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Note

Large-scale isolation and purification of human apolipoproteins A-I and A-II[a]

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Plasma high-density lipoprotein (HDL) is a complex of lipids (largely phosphatidylcholine and cholesteryl esters) and proteins. The major proteins associated with HDL are designated apo A-I and apo A-II [1]. Isolation of apo A-I and apo A-II generally includes ultracentrifugation to isolate HDL, delipidation to obtain apo HDL proteins free of lipids and chromatographic separation of apo A-I and apo A-II from other HDL proteins [2]. An alternative approach for isolation of apo A-I and apo A-II with scale-up potential far beyond the practical application of ultracentrifugation became available when a chromatographic procedure utilizing phenyl-Sepharose to adsorb lipoproteins from plasma was described [3,4]. Immunological and electrophoretic characterization in addition to amino acid determination appeared sufficient to determine the quality of these proteins.

Purified apo A-I and apo A-II proteins are most often used as tracers, standards and immunogens in conjunction with apo A-I and apo A-II immunoassays.

This paper describes a modified large-scale method for the isolation of purified apo A-I and apo A-II from human serum.

EXPERIMENTAL

Materials and standards

Ammonium hydrogencarbonate, sodium chloride, urea, Coomassie Brilliant Blue R-250, sodium dodecyl sulphate (SDS), ammonium peroxodisulphate, N,N-methylenebisacrylamide, acrylamide and N,N,N′,N′-tetramethylethylenediamine were obtained from Serva (Heidelberg, F.R.G.). Protein molecular mass standards were purchased from Pharmacia (Uppsala, Sweden).

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Isolation and delipidation of HDL from human serum

HDL of density 1.063–1.210 g/ml was isolated from fasting serum of normolipidaemic donors by sequential ultracentrifugation [5] in a Beckman (Berkeley, CA, U.S.A.) Model L8-70 ultracentrifuge using a 45.Ti rotor. The isolated HDL fraction was extensively dialysed against 50 m*M* ammonium hydrogencarbonate buffer (pH 8.2), lyophilized and delipidated by an original procedure using a chloroform–methanol–diethyl ether solvent system [6].

Gel permeation and anion-exchange chromatography

The proteins were solubilized in 10 m*M* Tris–HCl buffer (pH 8.6) containing 8 *M* urea (Tris–urea buffer) and applied to a Toyopearl HW-55F (Toyo Soda, Tokyo, Japan) column (90.0 × 3.5 cm I.D.). Elution was carried out with the same buffer at a flow-rate of 40 ml/h and 8-ml fractions were collected. Following analysis by polyacrylamide gel electrophoresis (PAGE) [7], fractions containing apo A-I and apo A-II were pooled. The apolipoprotein pool was applied to a DEAE-Toyopearl 650M (Toyo Soda) column (40.0 × 3.2 cm I.D.), equilibrated with Tris–urea buffer. Elution was effected with a linear gradient of sodium chloride from 0.02 to 0.15 *M* in Tris–urea buffer (1000 ml total gradient volume) at a flow-rate of 60 ml/h. Fractions of 6 ml each were collected. Those containing apo A-I and apo A-II were pooled separately and extensively dialysed against 50 m*M* ammonium hydrogencarbonate buffer (pH 8.2). After dialysis, the samples were desalted by gel permeation chromatography using a Toyopearl HW-40F (Toyo Soda) column (70.0 × 2.2 cm I.D.) with the same buffer at a flow-rate of 80 ml/h and finally lyophilized.

Characterization of proteins

Proteins were quantified according to Lowry *et al.* [8] and spectrophotometrically at 280 nm using extinction coefficients of 1.22 and 1.82 AU/mg protein · ml for apo A-I and apo A-II, respectively [9]. Lipid phosphorus analysis was performed utilizing the method of Bartlett [10]. Homogeneity was confirmed by SDS-PAGE on 15% polyacrylamide gels under both reducing and non-reducing conditions [7] and by urea-PAGE [11]. Identification of apo A-I and apo A-II was confirmed by electrophoretic mobility and immunoelectrophoresis [12]. Amino acid analyses of purified apo A-I and apo A-II were made in a Beckman 6300 amino acid analyser after 72 h of acid hydrolysis.

RESULTS AND DISCUSSION

A modified scheme for the preparative isolation and purification of apolipoproteins A-I and A-II, the major HDL proteins [1], from the 1.063–1.210 g/ml HDL material has been developed. The main aim of this work was to increase the efficiency of the method and to obtain apo A-I and apo A-II with the highest degree of purity. For this reason, we modified the conditions of the HDL delipidation (the total time of the modified procedure was 1 h) [6] and, as reported here, used Toyopearl HW-55F and DEAE-Toyopearl 650M for gel permeation and anion-exchange chromatography, respectively. The use of these packings allowed increased speed of elution without worsening the separation. Typical elution profiles are shown in Fig. 1. After the anion-exchange chromatography and dialysis steps, apo A-I and apo A-II proteins

