Volume 215, number 1, 105–108 FEB 04610 May 1987

Apolipoprotein A-I-binding protein from human term placenta

Purification and partial characterization

Leila Keso, Matti Lukka, Christian Ehnholm, Marc Baumann*, Pirkko Vihko[+] and Maritta Olkinuora[+]

*National Public Health Institute, Mannerheimintie 166, *Recombinant DNA-laboratory, University of Helsinki, Helsinki* and [+]*Department of Clinical Chemistry, University of Oulu, Oulu, Finland*

Received 6 February 1987

A protein that binds to the main apoprotein, apoA-I, of human high density lipoprotein (HDL) has been isolated from human placenta. Ligand blotting after SDS gel electrophoresis indicated that the 120 kDa protein in the absence of reducing agents binds apoA-I. If gel electrophoresis was performed under reducing conditions two main bands, approx. 50 and 30 kDa that did not bind apoA-I, were evident. In an enzyme-linked immunosorbent assay the binding protein specifically bound apoA-I, delipidated or as HDL. ApoA-II, apo E and LDL did not compete with apoA-I for binding to this protein.

Apolipoprotein-binding protein; Apolipoprotein A-I; Purification; (Human placenta)

1. INTRODUCTION

The concept that high density lipoproteins (HDL) have a protective role against development of coronary artery disease [1,2] has greatly stimulated the study of this lipoprotein class. HDL are initially secreted into circulation as precursor lipoproteins which are transformed intravascularly to spherical HDL [3]. This circulating HDL is a heterogeneous class of lipoproteins [4], in which the ratio of the two main apoproteins, apoA-I and apoA-II, varies among the subclasses [5]. Several studies have indicated that HDL are involved in the net movement of cholesterol out of cells [6] and in the delivery of cholesterol to hepatic and steroidogenic cells [7,8]. In cultured macrophages a receptor-mediated retroendocytosis pathway for HDL has been demonstrated [9,10]. Proteins that bind HDL or HDL apoproteins have been partially purified from rat liver and kidney [11] and adrenal membranes [12]. It has also been demonstrated that placental cells display specific binding of HDL [13].

We recently reported that acid phosphatases avidly bind to the main high density lipoprotein apoprotein A-I [14]. However, further studies indicate that apoA-I does not bind to the enzyme but to a protein that co-elutes with the enzyme during affinity chromatography on L-(+)-tartrate. We now describe the isolation and partial characterization of this apoA-I-binding protein (apoA-I BP) from human placenta.

Correspondence address: C. Ehnholm, National Public Health Institute, Mannerheimintie 166, SF-00280 Helsinki, Finland

2. MATERIALS AND METHODS

2.1. *Purification of human apoA-I-binding protein*

2.1.1. Ammonium sulphate precipitation

Full-term human placenta (obtained from the Department of Gynaecology, University of

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

Helsinki) was homogenized in distilled water containing 1 ml/l Tween 20 Atlas. After 12 h at 4°C the homogenate was centrifuged to remove cellular debris and the supernatant was 30% saturated with ammonium sulphate. After 4 h the homogenate was centrifuged for 20 min at 15000 × g. The ammonium sulphate concentration in the supernatant was increased to 70%. The precipitate was recovered, dissolved in 50 mM sodium acetate, pH 5.0, and dialyzed against the same buffer.

2.1.2. Chromatography on L-(+)-tartrate–AH-Sepharose 4B

L-(+)-Tartrate was coupled to Sepharose 4B [15]. The dialyzed ammonium sulphate precipitate was applied to a 2.6 × 40 cm column of tartrate–AH-Sepharose, equilibrated with 50 mM Na-acetate, pH 5.0, at a flow rate of 38 ml/h. The column was washed with equilibration buffer until no protein could be recorded in the eluate (A_{280}) and then eluted stepwise with equilibration buffer containing 10 and 25 mM L-(+)-tartrate.

2.1.3. Chromatography on apoA-I–Sepharose 4B

ApoA-I was coupled to CNBr-activated Sepharose 4B according to the instructions of Pharmacia Fine Chemicals (Sweden). Fractions eluted from the tartrate–AH-Sepharose column were pooled, neutralized with 1 M Tris and subjected to affinity chromatography on an apoA-I-Sepharose column (1.5 × 7 cm). The column was washed with PBS and then eluted with 0.1 M glycine, pH 2.0.

