Biochimica et Biophysica Acta, 1126 (1992) 105–113

BBALIP 53919

Isolation and partial characterization of lipoprotein A-II (LP-A-II) particles of human plasma

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(Received 14 August 1991)

Key words: Lipoprotein; Lipoprotein A-II; Lipid; Apolipoprotein; Immunoaffinity chromatography; (Human plasma)

High density lipoproteins (HDL) consist of a mixture of chemically and functionally distinct families of particles defined by their characteristic apolipoprotein (Apo) composition. The two major lipoprotein families are lipoprotein A-I (LP-A-I) and lipoprotein A-I:A-II (LP-A-I:A-II). This study describes the isolation of a third minor HDL family of particles referred to as lipoprotein A-II (LP-A-II) because it lacks ApoA-I and contains ApoA-II as its main or sole apolipoprotein constituent. Because ApoA-II is an integral protein constituent of three distinct lipoprotein families (LP-A-I:A-II, LP-A-II:B:C:D:E and LP-A-II), LP-A-II particles were isolated from whole plasma by sequential immunoaffinity chromatography on immunosorbers with antisera to ApoA-II, ApoB and ApoA-I, respectively. In normolipidemic subjects, the concentration of LP-A-II particles, based on ApoA-II content, is 4–18 mg/dl accounting for 5–20% of the total ApoA-II not associated with ApoB-containing lipoproteins. The lipid composition of LP-A-II particles is characterized by low percentages of triglycerides and cholesterol esters and a high percentage of phospholipids in comparison with lipid composition of LP-A-I and LP-A-I:A-II. The major part of LP-A-II particles contain ApoA-II as the sole apolipoprotein constituent; however, small subsets of LP-A-II particles may also contain ApoD and other minor apolipoproteins. The lipid/protein ratio of LP-A-II is higher than those of LP-A-I and LP-A-I:A-II. In homozygous ApoA-I and ApoA-I/ApoC-III deficiencies, LP-A-II particles are the only ApoA-containing high density lipoprotein with levels found to be within the same range (7–13 mg/dl) as those of normolipidemic subjects. However, in contrast to normal LP-A-II, their lipid composition is characterized by higher percentages of triglycerides and cholesterol esters and a lower percentage of phospholipids and their apolipoprotein composition by the presence of ApoC-peptides and ApoE in addition to ApoA-II and ApoD. These results show that LP-A-II particles are a minor HDL family and suggest that, in the absence of ApoA-I-containing lipoproteins, they become an efficient acceptor/donor of ApoC-peptides and ApoE required for a normal metabolism of triglyceride-rich lipoproteins. Their other possible functional roles in lipid transport remain to be established in future experiments.

Introduction

Apolipoprotein (Apo) A-II is the second most abundant protein of high density lipoproteins (HDL) in human plasma accounting for approx. 30% of their total protein mass [1–3]. Its plasma concentration has been reported to range between 25–80 mg/dl [4], with more than 90% occurring in the density range 1.063–1.25 g/ml [3,5]. In man, ApoA-II mainly consists of two identical polypeptide chains of M_r 8707 covalently linked by a disulfide bond between the cysteine residues at position 6 [1,6]; it occurs, however, in smaller amounts in its monomeric form and as a heterodimer with ApoE [6,7]. Like ApoA-I, ApoA-II is a secretory protein synthesized as a larger precursor (preproprotein) containing a prepeptide and a signal peptide with 18 and five amino acid residues, respectively [8]. Whereas the cleavage of prepeptide occurs intracellularly, the conversion of proprotein to mature ApoA-II mainly occurs extracellularly in a reaction catalyzed by a procathepsin B-like proteinase [6,9].

[1] Deceased 12 January 1992.

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LP, lipoprotein; Apo, apolipoprotein.

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ApoA-II exists in plasma in several polymorphic forms [10,11].

Due to its high lipid-binding affinity, ApoA-II plays a significant role in the formation and structural stability of HDL particles [12–16]. It has been suggested from in vitro experiments that ApoA-II inhibits the activation of lecithin:cholesterol acyltransferase by ApoA-I [17–19] and modulates the activity of hepatic triglyceride lipase [20–22]. In a recent study, Carson has demonstrated that ApoA-II inhibits factor X activation by interfering with the association of tissue factor (coagulation factor III) with factor VIIa [23].

A number of studies have demonstrated that HDL consist of several distinct lipoprotein particles differing with respect to their particles sizes, densities, chemical composition and metabolic properties [24–27]. Among these lipoprotein particles identified and differentiated on the basis of their apolipoprotein composition [24], ApoA-II mainly occurs in association with ApoA-I as lipoprotein A-I:A-II (LP-A-I:A-II) [28–34]. The LP-A-I:A-II family is a polydisperse system of particles present throughout the HDL density range with the major part occurring in the HDL_3 subfraction [33,35]. Another lipoprotein form of ApoA-II has been recently identified and characterized as lipoprotein A-II:B:C:D:E (LP-A-II:B:C:D:E or LP-A-II:B complex) in VLDL of patients with Tangier disease or phenotype V hyperlipoproteinemia [36]. There are also a few reports indicating the existence of LP-A-II particles containing ApoA-II as the major protein constituent but devoid of ApoA-I and ApoB and first detected in HDL of patients with Tangier disease [37]. Subsequently, these particles have also been identified in low density lipoproteins (LDL) of a patient with ApoA-I deficiency and planar xanthoma [38], in HDL of normolipidemic subjects [39] and patients with visceral Leishmaniasis [40].

