Quantitation and Distribution of γ-Zein in the Endosperm of Maize Kernels

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ABSTRACT

Changes in the content of γ-zein (M_r 27,000) in the endosperm of normal, opaque-2, floury-2, and modified opaque-2 maize varieties known as quality protein maize (QPM) were evaluated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, protein fractionation schemes, and an immunoabsorbance assay. QPM genotypes, which combine high levels of lysine and tryptophan with high yields, traditional appearance, and conventional kernel hardness, were shown to contain two to five times more γ-zein than normal, opaque-2, or floury-2 genotypes. Both soft and hard regions of QPM endosperm are enriched in γ-zein; the latter show higher levels of α-zeins. Relationships between amounts of zeins, kernel hardness, nutritional quality, and changes in other major protein components are discussed.

Maize prolamins, zeins, constitute a family of proteins that are soluble in aqueous alcohol solutions containing a reducing agent. Zeins are the major type of protein in the corn kernel, accounting for 50–60% of the total seed protein (Wilson 1983).

Researchers have used different procedures for isolating zeins, and this has resulted in a complex system of nomenclature. Esen (1987) proposed a nomenclature for zeins in which they are separated into three distinct classes (α, β, and γ) on the basis of their solubility in 2-propanol in the presence or absence of a reducing agent. In this system, γ-zein is made up of two proteins, a M_r 27,000 proline-rich polypeptide and a structurally related M_r 16,000 protein (Prat et al 1987). The M_r 27,000 γ-zein has also been referred to as reduced soluble protein (Wilson et al 1981); water-soluble, alcohol-soluble reduced glutelin (wsASG) (Paulis and Wall 1977); and glutelin-2 (Landry and Moureaux 1970). γ-Zeins account for approximately 5–10% of the total zein in normal maize and require a reducing agent for extraction (Esen 1986). They are distributed at the periphery of protein bodies (Lending et al 1988, Lending and Larkins 1989) and have a high content of cysteine and histidine (Gianazza et al 1977). Although charge variants of the M_r 27,000 γ-zein have been isolated, the protein is probably a single species (Esen et al 1982, Prat et al 1985, Wang and Esen 1986).

Zeins are deficient in nutritionally important amino acids, such as lysine and tryptophan, and this causes traditional maize varieties to be poor in protein quality. Mutations, such as opaque-2 and floury-2, decrease zein synthesis, thereby increasing the percentage of lysine and tryptophan (Mertz et al 1964, Nelson et al 1965, Nelson 1969, Paulis et al 1969, Jones et al 1977, Pedersen et al 1980). Although opaque-2 cultivars are twice as nutritious as normal maize, they have not been accepted by farmers because they have a soft, chalky endosperm texture, which results in lower yields due to decreased kernel density (Larkins et al 1982). The opaque-2 mutation also causes a higher susceptibility of the developing ear to diseases and insect damage (National Research Council 1988).

Different approaches have been used by maize breeders to improve the agronomic quality of opaque-2 genotypes. By using recurrent selection for opaque-2 modifier genes, breeders at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico converted opaque-2 maize into varieties that have high nutritional quality, high yields, traditional appearance, and conventional hardness (Vasal 1975, Vasal et al 1980). These modified opaque-2s are called quality protein maize (QPM). The lysine content of two QPM populations was found to be intermediate between those of normal and soft opaque-2 maize endosperm (Ortega and Bates 1983). Protein fractionation studies using a modified Landry-Moureaux procedure (Landry and Moureaux 1970) demonstrated that QPM genotypes are higher in fraction III protein (Gentinetta et al 1975, Ortega and Bates 1983). In the above-modified Landry-Moureaux procedure, fraction III is made up of proteins extracted using 70% isopropanol plus 0.6% 2-mercaptoethanol (2-ME) and contains proteins referred to as zeinlike proteins. Wallace et al (1990) presented data showing two to four times more γ-zein in QPM than in opaque-2 and normal maize varieties.

To further characterize qualitative and quantitative differences in zein content in normal, opaque-2, and QPM genotypes, we used sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and enzyme-linked immunosorbent assays (ELISA) to characterize the distribution of zeins in the endosperm of the three genotypes. We have also characterized the distribution of zeins in the hard and soft regions of mature endosperm of these genotypes.

