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Some Physical and Chemical Studies on Two Polypeptide Components of High-Density Lipoproteins of Human Serum*

V. Shore and B. Shore

ABSTRACT: Two different polypeptides separated from the protein moiety of high-density lipoproteins of human serum were found to be similar in molecular weight but very different in amino acid composition. One of the peptides, characterized by carboxyl-terminal glutamine, contains no histidine, arginine, tryptophan, or cysteine. Its amino acid composition is: Lys_{15},-Cys_2,Asp_5,Thr_{11},Ser_{11},Glu_{26},Pro_7,Gly_6,Ala_9,Val_{10},Met_2,-Ile_2,Leu_{14},Tyr_6,Phe_7; the total number of residues is 133 and the molecular weight from amino acid composition is 14,900. Sedimentation equilibrium experiments yielded molecular weight values of 14,300 and 14,900 for the polypeptide R-Gln in urea solutions and in guanidine hydrochloride solutions, respectively. The other polypeptide, characterized by carboxyl-terminal threonine, contains no isoleucine, cystine, or cysteine. Its amino acid composition is: Lys_{10},His_3,Arg_9,-Asp_{13},Thr_5,Ser_8,Glu_{24},Pro_5,Gly_6,Ala_{10},Val_7,Met_2,Leu_{20},-Tyr_4,Phe_3,Trp_4; the total number of residues is 133 and the molecular weight from amino acid composition is 15,500.

Sedimentation equilibrium experiments on the polypeptide R-Thr in guanidine hydrochloride solutions indicated homogeneity with respect to molecular weight; however, the molecular weight value 31,400 indicates that the polypeptide R-Thr exists as a dimer in guanidine hydrochloride solutions. Sedimentation equilibrium experiments on the polypeptide R-Thr in urea solutions and in dilute salt solutions containing sodium dodecyl sulfate indicated heterogeneity with respect to molecular weight and weight-average molecular weights of approximately 20,000.

The protein moiety of high-density lipoproteins (1.065–1.195 g/cc) of human serum is heterogeneous (Shore and Shore, 1968a,b). Polypeptides with carboxyl-terminal glutamine and threonine were found in comparable amounts by hydrazinolysis and by carboxypeptidase experiments on the lipid-free proteins from the 1.126–1.195-g/cc (HDL_3) and the 1.083–1.124-g/cc (HDL_2) lipoprotein fractions.

The present report describes some physical properties, obtained principally by the method of equilibrium sedimentation, and the amino acid compositions of these two polypeptide subunits which were separated by DEAE-cellulose chromatography in 8 M urea.

Experimental Section

Materials. The lipoproteins of density 1.126–1.195 g/cc (HDL_3), which were used as the source of protein in these experiments, were isolated from human serum by ultracentrifugation as described previously (Shore and Shore, 1967). The protein moiety was obtained with essentially complete recovery in lipid-free (<0.01% P; <0.02% cholesterol), water-soluble form by extraction with mixtures of ether and ethanol as described before (Shore and Shore, 1967). Ultra Pure urea and guanidine hydrochloride from Mann Research Laboratories, New York, N. Y., were used without further purification. Triton X-100 (alkylphenoxypolyethoxy ethanol) is a product of Rohm and Haas, Philadelphia, Pa. DEAE-cellulose in a microgranular, fully swollen, and wet form was obtained from Reeve Angel and Co., Clifton, N. J. Carboxypeptidases A (COA-DFP) and B (COB-DFP) were obtained from Worthington Biochemical Corp., Freehold, N. J.

DEAE-cellulose Chromatography. The protein moiety of HDL_3 lipoproteins was chromatographed at 6° on DEAE-cellulose columns (0.9 × 27 cm) which had been equilibrated with the starting buffer, 0.005 M Tris-HCl (pH 8.0) containing 8 M urea. The ion binding capacity was 0.2 mequiv/ml; the capacity for the protein under investigation was not established although it was found to be at least twice the amount used in these experiments. A linear gradient for elution of the peptides was formed by pumping 0.125 M Tris-HCl (pH

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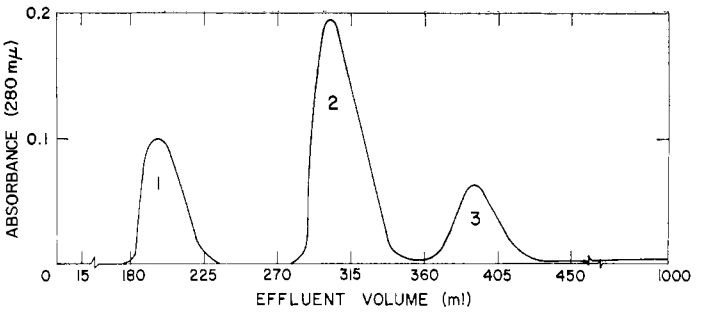

FIGURE 1: Chromatographic separation of the lipid-free protein of serum high-density lipoproteins. A DEAE-cellulose column (0.9 × 27 cm) was equilibrated with 0.005 M Tris-HCl buffer (pH 8.0) in 8 M urea (starting buffer). The protein (8.8 mg in 1.7 ml of starting buffer) was added to the column and eluted with a linear buffer gradient formed by pumping 0.125 M Tris-HCl buffer (pH 8.0) in 8 M urea at 15 ml/hr into a 250-ml mixing flask containing starting buffer.

