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INTERACTION OF HUMAN SERUM APOLIPOPROTEIN B WITH SODIUM DEOXYCHOLATE

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Preparation of apolipoprotein B (Apo B)-deoxycholate (DOC) complexes by gel filtration chromatography in the presence of 20 mM DOC, pH 8.5, gave two populations of particles with 5% (peak I) and 13% (peak II) lipid remaining bound. These complexes were initially shown to be very large and elongated, with partition radii of approx. 131 ± 0.5 Å, weight average molecular weights of approx. 164000 ± 1000, and an intrinsic viscosity of 80.19 ± 2.21 ml/g. Additionally, they appeared very similar to native low-density lipoprotein on sodium dodecyl sulfate-polyacrylamide gels, giving one major band. Incubation of these samples for 10 days under nitrogen at 4°C in the presence of antibiotics and protease inhibitor resulted in dissociation to many smaller subunits. Results of scanning molecular sieve chromatography and analytical ultracentrifugation showed that dissociation of these complexes was relatively slow and indicated the presence of at least two classes of components in fresh samples: one a very elongated complex with a radius directly correlated to the DOC/Apo B ratio and inversely correlated to sample aging; and another of much smaller radius which was independent of DOC/Apo B ratio but directly correlated to sample aging; indicating that these dissociated subunits interact with each other to an appreciable extent. Furthermore, these complexes were found to undergo a preferential hydration upon interaction with DOC, which may contribute to large changes in their effective specific volumes, as well as to dissociation of subunits.

1. Introduction

Surfactants such as sodium dodecyl sulfate (SDS), Tween 80, Triton X-100, Brij 58, *N*-dodecyloctaethylene glycol monoether ($C_{12}E_8$), sodium deoxycholate (DOC) and Lubrol WX are know to provide a structure-forming environment in which the native-order conformation of solubilized protein can be induced [1–3]. It has also been shown that the surfactant micelle can effectively strip the lipid from low-density lipoprotein particles (LDL) [4–12], and alter the conformation of protein in solution.

Below the critical micellar concentration (CMC) of SDS, for example, globular proteins retain the same conformation, although there is maximum interaction with SDS monomers. At SDS concentrations above the CMC, the presence of micelles induces a conformational change in the proteins in solution. There is, however, no consensus of opinion as to the shape of these protein-surfactant complexes [1,2,4,5,9–14], nor do any of the proposed models provide a sound molecular explanation for the ability of diverse hydrophilic proteins to form stable complexes of approx. 1.4 g SDS per g protein. Furthermore, the binding propensity of SDS for lipophobic proteins is much higher. For example, apolipoprotein B (Apo B)-SDS complex isolated from low-density lipopro-

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tein consists of about 2.6 g SDS per g Apo B [8].

The subunit structure – whether single or multiple chain – and relative stability of Apo B in surfactant solution have been the subject of study by a number of laboratories, with conflicting results being reported [15–18]. It would seem essential in examining the issue of Apo B's structure and stability to use a surfactant solution which is mild, anionic and capable of solubilizing proteins without substantially altering their biological activity. Sodium deoxycholate, used in these studies, have proven particularly effective in this regard. The DOC monomer is small and forms stable micelles above the CMC with a low aggregation number.

At concentrations below the CMC, DOC exhibits many characteristic regions, with the change in aggregate shape and aggregation number reflecting a continuous process. When a large aggregation number (15–20 monomeric units) is reached, it is generally assumed that superaggregation occurs. As a natural amphiphile, it readily forms small mixed micelles with cholesterol and other lipids, effectively removing them from LDL [19].

In this communication, we examine the stability of Apo B in surfactant complexes, particularly in sodium deoxycholate. The physical states of Apo B-DOC complexes prepared by gel filtration chromatography are compared with those prepared by organic delipidation [20].

2. Materials and methods

2.1. LDL preparation

Freshly drawn plasma was obtained from the blood bank and 1 ml of a solution containing 0.01% Na_2EDTA, 0.02% NaN_3, 0.01% Thimerosal (Sigma Chemical Co., St. Louis, MO), 0.002% phenylmethylsulfonyl fluoride (PMSF, Sigma Chemical Co.) added to inhibit potential proteolytic activity per 100 ml plasma. Solid KBr was used to adjust the desired background density, and LDL was obtained using two centrifugation runs for 20 h (33000 rpm, 105000 × g) at 4°C at each of the 1.019 and the 1.063 g/ml density cuts in order to prepare the lipoprotein free of very-low-density lipoprotein (VLDL) and high-density lipoprotein (HDL). In all runs a 70 Ti rotor (Beckman Instruments, Palo Alto, CA) was used. The isolated LDL was further fractionated by gel filtration chromatography over Sepharose 6B (Pharmacia Chemicals) as described elsewhere [20,21].

The solvent systems used in this study were 0.2 M $NaHCO_3$ (Mallinckrodt), 0.02% NaN_3, pH 8.5 and pH 9.7 (pH 8.5 and pH 9.7 buffer), ionic strength 0.2. In some cases the pH 8.5 buffer contained 0.01% Thimerosal, 0.01% EDTA and 0.002% PMSF. Sodium deoxycholate was obtained from Schwartz/Mann (Catalog No. 804312) and was used without further purification.

2.2. Isolation of Apo B-DOC complexes

2.2.1. Organic delipidation and dialysis

Apo B was prepared from LDL by organic delipidation at 4°C in the presence of 6 M guanidine, 0.2 M ammonium bicarbonate, pH 8.0, as described elsewhere [20]. This Apo B solution was placed into a dialysis bag and dialyzed first against 6 M urea in pH 8.5 buffer at a 1:50 (v/v) ratio for 1 day at 4°C with three changes. The Apo B solution was removed to a sealable plastic tube and solid sodium deoxycholate was added to a final concentration of 20 mM. The tube was sealed and incubated at 6°C until all of the DOC was dissolved (about 1 h). This solution was placed into a dialysis bag and dialyzed at 1:50 (v/v) ratio against 20 mM DOC/pH 8.5 buffer at 4°C for 3 days with six changes.

2.2.2. Gel filtration chromatography

A solution of LDL (≈ 4 mg/ml) in pH 8.5 buffer was added to an equal volume of 0.2 M DOC/pH 8.5 buffer, which corresponded to a weight ratio of approx. 90 g DOC/g protein, and mixed. This solution was incubated for 24 h at 6°C after which 7.0 ml were layered onto a 2.6 × 40 cm Pharmacia C26/40 column maintained at 6°C and containing Biogel A-1.5 m (200–400 mesh, BioRad, Control No. 207153) equilibrated with 20 mM DOC/pH 8.5 buffer; and flow started. The flow rate was held constant at 8.0 ml/h by an

LKB Model 10202-1 peristaltic pump and 2.0-ml eluant fractions were collected using a Gilson microfractionator (Serial No. 34957).

Using the column buffer as a blank, the absorbance of each fraction was determined at 280 and 484 nm by a Gilford Model 222 spectrophotometer and A_{280} and A_{484} were plotted against elution volume, as shown in fig. 1. Those fractions constituting a peak in absorbance at either wavelength were pooled separately and concentrated approx. 5-fold by ultrafiltration using an Amicon XM-50 filter at 4°C under nitrogen. Each peak was then dialyzed at a 1 : 25 (v/v) ratio against 20 mM DOC/pH 8.5 buffer at 4°C for 1 day with four changes.

2.3. Scanning molecular sieve chromatography (SMSC)

All SMSC experiments were done using a direct ultraviolet gel column scanning system which is essentially the same as that described elsewhere [22,23] with the exception of the monochromator and light source module, which are Heath EU-700 and EU 7[illegible]1-50, respectively.

The basic operating routine consists of moving a 24 × 0.90 cm quartz column, packed with a suitable gel (i.e., one with minimum scattering), through a horizontally collimated beam of monochromatic light (visible or ultraviolet) at a constant rate of 0.19 cm/s for 98 s, during which time transmittance is detected by an Aminco 10-267 solid-state, blank-subtract photomultiplier microphotometer through an end-on photomultiplier tube (RCA 6903 for ultraviolet or RCA C7164 for visible range).

