Isolation and characterization of the two major apoproteins in human lipoprotein[a]

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Abstract Human Lp[a] was isolated in preparative amounts from two donors; the native lipoprotein and its constituent apoproteins, apo[a] and apoB, were characterized extensively. Based on differences in apparent molecular weight, four different isoforms of apo[a], a_1–a_4, were observed between the two donors. The number and relative distribution of these isoforms varied between donors but were constant for each donor. Each apo[a] isoform was shown to be derived from a discrete apo[a]-B100 disulfide-linked complex present before reduction. Complete delipidation of Lp[a] was followed by solubilization, reduction, and carboxamidomethylation of the constituent apoproteins. These apoproteins were then separated by immunoaffinity chromatography using anti-apo[a]- or anti-apoB-Sepharose; their purity and structural integrity were demonstrated by Western blot analysis. ApoB isolated by this procedure was essentially identical to apoB from autologous LDL with respect to molecular weight, secondary structure, amino acid composition, and sialic acid content. However, apo[a] differed from apoB in that it exhibited: *1)* a much less α-helical, less β, but much more disordered structure; *2)* a lower proportion of aspartate, isoleucine, leucine, phenylalanine, and lysine, but a higher proportion of proline, glycine, and threonine; and *3)* a much higher content of sialic acid. These results indicate that apo[a] is not a superglycosylated form of apoB but is distinctly different in its composition and structure.—**Gaubatz, J. W., M. V. Chari, M. L. Nava, J. R. Guyton, and J. D. Morrisett.** Isolation and characterization of the two major apoproteins in human lipoprotein[a]. *J. Lipid Res.* 1987. **28:** 69–79.

Supplementary key words Lp[a] • apo[a] • apoB • Western blot • circular dichroism • amino acid analysis • isoforms • SDS-PAGE • immunoaffinity chromatography • secondary structure

Human Lp[a] is a lipoprotein whose plasma levels have been highly correlated with coronary heart disease (1–3). Although this important correlation was demonstrated a number of years ago, only rather recently has the structure of this lipoprotein received intensive study. This has been due, in part, to the lack of reliable methods for isolating preparative amounts of the lipoprotein in pure form, and to its relatively low plasma concentration compared to most other naturally occurring lipoproteins. The properties of apo[a], the apoprotein moiety unique to Lp[a], had been rather difficult to study until it was shown that this protein was cross-linked to apoB in the same particle by one or more disulfide bonds (4–6). Reductive cleavage of these disulfides allows selective removal of apo[a] from the lipoprotein by ultracentrifugation or heparin-Sepharose chromatography, but not by gel filtration (7, 8). The resulting apo[a]-free lipoprotein closely resembles autologous LDL with respect to electrophoretic mobility, chemical composition, size, immunochemical reactivity, and LDL receptor binding (8). However, a rigorous comparison of the chemical and physical properties of apoB from these two populations has not been reported. The present study provides a detailed comparison of this apoprotein from these two sources.

Apo[a] released from either Lp[a] or apoLp[a] exhibits molecular weight heterogeneity as determined by SDS-PAGE (4). The chemical nature of this heterogeneity has not been firmly established, although preliminary data suggest it may be attributed to apoprotein molecules carrying different amounts of carbohydrate. The occurence of apo[a] and apoB in the same lipoprotein particle raises the important question of how these two apoproteins relate to each other chemically and physically. In this report, we present experimental evidence demonstrating that they are distinctly different apoproteins.

Abbreviations: apo[a], apolipoprotein[a]; apoB, apolipoprotein B; CD, circular dichroism; d, density; dss, decyl sodium sulfate; DTT, dithiothreitol; EDTA, ethylenediamine tetraacetate; HDL, high density lipoprotein; KIU, kallikrein inactivator unit; LDL, low density lipoprotein; Lp[a], lipoprotein[a]; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethanesulfonyl fluoride; RCAM, reduced and carboxamidomethylated; SDS, sodium dodecylsulfate.

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METHODS

Isolation of lipoproteins

Two healthy subjects served as donors for lipoprotein isolation. These donors, A and B, were selected on the basis of their high plasma Lp[a] protein concentrations (37.7 and 41.0 mg/dl, respectively), and their willingness to submit to plasmapheresis on a continuing basis. Donor A was a 36-year-old black female whose plasma lipid levels were 218 mg cholesterol/dl, 113 mg triglycerides/dl, and 65 mg HDL-cholesterol/dl. Donor B was a 30-year-old white female whose plasma lipid levels were 210 mg cholesterol/dl, 163 mg triglycerides/dl, and 58 mg HDL-cholesterol/dl. Aprotinin (Trasylol, Mobay Chemical Corp., 10,000 kallikrein inactivator units/ml) was added as a proteolytic inhibitor to the plasma collection bag, and hence was present at the end of plasmapheresis at a concentration of 100 KIU/ml. Immediately after collection, the plasma was made 0.01% in NaN_3, 1 mM in EDTA, and 0.001% in PMSF. The freshly obtained plasma was subjected to differential ultracentrifugation to isolate the desired lipoprotein fractions (9) (**Fig. 1**). Typically, the following fractionations were performed: *1)* d < 1.12 g/ml to float the major Lp[a]-containing fraction and LDL; *2)* d 1.06–1.12 g/ml to exclude most of the LDL while including most of the Lp[a]; and *3)* d 1.02–1.06 g/ml to obtain LDL.

Immunoaffinity chromatography involving an anti-apo[a]-Sepharose affinity column was used to purify further the Lp[a] and LDL isolated ultracentrifugally (Fig. 1). The d 1.02–1.06 g/ml fraction was chromatographed in order to adsorb the small amount of Lp[a] and separate it from the more abundant LDL present. The d 1.06–1.12 g/ml fraction was chromatographed to separate the more abundant Lp[a] from lesser amounts of unbound LDL and HDL. After the column had been thoroughly washed, the Lp[a] was displaced from it by elution with 0.15 M NaCl adjusted to pH 10.5 with ammonium hydroxide. The Lp[a] was collected into tubes containing 0.5 M Tris (pH 7.0) which immediately lowered the pH of the highly basic effluent. The purity and chemical stability of the isolated apoproteins were determined as previously described (4).

