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The apolipoprotein(a) component of lipoprotein(a) mediates binding to laminin: contribution to selective retention of lipoprotein(a) in atherosclerotic lesions

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Abstract

Lipoprotein(a) [Lp(a)] entrapment by vascular extracellular matrix may be important in atherogenesis. We sought to determine whether laminin, a major component of the basal membrane, may contribute to Lp(a) retention in the arterial wall. First, immunohistochemistry experiments were performed to examine the relative distribution of Lp(a) and laminin in human carotid artery specimens. There was a high degree of co-localization of Lp(a) and laminin in atherosclerotic specimens, but not in non-atherosclerotic sections. We then studied the binding interaction between Lp(a) and laminin in vitro. ELISA experiments showed that native Lp(a) particles and 17K and 12K recombinant apolipoprotein(a) [r-apo(a)] variants interacted strongly with laminin whereas LDL, apoB-100, and the truncated $KIV_{6\text{-}P}$, $KIV_{8\text{-}P}$, and $KIV_{9\text{-}P}$ r-apo(a) variants did not. Overall, the ELISA data demonstrated that Lp(a) binding to laminin is mediated by apo(a) and a combination of the lysine analogue ε-aminocaproic acid and salt effectively decreases apo(a) binding to laminin. Secondary binding analyses with ^{125}I-labeled r-apo(a) revealed equilibrium dissociation constants (K_d) of 180 and 360 nM for the 17K and 12K variants binding to laminin, respectively. Such similar K_d values between these two r-apo(a) variants suggest that isoform size does not appear to influence apo(a) binding to laminin. In summary, our data suggest that laminin may bind to apo(a) in the atherosclerotic intima, thus contributing to the selective retention of Lp(a) in this milieu.

Keywords: Lipoprotein(a); Apolipoprotein(a); Laminin; Atherosclerosis

1. Introduction

Lipoprotein(a) is a complex lipoprotein consisting of an LDL particle and apolipoprotein(a). Apo(a) is covalently linked to apolipoprotein B-100 by a single disulfide linkage [1] and confers the unique structural, functional, and clinical features attributed to Lp(a) [2]. Apo(a) contains multiple tandem repeats of sequence closely resembling

Abbreviations: Lp(a), lipoprotein(a); LDL, low density lipoprotein; apo(a), apolipoprotein(a); apoB-100, apolipoprotein B-100; ECM, extracellular matrix components; KIV, kringle IV; KV, kringle V; LBS, lysine binding site(s); mAb, monoclonal antibody; ε-ACA, ε-aminocaproic acid; BSA, bovine serum albumin; PMFS, phenylmethylsulfonyl fluoride; PBS, phosphate-buffered saline; PB, phosphate-buffered; PAGE, polyacrylamide gel electrophoresis; r-apo(a), recombinant apolipoprotein(a); PVDF, polyvinylidene difluoride; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay

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plasminogen kringle IV (KIV), followed by sequences exhibiting ~90% identity to the kringle V (KV) and protease regions of plasminogen [3]. The apo(a) KIV domains can be classified into 10 types (KIV_1–KIV_{10}) on the basis of amino acid sequence [4]. The apo(a) kringle IV type 2 domain (the major repeat kringle) is present in varying numbers of copies, which constitutes the basis of Lp(a) isoform size heterogeneity in humans [5]. Of the kringle IV sequences in apo(a), the sequence of apo(a) kringle IV type 10 (KIV_{10}) most closely resembles that of plasminogen kringle IV. Like plasminogen kringle IV, apo(a) KIV_{10} contains a canonical lysine-binding site (LBS) [6]. In addition, molecular modeling studies indicated the presence of weak LBS in each of apo(a) KIV_5–KIV_8. Subsequent biochemical studies have revealed contributions of these kringles to lysine-mediated interactions involving apo(a) [4]. Importantly, these LBS mediate the initial noncovalent interaction between apo(a) and apoB-100 and appear to be masked in the context of the Lp(a) particle [6].