2.1.4. Isolation of lipoproteins and apolipoproteins

Lipoproteins were isolated by ultracentrifugation [16] and the apolipoproteins purified [17].

2.2. *Electrophoresis and ligand blotting*

After electrophoresis [18], the apoA-I-binding protein was transferred to 0.45 μm nitrocellulose membranes in 25 mM Tris/192 mM glycine/20% methanol (pH 8.3) for 1.5 h and 0.6 A. The membrane was then blocked, incubated overnight with 3% gelatin in 10 mM Tris/saline, pH 7.4 (TBS), and thereafter incubated with purified apoA-I (10 μg/ml in TBS containing 1% gelatin) for 8 h at room temperature. After washing twice for 20 min with TBS containing 0.05% Tween incubation with a monoclonal apoA-I antibody (2AI), second antibody incubation and peroxidase staining were performed [19]. Coating of microtiter-plates with the apoA-I-binding protein was done as described [19], using a protein concentration of 5 μg/ml.

2.3. *Binding studies*

Purified apolipoproteins were diluted in 1% gelatin/TBS (pH 7.4) to contain 0–50 μg/ml of protein and 100 μl of the dilutions were applied on microtiter plates coated with apoA-I BP. The plates were incubated for 2 h at room temperature and thereafter washed twice with 0.05% Tween/PBS. Then 100 μl of the respective antibodies, diluted in 1% gelatin/TBS (pH 7.4) were added to each well. The plates were incubated for 1 h at room temperature and washed twice. The plates were treated with second antibodies (anti-mouse IgG and anti-rabbit IgG conjugated to alkaline phosphatase) and developed as in [19].

3. RESULTS

3.1. *Purification of apoA-I-binding protein*

To isolate the apoA-I-binding protein ammonium sulphate fractionation of the placental homogenate was performed following by affinity chromatography on AH-Sepharose 4B gels containing L-(+)-tartrate. Fig.1 illustrates the elution profile obtained after stepwise elution of the column with 10 and 25 mM L-(+)-tartrate. The

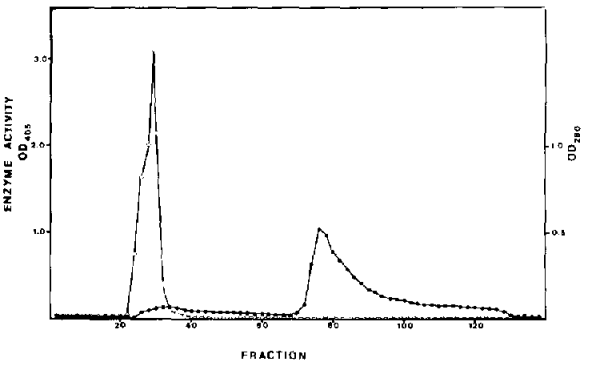

Fig.1. Affinity chromatography on L-(+)-tartrate–AH-Sepharose 4B. The eluting buffers, 10 and 25 mM L-(+)-tartrate in 50 mM acetate buffer, pH 5.0, are indicated. (○—○) Acid phosphatase activity as absorbance at 405 nm; (●—●) absorbance at 280 nm.

lysosomal acid phosphatase activity (LAP) eluted with 10 mM tartrate. Increasing the tartrate concentration to 25 mM resulted in the elution of protein but no further acid phosphatase activity. Affinity chromatography of the fractions with LAP activity on Sepharose gels containing immobilized apoA-I indicated that of the enzyme activity applied less than 1% was retained on the affinity matrix and 99% passed through the column.

When the fractions eluting with 25 mM tartrate (fig.1) were combined and subjected to affinity chromatography on apoA-I–Sepharose apoA-I-binding protein could be recovered by eluting the column with 0.1 M glycine buffer (pH 2.0).

