Lipoprotein(a) (Lp(a)) consists of a low density lipoprotein (LDL) particle covalently linked through a single disulfide bond to apolipoprotein(a) (apo(a)). Apo(a) contains multikringle domain glycopolypeptide that exists covalently linked to apolipoprotein B100 of low density lipoprotein, to form the lipoprotein(a) (Lp(a)) particle, or as proteolytic fragments. Elevated plasma concentrations of apo(a) and its fragments may promote atherosclerosis, but the underlying mechanisms are incompletely understood. The factors influencing apo(a) proteolysis are also uncertain. Here we have used exoglycosidase digestion and mass spectrometry to sequence the Asn (N)-linked and Ser/Thr (O)-linked oligosaccharides of human apo(a). We also assessed the potential role of apo(a) O-glycans in protecting thermolysin-sensitive regions of the polypeptide. Apo(a) contained two major N-glycans that accounted for 17% of the total oligosaccharide structures. The N-glycans were complex biantennary structures present in either a mono- or disialylated state. The O-glycans were mostly (80%) represented by the monosialylated core type 1 structure, NeuNaco2–3Galβ1–3GalNAc, with smaller amounts of disialylated and non-sialylated O-glycans also detected. Removal of apo(a) O-glycans by sialidase and O-glycosidase treatment dramatically increased the sensitivity of the polypeptide to thermolysin digestion. These studies provide the first direct sequencing data for apo(a) glycans and indicate a novel function for apo(a) O-glycans that is potentially related to the atherogenicity of Lp(a).
UK The Atherobacter ureafaciens sialidase (EC 3.2.1.18, ABS) and bovine testes galactosidase (EC 3.2.1.23, BTG) were from Glyko (Novato, CA), Clostridium perfringens sialidase (EC 3.2.1.18, CPS) was from Sigma (catalog number N2133). All other reagents were of the highest quality available and were purchased from Sigma.

**Table 1**

| Subject | Sex | Age | TC | HDL-C | LDL-C | TG | Lp(a) | Apo(a) ISO |
|---------|-----|-----|----|-------|-------|----|-------|------------|
| 1       | M   | 42  | 214| 45    | 131   | 190| >120  | 20         |
| 2       | F   | 28  | 240| 90    | 141   | 45 | >120  | 19/28*     |
| 3       | M   | 59  | 167| 48    | 95    | 42 | 50    | 29         |

* TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; Lp(a), lipoprotein (a); apo(a) ISO, apolipoprotein(a) isotype, given as number of kringle IV repeats.

a Subject 2 displayed two apo(a) isotypes.

**Isolation and Purification of Lp(a) and Apo(a)**—Lp(a) and apo(a) were isolated by sequential ultracentrifugation and lysine-Sepharose affinity chromatography as described previously (12). After purification, Lp(a) (1 mg/ml) was incubated with dithiothreitol (10 mM) for 3 h at 37 °C. After this treatment, apo(a) was separated from the remaining LDL-like particles on heparin-Sepharose according to Ref. 11 using 1 mg of Lp(a) per 2 ml of resin. All subsequent analyses were performed on individual Lp(a) or apo(a) samples.

**Apo(a) Glycan Structure and Protease Resistance**—A Waters CapLC system was interfaced with a QTOF hybrid quadrupole time-of-flight mass spectrometer with electrospray ionization in positive mode, fitted with a Z-spray ion source (Micromass UK, Ltd., Wythenshawe, Manchester, UK). A 1 × 150 mm microbore NP-HPLC column was packed with stationary phase material from a GlycoSepN column (Glyko). The same solvents were used as for standard NP-HPLC with a gradient from 80 to 50% acetonitrile over 120 min at a flow rate of 10 μl/min. Positive ion mass spectra of the sialylated O-glycans were recorded under the following conditions: source temperature 90 °C; desolvation temperature 150 °C; desolvation gas flow 200 liters/h; nebulizer gas 40 liters/h at 3000 V; cone voltage 30 V; mass range 50–3500.

