AN APOLIPOPROTEIN PREFERENTIALLY ENRICHED IN CHOLESTERYL ESTER-RICH VERY LOW DENSITY LIPOPROTEINS[1]

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Summary: One of the plasma apolipoproteins, rich in arginine and predominantly alpha-helical in conformation, is preferentially enriched in cholesteryl ester-rich very low density lipoproteins. The incidence of plasma lipoproteins that are enriched in the arginine-rich apolipoprotein appears to be a function of genetic, hormonal, and dietary factors and is high in hypothyroidism and type III hyperlipoproteinemia and in rabbits fed excess cholesterol. This apolipoprotein appears to be one of several involved primarily in the transport and metabolism of cholesteryl esters and/or cholesterol.

The very low density lipoproteins (VLDL)* of human plasma are heterogeneous in the kinds of proteins present (1,2) as well as in size, density, and lipid composition (3,4). The proportions of the various apolipoproteins vary considerably among fasting normal and hyperlipemic persons (1). Variation in a given apolipoprotein could reflect specific genetic, physiological, dietary, and other factors that perturb the homeostasis of lipid metabolism and could be associated with characteristic variations in lipid composition, structure, and properties of the lipoprotein.

In this study, our objectives were to elucidate factors that regulate the proportion of the various apolipoproteins in VLDL and the relation of apolipoprotein composition to lipid composition. We investigated the variability of apolipoproteins in VLDL and LDL in several different clinical and experimental

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* Abbreviations: VLDL, very low density lipoproteins, d 0.94-1.006 g/ml; LDL, low density lipoproteins; LDL_1, d 1.019-1.045 g/ml; LDL_2, d 1.006-1.019 g/ml.

conditions, particularly those in which there are both absolute and relative enrichments in cholesterol and cholesteryl esters.

MATERIALS AND METHODS

Lipoproteins were isolated according to density by centrifugation of serum diluted 4-8 fold and adjusted to the appropriate density with NaCl and 0.01 M tris buffer and 0.0008 M EDTA at pH 7.4 (5). The VLDL, LDL_2, and LDL_1 were isolated from individual sera of fasting human subjects and unfasted rabbits. The isolation was begun within one day after drawing the blood.

The subjects included two phenotypes of hyperlipoproteinemia (type II or familial hyperbetalipoproteinemia and type III or primary dysbetalipoproteinemia (6-9) and hypothyroidism, all in humans, and cholesteremic rabbits (10). The latter were New Zealand whites bled at 10 and 24 days after initiation of a diet of 1% cholesterol added to rabbit pellets. As has been previously reported, the type II (6-8) and hypothyroid (6) subjects had elevated serum LDL_1 and LDL_2, the type III subjects (6-9) had elevated VLDL and LDL_2, and the cholesteremic rabbits had greatly elevated VLDL (20-40 fold) and moderately elevated LDL (4-5 fold). The serum lipoproteins of three type II (one male and two female), five type III (four male and one female), four hypothyroid (two male and two female) subjects and of five cholesteremic rabbits were compared with the previously examined lipoproteins of a number of normolipemic and hyperlipemic individuals, including type IV or hypertriglyceridemic patients, and with the triglyceride-rich VLDL of normal rabbit serum.

Sera and isolated lipoproteins were electrophoresed in agarose gels (11). Procedures for delipidation of lipoproteins and isolation of their apolipoproteins are those used previously (1). Disc electrophoresis of apolipoproteins with tris-glycine buffer at pH 8.4 was essentially as described by Davis (12). Ten percent polyacrylamide gels containing 8 M urea were used. Lipoproteins were analyzed as follows: percentage protein was estimated from amino acid analysis of a measured aliquot of the sample; the total lipids were determined by weighing the lipids extracted from the sample; the phospholipid content was assumed to be 25 times the phosphorus content (13); the distribution of the total lipids among cholesterol, cholesteryl esters and triglycerides was estimated by thin layer chromatography (14).

RESULTS

The protein patterns from disc electrophoresis in polyacrylamide gels show relatively large amounts of one particular apolipoprotein in certain of the lipoproteins (Fig. 1). These are the VLDL and LDL_2 of hypothyroid and type III individuals and of cholesteremic rabbits. The proportion of this protein to total protein was highest in the VLDL, less in the LDL_2, and very much less in the LDL_1. In humans, it (band H2 in Fig. 1) contains the highest percentage of arginine of any apolipoprotein that has been isolated; it is rich also in glutamic acid, alanine, and leucine (1,15). The arginine-rich apolipoproteins of the rabbit (bands R2 and R3 in Fig. 1) make up about half

