THE PROTEIN SUBUNIT OF HUMAN SERUM LIPOPROTEINS OF DENSITY 1.125-1.200 GRAM/ML*

V. Shore and B. Shore

Department of Physiology, Washington University School of Medicine, St. Louis, Missouri

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Although much work has been done on the metabolism of human serum lipoproteins, and some work has been done on their structure, comparatively little is known about protein-protein, protein-lipid, and lipid-lipid interactions in the lipoprotein molecule. The physical and chemical data presented here on the lipoproteins of density 1.125-1.200 gm/ml and on the protein moiety obtained by delipidation indicate that this family of lipoproteins contains three peptide chains per molecule and that these protein subunits are not held together by covalent interchain bonds.

Density 1.125-1.200 gm/ml lipoproteins were isolated essentially as described previously (Shore, 1957), with two 40 hour centrifugations at 110,000 X g at a solvent density of 1.200 gm/ml following the removal of lipoproteins of density less than 1.125 gm/ml. A plot of η s against solutions of varying density, in which D_2O was used to achieve the necessary density increment, indicated an average density of 1.151 gm/ml for this fraction. The ultracentrifugal (Spinco Model E) and electrophoretic (Spinco Model H) patterns indicated the absence of contaminating proteins. A 3.5 mg/ml solution of the lipoprotein had an $s_{20,w}$ of 4.83, and a 7.5 mg/ml

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solution had an electrophoretic mobility (descending limb) of -6.1×10^{-5} $cm^2 sec^{-1} volt^{-1}$ in pH 8.6 buffer, 0.1μ in veronal and 0.0008M in EDTA.

The molecular weights of this lipoprotein fraction and its delipidized protein moiety were determined by the "approach to sedimentation equilibrium" method (Archibald, 1947); the data were evaluated in the manner suggested by Schachman (1957). The native lipoprotein, 8 mg/ml in a solution 0.0008M in EDTA, 0.08M in KCl, and 0.04M, pH 8.3 in veronal buffer, had a molecular weight of 200,000 ± 11,000. Since the lipoprotein contained 54% protein, a mole of lipoprotein contains about 110,000 grams protein.

Before the molecular weight of the protein component could be determined by the Archibald method, it was necessary to obtain a soluble preparation essentially free of lipid and apparently homogeneous. For delipidation, the lipoprotein (12 mg/ml), which had been dialyzed against a solution 0.1M in NaCl and 0.01M in pH 8.0 tris·HCl, was extracted vigorously at 2°C with an equal volume of Et_2O-EtOH (3:2, v:v) solution followed by two extractions with a volume of Et_2O-EtOH (3:1, v:v) equal to the original volume of the aqueous solution. The organic phase was separated by centrifugation after each of the three extractions. After the final extraction, the organic solvent dissolved in the aqueous phase was removed in vacuo, and the clear aqueous solution was dialyzed for 18 hours against a solution 0.04M, pH 8.6 in veronal and 0.08M in KCl. During the dialysis, about 1/3 of the protein formed a clear gel. Analysis of the mixture for phosphorus by the method of Knight and Woody (1958) indicated a P content of 0.01%, and analysis for cholesterol by the method of Abell et al. (1952) indicated a total (free and ester) cholesterol content of less than 0.035%. Saponification of the protein preparation with ethanolic KOH and subsequent acidification and extraction of the fatty acids indicated a cholesterol ester and glyceride

content of less than 0.1%. Thus the physical and chemical studies reported below were made on a protein which was almost completely free of lipid. Protein determination by the method of Lowry et al. (1951) indicated that essentially all of the protein moiety had been recovered.

The NH_2-terminal amino acid composition of this preparation was determined as described previously (Shore and Shore, 1960). After correctio for recoveries of 73 and 80%, respectively, for NH_2-aspartic acid and threonine, the protein was found to contain 1 mole of NH_2-aspartic acid per 38,000 gms and a trace amount of NH_2-threonine (1 mole per 600,000 gms), irrespective of whether the intact lipoprotein or the delipidized protein was taken for dinitrophenylation.

The gel formed during dialysis could be solubilized by the nonionic detergent Triton X-100 or by sodium dodecyl sulfate. Electrophoretic analysis of a solution containing both soluble protein and gel solubilized by 0.01% Triton X-100 showed a single peak in 0.1 μ, pH 8.6 veronal with a mobility (descending limb) of -4.7 Tiselius units, a value about 23% lower than that of the native, undelipidized lipoprotein. In the absence of detergent, the soluble protein showed a single peak with a mobility of -4.8 Tiselius units. The soluble protein obtained from several preparations of lipoprotein characteristically showed two peaks in the ultracentrifuge, the major having values of $s_{20,w}$ ranging from 2.3 to 2.6 and the minor having values ranging from 4.2 to 4.6.

