ISOLATION OF LIPOPROTEIN A WITH HYDRATED DENSITY CHARACTERISTIC FOR LOW DENSITY LIPOPROTEINS

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1. Introduction

In recent years great advances have been made in the field of lipoprotein research due to improvements of analytical and preparative methods. Originally only α_1 and β-lipoproteins were found in the blood serum and for a long time each of the characteristic protein parts of those lipoproteins was believed to consist of identical polypeptide subunits [1,2]. In 1964, Gustafson et al. [3] succeeded in discovering a third class of apolipoproteins named apo-LpC. Following this discovery Alaupovic [4,5] proposed a new classification system as a conceptual basis for the better understanding of the structural and functional complexities of plasma lipoproteins in health and disease. Human serum lipoproteins were subdivided into 3 lipoprotein families characterized by the presence of apolipoprotein A,B and C, respectively.

Since that time a great deal of investigation has been done to characterize different polypeptides in each density class and family of human serum lipoproteins. Shore and Shore [6] found 2 major peptides in high density lipoproteins (HDL) which were called R–Thr and R–Gln according to their presumed C-terminal amino acids. A reinvestigation showed glutamine to be the only C-terminal amino acid for both immunochemically different apolipoprotein A (apoLpA) peptides, which are called since ApoAI and ApoAII [7]. Although several investigators have reported the heterogeneity of the protein moiety of low density lipoproteins (LDL) [8, 9], the presence of different polypeptides in lipoprotein B (LpB) has not been demonstrated so far, with the exception of genetically determined polymorphic forms (allotypes). R–Val, R–Glu and R–Ala were found to represent the major peptides in the LpC family [10].

LpA is the major lipoprotein in the HDL class, LpB the major lipoprotein in the LDL class and LpC the major lipoprotein in the VLDL class. Some small amounts of apoLpC and apoLbB peptides are present in each HDL fraction capable of forming their own family as separate molecules within this density class [11]. The occurrence of LpA in 2 different subgroups could be shown [11,12], one containing only ApoAI and the other containing ApoAI and ApoAII as constitutive polypeptides. Human serum LDL (d 1.006–1.063) contain a few percent of apoLpA and ApoLpC peptides and because of the small amount of these "contaminations", the isolation of LpA or LpC with hydrated density properties of LDL failed to succeed so far.

The LpA of human chyle has been reported to contain a much higher percentage of triglycerides compared to serum LpA [13]. This seems to be the reason why a relatively high concentration of LpA can be found in the LDL class. It was the aim of this work to isolate and characterize this LpA from the LDL fraction, called LpA_{LDL} in this paper. A flotation rate $F_{1.063}$ of 3–4 was found and the lipid composition differed significantly from LpB isolated from the same density class. Both apoA peptides were constituents of LpA_{LDL}.

2. Material and methods

Human fasting chyle was obtained by cannulation of the thoracic duct via the pleural cavity of 2 female subjects. A normal pattern of serum lipoproteins was found by agarose gel electrophoresis and the values for serum lipids, too, were within the range of normals. The isolation of VLDL+LDL was performed in a preparative ultracentrifuge (Beckmann L4) at a density of 1.063 adjusted by adding solid NaBr, and spinning for 24 hr at 140,000 g as described elsewhere [14]. The removal of VLDL was carried out at a density of 1.023 and the LDL sedimenting at this density were washed twice at d 1.063. In a separate experiment, VLDL and LDL were precipitated from chyle with sodium phosphotungstate using Burnstein's method [15] or with Dextran Sulfate according to Cornwell and Krüger [16]. For isolating the remaining LDL the supernatant was centrifuged at a density of 1.063 and washed as described above.

For removal of all of the LpB-containing lipoproteins from LDL, an immunoadsorber was prepared according to Kostner et al. [17] labelled with pure antibodies to LpB [18] and the adsorption carried out on a column (1.5 × 10 cm) in 0.9% NaCl, pH 7.4. Immunoelectrophoresis, agarose gel electrophoresis and immunodiffusion were performed in 1% agarose in Barbital buffer, pH 8.2, and disc electrophoresis in 5% polyacrylamide gel (PAG) or 10% PAG containing 8 M urea at pH 9.2 as described earlier [7]. The lyophilized lipoprotein preparations were delipidized by extractions with ethanol–ether, 3:1 [7]. Cholesterol was determined according to Michaelis et al. [19], phospholipids according to Gerlach and Deuticke [20] and triglycerides according to Eggstein and Kreutz [21]. The protein content was calculated from the residue weight after extraction of lyophilized lipoproteins with chloroform–methanol, 2:1. All chemicals were p.a. reagents from E. Merck. Analytical ultracentrifugations were carried out in a Beckman Spinco Model E.

