain electron microscopy, all complexes appeared as lipid bilayer discs forming 'stacks' on the grid (Fig. 8). The apoprotein A-I-lipid complex had a size of about 120—150 Å × 55 Å (Fig. 8A), comparable to that of the smaller apoprotein A-II-lipid complex (Fig. 8B). The lipid-rich particle ob-

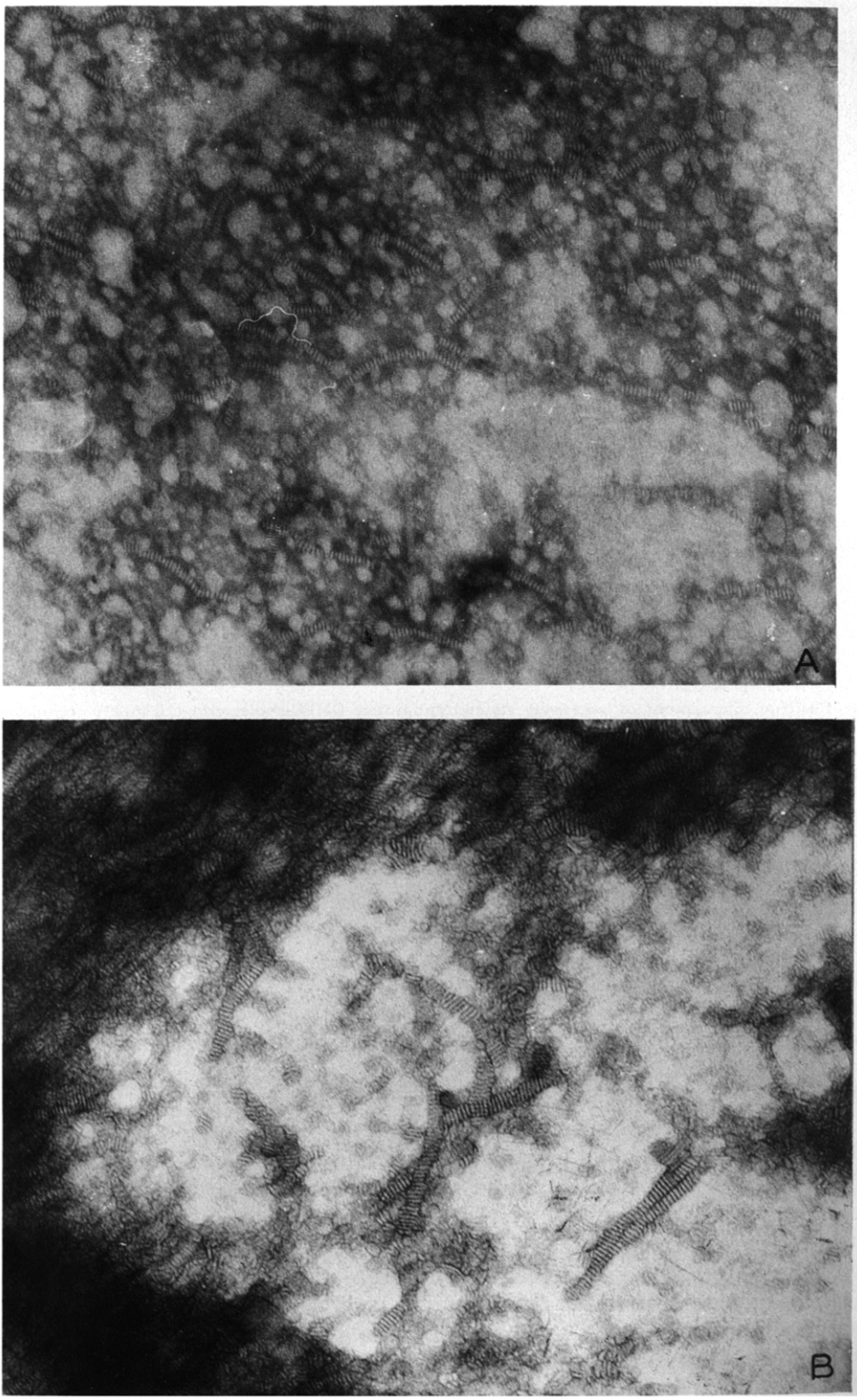

Fig. 8A and B.

