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Isolation and Characterization of Polypeptides of Human Serum Lipoproteins*

B. Shore and V. Shore

ABSTRACT: Two or more different polypeptides were isolated by DEAE-cellulose chromatography from each of the protein moieties of several fractions of human serum lipoproteins. The polypeptides were characterized by amino acid composition, carboxyl-terminal analysis, and polyacrylamide gel electrophoresis. The high-density lipoproteins of density 1.083–1.124 g/cc (HDL_2) were more heterogeneous than those of density 1.126–1.195 g/cc (HDL_3) with respect to polypeptide content and contained several polypeptides in addition to the two which comprise most of the protein of the HDL_3 fraction.

The low-density lipoprotein fraction of density 0.98–1.006 g/cc (S_f 20–100) also contains several polypeptides, two of which are similar to if not identical with peptides found as minor components in the high-density lipoproteins. The protein of the low-density lipoprotein fraction of density 1.029–1.039 g/cc (S_f 4–8 lipoproteins) yielded two polypeptides, which were different from the peptides of high-density lipoproteins and the S_f 20–100 fraction of low-density lipoproteins. Multiple forms, differing slightly in amino acid composition, of some of the peptides were found. The high-density lipoproteins and the low-density lipoproteins may be structurally and metabolically related by their content of lipid complexes of two polypeptides, one of which has carboxyl-terminal R-Ala-Val-Ala-Ala and one of which has an unusually high content of glycine, serine, and glutamic acid, which are major components of S_f 20–100 lipoproteins and minor components of HDL_2 lipoproteins.

The high-density lipoproteins of human serum contain two different polypeptides which have been isolated and characterized with respect to molecular weight, amino acid composition, and carboxyl-terminal sequence (Shore and Shore, 1968a,b). In the present work, the study is extended to the minor components of high-density lipoproteins (HDL)[1] and the proteins of low-density lipoproteins. Possible structural and metabolic relationships between high-density lipoproteins and low-density lipoproteins were explored on the basis of polypeptide composition of the protein moieties.

In this paper, we report the isolation and composition of several minor polypeptide components of HDL_2 (1.083–1.124 g/cc of lipoproteins) in addition to the two major ones previously isolated from the denser HDL_3 (1.126–1.195 g/cc) lipoproteins, which are less heterogeneous with respect to polypeptides. The protein moiety of S_f[2] 4–8 lipoproteins (1.029–1.039 g/cc) yielded none of the polypeptides found in HDL, but the very low-density lipoprotein yielded two polypeptides similar to if not identical in composition with two of the minor HDL components in addition to three other peptides not found in HDL or S_f 4–8 lipoproteins. Some of the polypeptides of HDL and the very low-density lipoprotein were found in multiple forms very similar but not identical in composition.

Experimental Section[3]

Materials. Human serum lipoprotein fractions were isolated from the serum of individual male and female donors, apparently healthy and nonfasting, by preparative centrifugation at 11–12° in a Spinco–Beckman Model L-2-65B centrifuge. NaEDTA (8×10^{-4} M) and Tris-HCl (0.015 M) at pH 7.4 were present at all stages of lipoprotein isolation. The fractions were characterized by density, per cent lipid, and S_f rate, which was calculated from the rate of movement of the maximum ordinate of the schlieren peak. Carbohydrates, present in small amounts in some human serum lipoproteins (Marshall and Kummerow, 1962; Scanu, 1966), were not determined. The amounts of the various lipoprotein fractions obtained from the serum samples were within the normal ranges reported by Gofman *et al.* (1954).

The S_f 20–100 lipoproteins (<1.007 g/cc) were isolated by centrifugation of 180–200 ml of serum for 36 hr at 30,000 rpm in a 30.2 rotor. The lipoproteins in the top 1-ml portions were combined, diluted to 180 ml with a sodium chloride solution

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[1] Abbreviation used is: HDL, high-density lipoprotein.

[2] S_f denotes the negative sedimentation coefficient in Svedbergs in density 1.063 g/cc NaCl solution at 26°.

[3] Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Atomic Energy Commission to the exclusion of others that may be suitable.

(1.007 g/cc), and centrifuged 24 hr at 30,000 rpm in a 30.2 rotor. The top 1-ml portions were combined, diluted, and centrifuged as above. The top 0.5-ml fractions from the final centrifugation contained the lipoprotein preparation of interest. The S_f values for individual lipoprotein preparations were in the range 40–45; the per cent protein (or glycoprotein) was 8–9 and the per cent lipid was 91–92.

