SUBUNIT STRUCTURE OF THE APOPROTEIN OF HUMAN SERUM LOW DENSITY LIPOPROTEINS*

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SUMMARY

Human serum low density lipoproteins (d 1.027-1.043 g/cm^3) were prepared by preparative ultracentrifugation and delipidated with sodium deoxycholate. By electrophoresis in sodium dodecyl sulfate polyacrylamide gel, the apoprotein was fractionated into major components with apparent molecular weights of 77,000, 66,000, 47,000, 33,500, 21,500, 13,000, and 9,500, respectively; and minor components of higher molecular weight. The data indicate the existence of at least two fundamental subunits of molecular weights of approximately 9,500 and 13,000 daltons.

INTRODUCTION

The subunit structure of LDL‡ has been the subject of many investigations and rather different results have been obtained (1-7). Varied strategies have been employed by different investigators to render the apoLDL soluble in aqueous solution; viz., chemical modification, alkaline pH, and the use of strong anionic detergents, all of which further increased the differences between experimental data.

In the current study, we used the bile salt sodium deoxycholate to delipidate LDL. The apoprotein was then separated into constituent components by polyacrylamide gel electrophoresis in the presence of SDS.

MATERIALS AND METHODS

LDL of d 1.027-1.043 g/cm^3 was isolated from normal human serum by sequential preparative ultracentrifugation at 16°C, and characterized

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‡Abbreviations used: LDL, low density lipoproteins; apoLDL, the delipidated apoprotein of LDL; SDS, sodium dodecyl sulfate; NaDOC, sodium deoxycholate; DTT, dithiothreitol.

by analytical ultracentrifugation, and by immunodiffusion (8). Since it has been suggested that LDL contains protease-like activity (9), we used the proteolytic enzyme substrate Azocoll (Calbiochem) to ascertain the presence of proteolytic activity in LDL preparations. Azocoll was incubated with LDL at 37°C for 48 hours. No proteolytic activity was detected. Furthermore, contrary to that report, we observed no difference in SDS-gel band patterns between LDL freshly isolated, LDL stored in the refrigerator for up to 24 days and LDL stored in the refrigerator in the presence of 1 mM phenylmethyl sulfonyl fluoride for 8 days.

ApoLDL was prepared by the method of Helenius and Simons (10) with minor modifications. The protein peak, which was well separated from all lipids, was concentrated by ultrafiltration with PM-30 membranes (Amicon). To remove bile salt, the concentrated material from Sephadex G-200 column was passed through a Sephadex G-75 column and eluted with 0.1 M Tris-HCl buffer; the protein peak was then concentrated by either ultrafiltration with PM-10 membrane or lyophilization after desalting. In control experiments LDL was delipidated by organic solvents as described by Albers and Scanu (11).

SDS-polyacrylamide gel electrophoresis was performed according to the procedure of Fairbanks, et al. (12). Samples were treated by adding SDS to 1%, EDTA to 2 mM, DTT to 50 mM, heated in boiling water for 2-3 minutes, then transferred to a 37° water bath and incubated overnight before application. Gels were stained for 16 hours in 0.2% Coomassie Brilliant Blue R 250 dissolved in 50% methanol, with 7% acetic acid added just before use. Destaining was carried out in methanol-water-acetic acid (5:5:1, v/v) in a diffusion destainer (Hoefner Scientific Instrument) for 36 hours. The molecular weights of protein bands were determined from semi-logarithmic plots of molecular weight versus relative mobility, using as standards: phosphorylase a, bovine serum albumin, glutamate dehydrogenase, ovalbumin, pepsin, chymotrypsinogen A, myoglobin, and lysozyme.

The individual bands from unstained SDS-gel were eluted following the procedure described by Weber, et al. (13). The localization of each band was facilitated by the fact that the mobility of each component that resolved in SDS-gel is fixed under constant defined experimental conditions so that no staining and destaining is needed. The eluting solution was 5 mM NH_4HCO_3 containing 0.05% SDS.

