Isolation and Characterization of the Tryptic and Cyanogen Bromide Peptides of ApoLp-Gln-II (ApoA-II), a Plasma High Density Apolipoprotein

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SUMMARY

ApoLp-Gln-II (or apoA-II), one of the two major apolipoprotein components of human plasma high density lipoproteins, was subjected to enzymatic and nonenzymatic digestion with trypsin and cyanogen bromide. The individual peptides were isolated to homogeneity by chromatography on DEAE-cellulose, Cm-Sephadex, Sephadex G-150, Sephadex G-75, preparative thin layer plates, and by peptide mapping. Isolated peptides were assayed for purity by amino acid analysis, thin layer chromatography, polycarboxylate gel electrophoresis, and Edman NH₂-terminal analysis. Cyanogen bromide cleavage followed by reduction and S-carboxymethylmentation produced two peptides, C-III and C-IV, of 51 and 26 residues, respectively. The smaller peptide (C-IV) contained homoserine and homoserine lactone and had NH₂-terminal pyrrolidone carboxylic acid. It was the NH₂-terminal cyanogen bromide peptide. This peptide contained the single S-carboxymethylcysteine residue. C-III was the COOH-terminal peptide. It contained COOH-terminal glutamine and a penultimate threonine residue. Tryptic digestion of reduced and alkylated apoLp-Gln-II yielded eight major tryptic peptides (T-I-1, T-I-2, T-I-3, T-III, T-IV, T-VI-1, T-VII, and T-VIII) and seven additional tryptic peptides which either contained or were contained in two or more of the eight major peptides. Only single unique NH₂-terminal (T-I-2), COOH-terminal (T-VII), S-carboxymethylcysteine-containing (T-VIII), methionine-containing (T-VI-1), and isoleucine-containing (T-I-3) peptides were found. The combined compositions of the tryptic or cyanogen bromide peptides were equal to the composition of S-carboxymethyl-apoLp-Gln-II. These results confirm previous observations from this laboratory on intact and reduced and alkylated apoLp-Gln-II and support the concept that the molecule is composed of two identical chains connected by a disulfide bond. The data available allow a partial alignment of these peptides and indicate the disulfide bridge is in the NH₂-terminal one-third of the molecule. The first three tryptic peptides in the sequence are T-I-2, T-VIII, and T-VI-1. T-VII is the COOH-terminal tryptic peptide. These assignments have been confirmed by manual and automated Edman degradations of the isolated tryptic and cyanogen bromide peptides performed in parallel with the present studies and published elsewhere (BREWER, H. B., JR., LUX, S. E., RONAN, R., AND JOHN, K. M. (1972) Proc. Nat. Acad. Sci. U. S. A. 69, 1304–1308).

We are presently undertaking a series of studies designed to explore the interaction between lipids and proteins in human plasma lipoproteins, using as a model system the interaction between phospholipids and apoLp-Gln-II, one of the two major apolipoproteins of human plasma high density lipoproteins. A necessary antecedent to such a study is a thorough knowledge of the structure of apoLp-Gln-II including the amino acid sequence. We have recently reported on the isolation and characterization of apoA-II (10). These studies indicate that apoA-II is composed of two identical chains connected by a single disulfide bond. Each chain has an NH₂-terminal Cm residue and a COOH-terminal Thr-Gln sequence. There are 77 amino acid residues per chain with single residues of methionine and isoleucine. The protein contains no histidine, arginine, or tryptophan. No carbohydrate or covalently bound lipid is detectable. The molecular weight of each chain, estimated from the amino acid composition and from gel filtration in 6 M guanidine hydrochloride, is 8600 (10).

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In the present study we report the isolation, purification, and characterization of the tryptic and cyanogen bromide peptides from Cys(Cm)-apoLp-Gln-II. These studies confirm previous observations on the composition and structure of intact and reduced and alkylated apoLp-Gln-II and support the concept that the molecule is composed of two identical chains linked by a disulfide bond. The data available allow a partial alignment of these peptides and indicate that the disulfide bridge is in the NH₂-terminal one-third of the molecule.

