Lipoprotein(a) (Lp(a)) consists of apolipoprotein(a) (apo(a)) covalently linked to apolipoproteinB100 (apoB100) in a lipoprotein particle with similar lipid composition to LDL. Fundamental questions regarding the biogenesis and metabolism of Lp(a) remain unresolved, particularly the site of Lp(a) assembly and the nature of the apoB100-containing species involved. Reports of small amounts of apo(a) in plasma, kinetic data suggesting dissociation of apo(a) from Lp(a) with possible reassociation with triglyceride-rich lipoproteins (TRLs), and the appearance of Lp(a) in TRL-containing fractions in hypertriglyceridemic subjects all complicate the picture. In this study, we have subjected fasting plasma from two individuals with high or low Lp(a) levels to fast protein liquid chromatography (FPLC) over a Superose 6 column to separate lipoprotein classes by size under native conditions. We found, using an apo(a)-specific ELISA, that Lp(a) eluted in a broad peak, centered approximately on the LDL-containing peak but also extending into the VLDL-size range; the latter characteristic was particularly evident for the subject with high Lp(a) levels. No apo(a) was detectable in later fractions where free apo(a) would be expected to elute. Addition of ε-aminocaproic acid (ε-ACA, a lysine analog) had no impact on the elution profile of apo(a), indicating a lack of non-covalent apo(a) complexes with apoB100-containing particles. We also supplemented the plasma with purified, recombinant 17-kringle apo(a) (17K). The majority of this material eluted in a pattern identical to Lp(a) itself, suggesting that the added apo(a) became associated with predominantly LDL-size particles. Western blot analysis of column fractions revealed that at least some of the added apo(a) was both non-covalently and non-covalently associated with both VLDL- and LDL-sized particles. Interestingly, we achieved the same results as for 17K when we used an apo(a) variant lacking the strong lysine-binding sites in kringle KIV types 7 and 8 (17KΔLBS7,8), indicating that the complexes do not depend on these lysine binding sites, which are otherwise key for Lp(a) assembly. A small peak of apo(a) in fractions spanning a size range smaller than HDL was detected by ELISA, although this material was not observable on western blots. As before, addition of ε-ACA did not materially affect the elution profiles of either apo(a) variant. It did, however, appear to diminish covalent complexes of apo(a) with particles in the VLDL-size range. Our findings are consistent with the essential absence of uncomplexed apo(a) in plasma as well as with the non-covalent association of Lp(a) with TRLs being mediated by the apo(a) component.
Distribution of Apolipoprotein(a)-containing Species in Human Plasma Assessed by Fast Protein Liquid Chromatography

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Methods

- Lipoprotein(a) (Lp(a)) consists of apolipoprotein(a) (apo(a)) covalently linked to apolipoproteinB100 (apoB100) in a lipoprotein particle with similar lipid composition to LDL.
- Fundamental questions regarding the biogenesis and metabolism of Lp(a) remain unresolved, particularly the site of Lp(a) assembly and the nature of the apoB100-containing species involved [1].
- Apo(a) and apoB100 are both produced in the liver of humans, apes, and Old World Monkeys.
- Assembly follows a two-step model (Fig. 1) where initial non-covalent interactions between the weak lysine binding sites in KIV7 and KIV8 and specific lysines on apoB100 precede covalent assembly through a single disulfide bond [2].
- In vivo human kinetic studies suggest that covalent assembly occurs either inside the hepatocyte or on the cell surface from an apoB100-containing lipoprotein distinct from VLDL (or LDL) [3]; some circulating LDL may also be involved [4].
- Evidence has also been presented for recycling of apo(a) and/or apoB100 into new Lp(a) particles after hepatic uptake of Lp(a) [5-7] or for exchange of apo(a) between apoB100-containing lipoproteins in plasma [7].
- A pool of free apo(a) in plasma has also been reported [8].

References:

Figure 1. Two-step model for Lp(a) assembly [2].
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Abstract

Figure 2. Scheme for the design of this study.
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Abstract

Figure 3. Plasma lipoprotein profile (left y-axis) from FPLC of (A) low Lp(a) and (B) high Lp(a) individuals. Cholesterol and triglyceride concentrations (μg/mL) were measured in eluted fractions. Apo(a) concentrations in each fraction (right y-axis) were measured using an apo(a)-specific ELISA.

Figure 4. Plasma apo(a) concentrations in individuals with either (A&B) low Lp(a) or (C&D) high Lp(a) concentrations. Freshly isolated plasma from each individual was supplemented with 2 µg/ml recombinant 17-kirgine apo(a) (17K) (A&C) or 2 µg/ml 17KΔLBS7,8 (B&D) (an apo(a) variant lacking the strong lysine-binding sites in kringle KIV types 7 and 8), and/or 50 mM ε-aminocaproic acid (ε-ACA, a lysine analog) and FPLC was performed. Apo(a) concentrations in each eluted fraction were measured using an apo(a)-specific ELISA.

Figure 5. Total Area Under the Curve (AUC) of the FPLC for individuals with either (A) low Lp(a) or (B) high Lp(a) concentrations. Area under the curve was calculated for all FPLC curves from Fig. 4, and for each segment of the curve (Pre-VLDL, VLDL, LDL, HDL or smaller than HDL) was expressed as a percentage of the total AUC.
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Abstract

17K Apo(a) alone

A B C

Western Blot Analysis of FPLC Column Fractions

Figure 6. Analysis of apo(a) and Lp(a) in eluted fractions from FPLC from low and high Lp(a) plasma samples. Fractions corresponding to major peaks (fractions 10, 15, 20, 25, 30 and 35) of the apo(a) ELISA of FPLC fractions (Fig. 2) were immunoprecipitated using an anti-apo(a) (α1-4) antibody and were resolved on SDS-PAGE and immunoblotted using anti-apo(a) (AS) antibody. Various plasma samples were supplemented with either 17K apo(a) or εACA. (A) Plasma alone, (B) Plasma + 17K apo(a) (50 µg/mL), (C) Plasma + 17K apo(a) (50 µg/mL) + εACA (200 mM). (D) High Lp(a) plasma supplemented with 17KΔLBS7,8, (an apo(a) variant lacking the strong lysine-binding sites in kringle KIV types 7 and 8) or (E) with this apo(a) variant plus εACA (200 mM). (F) 17K apo(a) (50 µg/mL) alone at eluted at later FPLC fractions.
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Abstract

• Lp(a) elutes almost entirely within the LDL range, particularly as revealed by Western blot analysis of FPLC column fractions.

• Our findings are consistent with the essential absence of uncomplexed apo(a) in plasma, as even the addition of εACA to plasma did not result in the appearance of significant free apo(a).

• Addition of apo(a) to plasma resulted in association in both a covalent and non-covalent manner with lipoproteins in the VLDL and LDL size ranges.

• The association with VLDL was most prominent in the subject with high Lp(a) who also had higher triglycerides.

• These associations with VLDL were sensitive to the addition of εACA, but did not appear to be mediated by the weak lysine binding sites in KIV types 7 and 8.

• The lack of a significant free apo(a) pool and the rapid association with apoB100-containing lipoproteins suggests that the biosynthesis of apo(a) is closely coupled to that of apoB100 in the hepatocyte or on the hepatocyte surface.

We gratefully acknowledge the assistance of Dr. Henian Cao (Hegele laboratory, Robarts Research Institute) and Ms. Dawn Telford (Huff laboratory, Robarts Research Institute) for assistance with the FPLC and clinical analyzer, and Ms. Brooke Kennedy (Hegele laboratory) for phlebotomy.