Isolation of apo[a] and apoB from Lp[a]

Delipidated, water-soluble preparations of the apoproteins from Lp[a] and LDL were obtained by a modification of the procedure described by Cardin et al. (10) for apoLDL. **Fig. 2** shows the steps in the procedure.

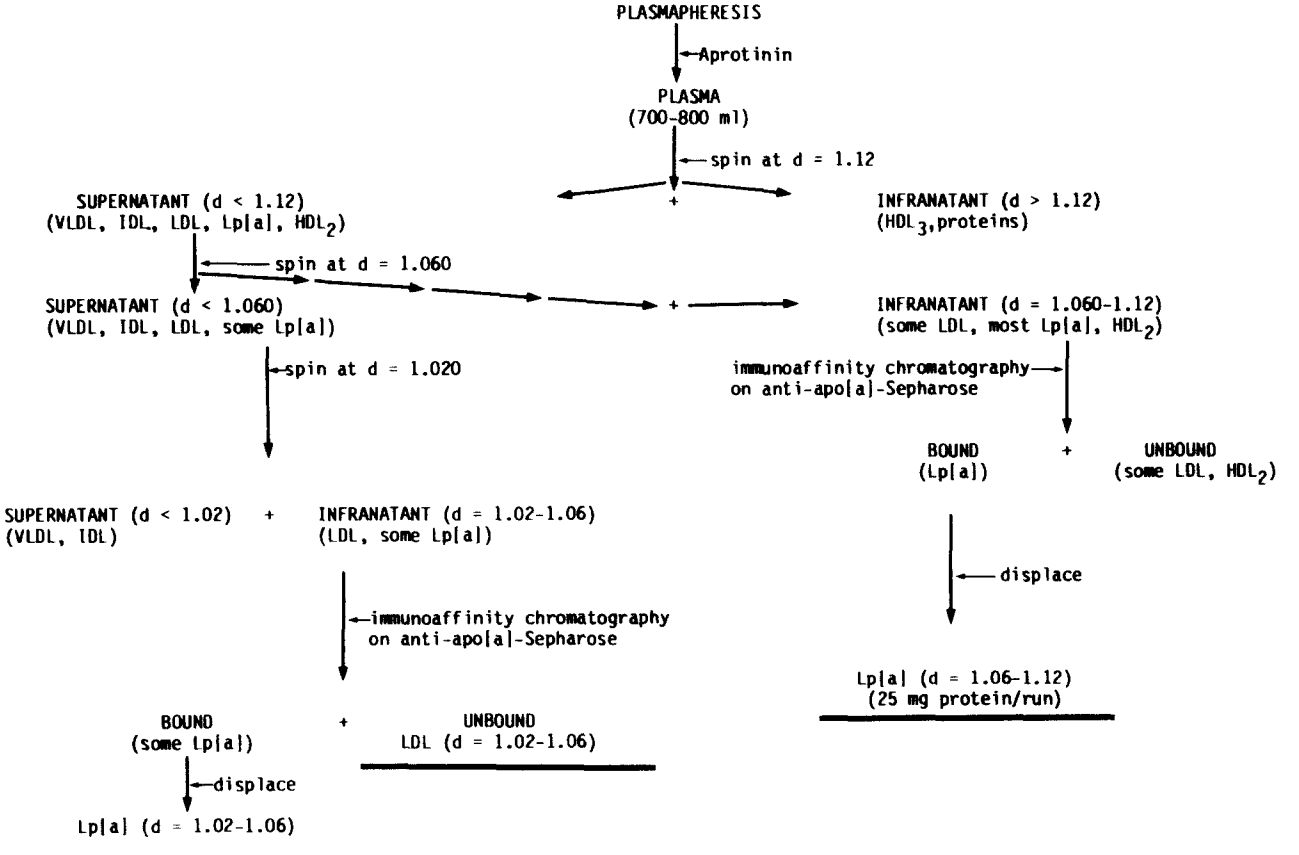

Fig. 1. Flow diagram for the preparative isolation of Lp[a] and LDL from human plasma. The purified fractions of greatest interest are underscored.

