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Recent Developments in Corn Protein Research

Jerrold W. Paulis

Corn grain consists predominantly of zein and other alcohol-soluble components that are deficient in lysine. Protein of high-lysine corns such as *opaque*-2 and *floury*-2 contains a smaller proportion of these alcohol-soluble proteins. Recent studies were carried out by our laboratory of the structure of zein and its genetic variations as means to identify various races and ancestral lines of corns. An alcohol-soluble fraction was isolated from the reduced glutelin and shown to be distinct from zein by amino acid analysis and electrophoresis. The increase in this lysine-deficient but methionine-rich alcohol-soluble glutelin fraction in su_1 lines enhances the methionine content of the grain—a desirable characteristic in feed formulations.

Corn is a principal source of food for millions of people, particularly in Latin America and Africa. It is an excellent source of carbohydrates, but its protein quality is relatively poor because it is deficient in the essential amino acids lysine and tryptophan. Mertz et al. (1964) showed that the endosperm of corn seeds homozygous for the *opaque*-2 (o_2) mutant gene had a higher lysine content than normal endosperm. Mutation at the o_2 locus dramatically alters the relative amounts of the different endosperm proteins, which vary in amino acid compositions. This finding that introduction of the o_2 mutant gene into corn improved the

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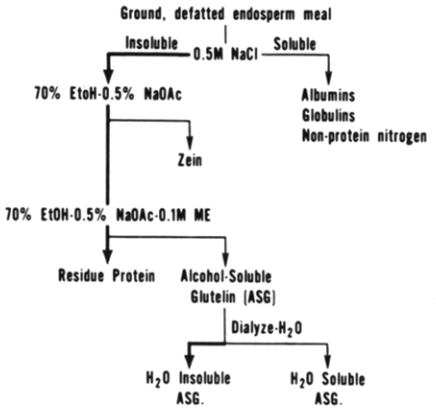


Figure 1. Procedure for preparation of some protein fractions from corn endosperm.

nutritional value of the proteins stimulated a large amount of work in the United States, Mexico, and other countries on the biochemical changes occurring upon introduction of this gene. Powerful and sensitive methods of isolation and characterization have been employed that are accelerating our knowledge of corn proteins. I will describe progress being made using these tools to characterize corn proteins and establish differences among them in various genotypes.

Osborne and Mendel (1914) developed a sequential extraction process that separates protein classes from a single corn sample based on solubility differences. First, extraction with water yields albumins and some globulins, since there is some salt in the meal. Aqueous (70%) ethanol solubilizes the zein, and, finally, dilute alkali is used to extract glutelin. In recent years this method has been extended by us (Figure 1) and others (Landry and Moureaux, 1970) to improve extraction efficiency and to further fractionate the complex glutelin fraction.

Zein is classified as a prolamin, a major class of storage protein of corn accounting for about 50% of the total endosperm protein. Since the discovery that the high-lysine mutant o_2 corn was dificient in zein (Mossé, 1966), considerable interest has arisen in better elucidation of zein's structure (Ganchev et al., 1976; Gianazza et al., 1977; Paulis, 1981), function (Sodek and Wilson, 1973; Harvey and Oaks, 1974), and synthesis (Burr and Burr, 1976; Larkins et al., 1976). This information could provide keys to the genetics of regulation of zein production in corn. In this paper I describe recent studies by our laboratory of the structure of zein and its genetic variations as means to identify various races and ancestral lines of corns. A type of protein thought by many researchers (Gianazza et al., 1976; Nucca et al., 1978; Soave et al., 1976, 1978; Tsai et al., 1978a,b) also to be zein, and, therefore, combined with zein in aqueous alcohol–mercaptoethanol extracts, will be shown to mainly differ from zein by several biochemical methods of analysis. Inclusion of this protein with the zein extracts can result in misinterpretation of results in genetic, taxonomic, or structure studies involving zeins. Accordingly, we have classified these proteins as components of glutelin, termed alcohol-soluble reduced glutelin (ASG), and have studied it as a separate class of proteins.