3. Results and discussion

The high salt flotation in the analytical ultracentrifuge of the isolated chyle LDL (d 1.023–1.063), at a solvent density of 1.200, revealed the presence of 2 peaks (fig. 1). After removal of the LpB-containing

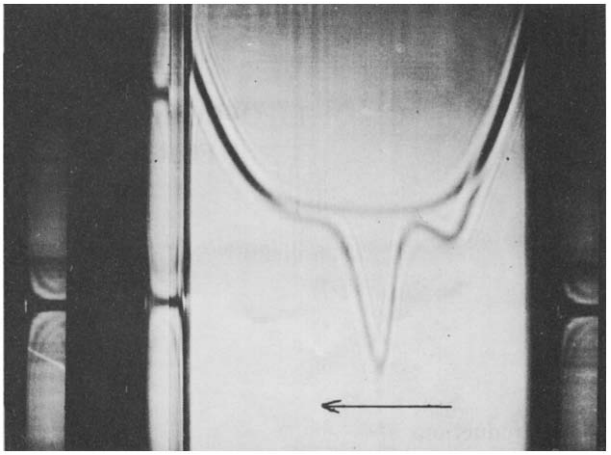


Fig. 1. High salt flotation of human chyle LDL (d 1.023–1.063) in the analytical ultracentrifuge at a solvent density of 1.200. 48,000 rpm 20 min after start.

lipoproteins by immunoadsorption, only the slower floating peak was observed, indicating that the faster migrating peak belonged to LpB.

After the immunoadsorber experiment, the fate of the LpC peptides was checked by immunochemistry and PAG electrophoresis in 10% gels after total delipidization of the lipoprotein fractions. Most of the apoLpC peptides were found in the eluate with glycine-HCl buffer, pH 3.2, from immunoadsorber specific for LpB indicating a certain association between LpB and LpC molecules. Fig. 2 shows the material of totally delipidized LpA_{LDL} compared to an apo-HDL_2 fraction from serum (d 1.063–1.125) in 10% PAG containing 8 M urea. Many more fast migrating bands indicating apoLpC peptides can be visualized in the serum HDL fraction. This small amount of LpC remaining in LpA_{LDL} could be completely removed by passing the material through a column (1.2 × 6 cm) packed with hydroxylapatite in 0.1 M K-phosphate buffer pH 6.8 [11]. The intact LpA_{LDL} was investigated by agarose gel electrophoresis, PAG electrophoresis and immunochemical methods. Both preparations had migrated the same distance on agarose gel electrophoresis and were found in the α_1 region. Fig. 3A shows the pattern of LpA_{LDL} in 5% PAG compared to HDL_3 isolated from human serum at a density of 1.125–1.21. A much slower migration rate of LpA_{LDL} was observed, indicating a larger particle size of this molecule. Immuno-

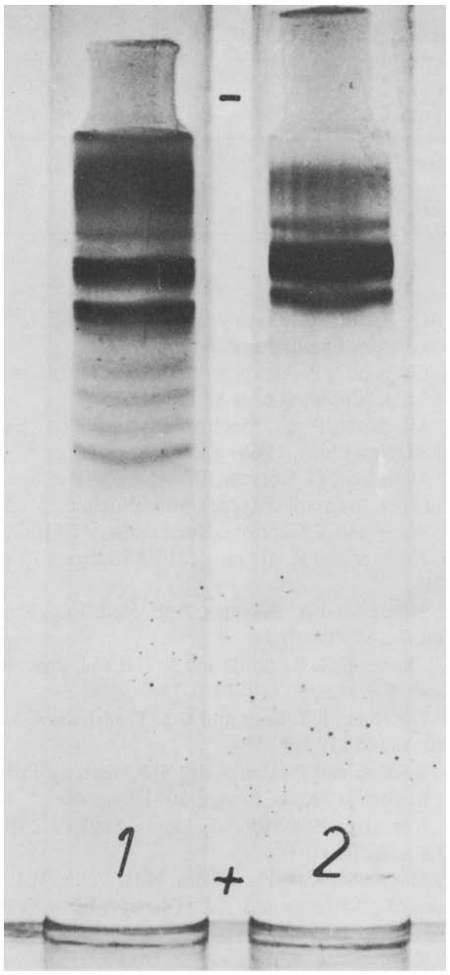
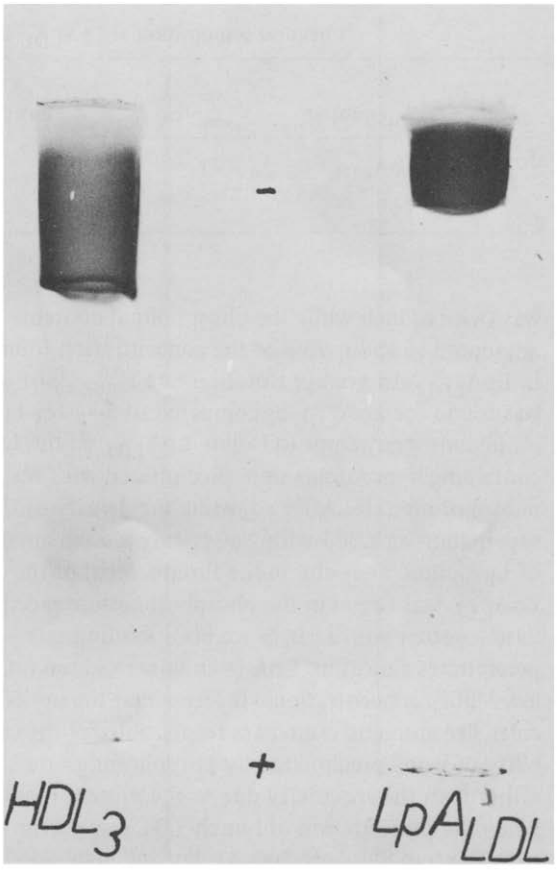


