

# Sorghum Protein Body Composition and Ultrastructure

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## ABSTRACT

Cereal Chem. 61(1):69-73

The protein composition and ultrastructure of sorghum protein bodies isolated from mature starchy endosperm material were studied. Transmission electron microscopy showed that the protein bodies were largely circular in section and varied between 0.4 and 2.0  $\mu\text{m}$  across. The isolated protein bodies were subjected to an Osborne-type protein-fractionation procedure, which revealed that they are composed mainly of prolamin protein. Protein body prolamin had a virtually identical amino acid composition and gave the same electrophoretic pattern as kafirin

(sorghum prolamin). This proves that starchy endosperm protein bodies are the site of kafirin storage in sorghum, as was suggested by electron microscope observations. The protein bodies were embedded in and, in some cases, penetrated by a matrix of mainly glutelin protein. This matrix protein consisted of many proteins, none of which predominated. Maltase activity was associated with the protein, and the matrix may function as a source of certain hydrolytic enzymes involved in the mobilization of endosperm reserves during germination.

Duvick (1961) proposed that the major site of zein (maize prolamin protein) storage was the protein granules in the maize endosperm cells. This proposition was confirmed by Christianson et al (1969), who isolated these granules or protein bodies from maize and compared their composition with that of zein prepared from mature endosperm. They found that the protein bodies were largely soluble in 70% ethanol and had amino acid compositions and electrophoretic patterns very similar to zein. These authors also reported that some protein bodies were surrounded by a protein matrix. This matrix protein resembled glutelin in that it was only soluble in dilute NaOH. However, it differed significantly in amino acid composition from glutelin extracted from mature endosperm.

The protein bodies of sorghum, which in many respects are similar to those of maize, have also been studied by several workers. Seckinger and Wolf (1973) used electron microscopy to observe the effect of various solvents on endosperm sections. They reported that 60% tert-butanol dissolved most of the protein body material, leaving a residual central core. This indicated that sorghum protein bodies, like those of maize, consist mainly of prolamin protein.

Sorghum protein bodies were observed to be surrounded by a protein matrix that had a staining density similar to that of the central core material. Matrix protein was unaffected by treatment with water of 1% NaCl but was preferentially degraded by pronase. From this evidence, Seckinger and Wolf (1973) concluded that matrix protein consisted mostly of glutelin. However, Subramanyan et al (1980), using scanning electron microscopy, reported that treatment of sorghum half-kernels with 5% NaCl resulted in the removal of some of the material between the protein bodies. This suggests that at least some of the protein matrix is albumin or globulin.

It is evident from these studies that simple observation of the effects of various treatments on sorghum protein bodies in situ has not led to a complete understanding of their protein composition. In the work reported here, we utilized a method developed in this laboratory for isolating protein bodies from cereal grains (Adams et al 1976). This method has been improved by the addition of a purification step. After isolation and purification, sorghum protein bodies were subjected to an Osborne-type protein-fractionation procedure. The amino acid compositions and electrophoretic patterns of the fractionated protein body proteins were then compared with those of proteins of the same class extracted from whole grain. To determine the location of each protein fraction, the protein bodies were examined by transmission electron microscopy (TEM) after each solvent extraction.

## MATERIALS AND METHODS

### Isolation of Protein Bodies

Sorghum grain cultivar Barnard Red was passed through a rice-pearling machine until 20% of the kernel's weight had been removed. Kernels free of aleurone layers were selected and the embryos removed with forceps. Five 20-g aliquots of the remaining starchy endosperm fraction were ground for 2 min in a Janke and Kunkel beater-type mill. The resulting flour was stirred for 1 hr at room temperature in 500 ml of 1% (w/v) sodium metabisulfite. This mixture was then passed three times through a Fryma mill (Rheinfelden, West Germany) to break the starch-protein complex. The resulting slurry was passed through a Sharples Super Centrifuge at a rate of 2 L/min and a barrel speed of 5,000 rpm. This pelleted the starch grains in the barrel while the protein bodies remained in the supernatant fraction. These were pelleted by centrifugation at 16,000  $\times g$  for 30 min, using a Sorvall RC-2 centrifuge. This protein body preparation was then purified by density gradient centrifugation. The protein bodies were resuspended in 100 ml of distilled water and 5-ml aliquots loaded onto 30-ml linear sucrose density gradients, 1.2–1.3 g/ml. Centrifugation was for 90 min at 16,000  $\times g$  in a Sorvall HB-4 Swinging Bucket Rotor. The protein bodies formed a single sharp band at a density of 1.27 g/ml. They were removed using a Pasteur pipette, washed four times with distilled water, and then freeze-dried.

