The apolipoprotein B polypeptide of human serum low density lipoprotein exists (after reduction of disulfide bonds) as a random coil with a molecular weight of 250,000 in concentrated solutions of guanidine hydrochloride. With intact disulfide bonds, there is a limited restraint on the polypeptide conformation in this denaturing solvent. In the presence of saturating amounts of bound sodium dodecyl sulfate, the apolipoprotein is dimeric and highly asymmetric. This work substantiates the molecular weight of 250,000 for apolipoprotein B reported by others (Smith, R., Dawson, J. R., and Tanford, C. (1972) J. Biol. Chem. 247, 3376-3381) and demonstrates that the dimeric state of the polypeptide extant in vivo is maintained in micellar detergent solution.

Serum lipoproteins are complexes of protein, free and esterified cholesterol, phospholipid, glycerides, and other lipids, and serve a variety of roles in lipid transport and metabolism in many species, including man (2). They are generally isolated from serum or plasma into several operational classes by ultracentrifugal procedures employing solutions of varying density (3). In order to elucidate the structure of these particles, characterization of their protein constituents (termed apolipoproteins) has been undertaken by several groups. With apolipoproteins A-I and A-II (found primarily in high density lipoprotein) and the C-peptides (found mainly in high density and very low density lipoproteins), this work has led to a knowledge of the amino acid sequences and lipid-combining properties of the apoprotein (4, 5) and has permitted the development of models which attempt to explain the structural bases of the lipoprotein particles (6). However, apolipoprotein B (apoB), the polypeptide moiety of low density lipoprotein (LDL) and one of the major apolipoproteins of very low density lipoprotein, has remained poorly characterized to date, primarily because of its marked insolubility in aqueous solution in the absence of amphipathic or denaturants. Many of its properties continue to be disputed; there is not even agreement on the number and size of apoB chains in the LDL particle (4, 7). In the preceding paper (8), the molecular and conformational stability and the amphiphilic associating capacity of the apoB polypeptide have been characterized; the present work considers the question of the molecular weight and hydrodynamic properties of this apolipoprotein.

Estimates of the molecular weight of apolipoprotein B have ranged from 8,000 to 340,000 (4, 9). However, many of these measurements were made using gel exclusion chromatography or sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, techniques entirely dependent upon similar behavior by the polypeptide under investigation and the calibrating proteins; in these investigations, this similarity has been assumed but not proven. Moreover, until recently, the molecular instability of the apoB polypeptide (8, 10) and its susceptibility to proteolysis (11) have not been appreciated; hence, the possibility of artifactual degradation of apoB in these studies must be considered. Tanford and co-workers (12) demonstrated by rigorous techniques that organically delipidated reduced and carboxymethylated apoB exists as a random coil with a molecular weight of 255,000 in concentrated guanidine hydrochloride (GdnHCl) solution. Others have suggested (4, 7) that this result may be due to an irreversible aggregation of the polypeptide during the organic delipidation procedure, although the agreement between values determined by techniques dependent on mass and on size (the latter subject to the assumption mentioned above) found by Smith et al. (13) makes this extremely unlikely. Given the central importance of the molecular size of the apoB polypeptide, this question has been reinvestigated using a different procedure to delipidate the lipoprotein. The hydrodynamic properties of the resultant apolipoprotein in GdnHCl and in SDS solutions have been determined and are presented here.

MATERIALS AND METHODS

Sodium Dodecyl Sulfate Studies—Samples of apoB in 2.5 mM SDS, 0.3 phosphate buffer (SDS buffer) were obtained by gel exclusion chromatography as described in the preceding paper (8); free sulphydryl groups were blocked using iodoacetamide as described there, resulting in carboxamidomethylated apoB (CAM-apoB). Selected protein-containing fractions (from different portions of the eluted protein peak) were dialyzed overnight versus protein-free SDS buffer. Protein concentrations were determined by a modified Lowry assay (8). The density of SDS buffer at 25.0°C was measured using a DMA-02C densitometer (Anton Paar, Graz, Austria); its value was 1.0115 g/ml. The viscosity of SDS buffer was determined to be 0.0486

* This investigation was supported by National Institutes of Health Grant HL 14882 (J. A. R.) and by United States Public Health Service Predoctoral Fellowship 5-TO-5-GM-01678 (J. C. H. S.). A preliminary report of portions of this work has appeared (1). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† The abbreviations used are: apoB, apolipoprotein B; LDL, low density lipoprotein, isolated as specified (8); SDS, sodium dodecyl sulfate; GdnHCl, guanidine hydrochloride; CAM-apoB, carboxamidomethylated apolipoprotein B; e, sedimentation coefficient at 25.0°C in SDS buffer, extrapolated to zero protein concentration; Rn; Stokes radius; Rmin; minimum Stokes radius of an unhydrated, spherical particle; Rf, the square of the distance (in centimeters) from the center of rotation in ultracentrifugal experiments; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; VLDL, very low density lipoprotein.

