Conformation of apolipoprotein B after lipid extraction of low density lipoproteins attached to an electron microscope grid

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Abstract We have examined the shape of apolipoprotein B (apoB) from low density lipoproteins (LDL) using a new method to prepare the electron microscope grids. After adsorption of the lipoproteins to a carbon-coated copper grid, lipids were extracted with ethanol-ether 4:1; an aqueous negative stain was then applied. When the LDL residue was examined after this treatment, apoB, together with residual lipid, appeared as an elongated flexible structure about 600-700 Å in length consisting of multiple domains of variable width from 20-70 Å. Occasionally, the elongated apoB formed an irregular ring-shaped structure, but most of the rings were open. When LDL were pretreated with glutaraldehyde, then adsorbed, extracted, and stained, most of the images were closed rings with an average contour length of 700 Å, again consisting of multiple domains of variable sizes. These results are consistent with apoB being composed of multiple domains arranged in an elongated structure on the surface of the LDL, and with distant domains possessing a mutual affinity that favors their cross-linking. —**Phillips, M. L., and V. N. Schumaker.** Conformation of apolipoprotein B after lipid extraction of low density lipoproteins attached to an electron microscope grid. *J. Lipid Res.* 1989. **30:** 415-422.

Supplementary key words LDL • glutaraldehyde cross-linking • IDL • protein conformation • lipid extraction

Apolipoprotein B (apoB) is the major apolipoprotein of both low density lipoproteins (LDL) and intermediate density lipoproteins (IDL), constituting greater than 95% by weight of LDL protein and approximately 60% of IDL protein (1). It is also a major component of the very low density lipoproteins (VLDL). ApoB is an integral non-exchangeable component of these lipoproteins, and its presence is probably essential for export of triglycerides as VLDL and chylomicrons from liver and intestinal mucosal cells. ApoB also contains a site through which LDL, IDL and possibly small VLDL are bound to the LDL receptor (2-4). Large VLDL do not bind to the LDL receptor, perhaps due to a conformational change in apoB (5). The primary sequence of apoB has been deduced from the nucleotide sequence for the cDNA (6, 7). The processed polypeptide chain consists of 4,536 amino acids, with a calculated molecular weight of 512,000. ApoB also contains 8-10% carbohydrate (8), giving a total molecular weight of 550,000-560,000. There is one apoB per LDL particle, and probably only one apoB per IDL and VLDL (9-11).

At least a portion of apoB is exposed to the aqueous environment at the surface of low density lipoproteins, since approximately 30% is liberated as peptide fragments on digestion with trypsin (7, 12). Nonetheless, detailed surface morphology is usually not observed on examining LDL in the electron microscope. There have been some reports of symmetrical globular (13, 14), or strandlike (15) substructures, but to date there is little agreement on the organization of apoB on the LDL particle.

Lipids can be removed from LDL using organic solvents or detergents. Ether treatment removes primarily the nonpolar lipid core (16) and the protein-phospholipid complex resulting from ether extraction of LDL has been visualized in the electron microscope as "strings of up to 500 Å in length" (13) which in LDL may form "strandlike substructures, possibly forming a surface network" (15). Electron microscope examination of apoB-detergent complexes has been reported for the detergents sodium deoxycholate (17), *n*-dodecyl octaethyleneglycol monoether (18), and Tween 80 (19). In all of these studies, the apoB-detergent complexes appeared as highly asymmetric and apparently flexible strings.

Our approach to the study of the structure of apoB by electron microscopy was to first attach individual LDL to

Abbreviations: VLDL, very low density lipoprotein, density less than 1.006 g/ml; IDL, intermediate density lipoprotein, density between 1.006 and 1.020 g/ml; LDL, low density lipoprotein, density between 1.020 and 1.050 g/ml; EDTA, ethylenediamine tetraacetic acid; SDS, sodium dodecyl sulfate; SD, standard deviation; SEM, standard error of the mean; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; KPT, potassium phosphotungstate.