The S_f 4–8 lipoproteins (1.029–1.039 g/cc) were isolated from serum, or in some cases from serum from which the lipoproteins of lesser density had been removed, which was adjusted to solvent density 1.029 g/cc with solid sodium chloride and centrifuged 48 hr at 39,000 rpm in a 40.3 rotor. The bottom 3-ml portions in the tubes were combined, diluted with an equal volume of a sodium chloride solution of density 1.029 g/cc, and centrifuged as before. The bottom 3-ml portions were combined, adjusted to solvent density 1.039 g/cc with solid sodium chloride, and centrifuged as before. The top 1-ml portions in these centrifuge tubes were combined, diluted with five volumes of sodium chloride solution of density 1.039 g/cc, and centrifuged as before. The top 1-ml portions from the final centrifugation contained the S_f 4–8 lipoproteins. The S_f values of individual lipoprotein preparations were 6.4–7.3; the per cent protein (or glycoprotein) was 22–23 and the per cent lipid was 77–78.

The HDL_2 lipoproteins (1.083–1.124 g/cc) and their lipid-free (<0.02% P; <0.02% cholesterol) protein moieties were prepared as described previously (Shore and Shore, 1968a). The per cent protein (or glycoprotein) of HDL_2 was 32–33 and the per cent lipid was 67–68.

The protein moieties of S_f 20–100 and S_f 4–8 lipoproteins were prepared by extracting the lipids with diethyl ether and mixtures of ether and ethanol at 0–6°. In the first step of the extraction procedure, the lipoproteins in 1 M NaCl at protein concentration 1–1.5 mg/ml were extracted repeatedly with ether (1:3, v/v) over a period of 5–6 hr to remove most of the cholesterol, cholesterol esters, and triglycerides. The salt was then removed by dialysis against water, and the remainder of the lipid was removed by six successive extractions with mixtures of ether and ethanol in which the ethanol was gradually increased from 20 to 40%. The ratio of water and organic phases was 1:1 (v/v). The protein was dialyzed against water to remove dissolved ethanol and ether. Oxygen (air) was excluded by using a nitrogen atmosphere during extraction and nitrogen-bubbled water for dialysis.

The proteins in water solution were prepared for column chromatography by dialysis for 5–6 hr at 4° against 8 M urea containing the starting concentration (0.01 M) of Tris-HCl at pH 8.0. Ultra Pure urea and Tris from Mann Research Laboratories were used without further purification. DEAE-cellulose in microgranular, fully swollen, wet form was obtained from Reeve Angel and Co. Visking 18 dialysis tubing from Union Carbide Corp. was cleaned by soaking in 1% $NaHCO_3$ at 80°, followed by 1% $NaHCO_3$ at room temperature, followed by glass-distilled water. Fractions from the column chromatography experiments were subjected to ultrafiltration on UM-2 filters (Amicon Corp.), which retained substances of mol wt > 1000. Carboxypeptidases A and B were obtained from Worthington Biochemical Corp.

Carboxyl-Terminal Amino Acid Analysis. Carboxypeptidase digests of proteins and polypeptides were made and analyzed for amino acids as described previously (Shore and Shore, 1968a) except that the substrate to enzyme ratio was 100:1 (w/w). Carboxyl-terminal amino acids were also determined by the hydrazinolysis procedure of Braun and Schroeder (1967) with subsequent quantitative amino acid analysis with the Spinco–Beckman Model 120B analyzer or by the method of Kawanishi *et al.* (1964).

Disc Electrophoresis. Polyacrylamide gel disc electrophoresis at pH 8.8 in 8 M urea by the method of Davis (1964) was carried out as described previously (Shore and Shore, 1968a,b).

DEAE-cellulose Chromatography. The protein moieties of HDL_2, S_f 4–8 lipoproteins, and S_f 20–100 lipoproteins were chromatographed on DEAE-cellulose columns essentially as described previously for HDL_3 protein (Shore and Shore, 1968b). All solutions contained 8 M urea and Tris-HCl buffers at pH 8.0. Gradients with respect to Tris-HCl concentration were formed by pumping limiting buffer at 16 ml/hr into a 300-ml mixing reservoir containing initially a buffer of 0.01 M Tris. The mixing reservoir was open only to an inlet from the limiting buffer reservoir and to an outlet to the pump. With proteins of S_f 20–100 lipoproteins and S_f 4–8 lipoproteins, limiting buffers of 0.2 M Tris (from 0 to 1040 min on the run timer) and 0.4 M Tris were used. With HDL_2 protein, limiting buffers of 0.1 M Tris (0–1400 min on the run timer), 0.2 M Tris (1400–2200 min), and 0.4 M Tris were used. Resin beds of 0.9 × 27 cm were satisfactory for the low-density lipoprotein proteins; 0.9 × 40 cm columns gave better resolution of the HDL_2 polypeptides than the shorter column.

