Journal of Chromatography, 583 (1992) 111–115
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6562

Short Communication

Characterization of plasma apolipoproteins by capillary electrophoresis

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(First received May 27th, 1992; revised manuscript received September 1st, 1992)

ABSTRACT

The main apolipoproteins of plasma high-density lipoproteins (HDL) and low-density lipoproteins (LDL) were analyzed by capillary electrophoresis. Where possible the results were compared with slab sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Addition of the detergent SDS to the running buffer was essential for separation. Separations were carried out in bare silica and polyacrylamide-coated capillaries. The main apolipoproteins of HDL could be separated in an uncoated capillary filled with borax buffer containing 0.1% SDS. Using the coated capillary, a mixture of HDL and LDL apolipoproteins was resolved in less than 12 min. These preliminary studies indicate that capillary electrophoresis is a promising technique for screening plasma apolipoproteins.

INTRODUCTION

The isolation and characterization of plasma apolipoproteins remains an important problem in clinical analysis. The apolipoproteins, along with cholesterol, phospholipids and triglycerides, make up lipoprotein particles. They play an important role in regulating lipid transport and metabolism [1]. Recent studies also indicate that plasma apolipoprotein distribution is actually a better indicator of coronary heart disease (CHD) than cholesterol or lipoprotein profiling [2–4]. Apolipoproteins appear to be more precise and sensitive in assessing CHD risk. Specifically, obtaining accurate profiles of apolipoproteins A and B (apo A and apo B, respectively) is important since these apolipoproteins exert opposite atherogenic effects [2].

Conventional techniques for separating apolipoproteins include slab gel electrophoresis [5–8], gel permeation [9,10], anion exchange [11] and reversed-phase [12–14] chromatography. However, these methods for apolipoprotein analysis tend to exhibit one or more of the following disadvantages: low reproducibility, long analysis times or poor resolution. Furthermore, protein profiles from the different lipoprotein fractions, very-low-, low- and high-density lipoproteins (VLDL, LDL and HDL, respectively), cannot be separated in one run.

Recently, capillary electrophoresis (CE) has demonstrated a potential for clinical diagnostic applications. The technique delivers the high resolving power of traditional electrophoresis in a

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0378-4347/92/$05.00

rapid, highly reproducible format. It is also better suited than high-performance liquid chromatography (HPLC) for separation of large biomolecules such as apolipoproteins [15]. To date clinical applications of CE include separations of components found in serum [16–18], urine [16] and bovine brain [19]. These studies suggest that CE is well suited for rapid screening of plasma apolipoproteins.

This paper describes the rapid, high-resolution separation of apolipoproteins by CE. Specifically, apolipoproteins from plasma HDL and LDL fractions are analyzed since they are the main sources of apo A and apo B. Apolipoprotein separations are performed in uncoated and polyacrylamide-coated capillaries.

EXPERIMENTAL

Apparatus

The CE system used a Bertan Model 230R (Bertan Assoc., Hicksville, NY, USA) power supply and an Isco CV^4 (Isco, Lincoln, NE, USA) detector. The output of the power supply was connected to the buffer reservoir via platinum electrodes (Bioanalytical Systems, West Lafayette, IN, USA). Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 50 μm I.D. and 375 μm O.D. were used. Electropherograms were collected on a Model SE120 (Goertz Electro, Vienna, Austria) strip chart recorder.

Slab-gel electrophoresis was carried out on a Mini-Protean II electrophoresis cell powered by a Model 200/2.0 power supply (Bio-Rad, Richmond, CA, USA).

Chemicals

Doubly distilled, deionized water was used for all experiments. Ultrapure sodium dodecyl sulfate (SDS), urea, tris(hydroxymethyl)methylamine and acrylamide were obtained from ICN Biochemicals (Montreal, Canada). Sodium tetraborate (borax) and 3-(trimethoxysilyl)propyl methacrylate were from Aldrich (Milwaukee, WI, USA). Electrophoresis grade N,N,N′,N′-tetramethylethylenediamine (TEMED), ammonium persulfate and Coomassie Blue stain (Bio-Rad, Mississauga, Canada) were used. Glacial acetic acid and hydrochloric acid were purchased from J.T. Baker (Phillipsburg, NJ, USA). High- and low-molecular-mass protein standard kits MW-SDS-200 and MW-SDS-70 from Sigma (St. Louis, MO, USA) were used in the slab-gel SDS polyacrylamide gel electrophoresis (PAGE) experiments. Apolipoprotein A-I and A-II standards were also purchased from Sigma.