MATERIALS AND METHODS

Isolation and Purity of apoLp-Gln-II—All of the apoLp-Gln-II used in this study was obtained from the plasma of a single, healthy, Negro female volunteer (M.H.). HDL was isolated by preparative ultracentrifugation between salt densities of 1.068 and 1.210 g per ml and dialyzed with ethanol-diethyl ether (1:3, v/v) or chloroform-methanol (2:1, v/v) as previously described (10). ApoLp-Gln-II was isolated from apoHDL, by chromatography on DEAE-cellulose in 6 M urea using a modification of the method of Shore and Shore (3). Details of the chromatographic procedure have been described (10).

Each preparation gave a single band on polyacrylamide gel electrophoresis in 8 M urea at pH 9.4 (10% gels, 20 µg protein load). All preparations contained less than 0.3% of histidine or arginine per 100 moles of amino acids, and frequently no histidine or arginine was detected. Selected preparations were also shown to be homogeneous by polyacrylamide gel electrophoresis in 8 M urea at pH 2.9 (10% gels, 20 µg protein load) and by immunoelectrophoresis in 1% agarose at pH 8.6 against six HDL antisera with reactivities to both apoLp-Gln-I and apoLp-Gln-II. The microheterogeneity of apoLp-Gln-II used in this study was obtained from the plasma of a single, healthy, Negro female volunteer (M.H.). The microheterogeneity of apoLp-Gln-II used in this study was obtained from the plasma of a single, healthy, Negro female volunteer (M.H.). HDL was isolated by preparative ultracentrifugation between salt densities of 1.068 and 1.210 g per ml and dialyzed with ethanol-diethyl ether (1:3, v/v) or chloroform-methanol (2:1, v/v) as previously described (10). ApoLp-Gln-II was isolated from apoHDL, by chromatography on DEAE-cellulose in 6 M urea using a modification of the method of Shore and Shore (3). Details of the chromatographic procedure have been described (10).

Cyanogen Bromide Cleavage of apoLp-Gln-II—Cyanogen bromide cleavages of apoLp-Gln-II were performed at room temperature in 50% formic acid with a 500-fold molar excess of cyanogen bromide at 97 min. The reaction mixture was titrated to pH 2.0 with HCl and repeatedly lyophilized to remove all traces of trimethylamine.

In a preliminary experiment aliquots taken from 2 to 360 min after the addition of trypsin were subjected to high voltage paper electrophoresis at pH 3.6 (pyridine-acetic acid-water, 1:10:289) and stained with ninhydrin. The amount of each peptide increased rapidly during the first 5 min of incubation. Forty-five minutes after the addition of trypsin, nine peptide spots were well resolved. After 60 min of incubation one of these spots gradually disappeared and three new spots gradually appeared. On the basis of this experiment 45 min was chosen as the incubation time for subsequent trypsin cleavages.

Isolation of Peptides—Cyanogen bromide peptides (10 to 40 mg) were reduced and S-carboxymethylated and then separated by gel filtration on a column (2 x 86 cm) of Sephadex G-75 (superfine, Pharmacia), equilibrated in 0.1 M Tris-HCl, 6 M urea, pH 8.0, and eluted with the same buffer at 10 ml per hour. The eluate was monitored by absorbance at 280 nm. The isolated fractions were rechromatographed on a column (2 x 45 cm) of Sephadex G-25 equilibrated in 0.01 M ammonium bicarbonate to remove urea and Tris-HCl.

Tryptic peptides (5 to 30 mg) were separated by chromatography on a column (0.9 x 45 cm) of DEAE-cellulose (Whatman DE52, microgranular). In a typical experiment the column was equilibrated in 0.01 M ammonium bicarbonate and eluted with this buffer until peptide T-III emerged. Then a linear gradient of ammonium bicarbonate (4000 ml, from 0.01 M to 0.10 M) was begun and continued until peptide T-IX was eluted. The eluate was monitored by measurement of absorbance at 220 nm.

