Journal of Chromatography, 225 (1981) 185—188
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 920

Note

Separation of human C-apolipoproteins by high-performance liquid chromatography*

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(First received January 15th, 1981; revised manuscript received March 23rd, 1981)

The C-apolipoproteins (apo C) are part of the protein moiety of very low-density lipoproteins (VLDL) which are involved in the metabolism of lipoproteins [1]. They are a heterogeneous class of proteins and can be separated into apo C-I, C-II, C-III by column chromatography [2]. Since isoelectric focusing results in additional C-apolipoprotein bands [3, 4] the separation of apo C by column chromatography seems to be incomplete. The increasing interest in specific antisera for the immunochemical quantification of apolipoproteins needs highly purified antigens. Therefore we tried to separate the C-apolipoproteins by high-performance liquid chromatography (HPLC).

MATERIAL AND METHODS

From pooled sera of patients with primary type IV hyperlipoproteinemia very low-density lipoproteins (VLDL) were prepared by preparative ultracentrifugation (d = 1.006 g/ml, 125,000 g, 48 h, 4°C). VLDL were separated by tube slicing, delipidated with an equal volume of tetramethylurea (TMU), dialyzed against 0.05 M NH_4HCO_3 buffer (pH 8.6) for 24 h, and lyophilized. For column chromatography (Sephadex G-200 fine, 100 × 2.5 cm, 4°C) 50 mg of the TMU-soluble apolipoproteins were resolubilized in 0.05 M NH_4HCO_3 buffer (pH 8.6) containing 6 M urea [5]. The apo C-containing fractions were pooled, dialyzed and lyophilized. They were, furthermore, analyzed by polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS) (10%) and 8 M urea (7.5%, pH 8.6). Fractionation of the C-apolipoproteins was performed by HPLC.

*Dedicated to Prof. Dr. H. Schwiegk on his 75th birthday.

0378-4347/81/0000—0000/$02.50

The HPLC experiments were performed using an Altex programmer Model 420 and two Altex pumps (Model 110A) with a variable-wavelength UV detector (Kontron Corp., Munich, G.F.R.).

Solvent methanol (HPLC grade) and reagent grade sodium phosphate were obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.). Water was glass-distilled from the deionized water supply of the laboratory. The HPLC column was purchased from Kontron; a pre-column was prepared in this laboratory. The column dimensions were 250 × 4.2 mm I.D., the pre-column dimensions 40 × 4.2 mm I.D. The packing was reversed-phase C-18 material (particle size 10 μm).

Chromatographic conditions were: sample injection as 15 mg/ml solutions in phosphate buffer; detection in a 10 mm path length cell (volume 7 μl) at 210 nm. Buffer A was methanol, buffer B 0.01 *M* phosphate buffer (pH 6.0). A linear gradient was supplied from 40 to 28% buffer B in 120 min. The flow-rate was 1 ml/min. Methanol was removed by drying under vacuum. The identification of the separated peaks was done by amino acid analysis and determination of the N-terminal amino acid.

RESULTS

The gel permeation chromatogram of the TMU-soluble VLDL proteins is shown in Fig. 1. The pooled C-apolipoproteins typically [6] could be separated by analytical polyacrylamide gel electrophoresis in urea (Fig. 2a) and gave a broad band in SDS (Fig. 2b). The preparative separation by HPLC resulted in 19 peaks with a yield of 80%; 17 peaks (Fig. 3) turned out to be C-apolipoproteins on the basis of amino acid analyses [7—9]. The remaining two peaks had amino acid compositions which do not correspond to any known apolipoprotein. While apo C-I represented one peak (typical amino acid composition, for example, the lack of half-cystine, tyrosine and histidine, one N-terminal amino acid residue threonine and the second residue proline from the N-terminus), apo C-II could be separated into 4 peaks (five tyrosine and one isoleucine residue, threonine as the single N-terminal amino acid and glutamic acid as the second N-terminal residue), and apo C-III into 12 peaks (two tyrosine residues, the lack of half-cystine and isoleucine, serine as the single N-terminal amino acid and glutamic acid as the second N-terminal residue). All the apo C-II or apo C-III peaks resulted in identical amino acid analyses and no atypical amino acids such as homocitrulline have been found.