The purpose of this study was to provide information on the concentration, composition and lipoprotein subspecies of LP-A-II particles isolated by immunoaffinity chromatography from whole plasma of normolipidemic subjects and to compare their properties with those of LP-A-II particles isolated from patients with severe forms of ApoA-I deficiency.

Materials and Methods

Subjects

Six normolipidemic subjects, two subjects homozygous and two subjects heterozygous for ApoA-I/ApoC-III deficiency, two subjects homozygous and three subjects heterozygous for ApoA-I deficiency and planar xanthoma and one subject homozygous for Tangier disease were studied (Table I). The normolipidemic subjects were healthy, asymptomatic Caucasians (two women and four men with a mean age of 47 ± 14 years) without any history of familial hyperlipoproteinemia or diabetes mellitus. They were non-obese and non-smokers except for one subject consuming ten cigarettes per day. The alcohol consumption was below 50 g per week. One of the women was premenopausal and the other was postmenopausal; the latter was taking Premarin (1.25 mg per day) and was characterized by very high levels of ApoA-I and ApoA-II. The clinical features of two female patients with ApoA-I/ApoC-III deficiency and their heterozygous

TABLE I

Concentrations of lipids, apolipoproteins and LP-A-II particles

Subjects	Total cholesterol	Triglycerides	ApoA-I	ApoA-II	ApoA-II associated with LP-A-II particles
Normals ($n = 6$)	194 ± 37 [a]	86 ± 49	162 ± 73	86 ± 13	8.9 ± 4.9
ApoA-I/ApoC-III deficiency					
Homozygotes	129	15	0	12	10.5
($n = 2$)	(108–149)	(15–16)		(10–13)	(9–12)
Heterozygotes	128	21	88	53	8.5
($n = 2$)	(92–163)	(13–29)	(73–102)	(49–57)	(6–11)
ApoA-I deficiency					
Homozygotes	133	55	0	11	10.0
($n = 2$)	(128–137)	(35–74)		(8–14)	(7–13)
Heterozygotes ($n = 3$)	193 ± 16	111 ± 65	108 ± 10	78 ± 2	10.7 ± 5.5
Tangier disease ($n = 1$)	81	362	2	12	0.6

Values are expressed in mg/dl.

[a] Mean ± S.D.

relatives (patients' mother and son of one of the patients) have been previously reported [41]. Both homozygous patients had low levels of total cholesterol and triglycerides, very low levels of ApoA-II and immunochemically undetectable concentrations of ApoA-I and ApoC-III. The heterozygous relatives also had low levels of total cholesterol and triglycerides. Their ApoA-I levels were between those of the patients and normals (Table I) and their ApoC-III concentrations (2.2 mg/dl) were approximately one-third to one-fourth of those considered normal for their age and sex (7.0 ± 0.2 mg/dl for males aged 11–14 and 11.3 ± 0.4 mg/dl for females aged 51–60) [42]. In another kindred, two apparently homozygous patients and three of their relatives belong to a recently described variant of ApoA-I deficiency [43]. Clinically, both patients were characterized by planar xanthoma, xanthelasma and corneal opacities, but normal appearance of tonsils and no tendon xanthoma; one of the patients had a coronary bypass surgery when she was 58 years of age. Both patients had moderately low levels of total cholesterol and normal levels of triglyceride (Table I). The main biochemical abnormalities were the severe deficiency, if not the absence, of ApoA-I and low concentrations of ApoA-II, ApoC-II (1.1 and 1.4 mg/dl), ApoC-III (2.2 and 3.3 mg/dl) and ApoD (6.7 mg/dl) but normal levels of ApoE (11.2 and 11.7 mg/dl). The apparently heterozygous relatives had normal concentrations of total cholesterol and triglycerides, but their levels of ApoA-I were more than one standard deviation below the normal mean values for their age and sex [42]; the concentrations of ApoC peptides, ApoD and ApoE were in the upper normal range for their age and sex [42]. The clinical and biochemical features of the homozygous Tangier patient have been previously described [36]. None of the dyslipoproteinemic subjects was taking drugs known to influence lipid concentrations with the exception of patients with ApoA-I/ApoC-III deficiency who were treated daily with β-blockers and soya lecithin.

Venous blood was drawn into EDTA-containing Vacutainer tubes from normolipidemic and dyslipoproteinemic subjects after an overnight fast and the plasma samples were collected by low-speed centrifugation. Plasma samples collected at the Henry Ford Hospital, Detroit, MI (ApoA-I/ApoC-III deficiency), University Health Center, Ann Arbor, MI (ApoA-I deficiency) and Molecular Disease Branch, NIH, Bethesda, MD (Tangier disease) were immediately shipped at 4°C and received in Oklahoma City, OK, the next morning. Upon receipt, preservatives were added to final concentrations of 500 units/ml penicillin-G, 50 μg/ml streptomycin sulfate, 1.3 mg/ml ε-amino caproic acid and 0.5 mg/ml reduced glutathione [44]. All blood donors signed written informed consents. The protocols were approved by the Institutional Review Boards.

