Vol. 112, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1983 Pages 257-264

cDNA CLONING OF HUMAN apoA-I: AMINO ACID SEQUENCE OF PreproapoA-I

Simon W. Law, Glenn Gray, and H. Bryan Brewer, Jr.

Molecular Disease Branch
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, MD

Received February 28, 1983

SUMMARY: ds-cDNA to human liver apoA-I mRNA has been cloned. Nucleic acid sequence analysis revealed that apoA-I mRNA codes for a precursor apolipoprotein, preproapoA-I, which contains 24 amino acids on the NH_2-terminal end of the mature plasma 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). Our results on human apoA-I are in agreement with the partial sequence of the precursor of rat apoA-I with respect to the length of the precursor sequence, location of the prepeptide, and the presence of an unusual propeptide sequence terminating in a neutral dipeptide Gln-Gln. A detailed analysis of the primary structure of normal human apoA-I mRNA will be essential to our ultimate understanding of the processing of human apoA-I and diseases characterized by molecular defects in apoA-I structure and function.

Human apolipoprotein A-I[+] contains 243 amino acids (1,2) and is a major protein constituent of plasma HDL. Clinically, low plasma levels of apoA-I and HDL have been associated with an increased risk of the development of premature cardiovascular disease (3-6). Several structural variants of apoA-I have been recognized which are associated with low levels of plasma HDL including apoA-$I_{Tangier}$ (7,8), apoA-I_{Milano} (9), and and apoA-$I_{Marburg}$ (10).

Human apoA-I is polymorphic in plasma (2,11,12), and changes in the apoA-I isoforms have been observed after fat feeding (13), in the Tangier, Milano, Marburg, and Eiessen apoA-I variants (7-10,14), and in cerebrotendinous xanthomatosis (15). In order to elucidate the molecular mechanism involved

[+] The abbreviations used are: apo, apolipoprotein; HDL, high density lipoproteins; dTTP, 2'-deoxythymidine 5'-triphosphate; dGTP, 2'-deoxyguanosine 5'-triphosphate; dCTP, 2'-deoxycytidine 5-triphosphate; dATP, 2-deoxyadenosine 5'triphosphate; ds-cDNA, double stranded complementary DNA; DTT, dithiothreitol.

0006-291X/83 $1.50

in the biosynthesis and metabolism of apoA-I we have initiated a systematic analysis of the structure of cloned apoA-I. The present report describes the molecular cloning of ds-cDNA to human liver apoA-I mRNA. Human apoA-I mRNA was shown to code for a precursor apolipoprotein, preproapoA-I, which contains 24 amino acids on the NH_2-terminal end of the mature plasma apoA-I. Eighteen amino acids are contained within the prepeptide followed by a 6 amino acid propeptide.

MATERIALS AND METHODS

Preparation of Human Liver Polyadenylated RNA. RNA was isolated from adult human liver obtained from a 34 year-old female automobile accident victim (protocol approved by Human Research Committee) using quanidine thiocyanate as described by Chirqwin et al (16). Polyadenylated RNA was isolated from total liver RNA by binding to oligo-d(T) cellulose (Collaborative Research, type II) (17) and elution with 10 mM Tris-HCl and 1 mM EDTA (pH 7.4). 75 ug of polyadenylated RNA was isolated per one gram of liver tissue.

Preparation of a ds-cDNA Library of Human Liver mRNA Sequences. Polyadenylated RNA was used for the construction of a ds-cDNA library as described by Law and Dugaiczyk (18) and the ds-cDNA was inserted into the Pst 1 site of the plasmid pBR322 before transforming the gram negative bacteria Escherichia coli strain RRI. 1 ug of ds-cDNA yielded approximately 10^4 recombinants. All experiments with bacteria containing recombinant plasmids were conducted in accordance with the National Institutes of Health Guidelines for Recombinant DNA Research.

