ApoLDL: evidence for an aggregating system of heterogeneous subunits[1]

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Abstract The protein moieties of low density lipoprotein (LDL) (d 1.019–1.063 g/ml) and subfractions: I (d 1.018–1.023 g/ml), II (d 1.023–1.028 g/ml), III (d 1.028–1.034 g/ml), and IV (d 1.034–1.052 g/ml) were obtained by a mild delipidation procedure involving attachment of LDL to an ionic-exchange column and a gradient of the non-ionic detergent Brij-36T (*J. Lipid Res.* 1979. **20:** 631.). The apoLDL, eluted in the presence of 6 M urea, 0.1% SDS, or 4 M guanidine-HCl and analyzed by pore-gradient polyacrylamide gel electrophoresis and high performance gel exclusion chromatography, appeared to be made up of several polypeptide components with molecular weights between 250,000 and 14,000. The complex pattern was observed in the apoprotein of LDL subfractions I to IV; however the distribution of the most prominent components was different for each density range. These results, those from immunoelectrophoresis, N-terminal analysis, and measurements of tetramethylurea-soluble apoproteins discounted the possibility that the presence of bands with molecular weights below 250,000 could be caused by contamination with VLDL or HDL or nonspecific proteolysis. Delipidation of subfractions I to IV with organic solvents produced, on the other hand, simple patterns of highly aggregated apoLDL subfractions, where most of the protein had molecular weights above 250,000. The more disaggregated apoLDL preparations were those obtained from LDL rapidly isolated by single-spin centrifugation in KBr gradients, from fresh plasma immediately mixed with EDTA, phenylmethyl sulfonylfluoride, and chloramphenicol. ApoLDL fractionated by single-pore polyacrylamide gel electrophoresis appeared to be a system of heterogeneous antigens unevenly distributed on the different polypeptide components. These results, and those obtained by peptide maps of tryptic hydrolyzates from the major constituents, suggest that apoLDL is made of several polypeptides of different lengths with similar repetitive sequences but with some dissimilar segments specific for the major protein constituent of LDL. These polypeptides show a high tendency to form multiple aggregates with size distribution dependent on the preparation history.—**Socorro, L., F. López, A. López, and G. Camejo.** ApoLDL: evidence for an aggregating system of heterogeneous subunits. *J. Lipid Res.* 1982. **23:** 1283–1291.

Supplementary key words immunoelectrophoresis • column delipidation • LDL • pore-gradient polyacrylamide gel electrophoresis

Recent efforts to study LDL have given conflicting results about the size and homogeneity of its polypeptide constituents. Olofsson et al. (1), using chloroform–methanol extraction, have obtained a multicomponent preparation from which a major protein with molecular weight between 63,000 and 76,000 was isolated and partially characterized. Smaller components were also detected and isolated. Huang and Lee (2), using ethanol–diethylether extraction and guanidine–$Cu(NH_3)_4^{2+}$ treatment, have isolated proteins with molecular weights of 68,000 and 136,000. On the other hand, Kane, Hardman, and Paulus (3) found after ether–ethanol delipidation that apoLDL from human plasma appeared to be made up of a large component (mol wt 549,000) and two complementary ones of 407,000 and 144,500. Steele and Reynolds (4), have interpreted their evidence, obtained with solvent and detergent delipidated apoLDL, as indicative of a single homogeneous apoLDL protein of 250,000 daltons. These authors have suggested also that low molecular weight components in apoLDL are caused by nonspecific proteolysis or oxidative breakdown, taking place when no precautions are used during LDL and/or apoLDL preparation (4, 5). A detailed account of the difficulties involved in efforts to characterize the apoLDL or apoB, has been published by Bradley, Rhode, and Gotto (6).

In our laboratory (7, 8), we have developed mild delipidation procedures that allow almost quantitative recoveries of soluble, immunologically active apoLDL. Polyacrylamide gel electrophoresis and gel filtration of this detergent-delipidated LDL indicated the presence of multiple components ranging in apparent molecular

Abbreviations: VLDL, very low density lipoprotein, d 1.006–1.019 g/ml; HDL, high density lipoprotein, d 1.063–1.210 g/ml; LDL, low density lipoprotein, d 1.019–1.063 g/ml; PMSF, phenylmethyl sulfonyl fluoride; Na_2EDTA, sodium salt of ethylenediamine tetraacetic acid; SDS, sodium dodecyl sulfate; Brij-36T, 10-polyoxyethylene lauryl ether; Nonidet P-40, iso-octyl-phenoxypolyethoxyethanol; DNS, 1-dimethylaminonaphthalene-5-sulfonyl-chloride; HPLC, high performance liquid chromatography; Tris, Tris(hydroxymethyl) aminomethane; PAGE, polyacrylamide gel electrophoresis.

[1] A preliminary account of this work was presented in November 1979 at the Fifth International Symposium on Atherosclerosis, Houston, TX, USA.

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weights from approximately 250,000 to 10,000. The results to be presented indicated that when this mild delipidation method is used in combination with urea, SDS, and guanidine–HCl treatment, distinct and apparently immunological heterogeneous aggregates can be demonstrated in apoLDL without evidence of proteolytic breakdown. The size distribution of these aggregates was found to be affected by several factors and the relative proportion of small and large components changes along the density range of LDL (1.019–1.063 g/ml).