** J. C. H. Steele, Jr., manuscript in preparation.
centipoise at 25.0°C using a Cannon-Fenske size 50 viscometer. Deionized water was used to make all solutions.

**Guani dine Hydrochloride Studies**—ApoB in SDS buffer was transferred to GdnHCl solutions of greater than 7 M concentration by dialysis under nitrogen-saturated conditions (8). Reduced and carboxamidomethylated apoB (CAM-apoB) was obtained by reduction and carboxamidomethylation of CAM-apoB in this solvent. Before use, apoB was dialyzed overnight against the desired concentration of GdnHCl solution. Protein and GdnHCl concentration were calculated as described previously (8). The densities of GdnHCl solutions were obtained from tables prepared by Dr. Y. Nozaki (this laboratory), while solution viscosities were calculated from the concentration (13). All solutions were made using deionized water and were adjusted to a pH between 6.5 and 7.0 by the addition of sodium hydroxide.

**Sedimentation Velocity Experiments**—Three hundred-microliter samples were used in double sector cells with aluminum-filled Epon centerpieces. Centrifugation was at 48,000 to 56,000 rpm in an AND rotor in a model E analytical ultracentrifuge equipped with a photodetector scanner (all equipment from Spinco); the average temperature during the run was always close to 25°C and showed only slight variation during a single experiment. When more than one boundary was observed, the relative amount of each species was calculated as the percentage of the total optical density each species contributed. Sedimentation coefficients were calculated by standard techniques (14). For the SDS experiments, it was calculated (15) from the measured binding of SDS to apoB (8), the partial specific volume of SDS (0.863 ml/g; Ref. 16), and the calculated partial specific volume of apoB (0.725 ml/g) based on its amino acid composition (12, 17) and its carbohydrate content (18). The theoretical Stokes radius of a random coil of molecular weight (M) as GdnHCl solution was calculated (19) as \( R = 0.157M^{0.28} \). The minimum Stokes radius \( R_m \), of an unhydrated, spherical apoB-SDS complex was calculated as described elsewhere (15); the ratio of the measured Stokes radius to this minimum value provides a measure of the asymmetry, or the hydration of the sedimenting particle, or both.

**Sedimentation Equilibrium Studies**—One hundred-microliter samples were placed in double sector centrifuge cells in AND or ANF rotors and centrifuged at speeds between 6000 and 12,000 rpm in the Spinco model E ultracentrifuge described above. In some instances, a preliminary overspeeding at 20,000 to 30,000 rpm for 2 to 4 h was employed to hasten the attainment of equilibrium. When necessary, the solution meniscus was depleted following a run by overspeeding at 30,000 to 40,000 rpm for 30 to 90 min. Photoelectric scans at 280 nm were made after 36 or more h at a given speed; when repeat scans made 6 to 8 h (SDS) or 12 h (GdnHCl) later showed no significant differences in plots (14) of \( A-versus-R^2 \), the system was considered to be at equilibrium and the run was ended. Data were analyzed as described under "Results."

**RESULTS**

Sedimentation velocity experiments using CAM-apoB and RCAM-apoB in 7.8 M GdnHCl solution showed that each was heterogeneous. Seventy to eighty per cent of the initial optical density present sedimented slowly with a sharp boundary, while the remainder of the sample displayed a much broader, faster moving boundary. The measured sedimentation coefficients in each case are listed in Table I. Based on molecular weights determined by sedimentation equilibrium runs on the same samples (see below), Stokes radii were calculated for the two species present, these are given in Table I, along with those theoretically expected for a random coil with a molecular weight of 250,000 (the molecular weight of the predominant species). The close agreement between the predicted Stokes radius and that measured for the fully reduced polypeptide indicates that the RCAM-apoB monomer exists as a random coil in concentrated GdnHCl. CAM-apoB, with intact disulfide bonds, demonstrated a somewhat smaller size, indicating conformational restraints imposed by these bonds.