Amino Acid Analyses. Amino acid analyses were as described previously (Shore and Shore, 1968b).

Protein Determination. The method of Lowry *et al.* (1951) was used to determine protein concentrations in solutions of lipoproteins. Protein concentrations in the lipid-free proteins and in the polypeptide fractions derived from them were obtained by hydrolyzing a known volume of the protein solution and summing the quantities of each of the amino acids found by the amino acid analyzer.

Results

Carboxyl-Terminal Analysis. The protein moieties of four major lipoprotein fractions (HDL_3, HDL_2, S_f 4–8, and S_f 20–100) from sera of apparently healthy young adults were obtained in lipid-free (<0.1% lipid), water-soluble form in essentially complete yield. Before fractionation of these proteins, portions were taken for carboxyl-terminal analysis by incubation with carboxypeptidases and by hydrazinolysis.

No carboxyl-terminal groups other than glutamine, threonine, and alanine were identified in these unfractionated proteins. Carboxypeptidase digests of water-soluble phospholipid–protein complexes obtained by ether extraction of the neutral lipids from S_f 4–8 and S_f 20–100 lipoprotein fractions gave the same results as digests of the lipid-free protein. The yields of carboxyl-terminal amino acids from the various lipoprotein proteins and, for comparison, from the purified polypeptides derived from them are shown in Table I. These results indicate the presence of polypeptides whose carboxyl ends were not identified by carboxypeptidase digestion or by hydrazinolysis; the protein moiety of S_f 4–8 lipoproteins yielded no end group and the S_f 20–100 fractions varied in content of carboxyl-terminal alanine and therefore in content of the peptide giving rise to it.

Glutamine and threonine, the carboxyl-terminal amino acids of the two major peptides of HDL_2 and HDL_3 (Shore

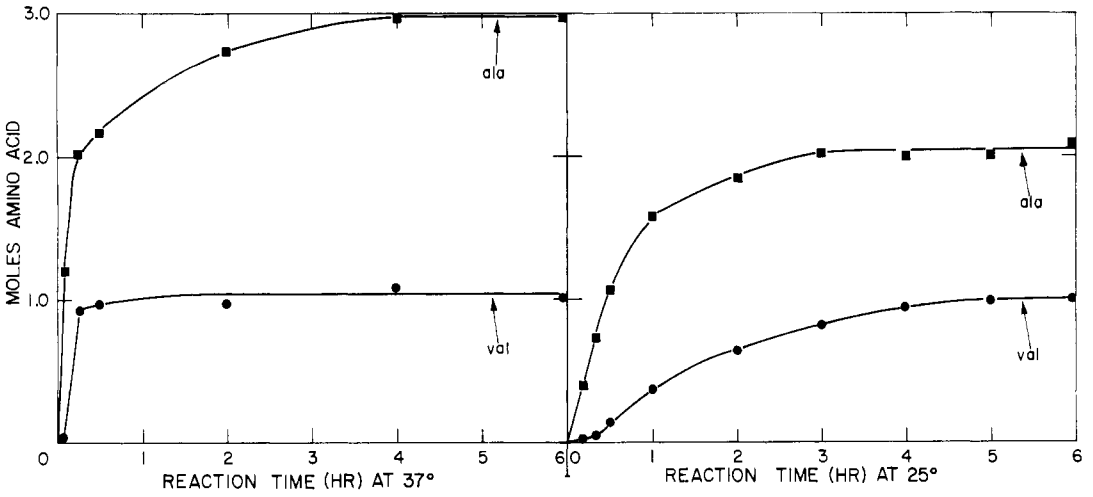

FIGURE 1: Action of carboxypeptidase A at 37° (left) and carboxypeptidase B at 25° (right) on the protein moiety of serum S_f 20–100 lipoprotein.

