FRACTIONATION AND CHARACTERIZATION OF ALCOHOL-SOLUBLE REDUCED CORN ENDOSPERM GLUTELIN PROTEINS

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ABSTRACT

Alcohol-soluble reduced glutelin (ASG) from corn has been separated into two fractions having distinctly different properties. The ASG was prepared from defatted corn endosperm meal previously extracted with saline and 70% ethanol-0.5% sodium acetate by extracting with 70% ethanol containing 0.5% sodium acetate and 0.1*M* β-mercaptoethanol (ME). Upon dialysis against water, the ASG separated into water-soluble and insoluble fractions. Molecular weights and subunit composition of each fraction were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and polyacrylamide gel electrophoresis (PAGE) in aluminum lactate-8*M* urea pH 3.1 buffer, respectively. Amino acid compositions of these preparations were different; the water-insoluble fraction has five times as much methionine as the soluble protein.

Glutelin, a major protein class in corn endosperm, contains most of the nitrogenous constituents remaining in the grain after extraction with saline and aqueous ethanol solvents. The proteins have generally been extracted by alkali with evident degradation. Glutelin is highly insoluble in the most potent protein-dissociating solvents, but upon reduction of its disulfide bonds it yields polypeptides which are soluble in 8*M* urea, 6*M* guanidine hydrochloride or sodium dodecyl sulfate (SDS) solutions (1–4). Using these solvents it has been possible to demonstrate heterogeneity of these reduced glutelin polypeptides by starch gel electrophoresis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and gel filtration chromatography (2–4).

The polypeptides of reduced corn glutelin may be fractionated by differences in solubility (1,3). A fraction has been isolated which is soluble in alcohol containing 0.1*M* β-mercaptoethanol (ME). This fraction has been referred to as glutelin-1 (1), zein-2 (5), or alcohol-soluble reduced glutelin (ASG) (2). It is very different from the alcohol-insoluble fraction of reduced glutelin which has a

much higher lysine content and lower leucine level (2). It has zein-like properties, being soluble in alcohol, but its amino acid composition and gel filtration pattern show that it is composed of unique proteins. Because of its deficiency in lysine, its quantitative extraction has been used in fractionation schemes for studying proteins of high-lysine corns (3–7). By dialyzing the ASG preparation against water, it can be separated into two fractions, distinctly different from each other by amino acid analysis, SDS-PAGE, and polyacrylamide gel electrophoresis (PAGE).

MATERIALS AND METHODS

Fractionation of ASG

Saline-soluble and zein proteins were extracted from 20 g of a defatted corn endosperm meal with 0.5 *M* NaCl and 70% ethanol, respectively (4). Next, ASG was extracted from the meal residue twice with 200 ml 70% ethanol-0.5% sodium acetate-0.1 *M* ME by shaking for 30 min (4). Three-fourths of the combined ASG extract was dialyzed against water at 4°C in a Spectrapor membrane tubing having a mol wt cutoff of 6000–8000 daltons. The retentate was centrifuged at room temperature at 1000 × *g* for 15 min and the precipitated protein and supernatant were separated. The precipitated protein was washed twice with cold water, centrifuged, and the washes combined with the original supernatant. The supernatant and precipitate were separately lyophilized to dryness. The remaining 25% of extract was dialyzed against water, and lyophilized in its entirety. This protein represented the total unfractionated ASG.

TABLE I
Amino Acid Composition of Alcohol-Soluble Reduced Glutelin Preparations and Zein

	g/100 g Protein			
Amino Acid	Zein	ASG	Water-soluble ASG	Water-insoluble ASG
Lysine	0.1	0.3	0.3	0.1
Histidine	1.5	3.5	8.1	2.3
Ammonia	3.4	3.3	2.3	2.7
Arginine	1.9	2.8	3.5	2.8
Aspartic acid	5.8	2.9	0.8	3.9
Threonine	3.2	3.4	4.1	3.4
Serine	5.9	5.0	3.9	5.3
Glutamic acid	20.8	17.1	15.1	18.6
Proline	10.7	15.2	23.5	13.5
Glycine	1.6	3.7	4.2	3.6
Alanine	11.2	7.7	4.5	9.3
Valine	4.1	4.2	6.4	3.9
Methionine	2.0	6.3	1.7	8.6
Isoleucine	4.2	2.4	2.0	2.7
Leucine	22.1	14.1	10.9	16.4
Tyrosine	5.7	5.8	3.6	7.2
Phenylalanine	8.1	4.3	2.0	5.5

Analytical Methods

Aliquots of extracts or portions of weighed, dried materials were assayed for nitrogen by a semimicro Kjeldahl method. Crude protein was estimated by multiplying nitrogen content by 6.25 and is given on an as-is basis.

Samples for amino acid analysis were hydrolyzed by refluxing in 6*N* HCl (2 ml/mg sample) for 24 hr and analyzed with a Beckman amino acid analyzer following a previously described procedure for quantitation (8). All amino acids were corrected to 97% recovery of nitrogen for comparison between samples.

SDS-PAGE of the polypeptides from zein and ASG preparations was carried out in 10% gels in SDS-borate pH 8.9 while PAGE was conducted in 5% gels in aluminum lactate-8*M* urea pH 3.1 (8). The proteins for PAGE were reduced with ME and alkylated with acrylonitrile (8).

