EFFECT OF SODIUM DODECYL SULPHATE ON HUMAN PLASMA LOW DENSITY LIPOPROTEINS

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1. Introduction

Sodium dodecyl sulphate (SDS) and other detergents are unique protein denaturants because they are able to produce drastic changes in protein structure at low concentrations of detergent leading to conformational changes [1], and to dissociation of reduced proteins into their constituent polypeptide chains [2].

Gotto et al. [3] recently reported partial separation of lipid from human plasma low density lipoproteins (LDL) by density gradient centrifugation after treatment with SDS. This communication reports the effect of SDS on LDL. The lipids are completely separated from the protein component when LDL is treated with 0.2 M SDS followed by gel filtration on Sepharose 4B using a buffer containing SDS.

2. Material and methods

LDL (density: 1.019–1.063) was prepared from the plasma of normal human males by preparative ultracentrifugation as described by Havel et al. [4]. Ultracentrifugation at density 1.063 was repeated at least once. The isolated LDL was dialyzed against 0.15 M NaCl containing 0.05% EDTA. The preparations were homogeneous when studied in immunoelectrophoresis and double diffusion in agar gel using rabbit anti-human serum. LDL was maleylated with maleic anhydride as described by Butler et al. [5]. SDS (Fluka) was recrystallized three times before use. Gel filtration was done on Sephadex G-200 or Sepharose 4B equilibrated with 0.1 M tris pH 7.7 and 0.1% SDS at room temperature. The columns were calibrated with blue dextran and 2-mercaptoethanol. SDS-disc electrophoresis with tris-glycine buffer was performed as described by Davis [6] except that the gels and buffers contained 0.1% SDS. In some runs, the upper buffer contained 0.1% 2-mercaptoethanol. The lower gel contained 6.25% polyacrylamide. Molecular weights were determined in this electrophoresis system with no mercaptoethanol in the upper buffer. Bovine albumin, human transferrin and human IgG were used as reference proteins. The albumin preparation contained monomer, dimer and trimer forms when examined by SDS electrophoresis, cf. [7]. Relative mo-

Table 1

	Protein (mg)	Phospholipid (mg)	Total cholesterol (mg)	Triglyceride
LDL prior to SDS-treatment	8.6	8.4	13.5	not determined
Fraction I from Sepharose 4B	7.6	0.03	0.02	traces

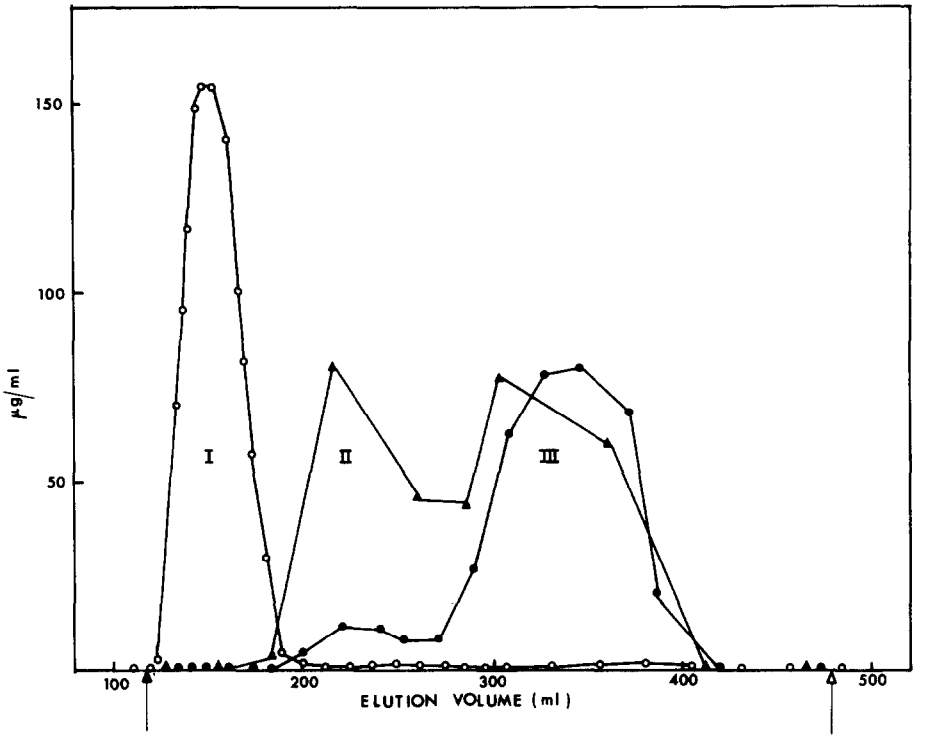

Fig. 1. Gel filtration of SDS-treated LDL (8.6 mg protein) in Sepharose 4B (2.5 X 90 cm column). Buffer: 0.1 M tris-HCl, pH 7.7 containing 0.1% SDS. Elution rate: 6 ml/hr. Protein o——o, phospholipid •——•, total cholesterol ▲——▲. Elution volume of of blue dextran marked with a solid arrow and that of mercaptoethanol with an open arrow.

