# Structural studies on apolipoprotein B: controllable heterogeneity of the complex formed with the surfactant, Triton X-100

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**Abstract** Apolipoprotein B complexed with Triton X-100 (T-ApoB) has been isolated from human low density lipoprotein (LDL). Preparations are heterogeneous when analyzed by sedimentation velocity, with a major 12 S species and minor 17 S species present. The 12 S T-ApoB complex possesses a molecular weight of 880,000 containing 400,000 daltons of protein. Hydrodynamic measurements on this complex are consistent with a prolate ellipsoid model having an axial ratio of 13:1 and 0.22 g/g of bound water. Heterogeneity results from the irreversible aggregation of 12 S complexes into discrete 17 S and faster sedimenting components. A significant finding is that three determinants of this T-ApoB heterogeneity could be elucidated and controlled. First, the initial state of aggregation is mainly influenced by the technique by which Triton and LDL are mixed. Second, once isolated, T-ApoB complexes slowly but spontaneously undergo further aggregation at 4°C; the rate and extent of aggregation is enhanced remarkably with increasing temperature. Finally, reagents that unfold and expose protein structure (perchlorate, thiocyanate, and reducing reagents) lead to increased aggregation. The ability to control heterogeneity carries important implications for other studies concerning interactions of apoB with surfactants and lipids.—**Patterson, B. W., L. L. Kilgore, P. W. Chun, and W. R. Fisher.** Structural studies on apolipoprotein B: controllable heterogeneity of the complex formed with the surfactant, Triton X-100. *J. Lipid Res.* 1984. **25:** 763–769.

**Supplementary key words** low density lipoproteins

Apolipoprotein B (apoB), the structural protein of plasma low density lipoprotein, plays a crucial biological role in intercellular transport and delivery of cholesterol and in the pathogenesis of atherosclerosis (1). A detailed knowledge of apoB/surfactant interactions is a requisite for many studies involving immunologic structural probes (2), proteolytic digestion (3), and reconstitution with natural lipids (4–6). Tanford et al. (7) and Reynolds and Tanford (8) have stressed the importance of characterizing protein/detergent complexes, for proteins which natively associate strongly with lipids, in order to determine protein molecular weight and native states of aggregation. A number of laboratories have examined the interaction of apoB with a variety of synthetic and natural surfactants including sodium dodecyl sulfate (SDS) (9–11), Triton X-100 (10, 12–14), Tween 80 (15), n-dodecyl octaethylene glycol monoether ($C_{12}E_8$) (16), sodium deoxycholate (10, 12), and cetyltrimethylammonium bromide (10).

In this report, we describe conditions that promote the aggregation of Triton/apoB (T-ApoB) complexes. The ability to reduce or eliminate such undesirable aggregation is crucial when studying the physical, biologic, or immunologic properties of apoB/surfactant or apoB/lipid complexes.

## MATERIALS AND METHODS

### Preparation of apoB

LDL was isolated preferably from fasting subjects or alternatively from refrigerated plasma less than 5 days old. To plasma were added 20 mg of soybean trypsin inhibitor, 20 mg of sodium azide, 10 mg of Merthiolate, and 10 mg of EDTA per dl, and to unclotted blood was also added 50 units of heparin, followed by immersion in ice. After separating the plasma, LDL was isolated ultracentrifugally between densities of 1.006 and 1.06 g/ml and passed through a 2.5 × 100 cm column of Sepharose CL-4B using a phosphate-buffered saline solvent. The LDL peak fractions were concentrated on an Amicon XM-100 membrane and stored frozen in 20% sucrose. LDL electrophoresed on 4 or 5% acrylamide gels in SDS showed a major Coomassie Blue staining band or a doublet with the bands lying in apposition near the top of the gel. Several small bands may occur immediately below the major protein; however, upon scanning at 590

Abbreviations: LDL, low density lipoprotein; apoB, apolipoprotein B from LDL; T-ApoB, Triton/apoB complexes; SDS, sodium dodecyl sulfate; $C_{12}E_8$, n-dodecyl octaethylene glycol monoether.

nm, these comprise less than 5% of the total protein. All preparations used lacked identifiable bands of other apolipoproteins or albumin.

