Comparison of Coomassie Brilliant Blue Protein Dye-Binding Assays for Determination of Urinary Protein Concentration

Thomas Marshall and Katherine M. Williams

We investigated the influence of assay protocol upon results of Coomassie Brilliant Blue protein dye-binding assays for urinary protein. Sensitivity and working range for, and linearity of response (including dilution dependence) of, three standard assays and four micro assays to bovine albumin, gamma-globulin, and urine are compared. The Read and Northcote standard assay (Anal Biochem 1981;116:53–64) appears to be the method of choice.

The use of the Bradford Coomassie Brilliant Blue (CBB) protein dye-binding assay (1) for determination of the total protein content of urine has been favorably assessed relative to alternative methods (2, 3), but there are discrepancies reported in the protein values so determined (4–8). Concern has been expressed over the positive response of the assay to mucoproteins relative to turbidimetric methods (5, 6), its variable response to different proteins (2, 9), and its apparent dilution dependence (4, 7, 8). In the present report, the influence of assay protocol upon assay performance with urine is assessed. The sensitivity and response to urine of seven such assays have been compared, with both bovine albumin and gamma-globulin as protein standards, and the dilution dependence of the assays with urine has been investigated.

Materials and Methods

Materials

Water. Glassware was extensively washed, and reagents were prepared with “ultrapure” water (MilliQ Water System; Millipore, U.K.).

Proteins. Bovine albumin (Cohn Fraction V, crystallized, essentially globulin free; Sigma Chemical Co.) was freshly dissolved at 1 g/L [based on 1% A280 = 6.6 (1)]. Bovine gamma-globulin protein standard, 1.4 g/L, was purchased from Bio-Rad Labs. Both protein standards were used at 1 g/L concentration in standard protein assays but were appropriately diluted for use in the micro assays.

Urine. Two urine pools were prepared by mixing equal volumes of early morning mid-stream specimens, classified by dipstick (Miles Laboratories Ltd., U.K.) as either protein-uric or normal. These are designated the high- and low-protein pools, respectively.

Methods

Protein content was determined from the increase in absorbance (A950) measured (Pye Unicam SP6-500 UV/VIS Spectrophotometer) against a reagent blank 10 to 60 min after mixing the sample with the appropriate dye reagent, as follows.

Bradford standard assay: We added 5 mL of Bradford dye reagent (0.1 g/L Sigma Coomassie Brilliant Blue G-250, 1.6 mol/L phosphoric acid, 0.8 mol/L ethanol) to 10–100 μg aliquots of the standard proteins (1 g/L) or 25–100 μL urine in a sample volume of 0.1 mL (1).

Bradford micro assay: We added 1 mL of Bradford dye reagent to 2–20 μg aliquots of the standard proteins (0.2 g/L) or 25–100 μL of low-protein urine pool in a sample volume of 0.1 mL (1).

Spector micro assay: We added 0.5 mL of Bradford dye reagent to 1–10 μg of bovine albumin (0.1 g/L) or 2–20 μg aliquots of the standard proteins (1 g/L) or 25–100 μL of urine in a sample volume of 0.1 mL (1).

Read and Northcote standard assay: We added 950 μL of dye reagent (0.1 g/L Serva Blue G, 1.6 mol/L phosphoric acid, 0.8 mol/L ethanol) to 1–20 μg aliquots of the standard proteins (0.4 g/L) or 10–50 μL of urine in a sample volume of 50 μL (11).

Read and Northcote micro assay: We added 300 μL of dye reagent concentrate (0.32 g/L Serva Blue G, 5.12 mol/L phosphoric acid, 2.56 mol/L ethanol) to 1–10 μg of bovine albumin (0.015 g/L) or 2–20 μg of bovine gamma-globulin (0.03 g/L) or 25–200 μL of low-protein urine pool in a sample volume of 700 μL (11).

Bio-Rad standard assay: We added 5 mL of a fivefold dilution of the commercial dye reagent concentrate to 10–100 μg aliquots of the standard proteins (1 g/L) or 25–100 μL of urine in a sample volume of 0.1 mL (12).

Bio-Rad micro assay: We added 0.2 mL of the commercial dye reagent concentrate to 1–10 μg aliquots of bovine albumin (0.015 g/L) or 2–20 μg of bovine gamma-globulin (0.03 g/L) or 20–200 μL of low-protein urine pool in a sample volume of 0.8 mL (12).

Results and Discussion

Figure 1 indicates the color yield response of the standard assays (Bradford, Read and Northcote, Bio-Rad) to increasing concentrations of bovine albumin or gamma-globulin in the appropriate recommended sample volume. The use of sample protein concentration (rather than amount of protein) as a sensitivity parameter overcame differences in assay protocol (i.e., assay volume and proportion of sample to dye reagent) to allow comparison of the working range of each assay and give a direct correlation between sample absorbance values and the required protein concentration. All the assays tested gave less color with gamma-globulin than with bovine albumin. The Read and Northcote standard assay proved to be the most sensitive, with a linear working range equivalent to 0.05–0.4 g of bovine albumin or 0.1–0.6 g of gamma-globulin per liter. It gave an optimal color yield response with both the high- and low-protein urine pools and was not adversely affected, in either case, by urine dilution (Figure 1). The Bio-Rad standard assay demonstrated an essentially linear working range equivalent to 0.1–0.75 g of bovine albumin per liter, while the standard Bradford assay (with similar working range) gave poorer color yields and impaired linearity of response (Figure 1). Both assays responded poorly to the urine pools and
High-protein urine pool: The standard assay values were 0.35 (Bradford), 0.32 (Bio-Rad), and 0.33 g/L (Read and Northcote) when calibrated against bovine albumin and 0.48, 0.73, and 0.62 g/L, respectively, when calibrated against gamma-globulin. Thus at relatively high protein load these assays gave comparable values with bovine albumin as a standard.

Low-protein urine pool: The standard assay values were 80 (Bradford, Bio-Rad) and 40 mg/L (Read and Northcote) when calibrated against bovine albumin and 140, 200, and 90 mg/L, respectively, when calibrated against gamma-globulin. The Bradford, Spector, Bio-Rad, and Read and Northcote micro assays all gave values of 40 mg/L when calibrated against bovine albumin and 90 mg/L when calibrated against gamma-globulin.

Thus the Read and Northcote standard assay gave both a value for high protein in urine consistent with other standard assays and a value for low protein in urine consistent with all four micro assays. This wide range of reliable response to urine without dilution dependence (Figure 1) favors its selection as the CBB protein dye-binding assay of choice. The micro assays are suitable for determination of low protein contents, but enhanced sensitivity limits their working range and the high proportion of sample to dye reagent increases the susceptibility to interference from other sample constituents.

References