The isolated tryptic and cyanogen bromide peptides were assayed for purity by amino acid analysis, thin layer chromatography, polyacrylamide gel electrophoresis, and Edman NH₂-terminal analysis. When indicated, fractions were further purified by chromatography on Sephadex G-10, Cm-Sephades A-25, preparative thin layer plates, and by peptide mapping. The procedures for routine amino acid analyses (10), analysis of glutamine and asparagine (15), acid hydrolyses in 2-mercaptoethanol-16 M urea (14), and NH₂-terminal analyses (18, 19) were described in detail in the previous report (10).

Homoserine was separated from other acidic and neutral amino acids on a column (0.9 x 52 cm) of Beckman AA-15 resin by elution with 0.2 M sodium citrate, pH 2.9. In this system, homoserine eluted between serine and glutamic acid at 82 min. Homoserine lactone was determined using a column (0.9 x 20 cm) of Beckman PA-35 resin eluted with 0.35 M sodium citrate buffer, pH 5.29, at 53°. It eluted immediately after ammonia at 97 min.

Methionine sulfoxide and methionine sulfone were separated from aspartic acid and the other acidic and neutral amino acids on a column (0.9 x 52 cm) of Beckman AA-15 resin by elution with 0.2 M sodium citrate, pH 3.10. With this buffer methionine sulfoxide, aspartic acid, methionine sulfone, threonine, serine, and glutamic acid eluted at 42, 49, 53, 62, and 77 min, respectively.

Analytical thin layer chromatography was performed on 100-µm thick plates of cellulose F (Brinkmann Instruments) developed with butanol-pyridine-acetic acid-water (30:20:6:24) (14). The plates were sprayed with 1.25% ninhydrin in acetone-water (16:1) containing 0.125% cadmium acetate and developed at 110° for 10 min. For preparative purposes 150 nmoles of pep-
tides were streaked on the 100-μ plates of cellulose F, developed as above, and eluted with 0.1 M ammonium hydroxide. Peptide mapping was performed on Whatman No. 3MM paper by descending paper chromatography in butanol-acetic acid-water (4:1:5) for 14 hours followed by high voltage electrophoresis in pyridine-acetic acid-water (1:10:280), pH 3.6, for 90 min at 2000 volts as described by Katz et al. (20). For analytical runs 30 nmoles of peptides were applied and the chromatograms developed by dipping in 0.5% ninhydrin in ethanol. For preparative runs 150 nmoles of peptides were separated, and the maps were sprayed lightly with 0.025% ninhydrin in ethanol-acetic acid (3:1) and developed at 110° for 5 to 10 min. Stained areas were cut from the map and the process was repeated several times until all of the peptides were visualized. In order to ensure complete visualization of all peptides, the chromatogram was then dipped in 0.5% ninhydrin in ethanol. The peptides were eluted from the paper with 0.1 M acetic acid or 0.1 M ammonium hydroxide in an apparatus similar to that described by Kasper (21).

Other Procedures—The procedures for reduction and alkyla-
tion with isoaoicetic acid (10), hexosamine determinations (22), COOH-terminal analyses with carboxypeptidase A (18) or hydrazinolysis (10, 24), molecular weight determinations in 6 M guanidine hydrochloride (23), and enzymatic digestion with papain, aminopeptidase M, and prolidase were performed as previously described (10). The trypsin digestion step was omitted for tryptic peptides, and the prolidase digestion was omitted for peptides which did not contain proline. Pyrroldione carboxylic acid was identified by isobutane chemi-
tronization mass spectrometry (Finnigan quadrupole spec-