MATERIALS AND METHODS

Human plasma preparations

Plasma was obtained immediately from freshly withdrawn blood and from blood-bank samples that had been stored at 5°C between 2 and 7 days under sterile conditions. In the first case, the blood from apparently healthy laboratory personnel was collected in tubes containing, per ml of blood, 1 mg of EDTA, 0.1 mg of chloramphenicol, and a final concentration of 0.2 nM PMSF. Blood donor samples were also mixed with the same compounds, once the plasma was separated. In all cases lipoprotein fractionation was started as soon as the plasma was obtained to reduce the possibility of microbial growth.

Lipoprotein fractionation

Two procedures were used for total LDL preparation. Differential centrifugation in a 60Ti rotor (Beckman Instruments, Palo Alto, CA), using solid KBr to adjust densities at 4°C (7), was based upon the principles developed by Havel, Eder, and Bragdon (9). For rapid fractionation, we resorted to exponential density gradient centrifugation in a SW-41 rotor (Beckman Instruments), as described by Redgrave, Roberts, and West (10). With this latter method, highly purified apoLDL could be obtained within 24 hr of blood withdrawal. In the two procedures used, the density range 1.019–1.063 g/ml was obtained. A shallower exponential density gradient (1.015–1.080 g/ml) was used to obtain from LDL (single spin) the subfractions I (d 1.018–1.023 g/ml), II (d 1.023–1.028 g/ml), III (d 1.028–1.034 g/ml), and IV (d 1.034–1.052 g/ml) after centrifugation for 24 hr at 38,000 rpm in a SW-41 rotor. In all cases, the fractionated lipoproteins were stored at 4°C in KBr solutions containing 1 mg/ml EDTA and 0.2 mM PMSF in order to reduce the possibility of microbial growth, oxidation, and proteolysis.

Homogeneity of LDL preparations

To investigate the possibility of contamination of total LDL and its subfractions with other lipoproteins, three procedures were used: *1*) bidimensional immunoelectrophoresis of total LDL; *2*) fused rocket immunoelectrophoresis of LDL subfractions; and *3*) quantitative evaluation of soluble apolipoproteins after delipidation with tetramethylurea. The last procedure was validated by Kane et al. (11) for the measurement of apoA-I, A-II, apoE, and apoC's in apoB-containing lipoproteins. This method, in which apoB remains insoluble and the other apoproteins are solubilized after delipidation with the tetramethylurea, was applied to total LDL, LDL subfractions, and HDL and VLDL, and the proportion of soluble and insoluble apoproteins was quantitatively evaluated.

Preparation of apoLDL

The LDL or fractions I to IV were equilibrated with 50 mM Tris-HCl, pH 8.8 (buffer 1) by passage through a Sephadex G-25 column (Pharmacia Fine Chemicals, Uppsala, Sweden), or by dialysis. In both cases buffer-1 was made 0.2 mM in PMSF and contained 5 mM EDTA. The LDL, or its fractions, at concentrations between 0.5 and 5 mg of protein/ml was added to a 15 × 1.5 cm column packed with DEAE-Bio-Gel A (Bio-Rad Laboratories, Richmond, CA), that had been equilibrated with buffer-1 containing PMSF and 1 mM EDTA. Delipidation was accomplished with 80 ml of a 0 to 2% (w/v) gradient of Brij-36T (generous gift of Imperial Chemicals, Mexico), prepared in buffer-1. With this gradient more than 99% of LDL-lipids were separated from the apoLDL and the protein remained attached to the DEAE-agarose. The apoLDL was eluted from the column with three different solutions: *a*) 1 M NaCl in 50 mM Tris-HCl (pH 7.4), 0.2 nM PMSF; *b*) 1 M NaCl, 6 M urea, 50 mM Tris-HCl (pH 8.4), 0.1% SDS, 0.2 nM PMSF, and *c*) 4 M guanidine–HCl in 25 mM Tris HCl (pH 7.2), 0.2 nM PMSF.

Gel exclusion and reverse phase liquid chromatography

A Varian 5000 system was used (Varian Instruments, Palo Alto, CA) in experiments of gel exclusion chromatography using TSK 3000 SW and 4000 SW columns of 50 × 0.7 cm. The columns were equilibrated either with 6 M urea, 0.1% SDS (w/v) in 50 mM Tris–HCl (pH 7.2), or with 4 M guanidine–HCl in 25 mM Tris–HCl (pH 7.2). The columns were run between 0.1 and 0.5 ml/min and followed at 280 nm or 230 nm. Calibration curves were obtained with standard protein mixtures purchased from Pharmacia Fine Chemicals.

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Automated fingerprinting of tryptic hydrolyzates of apoLDL fractions were obtained according to Fulmer and Wasserman (12), using reverse phase liquid chromatography on a 30 × 0.4 cm Micropack MCH-10 column (Varian Instruments) run with a gradient of 0 to 50% v/v acetonitrile in 0.1% w/v aqueous solution of phosphoric acid. The flow was 2 ml/min, and the eluent was monitored at 210 nm.

Production of tryptic hydrolyzates

Trypsin treatment of apoLDL fractions separated by gel electrophoresis was performed according to Elder et al. (13) using enzyme to protein ratios between 20/1 and 50/1. Trypsin, type XI; DPCC-treated, was purchased from Sigma Chemical Co. (St. Louis, MO). Radioiodination was omitted, and automatic fingerprinting in reverse phase liquid chromatography was used instead of peptide maps (12).

