IDENTIFICATION OF APOLIPOPROTEINS IN LIPOPROTEIN DENSITY CLASSES OF HYPERCHOLESTEROLEMIA (TYPE IIa)

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1. Introduction

Familial hypercholesterolemia (type IIa hyperlipoproteinemia) is a lipid transport disorder characterized by increased concentrations of plasma cholesterol and β-lipoproteins [1,2]. This type of hyperlipoproteinemia is often accompanied by xanthomatosis and premature coronary atherosclerosis [3]. Although several reports indicate that LDL* from hypercholesterolemic patients have normal hydrated densities and chemical composition [5–7], results of other studies show that LDL from type II hyperlipoproteinemic subjects differ in these parameters from normals [8,9]. Grant et al. [10] reported that water of hydration was significantly higher in LDL of a homozygote patient with hypercholesterolemia than in those of heterozygotes and normals. Agostini et al. [11] observed that under an electron microscope LDL of type II patients tended to form elongated or branched stacks, while LDL of normal subjects showed spherical shape.

In an attempt to resolve some of these differences, we have initiated a comparative study of the chemistry of LDL from normal [12] and hypercholesterolemic subjects. Here we present the identification of apolipoproteins and their polypeptides in each major lipoprotein density class in the plasma of patients with type IIa hyperlipoproteinemia. We found a great similarity in the polypeptide patterns of VLDL and LDL subfractions in the plasma of normal and type IIa hyperlipoproteinemic subjects. Minor differences were noted in the HDL subfractions.

* *Abbreviations:* VLDL, very low density lipoproteins, lipoproteins of d < 1.006 g/ml; LDL, low density lipoproteins, lipoproteins of d 1.006-1.063 g/ml; HDL, high density lipoproteins, lipoproteins of d 1.063-1.21 g/ml. Lipoprotein families and apolipoproteins were designated according to the ABC nomenclature [4].

2. Materials and methods

2.1. *Isolation of lipoprotein density classes*

Plasma was obtained by plasmapheresis from seven type IIa hyperlipoproteinemic donors. All donors were off medication for at least six weeks and were fasted for 12–14 hr before blood was drawn. Lipoprotein density fractions were isolated from individual plasma samples. The VLDL and three subfractions of LDL (LDL_1, d = 1.006–1.019 g/ml, LDL_2, d = 1.019–1.053 g/ml, and LDL_3, d = 1.053–1.063 g/ml) were isolated by ultracentrifugation [12]. To isolate two subfractions of HDL (HDL_2, d = 1.063–1.120 g/ml, and HDL_3, d = 1.120–1.21 g/ml), the plasma of d > 1.063 g/ml was adjusted with solid KBr to a solution density 1.12 and 1.21 g/ml successively, and centrifuged at 45 000 rev/min for 30 hr. All density fractions were recentrifuged until free of albumin as tested by double diffusion with antibodies to albumin.

An LDL_2 sample was subjected to a density gradient banding. The 9.6 ml gradient of KBr solution extending from 1.006 to 1.093 g/ml was layered on top of 3.5 ml LDL_2, which was pre-adjusted to a density of 1.21 g/ml with KBr. The gradients were centrifuged in swinging-bucket rotor SW40 at 40 000 rev/min for 24 hr at 10°C. Fractions were collected from the top of the tube with a hypodermic syringe.

Lipoproteins were dialyzed against several changes

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of 0.15 M NaCl containing 0.05% EDTA and 0.01 M $(NH_4)_2CO_3$ before use.

2.2. *Delipidization of lipoproteins*

Lipoproteins were delipidized with ethanol–ether and separated into soluble and insoluble apolipoproteins [12].

2.3. *Polyacrylamide-gel electrophoresis.*

The delipidized lipoprotein fractions were analyzed by electrophoresis on 7% polyacrylamide gel containing 8 M urea in basic [13] and acidic [14] buffers. Identification of the peptide bands in separating gels has been described previously [12,15].

2.4. *Immunochemical methods*

The immunochemical properties of lipoproteins and apolipoproteins were studied by double diffusion in 1% agarose. Antisera to LP-A; LP-B; LP-C; LP-D ('thin-line'); A-I; A-II; C-I; C-II, and C-III peptides were prepared and characterized as previously reported [4,16]. An antiserum to LP(a) was kindly supplied by Dr K. Walton, University of Birmingham, England. This antiserum was monospecific for Lp(a) lipoprotein and Lp(a) protein, and gave no reaction with LP-B or albumin. Antiserum to 'arginine-rich' peptide was supplied by Drs W. McConathy and P. Alaupovic of the Lipoprotein Laboratory, Oklahoma Medical Research Foundation, Oklahoma City. This antiserum gave a precipitin line only with 'arginine-rich' peptide that was isolated from VLDL according to Shelburne and Quarfordt [17].