The direct ultraviolet gel column scanner system [22–25] is interfaced with a North Star Horizon 780A microprocessor, a Sanyo DM50/2CX video display unit, and an Integral Data Systems Model 460 printer/plotter. Gel column scans may be made at any wavelength between 220 and 1000 nm. Scan rate was approx. 16 data points/cm, which is equivalent to 300 data points for a 98-s scan.

The partitioning properties of varying concentrations of Apo B in the presence of 20 mM DOC, as well as the partitioning properties of 20 mM DOC itself in Biogel A-1.5 m (100–200 mesh, exclusion limit $\simeq 1.5 \times 10^6$), were determined in large zone experiments at 280 nm at 25°C.

The more recent technique of difference profile analysis [22] was applied. This method permits analysis of the time-dependent properties of large zone profile at concentrations as low as 2–5 μg/ml.

The column was scanned at regular intervals as the solution/solvent boundary moved through the gel matrix at a constant flow rate. The rate of movement of the leading and trailing boundary was determined by locating the centroid position for each scan, $\bar{x}$, then plotting these distance values versus the time at which the scans were made. The baseline records of successive scans were subtracted from each other, yielding a difference profile. The partition coefficient was then calculated from

$$\sigma = \frac{(dt/d\bar{x})_p - (dt/d\bar{x})_o}{(dt/d\bar{x})_i - (dt/d\bar{x})_o} \quad (1)$$

where $(dt/d\bar{x})$ is the slope of a plot of t vs. $\bar{x}$ for a given sample marker, and the subscripts p, o and i refer to the species under investigation, the void volume marker and internal volume marker, respectively. The void volume marker used was tobacco mosaic virus (TMV, Miles Labs.) and the internal volume marker was provided by the difference in the index of refraction of the water/buffer interface from the same TMV solution. The partition radius of the solute was then calculated using the following expression [24,25]

$$a_i = a_o + b_o \mathrm{erf} c^{-1} \sigma_i \quad (2)$$

Here a_o and b_o are calibration constants for particles of known radii in a given gel and were determined independently [26,28] using thyroglobulin ($r = 85.0$ Å), ferritin ($r = 61.0$ Å) and catalase ($r = 52.2$ Å) – all from Sigma Chemical Co – in pH 8.5 buffer.

2.4. Sedimentation velocity and equilibrium measurements

All sedimentation velocity runs were made on Apo B-DOC complexes in 20 mM DOC/pH 8.5 buffer at 42 040 and 52 640 rpm at 8, 20 and 25°C in a Beckman Model E analytical ultracentrifuge

equipped with an RTIC unit using either a double-sector cell or standard cell. Particle radii for equivalent spheres were calculated from the following expression [26–28]:

$$r_o = \{9S^0\eta/2(\rho_p - \rho_o)\}^{1/2}$$

using the values $\eta = 0.010772$ P, $\rho_0 = 1.0118$ g/ml and $\rho_p = 1.3405$ g/ml for Apo B-20 mM DOC at 25°C, which may be taken as the inverse of either the partial specific volume of complex, $\bar{v}_c$, or the apparent partial specific volume, ϕ_c'.

All sedimentation equilibrium runs were made on Apo B in 20 mM DOC/pH 8.5 buffer, prepared by organic delipidation-DOC dialysis and by gel filtration from the LDL-DOC complex, at 8225 rpm at 8°C.

Calculation of the molecular weight distribution and $(d\ln C/dx^2)$ were based on the method of Yphantis [29], using a computer program which can be modified for use with an Amdahl 470/02 unit (modified IBM 360/1800), University of Florida's CIRCA computing facilities, plotting $\ln J$ or $\ln C$ as a function of X^2. The $\ln C$ versus X^2 data were fitted to a least-square polynomial and the values of $(d\ln C/dx^2)$ were calculated by a modification of the sliding three-point least-square quadratic treatment of Yphantis.

2.5. Evaluation of the preferential interaction term

The preferential interaction of solute (component 2) with solvent components 1 and 3 (water and DOC) may be determined from the expression [30,34]:

$$\xi_3 = \{(1 - \phi_2'\rho^0) - (1 - \bar{v}_2^0\rho^0)\}/(1 - \bar{v}_3\rho^0) \quad (4)$$

where ρ^0 is the density of the solvent mixture in the absence of component 2. The apparent partial specific volume, ϕ_2' or ϕ_c', is defined as $(\partial\rho/\partial C_2)\mu_3 = (1 - \phi_2'\rho^0)$ [31,32] where μ_3 is the chemical potential of component 3. $\bar{v}_2^0$ is the partial specific volume at infinite dilution, C_2 and the value of $\bar{v}_3$ corresponds to the finite concentration C_3. Reisler et al. [30] have shown that within a close approximation of zero protein concentration, assuming that the density of solution is a linear function of C_2 such that $\rho = \rho^0 + (\partial\rho/\partial C_2)_{\mu_3}C_2$, it becomes possible to determine the extent of preferential interaction.

The preferential interaction term is a measure of DOC binding to Apo B, in terms of the number of grams of DOC preferentially bound per gram of Apo B. Thus, ξ_3 from eq. 4 becomes

$$\xi_3 = (\bar{v}_c^0\rho^0 - \phi_c'\rho^0)/(1 - \bar{v}_3\rho^0) \quad (5)$$

Positive values of ξ_3 indicate preferential binding of DOC, while negative values indicate preferential hydration [30,33].

The apparent partial specific volume, ϕ_c', may be determined by the sedimentation velocity-viscosity method for each density as previously described [27,28].

2.6. Evaluation of the axial ratio, a/b

Once the true values of molecular weight and $\bar{v}$ are known, the radius of an equivalent sphere, r_o, may be calculated from

$$r_o = [(3\bar{M}_w\bar{v})/(4\pi N)]^{1/3} \quad (6)$$

The ratio of the particle is a relative measure of particle asymmetry. For a ratio of 1.0, the particle is of course a perfect sphere. For frictional ratios greater than 1.0, the particle may be considered ellipsoidal (either prolate or oblate) or even rod-like. The axial ratio, a/b, for a prolate ellipsoid or long rod may be determined from the axial ratio according to Van Holde [35].

2.7. Viscosity measurements

Solution viscosities were measured with an Ostwald-Cannon (Fisher No. 25) capillary viscometer of 5.0 ml capacity. This viscometer, which has an efflux time of approx. 500 s using distilled water, was immersed in a constant temperature bath at 25.00 ± 0.02°C.

The relative viscosities for varying concentrations of Apo B-DOC complex were calculated using the following values for 20 mM DOC/pH 8.5 buffer: $\rho_0 = 1.0114$ g/ml and $t_0 = 532.13$ s. The reduced viscosities [35] were calculated for each protein concentration from

$$\eta_{red} = \eta_{sp}/C = (\eta_r - 1)/C, \quad (7)$$

where η_{sp} is the specific viscosity and C the concentration of protein in g/ml. These η_{red} values were then plotted against concentration and fitted

to a straight line to determine the intercept, which was taken as the intrinsic viscosity, $[\eta]$ in ml/g, of the Apo B-DOC complex.

2.8. Density measurements

Density measurements were made with a bulb and capillary pycnometer of the Lipkin type, at 20 and 25°C. Triplicate measurements were made on each solvent and sample with a 5 ml pycnometer; the agreement between measured densities was 0.0001 g/ml or better in all cases.

