Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 7265–7269, November 1985
Biochemistry

Molecular cloning and expression of partial cDNAs and deduced amino acid sequence of a carboxyl-terminal fragment of human apolipoprotein B-100

(atherosclerosis/protein polymorphism/cDNA sequence/protein secondary structure/lipid binding)

CHIK-FONG WEI*†, SAN-HWAN CHEN*, CHAO-YUH YANG†, YVES L. MARCEL‡, ROSS W. MILNE‡, WEN-HSIUNG LI§, JAMES T. SPARROW†, ANTONIO M. GOTTO, JR.†, AND LAWRENCE CHAN*†

Departments of *Cell Biology and †Medicine, Baylor College of Medicine and The Methodist Hospital, Houston, TX 77030; ‡Laboratory of Lipoprotein Metabolism, Clinical Research Institute of Montreal, Montreal, Quebec H2W 1R7, Canada; and §Center for Demographic and Population Genetics, University of Texas, Houston, TX 77030

Communicated by Michael S. Brown, July 8, 1985

ABSTRACT **Apolipoprotein (apo) B-100 cDNAs were identified in a human liver cDNA library cloned in the expression vector λgt11. The β-galactosidase–apoB-100 fusion protein was detected by two independently produced low density lipoprotein polyclonal antisera and by three apoB-100 monoclonal antibodies that crossreact with apoB-74. It was not recognized by two apoB-100 monoclonal antibodies that cross-react with apoB-26. The longest clone, λB8, was completely sequenced. It contains a 2.8-kilobase DNA fragment containing the codons for the carboxyl-terminal 836 amino acid residues of apo-B-100, as well as the 3′ untranslated region of apoB-100 mRNA. We have thus mapped apoB-74 to the carboxyl-terminal portion of apoB-100. The deduced amino acid sequence of the cloned DNA matches the sequences of 14 apoB-100 peptides determined in our laboratory. Minor differences in amino acid sequence were noted in three of the peptides, suggesting polymorphism of apoB-100 at the protein and DNA levels. Secondary structure predictions reveal an unusual pattern for apolipoproteins, consisting of β-structure (24%), α-helical content (33%), and random structure (30%). Ten amphipathic helical regions of 10–24 residues were identified. This carboxyl-terminal fragment of apoB-100 is considerably more hydrophobic than other apolipoproteins with known structure. Its lipid binding regions might include stretches of highly hydrophobic β-sheets as well as amphipathic helices. Our findings on apoB structure might be important for understanding the role of apoB-100-containing lipoproteins in atherosclerosis.**

Apolipoprotein B (apoB) is the largest protein component in human lipoproteins. It is characterized by its extreme insolubility in aqueous buffers after removal of the lipid, by its inability to transfer among lipoprotein particles, and by its high molecular weight (1–3). Recently, it was recognized that human apoB is heterogeneous and that normal very low density lipoproteins and low density lipoproteins (LDL) contain a species identified on $NaDodSO_4$/PAGE with an apparent M_r of 549,000, designated B-100; whereas thoracic duct lymph chylomicrons contain another distinct protein, B-48, with an apparent M_r of 264,000 (4). Furthermore, LDL also contain two other proteins, designated B-74 and B-26, which appear to be complimentary fragments or constituents of the B-100 protein (4). Human apoB-100 and apoB-48 appear to be under separate genetic control, because a patient with apparently normal B-48 but with a selective deletion of B-100 has been described (5). There is evidence that B-100 and B-48 are of hepatic and intestinal origin, respectively (4, 6).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

While B-100 is an important protein determinant in the specific binding to LDL by cell-surface receptors (7) and is highly correlated with the prevalence of atherosclerosis (8), its primary structure has defied chemical elucidation, mainly because of its large size, insolubility, and tendency to aggregate (1, 9, 10).

In the present communication, we report the molecular cloning of cDNAs of human liver apoB-100, by using the expression vector λgt11 (11, 12). Sequence analysis of the clones reveals that we have isolated cloned cDNAs corresponding to a large fragment of the carboxyl-terminal portion of B-100. We present the nucleotide sequence of a 2.8-kilobase cloned apoB-100 cDNA, and the deduced amino acid sequence of 836 residues of human apoB-100 ending at its carboxyl-terminal residue. From our analysis, we can map B-74 to the carboxyl-terminal and B-26 to the amino-terminal ends of B-100, respectively (4).

