Isolation and Characterization of the Cyanogen Bromide Fragments from the High-Density Apolipoprotein Glutamine I†

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ABSTRACT: The major protein component of human plasma high-density lipoproteins (HDL), designated apoLP-Gln-I by the presence of carboxyl-terminal glutamine, was isolated from HDL by delipidation and chromatography on Sephadex G-150 and DEAE-cellulose in urea. Homogeneity of the isolated protein was established by amino acid analysis, by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, and by amino- and carboxyl-terminal analyses. By amino acid analysis, the isolated protein contained 242 amino acids including three residues of methionine. ApoLP-Gln-I was treated with a 500 molar excess of cyanogen bromide, resulting in cleavage of more than 95% of the methionine residues. Chromatography of the digest on Bio-Gel P-30 in 25% formic acid yielded three of the fragments, CNBr II, III, and IV, in essentially a pure state. Special pretreatment conditions were required prior to chromatography on Bio-Gel P-30 in order to achieve satisfactory separation of the fragments in good yield. The fourth fragment, CNBr I, was further purified on DEAE-cellulose in 6 M urea.

CNBr I contained 92 amino acids, was lacking homoserine or homoserine lactone, and had carboxyl-terminal glutamine, establishing this fragment as the carboxyl terminus of apoLP-Gln-I. The amino terminus of CNBr I was arginine. CNBr II contained 89 amino acid residues, amino-terminal aspartic acid, and carboxyl-terminal homoserine. Since only CNBr II contained amino-terminal aspartic acid, this fragment corresponded to the amino-terminal end of apoLP-Gln-I. CNBr III contained 37 amino acid residues, amino-terminal glutamic acid, and carboxyl-terminal homoserine. CNBr IV had 25 residues, amino-terminal serine, and carboxyl-terminal homoserine. Summation of the amino acid residues of the four fragments accounted for 243 residues, which is in good agreement with a value of 242 from amino acid analyses of the intact protein.

Human plasma high-density lipoproteins (HDL)[1] have been shown to contain two major and several minor protein or apoprotein components. The structures of these molecules have been discussed in several recent reviews (Scanu and Wisdom, 1972; Scanu, 1972; Shore and Shore, 1972; Fredrickson *et al.*, 1972). The two major apoproteins of HDL have been designated apoLP-Gln-I and apoLP-Gln-II based on their carboxyl-terminal amino acid (Lux *et al.*, 1972a). These two proteins have different chemical, physical, and immunochemical properties (Scanu, 1972). The complete amino acid sequence of apoLP-Gln-II has recently been reported (Brewer *et al.*, 1972a). A preliminary study has described the phospholipid-binding properties of the cyanogen bromide fragments of apoLP-Gln-II (Lux *et al.*, 1972b).

The other major HDL apoprotein, apoLP-Gln-I, has been shown to have a molecular weight of about 25,000–27,000 (Scanu *et al.*, 1971; Edelstein *et al.*, 1972), to contain amino-terminal aspartic acid (Edelstein *et al.*, 1972; Lux and John, 1972), and to be devoid of cysteine or cystine. There are conflicting reports about the carboxyl-terminal amino acid sequence of this protein (Kostner and Alaupovic, 1971; Rudman *et al.*, 1970; Lux and John, 1972, Edelstein *et al.*, 1972; Shore and Shore, 1972). It is, however, generally agreed that glutamine is the carboxyl-terminal amino acid. Shore and Shore (1972) have reported the amino acid sequence of the first 40 residues of apoLP-Gln-I. In a preliminary report we have recently (Jackson *et al.*, 1972) described the interaction between phosphatidylcholine and the fragment containing the 92 residues at the carboxyl terminus of the molecule. The purpose of this communication is to describe fully the isolation and characterization of the four cyanogen bromide fragments of apoLP-Gln-I. Such information is necessary in order to pursue a more detailed investigation of the primary structure and of the lipid-binding sites within the protein.

