The Molecular Weights of Porcine Plasma High Density Lipoprotein and Its Subunits*

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SUMMARY

The molecular weights of porcine plasma high density (1.1 to 1.2 g per ml) lipoprotein and its subunits were determined by sedimentation equilibrium. High density lipoprotein has a molecular weight of 210,000, of which 55% is protein composed of subunits of nearly identical or identical molecular weights: 28,000 to 29,000.

The molecular weights of the denser fraction of porcine plasma high density (1.1 to 1.2 g per ml) lipoprotein and its protein subunits were estimated to determine the number of chains and to characterize the molecular species for further studies. These estimates are compared with those reported for human HDL.

Shore and Shore (1) deduced that human plasma HDL, was composed of three identical protein subunits, each with a molecular weight of 37,000, and lipid complement. This deduction was based upon their estimates of the molecular weights by approach to sedimentation equilibrium, by determination of lipid composition, and by quantitative determination of NH₂-terminal amino acids. More recently, they have revised their estimate of the subunit's molecular weights from 37,000 to 32,000 as the result of more precise measurements (2, 3). Scanu (4) determined the molecular weight of all of the protein subunits for human plasma HDL₁, HDL₂, and HDL₃ to have an average value of 21,000, and he concluded that HDL₁ contains an average of five subunits, while HDL₂ contains an average of seven. Subsequently, Scanu (5) reported the subunit's molecular weights to be 24,000 ± 2,000; more recently, Scanu, Reader, and Edelstein (6) reported 25,800 ± 1,000. Shore and Shore (2) reported that HDL₃ contains a different protein, with a molecular weight of 23,000, and has a different amino acid composition.

* This work was supported by research grants from the National Science Foundation and from the National Institutes of Health, United States Public Health Service. It was abstracted from a portion of the thesis presented for the degree of Doctor of Philosophy by A. C. Cox, Duke University, 1966.

MATERIALS AND METHODS

Porcine plasma HDL₃ was isolated from blood collected directly from throat puncture of the animal. Approximately 25 ml of 0.25 M EDTA was immediately added to the blood as an anticoagulant. The blood was kept at 5° during the following steps. The red cells either were allowed to settle or were centrifuged at low speeds, after which the plasma was collected by aspiration. The plasma was then centrifuged for 30 min at 5000 × g to remove the remaining red cells, white cells, and some loosely aggregated fibrin.

Three different methods of isolating HDL₃ from this plasma were used, and the molecular weight of each HDL₃ preparation was then determined. For Preparation 1, the plasma of three young male pigs was pooled and the method of Hazelwood (7) was applied for HDL₃ preparation, with a modification: an additional flotation and sedimentation step was included to remove albumin. For Preparation 2, HDL₃ was sedimented from the plasma of one adult sow by centrifugation in a No. 30 rotor in the Spinco model J centrifuge at 30,000 rpm for 48 hours at 5°. Solvent density was adjusted to 1.11 g per ml by the addition of solid NaBr. The upper layer containing most of the other lipoproteins was removed by a transfer pipette. Then a 15- to 20-ml fraction was drawn from the center of the lower layer, leaving a viscous layer at the bottom and an almost clear layer on top. The solvent density of the pooled fractions was adjusted to 1.20 g per ml by addition of NaCl. Then a 15- to 20-ml fraction was drawn from the center of the lower layer, leaving a viscous layer at the bottom and an almost clear layer on top. The solvent density of the pooled fractions was adjusted to 1.20 g per ml and recentrifuged under the same conditions described above. The upper yellow layer was withdrawn (~1 ml from each tube) and was dialyzed against four changes (1 liter each) of 0.100 M NaCl-0.0010 M EDTA. The final 7 ml of solution contained 0.91 g of HDL₃ per 100 ml.