3. Results and discussion

Table 1 shows the immunochemical and electrophoretic identification of the intact and delipidized lipoproteins. All density fractions were found to be heterogeneous with respect to the protein moieties. Several peptides which had not been detected in the intact lipoproteins became detectable after delipidization. These included A-I and LDL_2 and LDL_3; A-II in VLDL, LDL_2 and LDL_3; and ApoD in LDL_1. Failure to detect these peptides in intact lipoproteins might have been due to their extremely low concen-

Table 1
Apolipoproteins and their polypeptides identified in intact and delipidized lipoproteins of density classes of type IIa hyperlipoproteinemia

Density fractions	A-I	A-II	ApoB	C-I	C-II	C-III	ApoD	'Arg.-rich' peptide	Lp(a) protein	Albumin
VLDL	–	–	+	+	+	+	–	+	–	–
apo VLDL	– (+)	– ±	+	+	+	+	–	+	–	–
LDL_1	–	–	+	+	+	+	–	+ (–)	–	–
apo LDL_1	– (+)	–	+	+	+	+	+ (–)	+ (–)	–	–
LDL_2	–	–	+	+	+	+	+	+	–	–
apo LDL_2	+	– ±	+	+	+	+	+	+	–	–
LDL_3	–	–	+	+	+	+	+	+	–	–
apo LDL_3	+	+	+	+	+	+	+	+	–	–
HDL_2	+	+	+	+	+	+	+ (–)	+	+	–
apo HDL_2	+	+	+	+	+	+	+	+	+	– (+)
HDL_3	+	+	–	+	+	+	+	–	–	–
apo HDL_3	+	+	–	+	+	+	+	– (+)	–	– (+)

Note: + Positive in all donors studied.
– Negative in all donors studied.
+ (–) Positive in all except one donor.
– (+) Negative in all except one donor.
– ± Negative in some donors and trace amounts in others, detectable with antibodies only after the immunodiffusion patterns were stained with protein stainings.

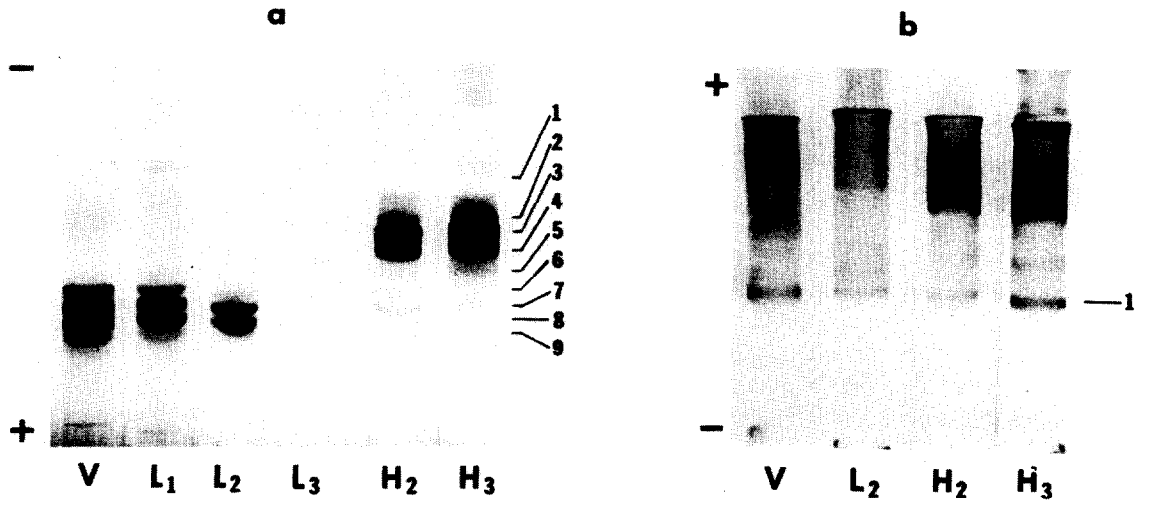

Fig.1. Basic (a) and acidic (b) polyacrylamide gel electrophoretic patterns of soluble apolipoproteins in VLDL (V), LDL_1 (L_1), LDL_2 (L_2), LDL_3 (L_3), HDL_2 (H_2) and HDL_3 (H_3) of a type IIa hyperlipoproteinemic patient. Identification of polypeptide bands: C-I (1), dimer of A-I or unidentified polypeptide (2), A-I (3), A-II (4), ApoD ('Thin line') (5), C-II (6), C-III-1 (7), C-III-2 (8) and C-III-3 (9).

tration and/or the masking of their antigenic sites.

As in normal subjects, A-I and A-II were also the major peptides in HDL_2 and HDL_3 of the type IIa hyperlipoproteinemics. ApoB was found in all density fractions except HDL_3. Similarly, the C-I, C-II and C-III polypeptides of LP-C family were found in all density fractions. The soluble apolipoproteins of each density fraction were seen in the basic 7% polyacrylamide gel electrophoresis (fig.1a). C-I polypeptide was not visible in basic polyacrylamide gel pattern; it was seen better in acidic polyacrylamide gel as the fastest moving band (fig.1b).

