Human apolipoprotein B: partial amino acid sequence

Renee C. LeBoeuf, Chad Miller*, John E. Shively*, Verne N. Schumaker†, Maria A. Balla and Aldons J. Lusis+

The Molecular Biology Institute, and the Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90024, +Departments of Medicine and Microbiology, UCLA, Los Angeles, CA 90024 and **Department of Immunology, City of Hope Hospital and Beckman Research Institute, Duarte, CA 91010, USA*

Received 28 February 1984

A successful approach has been developed for the sequencing of apolipoprotein B based upon the procedure of Cleveland et al. [(1977) J. Biol. Chem. 252, 1102–1106] involving limited proteolysis in the presence of sodium dodecyl sulfate. *Staphylococcus aureus* protease was employed to produce large peptides which were isolated in relatively pure form by preparative gel electrophoresis. Two peptides were partially sequenced using spinning-cup microsequencing techniques. The sequences are: Peptide R2-5, -Ala-Leu-Val-Gly-Ile-Asn-Gly-Glu-Ala-Asn-Leu-Asp-Phe-Leu-Asn-Ile-Pro-Leu-Arg-Ile-Pro-Pro-Met-Arg-(Arg)-; and Peptide R3-1, -Leu-Val-Ala-Lys-Pro-Ser-Val-Ser-Val-Glu-Phe-Val-Thr-Asn-Met-Gly-Ile-Ile-Ile-Pro-Lys-Phe-Ala-Arg-. Several stretches of residues suitable for the construction of oligonucleotide probes have been identified.

Apolipoprotein B *Amino acid sequence* *Proteolysis* *LDL* *Protein isolation* *Polypeptide composition*

1. INTRODUCTION

Apolipoprotein B is the major protein of human low density lipoproteins and it is composed of a single polypeptide chain with a molecular mass estimated to be 250–500 kDa [1,2]. Apolipoprotein B (apo-B) binds to lipid avidly, although its amino acid composition [3] is not particularly enriched in hydrophobic residues. Thus, apo-B may bind to lipid through stretches of hydrophobic amino acids, or even by covalent attachment to lipid.

It is of particular interest to examine the amino acid sequence of apo-B to investigate its lipid binding properties. Further, several forms of apo-B have been identified [3–7] and the structural relationships between these forms are not known. Sequence information would allow the construction of oligonucleotide probes which may ultimately be useful in examining its genetic structure in detail, as well as the processing of the apo-B message and the regulation of translation and transcription.

The hydrophobicity [8], large size and an apparently blocked N-terminus contribute to the difficulties in sequencing apo-B. Here we report that a successful approach has been developed for generating sequence information for apo-B based on the procedure in [9] involving limited proteolysis in the presence of sodium dodecyl sulfate (SDS). *Staphylococcus aureus* protease was employed to produce large peptides which were isolated by preparative gel electrophoresis in quantities sufficient for amino acid sequencing. These sequences are presented here, and several peptides suitable for the construction of oligonucleotide probes are indicated.

2. EXPERIMENTAL

2.1. *Isolation of low density lipoproteins*

Plasma was obtained from a normolipemic donor and LDL isolated by differential ultracen-

† To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

trifugation, using gentamycin, azide and EDTA [1]. The LDL used here were isolated between the densities of 1.02 and 1.05 g/ml.

2.2. *Proteolytic digestion of apolipoprotein B*

2.2.1. Analytical digestion

To find optimal conditions for limited proteolysis of apo-B, we varied protease concentration and the incubation time and examined the resulting peptide fragments by polyacrylamide gel electrophoresis in the presence of SDS. The proteolytic reaction was carried out by mixing 80 μg apo-B, as intact LDL, with a more concentrated reaction buffer to give a final volume of 100 μl in 125 mM Tris–HCl (pH 6.8) containing 0.5% SDS, 10% glycerol and 0.001% bromophenol blue. This mixture was heated to 100°C for 2 min and cooled on ice. Between 2 and 20 μg of *S. aureus* protease was added, and samples were incubated at 37°C for times ranging from 30 min to 3 h. The individual reactions were stopped by the addition of SDS and 2-mercaptoethanol to final concentrations of 2 and 10%, respectively. The mixtures were boiled for 2 min and cooled to room temperature before being applied to a 15% polyacrylamide SDS slab gel for electrophoretic separation of digestion peptides.