MATERIALS AND METHODS

Polyclonal Antisera Against Human apoB-100. Two separate rabbit antisera against two different preparations of purified LDL (shown to contain apoB-100 as the sole protein component) were used for the initial screening of the λgt11 library. Both antisera were demonstrated to be monospecific against apoB-100 by immunoblots of $NaDodSO_4$/PAGE and did not react against other apolipoproteins (apoE, apoA-I, apoCs) or albumin. However, both also reacted against apoB-48 (3). The antisera were purified by affinity chromatography over a column of apoB-100-Sepharose 4B constructed by coupling LDL-containing apoB-100 as its only protein component to CNBr-activated Sepharose 4B.

Monoclonal Antibodies Against Human LDL and apoB-100. The details of preparation of monoclonal antibodies against LDL have been published (13). Four of these antibodies have been used in this study. They include 1D1, 2D8, 4G3, and 3A8. All of them recognize LDL, but only one, 1D1, recognizes denatured apoB-100. This antibody, 1D1, is specific for B-100, but it also recognizes B-26 and B-48 (13, 14).

Monoclonal antibodies against solubilized apoB-100 were prepared by a previously described method (15). Purified human apoB-100 was solubilized according to the procedure of Cardin *et al.* (16). Mice were immunized with the soluble apoB-100. Fusion with SP2-0 cells and selection of clones were performed as described (17). We have used four of the monoclonal antibodies against soluble apoB-100 for this study: mAb2, mAb7, mAb13, and mAb16. By immunoblot analysis (18), they are all specific for B-100. In addition, mAb2, mAb7, and mAb16 also react against B-74, and

Abbreviations: apo, apolipoprotein; LDL, low density lipoproteins.

mAb13 also reacts against B-26. None of the antibodies that react against B-74 reacts against B-26 or vice versa.

Screening of Human Liver cDNA Library Constructed in λgt11. A human liver cDNA expression library cloned in λgt11 (11, 12) was a generous gift from S. L. C. Woo. This library contains 14×10^6 recombinant phage. We screened 3×10^5 recombinants initially by the method of Young and Davis (11, 12). For detection of the antigen–antibody complex, either ^{125}I-labeled protein A (for rabbit polyclonal antibodies; ICN), or ^{125}I-labeled Fab fragment of sheep anti-mouse IgG (for monoclonal antibodies, Amersham) was used. Autoradiography was performed with Kodak x-ray film, XAR-5, for 24–72 hr.

DNA Sequencing. Plaque DNA was purified (19), and DNA sequencing was performed by the dideoxynucleotide-chain-termination technique of Sanger *et al.* (20). Cloned DNA fragments were subcloned into the M13 vectors, mp18 or mp19, before sequencing. For the fragments ending in a restriction site (e.g., *Eco*RI), a 15-nucleotide universal primer was used. For sequences away from such sites, as sequencing primers, we synthesized oligonucleotides (16–22 nucleotides long) on an Applied Biosystems Model 380A DNA Synthesizer using the phosphoramidite technique (21).

Amino Acid Sequence Analysis of Human apoB-100. Human plasma was obtained by plasmaphoresis from a single patient with familial heterozygous type II hyperlipoproteinemia. The LDL was purified as described (22). It was reduced and alkylated before tryptic or peptic digestion or CNBr cleavage. The peptide mixture was fractionated on a 2.6×150 cm, Sephadex G-50 column. The fractions were pooled and purified by high performance liquid chromatography with a Vydac C_{18} reverse-phase column (4.6 × 250 mm) (23). The individual peptides were collected and sequenced either by a manual modified Edman degradation method (24) or by an automated gas-phase sequencer (25).

Secondary Structure Predictions. Three different algorithms were used to analyze the secondary structure of the apoB-100 carboxyl-terminal fragment: the helical hydrophobic moment of Eisenberg *et al.* (26), the hydropathy scale of Kyte and Doolittle (27), and the probability scale of Chou and Fasman (28).