Screening the Library for Recombinant Plasmids Bearing Human Liver Apolipoprotein A-I mRNA Sequences. Synthetic oligonucleotides were used for the screening of recombinant plasmids. A seventeen base-long oligonucleotide complementary to the mRNA region encoding for the known amino acid sequence of residues 108-113 (Trp-Gln-Glu-Glu-Met-Glu) of human apoA-I (1) was purchased from PL Biochemical, Inc. This oligonucleotide is a mixture of eight sequences designed to cover all possible codons for the amino acid sequence of residues 108-113. A radiolabeled hybridization probe with a specific activity of 2-4 x 10^8 cpm/ug DNA was prepared by phosphorylating the 5'-OH end group of the synthetic oligonucleotide with T_4 polynucleotide kinase (PL Biochemicals) and [γ^{32}P]ATP (New England Nuclear >3000 Ci/mmol) as previously reported (18,19). Bacterial colonies were allowed to grow on tetracycline (12 ug/ml) plates for 12 hours at 37°C, after which they were transferred to chloramphemicol (200 ug/ml) plates for plasmid DNA amplification (20) except that nitrocellulose filter papers were employed (22,23). Hybridization conditions were similar to previously published procedures (24-26).

Prime-Extension of apoA-I cDNA. Cloned cDNA fragment containing the oligonucleotide sequence of the NH_2-terminal end of apoA-I were 5' end labelled and primed to human liver polyadenylated RNA as previously described (18,21). DNA extension was performed with reverse transcriptase (1 ug primer/7 units enzyme, Life Science) on human liver polyadenylated RNA in the presence of 1 mM of dATP, dGTP, dCTP, and dTTP (PL Biochemicals), 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, and 50 mM Tris-HCl (pH 8.3). The primer and extension product were separated from the free dNTP and enzyme by fractionation on a Sephadex G-100 column (0.7 x 20 cm; buffer, 10 mM Tris-HCl, 1 mM EDTA, and .3 M NaCl, pH 7.4), ethanol precipitated, and sequenced by the Maxam and Gilbert procedure (27).

Additional Methods and Materials. Restriction enzymes Sau3A, Fnu4H I and Msp I were purchased from Boehringer Mannheim or New England Biolabs. One unit or 2 units of enzyme were employed per ug of whole plasmid DNA or insert DNA, respectively, and the reaction was performed for 60' at 37°C. Polyacrylamide gel and agarose gel electrophoresis were performed as previously described (18).

RESULTS

The major objective of this study was to determine if human apoA-I was synthesized as a precursor protein. A ds-cDNA library of human liver mRNA was prepared, and the recombinant plasmids were screened with a 17 base-long synthetic oligonucleotide prepared from residues 108-113 of the amino acid sequence of apoA-I (1). This 17 base-long oligonucleotide was selected since it contained little codon degeneracy. Approximately twenty out of three thousand independent clones hybridized to the seventeen base-long oligonucleotide at 37°C and the hybrids were stable following washing at 51°C. Eight positive clones were selected and the plasmid DNA analyzed by restriction enzyme mapping. Restriction patterns for these inserts were similar, however, they varied in the size of the insert DNA suggesting that they were independently derived apoA-I clones. One clone, designated pMDB-1408, was selected for sequence analysis. This clone contained a nucleotide sequence which coded for ten additional amino acids (Gly-Ser-Gln-Ala-Arg-His-Phe-Trp-Gln-Gln-apoA-I) on the NH_2-terminal end of the mature apoA-I (Figure 1). These results indicated that human liver apoA-I mRNA coded for a precursor protein.

In order to determine the remainder of the nucleotide sequence which encodes for the precursor sequence of apoA-I, a 119 base pair-long DNA fragment was excised from the plasmid pMDB-1408 DNA by cleavage with the restriction enzymes Sau3A and Msp I and 5' end labeled with T_4 polynucleotide kinase. After cleavage of the radiolabeled DNA fragment with the restriction enzyme Fnu4H I, a 32 base pair-long DNA fragment with the label at the Sau3A end was isolated by preparative polyacrylamide gel electrophoresis and sequenced. The oligonucleotide sequence of the 32 base pair DNA fragment encoded the amino acid residues of 9 to 1 of the mature apoA-I protein and 2 residues (Gln-Gln-apoA-I) of the precursor sequence. To establish the remainder of the apoA-I

