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Degradation of human apolipoprotein B-100 by apolipoprotein(a)

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Human plasma low density lipoproteins (LDL) contain a very high molecular weight protein termed apoB-100 (M_r = 550,000). In many samples of LDL, minor components designated as apoB-74 (M_r = 407,000) and apoB-26 (M_r = 145,000) are present. It has been shown that they can arise as a result of proteolytic degradation of apoB-100. Our earlier studies demonstrated that the active forms of lipoprotein(a) (LP(a)) and apolipoprotein(a) (apo(a)) possess proteolytic activity. In the present study we investigated the possibility of apoB-100 degradation in the presence of activated apo(a). LDL were incubated with the purified apo(a) and analyzed by SDS-polyacrylamide gel electrophoresis. It was found that apoB is cleaved by apo(a) with formation of proteolytic fragments including B-74 and B-26. The physiological significance of apoB degradation under the action of apo(a) is considered.

Lipoprotein(a); Apolipoprotein(a); Apolipoprotein B-100; Proteolytic degradation

1. INTRODUCTION

Apolipoprotein B-100 (apoB-100) is a major protein of plasma low density lipoproteins (LDL). ApoB-100 plays a key metabolic role in its function as a ligand for high affinity receptors on cell membranes, which endocytose LDL to provide sterol for membranes biogenesis and hormone synthesis [1].

The apparent molecular weight of apoB-100 by sodium dodecylsulfate (SDS) electrophoresis has been reported to be 550,000. Kane et al. [2] have reported the presence of smaller fragments of apoB in LDL and designated these fragments as B-100, B-74 and B-26. Significant amounts of B-74 and B-26, which may be complementary constituents of B-100, have been detected in the LDL of many individuals. At present, the origin and physiological significance of B-74 and B-26 are unknown, although it has been shown that they can arise during blood collection as a result of proteolytic degradation. It was found that kallikrein can degrade apoB-100 and produce B-74 and B-26 [3].

All human plasmas contain a variant of LDL called lipoprotein(a) (Lp(a)). Lp(a) consist of apoprotein B plus an additional specific antigen called apoprotein(a) (apo(a)) [4]. The apo(a) is covalently linked to apoB via disulfide bridges [5].

Recently we have found that Lp(a) after treatment with trypsin-Sepharose or collagen-Sepharose possessed proteolytic activity [6]. The active form of Lp(a), as well as active apo(a), can split some synthetic substrates and active plasma prekallikrein to kallikrein. Based on these results, we suggested that apo(a) may be responsible for the degradation of apoB-100. The present study was undertaken to clarify whether apo(a) is capable of cleaving apoB-100.

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2. EXPERIMENTAL

A lipoprotein fraction (ρ = 1.05–1.12 g/ml), including lipoprotein(a) was isolated from human pooled Lp(a) positive plasma containing EDTA (1 mM) and sodium azide (0.01%) by density gradient ultracentrifugation (40,000 rpm, for 20 h, at 14°C, in Beckman 40.3 rotor) [7]. LDL were isolated by ultracentrifugation between densities of 1.02 and 1.05 g/ml.

Apo(a) was prepared from isolated lipoproteins of ρ = 1.12 g/ml by the method of Fless et al. [8]. Lipoproteins (5–10 mg/ml in 0.05 M Tris-HCl, pH 7.4) were treated with dithiothreitol (10 mM) for 1 h at 37°C. The solution was brought to a density of 1.12 g/ml and was centrifuged for 20 h at 14°C and 40,000 rpm. Remnant lipoprotein particles, containing apoB were recovered from floating layers at the top of the tubes. The infranatant fluids which contained apo(a) were pooled and purified by ultrafiltration using Amicon XM-300 membrane.

The purified apo(a) was displayed as a single band of molecular weight of more than 550,000 on SDS-polyacrylamide gel electrophoresis (Fig. 1A).

Apo(a) was assayed for a amidolytic activity using the fluorogenic substrate benzyloxycarbonyl-L-phenylalanyl-L-agrinyl-4-methylcoumaryl-7-amide (Cbz-Phe-Arg-MCA). Apo(a) (100 μl) in 0.05 M Tris-HCl, pH 7.4, was incubated with 10 μl of 1 mM Cbz-Phe-Arg- MCA at 37°C for 10 min. The reaction was stopped by addition of 50 μl of 50% acetic acid, and the increase in fluorescence of the leaving group 7-amino-4-methylcoumarine was determined from the fluorescence emission (460 nm), excitation at 370 nm, using Opton fluorescence spectrophotometer. The specific activity was the amount of substrate hydrolyzed per min per mg of apo(a) at 37°C. The amount of 7-amino-4-methylcoumarin released was read from a 7-amino-4- methylcoumarin calibration curve. In order to determine the degradation of apoB-100 by apo(a), LDL containing predominantly apoB-100 were incubated with apo(a) and the degradation products were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was performed on 5% homogeneous slab gels employing the buffer system of Laemmli [9].

