Protein composition of Lp(a) lipoprotein from human plasma

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The apolipoprotein composition of purified human Lp(a) lipoprotein was investigated by SDS–polyacrylamide gel electrophoresis and immunochemically. The lipoprotein contains two different polypeptides. One is identical by its app. M_r of ~250000 and immunologically with apolipoprotein B of LDL (B-100). The other polypeptide has a higher app. M_r (~350000) and stains strongly with the periodate-Schiff's reagent. This high-M_r glycoprotein contains the specific Lp(a) immunoreactivity but does not react with antibodies against apo B. Apo B and Lp(a)-protein seem to be linked by disulfide bonds in the native lipoprotein. The unreduced detergent delipidized protein moiety from Lp(a) lipoprotein shows a single band of M_r ~700000 in SDS–polyacrylamide gel electrophoresis and the immunoprecipitates formed against anti-Lp(a) and anti-apo B by the unreduced protein show a reaction of immunological identity.

Lp(a) lipoprotein *Lp(a) antigen* *Atherosclerosis*

1. INTRODUCTION

The Lp(a)-antigen was first demonstrated in human sera by use of heterologous antibodies and was described as a predominantly inherited variant of LDL [1]. Later investigations have shown, that Lp(a) is a quantitative genetic trait [2–5] and that the specific antigenicity resides in a separate lipoprotein population designated the Lp(a) lipoprotein [6–9]. The Lp(a) lipoprotein has been purified and characterized by several investigators [8–12]. It floats in the density range from 1.05–1.12 g/ml and has a higher M_r (~5 × 10^6) than plasma LDL. Upon agarose gel electrophoresis it exhibits so-called slow pre-beta mobility. The protein moiety of Lp(a) lipoprotein is rich in carbohydrate containing about 6-times more sialic acid than plasma LDL [11]. There are several reports on the protein composition of Lp(a) lipoprotein [11–15]. All investigators agree that apo B and Lp(a)-protein are components of the particle but apolipoproteins C [13] A-III [12] and albumin [11,14] were also reported to be integral components of the lipoprotein. Authors in [12] have reported a M_r of 35000–40000 for the Lp(a)-antigen.

The Lp(a) lipoprotein has recently attracted considerable interest due to reports on a positive correlation of Lp(a) levels in plasma with the risk for coronary heart disease [16–18] and it has been postulated, that Lp(a) lipoprotein is an independent risk factor for atherosclerosis [19]. The earliest report on a higher frequency of Lp(a) positive individuals among patients with myocardial infarction dates back to 1965 [20], but has been ignored by later investigators.

Here, we report that Lp(a) lipoprotein contains two polypeptides, one of which seems identical with apo B from plasma LDL. The other polypeptide is a high-M_r glycoprotein that has the immunochemical properties of Lp(a) antigen.

Abbreviations: LDL, low density lipoproteins; apo, apolipoprotein; SDS, sodium dodecylsulfate; PAGE, polyacrylamide gel electrophoresis; PAS, periodic acid Schiff; NaDOC, sodium desoxycholate

2. MATERIALS AND METHODS

Lp(a) lipoprotein was purified from the plasma of 6 individual healthy blood donors that exhibited a strong positive reaction against anti-Lp(a) serum

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in double diffusion experiments. The density fraction 1.05–1.125 g/ml was prepared from 200 ml of plasma from each individual donor by sequential ultracentrifugation [21] and Lp(a) lipoprotein was isolated from the density fraction by column chromatography on Sepharose 4B® essentially as in [9]. LDL was isolated from the same plasma specimens by ultracentrifugation between densities 1.019 and 1.05 g/ml. The purity of Lp(a) lipoprotein and of LDL was monitored by polyacrylamide gel electrophoresis of Sudan black prestained lipoprotein fractions [6]. Column fractions containing the pure Lp(a) lipoprotein were pooled, concentrated by ultrafiltration on an XM-100 membrane and delipidated by dropwise addition of the lipoprotein solution to acetone–ethanol 1:1 (v/v) at −20°C. In an alternative procedure Lp(a) lipoprotein was delipidized by column chromatography on Sephadex G-200® in the presence of sodium desoxycholate following the procedure in [22] for LDL. A 2–50 cm column was prepared and equilibrated with 0.05 M NaCl, 0.05 M Na_2CO_3, 10 mM sodium desoxycholate, pH 10. To the Lp(a) lipoprotein (7 mg protein in 5 ml buffer) 100 mg solid sodium desoxycholate was added. The solution was applied to the column that was eluted with the equilibrating buffer at a flow of 17 ml/h. The absorbance was monitored at 256 nm and fractions (2-ml) were analysed for protein by SDS-gel electrophoresis and for lipids by thin-layer chromatography. The protein, eluting in the void volume, was free of lipids and was dialysed against several changes of 0.02 M ethylmorpholine–HCl, pH 8.6 buffer, 0.1% sodium dodecylsulfate.

