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Note

Simple and rapid method for the purification of human apolipoprotein C II by reversed-phase high-performance liquid chromatography

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Apolipoprotein C II (apo-C II) is a small peptide of 79 amino acids (8800 D) which is present in almost all serum lipoproteins [1]. Its amino acid sequence and its structure have been determined [2]. Apo-C II is an exchangeable apolipoprotein since transfers between chylomicrons, very-low-density lipoproteins (VLDL) and high-density lipoproteins (HDL) have been described [3]. It is the only known physiological activator of lipoprotein lipase [4], which is the key enzyme in triglyceride-rich lipoprotein metabolism [5]. Its genetic as well as secondary deficiency is always associated with hypertriglyceridemia.

Different purification procedures of apo-C II have been already reported. However, these methods are time-consuming and usually require low-pressure gels, such as Sephadex G-200 and DEAE-cellulose [6,7]. We propose here a new method for apo-C II purification, which is entirely performed using high-

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performance liquid chromatography (HPLC) and removes any residual contaminants reported elsewhere [8,9].

EXPERIMENTAL

Materials

Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA), bovine serum albumin (BSA) and Coomassie Blue were obtained from Sigma (Lyon, France). Trishydroxyaminomethane (Tris), urea, ammonium acetate, acrylamide, methylene bisacrylamide, perchloric acid, diethyl ether and 2-propanol were purchased from Merck (Darmstadt, F.R.G.). Absolute ethanol was from Carlo Erba (Milan, Italy) and ampholines from LKB-Pharmacia (Uppsala, Sweden).

Preparation of VLDL apolipoproteins

VLDL were isolated from human pooled sera by ultracentrifugation ($d = 1.006$ g/ml) for 20 h at 100 000 *g* and 4 °C [10] in a Beckman L8-55 ultracentrifuge using a 60 Ti rotor. VLDL were dialysed against 0.27 m*M* Na_2EDTA, then lyophilized and delipidated with ethanol–diethyl ether [11]. VLDL proteins were solubilized in 0.01 *M* Tris–HCl (pH 8.2) containing 6 *M* urea and spun at 3000 *g* for 15 min to remove insoluble material (apolipoprotein B). The protein concentration was determined according to Lowry et al. [12] with BSA as standard.

Chromatographic purification of apo-C II

Preparative HPLC was performed on a Varian Model 5040 apparatus (Varian, Walnut Creek, CA, U.S.A.) equipped with a Valco automatic 1-ml loop injector, a Kratos Spectroflow 773 detector (Cunow, France) and an analytical reversed-phase column (Nucleosil 1000 C_4, 250 mm × 4.6 mm I.D., 7 μm, Chrompack, Middelburg, The Netherlands).

Protein separation was achieved at room temperature. Separations were carried out by gradient elution using 50 m*M* ammonium acetate (pH 6.0) (solvent A) and 2-propanol (solvent B). A 60-min linear gradient from 100% A to 50% A–50% B was used. The flow-rate was 1.0 ml/min. Mobile phase solutions were filtered through 0.22-μm Millipore filters before use. Effluent absorbance was monitored at 280 nm, and 0.5-min fractions were collected.

A two-step procedure was followed for the purification of apo-C II. Urea-soluble VLDL apolipoproteins (ca. 3 mg) were first chromatographed and separated into six fractions. Each fraction was evaporated using a Speed Vac concentrator. The fraction corresponding to apo-C II was then solubilized in 0.01 *M* Tris–HCl (pH 8.2) containing 6 *M* urea and rechromatographed as described above. Before injection, each sample was filtered through a 0.22-μm Millipore filter, type GS.