Experimental Section

ApoLP-Gln-I Preparation. Plasma HDL were isolated from normal fasting donors by flotation between densities 1.063 and 1.210 g/cm^3 in KBr in a Beckman Model L2-65B ultracentrifuge (Lux *et al.*, 1972a). The isolated HDL were dialyzed overnight against 0.01% EDTA (pH 8.0) and delipidated with diethyl ether–ethanol (3:1) at 4° (Lux *et al.*, 1972a). The apoproteins were solubilized in 0.1 M Tris-HCl (pH 8.0) containing 5.4 M urea and 0.01% EDTA at a concentration of approximately 20 mg/ml and were subjected to chromatography on Sephadex G-150 as described for apoLP-Gln-II (Jackson and Gotto, 1972). In a typical experiment, 200 mg was fractionated at room temperature on a 2.6 × 200 cm column in 0.1 M Tris-HCl (pH 8.0) containing 5.4 M urea and 0.01% EDTA. The fractions corresponding to apoLP-Gln-I, or fraction III of Scanu *et al.* (1969), were pooled and dialyzed 24 hr at 4° against 0.01% EDTA (pH 8.0). The desalted protein was lyophilized and stored in a dry state at

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[1] The abbreviations used are: HDL, human plasma high-density lipoproteins of d = 1.063 to 1.210 g/cm^3; VLDL, human plasma very low-density lipoproteins of density < 1.006 g/cm^3; apoLP-Gln-I and apoLP-Gln-II, the major apoproteins of HDL; CNBr I, II, III, and IV, the cyanogen bromide fragments of apoLP-Gln-I.

−50°. For most of the studies, apoLP-Gln-I was refractionated on DEAE-cellulose in urea as described by Shore and Shore (1969). The protein (60 mg) was applied to a column (1.6 × 30 cm) equilibrated with a solution of 0.01 M Tris-HCl (pH 8.0) containing 5.4 M urea and 0.01% EDTA, and was eluted with a linear gradient of NaCl from 0 to 0.125 M. The gradient was prepared by mixing 250 ml of the equilibrating buffer and 250 ml of the same buffer containing 0.125 M NaCl. The DEAE-cellulose was Whatman microgranular DE-52.

Cyanogen Bromide Cleavage. The salt-free, lyophilized protein was dissolved in 70% formic acid at a concentration of 10 mg/ml and was treated with a 500 molar excess (based on methionine content) of cyanogen bromide for 24 hr at 23°. The reaction mixture was then diluted with 250 ml of water and lyophilized. Amino acid analysis indicated that more than 95% of the methionine residues had been converted to homoserine.

Gel Filtration. The cyanogen bromide digest of apoLP-Gln-I was subjected to chromatography on a column (2.6 × 200 cm) of Bio-Gel P-30 (Bio-Rad Laboratories) equilibrated in 25% formic acid. The apoprotein (200 mg) was dissolved in 20 ml of 0.1 M Tris-HCl (pH 8.0) containing 6 M urea and was taken to dryness by lyophilization; 25% formic acid (10 ml) was added to the dry protein. The mixture was incubated for 2 hr at 37°, applied to the column, and eluted with 25% formic acid at 23°; the flow rate was adjusted to 25 ml/hr. Peptides were monitored by absorbance at 280 nm. The appropriate fractions were then pooled, diluted with a tenfold excess of water, and lyophilized. The Bio-Gel column was calibrated by chromatography of human β-lactoglobulin, mol wt 37,100; bovine α-chymotrypsinogen, mol wt 26,000; equine cytochrome *c*, mol wt 13,400; and an apoprotein with carboxyl-terminal alanine (apoLP-Ala) from human plasma VLDL, mol wt 9,300 (Brewer *et al.*, 1972b).

Amino Acid Analysis. Analyses were performed on a Beckman Model 117 amino acid analyzer equipped for single column methodology, high sensitivity (5 nmol), and automatic sample application. Samples were routinely hydrolyzed in 6 N HCl, 0.1% phenol, for 22 hr at 110° in sealed evacuated hydrolysis tubes. After hydrolysis, the acid was removed by evaporating under reduced pressure in a rotary evaporator. Tryptophan was determined by the method of Liu and Chang (1971); homoserine by analysis at pH 2.80; and glutamine by analysis in lithium citrate buffer at pH 2.80 and 38°.

