ISOLATION OF A HIGH DENSITY LIPOPROTEIN WITH HIGH CONTENTS OF ARGININE-RICH APOPROTEIN (apoE) FROM RAT PLASMA

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1. Introduction

Although the rat has been extensively used as an animal model for studies on the plasma lipoprotein metabolism, the protein components of the rat lipoprotein classes have not until recently been explored in greater detail. It has been shown [1,2] that the protein part of HDL which is the major lipoprotein density class of the rat is more complex than that of human HDL. The presence of C-proteins and of monomeric apoA-II in rat HDL was demonstrated [3]. Using SDS–polyacrylamide gel electrophoresis, a major apoprotein with mol. wt 27 000 was shown [4] to be analogous to human apoprotein A-I. Two additional components not present in normal human HDL were found in rat HDL. One of these, which had app. mol. wt 46 000, has no known human analogue. The other one which had approx. mol. wt 35 000, is homologous with human arginine-rich protein [5] also called apoE [6].

The presence of apoE in normal human HDL is under debate; however, it has been demonstrated that HDL from patients with familial LCAT deficiency or longstanding biliary obstruction contains considerable amounts of apoE [6,7]. In a current study of lipoprotein patterns of rats with induced hepatobiliary damage we have found that the normal rat contained an HDL species with an abundance of apoE but little or no apoA-I. The isolation by zonal ultracentrifugation and partial characterization of this lipoprotein will be described here.

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoprotein; LCAT, lecithin cholesterol acyl transferase; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; apoC, C apolipoproteins; apoE, arginine-rich apolipoprotein; SDS, sodium dodecylsulphate

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2. Materials and methods

2.1. *Animals*

Male Sprague-Dawley rats (250–300 g) were fed a standard laboratory diet ad libitum for at least 4 days. The animals were fasted for 16 h before aortic puncture under light ether anaesthesia. The blood was collected in the presence of Na_2EDTA (1 mg/ml) and plasma separated within 1 h. Plasma from 10–15 rats were pooled and ultracentrifuged within 5 h.

2.2. *Zonal ultracentrifugation*

Lipoprotein fractionation was performed in a Beckman-Spinco ultracentrifuge model L2-65 equipped with a Ti-14 rotor. A density gradient of NaBr (550 ml, 1.00–1.29 kg/l, linear to rotor volume) was used for the fractionation of lipoproteins from 20–30 ml rat plasma. An overlay of 30 ml 0.01 M Tris–HCl (pH 7.4) containing 1 mM Na_2EDTA (d = 1.0 kg/l) and a cushion of NaBr solution (d = 1.30 kg/l) were used to fill the rotor completely. Ultracentrifugation was carried out at 15°C for 15 h at 46 000 rev/min (max RCF = 158 000 × g). The rotor content was collected in 7.5 ml fractions and A_{280} determined for each fraction.

Pooled fractions were extensively dialyzed (Spectrapore tubing, mol. wt cut-off 3500, Spectrum Medical Industries, Los Angeles) to remove NaBr and concentrated by dialysis against a 40% (w/v) solution of polyethylene glycol (mean mol. wt 20 000) to a protein concentration of 0.5–1 g/l.

2.3. *Electroimmunoassay*

Electroimmunoassay was performed as in [8] with an antiserum to dog β-lipoprotein [9] showing distinct immunoreactivity against rat LDL.

2.4. *Polyacrylamide gel electrophoresis*

Polyacrylamide gel electrophoresis was performed in 8 M urea after delipidation with tetramethyl urea [10] or in SDS [11]. Samples were reduced with 2-mercaptoethanol before electrophoresis in SDS.

2.5. *Lipid analyses*

Lipids were extracted with chloroform/methanol, 2:1 (v/v). The main lipid classes were separated by thin-layer chromatography on silica gel with petroleum ether/ethyl ether/glacial acetic acid (90:10:1) as mobile phase. The lipids were localized by iodine vapour and eluted from the scraped areas by chloroform/methanol, 1:1.

Triglycerides were determined with an enzymatic method [12] with reagents from Boehringer Mannheim. Cholesterol and cholesterol esters were determined enzymatically [13]. Phospholipids were determined by analysis of phosphorus in the lipid extract [14].

2.6. *Electron microscopy*

Lipoprotein samples (0.5–1 g/l) were dialyzed overnight against 0.1 M ammonium acetate and then mixed with equal vol. 2% (w/v) sodium phosphotungstate, pH 7.4. The samples were immediately applied to carbon-coated copper grids and examined under a Philips EM 300 electron microscope.