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the carbon support by strong, non-covalent absorption, and then to extract most of the lipids with a mixed organic solvent comprised of ether and ethanol, which removes both polar and nonpolar lipids, as well as some of the smaller apolipoproteins (20). After negative staining, the preparation was directly examined by transmission electron microscopy. When the LDL were fixed with glutaraldehyde prior to absorption on the carbon support, closed ring-like structures were observed.

MATERIALS AND METHODS

Materials

Glutaraldehyde purified for electron microscopy was purchased from Ted Pella, Inc. as an 8% aqueous solution stored under nitrogen in 10-ml ampules and used immediately after opening. Sodium azide, Tris base, and Gentamicin were purchased from Sigma Chemical Company. Sodium chloride and EDTA (disodium salt) were from Mallinkrodt. Diethyl ether (reagent grade) was purchased from Fisher Scientific. Ethanol (95%) was from Gold Shield. All were used without further purification. Uranyl acetate was from Eastman Kodak. A 1% aqueous solution was prepared, filtered using a 0.45-μm Millipore filter, and stored in the dark.

Isolation of lipoproteins

Blood was drawn from a normolipemic, nonfasting donor at the UCLA Blood Donor Center and the citrated plasma was provided after 18 hr. Plasma was made 0.04% in EDTA, 0.05% in sodium azide, and 0.005% in gentamicin using stock solutions of 4%, 5%, and 1%, respectively. The concentrations of EDTA, sodium azide and gentamicin were maintained throughout the isolation by addition of aliquots of the concentrated stock solutions. The density of the plasma was adjusted to 1.020 g/ml using saturated sodium chloride and the solution was spun in the Ti70 rotor in a Beckman L5-65 ultracentrifuge at 40,000 rpm for 24 hr at 16°C. The top 3 ml of each centrifuge tube, containing chylomicrons, VLDL, and IDL, was then removed with a Pasteur pipet. Another 4 ml was removed and the pellet was resuspended in the remaining solution. This solution was adjusted to a density of 1.050 g/ml with saturated sodium chloride. The solution was then spun as before at 40,000 rpm for 24 hr at 16°C and the LDL was collected in the top 1 ml of each tube. The LDL were then dialyzed against 0.90% saline containing 0.04% EDTA, 0.05% sodium azide, and 0.005% gentamicin, pH 7.6 (buffer A) and stored at 4°C.

IDL were prepared by dialyzing the sample containing VLDL, IDL, and chylomicrons from the first centrifugation against buffer A and respinning in a Ti70 rotor at 40,000 rpm for 24 hr at 16°C. The top 5 ml of the tube, containing VLDL and chylomicrons, was removed with a Pasteur pipet. Most of the remaining infranatant was removed and the IDL pellet was resuspended in the bottom 3 ml of the tube.

Analytical ultracentrifugation

Analytical ultracentrifugation of the LDL was carried out at 52,000 rpm in a Beckman Model E analytical ultracentrifuge equipped with a photoelectric scanner and multiplexer. The signal, taken directly from the chart recorder, was sent to the A/D converter of a DEC PDP 11/03 computer and stored on floppy disks. Subsequent data analysis was performed on a VAX 11/780 computer. LDL were analyzed in solvents adjusted to densities of 1.006 and 1.063 g/ml with NaCl. The solvents also contained 0.04% EDTA, 0.05% sodium azide, and 0.005% gentamicin.

Compositional analysis

Protein concentrations were determined by a modification of the Lowry procedure (21). Total and free cholesterol were determined enzymatically (22). Cholesteryl ester was calculated as (total cholesterol − free cholesterol) × 1.68. Phospholipid was determined as phosphorus (23) using an average molecular weight of 775 for phospholipid. Triglyceride was determined enzymatically, using a commercially available kit (Periodochrome GPO Triglycerides, from Boehringer-Mannheim, Diagnostics Division). SDS polyacrylamide gel electrophoresis was performed by the method of Laemmli (24) using a 9% gel. A Zeineh Soft Laser Scanning Densitometer (Biomed Instruments, Inc., Fullerton, CA) was used to quantitate the Coomassie-stained bands.