![Fig. 1 (left). Fractionation of the tryptic peptides of Cys(Cm)-apoLp-Gln-II by DEAE-cellulose chromatography. Following tryptic digestion, 30 mg of Cys(Cm)-apoLp-Gln-II were applied to a column of DEAE-cellulose equilibrated in 0.01 M ammonium bicarbonate, pH 8.0, and eluted with the same buffer until peptide T-I was eluted. Then a linear gradient of ammonium bicarbonate (4000 ml, from 0.01 M to 0.10 M) was begun and continued until peptide T-IX was eluted. No additional peptides were eluted when the column was washed with 1 M ammonium bicarbonate. Fractions 1 to 50 contained approximately 1 ml. Subsequently, 5-ml fractions were collected. The fractions were pooled as indicated by the heavy black bars.](http://www.jbc.org/)

![Fig. 2 (right). Chromatography of tryptic peptide fraction T-I on Sephadex G-10. The pooled fraction T-I from an experiment similar to that described in Fig. 1 was applied to a column (1.2 X 95 cm) of Sephadex G-10 equilibrated in 0.01 M ammonium bicarbonate, pH 8.0, and eluted with the same buffer. The fractions were pooled as indicated by the heavy black bars.](http://www.jbc.org/)
The two minor peptides T-VI-2 and T-VI-3 were separated from T-VI-1 by preparative thin layer chromatography. Fractions T-VII and T-VIII remained at the origin on thin layer chromatography but gave single bands on polyacrylamide gel electrophoresis (Fig. 3B).

The tryptic peptides were also isolated by two-dimensional paper chromatography and high voltage electrophoresis (Fig. 4). Eluted peptides were identified by amino acid analysis and compared to the peptides isolated by DEAE chromatography. All of the chromatographically separated peptides, except T-IX, were identified on the peptide maps stained with ninhydrin. The inability to detect T-IX was attributed to the poor yield of this peptide. Purified T-IX, isolated by DEAE chromatography, migrated in the area denoted by the dotted circle in Fig. 4. Several additional faint ninhydrin-positive spots were seen on the peptide maps (Fig. 4), but these areas yielded no additional peptides.

The amino acid compositions, NH$_{2}$-terminal analyses, and approximate yields of the purified tryptic peptides are shown in Table I. The mole ratios were computed using the method of Black and Hogness (27). Each purified tryptic peptide tested, except T-I-2, released a single phenylthiohydantoin amino acid on phenylisothiocyanate degradation (Table I). Since apoLp-Gln-II has been shown to contain NH$_{2}$-terminal pyrrolidone carboxylic acid (10), which was not reactive with phenylisothiocyanate, this suggested that T-I-2 was the NH$_{2}$-terminal tryptic peptide. T-I-2 contained 1 residue of glutamic acid on acid hydrolysis and was not hydrolyzed by papain and aminopeptidase M. Digestion with pyrrolidonecarboxylyl peptidase released pyrrolidone carboxylic acid (identified by gas-liquid chromatography and chemical-ionization mass spectroscopy) (Fig. 5). This confirmed the NH$_{2}$-terminal location of T-I-2.

A single tryptic peptide, T-VII, contained no basic residue and was presumed to be the COOH-terminal tryptic peptide. Digestion of T-VII for 17 hours with carboxypeptidase A released glutamine (0.91 mole per mole of peptide), consistent with carboxypeptidase A digestion of apoLp-Gln-II (1, 3, 10). Tryptic peptide T-I-1 contained only lysine and was proved to be monolysine rather than a polylysine peptide by thin layer chromatography (Fig. 6). No tryptic peptide contained any glucosamine or galactosamine.