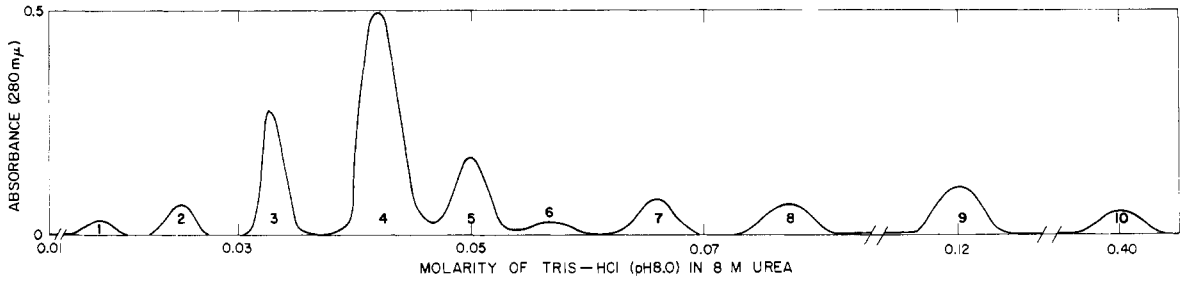

FIGURE 2: Chromatographic separation of the lipid-free protein of a serum high-density lipoprotein fraction of density 1.083–1.124 g/cc (HDL_2). A DEAE-cellulose column (0.9 × 40 cm) was equilibrated with 0.01 M Tris-HCl buffer (pH 8.0) in 8 M urea (starting buffer). The protein (27 mg in 5 ml of starting buffer) was added to the column and eluted with linear concentration gradients of buffer at constant pH, at 6–8° and at a constant flow rate of 16 ml/hr.

TABLE I: Carboxyl-Terminal Amino Acids of Human Serum Lipoproteins and of Some of Their Polypeptide Components.

Protein Source	Polypeptide	g of Protein/mole of Amino Acid[a]		
		Alanine	Glutamine	Threonine
HDL_3	Unfractionated	$>75 \times 10^4$	$26–31 \times 10^3$	$30–36 \times 10^3$
HDL_2	Unfractionated	$10–50 \times 10^4$	$28–36 \times 10^3$	$30–38 \times 10^3$
S_f 4–8 lipoprotein	Unfractionated			
S_f 20–100	Unfractionated	$30–40 \times 10^3$		
HDL_2, HDL_3	R-Thr-Gln		14×10^3	
HDL_2, HDL_3	R_1-Thr, R_2-Thr			15×10^3
HDL_2	R_3-Thr			15×10^3
HDL_2	R-Gly-Gln		24×10^3	
HDL_2, S_f 20–100	R_1-Ala	12×10^3		
S_f 20–100	R_2-Ala	12×10^3		

[a] Amounts released by carboxypeptidases and qualitatively confirmed by hydrazinolysis. Blank spaces indicate the amino acid was not carboxyl terminal for the given polypeptide.

and Shore, 1968a,b), were not found as carboxyl terminals in S_f 4–8 lipoproteins or in S_f 20–100 lipoproteins. Carboxyl-terminal alanine occurred in high-density and S_f 20–100 lipoproteins but not in S_f 4–8 lipoproteins. Of the high-density lipoproteins, the less dense HDL_2 contained more terminal alanine than the HDL_3 fraction, which contained little or none.

The protein moiety of S_f 4–8 lipoprotein, for which serine

TABLE II: Amino Acid Composition (moles/10^3 moles of Amino Acids[a]) of Polypeptides from High-Density Lipoproteins of Density 1.083–1.124 g/cc (HDL_2) from Human Serum.

	Peak No. from Figure 2								
Amino Acid	1	$2_{(1)}$[b]	$2_{(2)}$[b]	3	4	5, 6	7, 8	9	10
Lys	73.8	100.0	105.0	115.8	79.1	80.2	73.0	77.6	73.6
His	29.9	6.6	7.4	0	22.2	21.7	14.6	13.9	27.2
Arg	15.6	13.1	13.9	0	67.0	65.5	31.9	51.1	16.0
Asp	68.8	53.3	61.3	41.0	95.0	95.8	88.0	83.5	67.9
Thr	40.7	65.0	59.9	81.3	38.0	37.8	59.5	47.1	36.9
Ser	210.1	113.7	112.7	81.0	62.4	56.8	121.0	68.9	203.7
Glu	165.4	186.3	208.1	198.4	183.0	184.2	151.9	185.4	163.4
Pro	21.7	45.5	44.6	52.0	38.0	37.8	30.0	41.7	21.0
Gly	188.2	78.1	74.4	42.8	44.6	44.0	56.0	48.2	200.9
Ala	72.4	76.4	73.1	67.0	75.8	76.2	106.0	81.4	74.0
$^1/_2$-Cys[c]	7.6	14.6	14.8	14.7	0	0	0	0	6.0
Val	29.0	65.0	66.1	75.5	51.6	53.0	66.0	59.8	30.1
Met	7.4	8.0	6.0	14.4	15.0	15.1	20.9	13.8	6.6
Ile	20.0	14.1	16.0	14.7	0	6.6	6.7	6.7	19.6
Leu	32.1	90.5	94.4	102.4	148.8	147.9	75.0	138.0	31.1
Tyr	8.0	28.2	0	46.2	28.1	27.2	27.8	27.7	6.9
Phe	16.0	41.4	41.4	50.6	22.9	22.9	40.0	27.3	15.6
Trp[d]	0	0	0	0	28.8	29.0	28.9	27.5	0