8.0) in 8 M urea at 15 ml/hr into a 300-ml reservoir containing the starting buffer. The column effluent was monitored continuously for absorbance at 240, 260, and 280 mμ and for conductivity. The fractions were concentrated by ultrafiltration and freed of urea by dialysis against distilled water. Dialysis bags were of porosity such that substances of mol wt 1000 and greater were retained. The peptide composition of the fractions from the column was identified by acrylamide gel electrophoresis and by quantitative analysis of the amino acids released by carboxypeptidases A and B at 37° for 2 hr.

Disc Electrophoresis. Polyacrylamide gel disc electrophoresis (Davis, 1964) was carried out with a Canalco Model 12 apparatus (Canal Industrial Corp., Bethesda, Md.). The gels were made up in 8 M urea or in 1% Triton X-100. The buffer was 0.05 M Tris-glycine (pH 8.8) and the acrylamide monomer concentration was 10%. Electrophoresis was carried out at 2.5 mA/tube for 2 hr. The protein bands were stained with Amido Schwarz.

Reaction with Carboxypeptidases. Proteins were digested by carboxypeptidase A (dissolved in 2 M LiCl at pH 8 just before use) or carboxypeptidases A and B added to the substrate in water at pH 8. The pH was maintained between 7.8 and 8.2 by addition of 0.1 M LiOH. The ratio of substrate to enzyme was approximately 75:1 (w/w). The reaction was stopped by addition of 0.25 volume of either 1.5 N lithium citrate or 1.0 N sodium citrate buffer at pH 2.2 to an aliquot of the digestion mixture, which was then frozen and kept at −15° until amino acid analysis was performed. The blank levels of amino acids and the contribution from autodigestion were found to be negligible.

Amino Acid Analysis. Hydrolysates (20 and 40 hr at 110°) were made as described previously (Shore and Shore, 1967). For determination of cystine, samples of protein were oxidized with performic acid as described by Moore (1963) before hydrolysis for 18 hr at 110°. The amino acids were determined quantitatively as described in Bulletins A-TB-044 and A-TB-033 of Beckman Instruments, Palo Alto, Calif. A Beckman Model 120 B amino acid analyzer equipped with high-sensitivity cuvet, expanded-range recorder, and digital integrator was used. The short column on the amino acid analyzer was used also for total nitrogen determination on samples which had been completely digested with H_2SO_4 and 30% hydrogen peroxide. Tryptophan was determined spectrophotometrically by the method of Bencze and Schmid (1957).

Sedimentation Equilibrium. All sedimentation equilibrium experiments of the present investigation were carried out with the absorption optics and the photoelectric scanning and multiplex systems in the Beckman–Spinco Model E ultracentrifuge. The machine was equipped with an electronic speed control. The average rotor speed during the run was determined from the revolution counter and a stopwatch. Only the low-speed method was used. Each experiment was carried out at two or three solvent densities simultaneously. Rotors which accommodated three or five cells plus the counterbalance were used. In some cases, the density increment was achieved by using $H_2{}^{18}O$ and $D_2{}^{18}O$ as well as water in preparing the solution (Edelstein and Schachman, 1967); in others it was achieved by varying the concentration of a third component, urea or guanidine hydrochloride (Schachman and Edelstein, 1966). With the former, dialyzed samples were diluted about ninefold with the appropriate salt solution in H_2O, $H_2{}^{18}O$, or $D_2{}^{18}O$; with urea or guanidine hydrochloride, the proteins were dialyzed for 24–48 hr against the solvent solution. The protein concentrations were such that the initial optical density (with 12-mm cells) was in the range 0.4–0.6. A small volume (10–15 μl) of the fluorochemical FC 43 (from Beckman Instrument Co., Palo Alto, Calif.) was added to each sector of the double-sector cell. The solution side contained about 2–3 μl more of the oil than solvent side. Approximately 100 μl of protein solution and 107–110 μl of solvent were added to solution and solvent sectors, respectively. Scans were taken immediately after full speed had been obtained, after a time estimated by the method of Van Holde and Baldwin (1958) for achievement of equilibrium, and again some 6–10 hr later. If the last scan was identical with the previous one, it was assumed that equilibrium had been reached. At the end of the run, the cells were agitated to mix the contents and a zero-time picture was taken for comparison with that before the equilibrium centrifugation. In every case, no change was observed.