For Preparation 3, the plasma of one adult sow was collected, and the same techniques were applied as for Preparation 2 except that a different density fractionation was used. The plasma was fractionated first by sedimentation at a solvent density of 1.09 g per ml at 30,000 rpm for 48 hours at 5°. The lipoproteins were further fractionated by an initial flotation at 1.17 g per ml at 30,000 rpm for 48 hours at 5°, a second flotation...
at 1.17 g per ml at 44,770 rpm for 24 hours at 20° in a No. 32 rotor in a Spinco model E centrifuge, and finally a sedimentation at 1.12 g per ml under the same conditions as those of the second flotation. The lower layer was dialyzed against five changes (1 liter each) of 0.100 m NaCl-0.001 m EDTA, pH 6.2. The final modified Lipkin pycnometer of 6.6 cc volume. They were used to calculate partial specific volumes for HDL in 0.1 m NaCl-0.001 m EDTA, pH 6.2, according to the equation

\[ \phi = \frac{c + \rho - \rho_s}{\rho \phi} \]

where \( \rho \) is the density of the solution and \( c \) is the concentration in grams per ml (the other terms have been defined above). The partial specific volume under these conditions should not differ significantly from the value of \( \phi' \) required in Equation 1.

The \( \phi \) values determined for three different preparations (all prepared according to Procedure 2) of HDL3 in 0.10 m NaCl-0.001 m EDTA, pH 6.2, were 0.894, 0.880, and 0.873 ml per g. The average value of \( \phi \) weighted for the protein concentration in each determination was 0.880 ml per g and was used in the calculation of the molecular weights of native HDL3 reported in Table I.

The HDL3 samples were prepared by dilution with the dialysate to a concentration of 0.02 g/100 ml. The apo-HDL3 samples were diluted with appropriate concentrations of guanidine HCl to 0.1 m \( \beta \)-mercaptoethanol to a protein concentration of 0.02 g/100 ml and were dialyzed against 10 volumes of the guanidine HCl solution. The dialysates were used as reference solutions. Water blanks were used to correct for small optical distortions (8). The weight average molecular weights were calculated from the slope of a plot of log \( f \) (logarithm of the fringe displacement) against \( \phi' \) (square of the radius of rotation) according to:

\[ M_\omega = \frac{2RT(2.303) d \log f}{(1 - \phi' \omega \rho_0^2)} \]  

(1)

where \( \phi' \) is the effective specific volume of the macromolecule (9-11), \( \rho \) is the density of the solvent, and \( \omega \) is the angular velocity. Unless disaggregation or aggregation occurred, the compact macromolecules would be expected to behave ideally at the concentrations used in these studies, and therefore the calculated molecular weights should be the true molecular weights if the proper value of \( \phi' \) is used.

Pycnometric measurements were made at 25.0 ± 0.01° in a modified Lipkin pycnometer of 6.6-cc volume. They were used to calculate partial specific volumes for HDL in 0.1 m NaCl-0.001 m EDTA, pH 6.2, according to the equation

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Dry weights of HDL3, apo-HDL3, and lipid samples were obtained by drying the residues from their solutions at 107° in an circulating oven to constant weight. The lipid content of HDL3 was determined to be 45 ± 5% by weight for aliquots of approximately 10 mg.

**RESULTS**

**Sedimentation Velocity of HDL3**—The sedimentation velocity of porcine HDL was measured in 0.10 m NaCl-0.001 m EDTA, pH 6.2, at 25°. The sedimentation patterns of all preparations were not identical but varied from nearly symmetrical peaks to peaks having clearly detectable leading edges (Fig. 1). Preparations 1 and preparations obtained by the same method as that used for Preparation 2 gave nearly symmetrical peaks; the \( s_{20, w} \) values plotted in Fig. 2 were obtained from these preparations. The \( s_{20, w} \) obtained from the intercept of the plot was 4.9 \times 10^{-13} cm per sec. The concentration dependence, \( k \), determined from

\[ s_{20, w} = s_{20, w}^0 (1 - kc) \]

where \( c \) is the concentration of HDL3 in grams per ml, was 14 g per ml.

The combination of the equation

\[ g = \frac{M(1 - \rho_p)}{Nf} \]

with the Stokes equation for a hydrated spherical particle

\[ f = 6\pi\eta \left[ \frac{3M(\rho + \delta_o \rho_s)^{1/3}}{4\pi\eta N} \right]^{1/2} \]

yields an equation

\[ M = 12.62x10^6 \delta_o \rho_s (\phi + \delta_v \phi_s)^{1/3}/(1 - \psi_p)^{2/3} \]

which allows the molecular weight of a spherical particle to be calculated from its sedimentation coefficient. The symbols are \( N \) for Avogadro's number, \( f \) for the frictional coefficient, \( \delta \) for the weight fraction of solvent bound to particle, \( \eta \) for the solvent viscosity, and \( \phi_s \) for specific volume of the solvent; the other terms have been defined above. The molecular weight calcu-
lated from the $s_{50w}$ of HDL₄ is 213,000 if one assumes a hydration of 0.2 g of H₂O per 1 g of HDL₄. This is a minimum value because $f$ will increase if the protein is not spherical in shape.

