Maize Endosperm Proteins Compared by Sodium Dodecyl Sulfate Gel Electrophoresis and Isoelectric Focusing[1]

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ABSTRACT

Maize endosperm proteins were extracted by variations of the Osborne procedure. The protein components of each fraction were compared by sodium dodecyl sulfate polyacrylamide gel electrophoresis and isoelectric focusing. The salt-soluble fraction and the glutelins consisted of a large number of proteins with only a few bands in common. Only one of the glutelin bands was present in a large amount, and we do not consider this fraction to consist of true storage proteins. The zein fraction produced two major bands by gel electrophoresis but eight or more by isoelectric focusing. A two-dimensional separation revealed 22 different polypeptide spots from the zein-1 and zein-2 fractions. A minor protein fraction, named reduced-soluble protein, was extracted with either the salt-soluble fraction or the total zein fraction, depending upon which solvent contained a reducing agent. The reduced-soluble protein has a unique amino acid composition with much more proline, histidine, cysteine, glycine, and valine and less aspartate, alanine, glutamic acid, and leucine than zein. It is, however, also very low in lysine.

In 1908 Osborne reported on "present knowledge" of plant proteins and commented that

> The necessity of some scheme of classification for the proteins is recognized by all who write or teach about them, and although the present method of classifying proteins is wholly unsatisfactory from a purely chemical standpoint, it is practically the only one now available.

Since that time the classification by solubility of cereal seed proteins into albumins, globulins, prolamins (Osborne's spelling when he coined the word in his paper), and glutelins has proven useful. Now, however, additional approaches to the problems of identification, separation, and classification of proteins are available. Changes in reagents and procedures may produce large differences in the yields of the different protein solubility fractions (Preaux and Lontie 1975). Detergents and reducing agents were shown by Foster et al (1950) to be effective for extracting maize proteins but only entered into common usage when Landry and Moureaux (1970) separated the glutelin fraction (ie, proteins remaining in the starchy residue after extraction by water, salt solutions, and alcohol) into three components. These components are known as G_1, which is soluble in alcohol plus 2-mercaptoethanol (ME); G_2, soluble in pH 10 buffer with ME; and G_3, soluble in pH 10 buffer with ME plus sodium dodecyl sulfate (SDS) detergent. The G_1 fraction was termed the zein-2 fraction by Sodek and Wilson (1971) because of its high content of zeinlike polypeptides, although it also contained polypeptides different from classical zein. This fraction was also named alcohol-soluble reduced glutelin (ASG) with a subsequent division into water-soluble and water-insoluble fractions (Paulis and Wall 1977). Osborne's classification thus became more complex as it was stretched to fit new methods.

The glutelin fraction has been the most difficult fraction to work with because the alkaline extraction solvents generally used also degrade it. The major storage protein in rice may properly be classified as a glutelin (Juliano and Boulter 1976). Claims have been made that the glutelin fraction contains up to 40% of the total proteins of wheat, barley, and maize endosperms, but this probably reflects the incomplete extraction of the previous fractions (Shewry et al 1978b). In these cereals the glutelin fraction is probably not a storage protein per se (Miflin and Shewry 1979) but rather consists of those proteins that are insoluble by virtue of their location in membranes, microbodies, mitochondria, etc., or that become insoluble through formation of cross-links with other polypeptides during the final stages of maturation or during milling and extraction. These proteins may still be utilized during germination but as a secondary function.

Another approach to protein identification includes separations on the basis of size and charge by ion-exchange chromatography, gel exclusion chromatography, gel electrophoresis, and isoelectric focusing (IEF). These techniques show that the different solubility fractions are made up of large numbers of polypeptides. SDS-polyacrylamide gel electrophoresis (PAGE) reveals two major zein bands, but as many as five additional minor bands have been detected (Lee et al 1976, Soave et al 1976). IEF resolves zein into 8-15 polypeptides according to genotype, with a total of 27 different pIs having been identified (Valentini et al 1979). Combination of the techniques into a two-dimensional (2-D) separation revealed over 20 polypeptides from zein of a single variety (Miflin and Shewry 1979).

In this research the glutelin fraction of maize endosperm was examined to determine whether it consisted of a few or many polypeptides and whether these polypeptides were unique to the glutelin fraction.

MATERIALS AND METHODS

Maize Source

A dent hybrid (N28 × Oh7N) maize (*Zea mays* L.) was grown in Urbana, IL, and harvested at 50 days after pollination. The endosperms were separated from the hulls and embryos and freeze-dried. Mature samples of two inbreds (Oh45B and Oh7N) were air-dried at 40°C and then stored at 3°C. The silage variety Fronica, provided by the Plant Breeding Institute, Cambridge, England, was harvested when the endosperms contained 31% water and the black layer had begun to form in most kernels. The kernels were stored frozen.

Extraction

The frozen Fronica endosperms were separated and sliced, and the dried endosperms were crushed in a mortar and pestle. Then they were soaked overnight at 3°C in six volumes of 0.05*M* sodium phosphate buffer, pH 7.8, containing 0.5*M* NaCl and 0.01*M* ethylenediamine tetracetic acid. In certain experiments 2% ME or 0.05% dithiothreitol (DTT) were added to the buffer. The samples were homogenized with a Polytron homogenizer. The protein fractions were extracted and separated as outlined in Fig. 1, with all

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operations at 3°C unless otherwise noted. Three extractions were made at each step, using five or six parts of solvent per part of endosperm (v/w), with up to 1 hr of stirring by magnetic bars in 50-ml centrifuge tubes. Residues were sedimented by centrifugation at 15,000 × *g* for 15 min. Supernatant solutions were dialyzed where indicated against distilled water, then freeze-dried. The globulins were precipitated from the salt-soluble fraction by dialysis against water, but the albumins remained soluble. Precipitates and the final starchy glutelin residues were dispersed in water before freeze-drying. The initial zein fraction (extracted at room temperature) included both zein-1 and zein-2 (Sodek and Wilson 1971). Zein was precipitated from the alcoholic solution either by dialysis against water or by addition of two volumes of 4% NaCl. The protein that remained in solution after dialysis was termed "reduced-soluble protein" (RS-protein). In one experiment zein-1 was extracted using 50% (v/v) 1-propanol without ME and zein-2 by subsequent extraction with 50% (v/v) 1-propanol plus 2% ME.