Cys(Cm)-apoLp-Gln-II contains a single residue of Cys(Cm), methionine, and isoleucine per mole (Table I). Two Cys(Cm)-containing peptides (T-VIII and T-IX), two methionine-containing peptides (T-VI-1 and T-IX), and two isoleucine-containing peptides (T-I-3 and T-II) were found in the tryptic digests of Cys(Cm)-apoLp-Gln-II (Table I). However, three of these six peptides (T-I-3, T-III, and T-IX) contained 2 lysine residues. The amino acid composition of these three peptides indicated that each was a combination of two other tryptic peptides: T-I-3 = T-I-4 plus T-II, T-III = T-I-5 plus T-VI, and T-IX = T-VIII plus T-VI-1. NH$_{2}$-terminal analyses (Table I) established that T-I-4 precedes T-II, T-I-5 precedes T-V, and T-VIII precedes T-VI-1 in the sequence.

Peptides T-VI-2 and T-VI-3 were found to be derivatives of peptide T-VI-1 in which the methionine residue had been oxidized to methionine sulfoxide (T-VI-2) or methionine sulfone (T-VI-3). Excluding these two peptides and the three overlap peptides (T-I-3, T-III, and T-IX), the combined composition of the remaining tryptic peptides equals the composition of S-carboxymethyl-apoLp-Gln-II (Table I).2

1 It should be noted that although the combined results of acid and enzymatic hydrolyses indicate Cys(Cm)-apoLp-Gln-II contains 10 glutamic acid residues (counting the pyrrolidone carboxylic acid residue as a glutamic acid) and 6 glutamine residues (Table I) (10), manual and automated Edman degradations of the tryptic and cyanogen bromide peptides isolated from this study disclosed only 9 glutamic acids (including PCA) and 7 glutamine residues (28). This discrepancy arose because two of the glutamine residues were partially deamidated, perhaps in purification. These two glutamine residues were located in tryptic peptide T-V and its overlap peptide T-VI-3, and in cyanogen bromide peptides C-II and C-III (see below). Thus T-III and T-V

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Fig. 3 (left). A, thin layer chromatography of the fractions shown in Fig. 1. Five to 10 nmoles of each fraction and the unfraccionated tryptic digest (TD) were applied to a 100-μ thick plate of cellulose F, developed with butanol-pyridine-acetic acid-water (30:20:6:24), and sprayed with a ninhydrin-cadmium acetate reagent. B, polyacrylamide gel electrophoresis (pH 4.4, 10% gels) of approximately 150 nmoles of tryptic peptides T-VII and T-VIII. The gels were fixed in 20% trichloroacetic acid and stained with Coomassie blue.

Fig. 4 (right). Tryptic peptide map of Cys(Cm)-apoLp-Gln-II. Fifty nanomoles of Cys(Cm)-apoLp-Gln-II were digested with trypsin and separated by chromatography (C) in butanol-acetic acid-water (4:1:5) followed by high voltage electrophoresis (HVE) in pyridine-acetic acid-water (1:10:289), pH 3.6. All of the tryptic peptides except T-IX were detected. In a separate experiment purified T-IX was found in the area denoted by the dashed lines.
Cyanogen Bromide Cleavage—ApoLp-Gln-II was usually reduced and S-carboxymethylated after cyanogen bromide cleavage. Chromatography of the reduced and alkylated peptide mixture on Sephadex G-75 in 6 M urea at pH 8.0 yielded three major fractions (Fig. 7A), designated C-II, C-III, and C-IV. Fractions C-II and C-III gave single bands on polyacrylamide gel electrophoresis in 8 M urea at pH 9.2 (Fig. 8). Fraction C-IV did not stain well but was sometimes seen as a faint band near the top of the separating gel (not shown). A fourth fraction C-I, a polymeric form of C-IV which eluted before C-II, was found when gel filtration chromatography of unreduced apoLp-Gln-II which had not been reduced and alkylated, was performed. C-I contained 1 residue of glutamic acid and 2 partially deamidinated residues of glutamine (Table I) and C-III actually contains 6 glutamic acid residues and 5 glutamine residues, 2 of which are partially deamidated (Table II). This anomaly points up one of the pitfalls of enzymatic hydrolysis (or any other technique which determines amide content collectively) in glutamine and asparagine analysis and underscores the well known lability of these residues.

