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Note

Fractionation of serum lipoproteins by preparative electrophoresis in polyacrylamide gel

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Plasma lipoproteins are commonly separated by sequential ultracentrifugation[1], which is time consuming and may cause structural alterations. Alternative methods involve agarose column chromatography[2] or chemical techniques of precipitation[3], but electrophoretic methods have rarely been applied for this purpose. The high resolution obtained in the separation of lipoproteins by analytical electrophoresis in polyacrylamide gels[4] led us to develop an apparatus for use on a preparative scale. With this method, after removal of lipoproteins from plasma by ultracentrifugation, we have isolated LDL, HDL and Lp(a) lipoprotein** (sinking pre-β-lipoprotein) in the form of a purified lipoprotein class.

EXPERIMENTAL

Preparative electrophoresis in polyacrylamide gel

The apparatus is shown in Fig. 1. The main components are two Plexiglas tubes, a gel-holding tube and an inner tube that serves as a cooling reservoir.

To prepare the gel column for electrophoresis, the lower part of the tube is closed by screwing in a tightly fitting nylon stopper. Gel solutions are prepared according to Table I and polymerized in the order separating gel (100 ml), resolving gel (100 ml). The solutions are layered with water in order to prevent the formation of a curved meniscus during the polymerization. After completion of polymerization, usually achieved in 30 min at room temperature, the stopper is removed and the lower end of the column closed with a plastic membrane fastened with a securing O-ring made of silicone rubber tubing. The space formed between the lower surface of the

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** Abbreviations: LDL = low-density lipoprotein; VLDL = very-low-density lipoprotein; HDL = high-density lipoprotein.

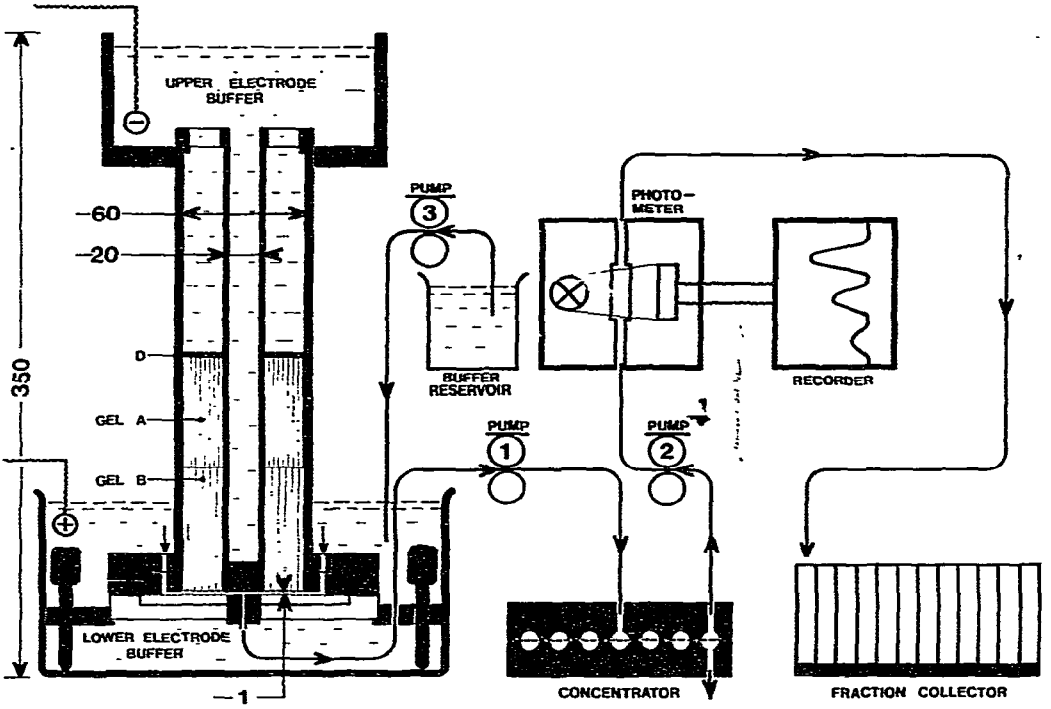

Fig. 1. Apparatus for preparative gel electrophoresis (all dimensions in millimetres).