Preparation of immunosorbers

To prepare immunosorbers, well-characterized 'pan' monoclonal antibodies to ApoA-I [45], ApoA-II [36] and ApoB [46] were coupled to the cross-linked agarose activated with *N*-hydroxysuccinimide (AffiGel 10, Bio-Rad, Richmond, CA) according to the previously described procedures [34,47]. The binding capacities of the anti-ApoA-I, anti-ApoA-II and anti-ApoB immunosorbers were 0.018 mg of ApoA-I, 0.027 mg of ApoA-II and 0.052 mg of ApoB per ml of gel, respectively. The immunosorbers were stable for more than 6 months at 4°C without loss of binding capacity.

Isolation of ApoA-II-containing lipoprotein particles

The fractionation of LP-A-I:A-II, LP-A-II:B complex and LP-A-II was performed by a recently described sequential, three-step immunoaffinity chromatography with anti-ApoA-II, anti-ApoB and anti-ApoA-I immunosorbers [43].

Crossed immunoelectrophoresis

Immunochemical characterization of LF-A-II particles was carried out by crossed immunoelectrophoresis performed according to a previously described procedure [40] using 1% agarose (Indubiose HA37, IBF Laboratories, Villeneuve-la-Garenne, France) as the supporting medium and a mixture (1 : 1, v/v) of 0.06 M barbital buffer (pH 8.6) and Tris-HCl, EDTA and boric acid solutions (10.5; 0.50; 0.65 g/l), pH 9.0. Starting material was either the ApoA-II-containing lipoprotein particles (retained fraction from the anti-ApoA-II immunosorber) or the isolated LP-A-II family (unretained fraction from the anti-ApoA-I immunosorber).

Lipid and apolipoprotein analyses

Neutral lipids were quantified by the gas-liquid chromatographic procedure of Kuksis et al. [48] and the phospholipid phosphorus content was determined by the method of Gerlach and Deuticke [49]. Quantitative determination of apolipoproteins A-I, A-II, B, C-II, C-III, D and E was performed by electroimmunoassays developed in this laboratory and described in several previous reports [34,36,38,47]. Monospecific, polyclonal antisera to apolipoproteins A-I, A-II, B, C-II, C-III, D and E were prepared in rabbits and goats and characterized as previously described [50].

Results

Plasma concentrations of ApoA-II associated with LP-A-I:A-II and LP-A-II particles

The fractionation of ApoA-II-containing lipoproteins was monitored by quantitative determination of ApoA-II in all retained and unretained column fractions. The absolute values of ApoA-II associated with LP-A-II particles were calculated on the basis of plasma

TABLE II

Lipid composition of LP-A-II particles

Subjects	Triglycerides	Cholesterol esters	Free cholesterol	Phospholipids
Normals ($n = 6$)	0.8 ± 1.1 [a]	11.8 ± 3.7	4.6 ± 2.1	82.8 ± 4.3
ApoA-I/ApoC-III deficiency				
Homozygotes	2.0	27.6	7.9	62.4
($n = 2$)	(1.2–2.8)	(15.7–39.6)	(7.7–8.2)	(49.9–74.9)
Heterozygotes	N.D.	8.9	5.2	85.9
($n = 2$)		(7.5–10.3)	(4.2–6.1)	(83.6–88.3)
ApoA-I deficiency				
Homozygotes	12.1	18.5	6.1	63.2
($n = 2$)	(5.3–19.0)	(15.8–21.3)	(5.0–7.2)	(60.2–66.2)
Heterozygotes	13.0	14.0	5.9	67.0
($n = 3$)	(3.9–19.8)	(11.2–16.2)	(4.8–7.5)	(61.2–75.9)
Tangier disease ($n = 1$)	0.4	6.1	3.9	89.6

Values are expressed in %.

[a] Mean ± S.D.

N.D., not detected by gas-liquid chromatography.

ApoA-II values and percent distribution of ApoA-II among ApoA-II-containing lipoproteins. After removal of LP-A-II:B complex, the most important criterion for the separation of LP-A-II from ApoA-I-containing lipoproteins was the immunochemically determined absence of ApoA-I in LP-A-II. In normolipidemic subjects, LP-A-II represented the minor (10.5 ± 5.5% of ApoA-II) and LP-A-I:A-II the major (89.5 ± 5.5% of ApoA-II) ApoA-II-containing lipoprotein devoid of ApoB; the concentration of LP-A-II particles ranged between 4–18 mg/dl and accounted for 5–20% of the total ApoA-II not associated with ApoB-containing lipoproteins (Table I). Although representing the only ApoA-containing lipoprotein family in homozygous patients with either ApoA-I/ApoC-III or ApoA-I deficiencies, the mean concentrations of LP-A-II particles (7–13 mg/dl) were only slightly higher than those of normolipidemic subjects (Table I). The levels of LP-A-II particles (5–16 mg/dl) in patients heterozygous for ApoA-I deficiencies were slightly higher than those of

TABLE III

Apolipoprotein composition of LP-A-II particles

Subjects	Apolipoproteins (%)				
	A-II	C-II	C-III	D	E
Normals					
($n = 3$)	77.4 ± 36[a]	N.D.	N.D.	22.6 ± 3.6	N.D.
($n = 2$)	100.0	N.D.	N.D.	N.D.	N.D.
($n = 1$)	88.7	N.D.	N.D.	3.8	7.5
ApoA-I/ApoC-III deficiency					
Homozygotes	64.3	N.D.	N.D.	4.9	30.8
($n = 2$)	(56.2–72.3)			(1.6–8.2)	(26.1–35.6)
Heterozygotes	86.3	N.D.	N.D.	13.7	N.D.
($n = 2$)	(85.7–86.9)			(13.1–14.3)	
ApoA-I deficiency					
Homozygotes	50.6	0.4	2.7	15.8	30.1
($n = 2$)	(47.0–54.6)	(0.2–0.6)	(2.0–3.5)	(12.3–19.3)	(21.8–38.6)
Heterozygotes	95.8	N.D.	N.D.	4.1	N.D.
($n = 3$)	(94.6–98.1)			(1.9–5.4)	

[a] Mean ± S.D.