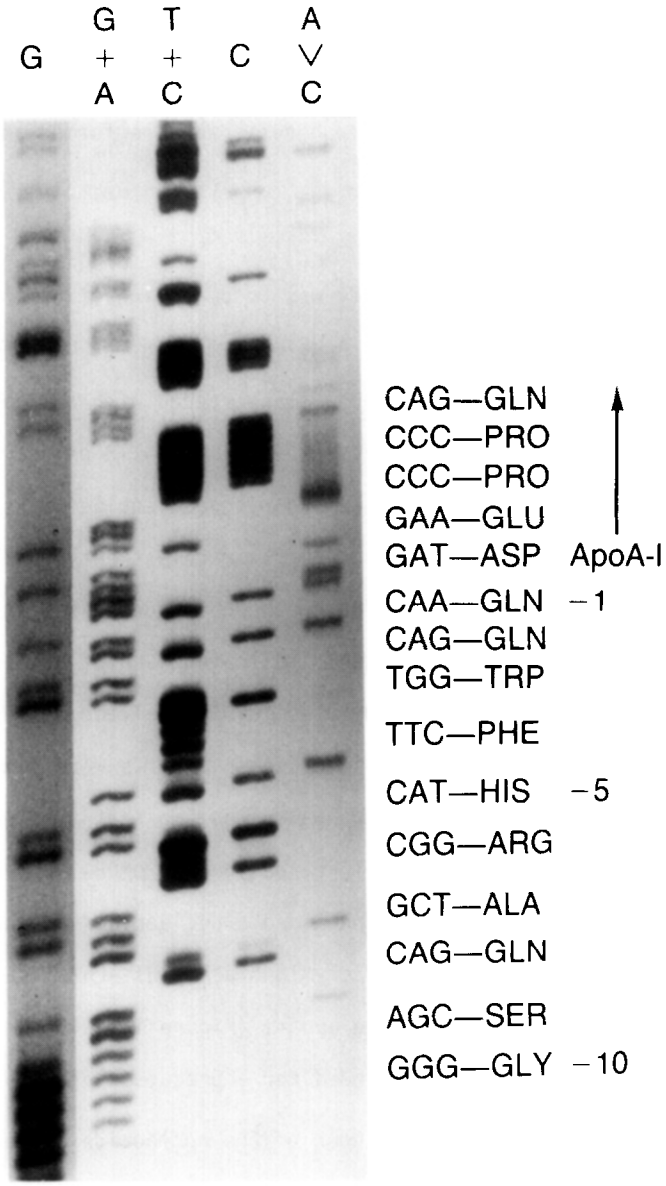


Figure 1: Autoradiogram of a DNA sequencing gel showing the 5' coding sequence (mRNA) of an apoA-I clone pMDB-1408 insert DNA. On this gel 10 additional amino acids were identified attached to the NH_2-terminal aspartic acid of human apoA-I (1).

precursor sequence, 0.5 ug of the 32 base pair-long DNA fragment labeled at the Sau3A site (1 x 10^7 cpm total) was used as a primer for prime extension of human polyadenylated liver mRNA. The synthesized product was isolated by Sephadex G-100 column chromatography (as described in Methods) and sequenced. The sequence of the prime extended cDNA fragment revealed that human apoA-I