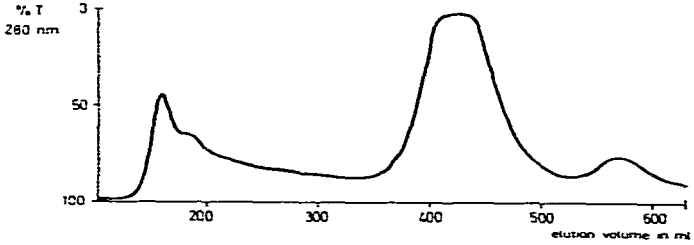

Fig. 1. Gel permeation chromatogram (percentage transmission at 280 nm) of 50 mg of TMU-soluble VLDL on Sephadex G-200 fine. The peak, containing the C-apolipoproteins, was eluted between 380 and 480 ml.

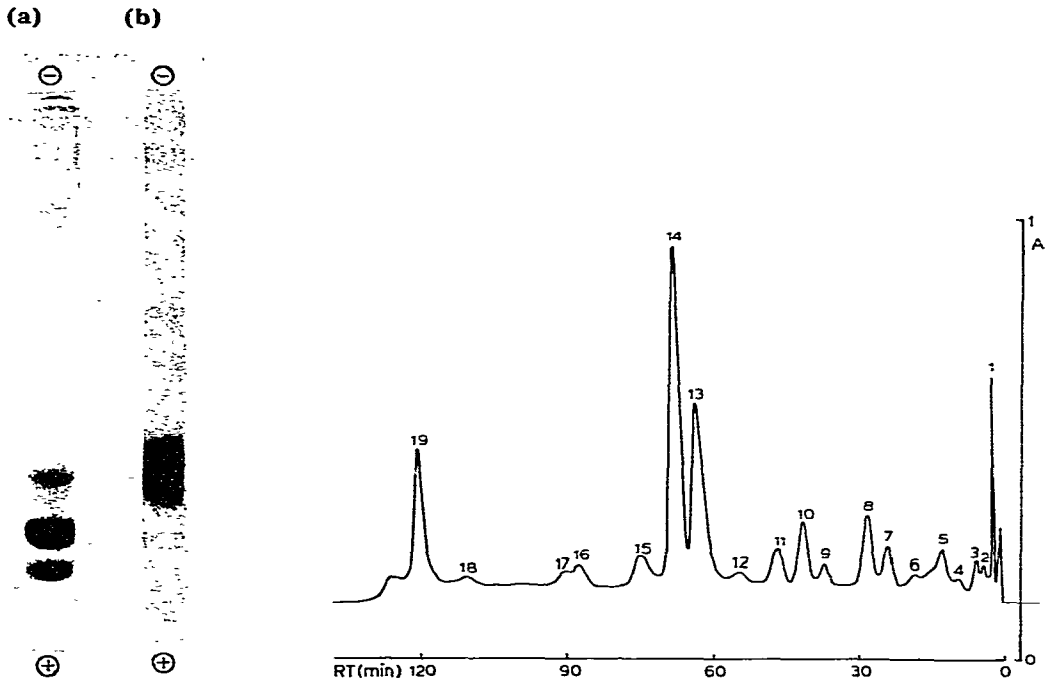

Fig. 2. Polyacrylamide gel electrophoresis (50 μg) in urea (a) and SDS (b) of the C-apolipoproteins. Bands from the cathode to the anode (in a): apo C-I, C-II, C-III$_0$, C-III$_1$, C-III$_2$.