N.D., not detected by electroimmunoassay.

normal subjects but essentially in the same range as those of homozygotes. Although in patients with Tangier disease the LP-A-II:B complex represented the major ApoA-II lipoprotein form [36], a small but measurable concentration of LP-A-II particles was also detected confirming the previously reported presence of this lipoprotein family in Tangier disease [37].

Lipid and apolipoprotein composition of LP-A-II particles

The percent lipid composition of LP-A-II particles from normolipidemic subjects, heterozygotes for ApoA-I/ApoC-III deficiency and a patient with Tangier disease was characterized by a negligible content of triglycerides, a very high content of phospholipids and a low ratio of cholesterol esters/free cholesterol (Table II) in comparison with the lipid composition of LP-A-I or LP-A-I:A-II particles [34]. In contrast, the lipid composition of LP-A-II from patients homozygous for either ApoA-I/ApoC-III or ApoA-I deficiency was characterized by increased percent contents of triglycerides and cholesterol esters and a decreased relative content of phospholipids (Table II) resembling the lipid composition of normal LP-A-I:A-II and, especially, LP-A-I particles rather than that of normal LP-A-II particles.

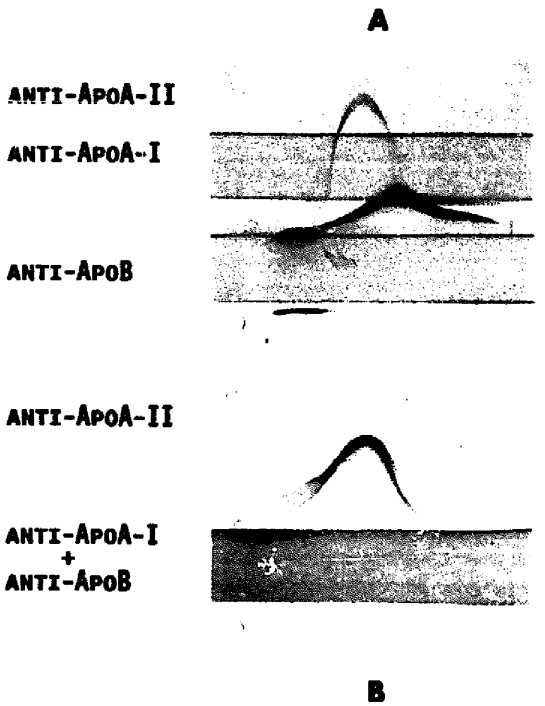

Fig. 1. Crossed immunoelectrophoresis of ApoA-II-containing lipoproteins isolated from whole plasma by affinity chromatography on an anti-ApoA-II immunosorber (the retained fraction). Panel A: normolipidemic plasma tested with anti-ApoB (a polyclonal antiserum to ApoB) incorporated in the lower gel, anti-ApoA-I (a polyclonal antiserum to ApoA-I) in the intermediate gel and anti-ApoA-II (a polyclonal antiserum to ApoA-II) in the upper gel. Panel B: plasma from a patient homozygous for ApoA-I/ApoC-III deficiency tested with a mixture of anti-ApoA-I and anti-ApoB incorporated in the lower gel and anti-ApoA-II in the upper gel. Experimental conditions are described in Materials and Methods.

In some normolipidemic subjects ApoA-II was the only apolipoprotein constituent of LP-A-II particles (Table III). However, in most normolipidemic subjects including heterozygotes for ApoA-I deficiencies, ApoA-II was found to be the major and ApoD the minor apolipoprotein. In contrast, the LP-A-II particles from homozygous patients with ApoA-I deficiencies contained ApoA-II, ApoE, ApoD and ApoC-peptides in descending order of their percent contents.

The lipid/apolipoprotein ratios of LP-A-II particles from normolipidemic subjexts (2.2) and heterozygotes for ApoA-I deficiency syndromes (2.2) were identical. However, the reduced lipid/apolipoprotein ratio (1.3) of LP-A-II particles from homozygous patients for ApoA-I deficiencies reflected increased apolipoprotein contents of these particles in the absence of ApoA-I-containing lipoproteins.

Immunochemical evidence for the heterogeneity of ApoA-II-containing lipoproteins and LP-A-II particles

To identify major ApoA-II-containing lipoprotein particles, the retained fraction from anti-ApoA-II immunosorber was tested by crossed immunoelectrophoresis with polyclonal antisera to apolipoproteins A-I, A-II and ApoB. The use of anti-ApoB in the lower gel, anti-ApoA-I in the intermediate gel and anti-ApoA-II in the upper agarose gel (Fig. 1, panel A) revealed the presence, in normolipidemic plasma, of three distinct ApoA-II-containing lipoprotein particles including the precipitin line of LP-A-II:B complex in the lower gel, LP-A-I:A-II rocket in the intermediate gel and LP-A-II rocket in the upper gel. On the other hand, in a patient homozygous for ApoA-I/ApoC-III deficiency, the immunoelectrophoretic pattern (Fig. 1, panel B) showed the presence of LP-A-II:B complex in the lower gel and LP-A-II in the upper gel, but the absence of LP-A-I:A-II in the intermediate gel.