Compositional analysis of LDL extracted with ethanol–ether 4:1 took advantage of the insolubility of the extracted product. Aliquots (25–100 μl) of LDL were extracted twice with a tenfold excess of cold (−10°C) ethanol–ether 4:1 and once with cold 95% ethanol, with the precipitate being collected by centrifugation, then assayed for lipid or protein. As a control, the organic solvent supernatant was dried at 95°C and extracted lipid or protein was assayed.

Radioimmunoassays

Microtiter plates (96-well) were coated with 40 μl of an LDL solution (PBS) containing 0.1 mg/ml protein for 2 hr. The LDL solution was removed and the plates were rinsed with 0.5% BSA. Some of the LDL-containing wells were extracted with ice cold ethanol–ether 4:1 for 1 min, then quickly rinsed with cold ethanol. Following two BSA-containing washes, 40 μl of about 1 μg/ml monoclonal antibody was added and incubated overnight. After removal of the monoclonal solution and thorough washing, ^{125}I-labeled rabbit anti-mouse polyclonal antibody in 0.5% BSA (in PBS) was added and allowed to bind over-

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night. The radioactive solution was removed and the plates were washed thoroughly before counting individual wells. Controls containing no LDL, or LDL but no monoclonal antibody, with and without extraction, were also run.

Electron microscopy

Carbon-coated copper grids were prepared by vacuum evaporation of carbon onto freshly cleaved mica, followed by floating the carbon off the mica onto distilled water, dropping copper grids (500 mesh) onto the film, and picking up the coated grids with plastic film (Saran Wrap). (Parlodion-carbon grids produced staining artifacts, probably due to etching of the plastic during the extraction with organic solvents.)

Lipoprotein samples were fixed with glutaraldehyde by first diluting to 10–20 μg protein/ml in 10 mM Tris, 0.15 M NaCl, pH 7.4, and then adding sufficient 8% aqueous glutaraldehyde to make the solution 0.8% in glutaraldehyde. In later experiments 10 mM phosphate, 0.15 M NaCl, pH 7.4 (PBS) was used with the same results. Lipoproteins were fixed for 1 hr at 4°C or at room temperature. Lipoproteins were bound to the electron microscope grid, extracted, and stained as follows. A 10-μl drop of sample containing 10–20 μg protein/ml was placed on a glow-discharged carbon-coated copper grid held by jewelers forceps. After 60 sec the drop was removed by gently tapping the forceps, and then the grid was touched to a 10-μl drop of TBS. After 10 sec this drop was removed, and the grid was touched to a 10 μl drop of distilled water. After 10 sec the water drop was removed and the grid was submerged in a beaker on a cold plate containing 5–10 ml of ethanol–ether 4:1 at −5°C. After 60–90 sec the grid was removed from the ethanol–ether, touched to a drop of 100% ethanol at −5°C, then touched for 10 sec each to four successive 10-μl drops of 1% aqueous uranyl acetate at room temperature. A final drop of uranyl acetate was left on the grid for 30 sec, then removed by touching a filter paper wedge (Whatman #4) to the edge of the drop. The grid was then placed face up on filter paper (Whatman #1) in a petri dish and allowed to dry. The 100% ethanol wash can be omitted, although the stain tends to clump when this is done. We initially employed a series of dehydration and rehydration steps using 20%, 40%, 60%, 80%, and 100% ethanol at −5°C before and after the ethanol–ether extraction. This does not appear to affect the results.