PROPERTIES AND COMPOSITION OF ZEIN

Prolamins have a low content of ionized amino acids, and their amino acid compositions are characteristically high in nonpolar amino acids and glutamine. Hydrolysates of zein, the prolamin fraction of corn protein, extracted with 70% ethanol–0.5% NaOAc, are devoid of lysine and tryptophan and contain much higher levels of leucine, proline, and glutamic acid than the other corn protein fractions (Table I). The glutamic and aspartic acids in

Table I. Amino Acid Composition of Corn Protein Fractions

	g/100 g of protein			
amino acid	albumins[a]	globulins[a]	zein[b]	glutelin[a]
lysine	5.6	5.3	0.2	3.1
histidine	2.2	3.4	4.3	3.3
ammonia	1.4	1.4	3.4	3.4
arginine	6.7	11.2	2.1	4.9
aspartic acid	8.4	6.7	4.8	7.2
threonine	4.5	2.8	3.4	4.2
serine	4.2	4.6	5.5	4.9
glutamic acid	11.1	14.7	26.5	19.1
proline	4.3	3.2	8.1	8.7
glycine	5.3	4.2	2.5	4.6
alanine	6.0	4.3	8.9	6.8
valine	5.0	4.9	3.6	5.2
methionine	1.3	0.9	1.5	3.7
isoleucine	3.2	2.6	3.9	4.2
leucine	5.6	5.1	19.4	11.4
tyrosine	3.1	2.8	5.8	5.4
phenylalanine	3.1	4.1	7.1	4.0

[a] From Paulis and Wall (1969). [b] From Paulis et al. (1969).

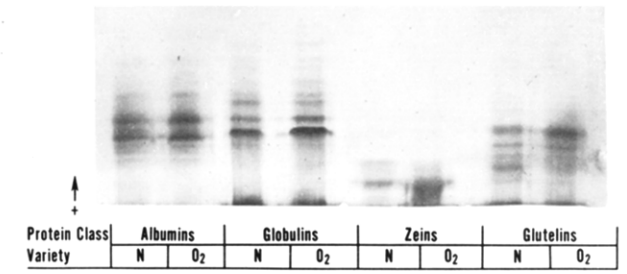


Figure 2. Starch gel electrophoretic (SGE) patterns of reduced and alkylated proteins [from Paulis et al. (1969)].

the hydrolysates are primarily from glutamine and asparagine, respectively, as evidenced by large amounts of ammonia. In contrast, the albumins and globulins are high in content of lysine and arginine. The glutelins are intermediate in amino acid composition between the zeins and the albumins and globulins.

After the disulfide bonds in the protein fractions of corn are reduced and the sulfhydryls are alkylated (A–R), the different protein fractions migrate at different rates in pH 3.1, aluminum lactate buffer during starch gel electrophoresis (SGE) (Figure 2). Zein, because of its much lower amount of ionizable amino acids, migrates more slowly than the albumins and globulins. Some of the glutelin fractions migrate at rates similar to globulins while others are slow moving like zein. No major differences can be seen in mobilities of the protein fraction from high-lysine and normal corn genotypes.

Turner et al. (1965) demonstrated than when zein was fractionated with 95% ethanol, the native soluble α zein separated into four distinct bands by SGE whereas the native insoluble β zein remained at the origin. Apparently, β zein is identical with the high molecular weight fraction of zein. After disulfide reduction, all zein subunits can migrate into a gel upon electrophoresis, giving evidence that disulfide bond cleavage reduces molecular size of the β zein.

In sodium dodecyl sulfate–polyacrylamide electrophoretic patterns of zein and its α and β fractions, the unreduced (native) whole and its α component consist mainly of M_r 45 000 and 68 000 bands, with a prominent band at M_r 24 000 (Figure 3). Native β zein does not migrate into the gel during electrophoresis. After reduction, α and β zeins exhibit both M_r 22 000 and 24 000 subunits. Data presented by Ganchev et al. (1976) suggest that the M_r

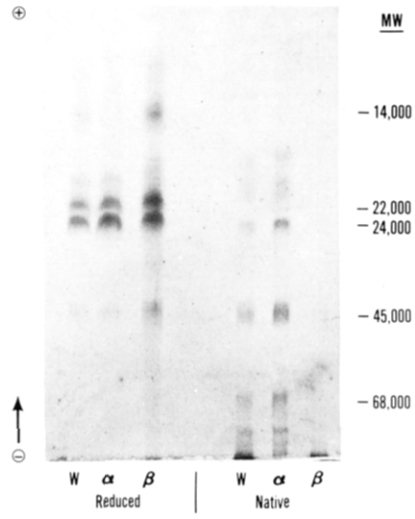


Figure 3. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis patterns of native and reduced whole zein (W), α zein (α), and β zein (β). The origin is at the bottom (Paulis, 1981).

