

BINDING OF LP(a) TO THE LOW DENSITY LIPOPROTEIN RECEPTOR OF HUMAN FIBROBLASTS

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1. Introduction

Several investigators have reported the presence in serum of a lipoprotein called lipoprotein(a) (Lp(a)) with a density between low density lipoprotein (LDL) and high density lipoprotein (HDL) (i.e., 1.055–1.110 g/ml) and with an electrophoretic mobility on agarose gel between β - and pre- β -lipoproteins (i.e., pre- β 1-mobility [1]). Although the lipid composition of Lp(a) is almost identical with that of LDL [1,2], its apolipoprotein composition differs from the apolipoprotein composition of LDL. The main apolipoprotein of Lp(a) consists of apolipoprotein B (apo B) as in LDL. In Lp(a), however, an additional specific Lp(a) antigen, dissociable from apo B, has been detected [3].

From [4] it is concluded that Lp(a) represents an independent additional risk factor for myocardial infarction once Lp(a) in serum is ≥ 30 mg/dl. It has been postulated that Lp(a) is not a metabolic product of other apo B-containing lipoproteins (chylomicrons, VLDL or LDL) and thus Lp(a) should be synthesized as a separate lipoprotein [5]. Furthermore Lp(a) is not converted into other lipoproteins [6]. At present, the site(s) of Lp(a) synthesis and catabolism are unknown. Here we describe the binding, uptake and degradation of Lp(a) by human fibroblasts in vitro. We conclude from biochemical and morphological studies that Lp(a) binds to the same receptor site as does LDL.

2. Materials and methods

2.1. Plasma samples

Blood from a 55 year old male patient, suffering from angina pectoris and tendinous xanthomas was

collected into ethylene diaminetetra-acetic acid disodium salt (EDTA, 1 mg/ml final conc.). Blood cells and platelets were separated by low speed centrifugation. Total and HDL-cholesterol was 10 and 1.15 mmol/l, respectively. Triglyceride concentration was 4 mmol/l. The patient was diagnosed as type IIB (Fredrickson). Plasma from apparently healthy subjects was also prepared by collecting blood into EDTA followed by low speed centrifugation.

2.2. Isolation and analysis of lipoproteins

LDL and Lp(a) were isolated by density gradient ultracentrifugation according to [7] followed by slicing off the respective lipoprotein band. Lipid analysis of the isolated Lp(a) fraction was done by thin-layer chromatography as in [8]. This fraction was analysed for the presence of apo B and apo E by double immunodiffusion (Ouchterlony), using monospecific rabbit anti-apo B and anti-apo E antiserum in serial dilutions. Electrophoretic mobility of LDL and Lp(a) was measured, after dialysis against saline, on agarose gel as in [9]. Protein was determined according to [10]. Cholesterol was measured using the method in [11].

2.3. Fibroblast cultures

Monolayers of human foreskin fibroblasts were cultured to confluency in 45 mm plastic Petri dishes with Ham's F 10 medium containing 15% (v/v) new born calf serum (NBCS). At confluence, the fibroblasts were preconditioned by incubation in the same medium containing 15% (v/v) lipoprotein-deficient NBCS (LPDS, $d > 1.215$ g/ml) for 18 h.

2.4. Binding of lipoproteins to fibroblasts

Isolated LDL and Lp(a) were labelled with ^{125}I as

in [12]. The specific activity for LDL and Lp(a) varied from 40–140 cpm/ng protein. Free ^{125}I and 10% (w/v) trichloroacetic acid-soluble radioactivity were <1% and <5%, respectively, of the total activity. However, the trichloroacetic acid-soluble fraction of Lp(a) increased dramatically (up to ~10%) after storage for 1 week at 4°C in the presence of bovine serum albumin (2% by wt). After incubation of the LPDS preconditioned cells with ^{125}I -labelled lipoprotein for 2 h at 37°C, binding, internalization and degradation of ^{125}I -labelled lipoproteins were measured essentially as in [13].

2.5. Morphological studies

For immunofluorescence microscopy, monolayers of fibroblasts were cultured on glass coverslips. After preconditioning of the cells in medium supplemented with 15% LPDS for 24 h at 37°C, the cells were incubated for 2 h at 4°C with LDL or Lp(a) at 50 µg protein/ml medium. After washing with an albumin containing buffer according to [13], the cells were stained with an indirect immunofluorescence technique. In the first immunological step a monospecific anti-apo B antiserum (diluted 1:20) was used [14]. In the second step goat anti-rabbit IgG conjugated with fluoresceine (Nordic, Tilburg) was added in a dilution of 1:80. The cells were examined in a Leitz orthoplan. For immunoelectronmicroscopy the procedure was exactly as in [15].