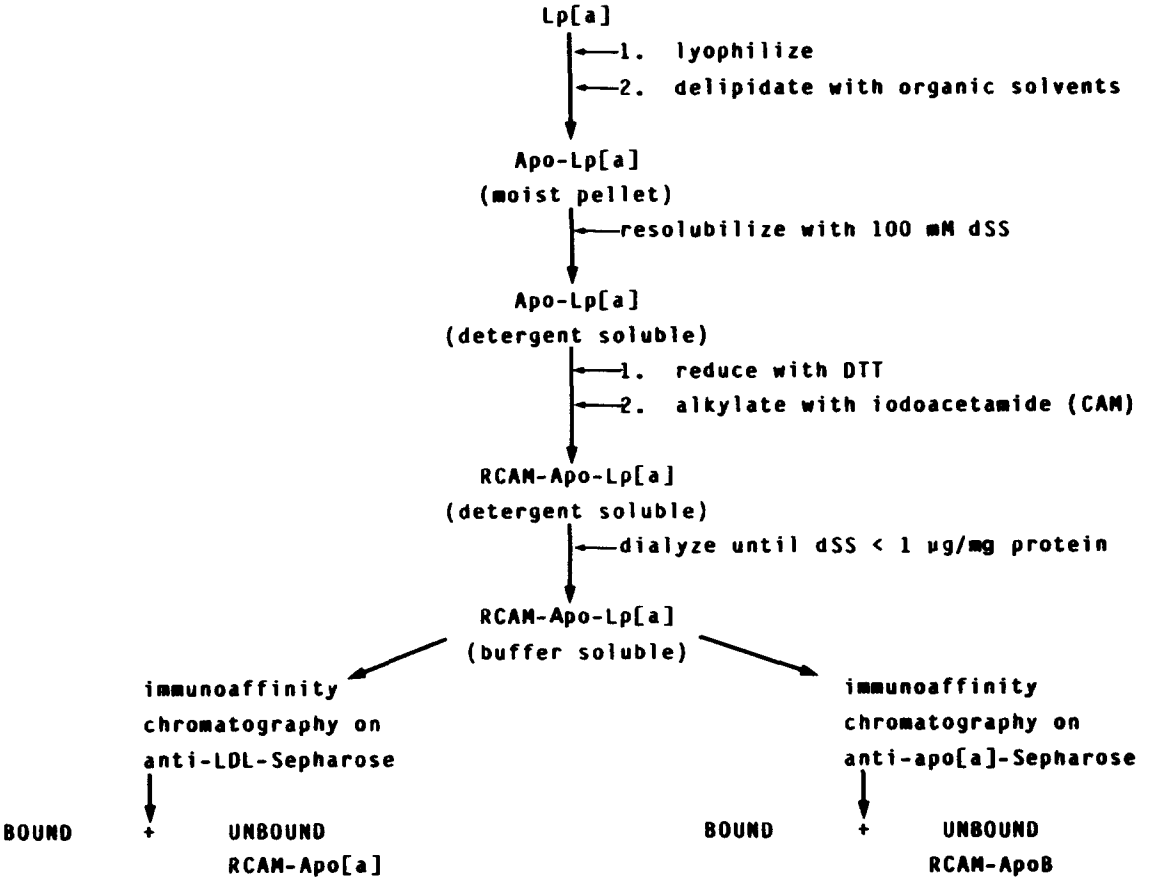

Fig. 2. Flow diagram for the isolation of apoprotein[a] and apoprotein B from Lp[a].

Lp[a] or LDL in 1 mM EDTA, 0.01% NaN_3, pH 7.2, were lyophilized in siliconized glass tubes. Samples were extracted at 4°C using a protein:solvent ratio of 1 mg:2.5 ml. The following extraction steps were performed: *1)* 2 × with ether–ethanol 3:1 (v/v) for 1 hr; *2)* 1 × with chloroform–methanol 2:1 (v/v) for 1 hr; and *3)* 1 × with ether for 0.5 hr. The apoprotein residue was solubilized in 100 mM dss, 10 mM Tris-HCl, 1 mM PMSF, 1 mM EDTA, 0.01% NaN_3, pH 8.5. The completely soluble protein was reduced with 20 mM DTT for 1 hr at room temperature in a N_2 atmosphere, then treated with 50 mM iodoacetamide for 1 hr. The reaction was stopped by addition of 100 mM DTT and incubation for 1 hr at room temperature. The sample was dialyzed against 10 mM Tris, 1 mM PMSF, 1 mM EDTA, 0.01% NaN_3, pH 8.5, until the dss concentration was < 1 µg/mg protein as determined by the method of Hayashi (11). For a 10-ml sample volume, this typically required five 2-liter changes over a 3-day period. The apoLp[a] proteins were then separated by immunoaffinity chromatography; apo[a] eluted as the unbound fraction from the anti-LDL-Sepharose column, and apoB eluted as the unbound fraction from the anti-apo[a]-Sepharose column.

Chemical analysis

Protein content was determined by the method of Lowry et al. (12). Free and total cholesterol were determined using the Boehringer-Mannheim assay kits based on the method of Roeschlau, Bernt, and Gruber (13). The difference between total and free cholesterol was multiplied by 1.68 to calculate the cholesteryl ester content. Triglyceride was determined using the Boehringer-Mannheim kit based on the method of Wahlefield (14). Lipid phosphorus was determined by the method of Bartlett (15); this was converted to phospholipid by using a multiplication factor of 25. Cholesterol, triglyceride, and HDL-cholesterol of the donor plasma samples were determined by the Atherosclerosis Clinical Laboratory (The Methodist Hospital, Houston, TX). Sialic acid was determined using both a kit from Boehringer-Mannheim involving enzymatic analysis and by the resorcinol method (16).

Electrophoretic methods

Homogeneous slab gels containing 2.75% acrylamide, 0.069% bisacrylamide were used for the SDS-PAGE ana-

lyses as previously described (4) except for the inclusion of 0.5% agarose (type C, Behring Diagnostics, La Jolla, CA) for the structural stability and increased resolution. Rocket immunoelectrophoresis and Western immunoblotting were performed as previously described (4).

Circular dichroism

Samples were dialyzed against 0.01 M borate buffer, pH 8.6, and the protein content was measured after dialysis. Spectra were recorded on a Jasco 500A spectropolarimeter equipped with a data processor for obtaining spectra corrected for buffer absorbance. Data were acquired using a 1.0-mm cell at 20°C in the far ultraviolet region from 190 to 240 nm. The mean residue ellipticities and the computed percentages of secondary structure were calculated as described by us previously (4).

Amino acid analysis

After careful degassing, samples were hydrolyzed for 20 hr at 100°C in 6 N HCl containing 0.1% phenol. An LKB 4400 amino acid analyzer equipped with an ion-exchange column packed with 8 μM BX-8 amino acid resin (Benson; Reno, NV) was used for the analyses.

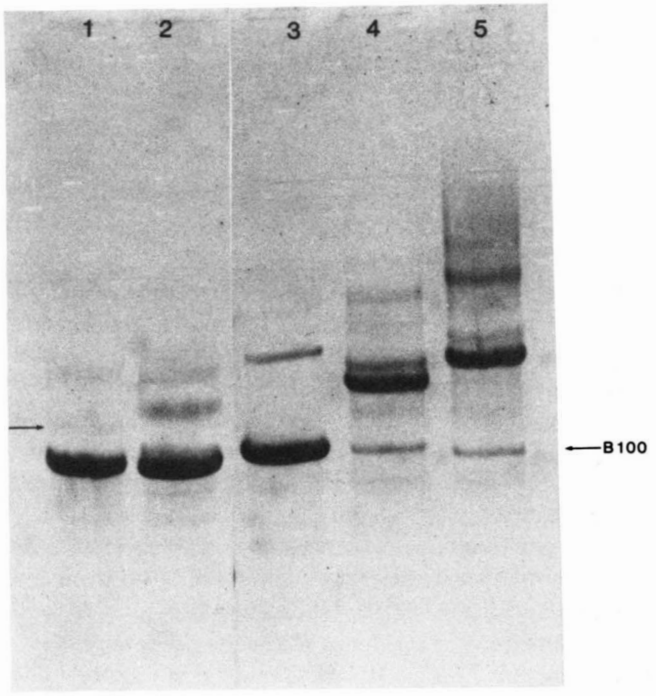

Fig. 3. SDS-PAGE on 2.75% slab gel stained with Coomassie Blue. Samples are the native lipoproteins which are the products of the purification scheme in Fig. 1. Lane 1, apoLp[a], reduced (donor B); lane 2, apoLp[a], reduced (donor A); lane 3, apoLDL; lane 4, apoLp[a], not reduced (donor B); lane 5, apoLp[a], not reduced (donor A). The native lipoproteins were delipidated by boiling for 3 min in 1% SDS. Reduced samples contained 1% mercaptoethanol. Sample load was 10 μg per lane.