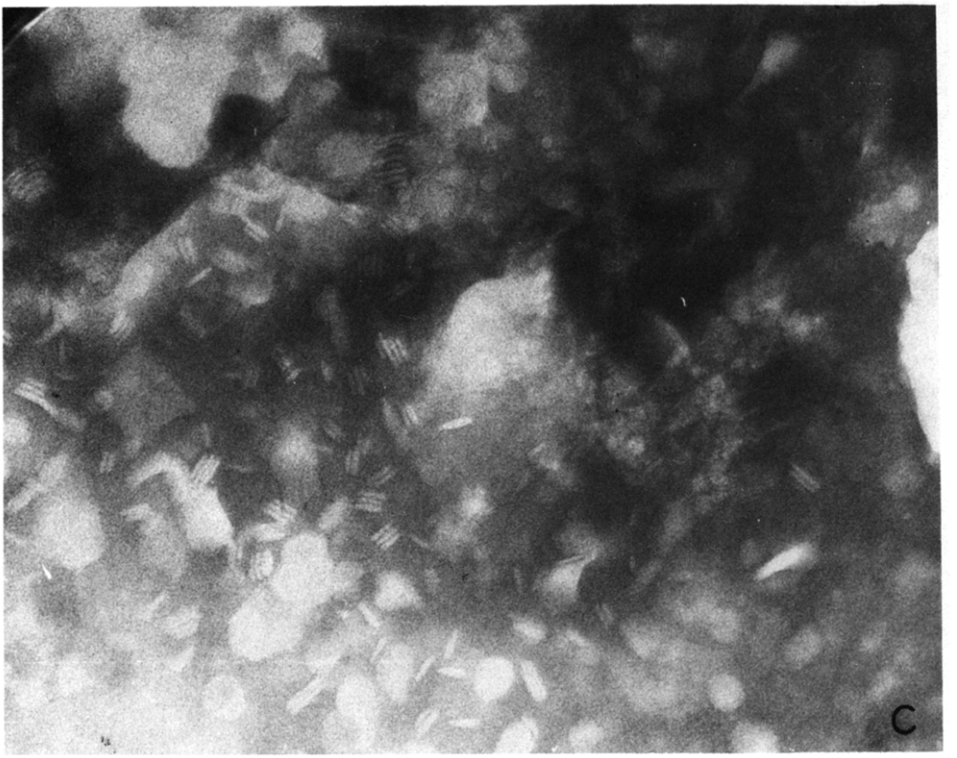

Fig. 8. Electron micrographs of negatively stained apoprotein-DMPC-cholesterol (10 mol%) complexes. The total magnification is 120 000-fold for the apoprotein A-I-lipid complex (A), and 180 000-fold for the apoprotein A-II-lipid complex containing 75 mol DMPC/mol A-II (B) and the apoprotein A-II-DMPC-cholesterol complex with 100 mol DMPC/mol A-II (C).

served with apoprotein A-II was about 250 × 55 Å (Fig. 8C). The dimensions of the smaller particles are comparable to those reported by Tall and Lange [7] for DMPC/cholesterol/apoprotein A-I mixtures.

Fluorescence polarization measurements

The fluorescence polarization ratio measured after labeling of the lipid phase with diphenyl hexatriene is dependent both on the lipid and protein composition of the particles [18].

As shown by Fig. 9A, the fluorescence polarization ratio of DMPC-cholesterol vesicles increases with increasing cholesterol content, especially above 24°C. This increase induces a progressive decrease of the amplitude and finally a disappearance of the gel to liquid crystalline transition of DMPC at cholesterol contents above 25 mol%, as previously observed by differential scanning calorimetry [19]. Up to this percentage of cholesterol the transition temperature of the mixed liposomes remains at 24°C.