For total amino acid analysis, the lipoprotein was delipidized with combinations of ethanol, ethyl ether, methanol, and methylal to yield an insoluble, granular precipitate. Its amino acid composition, which is presented in Table I, was determined by the Analytica Corporation (New York) by ion exchange chromatography (Moore, Spackman, and Stein, 1958).

From these data, a value of 0.734 can be calculated for $\bar{V}$. From the content of isoleucine, the least common amino acid, a value of 1 mole isoleucine per 18,600 grams protein or 2 moles per 37,200 grams can be calculated.

TABLE I

AMINO ACID COMPOSITION OF THE PROTEIN COMPONENT OF HUMAN SERUM LIPOPROTEINS OF DENSITY 1.125-1.200 GRAM/ML

Residues of Amino Acid/37,000 Grams Delipidized Protein			
Aspartic Acid	24.0	Histidine	5.9
Glutamic Acid	54.6	Cysteine	0.0*
Serine	19.8	Cystine	1.9*
Threonine	14.9	Methionine	4.0
Glycine	13.8	Valine	17.0
Alanine	23.4	Leucine	38.8
Proline	18.1	Isoleucine	2.0
Lysine	31.2	Phenylalanine	9.5
Arginine	16.1	Tyrosine	10.1

The sample was hydrolyzed for 16 hours at 110°C in a sealed, evacuated tube with constant boiling HCl.
*Cysteine and cystine were determined by us by amperometric titration with $AgNO_3$ of the delipidized protein in 8M urea before and after sulfitolysis.

For the determination of the molecular weight of the delipidized protein subunit, sodium dodecyl sulfate to a final concentration of 0.08% was added to the mixture of soluble protein and gel. This solution, which was clear at room temperature and at 4°, showed only one peak in the ultracentrifuge, with an $s_{20,w}$ of 2.59 at a protein concentration of 0.63%. From the data of Table II, a molecular weight of 38,500 $\pm$ 1,000 is indicated for the complex of protein and detergent.

More precise values of the molecular weight require knowledge of the binding of the detergent to the protein. However, if it is assumed that all of the detergent is bound to the protein and if the data are analyzed in the manner suggested by Hersh and Schachman (1958), it can be shown that the molecular

weight calculated without consideration of detergent binding is about 5% too high. Thus the molecular weight of the protein itself is about 36,500 ± 1,000. This value agrees fairly well with that obtained from the quantitative NH_2-terminal amino acid composition and from the isoleucine content, assuming 2 residues of this amino acid per protein monomer.

TABLE II

MOLECULAR WEIGHT OF THE COMPLEX OF DODECYL SULFATE AND THE PROTEIN SUBUNIT OF LIPOPROTEINS OF DENSITY 1.125-1.200 GM/ML

Time (min.)*	Mol. Wt. at Meniscus x 10^{-3}	Mol. Wt. at Cell Bottom x 10^{-3}
42	39	39
58	39	38
74	37	39
82	38	38
98	37	38
114	38	39

* Time after reaching 9343 rev./min. The protein concentration was 6.3 mg/ml, and the dodecyl sulfate concentration was 0.8 mg/ml.

From measurements of the sedimentation and diffusion coefficients, Scanu, Lewis, and Bumpus (1958) obtained a molecular weight of 75,000 for the protein component of delipidized 1.065-1.21 gm/ml lipoproteins. Their protein showed a single peak with $s_{20,w}$ (0.35% protein) of 4.11. On the other hand, as mentioned previously, our delipidized protein preparations have commonly shown two sedimenting peaks, the one of lower $s_{20,w}$ (2.3-2.6) probably corresponding to a monomeric unit. Indeed, the most plausible explanation for the differences between the two sets of data is that the protein studied by Scanu et al. may have been an aggregate -- perhaps a dimer -- of the monomeric unit which the detergent sodium dodecyl sulfate clearly reveals

Since a mole of the lipoprotein contains about 110,000 gms protein, the lipoprotein molecule contains three peptide chains. Although the ultracentri-

fugal data of Table II, the electrophoretic pattern, and the terminal amino acid composition do not indicate great heterogeneity, further studies are necessary before one can conclude that these chains are in fact identical. However, since the dodecyl sulfate is unlikely to split covalent bonds, it can be said that these protein chains, whether identical or not, very probably are held together in the native lipoprotein molecule by bonds which are not covalent. Whether these chains interact directly, or through lipid "bridges", in the lipoprotein molecule is also a subject for future study.

Our findings suggest that the 1.125-1.200 gm/ml lipoproteins contain identical protein components to which are bound different amounts and kinds of lipids (cf. Oncley and Allerton, 1961; Scanu and Hughes, 1962). Whether a similar situation also obtains in the 1.065-1.125 gm/ml lipoproteins, which we find to contain 1 mole NH_2-aspartic acid/38,000 gms protein, awaits further study.

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