3. Results

Zonal ultracentrifugation under the conditions described gave a separation of the main lipoprotein density classes and the bulk of plasma proteins as indicated in fig.1. Peak I consisted of VLDL followed by an LDL fraction (peak II) at a density of 1.04 kg/l. The LDL fraction was small in comparison with the HDL fraction (peak IV). The HDL peak, recovered at 1.13 kg/l, was homogeneous as judged from the density distribution, and well separated from the bulk of the plasma proteins. In contrast to human plasma, rat plasma contained a lipoprotein fraction with a density between LDL and the main HDL (peak III, fig.1). This lipoprotein fraction had an apparent density of 1.08 kg/l and was not totally separated from the other lipoprotein classes.

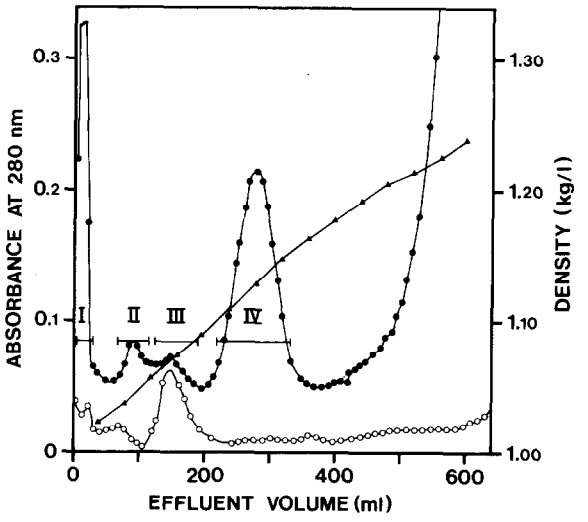

Fig.1. Lipoprotein profile of rat plasma separated by zonal ultracentrifugation. Plasma, 25 ml applied in a discontinuous sodium bromide gradient. For experimental details see section 2. (●——●) A_{280}; (▲——▲) density at 20°C; (○——○) A_{280} fraction III recentrifuged under the same conditions. Fractions were pooled as indicated by the horizontal bars.

Recentrifugation of fraction III was performed under the same conditions as the original centrifugation of rat plasma. As seen in fig.1 most material was recovered in a symmetrical peak in the density region around 1.08 kg/l. In electron microscopy, this fraction was shown to consist of spherical particles with an average diameter of about 14 nm (fig.2).

The apoprotein composition of the zonal ultracentrifugation fractions was studied with SDS–polyacrylamide gel electrophoresis. The HDL patterns were characterized by three main components with app. mol. wt 28 000, 36 000, 44 000 (fig.3d) and small amounts of low mol. wt (8000–12 000)

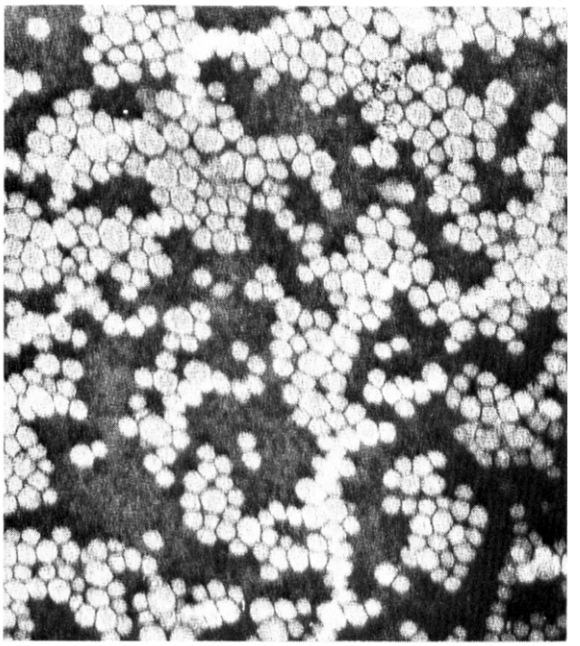

Fig.2. Negatively-stained HDL particles isolated at an average density of 1.08 kg/l (× 166 440).

