Rapid Sodium Dodecyl Sulfate/Polyacrylamide Gel Electrophoresis of Urinary Proteins

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We describe a procedure for the convenient separation of proteins by sodium dodecyl sulfate/polyacrylamide gel electrophoresis of urine from cases of renal disease. A precipitation method that requires no special apparatus was used to concentrate the urinary proteins; for electrophoretic separation we used a commercially supplied polyacrylamide/cellulose gel slab. This method seems to be valuable for investigation of proteinuria; we recommend it for routine use.

Additional Keyphrases: urine • renal disease • proteinuria

The nephron's handling of proteins depends greatly on their size. Electrophoresis on sodium dodecyl sulfate/polyacrylamide gel also separates proteins according to their size and is therefore especially appropriate for use in separating urinary proteins. Many methods make use of disc electrophoresis (1-6) after the urine is concentrated.

This article describes our particular adaptation of these methods. Our procedure was to concentrate urine by precipitation and separate the micellar protein/sodium dodecyl sulfate complexes on a new gel, Cellacryl (Sebia, 23, rue Maximilien Robespierre, 92130 Issy les Moulineaux, France), a hetero-copolymer of acrylamide and cellulose in the form of a film fixed to a rigid polyester support.

Materials and Methods

Preparation of Urinary Protein Concentrates

Urine was collected with sodium azide as preservative (0.5 g/L, final concentration) over a 24-h period. Under these conditions, it can be stored for several days at 4 °C or for several weeks at -20 °C. After filtration through Whatman no. 1 paper, a portion of the urine was treated with phosphotungstic acid reagent. The precipitated proteins were then measured by the biuret reaction (7). Each sample was concentrated, by either ultrafiltration or precipitation, depending on its protein content. For ultrafiltration, we used B15 Minicon microconcentrators (Amicon) to obtain a final concentration of 10 g/L. When precipitation was used, 1 mL of urine (diluted so as to obtain a protein concentration of 0.5 g/L) was mixed with 1 mL of modified Tauchiya reagent (ethanol/HCl/phosphotungstic acid), prepared according to Rice (7) and kept at 0 °C. After the mixture had been placed in crushed ice for 30 min, then centrifuged at 2000 × g for 10 min, the supernate was discarded and the protein precipitate was washed twice with a mixture of acetone/water (9/1 by vol). This is preferable to washing with ethanol, ensuring better elimination of mineral salts, which hamper electrophoretic migration.

Electrophoresis on Sodium Dodecyl Sulfate/ Polyacrylamide Gel

The protein pellet or 100 μL of the concentrated urine sample was mixed with 100 μL of imidazole/H3PO4 buffer, 10 mmol/L, pH 7.0, containing, per liter, 10 g of sodium dodecyl sulfate and 0.2 mol of iodoacetamide. This mixture was heated for 2 min at 100 °C, and 10 μL of the imidazole/H3PO4 buffer, containing 5 mg of bromphenol blue per liter as a marker, was then added. This preparation was centrifuged and an aliquot of the supernate was electrophoresed.

Electrophoresis was carried out as described previously (4), except that imidazole/H3PO4 buffer (50 mmol/L, pH 7.0) was used instead of phosphate buffer, and Coomassie Brilliant Blue G250 was used for staining, according to Blakesley and Boezi (8), instead of Amido Black.

Electrophoresis on Acrylamide Cellulose Slabs

Before experimentation, the dehydrated acrylamide slabs, ready for use, were immersed for 12 h in distilled water at 50 °C and then for another 12 h at room temperature in the 50 mmol/L buffer containing 1 g of sodium dodecyl sulfate per liter. After excess buffer has been removed with filter paper, an aliquot corresponding to 25 μg of protein was placed in each slot. Electrophoresis was carried out in the 50 mmol/L buffer at a constant current of 10 mA/slab for about 1 h, when the bromphenol blue had migrated to about 10 mm from the anodic edge. The slabs were then placed in a 0.25 g/L solution of Coomassie Brilliant Blue R250 in a solvent consisting of 225 mL of methanol, 150 mL of glacial acetic acid, and 670 mL of distilled water. After 3 h, the gels were transferred to be stained for 24 h in a mixture of distilled water/methanol/acetic acid/ethylene glycol (68/20/10/2 by vol).

The slabs were then placed for 2 h in a mixture of distilled water/ethanol/acetic acid/glycerol (50/30/10/10 by vol) and thoroughly dried at 37 °C. The dried slabs can be easily kept in files. They were scanned at 1.2 cm/s at 570 nm with a Cello-system Densitometer (Sebia, 23 rue Maximilien Robespierre, 92130 Issy les Moulineaux, France).

Results

Concentration procedure for electrophoresis. Urinary proteins must be concentrated before they can be examined by electrophoresis. For this, ultrafiltration is usually considered satisfactory (4) and has been extensively studied by Pesce et al. (9), who showed that analytical recovery could vary from

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one protein to another, with possible loss of proteins of low molecular mass (10). On the other hand, use of Tsuchiya reagent for the precipitation of the proteins, under the conditions described by Savory et al. (11), results in recovery of urinary proteins that is virtually complete and reproducible, although the precipitation procedure undoubtedly denatures proteins more than the ultrafiltration procedure does. However, this is unimportant, because the electrophoretic separation takes place in sodium dodecyl sulfate medium. Comparison of the patterns after the two concentration processes (Figure 1) shows them to be very similar, and such differences as there were did not change the clinical interpretation.

Electrophoretic conditions. Compared with the previously used phosphate buffer (6), the imidazole/H₃PO₄ buffer, which has a low ionic strength, allows for faster protein migration without the gel's becoming hot.

The iodoacetamide alkylates thiol groups, and thus stabilizes proteins against thermal denaturation (12, 13) in the presence of sodium dodecyl sulfate. Figure 2 shows the electrophoretic separation of myeloma proteins in urine. In tube 1, the samples were treated with iodoacetamide, and display the light chains from immunoglobulins (the fastest-migrating material). In tube 2, iodoacetamide treatment was omitted; most of the light chains seem to be aggregated, and probably migrate as the high-molecular-mass component above the transferrin band.

Ready-to-use slabs for electrophoresis have several advantages: they save time, the method can easily be carried out with conventional protein electrophoresis equipment, and the polyester support ensures good preservation of the slabs. Even though protein separation was not as sharp on slabs as in tubes, it was clear enough to allow the diagnosis of tubular proteinuria and evaluation of the degree of selectivity of glomerular proteinuria (Figure 3).

Moreover, the slabs can be scanned and quantitative data can be obtained rapidly with a good sensitivity (150 ng/fraction). Scanning reproducibility, tested at different protein concentrations, is sufficient for routine use.

Use of slabs that are ready for experimentation, combined with the technique of concentration of urine by precipitation in an acid medium, should enable more widespread adoption of sodium dodecyl sulfate/polyacrylamide gel electrophoresis for investigation of proteinuria.

References