RESULTS

Identification of apoB-100-Containing Clones. After an initial screening of 3×10^5 recombinants, we identified 16 positive clones that produce immunoreactive apoB that reacts with two independently produced polyclonal antibodies. Specificity of the antigen–antibody reaction was demonstrated by the absence of a signal when the antibodies were preadsorbed to purified apoB-100 (data not shown). All the clones selected by the polyclonal antisera were also positive with three of the monoclonal antibodies—mAb2, mAb7, and mAb16. They were, however, negative to two other monoclonal antibodies—1D1 and mAb13. They were also negative to three other monoclonal antibodies—2D8, 4G3, and 3A8, which only recognized LDL and not denatured apoB (13, 14).

Characterization of β-Galactosidase–apoB-100 Fusion Protein Produced by Recombinant Clones. The products of four of the recombinant clones—λB1, λB3, λB6, and λB8—were further studied by immunoblot analysis. Lysates of four lysogens containing these recombinants were fractionated on $NaDodSO_4$/3.5–12% polyacrylamide gel, transferred to nitrocellulose filter, and blotted to various antisera.

Immunoblot analysis confirms that the monoclonal antibodies, mAb2, mAb7, mAb16, mAb13, and 1D1 all recognize apoB-100. However, only mAb2, mAb7, and mAb16 detected protein bands from the recombinant lysates of M_r ≈160,000 (λB1 and λB3), ≈190,000 (λB6), and ≈210,000 (λB8), respectively. An antibody against β-galactosidase also reacted against the same bands detected by these monoclonal antibodies. In contrast, while mAb13 and 1D1 detected the apoB-100 bands, they did not react with the products from recombinant lysates (data not shown).

Characterization of apoB-100-Containing Recombinants. DNA was purified from the 16 recombinant clones identified by their immunoreactivity toward the poly- and monoclonal antibodies. Eight of these clones consist of only a single *Eco*RI fragment. The other 8 contain an additional 5′ *Eco*RI fragment (see below). The lengths of the individual clones were determined as follows: (*i*) by restriction mapping and sizing of the DNA fragments on polyacrylamide gels; and (*ii*) by DNA sequence analysis of the *Eco*RI fragments and comparison of the sequences. It is evident that all 16 clones have an identical 3′ *Eco*RI fragment and, in eight cases, an additional 5′ *Eco*RI fragment of various lengths that overlap one another in structure. As discussed below, all these clones represent various lengths of the 3′ end of apoB-100 mRNA. It is noted that the fusion proteins produced by all the clones react against three separate monoclonal antibodies—mAb2, mAb7, and mAb16—that are specific for B-74, but are not recognized by two different monoclonal antibodies—1D1 and mAb13—that are specific for B-26. Since the proteins produced by these clones cover the carboxyl-terminal sequences of B-100, we can conclude that B-74 corresponds to the carboxyl-terminal portion of B-100 (4).

DNA Sequence of apoB-100 cDNAs. The longest of the apoB-100 cDNA clones, λB8, was completely sequenced (Figs. 1 and 2). Sequencing was performed on both strands in entirety, which were found to be completely complementary to, and confirmatory of, each other. Both M13 primers and synthetic oligonucleotide primers were used in the sequencing. Significant overlaps between different sequence determinations were ensured in the analysis (Fig. 1).

Clone λB8 covers 2811 nucleotides and a poly(A) tail of 12 nucleotides. The translation termination codon TGA starts at nucleotide 2509, and there is a 3′ untranslated region of 303 nucleotides preceding the poly(A). Twenty-eight nucleotides upstream from the poly(A) is the beginning of a putative polyadenylylation signal, AATAAA.

Amino Acid Sequence of the Carboxyl-Terminal Portion of Human apoB-100. In the λB8 DNA sequence, a long single reading frame is uninterrupted by termination codons until nucleotide 2509. The reading frame is also the same as would be predicted from the reading frame of the β-galactosidase gene from all the clones characterized, indicating that the sequence represents a continuation of the β-galactosidase–apoB fusion gene. A total of 836 amino acids are encoded by this portion of apoB-100.

