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HUMAN PLASMA PROAPOA-I: ISOLATION AND AMINO-TERMINAL SEQUENCE

H. B. Brewer, Jr., T. Fairwell, L. Kay, M. Meng,
R. Ronan, S. Law, and J. A. Light*

Molecular Disease Branch, National Heart, Lung, and Blood Institute,
National Institutes of Health, Bethesda, Maryland, and
*Walter Reed Army Clinical Center, Bethesda, Maryland

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SUMMARY: Human apoA-I is synthesized as preproapoA-I, a 267 amino acid precursor apolipoprotein. PreproapoA-I initially undergoes intracellular co-translational proteolytic cleavage into proapoA-I. ProapoA-I is secreted from the cell and was isolated from thoracic duct lymph in the apoA-I_1 isoform position. The amino-terminal sequence of proapoA-I isolated from human lymph revealed the presence of 6 additional amino acids, Arg-His-Phe-Trp-Gln-Gln, on the amino-terminal end of apoA-I consistent with the proapoA-I sequence determined by nucleic acid sequence analysis of cloned apoA-I. Our results indicate that proapoA-I is present in human plasma, and undergoes post-translational proteolytic cleavage to mature plasma apoA-I.

INTRODUCTION: Plasma human apolipoprotein A-I[+] is a 243 amino acid major apolipoprotein of HDL (1,2) which has been reported to modulate the enzymic activity of LCAT, an enzyme which catalyzes the esterification of plasma cholesterol (3). Human plasma apoA-I is polymorphic (1,4-6) when analyzed by isoelectric focusing, and different qualitative patterns of apoA-I isoforms have been observed in cerebrotendinous xanthomatosis (7), after fat feeding (8,9) and in the Tangier, Milano, Marburg, and Giessen apoA-I variants (6,10-13). Analysis of human (14), rooster (15), and mouse (16) apoA-I have suggested that the basic isoforms separable by isoelectric focusing are precursors of the more acidic isoforms. An increase in the basic apoA-I isoform[‡], apoA-I_1, was also observed in human thoracic duct lymph following fat feeding (8,9). Previous studies utilizing

[+] The abbreviations used are: apo, apolipoprotein; HDL, high density lipoproteins; LCAT, lecithin cholesterol acyltransferase; $NaDodSO_4$, sodium dodecylsulfate; PTH, phenylthiohydantoin.

[‡] The human apoA-I isoforms have been designated apoA_1 to apoA-5 (basic to acidic) to be consistent with the nomenclature currently utilized for the rooster (15) and rat (17) apoA-I isoforms.

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cell-free translation techniques have established that rat and human apoA-I are initially synthesized as preproapolipoproteins and partial sequences of the rat and human precursor proteins have been reported (18,19). Recently, we have reported the complete amino acid sequence of the prepropeptide of human apoA-I determined by nucleic acid sequence analysis of cloned apoA-I (20). Human preproapoA-I has 24 additional amino acids attached to the amino-terminal end of mature apoA-I. Eighteen amino acids are contained within the hydrophobic prepeptide (Met-Lys-Ala-Ala-Val-Leu-Thr-Leu-Ala-Val-Leu-Phe-Leu-Thr-Gly-Ser-Gln-Ala) followed by a 6 amino acid propeptide (Arg-His-Phe-Trp-Gln-Gln) (20). The present study was initiated to determine if the apoA-I_1 isoform was proapoA-I thereby establishing the structural relationship of apoA-I_1 to the major acidic plasma apoA-I isoform (apoA-I_3).

MATERIALS AND METHODS

Lipoprotein and Apolipoprotein Isolation

Plasma was obtained from normolipidemic volunteers after an overnight fast for isolation of plasma lipoproteins. Lymph was obtained by cannulation of the thoracic duct in subjects undergoing lymph drainage for immunosuppression prior to kidney transplantation. Post-absorptive lymph was collected after a fat meal (100 g of cream) for six hours in sterile bags containing 3 mM Na_2EDTA (8,9). Plasma HDL (d = 1.063-1.21 g/ml) was isolated by ultracentrifugation in a Beckman 60 Ti rotor (Beckman, Inc., Fullerton, CA) (21). Lymph lipoproteins of d<1.006 g/ml were prepared by ultracentrifugation for 16 hrs (4°C) in a Beckman 60 Ti rotor. Lyophilized plasma HDL and lymph lipoproteins (d<1.006 g/ml) were delipidated by extraction with chloroform-methanol (3:1, v/v) and the apolipoprotein pellet dried under N_2 (10).