Digestion with Carboxypeptidase A. Digestions were carried out with 0.10 mg of carboxypeptidase A and 60–120 nmol of peptide in 0.20 ml of 0.1 M Tris-HCl (pH 8.0). Carboxypeptidase A (COADFP) was purchased from Worthington.

Tryptic Peptide Mapping. Cyanogen bromide fragments (0.1 μmol) were digested with 100 μg of L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone treated trypsin (Worthington) for 16 hr at 23°. The samples were applied directly to Whatman 3 mm paper and subjected to high-voltage electrophoresis at pH 3.6 in a Gilson apparatus for 1.5 hr at 2500 V. The paper was chromatographed with a buffer system containing pyridine–acetic acid–water (1:10:289, v/v) for 20 hr. The peptides were identified by dipping the papers in a solution of ninhydrin–collidine.

Other Methods. Polyacrylamide gel electrophoresis was performed in 0.1% sodium dodecyl sulfate–0.1 M sodium phosphate buffer (pH 7.0) (Weber and Osborn, 1969) or in urea (pH 8.9) (Davies, 1964). Amino-terminal amino acids were determined by manual Edman procedures (Edman, 1970). The PTH amino acids were identified by gas chromatographic techniques as described by Pisano *et al.* (1972). The Edman results were confirmed by the dansyl chloride method (Gray, 1967). The dansyl amino acids were separated and identified on polyamide sheets (Woods and Wang, 1967).

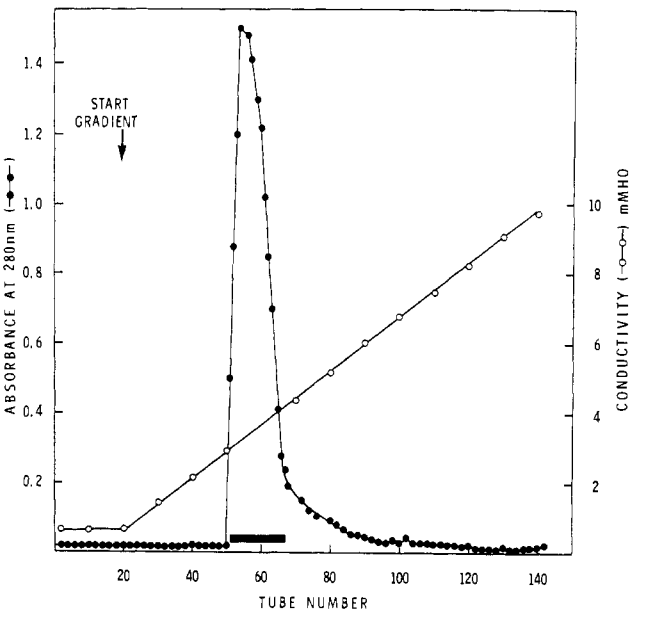

FIGURE 1: Chromatography of apoLP-Gln-I on DEAE-cellulose in 6 M urea. The column (1.6 × 50 cm) was equilibrated with 0.01 M Tris-HCl buffer (pH 8.0) containing 6.0 M urea and was operated at room temperature. The sample (60 mg) was applied and eluted with a NaCl gradient. To one side of the two-chambered gradient apparatus was added 250 ml of 0.01 M Tris-HCl (pH 8.0) containing 6 M urea. To the other side was added 250 ml of the same buffer, but also containing 0.125 M NaCl. The flow rate was adjusted to 30 ml/hr and 5-ml fractions were collected.

Results

Isolation and Characterization of ApoLP-Gln-I. Delipidated HDL were fractionated initially on Sephadex G-150 in 5.4 M urea. The peak corresponding to fraction III of Scanu *et al.* (1969) or apoLP-Gln-I in the present study was further purified on DEAE-cellulose in 6 M urea. ApoLP-Gln-I was eluted as a single peak (Figure 1) and gave a single major band on polyacrylamide gel electrophoresis in sodium dodecyl sulfate (Figure 2A). On the basis of comparison with standard proteins of known size, apoLP-Gln-I had an apparent molecular weight of about 27,000 on both Sephadex G-150 chromatography and on electrophoresis in sodium dodecyl sulfate. These results are consistent with published values (Edelstein *et al.*, 1972; Scanu *et al.*, 1971).