ApoD was found in all fractions except VLDL. In our experience, ApoD was never found in intact HDL_2 isolated centrifugally from over 100 normal donors of individual and pooled plasma. However, traces could be detected after delipidization of normal HDL_2.

The 'arginine-rich' peptide, originally isolated from VLDL of normal subjects [17,18], was detected in all density fractions of type IIa patients except HDL_3 (fig.2). In normal subjects, however, it was detected immunochemically in all density fractions. After delipidization, 'arginine-rich' peptide was mainly associated with the insoluble apolipoproteins. The trace amount present in the soluble apolipoproteins, was detectable by immunology but not visible on polyacrylamide gel.

Lp(a), the 'polymorphic' form of LP-B, was found only in HDL_2 (fig.3) of all seven type IIa donors studied. According to previous reports [19,20], and our own observation Lp(a) was not identified in HDL_2 of each normal subject. This observation is similar to that reported by Walton et al. [21]. They found that both the concentration and frequency of occurrence of Lp(a) were higher in type IIa patients than in normal subjects. The Lp(a) lipoprotein which correlates closely with angina pectoris has been suggested as a major risk factor in coronary heart disease [22,23].

Our study confirmed the report of Gotto et al. [24], that ApoB in type IIa hyperlipoproteinemia is immunochemically identical to ApoB from normal plasma. In addition, all the identified apolipoproteins and their polypeptides in type IIa hyperlipoproteinemia are immunochemically and electrophoretically identical to those from normal plasma. However, our findings in LDL subfractions do not agree with the report of Fisher et al. [7] that LDL of type IIa hyperlipoproteinemic patients is immunochemically homogeneous. It is possible that due to low titer of antibodies, their LDL preparations failed to react with

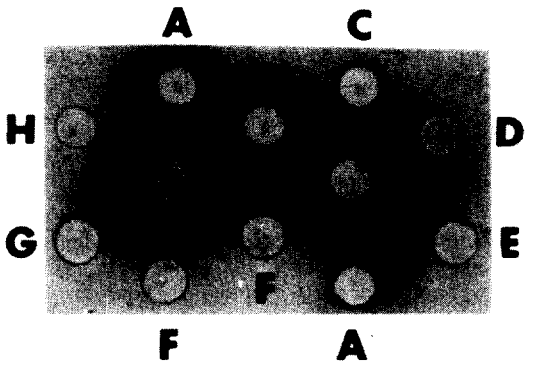

Fig.2. Immunodiffusion pattern of lipoprotein fractions in 1% agarose gel. Wells in the center contain antibodies to 'arginine-rich peptide. A, normal VLDL; B through H, lipoprotein fractions of a type IIa hyperlipoproteinemic patient. B, VLDL, C, LDL_1; D, LDL_2; E, LDL_3; F, HDL_2; G, HDL_3; H, 1.21 infranate.

antibodies to ApoC. Their LDL did react with antibodies to HDL and produced a 'faint line' of precipitation which was interpreted by the authors as ApoB reaction. Reaction of ApoC or ApoD cannot be ruled out, however, since normal HDL are known to contain ApoC and ApoD ('thin line' polypeptide) [4]. To eliminate the possibility that the disparity in results was due to the difference in isolation procedures, we subjected the purified LDL_2 to density gradient banding. We tested the recovered lipoproteins by double diffusion with antibodies to LP-C and LP-D. The results indicated that LDL_2 purified by density gradient banding still contained LP-C and LP-D.

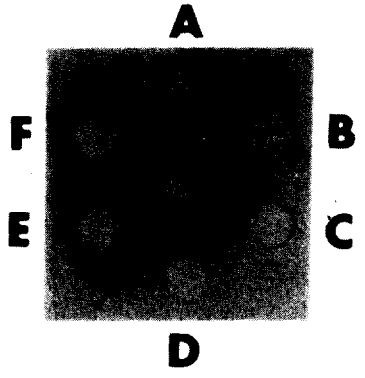

Fig.3. Immunodiffusion pattern of lipoprotein fractions of type IIa hyperlipoproteinemia. Well in the center contains antibodies to Lp(a). Outer wells contain lipoprotein samples. A, VLDL; B, LDL_1, C. LDL_2; D, LDL_3; E, HDL_2; and F, HDL_3.

Results of our study show a striking similarity between the polypeptide patterns of VLDL and LDL subfractions of normal donors, and patients with type IIa hyperlipoproteinemia. Minor differences were noted in HDL subfractions. These findings show that the chemical and physical-chemical differences observed between LDL from type IIa hyperlipoproteinemics and normal subjects [8–11] are not caused by the presence or absence of a particular apolipoprotein (such as ApoA, ApoB, ApoC, ApoD and 'arginine-rich' peptide), or its known constituent polypeptides (such as A-I, A-II, C-I, C-II or C-III). However, we have not ruled out the possibility that these differences may be associated with different concentrations of the various polypeptides. Indeed, some significant differences have been observed in the absolute and relative apolipoprotein concentrations of LDL subfractions of normal plasma and plasma of type IIa patients. These results will be presented in a separate communication.

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