Triton X-100 was purchased from Sigma, lot 40F0205, and used without further purification. A 1% aqueous solution exhibited a cloud point of 66.8°C, corresponding to an average of 10.1 ethylene oxide units.[1] [Phenyl-$^3$H]Triton X-100 (sp act 1.58 mCi/mg) was purchased from New England Nuclear. $D_2O$ (99.8% purity) was obtained from Mallinckrodt. All other reagents were standard reagent grade.

### Preparation of Triton-apoB complexes

Prior to delipidation, LDL was dialyzed into a standard buffer of 0.1 M $Na_2CO_3$, 0.01% Merthiolate, 0.02% $NaN_3$, pH 8.0. Triton X-100 was introduced into LDL by one of two methods as follows.

Method I: Triton was added to concentrated LDL (5–10 mg/ml protein) to provide a minimum weight ratio of 20:1 detergent to protein and vortexed at room temperature until dissolved (approximately 5–10 min).

Method II: Dilute LDL (0.5–1 mg/ml) was added dropwise with stirring at room temperature to sufficient volume of a 10% Triton solution to provide a 20:1 weight ratio and concentrated via ultrafiltration (XM-100 membrane, Amicon Corporation) at 4°C to a final detergent concentration of approximately 15%.

Triton-apoB complexes (T-ApoB) were then isolated from detergent/lipid mixed micelles by one of two procedures.

*1*) Gel filtration at 5°C essentially by the method of Helenius and Simons (10), using a Sepharose CL-6B (Pharmacia) resin (1.5 × 94 cm) equilibrated with Standard Buffer containing 0.2% w/v Triton X-100.

*2*) Ultracentrifugation through a KBr gradient. Gradients were prepared by hand-layering the following solutions (all prepared in standard buffer) into $^9/_{16}$″ × $3^1/_2$″ cellulose nitrate tubes and allowing to diffuse overnight at 5°C: 3.2 ml of 22.0% w/w KBr, 0.02% (w/v) Triton X-100; 3.2 ml of 17.0% w/w KBr, 0.20% (w/v) Triton X-100; 3.2 ml of 11.5% w/w KBr, 0.32% (w/v) Triton X-100.

The gradients were then overlayered with sufficient LDL/Triton mixture to fill the tube to capacity, spun in a Beckman SW 41 Ti Rotor for 24 hr at 40,000 rpm, 5°C, and fractionated into 1-ml aliquots from the top.

### Analytical ultracentrifugation

Sedimentation velocity experiments were routinely performed at 42,040 rpm, 25°C, in a Spinco Model E ultracentrifuge employing double sector cells with standard and wedge windows. Sedimentation equilibrium analysis was performed at 8°C using a six-chamber Yphantis cell on samples isolated by gel filtration to control for background Triton concentration. Interference fringe patterns were analyzed by the method of Richards and Schachman (17). The partial specific volume of Triton-apoB complexes was developed by measuring their sedimentation coefficient in $H_2O$ buffer and $D_2O$ buffer as outlined for LDL by Fisher, Granade, and Mauldin (18).

### Viscometry studies

A capillary viscometer was employed providing an outflow time for water at 25°C of approximately 200 seconds. Temperatures were maintained within 0.1°C.

### Chemical assays

Protein was measured using a modified Lowry assay (19) using bovine serum albumin as a standard. Phospholipid was calculated from the measured phosphorus content (20); total cholesterol was assayed spectrophotometrically (21).

## RESULTS

T-ApoB complexes were isolated from Triton/lipid mixed micelles by gel filtration chromatography as illustrated in **Fig. 1A.** Protein complexes eluted with a partition coefficient $\sigma = 0.13$. Detergent/lipid mixed micelles are well resolved at $\sigma = 0.53$, as marked by the absorbance at 456 nm, indicative of yellow carotenoids and lipids. An alternative method for isolation of T-ApoB complexes involves sedimentation through a salt gradient, illustrated in Fig. 1B. T-ApoB complexes sediment faster than detergent/lipid mixed micelles and are isolated in the bottom one-third of the gradient. This procedure has the potential advantage of being able to process larger volumes of LDL than does gel filtration, and physical properties of T-ApoB complexes are essentially the same for either technique of isolation. Compositional analysis on isolated T-ApoB complexes revealed virtually no cholesterol (detection limit = 0.01 g/g of protein) and very low levels of retained phospholipid (variable, but typically <0.10 g/g of protein).