The amino acid composition of apoLP-Gln-I was also in reasonable agreement with those from other laboratories (Table I). Extended time hydrolyses were performed to determine the extent of hydrolysis of the apolar amino acids and the destruction of threonine and serine. The extrapolation of these values is presented as the assumed values (Table I). From the amino acid content, it was calculated that apoLP-Gln-I contains 242 amino acids with a molecular weight of 27,986. The protein contained no detectable half-cystine and less than 0.05 residue of isoleucine. Compared to published values, our findings agreed most closely with those of Edelstein *et al.* (1972) and Lux and John (1972).

Preparation and Isolation of the Cyanogen Bromide Frag-

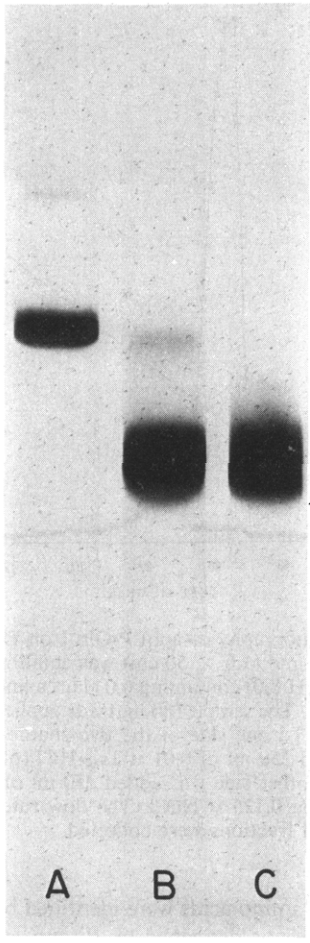

FIGURE 2: Polyacrylamide gel electrophoresis in sodium dodecyl sulfate of apoLP-Gln-I (A), CNBr I (B), and CNBr II (C). Twenty micrograms of each sample was applied to the gel for electrophoresis. The gels were stained with Coomassie Blue.

TABLE I: Amino Acid Composition of ApoLP-Gln-I.[a]

	Time of Hydrolysis[d]			Assumed Values	Range of Reported Values[b]
	22 hr	48 hr	96 hr		
Tryptophan[c]	3.5	N.D.	N.D.	4	3.7–7.2
Lysine	19.1	18.5	18.6	19	18.0–23.8
Histidine	4.7	4.4	4.1	5	3.8–5.6
Arginine	14.9	14.8	14.6	15	14.4–16.6
Aspartic acid	20.3	20.2	19.9	20	17.8–23.8
Threonine	9.8	8.4	7.4	10	9.1–10.6
Serine	14.1	12.3	10.7	15	11.8–15.1
Glutamic acid	48.4	48.4	48.2	49	36.3–48.0
Proline	9.2	9.5	9.7	10	8.9–12.0
Glycine	9.6	9.6	9.5	10	9.6–11.5
Alanine	18.3	18.4	18.6	18	16.6–20.1
Cystine	*c*	*c*	*c*	0	0–0.3
Valine	12.3	12.6	12.5	13	12.0–13.9
Methionine	2.8	2.5	1.9	3	1.9–3.6
Isoleucine	*c*	*c*	*c*	0	0–1.0
Leucine	38.1	37.7	37.5	38	35.0–42.0
Tyrosine	6.4	6.6	6.3	7	5.3–7.9
Phenylalanine	6.0	5.5	5.0	6	5.5–7.4

[a] The compositions are expressed as moles/mole of protein using an assumed molecular weight of 27,000. [b] Values obtained from Edelstein *et al.* (1972), Lux and John (1972), Shore and Shore (1969), and Rudman *et al.* (1970). [c] <0.05 mol/mol of protein. [d] N.D. = not determined.