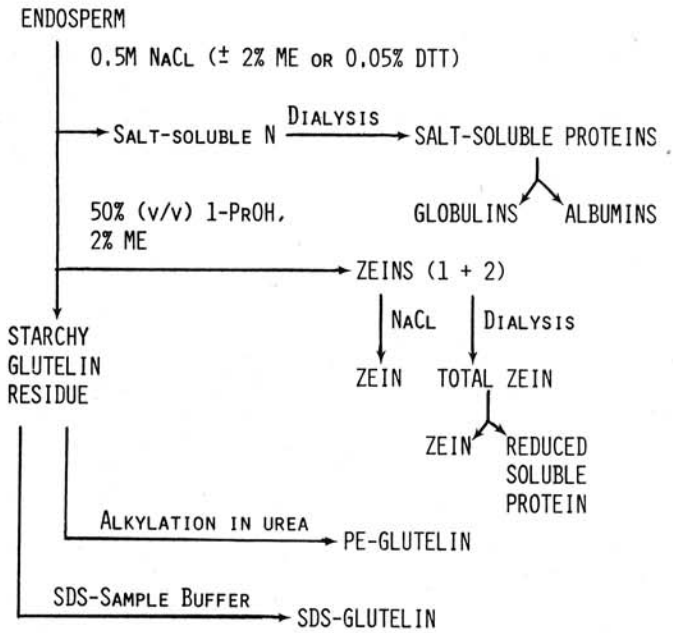

Fig. 1. Procedure for extraction and separation of maize endosperm protein fractions.

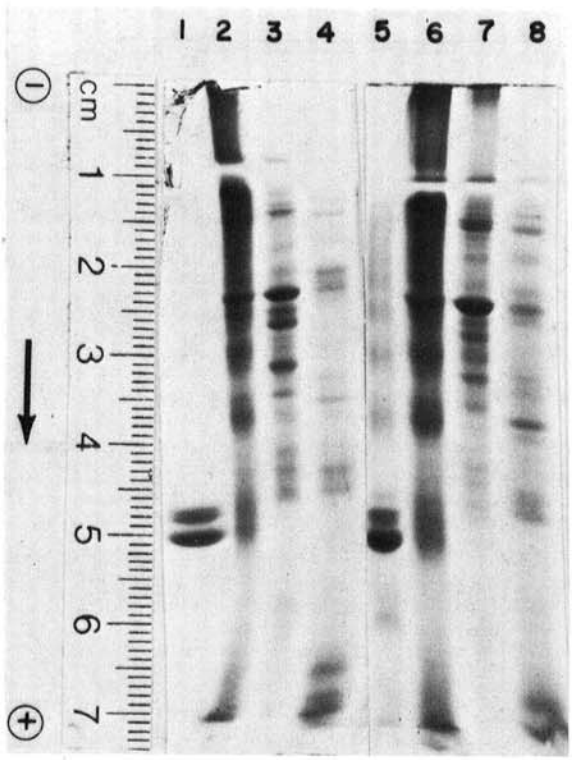

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of salt-soluble proteins, zein, and glutelins from two mature inbreds. Lanes **1** and **5,** zein; lanes **2** and **6,** PE-glutelins alkylated in the presence of mercaptoethanol; lanes **3** and **7,** PE-glutelins alkylated in the presence of dithiothreitol; lanes **4** and **8,** salt-soluble proteins. Lanes 1–4, Oh45B; lanes 5–8, Oh7N.

Glutelin Isolation

Alkylated glutelins were extracted from the starchy residue by a modification (Shewry et al 1978b) of the method of Friedman et al (1970). Briefly, the residue was suspended in pH 7.5 tris-nitrate buffer with 6*M* urea and either 1% ME or 0.07% DTT. The mixture was stirred overnight at 5°C. A threefold excess of 4-vinyl pyridine over total sulfhydryl groups was added at room temperature to convert the cysteine to S-β-(4-pyridylethyl)cysteine. After acidification with acetic acid, cooling to 5°C, and centrifugation, the protein recovered from the supernatant solution was termed pyridylethyl (PE)-glutelin. In one experiment 0.5% SDS was substituted for urea in the preparation of alkylated glutelins, but recovery of protein was poor and the results are not reported.

A fraction termed SDS-glutelin (nonalkylated) was extracted just before electrophoresis by mixing the starchy glutelin residue with 15 parts (w/v) of SDS-sample buffer containing 1% ME or 0.05% DTT and sometimes with 6*M* urea. The SDS-sample buffer consisted of 0.05*M* pH 7.0 tris buffer with 2m*M* ethylenediamine tetraacetic acid, 1% SDS, and 10% glycerol.

Protein Separation

SDS-PAGE in 12.5% polyacrylamide slab gels at pH 8.9 was as previously described (Shewry et al 1977). Freeze-dried protein samples (1–2 mg/ml) were dissolved in SDS-sample buffer with 2% ME (except where noted) by heating at 75°C for 10 min. Samples of 10–20 μl were applied to the gels.