**MALDI-TOF MS**—Positive ion MALDI-TOF mass spectra were recorded with a Micromass TOF Spec 2E reflector-TOF mass spectrometer (Micromass UK, Ltd., Wythenshawe, Manchester, UK) fitted with a de-oxidizing source temperature 190 °C; nitrogen source and nebulizer gas flow 0.2 and 5 l/min, respectively. The voltage was 20 kV; the pulse voltage was 2300 V, and the delay for the detected ion extraction was 500 ns. Samples were prepared by mixing 0.5 μl of the aqueous solution and 0.5 μl of a saturated solution of 2,5-dihydroxybenzoic acid on the stainless steel MALDI target plate and allowing the mixture to dry at room temperature. The sample: matrix mixture was then recrystallized from ethanol (30).

**Deglycosylation and Proteolytic Fragmentation of Apo(a)**—Limited proteolysis of apo(a) was carried out by incubation with thermolysin (from Bacillus thermostreptolyticus, 40 units/mg, Sigma) at a mass ratio of enzyme to substrate of 1:200 in 0.125 M Tris-HCl, 0.15 M NaCl, 10 mM CaCl₂ (pH 7.8) for 30 min at 37 °C (15). The reaction was stopped by addition of the polycrystalline gel electrophoresis loading buffer. Deglycosylation of Lp(a) was performed as follows: Lp(a) (10 μg) was incubated with CPS (0.05 units) and/or O-glycosidase (5 milliunits, Roche Molecular Biochemicals) in 100 μl of phosphate buffer (pH 7.3) overnight at 37 °C.

**Electrophoresis and Immunoblotting**—Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed on 4–10% acrylamide gradient slab minigels (Mini Protean II, Bio-Rad), using a discontinuous buffer system (31). Before electrophoresis, samples (50–200 ng of apo(a) protein) were combined with glycerol, bromophenol blue, and EDTA to final concentrations of 2%, 0.01%, and 0.05 mM, respectively. Reduced samples were prepared by boiling at 100 °C for 4 min in the presence of 200 mM dithiothreitol and 2% sodium dodecyl sulfate. Proteins were then electrobotted onto nitrocellulose and revealed by immunoblotting (15). The uniform transfer of all material was confirmed by Coomassie staining gels after electrophoresis and by reversible staining procedures. Polyclonal antibodies to apo(a) were revealed using a peroxidase-conjugated polyclonal anti-apo(a) antibody (32). The polyclonal antibody reacts strongly with all apo(a) fragments generated either in vivo or in vitro (15, 31). Visualization was achieved by enhanced chemiluminescence detection (ECL, Amersham Pharmacia Biotech).

**Prediction of Potential Apo(a) O-Glycan Sites**—Potential sites for O-linked glycosylation on apo(a) were predicted by comparison of the amino acid sequence of the specified interkringle linker domains with known O-glycosylated proteins listed in the O-GlycBASE and using the NetOglyc software (33).
RESULTS

Assignment of Apo(a) N- and O-Glycan Structures by Exoglycosidase Sequencing—Human Lp(a) was isolated from fasted plasma and apo(a) purified as described previously (12). Apo(a) was then subjected first to automated hydrazinolysis to remove all N-linked glycans, and the released sugars were fluorescently labeled and analyzed by NP-HPLC. Two predominant N-linked glycans were detected that accounted for more than 85% of the material eluting in positions of common N-linked glycans (Fig. 1). The G.U. values of the two major peaks and the products resulting from their digestion with the listed exoglycosidases indicated that they were biantennary complex oligosaccharides present in either a mono- (A2G2S1) or disialylated (A2G2S2) state (Fig. 1A). Treatment of the N-glycans with ABS results in the loss of both peaks and formation of a single peak with a G.U. value identical to the neutral biantennary structure, A2G2 (Fig. 1B). In the presence of both ABS and BTG, the terminal galactose residues were also removed leading to the formation of the common core structure GlcNAc2Man3GlcNAc2, which is also abbreviated as A2G0 (Fig. 1C). Because the conditions employed for hydrazinolysis of N-glycans can lead to the partial destruction of O-glycans, apoa) was also subjected to a milder manual hydrazinolysis method to maximize the recovery of O-glycans. This procedure yielded several compounds that were labeled with 2-AB and analyzed by HPLC. An NP-HPLC profile of the recovered peaks is shown in Fig. 2. The peaks labeled with asterisks (as well as a compound that coeluted with peak 1) were also detected in hydrazinolysis “blanks” and were subsequently found to be derived from carbohydrates associated with the filter paper used in the glycan purification method. The apo(a) O-glycans detected were all of the core type 1 structure (Galβ1-3GalNAc1-R) and were found to be present in different sialylated states. The predominant O-glycan detected was a monosialylated trisaccharide (peak 3) that was closely related to the disialylated (peak 4) and nonsialylated (peak 1) structures that were also present (Fig. 2A). Peak 2 accounted for ~4% of the O-glycan pool and was identified as NeuNAcO2-3Gal which is a degradation product caused via a β-elimination reaction occurring at the glycosidic linkage between the core GalNAc and sialylated Gal residues, a recognized characteristic of O-glycan instability also known as “peeling” (23, 24).

