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Primary structure of very low density apolipoprotein C-II of human plasma

(structure of lipoproteins/amino acid sequence/lipoprotein lipase activator)

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ABSTRACT **Apolipoprotein C-II (apoC-II), a protein constituent of very low density lipoproteins of human plasma and the activator protein of lipoprotein lipase, has been isolated and its amino acid sequence has been studied. The protein has 78 amino acid residues and is lacking cysteine, cystine, and histidine. Chromatography on Bio-Gel P-30 in 25% formic acid of the cyanogen bromide digest of apoC-II yields three fragments designated as CNBr-I, -II, and -III. They contained 50, 19, and 9 residues, respectively. The alignment of the cyanogen bromide fragments has been established as CNBr-III-I-II by isolation and sequence of the tryptic peptides of the intact protein. The amino acid sequences of the tryptic and CNBr peptides were determined by conventional methods. With this information, it was possible to establish the complete amino acid sequence of apoC-II.**

Very low density lipoproteins (VLDL) of human plasma contain approximately 90% lipid and 10% protein and are the major vehicles responsible for the transport of endogenous triglycerides. Lipid-free VLDL (apoVLDL) contain one major and several minor apoprotein constituents. The major one, comprising 40–50% of the protein by weight, is designated apoB and is identical to the major protein constituent of low density lipoproteins (1). The remaining proteins of VLDL are designated as the apoC proteins (2) (apoC-I, apoC-II, and apoC-III) and the "arginine-rich" protein (3). The physical, chemical, immunochemical, and physiologic properties of the various human apolipoproteins have been summarized in several recent reviews (4–7).

Of the three apoC proteins, the primary amino acid sequences of apoC-I (8, 9) and apoC-III (10, 11) have been determined. ApoC-II, the protein of study in this communication, has a reported molecular weight of approximately 10,000 (12), is devoid of cysteine, cystine, and histidine (13), has COOH-terminal glutamic acid (12–14), and plays an important role in triglyceride metabolism by serving as an activator protein of several triacylglycerol lipase enzymes (15, 16). Since apoC-II plays such an important role in lipoprotein metabolism, it was of considerable interest to determine the primary structure of this apoprotein. In this communication, we present a preliminary report of the complete sequence of apoC-II.

MATERIALS AND METHODS

Source and Isolation of ApoC-II. Plasma was obtained from fasting patients with primary type IV or type V hyperlipoproteinemia (17). The VLDL were isolated by ultracentrifugal flotation at plasma density and were delipidated with diethyl ether/ethanol (3:1) at 4° (18). The apoC proteins were separated from apoB and the "arginine-rich" protein by chromatography of apoVLDL on Sephadex G-150 in 1 mM sodium decyl sulfate (19). The fraction corresponding to the apoC proteins was pooled and rechromatographed on DEAE-cellulose in 8 M urea (19, 20); the fractions containing apoC-II were pooled, desalted on Bio-Gel P-2 (Bio-Rad) in 0.10 M ammonium bicarbonate, and lyophilized. ApoC-II was further purified by chromatography on Sephadex G-75 in a buffer containing 0.01 M Tris·HCl/5.4 M urea at pH 8.6. The purity of the isolated apoprotein was established by polyacrylamide gel electrophoresis in urea and sodium dodecyl sulfate, by amino acid analysis, and by the absence of histidine.

Determination of Amino Acid Sequence. Edman degradations on the intact apoprotein and cyanogen bromide fragments were performed automatically with a Beckman Sequencer, model 890B. *N,N*-Dimethylbenzylamine (Pierce Chemical) was used as the coupling buffer (21). The program for the degradation was the same as that described by Hermodson *et al.* (21) with the exception that the benzene extaction step was eliminated. The reagents were sequence grade and were obtained from Beckman Instruments or Pierce Chemical Co. The phenylthiohydantoin derivatives from each step were identified by gas-liquid chromatography (22) on a support of SP-400 with a Beckman GC-65 gas chromatograph apparatus and by thin-layer chromatography (23). Edman degradations on the tryptic, chymotryptic, and thermolytic peptides were performed by the subtractive method (24).