Polyacrylamide gel electrophoresis

Separation in gels was used both as an analytical and preparative tool. The analytical slabs were 1.5-mm thick and the preparative ones were 3.0-mm thick. In both cases 5% gels, 2 to 20% or 2 to 30% polyacrylamide gradients were used in a discontinuous buffer system. The gel was prepared in 0.25 M Tris-HCl (pH 8.8), 6 M urea, 0.1% (w/v) SDS. The electrode compartments were filled with buffer: 25 mM Tris, 0.2 M glycine (pH 8.4), 0.1% (w/v) SDS. The samples were dissolved in a dissociating solution containing 0.15 ml of 1 M Tris-HCl (pH 6.8), 10 mg of dithiothreitol, 2 g of sucrose, 3.6 g of urea, 5 ml of 10% (w/v) SDS, and water to 10 ml. The separation was carried out at 20 mA/slab for 1.5-mm thick gels and 40 mA/slab for the 3.0-mm gels. The gels were washed, fixed, and stained as described (7). To study the effect of a different high-resolution electrophoretic system on the patterns of apoLDL subfractions, the method of Bury (14) was also used in pore-gradient slabs.

Immunoelectrophoresis

Fused-rocket and rocket immunoelectrophoresis were carried out as suggested by Axelson, Krøll, and Weeke (15). Antisera were prepared against pooled human plasma, intact LDL, apoLDL, apoA-I, and apoE in New Zealand white rabbits. The apoLDL was extensively dialyzed against physiological saline and was injected as an aggregated suspension. ApoA-I was prepared from delipidated HDL and apoE from VLDL. The specific apoproteins were obtained by preparative acrylamide gel electrophoresis of the delipidated apoproteins.

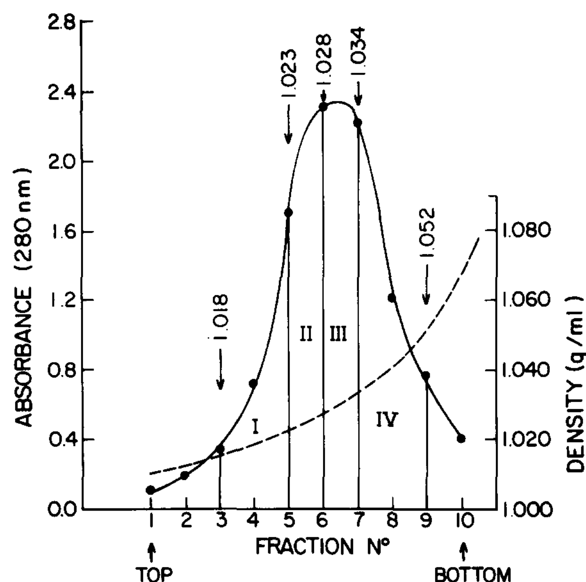

Fig. 1. Distribution in a KBr exponential gradient of LDL (d 1.019–1.063 g/ml) that had been previously isolated by differential centrifugation. The preformed gradient was centrifuged at 114000 *g* for 24 hr at 20°C. Fractions I–IV are indicated within their respective density limits.

Other analytical procedures

The methods for protein, cholesterol, and phospholipid determinations, as well as those used for dansylation of the proteins, have been previously indicated (7).

Reagents

All reagents were analytical grade; the solvents for HPLC were purchased from Burdick and Jackson Laboratories (Muskegon, MI).

RESULTS

Properties of total LDL and subfractions

In any effort to characterize the apoproteins of plasma lipoproteins it is important to define the properties of the population used. In **Fig. 1** is presented the distribution along an exponential KBr gradient of the LDL fraction isolated by conventional differential centrifugation. No evidence of significant contamination with lipoproteins with density below 1.015 g/ml or above 1.063 g/ml was detected. In the same figure are indicated the density ranges of LDL subfractions that were used to investigate apoprotein patterns. Using anti-LDL and anti-plasma sera in fused rocket experiments, no other antigen-inducing plasma proteins or lipoproteins besides those present in LDL were detected (**Fig. 2**). Fused rocket immunoelectrophoresis of fractions 3

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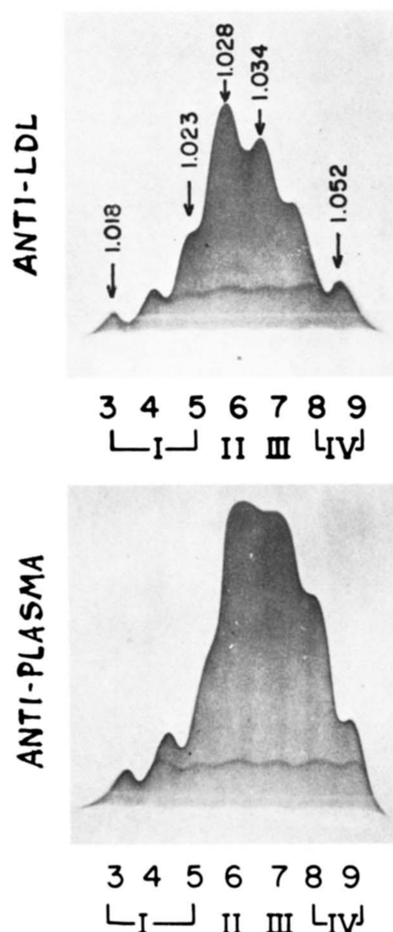