### Table I

**Amino acid composition of the tryptic peptides of S-carboxymethyl-apoLp-Gln-II**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>T-I</th>
<th>T-II</th>
<th>T-IV</th>
<th>T-V</th>
<th>T-VI</th>
<th>T-VII</th>
<th>T-VIII</th>
<th>T-VI &amp; T-VII</th>
<th>T-VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>0.96(1)</td>
<td>0.94(1)</td>
<td>0.94(1)</td>
<td>0.94(1)</td>
<td>0.94(1)</td>
<td>0.94(1)</td>
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<td>0.94(1)</td>
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<tr>
<td>Asparagine</td>
<td>0.93(1)</td>
<td>0.93(1)</td>
<td>0.93(1)</td>
<td>0.93(1)</td>
<td>0.93(1)</td>
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</tr>
<tr>
<td>Threonine</td>
<td>0.96(1)</td>
<td>0.96(1)</td>
<td>0.96(1)</td>
<td>0.96(1)</td>
<td>0.96(1)</td>
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<tr>
<td>Glutamic acid</td>
<td>0.99(1)</td>
<td>0.99(1)</td>
<td>0.99(1)</td>
<td>0.99(1)</td>
<td>0.99(1)</td>
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<tr>
<td>Phenylalanine</td>
<td>0.99(1)</td>
<td>0.99(1)</td>
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<tr>
<td>Tyrosine</td>
<td>0.99(1)</td>
<td>0.99(1)</td>
<td>0.99(1)</td>
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<tr>
<td>Lysine</td>
<td>0.99(1)</td>
<td>0.99(1)</td>
<td>0.99(1)</td>
<td>0.99(1)</td>
<td>0.99(1)</td>
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<tr>
<td>Met. Sulfone</td>
<td>0.99(1)</td>
<td>0.99(1)</td>
<td>0.99(1)</td>
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<td>0.99(1)</td>
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* The amino acid compositions are expressed in terms of the molar ratios of the constituent amino acids, calculated by the method of Black and Hogness (27). The numbers in parentheses are the assumed number of residues per mole.

b. Overlap peptide: T-I-3 = T-I-4 + T-II.

c. Overlap peptide: T-III = T-I-5 + T-V.


e. The glutamic acid residue of peptide T-I-2 is present as pyridolone carboxylic acid (see text).

A. As discussed in footnote 2, peptide T-V and its overlap peptide, T-III, actually contain 1 residue of glutamic acid and two residues of glutamine. This discrepancy arises because the two glutamine residues are partially deamidated. Thus, Cys (Cm)-apoLp-Gln-II actually contains 9 glutamic acid residues (including PCA) and 7 glutamine residues.

1. Proven to be a single residue of lysine by thin-layer chromatography.

2. Determined as the PTH-derivative by gas-liquid chromatography.

3. Determined as the PTH-derivative by gas-liquid chromatography.

4. N.D. = not determined.

5. DEAE, chromatography on DEAE cellulose; MAP, peptide or "fingerprint" with elution of the peptide; CMS, chromatography on CM-Sephadex; GIO chromatography on Sephadex G-10; PTLC, preparative thin-layer chromatography. See the text for details of these procedures.

* S. E. Lux, K. M. John, R. L. Jackson, S. Fleischer, and A. M. Gotto, manuscript in preparation.
FIG. 5. Analysis of the residue released by digestion of tryptic peptide T-1-2 with pyrrolidonecarboxylyl peptidase. A, gas-liquid chromatogram of the trimethylsilyl derivative of the released residue. Trimethylsilyl-PCA co-eluted with the principal peak at 6 min. B, chemical ionization mass spectrum of the released material. The principal species is the molecular ion of m/e 130.