Preparation of apolipoprotein samples

Blood from a fasting male donor was collected into tubes containing 0.01% EDTA. Plasma was isolated by centrifugation at 1000 *g* and 4°C for 20 min. Plasma lipoprotein fractions were obtained using standard procedures [20]. Ultracentrifugation was performed at 142 200 *g* and 5°C using a Beckman L8-80 centrifuge equipped with a Ti-50.3 rotor. The lipoproteins were isolated at the following density ranges: LDL, $d = 1.019$–1.063; HDL, $d = 1.063$–1.21. To remove contamination due to serum albumin, the HDL fraction was diluted 1:3 with a NaCl–NaBr solution of $d = 1.21$ and recentrifuged for 20 h. All lipoprotein fraction were dialysed against 0.9% NaCl.

The HDL fraction was delipidated using diethyl ether and ethanol [21]. The LDL fraction was not delipidated since solubility in aqueous solution decreased dramatically upon dilipidation.

Preparation of polyacrylamide-coated capillaries

The method of Hjertén [22] was used with some modifications. The capillary was first conditioned using a 15-min rinse with NaOH followed by a 5-min rinse with water. A solution containing 30 μl of 3-(trimethoxysilyl)propyl methacrylate in 1 ml of 1:1 (v/v) acetic acid and water was drawn through the capillary using house vacuum. After 1 h, this solution was removed. Next, the capillary was filled with a degassed 4% acrylamide solution containing 1 μl/ml TEMED and 10 μl/ml ammonium persulfate. After polymerization was completed (about 45 min), the excess polyacrylamide was removed and the capillary was rinsed with water.

CE procedures

The capillaries had a total length of 75 cm and a separation length of 50 cm. A wavelength of 220 nm was used for detection. A field strength of 333 V/cm was used for all separations. The pH of borax buffers was adjusted by titrating with appropriate amounts of HCl and NaOH. Bare-silica capillaries were conditioned between runs by 2-min rinses of 0.1 *M* NaOH and water. Samples were injected hydrodynamically at a height of 15 cm and an injection time of 8–10 s.

RESULTS AND DISCUSSION

In this work, the development of a method for characterization of plasma apolipoproteins by CE is described. Fig. 1 shows the CE and slab-gel SDS-PAGE results obtained for a sample of delipidated HDL from fresh plasma. A borax buffer of pH 8.3 was used as the running electrolyte. Comparison of the CE results with those obtained by slab-gel SDS-PAGE (Fig. 1c) indicates that the two main proteins of HDL, apo A-I and apo A-II, are only resolved when SDS is present in the running buffer. In fact, in the presence of SDS two different isoforms of apo A-II could also be distinguished.

It appears that SDS has a strong influence on the electrophoretic behaviour of apolipoproteins. Previous studies [23,24] have shown that both apo A-I and apo A-II have discrete binding sites for SDS which are primarily hydrophobic in nature. These same sites are also involved in protein–protein association. The effect of SDS on apolipoprotein CE is thus two-fold. First, it competes effectively with protein–protein interaction and eliminates aggregation. It also binds to the proteins and changes their electrophoretic mobilities by altering size, shape and net charge. It is interesting to note that among other serum proteins, only albumin and β-lactoglobulin interact with SDS in the native state [23]. Most proteins only bind SDS in the presence of heat.