Treatment of the O-glycan pool with ABS resulted in the loss of the sialylated structures and a concomitant increase in the level of the nonsialylated core type 1 structure, Galβ1-3GalNAc (Fig. 2B). In the presence of ABS and BTG the Galβ1-3GalNAc peak was partially removed, and the remaining non-digestible material appeared to be due to a contaminant introduced during the hydrazinolysis procedure. In order to achieve an accurate determination of the amount of Galβ1-3GalNAc normally present on apo(a), the glycan mixture was also run on RP-HPLC to separate the contaminant from Galβ1-3GalNAc. The RP-HPLC profiles are shown in Fig. 3. The compounds labeled with asterisks again represent peaks that were present in the hydrazinolysis “blank,” and the remaining peaks are numbered using the same nomenclature as shown in Fig. 2A. The Galβ1-3GalNAc (Fig. 3A, peak 1) was clearly separated from the other compounds and was found to account for ~11% of the O-glycan pool. Note that the order of elution of peaks 2 and 3 changed under the RP-HPLC conditions as compared with the NP-HPLC conditions (Figs. 3A and 2A, respectively). The identity of peak 1 was confirmed by ABS digestion of the sialylated structures (peaks 3 and 4) that were quantitatively recovered as Galβ1-3GalNAc on the RP-HPLC system (Fig. 3B).
determined by analyzing the glycan pool by MALDI-TOF MS, and the masses of the most abundant ions detected are given in Table II along with their corresponding glycan structures.

The structures of apo(a) glycans have not been determined previously; however, predictions based on apo(a) monosaccharide composition have been made (18, 34). A comparison of the published monosaccharide composition of apo(a) compared with the predicted monosaccharide composition based on our analysis of intact N- and O-glycans is given in Table III. The values for the three studies listed in Table III are in close agreement and indicate a mean apo(a) monosaccharide molar ratio of 3:7:5:4:7 for Man:Gal:GalNAc:GlcNAc:NeuNAc, respectively. However, the previous assertion that the predominant apo(a) O-glycan structures are Gal-GalNAc and NeuNAc-(NeuNAc-Gal)-GalNAc (18) appear to be incorrect as our glycan sequencing and mass spectrometry data indicate that NeuNAc-Gal-GalNAc is the most abundant O-glycan (Fig. 2). Comparison of the amounts of apo(a) N- and O-glycans recovered from apo(a) revealed that they were present at a molar ratio of 1:5 (N/O-glycans) consistent with the previously published monosaccharide data (data not shown).

Role of Apo(a) O-Glycans in Conferring Resistance to Thromolysis Digestion—Since apo(a) O-glycans are present in the interkringle linker domains (20), we speculated that they may

FIG. 2. Normal phase HPLC profile of apo(a) O-linked oligosaccharides. Apo(a) samples were subjected to a manual hydrazinolysis procedure at 60 °C for 6 h and the recovered glycans 2-AB labeled and analyzed by NP-HPLC. The structures illustrated were sequenced by exoglycosidase digestions (as indicated in the legend to Fig. 1) and by comparison of the product and precursor G.U. values with those of known standards. Conversion of the sialylated structures to the neutral Gal-GalNAc product after ABS treatment is indicated by the arrows. The glycan structures are represented by the symbols described in the legend to Fig. 1. See main text for further details.