Fig. 2. Fused rocket immunoelectrophoresis of the gradient distribution of intact LDL shown in Fig. 1. Antisera against LDL and total plasma were used. The figures on top of the immunoprecipitation pattern indicate the density limits of LDL fractions I–IV. The lipoprotein was dialyzed against buffer-1 but was not treated with detergents before the analysis.

to 9 of the same gradients showed no reaction against anti-apoA-I and anti-apoE sera. The observation of two apparent antigen envelopes in the intact LDL was a

TABLE 1. Content of tetramethylurea-soluble apolipoproteins in LDL, LDL density subfractions, VLDL, and HDL[a]

Lipoprotein	Percentage of Tetramethylurea-soluble Apolipoproteins
LDL (1.019–1.063 g/ml)	2.5
LDL-I (1.019–1.023 g/ml)	4.5
LDL-II (1.023–1.028 g/ml)	1.3
LDL-III (1.028–1.034 g/ml)	0.1
LDL-IV (1.034–1.052 g/ml)	1.2
VLDL (1.006–1.019 g/ml)	39.5
HDL (1.063–1210 g/ml)	91.6

[a] Lipoprotein fractions and subfractions containing between 450 and 500 mg of apolipoproteins were subjected to the delipidation procedure. The amount of tetramethylurea-soluble apolipoprotein was measured by the method of Lowry et al. (11).

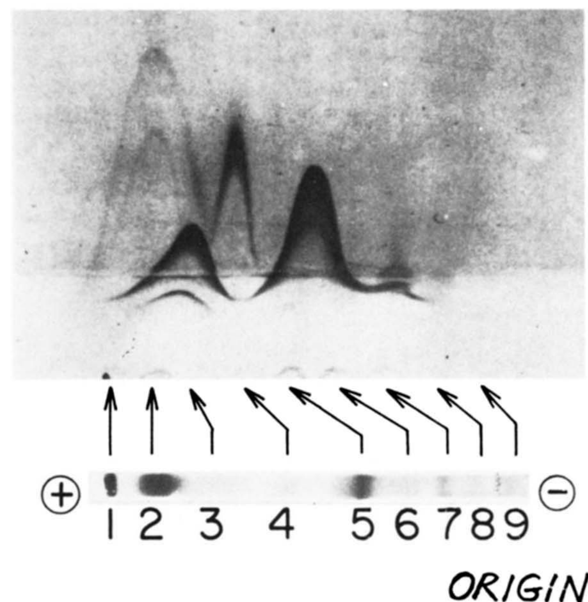

Fig. 3. Fused rocket immunoelectrophoresis of the main bands of apoLDL that were electro-eluted from a single-pore SDS acrylamide gel. Antisera against apoLDL were present (0.5% v/v) in the agarose gel.

constant finding in this type of experiment which appears not to be related to contamination.

A more quantitative evaluation of non-apoB-related apoproteins in LDL and the subfractions I–IV from the gradient was obtained by measurements of the soluble apoproteins present after delipidation with tetramethylurea. **Table 1** presents the results obtained from triplicate analysis from an individual LDL preparation and its subfractions. As control, the values obtained for VLDL and HDL from the same serum are included. To validate these results further the supernatants from the tetramethylurea samples were analyzed by polyacrylamide gel electrophoresis. In fraction I, a faint band corresponding to the C apoproteins was detected. In fraction IV, a very faint component moving as apoA-I was visualized. These results showed that in total LDL and its subfractions more than 95% of the protein was tetramethylurea-insoluble apoB and that the components with apparent molecular weights below 250,000, to be described in the next paragraphs, seemed not to be related to the apoA-I, A-II, E, or C polypeptides present as very minor constituents of the fractions.

The apoproteins of LDL

When apoLDL is obtained by delipidation with Brij-36T and eluted from the column in the presence of 6 M urea and 0.1% SDS, its polyacrylamide gel pattern is rather complex. **Fig. 3** shows the image obtained with a single-pore 5% gel. The major bands from this gel were eluted by electrophoresis from nonstained gel seg-

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ments and were analyzed by fused-rocket immunoelectrophoresis. Several independent antigenic components apparently related to the major bands are visible. A strong arc, present in low and high molecular weight regions, is the most prominent, but three other arcs restricted to low molecular weight components can also be observed. Since the anti-apoLDL antiserum used gave a much simpler pattern with intact LDL, we suggest that the apparent antigenic heterogeneity was uncovered by the delipidation procedure used. However, we cannot rule out the possibility that detergent-apoprotein associations could cause the complex pattern in this experiment.

We have observed several conditions that affect the electrophoretic fractionation of apoLDL in 6 M urea and 0.1% w/v SDS. One is storage of the blood or plasma. The patterns of LDL obtained from stored blood showed more aggregation than those obtained from apoLDL prepared by delipidation of LDL isolated by the fast density-gradient procedure. Another factor controlling aggregation is the solution used for elution of apoLDL from the delipidation column. Addition of 6 M urea, 0.1% SDS, to the eluent containing 1 M NaCl, or eluting the apoLDL with 4 M guanidine–HCl, led to preparations that entered completely 3 to 20% acrylamide gels showing appreciable amounts of components with molecular wights below 250,000; 6 M urea and 0.1% SDS were more efficient deaggregating agents than 4 M guanidine-HCl. However, even with these preparations, overloading of the delipidation columns or gels, induced the aggregation of apoLDL into components with molecular weights above 3×10^5.