Isoelectric focusing in 5% polyacrylamide slab gels was performed as described previously (Shewry et al 1978a). The gels

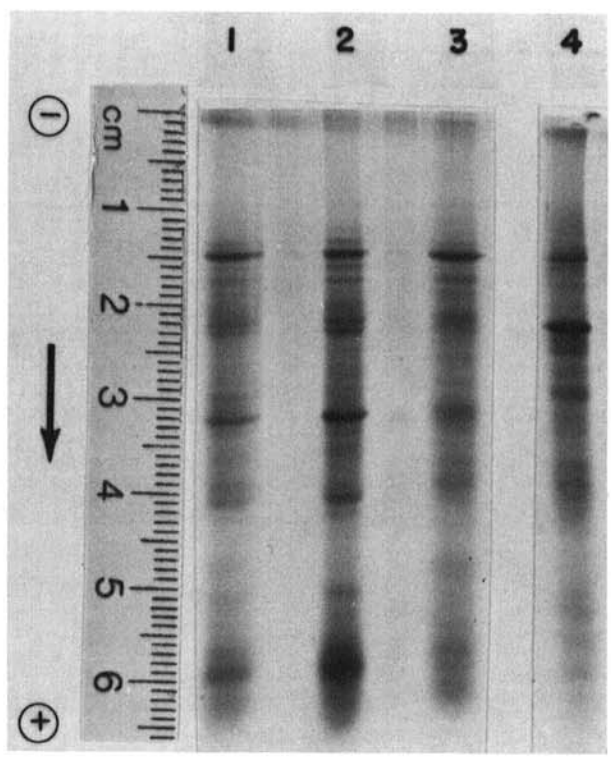

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of salt-soluble proteins. Lane **1,** total salt-soluble protein fraction; lane **2,** albumins; lane **3,** globulins; lane **4,** PE-glutelins. Variety Fronica.

were fixed in a solution of 10% trichloroacetic acid (TCA) plus 5% sulfosalicylic acid, were washed with 5% TCA, and were stained to equilibrium with 0.002% Coomassie blue G250 in 15% TCA. Destaining was not required.

Two-dimensional separations were performed first as IEF, then as SDS-PAGE on 17.5% acrylamide gels (Shewry et al 1978a).

Protein Analyses

The amino acids in two samples of RS-protein were determined after acid hydrolysis. One sample was assayed once at the Rothamsted Experimental Station on a Technicon TSM-1 analyzer as previously described (Shewry et al 1978b). The second sample was determined in the Agronomy Department, University of Illinois, on a Beckman 119CL analyzer using a lithium citrate buffer system. PE-cysteine could not be determined, and threonine and serine appeared to have been partially destroyed by the hydrolysis procedure used for the second sample. Duplicate analyses of all amino acids agreed within 0.1% for the second sample.

RESULTS AND DISCUSSION

Protein Extraction

The proteins were extracted under mild conditions from endosperms that had not been subjected to high temperatures during storage. To prevent denaturation of the glutelin fraction, none of the extracts were heated, although prolamins are most efficiently extracted with hot alcohol (Miflin and Shewry 1977, 1979). When the buffered salt solutions did not contain a reducing agent, 12–16% of the recovered nitrogen was extracted, and 65–72% of the nitrogen occurred in the subsequent zein fraction, which includes the zein-2 fraction. Addition of a reducing agent to the salt solution increased the nitrogen extracted to about 25% of recovered N, with a corresponding decrease of N in the zein fraction. The starchy glutelin residue contained between 12 and 22% of the recovered nitrogen.

When the residue from the zein extract was treated with urea (before alkylation), the starch became swollen and thus occluded much of the protein (ie, only a small portion of the protein was recovered in the supernatant solution after centrifugation). Starch doesn't swell when SDS is used, but SDS may interfere with some subsequent analyses such as IEF.

SDS-PAGE of Major Fractions

The three major Osborne classes of protein are compared in Fig. 2. Zein is easily recognized by the presence of two major bands, although the upper band may be a doublet (lanes 1 and 5). Doublets have been noted before and appear to be genetically determined (Soave et al 1976, Tsai et al 1978). The zein in lane 5 appears to be contaminated with high molecular weight material. The PE-glutelins alkylated in the presence of DTT (lanes 3 and 7) gave much sharper bands than those alkylated in the presence of ME (lanes 2 and 6). In some experiments ME gave satisfactory results but never better than those obtained with DTT. The salt-soluble fraction, as well as the glutelins, contained a large number of proteins distributed over a wide range of sizes. Some bands in these two fractions appeared to coincide, but major differences between them are obvious. Small genotypic differences were seen between these two inbreds in all three fractions, but careful comparisons were not made in this study.

The salt-soluble fraction gave 20 or more bands upon SDS-PAGE (Fig. 3). Most of the bands appeared in both the albumin and the globulin fractions. Two major bands at 32 and 40 mm were concentrated in albumin. The amino acid composition of albumins and globulins are not much different (Sodek and Wilson 1971). The dividing line between them is rather arbitrary and may be useful only for specialized purposes. PE-glutelins (lane 4) are included for comparison.

In Fig. 4, nonalkylated glutelins extracted with SDS-sample buffer with (lane 3) and without (lanes 1 and 2) urea are compared to PE-glutelins extracted with urea (lane 5). Most of the protein bands were the same in the four preparations because alkylation had little effect on the apparent molecular weights. ME and DTT were equally effective in the extraction of SDS-glutelin. The arrow indicates a major band found in all glutelin extracts when urea was used (lanes 3 and 5) and in much smaller amounts when urea was not used (lanes 1 and 2). This protein had a molecular weight of 70,000 or above, as determined on other runs. Some of this protein also appeared in the salt-soluble fraction (lane 4). Several of the high molecular weight proteins occurred in both salt-soluble and glutelin samples, but further work, such as 2-D gel electrophoresis and IEF should be employed before the conclusion is drawn that certain proteins are identical.