ments of ApoLP-Gln-I. Based on 242 amino acids, apoLP-Gln-I was expected to contain three residues of methionine and to yield four fragments on treatment with cyanogen bromide. After treatment of apoLP-Gln-I with cyanogen bromide, the conversion of methionine to homoserine was always greater than 95% and in most cases was greater than 99% as determined by amino acid analysis. Isolation of the cyanogen bromide fragments in good yield required several preliminary experiments. The following conditions were empirically found to yield the optimal separation and highest yields of the cyanogen bromide fragments. The digest from the cyanogen bromide treatment was dissolved in 0.10 M Tris-HCl buffer (pH 8.0) containing 6 M urea, lyophilized, and redissolved in 25% formic acid. Omission of these specific steps led to significant changes in the chromatographic properties of the fragments on Bio-Gel P-30 in 25% formic acid. When these conditions of pretreatment of the digest were employed prior to chromatography, the elution profile shown in Figure 3 was obtained. The peptides eluting in peaks I_a, I_b, and I_c from the P-30 column differed in amino acid analyses and in mobility on polyacrylamide gel electrophoresis in urea. Peak I_b contained no methionine, homoserine, or homoserine lactone. Peaks I_a and I_c contained homoserine and homoserine lactone. On polyacrylamide electrophoresis in standard urea gels, peaks I_a and I_c contained a slower migrating component (CNBr I) of R_F 0.2 and a faster migrating component (CNBr II) of R_F 0.5. Peak I_b contained entirely the slower migrating component. These analyses suggested that peaks I_a and I_c contained CNBr I and CNBr II and that peak I_b contained only CNBr I. By amino acid analysis, the total peak I_c contained about 75% CNBr II and 25% CNBr I while the down slope or more retarded half of peak I_c contained entirely CNBr II. The apparent contribution of CNBr II in peak I_c to that of CNBr I was exaggerated by absorbancy measurements at 280 nm, owing to the high tryptophan content of CNBr II (Table II). Isolation of CNBr I required further chromatography of peaks I_a, I_b, and I_c on DEAE-cellulose in 6 M urea (Figure 4); the fragment eluted from the column unretarded. CNBr II was retained by the column and could be eluted with a linear gradient of sodium chloride at a molarity greater than 0.05. Approximately 33% of the initial CNBr II could be recovered by this procedure. CNBr I was obtained in highly purified form at a yield of 64–85% of the original material. Omission of the specific pretreatment steps including lyophilization of the digest in urea led to a significant shift of CNBr II from peak II to peak I_c of Figure 3. This shift reduced the yield of CNBr II in peak II from 65 to 30%.

Peaks II, III, and IV from the P-30 column were clearly separated (Figure 3), and corresponded to unique cyanogen bromide fragments. These peptides were obtained from the P-30 column in nearly pure form and required only rechromatography on Sephadex G-50 in 0.10 M ammonium bicarbonate to remove the last traces of impurities. Peaks II, III, and IV were designated CNBr II, III, and IV, respectively, while the major peptide isolated from peaks I_a, I_b, and I_c was designated CNBr I.

Peaks I_b and I_c eluted from a calibrated P-30 column at a retention volume consistent with a molecular weight of about

TABLE II: Amino Acid Compositions of Cyanogen Bromide Fragments.[a]

Amino Acid	CNBr I	CNBr II	CNBr III	CNBr IV	Total	ApoLP-Gln-I
Tryptophan	*c*	2.6 (3)	*c*	0.8 (1)	4	4
Lysine	6.1 (6)	6.0 (6)	2.9 (3)	4.3 (4)	19	19
Histidine	3.3 (4)	*c*	0.9 (1)	*c*	5	5
Arginine	8.0 (8)	3.9 (4)	3.0 (3)	*c*	15	15
Aspartic acid	5.8 (6)	10.3 (11)	0.3 (0)	3.1 (3)	20	20
Threonine	4.4 (5)	4.3 (5)	*c*	*c*	10	10
Serine	5.4 (6)	6.1 (7)	1.0 (1)	1.0 (1)	15	15
Glutamic acid	14.9 (15)	17.2 (17)	12.2 (12)	7.1 (7)	51	49
Proline	2.8 (3)	3.9 (4)	1.8 (2)	1.1 (1)	10	10
Glycine	3.0 (3)	4.6 (5)	2.0 (2)	*c*	10	10
Alanine	11.9 (12)	2.7 (3)	2.0 (2)	1.0 (1)	18	18
Valine	2.7 (3)	6.1 (6)	1.0 (1)	1.8 (2)	12	13
Methionine[b]	*c*	0.4 (1)	0.5 (1)	0.6 (1)	3	3
Leucine	15.7 (16)	12.1 (12)	7.2 (8)	2.2 (2)	38	38
Tyrosine	2.6 (3)	2.0 (2)	0.8 (1)	0.9 (1)	7	7
Phenylalanine	1.7 (2)	2.7 (3)	*c*	0.9 (1)	6	6
Total Residues	92	89	37	25	243	242
Yield (%)	64	71	66	34		
Molecular weight	10,431	10,268	4376	3070	28,145	27,986