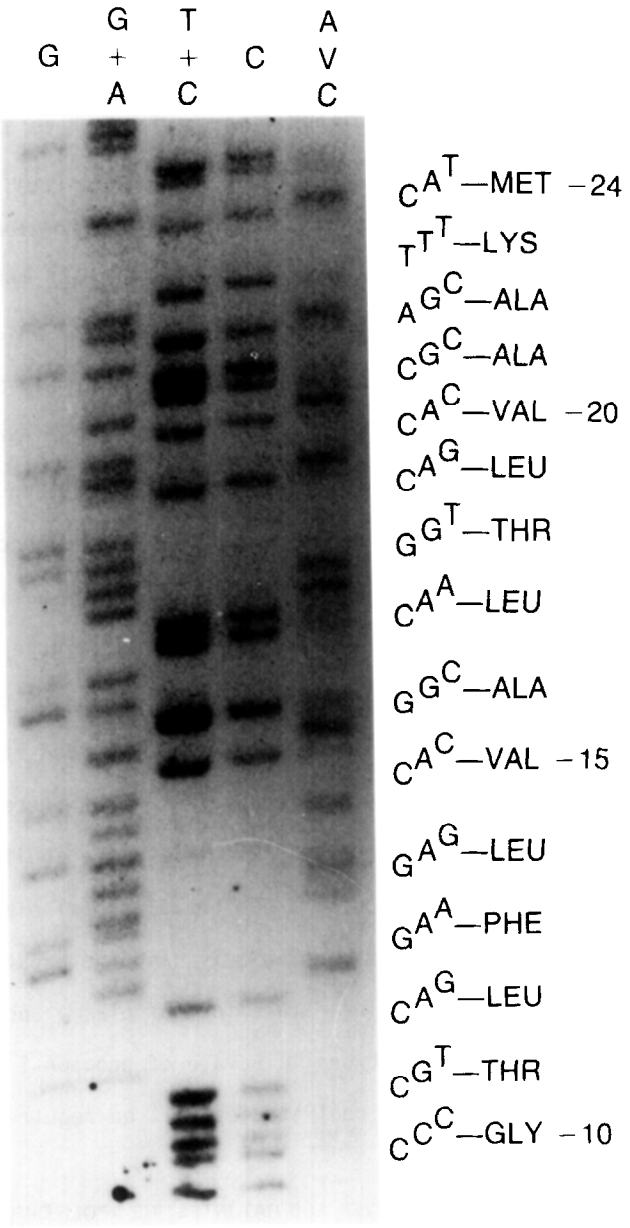


Figure 2: Autoradiogram of a DNA sequencing gel showing a portion of the complementary sequence to the 5' end of apoA-I mRNA as detailed in the text. The sequence shown is from amino acids -10 to -24 of the prepeptide.

mRNA encodes for a precursor sequence of 24 amino acids attached to the NH_2-terminal end of the mature protein (Figure 2).

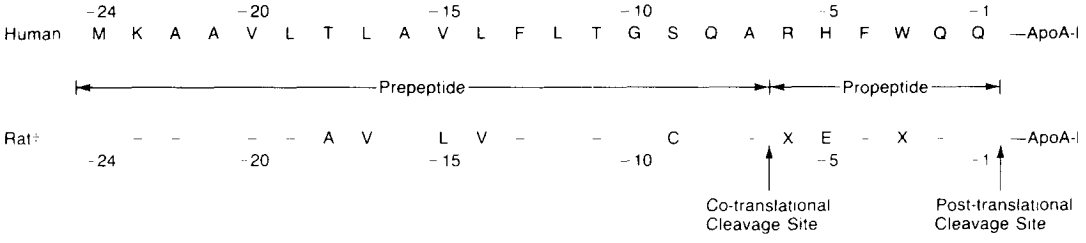
DISCUSSION

Partial sequence analysis of the cell-free translated product of rat intestinal apoA-I mRNA by Gordon et al (28) demonstrated that the rat A-I

apolipoprotein was synthesized as a 24 amino acid preproapolipoprotein with 18 amino acids in the prepeptide and 6 amino acids in the propeptide. Previous studies with human intestinal and liver cultures have also suggested that apoA-I was synthesized as a precursor protein (29). The results from the structural analysis of human apoA-I mRNA by molecular cloning and sequence analysis described in the present report indicate that human apoA-I is synthesized as a preproapolipoprotein with an 18 amino acid prepeptide (Met-Lys-Ala-Ala-Val-Leu-Thr-Leu-Ala-Val-Leu-Phe-Leu-Thr-Gly-Ser-Gln-Ala) and a 6 amino acid pro-peptide (Arg-His-Phe-Trp-Gln-Gln). Our results on human liver preproapoA-I are in agreement with Gordon et al (28) on the size, location of the prepeptide, and the presence of an unusual propeptide terminating in a neutral dipeptide Gln-Gln instead of the basic dipeptide which is characteristic of many secretary proproteins (Figure 3) (30).

Human apoA-I is polymorphic in plasma (2,11,12), and of particular interest is the secretion into plasma of the proapoA-I, and its increase following fat feeding (13). These studies indicate that there are two sites of processing of the A-I apolipoprotein. Initially, the apolipoprotein is synthesized as a preproapoA-I precursor. Co-translational cleavage of the prepeptide occurs predominately intracellularly, and the proapoA-I may be secreted into plasma and lymph. The mature form of plasma apoA-I is produced by a second post-translational cleavage of proapoA-I to yield the mature A-I apolipoprotein. Additional studies will be required to establish

PREPRO-SEQUENCE OF HUMAN LIVER AND RAT INTESTINE APOLIPOPROTEIN A-I



*As reported in reference (28); ( ) indicates amino acid sequence identical to human sequence. (X) amino acid residue not determined

Figure 3

the site(s) of cleavage and the factors which modulate the cleavage of preproapoA-I and proapoA-I and the importance of the precursor forms in the metabolism of apoA-I.

ACKNOWLEDGEMENTS

We wish to express our appreciation to Ms. Hilde Kahn for expert technical assistance, and Ms. Barbara Ziarnik for preparation of the manuscript.