When LDL is delipidated by the addition of Triton to concentrated LDL (Method I), Distinct heterogeneity is apparent upon sedimentation velocity analysis (lower panel of **Fig. 2A**). However, when LDL is delipidated by adding dilute LDL to a 10% solution of Triton (Method II), the schlieren profile of T-ApoB appears nearly homogeneous (lower panel of Fig. 2B). The complex for Fig. 2B and the major peak of heterogeneous T-ApoB (Fig. 2A) have sedimentation coefficients $s^0_{25°,\ buffer} = 12.0$ S; the faster component in the latter has an $s^0$ of 17 S.

[1] Cloud point standard curve courtesy of James D. Burke, Rohm & Haas, Spring House, PA.

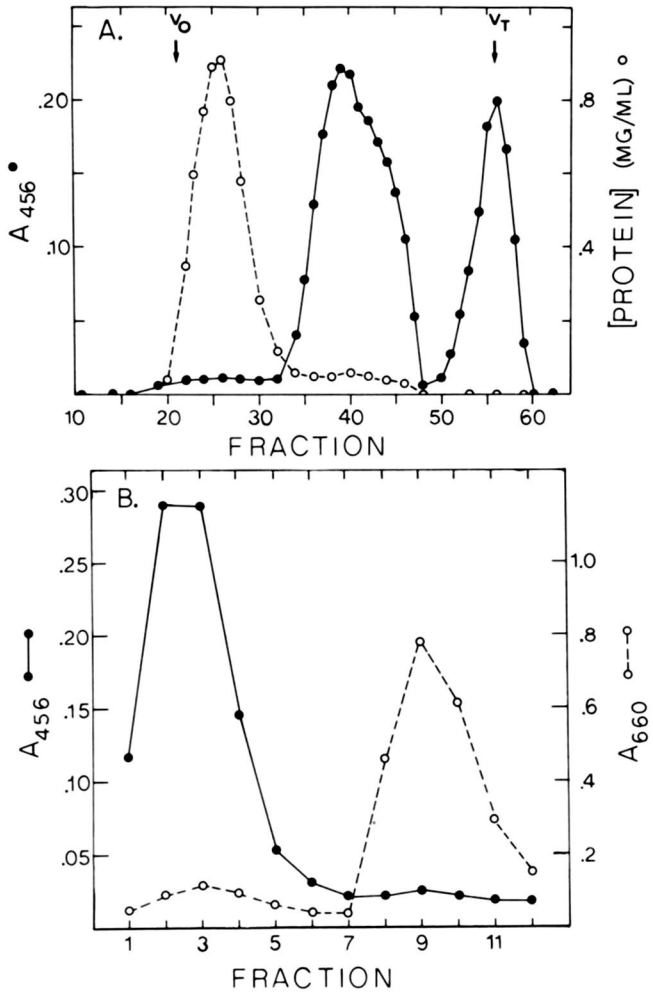


**Fig. 1.** A: Gel filtration chromatography of LDL/Triton mixture over Sepharose CL-6B; 24 mg of protein was applied; (O) assayed protein concentration; (●) absorbance at 456 nm, monitoring yellow lipids and a tracer of 10 mg $K_3Fe(CN)_6$ to mark the total volume. B: Density gradient sedimentation isolation of T-ApoB; 7 mg of protein was applied; (O) absorbance at 660 nm from protein assay; (●) absorbance at 456 nm to monitor LDL lipids.

The extent of detergent binding in $H_2O$ and $D_2O$ was directly assessed by gel filtration using $^3H$-labeled Triton for quantitation (column profiles not shown; essentially identical to Fig. 1A). Two columns (0.4 × 24 cm) were packed with Sepharose CL-6B and equilibrated with 0.04% $^3H$-labeled Triton standard buffers in either $H_2O$ or $D_2O$, using a Triton specific activity of 69,000 dpm/mg. Duplicate samples from a Triton/LDL mixture (same $^3H$-labeled Triton specific activity) were applied to each column. The degree of detergent binding, $\delta_D$, in $H_2O$ was measured at 1.18 ± 0.11 g/g of apoB, and in $D_2O$ was insignificantly higher at $\delta_D$ = 1.22 ± 0.12. An average $\delta_D$ = 1.20 is used for subsequent calculations.