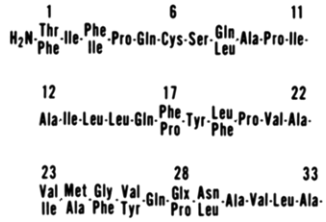


Figure 4. Amino-terminal amino acid sequence of whole pyridylethylated zein isolated from normal corn endosperm [from Bietz et al. (1979)].

22 000 subunits may exist only in intermolecular disulfide-linked chains in native zein as oligomers in the 45 000, 68 000, and higher molecular weight bands.

Native whole and α zeins exhibit about nine components by polyacrylamide gel electrophoresis in 8 M urea–aluminum lactate, pH 3.1 (Paulis, 1981). Most of the β fractions do not enter the gel but streak from the origin, probably due to high molecular weight and poor solubility. After reduction, whole and β zeins migrated completely into the gel. The streaked polyacrylamide gel electrophoretic portion of native zeins may be due to disulfide-linked oligomers containing M_r 22 000 and 24 000 subunits.

Isoelectric focusing (IEF) on polyacrylamide gels resolves 15 A–R zeins compared to about 9 by polyacrylamide gel electrophoresis (Paulis, 1981). Faint bands in native whole and α zeins appear at the same mobilities corresponding to the A–R forms. Again, native β zein does not migrate into the gel until it is reduced in size by breaking disulfide bonds.

The different zein subunits are mainly homologous in structure as shown by similar N-terminal amino acid sequences (Figure 4). Zein's very hydrophobic N-terminal amino acid sequence is consistent with its solubility. Of the major amino acids identified in the first 33 positions, 20 (60.6%) are hydrophobic (alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, and methionine) (Bietz et al., 1979).

In zeins from two normal corn hybrids, amino acid sequences were similar, but two significant differences occurred; the second zein lacked the Phe at position 1 and had a Pro/Gln substitution at position 16. Six minor differences in sequence were noted between normal zein and its near-isogenic o_2 counterpart, but otherwise the protein fractions were identical. These data suggest that

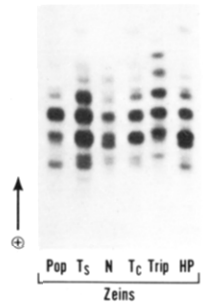


Figure 5. Polyacrylamide gel electrophoresis of zeins from the following grains: Pop = argentine popcorn; T_S = teosinte El Salada; N = normal (PAG SX52 hybrid) corn; T_C = teosinte Chalco; Trip = *Tripsacum dactyloides* (tetraploid); HP = Illinois high protein corn. The origin is at the bottom [from Paulis and Wall (1977b)].

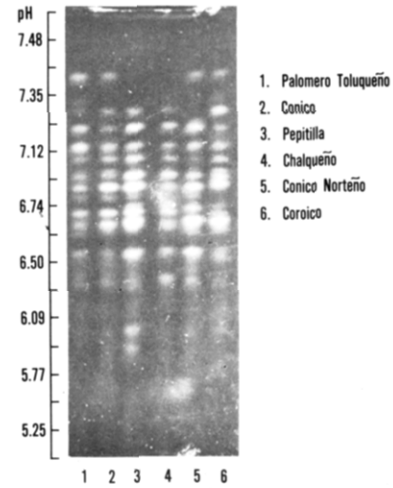


Figure 6. Isoelectric focusing (IEF) patterns on a polyacrylamide gel slab of reduced and alkylated zein extracts from ground endosperm meals of single kernels from selected races of corn [Paulis and Wall (1979b)].

zein polypeptides chains of M_r 21 000 and 23 000 have similar amino-terminal sequences and that the M_r 23 000 chains have internal insertions or a C-terminal extension of about 20 amino acid residues.