Protein characterization

Chromatographic fractions were analysed by isoelectric focusing [13] modified as follows: 7.5% polyacrylamide gel containing 6 *M* urea and 2% ampholines (pH 3.5–8.0 gradient) was prepared. Isofocusing was performed over-

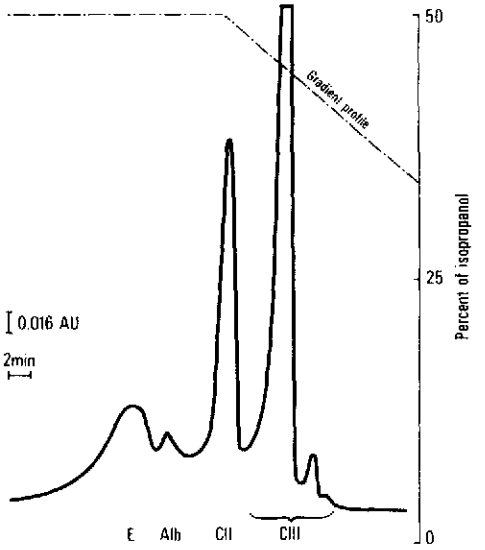

Fig. 1. Reversed-phase HPLC of urea-soluble VLDL apolipoproteins (ca. 3 mg).

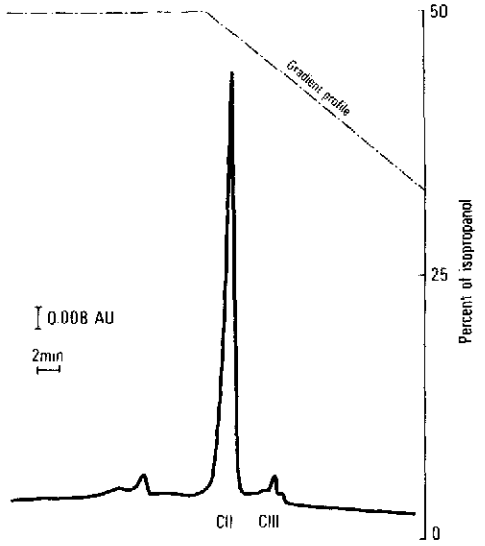

Fig. 2. Reversed-phase HPLC of apo-C II fraction obtained from the first-step HPLC (see Fig. 1).

night at 10°C. Gels were stained with 0.04% (w/v) Coomassie Blue G250 in 3.5% (w/v) perchloric acid. Apolipoproteins were identified according to their isoelectric point [13].

For the NH_2 terminal sequence analysis of apo-C II, Edman degradations were carried out with an Applied Biosystems sequencer (Model 470 A) equipped with on-line phenylthiohydantoin identification on the 120 A analyser using programs provided by the manufacturer (Applied Biosystems, Foster City, CA, U.S.A.).

RESULTS AND DISCUSSION

The first chromatographic separation efficiently separated VLDL apolipoproteins into five fractions (Fig. 1), corresponding to the three isoforms of apo-

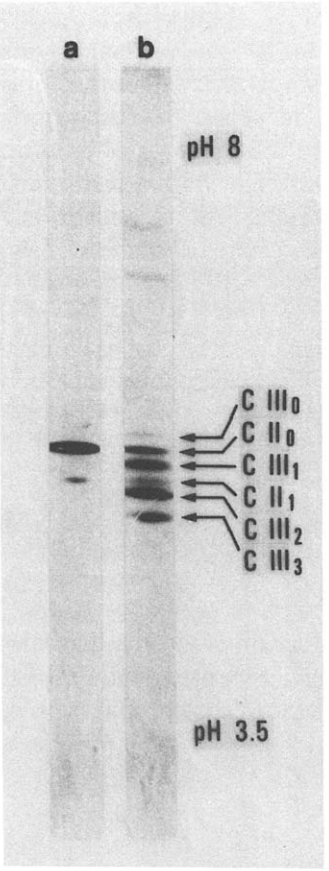

Fig. 3. Focusing gel (pH 3.5–8.0) showing the two isoforms of pure apo-C II (C II_0 and C II_1, line a) compared with total apo-C (line b, 50 μg) obtained by gel permeation chromatography on Sephacryl S 200 (LKB-Pharmacia).