bilities were calculated as described by Weber and Osborn [8] using bromphenol blue as reference dye. After electrophoresis gels were fixed with 20% sulphosalisylic acid for 16 hr, stained with coomassie blue and destained. Protein was determined by the Lowry method [9] with bovine serum albumin as standard; 0.1% SDS was included in the reaction mixture. Additional methods were: phosphorus analysis according to Bartlett [10], total cholesterol according to Abell et al. [11], qualitative lipid analysis using thin-layer chromatography [12], SDS determinations according to Karush and Sonenberg [13].

3. Results

SFS at final concentration of 0.2 M was added to 4 ml (8.6–15 mg protein) of dialyzed LDL. After 4 hr at 37°C with slow stirring, the sample was applied to an upward flowing column of Sepharose 4B, and eluted with 0.1 M tris, pH 7.7, and 0.1% SDS. A typical elution pattern is shown in fig. 1. There was a single protein peak (I) and two phospholipid-containing fractions (II and III). Fraction II had a cholesterol:phospholipid ratio of 8.0 whereas this ratio for fraction III was 1.3. Qualitative lipid analysis showed that the same phospholipids were present in both fractions. Table 1 shows the analysis of the protein fraction which contained less than 1% lipid in 4 different experiments. LDL preparations that were maleylated before incubation with SDS had an elution pattern similar to that shown in fig. 1. Thus anionic SDS was still effective when positive lysine amino groups were blocked with negative maleyl groups. If Sephadex G-200 was used instead of Sepharose 4B, the protein peak (I) was eluted together with lipid fraction (II) in the void volume.

SDS-disc electrophoresis of LDL treated with 0.2 M

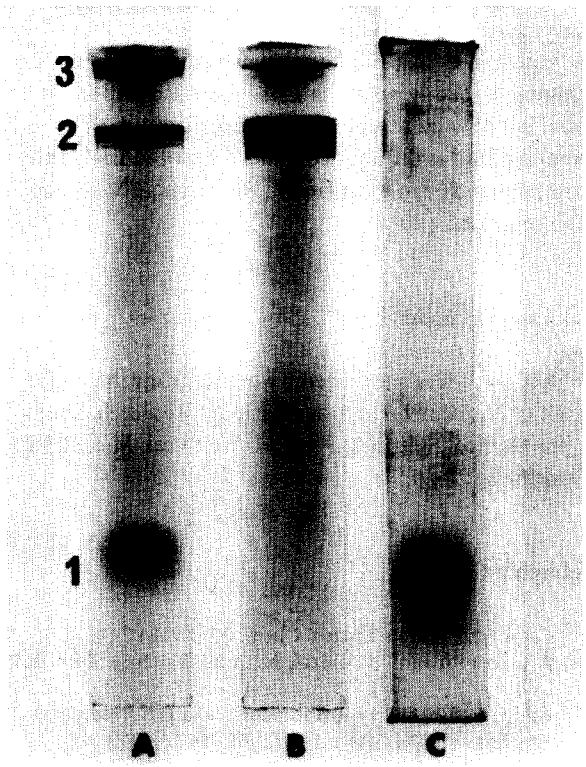

Fig. 2. SDS-polyacrylamide disc electrophoresis of A. LDL (10 μg protein) treated with 0.2 M SDS and 0.1% mercaptoethanol, B. fraction I (40 μg protein) and C. fraction III obtained after SDS-treatment of LDL and subsequent Sepharose 4B gel filtration (see fig. 1). The upper buffer contained 0.1% mercaptoethanol. Anode at the bottom. 1 mA per tube 30 min and 2.5 mA for 3 hr. Stain: coomassie blue.

