COMPARISON OF THREE METHODS OF ISOLATING LIPOPROTEIN (A) FROM HUMAN BLOOD PLASMA

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UDC 616.153.96:577.112.856]-074

KEY WORDS: lipoprotein (a); affinity chromatography; atherosclerosis; immunosorbent

Lipoprotein (a) – Lp (a) – of human blood plasma, discovered in 1963 [5], has been the object of the closest attention of biochemists and cardiologists [9, 13, 15]. It has been shown that a high Lp (a) level can be regarded as an independent risk factor of the development of atherosclerosis [4, 9, 10, 13]. In its physicochemical properties Lp (a) resembles a particle of low-density lipoproteins (LDL), but besides apoB 100 it also contains a unique apoprotein, namely apo(a), which is covalently bound with apoB 100 by a disulfide bond [6, 8]. Because of the structural similarity of apo(a) and plasminogen, Lp (a) can evidently participate in microthrombus formation and, at the same time, like LDL particles, in the accumulation of atherogenic cholesterol by vascular wall cells [11, 13, 15]. Unlike LDL, it is virtually impossible to obtain a highly purified preparation of Lp (a) by the standard method of stepwise ultracentrifugation [12]. At the present time, to isolate Lp (a), multistage methods are used as a rule, in which a fraction of blood plasma rich in Lp (a) is obtained in the first stages by ultracentrifugation, after which the Lp (a) preparation is purified by gel-filtration [7, 8]. In the present study, three methods of isolation of Lp (a) from human blood plasma are compared. Method I is the standard method used to isolate Lp (a), and methods II and III have been modified and worked out in our laboratory.

EXPERIMENTAL METHOD

Human blood plasma was obtained after plasmapherisis, treated with 0.04% NaN_3, 2 mM PMSF, and 1.5 mM EDTA and kept at −70°C. Ultracentrifugation was carried out in a density gradient of the neutral salt NaBr at 15°C and 105,000 g, in a Ti 45 fixed-angle rotor for 24 h in an L8-55 centrifuge ("Beckman," Austria). A 2.6 × 90 cm column, packed with Sepharose CL-4B ("Pharmacia Fine Chemicals," Sweden), equilibrated with 0.1 M Tris-HCl buffer with 0.15 M NaCl, 0.001 M EDTA, pH 8.2, was used for gel-filtration. The immunosorbents were obtained as described previously [3, 14]. Affinity chromatography of human blood plasma on an anti-Lp (a)-immunosorbent was carried out as follows. Through a column containing 3 ml of immunosorbent was passed 15 ml of plasma with an initial Lp (a) concentration of 23.0 ± 2.1 mg/dl, on recycling mode for 3 h. The Lp (a) was eluted from the sorbent with 0.2 M glycine buffer with 0.15 M NaCl, pH 3.0. Immunoelectrophoresis was carried out in 1% agarose gel for 2 h at 4°C, 200 V, in Tris-acetate buffer, pH 8.0. Antibodies to LDL and Lp (a) were added in a concentration of 1 mg/ml and antiserum to human blood proteins in a dilution of 1:4. The Lp (a) concentration was determined by radial immunodiffusion [1].

EXPERIMENTAL RESULTS

Lp (a) was isolated from human blood plasma by three methods (Fig. 1). Method I has three stages. In the first stage of ultracentrifugation the lipoprotein fraction floating in a density of 1.063 g/ml was removed from the plasma, in the second stage, the fraction floating at densities of 1.063-1.090 g/ml was separated by the same method, and this fraction was

Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 3, pp. 268-270, March, 1992. Original article submitted July 25, 1991.