Results

The lipid-free protein moiety of HDL_3 that gave two bands in polyacrylamide gel electrophoresis was separated into three fractions by chromatography on DEAE-cellulose (Figure 1). The first fraction eluted from the

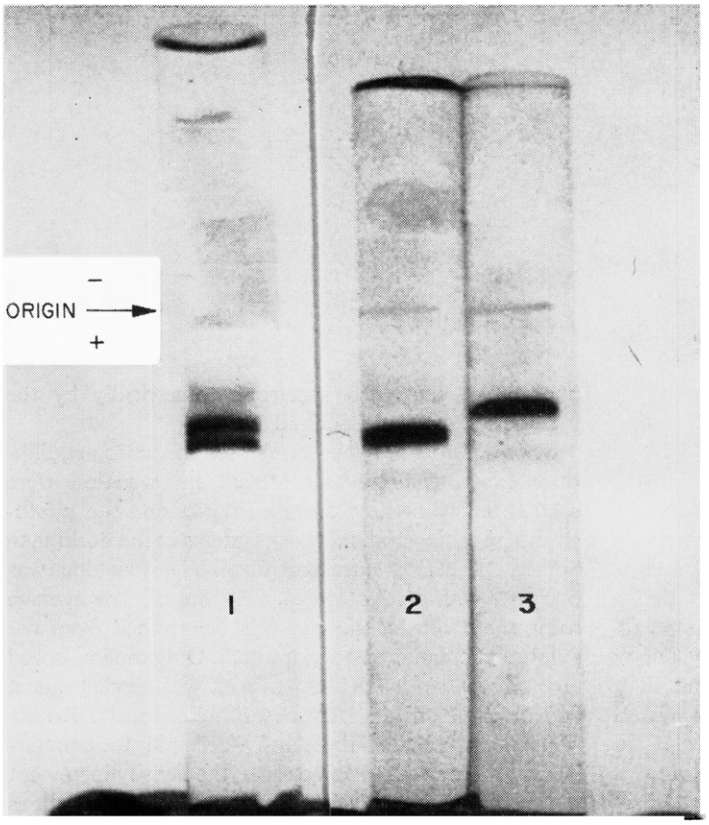

FIGURE 2: Patterns from disc electrophoresis at pH 8.8 in polyacrylamide gels containing 8 M urea of high-density lipoprotein protein and the polypeptides obtained from it by DEAE-cellulose chromatography. (1) Unfractionated protein, (2) the polypeptide R-Gln (peak 1), and (3) the polypeptide R-Thr (peak 2).

column, after concentration and dialysis, gave one band in polyacrylamide gel disc electrophoresis (Figure 2) and contained carboxyl-terminal glutamine. As was found previously in a similar experiment on the high-density lipoprotein protein fractions from DEAE-cellulose chromatography (Shore and Shore, 1968b), only glutamine and threonine were released from fraction 1 by carboxypeptidases A and B; after 2 hr at 37°, 1 mole of each amino acid/14,000 g of protein was released; after 1 min, 1 mole of glutamine and 0.5 mole of threonine per 14,000 g of protein were released. Fraction 2 contained a peptide (R-Thr) with carboxyl-terminal threonine although it usually was not completely free of the peptide (R-Gln) found in fraction 1. It was rechromatographed to give a fraction which gave only one band in disc electrophoresis (Figure 2) and yielded carboxyl-terminal threonine (1 mole/16,000 g) on incubation with carboxypeptidase. Fraction 3 appears to be a mixture of the two peptides found in fractions 1 and 2; the results of disc electrophoresis, carboxypeptidase action, and total amino acid analyses are consistent with this explanation. Fraction 3 gave two bands as did the unfractionated material in disc electrophoresis. Polyacrylamide gel disc electrophoresis in which 1% Triton X-100 was used as the dissociating agent instead of 8 M urea also gave patterns very similar to those shown in Figure 2, except that the faster and slower bands corresponded to R-Thr and R-Gln, respectively.

Sedimentation Equilibrium. Although the unfractionated protein moiety of HDL_3 is heterogeneous with respect to polypeptide content, sedimentation equilibrium analysis of the protein as a sodium dodecyl sulfate complex in 0.1 M NaCl–0.02 M Tris-HCl (pH 8.6) indicated homogeneity with respect to molecular weight (Shore and Shore, 1967). In similar experiments, the peptides R-Gln and R-Thr from DEAE-cellulose chromatography were both quite inhomogeneous with respect to molecular weight. In the present study, protein concentrations of about one-tenth that in the former study were used, since the absorption optical system (with scanner) required less material than did the interference optical system. Sedimentation equilibrium analysis with the scanner on the unfractionated protein in H_2O and in $H_2{}^{18}O$ solutions, both containing 0.1 M NaCl, 0.02 M Tris-HCl (pH 8.6), and sodium dodecyl sulfate (protein/detergent $\cong$ 3:1), indicated homogeneity (since log c *vs.* radius^2 gives a linear plot) with respect to molecular weight (Figure 3), and a molecular weight of 30,500 after correction for 20% bound detergent. In this experiment, the solution of protein–detergent complex was prepared by dialysis of a mixture of protein (3.12 mg/ml) and detergent (6.1 mg/ml) against water for 20 hr and subsequent dilution of the dialyzed solution with the appropriate salt solution in H_2O or $H_2{}^{18}O$. Detergent concentrations after dialysis were determined as described by Karush and Sonenberg (1950). The use of both H_2O and $H_2{}^{18}O$ solvents permits the measurement of the partial specific volume, $\bar{v}$, of the sedimenting protein during the experiment (Edelstein and Schachman, 1967). The measured value of $\bar{v}$ was 0.766 ml/g. If it is assumed that all the detergent is bound, a $\bar{v}$ of 0.772 ml/g can be calculated from the protein and detergent content