Plasma apoA-I was isolated to homogeneity by gel permeation and ion exchange chromatography as previously reported (1). ApoA-I_1 was isolated from post-absorptive lymph lipoproteins (d<1.006 g/ml) because of the relative increase of this isoform following fat feeding (8,9). Individual apoA-I_1 and apoA-I_3 isoforms were isolated by preparative isoelectric focusing from lymph lipoproteins (d<1.006 g/ml) and purified plasma apoA-I respectively. Lyophilized apolipoprotein samples (1.5-2.0 mg) were dissolved in aqueous 10 M urea (2 mg/ml) and fractionated by isoelectric focusing on a Bio-Rad 220 Dual Slab Cell (Bio-Rad, Richmond, CA). A 5.0-6.0 pH gradient (Serva ampholines, Serva AB, Heidelberg, FRG), and 3.0 x 100 mm gels (7.5% acrylamide, 0.2% bisacrylamide) were employed. The upper tray buffer was 0.02 M NaOH and the lower buffer, 0.01 M H_3PO_4. The gels were electrophoresed for 16 hr at 250 volts. After completion of the run, the isoforms were identified by cutting a 1 cm section from the side of the gel, and staining with 0.1% Coomassie Blue G 250 and destaining in 7.5% acetic acid acid solution. The corresponding gel segments containing the individual apoA-I isoforms were cut, and the protein eluted by electrophoresis (60 volts, 5 hrs) into Spectra-Por 3 (Spectrum Medical, Inc., Los Angeles, CA) dialysis bags. The isolated isoforms were desalted on Bio-Gel P-30 (1.2 x 90 cm) in 0.01 M NH_4HCO_3 (pH 8.2). The isolated isoforms (1 to 5 ug) were analyzed by $NaDodSO_4$ gel electrophoresis (15% acrylamide), and urea polyacrylamide gel electrophoresis (7.5% acrylamide, pH 8.9) as previously described (10). Analytical two-

dimensional gel electrophoresis was carried out with isoelectric focusing in the first dimension followed by $NaDodSO_4$ slab gel electrophoresis in the second dimension. Five to 10 ug of apolipoprotein were focused at 4°C for 18 hr in 1.5 x 95 mm cylindrical tubes containing 7.5% acrylamide, 0.2% bisacrylamide, 8 M urea, and 3% ampholines (pH 5.0 to 6.0). After isoelectric focusing the gel was removed from the tube and electrophoresed in a 1.5 x 100 mm slab gel containing 15% acrylamide, 0.085% bisacrylamide, and 0.1% $NaDodSO_4$. Samples were stained with 0.1% Coomassie Blue and destained as outlined above.

Phenylisothiocyanate Degradations

Automated degradations were performed with 1 to 5 nmoles of protein in a Beckman Sequencer Model 890B (Beckman, Inc., Palo Alto, CA) utilizing a 0.1 M Quadrol buffer system. The PTH amino acids were identified by high pressure-liquid chromatography (Zorbax ODS) (22), and mass spectrometry (Finnigan 4510 Mass Spectrometer) (23). PTH amino acids were quantitated by high pressure-liquid chromatography.

RESULTS: The pattern of major and minor human apoA-I isoforms present in plasma HDL and in the thoracic duct lymph (d<1.006 g/ml) of a subject following fat feeding are illustrated in Figure 1. The major apoA-I isoforms in plasma are apoA-I_3 and apoA-I_4. The apoA-I_1 isoform was a major apoA-I isoform in thoracic duct lymph lipoproteins (d<1.006 g/ml) obtained after fat feeding (Figure 1) (8,9). ApoA-I_1 was isolated to homogeneity by preparative isoelectric focusing