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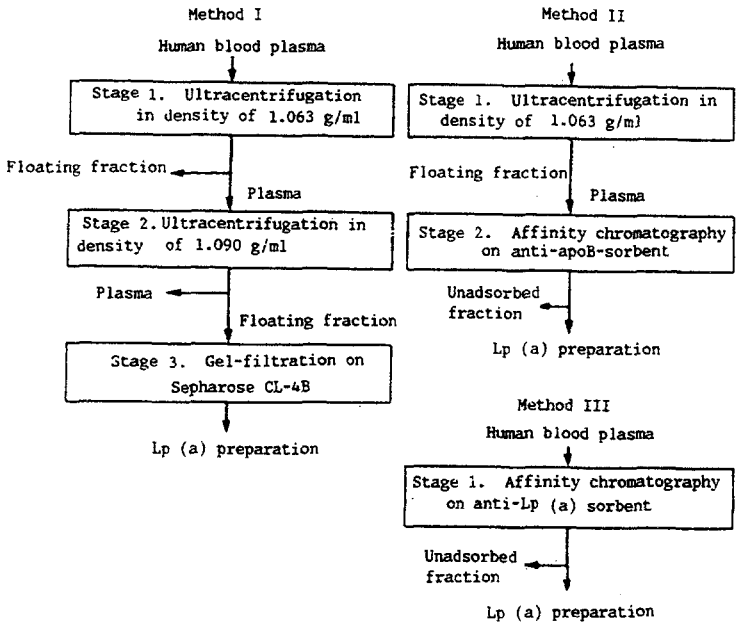

Fig. 1. Basic stages of three methods of isolating lipoprotein (a).

TABLE 1. Quantitative Comparison of Three Methods of Isolation of Lp (a) (M ± m)

Method	Stage	Loss during isolation, %	Yield of Lp (a), %
I	1 and 2	50,0±3,7	
	3	14,7±5,4	35,3±7,9
II	1	1,7±1,8	
	2	48,5±4,5	54,4±4,4
III	1	61,7±1,9	41,2±3,0

Legend. Here and in Table 2 results of six experiments are given. Initial Lp (a) concentration in plasma 23.0 ± 2.1 mg/dl.

used for further purification by the gel-filtration method (third stage). The quantity of the pure preparation of Lp (a) obtained by this method, depending on the original Lp (a) concentration in the plasma (20-50 g/dl), was 10-20 mg from 240 ml plasma. Disadvantages of the method, in our opinion, are the multiplicity of its stages, its duration, and also the fact that all stages impose limitations on the yield of preparative quantities of Lp (a). The principal losses are due to the fact that during ultracentrifugation a narrower fraction of plasma (1.063-1.090 g/ml) than the range of flotation densities of Lp (a) is obtained. However, this is essential for complete removal of LDL, which are difficult to separate at the gel-filtration stage.

To reduce losses, known methods were modified and method II developed. The first stage consisted of ultracentrifugation in a density of 1.063 g/ml, as a result of which all apoB-containing lipoproteins except Lp (a) were removed from the plasma. This was followed by chromatography of the apoB-deprived plasma on an anti-apoB-sorbent (second stage). We showed that Lp (a) is completely extracted under these conditions from the plasma. However, during elution by a buffer with low pH, aggregation of Lp (a) particles was observed, with the formation of a residue, and for that reason, method II has no significant advantages from the standpoint of yield of the end product (Table 1). Moreover, the stage of ultracentrifugation, corresponding to the first stage of method I, with its attendant disadvantages is still included. At the same time, it does contain one fewer stage, and this undoubtedly is a benefit.

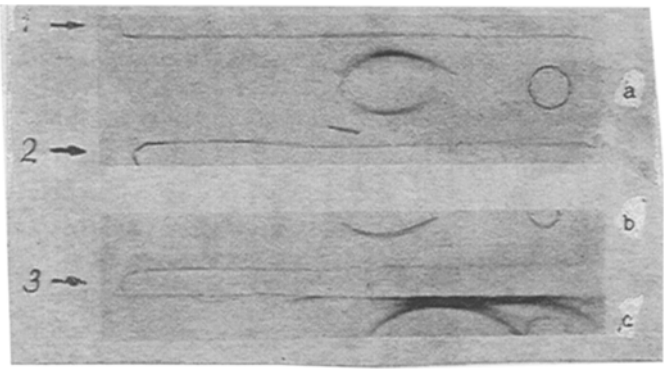

Fig. 2. Immunoelectrophoresis of Lp (a) preparation obtained by method III. Wells contain: a and b) Lp (a) preparation, c) human blood plasma. Gutters, indicated by arrows, contained: 1) antibodies to LDL, 2) antibodies to Lp (a), 3) antiserum to human blood proteins.