In efforts to use the high resolving pore-gradient gels as preparative tools without necessarily overloading them, we resorted to dansylation of the soluble apoLDL. By this technique we could conveniently follow the fractionation and isolation, using dilute solutions of the dansylated fractions. The results of the preparative run with diluted dansylated apoproteins from LDL obtained from fresh plasma and the re-running of the isolated bands in an analytical gel are presented in **Fig. 4.** Although band 1 appears as a continuum, and band 4 seemed to be a doublet, bands 2, 3, 5, 6, and 7 appeared well defined. These results indicate that it is possible to isolate from the apoLDL complex, fractionated by SDS-urea polyacrylamide gel electrophoresis, prominent components that resisted further resolution and that retained their original apparent size.

Although the experiment with tetramethylurea indicated that our preparations contained more than 97.5% of apoB, it is possible, at least theoretically, that the multiple band pattern obtained could have been due to contamination with apoproteins from other density classes, inasmuch as we used LDL within a wide range of densities. To further explore this possibility, narrow density cuts of LDL were delipidated with the DEAE column-Brij-36T procedure and also by ethanol–acetone extraction. The apoproteins obtained from subfractions I (d 1.018–1.023 g/ml), II (d 1.023–1.028 g/ml), III (d 1.028–1.034 g/ml), and IV (d 1.034–1.052 g/ml) from LDL of a single donor (defined in Fig. 1) were analyzed by pore gradient polyacrylamide gel electrophoresis. The results are presented in **Fig. 5.** A pattern of multiple bands was observed in the four subfractions of LDL when delipidated with the nonionic detergent (DD), indicating the presence of appreciable quantities of apoproteins with molecular weights below 250,000 in the whole density range. However, when the same fractions were delipidated with organic solvents (OS), most of the apoprotein remained at the top of the gel, as aggregates with molecular weights above 250,000.

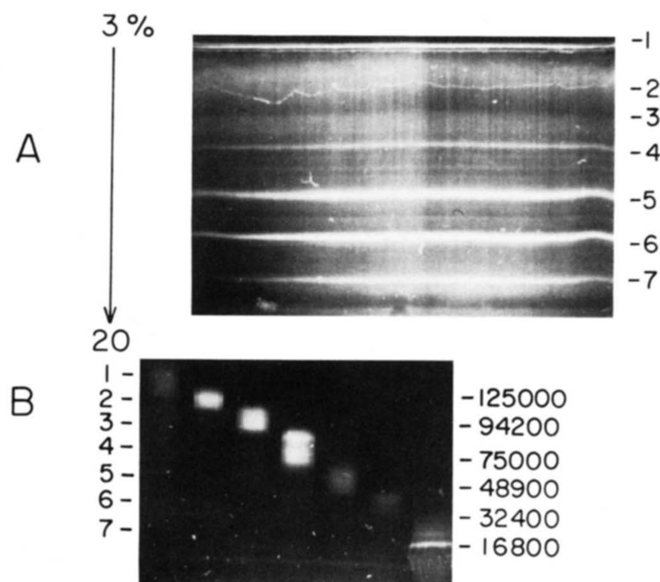

Fig. 4. Preparative and analytical SDS-urea, pore-gradient polyacrylamide electrophoresis of dansylated apoLDL. A, Preparative gel: bands 1 to 7 were eluted from the gel segments by electrophoresis, dialyzed, lyophylized, taken up in dissociating solution, and applied to an analytical gel. B, Photography taken with UV light and a 390 nm cut-off filter. Both gels were 3–20% w/s in acrylamide (see Methods).

It is important to notice that within the detergent-delipidated fractions, appreciable quantitative differences and some qualitative differences in electrophoretic patterns could be observed. Fractions I and II appeared mainly as disaggregated components; those with apparent molecular weights from 125,000 to 14,000 were the most prominent. In fraction III (d 1.028–1.034 g/ml), most of the apoproteins had apparent molecular weights between 300,000 and 125,000. The fraction IV (d 1.034–1.052 g/ml) apoproteins, although qualitatively similar to fraction III, appeared to

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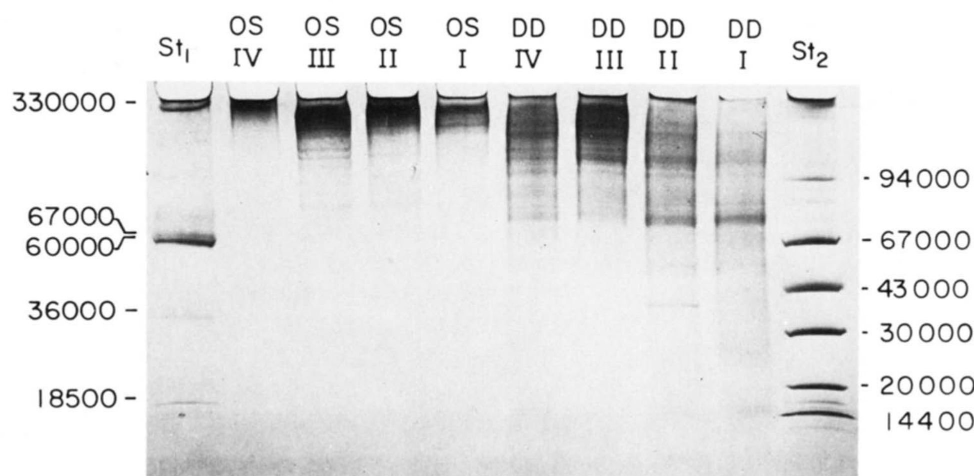