2.9. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Apo B-urea and Apo B-DOC complexes were electrophoresed on 5% polyacrylamide gels in the presence of 0.1% SDS, 0.1 M Tris, 0.2 M sodium acetate and 20 mM EDTA, pH 7.4 (electrophoresis buffer), according to the method of Weber and Osborn [36]. Samples were prepared by heating to 55°C for 1 h (unless otherwise specified) in the presence of 1% SDS and 1% β-mercaptoethanol in electrophoresis buffer. The gels were fixed overnight in 50% methanol/9% acetic acid and then stained with 0.125% Coomassie brilliant blue R dye in fixing solution. These gels were scanned at 590 nm using a Beckman Model 24 scanning spectrophotometer and the relative mobilities of the bands on each gel determined from the corresponding peaks. The relative amount of protein in all bands was determined by estimating the area of each peak from the product of its height times the half-width at half-height, summing these areas, and then determining the weight fraction of each peak. Major bands were than distinguished as those which constituted more than 15% of the total protein on the gel.

2.10. Chemical assays

The protein content of all samples in mg/ml was determined by the method of Lowry et al. [37], using a human serum albumin fraction V (Calbiochem Corp.) as a standard.

2.11. Triglyceride and total cholesterol

Triglyceride and total cholesterol were determined using an Oxford Tri-Chol Reagent Set with cholesterol and triolein as standards, as described elsewhere [38].

The triglyceride assay involved saponification to fatty acids and glycerol, oxidation of the glycerol to formaldehyde, and quantitation of the formaldehyde colorimetrically, as based on the Hantzsch reaction described by Fletcher [39]. The colorimetric determination of total cholesterol is based on the Kiliani-Zak reaction as described by Martinek [40], and does not distinguish between unesterified cholesterol and cholesteryl ester.

A control solution containing 20 mM DOC/pH 8.5 buffer was also assayed each time DOC was present in the samples. Interferences in either assay due to the presence of DOC were small and were corrected for.

2.12. Phospholipid

Phospholipid content was determined by the method of Rouser et al. [41] using potassium phosphate as a standard. Samples were treated twice with 2 vol. of 2:1 $CHCl_3/CH_3OH$ to extract the phospholipid. The assay involved the oxidation by 70% $HClO_4$ of phospholipid to P_i, which was detected colorimetrically at 820 nm after adding equal volumes of 2.5% ammonium molybdate and 10% ascorbic acid.

3. Results

3.1. Isolation of Apo B-DOC complexes

The elution profile of Apo B-DOC complexes prepared by DOC gel filtration chromatography is illustrated in fig. 1. Compositional analysis revealed that essentially all of the protein eluted in peaks I and II, while most of the lipid eluted in peak III (table 1). Peak II still contained a significant amount of lipid although it eluted later than peak I, which contained very little lipid.

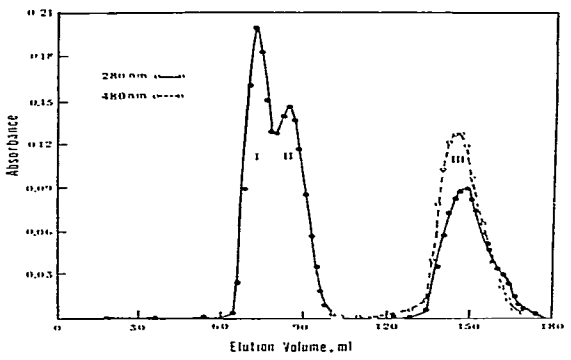

Fig. 1. Gel filtration elution profile of Apo B-DOC complexes. An LDL/0.10 M DOC solution was layered onto a 2.6×40 cm column packed with Biogel A-1.5 m (200–400 mesh) equilibrated with 20 mM DOC/pH 8.5 buffer at 6°C. 2-ml fractions were collected at a flow rate of 8.0 ml/h. Fractions corresponding to the three major peaks were pooled separately and concentrated approx. 5-fold. Absorbance at 484 nm indicates the presence of carotenoids. The column void volume, determined using TMV in pH 8.5 buffer, was approx. 68 ml, as indicted by the arrow. The internal volume (not shown); determined using potassium ferricyanide, was approx. 185 ml.

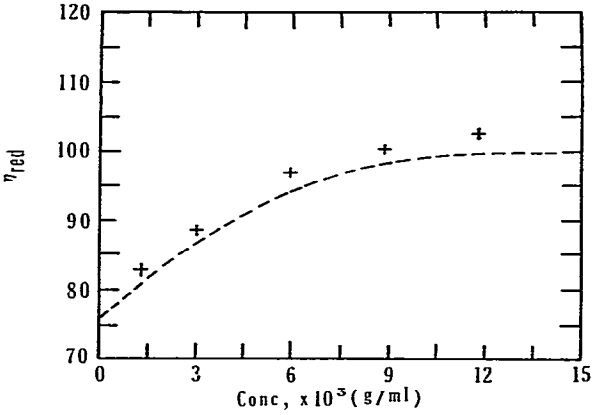

Fig. 2. Reduced viscosity versus concentration of Apo B. The values of reduced viscosity were calculated for Apo B-20 mM DOC complex (peak II in fig. 1) and are given in ml/g, while the values of protein concentration are given in g/ml. The dotted-line fit to the data gave a linear correlation coefficient of 0.9619 with a standard deviation of 2.62 ml/g in the reduced viscosities. The intercept was taken as the intrinsic viscosity of the complex, $[\eta] = 80.18 \pm 2.21$ ml/g. The data have been fitted to first- and second-order polynomials. For a first-order fit, $y = 80.1897 + 18863X$, correlation coefficient 0.96186. For a second-order fit, $y = 75.5506 + 41646X + 17947X^2$, correlation coefficient = 0.9978.

Table I

Lipid composition of LDL, Apo B-DOC and lipid-DOC complexes

The amounts of lipid are expressed as the number of lipid molecules per 637100 M_w unit of LDL protein. [a]

Particle	Triglyceride/ unit protein	Total cholesterol/ unit protein	Phospholipid/ unit protein	DOC/ unit protein
LDL	270	1995	740	–
Apo B-DOC organically delipidated	–	–	16	984
Apo B-DOC peak I	52	83	6	
Apo B-DOC peak II	97	260	16	
Lipid-DOC [b] peak III	5809	40190	> 8706	–

[a] One unit of LDL protein was calculated from: $(2.77 \times 10^6)(0.23) = 637100$ (taken from refs. 28 and 45). The molecular weight of DOC is 414.5. The average molecular weights of the lipids are triglyceride, 850; total cholesterol, 613 for LDL; and phospholipid 775 (taken from ref. 52).

[b] The amount of phospholipid in peak III was beyond the linearity limit of the assay and the amount of protein present was approx. 0.02 mg/ml, as compared to 1.17 and 0.54 mg protein/ml in peaks I and II.

3.2. Pycnometry and viscometry

The reduced viscosity at 25°C for Apo B-DOC complex freshly prepared by DOC gel filtration chromatography (peak II) is shown in fig. 2. The intercept of a plot of the reduced viscosity versus the concentration of Apo B was taken as the intrinsic viscosity, $[\eta]$, for the Apo B-DOC complex, which was 80.19 ± 2.21 ml/g. This value does not taken into account the amount of DOC bound. However, even if there were 2 g DOC bound per g Apo B, the intrinsic viscosity would still be large (≈ 27 ml/g). Such a large value indicates that this complex is not globular or spherical, but resembles a rod or random coil [35].

3.3. Scanning molecular sieve chromatography

Representative difference profiles of Apo B-DOC and lipid-DOC complexes in 20 mM DOC/pH 8.5 buffer on Biogel A-1.5m at 280 nm, as well as other subsequent data, are illustrated in fig. 3 and 4 and summarized in tables 2 and 3. The micelle radius was determined to be 28–30 Å.

Apolipoprotein B-DOC complexes prepared by DOC gel filtration chromatography (peaks I and II from fig. 1) revealed a time dependence on particle size in SMSC. Initially, these complexes gave profiles very similar to those for Apo B-DOC prepared by organic delipidation-DOC dialysis. For example, fresh samples of peak I, which contained very little lipid (table 1), produced a broad major peak and a very sharp minor peak (not shown). The major peak was the same as that for the organically delipidated-DOC dialyzed complex and the minor peak represented particles of approximately the same size as DOC micelles (table 3).