For SDS–polyacrylamide gel electrophoresis apolipoproteins were solubilized in 0.02 M ethylmorpholine–HCl (pH 8.6), 5% SDS, 5% β-mercaptoethanol and boiled for 3 min in a water bath. Electrophoresis was performed on 3.3% polyacrylamide gels [23]. In some experiments β-mercaptoethanol was omitted. The gels were stained either with Coomassie brilliant blue for protein or with the periodate-Schiff's reagent for carbohydrate [24]. Protein standards for M_r estimates were BSA, phosphorylase B, β-galactosidase and myosin (all from Sigma, München). For immunochemical analysis the regions corresponding to the slow and fast protein bands (see section 3) were cut with a razor blade from unstained gels running parallel. The proteins were eluted from the gel slices by incubation with 1 ml 0.02 M ethylmorpholine–HCl (pH 8.6), 0.1% SDS at 37°C under gentle agitation overnight. The eluates and the detergent delipidized apolipoproteins were analyzed by double immunodiffusion in 1% agarose containing 0.5% Triton X-100. Under these conditions no artefactual precipitates due to the presence of SDS were seen in control experiments.

3. RESULTS

In agreement with previous reports, the Lp(a) lipoprotein can be easily purified by a combination of ultracentrifugation and chromatography on an agarose column. The elution profile was essentially identical with that obtained in [9,11]. The purified lipoprotein migrates as a single band in polyacrylamide gel electrophoresis with a mobility clearly different from LDL and α_1-HDL (fig.1).

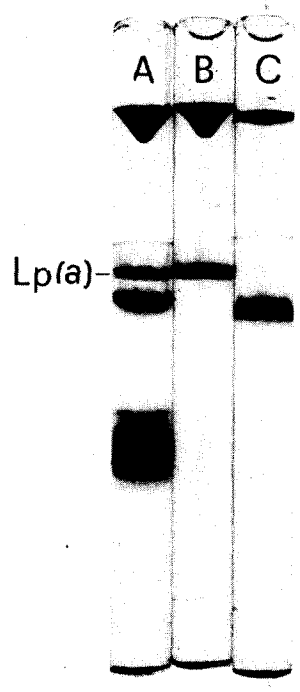

Fig.1. Polyacrylamide gel electrophoresis (3.75%) of Sudan black B prestained lipoproteins. (A) density fraction 1.05–1.125 g/ml; (B) Lp(a) lipoprotein from the Sepharose 4B column; (C) LDL (density 1.019–1.05 g/ml).

3.1. *SDS–polyacrylamide gel electrophoresis*

After extraction of lipids by organic solvent the protein moiety of Lp(a) lipoprotein was analyzed by 3.3% SDS-gel electrophoresis and compared to that of LDL. Coomassie staining for protein revealed a major band of M_r ~250000 in Lp(a) that corresponded in mobility to the single protein in human LDL (B-100). In addition, a less intensive staining protein band of M_r ~350000 was present in Lp(a) lipoprotein but not in LDL (fig.2). No other protein bands were present even when the samples were analyzed on 10% polyacrylamide gels (not shown). Staining of the gels with the periodate-Schiff's reagent demonstrated the same pattern of bands but with different intensities. The M_r 350000 protein in Lp(a) stained strongly with the Schiff's reagent, indicating that it has a high carbohydrate content. The M_r 250000 band stained poorly with the carbohydrate stain. When the protein moiety of Lp(a) lipoprotein was subjected to SDS–PAGE in the absence of a disulfide reducing agent it formed a single band of M_r 700000. Identical patterns were observed for all 6 individual Lp(a) preparations.

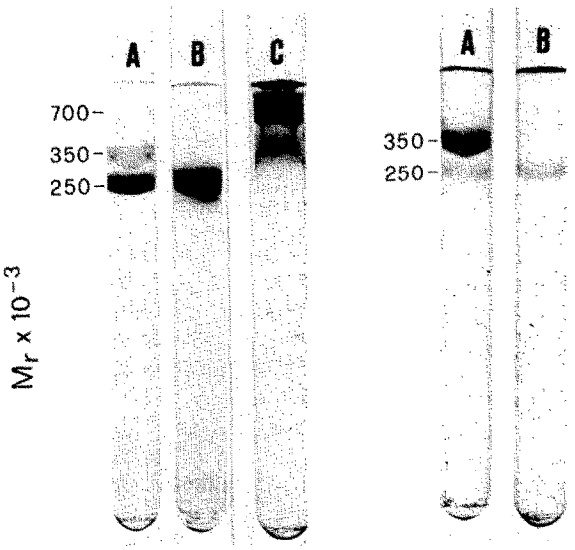

Fig.2. SDS–polyacrylamide gel electrophoresis (3.3%) of organic solvent delipidized apoproteins from Lp(a) lipoprotein (A,C) and LDL (B). Samples in A and B were reduced with β-mercaptoethanol, sample C was not reduced. Left: Coomassie staining. Right: periodate-Schiff-staining.