RESULTS

All results were obtained only on non-pooled plasmas and the resulting isolated fractions. The proportions of phospholipid, cholesterol, cholesteryl ester, and triglyceride (expressed as a fraction of total lipid) in Lp[a] and LDL were quite similar. However, the proportion of protein (expressed as a fraction of total lipoprotein) was significantly higher in Lp[a] (28.0 and 29.3%) than in LDL (18.9 and 20.3%) for both donors (A and B, respectively).

The electrophoretic banding pattern for the apoprotein(s) of LDL and Lp[a] are shown in **Fig. 3.** The apoLDL from both donors migrated with typical apoB-100 mobility; one representative sample [a] is presented (lane 3). In addition to B100, there was also present a much less abundant, higher molecular weight protein component. This component was also present in reduced apoLp[a] (lanes 1 and 2) and reacted with anti-apoB by Western immunoblotting. The differences in the apoprotein banding patterns for apoLp[a] from the two donors are observable in lanes 4 and 5. The apo[a]-B100 disulfide-linked complexes (labeled) were resolved into doublets; the one for donor A (lane 5) migrated to a higher apparent molecular weight position than the one for donor B (lane 4). After reduction (lanes 1 and 2), most of the Coomassie Blue-staining material was present at the position corresponding to B100. In addition, there was an observable weaker staining band in lane 2 for donor A and a faint band in lane 1 for donor B (arrow).

In order to determine the relationship of a band representing a unique free apo[a] isoform detectable after reduction to a particular apo[a]-B100 disulfide-linked complex present before reduction, a Western immunoblot experiment was performed (**Fig. 4**). In the left hand panel, only apo[a] was localized; in the right hand panel, only apoB. Specificity is demonstrated by the banding patterns observed with apoLDL loaded in lanes 3 and 4; no bands were observed with anti-apo[a] (Fig. 4A), and single sharp bands were seen with anti-apoB (Fig. 4B). Following reduction of apoLp[a], two apo[a] bands appeared in lane 1 (donor B) and three in lane 2 (donor A) (Fig. 4A). The four different bands observed have been designated $a_1 \rightarrow a_4$ corresponding to the highest → lowest molecular weight species. According to this nomenclature, Lp[a] from donor B contains predominantly a_4 with a lesser amount of a_3, while Lp[a] from donor A contains predominantly a_2, with lesser amounts of a_1 and a_4. The assignment of a specific apo[a] isoform disulfide-linked with B100, to a specific band (as shown in Fig. 4) was based upon the following rationale. *1)* The apparent molecular weight ($M_{r\ app}$) for B100 was the same for each donor (lanes 1, 2; Fig. 4B), so differences in the $M_{r\ app}$ of the complexes should be attributable solely to the $M_{r\ app}$ of the apo[a] species. *2)* The relative staining intensity of the complexes should be proportional to the

staining intensities of the apo[a] isoforms following reduction. Assignment of the complexes in lane 5 (donor B) is uncomplicated; the upper, less intense band in the doublet is designated as a_3-B100, and the lower, more intense band is designated as a_4-B100 complex. In lane 6 (donor A), the lower, more intense band in the doublet is designated as the a_2-B100 complex, and the upper band as the a_1-B100 complex. In this sample there was no band present corresponding to the a_4-B100 complex as one would expect. However, there were several weak, higher molecular weight mixed disulfides present in this sample; perhaps one of these corresponds to a higher order disulfide complex containing a_4.

Apo[a] isolated from apoLp[a] by the method outlined in the scheme of Fig. 2, would be a mixture of those isoforms present for a particular donor, while apoB would be essentially all B100. Some important properties of these buffer-soluble apoLp[a] species are demonstrated by double-decker rocket electrophoresis (**Fig. 5**). Samples that contain no free apo[a] or apo[a] disulfide-linked to apoB (e.g., lanes 3 and 4) form a rocket only in the lower deck. Samples that contain free apo[a] form a rocket in the upper deck, the height of which is proportional to the concentration. There is very little free apo[a] present in apoLp[a] before reductive cleavage of the connecting disulfide(s) as evidenced by the very small rockets in the upper anti-apo[a] deck (lanes 5 and 6). After reduction, the tall rockets formed in the upper deck (lanes 1 and 2) represent the large amount of free apo[a] produced by this treatment. In addition, this technique illustrates that these delipidated, detergent-free samples retain immunoreactivity, an important property utilized in the last step of the purification procedure (Fig. 2). Apoproteins isolated by this procedure were examined for purity and chemical stability by Western immunoblotting (**Fig. 6**). In lanes 1 and 2 were electrophoresed the apoLp[a] that had been reduced and carboxamidomethylated, the starting material for the immunoaffinity isolation. In lanes 3 were run apoB isolated from this starting material; it is clear that no apo[a] and only B100 is present. In lanes 4 were run isolated apo[a] that contained a_3 and a_4 isoforms, but not apoB. In lanes 6 were run the apoB isolated from the starting material shown in lane 2, Fig. 6B. In lanes 5, apo[a] isolated from donor A was run overloaded; however, no apoB was detectable in the sample under these overload conditions. When this same apo[a] was rerun at a lower load (cut-out at far left), the a_1, a_2, and a_4 isoforms were well resolved.

The secondary structures of these isolated apoproteins were studied by circular dichroism. The spectra obtained for the apoproteins isolated from the two donors were very similar; hence only that from donor A is shown (**Fig. 7**).