MATERIALS AND METHODS

The sample analyzed consisted of a normal maize genotype, W64A; its opaque-2 and floury-2 versions, W64Ao2 and W64Afl2, respectively; and three QPM genotypes, Blanco Dentado, Yellow Flint, and Pool 25. The QPM genotypes were provided by CIMMYT. Endosperm meal was prepared from kernels from which the germs and pericarps had been removed by soaking in water for 20 min. The vitreous and soft portions of the kernels were separated with a scalpel. Endosperms were ground in a cyclone mill or by mortar and pestle to obtain meal that would pass through a 0.35-mm screen.

Protein concentrations in meals and extracts were determined by the micro-Kjeldahl method (AOAC 1980) by multiplying the nitrogen content by the conversion factor 6.25.

Tryptophan and lysine in the protein samples were analyzed by the method of Hernandez and Bates (1969), using papain to hydrolyze the proteins and determining lysine according to its correlation with the tryptophan content.

Relative quantitation of γ-zein was by ELISA. The method employed was based on that described by Conroy and Esen (1984) with the following modifications: total zeins were extracted from 50 mg of meal for 2 hr at room temperature using 500 μl of 50% ethanol and 5% 2-ME, with periodic vortexing. Extracts were diluted 400-fold; then protein samples were adsorbed to the wells of microtiter plates (Immulon 2, Dynatech), with phosphate-buffered saline (PBS), pH 7.4, containing 1% 2-ME. An aliquot (300 ml) of each dilution was added to a well of an ELISA plate, and 10 twofold dilutions were made into adjacent wells containing PBS/2-ME. The antigen was allowed

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to bind for 2 hr. Rabbit anti-γ-zein serum (1:600) and goat antirabbit immunoglobulin G conjugated to alkaline phosphatase (1:1000) were diluted in PBS containing 0.1% Tween 20. The alkaline phosphatase substrate mixture contained *p*-nitrophenyl disodium phosphate (0.66 mg/ml) in a 1*M* solution of diethanolamin, pH 9.8. The alkaline phosphatase conjugate and substrate were obtained from Sigma Chemical Company. After 15 min of incubation, substrate hydrolysis was stopped by the addition of 50 μl of 3*M* NaOH per well. Color intensity was determined spectrophotometrically at 405 nm.

Total SDS-soluble protein was extracted from 50 mg of meal in 950 μl of SDS-extraction buffer (62.5 m*M* tris-HCl [pH 6.8], 2.3% SDS, 5% 2-ME, 10% glycerol, 0.1% bromophenol blue), for 1 hr at room temperature. The extract was clarified by centrifugation for 5 min at 3,000 × *g*.

For zein extractions, 20 mg of meal from either vitreous or soft portions of the endosperm was dissolved in 200 μl of 0.5*M* NaCl for 10 min. After a 10-min centrifugation at 3,000 × *g*, the supernatant was discarded. The pellet was washed with water, dissolved in 200 μl of 2-propanol (2-PROH) and 3% 2-ME for 2 hr, then centrifuged at 3,000 × *g* for 10 min. The resulting supernatant was then mixed with SDS-extraction buffer (1:1).

Protein fractionation was done according to a modification of the scheme proposed by Landry and Moureaux (1970). Albumins and globulins were extracted first with 0.5*M* NaCl (fraction I). Zeins were then extracted using 70% 2-PROH (fraction II) and 70% 2-PROH plus 0.6% 2-ME (fraction III). Finally, the glutelins were extracted using 0.018*M* borate buffer (BB), pH 10, plus 0.6% 2-ME (fraction IV) and BB (pH 10), 0.6% 2-ME, and 0.5% SDS (fraction V). A 1:10 ratio of sample weight to solvent volume was maintained for all extractions. For gel electrophoresis, extracts of proteins from fractions II and III were mixed with SDS-extraction buffer for total protein (1:1). All steps were performed at room temperature.

SDS-PAGE was perfomed in a discontinuous system essentially as described by Laemmli (1970). Protein extract mixtures were heated in boiling water for 3 min before electrophoresis.

RESULTS AND DISCUSSION

The SDS-PAGE patterns of total proteins (Fig. 1) showed a clear distinction between the major zeins of the genotypes investigated. The γ-zein at M_r 27,000 was present in markedly higher amounts in the QPM materials than in normal and opaque-2 or in the floury-2 genotype, which was more diffuse and appeared as a doublet. QPM genotypes contained very little of the M_r 22,000 α-zeins and showed amounts of the M_r 19,000 α-zeins significantly reduced from normal.