Separation of LDL apolipoproteins

The main protein found in LDL particles, apo B-100, is one of the largest proteins known. Due to its size and low solubility in aqueous solutions its structure has not been completely elucidated. Reported molecular masses range from 250 000 to 550 000, though 500 000 is the most commonly used [7]. The electropherograms obtained from an injection of LDL are shown in Fig. 2. Results from slab SDS-PAGE are also shown for com-

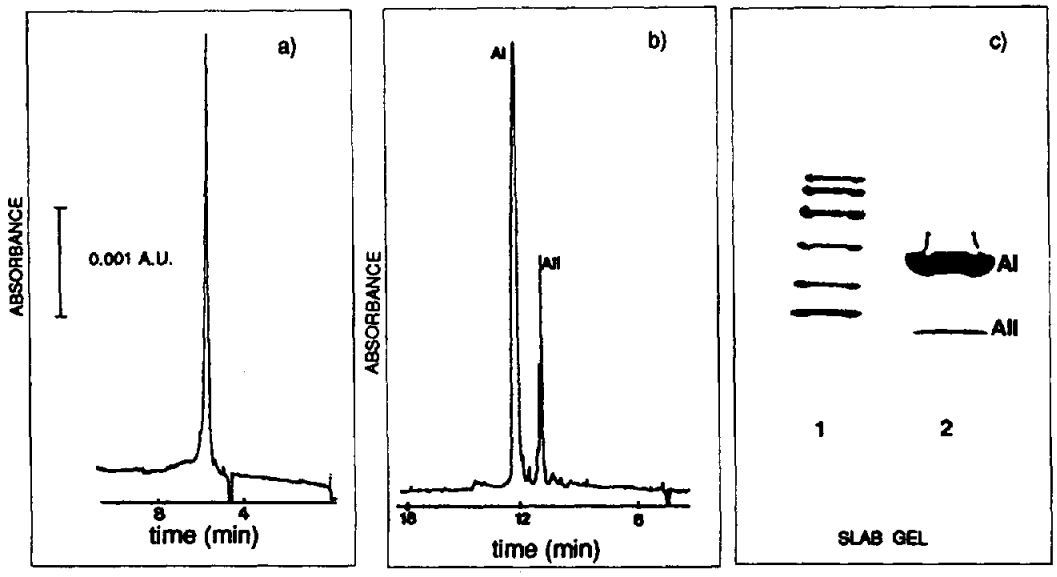

Fig. 1. Electropherograms of HDL apolipoproteins using 30 m*M* borax, pH 8.3, as the running buffer. Conditions: (a) no SDS in the buffer; (b) buffer containing 0.1% SDS; (c) slab-gel SDS-PAGE. Lane 1 = molecular-mass standards; lane 2 = HDL apolipoproteins.

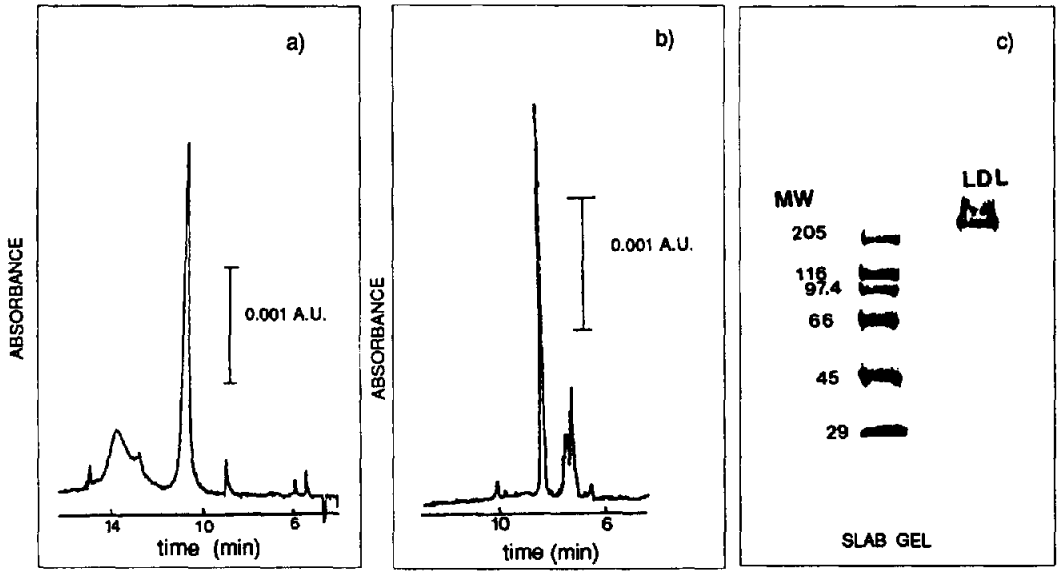

Fig. 2. Separation of LDL apolipoproteins. Conditions: 30 m*M* borax buffer, 0.1% SDS, pH 9. (a) Uncoated capillary; (b) polyacrylamide-coated capillary; (c) slab-gel SDS-PAGE.

parison. The slab-gel results indicate that the LDL sample was composed of a single high-molecular-mass protein.

