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THE HETEROGENEITY OF RAT HIGH DENSITY LIPOPROTEINS

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SUMMARY

Both the Apo A-I and Apo E proteins are major components of the rat high density lipoproteins. High density lipoproteins containing predominantly Apo A-I but little Apo E were separated from high density lipoproteins containing mainly Apo E and virtually no Apo A-I by heparin affinity chromatography. High density lipoproteins with Apo A-I had the chemical composition, electrophoretic and ultracentrifugal properties previously noted for rat high density lipoproteins. The Apo E high density lipoproteins contained relatively more triglyceride and free cholesterol than Apo A-I high density lipoproteins but exhibited similar ultracentrifugal and identical electrophoretic properties. In the fasting rat, the Apo A-I fraction accounted for approximately 90% of the total high density lipoproteins and the Apo E for 10%.

INTRODUCTION

One of the major differences between human high density lipoproteins (HDL) and that of the rat is the substantial content of Apo E (arginine rich apoprotein) in the rat lipoprotein (1), whereas human HDL contains very little of this apoprotein (2). Triglyceride rich lipoproteins of humans (3) and rats (4) have been observed to have an affinity for heparin in proportion to their content of Apo E. Heparin affinity chromatography was used to isolate rat high density lipoproteins rich in Apo E from the bulk of the high density lipoproteins which contained little Apo E. The properties of these two lipoproteins are described in this communication.

MATERIALS AND METHODS

Blood was obtained (in EDTA 1 mg/ml) from both male and female Sprague-Dawley rats purchased from Holtzman, Inc. and maintained on regular chow diets. The red cells were removed from the plasma by a 15 min. 3,000 rpm centrifugation in a PR-2 Sorvall Centrifuge at 10°. All very low density and low density lipoproteins were removed from the plasma by two successive 20 h ultracentrifugations at density 1.080 and 47,000 rpm in a Beckman 65-2 ultracentrifuge using a 50 Ti rotor. The bottom fraction was retrieved, made to density 1.21 and centrifuged for 48 hours at 47,000 rpm. The top fraction was respun

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and subsequently dialyzed against 2 mM phosphate buffer, pH 7.4 in preparation for heparin affinity chromatography. The high density lipoproteins were also isolated from whole plasma by molecular sieve chromatography using a BioGel A 0.5 m Agarose column as previously described (5).

Heparin affinity chromatography was performed exactly as previously described (3). The heparin affinity column was prepared by the method of Iverius (6). A buffer containing 2 mM phosphate (pH 7.4) with an initial NaCl concentration of 0.05 M and a final salt concentration of 1.5 M was used in each of these column separations. The eluent fractions were read at 280 nm on a Beckman DU spectrophotometer. Greater than 90% of the lipoprotein was recovered from the column.

Proteins were determined on the HDL eluates by the method of Lowry (7). The lipoprotein fractions eluted from the column were dialyzed and delipidated using 3:1 ethanol:ether by a conventional method (8). Sodium dodecylsulfate polyacrylamide gel electrophoresis was performed as described by Weber and Osborne (9) with the addition that on occasion the proteins were dansylated for visualization and subsequently eluted from the gels for amino acid analysis (10). Triglyceride, cholesterol and phospholipid were determined by conventional techniques (11, 12, 13). Free cholesterol was determined by a digitonide precipitation procedure (14). Thin layer chromatography was performed on the lipoprotein fractions using standard systems (15) for neutral lipids and phospholipids. Electron microscopy was performed as previously described (16) on a Philips Model 300 scope using carbon coated grids and 1% phosphotungstic acid as negative stain. Agar gel electrophoresis of the lipoprotein fractions was performed by a standard method (17) and stained for lipid and protein.

RESULTS

When rat HDL which had been isolated by either gel filtration or ultracentrifugation was applied to a heparin affinity column, the bulk of the HDL (≈90%) eluted promptly from the column in the unbound fraction (Figure 1). Approximately 10% of the lipoprotein eluted in the bound fraction at NaCl concentrations of 0.2 M (Figure 1). The apoprotein contents of the bound and unbound HDL fractions were entirely different (Figure 1 insets). The major apoprotein in the unbound HDL was a 27,000 dalton protein with an amino acid composition identical with Apo A-I. Lesser amounts of higher and lower molecular weight apoproteins were observed along with a small amount of Apo E. In contrast, the bound HDL contained Apo E as its major constituent with lesser quantities of smaller-sized apoproteins and little or no Apo A-I. Apo E was identified by its molecular size and the amino acid composition which was rich in arginine residues similar to that previously reported for the apoprotein (3). The smaller molecular weight proteins (≈10,000 daltons) of

