Simplified Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis of Unconcentrated Urine with Enhanced Resolution and Detection Sensitivity

Thomas Marshall and Katherine M. Williams

We applied a simple sodium dodecyl sulfate–polyacrylamide gel electrophoresis method to urine. The method, developed for serum protein analysis (Clin Chem 1984;30:475–9), has a high sample throughput and gives excellent resolution with unconcentrated urine. It clearly distinguishes and characterizes proteinuric urine (7.5 μL) by Coomassie Blue staining and gives complex silver-stained patterns with nonproteinuric urine (2 μL). The former is recommended for routine clinical screening, the latter for research purposes.

Additional Keyphrases: proteins • Coomassie Blue • silver stain

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) has been favorably assessed relative to gel filtration and other electrophoretic methods as a technique likely to yield urinary protein distribution profiles consistent with a patient’s clinical status (1–5). However, the methods recommended (1–7) are often cumbersome and technically demanding, with low sample throughput. Consequently they are not widely used in the routine clinical laboratory. We report application of a simplified SDS-PAGE method (8) for this purpose. Slab polyacrylamide gradient gels are used, throughput is 200 samples per day, and “high-resolution” protein-distribution profiles are obtained with unconcentrated proteinuric (7.5 μL) or nonproteinuric (2 μL) urines after Coomassie Blue or silver staining, respectively.

Materials and Methods

Reagents. Acrylamide, N,N’-methylenebisacrylamide, N,N,N’,N’-tetramethylethylene diamine, Tris, sds, glycine, ammonium persulfate, glycerol, methanol, and acetic acid were purchased from BDH Chemicals, Poole, Dorset, U.K.

All were of “Electran” or “AnalaR” grade. Agarose (Type 1) was obtained from Sigma Chemical Co., and Serva Blue R from Serva Fine Chemicals, Heidelberg, F.R.G. Ultrapure water (MilliQ Water Purification System, Millipore, Harrow, Middlesex, U.K.) was used throughout.

Procedure. Nine volumes of each noncentrifuged urine was mixed with two volumes of glycerol and one volume of sample denaturing solution (per liter, 10 g of sds in 625 mmol/L Tris HCl buffer, pH 6.8) and heated to 95 °C for 5 min (9). The denatured samples (2.5 μL or 10 μL = 1.9 μL or 7.5 μL of urine) were loaded into agarose wells on polyacrylamide-gradient gels and electrophoresed in sds buffer at 50 mA per gel for 1 h or until the bromphenol blue dye front reached the bottom of each gel (8). The protein bands were then made visible by staining with Coomassie Blue (8) or silver (10).

Biochemistry Research Laboratory, Biology Department, The University of Ulster at Coleraine, Cromore Road, Coleraine BT52 1SA, Northern Ireland.

Received May 19, 1987; accepted June 29, 1987.

1886 CLINICAL CHEMISTRY, Vol. 33, No. 10, 1987
Results and Discussion

Representative SDS-PAGE patterns of unconcentrated urine are shown in Figure 1. Coomassie Blue staining, after electrophoresis of 7.5 µL of sample, was adequate to confirm the enhanced protein content of proteinuric (Uristix-positive) urines and reveal their characteristic "tubular" and "glomerular"-type protein-distribution profiles (Figure 1A). This staining method revealed only traces of albumin, transferrin, and Tamm–Horsfall mucoprotein in nonproteinuric urines (Figure 1A). However, when stained with silver (Figure 1B), 2 µL of these urines revealed proteins in the IgG, haptoglobin, transferrin, albumin, α1-acid glycoprotein, and Tamm–Horsfall mucoprotein banding positions. (The latter two proteins stained more strongly with silver than with Coomassie Blue, relative to albumin and transferrin.) Additional unidentified proteins, apparently not of serum origin, were also detected (Figure 1B).

The method is undoubtedly of higher resolution and sensitivity than alternative SDS-PAGE methods (1–7); e.g., silver reportedly detects only traces of albumin and Tamm–Horsfall mucoprotein in unconcentrated nonproteinuric urine (7). Furthermore it is better suited to the routine clinical laboratory because of the high sample throughput (200 samples per batch of 15 identical precast polyacrylamide gradient gels) and because it avoids (a) sample concentration (1–5), (b) cumbersome casting of multiple cylindrical gels (2, 5) or discontinuous stacking/layered separation gels (1, 3, 6, 7), and (c) expensive or technically difficult silver stains (6, 7); i.e., Coomassie Blue staining is adequate for most clinical purposes. In conjunction with silver staining, the electrophoretic method provides a research tool of unprecedented sensitivity for assessing kidney function in health and disease.

References