We have determined the amino acid sequence of >1000 residues from tryptic, peptic, and CNBr peptides of purified human apoB-100. As shown in Fig. 2, there are 13 peptides in λB8 that match the sequence of 14 apoB-100 peptides. The carboxyl-terminal end of the λB8-derived sequence matches those of two overlapping peptides, one from CNBr cleavage

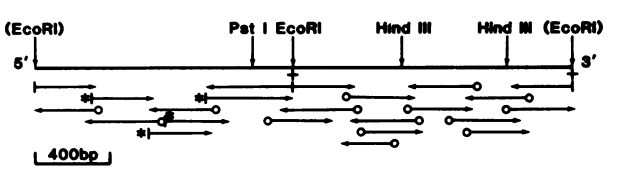

FIG. 1. Partial restriction map and sequencing strategy of λB8. Horizontal arrows indicate the direction and extent of each sequence determination. Vertical bars denote the use of the M13 universal primer for the reactions. Open circles indicate the use of synthetic oligonucleotides as primers. Asterisks denote the use of clones other than λB8 in the reaction; these sequences all match the λB8 sequence. Crosses indicate that the fragments have been sequenced multiple times for the clones depicted. bp, Base pairs.

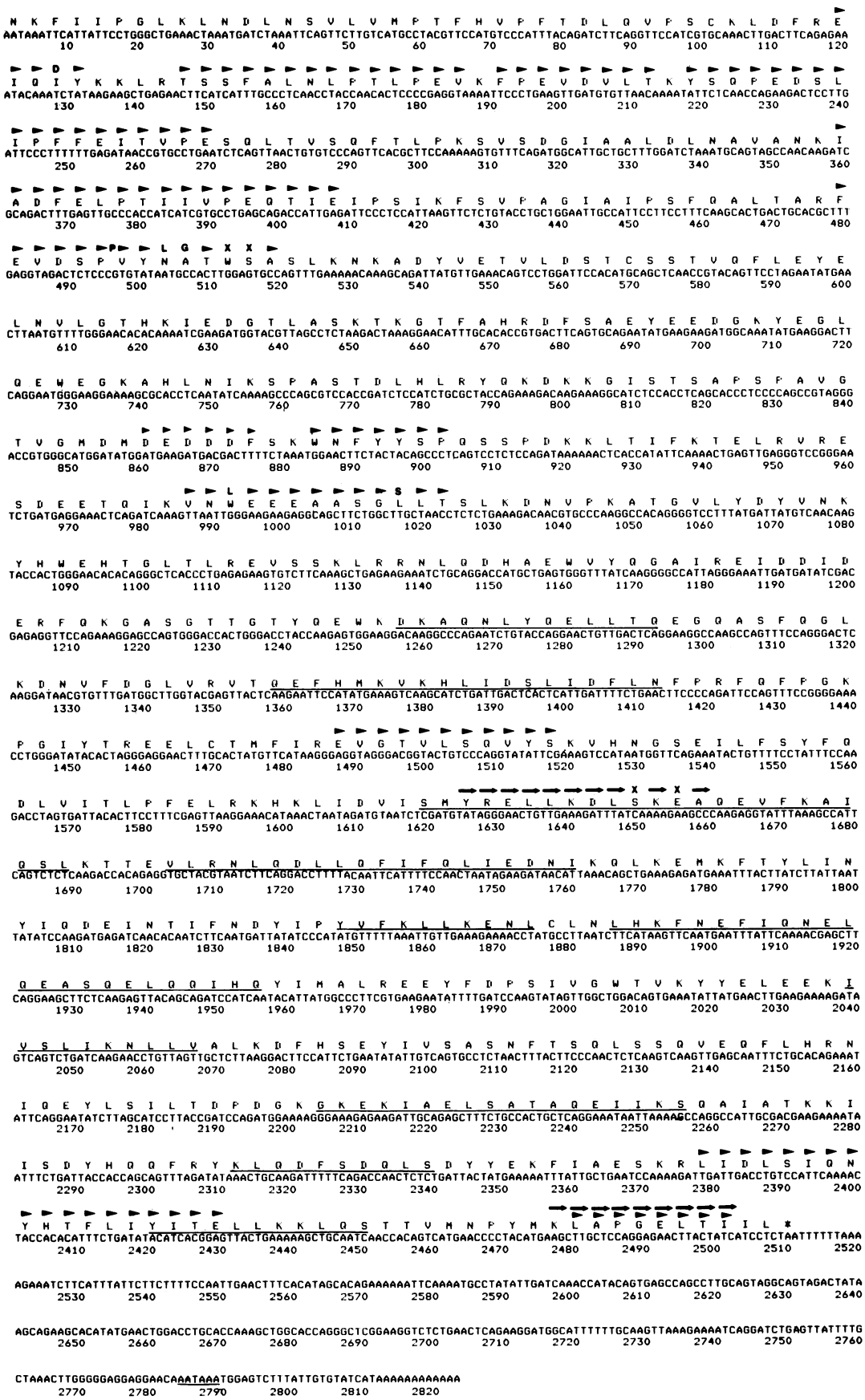

FIG. 2. DNA sequence of λB8, deduced amino acid sequence (identified by the single-letter code) of carboxyl-terminal fragment of apoB-100, and matching sequences of purified apoB-100 peptides. The deduced amino acid sequence is displayed above the DNA sequence. The sequences of the matching peptides are displayed above the DNA-deduced sequence. Short arrowheads indicate the sequence of a peptic peptide (DEDDDF). Long arrowheads indicate the sequences of 11 tryptic peptides. Arrows indicate the sequences of two CNBr peptides. × indicates that the residue has not been identified. The amphipathic helical regions are underlined. The polyadenylylation signal, AATAAA, is also underlined.

Table 1. Amino acid composition of cloned apoB fragment and apoB-100

Amino acid	LDL B-100	λB8	
Lys	79.85	78.9	
His	25.70	21.5	
Arg	33.77	27.5	
Asp	106.56	59.8	99.3
Asn		39.5	
Thr	66.44	62.2	
Ser	86.43	75.3	
Glu	116.16	82.5	142.3
Gln		59.8	
Pro	38.54	38.3	
Gly	47.31	35.9	
Ala	60.50	49.0	
Cys	4.45	4.8	
Val	55.60	57.4	
Met	15.95	12.0	
Ile	60.27	74.2	
Leu	118.46	111.2	
Tyr	33.58	44.3	
Phe	50.46	56.2	
Trp	ND	9.6	

Results are expressed as residues per 10^3 residues. The LDL B-100 composition is from Kane *et al.* (4). λB8 data are deduced from nucleotide sequence of λB8, which covers 836 amino acids. ND, not determined.