Since $D_2O$ does not alter $\delta_D$, the hydrated densities of 12 S and 17 S T-ApoB complexes can be determined directly by sedimentation in $H_2O$ and $D_2O$ standard buffer solutions containing 0.2% Triton. A proper evaluation by this method requires that an estimate be made for the effect of deuterium exchange on the protein and deter-

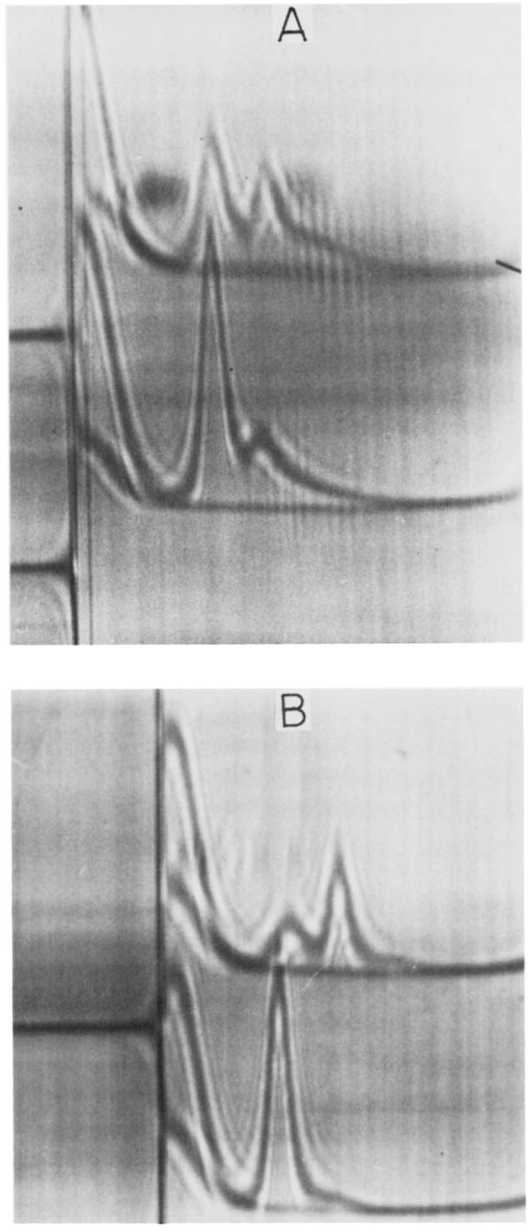


**Fig. 2.** Sedimentation velocity schlieren profile of T-ApoB complexes. The large peak near the meniscus is Triton micelles. A: Lower panel, T-ApoB prepared by Method I and isolated by density gradient ultracentrifugation. Protein concentration = 3.1 mg/ml; photo taken 45 min into run; blank = 0.2% Triton buffer. Upper panel, same as lower, except heat-incubated at 40°C for 2.5 hr. B: Lower panel, T-ApoB prepared by Method II and isolated by gel filtration, then concentrated threefold on Amicon XM-100 membrane. Protein concentration = 0.92 mg/ml; photo taken 37 min into run; blank = 0.2% Triton buffer. Upper panel, same as lower, except heat-incubated at 40°C for 3.5 hr.