3. Results and discussion

In the density gradient ultracentrifugation pattern of plasma from the atherosclerotic patient (section 2) an additional lipoprotein class with density between LDL and HDL was found. For further analysis this additional lipoprotein was isolated from the density gradient tube by slicing off the corresponding lipoprotein band to avoid contamination with other lipoproteins like LDL and HDL₂. In fig.1 it is shown that the electrophoretic mobility of this particular lipoprotein lies between β -LDL and pre- β (very low density lipoprotein, VLDL). Lipid analyses showed that the lipid component of this lipoprotein consisted of 76% of neutral lipid and 24% of phospholipid. The total lipid consisted of 55% and 15% of cholesterol-esters and free cholesterol, respectively. The lipid composition of this lipoprotein resembles the lipid composition of Lp(a) as described in [1]. Double

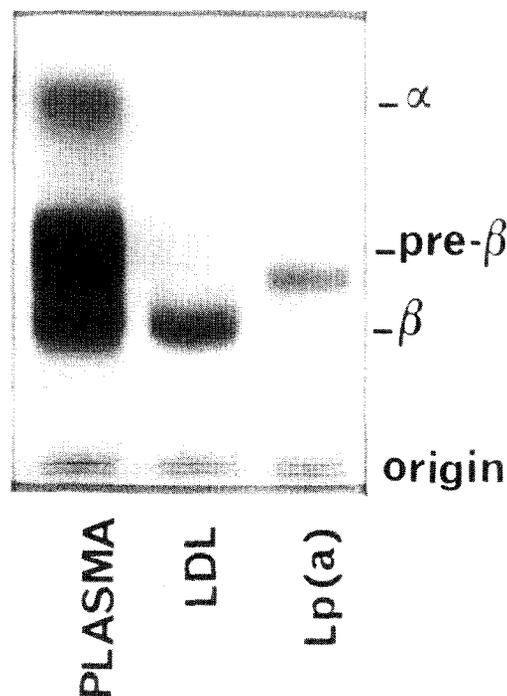


Fig.1. Agarose electrophoresis of plasma and lipoprotein samples. Lipoprotein samples were isolated by density gradient ultracentrifugation followed by slicing. Prior to electrophoresis the lipoprotein samples were dialyzed against saline.

immunodiffusion techniques (Ouchterlony) with monospecific anti-apo B and anti-apo E antiserum in serial dilutions showed only the presence of apo B.

HDL_c also has pre- β mobility and also covers the density between the density of LDL and HDL. However, since HDL_c does not contain apo B, whereas in HDL_c apo E is one of the major apolipoproteins [16] we conclude that the apo B-containing and apo E-free pre- β 1 lipoprotein found in our patient is Lp(a). Further analysis of this Lp(a) lipoprotein by specific anti-Lp(a) antiserum could not be done since this antiserum was not available.

In fig.2 the binding, internalization and degradation of ^{125}I -labelled Lp(a) with fibroblasts is shown. With Lp(a) >15 µg/ml medium the binding reaches saturation comparable to the binding of LDL to fibroblasts [13]. Fig.3A shows that unlabelled LDL competes with ^{125}I -labelled Lp(a) for degradation by fibroblasts. Fig.3B shows the opposite experiment, i.e., Lp(a) competes with ^{125}I -labelled LDL for degradation. The results shown in fig.3A,B present biochemical evidence that Lp(a) binds to the LDL