The large number of proteins occurring in the glutelin fraction suggested that these proteins may not meet the criteria for classification as storage proteins (Miflin and Shewry 1979), with the possible exception of the protein made soluble by urea.

RS-Protein

In Fig. 5 the SDS-PAGE patterns produced by a total zein fraction (zein 1 + 2) are compared with those of zeins precipitated by NaCl and those of the RS-protein. (Zein precipitated by water dialysis was not run on this SDS gel.) Equal weights of the zein preparation (60% protein) and of RS-protein (N content not determined) were applied to the gels. In this run four minor bands are visible in the total zein sample, but the minor band at 31 mm is missing from the salt-precipitated zein. A minor band at 52 mm was present. The RS-protein consisted almost entirely of that minor

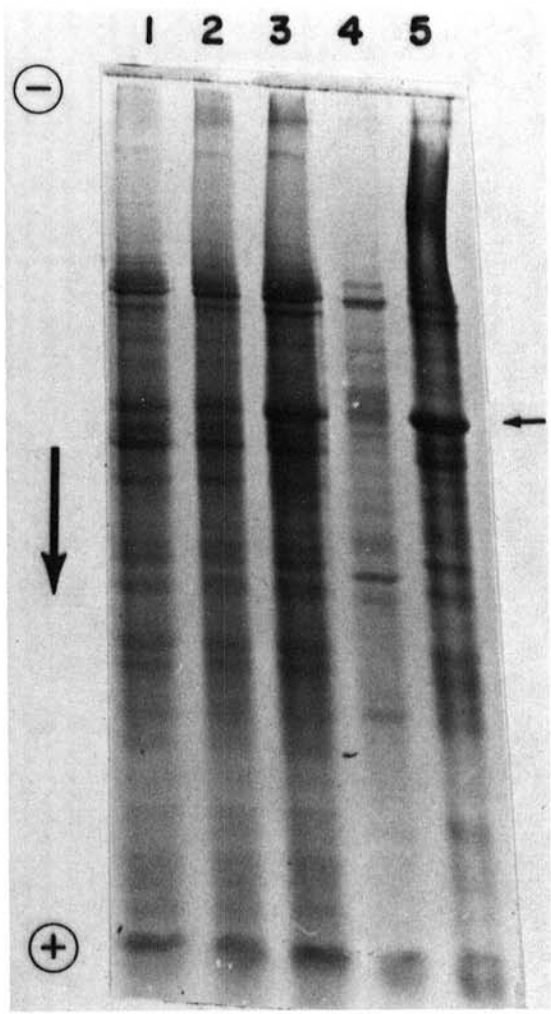

Fig. 4. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis of nonalkylated and alkylated glutelin compared to nonalkylated salt-soluble proteins. Lanes **1–3,** SDS-glutelin extracted in the presence of 2% mercaptoethanol (ME), 0.05% dithiothreitol, and 2% ME with 6*M* urea, respectively; lane **4,** salt-soluble protein; lane **5,** PE-glutelin. Variety Fronica.

band, which appeared at 31 mm in the total zein sample, and a small amount of high molecular weight protein. The major RS-protein band is usually rather broad and diffuse whether the sample load is large (lane 3) or small (not shown).

When DTT was included in the initial salt buffer, the RS-protein was extracted in the salt-soluble protein fraction (Fig. 6, lanes 3 and 4, the band at 43 mm). The high molecular weight material present in the RS-protein (Fig. 5) was also extracted (26 mm). Small amounts of RS-protein were present in the zein fraction, especially if no reducing agent was used in the salt buffer (lane 5). This experiment illustrated two aspects of protein alkylation (Fig. 6). Most of the salt-soluble proteins were not recovered when the alkylation was performed with ME (lanes 2 and 4) rather than with DTT (lanes 1 and 3). However, the RS-protein was recovered in the presence of either reducing agent.

The results in Figs. 5 and 6 and the recoveries of N in the different fractions suggest that a proportion (up to 7% of the total recoverable N) of the endosperm protein is solubilized when a reducing agent is in the extraction solvent. After reduction, this fraction is soluble in both aqueous and aqueous-alcohol solvents. We therefore chose to call this the "reduced-soluble" protein. The amino acid composition of this fraction was determined (Table I); it corresponded with the protein termed "water-soluble alcohol-soluble reduced glutelin" (water-soluble ASG) by Paulis and Wall (1977). This protein is characterized by small amounts of lysine, aspartic acid, and alanine; by large amounts of histidine and proline; and by moderately large amounts of glutamic acid and cysteine. The proline content is particularly notable. The high histidine may contribute to the high mobility with electrophoresis at pH 3.1 (Paulis and Wall 1977). The differences between the two samples of RS-protein may be partially due to hydrolysis losses in one sample, but the two samples also show slightly different IEF profiles and may contain different amounts of other proteins with different amino acid compositions. The RS-protein amino acid levels are quite different from those of either the salt-soluble proteins or zein. In contrast, the low molecular weight minor zein bands have amino acid compositions with some characteristics of zein (Gianazza et al 1977).