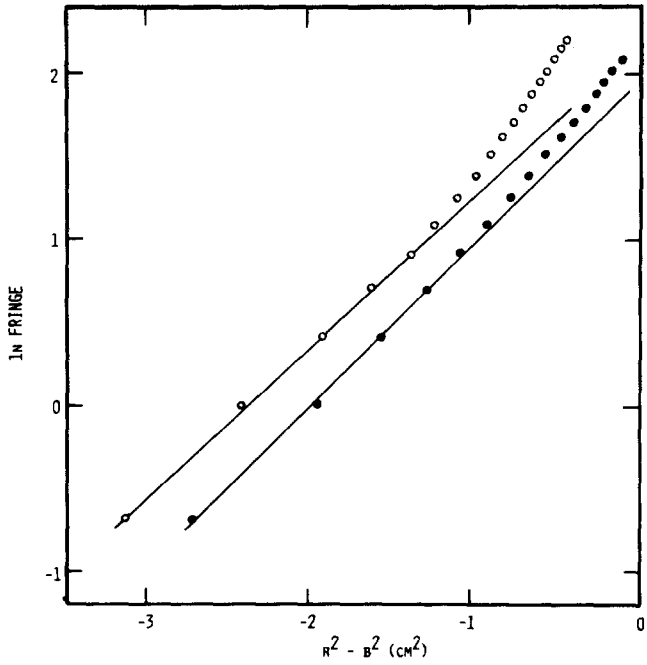


**Fig. 3.** Sedimentation equilibrium analysis of T-ApoB isolated by gel filtration; protein concentration = 0.23 mg/ml. Model E run at 5227 rpm, 8°C; (●) control cold T-ApoB; (○) heat-incubated T-ApoB (40°C, 2.5 hr). Lines show initial limiting slope, determined by 2.5 fringes.

gent components. For 100% $D_2O$, the exchangeable hydrogens on apoB are 0.0130 g/g (18); from the chemical structure of Triton X-100, there is one exchangeable proton per molecule, or 0.0015 g/g for an average molecular weight of 650.4. Given the constraint that $\delta_D$ = 1.20, T-ApoB complexes are 45% protein and 55% Triton by weight. Thus, the total exchangeable protons on T-ApoB are 0.0067 g/g. It follows from this analysis that $\bar{v}_{12\,S}$ = 0.824 and $\bar{v}_{17\,S}$ = 0.821 ml/g. An average partial specific volume for the complex ($\bar{v}_c$) of 0.823 is used for all computations.

Sedimentation equilibrium analysis was applied to determine the molecular weight of T-ApoB (**Fig. 3**). Upward curvature results in the log fringe versus $r^2$ plot as expected for samples that are not homogeneous (Fig. 2). The extent of curvature is increased by a mild heat incubation (40°C, 2.5 hr), yet the initial slopes are virtually parallel. Using a partial specific volume of $\bar{v}_c$ = 0.823, the molecular weight from the initial limiting slope (the first 2.5 fringes) for the illustrated set of data are 896,000 for the control sample and 826,000 after heat incubation. The average molecular weight obtained from the initial limiting slopes of 12 analyses was 880,000 ± 86,000 (SD). The molecular weight obtained using the entire range of data from the same 12 analyses was insignificantly higher at 930,000 ± 111,000 (SD); however, the molecular weight resulting from the limiting slopes near the bottom of the cell for five heat-incubated samples was 1,360,000 ± 24,000 (SD), demonstrating that heat incubation induced T-ApoB aggregation.

This increase in aggregation results in a marked increase in heterogeneity visible during sedimentation velocity analysis of heat-incubated T-ApoB (upper panels of Fig. 2A and B). The relative proportion of 12 S complexes is depleted, while the 17 S complex is enriched; additional faster components (22–23 S) also appear. Such heat-induced aggregation was not found to be temperature reversible; i.e., a heat-incubated sample does not undergo dissociation when stored up to 3 days at 4°C, and the subsequent sedimentation velocity analysis is performed at 5°C.

The heat-induced aggregation of T-ApoB is a slow kinetic process. Time course profiles are presented in **Fig. 4.** Heterogeneity induced at 40°C, as assessed directly