RESULTS

ApoLDL prepared by the NaDOC delipidation method was fractionated by electrophoresis in SDS-gel into five major components and several minor components (Fig. 1, B). The five major components have apparent molecular weights of 77,000 (Band III), 66,000 (Band IV), 47,000 (Band V), 33,500

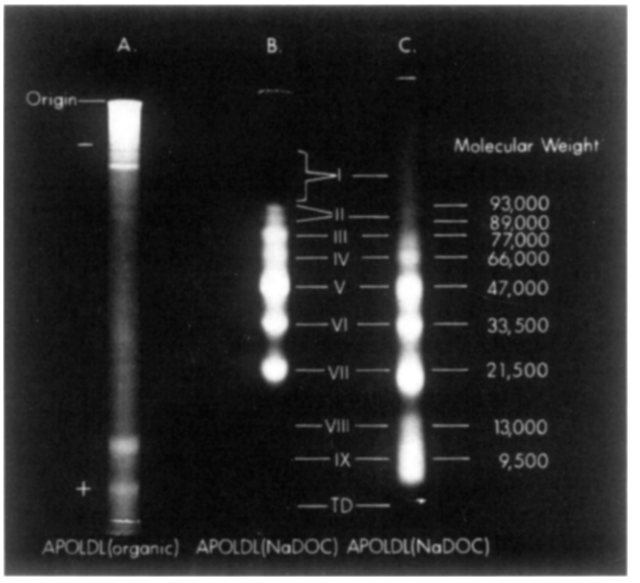

Figure 1: SDS-polyacrylamide gel electrophoresis of apoLDL. (A) ApoLDL prepared by organic solvents extraction. (B) ApoLDL prepared by NaDOC delipidation. (C) Same as (B) but dialyzed against a low ionic strength buffer and retreated with SDS, EDTA and DTT.

(Band VI), and 21,500 (Band VII), respectively. One or two minor components were sometimes observed in the region between bands V and VI. The materials in region II consist of two components, one with molecular weight of approximately 89,000, the other 93,000. Inclusion of dithiothreitol during sample treatment did not cause any apparent qualitative change in the band pattern of apoLDL in SDS-gel. It did, however, reduce significantly the relatively staining intensity in regions I and II. The region labeled VIII was a diffuse, weakly stained zone.

In the early phase of our studies, the bile salt was removed from the apoLDL before SDS polyacrylamide gel electrophoresis was performed on the sample. It was found later, however, that equivalent SDS-gel band patterns could be obtained if the detergent-removal step was omitted and the apoLDL treated directly with SDS, EDTA and DTT. In this case, the complexed NaDOC was found to dissociate from the apoprotein during electrophoresis and migrate just in front of the tracking dye.

For comparison, the electrophoretic pattern of apoLDL obtained by organic solvents extraction is shown in Figure 1, A. While discrete bands are present, most of the material had molecular weight higher than 100,000 and remained at the top of the gel. Two components with mobilities similar to bands VIII and IX (Fig. 1, C) are apparent.

When the treated apoLDL (from Sephadex G-200 column) was dialyzed against a low ionic strength buffer (0.01 M Tris-HCl-0.1% SDS, pH 8.0), concentrated and retreated with SDS, EDTA and DTT, a somewhat different result was obtained upon SDS-gel electrophoresis (Fig. 1, C): the relative staining intensity of bands III and IV was greatly diminished, while that in regions VIII and IX was greatly increased. The materials in these regions were resolved into two components: band VIII with apparent molecular weight of 13,000; and band IX with apparent molecular weight of 9,500.

Upon elution and re-electrophoresis in SDS-gel each major component of NaDOC delipidated material was found to migrate as a single band with its original mobility (Fig. 2). The constituent components, once isolated from SDS-gel are stable, and do not reassociate upon mixing. This is demonstrated in Figure 3. Several pairs of eluted components were co-electrophoresed. Each component was found to retain its original electrophoretic mobility. The pattern on the left was a mixture of bands VI and VIII; the pattern on the right was a mixture of bands VII and IX.