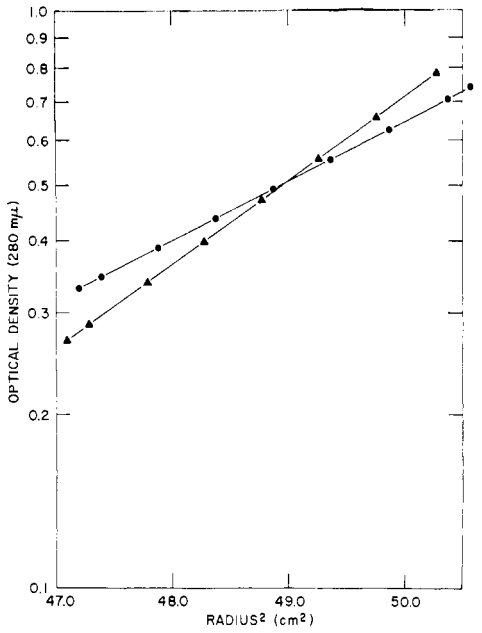

FIGURE 3: Plot of sedimentation equilibrium data from high-density lipoprotein protein in (▲) H_2O and (●) $H_2{}^{18}O$ solutions. Each solution contained the protein as a sodium dodecyl sulfate complex (0.36 mg of protein and 0.11 mg of SDS per ml) in 0.1 M NaCl–0.02 M Tris-HCl at pH 8.6 and were prepared by diluting 8.6-fold a dialyzed solution of the protein–detergent complex with the appropriate solvent. The experiment was carried out at a rotor speed of 13,000 rpm and a temperature of 20.0°. (▲) ρ 1.0030 g/ml and d ln $c/dr^2 = 0.337$. (●) ρ 1.0932 g/ml and d ln $c/dr^2 = 0.239$.

from $\bar{v}_c = (1 - x)\bar{v}_p + x\bar{v}_{SDS}$, where c refers to the complex, p the protein, SDS the detergent, and x is the fraction of the complex that is detergent ($\bar{v}_p = 0.737$ ml/g; $\bar{v}_{SDS} = 0.885$ ml/g). The small difference between measured and calculated values for the partial specific volume of the complex may be caused by incomplete binding of detergent to protein under the conditions of the experiment. The solutions used for sedimentation equilibrium analysis were not dialyzed to equilibrium since dilutions of the dialyzed solutions were made. It is also possible that the difference may arise, in part at least, from a decrease in the partial specific volume of the protein caused by denaturation (Schachman and Edelstein, 1966). The value of 20% for the detergent content of the protein–detergent complex was obtained by assuming that the difference between the measured and calculated values of partial specific volume was due entirely to incomplete binding. If it is assumed that the difference is attributed entirely to a lower partial specific volume of the protein than that calculated from its composition, the molecular weight is 29,300.

Sedimentation equilibrium experiments on the polypeptide R-Gln (no detergent) in H_2O and in D_2O solutions, both containing 0.1 M NaCl and 0.02 M Tris-HCl (pH 8.6), indicated heterogeneity in molecular weight. The weight-average molecular weight varied from about 18,000 to 30,000 from top to bottom, respectively, of the solution column (0.265 cm in height) in the centrifuge cell. The experiment was carried out at 20.0°

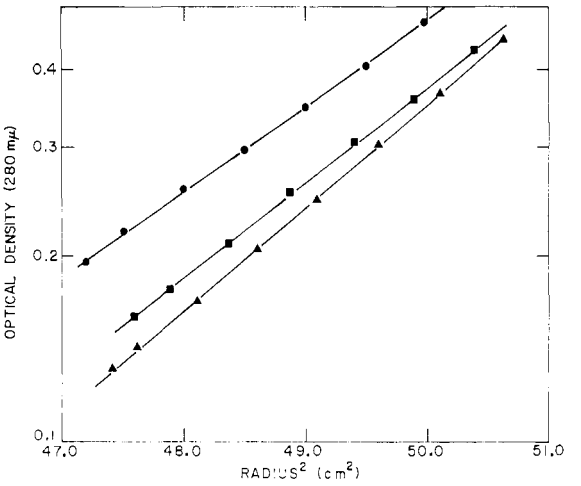

FIGURE 4: Results of sedimentation equilibrium experiments on urea solutions of the polypeptide R-Gln from high-density lipoproteins. The rotor speed was 26,500 rpm and the temperature, 20.0°. (●) In 8.3 M urea; (■) in 7.0 M urea; (▲) in 5.5 M urea.

and a rotor speed of 16,000 rpm. The protein concentration was 0.32 mg/ml. The dissociation of R-Gln was not noticeably increased by addition of sodium dodecyl sulfate before the centrifuge experiment. Sedimentation experiments on the polypeptide R-Gln in urea solutions (5.5, 7, and 8.3 M) and in guanidine hydrochloride solutions (4 and 7 M) yielded linear plots of log concentration (or optical density) *vs.* the square of the distance from the center of rotation, which indicated homogeneity with respect to molecular weight of the polypeptide in these solvents. Data from experiments in which urea solutions were used as solvents are shown in Figures 4 and 5 and in Table I. The results of experiments with guanidine solutions as solvents are shown in Table I. From the apparent partial specific