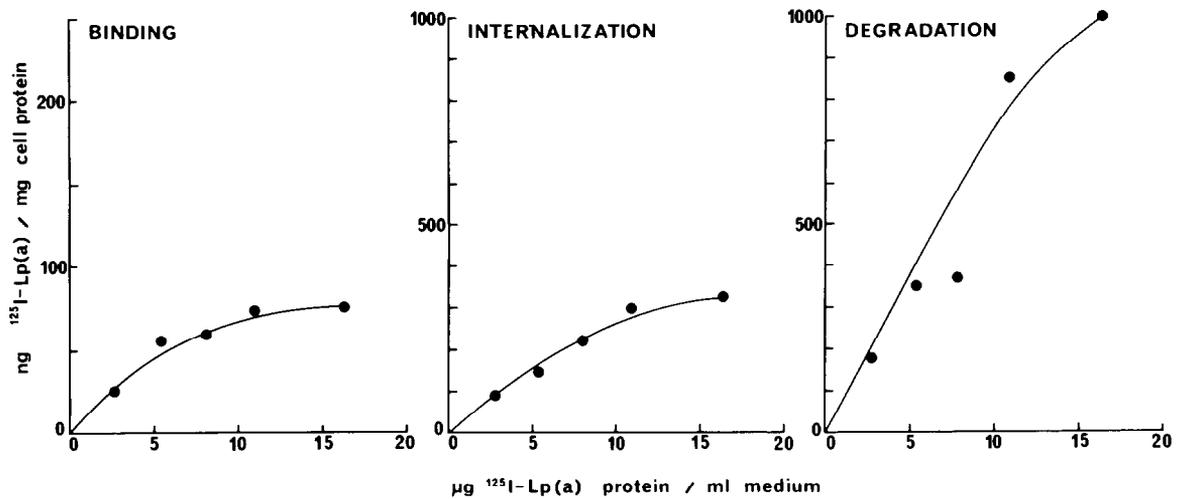


Fig.2. Interaction of Lp(a) with fibroblasts: Binding, internalization and degradation of ^{125}I -labelled Lp(a) (57 cpm/ng Lp(a) protein) was performed as referred to in section 2.

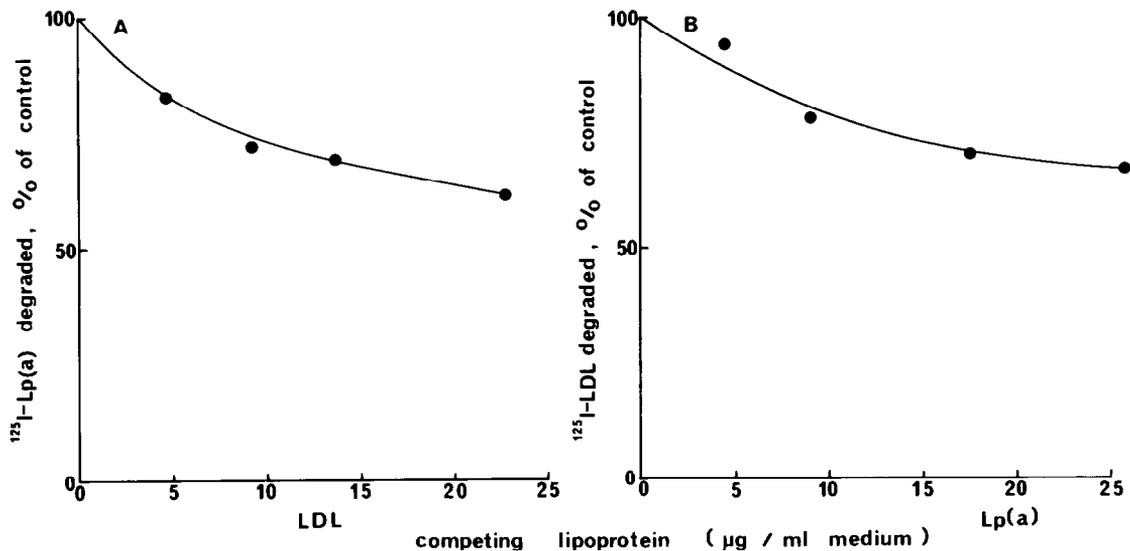


Fig.3. Ability of non-radioactive LDL and Lp(a) to inhibit the degradation of respectively ^{125}I -labelled Lp(a) and ^{125}I -labelled LDL by fibroblasts. (A) competition of ^{125}I -labelled Lp(a) (57 cpm/ng) by unlabelled LDL for degradation. Concentration of unlabelled LDL is as indicated. Without the addition of unlabelled LDL 968 ng ^{125}I -labelled Lp(a) was degraded/mg cell protein (=100%). (B) Competition of ^{125}I -labelled LDL (68 cpm/ng) by unlabelled Lp(a) for degradation. Concentration of ^{125}I -labelled LDL is 22 $\mu\text{g/ml}$ medium, Concentration of unlabelled Lp(a) is as indicated. Without the addition of unlabelled Lp(a) 690 ng ^{125}I -labelled LDL was degraded/mg cell protein (=100%).