Cyanogen Bromide Cleavage. ApoC-II (10 mg) was treated with a 400 molar excess of cyanogen bromide in 2 ml of 70% formic acid. After 24 hr at 23°, the digest was lyophilized to dryness and redissolved in 1 ml of 25% formic acid for chromatography on a column (1.6 × 200 cm) of Bio-Gel P-30 (Bio-Rad) equilibrated with 25% formic acid. The fractions were identified by measuring the absorbance at 280 nm and by the ninhydrin procedure after alkaline hydrolysis (25). Fractions containing peptide were pooled, diluted with water, and lyophilized.

Digestion of ApoC-II with Proteolytic Enzymes. ApoC-II was dissolved in 0.1 M ammonium bicarbonate, pH 8.0, to give a concentration of 10 mg/ml. Trypsin (TRTPCK, Worthington) was added to 2% (wt/wt), and the digestion was allowed to proceed for 6 hr at 23°. After the reaction mixture was acidified with 0.1 ml of glacial acetic acid, a precipitate formed which was removed by centrifugation. This acid-insoluble material represented tryptic peptide T-7. ApoC-II was also digested with chymotrypsin (Worthington) under the same conditions as trypsin with the exception that the reactions were allowed to proceed for 15 hr at 23°. Some of the tryptic and CNBr peptides were also digested with thermolysin.

Isolation of Peptides. Proteolytic digestion mixtures were fractionated on columns (1.6 × 200 cm) of Sephadex G-50 (Pharmacia), using 0.1 M ammonium bicarbonate/1.0% pyri-

Abbreviations: VLDL, very low density lipoproteins; apoC-I, apoC-II, and apoC-III, apolipoproteins from human VLDL.

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Table 1. Amino acid composition of apoC-II and cyanogen bromide fragments*

Amino acid	CNBr-I	CNBr-II	CNBr-III	ApoC-II	Range of reported values†
Aspartic acid	3.2 (3)	1.1 (1)	1.0 (1)	5.1 (5)	5.4–5.8
Threonine	4.8 (5)	2.9 (3)	0.8 (1)	7.6 (9)	7.9–9.2
Serine	6.0 (6)	2.0 (2)	0.2	8.0 (8)	9.0–9.1
Glutamic acid	6.4 (6)	3.1 (3)	4.9 (5)	14.2 (14)	9.5–18.5
Proline	3.1 (3)	— —	1.1 (1)	3.6 (4)	2.4–3.6
Glycine	0.4 —	2.1 (2)	— —	2.1 (2)	2.3–2.5
Alanine	5.8 (6)	— —	— —	6.3 (6)	6.6–6.8
Half-cysteine‡	— —	— —	— —	— —	—
Valine	2.2 (2)	1.9 (2)	— —	4.1 (4)	4.0–4.3
Methionine	0.8§ (1)	— —	0.7 (1)§	1.7 (2)	1.9–2.0
Isoleucine	— —	0.9 (1)	— —	0.7 (1)	0.9–1.0
Leucine	6.0 (6)	2.0 (2)	— —	7.8 (8)	7.4–8.5
Tyrosine	3.9 (4)	1.0 (1)	— —	4.7 (5)	4.2–4.9
Phenylalanine	0.8 (1)	1.0 (1)	— —	1.9 (2)	2.0–2.1
Tryptophan‡	0.8 (1)	— —	— —	0.8 (1)	1.8¶
Lysine	5.0 (5)	1.0 (1)	— —	6.0 (6)	5.5–6.3
Histidine	— —	— —	— —	— —	—
Arginine	1.2 (1)	— —	— —	1.1 (1)	1.0–1.2
Total	50	19	9	78	
Yield %	66	39	92		

* The two columns of numbers represent the values obtained from 22-hr hydrolysates and from the sequence (in parentheses). The CNBr fragments are those shown in Fig. 1.
† Obtained from refs. 3, 12, 14, and 34.
‡ Determined after hydrolysis with methane sulfonic acid.
§ As homoserine.
¶ From ref. 3.

dine. The columns were operated at ambient temperature; effluents were monitored by the ninhydrin reaction after alkaline hydrolysis (25). The peptides from each zone of the Sephadex fractionation were further purified by a combination of electrophoresis and chromatography on paper using the same methods described previously (26).