ZEIN AS MARKERS FOR CORNS

The polyacrylamide gel electrophoretic zein spectra of mutants are specific in terms of the number and composition of the components as well as the general structure of the spectrum (Yamaleyeva and Kissel, 1973). The specificity of the electrophoretic spectrum of the zein of endosperm mutants and their derivatives (double recessives) makes it possible to use them in the identification and genetic analysis of original and selectively bred material. Zein polypeptides as separated by isoelectric focusing (IEF) from 50 inbreds lines of corn show that the obtained grouping of the inbreds agrees closely with the already known genetic relationship among the genotypes (Nucca et al., 1978). On the basis of these results, IEF patterns of zein were suggested as aids in taxonomic studies of corn strains or varieties.

So that evolutionary and genetic relationships could be established, the proteins of several corns were compared to those of two wild relatives, teosinte and *Tripsacum* (Paulis and Wall, 1977b). Polyacrylamide gel patterns of zein polypeptides of the teosintes were similar to those of their counterparts in the corns but those of *Tripsacum* showed marked differences (Figure 5). These data support other evidence that teosinte is closely related to corn and

Table II. Amounts of Alcohol-Soluble Protein Subunits in Reduced Glutelins from Normal and *Opaque*-2 Maize[a]

		N	O_2
(1)	amount of glutelin, % of total protein	37.5	46.2
(2)	alcohol-soluble protein subunits in reduced glutelin, %	17.8	7.8
(3)	alcohol-soluble glutelin in total protein, % (1 × 2)	6.7	3.6
(4)	zein in total protein, %	45.8	24.4
(5)	zein + alcohol-soluble glutelin subunits, % of total protein (3 + 4)	52.8	28.7

[a] From Paulis et al. (1969).

may be its direct ancestor, whereas tripsacum is less closely related.

Figure 6 shows that zein polypeptides from ground endosperm meals of single kernels of various races of corn exhibit sufficient differences in the IEF pattern to fulfill the conditions for being considered almost perfect traits for racial classification (Paulis and Wall, 1979a). Detailed studies of IEF patterns of zeins from the corn races might be useful in following their introgression into the present hydrids.

ALCOHOL-SOLUBLE REDUCED GLUTELIN (ASG) VS. ZEIN

In an effort to study the residual glutelin after albumins, globulins, and zein proteins were removed from core endosperm meal, it was found that the addition of mercaptoethanol to an 70% ethanol–0.5% sodium acetate solvent removed protein from normal and an *opaque*-2 counterpart corn (Table II) (Paulis et al., 1969). This protein was thought to be zein-like in solubility, but later it was termed alcohol-soluble reduced glutelin (ASG) based on the definition that all proteins remaining after removal of salt and alcohol protein were glutelins (Paulis and Wall, 1971). In normal corn, ASG accounts for about 20% of the glutelin or 8% of total protein; about half this yield was obtained from *opaque*-2 corn.

The observation that an alcohol-soluble fraction of glutelin (ASG) can be extracted from reduced glutelin was also made independently by Landry and Moureaux (1970). They referred to this fraction as G_1. Differences between normal and *opaque*-2 corn in amounts of ASG subunits were confirmed by Sodek and Wilson (1971) who referred to this fraction as zein-2.

SGE patterns of alkylated–reduced (A–R) glutelin showed an absence of lower migrating bands after extraction of the protein with 70% ethanol (Paulis et al., 1969). This slow-migrating protein had a mobility similar to that of A–R zein. High-lysine SGE pattern of A–R glutelin contained less of this slower migrating protein than did normal corn.

Later, a comparison of SGE patterns of different A–R glutelin preparations showed that the ethanol-extracted product yielded about four slow-moving electrophoretic bands that corresponded in mobility to that of A–R zein and two bands having greater mobility (Paulis and Wall, 1971). The 70% ethanol insoluble A–R glutelin exhibited bands with more rapid migration than those of the zeins.

