ANALYTICAL BIOCHEMISTRY 88, 695–698 (1978)

A One-Step Biuret Assay for Protein in the Presence of Detergent

An alkaline–copper reagent is described which forms a soluble, stable complex with protein in the presence of detergent. The modified reagent produces a relatively uniform absorbance for six different proteins, and it may be used to determine the protein content of such heterogeneous samples as plasma membranes and mitochondria.

Ease of operation as well as linearity and uniformity of spectrophotometric response make the biuret procedure the best protein assay for those applications in which its relatively low sensitivity is not a problem. Increasingly, however, proteins are extracted or prepared with detergents, and subsequent assays of such preparations often develop turbidity. Modifications of the biuret method reported to circumvent this problem have not always been successful, and at the very least, the addition of centrifugation or other clarification steps makes the procedure more cumbersome (1).

Knowledge that, in principle, many different formations of the biuret reagent are possible (2) stimulated our search for a modification that would preserve both the simplicity and the linearity of the one-step assay. We report here the formulation of a reagent that produces *no* turbidity in the presence of three widely used detergents but does generate a linear change in absorbance for variations in protein concentration of both homogeneous and heterogeneous samples. The reagent also produces a uniform absorbance for a standard concentration of six different proteins.

While maintaining an optimum balance among copper, tartrate, and hydrogen ions (2), the traditional biuret reagent has been modified in three ways: (i) since potassium reacts with dodecyl sulfate in many solutions to form a precipitate (e.g., 3), sodium potassium tartrate has been replaced with an equivalent amount of sodium tartrate; (ii) without significantly affecting color development (2), the concentration of NaOH has been reduced by 0.75× to prevent the precipitation of the detergent or the copper–protein–detergent complex; and (iii) iodide has been deleted because the reagent is stable without it being present (2). One liter of modified reagent (MBR) contains 1.5 g of $CuSO_4 \cdot 5H_2O$, 4.9 g of $Na_2C_4H_4O_6 \cdot 2H_2O$, and 7.5 g of NaOH; it is stable for at least 4 months when stored in a plastic bottle at room temperature. The assay consists simply in adding 0.5 ml of solubilized sample, which contains 2% detergent and between 0.2 and 4 mg of protein, to 2.5 ml MBR. The mixture is allowed to stand

0003-2697/78/0882-0695$02.00/0

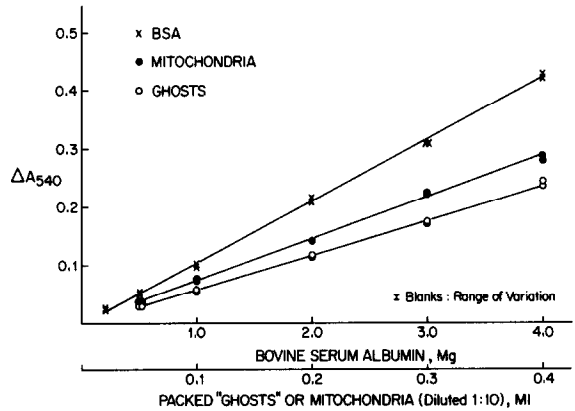

FIG. 1. Beer–Lambert curves for the modified biuret assay of bovine serum albumin (×), human red cell "ghosts" (○), and rat liver mitochondria (●). Assays were performed as described in Table 1, on 0.5-ml samples that contained 2% SDS and the amounts of material indicated. Mitochondria, packed by centrifugation, were resuspended in a 9× volume of 0.25 M sucrose prior to being assayed.

at room temperature for 30 min, and the absorbance is then measured at 540 nm against a blank containing 0.5 ml of 2% detergent and 2.5 ml of MBR. Color develops more slowly in the presence of detergent, but the absorbance does not change significantly beyond 30 min.