and one from tryptic digestion of apoB-100. In total, there are 143 identical residues between the DNA-deduced sequence and the amino acid sequences determined on the various purified B-100 peptides. Furthermore, the tryptic cleavage sites are always preceded by lysine or arginine, whereas the 2 CNBr peptides are preceded by methionine.

DISCUSSION

In this paper, we have presented the sequence of a 2.8-kilobase fragment of human B-100 cDNA cloned in the expression vector λgt11. The following pieces of evidence indicate that the various clones are authentic apoB-100 cDNAs: (*i*) the expressed protein fragments are recognized by two independently produced polyclonal antisera; (*ii*) they are also recognized by three distinct monoclonal antibodies against B-100, which react with the fusion proteins in the recombinant plaques *in situ* and on immunoblots; and (*iii*) the DNA-deduced amino acid sequence matches 14 apoB-100 peptide sequences determined in our laboratory. The match extends all the way to the carboxyl-terminal end of the predicted sequence, which is identical to the sequences of a B-100 tryptic peptide and a CNBr peptide (Fig. 2). This, together with the fact that the sequence matches are interspersed all over the predicted peptide sequence, indicates that we have correctly identified the reading frame of the apoB-100 cDNA clone and that the 836 amino acid sequence is that of a continuous fragment of the carboxyl-terminal part of apoB-100, which we shall designate as apoB-100–COOH. The molecular weight of apoB-100–COOH is ≈96,000 and comprises some 17–25% of apoB-100, depending on the actual molecular weight of the latter (1, 3, 9, 10, 29, 30). Furthermore, we have mapped B-74 to the carboxyl-terminal part of apoB-100, based on the binding of apoB-100–COOH

Table 2. Possible polymorphism of amino acid sequence in apoB-100 peptides

Peptide	Amino acid sequence
1a	E I Q I Y K
b	→→→D→
2a	F E V D S P – V Y N A T W S A
b	→→→→→→P →→L G →X X →
3a	V N W E E E A A S G L L T
b	→→L →→→→→→→→→S →→

Amino acids are identified by the single-letter code. →, Identical residue; –, a deletion in the DNA-deduced sequence or an insertion in the tryptic peptide sequence; X, the residue has not been identified. a, DNA-deduced sequence; b, tryptic peptide sequence.