Samples were examined by transmission electron microscopy with a JEOL JEM 100B electron microscope at a magnification of 48,500. A condenser aperture of 200 μm and an objective aperture of 50 μm were used. The magnification was calibrated with a diffraction grating replica (54,800 lines/inch). Particle sizes were determined from the enlarged prints using a 7× measuring magnifier with a reticule with 0.1-mm divisions. Diameters were measured in two directions, 90° apart, and the average was used. Contour lengths were obtained by tracing molecules from the enlarged prints onto an acetate sheet and measuring the tracings with a DNA length measuring device (25). For ring-shaped structures, contour lengths were measured midway between the outer and inner edges of the ring.

RESULTS

The properties of our LDL preparation are summarized in **Table 1.** These LDL were well within the normal range for LDL, but were somewhat larger than usually reported. From the percentage by weight of protein and the known molecular weight of apoB protein we can calculate an average molecular weight for the LDL. The value 3.14×10^6, is not significantly different from the hydrodynamically determined average molecular weight, 3.35×10^6, computed from the peak S^0_f and buoyant density for particles with a frictional ratio, f/fo = 1.11.

The LDL used in this study, as visualized in the electron microscope, are shown in **Fig. 1A.** All images examined were approximately circular, consistent with a semi-spherical shape. Particles contacting each other were frequently distorted along the line of contact, indicating that they are readily deformable. Glutaraldehyde treatment did not affect this appearance, nor did the particles appear substantially aggregated (Fig. 1C). Glutaraldehyde-treated LDL, with an average measured diameter of

TABLE 1. Properties of low density lipoproteins

	Percent Composition (by weight) ± SEM	
	Before extraction	After extraction
	%	
Protein[a]	16.3 ± 0.4	99.2 ± 1.0
Phospholipid	24.4 ± 0.3	0.5 ± 0.3
Cholesterol	11.3 ± 0.1	0.7 ± 0.4
Cholesterol Ester	43.2 ± 0.4	0.3 ± 0.8
Triglyceride	4.9 ± 0.1	0.6 ± 0.2
Hydrodynamic Properties		
$S^0_{26,1.063}$ = 7.35 Svedbergs[b]		
ϱ = 1.031 g/ml[c]		
M.W. = 3.35×10^6[d]		
Diameter = 218 Å[e]		

[a]Using a factor of 0.78 ± 0.3 to correct for the difference in chromogenicity of BSA and apoB (26).
[b]A small concentration correction of 1.1% was employed to adjust the observed flotation coefficient to infinite dilution (27).
[c]Sedimentation and flotation coefficients were measured at two different densities. The buoyant density was determined from an ηs vs ϱ plot.
[d]Assuming that the frictional ratio, f/fo = 1.11 (28).
[e]Assuming a spherical particle with the indicated molecular weight and density.