After 4 days, broadening was observed for both SMSC peaks, from peak I, with a decreased amount of the major SMSC peak present (fig. 4a). The weight average radii of the two peaks also increased. This increase in size and heterogeneity is a reflection of the high degree of interaction which is present between the complexes responsible for these peaks. The decreased amount of the major peak also indicates a precursor-product relationship between the major and minor peak complexes. This relationship is even more apparent in fig. 4b and c, which shows the same results for the time-dependent behavior of SMSC profiles for complexes produced by peak II from DOC gel filtration chromatography.

Lipid-DOC complexes, produced from LDL by DOC gel filtration chromatography (peak III from fig. 1), remained essentially unaltered in their SMSC profiles over the course of 1 week. One fairly sharp, symmetrical peak was observed both 2 days (fig. 4d) and 7 days after isolation, corresponding to particles with a weight average parti-

Table 2

Partition coefficient and partition radius vs. concentration of Apo B in 20 mM DOC/pH 8.5 buffer

These data were obtained in large zone experiments from Apo B prepared by organic delipidation and dialysis into DOC and Biogel A-1.5m (100–200 mesh) saturated with 20 mM DOC/pH 8.5 buffer at 25°C at 280 nm. All Apo B samples were incubated for 4 h at room temperature before applying to the column.

Relative dilution [a]	(dt/dx_o) [b]	Correlation coefficient	$\bar{\sigma}_w$	$\mathrm{erfc}^{-1}\bar{\sigma}_w$	Radius (Å) [c]
1.00	0.2070	0.99986	0.0457	1.41	127.81
0.50	0.1938	0.99984	0.0134	1.75	153.83
	0.5301	0.99976	0.8358	0.15	31.37
0.25	0.1949	0.99966	0.0163	1.70	150.00
	0.5337	0.99987	0.8446	0.14	30.60
20 mM DOC alone	0.5369	0.99996	0.8524	0.13	29.84

[a] The absolute protein concentration was not determined (note: 120 μg/ml could not be detected at 280 nm).
[b] The slopes of the void and internal volume markers were 0.1883 and 0.5973, respectively.
[c] Calculated from the fitted values where $a_o = 19.89$ and $b_o = 76.59$. The standard deviation in the fitted radii in each case is 5.89 Å.

tion radius of approx. 43 Å (table 3). Such particles are apparently large mixed micelles containing approx. 82% of all of the lipid, both polar and nonpolar, from the original LDL particles, and very little or no protein (table 1).

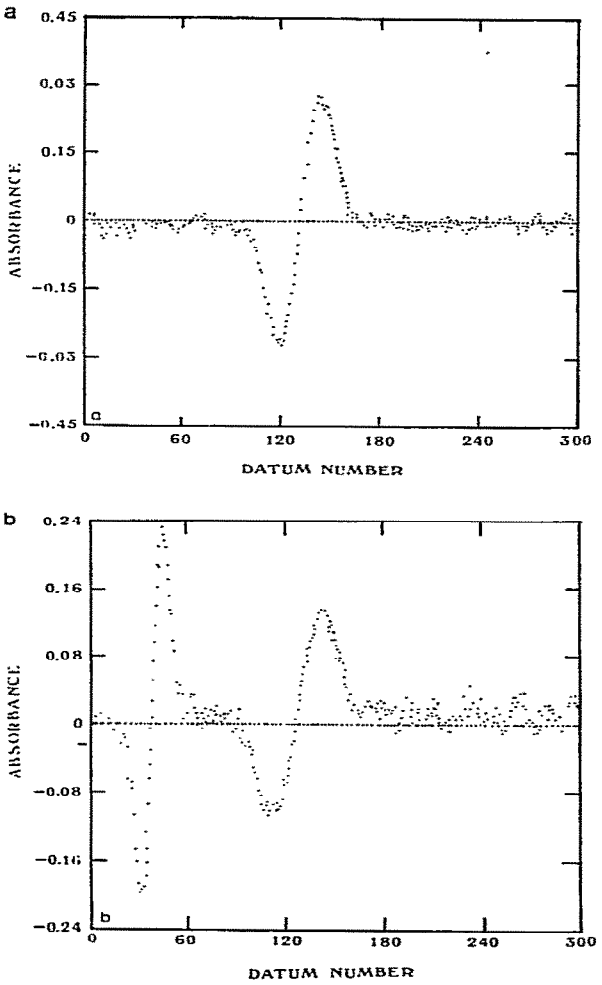

Fig. 3. Difference profiles from SMSC − Apo B in 20 mM DOC/pH 8.5 buffer. These profiles were taken from two small zone profiles 30 min after initial sample application (0.2 ml) over Biogel A-1.5m (100–200 mesh). The incubation time in DOC prior to sample application was 4 h at room temperature. (a) Undiluted sample; (b) 1/4 dilution. The two sets of peaks represent the leading and trailing edges of two classes of Apo B-DOC complexes in which the physical states of the protein are different.

Apo B-DOC complex prepared by organic delipidation-DOC dialysis showed a marked concentration dependence on particle size for the major peak, which moved much faster than expected, representing particles of radius approx. 130 Å and greater. When taken with previous results for LDL in 20 mM DOC [10], these data indicate that the protein does indeed unfold as more lipid is removed, in response to increasing the DOC-to-LDL weight ratio.

By contrast, the minor SMSC peak represents very small complexes with radii on the order of 30 Å or less.

3.4. Sedimentation velocity experiments

As seen in fig. 5, in 6 M urea/0.2 M Na_2CO_3 buffer, pH 9.7, Apo B shows an apparent sedimentation coefficient of 1.4 S, and the value of the

Table 3

Partition radii of Apo B-DOC and lipid-DOC complexes prepared from LDL by DOC gel filtration chromatography

The radii were calculated from (dI/dx) data using a $a_i = a_o + b_o\ \mathrm{erfc}^{-1}\sigma_i$, where $a_o = 19.89$ and $b_o = 76.54$. The standard deviation in the fitted radii in each case is 5.89 Å.

Source	[Apo-LDL] (mg/ml)	Incubation time [a]	Radius (Å)
Organically delipidated-DOC dialysis	2.00	–	130.1
	1.00	–	137.0
			25.3
Peak I	1.17	–	128.6
	1.17	4 days	29.1
			150.0
			38.3
Peak II	0.54	1 day	133.9
	0.54	4 days	40.6
Peak III	0.02	2 days	41.3
	0.02	7 days	44.4
20 mM DOC alone	0	–	28.3 [b]

[a] The indicated time at 4°C.
[b] Micelle partition radius of 28.3 ± 0.5 Å.