The ethanol-soluble fraction of A–R glutelin was much different in amino acid composition than the ethanol-insoluble A–R glutelin (AIG) (Paulis and Wall, 1971). The ASG contained much less lysine, arginine, aspartic acid, and glycine and more proline, leucine, methionine, and half-cystine than the AIG. It was also not identical to A–R zein as evidenced by its higher content of histidine, arginine, proline, glycine, methionine, and half-cystine and its lower amount of aspartic acid, alanine, and leucine (Table III). The presence of 3 times more half-cystine in the ASG than in A–R zein may account for a high degree of disulfide cross-linking, which could render the protein insoluble in 70% ethanol before disulfide cleavage as compared to native zein's solubility in 70% ethanol.

Table III. Amino Acid Composition of Alcohol-Soluble Reduced Glutelin Preparations and Zein[a]

	g/100 g of 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

[a] From Paulis and Wall (1977a).

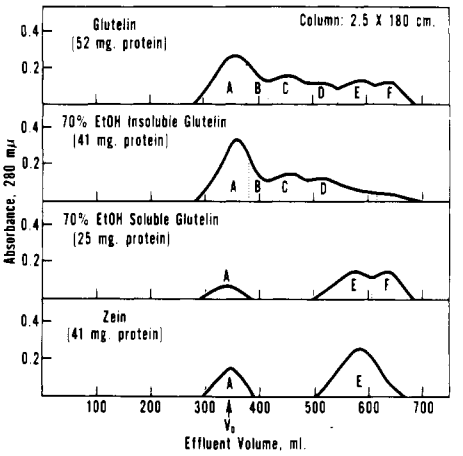


Figure 7. Effect of 70% ethanol extraction of alkylated–reduced glutelin on Sephadex G-200 chromatography [Paulis and Wall (1971)].

When A–R glutelin is passed through two columns of Sephadex G-200 in 6 M guanidine hydrochloride, it is resolved into six components (Figure 7). In this solvent, the ASG fraction of A–R glutelin elutes like the last two fractions, corresponding to molecular weights of 25 000 and 17 500. The second to the last fraction, peak E (M_r 25 000) corresponds to the elution position of A–R zein (Figure 7).

My colleagues at the Northern Center asked how ASG exists in the microscopic structure of the glutelin matrix. Wolf (1973) made an interesting observation that some parts of protein bodies, especially near the core, stain darker with uranyl acetate (Figure 8). When he extracted endosperm tissue with 70% ethanol, most of the ground proteins in the bodies (pb) were removed but the core and other dark-staining materials remained. When similar sections were extracted with 2-mercaptoethanol (ME) in 70% ethanol, the core material was solubilized and removed. It is probable that at least a portion of ASG has

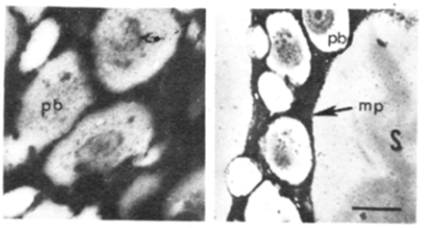


Figure 8. Transmission microscopic view of the protein bodies of corn endosperm (courtesy of Dr. M. Wolf, formerly of Northern Regional Research Center, U.S. Department of Agriculture).

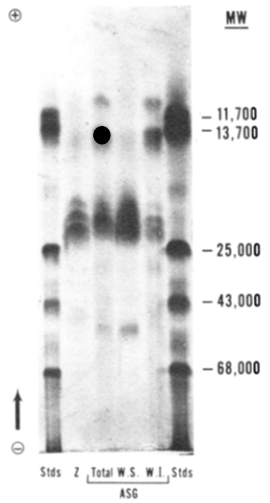


Figure 9. $NaDodSO_4$–polyacrylamide gel electrophoretic patterns: (1) 6 M_r (MW in figure) calibration mixture; (2) zein; (3) whole alcohol-soluble reduced glutelin (ASG); (4) water-soluble ASG; (5) water-insoluble ASG. The origin is at the bottom [from Paulis and Wall (1977a)].

its origin in the protein bodies, as does zein.

ALCOHOL-SOLUBLE REDUCED GLUTELIN

The procedure for preparation of ASG fractions from corn endosperm is given in Figure 1. Fractionation of ASG by dialysis against water produced a soluble and insoluble protein that represented 7% and 13% of the total protein (Paulis and Wall, 1977a). The remaining residue or AIG represented 35% of the total protein.