The RS-protein can be traced in other references. The G_2 fraction

TABLE I
Amino Acid Composition[a] of Reduced-Soluble (RS) Protein, Water-Soluble Alcohol-Soluble Reduced Glutelin (ASG),[b] Zein,[c] and Albumins plus Globulins[c]

Amino Acid	RS-Protein M[d]	RS-Protein J[e]	Water-Soluble ASG	Zein	Albumin + Globulin
Lysine	0.4	0.1	0.3	0.1	5.2
Histidine	7.0	8.0	6.7	0.9	2.0
Ammonia			(17.4)		
Arginine	2.2	2.9	2.6	1.0	5.4
Aspartic acid	0.7	0.2	0.8	5.1	8.4
Threonine	4.3	1.7	4.4	2.9	5.0
Serine	4.3	0.3	4.8	6.0	5.6
Glutamic acid	16.5	18.5	13.2	20.7	12.0
Proline	23.4	28.1	26.3	10.8	7.1
Glycine	7.8	8.1	7.2	2.2	10.7
Alanine	5.3	6.2	6.5	13.7	10.7
(PE) Cysteine	3.7	···	···	0.3	0.7
Valine	6.4	9.1	8.7	4.4	7.0
Methionine	0.5	0.2	1.5	0.8	1.2
Isoleucine	2.9	2.3	2.0	4.0	4.3
Leucine	9.5	11.9	10.7	19.2	8.7
Tyrosine	3.1	0.9	2.8	3.2	2.6
Phenylalanine	2.0	1.4	1.6	4.9	3.4

[a] In μmoles/100 μmoles.
[b] From Paulis and Wall, 1977.
[c] From Sodek and Wilson, 1971.
[d] The sample run in lane 5 of Fig. 8 was assayed at Rothamsted Experimental Station.
[e] The sample run in lane 7 of Fig. 8 was assayed at the Agronomy Department, University of Illinois.

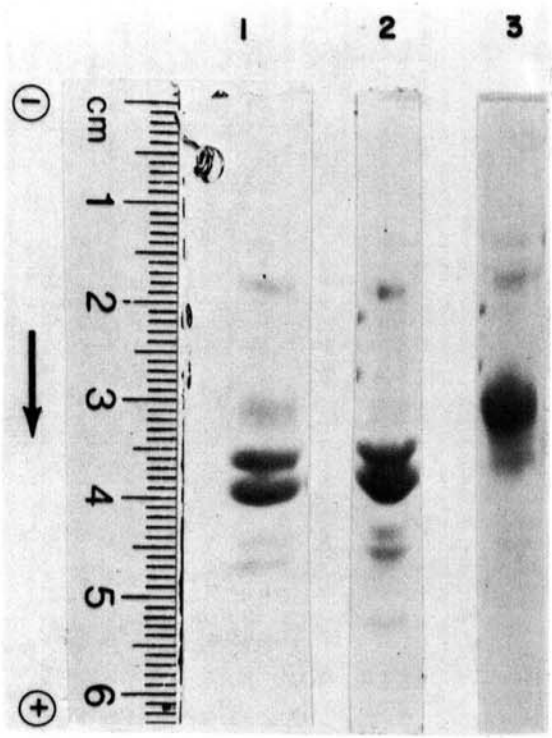

Fig. 5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of zein fractions. Lane **1,** total zein; lane **2,** zein precipitated by NaCl; and lane **3,** reduced-soluble protein remaining in solution after dialysis of total zein against water. Variety Fronica.

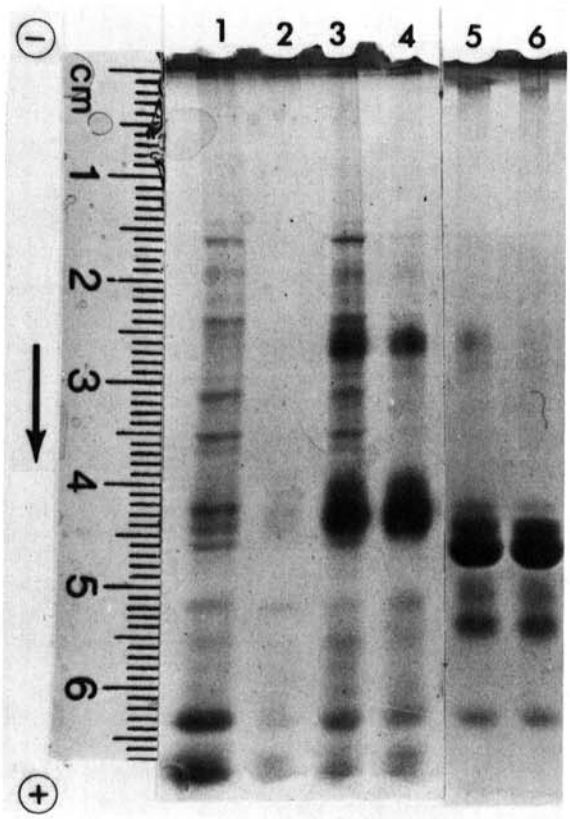

Fig. 6. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of salt-soluble and zein fractions. Lanes **1–4,** salt-soluble proteins—1 and 2 extracted without reducing agent, 3 and 4 extracted with 0.05% dithiothreitol (DTT); lanes **5** and **6,** water-precipitated zeins extracted from the samples for lanes 1 and 2 and for lanes 3 and 4, respectively. All samples alkylated, with mercaptoethanol used for samples for lanes 2 and 4–6 and DTT for lanes 1 and 3. Hybrid N28 × Oh7N.

isolated by Landry and Moureaux (1970) has an amino acid composition tending toward that of RS-protein. A G_2 fraction isolated by the Landry and Moureaux technique gave a band similar to that in Fig. 5 (Di Fonzo et al 1977). A small amount of RS-protein was also found in the zein-2 fraction. A G_2 fraction was isolated by Landry and Moureaux (1970) by following the usual salt extraction with an extraction of salt plus ME. Thus, this protein can be isolated without using alcohol. Fractions with amino acid compositions suggesting that the RS-protein is a major component (very high proline, high histidine, low lysine, and aspartic acid) have been isolated by various techniques by Christianson (1969), Jimenez (1968), and Popovic et al (1974).

Prolamins were originally defined as being alcohol-soluble and water-insoluble (Osborne 1908). Thus RS-protein should not be classified with the zeins. As Paulis and Wall (1977) noted, this protein would occur in the fraction originally termed glutelin. However, as far as maize is concerned, "glutelin fraction" should be considered a catch-all term for those proteins not previously extracted. As new extractants are used, and as new means to characterize proteins are developed, we should expect that one by one the proteins formerly classified as glutelins will be better characterized. We are using the new name of RS-protein because it is descriptive.

