IDENTIFICATION OF THE PROTEIN MOIETY OF THE LP(a)-LIPOPROTEIN IN HUMAN PLASMA

D. SEIDEL, H.P. GEISEN and D. ROELCKE

Medizinische Universitäts-Klinik (Ludolf-Krehl-Klinik) Heidelberg, Germany

and

Institut für Immunologie und Serilogie der Universität Heidelberg, Heidelberg, Germany

1. Introduction

The structure and function of plasma lipoproteins have received increasing attention in the past few years. The recognition of the importance of the protein moiety for the structural stability and metabolism of these conjugated macromolecules has stimulated this field of research and has emphasized the careful characterization of the apo-lipoproteins for a better understanding of lipid transport and metabolism.

The classification and nomenclature in the lipoprotein field have been commonly based on two sets of operational terms. Differences in the hydrated density of plasma lipoproteins, which are largely a consequence of their lipid composition and differences in their electrophoretic mobility, which is primarily a consequence of their protein moiety, have provided the basis of a widely accepted classification system, comprising four major classes, each of which is characterized by a relatively narrow density range and a distinguished electrophoretic band. These are the very low density lipoproteins (VLDL; d < 1.006 g/ml), which can be further subdivided into chylomicrons and pre-β-lipoproteins, the low density lipoproteins (LDL; d 1.006–1.063 g/ml) with β-mobility and the high density lipoproteins (HDL; d 1.063–1.21 g/ml) exhibiting α-mobility on electrophoresis.

Since we know more about the protein moiety of the plasma lipoproteins, the so-called apo-lipoproteins and their distribution between the different classes, so far three distinct are characterized (apo-A, apo-B and apo-C) each of which consists of subunits,

no one of the two classification systems based on density or electrophoretic mobility is any more adequate to describe the different classes of lipoproteins from a physiological-chemical point of view. This is particularly true for dyslipoproteinemias. It is also true for the LP(a)-lipoprotein first described by Berg [1]. It has been suggested by Berg [2] that the LP(a)-lipoprotein represents an inherited antigenic polymorphic system of the β-lipoproteins which can be found in 30–35% of European population. In 1968, Wiegand et al. [3] and Schultz et al. [4] were the first to describe the hydrated density of the LP(a)-lipoprotein within the density range of the high density lipoproteins (d 1.063–1.21 g/ml). The isolated LP(a)-lipoprotein [5] reacted immunochemically only with anti-β-lipoprotein serum and anti-LP(a)-lipoprotein serum, but not with anti-α-lipoprotein serum or anti-albumin serum. It contained less lipids than normal LDL. The aminoacid composition of apo-LP(a)-lipoprotein differs from that of apo-B and apo-A, the protein moieties of β- and α-lipoproteins [6]. Riedel et al. [7] and Simons et al. [6] demonstrated that the LP(a)-lipoprotein develops pre-β-mobility on agarose electrophoresis.

Since the pre-β-lipoprotein fraction normally consists predominantly of apo-lipoprotein C, the third apo-lipoprotein first described by Gustafson et al. [8], it seemed justified and was the purpose of this study to analyze the LP(a)-lipoprotein in more detail with regard to its composition of apo-lipo-. proteins.

2. Material and methods

2.1. Isolation of the LP(a)-lipoprotein

Blood samples were obtained from healthy male and female volunteers, 24 to 45 years of age. The blood was usually drawn 10 hr after a meal, but never less than 5 hr after. It was collected for preparative isolation into flasks containing sodium citrate and the plasma was recovered by low speed centrifugation.

The plasma was adjusted to a solution density of d 1.063 g/ml by adding sodium bromide and layered under equal volumes of a sodium bromide solution with the density d 1.063 g/ml and centrifuged in the type Ti 50 Rotor of the Spinco Model L-2 ultracentrifuge for 44 hr at 105,000 g and 4°.

The top fraction was removed by a tube slicing technique and recentrifuged under the same conditions at the solution density of 1.12 g/ml. The top fraction after the second centrifuge run was dialyzed for 12 hr against 0.9% NaCl and further fractionated by column chromatography (2.1 X 30 cm) with hydroxylapatite according to the method of Tiselius et al. [9], Hjertén et al. [10] and Cramér and Brattstein [11] in order to separate the LP(a)-lipoprotein from the α-lipoproteins. The LP(a)-lipoprotein was diluted with a K-phosphate buffer, pH 8.6, 0.65 M. For details see Roelcke [12].

2.2. Immunological methods

The immuno-chemical properties of the isolated LP(a)-lipoprotein were studied by double immuno-diffusion [13] and immuno-electrophoresis [14] in 1% agarose gel employing a barbital buffer, pH 8.6, ionic strength 0.05. Rabbit anti-human sera to α-lipoproteins, β-lipoproteins and whole human serum (Behring Werke AG, Marburg/Lahn, Germany) were used. The rabbit anti-sera against lipoprotein-C and LP(a)-lipoprotein were prepared in our laboratories as described earlier [1, 15]. As apo-C antigen either very low density lipoproteins or isolated LP(x) [15, 16] was used.

2.3. Electrophoresis

Lipid electrophoresis was performed according to the technique by Noble [17] with minor modifications [18].

2.4. Delipidization

The isolated LP(a)-lipoprotein was delipidized according to a modification of the procedure of Scanu et al. [19]. Five successive extractions with ethanol-diethyl ether (3:1, v/v) followed by five extractions with diethyl ether at 0° were performed. After the final solvent treatment the essentially lipid-free protein residues were removed by low speed centrifugation and were dried under nitrogen for several hours. 0.9% NaCl was added to redissolve the apo-lipoprotein C compound.