TABLE I

PREPARATION OF GEL SOLUTIONS AND ELECTROPHORESIS BUFFER

Part of system	*Component*	*Amount*
Electrophoresis buffer	Tris(hydroxymethyl)aminomethane	15 g
	Glycine	72 g
	Distilled water	to 5000 ml
Resolving gel solution	Acrylamide	3.0 g
	Bisacrylamide	0.25 g
	Electrophoresis buffer	80 ml
	Dissolve by mixing, and remove air by stirring under vacuum	
	Ammonium persulphate (100 g/l)	0.7 ml
	N,N,N′,N′-Tetramethylethylenediamine	0.03 ml
	Electrophoresis buffer	to 100 ml
Separating gel solution	Acrylamide	2.0 g
	Bisacrylamide	0.5 g
	Electrophoresis buffer	80 ml
	Dissolve by mixing and remove air by stirring under vacuum	
	Ammonium persulphate (100 g/l)	0.7 ml
	N,N,N′,N′-Tetramethylethylenediamine	0.03 ml
	Electrophoresis buffer	to 100 ml

gel, which rests on a woven nylon disk, and the upper surface of the plastic membrane forms the elution chamber. The electrophoresis apparatus is then assembled and electrode vessels are filled with electrode buffer prepared according to Table I. Lipoproteins can be pre-stained with the diformazan of nitroblue tetrazolium[4]. Samples (1–3 ml) are layered on top of the resolving gel through the upper electrode buffer.

Electrophoresis is performed at a constant voltage of 200 V at 4°. When emerging from the gel, the separated fractions pass into the elution chamber, from which they are extracted. Electrode buffer is drawn through radial notches (Fig. 1) and flows perpendicularly to the direction of electrophoretic migration. The eluted fraction is swept from the gel into a concentrator. From the concentrator (Amicon, Oosterhout, The Netherlands), the eluate is pumped by a peristaltic pump into a continuous-flow cell in a photometer and then to a fraction collector. The eluates are monitored at 280 nm or, when lipoproteins are pre-stained, at 520 nm. Fractions of 4 ml per tube are collected. When necessary, the lipoprotein solutions are concentrated to the desired volume by filtration in a Diaflo apparatus (Amicon) equipped with a PM 10 membrane.

Analytical methods

Lipoproteins were isolated from the plasma of individual donors by preparative ultracentrifugation[1], carried out in a 50 Ti rotor at 100,000 *g* at 10° for 24 h. For separating the lipoproteins from the other plasma proteins, the solvent density of the plasma was increased to 1.21 g/ml by adding sodium chloride–potassium bromide solution. After ultracentrifugation, the supernatant was removed and "washed" by an additional centrifugation in the 1.21 g/ml solvent.

The preparation of the Lp(a) lipoprotein required two steps. In the first step, the density of the plasma containing this lipoprotein was adjusted to 1.063 g/ml with sodium chloride–potassium bromide solution. After ultracentrifugation, the top 3 ml was removed and the bottom fraction was re-adjusted to 1.12 g/ml with sodium chloride–potassium bromide solution and re-centrifuged. After the second ultracentrifugation, the top fraction (density 1.063–1.12 g/ml) was removed and layered on the gel column for subfractionation.

The fractions eluted from the column were identified by electrophoresis on polyacrylamide gradient gel[4] and by agarose electrophoresis using the Biogram A kit (Bio-Rad Labs., Richmond, Calif., U.S.A.). The homogeneity of their protein composition was determined by immunoprecipitation in agar gel using rabbit antisera against human serum [anti-α- and -β-lipoproteins (Behringwerke, Paris, France), anti-Lp(a) (a gift from Dr. Burstein, Centre National de Transfusion Sanguine, Paris)].