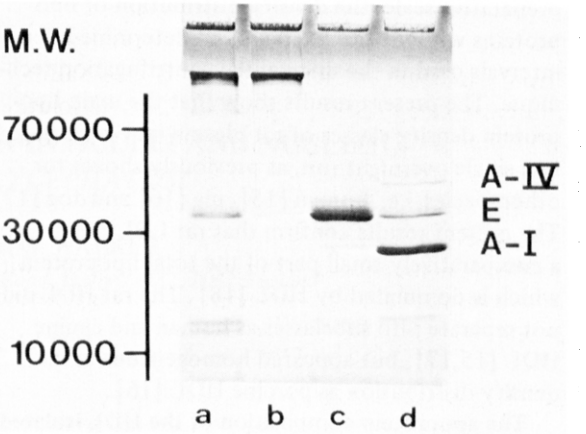

Fig.3. SDS–polyacrylamide gel electrophoresis of lipoproteins separated by zonal ultracentrifugation (cf. fig.1). Separation performed in 12% polyacrylamide. The approximate molecular weight scale was deduced from the position of 9 standard proteins. (a) Fraction I (VLDL); (b) Fraction II (LDL); (c) Fraction III ('HDL_1'); (d) Fraction IV (HDL).

material. The component with mol. wt 28 000 was the most prominent one and constituted more than half of the protein part of HDL.

The material in the fraction collected between LDL and HDL (fraction III in fig.1) had an apoprotein composition quite different from that of HDL. As seen in fig.3c the component with mol. wt 35 000 was very prominent, while the component with mol. wt 28 000 was present in much smaller amounts. Small amounts of low molecular weight components were also present as in HDL. The presence of an apoprotein analogous to dog B-protein in fractions I and II was established by the distinct immunoprecipitates obtained on electroimmunoassay against an antiserum to dog B-lipoprotein. Such material was present in, at most, trace amounts in fractions III and IV.

The total protein concentration of fraction III was 25–35% (w/w) compared to 44% (w/w) for HDL (fraction IV). The lipid composition of rat HDL and the intermediate fraction (fraction III) is shown in table 1. No striking differences were observed.

4. Discussion

Rat plasma lipoproteins were prepared by differential ultracentrifugation [1–4]. The gradient centrifugation in zonal rotors makes it possible to study, on a

Table 1
Lipid composition of the intermediate fraction (III) and HDL (IV) (%, w/w)

	Triglyceride	Cholesteryl esters	Free cholesterol	Phospholipids
Fraction III Exp. 1	6.3	38.9	12.6	42.0
Exp. 2	4.7	35.5	9.4	50.4
HDL (Fraction IV)	4.9	38.0	4.8	52.3

preparative scale, the density distribution of lipoproteins without the fixed and predetermined density intervals used in the differential centrifugation technique. The present results show that the main lipoprotein density classes of rat plasma may be isolated in a single overnight run, as previously shown for other species, i.e., human [15], pig [16] and dog [17]. The present results confirm that rat LDL constitutes a comparatively small part of the total lipoprotein, which is dominated by HDL [18]. The rat HDL did not separate into subclasses as human and canine HDL [15,17], but appeared homogeneous in its density distribution as porcine HDL [16].

The apoprotein composition of the HDL isolated by zonal ultracentrifugation was in accordance with [4] where apoA-I, arginine-rich protein (apoE) and a component with app. mol. wt 46 000 (A-IV) were found.

The fraction between LDL and HDL (fraction III) contained only small amounts of apoA-I. The major apoprotein in this fraction was most probably identical with apoE. This apoprotein composition is of interest in relation to [19] where separate distributions of apoE and apoA-I on Sepharose gel filtration of rat plasma were found, consistent with the appearance of a lipoprotein E in the present zonal ultracentrifugal experiments. The rat lipoprotein described herein has no known counterpart in the normal human lipoprotein spectrum. A human lipoprotein with a similar apparent density of 1.08 kg/l has been isolated from patients with longstanding cholestasis [15] and is most probably also present in plasma from individuals with alcoholic liver disease [20]. The human abnormal lipoprotein, tentatively called HDL_1-C [15] also contains apoE as the major apoprotein, but the unique lipid composition of HDL_1-C is quite different from the rat lipoprotein which has approximately the same lipid composition as normal rat HDL with a major part of its cholesterol esterified. This fact also excludes the possibility that the lipoprotein described here is closely related to 'nascent' rat HDL described [21]. In addition, electron microscopic images obtained by us reveal a 'normal' spherical appearance of the lipoprotein particles rather than the disc-like rouleaux forming particles demonstrated [21].

Acknowledgements

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