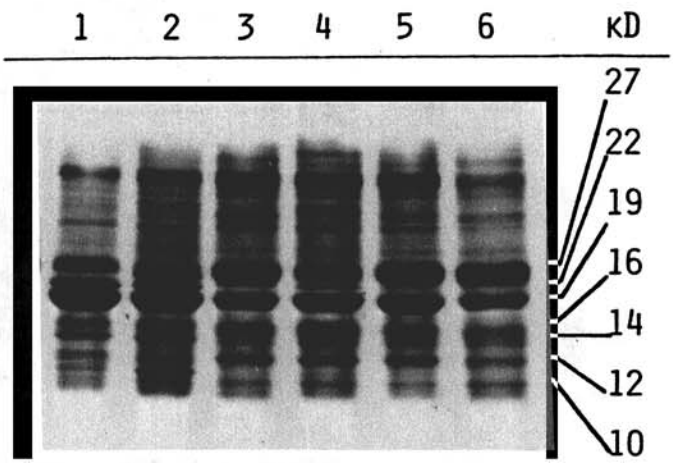

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of total protein extracts from meals of W64A normal (1), W64A floury-2 (2), Yellow Flint-QPM (3), W64A opaque-2 (4), Blanco Dentado-QPM (5), and Pool 25-QPM (6) maize genotypes. The amount of sample loaded onto the gel corresponded to 5% of the total extract (from 100 mg of flour).

The amounts (mg/100 mg of endosperm meal) of tryptophan and lysine (Table I) in the endosperms of the QPM kernels are 15–72% higher than that in the normal genotype and about one third less than those found in opaque-2 and floury-2. The γ-zein content, as measured by a quantitative ELISA assay, is also shown in Table I. The QPM types contain two to five times more γ-zein than do normal, opaque-2, or floury-2 genotypes, which confirms the results indicated by SDS-PAGE (Fig. 1). Therefore, a positive correlation exists between the content of γ-zein and the content of tryptophan and lysine in the endosperm of QPM genotypes.

The six maize genotypes show great divergence in the distribution of protein, based on the Landry and Moureaux (1970) protein fractionation scheme (Table II). As in opaque-2 and floury-2 genotypes, QPM genotypes have one fourth to twice more albumins and globulins (fraction I) than the normal genotype. In fraction II (prolamins), the normal genotype showed about twice more protein than floury-2, three times more than opaque-2, and up to seven times more than one of the QPM types. For fraction III ("prolaminlike"), the situation was the opposite: the opaque-2, the floury-2, and the QPMs showed twice as much protein as the normal genotype. All genotypes but floury-2 contained about the same amount of fraction IV glutelin compared to the normal genotype, whereas all contained more fraction V glutelin than normal W64A.

To better characterize the prolamins from fractions II and III of the Landry and Moureaux fractionation scheme, the proteins in these fractions were separated by SDS-PAGE (Fig. 2). The protein bands of fraction II consisted of two polypeptides, corresponding to α-zeins (M_r 22,000 and M_r 19,000). In addition to residual α-zeins, fraction III contained protein bands corresponding to γ-zeins (M_r 27,000 and M_r 16,000), β-zein (M_r 14,000), δ-zein (M_r 10,000), and a minor polypeptide of M_r 12,000.

The protein profiles of fractions II and III (Fig. 2) show that β-zeins are significantly reduced in opaque-2 and in the three QPM materials. Interestingly, higher amounts of the γ-zein polypeptide are present in fraction III of the QPM genoytpes, confirming the results obtained on total protein extracts (Fig. 1 and Table I). Thus, the higher protein contents in the QPM fraction III extracts (Table II), which have also been reported

TABLE I
Total Protein, γ-Zein, Tryptophan, and Lysine in the Endosperm of Normal, Opaque-2, Floury-2, and Three Quality Protein Maize (QPM) Genotypes

Genotypes	Total Protein[a]	Gamma Zein[b]	Tryptophan[a]	Lysine[a]
W64A				
Normal	13.3	100	0.047	0.242
Opaque 2	11.0	89	0.113	0.501
Floury 2	13.4	55	0.110	0.457
Blanco Dentado (QPM)	11.1	192	0.078	0.355
Yellow Flint (QPM)	10.2	251	0.081	0.365
Pool 25 (QPM)	9.0	257	0.062	0.279

[a] Milligrams per 100 mg of endosperm meal.
[b] Values normalized to those of W64A, normal.