Fig. 2. Pattern of totally delipidized apoHDL$_2$ from human serum (1) and totally delipidized apoLpA$_{LDL}$ (2) in 10% polyacrylamide gels containing 8 M urea.



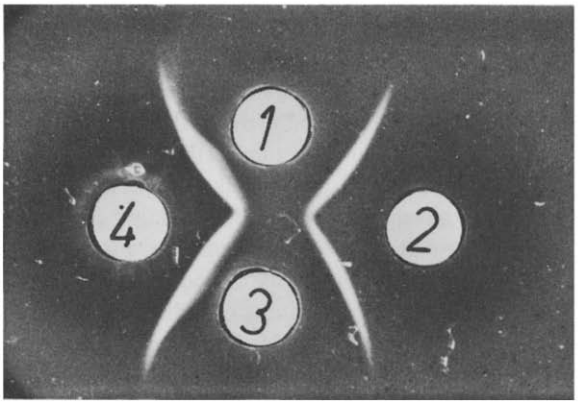


Fig. 3. A: Comparison of human serum HDL$_3$ with LpA$_{LDL}$ from chyle in 5% polyacrylamide gels. B: Immunochemical identity reaction of LpA$_{LDL}$ with HDL$_3$ using monospecific antisera to ApoAI and ApoAII peptides: 1:LpA$_{LDL}$, 2:anti-ApoAII, 3:HDL$_3$, 4:anti-ApoAI.

chemically there was complete identity between LpA$_{LDL}$ and HDL$_3$ using monospecific antisera to ApoAI and ApoAII peptides (fig. 3B). No reaction with antisera to LpB or LpC was observed. These findings demonstrate the existence of both major ApoA peptides in lymph LpA$_{LDL}$ and LpB eluted evaluation showed the presence of 17% and 20% LpA in lymph LDL preparations from 2 different persons.

The chemical analysis of LpA$_{LDL}$ and LpB eluted from immunoadsorber with 0.2 M glycine-HCl buffer, pH 3.2, showed some significant differences (Table 1). The amount of total cholesterol in the LpB fraction

Table 1
Chemical composition of LpA_{LDL} and LpB isolated from chyle LDL (d=1.023-1.063).

Lipoprotein	Composition (%) Protein	Phospholipids	Total Cholesterol	Triglycerides
LpA_{LDL}	31	38	9	18
LpB	25	29	19	22

was twice as high while the phospholipid content amounted to about 75% of the concentration found in LpA_{LDL}. An average flotation rate $F^{1\%}_{1,0630}$ of 3–4 was found for LpA_{LDL} as compared to 5–7 for LpB.

In another attempt to isolate LpA_{LDL}, all the LpB-containing lipoproteins were precipitated with Na-phosphotungstate. After adjusting the density of the supernatant at 1.063 with NaCl, only a small amount of LpA could be found in the flotate. Most of the LpA_{LDL} was found in the phosphotungstate precipitate together with LpB. Since phosphotungstate precipitates almost no LpA from human serum (at low $MgCl_2$ concentrations) it seems that the molecular size and lipid content is responsible for the capability of being precipitated by phosphotungstate rather than the specificity due to the protein moiety. When the precipitation of lymph LDL was performed with Dextran Sulphate 500, a relatively high amount of LpA was also found in the precipitate. The difference compared to human serum, however, was not so striking, as some LpA is also found in the Dextran Sulfate precipitate from human serum.

This work represents the first success in the isolation and characterization of LpA, the major lipoprotein family in human serum HDL with a hydrated density characteristic for the bulk of LpB, and expresses the advantage in using the "family" classification system as an operational concept. Furthermore, it demonstrates the capability of apoLpA to handle significantly different amounts of individual lipids forming lipoproteins of a wide hydrated density range.

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