RESULTS

The elution pattern of lipoproteins removed from plasma at a density of 1.21 g/ml and applied to the gel column is shown in Fig. 2. By means of the procedure described, the separation of HDL and LDL from VLDL is possible. HDL are eluted first (peak I) and LDL are then recovered (peak II), while VLDL remain on the gel column.

Both the LDL and HDL isolated by this preparative electrophoresis method showed a single band on polyacrylamide gel electrophoresis (Fig. 3B and C). They

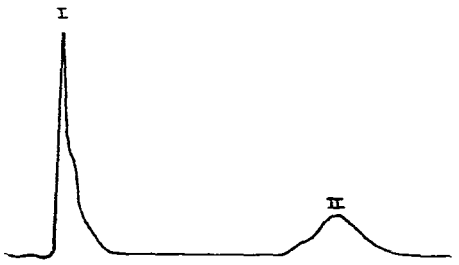

Fig. 2. Separation of plasma lipoprotein classes by preparative electrophoresis in polyacrylamide gel. Elution profile from the gel column of plasma lipoproteins removed by ultracentrifugation at a density of 1.21 g/ml. Peaks: I = HDL; II = LDL.

had the characteristic immunoprecipitin arcs when they reacted with anti-LDL and anti-HDL sera (Fig. 4A and B).

Lp(a) lipoprotein was separated from HDL by the same procedure. After concentration, the first fraction eluted from the column constituted purified Lp(a) lipoprotein. It showed a single band on polyacrylamide gel electrophoresis (Fig. 3D)

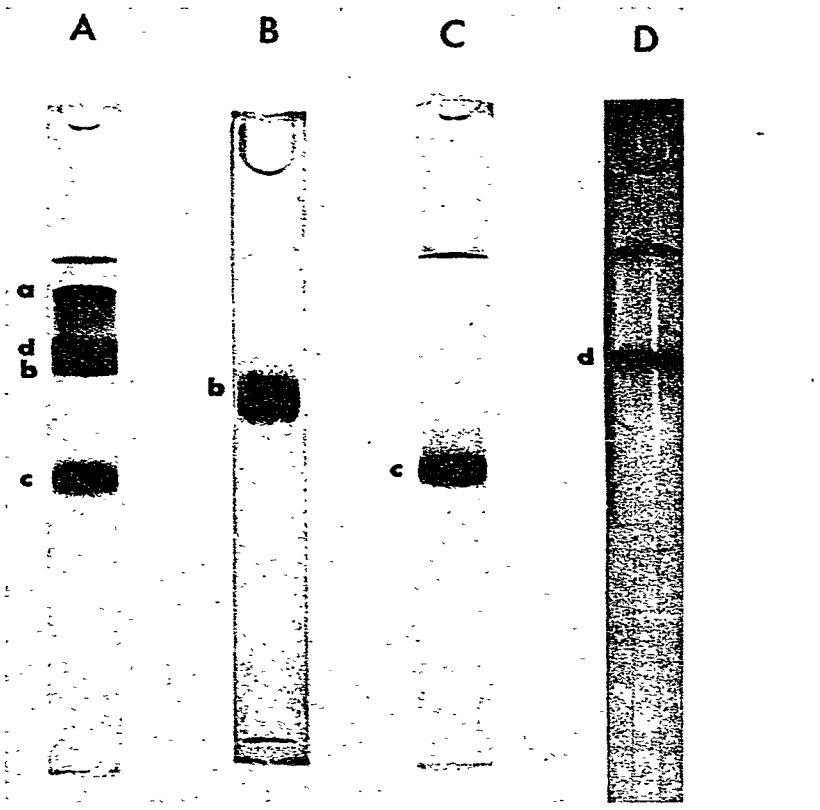

Fig. 3. Acrylamide electrophoresis patterns of human lipoprotein classes separated by preparative electrophoresis in polyacrylamide gel. A, Human whole plasma sample with high Lp(a) concentration; B, peak II (LDL); C, peak I (HDL); D, Lp(a) lipoprotein. a, VLDL; b, LDL; c, HDL; d, Lp(a).