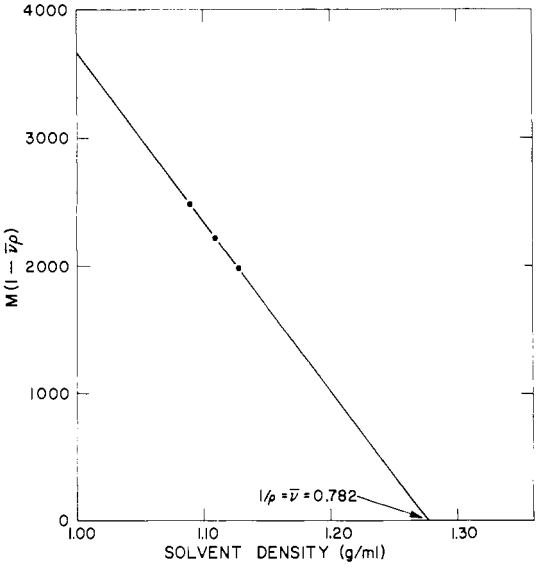

FIGURE 5: Sedimentation equilibrium of the polypeptide R-Gln from high-density lipoproteins. $M(1 - \bar{v}\rho)$ values were calculated from the slopes of the lines shown in Figure 4 and plotted against the corresponding solvent density.

TABLE I: Molecular Weights from Sedimentation Equilibrium Experiments of the Polypeptide R-Gln from High-Density Lipoproteins.

Expt	ρ (g/ml)	d ln c/dr^2	$M(1 - \bar{v}\rho)$	M_{obsd}[d]	M_{cor}[e]
1[a]	1.0895	0.395	2,500	17,000	14,300
1	1.1100	0.355	2,240	17,000	14,300
1	1.1278	0.320	2,020	17,100	14,400
1	1.00		3,700[c]	14,300	14,300
2[b]	1.0991	0.357	2,660	15,660	14,800
2	1.1659	0.247	1,880	15,800	14,900
2	1.00		3,840[c]	14,800	14,800

[a] Sedimentation equilibrium in urea solutions at 20.0° and 26,500 rpm. [b] Sedimentation equilibrium in guanidine hydrochloride solutions at 20.0° and 24,400 rpm. [c] Extrapolated value from plot of $M(1 - \bar{v}\rho)$ *vs.* ρ. [d] From $M = (2RT/(1 - \bar{v}\rho)\omega^2)(\text{d ln } c/\text{d}r^2)$. [e] Corrected for preferential interaction of the polypeptide with water as described by Schachman and Edelstein (1966).

volumes of 0.782 ml/g in urea and 0.755 ml/g in guanidine hydrochloride [from $1/\rho$ at $M(1 - \bar{v}\rho) = 0$] and the value of 0.741 ml/g which was calculated from amino acid composition, it was estimated that the polypeptide R-Gln shows a preferential interaction with water to the extent of 16% in urea solutions and 5% in guanidine hydrochloride solutions.

Sedimentation equilibrium experiments on the polypeptide R-Thr in H_2O and $D_2{}^{18}O$ solutions containing 0.1 M NaCl and 0.02 M Tris-HCl (pH 8.6), in 6 and 8.3 M urea solutions, and in 3, 5, and 7 M guanidine hydrochloride solutions were carried out. In the absence of detergents, R-Thr in water or dilute salt solutions forms aggregates. Also, in the presence of sodium dodecyl sulfate (protein/SDS = 3:1, w/w), inhomogeneity with respect to molecular weight of R-Thr was seen in sedimentation equilibrium experiments. In the upper two-thirds of the solution column in the centrifuge cell, the weight-average molecular weight was approximately 20,000. Sedimentation equilibrium experiments on R-Thr in 6 and 8.3 M urea solutions indicated some heterogeneity with respect to molecular weight. A weight-average molecular weight of about 20,000 was determined from an experiment which was carried out at 20.0°, a rotor speed of 16,000, and a

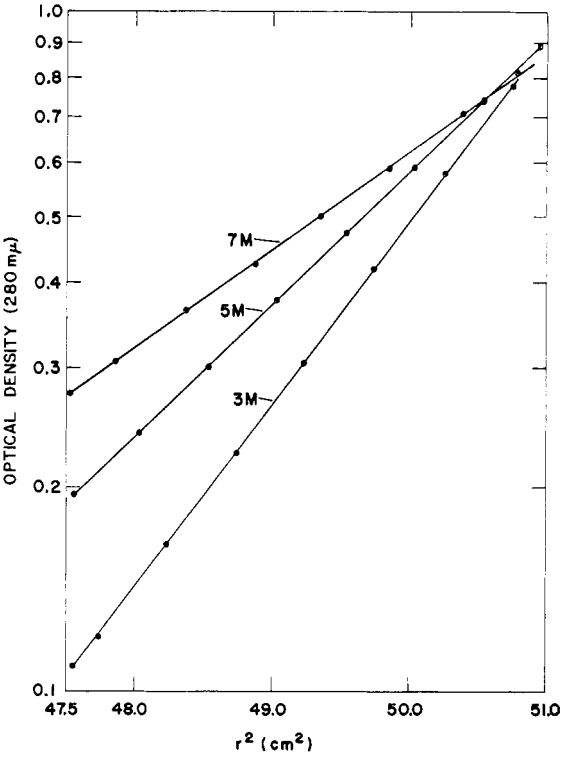

FIGURE 6: Results of sedimentation equilibrium experiments on guanidine hydrochloride solutions containing the polypeptide R-Thr from high-density lipoproteins. The rotor speed was 20,000 rpm and the temperature 20.0°.