FIG. 3. Reversed phase HPLC profile of apo(a) O-linked oligosaccharides. Apo(a) samples were subjected to a manual hydrazinolysis procedure at 60 °C for 6 h and the recovered glycans 2-AB labeled and analyzed by RP-HPLC. The peaks were assigned according to the numbering scheme for the NP-HPLC analysis (Fig. 2). Identity of the structures was confirmed by digestion with ABS and by comparison of the product and precursor arabinose unit (A.U.) values with those of known standards (29). See main text for further details.
In order to test this hypothesis, we pretreated Lp(a) with CPS or CPS plus O-glycosidase (which removes core Gal-GalNAc from Ser and Thr residues) and assessed apo(a) susceptibility to limited thermolysin digestion. In the presence of thermolysin, apo(a) was degraded to form two peptides (Fig. 6A, lane 2) that have been characterized previously and found to be the result of a cleavage between Ala3532 and Phe3533 of the linker 4 domain that links kringle IV4 and kringle IV5 (12). Our present data suggest that desialylation of apo(a) results in a partial increase in its sensitivity to thermolysin digestion, whereas complete removal of apo(a) O-glycans dramatically increases its subsequent fragmentation.

The pretreatment of apo(a) with O-glycosidase (which specifically cleaves Gal-GalNAc) alone did not alter apo(a) susceptibility to thermolysin digestion nor did it result in a change in molecular weight of the two thermolysin proteolytic products (Fig. 6A, compare lanes 2 and 5). This final result indicates that only a very small proportion of the apo(a) glycans was present as Gal-GalNAc and that their removal does not make a significant impact on the susceptibility of apo(a) to thermolysin digestion. The fact that the molecular weight of the initial N-terminal and C-terminal peptides remains constant after O-glycosidase treatment also supports our oligosaccharide sequencing and mass spectrometry data (Fig. 3 and Table II) showing that the predominant O-glycan present is NeuNAc-Gal-GalNAc (which is not a substrate for O-glycosidase).

Fig. 6B shows an additional Western blot from a separate experiment. In this case the gel was intentionally overloaded in order to detect any minor apo(a) fragments generated under the different digestion conditions. The data are in close agreement with those shown in Fig. 6A and confirm that desialylation of apo(a) results in a partial increase in its sensitivity to thermolysin digestion, whereas complete removal of apo(a) O-glycans dramatically increases its subsequent fragmentation.

**DISCUSSION**

The present studies utilized exoglycosidase sequencing techniques and mass spectrometry to determine the complete structure (including glycosidic linkage analysis) of human apo(a) oligosaccharides. The predicted monosaccharide composition was in general agreement with data published previously (18, 34). However, the desialylated and non-sialylated O-glycan structures that were predicted previously to occur (18) were found to account for only 10–20% of the apo(a) O-glycan pool (Fig. 3). The most abundant oligosaccharide was, in fact, a monosialylated core type I O-glycan (Fig. 5), a structure that can also be deduced from the previous monosaccharide analysis (18, 34).

The slightly lower ratio of NeuNAc:Gal observed in our study is due to the finding that almost half of the biantennary N-glycans (which accounted for ~17% of total apo(a) glycans) were present in a monosialylated state. In earlier studies (18), NeuNAc and Gal were present at equimolar ratios, suggesting an absence of monosialylated N-glycans. Possible reasons for this discrepancy may be related to the high variation in sialic acid concentrations reported (as determined by gas chromatography) or due to the nonspecificity of the thiobarbituric acid method used in the NeuNAc quantitation (18). Use of the thiobarbituric acid or “Warren” method can lead to an overestimation of lipoprotein sialic acid levels (35, 36). It is unlikely that the apo(a) N-glycans were desialylated during processing.
in the present work as the O-glycan pool (which also contained NeuNAc in the α2–3 linkage to Gal) did not contain a predominant nonsialylated structure. We have also observed that human apoB100 contains a high proportion of its N-glycans as a monosialylated complex biantennary structure, and this was noted when the glycans were removed from apoB100 by either hydrazinolysis or treatment with peptide N-glycosidase F, indicating that under our hydrazinolysis conditions the NeuNAcα2–3Gal glycosidic linkage is stable.