### Extraction of Proteins

The purified protein body preparation (1 g) was extracted sequentially by stirring for three 1-hr periods with 25-ml aliquots of 1.25M NaCl at 4°C and then with 60% (v/v) tert-butanol plus 0.05% (w/v) dithiothreitol (DTT) at room temperature. This extracted the albumin plus globulin and prolamin proteins, respectively. The protein remaining after extraction by saline and aqueous-alcohol was designated glutelin (Wilson 1981). Glutelin proteins were also extracted directly from the original protein body preparation by stirring with 0.1M NaOH for 1 hr at room temperature.

Albumin plus globulin and prolamin proteins were also extracted from ground whole grain, using the same solvent sequence. To facilitate electrophoresis of the residual glutelin fraction, it was freed from contaminating starch by incubating for 24 hr at 35°C (pH 5.5) with 0.5% (w/w)  $\alpha$ -amylase (*Bacillus subtilis*, Boehringer Mannheim).

After extraction, all the protein fractions were dialyzed overnight against distilled water and freeze-dried.

### Analyses

Protein (N  $\times$  6.25) was determined using the method of Thomas et al (1967), except that the digestion mixture consisted of 20% (w/v) K<sub>2</sub>SO<sub>4</sub> and 0.1% (w/v) SeO<sub>2</sub> in concentrated H<sub>2</sub>SO<sub>4</sub>.

Samples for amino acid analysis were hydrolyzed by refluxing under vacuum with 6M HCl (2 ml per milligram of sample) for 22 hr at 110°C and analyzed using a Technicon TSM amino acid

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analyzer.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in 12.4% gels was performed as described by Shewry et al (1977).

Protein body samples were prepared for TEM as described by Adams et al (1976).

Maltase activity was determined by incubating with agitation 100  $\mu$ l (1 mg protein per milliliter) of the protein body preparations and 300  $\mu$ l maltose (8.5 mg/ml) in 0.2M citrate-phosphate buffer, pH 3.75, for 30 min at 37°C. The reaction was terminated by immersion in boiling water for 15 min. A clear supernatant that contained glucose, the product of maltase activity, was obtained by centrifugation at 16,000  $\times$  g for 10 min. Glucose was estimated by the glucose oxidase method (Fleming and Pegler 1963).

## RESULTS AND DISCUSSION

### Description of Isolated Protein Bodies

The protein body preparation contained 89.6% protein (Table I). TEM showed that it consisted almost entirely of clusters of protein bodies, some of which were adhered to pieces of cell wall. The protein bodies, which were largely circular in section, varied between 0.4 and 2.0  $\mu$ m across. They were embedded in a matrix of darker-staining material. Some of the protein bodies contained inclusions that had a staining density similar to the matrix material. These findings are in close agreement with those of Seckinger and Wolf (1973), who studied sorghum protein bodies using sections of endosperm. This shows that the isolated and purified protein bodies were representative of those in situ. A typical cluster of protein bodies is shown in Fig. 1. Its concave surfaces suggest that it was embedded between several starch grains.

### Albumin and Globulin Proteins

Extraction of the protein body preparation with 1.25M NaCl resulted in no visible loss in material from either the protein bodies or the matrix (Fig. 2). Nevertheless, some protein had been extracted by this solvent, accounting for 1.5% of the protein body

preparation (Table I). The loss of such a small amount was apparently below the level of visual differentiation. This result agrees with the findings of Seckinger and Wolf (1973), who also did not observe any loss in material from sections of protein bodies extracted with NaCl. A possible explanation for the visible loss in matrix material from protein bodies extracted with salt solution reported by Subramanyan et al (1980) is that the extraction temperature employed by these authors (60°C) resulted in some proteolysis. Adams and Novellie (1975) found that, at a temperature of 55°C, isolated sorghum protein bodies exhibited considerable proteolytic activity.