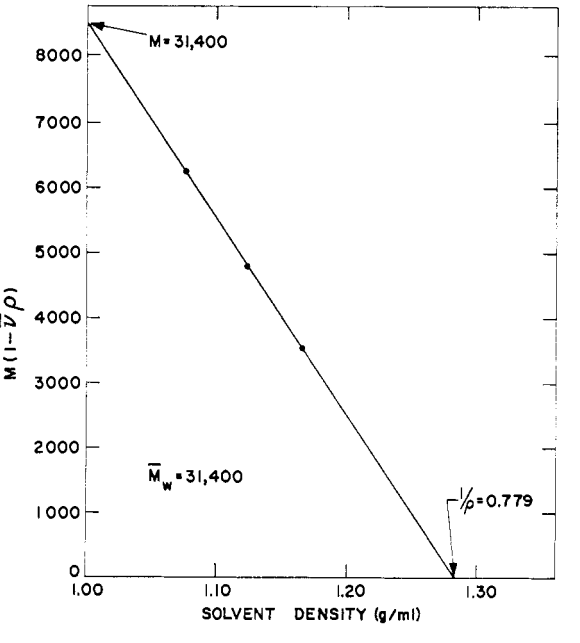

FIGURE 7: Results of sedimentation equilibrium of the polypeptide R-Thr from high-density lipoproteins. $M(1 - \bar{v}\rho)$ values were calculated from the slopes of the lines shown in Figure 7 and plotted against the corresponding solvent density.

TABLE II: Amino Acid Composition of Polypeptides from High-Density Lipoproteins of Human Serum.

Amino Acid	Moles/10^3 Moles of Amino Acids[a]			Moles/Mole of Peptide	
	R-Gln	R-Thr	Unfractionated Protein	R-Gln	R-Thr
Lysine	115.0 ± 1.0	77.7 ± 1.0	91.7	15.4	10.4
Histidine	0	22.6 ± 1.1	14.8	0	3.0
Arginine	0	67.6 ± 1.7	45.5	0	9.0
Aspartic acid	40.6 ± 0.6	98.0 ± 0.8	77.2	5.4	13.1
Threonine	81.9 ± 1.1	38.0 ± 1.1	51.3	10.9	5.1
Serine	82.3 ± 2.2	62.5 ± 1.3	67.6	11.0	8.3
Glutamic acid	197.6 ± 0.8	181.0 ± 1.5	185.0	26.3	24.1
Proline	52.3 ± 0.6	36.9 ± 0.8	47.2	7.0	4.9
Glycine	42.5 ± 1.2	44.3 ± 1.4	43.6	5.7	5.9
Alanine	68.1 ± 1.4	75.8 ± 1.0	74.6	9.1	10.1
Cystine (half)[b]	15.0 ± 0.3	0	7.4	2.0	0
Cysteine	0	0	0	0	0
Valine	76.8 ± 1.4	51.3 ± 1.5	59.5	10.2	6.9
Methionine	14.8 ± 0.4	15.0 ± 0.4	15.0	2.0	2.0
Isoleucine	15.0 ± 0.2	0	7.6	2.0	0
Leucine	103.2 ± 1.0	148.1 ± 0.8	133.6	13.8	19.8
Tyrosine	45.2 ± 0.4	28.4 ± 0.7	37.7	6.0	3.8
Phenylalanine	49.9 ± 0.5	22.9 ± 0.8	33.7	6.7	3.1
Tryptophan	0	30.0 ± 0.6	14.5	0	4.0

[a] The values for the peptides R-Gln and R-Thr are the averages plus and minus standard deviation of hydrolysates from two different preparations which were analyzed in triplicate. The values for the unfractionated protein are from a previous study (Shore and Shore, 1967). Experimental values for cysteic acid, serine, and threonine were corrected for losses of 8, 10, and 5%, respectively. [b] Determined as cysteic acid.

protein concentration of 0.23 mg/ml. R-Thr in guanidine hydrochloride solutions exhibited homogeneity with respect to molecular weight (Figure 6). However, the values for the molecular weight (31,400 at ρ 1.00), 31,600, 31,500, and 31,400 in 3, 5, and 7 M guanidine hydrochloride, respectively (after correction for 15% bound water), are approximately twice the value expected from the yield of threonine (and other amino acids) released by carboxypeptidase. The determination of the apparent partial specific volume (Schachman and Edelstein, 1966) is shown in Figure 7.