The influence of apoproteins A-I and A-II on the fluidity of the vesicles is depicted in Fig. 9B. The complexes, containing 0, 5 or 10 mol% cholesterol were isolated either by ultracentrifugation or gel filtration. At 20 mol% cholesterol, the curve on Fig. 9B corresponds to a lipid-apoprotein mixture contain-

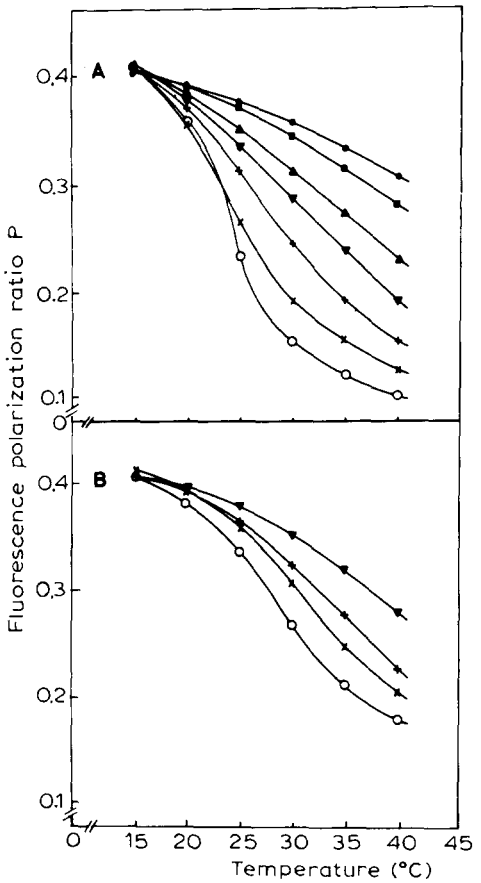

Fig. 9. Fluorescence polarization ratio, p, as a function of temperature for: A, cholesterol/DMPC mixtures with a cholesterol content of 0 (○———○); 5 (X———X); 10 (+———+); 20 (▼———▼); 28 (▲———▲); 43 (■———■); 53 (●———●) mol%; B, Isolated apoprotein (A-I and A-II)-lipid complexes at cholesterol-DMPC contents of: 0 (○———○); 5 (X———X); 10 (+———+) mol% cholesterol. At 20 mol% cholesterol (▼———▼) the curve is representative of an apoprotein-lipid mixture containing an excess of apoprotein.

ing an excess of apoprotein. At a given cholesterol content, the complexes generated with either apoprotein A-I or apoprotein A-II have the same fluorescence polarization pattern as a function of temperature. Compared to the original vesicles (Fig. 9A), the gel-to-liquid-crystalline transition of DMPC in the complex is characterized by a reduced amplitude and a shift of the transition to 28°C at 0 and 5 mol% cholesterol. Above 10 mol% cholesterol, the phospholipid transition disappears in the complexes, whilst it remains apparent up to 25 mol% in the pure lipid vesicles.

At cholesterol ratios higher than 25 mol%, the fluorescence polarization pattern measured for the apoprotein-lipid mixture was identical to that of the pure vesicles, suggesting that no interaction takes place when the gel-to-liquid-crystalline transition of the vesicles has disappeared. This hypothesis is supported by the ultracentrifugation and gel filtration data described above.

Discussion

Comparison of the kinetics of association between human apoproteins A-I and A-II and mixed cholesterol-DMPC liposomes documents the specificity of the apoprotein-lipid binding.

The optimal association seems to take place around the transition temperature of the phospholipid, where crystalline and liquid-crystalline structures coexist and where maximal disorder and structural lability enhance and facilitate the lipid-apoprotein association. This effect is specially pronounced for the apoprotein A-I-lipid association where the reaction rate decreases by a factor of 10 between 24 and 28°C compared to a factor of 2 for the apoprotein A-II. The addition of cholesterol to the DMPC liposomes enhances and accelerates the apoprotein-lipid association and this effect is especially pronounced for the apoprotein A-I.

The comparison of the kinetics of association of the two major HDL apoproteins suggest that the apoprotein A-II is able to interact with cholesterol-lecithin bilayers under a wide range of composition, temperature and crystalline state. The apoprotein A-I-lipid interaction is restricted to disordered structures presenting 'holes' of discontinuities [6] where the apoprotein can approach the phospholipid acyl chains. This difference might be related to the size and tertiary conformation of the two apoproteins; apoprotein A-II as a dimer contains short amphipatic helices [20] and can interact more easily with the bilayer than apoprotein A-I, which is easily self-associated and contains longer 'lipid-binding' segments.

