THE PROTEIN MOIETY OF HUMAN SERUM β-LIPOPROTEINS

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The protein moiety of human serum β-lipoproteins, unlike that of the serum α-lipoproteins, has proved somewhat difficult to obtain in a lipid-free, soluble, monodisperse form suitable for molecular weight measurements. The present communication describes a method for such a preparation and the results of sedimentation equilibrium experiments on the protein subunit from a β-lipoprotein fraction of density 1.029-1.039 g/ml. The molecular weight of this fraction is $2.8\text{-}3.0 \times 10^6$ (Bjorklund and Katz, 1956), and it contains 20-21% protein. A molecular weight of 6.4×10^4 was obtained for the protein subunit in 8M urea after removal of the lipids with ether and ether-ethanol mixtures and treatment of the lipid-free protein with sodium borohydride and iodoacetamide in the presence of sodium dodecyl sulfate (SDS). Essentially all (>95%) of the protein present in the lipoprotein preparation was recovered in soluble form.

METHODS

The β-lipoprotein fraction was prepared essentially as described by de Lalla and Gofman (1954). It contained the serum lipoproteins of density between 1.029 and 1.039 g/ml. A plot of ηs versus ρ indicated a value of 1.032 g/ml corresponding to the density of zero sedimentation rate of the lipoprotein fraction.

For removal of lipids, the lipoprotein was adjusted to a concentration of 8-10 mg/ml in 1M NaCl. Repeated extraction with cold,

peroxide-free ether removed 80% of the total lipids. Before extraction of the remaining lipids with mixtures of ether and ethanol, the salt was removed from the ether-extracted lipoprotein by dialysis against H_2O (O_2-free) and the pH was adjusted to 3.4 with HCl. The initial ratio of ether to ethanol was 6 to 1 (v/v) and this was gradually decreased to 3 to 2 (v/v). Two phases were present throughout the extraction. The total lipids extracted accounted for 79.2-79.7% by weight of the lipoprotein preparations taken for removal of lipids. Organic solvents dissolved in the protein solution were removed by dialysis against H_2O at 20-22°. The phosphorus remaining with the protein after extraction was found by the method of Chen, Toribara and Warner (1956) to be 0.015 g/100 g protein; the cholesterol found by gas chromatography after saponification and extraction of the lipids into hexane was 0.08 g/100 g protein.

Although addition of SDS to the lipid-free protein (3/1, w/w) was quite effective in reducing the sedimentation coefficient of the protein, sedimentation velocity patterns of the SDS-protein in 0.1M NaCl indicated heterogeneity. The sedimentation coefficient could be further reduced by reacting the SDS-protein in water (3 mg protein/ml) with $NaBH_4$ (1 mg/mg protein) for 1 hr at 20-22° followed by iodoacetamide (1 mg/mg protein) for an additional 2 hr at 20-22°. However, it was necessary to dialyze the protein against 8M urea to achieve a monodispersity appropriate for molecular weight measurement. After dialysis of the borohydride- and iodoacetamide-treated protein against H_2O for 16 hr, it was dialyzed against 8M urea for 60 hr. After dialysis, the amount of SDS present, determined by the method of Karush and Sonenberg (1950), corresponded to 3% of the protein by weight. The partial specific volume ($\bar{V}$) of the protein in 8M urea was calculated according to Casassa and Eisenberg (1964) from the densities obtained by pycnometry of the 8M urea and of the protein in 8M urea. Sedimentation equilibrium experiments were carried out with the

interference optical system of the Model E Spinco analytical ultracentrifuge as described by Richards and Schachman (1959). In these experiments, the centrifuge cell was filled so that the menisci at the top and bottom of the solution column were superimposed on the corresponding ones in the solvent compartment.

RESULTS

The protein, at a concentration of 2.5 mg/ml, gave a single boundary in 8M urea and in 6M urea in sedimentation velocity experiments; s_{20} was 0.79 in 8M urea and 1.41 in 6M urea. $\bar{V}$ for the protein in 8M urea was found to be 0.82 ml/g, in contrast to a value of 0.725 in water which can be calculated from its amino acid and carbohydrate composition. These data suggest that water is preferentially bound by this protein in 8M urea to the extent of 0.51 g/g protein.

Sedimentation equilibrium experiments on the protein moiety of the β-lipoprotein in 6M urea and in 8M urea indicated some aggregation at the lower urea concentration. Thus, it was not possible to evaluate $\bar{V}$ for the protein in urea solutions by the method described by Schachman and Edelstein (1966) in which $M(1 - \bar{V}\rho)$ is plotted against the solvent density. Although the plots of the log of the concentration against the square of the distance R from the center of rotation curved upward for the protein in 6M urea, they were linear for the protein in 8M urea (Fig. 1).

From the slope of the curve in Fig. 1 and a $\bar{V}$ of 0.82, a molecular weight of 6.4×10^4 for the protein subunit in 8M urea is calculated. It was assumed that the experimental data give $M_c(1 - \bar{V}_c\rho)$ and that $M_c = M_p(1 + x)$, where M_c and M_p are the molecular weights of the protein before and after correction for preferentially bound water, $\bar{V}_c$ is the partial specific volume of the hydrated protein complex and x is

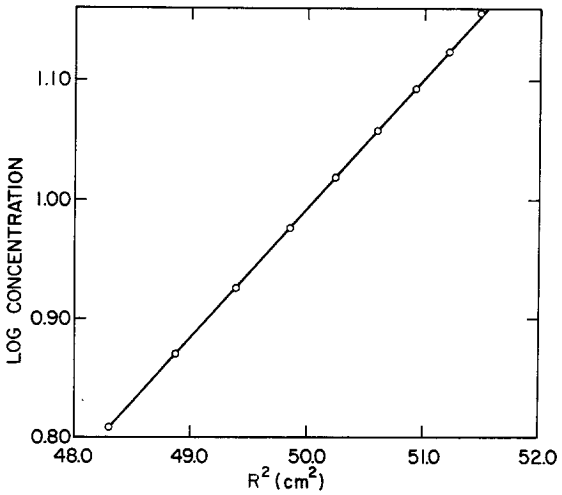

Fig. 1. Sedimentation equilibrium of the protein moiety of human serum β-lipoprotein in 8M urea. The initial protein concentration was 2.62 mg/ml (10.10 fringes), the solution column height in the centrifuge cell was 2.750 mm, the rotor speed was 12,000 rpm, and the temperature was 20.0°. R is the distance from the center of rotation.

the number of grams of water bound by one gram of protein (Schachman and Edelstein, 1966). A similar correction was also made for the SDS, which was assumed to be protein-bound. If we assume no preferential interaction of the protein with water, a value of 4.2×10^4 is obtained for the molecular weight.

It appears that β-lipoproteins of density 1.029-1.039 g/ml contain 9 or 10 protein subunits that are identical or very similar in molecular weight.

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