IDENTIFICATION OF THE PROTEIN MOIETY OF AN ABNORMAL HUMAN PLASMA LOW-DENSITY LIPOPROTEIN IN OBSTRUCTIVE JAUNDICE

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1. Introduction

The characteristic elevation of plasma unesterified cholesterol and phospholipid concentrations in subjects with biliary obstruction is due to the presence of an abnormal low-density lipoprotein, LP-X, which does not react with antibodies to normal LDL [1–4]. The isolated lipoprotein, LP-X, is characterized by a low content of protein (5%) and a very high content of phospholipid (61–66%) and unesterified cholesterol (23–26%). Since several investigators [5–7] demonstrated immunochemically the presence of LP-A in the LDL fraction, it has been suggested [8,9] that the increased concentration of LDL in subjects with biliary obstruction is caused by a shift of a particular LP-A from the HDL into the LDL fraction. To study individual lipoproteins in the LDL fraction from subjects with biliary obstruction a procedure was developed in our laboratory [4] which makes possible the isolation of three immunochemically distinct lipoproteins, LP-A, LP-B and LP-X. The abnormal lipoprotein LP-X and the normal LP-B together accounted for 98%, and the LP-A for only 2%, of the total protein content of the LDL fraction.

It has been demonstrated in our laboratory that human hyperlipemic plasma [10,11] and normal postprandial chyle VLDL [12] contain a protein moiety, apolipoprotein C, which differs immunochemically and chemically from the apolipoproteins A and B, the characteristic proteins of HDL and LDL, respectively. In this paper we are presenting evidence that the protein moiety of the abnormal low-density lipoproteins in biliary obstruction consists of a combination of albumin and apolipoprotein C.

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Abbreviations of lipoproteins: VLDL, very low-density lipoproteins, $d < 1.006$ g/ml; LDL, low-density lipoproteins, d 1.006–1.063 g/ml; HDL, high-density lipoproteins, d 1.063–1.21 g/ml; α_1-LP, α-lipoproteins characterized with an electrophoretic mobility of α_1-globulins; β-LP, β-lipoproteins characterized with an electrophoretic mobility of β-globulins; LP-A, lipoproteins characterized by the presence of apolipoprotein A (ApoA); LP-B, lipoproteins characterized by the presence of apolipoprotein B (ApoB); LP-C, lipoproteins characterized by the presence of apolipoprotein C (ApoC). Each apolipoprotein is characterized by its specific immunochemical characteristics and by such chemical properties as terminal amino acids.

2. Materials and methods

2.1. *Isolation of LP-X*

Blood samples from four fasting patients with extra-hepatic biliary obstruction were collected into plastic bags containing 2.2% Na citrate and the plasma was recovered by low-speed centrifugation. LP-X was isolated by a previously-described procedure which combines heparin precipitation, ethanol fractionation and ultracentrifugation [4].

2.2. *Isolation of apolipoprotein* C

Blood was obtained from subjects with hypertriglyceridemia (Types 3 or 5) 5 hr after ingestion of a heavy fat meal. Chyle was collected continuously over an 8 hr period following a fat meal by cannulation of the thoracic duct of a patient with normal fasting serum lipid levels (chyle was supplied by Dr. W.H.Falor, Akron City Hospital, Akron, Ohio). Plasma and chyle VLDL were isolated, purified and partially delipidized as previously described [11,12]. The ApoA- and ApoB-containing phospholipid residues were precipitated by adding successively to partially delipidized VLDL, solubilized in borate buffer, pH 9.0, equivalent amounts of rabbit antihuman sera LP-A (α_1-LP) and LP-B (β-LP) (to avoid contamination with rabbit lipoproteins, the antisera, adjusted to *d* 1.25 g/ml, were centrifuged at 105,000 × *g* for 44 hr and the floating lipoproteins were removed and discarded). The reaction mixtures were allowed to stand for 2 hr at 37° and the immunoprecipitates were sedimented by low-speed centrifugation (2,000 rpm) for 30 min. The density of the supernate remaining after the second immunoprecipitation was adjusted to 1.21 g/ml and the solution was centrifuged at 105,000 × *g* for 44 hr. The top fraction (upper 1 cm), removed by a tube-slicing technique, contained the ApoC-phospholipid residue and the bottom fraction consisted of a mixture of rabbit plasma proteins. ApoC (phosphorus content less than 0.1%) was obtained by five successive extractions of its protein-phospholipid residue with ethanol-diethyl ether (3:1, v/v) at 0°.

