THE STRUCTURE AND PROPERTIES OF HUMAN BETA-LIPOPROTEIN AND BETA-APOPROTEIN

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Little is known about the structure of the protein of serum beta-lipoprotein owing to the insolubility of the delipidated product (beta-apoprotein). Attempts have been made to solubilize the apoprotein (Granda and Scanu, 1966) or a chemical derivative of the apoprotein (Gotto et al., 1968a, b, c; Scanu et al., 1967; Shore and Shore, 1967). This communication describes the preparation of beta-apoprotein by solubilization with sodium decyl sulfate. The apoprotein closely resembles the parent lipoprotein in immunological properties and structural conformation. The circular dichroism and infrared spectrum of beta-lipoprotein indicate a significant amount of pleated sheet, anti-parallel chain (APC) β-structure. This is also true for the apoprotein, except that the molar ellipticity at 216 mμ ($[\theta]_{216}$) is reduced by about 25%. The apoprotein has a fibrillar appearance when viewed with the electron microscope by the technique of negative staining.

MATERIALS AND METHODS

Preparation and Delipidation of Beta-lipoprotein

Beta-lipoprotein was prepared by precipitation with heparin and manganese and ultracentrifugation between densities 1.019 and

1.063 as previously described (Gotto et al., 1968b), and was pure by immunochemical criteria (Levy and Fredrickson, 1965). After delipidation with ether-ethanol (3:1), the product contained no detectable cholesterol or triglyceride and approximately 1% phospholipid (Gotto et al., 1968b).

Investigational Techniques

Circular dichroism was measured in quartz cells (0.5 mm light path) using a Cary 60 spectropolarimeter at 24°. The solvent employed was 0.05 M potassium phosphate (with 0.01% EDTA), pH 7.9, and the protein concentrations varied from 0.2 to 1.0 mg per ml. Values were corrected for the refractive index of the solvent. Infrared spectra were obtained with an IR7 Infrared Spectrometer in calcium fluoride cells (0.1 mm path length). The solvent system was 0.05 M potassium phosphate (with 0.01% EDTA) in 100% D_2O at pD 7.9 and the protein concentrations varied from 15 to 30 mg per ml.

Native beta-lipoprotein (0.1 mg/ml in 5 mM Tris buffer, 0.01% EDTA, pH 8.3) and beta-apoprotein (0.1 mg/ml in 5 mM Tris buffer, 0.01% EDTA, 0.5 mM sodium decyl sulfate, pH 8.3) were placed on Formvar carbon-coated 400 mesh grids negatively stained with 1% potassium phosphotungstate, pH 7.0, and observed with a Phillips EM 200 electron microscope.

RESULTS AND DISCUSSION

Solubilization of Beta-apoprotein

The dry residue of beta-apoprotein was completely dissolved by incubation for 16 hours at 37° with 0.13 M Tris (0.01% EDTA) buffer containing 60 mM sodium decyl sulfate. The preparation

was then dialyzed against the same buffer system at a 2 mM concentration of sodium decyl sulfate. It was stable for several weeks at 4^{o} as judged by solubility and circular dichroism.

Immunochemical Studies

On immunoelectrophoresis in 0.5% agarose (Levy et al., 1966) beta-apoprotein reacted with ten rabbit antisera to native beta-lipoprotein and with a commercial anti beta-lipoprotein serum (Hyland) (Levy and Fredrickson, 1965). Rabbit antisera to the apoprotein reacted with beta-apoprotein and beta-lipoprotein. It formed a single precipitin line of beta mobility with normal plasma. The antisera to β-apoprotein did not react with plasma from a patient with abetalipoproteinemia or with the infranate derived from this or normal plasma by centrifugation at a density of 1.21. In double diffusion studies beta-apoprotein was immunologically identical to native beta-lipoprotein.

Conformation of Beta-lipoprotein and Beta-apoprotein

Previous studies employing optical rotatory dispersion and infrared spectroscopy had led to the conclusions that beta-lipoprotein contained a mixture of α-helix and random structure having no detectable β-structure (Scanu and Granda, 1967). On the basis of circular dichroism measurements, it has been suggested that removal of the lipids from beta-lipoprotein changes the conformation of the molecule (Scanu and Hirz, 1968). These conclusions were not borne out by the present studies.

Our findings were as follows. 1) Both native beta-lipoprotein (in 0.05 M phosphate buffer, pH 7.9) and beta-apoprotein (in 0.05 M phosphate buffer, pH 7.9 with 0.2 to 0.4 mM sodium decyl sulfate)