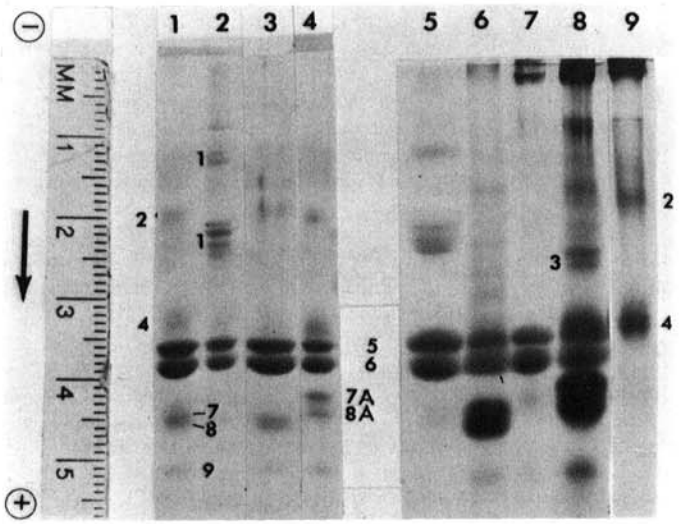

Fig. 7. Variable banding patterns (**1–9**) of different zein samples in sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Lane **1,** total zein; lane **2,** zein precipitated by dialysis and dissolved in SDS-sample buffer without mercaptoethanol; lane **3,** zein precipitated by dialysis; lane **4,** total PE-zein; lane **5,** zein-1; lane **6,** zein-2; lane **7,** PE-zein-1; lane **8,** PE-zein-2; lane **9,** PE-reduced-soluble protein. Lanes 1–4 were run in a 12.5% acrylamide gel, lanes 5–9 in a 17.5% acrylamide gel. Variety Fronica, except for hybrid N28 × Oh7N in lane 9.

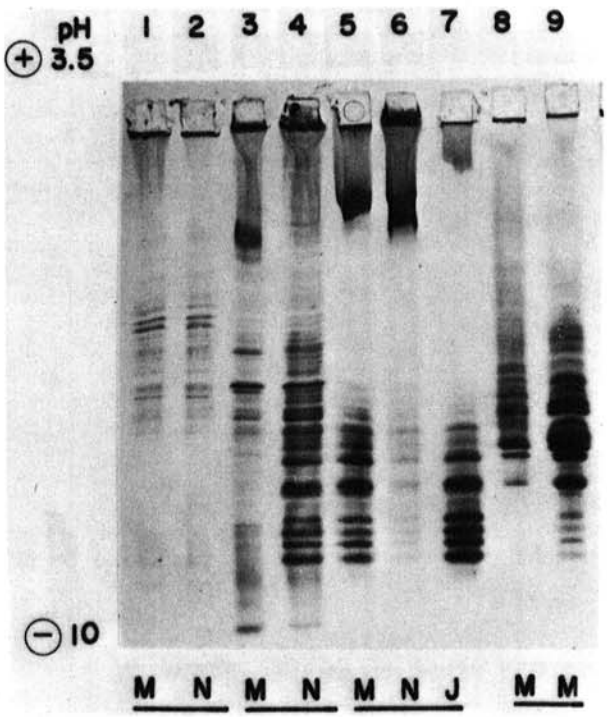

Fig. 8. Isoelectric focusing of four sequentially extracted protein fractions. The endosperms for samples marked **M** and **J** were extracted without reducing agent in the salt buffer; samples marked **N** were extracted with 0.05% dithiothreitol. Lanes **1** and **2,** PE-glutelin; lanes **3** and **4,** PE-salt-soluble protein; lanes **5–7,** PE-reduced-soluble protein; lane **8,** nonalkylated zein; lane **9,** PE-zein. Samples M and N, N28 × Oh7N; J, Fronica.

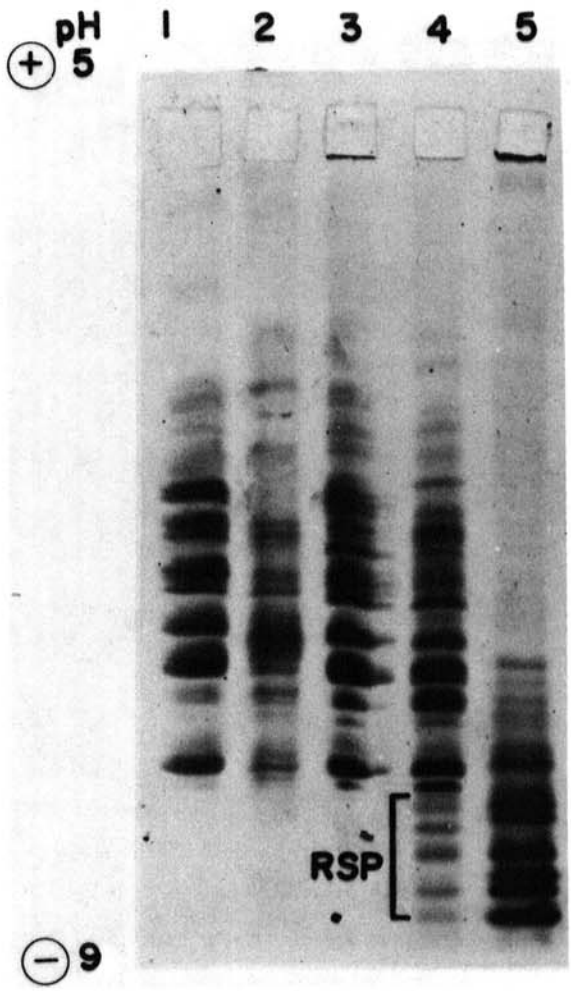

Fig. 9. Isoelectric focusing of zein-1, zein-2, and reduced-soluble protein (**RSP**). Lanes **1** and **3,** zein-1; lanes **2** and **4,** zein-2; lane **5,** RS-protein. Proteins in lanes 3–5 were alkylated. Variety Fronica.