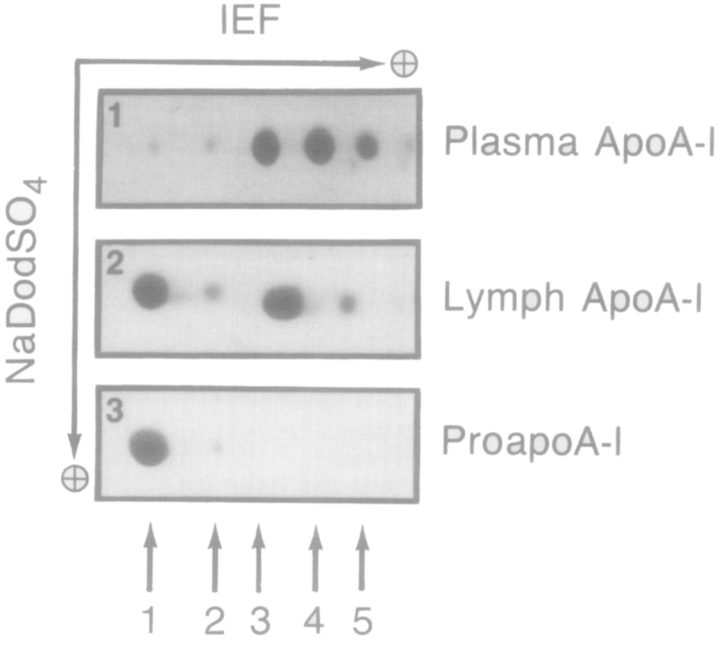

Figure 1. Two-dimensional gel electrophoresis of human plasma apoA-I (1), lymph apoA-I (2), and purified proapoA-I (apoA-I_1) (3). Only the area of the gel corresponding to the apoA-I is illustrated.

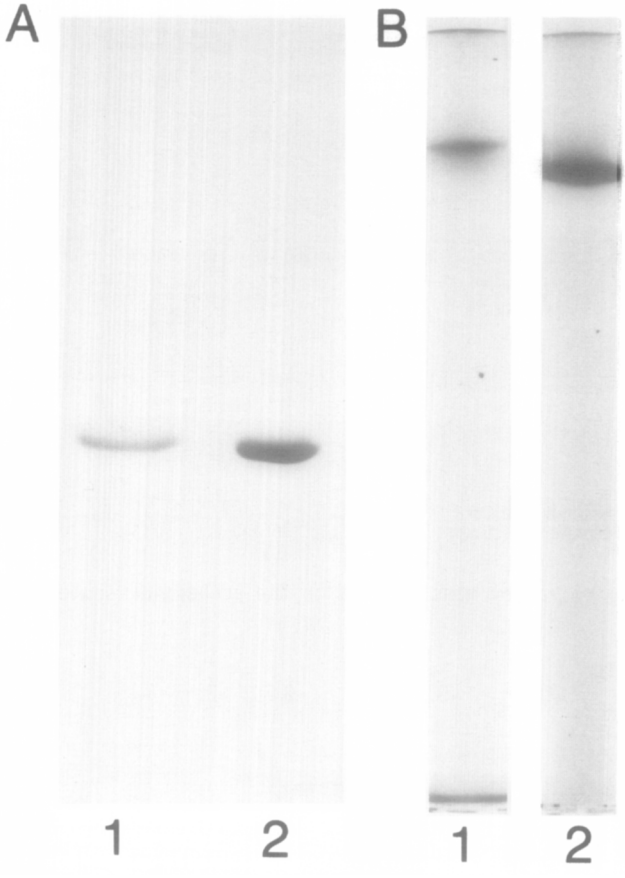

Figure 2. Purified proapoA-I (apoA-I_1) (1) and mature apoA-I (apoA-I_3) (2) analyzed on $NaDodSo_4$ (A) and urea polyacrylamide, pH 8.9, (B) gel electrophoresis.

of lymph lipoproteins (d<1.006 g/ml), and is illustrated in Figure 1. ApoA-I_3 was isolated by preparative isoelectric focusing from purified apoA-I initially isolated from plasma HDL (1). Purified apoA-I_1 and apoA-I_3 migrated as single electrophoretic bands in $NaDodSO_4$ electrophoresis with a similar apparent molecular weight (Figure 2A). ApoA-I_1 and apoA-I_3 were single electrophoretic bands of different relative migration on urea polyacrylamide gel electrophoresis at pH 8.9 (Figure 2B).

The amino-terminal sequence of apoA-I_1 (3 nmol) and apoA-I_3 (1 nmol) were determined on an automated Beckman 890B sequencer, and the PTH amino acids were quantitated and identified by high pressure liquid chromatography and mass spectrometry. The amino acid sequences are as follows:

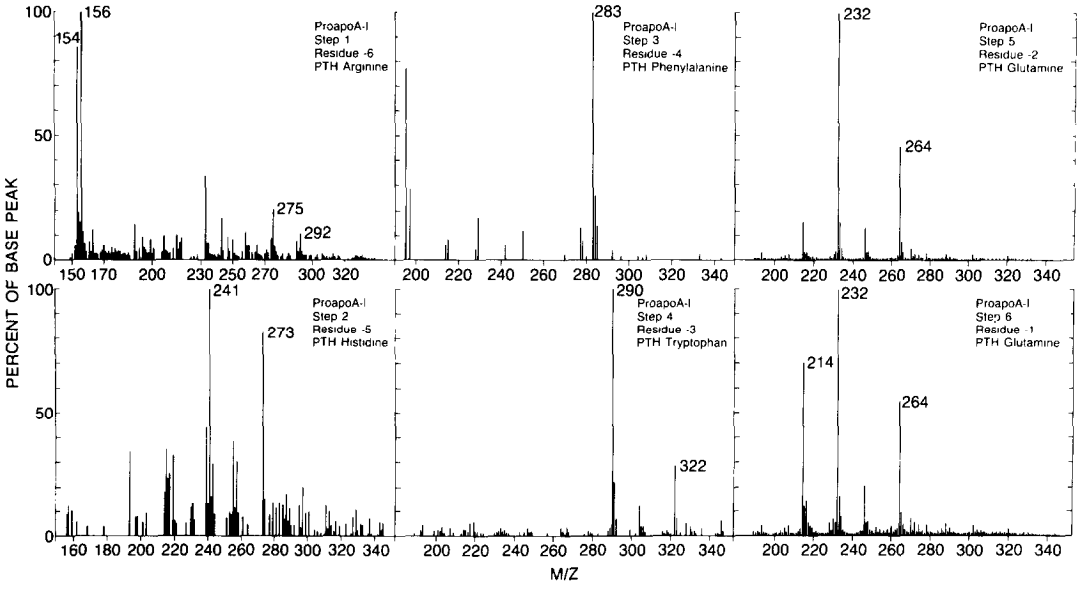

Figure 3. Chemical ionization mass spectra of the propeptide sequence of proapoA-I (apoA-I_1).

Residue in Mature ApoA-I Sequence	-6	-5	-4	-3	-2	-1	1	2	3
ApoA-I_1									
Edman Step	1	2	3	4	5	6	7	8	9
Amino Acid	Arg	His	Phe	Trp	Gln	Gln	Asp	Glu	Pro
Yield (nmol)	2.0	2.0	4.2	1.1	3.7	3.6	1.8	1.8	0.4
ApoA-I_3									
Edman Step							1	2	3
Amino Acid							Asp	Glu	Pro
Yield (nmol)							0.3	0.6	0.3

The amino acid sequence of the initial 6 amino acids in apoA-I_1 was identical to the propeptide sequence of human apoA-I which we have previously determined by nucleic acid sequence analysis of cloned human apoA-I (20). The mass spectra of each of the 6 steps of the propeptide sequence of apoA-I_1 are shown in Figure 3. The amino-terminal sequence of apoA-I_3 began with aspartic acid and was the sequence of the mature 243 amino acid apoA-I which we have previously reported (1). These studies definitively establish for the first time that human plasma contains both proapoA-I (apoA-I_1 isoform) as well as mature apoA-I.

DISCUSSION: The data from this study and our previous report (20) have now established that human apoA-I is synthesized as a 267 amino acid preproapoA-I.

The 18 amino acid prepeptide is cleaved by an intracellular protease, and proapoA-I may be secreted into lymph and plasma. ProapoA-I contains 6 additional amino acids and undergoes post-translational proteolytic cleavage after secretion. ProapoA-I (apoA-I_1 isoform) increases in human lymph following diets enriched in fat, but not protein or carbohydrates (8,9). The metabolism of proapoA-I isolated from human lymph was recently studied in man utilizing radiolabelled proapoA-I (24). Following injection proapoA-I was rapidly converted to the mature acidic isoforms, demonstrating directly the conversion of proapoA-I to mature apoA-I in plasma (24).

The proteolytic cleavage of proapoA-I to mature apoA-I occurs at an unusual site between glutamine and aspartic acid. The presence of two neutral amino acids residues (glutamine and glutamine; residues -2 and -1) (20) and two adjacent negatively charged residues (aspartic acid and glutamic acid; residues 1 and 2) (1) may be important in the specificity of the cleavage site by a specific protease which we have designated apoA-I propeptidase. The combined results from these studies have established a complex system of co-translational and post-translational proteolytic processing of human apoA-I. A detailed understanding of these processes will be necessary to evaluate the factors which modulate the conversion of proapoA-I to apoA-I, and the importance of the apoA-I isoproteins in normal HDL metabolism, and disease associated with apoA-I variants and low plasma concentrations of HDL.

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