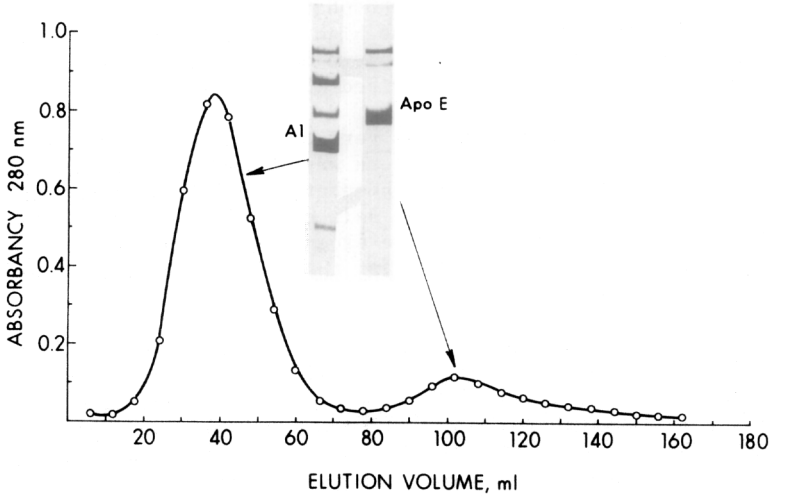

Figure 1. The elution of rat HDL (δ1.080 - 1.21) from a heparin affinity column using a linear NaCl gradient from 0.05 M to 1.5 M. The insets are the proteins of each peak evaluated by sodium dodecyl sulfate polyacrylamide electrophoresis.

the bound HDL had amino acid compositions consistent with a mixture of C proteins (7). The only difference between the HDL prepared by ultracentrifugation and gel filtration was the significant presence of contaminant albumin in the unbound fraction of the latter.

The lipid compositions of the bound and unbound rat HDL were also considerably different (Table I). One of the greatest differences between the two was the significant presence of triglyceride (6%) in the bound HDL with little found in the unbound fraction (<1%). Analysis of the cholesteryl ester and free cholesterol contents of the two lipoproteins showed relatively more free cholesterol for the bound lipoproteins. There was relatively more phospholipid in the unbound HDL, and by thin layer chromatography the major phospholipid was lecithin for both lipoproteins. The bound HDL contained no lysophospholipid, whereas this was a readily detectable band on the thin layer chromatograms of all unbound lipoproteins. Both bound and unbound HDL migrated in the α_2 region on zonal electrophoresis (Figure 2). They were both ultracentrifugally isolated in the δ1.080 - 1.21 although the composition of the Apo E HDL would indicate a somewhat lower hydrated density than Apo A-I

TABLE I

Percent Composition of Rat High Density Lipoprotein Fractions[a]

Protein	Phospholipid	Total Cholesterol	Free Cholesterol[b]	Triglyceride
Apo A-I HDL				
56 (62-44)	28 (22-36)	15 (12-20)	19 (18-20)	<1 (0-1)
Apo-E HDL				
41 (38-48)[c]	36 (30-40)[c]	19 (15-23)	33 (26-39)[c]	6 (4-8)[c]

[a]The values represent the means from the analysis of seven lipoprotein lots (6 animals per lot) for each specie; the ranges are within parentheses.

[b]The free cholesterol is expressed as a percentage of total cholesterol.

[c]Significantly different from A-I HDL at $p < 0.01$.

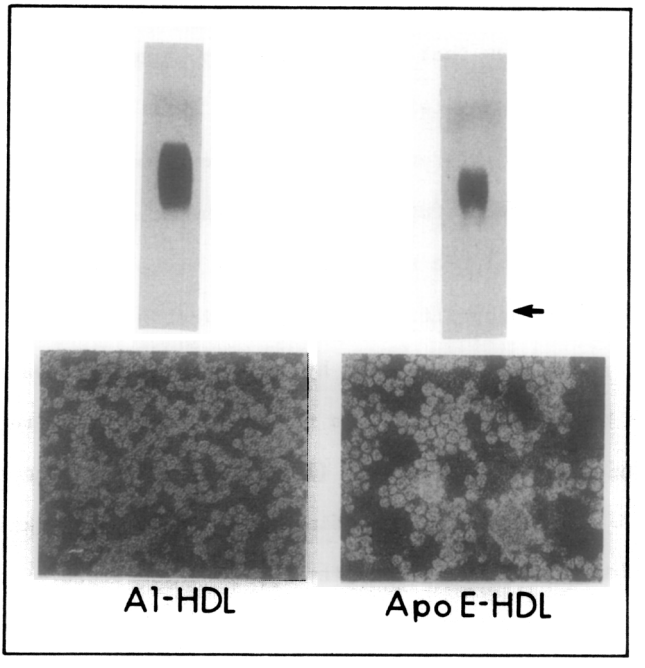

Figure 2. The agar gel migration of Apo E HDL and A-I HDL stained with Fat Red 7B. The bottom panels are the negatively stained (1% phosphotungstic acid, pH 6.8) electron micrographs at a magnification of 250,000. The arrow indicates the site of application.