Amino Acid Composition. Although the polypeptides R-Gln and R-Thr are similar in size, they are very different in amino acid composition (Table II). For comparison, the composition of the unfractionated protein moiety of HDL_3 lipoproteins determined previously (Shore and Shore, 1967) is shown. R-Gln contains no histidine, arginine, tryptophan, or cysteine; R-Thr contains no cystine, cysteine, or isoleucine. They differ considerably also with respect to the percentage of most of the other amino acids except glycine and methionine. The amino acids recovered after hydrolysis accounted for 97.0% of the nitrogen in the R-Gln samples and 96.6% of the nitrogen in the R-Thr samples.

The spectrophotometric method for tryptophan suffers from the difficulty in that the absorbancy indices for free tryptophan may not be applicable to tryptophan residues in intact proteins. However, the spectrum for R-Gln in 0.1 N NaOH (Figure 8) indicated no tryptophan. A synthetic mixture of tyrosine and tryptophan containing tyrosine at the concentration in the protein and tryptophan in amount corresponding to 1 mole/30,000 g of protein gave a spectrum from which the tryptophan content was easily determined. The spectra of R-Thr and of the unfractionated protein in 0.1 N NaOH are also shown in Figure 8.

The molecular weight calculated by summing the products of the nearest integral number of residues of each amino acid and the molecular weight of the respective residue gave molecular weights of 14,885 for R-Gln and 15,493 for R-Thr. Average values of 14,960 ± 300 for R-Gln and 15,520 ± 250 for R-Thr were calculated from the relationship, [(amino acid residue molecular weight) × 100/percentage of amino acid residue in the protein] multiplied by the nearest integral number of amino acid residues consistent with one cystine residue per R-Gln molecule and with two methionine residues per R-Thr molecule. The minimum molecular weight of R-Gln calculated from the amino acid composition agrees with the values obtained from sedimentation equilibrium experiments and from the amount of carboxyl-terminal glutamine released by carboxypeptidase. The calculated molecular weight for R-Thr is approximately equal to that obtained from carboxyl-terminal analysis and one-half that obtained

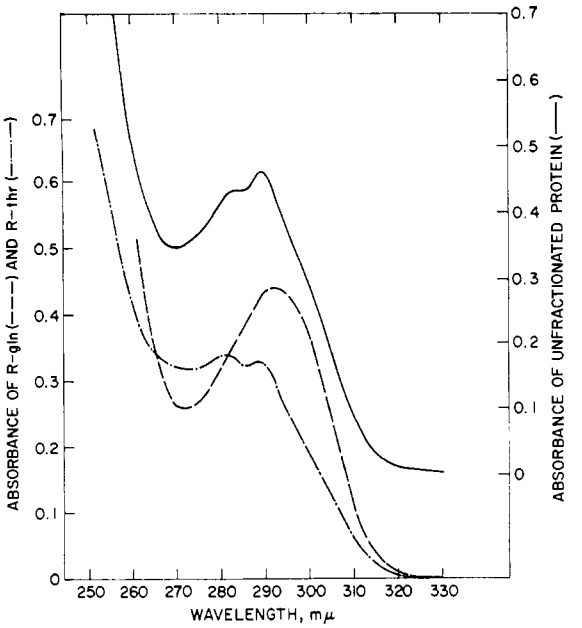

FIGURE 8: Absorption spectra of the unfractionated protein moiety of HDL_3 and its polypeptide components R-Gln and R-Thr in 0.1 N NaOH at concentrations of 0.35, 0.44, and 0.22 mg/ml, respectively.

by sedimentation equilibrium in guanidine hydrochloride solutions. The partial specific volumes, $\bar{v}$, calculated as described by Cohn and Edsall (1943) from amino acid composition were 0.741 and 0.732 ml/g, respectively, for R-Gln and R-Thr.

Discussion

The role of each of the two polypeptides R-Gln and R-Thr, which are so strikingly different in composition, in the structure of high-density lipoproteins of serum appears now as a problem of major interest with regard to the physiological function of lipid transport by these molecules. The two peptides would be expected, on the basis of differences in composition, to have different affinities for the lipids found in the high-density lipoprotein spectrum. It is also possible that the basic lipid binding protein subunit is a mixed dimer. The HDL_3 lipoprotein molecule, of average mol wt 175,000 (Hazelwood, 1958) and 53% protein (Shore and Shore, 1967), probably contains a total of about six polypeptide units of 15,000 in molecular weight. Protein–lipid interactions of the type P_1-L-P_1, P_1-L-P_2, P_2-L-P_2, and P_1-P_2-L (P = peptide; L = lipid) are all possible. It is also possible that the two peptides R-Gln and R-Thr are not derived from the same parent lipoprotein molecule. Polyacrylamide gel (7.5% monomer) disc electrophoresis at pH 8.8 of the lipoprotein fraction HDL_3 in 8 M urea or in 1% Triton X-100 gave two bands which were resolved to the same extent as in electrophoresis of the lipid-free protein moiety in 10% gels (unpublished observation). The lipoproteins were not preincubated with urea or the detergent. Gels of HDL_3 lipoproteins developed with Amido Schwarz were indistinguishable from those of the lipid-free protein. It is possible, however, that urea and Triton X-100 dissociate the lipoprotein molecule, just as they do the lipid-free protein moiety.