TABLE II
Protein[a] in the Fractions Obtained by the Landry and Moureaux[b] Protein Fractionation Scheme

Genotypes	Protein Fraction[c] I	II	III	IV	V	Total Protein	Residue Protein
W64A							
Normal	0.76	7.19	0.87	1.10	2.22	12.15	1.15
Opaque 2	1.74	2.17	1.71	1.21	3.71	10.54	0.46
Floury 2	1.76	3.82	2.02	0.66	4.28	12.54	0.86
Blanco Dentado (QPM)	1.69	1.96	2.32	1.01	3.33	10.31	0.78
Yellow Flint (QPM)	1.00	1.51	2.04	1.37	3.79	9.71	0.49
Pool 25 (QPM)	0.95	1.04	1.98	1.28	3.34	8.50	0.50

[a] Milligrams per 100 mg of endosperm meal.
[b] Landry and Moureaux (1970).
[c] I, albumins and globulins; II and III, prolamins; IV and V, glutelins.

by Ortega and Bates (1983) and Gentinetta et al (1975), can be attributed to the higher level of γ-zein present in the endosperm of the QPM materials.

To explore the distribution of γ-zein in these various genotypes, soft and hard portions of the endosperm were manually separated. The QPMs and the normal genotypes contained approximately a 3:1 ratio of hard to soft endosperm regions, while the mutants opaque-2 and floury-2 had only a very thin hard endosperm region just below the aleurone layer. The electrophoretic patterns of zeins extracted from the two different endosperm regions are presented in Figure 3. The protein profiles show quantitative changes in the zein components among the six genotypes and a higher zein content in the hard endosperm regions regardless of the genotype. For the normal and floury-2 genotypes, the reduction in zein content in going from hard to soft regions is equivalent for all major zein components. However, QPMs and opaque-2 show a stronger reduction of α-zein (M_r 19,000) in going from hard to soft endosperm regions. Again, the γ-zein content of the QPMs is much higher than that in the other genotypes, regardless of the endosperm region. γ-Zein appears as the major prolamin in the soft region of the QPM endosperm, whereas the high-lysine and high-tryptophan genotypes (opaque-2 and floury-2), which have a very soft endosperm, show only trace amounts of γ-zein in the soft endosperm region (Fig. 3).

The percentages of total protein and zein in the hard and soft endosperm regions of these genotypes are shown in Table III. The hard endosperm region is also enriched in proteins other than zeins. The floury-2 and the normal genotypes show the highest levels of total protein and zeins in the hard endosperm region. However, one needs to consider that floury-2 genotypes are reported to have only a thin layer of high-protein subaleurone cells (Wolf et al 1969), which in this study correspond to the hard region of the floury-2-endosperm. The content of total protein in the soft region is about the same for all genotypes.

The percentages of total zein in the hard and soft endosperm regions of QPMs and opaque-2 are about the same (Table III). However, major quantitative differences exist in the type of zeins present (Fig. 3), in protein quality (Table I), and in the hardness of the kernels.

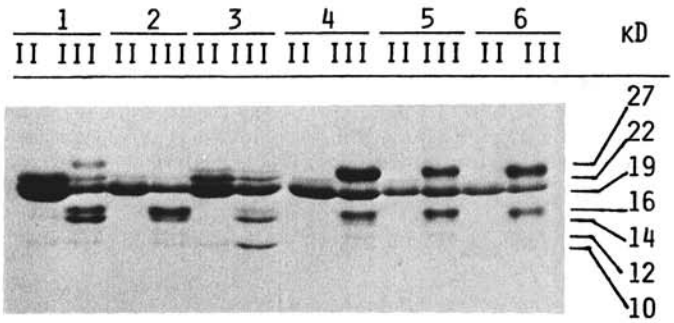

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of prolamins (fractions II and III) obtained by the Landry and Moureaux protein fractionation scheme from meals of W64A normal (1), W64A opaque-2 (2), W64A floury-2 (3), Blanco Dentado-QPM (4), Yellow Flint-QPM (5), and Pool 25-QPM (6) maize genotypes. The amount of sample loaded onto the gel corresponded to 2.5% of the total extract (from 100 mg of flour).