As a test for heterogeneity, apparent diffusion coefficients were calculated from boundary spreading during sedimentation runs, with the method of Fujita (12). One would generally expect anomalously high values for the diffusion coefficient of lipoproteins because of their heterogeneity. However, a reasonable value, $D_{av} = 4.9 \times 10^{-7}$ cm² per sec, was obtained for Preparation 1. In combination with the sedimentation coefficient given earlier, this value led to a molecular weight of 232,000, in quite reasonable agreement with the more precise molecular weights measured by other procedures. This preparation therefore must be quite homogeneous. Somewhat more rapid boundary spreading, indicative of some heterogeneity with respect to sedimentation properties, was observed for the other preparations of HDL₄. This heterogeneity was apparently not sufficient to affect seriously the sedimentation equilibrium runs reported below: the plots of log $f$ against $r^2$ were nearly as linear for Preparations 2 and 3 as for Preparation 1.

**Molecular Weight of Native HDL₄—**The sedimentation equilibrium experiments reported here were performed according to the method of Yphantis (8), except that the heights of the solution and reference columns were at least 5 mm. The longer columns were used in an attempt to resolve better the heterogeneity in molecular weights.

The initial concentrations were about 0.02 g/100 ml and the time necessary to reach equilibrium was about 1 to 3 days, depending mainly on the column height. In a different study on HDL₄, it was noted that some decrease usually occurred in the specific optical rotation measured at 233 nm for samples at such low concentrations when left at 25° for periods of several days. However, the difference of the fringe displacement in the Rayleigh interference pattern between the top and the bottom of the column remained the same for several hours after the maximum value was obtained. This was the method used to ensure that equilibrium had been reached. Also, there was no evidence of any lipid concentrating at the upper meniscus. Most of the sample in the centrifuge cell was concentrated at the bottom, which may account for the apparent greater stability of HDL₄ there.

**Fig. 1.** Sedimentation pattern of HDL₄ in 0.1 M NaCl-0.001 M EDTA, pH 6.2. This picture was taken 64 min after a rotor speed of 59,780 rpm had been reached.

**Fig. 2.** Sedimentation velocity of HDL₄ in 0.1 M NaCl-0.011 M EDTA, pH 6.2, at 25°. Rotor speed was 59,780 rpm.

**Fig. 3.** Sedimentation equilibrium of HDL₄ in 0.1 M NaCl-0.001 M EDTA, pH 6.16. Rotor speed was 14,290 rpm.

The molecular weight was determined for three separate preparations of HDL₄. Fig. 3 shows the plot of log $f$ against $r^2$ from Preparation 1, which was found to be the most homogeneous of our preparations by the boundary-spreading experiments cited above. The slope of this plot, as indicated by Equation 1, is equal to $M(1 - \varphi\rho)$; the linearity over most of the plot indicates the homogeneity of $M(1 - \varphi\rho)$ for the various HDL₄ molecules. The deviation from linearity seen in the plot near the lower meniscus (i.e. near $r^2$) represents only 2% of the total protein concentration. The plots of log $f$ against $r^2$ for the sedimentation equilibrium runs of Preparations 2 and 3 were also very close to linear, but they contained about 5% of the heavier component.

Since the distribution of the hydrated density of the different molecules is probably narrow, and the sedimentation equilibrium results themselves indicate little variation in $M(1 - \varphi\rho)$ with the position in the cell, it is reasonable to treat the results in terms of the properties of a single homogeneous protein and to extract just a single molecular weight from each equilibrium run. The molecular weights so obtained are listed in Table I.