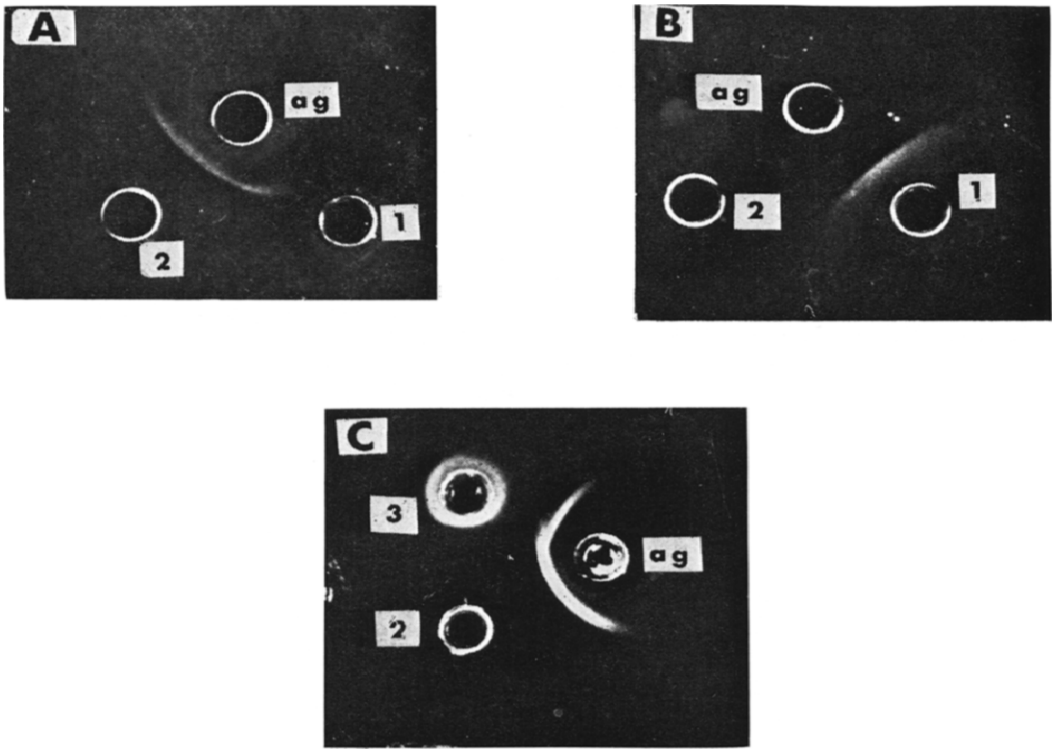

Fig. 4. Immunodiffusion of human lipoproteins separated by preparative electrophoresis in polyacrylamide gel. Centre wells (ag) contained the equivalent of 30 μg of lipoprotein protein. For both plates, the outside wells contained 20 μl of rabbit antiserum: 1, anti-HDL; 2, anti-LDL; 3-anti Lp(a). Diffusion was complete after 96 h and photographs were taken. A, ag = peak I; B, ag = peak II; C, ag = peak III.

and reacted only with anti-Lp(a) and anti-LDL sera (Fig. 4C). Its mobility on agarose gel electrophoresis was that of pre-beta.

DISCUSSION

Preparative electrophoresis in polyacrylamide gel has been used by several investigators to isolate plasma proteins. This procedure is preferred for its relative simplicity, and to our knowledge, it has not been applied to the purification of plasma lipoproteins. The method reported here allows the isolation of human LDL and HDL with good recoveries. In addition, it can be used when the ultracentrifugation method is unable to separate the lipoproteins in a pure form because of overlapping densities, as occurs with the Lp(a) and HDL lipoproteins.

Lipoproteins isolated by this procedure give characteristic immunoprecipitin reactions, and so do not seem to be substantially altered in their structural integrity or antigenic properties. The results described here suggest that preparative electrophoresis in polyacrylamide gel, which can be performed in about 1 day, may prove to be a valuable technique for the preparation of discrete lipoprotein fractions.

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