SDS-PAGE Comparisons of Alcohol-Soluble Proteins

Different SDS-PAGE banding patterns produced by several zein and zeinlike fractions are illustrated in Fig. 7. Two sets of bands (band 1) were found in samples of total zein dissolved in sample buffer without ME (lane 2) or in a sample of zein-1 extracted from the endosperm without ME (lane 5). Landry (1979) isolated a "native" zein fraction with similar bands by extracting without a reducing agent and preparing the sample for SDS-PAGE without a reducing agent. Paulis and Wall (1979) suggested that these bands could represent native dimers among the different zein units. Our results suggested that the dimers may reform after extraction of the separated zein subunits. Whether dimers originally existed as such was not determined. Band 2 is present in a number of zein fractions

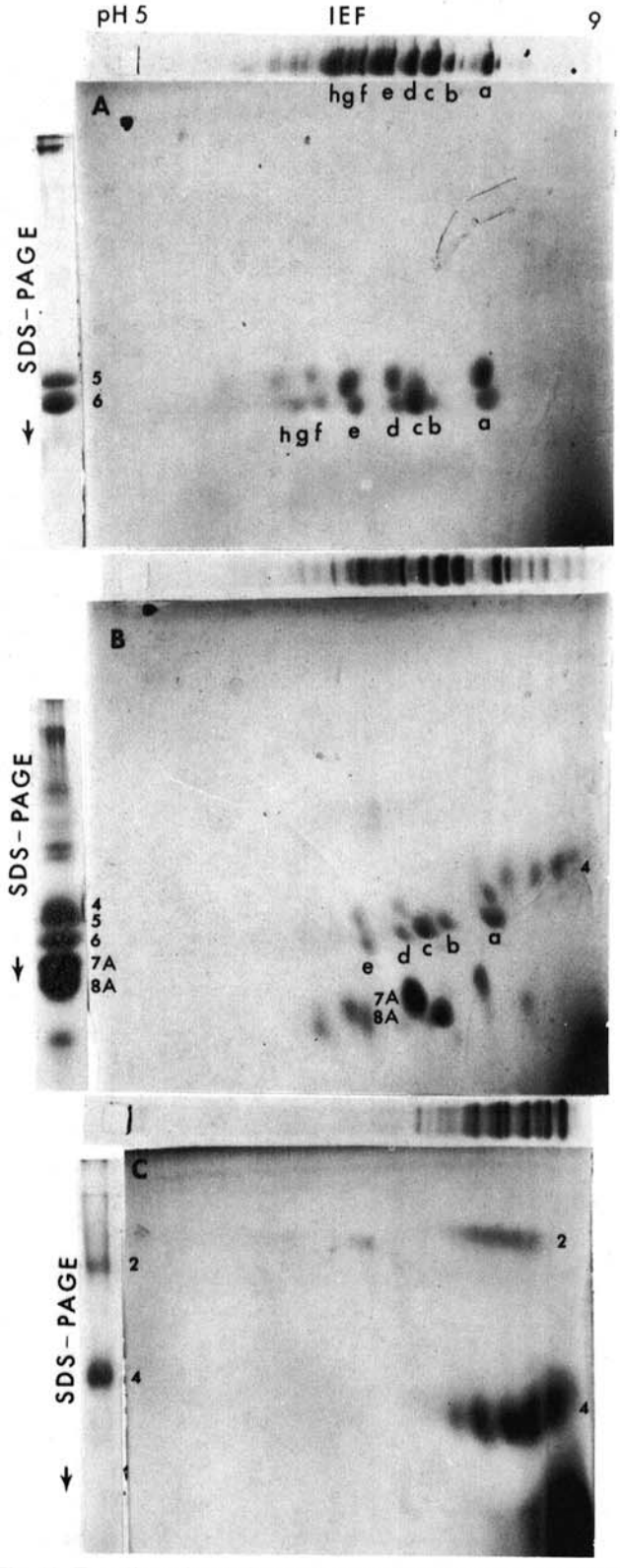

Fig. 10. Two-dimensional (2-D) separation of proteins in the total zein fraction. **A,** 2-D separation of Fronica PE-zein-1. First dimension, by isoelectric focusing (IEF), represented by lane 3 from Fig. 9, showing bands **a–h**. Second dimension, by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), represented by lane 7 from Fig. 7, showing bands **5** and **6**. **B,** 2-D separation of Fronica PE-zein-2. IEF represented by lane 4 of Fig. 9, showing bands **a–e**; SDS-PAGE by lane 8 of Fig. 7, showing bands **4, 7A,** and **8A**. **C,** 2-D separation of Fronica reduced-soluble protein. IEF represented by lane 5 of Fig. 9, SDS-PAGE by lane 9 of Fig. 7, showing bands **2** and **4**. The one-dimension samples were run in separate experiments and migration distances do not correspond directly to those in the 2-D gels.