To ascertain whether the multiple bands observed in SDS-gel were due to differential SDS binding, apoLDL was run at three different acrylamide gel concentrations, and a plot of relative mobility versus percentage of

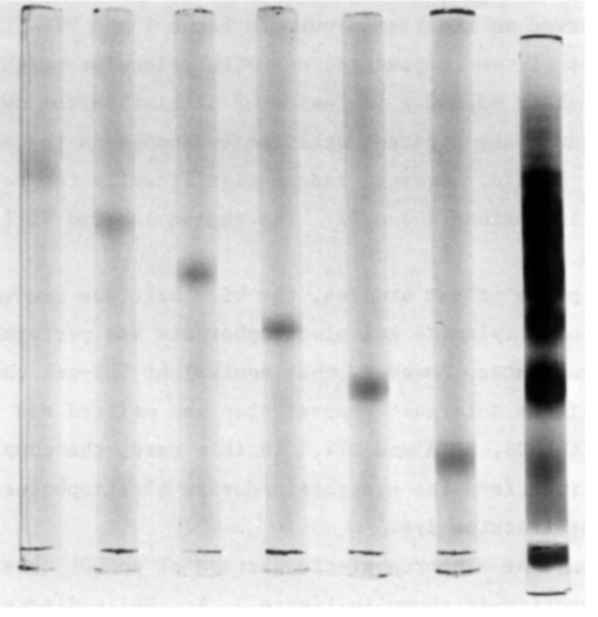

Figure 2: SDS-polyacrylamide gel electrophoresis of apoLDL and its isolated single components. The samples were, from left to right: bands II, IV, V, VI, VII, VIII and apoLDL.

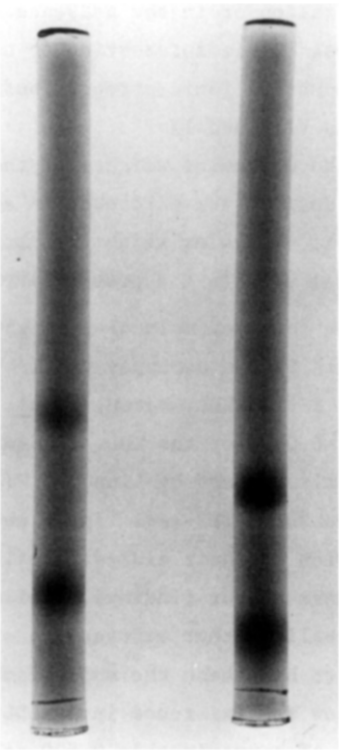

Figure 3: Co-electrophoresis of eluted components of apoLDL. The sample for the gel at left was a mixture of bands VI and VIII, the sample for the gel at right was a mixture of bands VII and IX.

acrylamide was made. The data points for bands II through VI all extrapolated to the same intercept on the ordinate, indicating that these components bound essentially the same amounts of SDS. Bands VII and VIII bound slightly different amounts of SDS. The actual molecular weights of these components might, therefore, be slightly different from the values given in Figure 1.

DISCUSSION

The results in Figure 1 demonstrate clearly that the method of delipidation greatly affects the physical state of apoLDL. Delipidation by organic solvents induces irreversible aggregation of apoLDL, even in the presence of SDS and DTT. This may explain the wide variation in reported values for the subunit molecular weight of apoLDL.

The fact that inclusion of dithiothreitol during sample treatment did not cause any apparent qualitative change in the band pattern of apoLDL in SDS-gel suggests that intermolecular disulfide bonds are probably not involved in the maintenance of the subunit structure of apoLDL.

At high protein concentration or in the presence of salt, the lipid-free apoLDL readily aggregates. It is interesting to note that dialysis of NaDOC-delipidated apoLDL against low ionic strength buffer enhances substantially the resolution of bands VIII and IX.

As shown in Figure 1, the molecular weights of the five major components (Band III through VII) suggest the existence in apoLDL of an oligomeric series with a monomeric subunit molecular weight of about 11,000. Also compatible with these experimental data is the possibility of having two different subunits in apoLDL, with their combined molecular weights of about 22,000. Our observation of band VIII and IX favors strongly the latter alternative. Based upon X-ray scattering work on native LDL, Mateu, et al. (7) recently suggested a subunit molecular weight of 8,000 for the LDL. A minimum molecular weight of about 10,000 was subsequently deduced by Lipp and Wiegandt (14) from their electrophoresis studies on apoLDL in SDS-gel. These authors, however, observed reaggregation and disaggregation of their eluted fractions upon re-electrophoresis. This is in marked contrast to our findings. While we do not know the cause for the difference, we believe that exposure to organic solvents during sample preparation is likely to have been the major factor.

In summary, our data show the existence in apoLDL of two fundamental subunits of molecular weights of approximately 9,500 and 13,000 daltons, respectively.

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