Parallel sedimentation equilibrium experiments on RCAM-apoB and CAM-apoB in 7.8 M GdnHCl confirmed the existence of monomeric and aggregated species in these samples. The experimental data (Fig. 1A) were closely fit by the line predicted (20) for two nonassociating species with molecular weights of 250,000 and 1,500,000; the amounts (optical density) of each determined by sedimentation velocity runs. Similar experiments on other samples in 7.6 M GdnHCl solutions gave comparable plots; although the relative amount of aggregated material varied somewhat, a monomeric species with an approximate molecular weight of 250,000 was always observed at centrifuge times less than 100 h (Table II). At no time was there evidence for any smaller polypeptide. In one case, a scan made after 160 h of centrifugation revealed a different pattern (Fig. 1B). The experimental data no longer agreed with that expected for the two nonassociating species described above; instead, it appears that aggregation of the previously monomeric species has begun to occur after this long time.

Hydrodynamic studies of apoB in SDS buffer were complicated by the instability of the delipidated polypeptide in this solution, as documented in the preceding paper (8). Using freshly prepared CAM-apoB, sedimentation velocity experiments revealed a marked dependence of the sedimentation

---

### Table I

<table>
<thead>
<tr>
<th>Sample</th>
<th>Species</th>
<th>( S )</th>
<th>( A )</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCAM-apoB(^a)</td>
<td>Monomer</td>
<td>80</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>Aggregate</td>
<td>20</td>
<td>2.64</td>
</tr>
<tr>
<td>CAM-apoB(^b)</td>
<td>Monomer</td>
<td>70</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td>Aggregate</td>
<td>30</td>
<td>2.89</td>
</tr>
</tbody>
</table>

\(^a\) Monomer indicates the 250,000-dalton species, which exhibited a very sharp boundary throughout centrifugation. Aggregate refers to the midpoint of the broad boundary observed during centrifugation, with an average approximate molecular weight of 1,500,000.

\(^b\) Reduced and carboxamidomethylated apolipoprotein B. Initial optical density at 280 nm was 0.19; solvent was 7.7 M guanidine hydrochloride.

---

\(^1\) Carboxamidomethylated apolipoprotein B. Initial optical density at 280 nm was 0.20; solvent was 7.8 M guanidine hydrochloride.

---

\(^2\) The broad boundary of the aggregate observed in sedimentation velocity experiments suggests that a mixture of aggregated species of different molecular weight was actually present. However, the theoretical boundaries for each of these overlapped to such an extent that it was not possible to assess the relative amounts of each. Consequently an "average" molecular weight of 1,500,000, estimated from a curve-fitting procedure has been used as the approximate molecular weight of the aggregated species. The aggregate presumably results from an irreversible process occurring while the protein is in monomeric SDS solution, during the transfer from micellar SDS to concentrated GdnHCl solution.
Hydrodynamic Properties of Apolipoprotein B in GdnHCl and SDS

FIG. 1. Sedimentation equilibrium of apolipoprotein B in guanidine hydrochloride solution. Experimental points of CAM-apoB at 8972 rpm for 51 h (A), then at 11992 rpm for an additional 109 h (B) are shown as filled circles. The line represents the pattern expected at equilibrium for a sample composed of 70% 250,000-dalton species and 30% 1,500,000-dalton species.

TABLE II

<table>
<thead>
<tr>
<th>Sample</th>
<th>Speed (rpm)</th>
<th>Time (h)</th>
<th>Initial A$_{280}$</th>
<th>Temperature (°C)</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCAM-apoB</td>
<td>8,880</td>
<td>93</td>
<td>0.416</td>
<td>19.85</td>
<td>254,000</td>
</tr>
<tr>
<td>CAM-apoB</td>
<td>7,203</td>
<td>71</td>
<td>0.185</td>
<td>22.70</td>
<td>252,000</td>
</tr>
<tr>
<td></td>
<td>8,972</td>
<td>01</td>
<td>0.197</td>
<td>22.05</td>
<td>248,000</td>
</tr>
<tr>
<td></td>
<td>11,996</td>
<td>90</td>
<td>0.230</td>
<td>24.88</td>
<td>232,000</td>
</tr>
<tr>
<td></td>
<td>11,992</td>
<td>160°</td>
<td>0.197</td>
<td>21.85</td>
<td>358,000</td>
</tr>
</tbody>
</table>

*±10%.

*Including 51 h at 8972 rpm.

