HUMAN SERUM BETA-LIPOPROTEIN: PREPARATION AND PROPERTIES OF A DELIPIDATED, SOLUBLE DERIVATIVE

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Received December 22, 1967

An investigation of the protein moiety of human serum β-lipoprotein has been hampered by the insolubility of the delipidated protein in aqueous media (Scanu et al., 1958; Banasak and McDonald, 1962; Margolis and Langdon, 1966). The purpose of this communication is to describe a method which can conveniently be used for the preparation in high yield of a delipidated form of β-lipoprotein. This is accomplished by succinylation, delipidation and solubilization of the protein moiety in the presence of relatively low concentrations of decyl sulfate, an ionic detergent which may be removed readily by dialysis. Pertinent properties of this delipidated soluble protein are presented.

EXPERIMENTAL

Materials

Succinic anhydride was obtained from Eastman Kodak Distillation Products Industries, Rochester, New York. Recrystallized sodium decyl sulfate and sodium dodecyl sulfate were generous gifts from Dr. Ralph Reisfeld and the E. I du Pont Co., respectively.

Methods

The delipidated protein was extracted in chloroform:methanol (2:1) for the estimation of triglyceride (Kessler and Lederer, 1966), cholesterol (Autoanalyzer Manual, 1964) and phospholipid (Stewart and Hendry, 1935).

The extract was also examined for lipid by thin-layer chromatography (Tschesche, 1961). Protein was estimated by the method of Lowry et al. (1951). Immunological techniques have been described previously (Levy and Fredrickson, 1965; Levy et al., 1966).

Sedimentation velocity experiments were performed with a Spinco Model E analytical ultracentrifuge in an aluminum epon single sector cell. The rotor speed was 59,780 rpm and Schlieren optics were utilized. The values of $s_{20,w}$ were extrapolated to infinite dilution.

Preparation of apoprotein from human serum β-lipoprotein.

Plasma was collected in EDTA, 1 gm/l, pH 7.0, from fasting normal subjects and stored at 4°. All subsequent procedures were performed at 4° unless specified otherwise. Fibrinogen was removed by the addition of 3,000 N.I.H. units of bovine thrombin to each liter of plasma, stirring for 2 h and centrifugation for 15 min at 10,000 rpm. β-Lipoprotein was precipitated with heparin (2,000 units per 1 of plasma) and 1 M manganese chloride (45 ml per 1 of plasma). The precipitate was dissolved in 10% sodium citrate (pH 7.0) and dialyzed against 0.15 M saline containing 0.001 M EDTA, pH 7.0. The lipoprotein fraction of density 1.019 to 1.063 was isolated by density gradient centrifugation against KBr-NaCl (Havel et al., 1955). The preparation was washed by recentrifugation with KBr-NaCl of density 1.063.

The β-lipoprotein was succinylated with succinic anhydride as described by Hass (1964), except that a 60 to 1 ratio of succinic anhydride to lysine (based on the data of Granda and Scanu, 1966) was employed. The succinylated β-lipoprotein was dialyzed 48 h against 0.15 M NaCl 0.001 M EDTA, pH 7.0.

For purpose of delipidation, 1.0 ml of dialyzed succinylated β-lipoprotein (4 to 6 mg of protein) was shaken for 8 h at 4° in 50 ml stoppered, conical centrifuge tubes containing ether:ethanol (3:1). For some preparations, as indicated later, sodium decyl sulfate (1×10^{-2} M) was added to the protein prior to extraction. The supernatant was removed by centrifugation and the extraction was repeated for a further 12 h. The

precipitate was dried at room temperature under a stream of nitrogen.

RESULTS: Properties of the Apoprotein Moiety of β-Lipoprotein

Solubility

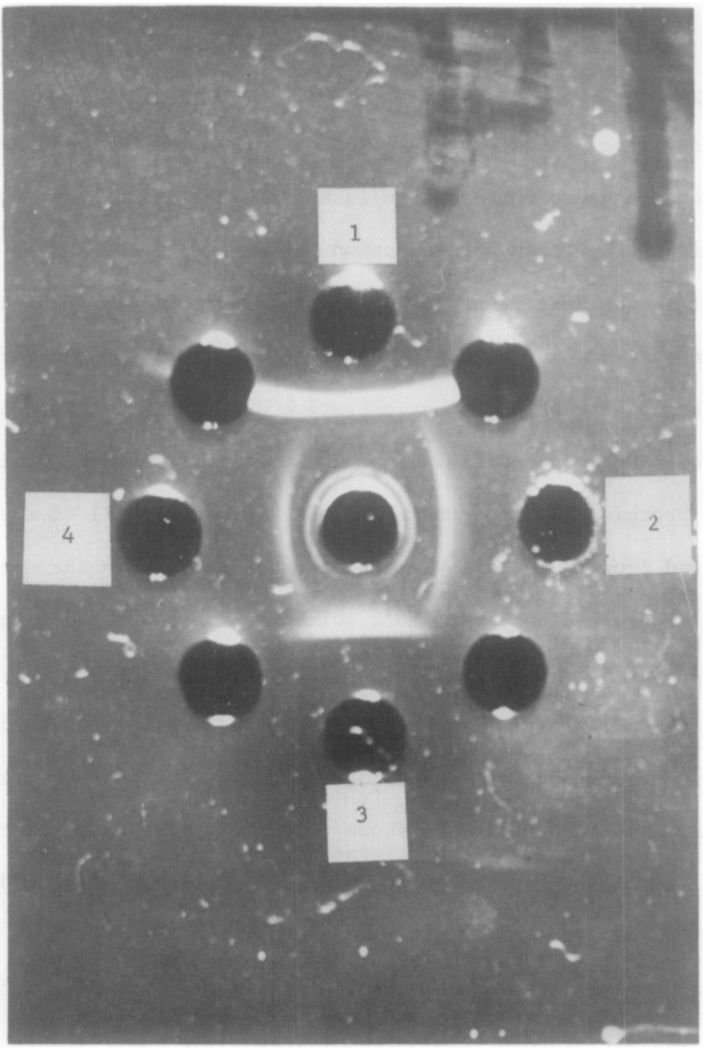
The dry precipitate was dissolved by adding 1 ml of 0.13 M Tris buffer pH 8.0, containing sodium decyl sulfate, and warming for 2 to 4 h at 40°. When the initial protein concentration was 5 mg or less, 2×10^{-3} M sodium decyl sulfate was sufficient to achieve solubilization. At higher protein concentrations, 2×10^{-2} M sodium decyl sulfate was required for complete solubilization. This latter concentration was routinely employed for preparation of the apoprotein. In the experiments shown in Table I, 4.5 and 4.3 mg of protein (as lipoprotein) were delipidated. All of the visible precipitated protein following extraction was soluble in the detergent solution. There was no appreciable solubilization by the detergent unless the protein was succinylated. The succinylated protein was only slightly soluble after delipidation in the absence of detergent. Both sodium decyl sulfate and sodium dodecyl sulfate were effective in achieving solubilization. In separate experiments, it was shown that the yield of soluble protein was not increased by having the detergent present during the delipidation process.