Elevated plasma levels of Lp(a) are an established risk factor for atherothrombogenesis [7,8]. Lp(a) may be prothrombotic through its competition with plasminogen at sites of fibrin deposition, thus inhibiting plasmin formation and clot lysis [9,10]. Additionally, Lp(a) could be proatherogenic through its binding affinity for components of the extracellular intima [11,12]. The preferential retention of Lp(a) in atherosclerotic lesions may occur as Lp(a) has been shown, in vitro, to bind to several components of the extracellular matrix, including chondroitin sulfate A, chondroitin sulfate C, heparan sulfate [11], mixed aortic proteoglycans [13], and to fibrin(ogen), fibronectin, and laminin [3,5,14–19].

Specifically, the laminins are a family of extracellular matrix glycoproteins that are integral to the structural scaffolding of basement membranes in almost every animal tissue [20]. Each laminin is a heterotrimer assembled from alpha, beta, and gamma chain subunits, secreted and incorporated into cell-associated extracellular matrices. Evidence is mounting that laminins play a central role in the formation, the architecture, and the stability of basement membranes [20]. Laminins also provide adjacent cells with a mechanical scaffold and biological information either directly by interacting with cell surface components, or indirectly by trapping growth factors. In addition, laminin represents a relevant constituent of the atherosclerotic plaque produced from smooth muscle cells that have taken on a secretory phenotype [21].

It has not yet been established whether laminin may contribute to Lp(a) retention at sites of vascular injury. To address this issue, we performed immunohistochemistry experiments to examine the relative distribution of Lp(a) and laminin in human atherosclerotic and non-atherosclerotic carotid specimens. We also studied the binding of Lp(a) to laminin in vitro and examined the role that apo(a) plays in mediating this interaction.

2. Materials and methods

2.1. Materials

Human plasma fibronectin and type II collagen were purified as previously described [22,23]. Ultrapure murine laminin was purchased from Becton Dickinson (Two Oak Park, Bedford, MA, USA). Chondroitin sulfate A (C4S), chondroitin sulfate C (C6S), keratan sulfate, dermatan sulfate and heparan sulfate were from Seikagaku Co. (Tokyo, Japan).

ε-Aminocaproic acid (ε-ACA), bovine serum albumin (BSA), human plasminogen, rabbit anti-mouse laminin polyclonal antibody, phenylmethylsulfonyl fluoride (PMSF), and human fibrinogen were purchased from Sigma (St. Louis, MO, USA). Human fibrinogen was purified further over a gelatin-Sepharose column (Amersham Biosciences, Uppsala, Sweden) to remove residual traces of fibronectin. Human apolipoprotein B-100 (apoB-100) and apoB-100-specific monoclonal antibody, 6H12, were from ICN Biomedicals (Irvine, CA, USA). Human apoB-100 was completely delipidated and aggregated. Rabbit anti-human lipoprotein(a) polyclonal antibody, horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG, and rabbit anti-mouse IgG were purchased from DakoCytomation (Glostrup, Denmark). HRP-conjugated anti-mouse IgG was from Amersham Biosciences. Lp(a) concentrations in human plasma were measured using a commercial enzyme-linked immunoassay (Terumo Medical Corporation Diagnostic Division, Elkron, MD, USA). Super Sensitive MultiLink Kit containing biotinylated secondary IgG antibody and Label Super Sensitive Kit containing the HRP-labeled streptavidin were from Bio-Genex (San Ramon, CA, USA). Carrier-free ^{125}I (specific activity 15 mCi/μg) was purchased from Radiochemicals Centre (Amersham Biosciences).

2.2. Protein purification

LDL (density 1.01 to 1.05 g/mL) was prepared from normolipidemic subjects [5] and contained no Lp(a) contamination as assessed by Western blot analysis.

Native Lp(a) particles (n=12) were purified from human plasma of several donors of varying apo(a) phenotypes ranging from about 280 to 820 kDa molecular weight [24]. Plasma was prepared by centrifugation of whole blood (2000×g for 20 min) and PMSF was added to 1 mM (final concentration) to prevent endogenous proteolysis. Sepharose 4B CNBr-activated (Amersham Biosciences) was coupled with the rabbit anti-human lipoprotein(a) polyclonal antibody. The plasma was incubated with the anti-Lp(a) affinity matrix, previously equilibrated with PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4), in an end-over-end mixer overnight at 4 °C. The gel was loaded into a column support and washed with PBS (containing 0.35 M NaCl, pH 7.0). Lp(a) was eluted with 100 mM glycine pH 3.0 and 2-mL fractions containing 200