The equation fit was ln A = α(R')^2 + βR' + c, where α, β, and c are coefficients obtained by the least squares fitting procedure.

coefficient upon protein concentration, indicative of nonideal behavior (Fig. 2). A least squares linear fit of the data yielded an s$_{20}$ value of 9.07 S, from which a Stokes radius of 243 Å for the polypeptide SDS complex could be calculated assuming a protein molecular weight in the particle of 500,000 (see below). From the theoretical minimum Stokes radius (R$_{min}$) of an unhydrated sphere of identical composition, 75.2 Å, a value of 3.23 for R/R$_{min}$ can be calculated. As discussed elsewhere (15), such a large ratio indicates that the sedimenting particle is asymmetric and provides an explanation for the observed nonideality.

Initial sedimentation equilibrium measurements on CAM-apoB in SDS buffer gave variable results, which were eventually recognized as reflecting nonideal behavior of a protein-detergent complex whose protein moiety was slowly degrading. (Attempts to circumvent this instability of the polypeptide by use of the Archibald technique were unsuccessful due to the low concentration of protein necessitated by the particle's nonideality.) This assessment was documented using freshly detergent-delipidated CAM-apoB. Centrifugation at 7200 rpm for 52 h yielded a plot of ln A versus R' which could be closely fit by least squares techniques to a second order equation.* From this, the slope (and thereby the apparent molecular weight) and the optical density at various appropriate values of R' could be calculated. This permitted a plot of apparent molecular weight versus A to be made (Fig. 3); extrapolation to zero A (zero protein concentration) then yielded the true molecular weight of the polypeptide in the sedimenting species. At 52 h at 7200 rpm, essentially no breakdown of the protein had occurred (confirmed by SDS-polyacrylamide gel electrophoresis) and a molecular weight of

FIG. 2. Sedimentation coefficient of apolipoprotein B in sodium dodecyl sulfate solution. The measured sedimentation coefficients, s$_{20}$b, corrected to 25.0°C in SDS buffer, at various protein concentrations are shown as open circles. The least squares line through the data is indicated.

FIG. 3. Sedimentation equilibrium of apolipoprotein B in sodium dodecyl sulfate solution. The apparent protein molecular weight of the sedimenting species, M$_{app}$, as a function of optical density at 200 nm is shown, the data being derived from sedimentation equilibrium experiments in SDS buffer. Centrifugation was at 7199 rpm for 52 h (○), next at 8993 rpm for 39 h (●), and then at 5991 rpm for 77 h (●). The lines indicate extrapolation to zero optical density.

*The equation fit was ln A = α(R')^2 + βR' + c, where α, β, and c are coefficients obtained by the least squares fitting procedure.
Hydrodynamic Properties of Apolipoprotein B in GdnHCl and SDS

about 500,000 was indicated. Scans made at later times (91 total h at the time of the 9000 rpm scan, 168 total h for the 6000 rpm scan) showed that degradation of the protein had begun to influence the measured molecular weight, especially at the later time. SDS-polyacrylamide gel electrophoresis of the protein sample at the end of centrifugation appeared similar to Fig. 1C in the preceding paper (8), indicating that breakdown had indeed occurred. On the basis of the 32-h results, it can be stated that the sedimenting particle in 2.5 mm SDS solution contains two polypeptide chains, each with a molecular weight of 250,000 (above and Ref. 12). Samples from various fractions of the eluted protein peak in SDS gel exclusion chromatography (8) behaved similarly, indicating that the peak consisted of a homogeneous species, a dimer.

The fact that the polypeptides were not disulfide-bonded to form a dimer was demonstrated by transferring the dimeric species to GdnHCl solution; as described above, the resultant CAM-apoB in concentrated GdnHCl solution existed primarily as a 250,000-dalton monomer.

**DISCUSSION**

Apolipoprotein B prepared by delipidation using SDS exists predominantly as a monomeric species with a molecular weight of 250,000 (±10%) in concentrated guanidine hydrochloride solution, as shown by the studies using fully reduced and carboxymethylated apoB. That this represents the apoB polypeptide monomer is indicated by the lack of any residual structure in the polypeptide in 7.6 to 8.0 M GdnHCl as judged by CD criteria (8) and by the close agreement of the measured Stokes radius (147 Å) with that expected for a random coil of this molecular weight (156 Å), based on a relation derived using much smaller polypeptides. This value is the same found by Tanford and co-workers (12) using organically delipidated LDL, and combined with their results argues strongly against this large molecular weight being the result of artificial aggregation, as some have suggested (4, 7). (The only possible structure composed of smaller subunits consistent with these studies would be an end-to-end linkage, either covalent or otherwise, of the subunits to form a long chain of essentially the same length an a single, continuous amino acid chain would possess. These hypothetical cross-links could not be disulfide bonds, since the fully reduced and carboxymethylated polypeptide was studied. Such an arrangement of subunits is unprecedented and is considered highly unlikely.)