Fig. 5. Analytical pore-gradient polyacrylamide gel electrophoresis of the protein moieties of LDL fractions I (d 1.018–1.023 g/ml), II (d 1.023–1.028 g/ml), III (d 1.028–1.034 g/ml), and IV (d 1.034–1.052 g/ml) obtained by organic solvent delipidation (OS) and the column-detergent procedure (DD). The gradient was 2 to 30% acrylamide.

contain more components in the 125,000 to 75,000 apparent molecular weight range. Definitive apparent molecular weight assignments in the apoLDL aggregating-system are not possible, since so many factors appear to affect distribution in pore-gradient gels. We have used the high resolution system-I of Bury (14) with the same Brij 36T-delipidated fractions of Figs. 1, 2, and 5, and the results are presented in **Fig. 6.** These gels allow a more detailed fractionation of the upper molecular weight components and it seems that the differences in the apoLDL multiple-component pattern, along the density range of LDL, are due to changes in the proportion of the same aggregates rather than to the presence of dissimilar constituents. However, the large proportion of components with apparent molecular weights below 250,000 is clearly visible. Densitometric tracings of this gel showed that in fractions I and II more than 90% of the components had molecular weights below 160,000. In fraction III, more than 85% of the stained bands ranged between 250,000 and 94,000, whereas the banding in fraction IV extended more evenly down to 60,000.

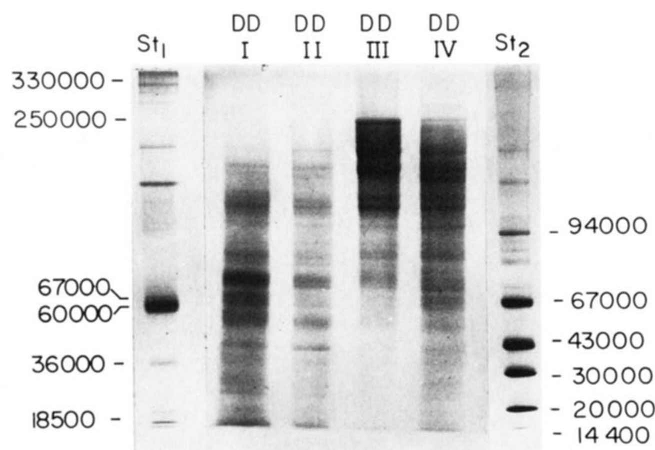

Fig. 6. Analytical pore-gradient polyacrylamide gel electrophoresis of the protein moieties of LDL fractions I, II, III, and IV obtained by the column-detergent procedure. The gradient was from 2 to 20% acrylamide and the discontinuous buffer system was the one suggested by Bury (14). The 250,000 value was calculated from the molecular weight-distance calibration curve.

Preparative and analytical gel exclusion chromatography

ApoLDL eluted from the delipidation column with 6 M urea and 0.1% SDS or 5 M guanidine–HCl was fractionated by gel exclusion chromatography in columns equilibrated with the above dissociating solutions. These columns have a high resolution and can be adapted to small scale preparative purposes. **Fig. 7** shows a calibration run and the fractionation of apoLDL in 4 M guanidine–HCl. Similar results were obtained with 6 M urea–0.1% SDS in these gel exclusion columns. The apoLDL gave multiple peaks, the most prominent being the one made of components above 90,000 molecular weight, the exclusion limit of the TSK-3000 column in these conditions. Other important peaks appeared with K_d corresponding to apparent molecular weights of 66K, 45K, 28K, and 14K. The combined area of the 280 nm absorption curves of the components with molecular weights below 90,000 was approximately 40% of the total. Fractions collected from this column, after dialysis, as well as the parent apoLDL were analyzed by pore-gradient polyacrylamide gel electrophoresis. The results, shown in Fig. 7, suggest that it is possible to isolate fractions containing the more