[a] Values are the averages of two to six determinations for which standard errors were comparable to those in previous amino acid analyses (Shore and Shore, 1968b). The compositions of fractions 5 and 6 were not significantly different, nor were those of fractions 7 and 8. Experimental values for cysteic acid, serine, and threonine were corrected for losses of 8, 10, and 5%, respectively. [b] Different samples. [c] Determined as cysteic acid. [d] Determined by the method of Bencze and Schmid (1957).

has been reported from other hydrazinolysis experiments to be carboxyl terminal (Shore, 1957), did not yield serine under hydrazinolysis conditions (40 or 60 hr at 80°) in which Amberlite CG 50 resin was used as catalyst.

The protein fractions which gave alanine on incubation with carboxypeptidase A or B also gave valine (Figure 1). No other amino acids were released from the protein of S_f 20–100 lipoproteins by either enzyme or by a mixture of the two. Hydrazinolysis indicated that valine was not carboxyl terminal. From these experiments, it appeared that a polypeptide R-Ala may occur in both high- and low-density lipoprotein fractions. However, isolation and characterization of R-Ala and of other polypeptides not indicated by end-group analysis on the unfractionated protein were necessary for definite conclusions relating different lipoprotein classes on the basis of a common polypeptide moiety.

HDL_2 Protein. Chromatography on DEAE-cellulose of the lipid-free protein moieties of HDL_2 lipoproteins usually gave several polypeptide fractions in addition to the three fractions obtained from HDL_3 protein. In a representative chromatogram (Figure 2), peaks 3, 4, and 5 correspond to fractions 1, 2, and 3 obtained from HDL_3 protein (Shore and Shore, 1968b). Fractions 4, 5, 6, 7, and 8 were rechromatographed (separately) in order to assure one component per fraction. The major component of each fraction on rechromatography was eluted at the position (Figure 2) of the fraction from which it was derived. Fractions 1, 2, and 10 did not yield any carboxyl-terminal group. Fraction 3 contained a polypeptide with the carboxyl-terminal sequence R-Thr-Gln (1 mole/14,000 g). Fractions 4, 5, and 6 contained peptide(s) (R_1-Thr, R_2-Thr, and R_3-Thr, respectively) with carboxyl-terminal threonine (1 mole/15,000 g). Fractions 7 and 8 contained peptide(s) with carboxyl-terminal alanine (1 mole/12,000 g) and yielded both alanine and valine (3:1) on digestion with carboxypeptidase A at 37°. Although the polyacrylamide gel pattern of fraction 9 was not a sharp band, suggesting hetero-

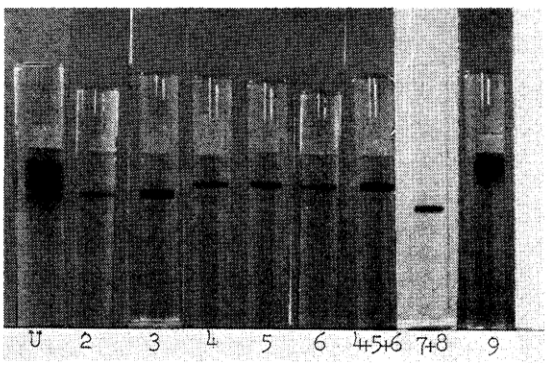

FIGURE 3: Patterns from disc electrophoresis at pH 8.8 in 12.5% polyacrylamide gels containing 8 M urea of the protein moiety of a serum high-density lipoprotein fraction (*d* 1.083–1.124 g/cc or HDL_2) and of polypeptides obtained from it by DEAE-cellulose chromatography. U, unfractionated protein. The other patterns are designated by the appropriate fraction numbers from Figure 2: 2, R-X; 3, R-Thr-Gln; 4, R_1-Thr; 5, R_2-Thr; 6, R_3-Thr; 8, R-Ala from fractions 7 and 8; 9, R-Gly-Gln.