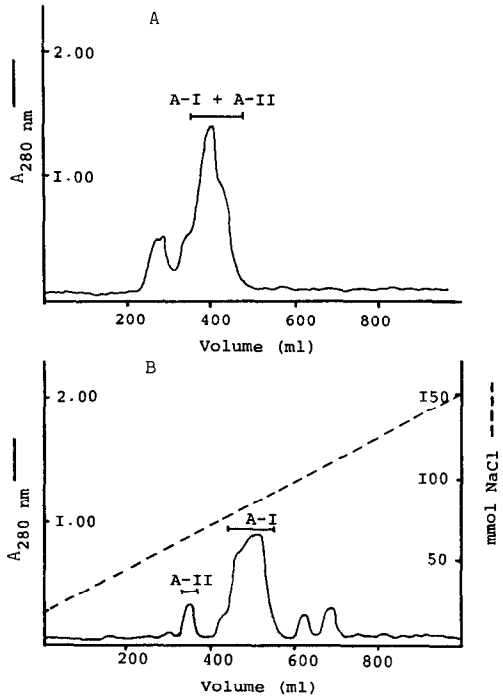

Fig. 1. (A) Toyopearl HW-55F chromatography of delipidated 1.063–1.210 g/ml HDL. Elution buffer, 10 m*M* Tris–HCl (pH 8.6)–8 *M* urea; (B) DEAE-Toyopearl 650M chromatography of pooled A-I + A-II-containing fractions. Starting buffer, 10 m*M* Tris–HCl (pH 8.6)–8 *M* urea–0.02 *M* sodium chloride; linear gradient of NaCl from 0.02 to 0.15 *M* in the same buffer, total gradient volume 1000 ml; flow-rate, 60 ml/h.

proved to be homogeneous according to the SDS-PAGE and urea-PAGE data (not shown).

The apo A-I and apo A-II proteins were desalted using a Toyopearl HW-40F column (both proteins were eluted with the void volume) for the complete removal of urea and inorganic salts.

The homogeneity apo A-I and apo A-II proteins purified by this procedure was confirmed by SDS-PAGE (see Fig. 2, lanes 2, 4, 5 and 7) and urea-PAGE (not shown). Densitometric scanning of these gels gave a single symmetrical peak as an indication of homogeneity. The molecular mass of apo A-I was determined electrophoretically to be near 28 000 and was unaffected by reduction (see Fig. 2, lane 3). The molecular mass of apo A-II was estimated in the same way to be near 17 000, but after reduction gave an estimated molecular mass of about 8600 (see Fig. 2, lanes 5 and 6). These characteristics were appropriate for apo A-I [13] and for apo A-II [14]. No phosholipid was detected in either the isolated apo A-I or apo A-II.

The identity of the isolated proteins with apo A-I and apo A-II was confirmed by immunoelectrophoresis with monospecific antibodies to human apo A-I and apo A-II, respectively [12]. Furthermore, amino acid analyses of the purified apo A-I and apo

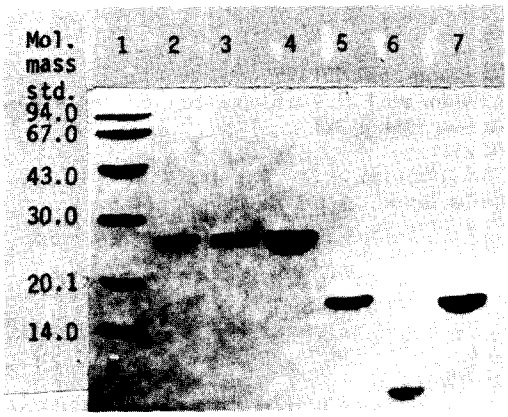

Fig. 2. SDS-PAGE in 15% polyacrylamide gel of purified apo A-I and apo A-II. Lanes: 1 = low-molecular-mass standards from Pharmacia (molecular masses of the standards are those provided by Pharmacia ($\times 10^{-3}$); 2, 3, 4 = 10 μg, 10 μg (with 2% of 2-mercaptoethanol) and 30 μg of purified apo A-I, respectively; 5, 6, 7 = 10 μg, 10 μg (with 2% of 2-mercaptoethanol) and 30 μg of purified apo A-II, respectively. Apo A-I and apo A-II were identified by comparison with known standards.

A-II proteins were compatible with the compositions derived from published sequences (data not shown) [14,15].

Isolated apo A-I and apo A-II proteins were successfully used as immunogens and as standards for assigning values to sera for use as secondary standards.

The efficiency of the described procedure was determined by the real yield of the lyophilized apo A-I and apo A-II proteins. A full cycle of isolation of these proteins from human serum lasted 10–14 days, including the preparation of 1.063–1.210 g/ml HDL. The average amount of the recovered lyophilized protein was about 300 mg per cycle for apo A-I and 60 mg per cycle for apo A-II. The average concentrations of apo A-I and apo A-II in pooled unextracted human serum were 1.5 and 0.4 mg/ml, respectively, according an immunoturbidimetric assay. Hence, the real yields of purified proteins were about 50% and 40% for apo A-I and apo A-II, respectively, taking into account the volume of the initial human serum (about 400 ml).

Unfortunately, in many papers [2,16–18] no data are given that allow the calculation of the real yield of purified apo A-I and apo A-II proteins, which is particularly important when procedures alternative to ultracentrifugation are used [3,4]. The lack of such data makes it impossible to compare these methods with our technique with respect to efficiency.

In conclusion, we have described a method suitable for the large-scale preparation of lyophilized apo A-I and apo A-II from human serum with real yields of purified proteins of about 50% and 40%, respectively. The high degree of purity makes it possible to use the recovered proteins as primary standards for apo A-I and apo A-II immunoassays.

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