FIG. 6. Thin layer chromatography of (1) tryptic peptide T-1-1, (2) lysine, (3) lysyl lysine, and (4) lysyl lysyl lysine. The conditions of chromatography were the same as described in Fig. 3.

The combined composition of C-III plus C-IV was equal to the composition of Cys(Cm)-apoLp-Gln-II.

C-IV contained homoserine and revealed no NH₂-terminal amino acid by the Edman or dansyl chloride techniques suggesting that it was the NH₂-terminal cyanogen bromide peptide. Digestion of C-IV with pyrrolidonecarboxylyl peptidase released PCA, confirming this assignment. C-III contained no homoserine or homoserine lactone, identifying it as the COOH-terminal cyanogen bromide peptide. Carboxypeptidase A digestion of C-III for 16 hours released glutamine (0.83 mole per mole of peptide) and threonine (0.46 mole per mole of peptide) as expected. The NH₂-terminal residue of C-III was shown to be glutamic acid with both the Edman and dansyl chloride methods.

The amino acid composition, NH₂-terminal analysis, and yield (Table II) of fraction C-II indicated that it was apoLp-Gln-II which had resisted cleavage by cyanogen bromide. However, on polyacrylamide gel electrophoresis at pH 2.9, C-II had a greater mobility than the major apoLp-Gln-II band and migrated with a minor band which, as previously described (10, 29), was not present initially in apoLp-Gln-II preparations but appeared within days to weeks on storage in solution at 4°. Amino acid analysis following complete enzymatic digestion of C-II with trypsin, papain, aminopeptidase M, and prolidase indicated that the methionine in C-II was predominately in the
sulfoxide form. We concluded that fraction C-II was primarily apoLp-Gln-II which was resistant to cyanogen bromide because of the altered methionine residue. Small amounts of homoserine and homoserine lactone were also present in fraction C-II (Table II). These residues may have been derived from apoLp-Gln-II which reacted with cyanogen bromide but was not cleaved or from contamination with peptide C-IV.

**DISCUSSION**

From these combined results certain conclusions about the structure of apoLp-Gln-II can be deduced. First, the results are entirely consistent with the evidence presented in the previous paper (10) that apoLp-Gln-II is composed of two identical chains linked by a single disulfide bond containing single NH$_2$-terminal (T-I-2), COOH-terminal (T-VII), Cys(Cm)-containing (T-VIII), methionine-containing (T-VI-1), and isoleucine-containing (T-I-3) tryptic peptides were found. These peptides account for 60 of the 77 residues of Cys(Cm)-apoLp-Gln-II. The other 17 residues are accounted for by tryptic peptides T-I-1, T-I-3, and T-IV. The remaining tryptic peptides (T-I-4, T-I-5, T-II, T-V, T-VI-2, T-VI-3, and T-IX) all either contain or are contained in the tryptic peptides noted above. In addition the combined compositions of the single NH$_2$-terminal and COOH-terminal cyanogen bromide peptides equal the composition of Cys(Cm)-apoLp-Gln-II.

Second, the results allow us to align the cyanogen bromide peptides and partially align the tryptic peptides, as shown in Fig. 9. C-IV is the NH$_2$-terminal cyanogen bromide peptide by virtue of its NH$_2$-terminal PCA residue. The peptide is 26 residues long and contains homoserine, assigning methionine to position 26 in the sequence. The amino acid composition of C-IV corresponds to the compositions of tryptic peptides T-I-2 (the NH$_2$-terminal tryptic peptide) and T-VIII plus aspartic acid, leucine, and methionine (as homoserine) of tryptic peptide T-VI-1. Since T-I-2 contains NH$_2$-terminal PCA and is a tryptic peptide, its sequence would be predicted to be PCA-Ala-Lys. Tryptic peptide T-VIII must be the second and tryptic peptide T-VI-1 the third tryptic peptide (a) because all of T-VIII and only a portion of T-VI-1 are contained in C-IV and (b) because overlap peptide T-IX (which contains both T-VIII and T-VI-1) has NH$_2$-terminal glutamic acid, the NH$_2$-terminal residue of T-VIII. Since T-VIII contains the single Cys(Cm) residue, the interchain disulfide bond occurs in the NH$_2$-terminal one-third of the molecule.