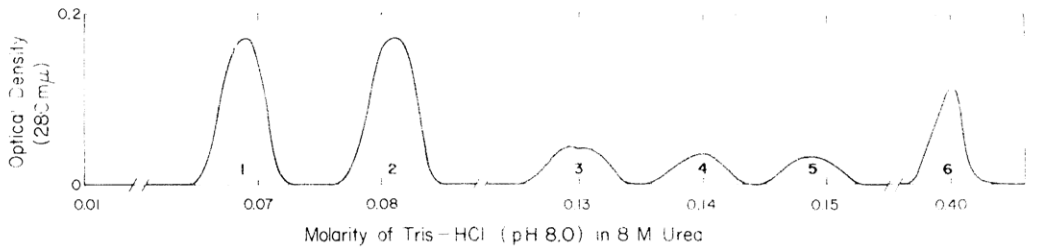

FIGURE 4: Chromatographic separation of the lipid-free protein moiety of a serum low-density lipoprotein fraction (S_f 20–100 or 0.98–1.006 g/cc) on a DEAE-cellulose column (0.9 × 27 cm). The conditions are the same as in Figure 2 except that 5 mg of protein was applied to the column.

geneity, the gel pattern and the amino acid composition were not changed by rechromatography. Incubation of fraction 9 with carboxypeptidases (30 min at 25°) released only two amino acids, glycine and glutamine (1 mole each/24,000 g), of which glutamine was terminal.

Polyacrylamide gel electrophoretic patterns are shown in Figure 3 for the sample of HDL_2 protein which gave the fractions shown in Figure 2 and for the polypeptides obtained from it. Fractions 1 and 10 (peptides) were not fixed by the solution (1% Amido Schwarz, 50% methanol, and 10% acetic acid in water or 1% stain in 7% acetic acid) used to fix and stain the gels. The polypeptides from fractions 4, 5, and 6 were unresolved when electrophoresed as a mixture in the gels (Figure 3). They were also very similar in amino acid composition (Table II) except that R_2-Thr and R_3-Thr (fractions 5 and 6) were not lacking in isoleucine as was R_1-Thr (fraction 4). The polypeptides from fractions 3 and 4 appear to be identical with the R-Gln and R-Thr polypeptides, respectively, which were isolated previously from HDL_3 lipoproteins (Shore and Shore, 1968b). The polypeptides of fractions 1 and 10 appear very similar in amino acid composition but more evidence is needed to establish that they are related with respect to amino acid sequence. The polypeptides of the two fraction 2 samples were eluted at the same position (peak 2 of Figure 2), but from different preparations of HDL_2. They appear similar in composition except with respect to tyrosine content. The difference is not due to destruction of tyrosine during hydrolysis of the polypeptide, since the absorption spectrum of the unhydrolyzed peptide indicated no tyrosine. Although two fractions from the column (7 and 8) yielded R-Ala, they appear to have the same composition which is given in one column in Table II; there is one isoleucine residue per polypeptide molecule since end-group analysis indicates the molecular weight of the polypeptide to be 12,000 to 13,000. Fraction 9 (R_2-Gln or R-Gly-Gln) does not appear to be related in composition to R_1-Gln or R-Thr-Gln (fraction 3).

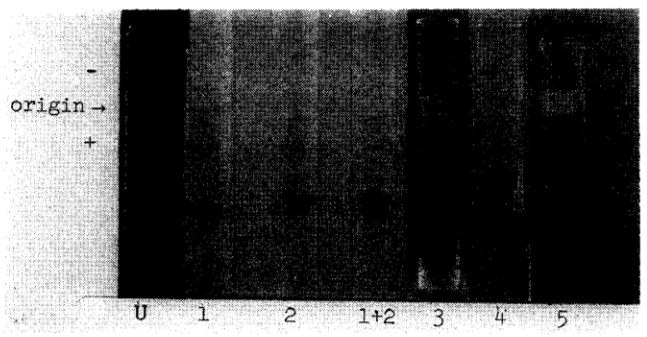

FIGURE 5: Patterns from disc electrophoresis of the protein moiety of S_f 20–100 lipoproteins and of polypeptides obtained from it by DEAE-cellulose chromatography; at pH 8.8, in polyacrylamide gels containing 8 M urea. U, unfractionated protein in 7.5% gel. The other patterns, designated by the appropriate fraction numbers from Figure 4, are in 12.5 % gels.