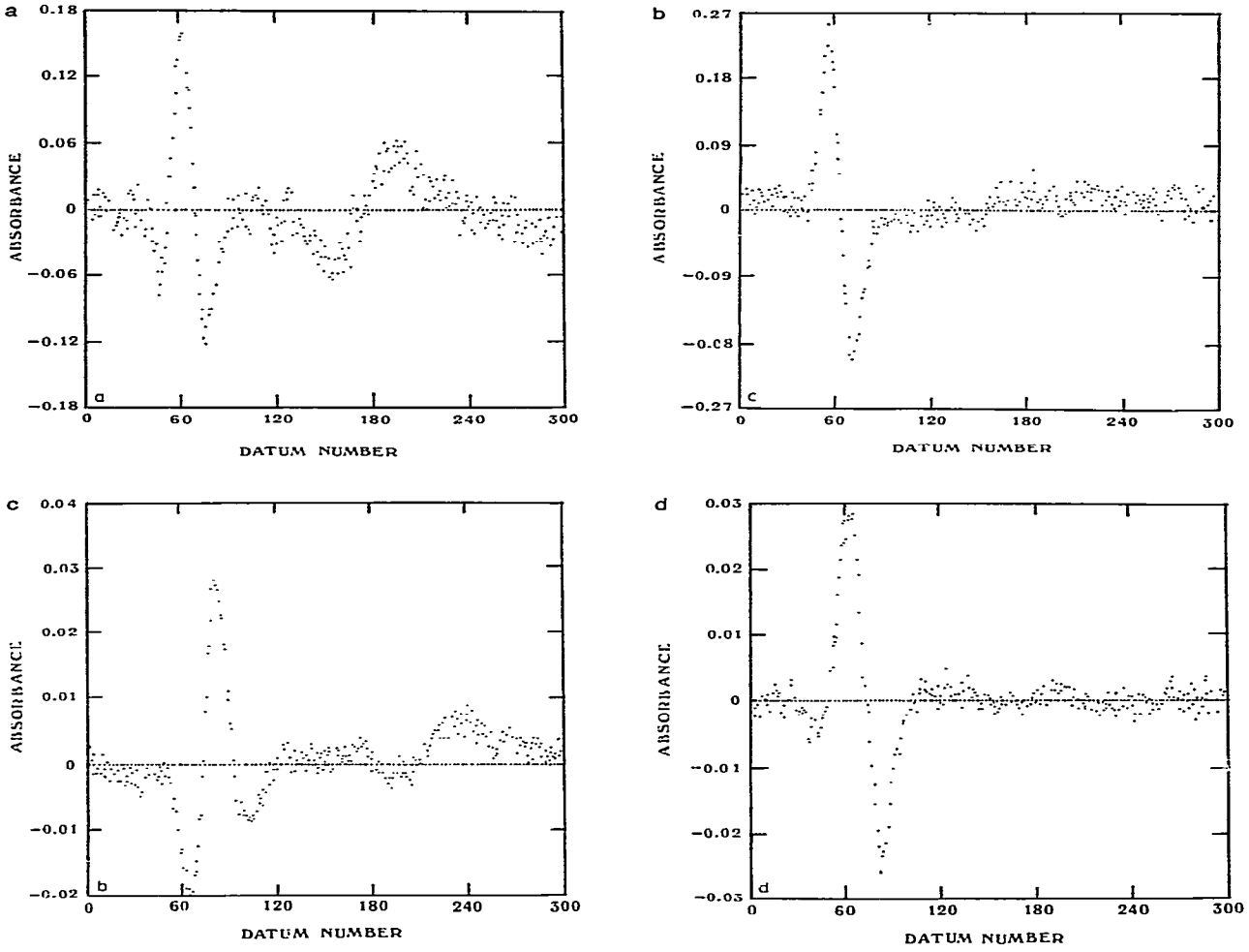

Fig. 4. Difference profiles from SMSC – Apo B- and Lipid-20 mM DOC complexes prepared from LDL by DOC gel filtration chromatography as shown in fig. 1. These profiles were obtained from two small zone profiles 30 min after initial sample application (0.2 ml) over Biogel A-1.5m (200–400 mesh). Scans were made at 280 nm. All samples were incubated for 30 min at room temperature before each column run. Prior incubation at 4°C is as indicated below. Two sets of peaks, when present, represent the leading and trailing boundaries of two classes of Apo B-DOC complexes in which the physical states of the protein are different. (a) Peak I, 1.17 mg protein/ml, 4-day incubation; (b) peak II, 0.54 mg protein/ml, 1-day incubation; (c) peak II, 0.54 mg protein/ml, 4-day incubation; (d) peak III, 0.02 mg protein/ml, 2-day incubation.

sedimentation coefficient diminishes as the length of the run is increased. The boundary disappears after an elapsed time of 3500 s at 42040 rpm. A similar phenomenon is observed even in the presence of 20 mM dithiothreitol. These results indicate either heterogeneity or aggregated polydispersity in the Apo B preparation.

Runs on the Apo B-20 mM DOC complexes

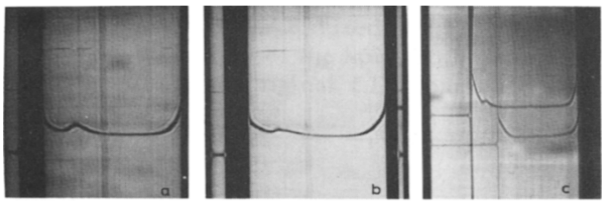

Fig. 5. Schlieren patterns of Apo B in urea and sodium deoxycholate, under various experimental conditions at 20°C and 42040 rpm. (a) Apo B in 6 M urea, Na_2CO_3 buffer, pH 9.7. Synthetic boundary cell = 1.86 mg/ml. The elapsed time was 1076 s after reaching speed. Bar angle of 50°C. (b) Apo B in 2 M urea, 0.05 M sodium phosphate buffer, pH 7.4. Synthetic boundary cell = 2.5 mg/ml. The elapsed time was 905 s after reaching speed. Bar angle of 45°C. (c) Apo B (organically delipidated), primed with a small amount of 20 mM sodium deoxycholate in 0.2 M $NaHCO_3$, pH 8.5, dialyzed against buffer for 5 days at 4°C with several changes per day. Standard cell = 1.0 mg/ml. Wedge cell = 2.0 mg/ml. The elapsed time was 1464 s after reaching speed. Bar angle of 65°.

prepared by DOC gel filtration chromatography at 8 and 20°C revealed two peaks as shown in fig. 6, but with large variability in s values among different preparations. These values ranged from 4.8 to 8.4 S for the slower moving peak and 8.5 to 14.0 S for the faster moving peak, corresponding to particles with equivalent Stokes radii [28] of 27.0 ~ 35.0 ± 0.2 and 35.0 ~ 45 ± 0.4 Å, respectively. Such variability indicates a high degree of heterogeneity in these preparations. However, the ranges of particle radii, which compare with the slower moving components in SMSC, indicate that complexes in this class are more globular than those with radii approaching 130 Å. Additionally, changes in s value may arise either through changes in particle shape or particle size or both.

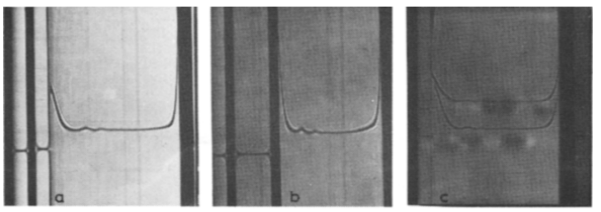

Fig. 6. Schlieren patterns of LDL and Apo B in sodium deoxycholate under various experimental conditions at 20°C. (a) LDL in 20 mM sodium deoxycholate, 0.2 M $NaHCO_3$, pH 8.5 (dialyzed against buffer for 3 days at 4°C). LDL concentrations, 4.0 mg/ml. Run made at 52640 rpm, elapsed time of 2006 s after reaching speed. Bar angle of 60°. Two sedimenting species: $s_{slow} = 6.50$, $s_{fast} = 8.50$. (b) Apo B-sodium deoxycholate (Apo B-DOC) complex, isolated from Sepharose 6B column (before fractionation LDL was incubated with 0.2 M DOC, 0.2 M $NaHCO_3$, pH 8.5, containing NaN_3, EDTA, PMSF and thimerosal for 11 h at 5°C). Run made at 52640 rpm, elapsed time of 2823 s after reaching speed. Bar angle of 60°. Initial concentration 3.0 mg/ml. (c) Apo B-DOC complex, isolated from Biogel 1.5m column (LDL was incubated with 0.1 M DOC, 0.2 M $NaHCO_3$, pH 8.5, containing NaN_3, EDTA, PMSF and thimerosal for 11 h at room temperature). LDL concentration of 3.0 mg/ml. Run made at 42040 rpm, elapsed time of 2630 s after reaching speed. Bar angle of 70°.