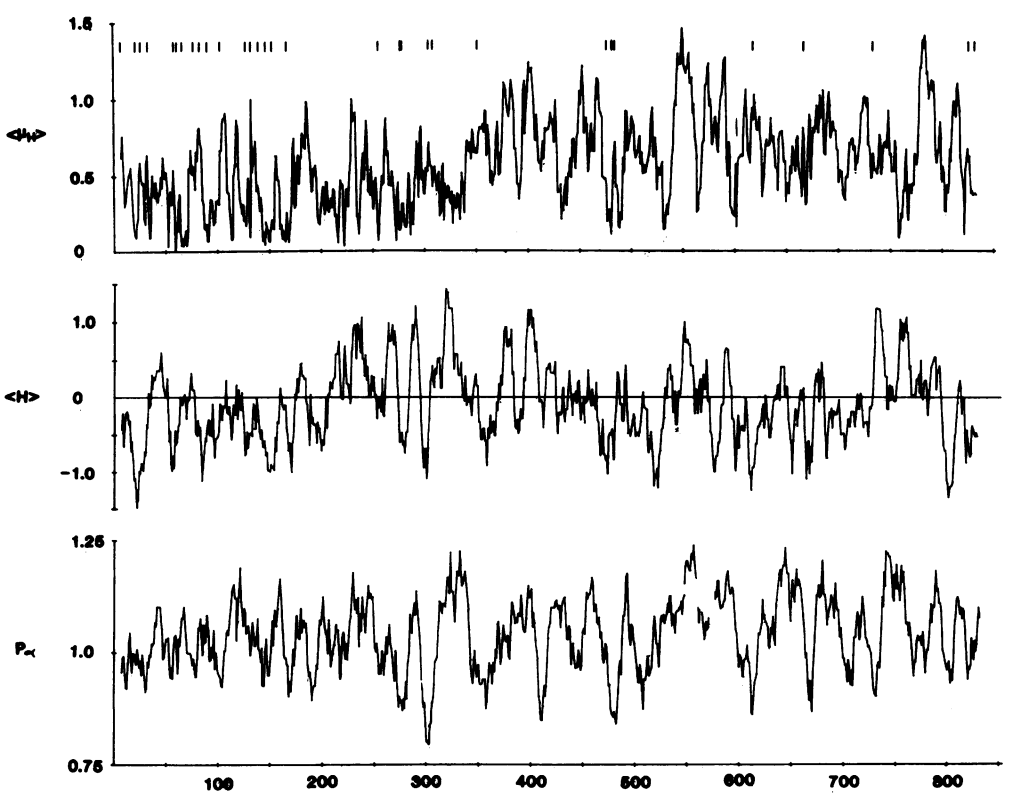

FIG. 3. Secondary structure analysis of apoB-100–COOH. The structure is analyzed by the helical hydrophobic moment, $\langle\mu_H\rangle$; the hydropathy, $\langle H\rangle$; and the α-helical probability, P_α. Each analysis was performed on 11 residue fragments and the respective parameters were plotted at the midpoint of each fragment. The units on the ordinates of $\langle\mu_H\rangle$ and $\langle H\rangle$ are in kcal. On the P_α plot, the ordinate represents the probability scale according to Chou and Fasman (28). The abscissa represents the amino acid residues, with position 836 being the carboxyl-terminal leucine. Vertical bars at the top of the figure indicate the positions of proline residues.

to a set of monoclonal antibodies demonstrated to specifically interact with B-74.