2 B. Garner, D. J. Harvey, M. Frischmann, F. Nigon, M. J. Chapman, and P. M. Rudd, unpublished data.

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**TABLE II**

| Peak | G.U. Value | A.U. Value | Mass | Structure |
|------|------------|------------|------|-----------|
| N-glycans | | | | |
| 1. | 7.96 | 2074.6<sup>a</sup> | 2074.7 | Galβ1-4GlcNAcβ1-2Man<sub>1</sub> |
| | | | | |
| 2. | 8.78 | 2409.8<sup>b</sup> | 2409.8 | Manβ1-4GlcNAcβ1-4GlcNAc(2-AB) |

| O-glycans | | | | |
| 1. | 1.77 | 1.38 | 526.1<sup>c</sup> | 526.2 | Galβ1-3GlcNAc(2-AB) |
| 2. | 2.24 | 2.22 | 614.1<sup>c</sup> | 614.2 | NeuNAcβ2-3Gal(2-AB) |
| 3. | 2.83 | 2.07 | 817.3<sup>c</sup> | 817.3 | NeuNAcβ2-3Galβ1-3GlcNAc(2-AB) |
| 4. | 4.26 | 2.75 | 1086.4<sup>d</sup> | 1086.4 | NeuNAcβ2-6(NeuNAcβ2-3Galβ1-3)GlcNAc(2-AB) |

<sup>a</sup> Major ion was detected by MALDI-TOF as [M + Na]<sup>+</sup> ion.

<sup>b</sup> Major ion was detected by MALDI-TOF as [M – 2H + 3Na]<sup>+</sup> ion.

<sup>c</sup> Degradation product was caused by peeling reaction during hydrazinolysis.

<sup>d</sup> Major ion was detected by LC-QTOF as [M + H]<sup>+</sup> ion. Note: the NeuNAc residue of N-glycan 1 may also be present on the Manα1–6Man arm. There were no significant differences observed in glycan composition among the apo(a) isotypes analyzed.
When apo(a) was treated with O-glycosidase (which cleaves Gal-GalNAc but not NeuNAc-Gal-GalNAc from Thr/Ser) followed by thermolysin, there was no significant change in the molecular weight of the resulting peptides compared with the peptides resulting from treatment of apo(a) with thermolysin alone. This supports our exoglycosidase sequencing data indicating that the Gal-GalNAc content of the original apo(a) samples was low.

Having established the apo(a) oligosaccharide structures, we went on to investigate the potential influence of the O-glycans on apo(a) sensitivity to protease digestion. It is well known that human plasma and urine contains apo(a) fragments (31, 37). The factors that control the formation of these fragments are, however, poorly understood. It appears that specific domains of apo(a) are more susceptible to proteolytic cleavage than others (12). For example, the major cut site for both thermolysin and neutrophil elastase (both serine proteases) is in the kringle IV4 linker (12, 13). This domain is predicted to be devoid of glycan chains (Fig. 7B), and we hypothesized that apo(a) fragments resulting from digestion at this site may be more susceptible to proteolysis than those resulting from digestion at other sites.

### Table III

| Monosaccharide                  | This study (n = 2) | Fless et al. (18) (n = 1) | Seman and Breckenridge (34) (n = 2) | Mean |
|--------------------------------|-------------------|--------------------------|-----------------------------------|------|
| Mannose                        | 3                 | 3                        | 3                                 | 3    |
| Galactose                      | 7.2 (2.4)         | 7                        | 7.1 (0.3)                         | 7.1  |
| N-Acetylglactosamine           | 4.2 (0.3)         | 5                        | 4.3 (0.5)                         | 4.5  |
| N-Acetylglucosamine            | 4.0 (0.0)         | 4                        | 3.4 (0.8)                         | 3.8  |
| N-Acetylneuraminic acid        | 6.3 (2.1)         | 7                        | 8.8 (3.7)                         | 7.4  |

*Where n = 2, values are means with range shown in parentheses.

*Values quoted in Ref. 34 were originally given as masses for each monosaccharide.

![Fig. 6. Influence of O-glycans on apo(a) susceptibility to thermolysin digestion.](image)

Fig. 6. Influence of O-glycans on apo(a) susceptibility to thermolysin digestion. Purified Lp(a) was analyzed by Western blotting without enzymatic treatment (lane 1) or after partial thermolysin digestion in a fully glycosylated state (lane 2) or after an initial treatment with CPS to remove terminal sialic acids (lane 3) or CPS + O-glycosidase to remove sialylated core type 1 structures (lane 4). Lane 5 represents Western blots from two independent experiments. A, Lp(a) was also pretreated with O-glycosidase to remove non-sialylated core type 1 (Gal-GalNAc-R) structures prior to thermolysin digestion (A, lane 5). B, the gels were overloaded in order to detect the quantitatively minor apo(a) fragments. The presence of naturally occurring apo(a) fragments associated with the non-digested Lp(a) is also evident in the overloaded condition (B, lane 1). Enzymatic deglycosylation of Lp(a) without thermolysin digestion did not reduce antibody binding to apo(a) (not shown). Results are representative of three independent experiments. See "Experimental Procedures" for further details.