[a] The compositions were obtained from 22 hr hydrolysates and are expressed as moles/mole of protein. The assumed integral values are given in parentheses. [b] As homoserine. [c] <0.05 mole/mole of protein.

20,000 (Figure 3). Based on polyacrylamide gel electrophoresis in sodium dodecyl sulfate and on the quantity of leucine, threonine, and glutamine released by carboxypeptidase A (Table III) a molecular weight of 10,400 was obtained for CNBr I. These findings indicated that CNBr I eluted from P-30 in 25% formic acid as dimeric, trimeric, and possibly even tetrameric aggregates. Attempts to dissociate CNBr I into monomeric forms were unsuccessful except with polyacrylamide gel electrophoresis in sodium dodecyl sulfate (Figure 2B). Peak II, however, eluted from a calibrated P-30 column at a retention volume consistent with an approximate molecular weight of 10,000 (Figure 3).

Although CNBr I and II eluted at different positions from Bio-Gel columns, they appeared to be of about the same size as assessed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (Figure 2B,C). CNBr I and CNBr II each had an apparent molecular weight of about 10,000 based on comparisons with standard proteins of known molecular weight. This value was in close agreement with the molecular weight obtained from amino acid analysis (Table II), from the elution

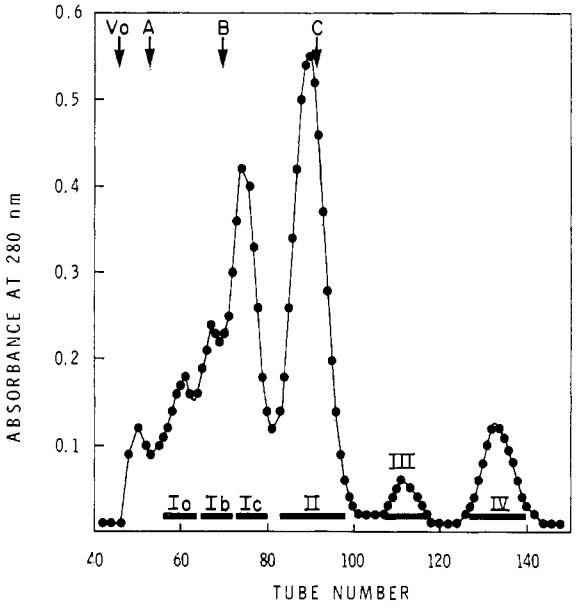

FIGURE 3: Chromatography of the cyanogen bromide digest of apoLP-Gln-I on Bio-Gel P-30. The column (2.6 × 200 cm) was equilibrated with degassed 25% formic acid. The sample, 200 mg, was dissolved in 20 ml of 6 M urea and 0.1 M Tris-HCl (pH 8.0) and lyophilized. 25% formic acid (10 ml) was added to the dry sample, which was then incubated for 2 hr at 37° prior to application to the column. The column was eluted with 25% formic acid at a flow rate of 25 ml/hr. Fractions (4 ml) were collected. V_0 corresponded to the void volume of the column. A, B, and C corresponded to the elution volume of β-lactoglobulin (mol wt, 37,100), chymotrypsinogen (mol wt 26,000) and the VLDL apoprotein, apoLP-Ala (mol wt 9300), respectively.

