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Note

Analytical electrophoretic separation of undelipidated rat plasma apolipoproteins

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Analysis of plasma lipoproteins is most conveniently performed by various methods of acrylamide electrophoresis. Complete resolution of the apolipoproteins (including isomorphic forms) requires two-dimensional electrophoresis [1]. The separation of rat apoproteins by molecular weight is sufficient for many studies. While sodium dodecyl sulphate (SDS) electrophoresis has been used to resolve apoproteins with molecular weights between 15,000 and 80,000 daltons, the apoC proteins remain unresolved [2]. These low molecular weight apoproteins can be resolved by urea—acrylamide electrophoresis [3] or isoelectric focusing [1], methods which do not yield complete resolution of the higher molecular weight apoproteins (in particular, rat apoE and ApoA-IV). The complete spectrum of apolipoproteins can be separated according to molecular weight, provided the samples are first delipidated, by SDS—acrylamide gradient gel electrophoresis [4]. The present paper describes the resolution of rat apoproteins in the molecular weight range from 6000—80,000 daltons using non-gradient SDS—urea polyacrylamide gel electrophoresis without prior delipidation of the lipoprotein samples and illustrates the advantages of this method.

EXPERIMENTAL

Acrylamide, N,N,N,N-tetramethylethylenediamine (TEMED), N,N′-methylene-bisacrylamide, and ammonium persulfate (electrophoresis grade) were obtained from Bio-Rad Labs. (Richmond, CA, U.S.A.). Ultrapure urea was purchased from Schwarz-Mann (Orangeburg, NY, U.S.A.), and Ampholines, pH 4—6, were supplied by LKB (Stockholm, Sweden). D,L-Dithiothreitol was purchased from Sigma (St. Louis, MO, U.S.A.). Tetramethylurea was

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purchased from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). The animals were male rats of the Wistar strain (250—400 g) fed ad libitum standard Purina Rat Chow, Purina Foods (Toronto, Canada). Blood samples were drawn in tubes containing EDTA (1 mg/ml) and plasma was separated by low speed centrifugation (2000 *g*) for 20 min.

Isolation and analysis of rat plasma lipoproteins

Rat plasma lipoproteins were isolated by sequential ultracentrifugation at the following densities: very low density lipoproteins (VLDL), d = 1.006 g/ml; low density lipoproteins (LDL), d = 1.006—1.040 g/ml; and high density lipoproteins (HDL), d = 1.040—1.21 g/ml [5, 6]. Prior to electrophoresis each lipoprotein fraction was dialyzed against 0.15 *M* sodium chloride, 0.04% EDTA, pH 8.6, at 4°C.

The lipoprotein fractions were characterized by lipid and protein analyses. Lipids were quantitated by high temperature gas—liquid chromatography after dephosphorylation and trimethylsilylation, using tridecanoylglycerol as internal standard [7]. Protein was measured according to Markwell et al. [8] using bovine serum albumin (Fraction V, Sigma) as reference standard.

Electrophoresis

Polyacrylamide gel electrophoresis was carried out in a Model DE-102 tube gel apparatus (Hoffer Scientific Instruments, San Francisco, CA, U.S.A.) using a Bio-Rad Model 1420 power supply. The electrophoretic system of Swank and Munkres [9] was used as modified by Butkowski [10]. Twenty-five ml of gel solution was sufficient to make twelve tube gels 90 × 5 mm in 125 × 5 mm glass tubes. Acrylamide gels (15%) were made by mixing the following: 50% acrylamide, 2% bisacrylamide (7.5 ml); 0.8 *M* H_3PO_4, 0.8% SDS, 1.29 *M* tris (hydroxymethyl) aminomethane, pH 6.8 (3.13 ml); urea (9.38 g); and distilled water to 24.75 ml. The above were thoroughly mixed to dissolve the urea, and 1.0% ammonium persulfate (0.25 ml) and TEMED (0.01 ml) were added. The solution was degassed and gels poured and overlayed with distilled water. Polymerization took place within 40 min and gels were used 1—2 h after preparation. After mounting the tubes in the electrophoresis apparatus the upper and lower reservoirs were filled with 0.1% SDS, 0.1 *M* sodium phosphate buffer, pH 7.0 [10, 11]. Electrophoresis was performed at 5 mA per gel constant current for 10—12 h or at 2.5 mA per gel overnight until the tracking dye was 1 cm from the end of each gel. Gels were stained in 9.2% acetic acid, 45.4% methanol, 0.025% Coomassie Brilliant Blue G-250 (filtered before use) for a minimum of 6 h and destained in 7.5% acetic acid, 5.0% methanol. Urea polyacrylamide gel electrophoresis was carried out as described [4]. Isoelectric focusing and two-dimensional gel electrophoresis were performed according to Swaney and Gidez [1]. The pH of the isoelectric focusing gels was determined using a combination pH electrode Model 1885, manufactured by the Markson Scientific Company (Del Mar, CA, U.S.A.).