This hypothesis is supported by the compositional data on the isolated apoprotein-lipid complexes. On a molar basis the apoprotein A-I-lipid complexes contain half as much protein as the apoprotein A-II-lipid complex, in agreement with the data of Scanu et al. [21] who could replace one apoprotein A-I by two apoprotein A-II molecules in the canine HDL. Above 24°C the conformation of the lipid is more ordered in the complexes than in the original vesicles as the phospholipid acyl chains are partially immobilized by the apoprotein.

Based on these observations, one can speculate upon the distribution of the apoproteins A-I and A-II in HDL. The cholesterol/phospholipid ratio in HDL varies between 25 mol% for HDL_3 and 36 mol% for HDL_2. In the hypothesis that apoprotein A-I preferentially exists at a discontinuity between phospholipid/cholesterol phases, the apoprotein A-II could be more evenly distributed and interact with either phospholipid or phospholipid/cholesterol.

For that which concerns the cholesterol uptake by HDL, the above data suggest that the apolar lipid would be preferentially incorporated in the vicinity of apoprotein A-II and that this particular apoprotein might play a preferential role in the cholesterol exchange and transport by HDL, in view of the extent of lipid-apoprotein A-II association and the stability of the complexes.

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References

1 Stein, Y., Glangeaud, M.C., Fainaru, M. and Stein, O. (1975) Biochim. Biophys. Acta 380, 106—118
2 Carew, T.E., Hayes, S.B., Koschinsky, T. and Steinberg, D. (1974) Lancet, 1315—1317
3 Assmann, G. and Brewer, H.B. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 989—993
4 Morrisett, J.D., Jackson, R.L. and Gotto, A.M. (1977) Biochim. Biophys. Acta 472, 93—133
5 Rosseneu, M., Soetewey, F., Middelhoff, G., Peeters, H. and Brown, W.V. (1976) Biochim. Biophys. Acta 441, 68—80
6 Pownall, H.J., Massey, J.B., Kusserow, S.K. and Gotto, A.M. (1979) Biochemistry 18, 574—579
7 Tall, A.R. and Lange, Y. (1978) Biochem. Biophys. Res. Commun. 80, 206—210
8 Jonas, A. and Krajnovich, D.J. (1978) J. Biol. Chem. 253, 5758—5763
9 Blaton, V., Vercaemst, R., Rosseneu, M., Mortelmans, J., Jackson, R.L., Gotto, A.M. and Peeters, H. (1979) Biochemistry 16, 2157—2163
10 Rosseneu, M. (1978) in The Lipoprotein Molecule (Peeters, H., ed.), pp. 129—154, Plenum Press, New York
11 Middelhoff, G., Rosseneu, M., Peeters, H. and Brown, W.V. (1976) Biochim. Biophys. Acta 441, 57—67
12 Rosseneu, M., Blaton, V., Vercaemst, R., Soetewey, F. and Peeters, H. (1977) Eur. J. Biochem. 74, 83—88
13 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, P.J. (1951) J. Biol. Chem. 193, 265—275
14 Eibl, H. and Lands, W.E.M. (1969) Anal. Biochem. 30, 51—57
15 Gamble, W., Vaughan, M., Kruth, H.S. and Avignan, J. (1978) J. Lipid Res. 19, 1068—1070
16 Rosseneu, M., Soetewey, F., Vercaemst, R., Lievens, M. and Peeters, H. (1977) Protides Biol. Fluids 25, 47—54
17 Jonas, A. (1975) Biochim. Biophys. Acta 393, 471—482
18 Rosseneu, M., Vercaemst, R., Van Tornout, P., Caster, H., Lievens, M.J. and Herbert, P.N. (1979) Eur. J. Biochem. 96, 357—362
19 Mabrey, S., Mateo, P.L. and Sturtevant, J.M. (1978) Biochemistry 17, 2464—2468
20 Segrest, J.D., Jackson, R.L., Morrisett, J.D. and Gotto, A.M. (1974) FEBS Lett. 38, 247—249
21 Lagocki, P.A. and Scanu, A.M. (1980) J. Biol. Chem. 255, 3701—3706