Lipoprotein Lp(a): Structure and Metabolism

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Introduction

The Lp(a) antigen was first detected in human plasma by Berg [1]. Although initially thought to represent a genetic variant of LDL, it was later shown to be associated with a specific lipoprotein fraction that is now commonly termed Lp(a) (for recent review see [2]). This lipoprotein has pre-β-mobility on zone electrophoresis as opposed to the β-mobility of LDL, and it has a higher hydrated density of 1.06–1.12 g/ml.

Numerous clinical studies have demonstrated a strong association between high Lp(a) concentrations and both cardiovascular and cerebrovascular disease [3–8]. The relative risk for the development of coronary heart disease has been estimated to be around 2, at Lp(a) concentrations of 30 mg/dl and greater [3–5], increasing to a value of approximately 5, however, in angiographically documented coronary sclerosis [5] if LDL concentrations are also elevated.

Structure of the Lipoprotein Lp(a)

The lipoprotein Lp(a) contains two distinct proteins: apo B-100 and a unique carbohydrate-rich protein apo(a). Although the two proteins are linked by one or more disulfide bonds in the lipoprotein [9–11], apo(a) can be readily separated from the lipoprotein particle by mild reduction with dithiothreitol (DTT) or 2-mercaptoethanol followed by one of the following procedures: electrophoresis [12], ultracentrifugation [12, 13], heparin-Sepharose affinity chromatography [12], or chromatography on anti-apo(a)-Sepharose [14]. The residual apo(a)-free lipoprotein – designated Lp(a−) – is similar to LDL in many of its physicochemical and immunochemical properties (Table 1), although it appears to be slightly larger in mass than autologous LDL [15]. Furthermore, Lp(a−) is bound and degraded as efficiently as LDL by the LDL receptor in cultured human fibroblasts, whereas unreduced Lp(a) displays a much reduced capacity for binding and degradation [12] by this receptor. The structure of Lp(a) is therefore essentially that of an LDL particle to which the glycoprotein apo(a) is attached through disulfide bonds to apo B-100. This additional protein conveys the distinctive immunochemical and physicochemical properties of Lp(a).

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Table 1. Comparison of the physicochemical properties of Lp(a), Lp(a−) and LDL

	Lp(a)	Lp(a−)[a]	LDL
Electrophoretic mobility	pre-β	β	β
Hydrated density	1.055–1.12	1.02–1.063	1.02–1.063
Apoprotein content	B-100, (a)	B-100	B-100
Lipid protein composition %			
Cholesterol	7.9	9.5	8.5
Cholesteryl ester	37.1	42.8	40.7
Triacylglycerol	19.0	21.2	21.3
Phospholipid	5.0	4.6	7.1
Protein	30.9	21.8	22.4

[a] Prepared by reduction of Lp(a) with DTT followed by heparin-Sepharose affinity chromatography [12].

Structure of Apo(a)

Apo(a) is a high molecular weight glycoprotein with a high carbohydrate content [15, 16] and displaying remarkable size heterogeneity. Several molecular weight forms have been described with masses ranging from 280000 to 700000 daltons. The genetic basis for this polymorphism has been clarified by Utermann and coworkers [17], who observed six different apo(a) phenotypes in the study of a large population. They have postulated that apo(a) phenotypes are controlled by a series of autosomal alleles at a single locus.

The complete primary amino acid sequence of an apo(a) of unknown phenotype has been recently derived by sequencing cloned human apo(a) cDNA [18]. Initial protein sequencing studies had revealed a high degree of homology between peptides from apo(a) and human plasminogen [19]. The latter is a single-chain protein of M_r 92000 containing several distinct structural regions [20]: an N-terminal sequence of 76 amino acids is followed by five tandemly arranged kringle domains having approximately 40%–50% homology with each other. Each kringle contains 78–80 amino acids and includes six Cys residues with disulfide bridges between the first and sixth, second and fourth, and third and fifth cysteines in each kringle sequence, giving it a characteristic triple-loop structure. Similar kringle structures have been observed in prothrombin [21], tissue plasminogen activator [22], urokinase [23], and factor XII [24]. The structure of plasminogen is completed by a trypsin-like protease region. The complete apo(a) sequence reported by McLean et al. [18] is that of a giant, abnormal plasminogen molecule (Fig. 1) containing one copy each of sequences homologous to the kringle V and protease regions from plasminogen and 37 sequences homologous to the kringle IV domain, 28 of which are identical.