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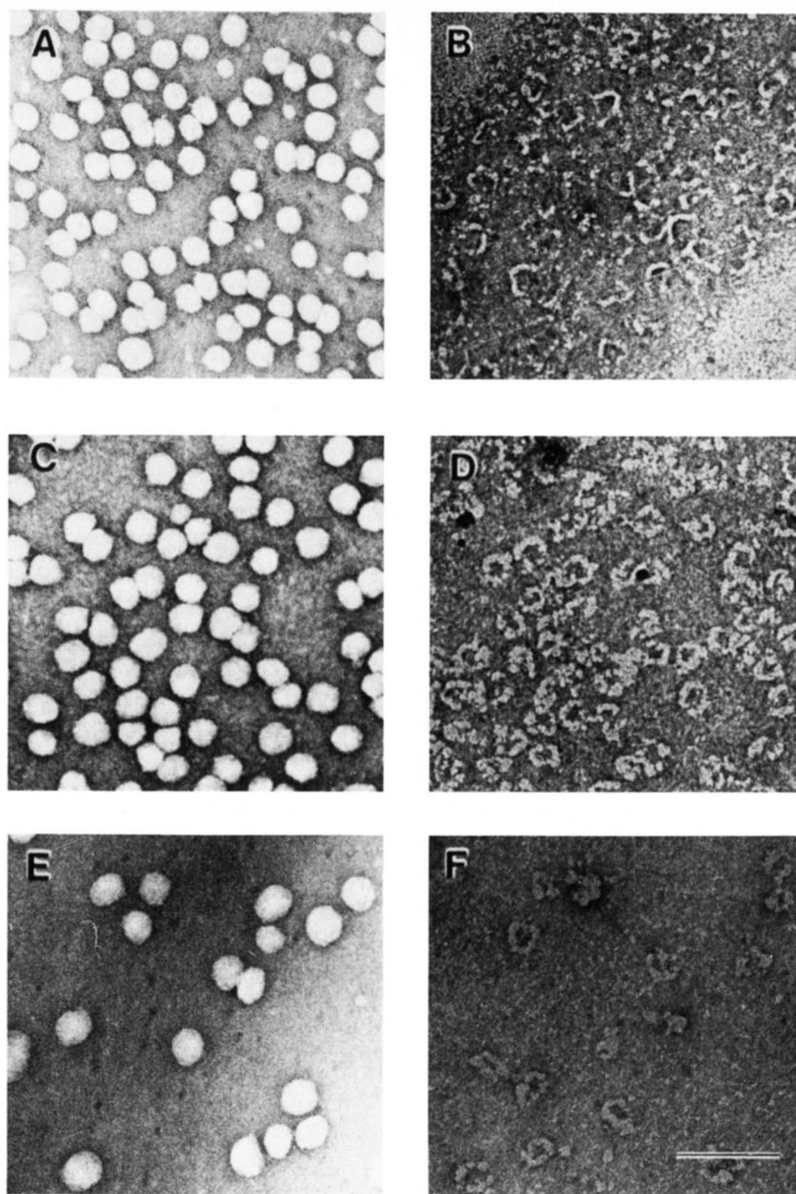

Fig. 1. Electron microscopy of lipoproteins and lipid-extracted lipoproteins. Lipoproteins were bound to the electron microscope grid and negatively stained with uranyl acetate, or bound to the grid, extracted with ethanol-ether 4:1, and stained. A: LDL; B: LDL extracted with ethanol-ether 4:1; C: LDL treated with 0.8% glutaraldehyde; D: LDL treated with 0.8% glutaraldehyde and extracted with ethanol-ether 4:1; E: IDL treated with 0.8% glutaraldehyde; F: IDL treated with 0.8% glutaraldehyde and extracted with ethanol-ether 4:1. The bar corresponds to 1000 Å.

303 ± 3.4 (SEM) Å, were significantly larger than untreated LDL, which had an average diameter of 281 ± 2.3 (SEM) Å. The average diameter of the LDL determined by electron microscopy, 281 ± 16 (SD) Å, was very much larger than the hydrodynamic diameter of 218 Å, computed for a sphere with a molecular weight of 3.35×10^6 Da and a density of 1.031. The apparent diameter of the LDL is very sensitive to the type of negative stain and its concentration. Thus, these LDL were stained with 1% uranyl acetate for the electron microscope measurements. When the LDL were stained with 2% potassium phosphotungstate (KPT) a diameter of 215 ± 6.2 Å was found. As the concentration of KPT was decreased the apparent size of the LDL increased (1% KPT, LDL diameter = 242 ± 8.3 Å; 0.5% KPT, LDL diameter = 276 ± 10.1 Å).

Glutaraldehyde-treated IDL were similar in appearance to glutaraldehyde-treated LDL (Fig. 1E) but were substantially larger (IDL diameter = 339 ± 3.0 (SEM) Å when stained with 1% uranyl acetate).