RESULTS AND DISCUSSION

Extraction of defatted corn endosperm meal with 0.5*M* NaCl and 70% ethanol-0.5% sodium acetate removed 4 and 41% of the total nitrogen,

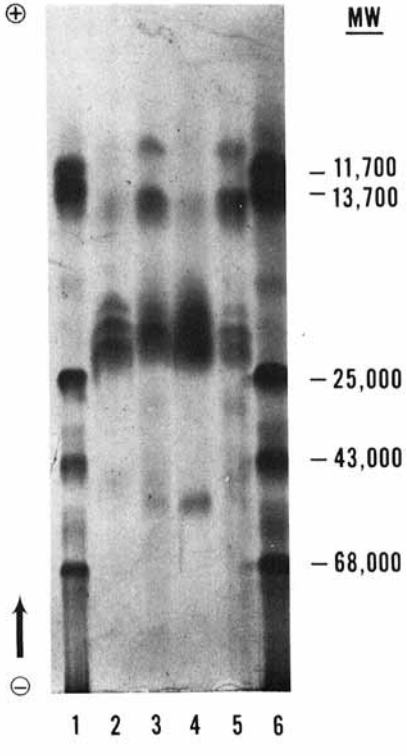

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns. 1, 6, mol wt (MW) calibration mixture; 2, zein; 3, whole alcohol-soluble reduced glutelin (ASG); 4, water-soluble ASG; 5, water-insoluble ASG. Origin at bottom.

respectively. The ASG contained 20% of the total nitrogen.

As previously reported by the authors (2) and others (5,9), ASG is quite different from zein in amino acid composition. It contains much more histidine, proline, glycine, and methionine and less aspartic acid, glutamic acid, isoleucine, leucine, and phenylalanine than zein (Table I).

Fractionation of ASG by dialysis against water produced a soluble protein that represented 33.0% of the total ASG. Except for the small amount of lysine, this protein had an amino acid composition different from total ASG, zein, or water-insoluble ASG (Table I). The biggest differences were in its high histidine, proline, and valine contents and low aspartic acid, alanine, tyrosine, phenylalanine, and methionine.

Although water-insoluble ASG is closer in amino acid composition to zein than to that of the soluble ASG, it exhibits large differences from zein in content of proline, glycine, methionine isoleucine, leucine, and phenylalanine (Table I). There is more than 4 times as much methionine in this fraction as in zein.

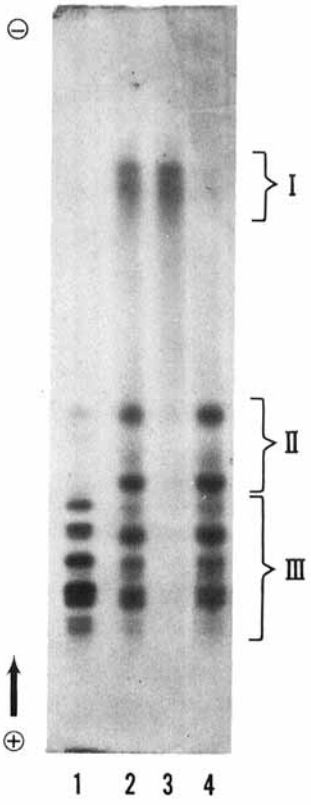

Fig. 2. Polyacrylamide gel electrophoresis (PAGE) patterns of 1, zein; 2, whole alcohol-soluble reduced glutelin (ASG); 3, water-soluble ASG; 4, water-insoluble ASG. Origin at bottom.

The SDS-PAGE patterns of zein show principally three bands in the 22,000–24,000 dalton region (Fig. 1). These bands correspond in mobility to prominent bands observed during SDS-PAGE of whole ASG and water-soluble ASG. There are also faint bands in water-insoluble ASG in this region. Some of these bands might be some residual water-soluble ASG. Both ASG and water-soluble ASG have a faint band around 50,000 daltons which is absent in zein and water-insoluble ASG. The water-insoluble ASG has two prominent bands in the 10,000–14,000 dalton region which are present only as traces in zein and water-soluble ASG.

PAGE in aluminum lactate-8*M* urea-pH 3.1 buffer (Fig. 2) gave patterns showing different relations than the SDS-PAGE patterns. The pattern of water-soluble ASG in PAGE differs considerably from that of zein and the water-insoluble fraction. The water-soluble fraction contains two or more components of high mobility (region I). These components are absent from zein or water-insoluble fraction. In contrast, the water-insoluble fraction contains components having mobilities similar to those of zein in region III. Two additional prominent fast-moving components of the water-insoluble fraction (region II) are very faint in the zein pattern.

The results of SDS-PAGE and PAGE in aluminum lactate-8*M* urea buffer appear contradictory. The water-soluble fraction resembles zein in mol wt by SDS-PAGE but not in mobilities in PAGE. Most of the water-insoluble proteins migrate like the zein in PAGE but have much lower mol wt. Thus, while the whole mixture of these proteins (whole ASG) apparently has components with mol wt and electrophoretic mobilities resembling zein, none of the fractionated proteins is completely like zein in both SDS-PAGE and PAGE.

The water-soluble ASG may be soluble in both water and alcohol since its amino acid composition contains less nonpolar amino acids than zein (Table I). The fraction of ASG which is soluble in low concentration in water becomes insoluble after lyophilization. Probably, upon concentration, the disulfide bonds of water-soluble ASG are reformed, which renders the proteins insoluble in water.

The high-methionine portion of ASG precipitates readily upon dialysis against water and thereby separates from the rest of ASG. This fraction contains mainly low-mol wt components. In earlier studies of the fractionation of glutelin by gel filtration (2) and SDS-PAGE (4), the high methionine protein was also shown to be low mol wt.

Fractionation into water-insoluble and soluble ASG goes one step further in the solvent fractionation of glutelin beyond the Moureaux and Landry (1) and Paulis *et al.* (3) schemes. The previous partial fractionation of ASG into two fractions achieved by gel filtration chromatography required much more effort (2) and now can be done by dialysis against water.

Acknowledgment

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