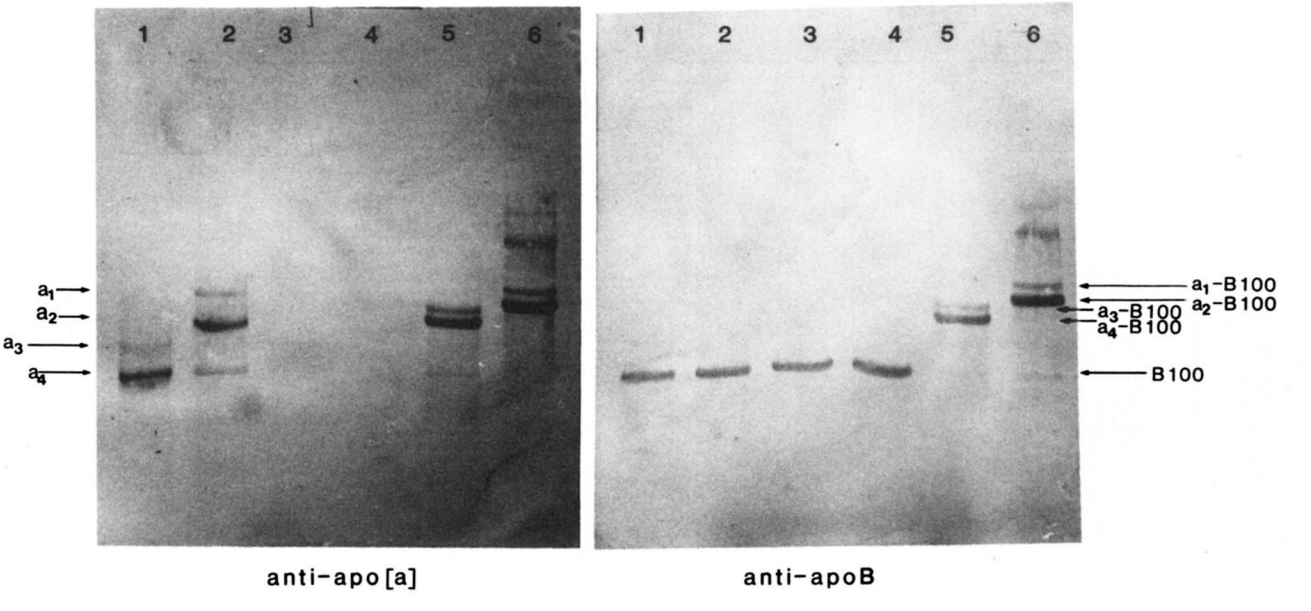

Fig. 4. Western immunoblot to apoproteins after 2.75% SDS-PAGE. Apo[a] was localized in the blot on the left and apoB in the duplicate blot on the right. Samples were prepared as described in Fig. 3. Lane 1, apoLp[a], reduced (donor B); lane 2, apoLp[a], reduced (donor A); lane 3, apoLDL (donor B); lane 4, apoLDL (donor A); lane 5, apoLp[a], not reduced (donor B); lane 6, apoLp[a], not reduced (donor A). Sample load was 1 μg per lane.

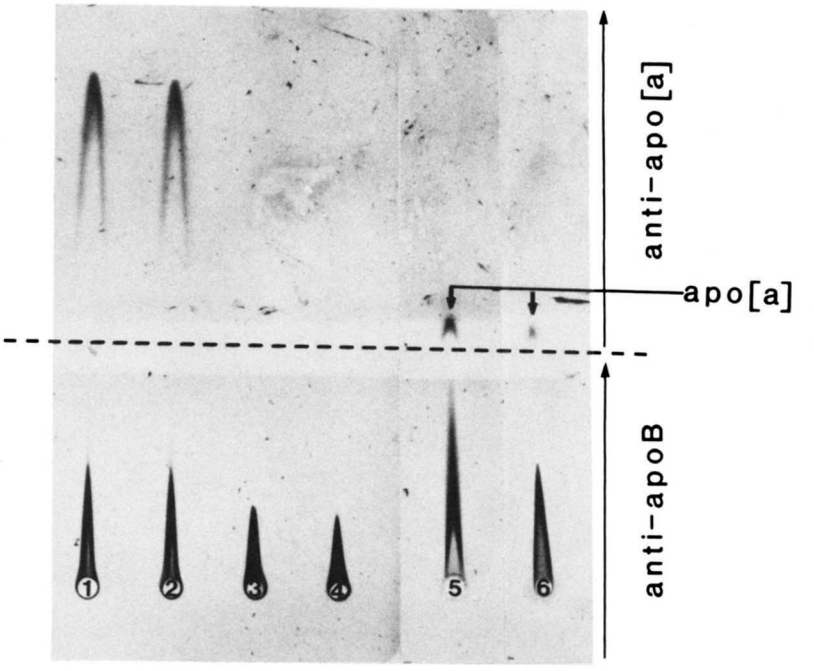

Fig. 5. Double-decker rocket immunoelectrophoresis of samples taken from the various steps of the flow diagram shown in Fig. 2. The lower agarose deck contains anti-apoB and the upper deck contains anti-apo[a]. Lanes 1-2, RCAM-apoLp[a]; lanes 3-4, RCAM-apoLDL; lanes 5-6, apoLp[a].

The apoB isolated from Lp[a] gave a spectrum identical to that isolated from LDL. Apo[a], however, exhibited a very different spectral curve. The fractional secondary structures for these apoproteins were calculated from spectral ellipticities; the percentages are shown in **Table 1.** ApoB from Lp[a] had a distribution of secondary structure almost identical to that of LDL. In contrast, apo[a] had a much lower fraction of α-helical structure (16% and 12%) and