The relatively high percentage contents of ApoD and ApoE in LP-A-II particles of homozygous patients with ApoA-I deficiency syndromes suggested the possible heterogeneity of this lipoprotein family, i.e., the occurrence of several LP-A-II subfamilies differing in their apolipoprotein composition. Testing of LP-A-II with anti-ApoA-I and anti-ApoB sera incorporated into the lower gel and anti-ApoA-II serum in the upper gel showed that these particles were devoid of ApoA-I and ApoB (Fig. 2, panel A). The use of anti-ApoD and anti-ApoE in the lower agarose gel and anti-ApoA-II in the upper agarose gel revealed that LP-A-II family consisted of the so-called simple LP-A-

II particles (LP-A-II particles only containing ApoA-II as their protein moiety) forming a rocket in the upper gel and complex LP-A-II : D and LP-A-II : E particles precipitated as two separate lines in the lower gel (Fig. 2, panel B). The simple LP-A-II particles migrated faster in the first dimension than complex LP-A-II : D and LP-A-II : E particles. To provide additional evidence for the existence of LP-A-II : D and LP-A-II : E particles, the anti-ApoE serum was incorporated into the lower gel and anti-ApoD serum in the upper gel (Fig. 2, panel C); ApoE-containing particles were precipitated in the lower gel and the LP-A-II : D particles devoid of ApoE formed a precipitin line in the intermediate gel. When the antisera were reversed (Fig. 2, panel D) LP-A-II : D formed a precipitin line in the lower gel and LP-A-II : E formed a rocket in the intermediate gel. These experiments, however, have not excluded the possible, albeit unlikely, existence of LP-A-II : D : E particles. The distribution of ApoC-peptides among these LP-A-II subfractions in homozygous patients with ApoA-I deficiency remains to be established in future experiments.

Discussion

The chemical and metabolic heterogeneity of HDL is due to the presence of discrete lipoprotein families of particles defined by their characteristic apolipoprotein composition. The application of immunoaffinity chromatography to the fractionation of HDL particles has resulted in the recognition and isolation of LP-A-I and LP-A-I : A-II as two major ApoA-I-containing lipoprotein families. The chemical and metabolic uniqueness of these two lipoprotein families has been demonstrated by several independent studies [26–34]. The present report confirms and extends previous observations [37–40] of a third minor ApoA-containing lipoprotein family, LP-A-II, characterized by ApoA-II as its major protein constituent.

In normolipidemic subjects, the concentration of LP-A-II accounted for 5–20% of the total plasma ApoA-II content not associated with ApoB. A comparison between the chemical characteristics of LP-A-II and LP-A-I and LP-A-I : A-II particles isolated from the same normolipidemic subjects [34] attested to the chemical uniqueness of this minor lipoprotein family. In contrast to LP-A-I and LP-A-I : A-II particles, it only contained ApoD as an additional apolipoprotein constituent except for a rare presence of a small amount of ApoE. Its lipid composition was characterized by the virtual absence of triglycerides, a significantly higher percentage of phospholipids (82.8 ± 4.3 vs 66.7 ± 8.4 and $58.2 \pm 5.2\%$, $P < 0.01$–0.001) and a significantly

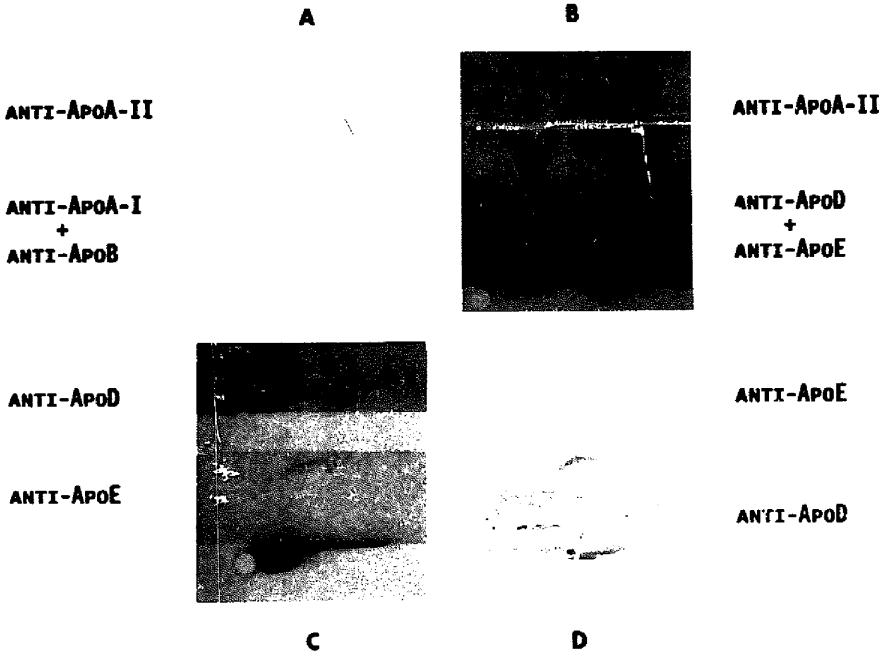

Fig. 2. Crossed immunoelectrophoresis of LP-A-II particles isolated from whole plasma of a patient homozygous for ApoA-I deficiency by sequential affinity chromatography on immunosorbers with anti-ApoA-II and anti-ApoB sera. Panel A: LP-A-II particles tested with anti-ApoA-I and anti-ApoB in the lower gel and anti-ApoA-II in the upper gel. Panel B: LP-A-II particles tested with anti-ApoD (a polyclonal anti serum to ApoD) and anti-ApoE (a polyclonal antiserum to ApoE) incorporated in the lower gel and anti-ApoA-II in the upper gel. Panel C: LP-A-II particles tested with anti-ApoE incorporated in the lower gel and anti-ApoD in the upper gel. Panel D: LP-A-II particles tested with anti-ApoD incorporated in the lower gel and anti-ApoE in the upper gel. Experimental conditions are described in Materials and Methods