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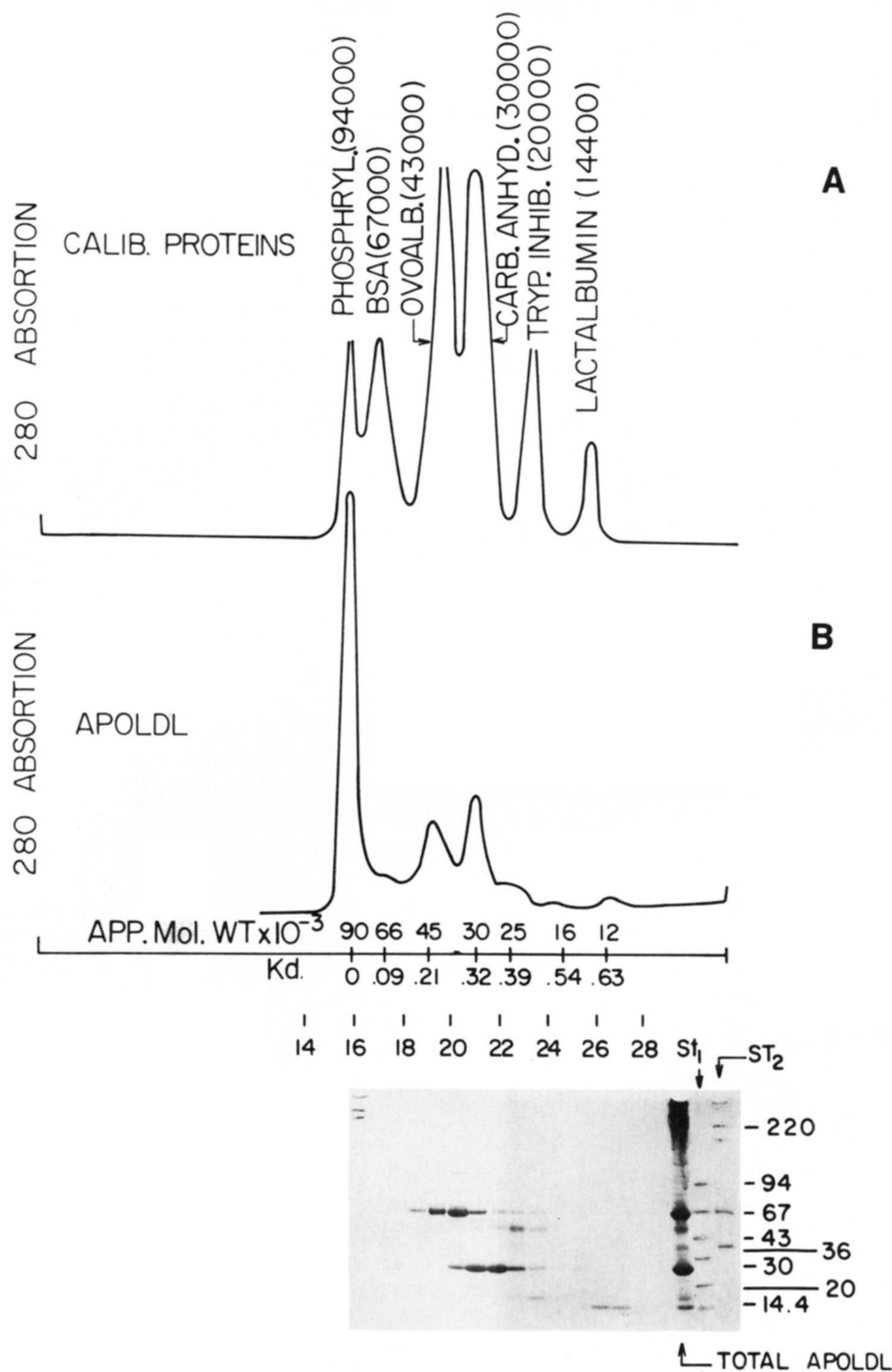

Fig. 7. Gel exclusion chromatography in TSK-3000 SW columns of apoLDL. A, Calibration run with standard proteins, column equilibrated with 4 M guanidine–HCl, flow 0.5 ml/min. B, Elution pattern of soluble apoB eluted with 4 M guanidine–HCl. Fractions of 0.5 ml (16 to 28) were collected, dialyzed, lyophylized, and analyzed by pore-gradient SDS-urea polyacrylamide gel electrophoresis.

abundant polypeptide components of apoLDL by steric exclusion chromatography, under dissociating conditions; their behavior in SDS-polyacrylamide electrophoresis is that expected from its chromatographic K_d. The stepwise fractionation procedure allowed a cleaner separation in the electrophoresis gel. However, most bands

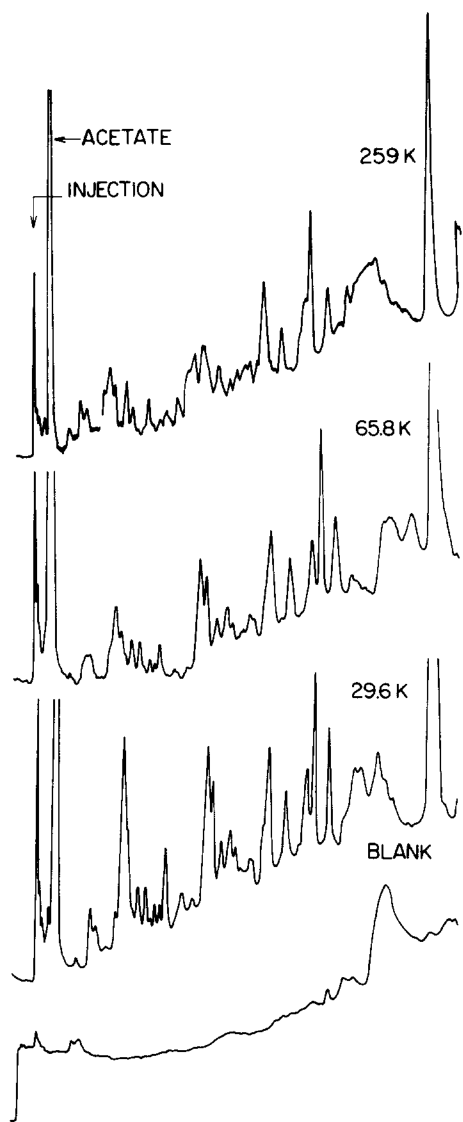

Fig. 8. Analytical peptide mapping of tryptic hydrolyzates of apoLDL fractions obtained by preparative polyacrylamide electrophoresis (see Methods). Solvent gradient 0 to 50% (v/v) acetonitrile in 0.1% (v/v) H_3PO_4 in water, flow 2 ml/min, monitored at 210 nm (0.1 AUFS).

could also be observed in the unfractionated apoLDL in the lane that was overloaded in order to facilitate the identification of as many bands as possible.