3. Results and discussion

Ultracentrifugation of the plasma from donors with immuno-chemically proven positive LP(a)-lipoprotein demonstrates that this plasma lipoprotein is within the density fraction d 1.063–1.12 g/ml (HDL). The 1.063 g/ml top fraction and the d 1.12 g/ml bottom fraction showed no immunoprecipitating reaction against the mono-specific anti-LP(a)-lipoprotein serum. The isolated LP(a)-lipoprotein develops pre-β-mobility on agarose electrophoresis (fig. 1).

Whereas the density fraction d 1.063–1.12 g/ml reacted immuno-chemically with anti-α-lipoprotein serum, the isolated LP(a)-lipoprotein showed no reac-
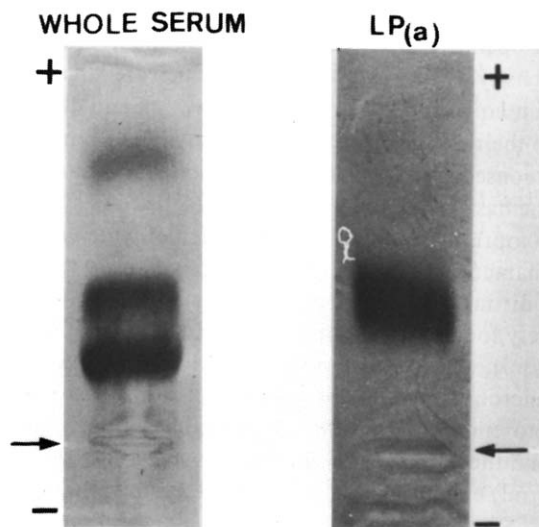

Fig. 1. Lipidelectrophoresis of whole plasma and the isolated LP(a)-lipoprotein fraction in 1% Agarosegel; Oil red 0 stained.
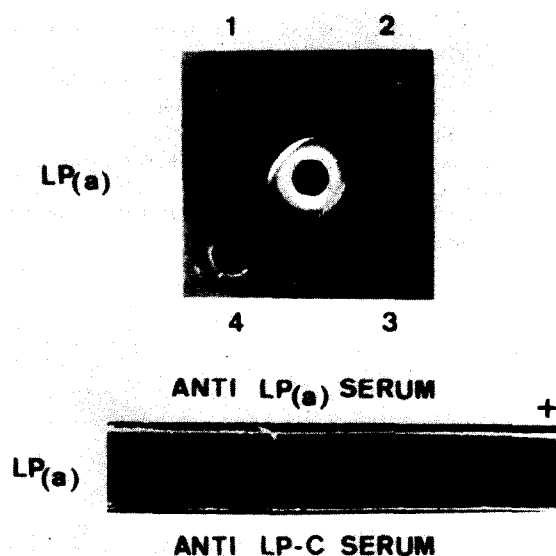
Fig. 2. Double immunodiffusion (1% Agar-gel) and immuno-electrophoresis (1% Agarose-gel) of isolated LP(a)-lipoprotein. 1 = anti-β-lipoprotein serum, 2 = anti-LP-(a)-lipoprotein serum, 3 = anti-LP-C serum and 4 = anti-α-lipoprotein serum.

tion against this anti-serum (fig. 2). However, the isolated LP(a)-lipoprotein reacted immuno-chemically not only with anti-serum to normal β-lipoproteins and with the specific anti-serum to the LP(a)-lipoprotein, but also with anti-serum to apo-lipoprotein C. By double immuno-diffusion (see fig. 2) full identity was obtained between anti-β-lipoprotein serum, anti-LP(a)-lipoprotein serum and anti-apo-C serum, clearly indicating that the LP(a)-lipoprotein carries all three antigenic determinants.

This finds support by the immuno-electrophoretic pattern of isolated LP(a)-lipoprotein, tested against anti-apo-C- and anti-LP(a)-lipoprotein serum. The immunoprecipitating reaction against the two different anti-bodies develops in the same (pre-β) position (see fig. 2).

After total delipidization of the isolated LP(a)-lipoprotein only the apo-lipoprotein C compound was soluble in 0.9% NaCl and detectable by immuno-chemical means. It seems therefore unlikely, that the specific antigenic determinant of the LP(a)-lipoprotein is located on or is a part of the apo-lipoprotein C protein moiety. Further studies are necessary to elucidate whether the specific antigenic determinant is part of the apo-B protein moiety or part of a

separate protein moiety. These studies are in progress in our laboratories.

Our data establish that the LP(a)-lipoprotein as other pre-β-lipoproteins consists of apo-lipoprotein C in addition to apo-lipoprotein B. However, with regard to the content of apo-lipoprotein A it seems to differ from the normal pre-β-lipoproteins of the VLDL fraction, since this density class generally contains apo-lipoprotein A in small amounts. We were unable to detect apo-lipoprotein A by immunological means neither in the intact LP(a)-lipoprotein nor in its delipidized form. Thus, the increase in mobility of the LP(a)-lipoprotein seems not to be due to the apo-lipoprotein A content, as discussed for the normal pre-β-lipoproteins. It may, however, be a result of its sialic acid content as indicated by Simons [6].

Although there is still much uncertainty about structural relations of the lipids to the proteins in the plasma lipoproteins, we feel that further emphasis on the characterization of the protein moieties of plasma lipoproteins, especially of those with abnormal physico-chemical characteristics, such as the LP(a)-lipoprotein, may provide a better understanding of lipid metabolism.

Acknowledgement

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