2.3. *Immunochemical methods*

The immunochemical properties of LP-X and ApoC were studied by double diffusion and/or immunoelectrophoresis as previously described [4,11,12]. Rabbit antihuman LP-A (α_1-LP), LP-B (β-LP), albumin and whole human serum were purchased from Behringwerke AG, Marburg-Lahn, Germany. Goat antirabbit whole serum and γ-globulin were obtained from Mann Research Laboratories, New York. Detailed procedures for the preparation and characterization of the purified rabbit antisera to LP-X [4] and VLDL [12] have been reported. The antibodies to LP-X reacted only with LP-X. The antisera to VLDL gave precipitin lines with partially delipidized VLDL; one of these precipitin lines gave an identity reaction with LP-A and the other with LP-B. The third precipitin line, characteristic of ApoC, showed no reaction with any available serum protein preparation.

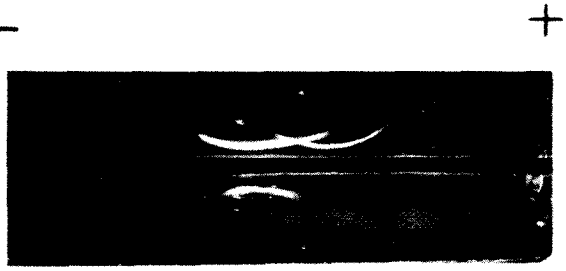

Fig. 1. Immunoelectrophoresis patterns of partially delipidized LP-X in 1% agar gel; partially delipidized LP-X before ultracentrifugation, upper well, and, lower well, after removal of albumin by ultracentrifugation. Central trough contains antibodies to LP-X and albumin.

2.4. *Electrophoresis*

The electrophoresis in 1% agar and 1% agarose gels was carried out as described under Immunochemical methods.

2.5. *Chemical methods*

Determination of C-terminal amino acids with carboxypeptidases A and B (Worthington Biochemical Corp. Freehold, New Jersey) was carried out according to the procedure by Ambler [13]. Lipid and protein analyses were performed according to methods previously described [4, 11]. The anthrone-positive carbohydrates were estimated by the method of Koehler [14].

3. Results

LP-X, characterized by a high phospholipid/protein ratio of 11.5 and single bands in agar and agarose electrophoresis, gave no reaction with antibodies to normal whole serum, albumin, LP-A or LP-B; it reacted only with antibodies to LP-X [4]. However, LP-X partially delipidized by successive extractions with *n*-heptane according to the procedure by Gustafson [15], gave a positive reaction not only with antibodies to LP-X, but also with antibodies to albumin (fig. 1,

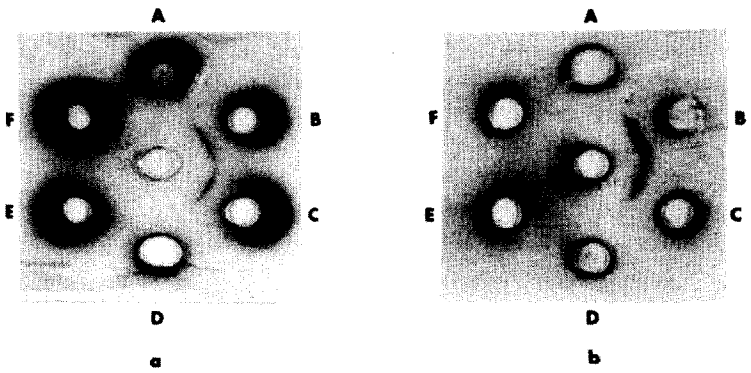

Fig. 2. Immunodiffusion patterns of partially delipidized non-albumin protein of LP-X (central well in pattern a) and ApoC (central well in pattern b). Outer wells contain antibodies to LP-A (A), VLDL (B), LP-X (C), LP-B(D), whole serum (E) and albumin (F). Plate is stained with Amido Black 10B.

upper portion). Separation of these two protein moieties was achieved by adjusting the density of partially delipidized lipoprotein solution to 1.21 g/ml and centrifuging the mixture at 105,000 × *g* for 44 hr. The top fraction contained the non-albumin protein moiety reactive with antibodies to LP-X (fig. 1, lower portion) and the bottom fraction contained only the immunochemically identified albumin. Protein analyses of three separate partially delipidized LP-X samples showed that albumin accounted for approximately 40% and the ApoX for 60% of the total protein content.