Samples for electrophoresis were prepared as follows: an aliquot containing 40 μg of protein (25—250 μl of each lipoprotein fraction) was added to an equal volume of glycerol followed by a 0.25 volume of isopropanol. SDS was added to each sample to a final concentration of 2% using a 20% stock solu-

tion. Some samples were reduced by the addition of 0.10 volume of a freshly prepared 400 m*M* dithiothreitol solution as described previously [12]. The samples were heated at 100°C in a boiling water bath for 2 min. Bromophenol Blue (10 μl of a 0.02% solution) was added to each sample as a tracking dye and each sample was layered under the running buffer.

RESULTS AND DISCUSSION

Fig. 1 shows the resolution obtained for the apoproteins of rat HDL with the SDS—urea system. The identity of the apoprotein bands was established by two-dimensional electrophoresis in which isoelectric focusing is used in the first dimension and the SDS-urea system in the second dimension. The p*I* values of the A-I, A-II, A-IV, E, and C-II, C-III-0 and C-III-3 components corresponded to those previously reported [1]. ApoA-II, which is present in low amounts in rat HDL, was detectable in this gel but too faint to reproduce photographically. Fig. 2 compares the separation obtained with the SDS-urea system to that realized with the urea or SDS systems run separately. The urea system resolves the apoC apoproteins; however, the use of tetramethylurea can produce artifactual bands in the apoC region, complicating interpretation of the results (data not shown). In addition, there is considerable overlap of the higher molecular weight apoproteins. Both SDS systems resolve the higher molecular weight apoproteins while only the SDS—urea system resolves the Apo A-II and the C apoproteins (apoC-II, ApoC-III-0 and apoC-III-3) routinely on the basis of apparent molecular weight. It was not possible to positively

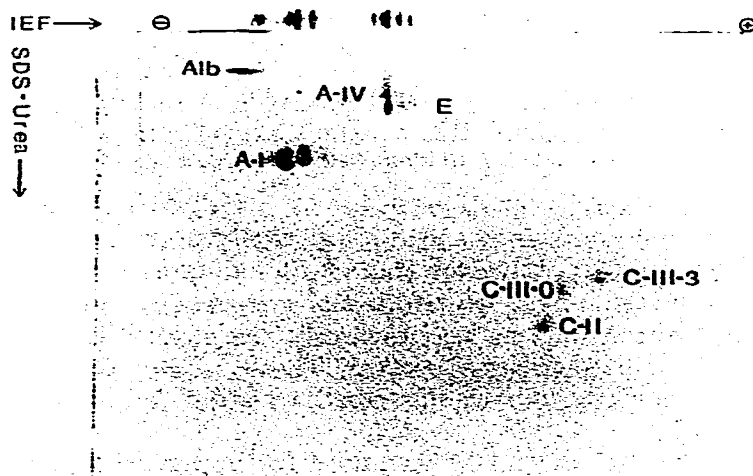

Fig. 1. Resolution of rat HDL apoproteins by SDS—urea gel electrophoresis and identification of components by two-dimensional electrophoresis. Isoelectric focusing was performed in a tube gel (top) using 80 μg of rat plasma HDL. A 1 mm thick slice of the gel was then incubated in 0.2% SDS—phosphate buffer, pH 7.0 (37°C, 1.5 h) and electrophoresis performed in the second dimension using the SDS—urea system. A-IV = apoprotein A-IV (p*I* 5.37—5.60); E = apoprotein E (p*I* 5.37—5.60); A-I = apoprotein A-I (p*I* 5.68—5.81); C-III-3 = apoprotein C-III-3 (p*I* 4.52); C-III-0 = apoprotein C-III-0 (p*I* 4.70); C-II = apoprotein C-II (p*I* 4.80).

