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Assay for Protein by Dye Binding

A proposed assay for protein [Bradford, M. M. (1976) *Anal. Biochem.* 72, 248–254] by binding of Coomassie brilliant blue G-250 has been evaluated. Some proteins give standard curves similar to those reported by Bradford, but others deviate widely: caution is urged in application of the procedure to general assay for protein concentration.

A method for rapid quantitation of microgram amounts of protein was presented by Bradford (1). Slight deviation from linearity was found, but a standard curve for five proteins was best described by a single line. On the basis of sample size and the time for assay, the method has great potential value during the purification and assay of proteins and enzymes. Of particular interest to us was its applicability to biological samples such as urine, cerebral spinal fluid, and duodenal aspirates.

MATERIALS AND METHODS

Coomassie brilliant blue G-250 and the proteins tested were obtained from Sigma (St. Louis, Missouri). Proteins were desiccated *in vacuo* at least overnight against P_2O_5 and were weighed on a microbalance. Concentrations were verified spectrophotometrically by use of published extinction coefficients; they were within 6%, which could represent instrument variation. All stock solutions and dilutions were made up in 0.1 M NaCl.

The "standard method" of Bradford was employed. To 0.1 ml of protein solution, 5 ml of reagent was added (0.01% dye, 4.7% ethanol, and 8.5% phosphoric acid) with rapid mixing. After 2 min and before 1 hr, absorbance at 595 nm was then measured. All samples were read in a Zeiss PMQ II spectrophotometer.

RESULTS

Ribonuclease was tested as an example of a nonproteolytic protein from the pancreas which is available commerically in highly purified form. As seen in Fig. 1, absorbance at 595 nm was very much lower than that for the proteins employed by Bradford (1). In contrast, Bradford's results were reproduced for bovine serum albumin and cytochrome *c* (Fig. 1). Both ribonuclease and cytochrome *c* are small, basic proteins; lysozyme was tested as a third example of such a protein. The standard curve for lysozyme had a greater slope than that for ribonuclease (Fig. 1) but, again, substantially less than serum albumin and cytochrome *c*. Similarly the stand-

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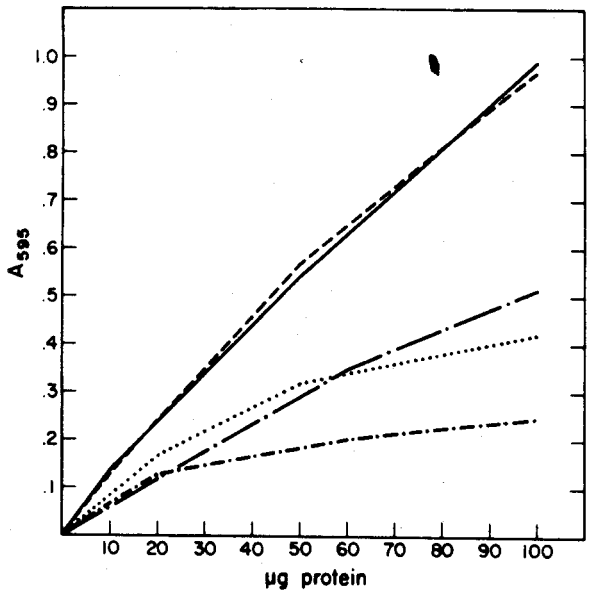

FIG. 1. Response of proteins in the dye-binding assay. (——), Bovine serum albumin; (– – –), cytochrome *c*; (· · ·) lysozyme; (——·——), egg albumin; (·–·–·), ribonuclease. The curves represent the mean value of samples run in triplicate. With experience a coefficient of variation of 2% or better was achieved.

ard curve for egg albumin was much lower than Bradford's results, with a slope about half that for serum albumin.

DISCUSSION

Quantitation of protein by binding of dye is applied more often in clinical chemistry than in research biochemistry (2). The method is frequently applied for selective binding, as in determining the albumin concentration in serum, rather than for total protein. It should be recognized that dye binding as well as most quantitative methods for protein are based on a specific property of the protein rather than on the mass of protein.

The method of Bradford (1) would have great advantages compared to the method of Lowry *et al.* (3) or other procedures, especially in terms of the time for analysis while retaining sensitivity. Bradford defined limits of interference in his assay for substances known to interfere in the assay of Lowry *et al.*

The dye reagent of Bradford contains strong acid so that almost every protein would be below its isoelectric pH and carry a charge consistent with interaction with an anionic dye. We were able to reproduce Bradford's results for the proteins reported but very different standard curves were found for other proteins. It may be possible to find alternative conditions

for the assay which will retain linearity and solubility; a critical relationship between concentration of dye and perchloric acid was found for staining of polyacrylamide gels with Coomassie brilliant blue G-250 (4).

The method of Bradford (1) may provide a rapid test for the presence of protein, as in chromatographic fractions, but it appears in its present form to have substantial shortcomings as a general method for quantitation. A standard curve prepared for a specific protein is reproducible and useful for that protein but cannot be generally applied without potential great error.

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