Sedimentation velocity runs at 20°C on Apo B-20 mM DOC complexes prepared by organic delipidation-DOC dialysis at 4°C revealed only one peak with an apparent s value of 12.0 S in water/pH 8.5 buffer and 9.10 S in 50% ^2H_2O/pH 8.5 buffer (fig. 5c). These values were used to calculate the apparent effective specific volume of these complexes as 0.741 ± 0.090 ml/g, with 0.42 g DOC bound/g Apo B. However, these values represent all classes of components present, in which there are at least two with different $\bar{v}_c$ values. It is assumed that the true $\bar{v}_c$ for each and all complexes present lies between that for Apo B, 0.725 ml/g, and DOC, 0.779 ml/g.

Interestingly, the preferential interaction term, ξ_3, is negative over this entire range from −0.007 to −0.268, indicating that the presence of DOC results in the preferential hydration of Apo B rather than a preferential DOC interaction. This suggests that the Apo B-DOC complexes bind more water than either Apo B or DOC alone. Since the molar ratio of DOC to Apo B is very large, some micelles may be used solely for removing and solubilizing residual lipid from Apo B. As this occurs, the protein may bind more DOC, and hence more water, and the partial specific volume of the complex will change. It was estimated that the change in equivalent spherical radius for such a large $\bar{v}_c$ change may be as great as 2.5 Å – the approximate thickness of one water molecule [27,28].

3.5. Sedimentation equilibrium

We examine the molecular weight distribution of Apo B (organically delipidated) as a function of

guanidine in 0.2 M NH_4HCO_3, pH 8.0, in the presence and absence of 20 mM dithiothreitol. It is apparent that the plot of ln C vs. X^2 is very similar for all experimental conditions (fig. 7). In 6 M guanidine in this buffer system, at a concentration of 0.6 mg/ml, Apo B has a weight average molecular weight of 183 900 ± 11 400 to 295 500 ± 4000 [42] (table 4), based on the apparent partial specific volume, ϕ_c', which was determined to be 0.722, consistent with published values [43–45].

For the Apo B-20 mM DOC complex prepared by organic delipidation and dialysis into DOC, the whole cell weight average molecular weight was determined to be $1.85 \pm 0.12 \times 10^5$ g/mol, assuming an effective specific volume of 0.746 ml/g, based on a binding of 0.64 g DOC/g Apo B [7]. A plot of ln C vs. X^2 for Apo B-20 mM DOC complexes at 8°C is shown in fig. 8. The molecular weight and radius of particle calculated therefrom are given in table 4. One of the characteristics of the interaction of Apo B with any surfactant is that a plot of ln C vs. X^2, such as that for Apo B with DOC shown in this figure, shows an initial linear slope which subsequently curves upward in the region approaching the bottom of the cell. This contrasts sharply with a similar plot for organically delipidated Apo B in 6 M guanidine in 0.2 M NH_4HCO_3 buffer, seen in fig. 7.

The largest values of $\overline{M}_w$ were consistently observed for Apo B-DOC samples prepared by organic delipidation-DOC dialysis (table 4), probably due to the predominant presence of the elongated complexes or aggregation. The $\overline{M}_w$ range for these samples was also the broadest, going from

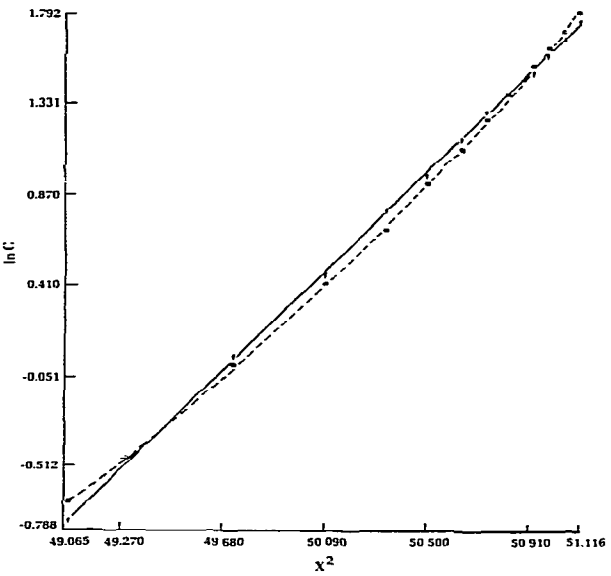

Fig. 7. Plot of ln C vs. X^2 of Apo B in 6 M guanidine/0.2 M NH_4HCO_3 buffer, pH 8.0. Run was made at 10589 rpm for 48 h. $\rho_0 = 1.1446$. Initial concentration = 1.20 mg/ml. (———) f = theoretical, computer representation of ideal case, (- - - - -) a = experimentally obtained.

Table 4

Molecular sizes of Apo B-20 mM DOC complexes

Molecular weight distributions were determined from plots of ln C vs. X^2 as previously described, from 72-h sedimentation equilibrium runs in 20 mM DOC/pH 8.5 buffer as 8225 rpm at 8°C, using $\bar{v}_c = 0.746$ ml/g and $\rho_0 = 1.0144$ g/ml. The molecular weight ranges indicated are from the top of an equivalent sphere, calculated from the whole cell weight average molecular weight using a rearrangement of eq. 2. At least one sample in each set contained 0.01% thimerosal and 0.02% PMSF.

Source	$\overline{M}_z$ range	$\overline{M}_n$ range	$\overline{M}_w$ range	Whole cell $\overline{M}_w$ [a]	Radius (Å)
Organically delipidated	232 800–567 100	110 900–211 000	183 900–295 500	235 900 ± 11 400	139.8 ± 0.1
Organically delipidated-DOC dialyzed		6570– 38 100	13 100–424 000	184 900 ± 12 300	38.0 ± 0.8
Peak I + II [b] (fresh)	133 000–133 400	47 130–104 500	142 000–604 500	163 600 ± 10 700	36.4 ± 0.8
Peak II (1-week old)		68 200–176 800	122 300–191 000	92 300 ± 6800	30.1 ± 0.7

[a] The mean value of three determinations in g/mol. The standard deviation is the largest whole cell S.D. in each set.
[b] From DOC gel filtration chromatography, as shown in fig. 1.

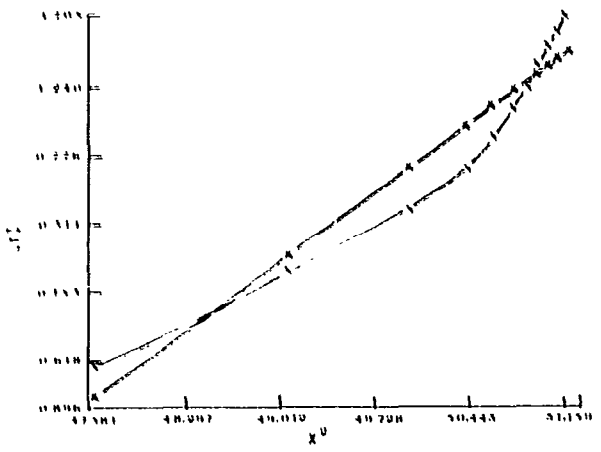

Fig. 8. $\ln C$ vs. X^2 Plot of Apo B-20 mM DOC – organically delipidated-DOC dialyzed. Apo B-DOC complex was prepared as previously described. Sedimentation equilibrium experiments were performed at 8225 rpm in an An-D rotor at 8°C using an Yphantis three-channel cell. $\ln C$ represents the natural logarithm of the fringe number (from interference optics) and X^2 represents the squared distance from the center of rotation after 45 h. The experimental values are denoted by X while the theoretical values for a linear fit are denoted by &. The concentration of protein was approx. 1 mg/ml

approx. 13000 to over 423000, indicating a greater amount of heterogeneity in these complexes. This heterogeneity is also evidenced by grossly different values of $\bar{M}_n$, $\bar{M}_z$, and $\bar{M}_w$ when extrapolated to zero concentration [46] for all sample preparations, regardless of method of preparation or aging. Also, the presence of antibiotics and protease inhibitor did not appear to have any significant effect on the molecular weight distributions.