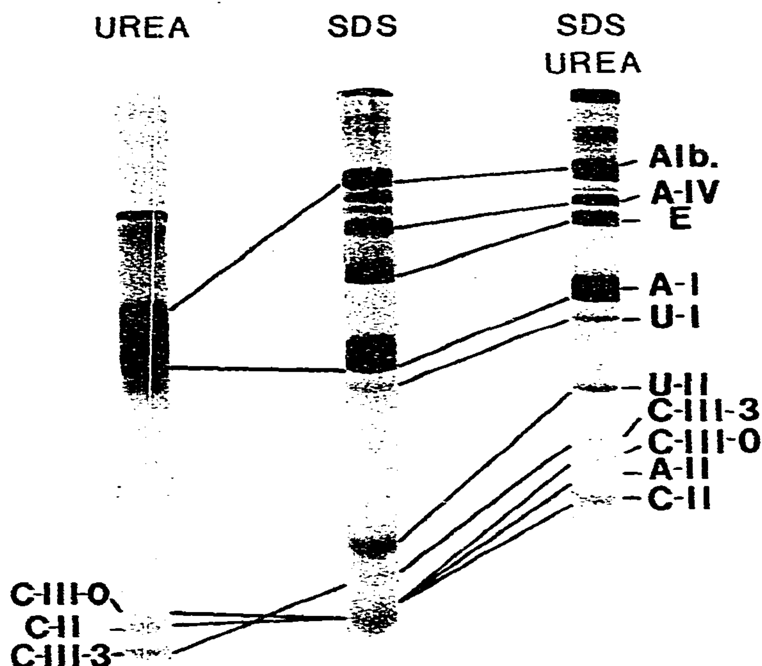

Fig. 2. Comparative resolution of the apoproteins of rat HDL by SDS, urea, and SDS—urea gel electrophoresis. A 40-μg sample of rat plasma HDL protein was applied to each gel. Bands were identified as in Fig. 1. U-I and U-II are unidentified components of rat HDL.

identify apoC-I due to the ambiguity in its position in pH 4—6 isoelectric focusing gels [1]. Under optimal conditions a band apparently corresponding to apoC-I was present below apoC-II, however there was incomplete resolution of apoC-I and apoC-II in most runs. Two additional components referred to as U-I and U-II and not corresponding to previously demonstrated apoproteins, are also resolved in the SDS—urea system. These unknowns were present in several rat HDL fractions run as described or after delipidation with ethanol—diethyl ether [13].

SDS—urea gel electrophoresis was originally proposed for improved separation of low molecular weight peptides because of the increased sieving effect and the improved handling characteristics due to the inclusion of urea in gels of high acrylamide concentration [9]. We have confirmed its suitability for rat apoproteins of low molecular weight. It was noted, however, that the stacking efficiency of the buffer system was inadequate for normal rat VLDL in which the protein concentration is often very low (in the range of 40 μg per 250 μl). The sharpness of the protein bands was considerably improved for sample volumes as large as 400 μl by the use of the buffer combination

described here. However, as previously noted [4], electrophoresis of intact lipoprotein samples caused distortion of the apoprotein bands and resulted in streaks of stainabie material along the length of the gel. The addition of glycerol and isopropanol to lipoprotein samples described here eliminates these problems and reproducibly yields distinct aproprotein bands. As well, intact lipoproteins prepared with glycerol—isopropanol—SDS gave the same apoprotein pattern as delipidated lipoproteins treated with SDS alone (data not shown). This method of sample preparation should be compatible with any SDS electrophoretic system. The present system is preferred by the authors due to the inherent ease of pouring these gels relative to gradient acrylamide gels.

In summary, the application of SDS—urea gel electrophoresis to rat plasma lipoproteins results in a resolution superior to that obtained with any previously described conventional system and equals that reported for gradient acrylamide gel electrophoresis. Due to the simplicity of this sytem and the method of sample preparation, it should be advantageous where rapid simultaneous resolution of high and low molecular weight peptides is required.

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