Since the intact LDL particle contains about 500,000 daltons of protein, there must be two polypeptides with a molecular weight of 250,000 in the lipoprotein (12). It has not been previously determined whether these two chains are covalently cross-linked by disulfide bonds (like the apoA-I-II peptides of high density lipoprotein) or not (like the apoA-I peptides of high density lipoprotein). SDS-polyacrylamide gel electrophoresis and SDS-gel exclusion chromatography studies of the apoB polypeptide in the presence and absence of 2-mercaptoethanol showed no significant differences (8, 21), which would suggest that the two chains are not disulfide-linked. However, this suggestion is based on the assumption that the polypeptide is monomeric in SDS, which is not the case (see below). The sedimentation equilibrium studies of CAM-apoB in GdnHCl reported above, in which only the free sulfhydryl groups of apoB have been carboxymethylated to prevent disulfide exchange during delipidation, provide conclusive evidence that no disulfide bonds between the two chains exist, for the predominant sedimenting species was monomeric. Sedimentation velocity experiments with CAM-apoB showed that, in spite of its intact disulfide bonds, this molecule has a Stokes radius of 137 Å, compared to one of 147 Å for RCAM-apoB. This finding indicates that the intact disulfide impose only a modest restraint on the denatured dimensions of the apoB polypeptide. Similar but less quantitative conclusions can be drawn from the nondetectable effect of disulfide bonds on the size of the apoB-SDS complex, as evidenced in electrophoresis and chromatographic experiments (8, 21). It would thus appear that the six or seven disulfide bonds present in the apoB molecule must link closely adjacent residues, so that the overall dimensions of the polypeptide are not substantially affected by such bonds.

The studies in sodium dodecyl sulfate solution show that the apoB-detergent complex is quite asymmetric and consequently its behavior in the ultracentrifuge is nonideal (and further complicated by the instability of the polypeptide in SDS). This finding is consistent with the observed asymmetry of other protein-SDS complexes (22). However, unlike almost all other polypeptides, which behave as monomers in SDS, apoB exists as a dimer in the homogeneous hydrodynamic particle formed in micellar SDS solution. (The coat protein of the Fl bacteriophage behaves similarly (23).) The possibility that this is due to disulfide bonding between two monomers can be ruled out by the GdnHCl experiments using CAM-apoB reported above. It would appear that this dimerization is not an artifact produced by the delipidation process employed (8), for the measured sedimentation coefficient of the dimer, 9.07 S, agrees closely with that observed by Ikai (24) in the final stage of a stepwise delipidation of LDL by SDS. As the intact LDL particle (as well as VLDL) contains two apoB polypeptides (25), the dimerization in SDS may reflect an inherent property of the polypeptide to self-associate when in the presence of natural or synthetic amphiphiles. Further studies using other amphiphilic ligands will be necessary to confirm this possibility.7

In view of the results reported here and elsewhere (12), the monomeric molecular weight of the apolipoprotein B polypeptide must be considered to be 250,000. Those workers (4) who have reported much smaller values presumably either were studying a degraded form of the apolipoprotein (see Ref. 8 regarding apoB stability) or were employing a technique based on an invalid assumption of similar behavior by apoB and the calibrating protein standards; no such assumption is necessary in ultracentrifugal measurements. Other researchers, who have reported higher molecular weights for apoB, e.g. 340,000 (9), have used SDS-polyacrylamide gel electrophoresis. Since apoB dimerizes in SDS, a comparison between its electrophoretic behavior and that of the calibrating standards is not possible. In addition, the accuracy of this technique for measuring molecular weights above 150,000 is poor (27), so that any agreement (12) between electrophoretic and ultracentrifugal measurements should be considered fortuitous.

**Acknowledgments**—We thank Dr. C. Tanford for many helpful discussions throughout the course of this work. Various computer programs for the Tektronix 31 calculator were used extensively in this work; our appreciation is extended to Darrell R. McCasin, who prepared most of these programs.

**REFERENCES**


7Preliminary, unpublished results by D. Goodenburger and J. A. Reynolds indicate that apoB exists as a dimer in sodium deoxycholate solution. Other workers have recently shown (26) that apoB is a dimer in Triton X-100 solution.
Hydrodynamic Properties of Apolipoprotein B in GdnHCl and SDS

Invest. 34, 1345-1353


Molecular weight and hydrodynamic properties of apolipoprotein B in guanidine hydrochloride and sodium dodecyl sulfate solutions.
J C Steele, Jr and J A Reynolds


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