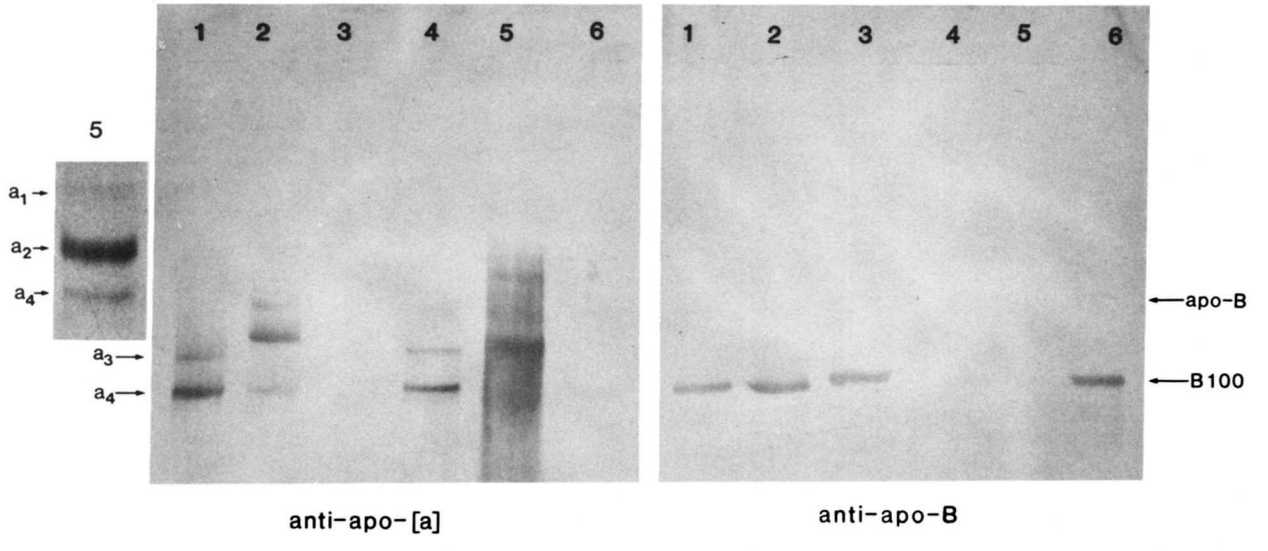

Fig. 6. Western immunoblot of apo[a] and apoB isolated from apoLp[a] as shown in Fig. 2. Apo[a] was localized in the blot on the left and apoB was localized in the duplicate blot on the right. Lane 1, RCAM-apoLp[a] (donor B); lane 2, RCAM-apoLp[a] (donor A); lane 3, unbound RCAM-apoB (donor B); lane 4, unbound RCAM-apo[a] (donor B); lane 5, unbound RCAM-apo[a] (donor A); lane 6, unbound RCAM-apoB (donor A).

a much greater proportion of disordered structure (64% and 68%) than apoB.

The amino acid compositions of samples isolated from the two donors are shown in **Table 2.** The values for apoLp[a] and apoLDL are in good agreement with those reported from other laboratories (10, 17). The apoB isolated from Lp[a] has an amino acid composition almost identical to that of apoB from LDL. The composition of apo[a] differs from that of apoB for a number of amino acids. For both donors, apo[a] contains a lower fraction of aspartate, isoleucine, leucine, phenylalanine, and lysine, but a higher fraction of proline, glycine, and threonine. The composition of apoLp[a] exhibits a similar trend for almost every one of these amino acids with a value intermediate between that for apoB and apo[a], but somewhat closer to that of apoB, which is what one would expect as a weighted average, since apoLp[a] consists of about 70% apoB and 30% apo[a] (J. W. Gaubatz, unpublished data).

The sialic acid content of native Lp[a] and LDL and their isolated apoproteins are shown in **Table 3.** Several different methods can be used for determination of sialic acid and a comparative study of these methods has shown that they sometimes give significantly different values for the same sample (18). In the present study, we have used both the resorcinol method and an enzymatic method for analysis; the enzymatic method consistently yielded lower values for sialic acid. The values for native LDL and Lp[a] are in excellent agreement with those obtained by others (19, 20). The sialic acid content of Lp[a] is from three- to sixfold greater than that of LDL. ApoB from Lp[a] has a much lower sialic acid content than does apo[a], and has a slightly greater content of sialic acid than apoB from LDL. The sialic acid content of apo[a] expressed as percent of protein was 25.3% for donor A and 23.7% for donor B (data from enzymatic analysis). When one uses the experimentally derived values for apo[a] and apoB to calculate the sialic acid content of Lp[a] based upon a 70%:30% ratio of apoB to apo[a], the values obtained are somewhat higher than the experimental values.

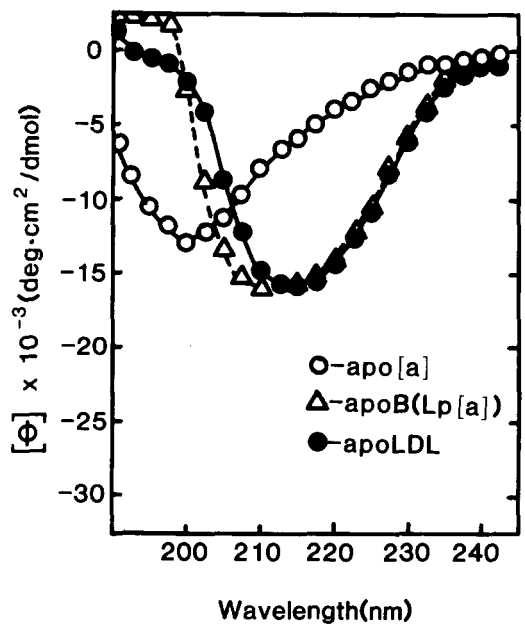

Fig. 7. Far ultraviolet circular dichroic spectra of apoproteins isolated from Lp[a] and LDL. Spectral accumulation conditions are given in the Methods section.

DISCUSSION

We have demonstrated previously that the only proteins present in Lp[a] are apo[a] and apoB, and that these are linked by one or more disulfide bonds (4). We have also characterized the native lipoprotein with respect to its density distribution, chemical composition, apparent molecular weight, and protein secondary structure. We and others (6) have emphasized the importance of including appropriate proteolytic inhibitors during the isolation of Lp[a], and of working with individual, non-pooled plasma samples because of their potential apoprotein heterogeneity. In the present study, we have carefully characterized Lp[a] and LDL from two donors, not only with respect to lipid composition, but also apoprotein distribution. Detergent-free, water-soluble derivatives of apo[a] and apoB have been prepared from Lp[a], and these modified apoproteins have been separated by immunoaffinity chromatography. The structural integrity of these isolated apoproteins was indicated by the similarity of their Western blot patterns to those of starting preparations. These apoproteins have been characterized with respect to their secondary structure, amino acid composition, and sialic acid content.