(lanes 1, 3, 4) and is also present in the RS-protein fraction (lane 9), although sometimes in low amounts (Fig. 5, lane 3). The bands labeled 3 in the PE-zein-2 fraction (lane 8) have not been characterized. Band 4 is the major RS-protein, which may be seen in total zein and PE-zein fractions (lanes 1 and 4), in an alkylated zein-2 fraction (lane 8, overlapping the main zein band), and in the isolated water-soluble fraction from an alcoholic extract (lane 9). Bands 2 and 4 are present in lesser amounts in dialyzed zein (lane 3). Bands 5 and 6 are the major zein bands always found on SDS-PAGE when nonmutant varieties are examined. Bands 7 and 8 are two minor bands that were concentrated in the zein-2 fraction (lane 6) but were also seen in total and dialyzed zeins (lanes 1 and 3). Reducing agents appear to increase their extractability. They were sometimes poorly separated, except after alkylation (lane 4). Band 9 is another small minor component. Alkylation appears to have the greatest effect on the movement of bands 7 and 8, as can be seen by comparing these bands in lanes 3 and 4 to the ones in lanes 6–8.

The minor proteins (bands 2, 4, 7–9) have all been noted elsewhere (Lee et al 1976, Soave et al 1976), and some may be the major components of the alcohol-soluble fraction in some double mutants (Tsai et al 1978) or of the water-insoluble ASG (Paulis and Wall 1977). Some variability may be noted among these reports, and also among the figures in this paper, as to the relative mobilities, the assigned molecular weights, and the number of bands observed. A discontinuity in the relative mobility of small molecular weight polypeptides with SDS-PAGE is affected by changing the properties of the gel and the electrophoresis buffers (Swank and Munkres 1971). Gomes et al (1979) reported an unexpectedly high mobility for soybean trypsin inhibitor, which would otherwise be a good standard for zein. As Rodbard (1976) has pointed out, the determination of molecular "sizes" by SDS-PAGE requires a number of assumptions that are not always met in gel and sample preparation. Work in progress by one author[4] has found that different SDS-PAGE systems and different protein standards may give different apparent molecular weights for zein. This may account for the variations in literature values for zein (Di Fonzo et al 1977, Lee et al 1976, Paulis and Wall 1977).

IEF

The common identity of the PE-RS-protein found in the different fractions was confirmed by IEF (Fig. 8). Equal weights of RS-protein preparations recovered after dialysis of total zein fractions were added to lanes 5–7, but much less protein was recovered if the salt buffer had included a reducing agent (lane 6). The corresponding bands were also found in the salt-soluble protein fraction extracted with a reducing agent (lane 4). The RS-protein from Fronica and N28 × Oh7N have the same banding pattern and are the most basic proteins in all the samples. The RS-protein bands were not seen in the total zein fraction (lane 8) unless the sample was alkylated (lane 9). The RS-proteins may separate from the major zein bands only after their positive charge is increased by the addition of PE groups to sulfhydryl groups. This would explain their absence from previous IEF zein separations (Righetti et al 1977). Some of the bands seen by IEF could be artifacts produced by partial alkylation of the multiple sulfhydryl groups on the RS-protein, but our experience suggests that this is unlikely. They make up only a small portion of the total zein pattern. No RS-protein bands were detected in the glutelin fraction (lanes 1 and 2). The PE-glutelin and PE-salt-soluble proteins are distributed over the same range of isoelectric points, with a few apparent matches but with obvious differences in relative band intensities.

When the zein-1 fraction was isolated separately from the zein-2 fraction (Fig. 9), IEF showed greater differences between the two fractions if they were alkylated (lanes 3–4) than if they were not (lanes 1–2). The RS-proteins were visible in the zein-2 fraction only after alkylation (lane 4). However, the banding in the alkaline region of the zein-2 sample was slightly different from that in the RS-protein sample (lane 5).

[4]C. M. Wilson, unpublished data.

2-D Separations

The two major zein bands found by SDS-PAGE are represented by a much larger number of bands and spots when separated by IEF and 2-D analysis. The 2-D patterns are similar to those reported earlier (Miflin and Shewry 1979), with minor differences that may be due to varietal differences or extraction procedures. Zein-1 produced eight or more major bands by IEF alone (Fig. 9, lanes 1 and 3; Fig. 10A a–h). Some of the bands (Fig. 10A, a, d–f, and h) were then separated into two spots on the second dimension, although the smaller protein of each pair appeared to have a slightly more negative charge. At least 12 polypeptide spots were detected. When the zein-2 fraction was separated on a 2-D gel (Fig. 10B), eight of the same spots seen on the zein-1 gel were detected plus 10 spots seen only with zein-2. The low molecular weight bands (7A and 8A) produced two major spots but also several minor spots over a range of isoelectric points similar to that of bands 5 and 6. The RS-protein bands are visible at the alkaline end of the gel. The RS-protein bands 2 and 4 separated on the 2-D gel (Fig. 10C) into spots with similar ranges of isoelectric points.

Conclusions

The maize endosperm proteins in the different Osborne solubility classes may be further sudivided by the use of SDS-PAGE and IEF. The alcohol-soluble fraction contained two major groups of proteins clearly distinct from all other proteins on SDS-PAGE. However, this fraction contained minor amounts of other proteins, which gave varying patterns under different conditions. Another group of proteins, here designated RS-protein, can be located in several different fractions and is soluble in water, salt, and alcoholic solutions after reduction. The high cysteine content of this protein might allow it to take part in disulfide interchanges that could form the aggregates found in some zein preparations. Wall (1971) discussed some of the ways in which the formation of disulfide bonds affects analyses of cereal proteins.

Alkylation to produce PE-cysteine was effective in allowing sharp resolution of glutelins by SDS-PAGE and IEF, but problems occurred in trying to reduce, alkylate, extract, and separate all of the proteins extracted from maize endosperm. Under the alkylation conditions used here, DTT often produced better results than did ME.

SDS-PAGE and IEF of the salt-soluble protein and glutelin fractions consistently gave large numbers of bands with similar ranges of molecular weights and isoelectric points. At least some bands were common to both fractions. The protein present in the largest amount in the glutelin fraction was most readily extracted by urea.

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