Amino acid analysis of the NaCl-soluble protein associated with the protein bodies shows that it differs considerably from the albumins and globulins extracted from the whole grain (Table II). The protein body protein was richer in glutamic acid, proline, half-cystine, leucine, and tyrosine and poorer in lysine, arginine, aspartic acid, and valine. This is consistent with the finding that the majority of albumin and globulin proteins is associated with the embryo (Chibber et al 1978).

### Prolamin Proteins

Extraction of the NaCl-treated protein body preparation with 60% tert-butanol plus 0.05% DTT (Fig. 3) solubilized virtually all the protein body material, leaving the darker-staining matrix and inclusions (arrows). The solubilized protein accounted for 76.1% of the preparation (Table I). Amino acid analysis of this protein (Table II) showed that it was a typical prolamin, rich in glutamic acid and proline but containing no lysine. Its amino acid composition is virtually identical to that of sorghum prolamin, kafirin extracted from whole grain (Table II). These results show that prolamin is the major component of sorghum protein bodies, as was indicated by the work of Seckinger and Wolf (1973).

SDS-PAGE (Fig. 4) gave a molecular weight for the major prolamin band of about 26,300, which is in reasonable agreement with the findings of Guiragossian et al (1978) and Paulis and Wall (1979), who reported molecular weights of 24,000–25,000 and 22,000, respectively. The fact that the prolamins extracted from the protein bodies gave the same pattern of bands as those from whole grain indicates that all the sorghum prolamin proteins are located within the protein bodies in the starchy endosperm. This apparently is not the case in maize, in which the reduced-soluble protein described by Wilson et al (1981) is not a protein body prolamin (Mifflin et al 1981).

### Glutelin Proteins

The material that remained after extraction of the protein body preparation with saline and aqueous-alcohol consisted almost entirely of matrix and a small amount of cell walls (Fig. 3). It was composed of some 71% protein, which accounted for 12% of the preparation (Table I). Extraction of the original protein body

TABLE I  
Protein Composition and Maltase Activity  
of Sorghum Protein Body Preparation

	Percentage Composition of Protein Body Preparation in Terms of Protein	Maltase Activity <sup>a</sup>
Protein body preparation	89.6	23.5
Albumins + globulins	1.5	28.5
Prolamins	76.1	ND <sup>b</sup>
Glutelins (residual protein)	12.0	69.3

<sup>a</sup>In nanomoles of maltose per minute per milligram of protein.

<sup>b</sup>ND = not detectable.

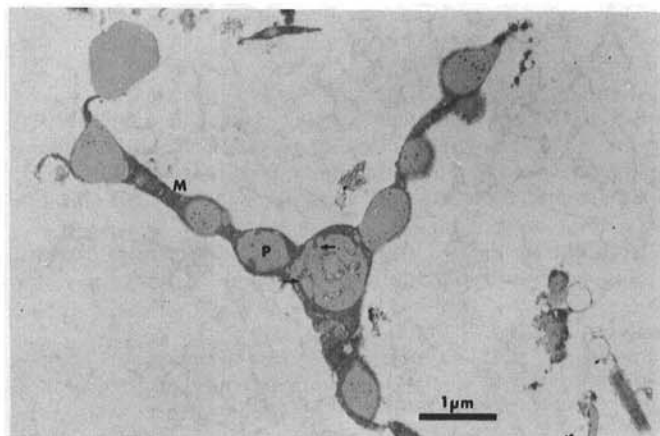


Fig. 1. Cluster of protein bodies. M = matrix, P = protein body. Arrows indicate position of inclusions. ( $\times 17,000$ )