Other Methods. COOH-terminal amino acids were determined by digestions with carboxypeptidase A or B (Worthington). The peptides were dissolved in 0.20 ml of 0.10 M Tris·HCl, pH 8.0, and 0.10 mg of the enzyme preparations was added. After an appropriate time, 0.10 ml of glacial acetic acid was added and the digest taken to dryness with a stream of nitrogen. The amino acids released were quantitated by amino acid analysis without acid hydrolysis.

RESULTS

Characterization of apoC-II

Chromatography of human apoVLDL on Sephadex G-150 in sodium decyl sulfate followed by DEAE-cellulose in 8 M urea usually yielded an impure preparation of apoC-II, as evidenced by the presence of histidine (usually 0.2–0.3 residue) and an arginine content greater than 1.0. However, chromatography on Sephadex G-75 in urea consistently gave a preparation of apoC-II that was void of histidine and contained 1.1 residues of arginine. The amino acid composition of the purified apoC-II is given in Table 1. The values are reasonably close to published ones and to those obtained from sequence analysis.

ApoC-II was subjected to 25 cycles of the automatic Edman degradation. Those residues that were positively identified by gas chromatography of the phenylthiohydantoin derivatives are shown in Fig. 1. Threonine was identified as the NH_2 terminus of apoC-II, and methionine was identified at residue 9.

Characterization of cyanogen bromide peptides

Since apoC-II contains two residues of methionine, three cyanogen bromide (CNBr) fragments were expected. Cleavage of apoC-II with CNBr followed by chromatography of the digest on Bio-Gel P-30 in 25% formic acid yielded the three fragments (Fig. 2). The amino acid compositions of the three fragments are shown in Table 1. Since methionine was found at step 9 of the intact apoC-II, this established CNBr-III as the NH_2-terminal CNBr fragment. CNBr-I was subjected to 16 cycles of the automatic degradation. As shown in Fig. 1, those residues between steps 10 and 25 that were positively identified in apoC-II were identical to the NH_2-terminal sequence of CNBr-I, thus establishing the order of the CNBr fragments; CNBr-II contained no homoserine and was the COOH-terminal peptide. The residues identified by Edman degradation of CNBr-II are shown in Fig. 1. The sequences of CNBr-I, -II, and -III were established (unpublished data) by conventional means using proteolytic digestions with trypsin, chymotrypsin, and thermolysin, isolation of peptides, and subtractive Edman degradation (24).

Characterization of tryptic peptides

All of the tryptic peptides of apoC-II were soluble at pH 3.7 with the exception of T-7 (Fig. 2); this peptide was removed from the incubation mixture by centrifugation. The soluble peptides were fractionated by high voltage paper electrophoresis at pH 3.7 and by paper chromatography. The sequence of each peptide (Fig. 1) was established by conventional methods (unpublished data). Peptides T-1, T-2, and T-3 were degraded further with chymotrypsin and thermolysin. The resulting peptides were isolated and their sequences determined.