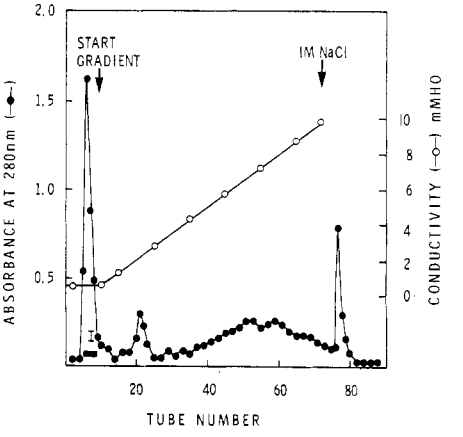

FIGURE 4: Chromatography of I_a, I_b, and I_c (Figure 3) on DEAE-cellulose in 6 M urea. Conditions were identical with the DEAE chromatography of apoLP-Gln-I as described in Figure 1. In later experiments, the gradient was omitted and CNBr II was eluted with 6 M urea and 0.01 M Tris-HCl (pH 8.0) containing 0.3 M NaCl.

TABLE III: Carboxypeptidase A Digestion of ApoLP-Gln-I and CNBr Fragments.[a]

Peptide	Time (min)	Amino Acid Released (mol/mol of peptide)						
		Thr	Gln	Glu	Leu	Lys	Homoserine	Asn
ApoLP-Gln-I	5	0.2	0.7					
	15	0.6	1.0		0.5			0.3
	30	0.9	0.9		0.7			0.6
	60	1.0	0.7		1.0			0.7
CNBr I	5	0.1	0.4					
	15	0.4	0.5		0.1			
	30	0.6	0.6		0.4			0.1
	60	0.8	0.9		0.7	0.1		0.3
CNBr II	60	0.1					0.1	
	180	0.2		0.2			0.5	
CNBr III	30			0.1			0.2	
	60			0.2			0.2	
	180			0.2			0.2	
	720			0.4			0.6	
CNBr IV	30	None released						
	60	None released						
	600	None released						

[a] ApoLP-Gln-I and the CNBr fragments were digested with carboxypeptidase A as described in the text. The results are expressed as moles of amino acid released/mole of peptide.

of CNBr II on P-30 (peak II), and from the treatment of CNBr I with carboxypeptidase A (Table III).

CNBr I was the only fragment devoid of homoserine or homoserine lactone and, therefore, corresponded to the carboxyl-terminal peptide of apoLP-Gln-I. CNBr I contained 92 amino acid residues. Approximately one-half of these residues were glutamic acid and/or glutamine, alanine, and leucine. Two-thirds of the alanine residues (12 of 18) and four of the five histidines of apoLP-Gln-I were in CNBr I. Tryptophan was completely absent. A tryptic peptide fingerprint map revealed 14 peptides and was consistent with the number of basic amino acids in CNBr I.

CNBr II had 88 amino acids, three of the four tryptophans, and more than half of the aspartic acid and/or asparagine residues of apoLP-Gln-I. Histidine was absent. CNBr II contained six lysine and four arginine residues consistent with 12 peptides obtained from a tryptic fingerprint.

CNBr III and CNBr IV contained respectively 37 and 25 amino acids. CNBr III had no tryptophan, aspartic acid, threonine, or phenylalanine. More than half of its residues were glutamine and/or glutamic acid and leucine. (CNBr IV was devoid of histidine, arginine, threonine, and glycine. By fingerprint analyses, CNBr III had seven ninhydrin spots and CNBr IV, five.)

The overall yields of CNBr I, II, III, and IV were 64, 71, 66, and 34%, respectively. These four CNBr peptides accounted for 243 amino acids or all of the amino acids present in apoLP-Gln-I. This is consistent with the number of tryptic peptides isolated from each of the individual cyanogen bromide fragments.[2]