In an independent investigation [25] we isolated an apo(a) of defined phenotype (S1 according to Utermann) with M_r 570000 and subjected it to limited proteolysis with trypsin and V8 protease. Peptides were purified by a combination of gel filtration and reverse phase HPLC and then sequenced. The majority of the peptides from apo(a) showed high homology to sequences from the kringle IV domain of human plasminogen. A further two peptides were homologous to the kringle V domain, and another three peptides corresponded to sequences from the protease region of plasminogen.

The results from our investigation are in agreement with the cDNA-derived structure of McLean et al. [18]. The complete amino acid sequence is given in Fig. 2, with those parts of the sequence that can be accounted for by the peptides from Kratzin et al. [25] highlighted by large lettering. Only three amino acid differences were observed between the two publications. These were located in peptides T1, T8 and T12 (see [25]). On reevaluation of our sequence data, we discovered that an interpretation error had been made in peptide T8, and that the correct amino acid at position 7 in this peptide is indeed Asn and not Asp. The original assignments of the amino acids Asp-50 and Thr-13 in tryptic peptides T1 and T12 respectively were, however, correct. The corresponding residues in human plasminogen are also Asp and Thr as determined from both protein sequencing [18] and cDNA analysis [26]. In the apo(a) sequence of McLean et al. [18], the equivalent residues are Asn (kringles 2–30) and Met (kringle 37).

Despite these minor differences, there is an extremely high degree of agreement between those apo(a) sequences derived from cDNA analysis [18] and those from the classical protein sequencing study reported by us [25]. On the other hand, peptides from the lower molecular weight form [19] of apo(a) show several amino acid differences to both the cDNA structure and our own peptide sequences. Further investigations are required to determine to what extent the amino acid sequence of apo(a) is conserved between the various apo(a) phenotypes. These studies do, however, suggest that the size heterogeneity observed in the different apo(a) phenotypes can be accounted for by variation in the number of kringle IV repeats.

In contrast to plasminogen, apo(a) has a high carbohydrate content [15, 16]. During our protein sequencing studies, a carbohydrate-rich peptide was isolated in high yield, which could be separated from the other apo(a) peptides by gel filtration alone. This peptide T1 [25] was 55 amino acids in length. The first nine amino acids displayed high homology to the last nine amino acids in the kringle IV domain

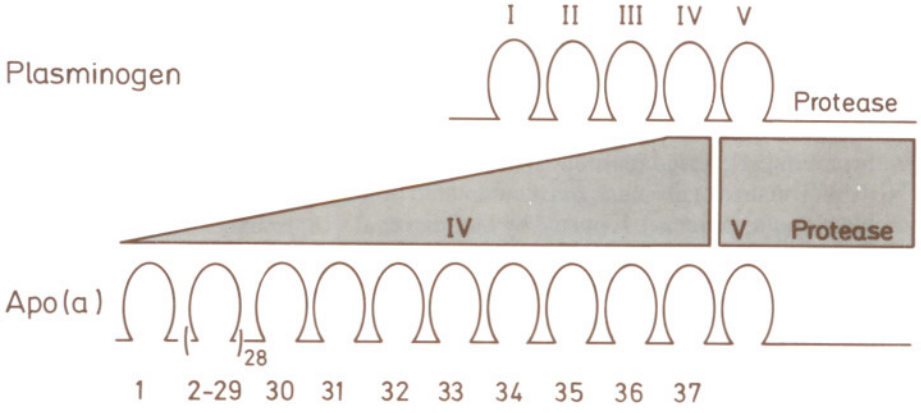

Fig. 1. Schematic respresentation of the structures of human plasminogen and apo(a). The latter is adapted from McLean et al. [18]. The exact number of duplicated kringles 2–29 in the apo(a) investigated is uncertain (28 ± 2). It appears likely that the size heterogeneity of apo(a) is due to individual variation in the number of these duplications

Fig. 2. Complete primary amino acid sequence of apo(a) adapted from McLean et al. [18], with the sequences of tryptic peptides isolated and sequenced by Kratzin et al. [25] illustrated by the large lettering. The amino acids marked ▼ differ from those found by McLean et al. [18]; in the case of the latter apo(a), an N was observed in place of the D and an M in place of the T. The amino acid marked ▽ originally designated as D [25] has now been corrected to N

of plasminogen, while the last ten C-terminal amino acids of T1 were identical to the first ten amino acids at the N terminus of kringle IV. This peptide therefore represents the sequence connecting two kringle IV-like domains and is found to be repeated in the cDNA derived sequence [18], with the one amino acid difference noted above, through kringles 2–30. Seven probable glycosylation sites could be identified in peptide T1: one N-linked oligosaccharide within the kringle and six O-linked oligosaccharides (four Thr and two Ser) in the connecting sequence. The latter is similar to the proline-rich hinge region of IgA [27] which contains several O-glycosides of the type Gal-GalNAc [28]. To what extent all potential glycosylation sites between kringles 2 and 30 are linked to carbohydrate cannot be ascertained at present. However, on the basis of the high yield of the peptide T1 and the fact that apo(a) contains a large amount of polysaccharide, it appears probable that the carbohydrate is distributed in clusters over a large part of the apo(a) sequence rendering it highly hydrophilic.