When LDL bound to the electron microscope grid were extracted with ethanol-ether 4:1 and then stained, the

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residual complexes appeared as flexible structures roughly 600 Å long and of variable thickness, ranging between 20 and 70 Å (Fig. 1B). The length is uncertain for two reasons. First, the ends of the molecule were frequently hard to establish, and the measured length is thus an underestimate. Second, the measured length does not include a subpopulation of highly variable, considerably shorter, molecules. SDS polyacrylamide gel electrophoresis indicated that the apolipoprotein in this LDL sample was approximately 75% intact, with the remainder of the apoB being present as fragments of 110,000 Da or greater. This might explain the presence of these shorter molecules. In addition to elongated structures, there were a few closed circles present, of approximately the size of an LDL particle. The average contour length (measured halfway between the inner and outer edges of the ring) of three clear examples was 660 Å.

When LDL were first treated with glutaraldehyde, then bound to the election microscope grid, extracted, and stained, ring-shaped structures were observed with a diameter of about 270 Å, measured from edge to edge (Fig. 1D). Frequently, the rings were better described as ellipses rather than as circles, with a major axis of about 270 Å and with a minor axis that was shortened, sometimes considerably. We repeated this experiment several times with LDL preparations from three different individuals and always observed this result. Fixation at room temperature instead of 4°C did not alter the appearance of these structures. The rings had a contour length (measured halfway between the inner and outer edges of the ring) of about 700 Å. Again, the thickness of the molecule was highly variable, in the range between 20 and 70 Å. The rings were not smooth but frequently gave hints of globular structure, as most clearly seen in Fig. 1F. This globular structure appeared to be better preserved in the glutaraldehyde-fixed material. Occasionally, finger-like protrusions could be seen from the outer edge of the ring.

When IDL were fixed with glutaraldehyde, bound to the electron microscope grid, extracted, and stained, ring-shaped structures were again observed (Fig. 1F). The contour length of these rings was slightly larger than the glutaraldehyde-treated, extracted LDL rings but the two structures were otherwise very similar.

As a control, high density lipoproteins, which do not contain apoB, were cross-linked, bound, extracted, and stained using an identical procedure. The HDL protein formed small discrete globules, a little smaller than native HDL. No ring-shaped structures were observed. When LDL and HDL were mixed prior to cross-linking, binding and extraction, a mixture of LDL rings and discrete HDL protein globules was observed.

In order to estimate the composition of these extracted structures, small aliquots of LDL solution were extracted with an excess of ethanol–ether 4:1. The insoluble pellet so formed contained 64% of the protein of the original LDL, less than 1% of the triglyceride and cholesterol, and essentially no cholesteryl ester or phospholipid (Table 1). Phospholipid and triglyceride were quantitatively recovered in the dried organic phase extract. The recovery of cholesteryl ester and cholesterol was 50–75%, probably due to reduced reactivity of the enzymatic assay with the dry extracts.

The relative binding of 12 different monoclonal antibodies to their respective epitopes before and after lipid extraction of LDL attached to microtiter wells was determined in a noncompetitive saturation assay (**Table 2**). The percentage listed in the last column reflects the percentage of each epitope that survived extraction and was exposed to the solvent.

DISCUSSION

When glutaraldehyde-treated LDL were bound to an electron microscope grid, extracted, and stained, the residual complexes appeared as closed circular structures (Fig. 1D). Presumably these were apoB with, perhaps, some lipid still attached. We interpret the ellipsoidal component of the ring shape as reflecting the degree of tilt of the apoB ring with respect to the carbon surface prior to extraction of the lipid from the LDL bound to the electron microscope grid. The contour length of the rings was approximately 700 Å. If used as a circumference, this would correspond to a sphere with a diameter of about 220 Å. Thus the dimensions of the rings are compatible with the apoB as a roughly equatorial structure surrounding the LDL.