The formation of a mixed dimer R-Gln-R-Thr in the unfractionated protein moiety of high-density lipoproteins after removal of lipids is suggested by the results of this investigation. The molecular species of mol wt 30–31 $\times 10^3$ which is observed in sedimentation equilibrium experiments on the unfractionated protein moiety is clearly a dimer. Similar experiments on the peptides R-Gln and R-Thr isolated from the protein moiety do not indicate the homogeneity with respect to molecular weight seen in the unfractionated material, and the weight-average molecular weight of the sedimenting species is considerably lower than 30,000. However, these experiments also indicate that aggregates of the type $(R\text{-Gln})_x$ and $(R\text{-Thr})_x$ certainly occur at least after separation of the peptides from each other.

Complete dissociation of the protein of HDL_3 by urea or guanidine hydrochloride, especially at protein concentrations greater than 3 mg/ml, is not readily achieved. It was previously observed that the unfractionated protein moiety (lipid-free) in 5 M guanidine hydrochloride was heterogeneous with respect to molecular weight (Shore and Shore, 1967). In the present study, R-Gln was a monomer but R-Thr was a dimer in guanidine solutions. Although R-Gln appears to be completely in the monomer form in urea solutions, R-Thr shows some aggregation. The results of the DEAE-cellulose chromatography experiments also suggest that the unfractionated sample (5–6 mg of protein/ml of 8 M urea) was not completely dissociated since the third fraction eluted from the column appears to be a mixture of the peptides found in fractions 1 and 2. It is unusual that the polypeptide R-Thr, which contains no cystine, appears not to be dissociated to the monomer in guanidine hydrochloride solutions, which usually are quite effective in unfolding and in dissociating proteins (Hade and Tanford, 1967). Furthermore, these peptides appear to show a preferential interaction with water rather than with guanidine as is frequently observed (Hade and Tanford, 1967). In similar experiments on the sedimentation equilibrium of aldolase in guanidine hydrochloride solutions, however, a preferential interaction of protein with water was observed (Schachman and Edelstein, 1966).

Further study on the lipoproteins themselves, with attempts to fractionate on the basis of peptide content and attempts to obtain lipoprotein subunits of the molecule, might well prove more fruitful than studies on the lipid-free protein in resolving some of the problems concerning the size and nature of protein subunits of the lipoproteins.

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Equilibrium Constant for the Reversible Deamination of Aspartic Acid*

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ABSTRACT: The equilibrium constant for the deamination of aspartic acid to fumaric acid and ammonia has been measured between 5 and 37° with the enzyme aspartase, and between 60 and 135° by the nonenzymatic reaction.

The equilibrium constant is given by log $K_{DL} = 8.188 - 2315.5/T - 0.01025T$. The equilibrium constant for the enzyme reaction, K_L, is $2K_{DL}$. The pH and ionic strength dependence of this equilibrium were also investigated.

Aspartic acid, unlike most amino acids which occur in proteins, decomposes by a reversible deamination yielding fumaric acid and ammonia (eq 1). This reac-

$$^{-}OOCCH_2\underset{\underset{NH_3^+}{|}}{C}HCOO^- = {}^{-}OOCCH{=}CHCOO^- + NH_4^+ \quad (1)$$

tion is catalyzed by the enzyme aspartase (L-aspartate ammonia-lyase EC 4.3.1.1).

The equilibrium constant for eq 1 has been measured a number of times using aspartases from several bacteria (Quastel and Woolf, 1926; Cook and Woolf, 1928; Woolf, 1929; Jacobsohn and Tapadinhas, 1935; Williams and McIntyre, 1955; Wilkinson and Williams, 1961; Sekijo *et al.*, 1965); the values reported range from 0.008 to 0.04 at 37°. These equilibrium constants show considerable disagreement, mainly because of the difficulty in obtaining preparations of aspartase free of fumarase and because only NH_3 in the equilibrium concentration mixture was measured and the other compounds were calculated by differences.

This reaction also occurs nonenzymatically at elevated temperatures at a rate sufficient to obtain equilibrium. It has recently been proposed that this equilibrium can be used to esitmate the minimum ammonium ion concentration in the oceans of the primitive earth (Bada and Miller, 1968). This estimate requires an accurate value of this equilibrium constant and its pH and temperature dependence. The equilibrium constant is also needed in an investigation of the kinetics and mechanism of the nonenzymatic reaction (J. L. Bada and S. L. Miller, unpublished results).

This paper reports an investigation of this equilibrium constant between 5 and 135°. The effect of pH and ionic strength was also investigated.

Experimental Section

Materials. The L- and DL-aspartic acids and the Good buffers (Good *et al.*, 1966) were purchased from Calbiochem. The fumaric acid (Eastman) was recrystallized three times from hot water. All other chemicals were reagent grade. The aspartase enzyme from *Enterobacter aerogenes* subspecies *alvei* (formerly *Bacterium cadaveris*) was given to us by Dr. V. R. Williams. It was in the form of the frozen ammonium sulfate precipitate and had been partly purified according to the procedure of Williams and Lartique (1967).

The reaction solutions were sealed in ampules con-

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