The Apo B-20 mM DOC complexes freshly prepared by DOC gel filtration chromatography had whole cell weight average molecular weights of $1.31 \pm 0.08 \times 10^5$ g/mol for peak I and $1.63 \pm 0.11 \times 10^5$ g/mol for peaks I and II. All of these complexes were unstable and exhibited a high degree of concentration-dependent self-association, regardless of the presence or absence of antibiotics and protease inhibitors. After 1 week, peak II had a whole cell weight average molecular weight of $0.92 \pm 0.07 \times 10^5$ g/mol, using the same $\bar{v}_c$ value. The value of $\bar{v}_c = 0.746$ ml/g was calculated using $\bar{v}_3 = 0.779$ ml/g for DOC and $\bar{v}_2 = 0.725$ ml/g for Apo B, and assuming the binding of DOC to Apo B to be $\bar{x}_3 = 0.64 \pm 0.11$ g DOC/g Apo B [7]. As has been mentioned, the effect on the radius of an equivalent sphere over this range

Table 5

Molecular size and shape of Apo B-DOC complexes

Partition radii were determined by SMSC in pH 8.5 buffer at 25°C and molecular weights were determined by analytical ultracentrifugation at 8°C.

Sample	$\bar{M}_w$ ($\times 10^{-6}$)	Radius (Å)	r/r_0 [a]	Approximate shape	a/b [b]
Apo B-DOC organically delipidated	0.185 ± 0.012	140 ± 0.7 [c]	3.68	Rod	424
Apo B-DOC peak I	0.131 ± 0.008	129 ± 0.6 [d]	3.80	Rod	450
		38 ± 0.2 [d]	1.10	Prolate ellipsoid	3.30
Apo B-DOC peak II fresh	0.164 ± 0.011	134 ± 0.4 [d]	3.68	Rod	422
Apo B-DOC peak II 1-week old	0.092 ± 0.006	41 ± 0.5 [d]	1.35	Prolate ellipsoid	6.50

[a] The frictional ratio, where r_0 is the radius of an equivalent sphere calculated from $\bar{M}_w$.
[b] The axial ratio, calculated from r/r_0 according to ref. 33.
[c] Averaged value from tables 2 and 3.
[d] From table 3.

is approx. 2.5 Å, which corresponds to a layer of water one molecule thick. Therefore, the values of spherical radii given in table 4 are representative of a major class of interacting species which may indeed be globular, since these radii compare very favorably with those of the slower moving components in SMSC (table 2), as indicated by the axial ratios given in table 5.

3.6. SDS-PAGE

Apo B prepared by organic delipidation and dialysis into 6 M urea/pH 8.5 buffer resulted in many bands on SDS-PAGE, as shown in fig. 9. Also notable was the appearance of two bands with very low mobilities. Such increases in particle size are consistent with the aggregation of Apo B in denaturant solutions observed by others [17,47]. The gel pattern of this sample, dialyzed into 20 mM DOC/pH 8.5 buffer, was not significantly different, indicating that DOC has no major effects on Apo B samples prepared by organic delipidation, except possibly for a slight decrease in the mobilities of all bands. However, this decrease may simply be the result of additional aging during dialysis or of differences in equilibration time between urea and DOC across the dialysis membrane.

Fresh samples of Apo B-DOC prepared by DOC gel filtration chromatography appeared very similar to native LDL and did not give bands of very low mobility like organically delipidated samples, even after aging. That is, fresh peak I samples routinely gave one or two major bands. One, a band corresponding to the major band of LDL protein, accounted for 16% of the total protein in the gel. The most prominent band ran slightly slower than this and accounted for 54% of the total protein (fig. 9d). Many of the minor bands were not significantly different from those of LDL preparations, except for the appearance of several very minor bands of very low molecular weight. Fresh peak II samples produced slightly more lower molecular weight material, although two major bands, similar to those from peak I, were still present (fig. 9e).

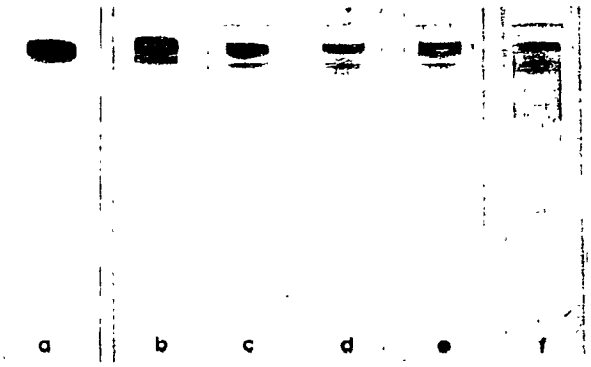

Fig. 9. SDS-PAGE of Apo B in urea and DOC. 5% polyacrylamide gels were prepared as previously described in the presence of 0.1% SDS. Samples were prepared by heating to 55°C for 1 h (unless otherwise indicated) in the presence of 1% SDS and 1% β-mercaptoetnanol and electrophoresed in the presence of 0.1% SDS, 0.1 M Tris, 0.2 M sodium acetate, and 20 mM EDTA, pH 7.4. Approx. 20–40 μg protein were layered onto each gel. The gels were stained with Coomassie blue. (a) LDL in pH 8.5 buffer; (b) Apo B in 6 M urea; (c) Apo B in 20 mM DOC (organically delipidated-DOC dialyzed); (d) Apo B in 20 mM DOC (peak I); (e) Apo B in 20 mM DOC (peak II); (f) Apo B in 20 mM DOC (peak II), 10 days old.

4. Discussion

4.1. Isolation of Apo B-DOC complexes

The profile shown in fig. 1 for isolation of Apo B-DOC complexes by gel filtration was obtained for each of seven preparations in which the DOC-to-Apo B ratio was greater than 60:1. Lower ratios have previously been reported to result in incomplete removal of lipid [1,48]. In this case, two protein peaks were obtained (I and II) which contained approx. 0.16 and 0.40 g lipid/g protein or a 95 and 87% removal of lipid, respectively, from LDL.

Helenius and Simons [6] have reported using 57 mg DOC/mg Apo B to obtain only one protein peak with approx. 1% lipid remaining bound, using this technique at high pH. Although the higher pH may have aided in lipid stripping, these workers used Sephadex G-200, which would not have separated protein-DOC complexes of varying compositions. Suzuki et al. [48] used the same approach for delipidation of human ascites plasma

LDL, which contains larger amounts of triglyceride than serum LDL. They found that complete delipidation occurred only when the DOC/Apo B weight ratio was 115 : 1, since a ratio of 57.5 : 1 resulted in a complex in which no triglyceride was removed while other lipids were.

In the present study, 90 mg DOC/mg Apo B was used to obtain the results shown in table 1. Use of Biogel A-1.5 m resulted in two peaks of differing composition. Although the removal of lipid from peak I was much greater than that from peak II, peak I eluted first. This result supports the premise that the Apo B is unfolded or elongated as more lipid is removed. Additionally, even though the peak II complex contained significant amounts of triglyceride and total cholesterol, it is apparent that the bulk of phospholipid is more easily removed. This phenomenon has been noted before using this and other detergents [6,44,48] and indicates that Apo B may interact more strongly with nonpolar lipids (cholesteryl ester and triglyceride) than with polar ones (phospholipid and unesterified cholesterol). It has also been shown that Apo B binds very tightly to free fatty acids, even after extensive extraction with organic solvents [49]. Since most or all of the protein in LDL is near the surface [6,27,28,50–56], this suggests that such interactions may give rise to a distinct interface region between the inner and outer hydrophobic core, consisting of protein and either triglyceride or cholesteryl and beginning at the ends of the phospholipid acyl chains [28]. If the Apo B is indeed one peptide chain, such partitioning would be expected to influence the conformation of Apo B at the LDL surface and, therefore, affect the binding propensity to LDL receptors.

