Rapid Solid-Phase Determination of Cereal Protein Using Bicinchoninic Acid[1]

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ABSTRACT

A modification of the bicinchoninic acid assay that can be used for the rapid determination of protein in cereal-based materials is described. Samples are directly suspended in bicinchoninic acid reagent, where under sonication the protein undergoes reaction and a soluble purple chromophore is released. After a clarification step, absorbance readings are obtained and protein quantified by comparison with a standard curve. In the various corn-derived fractions tested, the solid-phase assay was linear with the amount of sample added, and high reproducibility was observed between replicates. With two commercially prepared flours (Micro-Crisp and Pure n Thick), direct comparisons with the Kjeldahl assay showed general agreement. In two other samples (corn meal and zein), higher readings, possibly derived from the contribution of nonprotein nitrogen, were obtained by the Kjeldahl method. The solid-phase assay represents a rapid alternative to the Kjeldahl assay, especially when numerous insoluble proteinaceous materials require screening.

The importance of rapid methods for quantification of protein in biological tissue has long been recognized (Davis 1988). Colorimetric assays such as the Lowry assay (Lowry et al 1951, Peterson 1979) and newer methods such as the Bradford assay (Bradford 1976) and the bicinchoninic acid (BCA) protein assay (Smith et al 1985) are highly sensitive, and numerous variations have been designed to eliminate interferences (Brown et al 1989, Gates 1991). Although these methods are highly accurate when used to assay soluble proteins, the hydrophobic and largely insoluble nature of cereal proteins poses special problems that have generally precluded use of colorimetric assays. Even when urea, sodium dodecyl sulfate (SDS), and a reducing agent are used in combination, most but not all of the protein becomes soluble. Thus, the Kjeldahl method (AACC 1986) has remained the standard technique for assay of proteins in cereal-based materials. Although highly reliable, the Kjeldahl assay is labor-intensive, requiring separate digestion and titration steps; therefore, the number of samples that can be rapidly screened is limited. Moreover, protein levels can be significantly overestimated in samples containing significant amounts of nonprotein nitrogen.

Recently, Stich (1990) reported a variation of the BCA assay that directly assays protein bound to agarose beads, and Gates (1991) measured protein bound to nylon membranes. The ability to use this procedure to quantify insoluble proteins derives from the reduction of Cu^{2+} to Cu^{+} by the protein. Cu^{+} then complexes to BCA to form a purple chromophore (BCA-Cu^{+} complex) that is freely soluble in aqueous solution. Here, we demonstrate that with addition of a clarification step to remove suspended material, the BCA protein assay is a convenient and highly effective method for rapid screening of protein levels in corn meal.

MATERIALS AND METHODS

Solid-Phase Protein Assay

Colorimetric reactions were conducted under the conditions described by Smith et al (1985). Samples (corn meal, Lauhoff Grain Co., Danville, IL; Micro-Crisp (42% amylose, 18% amylopectin) and Pure n Thick (60% amylopectin), National Starch, Bridgewater, NJ; zein, Sigma, St. Louis, MO) ranging from 2 to 7 mg (ground in a mill to 40 mesh) were weighed into 1.5-ml amber microcentrifuge tubes and suspended in 1.0 ml of BCA reagent (Pierce Chemical Co., Rockford, IL). The BCA reagent was prepared by combining 49 ml of reagent A with 1 ml of reagent B as specified by the manufacturer (Pierce Chemical Co., Rockford, IL). The samples were then incubated at 37°C for 30 min in a thermostat-controlled ultrasonic cleaner (model 3200, Branson Ultrasonics Corp., Danbury, CT) with intermittent vortex mixing at least every 10 min. The samples were then cooled in an ice bath for 5 min and centrifuged at 13,600 × *g* for 10 min in a microcentrifuge at room temperature. A 0.2-ml aliquot of supernatant was removed and diluted with 0.8 ml of BCA reaction buffer A solution. This solution was thoroughly mixed and centrifuged at 13,600 × *g* for 30 sec, and its absorbance at 562 nm was read.

A standard curve (50–400 μg) was constructed using bovine serum albumin (Sigma, stock concentration 10 mg/ml) in 1% SDS and 12.5 m*M* sodium tetraborate, pH 10.0. Samples were brought to 1 ml with BCA reagent, incubated at 37°C for 30 min, cooled on ice, diluted, and read at 562 nm as described above.

Kjeldahl assays were conducted as described (AACC 1986). Protein, expressed as a weight percentage, was calculated as the product of Kjeldahl nitrogen × 6.25.

RESULTS AND DISCUSSION

The solid-phase assay consists of suspending samples directly in BCA reagent, where the protein undergoes reaction and a soluble purple chromophore is released. Sonication is employed to maximize interaction of the reactants with the insoluble sample. After clarification by microcentrifugation and dilution (Chan and Wasserman 1992), absorbance readings are obtained and protein is quantified by comparison with a standard curve.