exhibited similar spectra with a single negative band having a minimum at 215-217 mM. The $[\theta]_{216}$ for beta-lipoprotein and beta-apoprotein were 12,400 and 8,000, respectively. This spectrum has been described as characteristic of a pleated sheet β-structure (Sarkar and Doty, 1966). We did not find the negative Cotton effects at 208 and 222 mμ which are characteristic of the α-helix (Sarkar and Doty, 1966). 2) The addition of 20 mM sodium decyl sulfate significantly altered the spectra of both beta-lipoprotein and beta-apoprotein, leading in some experiments to a detectable shoulder (negative Cotton effect) at 222 mμ, but in all cases to a significant trough (negative Cotton effect) at 208 mμ. Similar changes were described for beta-lipoprotein by Scanu and Hirz (1968) when sodium dodecyl sulfate was added. The spectral shifts in an apoprotein preparation attributed by these workers to delipidation possibly were due to the presence of sodium dodecyl sulfate, since the spectrum of the apoprotein was similar to that of the parent lipoprotein in very low concentrations of detergent (0.2 to 0.4 mM in our studies). 3) In D_2O, beta-lipoprotein exhibited an asymmetric infrared absorption peak with a clear-cut maximum at 1625 to 1630 cm^{-1}. This is characteristic of the amide I frequency of the β-structure of polyglycine I (Suzuki et al., 1966). A shoulder was present at 1640 cm^{-1} and possibly at 1650 cm^{-1}, the two frequencies assigned respectively to random structure and α-helix. A reproducible, well-defined shoulder was seen at 1680 to 1685 cm^{-1}, characteristic of the anti-parallel chain (APC) type of β-structure (Suzuki et al., 1966; Krimm, 1962). 4) The addition of sodium decyl sulfate to a concentration of 100 mM shifted the previously

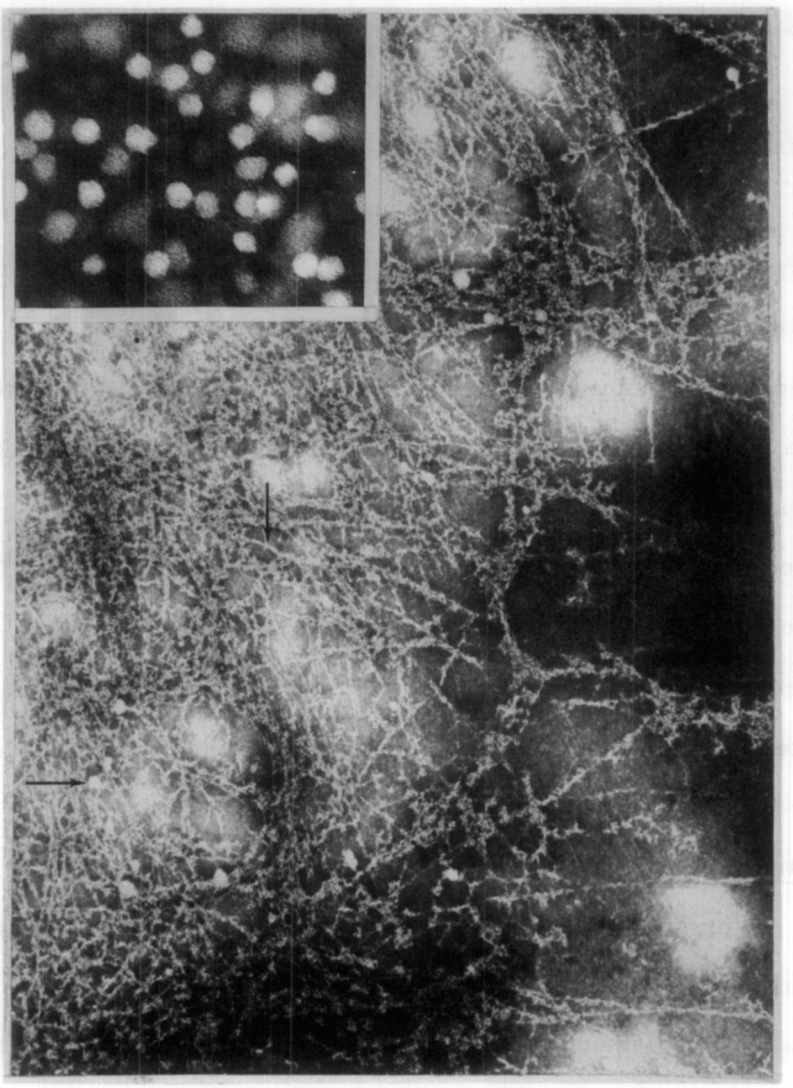

Figure 1. Electron micrograph of negatively stained beta-apoprotein. Note fibrous structure in apoprotein preparation and rare spherical particle resembling native beta-lipoprotein. Magnification x 68, 000.

Insert. Beta-lipoprotein prior to delipidation. Spherical structures have diameter ca. 220 A. Magnification x 200, 000.

described infrared spectrum of beta-lipoprotein to a more symmetrical peak with a well-defined maximum at 1650 cm^{-1} characteristic of the α-helix and an apparent shoulder at about 1640 cm^{-1}. The previous peak at 1625-1630 cm^{-1} and shoulder at 1680-1685 cm^{-1} were totally abolished.

These results therefore indicate a significant contribution of APC β-structure to the conformation of beta-lipoprotein. There probably also is some degree of random structure and we cannot rule out the possibility of some α-helical content, although this would seem to be less significant than the β-structure. We found no significant spectral shifts when the lipid was removed, although $[\theta]_{216}$ was reduced by approximately 25%. The addition of sodium decyl sulfate led to a loss of the β-configuration, an apparent shift to α-helical structure and possibly also to an increased random structure. A related detergent, sodium dodecyl sulfate, has been found to increase the α-helical content of other proteins (Jirgensons, 1966).

Electron Microscopy

With the technique of negative staining, beta-lipoprotein appeared as a uniform preparation of approximately spherical particles of 216-220 Å in diameter (Fig. 1, insert). Beta-apoprotein, on the other hand, consisted of long fibrillar chains (Fig. 1).

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