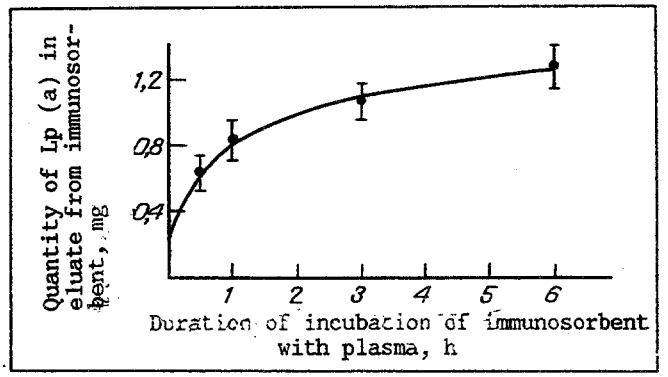

Fig. 3. Dependence of sorption capacity of anti-Lp (a)-sorbent on duration of incubation with human blood plasma. Affinity chromatography carried out as described in the section "Experimental Method." Abscissa, duration of incubation of immunosorbent with plasma (in h); ordinate, quantity of Lp (a) in eluate from immunosorbent (in mg).

TABLE 2. Dependence of Completeness of Elution of Lp (a) from Immunosorbent on Composition of Eluting Buffer (M ± m)

Eluting buffer	Quantity of Lp (a) eluted from 1 ml gel, mg
0,2 M glycine 0.15 M NaCl pH 3.5	0,15±0,03
0,2 M glycine 0.15 M NaCl pH 3.0	0,31±0,06
0,2 M glycine 0.15 M NaCl pH 2.5	0,49±0,11
5,0 M M sodium thiocyanate	0,40±0,10
5,0 M $MgCl_2$	0,07±0,02
0,15 M phosphate buffer with 5 M NaCl	0,01±0,00

Method III, in our view, is the most promising. This is a one-stage method of isolating Lp (a) by affinity chromatography on an immunosorbent with monospecific polyclonal antibodies to apo(a). Detailed characteristics of this sorbent were published by the writers previously [14]. The purity of the preparation obtained as a result of affinity chromatography of human blood plasma on anti-Lp (a)-sorbent was studied by immunoelectrophoresis. The experimental results are shown in Fig. 2. The isolated Lp (a) preparation interacts with antibodies to apoB and apo(a) and gives one precipitation band

with antiserum to human blood proteins, evidence of the immunochemical homogeneity of the isolated Lp (a) preparation and the absence of any contamination of it by other plasma antigens.

The quantity of Lp (a) bound by the immunosorbent depends on the concentration of immobilized antibodies and the duration of incubation of the sorbent with plasma. The sorbent binds Lp (a) most efficiently during the first hour of interaction (Fig. 3). With antibodies in a concentration of 9.8 mg/ml in the gel, 1 ml of sorbent binds 0.8 ± 0.2 mg Lp (a).

Table 2 gives the results of an experiment using these conditions of elution of Lp (a) after sorption on the anti-Lp (a)-sorbent. The largest amount of Lp (a) in the eluate was obtained by the use of glycine buffer, pH 2.5. However, exposure to low pH values leads to denaturation of the product obtained. The most acceptable eluant, in our view, is therefore sodium thiocyanate. Even in this case, however, some of the product is lost during elution (Table 1).

Thus our suggested one-stage method of isolation of Lp (a) by affinity chromatography on an immunosorbent is a simple method and enables preparative amounts of Lp (a) to be obtained quickly from large volumes of plasma.

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