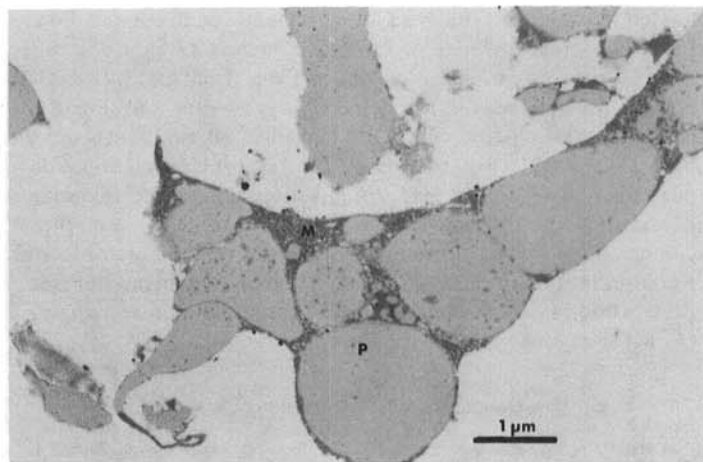


Fig. 2. Protein bodies after extraction with 1.25M NaCl. M = matrix, P = protein body. ( $\times 17,000$ )

preparation with 0.1M NaOH solubilized all the matrix, leaving protein bodies and pieces of cell wall (Fig. 5). These results show that the matrix between the protein bodies is primarily glutelin protein, as was concluded by Seckinger and Wolf (1973).

SDS-PAGE of protein body glutelins and glutelins prepared from whole grain showed them to have several common bands (Fig. 4, arrows). Also, the amino acid compositions of these two fractions are very similar (Table II). From this, it can be concluded that the protein body matrix protein makes up at least a portion of those cereal proteins usually referred to as glutelins. However, both the amino acid compositions and SDS-PAGE protein patterns of these fractions differ considerably from the values for sorghum glutelins found by Guiragossian et al (1978). The glutelins prepared by these workers were richer in glutamic acid, alanine, and leucine

and had a major protein band of molecular weight 24,500. These results may have been a reflection of contamination of the glutelin fraction by prolamin proteins, which are known to be rich in these amino acids and have a major protein band of approximately this molecular weight. This is supported by the fact that the glutelin

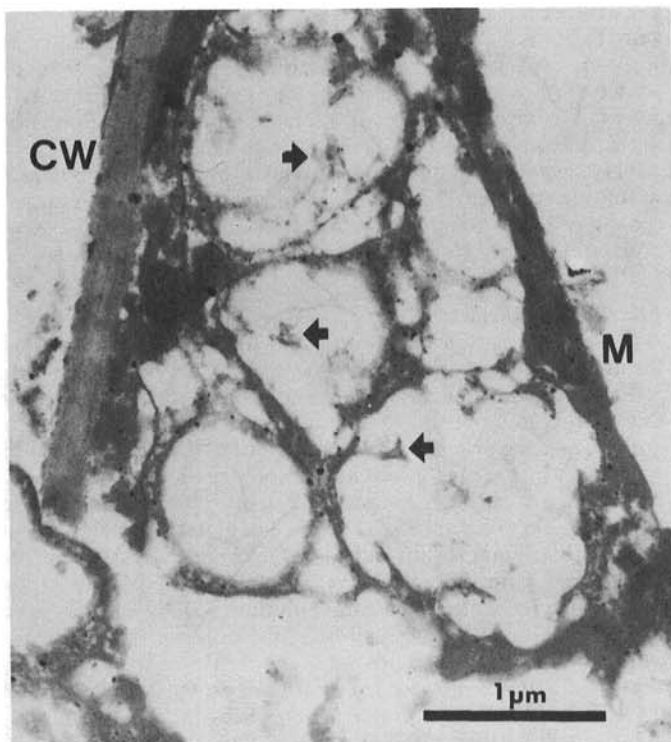


Fig. 3. Protein bodies after extraction with 1.25M NaCl and 60% tert-butanol + dithiothreitol. M = matrix, CW = cell wall. ( $\times 24,000$ )