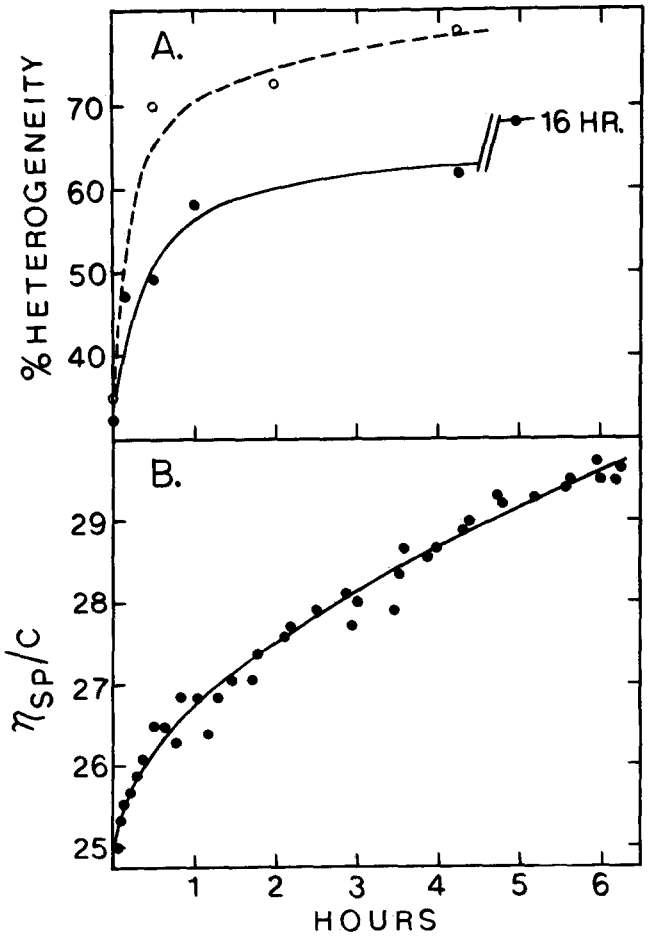


**Fig. 4.** Time course of heat-induced aggregation for T-ApoB. A: Change in schlieren profile heterogeneity with time; T-ApoB prepared by Method I (hence starts heterogeneous) and kept at 4°C until incubated at either 40°C (●) or 50°C (○) for the indicated times; sedimentation velocity analysis performed at 25°C; protein concentration = 1.4 mg/ml. B: Change in viscosity with time; T-ApoB prepared by Method I (hence starts heterogeneous) and kept at 4°C until being placed in viscometer equilibrated to 40°C; protein concentration = 3.8 mg/ml; C = whole complex concentration = 6.8 mg/ml.

from schlieren profiles, increases rapidly at first, then progresses more slowly over a longer period (Fig. 4A). The 16-hr time point is not yet at completion. At 50°C, aggregation occurs faster and proceeds to a further extent than at 40°C. A noticeable increase in heterogeneity occurs if a sample is stored at 4°C for a week, or allowed to sit at room temperature for 2 hr. An increase in viscosity attends the increase in heterogeneity during a 40°C incubation (Fig. 4B). An initial rapid increase in viscosity becomes attenuated at 1 hr, yet over 6 hr shows no indication of reaching completion.

Aggregation was also influenced by the presence of several common reagents (**Table 1**). Notably, 30 mM dithiothreitol and chaotropic agents (perchlorate and thiocyanate) were found to induce heterogeneity at 4°C. NaCl and phosphate did not induce heterogeneity, nor did phosphate protect the sample upon heat incubation. In an additional series of experiments (data not shown), significant heterogeneity was induced in only 1 hr at 4°C by 5% $\beta$-mercaptoethanol. Also, iodoacetylation of sulfhydryls on LDL by the method of Cardin et al. (22), with or without prior reduction by 5% $\beta$-mercaptoethanol, had no effect. Such modified LDL did not show enhanced aggregation upon the addition of Triton, nor were such samples protected against heat-induced aggregation to any significant extent.

Plots of $\eta_{sp}/c$ versus c for T-ApoB were found to be linear (results not shown). The intrinsic viscosity at 8°C of nearly homogeneous 12 S T-ApoB complexes (prepared by Method II) was determined to be 21 ml/g; the low temperature was chosen to retard aggregation during the course of the experiment. Heat incubation (40°C for 2.5 hr) caused the intrinsic viscosity at 8°C to increase to approximately 26 ml/g, consistent with the increase in $\eta_{sp}/c$ seen in Fig. 4B. Clearly, the molecular domain of T-ApoB is increased in the heat-incubated sample.

TABLE 1. Induction of T-ApoB heterogeneity

| Treatment | % Heterogeneity[a] |
|---|---|
| Fresh complexes | 34 (±5 SD)% (n = 3) |
| 40°C, 2.5 hr | 51% |
| 30 mM Dithiothreitol, stored at 4°C, 21 hr | 63% |
| 0.2 M KSCN, stored at 4°C, 21 hr | 54% |
| 0.2 M $NaClO_4$, stored at 4°C, 23 hr | 49% |
| 0.2 M NaCl, stored at 4°C, 23 hr | 32% |
| 0.1 M $PO_4$ buffer, dialyzed 4°C, 48 hr | 36% |
| 0.1 M $PO_4$ incubated 40°C, 2.5 hr | 57% |

T-ApoB prepared by Method I and isolated by the density gradient method (3.1 mg/ml) was made the stated concentration of solute by the addition of dry weight reagent, or was dialyzed into a 0.1 M Na phosphate buffer, 0.2% Triton, 0.01% Merthiolate, 0.02% azide, pH 8.10. All photos were taken 45 min into the run at 42,040 rpm.