Amino- and Carboxyl-Terminal Analysis of the CNBr Fragments. ApoLP-Gln-I and each of its CNBr peptides were examined for amino- and carboxyl-terminal amino acids by the Edman reaction, the dansyl method, and with carboxypeptidase A as described in the Experimental Section (Table III). Homoserine was released from CNBr II and III on treatment with carboxypeptidase A. No detectable amino acid was released from CNBr IV when digested with carboxypeptidase A. However, CNBr IV did contain a significant quantity of homoserine (Table II), which was presumed to be the carboxyl-terminal residue. The order of release of amino acids of CNBr I by carboxypeptidase A indicated carboxyl-terminal amino acid sequence of Leu-Thr-Gln. The amino-terminal amino acid from CNBr I was identified as arginine. The amino-terminal amino acid for CNBr II was aspartic acid; for CNBr III, glutamic acid; for CNBr IV, serine; and for apoLP-Gln-I, aspartic acid. These results were confirmed by both dansylation and the Edman procedure as described in the Experimental Section. These data were consistent with the following alignments of the CNBr peptides: II-III-IV-I or II-IV-III-I.[3]

Discussion

In contrast to published reports (Shore and Shore, 1969; Edelstein *et al.*, 1972; Lux and John, 1972), chromatography of apoLP-Gln-I on DEAE-cellulose as described in this communication reproducibly gave a single protein component eluting at a concentration of NaCl of approximately 0.04 M. Shore and Shore (1969) and Edelstein *et al.* (1972) each found a major protein and two minor components that had nearly identical amino acid content but differed with respect to content of isoleucine. Results of amino acid analysis in the present study showed that apoLP-Gln-I had little or no isoleucine, in agreement with the reports of Lux and John (1972) and of Albers *et al.* (1971). These apparent discrepancies could simply reflect differences in methodology or could be due to microheterogeneity of apoLP-Gln-I. There are also

[2] H. N. Baker, A. M. Gotto, and R. L. Jackson, manuscript in preparation.

[3] Unpublished results from this laboratory indicate that the correct alignment is II-IV-III-I: H. N. Baker, A. M. Gotto, and R. L. Jackson, manuscript in preparation.

considerable differences in the molecular weights which have been reported for apoLP-Gln-I; values have ranged from 15,000 (Shore and Shore, 1969) to 28,000 (Scanu *et al.*, 1971). Summing the amino acid compositions of the four cyanogen bromide fragments described in this report gave a value of approximately 28,000.

The fragment CNBr I lacked homoserine or homoserine lactone and, therefore, corresponded to the carboxyl terminus of apoLP-Gln-I. Of the cyanogen bromide fragments II, III, and IV, only CNBr II contained amino-terminal aspartic acid, and, thus, could be aligned as the amino terminus of apoLP-Gln-I. The amino acid composition of CNBr II was consistent with the composition of the first 40 residues of apoLP-Gln-I recently reported by Shore and Shore (1972). The amino-terminal fragment, CNBr II, contained nearly all of the tryptophans and one-half of the prolines of apoLP-Gln-I. Summation of the amino acid compositions of each of the cyanogen bromide fragments of apoLP-Gln-I gave 243 residues in good agreement with a value of 242 residues for the amino acid content of the native molecule.

The major technical difficulty encountered in this study was caused by the tendency of CNBr I to form molecular aggregates. It has not yet been determined if CNBr I also forms aggregates with CNBr II. Whether the ability of the carboxyl-terminal part of the molecule to aggregate contributes significantly to protein–protein or to protein–lipid interactions with native HDL remains to be established. It has been shown that the small proteins from VLDL are transferred both *in vivo* and *in vitro* to HDL (Bilheimer *et al.*, 1972; Eisenberg *et al.*, 1972). It is also known that apoLP-Gln-I may be dissociated from its complement of lipid during ultracentrifugation in salt solution (Albers and Aladjem, 1971) or by rotary evaporation (Nichols *et al.*, 1972). The potential role of apoLP-Gln-I in protein–protein interactions in HDL is worthy of further study. The carboxyl-terminal part of apoLP-Gln-I has been shown in a preliminary study to be capable of binding phosphatidylcholine (Jackson *et al.*, 1972). This fragment also retains a high proportion of the α-helical conformation (Jackson *et al.*, 1972). These pieces of data suggest the possibility that apoLP-Gln-I may be bimodal with a disordered amino terminus and a highly helical carboxyl terminus that is capable of binding phospholipid and of participating in protein–protein interactions. While such interactions are presently speculative, they could contribute significantly to the functional and structural integrity of HDL.

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