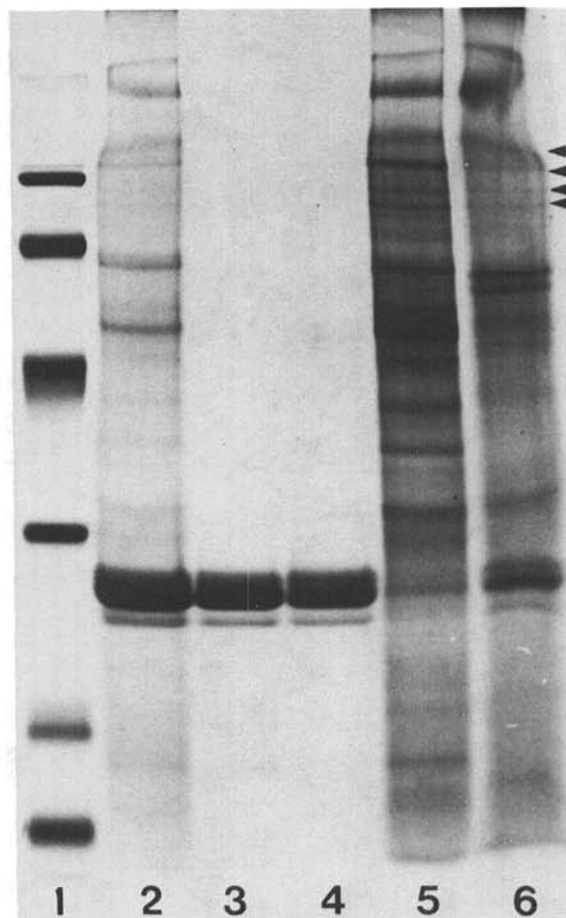


Fig. 4. SDS-PAGE of sorghum protein body and grain proteins. 1, molecular weight standards; 2, proteins in protein body preparation; 3, protein body prolamins; 4, whole grain prolamins; 5, protein body glutelins; 6, whole grain glutelins. Arrows indicate common bands.

TABLE II  
Amino Acid Composition of Proteins Extracted from Sorghum Protein Body Preparation and Whole Grain (g/100 g of Protein)

	Albumins + Globulins		Prolamins		Glutelins (residual protein)	
	Protein Body Preparation	Whole Grain	Protein Body Preparation	Whole Grain	Protein Body Preparation	Whole Grain
Lysine	3.2	5.5	0.0	0.0	4.6	5.8
Histidine	2.5	2.4	0.9	1.0	2.8	2.6
Ammonia	2.1	1.0	1.8	2.5	1.8	2.1
Arginine	5.4	7.3	2.3	2.3	4.6	4.5
Aspartic acid	6.1	9.2	5.5	4.8	7.3	7.6
Threonine	3.2	4.8	1.9	2.1	3.5	4.2
Serine	3.5	4.8	2.8	3.1	3.2	3.9
Glutamic acid	17.4	14.6	25.2	25.0	16.3	16.4
Proline	8.4	4.8	8.8	7.2	7.4	7.7
Half-cystine	6.1	3.2	1.8	1.9	4.3	2.5
Glycine	4.4	5.8	1.2	1.1	4.8	4.8
Alanine	8.1	8.7	12.9	12.3	7.9	7.9
Valine	5.6	7.6	5.1	5.8	6.1	7.3
Methionine	1.9	2.0	1.0	1.2	2.1	1.5
Isoleucine	4.4	4.3	4.5	5.2	5.5	5.3
Leucine	9.5	7.1	15.1	15.9	9.4	9.8
Tyrosine	4.4	2.6	4.2	3.7	4.0	3.1
Phenylalanine	3.7	4.3	5.2	5.1	4.5	4.9

fractions obtained by these workers made up more than 40% of the total endosperm protein. Shewry et al (1978) stated that such high values for glutelins probably reflect incomplete extraction of the previous fractions.

It has been stated that the glutelins, like the prolamins, function as storage proteins (Folkes and Yemm 1958). In the case of

sorghum and maize, this view is probably an oversimplification. SDS-PAGE of the sorghum protein body glutelins shows them to consist of many proteins, none of which predominate (Fig. 4), whereas the prolamins exhibit just one major and one minor band (Fig. 4). Similarly, SDS-PAGE and isoelectric focusing of maize endosperm glutelins revealed that they also consist of many proteins (Wilson et al 1981). This observation led these workers to conclude that the glutelin fraction did not consist of true storage proteins. From their work with sorghum, Wu and Wall (1980) provided other evidence that casts doubt on the idea that the sole function of glutelins is as storage proteins. These authors could find no definite trend in the changes in the proportion of glutelins relative to total protein during germination. This was in contrast to the prolamins, which declined from 48 to 16% over 10 days.