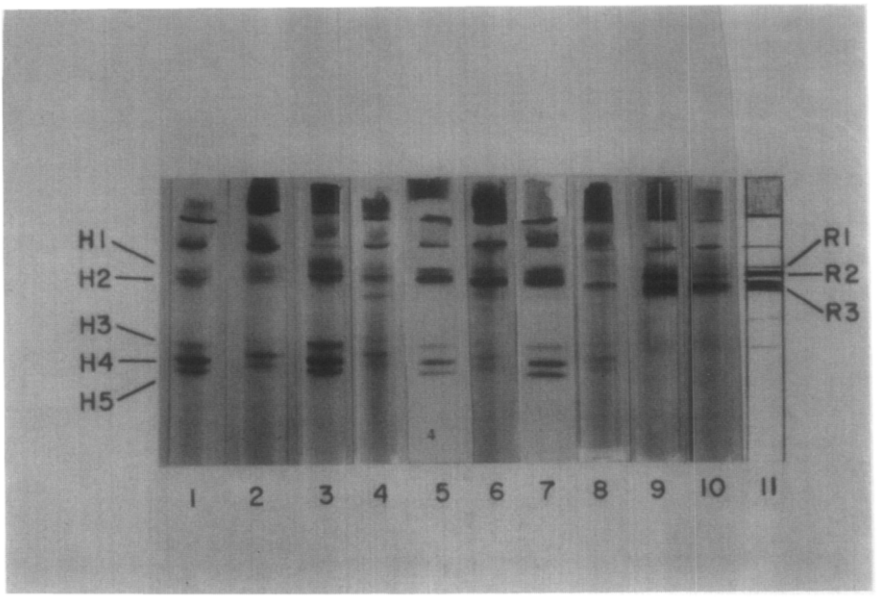

Figure 1. Apolipoprotein patterns from disc electrophoresis at pH 8.4 in 10% polyacrylamide gels containing 8M urea. The stain is aniline blue black. Origin of the proteins is as follows: patterns 1, 2, VLDL and LDL_2, respectively, of a normal human; 3, 4, of a type II human; 5, 6, of a type III human; 7, 8, of a hypothyroid human; and 9, 10, of a cholesteremic rabbit (diet-induced). The amount of protein taken for each gel was 100 μg for VLDL and 200 μg for LDL_2.

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of the cholesteremic VLDL protein; they were isolated from the cholesteremic VLDL by chromatography of its protein on DEAE-cellulose. The conditions were those used previously to isolate human apolipoproteins (1,15). The arginine-rich protein(s) of the rabbit VLDL (like that of humans), occurs in multiple forms that were separated on DEAE-cellulose; they are almost if not completely identical in their amino acid composition, which is very similar to that published for the corresponding human protein (1,15). The human and rabbit apolipoproteins appear to be very similar also in conformation; the circular dichroic spectra of the lipid-free arginine-rich proteins isolated from VLDL of human and rabbits (Fig. 2) indicate considerable amounts, perhaps two-thirds by comparison with poly-α-L-glutamic acid (16), of the molecule to be α-helix. It is more helical than other apolipoproteins isolated from human VLDL (4,17).

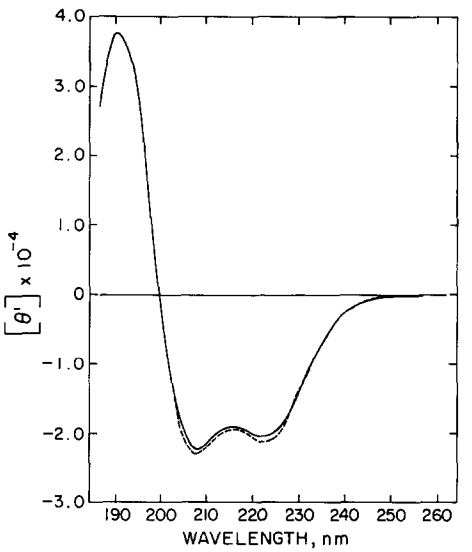

Figure 2. Circular dichroic spectra of the lipid-free arginine-rich apolipoproteins, 0.08 mg/ml in aqueous solution at pH 7, isolated from human (___) and rabbit (---) VLDL.

The rabbit and human VLDL with relatively large amounts of this arginine-rich protein are predominantly of β mobility in agarose electrophoresis, unlike the normal triglyceride-rich lipoproteins, which are mainly pre-β in mobility (Fig. 3). The decreased mobility could be due to a decrease in content of faster migrating apolipoproteins that are seen in disc electrophoresis. Type III VLDL, as previously reported (7-9), are mostly β in mobility, but significant amounts are fast migrating species; their triglyceride:cholesteryl-ester ratio is abnormally low (9). The hypothyroid VLDL also contain more than usual of the slower migrating species (Fig. 3). In our hypothyroid and type III subjects as well as our cholesteremic rabbits, the VLDL had a relatively lower triglyceride:cholesteryl-ester ratio than in type II and IV subjects or normolipemic humans and rabbits. Representative thin layer chromatograms of the VLDL lipids are shown in Figure 4.