Fractionation of ASG by dialysis against water produced a soluble protein that represented 33% of the total ASG. Except for containing a small amount of lysine, this protein has an amino acid composition different from total ASG, zein, or water-insoluble ASG (Table III). The biggest differences were in its high histidine, proline, and valine contents and low aspartic acid, alanine, tyrosine, phenylalanine, and methionine. The water-soluble ASG's dual water and alcohol solubilities may be due to its high content of histidine and proline residues. The histidine residues may be exposed in water, but in alcohol the conformation of the molecule could be changed to expose more proline groups.

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 III). There is more than 4 times as much methionine in this fraction as in zein.

The $NaDodSO_4$–polyacrylamide gel electrophoretic patterns of zein show principally three bands in the M_r 22 000–24 000 region (Figure 9). Water-soluble ASG has prominent bands also in this same mobility range. The water-insoluble ASG has lower migrating bands at ca. 9500

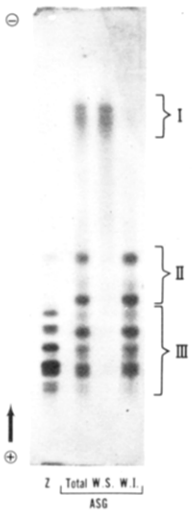


Figure 10. Polyacrylamide gel electrophoretic patterns of (1) zein, (2) whole alcohol-soluble reduced glutelin (ASG), (3) water-soluble ASG, and (4) water-insoluble ASG. The origin is at the bottom [from Paulis and Wall (1977)].

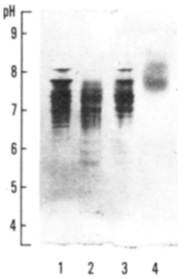


Figure 11. IEF patterns at pH 3–11 of (1) zein, (2) whole alcohol-soluble reduced glutelin (ASG), (3) water-insoluble ASG, and (4) water-soluble ASG.

and 13 500. The molecular weight range of water-soluble and -insoluble ASG corresponds to M_r 17 500 and 25 000 of peaks E and F determined from elution positions of ASG off Sephadex G-200 (Figure 7).

Polyacrylamide gel electrophoresis of the ASG fraction in aluminum lactate–8 M urea, pH 3.1 buffer (Figure 10) gave patterns showing different relationships than the $NaDodSO_4$–polyacrylamide gel electrophoretic patterns. The pattern of water-soluble ASG in polyacrylamide gel electrophoresis 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 the 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.

IEF patterns of the water-soluble and -insoluble ASG fractions (Figure 11) demonstrate that the soluble component's higher mobility bands in polyacrylamide gel electrophoresis (Figure 10) are due to their more basic range of isoelectric points, pH 8–9 vs. pH 7–8, respectively. The more basic charge of the water-soluble compared to that of water-insoluble components is probably due to the larger amount of histidine (Table III).

The low molecular weight region 9500–13 500) of the high-methionine, water-insoluble ASG was found to resist amino acid sequence analysis and apparently has a blocked N terminus (Bietz, 1980). The higher molecular weight (20 000–24 000), water-soluble ASG had an amino acid sequence that showed it to be a heterogeneous mixture of

```
   1  2  3  4  5  6  7  8  9  10 11
H2N- -His-    -Gly-Gly-  -Gly-  -Ser-Pro-

   12 13 14 15 16 17
Pro-Pro-Pro-Val-His-Leu-

   18 19 20 21 22 23
Pro-Pro-Pro-Val-His-Leu-

   24 25 26 27 28 29
Pro-Pro-Pro-Val-His-Leu-

   30 31 32 33 34 35
Pro-Pro-Pro-Val-His-Leu-

   36 37 38 39 40 41
Pro-Pro-Pro-Val-His-Leu-
```

Sample provided by Dr. A.Esen, Virginia Polytech University.

Figure 12. Amino-terminal amino acid sequence of pyridylethylated fraction 4 of ASG (courtesy of J. Bietz, Northern Regional Research Center, U.S. Department of Agriculture).