3.2. *Immunochemical analysis*

The Lp(a) lipoprotein was delipidated by column chromatography on Sephadex G-200 in the presence of sodium desoxycholate under non-reducing conditions. The protein moiety eluted as a single peak in the void volume of the column and was completely separated from the lipids essentially as shown for LDL by Helenius and Simons [22]. The apoprotein patterns of reduced and unreduced detergent delipidized apo Lp(a) were identical with that seen with organic solvent extracted apo Lp(a) (see above). The unreduced protein reacted with antibodies against Lp(a) antigen and apo B. Both

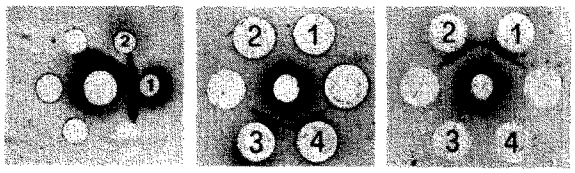

Fig.3. Double immunodiffusion analysis of apolipoproteins from Lp(a) lipoprotein in 1% agarose, 0.5% Triton X-100. Left: immunodiffusion of detergent delipidized unreduced protein from Lp(a) lipoprotein (center well) against anti Lp(a) (well 1) and anti apo B (well 2). Middle: immunodiffusion of M_r 250000 protein (wells 1,2) and M_r 350000 protein (wells 3,4) against anti Lp(a). Right: same proteins as in the middle plate except that center well contains anti apo B.

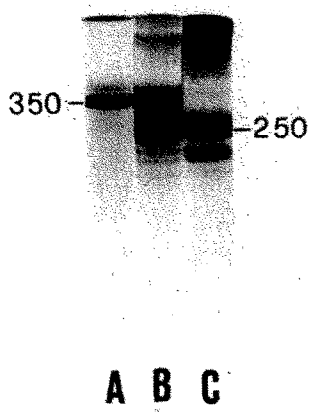

Fig.4. SDS–polyacrylamide gel electrophoresis (3.3%) of β-Me-reduced proteins from Lp(a) lipoprotein: (A) M_r 350000 protein eluted from unstained SDS–gels; (B) total protein from Lp(a) lipoprotein delipidized by Sephadex G-200 chromatography in the presence of NaDOC; (C) M_r 250000 protein eluted from unstained SDS–gels.

precipitates formed a line of identity in double immunodiffusion (fig.3).

The two protein species of Lp(a) lipoprotein were separated by SDS–PAGE under reducing conditions and were eluted from unstained gels into 0.02 M ethylmorpholine–HCl, pH 8.6 buffer, 0.1% SDS. Re-electrophoresis yielded single protein bands of M_r ~250000 and M_r ~350000 (fig.4). When tested by immunodiffusion the M_r 350000 protein reacted with anti-Lp(a) only, whereas the M_r 250000 protein reacted only with anti apo B (fig.3). Taken together, the immunochemical analysis and the results from SDS–PAGE under reducing and non-reducing conditions indicate that Lp(a) protein and apo B form a complex by disulfide bridge formation in Lp(a) lipoprotein.

4. DISCUSSION

Previous studies on the protein moiety of the Lp(a) lipoprotein demonstrated the occurrence of various proteins in this lipoprotein including apo B, apo A-III, apo C, albumin and the Lp(a) antigen [10–15]. Our results show that there are only two proteins present in purified Lp(a) lipoprotein, namely apo B and Lp(a)-antigen. The discrepancy between our findings and previous results may be related to differences in the purification procedures. Authors in [12] precipitated lipoproteins with phosphotungsten before further purification and this may have resulted in the coprecipitation of apo A-III. Albumin has been demonstrated in Lp(a) lipoprotein preparations only after ageing [11,15] and may have represented an artefact.

The apo B species in Lp(a) corresponds to the B-100 protein of LDL [25] by app. M_r and immunochemically (fig.1,3). No protein corresponding to the B-48 apoprotein (B-like protein) from lymph chylomicrons [25,26] was detected in any of the 6 Lp(a) preparations tested.

The Lp(a) antigen exhibits a higher app. M_r (~350000) in SDS–PAGE than apo B-100. This is in contrast to the report in [12] that determined an M_r of 35000–40000 for Lp(a) antigen. The app. M_r for the proteins of Lp(a) lipoprotein determined here should be considered with reservation. Authors in [27] have provided evidence, that apo B does form dimers even in the presence of SDS and exhibits anomalous mobility in SDS–PAGE. Authors in [11] have shown that the protein moiety of Lp(a) lipoprotein is rich in carbohydrate and contains 0.26 mg carbohydrate/mg protein. Our results from the periodate-Schiff staining indicate that most of the carbohydrate is associated with the Lp(a) antigen and not with apo B. This high carbohydrate content may result in an anomalous mobility also of the Lp(a) protein in SDS–PAGE.

Even though the M_r for Lp(a) antigen may not be precise, our data indicate that it is a high-M_r component that is bound to apo B-100 by disulfide bond formation. Further studies on the properties of this protein are required and may help to understand the structural and genetic basis of the quantitative Lp(a) trait. This seems important in view of the postulated involvement of Lp(a) lipoprotein in human atherosclerosis.

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