**Subunit Molecular Weight—**The polypeptide subunit molecular weights of four preparations of apo-HDL₄ were determined by sedimentation equilibrium in guanidine HCl solutions containing 0.1 M β-mercaptoethanol. Although β-mercaptoethanol is not needed to dissociate the subunits (1, 4, 13), it was added to prevent possible aggregation. The plots of log $f$ against $r^2$ obtained from the results of the sedimentation runs on Preparations 1 and 4 are shown in Figs. 4 and 5, respectively. The numerical results from all four preparations are listed in Table I.
TABLE I

Molecular weights of HDL₃ and apo-HDL₃ (in guanidine HCl solution with 0.1 M β-mercaptoethanol)

<table>
<thead>
<tr>
<th></th>
<th>Rotor speed</th>
<th>Column</th>
<th>ρ</th>
<th>d log f</th>
<th>Mol wt</th>
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<td></td>
<td>rpm</td>
<td>mm</td>
<td>g/ml</td>
<td>(g/cm²)</td>
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<tr>
<td>HDL₃</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Preparation 1</td>
<td>14,290</td>
<td>8.3</td>
<td>1.0014</td>
<td>0.518</td>
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<tr>
<td>Preparation 2</td>
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<td>0.575</td>
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<tr>
<td>Preparation 3</td>
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<td>5.0</td>
<td>1.0014</td>
<td>0.648</td>
<td>235,000</td>
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<tr>
<td>apo-HDL₃</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Preparation 1</td>
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<td>Preparation 4</td>
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<td>9.0</td>
<td>1.125</td>
<td>0.445</td>
<td>29,300</td>
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* Since native HDL₃ contains about 45% lipid, the results for HDL₃ correspond to protein contents of 117,000, 115,000, and 129,000 g per mole, respectively.

DISCUSSION

The molecular weights of porcine HDL₃ prepared under three different conditions were determined to be 213,000, 209,000, and 235,000. Levy and Fredrickson (15) have reported that a fraction of HDL is stripped of a portion of its lipids each time that the HDL is centrifuged in solutions of high salt concentration. Scanu and Granda (16) have confirmed and quantitatively determined these results; at least 3% of the HDL is rendered lipid-poor with each centrifugation. Preparation 3 undoubtedly contained some partially delipidized material, since it was collected in the final step of its isolation from the bottom layer of a centrifuge tube. Therefore, the 235,000 value is not considered representative of HDL₃. The molecular weights of the first two preparations indicate that HDL₃ has a molecular weight of about 210,000. After correction for lipid content, the portion of this molecular weight ascribable to protein is 116,000.

The molecular weight of 210,000 agrees with the values of 215,000 and 200,000 ± 10,000 for human HDL₃ reported by Oncley and Allerton (17) and Shore and Shore (1), respectively; the former value was determined by sedimentation equilibrium and the latter by approach to equilibrium. Hazelwood (7) obtained a molecular weight for human HDL₃ of 174,000, and Scanu and Granda (16) obtained 170,000, both by the method of approach to sedimentation to equilibrium. (The true equilibrium method is more reliable than the approach to equilibrium.) The discrepancy between the latter values and our value for porcine HDL₃ should not be attributable to differing methods of isolation, since Preparation 1 was prepared according to the procedure of Hazelwood and Preparation 2 was prepared by a method similar to that used by Scanu and Granda.

The agreement between the molecular weight of HDL₃ ob-
The molecular weight of the subunits of porcine apo-HDL₄ is 28,500 if the $v$ in guanidine HCl solutions is taken as 0.73 ml per g, as calculated from the amino acid composition and if the difference between $\phi$ and $\phi'$ is assumed to be similar to what has been found for other proteins. Incorporating a reasonable margin of error, the molecular weight should be within the range 28,500 ± 1,500. Shore and Shore (2) have reported a value of 31,000 for human HDL₄ subunits, determined by sedimentation equilibrium in a sodium dodecylsulfate solution. When a similar error is assumed in their value, the two values agree in the upper extreme limit of our value. However, the most recent and highest molecular weight for human HDL subunits reported by Scanu et al. (6) is 25,800 ± 1,000, as determined by sedimentation equilibrium, and nearly agrees with the lower extreme of our value.

All of these subunits must have nearly the same molecular weight, since they appeared homogeneous in our sedimentation runs. Shore and Shore (2, 3) also reported that the subunits appeared homogeneous in the sodium dodecylsulfate solutions; they had previously suggested (1) that the subunits were themselves identical, which is possible.

Our results for the weight of protein per mole of protein (116,000) and the molecular weights of the individual chains (28,500) indicate that there must be four polypeptide chains per native molecule.

Acknowledgments—We wish to thank Dr. S. Fleischer and Dr. B. Fleischer for giving us their procedure for delipidizing HDL. The procedure reported here was a slightly modified version.

REFERENCES

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