2.2.2. Preparative digestion

Six mg of apo-B, as intact LDL, was mixed with the reaction buffer given above in a final volume of 2.8 ml. This mixture was incubated at 100°C for 3 min and cooled on ice, then 87 μg of *S. aureus* protease was added, and the mixture incubated at 37°C for 75 min. The proteolysis was stopped with SDS and 2-mercaptoethanol as described above. The mixture was boiled for 3 min and applied to 2 slab polyacrylamide gels of 14 cm × 18 cm × 3 mm.

2.3. *Treatment of slab gels*

Electrophoresis of the preparative digestion samples proceeded until the tracking dye was within 1 cm of the gel bottom. The gels were stained for 45 min with 0.25% Coomassie brilliant blue in 50% methanol and 10% acetic acid and destained for about 45 min in 50% methanol and 7% acetic acid. The gels were soaked at 4°C overnight in distilled water.

Polyacrylamide gel strips containing peptide bands were sliced out of the preparative gels with a scalpel. In some cases, band slices were stored frozen in polypropylene tubes at −20°C until elution.

2.4. *Gel electroelution*

Protein was eluted from the acrylamide gel bands electrophoretically using an electroelution apparatus (CBS Scientific Co., Del Mar, CA) consisting of a bridge extending between 2 buffer chambers. The running buffer was 0.05 M Tris-acetate (pH 7.8) with 0.05% SDS. The bridge was bounded on both ends by dialysis membranes (Spectra/Por), and on one side was placed the strip of acrylamide gel containing the peptide band. Following elution for 24 h at 60 mA, protein plus Coomassie stain was collected from the anodal side of the bridge structure in a small volume (100 μl), retained by the dialysis membrane. The protein samples were dialyzed againsts 5 mM ammonium bicarbonate before further analysis.

2.5. *Amino acid sequencing*

The amino acid composition of digestion peptides and the amino acid sequencing were carried out as in [10–12].

3. RESULTS AND DISCUSSION

S. aureus protease cleaves peptide bonds on the C-terminal side of glutamic acid residues. Based on the amino acid composition of apo-B with about 400 glutamate residues, a large number of peptide fragments are expected upon complete proteolysis. By limiting the extent of proteolysis by including SDS in the reaction mixture and by limiting incubation time and enzyme concentration, fewer peptides are formed, and most of these are large enough to be retained by polyacrylamide gels. Further, since all peptide bonds are not equally sensitive to proteolysis, some peptides exist in disproportionately high concentrations.

Under our digestion conditions, apolipoprotein B was fragmented into at least 20 peptides of molecular masses between 15 and 70 kDa (fig.1). Digestion under varying conditions of time and protease concentrations yielded remarkably similar peptide patterns, with the steady loss of higher molecular mass peptides with increasing protease and incubation times. These patterns were

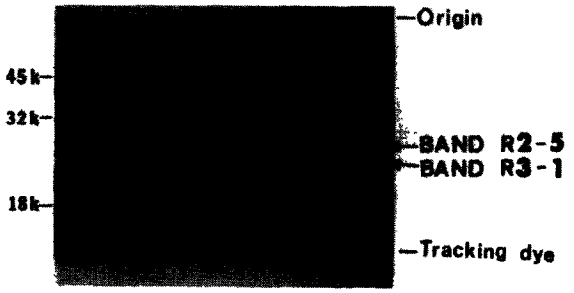

Fig.1. Preparative polyacrylamide gel electrophoresis of human apo-B peptides. *S. aureus* protease peptides were generated from apo-B and run on a 15% polyacrylamide gel as described in the text. The peptides R2-5 and R3-1 are indicated by arrows, and M_r markers are indicated on the left (ovalbumin, 45000; carbonic anhydrose, 32000; β-lactalbumin, 18000). k, kDa.

reproducible, at least using LDL isolated from the same individual. Apo-B incubated in the absence of the protease remained intact throughout the longest incubation period. At 60 min incubation time and 40 μg/ml protease, there appeared to be a slight increase in a peptide labeled R3-1 which appeared to be resolved from other peptides. We chose similar conditions for our large-scale digestion of human apo-B.

The purity and size of 7 isolated peptides are shown in table 1. The peptides ranged in size from about 22 to 68 kDa. While several peptide fractions (3,4) contained more than one band when rerun, most fractions appeared as a single peptide band on SDS-polyacrylamide gels. We did not explore whether or not several proteins of similar molecular mass but differing charge contributed to each band on our SDS gels, but as shown below, the two peptides selected for sequencing gave unique sequences.