S_f 20–100 Lipoprotein Protein. Chromatographic fractionation of the S_f 20–100 lipoprotein protein (Figure 4) did not yield detectable amounts of the major polypeptides of HDL protein, which are characterized by carboxyl-terminal glutamine and threonine and are eluted before 0.06 M Tris-HCl. Either one or two fractions (peaks 1 and 2 of Figure 4) which yielded carboxyl-terminal alanine (1 mole/12,000 g of protein) were obtained depending upon the individual source of the protein. Alanine and valine, as in the unfractionated protein, were released by carboxypeptidase A from the purified polypeptide in a 3:1 ratio; of these, two residues of alanine and the valine are not terminal. The kinetics of release by carboxypeptidase A at 37° did not distinguish between R-Ala-Val-Ala-Ala and R-Ala-Ala-Val-Ala. When the reaction was carried out with a carboxypeptidase B preparation at 25°, only two residues of alanine in the chain were released and the release of valine was relatively slower. Under these conditions the sequence R-Ala-Val-Ala-Ala appeared probable, since the ratio of alanine:valine was greater than $1 + X$, where X is the fraction of valine released (maximum valine release = 1) at some intervals of the reaction.

In addition to the peptides with carboxyl-terminal alanine, the HDL_2 and the S_f 20–100 lipoproteins contained in common a second polypeptide of very similar if not identical amino acid composition (fraction 6 of Figure 4) which is eluted at about 0.4 M Tris-HCl in 8 M urea. No other polypeptides common to HDL and S_f 20–100 lipoproteins were found. Three other peptides (fractions 3, 4, and 5 of Figure 4), which contained little or no tyrosine were found as minor components in S_f 20–100 lipoprotein protein but they differed from the HDL_2 polypeptide (fraction $2_{(2)}$) which contained no tyrosine.

Polyacrylamide gel electrophoresis patterns for the polypeptides separated on the DEAE-cellulose columns are shown in Figure 5. The polypeptide of fraction 6, Figure 4, was not fixed in the staining procedure following gel electrophoresis. The amino acid composition of the polypeptides isolated from S_f 20–100 lipoprotein protein is given in Table III. The column numbers correspond to the fraction numbers in Figure 4. Both gel electrophoresis (Figure 5) and amino

TABLE III: Amino Acid Composition (moles/10^3 moles of Amino Acids[a]) of Polypeptides from Low-Density Lipoproteins of Human Serum.

	S_f 20–100 Lipoprotein Fractions						S_f 4–8 Lipoprotein Fractions	
Amino Acid	1	2	3	4	5	6	1	2
Lys	72.6	69.6	84.7	75.6	59.2	75.5	75.0	73.1
His	14.7	14.0	14.8	22.9	19.2	29.5	21.0	19.5
Arg	28.0	27.8	29.4	22.5	29.0	13.6	28.0	26.8
Asp	93.0	91.0	88.0	84.7	90.1	72.0	97.4	74.8
Thr	59.1	61.6	45.0	49.4	40.7	40.3	58.6	47.5
Ser	128.0	125.3	124.6	169.3	146.0	203.5	101.6	148.0
Glu	143.7	148.8	160.5	151.1	163.4	159.8	149.8	146.7
Pro	29.7	29.2	29.7	24.8	19.6	20.2	31.8	26.6
Gly	57.8	59.2	83.6	136.7	182.5	193.5	102.1	158.3
Ala	107.0	109.1	130.5	93.0	68.2	71.4	68.5	72.8
Val	65.1	66.0	75.2	46.1	38.1	29.7	52.2	41.0
$^1/_2$-Cys[b]	0	0	7.0	14.2	12.8	7.0	7.4	6.7
Met	21.4	20.2	6.8	7.6	12.9	6.8	15.2	13.9
Ile	14.3	7.4	7.0	14.7	30.2	20.8	38.1	33.1
Leu	72.6	70.1	73.3	52.8	63.3	34.9	89.4	65.8
Tyr	26.7	25.0	0	6.8	0	6.8	27.6	19.5
Phe	44.0	42.7	42.7	35.8	25.0	15.1	36.3	26.8
Trp[c]	28.0	28.0				0		

[a] Experimental values for cysteic acid, serine, and threonine were corrected for losses of 8, 10, and 5%, respectively. [b] Determined as cysteic acid. [c] Determined by the method of Bencze and Schmid (1957).

acid composition (Table III) indicated that the polypeptide R-Ala exists in more than one form. The two forms (columns 1 and 2 of Table III) are very similar in composition; one form appears to contain one isoleucine residue per molecule and the other, two. The form found in HDL_2 contained one isoleucine residue per molecule.