While hydrodynamic and electron microscope measurements are both consistent with an approximately spherical shape for LDL, the measured size of our LDL,

TABLE 2. Radioimmunoassay of LDL and extracted LDL using 12 monoclonal antibodies

Monoclonal	Native LDL	Extracted LDL	Ratio × 100
	cpm – background		
B1	3128	705	23
B2	2421	320	13
B3	6932	3865	56
B11	2420	579	24
B14	3088	428	14
B16	2971	443	15
B17	4065	528	13
B19	3714	766	21
B20	6021	1429	24
B24	5696	2535	45
B47	10335	9115	88
H11G3	6783	2569	38

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stained with 1% uranyl acetate, was much greater than that calculated from the hydrodynamic data (LDL diameter = 281 Å by electron microscopy, LDL diameter = 218 Å hydrodynamically). We believe that this must be due to flattening of the LDL on drying on the electron microscope grid. To test this we stained LDL with 2%, 1%, or 0.5% potassium phosphotungstate solutions, reasoning that the lower KPT concentrations would provide less support for the particles on drying and, thus, a larger diameter would be observed. Indeed, this was found, consistent with a layer of stain providing lateral support for a soft molecule that otherwise tends to spread on a carbon surface during drying.

Electron microscopy of glutaraldehyde-treated LDL indicated that this treatment did not lead to aggregation of the LDL (Fig. 1C). When LDL were treated with glutaraldehyde, their diameter increased by about 8%. This difference is too small to correspond to the fusion of two particles, which would cause an increase of 26% in the diameter. It has been reported that dimethylsuberimidate-treated LDL are larger than untreated LDL (29).

When LDL were bound to an electron microscope grid, extracted, and stained, the appearance of the resulting complexes was consistent with reports of the structure of apoB-detergent complexes and of apoB-phospholipid complexes after partial delipidation with diethyl ether. We observed elongated flexible rods approximately 20-70 Å × 600 Å. Pollard, Scanu, and Taylor (13) reported that ether-extracted LDL consisted of subunits "predominantly arranged in strings of up to 500 Å in length." Forte and Nichols (15) also report observing "strandlike structures" on ether extraction of LDL. Gulik et al. (17) used freeze-fracture electron microscopy to visualize sodium deoxycholate-solubilized apoB as "a rod-shaped molecule." Ikai (19) used negative staining to visualize Tween 80-solubilized apoB as "a flexible string, a little less than 1000 Å in length and 50 to 60 Å in width." Zampighi, Reynolds, and Watt (18) report that apoB solubilized by *n*-dodecyl octaethyleneglycol monether and visualized by metal-shadowing "appears as a rod-shaped particle, 75-80 nm long and 4.5-5.5 nm wide." The dimensions quoted for the apoB-detergent or apoB-polar lipid complexes vary considerably, perhaps due to the different properties of the complexes, perhaps due to the different methods used for visualizing the complexes; nevertheless, all of these studies suggest that apoB is an elongated rod-like or flexible string. This description is also compatible with the material seen in Fig. 1B, suggesting that this material is predominantly apoB.

The average contour length measured for the apoB rods, about 600 Å, is substantially shorter than the average contour length for the glutaraldehyde-treated apoB rings, about 700 Å. Since glutaraldehyde treatment also increases the apparent size of the LDL, the question arises of whether glutaraldehyde treatment is substantially altering the structure of apoB. We do not believe this is the case, since ring-shaped structures can also be seen in the non-glutaraldehyde-treated control. The average contour length for three well-defined rings present in the non-glutaraldehyde-treated control was 660 Å, substantially larger than the length of linear structures present in the same fields. Careful comparison of the images, such as those seen in Fig. 1B, gives the impression that a portion of apoB is often rather diffuse and not included in the measurement of the linear structures, which may account for the shorter average contour length measured for the non-glutaraldehyde-treated molecules.

In addition to examining extracted LDL, we have performed two other controls. In the first we bound, extracted, and stained glutaraldehyde-treated high density lipoproteins, which do not contain apoB. No ring-shaped structures were observed. In the second we examined glutaraldehyde-treated IDL lipoproteins, larger than LDL, which contain apoB as well as smaller amounts of other apolipoproteins. After extraction, glutaraldehyde-treated IDL gave structures very similar to the LDL rings (Fig. 1F).