SDS gave three major components [14]: a rapidly migrating band (1) behind the buffer front, a slower band (2) and in some samples an additional faint band ahead of this component, and a band (3) that barely entered the gel, probably aggregate. Less of band 3 was

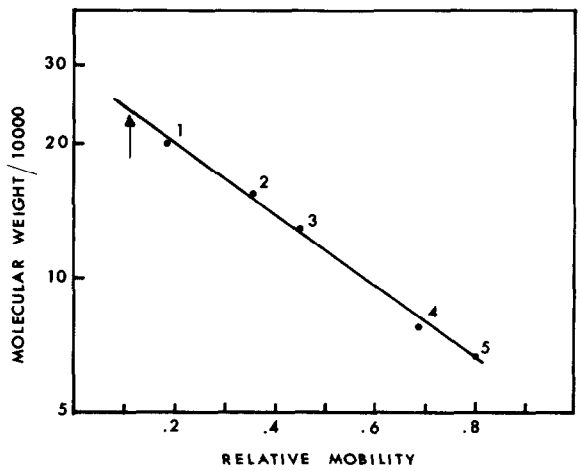

Fig. 3. Apparent molecular size of the LDL protein component (arrow) estimated by SDS-disc electrophoresis. 1. Bovine albumin trimer. 2. Human IgG. 3. Bovine albumin dimer. 4. Human transferrin. 5. Bovine albumin monomer.

seen when 0.1% 2-mercaptoethanol was present during SDS treatment and in the electrophoresis buffer (fig. 2A). Protein component I from Sepharose 4B gel filtration corresponded to bands 2 and 3 (fig. 2B). The lipid fraction III (fig. 2C) migrated rapidly behind the buffer front, whereas lipid fraction II also contained material which barely entered the gel. Calibration with marker proteins gave an apparent molecular weight of about 230,000 for the SDS-solubilized LDL protein component (fig. 3). Equilibrium dialysis against 0.1% SDS in 0.1 M tris, pH 7.7 at room temperature was used to find out how much SDS was bound by the protein component isolated by Sepharose 4B gel filtration; each mg of protein bound 2.6 mg of SDS. All attempts to remove the bound SDS (dialysis, electrodialysis, gel filtration, anion exchange) resulted in precipitation if the SDS:protein ratio was reduced to less than 0.25.

4. Discussion

We have shown that the lipid and protein of LDL can be completely separated without the use of organic solvents by SDS treatment followed by gel filtration in buffer containing SDS. Similar results have been re-

ported when the membranes of *Micrococcus lysodeicticus* [15], *Mycoplasma* [16], *Escherichia coli* [17], and Semliki Forest virus [18] were treated with detergent.

Two SDS lipid complexes of different sizes could be separated from the protein component of LDL by gel filtration on Sepharose 4B. The larger of these had a higher cholesterol-phospholipid ratio than the smaller complex. This may be explained by earlier findings that there is a pronounced increase in micelle molecular weight as the amount of solubilized cholesterol increases [19].

Previously, we found that maleylated and partially delipidated LDL gave two components on gel filtration in the presence of 0.1% SDS [14]. The present results show that the first component which had a higher molecular weight was the protein-SDS complex and the second smaller component was the residual lipid complexed to SDS.

SDS-disc electrophoresis indicated that the protein component of LDL had an apparent molecular weight of about 230,000 when compared with standard proteins. In the only study so far where detergents were not used in the delipidation procedure Scanu et al. [20] obtained a value of 36,000–38,000 for the molecular weight of the LDL polypeptide chain by sedimentation equilibrium analysis at pH 11.6 of the succinylated and delipidated LDL. This discrepancy is probably due to the unusually high SDS binding of the LDL protein and to complex formation involving more than one polypeptide chain. Equilibrium dialysis showed that the LDL protein bound 2.6 times its weight of SDS whereas most other proteins including albumin and IgG bind only 0.7 to 1.4 mg SDS per mg protein in similar conditions [21]. Therefore, the convenient SDS-disc electrophoresis method for determining molecular weights [2] cannot be used to estimate the molecular weight of the LDL polypeptide chain. This may be the case also for other lipophilic proteins.

The mechanism for the delipidating effect of SDS on LDL is not known. Optical rotatory dispersion and circular dichroism studies [22, 23] have shown that SDS above its critical micellar concentration produces significant conformational changes in the structure of LDL. This probably facilitates SDS penetration into the interior of the spherical LDL molecule. The LDL lipid would thus be displaced by SDS [cf. 24], transferred into SDS micelles and solubilized. SDS is bound by the protein to newly exposed sites which probably interact with the hydrophobic moieties of the detergent molecules to form micelle-like regions [1].

The solubility of the LDL protein seems to depend on bound lipid or detergent. We failed to produce a LDL protein which was water-soluble at neutral pH and free of both lipid and detergent; similar attempts using extraction with organic solvents were also unsuccessful [see 25]. SDS-dissociated lipid seems to reassociate readily with the LDL protein component under suitable conditions (unpublished results). We therefore find it difficult to believe that the LDL apoprotein as such can exist free in the circulation as has been proposed [26].

Acknowledgements

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