In any case, the immunological properties of Apo B-detergent complexes must certainly be dependent on the mechanism and efficiency of lipid displacement, as well as the mode of interaction of a particular detergent with Apo B [54]. Such properties have been studied by Helenius and Simons [6,7] for several Apo B-detergent preparations. They concluded that the use of DOC and the nonionic detergents NP40 and Triton X-100 results in the isolation of Apo-LDL which carries all the major immunological determinants of the intact LDL particle, and that these have not undergone conformational changes resulting in detectable immunochemical alterations, while the use of other detergents, such as SDS and cetyltrimethylammonium bromide, results in altered immunological properties. Apparently, DOC and the nonionic detergents are more successful at mimicking the lipid environment (especially cholesteryl ester and triglyceride) of Apo-LDL. However, in the presence of DOC, this delipidation protein is unstable and readily dissociates into subunits.

4.2. Interaction of Apo B and DOC

Apo B prepared by organic delipidation and dialysis into 20 mM DOC routinely gave two peaks in SMSC experiments. Such samples, upon increasing the DOC/Apo B weight ratio, exhibited significant increases in the radius of the major SMSC peak (130 to 150 Å), while the radius of the minor SMSC peak remained relatively constant (25 to 31 Å). The minor peak appears to represent small complexes which are the result of dissociation and the size of these complexes do not appear to be significantly affected by the presence of DOC. Although it was not possible to determine the composition of these complexes, it is not unreasonable to assume that this minor peak represents Apo B-DOC complexes in which the protein is in a different physical state from that in the major peak. However, it is also possible that this minor peak represents lipid-DOC complexes resulting from displacement by DOC of the residual lipid still bound to Apo B, even after organic delipidation. This is unlikely, since the amounts of lipid present may be too small to detect at 280 nm, and there is no carotenoid present to follow the absorbance at 484 nm. Additionally, this minor SMSC peak is sharp, indicating the presence of minimal heterogeneity. Such a particle may be a stable, more compact form of the Apo B-DOC complex which is formed from the relatively unstable elongated Apo B-DOC complex (table 5). However, the sizes of the large complexes are affected by the presence of DOC and such large increases may be the result of either DOC-induced reassociation of subunits or an unfolding or elongation of Apo B upon removal of residual lipid. Examination of fig. 3a and b shows a shift in the

relative amounts of the two peaks. indicating that the extent of dissociation is also dependent on the DOC/Apo B weight ratio.

The relative rates of this dissociation process for Apo B-DOC complexes prepared by DOC gel filtration were also assessed by SMSC (table 3). Fresh samples of peaks I and II appeared very similar to organically delipidated-DOC dialyzed samples with regard to the relative sizes and rates of movement of the major SMSC peaks (130–134 $\pm$ 0.5 Å) and minor SMSC peaks (27.0–29.0 $\pm$ 0.3 Å). Incubation of these samples for 4 days under nitrogen at 4°C seemed to have the same effect on the major peak as increasing the DOC/Apo B ratio did on organically delipidated-DOC dialyzed samples. That is, the major SMSC peak became broader, with a faster rate of movement while decreasing in amount, again indicating that both dissociation and aggregation or elongation processes were taking place. However, the minor SMSC peak not only increased in amount, but also increased in its rate of movement, suggesting the presence of a heterogeneous mixture of Apo B-DOC complexes containing small peptides and lipid-DOC complexes resulting from removal of residual lipid which was still bound to peaks I and II upon isolation. That lipid-DOC complexes were present was evidenced by the similarity of the SMSC profile for peak II after 4 days (fig. 4c) to the profile for peak III (fig. 4d), which was essentially unchanged over the course of 1 week.

The molecular weights of these complexes were assessed by sedimentation equilibrium, and the shapes of the ln C vs. X^2 plots indicate a great deal of heterogeneity and nonideal behavior as a result of the presence of two classes of highly interacting components. One major class is a very elongated Apo B-DOC complex and another is a smaller, more globular Apo B-DOC complex. The large curvature in these plots prohibited determination of an accurate value of $\bar{v}_c$ and further suggested that the dissociation and reassociation processes taking place may be accompanied by large volume changes as well. The degree of curvature was also dependent on method of delipidation and age of the sample. Although the value $\bar{v}_c = 0.741 \pm 0.090$ ml/g was provided by sedimentation velocity experiments, the large error in the sedimentation coefficients was again reflective of the high degree of heterogeneity present. The preferential interaction term, ξ_3, is negative over the entire range of anticipated $\bar{v}_c$ values, in contrast with the positive ξ_3 value determined for the LDL-1 mM DOC complex [19]. This suggests that delipidated Apo B undergoes a preferential hydration upon interaction with DOC. Such changes in preferential hydration, even if they are small, would result in large changes in $\bar{v}_c$ and may even be responsible, at least in part, for the dissociation of Apo B into subunits in the presence of DOC.

Using a value of $\bar{v}_c = 0.746$ ml/g with the sedimentation equilibrium data gave whole cell weight average molecular weights ranging from 142 000 to 605 000 $\pm$ 10 700 g/mol for freshly prepared samples of peaks I and II and 92 000 $\pm$ 6800 g/mol for the peak II complex after 1 week. These values were then combined with SMSC results to estimate the approximate shapes of the various species present (table 5). Although this approach was based on averages, it does illustrate that there may indeed be large volume differences between the two classes of components present. Additional evidence that the large species are elongated comes from the fact that fresh peak II complex had an intrinsic viscosity of approx. 80 ml/g. Reexamination of viscosity data (plot of reduced viscosity versus concentration) suggests the presence of two distinct components. If the curve is separated into two sets of straight lines, the intercepts yield intrinsic viscosities of approx. 77 and approx. 89 ml/g, suggesting that both components or peptides are elongated. It is not known whether the smaller $[\eta]$ value represents the slightly smaller of the two major bands seen in SDS-PAGE for such samples (fig. 9). If the smaller peptides (minor bands on SDS-PAGE) are indeed globular, one would expect an increased downward curvature of the reduced viscosity vs. concentration plot with time, with the upper portion of the curve remaining relatively constant.

The SDS-PAGE results for Apo B in 6 M urea and 20 mM DOC (fig. 9b and c) are not significantly different in appearance, although there are two more major bands for the DOC dialyzed sample, with slight decreases in the mobilities of all bands present. Again, this suggests that the

organic delipidation process or the use of denaturants, such as guanidine or urea, results in a certain amount of dissociation and irreversible aggregation of Apo B, as has been previously reported [17,47], with the increased incubation time in the presence of DOC possibly resulting in slightly more aggregation. This aggregation is also affected by temperature and the presence of reducing agents [19,57].

The differences in the effects of aging of peaks I and II in SDS-PAGE patterns are apparently due to differences in lipid composition. The lipid-rich peak II complexes may undergo further elongation as more lipid is removed with time, resulting in bands of lower mobility. However, the peak I complexes, which contain much less lipid and are already elongated, may expose certain labile protein-protein or protein-lipid domains which than become susceptible to disruption. Increases in hydration with DOC binding may also aid in dissociation into subunits.

The reason for the instability of Apo B in the presence of DOC is unknown, but it is not a result of protease contamination or lipid peroxidation. Furthermore, until a detailed analysis of the molecular composition of Apo B is made available, as well as its orientation in the LDL particle being elucidated with respect to other components, the question of whether Apo B exists as one or more subunits in the intact LDL article will remain unanswered.

In summary, then, our findings suggest that:

(1) Apo B-DOC complexes, isolated by DOC gel filtration chromatography, are initially very large and elongated with a radius of 131 Å and an intrinsic viscosity of 80 ml/g. However, these complexes readily dissociate into many smaller subunits of varying sizes and shapes, resulting in a very heterogeneous and highly interacting system.

(2) These Apo B-DOC complexes were also shown to undergo a preferential hydration upon DOC binding, which may contribute to large changes in v_{ξ}^{0}, as well as to dissociation into subunits.

(3) Finally, the instability of Apo B in the presence of DOC is not due to lipid peroxidation or to the presence of proteases, since samples were sealed under nitrogen at 4°C in the presence of azide, methiolate, EDTA and PMSF.

Acknowledgements

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