Using a combination of SDS-PAGE and Western immunoblotting, we have shown that apo[a] exists in at least four different molecular weight forms that react with a polyclonal antibody. For one donor (B), isoforms a_3 and a_4 were observed; for the other donor [a], isoforms a_1, a_2, and a_4 were present. These inter-donor differences illustrate the importance of performing such analyses on individual, non-pooled preparations. The lowest molecular weight isoform, a_4, exhibited the same apparent molecular weight as B100, and was present in both donors although in considerably different proportions. Fless, Rolih, and Scanu (6) have reported the existence of three apo[a] species from one donor; these were either smaller than, equal to, or larger than B100. The smaller apo[a]

TABLE 1. Secondary structure of apoproteins in Lp[a] and LDL as determined by circular dichroism

Sample	Percent of Total		
	α-Helix	β-Pleated Sheet	Disordered
Lp[a][a]	29	20	51
LDL[a]	48	25	27
Donor A			
ApoLDL	40	30	30
ApoB (Lp[a])	40	30	30
Apo[a]	16	20	64
Donor B			
ApoLDL	38	30	32
ApoB (Lp[a])	39	30	31
Apo[a]	12	20	68

[a]Values were taken from Gaubatz et al. (4).

was observed only in a low density Lp[a] particle population found in the 1.019–1.063 g/ml density range. For this reason, we examined the Lp[a] found in the d 1.02–1.06 g/ml fraction of donor B (see Fig. 2 for isolation). Still, both a_3 and a_4 isoforms were present in this fraction. The differences in the isoform distribution observed in these two studies may be attributed to intrinsic differences between the Lp[a] preparations from different donors and/or to differences in isolation techniques. For donor B, the a_3 and a_4 isoforms could be assigned to discrete, resolved apo[a]-B100 disulfide-linked complexes (Fig. 4, lane 5). For donor A, the a_4 isoform may occur as part of the higher order disulfide complexes represented by the higher molecular weight bands in the gel (Fig. 4, lane 6) although this assignment is not certain at present. The very weak Coomassie Blue staining of bands in which apo[a] alone is present (Fig. 3) is probably due to the apoprotein's large fraction of carbohydrate which decreases dye binding. This view is supported by the observation that the Bradford assay for protein (21), which is based on the binding of Coomassie Blue G-250, underestimates the protein content of apo[a] by a factor of 2.5, but that of apoB by only 1.2, compared to values determined by the Lowry method (J. W. Gaubatz, unpublished data).

The isoforms of apo[a] could result from: *1)* the synthesis of polypeptides of differing molecular weights; *2)* a single polypeptide that has undergone various degrees of post-translational glycosylation yielding glycoproteins of differing apparent molecular weights as judged by SDS-PAGE; or *3)* a combination of these phenomena. The second phenomenon is similar to that observed for the isoproteins of apoE that have slightly different molecular weights caused by attached carbohydrate chains of different lengths (22). The total number of different apo[a] isoforms present in a population, its physiological significance, and its genetic basis can be determined only by study of a larger number of subjects.

Initially, we evaluated several different methods for preparing apo[a] and apoB in water-soluble, detergent-free form. The methods of Walsh and Atkinson (23) and of Socorro and Camejo (24) in which ionic and non-ionic

TABLE 2. Amino acid compositions[a] of apolipoproteins isolated from Lp[a] and LDL[b]

Amino Acid	Donor A				Donor B			
	ApoLp[a]	ApoLDL	ApoB from Lp[a]	Apo[a]	ApoLp[a]	ApoLDL	ApoB from Lp[a]	Apo[a]
Asp	9.3	10.0	10.7	8.6	10.0	11.1	11.3	9.7
Thr	8.0	6.2	6.5	9.5	7.9	6.3	6.5	10.7
Ser	8.5	7.7	8.4	8.1	8.4	7.9	8.1	8.7
Glu	14.1	14.0	13.9	13.7	14.3	13.9	14.6	13.7
Pro	5.9	3.9	3.8	8.1	5.4	4.0	3.6	10.2
Gly	5.6	4.2	5.0	8.6	5.4	4.3	5.2	8.2
Ala	6.4	5.8	5.7	7.1	6.3	5.9	6.0	7.3
Val	5.9	5.4	4.6	6.7	5.4	5.5	5.7	6.8
Met	1.7	1.5	1.5	1.9	1.7	1.6	1.6	1.9
Iso	4.2	6.6	5.4	2.9	4.2	5.5	5.6	1.9
Leu	9.3	12.7	10.7	6.7	10.0	12.2	10.9	4.5
Tyr	3.8	3.9	3.1	4.8	4.6	3.2	3.2	5.3
Phe	4.7	4.6	5.7	2.4	3.8	5.1	4.8	1.0
His	2.5	2.3	2.3	2.4	1.7	2.4	2.4	2.9
Lys	5.9	7.7	9.6	3.3	6.3	7.9	7.7	1.9
Arg	4.2	3.5	3.1	5.2	4.6	3.2	2.8	5.3
Cys	N.D.[c]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

[a]Expressed in terms of mole percent.
[b]Mean of three determinations.
[c]N.D., not determined.

TABLE 3. Sialic acid content of apolipoproteins isolated from Lp[a] and LDL[a]

Sample	Percent of Protein	
	Resorcinol Method	Enzymatic Method
Donor A		
LDL	1.3	1.0
Lp[a]	7.3	5.2
ApoB (Lp[a])	2.9	N.D.[b]
Apo[a]	21.0	17.8
Donor B		
LDL	2.5	0.9
Lp[a]	7.6	4.2
ApoB (Lp[a])	3.4	N.D.
Apo[a]	25.3	23.7

[a]Values shown are means of duplicate or triplicate.
[b]N.D., not determined.

detergents, respectively, were used to delipidate LDL, yielded preparations that were rather incompletely delipidated. Armstrong, Walli, and Seidel (8) and Fless, ZumMallen, and Scanu (25) have reported using dithiothreitol at 37°C to reductively cleave apo[a]-$(S\text{-}S)_n$-apoB followed by heparin-Sepharose chromatography or ultracentrifugation to separate apo[a] from the lipoprotein. In our experience, the apo[a] isolated under these conditions exhibited significant loss of structural integrity as evidenced by the presence of several bands of lower molecular weight than those present in freshly delipidated Lp[a], although apoB in the Lp[a] particle retained its structural integrity. In contrast, the isolation procedure outlined in Fig. 2 provided both apo[a] and apoB that were completely lipid-free, immunoreactive, and without chemical degradation.