TABLE I

SEQUENTIAL EDMAN DEGRADATION

Results are expressed in pmol. The published amino acid sequence of apo-C II [2] is as follows: H_2N-Thr-Gln-Gln-Pro-Gln-Gln-Asp-Glu-Met-Pro-Ser...

Protein or peptide	Cycle No. 1	Cycle No. 2	Cycle No. 3
NH_2 terminal	Thr (4.5)	Gln (5.5)	Gln (6.2)
N-6	Asp (2.4)	Glu (1.5)	Met (0.3)
N-8	Met (+)	Pro (2)	Ser (+)

C III, apo-C II and apo-E. A trace amount of albumin was also present and was eluted as a distinct fraction between apo-C II and apo-E. When analysed by isoelectric focusing, the apo-C II fraction appeared to be slightly contaminated (ca. 5%) by apo-C III.

The second chromatographic separation further purified the apo-C II fraction. Chromatograms revealed a major peak of apo-C II with only minor contaminating fractions (Fig. 2). This apo-C II fraction was free of any contaminant as judged by isoelectric focusing (Fig. 3), and showed the two isoforms of apo-C II: 3 mg of urea-soluble VLDL apolipoproteins yielded ca. 250 μg of pure apo-C II.

The NH_2 terminal sequence determined by automated Edman degradation clearly identified the amino acid sequence of apo-C II [2] without any contamination by apo-C III, apo-E or albumin (Table 1). In addition to a major component (apo-C II_0) corresponding to the published sequence, the experiment indicated the presence of two minor components with sequences missing the first six and eight residues, respectively. The N-6 sequence was identified as apo-C $II_{1,2}$, according to Fojo et al. [14].

CONCLUSION

The technique described here allows a rapid purification of apo-C II directly from VLDL apolipoproteins using only two successive reversed-phase HPLC analyses. This simple procedure could be very useful for rapid preparation of sufficient amounts of pure apo-C II for analytical or immunological purposes.

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REFERENCES

1 E.J. Schaefer, S. Eisenberg and R.I. Levy, J. Lipid Res., 19 (1978) 667.
2 A.V. Hospattankar, T. Fairwell, R. Ronan and H.B. Brewer, J. Biol. Chem., 259 (1984) 318.
3 P. Alaupovic, Can. J. Biochem., 59 (1981) 565.
4 R. Ekman and P. Nilsson-Ehle, Clin. Chim. Acta, 63 (1975) 29.
5 P. Nilsson-Ehle, A.S. Garfinkel and M.C. Schotz, Ann. Rev. Biochem., 49 (1983) 667.
6 R. Ronan, L.L. Kay, M.S. Meng and H.B. Brewer, Biochim. Biophys. Acta, 713 (1982) 657.
7 P. Schwandt, W.O. Richter and P. Weisweiler, J. Chromatogr., 225 (1981) 185.
8 C.T. Wehr, R.L. Cunico, G.S. Ott and V.G. Shore, Anal. Biochem., 125 (1982) 386.
9 H. Mezdour, V. Clavey, I. Kora, M. Koffigan, A. Barkia and J.C. Fruchart, J. Chromatogr., 414 (1987) 35.
10 R.J. Havel, H.A. Eder and J.H. Bragdon, J. Clin. Invest., 34 (1955) 1345.
11 A.M. Scanu and C. Edelstein, Anal. Biochem., 44 (1971) 576.
12 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem., 193 (1951) 265.
13 S.W. Weidman, B. Suarez, J.M. Falko, J.L. Witztum, J. Kolar, M. Raben and G. Schonfeld, J. Lab. Clin. Med., 93 (1979) 549.
14 S.S. Fojo, L. Taam, T. Fairwell, R. Ronan, C. Bishop, M.S. Meng, J.M. Hoeg, D.L. Sprecher and H.B. Brewer, J. Biol. Chem., 261 (1986) 9591.