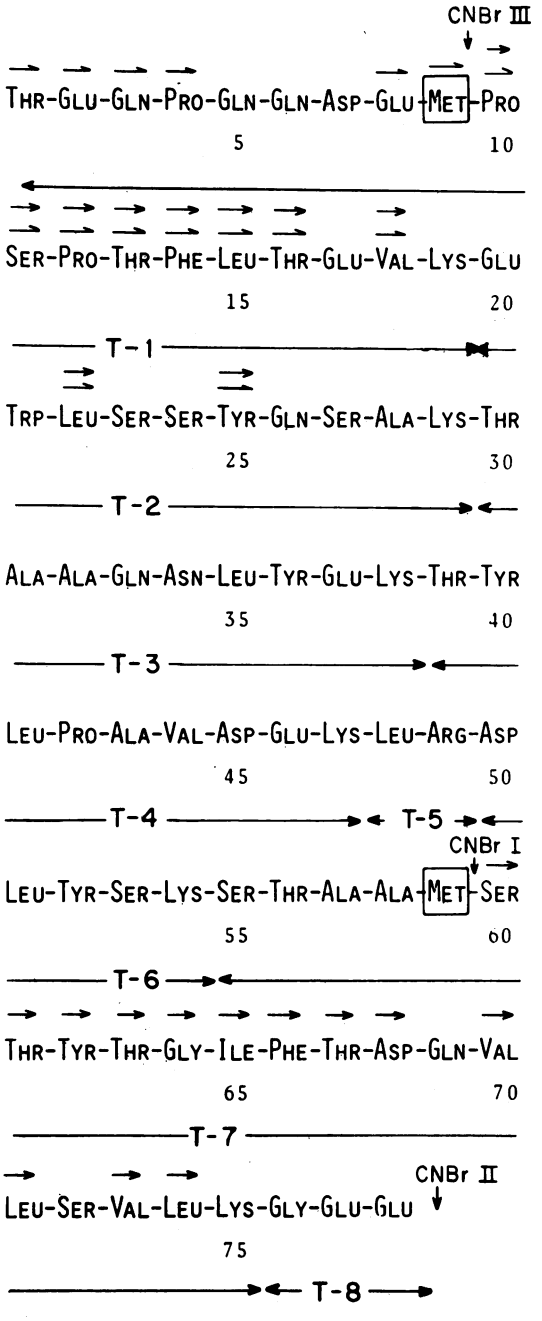

FIG. 1. The amino acid sequence of human plasma apoC-II. Arrows above the sequence represent those amino acids identified by direct Edman degradations of the intact protein (—) or of cyanogen bromide fragments (→). The tryptic (T) peptides of apoC-II are shown.

DISCUSSION

The complete amino acid sequence of human plasma very low density lipoprotein C-II (apoC-II) is presented in Fig. 1. The calculated molecular weight for the 78 amino acid residues is 8837, in close agreement with that obtained by equilibrium ultracentrifugation (12). The sequence contains several interesting features. For example, three of the four prolines of the molecule are contained within the first 12 residues; the other proline is at residue 42. As noted by ourselves and others (8–11, 27–33), the plasma apolipoproteins of known sequence have a number of basic and acidic residues that are either adjacent to each other (Lys-19 and Glu-20, Glu-37 and Lys-38, Glu-46 and Lys-47, and Arg-49 and Asp-50) or are in a 1 → 4 relationship (Lys-47 and Asp-50, Glu-46 and Arg-49, and Lys-75 and Glu-78). The importance of this distribution is related to a recent hypothesis (32, 33) concerning the lipid-binding determinants of the plasma apolipoproteins. It would be interesting to test the phospholipid-binding properties of apoC-II and to compare them to those of the CNBr fragments.

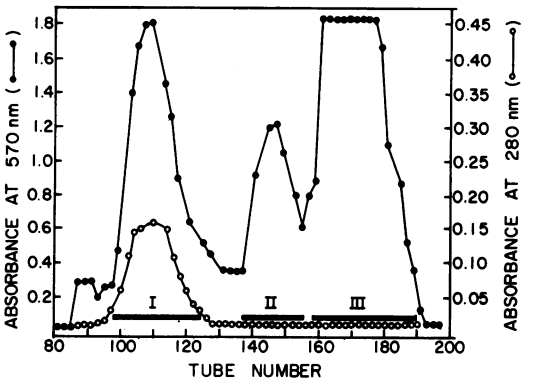

FIG. 2. Chromatography of the cyanogen bromide digest of apoC-II on Bio-Gel P-30. The column (1.6 × 200 cm) was equilibrated with 25% formic acid. The sample, 10 mg, was dissolved in 2 ml of 6 M urea and 0.1 M Tris·HCl, pH 8.0, and lyophilized. Two milliliters of 25% formic acid was added to the dry sample and the digest was applied to the column. The column was eluted with 25% formic acid at a flow rate of 25 ml/hr; 2.5-ml fractions were collected. Peptide was detected by absorbance at 280 nm and by ninhydrin after alkaline hydrolysis (25). Zones I, II, and III correspond to the three cyanogen bromide fragments (Table 1). The high abosrbance in zone III was due to urea. The peptide was desalted on Bio-Gel P-2.

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