An alternative explanation for our results could be proposed. Looking down on any quasi-spherical object, it appears as a circle. Since the density of surface components becomes greater from the center towards the edge, a network of protein evenly spread over the whole LDL surface, collapsing on removal of the core, would leave the greatest density of material at the edge, generating a ring. We do not believe this is the case for several reasons. First, the rings are sharply defined and the transition to the dark central staining region is abrupt; there is no shading toward the center to indicate a gradual diminution of mass. Second, the ellipsoidal shapes which we have interpreted as projections of tilted rings are not explained by this alternative hypothesis. Third, extraction of non-glutaraldehyde-treated LDL produces elongated linear structures, inconsistent with a model of apoB as a uniform coating over the surface of spherical LDL.

Glutaraldehyde primarily forms cross-links between lysine residues in proteins. If these residues were being randomly cross-linked, we would expect to see many different domains cross-linked together. This is not seen, suggesting the possibility that we are observing a specific domain-domain contact stabilized by glutaraldehyde cross-linking. It is possible that other domain-domain interactions might exist, but if so the lysines in the domains are not positioned so as to allow cross-linking.

The simplest explanation for our data is that apoB occupies an elongated, probably flexible, structure encircling the LDL particle. This model is distinctly different from the dodecahedral model of Pollard et al. (13) or the tetrahedral model of Luzzati, Tardieu, and Aggerbeck (30). Both of these models assume symmetrical arrangements of quasi-identical subunits. Our model is also dis-

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tinctly different from the globular structure recently (31) reported for apoB when extracted by a procedure that first denatures and then refolds the molecule into a configuration that allows it to be soluble in an aqueous environment. In this case it seems likely that the apoB is refolded into a globular configuration to satisfy the hydrophobic requirements of its lipid binding domains, which ordinarily interact with the LDL lipids, by their coming together to present only a hydrophilic surface to the aqueous solvent. Thus, the principal elements of secondary structure may reform, as indicated by circular dichroism measurements, while the molecule adopts a globular tertiary configuration appropriate to its aqueous environment.

Marcel et al. (32) found that extraction of apoB using organic solvents and then SDS, solubilization in 6 M guanidinium hydrochloride, and finally dialysis through urea into a dilute aqueous salt solution, eliminated significant binding of 5 out of 6 monoclonal antibodies. In contrast, lipid extraction of LDL attached to microtiter plates has been reported to reduce epitope expression by only 50% (33). Our results seem midway between these two extremes, with an average binding of 31%. Of the 12 monoclonal antibodies studied, we arbitrarily classify 1 as being a good binder to LDL after lipid extraction (88%), 3 as being fair binders (38–54%), 4 are only poor binders (21–24%), and 4 are rather marginal binders (13–15%). Thus one-third of the epitopes examined are both fairly exposed and recognized by the antibodies, one-third are buried or badly disturbed, and the remaining third are of intermediate quality and/or location. We would like to use this result to argue against apoB being a sheet-like structure covering the surface of the LDL which "rolls up" to form a flexible rod upon lipid extraction, burying most of the surface exposed epitopes. Actually, we imagine that the hydrophobic surface of the apoB that originally made contact with the LDL lipids becomes attached during lipid extraction to the hydrophobic carbon film coating the electron microscope grid. In this case, most of the epitopes would remain exposed to solvent, and the lower percentage expression would reflect differential protein denaturation suggested to occur as the lipid is extracted from the apoB and replaced with the carbon film.

We may speculate as to the significance of the ring-shaped conformation that we observe here. One possibility is that the ring shape reflects an elongated semicircular conformation of apoB when it is involved in the packaging of triglyceride into very low density lipoproteins, perhaps by sealing off a portion of the membrane after triglyceride is added. In this view, what we are seeing in LDL is a vestigial structure, reflecting the role of apoB in the formation of VLDL. Another possibility would be that the apoB represents a limiting structure, defining the final size of the smallest LDL formed during metabolism. In this view, apoB would represent a necessary structural element for the formation of LDL. A combination of these two functions would be, of course, entirely possible.

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