CONCLUSIONS

Changes in the major zein components were evident among the genotypes investigated. QPM varieties, which combine high levels of lysine and tryptophan with high yields, traditional appearance, and conventional kernel hardness, were also shown to contain relatively high amounts of γ-zein in the endosperm. γ-Zein was found in very small amounts in the endosperm of opaque-2 and floury-2 genotypes, which have a very soft endosperm texture. While the nature of the correlation is unclear, it seems that to have both a high lysine and tryptophan content and a hard, vitreous, kernel, the seed must also have a high level of γ-zein. Considering γ-zein's localization at the periphery of protein bodies (Lending and Larkins 1989), its high cysteine content (Prat et al 1985), and the fact that this protein requires a reducing agent for extraction, it is possible that γ-zein is involved in disulfide interactions that influence kernel hardness in the QPM genotypes.

We also found that when QPM corn meal is partially substituted for wheat flour in breadmaking, the result is a better quality dough with improved baking quality when compared with mixtures containing meal of traditional maize genotypes

TABLE III
Percentage of Total Protein and Zein in the Hard and Soft Endosperm Regions of Maize Genotypes

	Total Protein		Zein	
Genotypes	Hard	Soft	Hard	Soft
W64A				
Normal	13.5	7.5	6.3	3.7
Opaque 2	11.8	6.8	4.6	1.8
Floury 2	15.1	7.1	7.0	3.3
Blanco Dentado (QPM)	11.1	7.6	5.1	2.7
Yellow Flint (QPM)	10.1	6.7	3.4	1.8
Pool 25 (QPM)	9.5	7.0	3.8	1.9

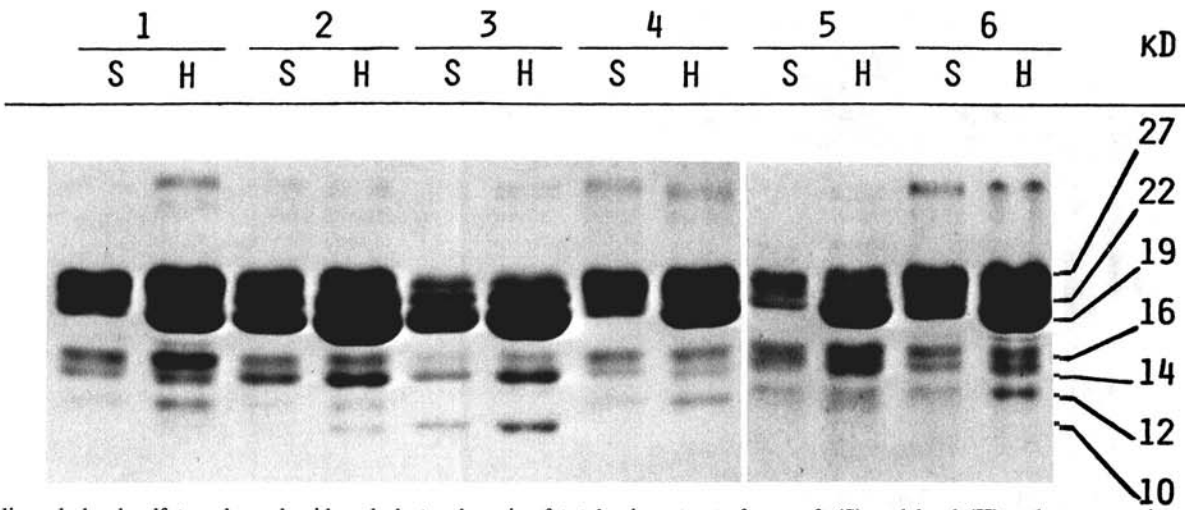

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of total zein extracts from soft (S) and hard (H) endosperm regions of Yellow Flint-QPM (1), W64A normal (2), W64A floury-2 (3), Pool 25-QPM (4), W64A opaque-2 (5), and Blanco Dentado-QPM (6) maize genotypes. The amount of sample loaded onto the gel corresponded to 15% of the total extract (from 100 mg of flour).

(unpublished data). This effect might be attributed to disulfide interactions made possible by the high content of cysteine in γ-zein.

Based on the finding that γ-zein genes isolated from normal maize do not code for any lysine or tryptophan (Prat et al 1985, Wang and Esen 1986), the relatively high lysine content of QPM cannot be explained by the increase in γ-zein. γ-Zein genes from QPM genotypes may, however, encode lysine and tryptophan. Molecular investigations of these genes should reveal whether this is so.

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