REFERENCES

1. Brewer, H. B., Jr., Fairwell, T., LaRue, A., Ronan, R., Houser, A., and Bronzert, T. J. (1978) Biochem. Biophy. Res. Comm. 80, 623-630.
2. Osborne, J. C., Jr., and Brewer, H. B., Jr. (1977) Adv. Prot. Chem. 31, 253-337.
3. Miller, N. E., Thelle, D. S., Forde, O. H., and Mjos, O.D. (1977) Lancet I, 965-967.
4. Rhoades, G. G., Gillbrandsen, C. L., and Kagan, A. (1976) N. Eng. J. Med. 294, 293-295.
5. Castelli, W. P., Doyle, J. Y., Gordon, T. G., Hames, C. G., Hjortland, M. C., Hylley, S. B., Kajaln, A., and Zukel, W. J. (1977) Circulation 55, 767-772.
6. Avagaro, P., Bittolo Bon, G., Cazzolato, G., Quinci, G. B., and Belussi, F. (1978) Artery 4, 385-394.
7. Kay, L. L., Ronan, R., Schaefer, E. J., and Brewer, H. B., Jr. (1982) Proc. Natl. Acad. Sci. USA 79, 2485-2489.
8. Schaefer, E. J., Kay, L. L., Zech, L. A., Lindgren, F. T., and Brewer, H. B., Jr. (1982) J. Clin. Invest. 70, 934-945.
9. Weisgraber, K. H., Bersot, T. P., Mahley, R. H., Franceschini, G., and Sirtori, C. R. (1980) J. Clin. Invest. 66, 901-907.
10. Utermann, G., Feussner, G., Franceschini, G., Haas, J., and Steinmetz, A. (1982) J. Biol. Chem. 257, 501-507.
11. Nestruck, A. C., Suzue, G., and Marcel, Y. L. (1980) Biochim. Biophys. Acta 617, 110-121.
12. Zannis, V. I., Breslow, J. L., and Katz, A. J. (1980) J. Biol. Chem. 255, 8612-8617.
13. Ghiselli, G., Schaefer, E. J., Zech, L. A., Light, J. A., and Brewer, H. B., Jr. (1982) Arteriosclerosis 2, 439a.
14. Zannis, V. I., Lees, A. M., Lees, R. S., and Breslow, J. L. (1982) J. Biol. Chem. 257, 4978-4986.
15. Shore, V., Salen, G., Cheng, F. W., Forte, T., Shefer, S., Tint, G. S., and Lindgren, F. T. (1981) J. Clin. Invest. 68, 1295-1304.
16. Chirqwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 24, 5294-5299.
17. Aviv, H., and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408-1412.
18. Law, S. W., and Dugaiczyk, A. (1981) Nature 291, 201-205.
19. van de Sande, J. H., Kleppe, K., and Khorana, H. G., (1973) Biochemistry 12, 5050-5055.
20. Gergen, J. P., Stern, R. H., and Wensink, P. C. (1979) Nucleic Acids Res. 7, 2115-2136.
21. Dugaiczyk, A., Law, S. W., and Dennison, O. E. (1982) Proc. Natl. Acad. Sci. USA 79, 71-75.
22. Hanahan, D., and Meselson, M. (1980) Gene 10, 63-67.
23. Grosveld, F. G., Henrik, H., Dahl, M., deBoer, E., and Flavell, R. A. (1981) Gene 13, 227-237.

24. Grunstein, M., and Hogness, D. S. (1975) Proc. Natl. Acad. Sci. USA 72, 3961-3965.
25. Wallace, R. B., Johnson, M. J., Hirose, T., Miyake, T., Kawashima, E. H., and Itakura, K. (1981) Nucleic Acids Res. 9, 879-894.
26. Suggs, S. K., Wallace, R. B., Hirose, T., Kawashima, E. H., and Itakura, K. (1981) Proc. Natl. Acad. Sci. USA (1981) 78, 6613-6617.
27. Maxam, A. M., and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560-564.
28. Gordon, J. I., Smith, D. P., Andy, R., Alpers, D. H., Schonfeld, G., and Strauss, A. W. (1982) J. Biol. Chem. 257, 971-978.
29. Zannis, V. I., Kurnit, D. M., and Breslow, J. L. (1982) J. Biol. Chem. 257, 536-544.
30. Steiner, D. F., Quinn, P. S., Chan, S. J., Marsh, J., and Tager, H. S. (1980) Ann. N. Y. Acad. Sci. 343, 1-15