For the LDL apolipoproteins, slightly better results were obtained using the polyacrylamide-coated capillary. Comparison of the electropherograms in Fig. 2 indicates improved resolution and narrower peaks in the coated capillary. This may be due to increased adsorption in the uncoated capillary. The two minor overlapping peaks in Fig. 2b are likely fragments of apo B-100. These are designated B-74 (molecular mass 300 000) and B-26 (molecular mass 100 000) [25].

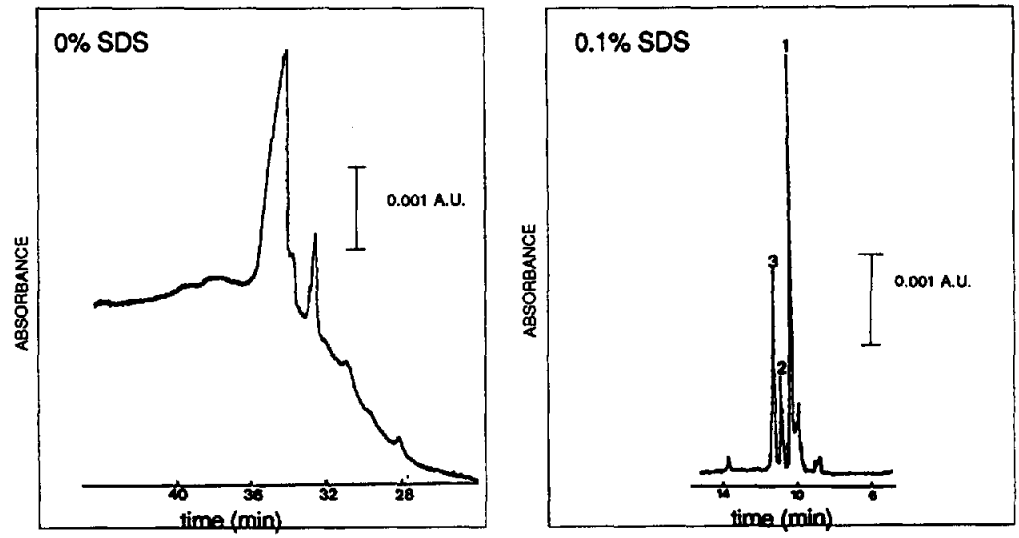

Fig. 3. Separation of HDL and LDL apolipoproteins in a polyacrylamide-coated capillary. Conditions: 30 m*M* borax, pH 9. Peaks 1, 2 and 3 are apolipoproteins A-I, A-II and B-100, respectively.

In assessing a patient's risk of developing coronary heart disease it is necessary to obtain a profile of the A and B apolipoproteins. Fig. 3 shows the separation of HDL and LDL apolipoproteins in a polyacrylamide-coated capillary. Once again, SDS must be present for separation to occur. Using only borax buffer, apo A-I and B-100 coelute as a single species at 36 min. However, when 0.1% SDS is added to the buffer, all of the major proteins of HDL and LDL are well resolved in about 12 min.

CONCLUSIONS

The main markers of atherosclerosis, apolipoproteins A and B, are separated in one run by CE. This is a significant improvement over HPLC and conventional electrophoresis where HDL and LDL apolipoproteins cannot be separated under the same conditions. The simplicity and speed of this method indicate the potential utility of CE for apolipoprotein analysis. Work is currently under way to characterize the VLDL apolipoproteins using this method. The effect of other detergents which bind to apolipoproteins is also being investigated.

ACKNOWLEDGEMENTS

The authors are indebted to the National Research Council of Canada for financial support of this work. The authors also wish to thank the Lipid Research Center of the Royal Victoria Hospital in Montreal for use of their facilities in preparing the lipoprotein fractions and Merck Frosst Canada for their assistance in purchasing the CE equipment.

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