A sample of LDL, containing 0.1 mg of protein in 0.1 ml of 0.05 M Tris-HCl, pH 7.4, was combined with 0.01 mg of apo(a) and incubated for 5 min, 30 min, and 3 h at 37°C. As control was used LDL incubated for 3 h at 37°C in 0.05 M Tris-HCl, pH 7.4, without apo(a). The enzymatic reaction was terminated by mixing the samples (20 μl) with electrophoresis sample buffer of final composition 60 mM Tris-HCl, pH 6.8, 1% SDS, 5% 2-mercaptoethanol, 20% sucrose, and 0.03% Bromophenol blue. Prior to electrophoresis, the samples were boiled for 1 min. The gels were stained with Coomassie brilliant blue R-250. The protein standards were ferritin (440,000 and 220,000), phosphorylase *b* (94,000), bovine serum albumin (68,000), ovalbumin (45,000).

Protein concentration was determined by the method of Lowry using bovine serum albumin as a standard [10].

3. RESULTS AND DISCUSSION

The purified preparation of apo(a) had an initial specific activity of 0.5 nmol/min/mg. Storage at 7°C (in the refrigirator) led to the activation of apo(a). The apo(a) kept in the cold for two weeks and having a specific activity of 20 nmol/min/mg was used in our experiments. When LDL was incubated with apo(a) and electrophoresed on SDS-PAGE, fragments of apoB-100 were observed with apparent molecular weights corresponding to B-74 and B-26, as well as other fragments with molecular weights in the range of 220,000, 80,000, 60,000, and 40,000. (Fig. 1B, lanes c–e). The predominant proteolytic products initially observed were peptides with apparent molecular weights 400,000, 220,000, 60,000, and 40,000 (Fig. 1B, lanes c,d). After 3 h incubation, apoB-100 was virtually absent (Fig. 1B, lane e). The control LDL, incubated at 37°C for 3 h without apo(a), showed no degradation (Fig. 1B, lane b). This rules out the presence of lipoprotein-associated protease(s) contaminant. Therefore, the degradation of apoB was due to the apo(a)-caused proteolysis.

The enzymatic properties of the active form of apo(a) – its substrate specificity, relation to inhibitors, ability to activate plasma prekallikrein [6] – provide evidence that apo(a) is a new plasma proteinase. The presently known activator of plasma prekallikrein (factor XIIa) differs from apo(a) by its physico-chemical properties and relation to inhibitors. The structure of apo(a) was established in 1987 [11] but its enzymatic activity and function remained obscure. The results of our earlier and present experiments showed that apo(a) is a zymogen. Catalytically active apo(a) can convert plasma prekallikrein to kallikrein and degrade apoB-100. Characteristically, apoB-100, the ligand for LDL receptor, occurs in the circulation being covalently bound to the proenzyme, the activation of which leads to the degradation of apoB-100. It is likely that specific linkage of apo(a) to apo-B has physiological significance. It may well be suggested that concurrently proceeding plasma kallikrein activation and apoB hydrolysis have a role in the regulation of the uptake and metabolism of lipoproteins by some type of cells, e.g. by granulose (steroidogenic) cells, which have cAMP-mediated mechanism for regulation of LDL receptor synthesis [12]. Bradykinin released by kallikrein is known to stimulate cAMP formation [13].

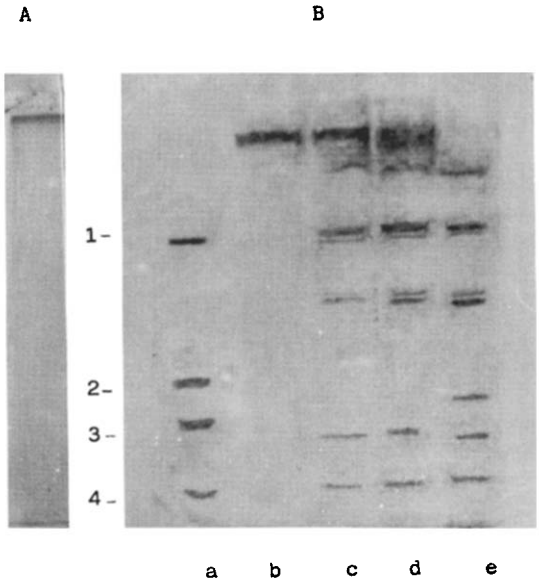

Fig. 1. SDS-PAGE analysis: (A) purified apo(a); (B) degradation of apoB-100 by apo(a). (Lane a) Standard proteins used as molecular mass markers: (1) ferritin (220,000); (2) phosphorylase *b* (94,000); (3) bovine serum albumin (68,000); (4) ovalbumin (43,000). (Lane b) Control LDL. (Lanes c–e) LDL (0.1 mg apoB-100) at various times (5 min, 30 min, 180 min) after incubation with apo(a) (0.1 mg). 20 μg of protein were electrophoresed in 5% PAG, containing 0.1% SDS. Experimental details are given in the text.

It is as yet unclear how and when the activation of apo(a) of Lp(a) occurs in vivo and what its physiological significance really is. It is apparent, however, that disturbances in the regulation of apo(a) activity may lead to such pathological consequences as thrombosis and atherosclerosis.

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