Partially and totally delipidized non-albumin protein of LP-X moved as single fast bands in agar and agarose electrophoresis. Partially delipidized LP-X gave no reaction with antibodies to LP-A, LP-B, whole serum or albumin, but showed identical immunoprecipitin lines with antibodies to VLDL and LP-X (fig. 2, pattern a). Totally delipidized non-albumin protein, showing identical immunochemical behavior, contained traces of phospholipids (less than 0.1%) and no detectable free fatty acids or anthrone-positive carbohydrates. The C-terminal amino acid of the non-albumin protein was identified by the action of carboxypeptidases A and B as alanine; kinetic studies indicated a C-terminal sequence: R-Val-Ala-Ala-COOH.

The finding of a positive immunoprecipitin reaction of non-albumin protein with antibodies to VLDL, but not with antibodies to LP-A or LP-B, indicated similarity with ApoC. The mobility of partially and totally delipidized ApoC in agar and agarose electrophoresis was the same as that of the non-albumin protein. Partially delipidized ApoC gave no reaction with antibodies to human LP-A, LP-B, whole serum and albumin or with antibodies to rabbit whole serum or γ-globulins, but showed total coalescence of precipitin lines with antibodies to VLDL and LP-X (fig. 2, pattern b). Its C-terminal amino acid and C-terminal sequence was the same as that of the non-albumin protein. A direct comparison of partially delipidized non-albumin protein and ApoC showed that these two proteins gave complete fusion of precipitin lines with antibodies to VLDL and LP-X (fig. 3). Totally delipidized non-albumin protein and ApoC gave identical immunochemical patterns.

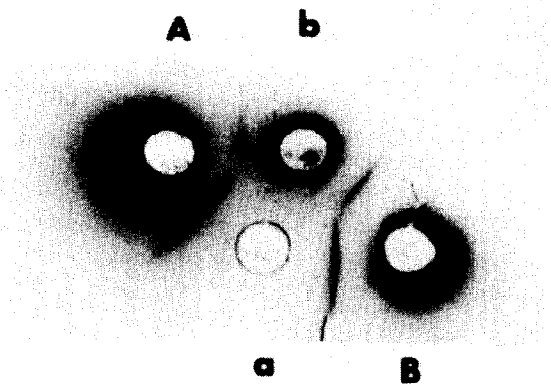

Fig. 3. Immunodiffusion patterns of partially delipidized non-albumin protein of LP-X (lower center well a) and ApoC (upper center well b). Outer wells contain antibodies to LP-X (A) and VLDL (B). Plate is stained with Amido Black 10B.

4. Discussion

Results of the present study indicate that the abnormal low-density lipoprotein in biliary obstruction contains a protein moiety of unique composition consisting of a combination of approximately 40% albumin and 60% of a specific apolipoprotein. This abnormal lipoprotein thus represents an albumin-lipoprotein complex in which the antigenic site of albumin is revealed by partial or total delipidization. Since immunochemical studies have shown that the non-albumin protein and ApoC have a common antigenic determinant, these two proteins are similar if not identical. This conclusion is further supported by the identical electrophoretic behavior on agar and agarose immunoelectrophoresis and C-terminal analyses of both protein preparations. Although the questions about the molecular arrangement in the albumin-LP-X complex, the site of its formation, the reasons for its apparently exclusive occurrence in patients with obstructive jaundice [4] and the additional chemical evidence for the identity of the non-albumin protein and ApoC remain to be answered by further investigations, we are proposing as a working hypothesis that the formation and accumulation in serum of LP-X may be the result of an impaired catabolism of ApoC-containing lipoproteins caused by inhibitory action of increased concentrations of bile salts in the liver.

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