lower percentage of cholesterol esters (11.8 ± 3.7 vs. 24.2 ± 6.7 and $32.3 \pm 4.5\%$, $P < 0.01$–0.001) than those of homologous LP-A-I and LP-A-I : A-II particles. As a consequence, the cholesterol ester/free cholesterol ratio of LP-A-II particles (2.5 ± 1.5) was significantly lower than those of LP-A-I (3.9 ± 1.3) and LP-A-I : A-II (5.3 ± 1.2). In addition, the lipid/apolipoprotein ratio of LP-A-II (2.26) was approx. 2-fold higher than the corresponding ratios of LP-A-I (1.24) and LP-A-I : A-II (0.90).

In the absence of ApoA-I, the only detectable ApoA-containing lipoproteins are LP-A-II particles, the concentration of which does not differ significantly from those of normolipidemic subjects or subjects heterozygous for A-I deficiency syndromes. However, there are marked differences in the chemical composition of LP-A-II. The lipid profile of LP-A-II from homozygous patients was characterized by a significantly higher percentage of triglycerides and a lower percentage of phospholipids in comparison with those of normal LP-A-II. The apolipoprotein composition of LP-A-II from homozygotes was characterized by a relatively high content of ApoE and, in the case of ApoA-I deficiency, presence of ApoC peptides accounting for up to 53% of the total protein mass; the protein content of ApoD was either within the normal range or markedly decreased. In hypercatabolic states such as Tangier disease, the HDL particles are quickly removed from the plasma [51,52] which, judged from the low concentration of LP-A-II, also seems to apply to this lipoprotein family. It should be pointed out, however, that in Tangier disease the major part of ApoA-II occurs in the LP-A-II : B complex particles of very low and low densities [36].

The presence of ApoD and other minor apolipoproteins in LP-A-II particles of normolipidemic and dyslipoproteinemic subjects suggested the possible subspecies heterogeneity of this lipoprotein family. As shown previously for LP-A-I and LP-A-I : A-II particles [34], minor apolipoproteins cannot be equally distributed on all LP-A-II particles, because they are not present in the same molar ratios. In normolipidemic subjects, LP-A-II particles with ApoA-II as the sole apolipoprotein constituent are frequently the only lipoprotein form. However, even in subjects whose LP-A-II particles contain both ApoA-II and ApoD, the major lipoprotein form is the simple LP-A-II with ApoA-II as the sole protein and the minor form is LP-A-II : D. The crossed immunoelectrophoretic testing of ApoA-II-containing lipoproteins in patients homozygous for ApoA-I deficiencies provided evidence for the occurrence not only of simple LP-A-II particles but also for the presence of separate LP-A-II: D and LP-A-II : E particles (Fig. 2). The distribution of ApoC peptides, when present, in these LP-A-II subspecies remains to be established. It should also be pointed out that the protein moiety of LP-A-II : E subspecies mainly consists of a mixed disulfide complex, ApoE-ApoA-II monomer, as previously reported for homozygous patients with ApoA-I/ApoC-III deficiency [53].

The origin of LP-A-II particles is not known. However, it is quite probable that these particles are formed primarily, if not exclusively, in the liver as nascent lipoproteins rich in phospholipid and free cholesterol. Fractionation of HepG2 cell medium by sequential affinity chromatography on concanavalin A and immunosorbers with anti-ApoA-II and anti-ApoA-I resulted in the identification of a small lipoprotein fraction containing ApoA-II, but not ApoA-I, suggestive of LP-A-II particles (Dashti, N., personal communication). The previously established usefulness of this cell line as a model for studying the formation of LP-A-I and LP-A-I : A-II particles [54,55] may prove to be equally useful for further studies on the origin and assembly of LP-A-II particles.

At the present time we can only speculate about the possible physiologic role(s) of ApoA-containing lipoproteins including LP-A-II. It has already been suggested that LP-A-I or LP-A-I : A-IV particles may function as the acceptors of peripheral cholesterol [25,56,57] and LP-A-I : A-II particles as the acceptors of lipids and apolipoproteins released during the lipolysis of triglyceride-rich lipoproteins or, conversely, as donors of these apolipoproteins to newly secreted triglyceride-rich lipoproteins of hepatic and intestinal origin [58,59]. Evidence presented in this study suggests that, in the absence of ApoA-I lipoproteins, LP-A-II particles may function as substitute acceptors and donors of lipids, ApoC-peptides and ApoE taking part in processes responsible for the catabolism of triglyceride-rich lipoproteins. In fact, the capacity of LP-A-II particles to bind and incorporate neutral lipids and minor apolipoproteins appears to be similar, if not greater, than those of LP-A-I and LP-A-I : A-II. However, due to their relatively low plasma concentration, the LP-A-II particles may not be functionally as efficient as LP-A-I : A-II particles, the most abundant ApoA-containing lipoprotein family. Some of the other possible roles for LP-A-II may be to function as a precursor, in conjunction with LP-B : C : E particles, in the formation of triglyceride-rich LP-A-II : B : C : D : E particles [36], as modulators of hepatic triglyceride lipase [20–22] and lecithin:cholesterol acyltransferase [17–19] activities, as inhibitors of factor X activation [23] and as antagonists in processes responsible for the removal of cholesterol from peripheral cells [56,57]. These possible functional roles remain to be examined in future experiments.