The amino acid composition of apoB-100–COOH is compared to that of apoB-100 (4) in Table 1. With a few exceptions, the two are quite similar. This is in agreement with previous observations that proteolytic peptides of incompletely digested apoB-100 have amino acid compositions very similar to that of the intact protein (1). While most of the B-100 peptide sequences are identical to the DNA-deduced amino acid sequences, some differences are noted in the three peptides shown in Table 2). With the exception of peptide 1, which is only represented in λB8, the sequences encoding peptides 2 and 3 have been confirmed in overlapping clones. We note that in peptide 3, the W→L and L→S changes are the result of single-base substitutions. In peptide 2, the A→G change can also result from a single-base substitution. However, the I→D, and N→L switches in peptides 1 and 2, respectively, require more than single-base substitutions, and the insertion of P in peptide 2 suggests a duplication of the P (or CCC at the DNA level) in this peptide. One possible reason for these observed differences is that the peptides are derived from entirely different regions of apoB-100 not covered by λB8, and they represent some internally repeated sequences. Another possible explanation is that apoB-100 is polymorphic at the DNA and amino acid levels. Immunochemical polymorphism of human apoB has been shown in various laboratories to exist in humans (31, 32). The early studies did not address the question of the B-100 vs. B-48 heterogeneity. However, a recent study (33) using monoclonal antibodies against purified LDL (containing B-100 as its only protein component) confirmed the existence of such immunochemical polymorphisms. Our observations suggest that the polymorphism extends to the amino acid and DNA levels.

The predicted secondary structure of apoB-100–COOH is displayed in Fig. 3. Inspection of this figure and a careful examination of the sequence reveals that apoB-100–COOH differs in many respects from other apolipoproteins of known structure. The average hydrophobicity of apoB-100–COOH is higher than any of the latter, at 0.967 kcal per residue (1 cal = 4.184 J), compared to 0.718, 0.772, 0.806, 0.863, 0.825, 0.838, and 0.752 kcal per residue for apoE, apoA-IV, apoA-I, apoA-II, apoC-I, apoC-II, and apoC-III, respectively. The Chou–Fasman analysis indicates that this fragment contains 33%, 24%, and 30% α-helical, β-, and random structures, and 13% (or a total of 27) β-turns, respectively. Thus, the predicted α-helical structure is somewhat lower, and β-structure is considerably higher, than that of other apolipoproteins. Almost identical values were obtained when LDL conformation was examined by circular dichroism and infrared absorption studies (34, 35). Another interesting feature of apoB-100–COOH is the relatively low number of amphipathic helical regions (36). We identified only 10 such regions involving 159 residues (underlined in Fig. 2) in the whole sequence of 836 residues. However, since the proportion of α-helical structure is relatively low, at least 60% of the α-helices appears to be involved in lipid binding. About one-third of the amphipathic helices are relatively short (11 residues or less) but quite hydrophobic. In addition, many highly hydrophobic β-sheet regions can be identified throughout the sequence. Therefore, we speculate that this fragment of apoB-100 can interact with lipids in two ways: (*i*) by hydrophobic interaction of amphipathic helical regions at the surface with phospholipids, and (*ii*) by hydrophobic interaction of β-sheets by penetration into the lipid core of LDL.

We thank Dr. Simon Mao for supplying one of the polyclonal antibodies, Dr. Henry Pownall for assistance with the computer analysis of protein secondary structure, Dr. Charles Lawrence for computer analysis of DNA data, Drs. William A. Bradley and Richard Weinberg for discussions, and Ms. Debra Kennedy for technical assistance. We gratefully acknowledge the support and interest of Mr. Larry Mathis and Mr. Albert Alkek. This work was supported by National Institutes of Health Grants HL-27341 for a Specialized Center of Research in Atherosclerosis and GM-30998, and by grants from the March of Dimes Birth Defects Foundation, The Methodist Hospital, and Texas Eastern Corporation.