The modified assay produces a linear change in absorbance for samples of bovine serum albumin (BSA) that vary in concentration from 0.4 to 8 mg/ml and contain 2% sodium dodecyl sulfate (SDS). Statistically indistinguishable curves are produced by the modified procedure for SDS-treated samples of BSA, deoxyribonuclease, and insulin (data not pre-

TABLE 1

RELATIVE EFFECT OF TRADITIONAL AND MODIFIED BIURET REAGENTS AND VARIOUS DETERGENTS ON A_{540} OF 4-MG/ML SAMPLES OF BOVINE SERUM ALBUMIN

Assay conditions[a]	Mean ± SE (N = 4)[b]
No detergent; traditional biuret reagent[c]	104 ± 1.4
No detergent; modified biuret reagent (MBR)	100 ± 0.9
2% sodium dodecyl sulfate; MBR	96 ± 0.7
2% sodium cholate; MBR	97 ± 1.4
2% Triton X-100; MBR	95 ± 1.5

[a] Assay entailed the addition of 0.4 ml of 0.5% bovine serum albumin (Sigma 2X crystalline) to 0.1 ml of deionized water or 10% detergent. This sample was then mixed with 2.5 ml of biuret reagent and allowed to stand at room temperature (20°C) for 30 min.

[b] Values are expressed as the percentage of the average ΔA_{540} of samples assayed with the modified reagent in the absence of detergent.

[c] Sigma formulation according to ref. (2): 0.15% copper sulfate, 0.6% sodium potassium tartrate, 3.0% sodium hydroxide, and 0.1% potassium iodide.

TABLE 2

COMPARATIVE ASSAY WITH THE MODIFIED BIURET REAGENT OF VARIOUS SDS-TREATED PROTEINS

Protein[a]	Mean ± SE (N = 4)[b]
Bovine serum albumin	100 ± 1.5
Bovine insulin	101 ± 1.5
Bovine pancreatic DNase I	102 ± 2.0
Ovalbumin	112 ± 1.5
Bovine chymotrypsin	119 ± 1.0
Bovine pancreatic RNase	132 ± 1.1

[a] Modified biuret assay performed, as described in Table 1, on 0.5-ml samples containing 2 mg of protein and 2% SDS.

[b] Values are expressed as the percentage of the average ΔA_{540} of the BSA samples.

sented). Linear responses also may be obtained for assays of human red cell "ghosts" and rat liver mitochondria that have been made soluble by treatment with SDS (Fig. 1). The estimated concentration of protein in packed ghosts (5.6 mg/ml) agrees well with literature values obtained with the Lowry method (4).

Standard assays of BSA made in the presence of either SDS, sodium cholate, or Triton X-100 produce identical absorbances (Table 1). Color development in these assays, however, seems to be slightly less than that obtained in the ones that lack detergent. More significantly, parallel determinations with any detergent and the traditional reagent [prepared according to ref. (2)] could not be completed because turbidity developed, usually within 15 min, in both the protein samples and the blanks. In contrast, no turbidity developed over a period of 3 hr (the longest period tested) in any assay involving the modified reagent.

When assayed with the modified reagent in the presence of SDS, different proteins produce color of fairly uniform optical density (Table 2). While not identical the absorbances vary much less than do those generated for the same proteins by the Lowry method (5). Moreover, this relatively small variation in color development is similar to that reported for different proteins assayed with the traditional reagent in the absence of detergent (2).

We conclude therefore that the modified biuret assay, like the traditional one, produces a linear and uniform response. Consequently, MBR can be used advantageously for protein determinations in the presence of detergent without the interpolation of corrective steps that reduce either the ease or reliability of the assay.

ACKNOWLEDGMENT

This study was supported with funds provided by the President and Fellows of Middlebury College.

REFERENCES

1. Futterman, S., and Rollins, M. H. (1973) *Anal. Biochem.* **51,** 443–447.
2. Gornall, A. G., Bardawill, C. J., and David, M. M. (1949) *J. Biol. Chem.* **117,** 751–766.
3. Nelles, L. P., and Bamburg, J. R. (1976) *Anal. Biochem.* **73,** 522–531.
4. Mitchell, C. D., Mitchell, W. B., and Hanahan, D. J. (1965) *Biochim. Biophys. Acta* **104,** 348–358.
5. Hartree, E. F. (1972) *Anal. Biochem.* **48,** 422–427.

CHRISTOPHER WATTERS

Department of Biology
Middlebury College
Middlebury, Vermont 05753
Received December 15, 1977