Acknowledgements

The authors thank Clinton Corder, M.D., Ph.D., Oklahoma Medical Research Foundation, for supply-

ing plasma samples from normolipidemic donors and H. Bryan Brewer, Jr., M.D., Molecular Disease Branch, National Heart, Lung and Blood Institute, National Institutes of Health, for a plasma sample from a patient with Tangier disease. The authors also wish to thank Ms. Carmen Quiroga, Mr. Jim Fesmire and Mr. Randy Whitmer for their technical assistance, and Ms. Margo French for preparation of the manuscript. This study was supported, in part, by grant HL-23281 from the National Institutes of Health and by the resources of the Oklahoma Medical Research Foundation.

References

1 Shore, V. and Shore, B. (1968) Biochemistry 7, 3396–3403.
2 Brewer, H.B., Jr., Lux, S.E., Ronan, R. and John, K.M. (1972) Proc. Natl. Acad. Sci. USA 69, 1304–1308.
3 Herbert, P.N., Assman, G., Gotto, A.M., Jr. and Fredrickson, D.S. (1983) in The Inherited Basis of Metabolic Diseases (Stanbury, J.B., Wyngaarden, J.B., Fredrickson, D.S., Goldstein, J.L. and Brown, M.S., eds.), 5th Edn., pp. 589–621, McGraw-Hill, New York.
4 Labeur, C., Shepherd, J. and Rosseneu, M. (1990) Clin. Chem. 36, 591–597.
5 Puchois, P., Luley, C. and Alaupovic, P. (1987) Clin. Chem. 33, 1597–1602.
6 Scanu, A. (1987) in The Plasma Proteins (Putman, F.W., ed.), Vol. V., pp. 141–190, Academic Press, New York.
7 Weisgraber, K.H. and Mahley, R.W. (1978) J. Biol. Chem. 253, 6281–6289.
8 Gordon, J.I., Budelier, K.A., Sims, H.F., Edelstein, C., Scanu, A.M. and Strauss, A.W. (1983) J. Biol. Chem. 258, 14054–14059.
9 Gordon, J.I., Sims, H.F., Edelstein, C., Scanu, A.M. and Strauss, A.W. (1985) J. Biol. Chem. 260, 14824–14831.
10 Schmitz, G., Ilsemann, K., Melnik, B. and Assmann, G. (1983) J. Lipid Res. 24, 1021–1029.
11 Sprecher, D.L., Taam, L. and Brewer, H.B., Jr. (1984) Clin. Chem. 30, 2084–2092.
12 Segrest, J.P., Jackson, R.L., Morrisett, J.D. and Gotto, A.M., Jr. (1974) FEBS Lett. 38, 247–253.
13 Ritter, M.C. and Scanu, A.M. (1979) J. Biol. Chem. 254, 2517–2525.
14 Van Tornout, P., Caster, H., Lievens, M.-J., Rosseneu, M. and Assmann, G. (1981) Biochim. Biophys. Acta 663, 630–636.
15 Ibdah, J.A., Krebs, K.E. and Phillips, M.C. (1989) Biochim. Biophys. Acta 1004, 300–308.
16 Shen, B.W. (1985) J. Biol. Chem. 260, 1032–1039.
17 Fielding, C.J., Shore, V.G. and Fielding, P.E. (1972) Biochem. Biophys. Res. Commun. 46, 1493–1498.
18 Soutar, A.K., Garner, C.W., Baker, H.N., Sparrow, J.T., Jackson, R.L., Gotto, A.M. and Smith, L.C. (1975) Biochemistry 14, 3057–3064.
19 Chung, J., Abano, D.A., Fless, G.M. and Scanu, A.M. (1979) J. Biol. Chem. 254, 7456–7464.
20 Jahn, C.E., Osborne, J.C., Jr., Shaefer, E.J. and Brewer, H.B., Jr. (1981) FEBS Lett. 131, 366–368.
21 Kubo, M., Matsuzawa, J., Yokoyama, S., Tajima, S., Ishikawa, K., Yamamoto, A. and Terui, S. (1982) J. Biochem. (Tokyo) 865–870.
22 Shinomiya, M., Sasaki, N., Barnhart, R.L., Shirai, K. and Jackson, R.L. (1982) Biochim. Biophys. Acta 713, 292–299.
23 Carson, S.D. (1987) J. Biol. Chem. 262, 718–721.
24 Alaupovic, P. (1984) in Clinical and Metabolic Aspects of High Density Lipoproteins (Miller, N.E. and Miller, G.J., eds.), pp. 1–44, Elsevier, Amsterdam.
25 Fielding, C.J. and Fielding, P.E. (1981) Proc. Natl. Acad. Sci. USA 78, 3911–3914.
26 Cheung, M.C., Wolf, A.C., Lum, K.D., Tollefson, J.H. and Albers, J.J. (1986) J. Lipid Res. 27, 1135–1144.
27 Brewer, H.B., Jr., Rader, D.J., Hoeg, J.M., Mann, A. and Tennyson, G. (1991) Adv. Exp. Med. Biol. 285, 237–244.