Figure 1 demonstrates that for the samples analyzed, the solid-phase assay exhibited an essentially linear response between 2 and 3.5 mg of sample. Samples less than 2 mg were not routinely assayed to avoid variations arising from weighing errors. It should be noted that addition of 1% SDS or 8*M* urea to protein suspensions had no significant effect on protein content, indicating that surfactants were not necessary to expose buried protein domains within the sample matrix that might be inaccessible to BCA.

Table I compares the solid-phase BCA assay with the Kjeldahl method. Of the four samples analyzed, protein values for the two purified starch products, Micro-Crisp and Pure n Thick, were in close agreement. In the other two samples, corn meal and zein, the solid-phase assay gave values that were 20–30% lower than those obtained using the Kjeldahl assay (Table I). A possible reason for the observed differences in corn meal and zein may lie in the relative purity of these two materials. Whereas the BCA assay measures the number of peptide bonds accessible to the color reagent, the Kjeldahl method quantifies total nitrogen of both proteinaceous and nonproteinaceous compounds. Thus, any sample containing significant levels of nonprotein nitrogen would yield higher values by the Kjeldahl assay. It is not known whether the apparent differences with zein and corn meal are due to the presence of interfering substances, masking of reactive sites, or differences in the reactivity between each respective set of assay reagents. Nonetheless, replicates of zein and corn meal exhibited the same low variability as both of the commercially prepared flours.

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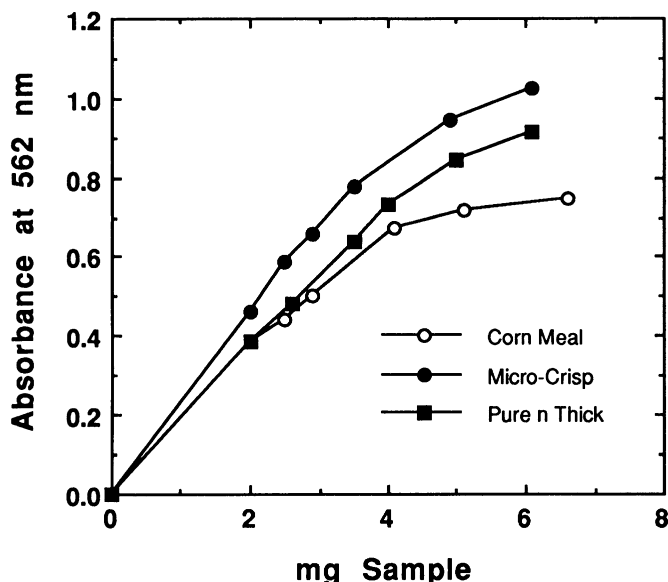

Fig. 1. Concentration-dependence of the solid-phase bicinchoninic acid protein assay.

TABLE I
Comparison of the Kjeldahl and Solid-Phase Bicinchoninic Acid (BCA) Protein Assays

	Starch, %			
Assay	**Corn Meal**	**Micro-Crisp**	**Pure n Thick**	**Zein**
Kjeldahl	4.64	6.22	4.05	65.9
	5.95	6.86	5.86	68.4
	6.52	7.22	6.01	71.2
	7.55	7.66	6.08	72.9
	6.16 ± 0.52[a]	6.99 ± 0.26	5.50 ± 0.42	69.6 ± 1.34
BCA solid-phase	4.32	6.60	4.62	53.0
	4.38	6.86	5.42	54.5
	4.40	7.25	5.90	55.0
	4.54	7.69	6.02	56.0
	4.41 ± 0.04	7.10 ± 0.20	5.49 ± 0.27	54.6 ± 0.54

[a] Means ± standard error.

The key features of the solid-phase BCA assay are release of the purple chromophore and the clarification step. Recently, a Coomassie blue-based assay was developed to quantify immobilized protein (Bonde et al 1992). In this assay, the immobilized protein binds the dye, and the decrease in the absorbance of the supernatant is measured. This method is based on the assumption that all of the protein remains tightly coupled to the matrix. Therefore, it would not be satisfactory for use with cereal proteins, which partially solubilize upon suspension in aqueous solution. Because the BCA assay produces a soluble chromophore, it does not matter how much of the protein solubilizes while the color reaction is conducted. However, it is critically important to clear suspensions of any turbidity before taking absorbance readings.

The solid-phase BCA protein assay is a technique that can be used as a supplement to the Kjeldahl assay for estimating the protein content of corn-based products. It enables rapid screening of large numbers of samples, and the variation between replicates is low. The solid-phase assay also avoids problems associated with the incomplete solubilization of protein. The applicability of the solid-phase BCA assay to protein-containing fractions from cereals other than corn remains to be determined.

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