S_f 4–8 Lipoprotein Protein. When the protein moiety of S_f 4–8 lipoproteins was chromatographed on a DEAE-cellulose column under the same conditions as the proteins of S_f 20–100 lipoproteins, none of the peptides obtained from high-density lipoproteins or S_f 20–100 lipoproteins was obtained. The only two polypeptides eluted were eluted by 0.08 and 0.4 M Tris-HCl. Their compositions (Table III) are not very similar to that of any polypeptides isolated from other lipoprotein classes.

Individual Variation and Recoveries. In HDL_3 protein, three polypeptide fractions (R-Gln, R_1-Thr, and R_2-Thr) comprised at least 90–95% of the total protein. In HDL_2, proteins from five individuals varied considerably in the per cent distribution of their polypeptides although these three polypeptides comprised over 60% of the total protein in every case. The proportion of R_1-Thr to R_2-Thr varied between approximately 2:1 and 5:1 among the samples fractionated. The fraction of the total protein found in the other fractions (1, 2, 7, 8, 9, and 10 of Figure 2) also varied considerably, and there was no consistent proportion among these fractions. In HDL_2 the R-Ala peptide(s) made up less than 10% of the total protein. Fraction 2 was very small (less than 5%) in some cases and as much as 15% of the total protein in one; in two individuals (both male) it contained no tyrosine ($2_{(2)}$ of Table II) and in others it contained the amino acids shown under $2_{(1)}$ of Table II. The amount of fraction 9 (R-Gly-Gln) in HDL_2 protein varied from about 5 to 15%. Essentially all of the protein of HDL_2 was eluted from the DEAE-cellulose column.

Three preparations of S_f 20–100 lipoprotein protein and one of S_f 4–8 lipoprotein protein were taken for DEAE-cellulose chromatography. With the former, 50–60% of the total protein was recovered in the fractions from the column, and with the latter the recovery was of the order of 30%. In both cases, the amount of protein not eluted from the column was considerable; some losses may have occurred in the concentration and dialysis procedures subsequent to fractionation.

Discussion

The high-density lipoproteins and the very low-density lipoproteins of serum have been reported to be related structurally and metabolically, on the basis of immunochemical and metabolic studies (reviewed by Schumaker and Adams, 1969). From the results of the present study it appears that these two lipoprotein classes are related by virtue of two polypeptides which are normally minor components of HDL protein but major components of S_f 20–100 lipoprotein protein. However, since normal serum contains more HDL protein (~100 mg/100 ml) than S_f 20–100 lipoprotein protein (~10 mg/100 ml) (Gofman *et al.*, 1954), the total amounts in the two lipoprotein fractions are similar. None of the high-density lipoprotein peptides were found in S_f 4–8 lipoproteins. The two fractions are related to each other (and to S_f 20–100 lipoproteins) by lipid-exchange processes (Schumaker and Adams, 1969). The S_f 4–8 lipoproteins and very low-density

lipoproteins may also be related by common polypeptides, as yet not isolated, since some of the protein from these two fractions was not eluted from the column. Direct interconversion of lipoproteins by removal or addition of lipids to a polypeptide or a polypeptide–lipid complex present in both HDL and S_f 20–100 lipoproteins seems most likely with the polypeptides R-Ala and R-X (HDL: fractions 7, 8, and 10 of Table II; S_f 20–100: fractions 2 and 6, Table III) and improbable with the major polypeptides R-Gln and R-Thr of HDL. Immunochemical and amino acid sequence studies are necessary for unequivocal establishment of identity, although the similarities in composition, in carboxyl-terminal sequence, in polyacrylamide gel patterns, and in DEAE-cellulose chromatography indicate that the peptides R-Ala and the peptides R-X from high-density and very low-density lipoproteins are very similar or identical. A role in lipid transport for these two polypeptides as lipid acceptors and donors is an interesting possibility and their roles in the structure of the lipoproteins should be studied further. Some HDL lipoprotein molecules do not contain the peptide R-Ala; a number of HDL_3 fractions which contain little or no R-Ala have been prepared. However, it is not clear whether R-Ala occurs in lipoprotein molecules distinct from those containing R-Thr and R-Gln, or whether lipid complexes of R-Ala associate with HDL_3 to give a larger, less dense HDL_2 molecule. The lipoproteins should be further studied with attempts to fractionate them on the basis of their peptide content and to obtain lipoprotein subunits of the molecule. The results should answer some of the questions about the lipoprotein molecule from which each of the polypeptides is derived.

The existence of multiple forms of polypeptides may be of considerable significance in the physiological and biochemical functions of the lipoproteins since minor changes in amino acid sequence might affect the binding of lipids.

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