Previous CD experiments from this laboratory (4) indicated that the apoproteins in Lp[a] exhibited much less combined α-helical structure and much more disordered structure than apoB in LDL. At that time, we proposed that this difference might be attributable primarily to the apo[a] component. The present studies support this proposal since they establish that the apoB from Lp[a] has a secondary structure almost identical to that from LDL, and that the apo[a] is very different, having an α-helical structure of only 14% and a disordered structure of 66% (Fig. 7, Table 1). The validity of comparing the structure of apoB within LDL and Lp[a] is dependent upon the assumption that the secondary structure of the apoproteins is similar in their lipid-free and native lipoprotein environments. This is clearly not the case for many of the other apoproteins that contain much more random coil structure in their delipidated form and more helical structure in their lipid-bound form. However, with apoB, this assumption seems to be reasonably valid as judged from a comparison of the secondary structure of apoB isolated in this study with that of apoB in the native lipoprotein. A similar comparison made by Cardin et al. (10) leads to the same conclusion. Also, the weighted average of the secondary structures of isolated apo[a] and apoB yields a value similar to that measured for native Lp[a].

The amino acid composition of apo[a] from donor B is nearly identical to that reported by Fless, ZumMallen, and Scanu (26) and Bersot et al. (27) but their results differ from the composition of apo[a] from donor A with respect to proline, leucine, phenylalanine, and lysine. This could be due to the differing number and proportion of isoforms present. However, the differences between donors for apo[a] appear small in comparison to that between apo[a] and apoB for these same amino acids plus aspartate, isoleucine, glycine, and threonine. These differences imply that apo[a] possesses a unique polypeptide backbone, and does not originate by the super-glycosylation of apoB. Compared to apoB, apo[a] has a higher content of glycine and proline, both of which destabilize α-helices (28). Asparagine is the site for N-glycosidic linkage of carbohydrate to the protein, while O-glycosidic linkages occur through serine or threonine (29). In apo[a], there is a larger fraction of threonine (but not of serine) compared to apoB. There is a lower content of aspartate, but it is difficult to assess the contribution of asparagine to this, since it is not determined separately. The much greater proportion of carbohydrate in apo[a] may be the result of either increased chain branching or length. Alternatively, only a fraction of the potential sites for glycosylation are utilized for oligosaccharide attachment in apoB (30), and the higher content of carbohydrate in apo[a] may be due to the glycosylation of a much greater percentage of these sites rather than to increased chain length or branching. Since glycosylation is a post-translational event, the large proportion of disordered structure may present a polypeptide backbone which sterically favors a more complete glycosylation, although it has been reported that the most probable conformation of a polypeptide in a region of N-glycosylation is the β-turn (29). Apo[a] has an extremely high content of sialic acid, with the exact value depending upon the individual and the method of assay, but averaging about 200 μg/mg protein (Table 3). The apoB in Lp[a] has a sialic acid content only one-eighth that of apo[a] but slightly greater than that of apoLDL. Sialic acids occur only as terminal components of oligosaccharides in mammals (31). In an Lp[a] particle there could be high local concentrations of negative charges due to sialic acid only on those parts of the surface occupied by apo[a]. Armstrong et al. (8) have demonstrated that the lipoprotein particle produced by the removal of apo[a] migrated with β-mobility on agarose gel electrophoresis. This resultant Lp[a-] parti-

cle bound to the LDL receptor of fibroblasts as effectively as LDL itself, while Lp[a] was a much poorer ligand. The forces necessary for receptor binding operate over relatively short distances (32); therefore, the repulsion between the negative charge on the Lp[a] particle and those on coated pits of the cell surface may result in binding that is energetically less favorable. In addition, the apo[a] in Lp[a] may sterically block certain regions of apoB involved in receptor binding, thereby diminishing their interaction.

The current findings afford some insight into the architecture of the protein in a "typical" Lp[a] particle. We have shown that individuals exhibit discrete isoforms of apo[a], based on apparent molecular weight, that differ in number and proportion but are rather constant for an individual. As demonstrated by SDS-PAGE and immunoblotting (Figs. 3 and 4), only a single isoform of apo[a] is disulfide-linked to B100 to produce each of the resultant a_n-B100 complexes. Since the immunoblots typically show a one band:one apo[a] isoform correspondence, then for any a_n, there is only a single molar ratio of B100 to a_n present as a disulfide-linked complex. Furthermore, if more than one a_n polypeptide were present in each complex, this would require the disulfide-linking of the same number of identical isoforms in the individual complexes, a requirement which seems unlikely. These arguments suggest the presence of only one polypeptide of apo[a] per disulfide-linked complex. This view, together with published data raises an important question about the number of B100 and apo[a] polypeptides in the Lp[a] particle. It is now generally agreed that for Lp[a]: *1)* apoB comprises 65–70% of the total protein (8, 19; Gaubatz, unpublished data); *2)* the average molecular weight of Lp[a] is about 4×10^6; *3)* the average protein plus carbohydrate content is 35–40%; *4)* the M_r of B100 is 513 k (30) and that of the apo[a] isoforms range from 0.8 to 1.5 times that of B100 based on SDS-PAGE (4, 6). Thus, in terms of protein plus carbohydrate, the M_r estimated in a typical Lp[a] particle is 1.5×10^6. The simplest ratio of apoB:apo[a] most compatible with this data is 2:1. A 1:1 ratio, while less likely, would also be possible with the uncertainties inherent in some of these measurements. Therefore, our current working model depicts a typical Lp[a] particle as containing a single apo[a] polypeptide, which may be any of the various isoforms, disulfide-linked to either one or two B100 polypeptides. The heterogeneity among Lp[a] particles is produced by the isoforms of apo[a] that have different $M_{r\ app}$, with further heterogeneity produced within these particles by differences in the fraction of lipid present, as has been shown for certain LDL populations (33).

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