Lipid content

The apoprotein contained no cholesterol or triglyceride detectable by chemical analysis or thin-layer chromatography. A small quantity of phospholipid (1-1.5% by weight) was not removed. There was a small increase in the quantity of phospholipid extracted when decyl sulfate was present (about 0.5%) prior to delipidation. When larger quantities of lipoprotein were extracted (greater than 5 mg of protein), the residual phospholipid content increased to 2-4% by weight.

Immunological properties

Immunological activity of the apoprotein was examined against rabbit antisera to human serum β-lipoprotein by the double diffusion technique of



Legend to Fig. 1. Immunodiffusion Patterns on agarose of Beta-lipoprotein, succinylated Beta-lipoprotein and succinylated apoprotein. The center well contained rabbit antiserum (R_{51}). The original Beta-lipoprotein was in well 1, succinylated Beta-lipoprotein in well 3, apoprotein in sodium decyl sulfate in well 2 and apoprotein in sodium dodecyl sulfate in well 4.

Oucterlony. Of the 15 antisera studied, 10 exhibited activity with the succinylated, delipidated derivative (Fig. 1). With certain of the antisera, two or more precipitation lines were observed.

Sedimentation studies

The succinylated β-lipoprotein sedimented as a single component in the ultracentrifuge with an $s_{20,w}$ value of 5.0. The succinylated apoprotein was examined in the presence of sodium decyl sulfate (2×10^{-2} M) and found to exhibit two components with $s_{20,w}$ values of 4.2 and 5.6.

DISCUSSION

Methods for the preparation of a soluble form of delipidated β-lipoprotein have been described previously which employ extraction with ethanol: ether (1:3) either in the presence of 0.2 M sodium dodecyl sulfate (Granda and Scanu, 1966) or after initial succinylation of the lipoprotein (Scanu, 1967). However, the insolubility of sodium dodecyl sulfate interferes with further studies of the protein and the residual detergent cannot be removed, even by dialysis for several days (Granda and Scanu, 1966). It appears from recent work of Scanu et al. (1967) and from the evidence presented in Table I that delipidation of the lipoprotein after its succinylation achieves a small yield of soluble protein (Scanu, 1967).

While the present work was in progress, Shore and Shore (1967) have described the preparation of the protein moiety of human serum β-lipoprotein by ether:ethanol delipidation followed by reduction and alkylation with sodium borohydride and iodoacetamide in the presence of sodium dodecyl sulfate. The modified apoprotein could then be solubilized in the presence of the residual 3% sodium dodecyl sulfate only if urea was added to a concentration of 6 to 8 M.

The method presented in this communication makes use both of succinylation and of a dialyzable detergent, viz. sodium decyl sulfate in such a way as to make possible the investigation of the apoprotein derivative by the established techniques of protein chemistry.

Table I. Solubility of β-Lipoprotein in Aqueous Solution: Effect of Succinylation, Delipidation, Sodium Decyl Sulfate and Sodium Dodecyl Sulfate

All flasks contained 4.5 (expt. 1) or 4.3 (expt. 2) mg of succinylated β-lipoprotein (as protein), except for flask 1 which contained 4.5 mg of β-lipoproteins which was not succinylated.

Delipidation was performed in 50 ml conical flasks as described in Methods. After delipidation, the dry residue was suspended in 1.0 ml of 0.13 M Tris buffer, pH 8.0, and incubated 40° for 2 to 4 h. The Tris buffer added to flasks 1 and 3 contained 2×10^{-2} M sodium decyl sulfate and that added to flask 4 contained 2×10^{-2} M sodium dodecyl sulfate. In flasks 3 and 4, all of the visible precipitated protein following extraction was soluble in the detergent solution.

Flask	Treatment	Protein Solubilized mg/ml	
		Expt. 1	Expt. 2
1	Delipidation + decyl sulfate	0.09	
2	Succinylation + delipidation	0.7, 0.8	1.3 (S.E. 0.1)
3	Succinylation + delipidation + decyl sulfate	3.7	3.3 (S.E. 0.1)
4	Succinylation + delipidation + dodecyl sulfate	3.5	

ACKNOWLEDGMENTS

We wish to thank Drs. R. Adelstein, A. Chrambach, H. Sloan and S. Margolis (The Johns Hopkins School of Medicine) for useful discussions.

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