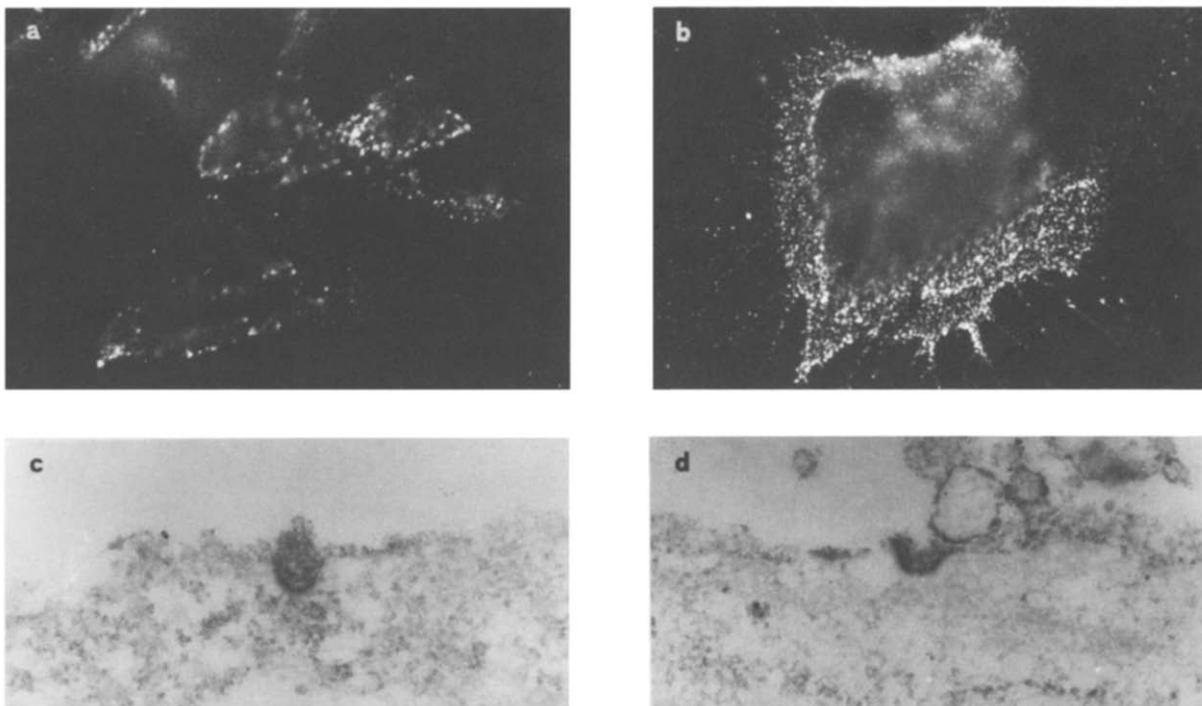


Fig.4. Visualization of binding of Lp(a) and LDL to fibroblasts: (a) Indirect immunofluorescence microscopy of binding of Lp(a), magnification 260X; (b) as (a) but LDL; (c) immunoelectronmicroscopy of binding of Lp(a), magnification 39 000X; (d) as (c) but LDL.

receptor on the plasma membrane. The possibility that the binding of Lp(a) to the LDL-receptor is due to the presence of apo E containing lipoproteins (like HDL_C) in the Lp(a) sample can be excluded because apo E could not be detected at all in the Lp(a) sample by double immunodiffusion. The binding capacity of the Lp(a) sample to the LDL-receptor was not due to a definite contamination with LDL as revealed by agarose electrophoresis (see fig.1).

To obtain more evidence for the binding of Lp(a) to the LDL-receptor we performed morphological studies. Using indirect immunofluorescence microscopy, both for Lp(a) and for LDL we were able to show the presence of apo B-immunoreactive sites on the cell surface (see fig.4a,b). With the technique of immunoelectronmicroscopy [15] we were able to visualize the binding of Lp(a) to coated pits (fig.4c) using anti-apo B as primary antiserum. The binding of Lp(a) to fibroblasts was morphologically indistinguishable from the binding of LDL to fibroblasts (fig.4d). In case an antiserum against apo E was used [17] no binding of Lp(a) was observed. No binding of Lp(a)

to fibroblasts derived from patients homozygous for familial hypercholesterolemia could be visualized. Since the cells derived from homozygous hypercholesterolemic patients lack LDL-receptors [18,19] these morphological studies confirm our biochemical results that Lp(a) binds to the LDL-receptor.

No information is currently available about the site of the catabolism of Lp(a). In [4], individuals with elevated LDL levels (e.g., familial hypercholesterolemic patients) generally exhibited higher Lp(a) levels. In [6], the kinetic parameters of Lp(a) were very similar to those of LDL in normal subjects. This paper presents evidence that Lp(a) is catabolized via the specific LDL receptor pathway. Whether the effect of Lp(a) on the intracellular cholesterol synthesis is identical with that of LDL remains to be investigated.

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