![Fig. 7. Potential sites for apo(a) O-linked glycosylation.](image)

Fig. 7. Potential sites for apo(a) O-linked glycosylation. Potential sites for O-linked glycosylation on the linker domains between kringle IV2–IV3 (A), kringle IV4–IV5 (B), and kringle IV7–IV8 (C) were predicted by amino acid sequence comparison with known O-glycosylated proteins listed in the O-GLYCBASE and using the NetOglyc software. The amino acid sequences for each of the kringle linkers is given on the x ordinate, and the potential for glycosylation is indicated on the y ordinate. The horizontal line on each panel represents the threshold value for glycosylation (i.e. if the bars on the histogram are higher than the threshold value, O-glycosylation is predicted to occur).
saccharide structures were removed by treatment with sialidase and particularly when the entire oligosaccharide structures were removed by treatment with sialidase. The role of O-glycans in protecting apo(a) from protease digestion may also explain why the apo(a) peptide generated by cleavage at the elastase cut site in the kringle IV7 linker requires an extended incubation time or increase in enzyme concentration (13).

It is likely that apo(a) O- and N-glycans play additional functional roles, for example in intracellular processing (39, 40), maintaining the tertiary structure of apo(a), and preventing aggregation (17). Because apo(a) O-glycans are hydrophilic, they may also play a role in ensuring that the bulk of apo(a) is extended out into the aqueous phase, as has been observed in structural studies of recombinant Lp(a) (41).

Megalin/gp 330 has recently been identified as an endocytic receptor for Lp(a) (42). This receptor also appears to be involved in the cellular uptake of other glycosylated apolipoproteins including apoE, apoD, apoH, and apoB100 (43–46). Since megalin is highly expressed in the brush border of renal proximal tubules and in the coated pits of glomerular epithelial cells (47, 48), it provides a plausible control mechanism for the generation of specific urinary apolipoproteins (49). Interestingly, plasminogen is also a ligand for megalin (50), yet its role is not yet fully elucidated. Since the kringle type IV and type V structures of apo(a) are homologous to plasminogen (2), it is tempting to speculate that the higher excretion of apo(a) may be related to the almost 14-fold higher content of carbohydrate associated with the apo(a) kringle linkers. This possibility has not been addressed as far as we are aware.

The presence of terminal Gal residues on apo(a) may also confer an ability to bind to the macrophage asialoglycoprotein receptor. An analogous pathway has been proposed for apoB100 (LDL) endocytosis by macrophages (51, 52). Arguing against such a pathway, one study has shown that the removal of sialic acid residues from recombinant apo(a) did not alter its binding to a partially characterized macrophage foam cell surface receptor (53). Investigation of the full range of functions for apo(a) oligosaccharides will clearly require further study.

The generation of apo(a) fragments in vivo has been suggested to be potentially atherogenic due to the possibility of C-terminal peptides interfering with fibrinolysis (15, 19). Of potential relevance, apo(a) fragments accumulate in atherosclerotic lesions where they may promote thrombogenesis (14). In the microenvironment of the artery wall, it is possible that macrophage-derived glycosidases and proteases act in concert to degrade apo(a). This might have the unfortunate consequence of generating macromolecular complexes between “mini-Lp(a)” and extracellular matrix components that could then be taken up by macrophages to form foam cells and thereby promote the development of the atherosclerotic lesion. Since macrophages can release proteases (54, 55) and glycosidases (56), our discovery that apo(a) glycans normally limit the extent of proteolytic fragmentation might explain why, in the macrophage-rich atherosclerotic lesion (57), apo(a) fragments are found to accumulate (14). It would be interesting to assess the degree of glycosylation present on such lesion-derived apo(a) fragments. In conclusion, the present study has directly elucidated the structure of human apo(a) oligosaccharides and shown that the O-glycans play an important role in maintaining the stability of apo(a) by conferring resistance to degradation by the serine protease, thermolysin.

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