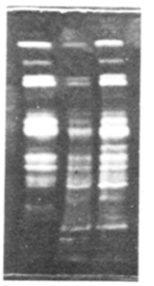
residues with a predominant amino acid arrangement similar to that of a purified ASG fraction isolated by Dr. A. Esen (Virginia Polytechnic Institute). The purified polypeptide fractions of water-soluble ASG have a highly unusual sequence of amino acid residues (Figure 12). Following an initial N-terminal sequence of 11 residues, a six-residue sequence containing three successive prolines was found which repeated 6 or more times.

Increases in the amount and methionine content of ASG contributed to the increased methionine in *sugary*-1 (su_1) corn endosperm (Paulis et al., 1978). The endosperm protein of B37 inbred had 36% more methionine in the su_1 strain than did the protein from the normal, and protein from the Oh 43 su_1 had 21% more methionine than did protein from its normal allelic sib. It has been suggested by Melcher and Fraij (1980) that production of specific storage proteins rich in sulfur amino acids in seeds may be an adaptation that allows them to survive variations in levels of available sulfur during germination.

The high level of proline in water-soluble ASG (Table III) may serve as a source for this amino acid, which has been shown to be needed for seed development (Gavazzi et al., 1975; Racchi et al., 1978). Studies have also revealed that proline formation by corn under salts stress (NaCl; KCl; decreased water potential) was dependent on sufficient oxygen supply and carbohydrates (Goering et al., 1978).

CLASSIFICATION OF ASG

Since the simultaneous discovery of ASG by Paulis et al. (1969) and Landry and Moureaux (1970), the protein fraction has been designated zein-like by others (Gianazza et al., 1976; Nucca et al., 1978; Soave et al., 1976, 1978; Tsai et al., 1978a,b) and even referred to as zein-2 (Sodek and Wilson, 1971). Because it is termed zein-like and is easily removed with alcohol and ME, both zein and ASG have been extracted simultaneously with this solvent mixture. The ASG fraction has been shown by us (Paulis et al., 1969, 1975, 1978; Paulis and Wall, 1971, 1977a) and others (Landry and Moureaux, 1970; Sodek and Wilson, 1971) to represent ca. 12–27% of the total protein and thereby to increase extraction yields of alcohol-soluble proteins from corn endosperm to ca. 60% of the total protein. Because of ease of extraction and increased yields of more zein-like protein, the aqueous alcohol–ME solvent mixture extract has been used in IEF to study gene loci involved in zein synthesis (Soave et al., 1978), taxonomic studies in corn breeding (Nucca et al., 1978), and zein polypeptide differences in normal and *opaque*-2 endosperms (Gianazza et al., 1976). The solvent mixture also has been used to evaluate the relationship of zein content with yield potential for corn (Tsai et al., 1978a) and to compare Na-



1 2 3

Figure 13. IEF patterns at pH 6–9 of (1) zein, (2) whole ASG, and (3) water-insoluble ASG.

$DodSO_4$–polyacrylamide gel electrophoretic patterns of reduced zeins in *opaque*-2 with starch-modifying mutant genes (Tsai et al., 1978b).

Typical IEF patterns for zein and ASG subunits by the system used by Soave et al. (1978) for zein synthesis studies are shown in Figure 13. Zein, total ASG, and water-insoluble ASG have similar IEF patterns. Therefore, if ME is used in the alcoholic solution, the mixture of ASG and zein will give the same pattern as zein. It is also easy to mistake ASG for zein since part of its components in polyacrylamide gel electrophoresis (Figure 10) and $NaDodSO_4$–polyacrylamide gel electrophoresis (Figure 9) resembles zein. The error arises when each electrophoretic method's patterns are used independently as criteria for identifying zein. $NaDodSO_4$–polyacrylamide gel electrophoresis show water-soluble ASG has a molecular weight close to that of zein (ca. 25 000–24 000), whereas it is uniquely different from zein by polyacrylamide gel electrophoresis and IEF. The water-insoluble ASG polypeptides have completely opposite electrophoretic characteristics: mainly different from zein by $NaDodSO_4$–polyacrylamide gel electrophoresis (Figure 9) and similar by polyacrylamide gel electrophoresis (Figure 10) and IEF (Figure 13). Comparing both the amino acid composition of ASG and its fractions to that the zein (Table III) and the amino acids sequence of water-soluble ASG (Figure 12) to that of zein (Figure 4) definitely shows dissimilarity. Evidence that the water-insoluble ASG lower molecular weight region (9500–13 500) cannot be N-terminal amino acid sequenced provides further evidence that it is mainly different from zein. Since all these data show that ASG and zein are mostly unique from each other, it would be wrong to base conclusions concerning zein genetics on studies employing IEF patterns of aqueous alcohol–ME extracts. Both ASG fractions and zein have to be studied independently in order to determine any meaningful information characteristic of the specific protein class. The ASG subunits are mainly a unique fraction of proteins that are insoluble in alcohol until their disulfide bonds are broken and, therefore, cannot be regarded as part of zein, which appears by N-terminal sequence analysis to consist of closely homologous subunits (Figure 4).