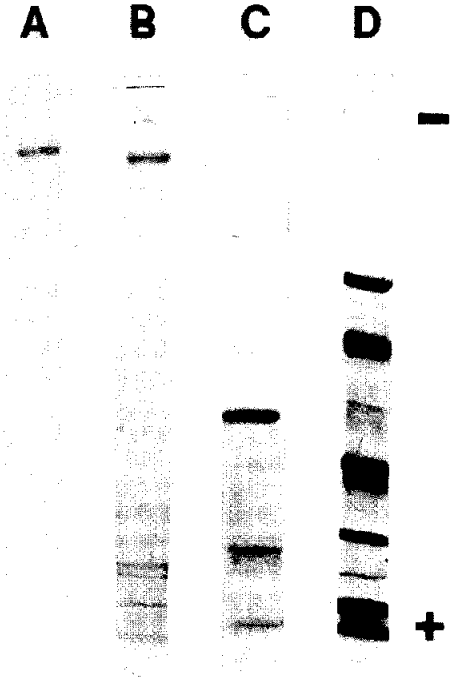

Fig.2. SDS gradient (5–20%) gel electrophoresis. Lanes: (A) ligand blotting of apoA-I-BP after SDS gel electrophoresis in the absence of reducing agent; (B–D) Coomassie blue-stained gels of: (B) apoA-I-BP, 10 μg applied in the absence of 2-mercaptoethanol; (C) the same amount of apoA-I-BP under reducing conditions; (D) low molecular mass standards (Pharmacia) – (from the top) phosphorylase *b* (92.5 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa).

3.2. *Characterization of apoA-I-binding protein*

The affinity-purified apoA-I-binding protein (apoA-I-BP) was characterized by SDS gradient gel electrophoresis followed by ligand blotting (fig.2). The protein that elutes from the apoA-I affinity column is a high-M_r protein or a protein aggregate as judged from SDS gel electrophoresis performed in the absence of reducing agents (fig.2B). This protein binds apoA-I as demonstrated by ligand blotting (fig.2A). Ligand blotting using apoA-II and the corresponding antibody did not result in any binding of apoA-II (not shown). When SDS gel electrophoresis was performed under reducing conditions two main bands, 50 and 30 kDa, are evident (fig.2C) (Coomassie staining). Neither reacts with apoA-I in ligand blotting.

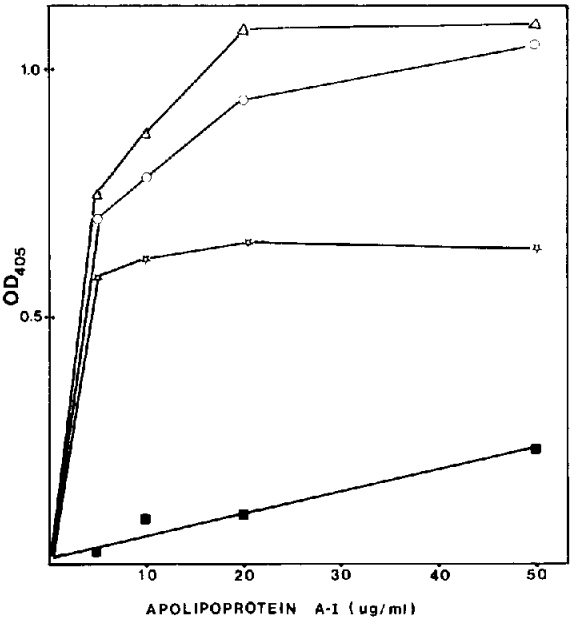

Fig.3. Binding of purified apoA-I and apoA-I derived from plasma or HDL to immobilized apoA-I-BP. Microtiter plates coated with apoA-I-BP were incubated with different amounts of purified apoA-I (☆—☆), plasma (○—○) or isolated HDL (△—△) containing the indicated amounts of apoA-I as determined by immunoassay. After a 3 h incubation the wells were emptied, washed and the bound apoA-I visualized using monoclonal apoA-I antibody and enzyme labeled anti-mouse γ-globulin antiserum. Control incubations in wells coated with bovine serum albumin showed no binding of apoA-I (■—■).

To study further the binding properties of apoA-I-BP, it was immobilized on microtitration plates. Plates coated with different concentrations of apoA-I-BP bound increasing amounts of apoA-I in a saturable manner. However, when no apoA-I was added to the plates coated with apoA-I-BP no binding of the monoclonal A-I antibody could be observed indicating that the isolated apoA-I-BP preparation did not contain apoA-I. Also the apoA-I present in HDL or in serum showed saturable binding to the immobilized apoA-I-BP (fig.3). In experiments with apoA-II, apoE and apoB (as LDL) only very low non-saturable binding could be demonstrated (not shown). To determine the specificity of the binding protein the binding of apoA-I (10 μg/ml) to microtitration wells coated with apoA-I-BP in the presence of increasing concentrations (10–100 μg/ml) of purified apoproteins A-II, E and B (as LDL) was studied. These apoproteins did not compete for binding of apoA-I to the binding protein (not shown). Thus the binding protein we have purified appears to be specific for apoA-I.