Fig. 3. Chromatogram of C-apolipoproteins separated by HPLC under the following conditions: linear gradient from 40% to 28% buffer B (0.01 *M* sodium phosphate, pH 6.0) eluent methanol; 120 min, flow-rate 1.0 ml/min. RT = retention time. Peak 1 = apo C-I; peaks 2 and 4 = unidentified; peaks 3 and 5—15 = apo C-III; peaks 16—19 = apo C-II.

Comparable chromatograms were obtained under more acidic conditions (down to pH 2.1).

DISCUSSION

These data demonstrate that HPLC is a suitable method for the preparative separation of the heterogeneous C-apolipoproteins. This method is superior to preparative isoelectric focusing of the C-apolipoproteins (close isoelectric points, low yield and difficulty in removing the ampholyte [4]).

Apo C-III has been reported to consist of three polymorphic forms [10], which is due to different sialic acid content [11]. Whether the six polymorphic forms in isoelectric focusing [4] or the 12 forms we found in HPLC are due to differences in the sialic acid portion or due to aggregation remains to be established. Carbamylation can be excluded, since chromatography was performed at 4°C [2], the ratio of the lysine residues was constant and no atypical amino acid residues have been found.

Apo C-II is considered to be homogeneous [8] though in isoelectric focusing two forms have been reported [4]. The demonstration of four apo C-II peaks in HPLC which are identical in their amino acid composition might be

explained by differences in their carbohydrate content [12]. Whether our two unidentified peaks correspond to apo C-IV and apo C-V [3] can not be decided.

The preparation of highly purified C-apolipoprotein might be of importance not only for raising specific antibodies but also of clinical importance [13—15].

ACKNOWLEDGEMENTS

The skilful technical assistance of Ms. Margit Klepsch, Ms. Waltraud Hilscher and Ms. Beate Schalk is greatly acknowledged.

REFERENCES

1 S. Eisenberg, Progr. Biochem. Pharmacol., 19 (1979) 139.
2 P.N. Herbert, R.S. Shalman, R.I. Levy and D.S. Fredrickson, J. Biol. Chem., 248 (1973) 4941.
3 A.L. Catapano, R.L. Jackson, E.B. Gilliam, A.M. Gotto, Jr. and L.C. Smith, J. Lipid Res., 19 (1978) 1047.
4 J.L. Marcel, M. Bergseth and A.C. Nestruck, Biochim. Biophys. Acta, 573 (1979) 175.
5 V.G. Shore and B. Shore, Biochemistry, 12 (1973) 502.
6 J.P. Kane, T. Sata, R.L. Hamilton and R.J. Havel, J. Clin. Invest., 56 (1975) 1622.
7 R.L. Jackson, J.T. Sparrow, H.N. Baker, J.D. Morrisett, O.D. Taunton and A.M. Gotto, Jr., J. Biol. Chem., 249 (1974) 5308.
8 R.L. Jackson, H.N. Baker, E.B. Gilliam and A.M. Gotto, Jr., Proc. Nat. Acad. Sci. U.S., 74 (1977) 1942.
9 H.B. Brewer, Jr., R. Shulman, P. Herbert, R. Ronan and K. Wehrly, J. Biol. Chem., 249 (1974) 4975.
10 W.V. Brown, R.I. Levy and D.S. Fredrickson, J. Biol. Chem., 245 (1970) 6588.
11 P. Vaith, G. Assmann and G. Uhlenbruck, Biochim. Biophys. Acta, 541 (1978) 234.
12 G.M. Kostner, in H. Schwiegk (Editor), Handbuch der Inneren Medizin, Vol. 7, Springer, Berlin, Heidelberg, New York, 5th ed., 1976, p. 125.
13 W.C. Breckenridge, J.A. Little, G. Steiner, A. Chow and M. Papst, N. Engl. J. Med., 298 (1978) 1265.
14 P. Dewailly, E. Dedonder, J.C. Fruchart, G. Sezille and J. Jaillard, Diabète et Métabolisme, 6 (1980) 13.
15 A.L. Catapano, Atherosclerosis, 35 (1980) 419.