1. Bradley, W. A., Rhode, M. F. & Gotto, A. M., Jr. (1980) *Ann. NY Acad. Sci.* **348**, 87–103.
2. Morrisett, J. D., Jackson, R. L. & Gotto, A. M., Jr. (1975) *Annu. Rev. Biochem.* **44**, 183–207.
3. Kane, J. P. (1983) *Annu. Rev. Physiol.* **45**, 637–650.
4. Kane, J. P., Hardman, D. A. & Paulus, H. E. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2465–2469.
5. Malloy, M. J., Kane, J. P., Hardman, D. A., Hamilton, R. L. & Dalal, K. B. (1981) *J. Clin. Invest.* **67**, 1441–1450.
6. Rash, J. M., Rothblat, G. M. & Sparks, C. E. (1981) *Biochim. Biophys. Acta* **666**, 294–298.
7. Brown, M. S. & Goldstein, J. (1975) *Cell* **6**, 307–316.
8. Goldstein, J. L. & Brown, M. S. (1977) *Annu. Rev. Biochem.* **46**, 897–930.
9. Bradley, W. A., Rhode, M. F., Gotto, A. M., Jr., & Jackson, R. L. (1978) *Biochem. Biophys. Res. Commun.* **81**, 928–935.
10. Deutsch, D. G., Heinrickson, R. L., Forman, J. & Scanu, A. M. (1978) *Biochim. Biophys. Acta* **529**, 342–350.
11. Young, R. A. & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1194–1198.
12. Young, R. A. & Davis, R. W. (1983) *Science* **222**, 778–782.
13. Marcel, Y. L., Hogue, M., Theolis, R., Jr., & Milne, R. W. (1982) *J. Biol. Chem.* **257**, 13165–13168.
14. Marcel, Y. L., Hogue, M., Weich, P. K. & Milne, R. W. (1984) *J. Biol. Chem.* **259**, 6952–6957.
15. Milne, R. W., Theolis, R., Jr., Verdery, R. B. & Marcel, Y. L. (1983) *Arteriosclerosis* **3**, 23–30.
16. Cardin, A. D., Witt, K. R., Barnhart, C. L. & Jackson, R. L. (1982) *Biochemistry* **21**, 4503–4511.
17. Schulman, M., Wilde, C. D. & Kohler, G. (1978) *Nature (London)* **276**, 269–270.
18. Theolis, R., Jr., Weech, P. K., Marcel, Y. L. & Milne, R. W. (1984) *Arteriosclerosis* **4**, 498–509.
19. Maniatis, T., Fritsch, Z. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
20. Sanger, F., Coulson, A., Barrell, B., Smith, A. & Roe, B. (1980) *J. Mol. Biol.* **143**, 161–178.
21. Beaucage, S. L. & Caruthers, M. H. (1981) *Tetrahedron Lett.* **22**, 1859–1862.
22. Patsch, J. R., Sailer, S., Kostner, G., Sandhofer, F., Holasex, A. & Braunsteiner, H. (1974) *J. Lipid Res.* **15**, 356–366.
23. Yang, C. Y., Pownall, H. J. & Gotto, A. M., Jr. (1985) *Anal. Biochemistry* **145**, 67–72.
24. Chang, J. Y., Brauer, D. & Wittmann-Liebold, B. (1978) *FEBS Lett.* **93**, 205–214.
25. Hewick, R. M., Hunkapiller, M. W., Hood, L. E. & Dreyer, W. J. (1981) *J. Biol. Chem.* **256**, 7990–8005.
26. Eisenberg, D., Weiss, R. M. & Terilliger, T. C. (1982) *Nature (London)* **299**, 371–374.
27. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157**, 105–132.
28. Chou, P. Y. & Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47**, 251–276.
29. Steele, J. C. H., Jr., & Reynolds, J. A. (1979) *J. Biol. Chem.* **254**, 1639–1643.
30. Lee, D. M., Valente, A. J., Kuo, W. H. & Maeda, H. (1981) *Biochim. Biophys. Acta* **666**, 133–146.
31. Blumberg, B. S., Dray, S. & Robinson, J. C. (1962) *Nature (London)* **194**, 656–658.
32. Berg, K. (1979) in *Biochemistry in Atherosclerosis*, eds. Scanu, A. M., Wissler, R. W. & Getz, G. S. (Dekker, New York), pp. 419–480.
33. Schumaker, V. N., Robinson, M. T., Curtiss, L. K., Butler, R. & Sparkes, R. S. (1984) *J. Biol. Chem.* **259**, 6423–6430.
34. Gotto, A. M., Jr., Levy, R. I. & Frederickson, D. S. (1968) *Proc. Natl. Acad. Sci. USA* **60**, 1436–1441.
35. Chen, G. C. & Kane, J. P. (1975) *Biochemistry* **14**, 3357–3362.
36. Segrest, J. P., Jackson, R. L., Morrisett, J. D. & Gotto, A. M., Jr. (1974) *FEBS Lett.* **38**, 247–253.