Analytical peptide mapping

The more intensely stained bands in apoLDL obtained after pore-gradient PAGE in SDS-urea, were subjected to tryptic hydrolysis within the gel segments. The eluted peptide fragments were analyzed by reverse phase liquid chromatography. The most relevant results are presented in **Fig. 8.** The high molecular weight band around 295K showed several peptides in common with those from the prominent 65.8K band, but they were not identical. The 25.9K band was also present in regions in common with the 65.8K polypeptide fraction and with the 295K one, although there was less overlapping between the 25.9K and 295K fraction.

DISCUSSION

The experiments described are part of an effort to gain knowledge of the properties of the protein moiety of total human LDL. We believe that because of the controversial results obtained with apoLDL (apoB), it is important to study the apoprotein of total LDL delipidated and quantitatively recovered under defined conditions, and concomitantly to isolate and characterize subunits of apoLDL from narrow density cuts of LDL (1, 2, 4). Furthermore, the interpretation of a large body of metabolic studies rests heavily on establishing the composition and structure of the apoproteins present in the total LDL and its density subfractions, especially now that it has been demonstrated that LDL is present in multiple distinct subpopulations (16–18).

Our results indicate that when mild delipidation procedures are used in combination with dissociating agents, the quantitatively recovered apoLDL is disaggregated into several polypeptides as described previously (7, 8). This dissociation appears not to be caused by hydrolytic processes since provisions were taken to inhibit proteases and microbial growth, and no formation of new N-terminals was detected. Moreover, the best dissociated preparations of apoLDL were those obtained from LDL isolated by fast density gradient centrifugation from immediately processed blood, collected over EDTA, chloramphenicol, and PMSF and maintained in KBR solutions with PMSF and EDTA until delipidated. Consistently, apoLDL showed the presence of polypeptides with apparent molecular weights below 250,000.

The proportion of the components varied from preparation to preparation and any effort to concentrate apoLDL above 1 mg/ml led to increased proportions of high molecular weight components. Our previous results indicate that antigenically, apoLDL is heterogeneous (7). In the experiment from Fig. 3, one major antigen, associated with low molecular weight polypeptides, was also present in some of the large components. We cannot yet ascribe specific antigens to specific polypeptides but the results indicate that they are unevenly distributed along the size spectrum of the apoLDL aggregates separated by preparative SDS-urea polyacrylamide gel electrophoresis. These polypeptides retain their apparent homogeneity after re-analysis on the same type of gels. Also, SDS-urea gel electrophoresis of the major fractions obtained from steric exclusion chromatography using dissociating buffers showed that the major components had apparent molecular weight that could be predicted from the chromatographic K_d.

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The results from immunological analysis of narrow density cuts of LDL, and their content of tetramethylurea-soluble apoproteins (11) do not support the possibility that the small molecular weight components, observed in total apoLDL, originated from contaminants of LDL. The differences in size-distribution along the density range of LDL do not favor the hypothesis of a proteolytic origin of the small size components, inasmuch as we would have to accept that the degradative action varied along the density range of LDL. Furthermore, since one can obtain highly aggregated apoproteins after organic solvent extraction, or disaggregated apoproteins after detergent delipidation from the same preparation of LDL or LDL fractions, it is unlikely that the presence of small molecular weight components is caused either by degradation or contamination, because these will be observed after any type of delipidation (7).

The results from the fingerprinting experiments and those discussed above, indicate that the polypeptides around 296K are not aggregates of the 65K or the 26K polypeptides since their patterns are not identical or complementary. One interpretation could be that the polypeptides are chains of different length with similar and repetitive sequences but with segments that are specific for the individual chains. These last segments could be the origin of the antigenic heterogeneity and differences in the fingerprinting; however differences in carbohydrate content and/or conformation could be responsible for some of the dissimilarities observed in the peptide maps and the isolated components could be very similar in their primary structure.

At this stage other hypotheses could be proposed to explain the complex pattern of apoLDL, such as the possibility of fragmentation of a very large polypeptide chain with repetitive sequences, without the production of N-terminals. We believe, however, that the experimental data are more consistent with the hypothesis that the protein moiety of LDL (d 1.019–1.063 g/ml) is made up of more than one protein component, with a high tendency to form multiple aggregates. The size distribution of these aggregates appears to depend on the delipidation procedure used, the concentration before and after delipidation, the dissociating power of the denaturant present, and the relative proportion of the apoLDL components forming the aggregates, some of which seem to be of small molecular weight.

This work was partially supported by grants SI-0594 and SI-1253 from the Consejo Venezolano de Investigaciones Científicas y Tecnológicas (CONICIT). Miss Lilian Socorro is a recipient of a scholarship from the Fundación Gran Mariscal de Ayacucho. We gratefully acknowledge the secretarial work of Mrs. Anita Istok and Lubomira de Sevcik.

Manuscript received 8 June 1981, in revised form 4 January 1982, and in re-revised form 19 July 1982.

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