HDL. In fact, a specie similar to Apo E HDL has recently been partially isolated from the bulk of the rat HDL by zonal centrifugation (18). Both lipoproteins had a similar appearance by negative staining electron microscopy. The Apo E HDL was somewhat larger, more heterogeneous and contained some particles (Figure 2) of a size compatible with triglyceride rich lipoproteins. This is compatible with the somewhat earlier elution of the antigenic determinant of rat HDL Apo E than A-I on a gel filtration system (5).

DISCUSSION

The differences in both the protein and lipid contents of Apo A-I HDL and Apo E HDL of fasting rats indicate that these are distinct lipoproteins. The previous observation (5) that the antigenic determinant for rat HDL Apo E chromatographed differently from that of HDL A-I when whole plasma was applied to a molecular sieve system strongly indicates that these lipoproteins exist in whole plasma and are not artifacts of centrifugation or affinity chromatography. The ability to partially purify the Apo E specie by zonal ultracentrifugation also indicates that heparin affinity chromatography did not spuriously generate this lipoprotein.

Although the chemical differences are great between the two lipoprotein species, their size, ultracentrifugal and electrophoretic behaviors are fairly similar. The similar size of the two lipoproteins makes their separation by molecular sieve chromatography difficult with considerable overlap for the two lipoproteins (5). The difference in hydrated density between the two lipoproteins makes zonal centrifugation a reasonable method for separating the species although overlap also exists (18). The ability to separate out lipoproteins rich in Apo E by heparin affinity chromatography makes possible the isolation of this distinct high density lipoprotein specie of the rat. Although the Apo E HDL comprises only about 10% of the total rat HDL mass in the fasting animal, the functional role of this high density lipoprotein may be quite important. The finding of different lipoprotein species in rat HDL

with a single predominant apoprotein is similar to observations in human HDL (19) and lends some credence to the "family" concept of lipoproteins (20).

The two major chemical differences of Apo E HDL, namely, a small content of triglyceride and a high free cholesterol content, suggest two possible functional roles for this HDL specie. The observation that triglyceride is associated with this lipoprotein may indicate its relevance to the plasma transport of triglycerides. It has been observed that chylomicrons, upon entering plasma, obtain an enrichment of Apo E and this enrichment appears to be derived in large measure from HDL in the rat (4). It is possible that the Apo E lipoprotein associates with the lymph chylomicron and passes its Apo E to the chylomicron; in so doing the HDL is enriched in triglyceride. Studies on the Apo E HDL after chylomicron exposure show a pronounced decrement in Apo E and lend support to this thesis (21). Although it is possible that triglyceride could be an intrinsic constituent of these lipoproteins, it is also quite plausible, in view of what appears to possibly be triglyceride rich particles on the electron micrograph, that the triglyceride in this fraction is really a reflection of a few chylomicrons interacting with Apo E HDL. We have tried to separate chylomicrons from this fraction by ultracentrifugal and molecular sieve techniques and have been unsuccessful in this regard.

If the Apo E protein has a role in directing plasma triglyceride to peripheral tissues (muscle, adipose tissue) (22), the amount of Apo E on this HDL particle as opposed to the triglyceride rich particle could have metabolic significance. The relative amount of triglyceride rich particles would distribute more of the Apo E protein onto these triglyceride rich particles in a manner possibly similar to the Apo C transfer between triglyceride rich lipoproteins and HDL (23). This would then facilitate the peripheral uptake of triglyceride from the chylomicron-like particles and remove the potential inhibitory role of the relatively triglyceride poor Apo E HDL.

The finding of a relatively high amount of free cholesterol on Apo E HDL

also suggests a role for the lipoprotein as a lecithin-cholesterol acyltransferase (LCAT) substrate. It is quite possible that the lymph chylomicron Apo E HDL complex has an association with LCAT activity. It has been observed that chylomicrons do stimulate the esterification of plasma cholesterol (24). Such a role for Apo E HDL needs further definition. In view of the affinity of the Apo E protein to a receptor responsible for initiating the uptake of cholesterol into cells, this particle may also have a role in the cellular homeostasis of cholesterol. Functional aspects of the newly described lipoprotein are being investigated.

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