Metabolism of Apo(a)

At present little is known about the metabolic origin of Lp(a). The liver would appear to be one site of synthesis since apo B-100 is predominantly of hepatic origin, and the cDNA of apo(a) was constructed from a library of hepatic RNAs [18]. This is also supported by the observation that patients with alcoholic cirrhosis have low plasma Lp(a) levels [29]. Apo(a) has also been detected after a fat-rich meal in large lipoprotein particles of a density less than 1.006 that are rich in apo E and apo B-48, suggesting an intestinal origin [30]. However, our own investigations (J. Schleef, V.W. Armstrong, D. Seidel, unpublished observations) have shown that plasma Lp(a) can bind to lipoproteins of $d < 1.006$ and, in particular, postprandial lipoproteins through a lysine binding site, a phenomenon that has also been found with plasminogen [31].

Krempler et al. [32] reported that Lp(a) is not derived from either VLDL, LDL, or chylomicrons, although these studies do not eliminate the possibility that Lp(a) can initially be secreted as a short-lived VLDL-like particle. Lp(a) is also not converted to LDL to any great extent in vivo.

Several investigators [32–34] reported a specific LDL receptor mediated uptake and degradation of native Lp(a) by cultured human fibroblasts. In contrast, Martmann-Moe and Berg [35] concluded that Lp(a) enters fibroblasts independently of the LDL receptor pathway. In this study total binding (specific and nonspecific) of both Lp(a) and LDL were compared in fibroblasts from normal persons as well as from individuals with the homozygous and heterozygous forms of familial hypercholesterolemia (FH). Lp(a) uptake and degradation were only slightly higher in normal cells as compared to cells from homozygous FH, whereas a tenfold difference was seen in the case of LDL. In a detailed study [12] we were able to show that the apo(a) protein does indeed impair binding, uptake, and degradation of Lp(a) through the LDL receptor mediated pathway without blocking it entirely. After reduction and separation of apo(a) from the lipoprotein particle, Lp(a−) was bound, internalized and degraded as efficiently as LDL by cultured human fibroblasts. Although native Lp(a) was also found to be a ligand for the LDL receptor,

it displayed a weaker affinity than either LDL or Lp(a−), and its maximum degradation capacity at saturation of the receptor was only around 25% that of LDL and Lp(a−).

The contribution of the LDL receptor pathway to the catabolism of Lp(a) in vivo is still unclear. Krempler et al. [32] found the mean fractional catabolic rate (FCR) of Lp(a) to be 69% that of LDL. Furthermore, there was a strong positive correlation between the FCRs of LDL and Lp(a) in the 12 normolipemic subjects studied, suggesting a common catabolic pathway. However, turnover studies carried out at the same time in an individual with homozygous FH revealed a FCR for Lp(a) which was 81% of the mean FCR in normal subjects, implying that the LDL receptor does not play a major role in the removal of Lp(a). This is also supported by studies with drugs that are known to lower plasma cholesterol by modulation of LDL receptor activity. Thus, administration of cholestyramine [36] does not alter serum Lp(a) concentrations although it causes a significant reduction in LDL cholesterol levels by increasing the number of LDL receptors. A new class of drugs that inhibit 3-hydroxy-3-methylglutaryl (HMG) CoA reductase also appear to exert their major LDL cholesterol lowering effect through increasing LDL receptor activity. In a study on 23 persons with severe type II hypercholesterolemia, an HMG CoA reductase inhibitor was found to have no consistent effect on serum Lp(a) concentrations at doses which brought about a mean 38% reduction in serum LDL cholesterol (J. Thiery, J. Schleef, V. W. Armstrong, D. Seidel, unpublished observations). Further research is obviously still required to explain fully these apparently contradictory observations and to establish finally the pathway(s) by which Lp(a) is catabolized.

Conclusion

During the past 5 years great progress has been made in our understanding of the structure of Lp(a), culminating in the elucidation of the complete amino acid sequence of apo(a). This has raised further intriging possibilities with regard to the potential atherogenicity of this lipoprotein; on account of the structural similarity of apo(a) to plasminogen it must now be considered whether Lp(a) might not affect the fibrinolytic system in vivo. The function of apo(a) is still unknown and poses a challenging problem for future research.

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