The possible combinations of ASG polypeptides linked in situ by disulfide bonds (–S–S–) are several. Either water-soluble and -insoluble ASG components could be –S–S– bonded only to similar subunits or both types could be combined intermolecularly. One or both of these proteins are probably located in the core portion of the protein body. In every case, the ASG fraction has been found totally insoluble in aqueous alcohol, 8 M urea, 6 M guanidine hydrochloride, and $NaDodSO_4$ until the –S–S– bonds are broken and thus, to be studied in the native state, will have to be isolated as particulates.

The original basis of classification of cereal proteins by Osborne and Mendel (1914) was solubility. This basis does

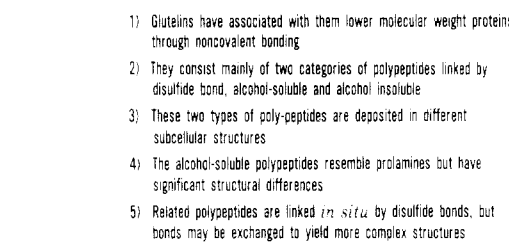


Figure 14. Corn glutelins—general thesis (courtesy of J. Wall, Northern Regional Research Center, U.S. Department of Agriculture).

not stand up as a criterion for protein class identification with present techniques for isolation and characterization. The ASG proteins described in this paper are differentiated from zeins by several of the present methods shown by us for characterization. More appropriately, any proteins insoluble after salt and aqueous alcohol extractions are corn glutelins as described by principles listed in Figure 14. Protein fractions soluble in an aqueous mixture of alcohol and reducing agent have been identified also in wheat (Bietz and Wall, 1973), sorghum (Jambunathan and Mertz, 1973), and barley (Lontie and Voets, 1959) and would also fall in the glutelin category (Figure 14). No significant structural differences have been found for sorghum ASG compared to the sorghum prolamin kafirin (Paulis and Wall, 1979b).

CONCLUSION

Our exploration of the different solubility classes of corn endosperm proteins by means of improved fractionation methods has established that each class is heterogeneous. Zein exhibits many components by isoelectric focusing or electrophoresis. Each zein component is coded genetically, and so these IEF and polyacrylamide gel electrophoretic patterns are useful keys to the geneology of inbred lines and are useful tracers in evolutionary development. The glutelin components are more complex in origin and composition. An elevation in the total amount of glutelin and the predominant alcohol-insoluble fraction in reduced glutelin is responsible for increased lysine in *opaque*-2 corn. An alcohol-soluble fraction was isolated from the reduced glutelin and shown to be mostly different from zein by amino acid analysis and electrophoresis. The increase in this lysine-deficient but methionine-rich alcohol-soluble glutelin fraction in su_1 lines enhances the methionine content of the grain—a desirable characteristic in feed formulations. Our studies on corn proteins suggest that we can manipulate their composition to attain desired nutritional benefits with suitable mutant genetic lines. However, many of these endosperm mutations cause extensive changes in kernel composition and properties. Additional work is therefore necessary to further understand the genetic control of protein synthesis in corn if optimal nutritional value is to be coupled with good grain yield and kernel structure.

There are several future research objectives for corn genetics and biochemistry. There is need for total sequence analysis to establish the precise nature of differences in these proteins. Additional work on the mechanism of biosynthesis is required in order to establish the mechanism whereby mutant genes such as o_2 and fl_2 regulate protein synthesis. Possible genetic engineering may eventually be used to produce more nutritious protein.

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