[a] % Heterogeneity is defined as % of schlieren peak area not represented by 12 S complexes (see Fig. 2).

## DISCUSSION

Delipidation of LDL by replacement with Triton X-100 yields a degree of delipidation equal to that of organic solvent extractions (23). The inability to remove all the phospholipid bound to apoB may be explained by the physical size of Triton micelles which may not allow complete penetration of protein structure. Alternatively, it has been suggested that approximately 20% of the phospholipid is immobilized within native LDL due to tight lipid-protein associations (24); perhaps such tightly bound lipid is resistant to any gentle delipidation procedure that does not degrade protein structure.

The gel filtration profile showing isolation of T-ApoB complexes (Fig. 1A) is similar to previous reports (10). An alternative method for accomplishing this separation using density gradient ultracentrifugation (Fig. 1B) has the principal advantage of processing large volumes at one time; as designed, each tube could contain approximately 3 ml of sample. However, the background Triton concentration is not easily controlled by this procedure.

The degree of Triton X-100 binding to apoB reported here is higher than two previously reported values. Helenius and Simons (12) obtained $\delta_D = 0.52 \pm 0.07$ g/g of apoB using a similar gel filtration technique. In their experiment, LDL was initially delipidated with sodium deoxycholate, and a sample of apoB containing unlabeled deoxycholate (10 mg per mg of protein) was applied to the radiolabeled Triton X-100 column. It was reported that no residual deoxycholate was detectable in the $^3$H-labeled Triton/apoB complexes (12). In contrast, our Triton binding ratio was determined for apoB derived directly from LDL by Triton delipidation. Clarke (14) also reported the binding of Triton to apoB, obtaining a value of 0.92 g/g in $H_2O$ buffers.

Our measurement of the partial specific volume of T-ApoB complexes is based on differential sedimentation rates in $H_2O$ and $D_2O$ buffers. The value of 0.823 ml/g compares favorably with the value of 0.84 obtained by Clarke (14) using a similar technique, except that sedimentation coefficients were determined by ultracentrifugation through sucrose gradients. It may be significant that Clarke measured less binding of Triton X-100 to apoB in $D_2O$ than in $H_2O$ (0.72 vs. 0.92 g/g of protein); similar behavior has been noted for Semliki forest virus coat protein (25). However, $D_2O$ clearly did not alter the binding of Triton to apoB in our experiments.

Another estimate for $\bar{v}_c$ can be made from our data. Assuming additivity of components, then:

$$\bar{v}_c = \frac{\bar{v}_p + \delta_D \bar{v}_D}{1 + \delta_D}$$

where $\bar{v}$ of protein ($\bar{v}_p$) = 0.725 based on amino acid and carbohydrate content (9), and $\bar{v}$ of Triton ($\bar{v}_D$) = 0.908

(7). Using our average value of $\delta_D = 1.20$, $\bar{v}_c$ is calculated to be 0.825 ml/g, in excellent agreement with our sedimentation-derived value of 0.823. Note that the incorporation of 5% residual phospholipid would raise this estimate by 0.004 ml/g.

ApoB appears to exist natively as a mass of approximately 500,000 dalton per LDL particle (26) as well as in complexes with Triton X-100 (13), SDS (9), Tween 80 (15), and $C_{12}E_8$ (16). Our measured 12 S complex molecular weight of 880,000 ± 86,000 leaves a residual protein mass (using $\delta_D = 1.20$) of 400,000 ± 39,000 daltons. The reported molecular weight of apoB in 6–7 M guanidine hydrochloride is 250,000 (9, 27). This value is not unambiguous, however, in that no consideration was made for nonideality. A random coil of this size must possess a significant second virial coefficient due to size exclusion (28). There is mounting experimental evidence[2,3] that suggests that nonideality of apoB in this solvent cannot be ignored; if this holds, the published molecular weight of 250,000 daltons must be an underestimate. In light of this, it may be possible that apoB exists as a monomer when complexed with detergents, rather than the usual interpretation of a dimer (9, 13, 15, 16).