28 Albers, J.J. and Aladjem, F. (1971) Biochemistry 10, 3436–3442.
29 Suenram, A. and Alaupovic, P. (1977) in Atherosclerosis IV (Schettler, G., Goto, I., Hata, I. and Klose, G., eds.), p. 248, Springer-Verlag, Berlin.
30 Atmeh, R.F., Shepherd, J. and Packard, C.J. (1983) Biochim. Biophys. Acta 751, 175–188.
31 Cheung, M.D. and Albers, J.J. (1984) J. Biol. Chem. 259, 12201–12209.
32 Ohta, T., Hattori, S., Nishiyama, S. and Matsuda, I. (1988) J. Lipid Res. 29, 721–728.
33 James, R.W., Proudfoot, A. and Pometta, D. (1989) Biochim. Biophys. Acta 1002, 292–301.
34 Bekaert, E.D., Alaupovic, P., Knight-Gibson, C., Blackett, P.R. and Ayrault-Jarrier, M. (1991) Pediatr. Res. 29, 315–321.
35 Cheung, M.D. and Albers, J.J. (1982) J. Lipid Res. 23, 747–753.
36 Alaupovic, P., Knight-Gibson, C., Wang, C.-S., Downs, D., Koren, E., Brewer, H.B., Jr. and Gregg, R.E. (1991) J. Lipid Res. 32, 9–19.
37 Assmann, G., Herbert, P.N., Fredrickson, D.S. and Forte, T. (1977) J. Clin. Invest. 60, 242–252.
38 Gustafson, A., McConathy, W.J., Alaupovic, P., Curry, M.D. and Persson, B. (1979) Scand. J. Clin. Lab. Invest. 39, 377–387.
39 März, W. and Gross, W. (1988) Biochim. Biophys. Acta 962, 155–158.
40 Bekaert, E.D., Kallel, R., Bouma, M.E., Lontie, J.F., Mebazza, A., Malmendier, C.L. and Ayrault-Jarrier, M. (1989) Clin. Chim. Acta 184, 181–192.
41 Norum, R.A., Lakier, J.B., Goldstein, S., Angel, A., Goldberg, R.B., Block, W.D., Noffze, D.K., Dolphin, P.J., Edelglass, J., Bogorad, D.D. and Alaupovic, P. (1982) N. Engl. J. Med. 306, 1513–1519.
42 Alaupovic, P., McConathy, W.J., Fesmire, J., Tavella, M. and Bard, J.M. (1988) Clin. Chem. 34, B13–B27.
43 Bekaert, E.D., Alaupovic, P., Knight-Gibson, C.S., Laux, M.J., Pelachyk, J.M. and Norum, R.A. (1991) J. Lipid Res. 32, 1587–1599.
44 Lee, D.M., Valente, A.J., Kuo, W.H. and Maeda, H. (1981) Biochim. Biophys. Acta 666, 133–146.
45 Bekaert, E.D., Ayrault-Jarrier, M., Petit, E., Betourne, C., Robin, H. and Polonovski, J. (1988) Clin. Chem. 34, 1030–1035.
46 Koren, E., Solter, D., Lee, D.M., Reiner, Z., McConathy, W.J., Dashti, N. and Alaupovic, P. (1986) Biochim. Biophys. Acta 876, 91–100.
47 Koren, E., Knight-Gibson, C., Wen, G., DeBault, L.E. and Alaupovic, P. (1986) Biochim. Biophys. Acta 876, 101–107.
48 Kuksis, A., Myher, J.J., Marai, L. and Geher, K. (1981) J. Chromatogr. Sci. 13, 423–430.
49 Gerlach, E. and Deuticke, B. (1963) Biochem. Z. 337, 477–479.
50 Alaupovic, P., Lee, D.M. and McConathy, W.J. (1972) Biochim. Biophys. Acta 260, 689–707.
51 Schaefer, E.J., Blum, C.B., Levy, R.I., Jenkins, L.L., Alaupovic, P., Foster, D. and Brewer, H.B., Jr. (1978) N. Engl. J. Med. 299, 905–910.
52 Bojanovski, D., Gregg, R.E., Zech, L.A., Meng, M.S., Bishop, C., Ronan, R. and Brewer, H.B., Jr. (1987) J. Clin. Invest. 80, 1742–1747.
53 Forte, T.M., Nichols, A.V., Krauss, R.M. and Norum, R.A. (1984) J. Clin. Invest. 74, 1601–1613.
54 Dashti, N., Koren, E. and Alaupovic, P. (1989) Biochem. Biophys. Res. Commun. 163, 574–580.

55 Cheung, M.C., Lum, K.D., Brouillette, C.G. and Bisgaier, C.L. (1989) J. Lipid Res. 30, 1429–1436.
56 Barbaras, R., Puchois, P., Fruchart, J.-C. and Ailhaud, G. (1987) Biochem. Biophys. Res. Commun. 142, 63–69.
57 Steinmetz, A., Barbaras, R., Ghalim, N., Clavey, V., Fruchart, J.-C. and Ailhaud, G. (1990) J. Biol. Chem. 265, 7859–7863.
58 Alaupovic, P. (1981) Can. J. Biochem. 59, 565–579.
59 Eisenberg, S. (1984) J. Lipid Res. 25, 1017–1058.