DISCUSSION

Possibly these VLDL of β mobility are not abnormal lipoproteins, but are rather greatly elevated in proportion to the other plasma lipoprotein species

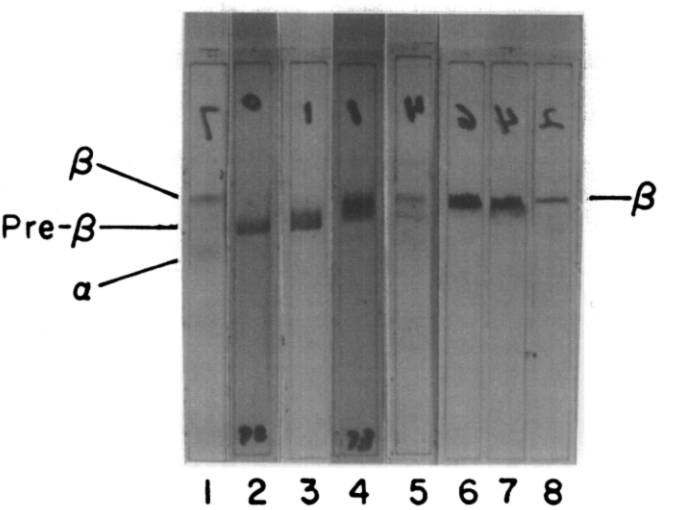

Figure 3. Electrophoresis of lipoproteins in agarose at pH 8.6. The stain is fat red 7B. Patterns 1, 2 correspond to normal human serum and VLDL, respectively; 3, 4, and 5 to VLDL of type II, type III, hypothyroid, respectively, humans; 6, 7, 8, to cholesteremic rabbit serum, VLDL, and d > 1.006 fraction of serum, respectively. The lipoproteins of patterns 2, 3, and 5 were concentrated above the level at which they occurred in serum.

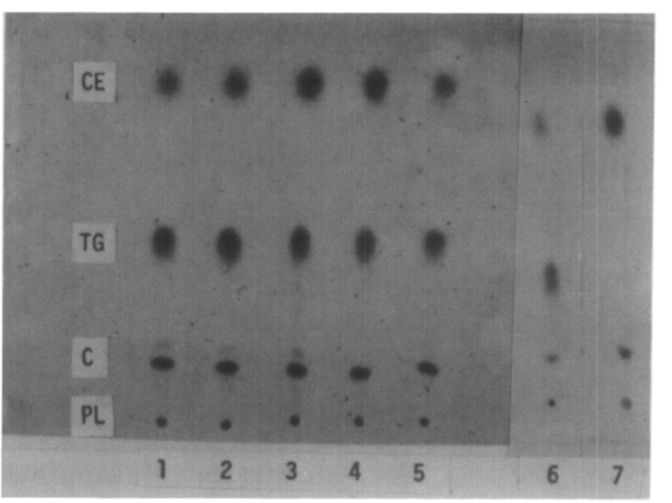

Figure 4. VLDL lipids separated by thin layer chromatography. 1, normal human; 2, type II; 3, type III; 4, hypothyroid; 5, type IV; 6, normal rabbit; 7, cholesteremic rabbit. CE, cholesteryl esters; TG, triglycerides; C, cholesterol; PL, phospholipids. The total lipids in each sample was 40 μg.

usually present in the same density range. The arginine-rich protein is indeed present in all the human VLDL, perhaps 50 or more, that we have examined by disc electrophoresis; and a molecular species of comparable polyacrylamide gel pattern was separated from human VLDL by affinity chromatography (1). The proportion of this arginine-rich protein in VLDL is quite variable among different individuals (1); frequently there is no excess in hypertriglyceridemic persons (1,18-20) and occasionally relatively very little (18,19). An excess appears to be characteristic but not unique to type III VLDL (18-20), even after a prolonged low-cholesterol diet (19). Also the LDL_2 of normal and hypertriglyceridemic persons usually contain no excess of the arginine-rich protein. Thus, the proportion of the arginine-rich protein to total apolipoproteins depends upon genetic factors, upon thyroid function, and from the rabbit experiments we suspect that it depends also upon the cholesterol content of the diet.

Our observations suggest that the arginine-rich protein is associated primarily with cholesterol and/or cholesteryl esters alone or in combination with phospholipids. The strongest evidence is its great increase in relative abundance from about 10 to 15% of the total protein in normal VLDL to more than 50% in cholesteremic rabbit VLDL (rich in cholesteryl esters and very poor in triglycerides). Other evidence is observation of relative abundance of the protein in the VLDL of hypothyroid and type III persons. These human VLDL are enriched in both cholesteryl esters and the arginine-rich protein(s), although not so much as are the cholesteremic rabbit VLDL. Since the arginine-rich protein appears to bind preferentially certain cholesterol-rich particles, it could be associated specifically with a metabolic pathway for utilization of cholesterol as well as with the synthesis of these particles in liver. Certainly other apolipoproteins (the β-proteins and the major high density apolipoproteins, one of which activates lecithin-cholesterol acyl transferase (21)), also have major roles in cholesterol transport and metabolism, but different mechanisms may be involved for each protein. However, it remains

to be seen how this apolipoprotein is related to the metabolism of cholesterol and of the lipoproteins.

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