C-III contains no homoserine or homoserine lactone, has COOH-terminal glutamine and a penultimate threonine, identifying it as the COOH-terminal cyanogen bromide peptide. It contains the residues of tryptic peptides T-I-1, T-I-3, T-III, T-IV, and T-VII plus glutamic acid and lysine of tryptic peptide T-VI-1. Tryptic peptide T-VII has COOH-terminal glutamine and contains no lysine and, hence, is the COOH-terminal tryptic peptide. The alignment of the other four tryptic peptides (T-I-1, T-I-3, T-III, and T-IV) could not be ascertained from the overlap peptides obtained by tryptic and cyanogen bromide cleavage. Since the NH$_2$-terminal residue of C-III is glutamic acid and the NH$_2$-terminal residue of T-VI-1 is aspartic acid, the sequence of T-VI-1 would be predicted to be Asp-Leu-Met-Glu-Lys.

These deductions were borne out by manual and automatic Edman degradations of the tryptic and cyanogen bromide peptides which were conducted in parallel with the present studies (Fig. 10). The results of these degradations have been published in detail elsewhere (28). Each of the tryptic peptides was confirmed by Edman degradation and then by automatic Edman degradations of the tryptic and cyanogen bromide peptides which were conducted in parallel with the present studies (Fig. 10).
pletely sequenced by manual Edman degradations. The alignment of peptides T-I-2 and T-VIII, deduced above, was confirmed by automated Edman degradations of Cys(Cm)-apoLp-Gln-II after treatment with pyrrolidonecarboxylyl peptidase to remove the NH₂-terminal PCA residue. The alignment of peptides T-VIII and T-VI-1 was confirmed by manual degradation of tryptic peptide T IX. Finally, an automated analysis of the first 36 residues of cyanogen bromide peptide C-III confirmed the COOH-terminal location of T-VII and oriented the four remaining tryptic peptides as (T-III)-(T-IV)-(T-I-3)-(T-I-1) (Fig. 10) (28).

Knowledge of the sequence of apoLp-Gln-II now permits detailed studies of the amino acid residues involved in lipid binding in this protein. Comparison of the sequences of apoLp-Glu-II and apoLp-Ala (30) with the sequences of proteins not known to bind lipids (31) discloses an unusually high frequency of basic lysine residues adjacent to a dicarboxylic acid in these two apolipoproteins. These occur at positions 3, 4; 23, 24; 27, 28; 43, 44; and 46, 47 in apoLp-Gln-II and at positions 24, 25; 51, 52; and 58, 59, 60 in apoLp-Ala (30). Unpublished studies indicate an even higher frequency of such acidic-basic amino acid pairing in apoLp-Ser, an apolipoprotein which, like apoLp-Ala, is common to both high density and very low density lipoproteins. These three proteins all have in common the ability to bind phospholipids such as phosphatidyl choline and phosphatidyl ethanolamine. Intriguingly, when molecular models of these phospholipids and lysine-aspartic acid or lysine-glutamic acid dipeptides are compared, there is a remarkable coaptation of the lipid and peptide models. The positively charged ε-amino group of the lysine residue abuts the negatively charged lipid phosphate, and the positively charged terminus of the choline and ethanolamine moieties aligns with the negatively charged dicarboxylic acid. We can only speculate as to whether a similar interaction occurs in the binding of phospholipid to these apoproteins. Additional studies involving the binding and, in some cases, affinity labeling of various phospholipids and phospholipid analogs to native and chemically modified apolipoproteins and to peptide fragments derived from these apolipoproteins will be required to substantiate or refute this hypothesis. These studies are currently in progress.

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