Earlier work in this laboratory (Adams et al 1975) had shown that maltase activity was associated with protein bodies of sorghum and other cereals. Table I shows that the major part of this enzymic activity is associated with the insoluble glutelin protein. In addition, this fraction exhibited the highest specific maltase activity. Other work (Adams et al 1976) showed that proteolytic activity was also associated with cereal protein bodies. Similarly, the activity was associated mainly with the insoluble fraction.

This suggests that the glutelin matrix protein functions as a source of certain enzymes involved in the hydrolysis of starch and protein reserves, rather than being solely a storage protein like the prolamins.

#### Protein Body Inclusions

Close examination of Fig. 1 shows that, in some cases, the inclusions in the protein bodies are continuous with the matrix protein. High magnification of the edge of a protein body shows this more clearly (Fig. 6). The inclusions appear to penetrate radially into the protein body from its surface.

The fact that the inclusions are, in some cases, continuous with the matrix protein and have a similar staining density (Figs. 1 and 6) suggests that they are also composed of glutelin proteins. Other evidence that supports this view is that, like the matrix protein, the inclusions remain after extraction of the protein body preparation with saline and aqueous-alcohol solutions (Fig. 3). It has been suggested that the inclusions in the protein bodies of developing wheat endosperm are composed of tonoplast membranes (Bechtel et al 1982). However, these inclusions are probably quite distinct from those in sorghum protein bodies because they are mainly limited to the periphery of the protein bodies, are vesicular shaped, and appear to be stained much more darkly. We have concluded from numerous examinations of both isolated protein bodies and endosperm sections, that the membranes surrounding developing protein bodies are not extant in the mature grain. Possibly, they are disrupted during grain maturation.

Extraction of the sorghum protein body preparation with 0.1M NaOH dissolved the matrix protein and created holes within the protein bodies (Fig. 5). If these holes represent the position of inclusions, it would further support the view that they are composed of glutelin proteins. Also, it would suggest that the inclusions in the middle of protein bodies are continuous with the matrix protein. Otherwise, the NaOH could not have penetrated through the prolamins of the protein bodies.

Together, these results indicate that some of the inclusions in sorghum protein bodies are in the form of invaginations of the matrix protein, rather than just isolated concentric rings, as was suggested earlier (Seckinger and Wolf 1973, Adams et al 1976). Because proteolytic activity is associated with the insoluble component of protein bodies (Adams et al 1976), such invaginations of the matrix protein into the protein bodies may have a role in protein body degradation. To confirm this hypothesis, the manner in which sorghum protein body breakdown occurs during germination is now being investigated.

#### ACKNOWLEDGMENT

We thank Ilze Meyer for the amino acid analysis.



Fig. 5. Protein bodies after extraction with 0.1M NaOH. CW = cell wall, P = protein body. Arrows indicate holes created within the protein bodies by extraction of the sorghum protein body preparation. ( $\times 15,000$ )

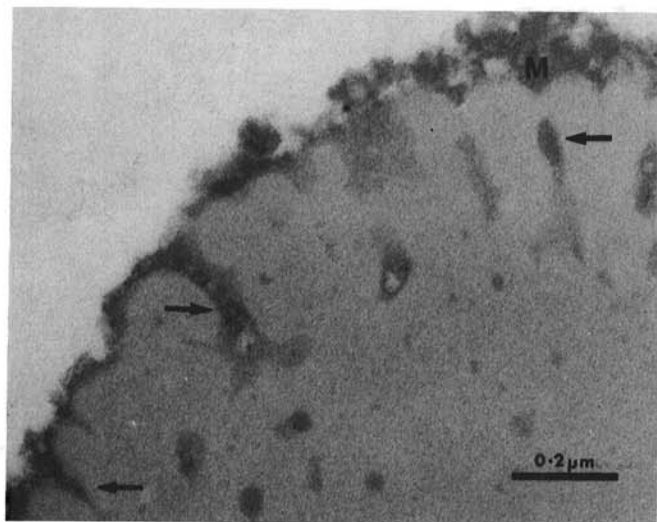


Fig. 6. Edge of a protein body. Arrows indicate that inclusions in the protein bodies are continuous with the matrix protein. ( $\times 100,000$ )

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[Received May 5, 1983. Accepted September 1, 1983]