The aggregation phenomenon described in this report is likely due to a dimerization of the 12 S T-ApoB complex. We have measured weight average molecular weights of approximately $1.4 \times 10^6$ from the limiting slope at the solution column bottom for heat-incubated T-ApoB samples. Recognizing that such a slope represents, at a minimum, a composite of 12 S and 17 S complexes, a suggested molecular weight for 17 S complexes of approximately $1.8 \times 10^6$ may not be unfounded. Clearly, however, any assignment of molecular weight to any species larger than 12 S will remain speculative until methods can be devised to isolate them in stable form.

Assuming that the initial limiting slope of ln fringe versus $r^2$ plots is primarily representative of the 12 S T-ApoB complex, it is possible to combine ultracentrifugational and viscometric results in order to obtain information concerning the shape and hydration of T-ApoB complexes. The frictional coefficient ratio (29), $f/f_{min}$, determined from a combination of ultracentrifugation parameters ($s^0$ and molecular weight) and $\bar{v}$, is 1.84. From this value, maximum limits of hydration (4.3 g water/g of symmetric complex) or asymmetry (axial ratio of 16:1 for an unhydrated prolate ellipsoid) can be estimated. Since the intrinsic viscosity measurement is independent of $s^0$ and molecular weight, it is appropriate to determine what shape and hydration terms will satisfy the Simha intrinsic viscosity expression (29) and frictional coefficient ratio simultaneously. This condition is satisfied at a prolate ellipsoid axial ratio of 13:1 and 0.22 g water/g complex, resulting in a frictional coefficient shape factor $f/f_0 = 1.70$ and a Simha shape factor $\nu = 20.3$. Taking an axial ratio of 13:1, the Scheraga-Mandelkern shape factor $\beta = 2.50 \times 10^6$ for prolate ellipsoids (30). As additional corroboration of the sedimentation equilibrium results, this value for $\beta$ can be used to estimate the complex molecular weight knowing $\bar{v}$, $s^0$, and intrinsic viscosity. This value, 880,000, is in perfect agreement with the value measured by sedimentation equilibrium.

The high degree of asymmetry apparent for T-ApoB is quite consistent with other apoB/detergent models. For example, previously reported values for the frictional coefficient ratio ($f/f_{min}$) of Triton-apoB complexes are 2.2 (13) and 1.8 (14). Similar values have been reported with other detergents: 2.2 in Tween 80 (15), 2.0–2.3 in $C_{12}E_8$ (16), and 3 in SDS (9). Long flexible rods of apoB in Tween 80 having an axial ratio of 20:1 have been viewed directly by negative stain electron microscopy (15), and in $C_{12}E_8$ (axial ratio 16:1) using shadow-casting electron microscopy (31). It is evident that apoB forms very asymmetric structures in all detergents yet investigated.

In this report, three major sources of heterogeneity of apoB in Triton have been elucidated: the manner in which LDL and detergent are initially mixed, the handling of complexes after isolation, and the influence of certain buffer components. Gross aggregation of apoB occurs when pure Triton X-100 is added directly to LDL (Method I). During the initial stages of Triton solubilization, it is unlikely that this method provides sufficient Triton to prevent protein aggregation during the delipidation process. In contrast, considerably less heterogeneity results when dilute LDL is slowly added to dissolved Triton (Method II).

It is apparent that warming of the sample or treatment with chaotropes or disulfide cleaving reagents potentiates the aggregation of T-ApoB complexes. So far as we know, this aggregation is essentially irreversible. One inference that could be made from these later causes of aggregation is that reagents which unfold and expose protein structure may induce aggregation. T-ApoB complex aggregation is promoted by fully reducing conditions (5% $\beta$-mercaptoethanol or 30 mM DTT), and prior alkylation of exposed sulfhydryl residues does not inhibit aggregation. Thus, sulfhydryl exchange, as outlined by Cardin et al. (22) for apoB during solvent extraction of LDL, does not appear to be essential for aggregation of apoB in Triton.

Studies of apoB interactions with surfactants or lipids are often hampered by excessive heterogeneity. Such investigations should benefit from the principles outlined herein for reducing heterogeneity. Once such heterogeneity can be controlled, it will be possible to generate

[2] Patterson, B. W., and W. R. Fisher. Unpublished results.
[3] Schumaker, V. N. Personal communication.

surfactant/apoB complexes suitable for systematic investigations of apoB molecular weight determination, lipid reconstitution, or protein heterogeneity screening.

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