Table 1

Quantitative analysis of isolated peptides

Band	Apparent	Quantity		Yield
	M_r	(μg)	nmol	(%)
1	68000	32	0.5	16
2	52000	8	0.2	9
3	50000	6	0.1	10
4	47000	30	0.6	18
5	43000	15	0.4	12
R2-5	24000	54	2.2	14
R3-1	22000	49	2.2	10

The mass of protein was initially estimated by comparing the amount of Coomassie stain within each band to that of bovine serum albumin standards run on the same acrylamide gel. On this basis, the yield of peptide protein was low, ranging from about 10 to 20% recovery. Subsequent to the work presented here, we have increased our yield 2-fold by increasing the time of elution from 24 h to 48 h. Two peptides, R2-5 and R3-1, each yielded about 2 nmol of protein (table 1) and were submitted for amino acid sequence determinations.

In fig.2 are given partial sequences of two peptides of apparent molecular masses of 22 and 24 kDa. Using microsequencing techniques, the sequence of the first 24 residues of each peptide were obtained, starting at the N-terminal end, from only 2 nmol of peptide protein. The sequences of our human apo-B peptides are not homologous to any of the sequences reported for other apolipoproteins, including apo-A-I, A-II, E and C-peptides [13,14]. The apo-B peptides are also not homologous to the sequence of *S. aureus* protease [15].

The thrust of much of the recent biochemical work concerning apolipoproteins has involved molecular cloning techniques. In the case of apo-B, this approach should prove particularly fruitful in terms of determining the sequence and absolute size of this protein through analysis of the DNA. We have identified several regions within the amino acid sequences presented for peptides R2-5 and R3-1, which are suitable for the construction of oligonucleotide probes. Two of these are indicated in fig.2. We have synthesized these and other probes and are currently characterizing several potential apo-B clones identified from our human liver cDNA and genomic libraries.

R2-5: -ALA-LEU-VAL-GLY-ILE-ASN-GLY-GLU-ALA-ASN-
LEU-ASP-PHE-LEU-ASN-ILE-PRO-LEU-ARG-ILE-
PRO-PRO-MET-ARG-(ARG)-

R3-1: -LEU-VAL-ALA-LYS-PRO-SER-VAL-SER-VAL-GLU-
PHE-VAL-THR-ASN-MET-GLY-ILE-ILE-ILE-PRO-
LYS-PHE-ALA-ARG-

Fig.2. Sequences of human apo-B peptides, R2-5 and R3-1. Underlined regions were used to construct oligonucleotide probes.

ACKNOWLEDGEMENTS

We wish to express our gratitude to the National Institute of Health for its generous support of these studies through research grants GM 13914 and HL/AM-28481.

REFERENCES

[1] Smith, R., Dawson, J.R. and Tanford, C. (1972) J. Biol. Chem. 247, 3376–3381.
[2] Milne, R.W. and Marcel, I.L. (1982) FEBS Lett. 146, 97–100.
[3] Kane, J.P., Hardman, D.A. and Paulus, H.E. (1980) Proc. Natl. Acad. Sci. USA 77, 2465–2469.
[4] Malloy, M.J., Kane, J.P., Hardman, D.A. and Dalal, K.B. (1982) J. Clin. Invest.
[5] Elovson, J., Huang, Y.O., Baker, N. and Kannan, R. (1981) Proc. Natl. Acad. Sci. USA 78, 157–161.
[6] Krishnaiah, K.V., Walker, L.F., Borensztajn, J., Schonfeld, G. and Getz, G.S. (1980) Proc. Natl. Acad. Sci. USA 77, 3806–3810.
[7] Sparkes, C.E. and Marsh, J.B. (1981) J. Lipid Res. 22, 514–518.
[8] Bradley, W.A., Rhode, M.F. and Gotto, A.M. (1980) Ann. NY Acad. Sci. 348, 87–103.
[9] Cleveland, D.W., Fischer, S.G., Kirschner, M.W. and Laemmli, U.K. (1977) J. Biol. Chem. 252, 1102–1106.
[10] Shively, J.E., Hawke, D. and Jones, B.N. (1982) Anal. Biochem. 120, 312–322.
[11] Hawke, D., Yuan, P.M. and Shively, J.E. (1982) Anal. Biochem. 120, 302–311.
[12] Yuan, P.M., Pande, H., Clark, B.R. and Shively, J.E. (1982) Anal. Biochem. 120, 282–288.
[13] Morrisett, J.D., Jackson, R.L. and Gotto, A.M. jr (1975) Annu. Rev. Biochem. 44, 183–207.
[14] Smith, L.C., Pownall, H.J. and Gotto, A.M. jr (1978) Annu. Rev. Biochem. 47, 751–777.
[15] Drapeau, G.R. (1978) Can. J. Biochem. 56, 534–544.