4. DISCUSSION

The protein we have isolated from human placenta by affinity chromatography on L-(+)-tartrate-AH-Sepharose and immobilized apoA-I binds specifically the main apolipoprotein of HDL, apoA-I. Why this protein also interacts with L-(+)-tartrate is not known at present. This latter interaction which has been considered rather specific for the tartrate-inhibitable lysosomal acid phosphatase (LAP) led us to believe that this enzyme also binds the apolipoprotein A-I [14]. However, the present study clearly demonstrates that the LAP activity can be separated from the apoA-I-BP. In studies with microtiter plates coated with apoA-I or apoA-I-BP no binding of purified LAP to these proteins could be demonstrated (not shown), neither did the enzyme bind to apoA-I covalently linked to Sepharose.

The presence of proteins involved in the binding of HDL to a number of different cell types has been demonstrated [10,11,20]. As HDL interacts differently with different cell types, delivers cholesterol to some and removes it from others, it is probable that the proteins mediating the lipoprotein-cell interaction are also different. Binding of HDL to human placental membrane fractions has been demonstrated [13], however, the binding site has not been isolated. The physiological function of the placental apoA-I-BP which we have isolated is not known and warrants further study.

REFERENCES

[1] Miller, G.J., Miller, N.E. and Ashcroft, M.T. (1976) Clin. Sci. Mol. Med. 51, 475–482.
[2] Heiss, G., Johnsson, N.J., Reiland, S., Davis, C.E. and Tyroler, H.A. (1980) Circulation 62, suppl.IV, 116–136.
[3] Hamilton, R.L., Williams, M.C., Fielding, C.J. and Havel, R.J. (1976) J. Clin. Invest. 58, 667–680.
[4] Eisenberg, S. (1984) J. Lipid Res. 25, 1017–1058.
[5] Cheung, M.C. and Albers, J.J. (1979) J. Lipid Res. 20, 200–207.
[6] Tall, A.R. and Small, D.M. (1980) Adv. Lipid Res. 7, 1–51.
[7] Rifici, V.A. and Eder, H.A. (1984) J. Biol. Chem. 259, 13814–13818.
[8] Glass, C., Pittman, R.C., Weinstein, D.B. and Steinberg, D. (1983) Proc. Natl. Acad. Sci. USA 80, 5435–5439.
[9] Schmitz, G.H., Robenek, H., Lohmann, V. and Assmann, G. (1985) EMBO J. 4, 613–622.
[10] Schmitz, G., Niemann, R., Brennhausen, B., Krause, R. and Assmann, G. (1985) EMBO J. 4, 2773–2779.
[11] Fidge, N.H. (1986) FEBS Lett. 199, 265–268.
[12] Fidge, N.H., Kagami, A. and O'Connor, M. (1985) Biochem. Biophys. Res. Commun. 129, 759–765.
[13] Cummings, S.W., Hatley, W., Simpson, E.R. and Ohashi, M. (1982) Clin. Endocrinol. Metab. 54, 903–908.
[14] Vihko, P., Wahlberg, L., Ehnholm, C., Lukka, M. and Vihko, R. (1986) FEBS Lett. 202, 309–313.
[15] Vihko, P., Kontturi, M. and Korhonen, L.K. (1978) Clin. Chem. 24, 466–470.
[16] Havel, R.J., Eder, H.A. and Bragdon, J.H. (1955) J. Clin. Invest. 34, 1345–1353.
[17] Albers, J.J. and Cheung, M.C. (1979) in: Proceedings of the High Density Lipoprotein Workshop, no.79, pp.29–42, DHEW, Bethesda, MD.
[18] Laemmli, U.K. (1970) Nature 227, 680–685.
[19] Ehnholm, C., Lukka, M., Rostedt, I. and Harper, K. (1986) J. Lipid Res. 27, 1259–1264.
[20] Sviridov, D.D., Safonova, I.G., Gusev, V.A., Talalaev, A.G., Tsibulsky, V.P., Ivanov, V.O., Preobrazensky, S.N., Repin, V.S. and Smirnov, V.N. (1986) Metabolism 35, 588–595.