Elevated Protein-Bound Methionine in Seeds of a Maize Line Resistant to Lysine Plus Threonine[1]

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ABSTRACT

Protein fractions were analyzed from inbred maize lines with a variety of seedling responses to lysine plus threonine (LT) inhibition. Previous work has shown that BSSS-53, an LT-resistant inbred, had an elevated whole kernel methionine concentration. This report shows that BSSS-53 has an elevated zein-2 methionine concentration accounted for by: 1) an increase in the proportion of sulfur-rich low-molecular weight zeins in zein-2 and 2) an increased methionine concentration in the 10-kd zein fraction.

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The search for maize (*Zea mays* L.) variants with improved protein quality has yielded several mutants with altered concentration of nutritionally limiting amino acids. An example of such a variant is the *opaque*-2 mutant, which has elevated lysine and tryptophan concentrations relative to normal inbreds (Mertz et al 1964). The pattern in mutants such as *opaque-2* is to reduce the amount of nutritionally poor storage protein (zein) relative to protein fractions that are nutritionally more valuable (albumins, globulins, and glutelins) (Misra et al 1972).

When used as a feed for poultry, maize supplemented with soybean meal is deficient in methionine; and synthetic methionine is used as a feed additive. *Floury-2* and *sugary-1* (su_1) are two recessive mutations that in the homozygous condition can increase the methionine concentration of maize. The *floury-2* mutant (Nelson et al 1965) increases endosperm methionine concentration approximately 50%; this is thought to be due to a depression of zein synthesis coupled with corresponding increases in glutelins and with an increase in glutelin methionine concentration (Hansel et al 1973). The su_1 mutant can increase methionine concentration up to 36% depending on the genetic background. In $su_1/su_1/su_1$ endosperm the increased methionine is due to depressed zein-1 synthesis coupled with increases in the methionine-rich zein-2 and glutelin fractions (Paulis et al 1978).

In 1974, Green and Phillips suggested that the pattern of regulation in the biosynthesis of lysine, threonine, and methionine provides the potential for screening cereals for overproducers of these amino acids. This screening technique revolves around the synergistic inhibition of seedling growth on lysine plus threonine (LT)-supplemented agar media. A paper from this laboratory (Phillips et al 1981) reported the results of a large-scale seedling screening program in which over 200 lines of maize were tested for resistance to LT inhibition. That study showed that BSSS-53, the most resistant inbred tested, had a methionine concentration 30% higher than a typically inhibited line (3.95 g methionine/100 g protein N in BSSS-53 vs. 3.05 g methionine/100 g protein N in W23, an inhibited inbred). The elevated methionine level in resistant inbreds was interpreted in the light of data (Green and Phillips 1974, Green and Donovan 1980) showing that LT inhibition in maize seedlings is relieved by externally supplied methionine. We have evidence that LT resistance in normal inbreds is related to homoserine content in the free amino acid pool (Phillips and McClure, *unpublished*). This work also showed that the elevated methionine level in BSSS-53 was not caused by elevated free pool methionine, as might have been expected based on the experience with LT-resistant variants derived from selection in tissue culture (Hibberd et al 1980, Hibberd and Green 1982). The increased methionine in BSSS-53 is protein bound.

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This paper addresses the distribution of protein-bound methionine in BSSS-53. Here we report: 1) a normal distribution of protein among the major Osborne fractions in BSSS-53 and three other inbred maize lines with varying resistance to LT, and 2) that variation in methionine concentration of zein-2 arises from an increasing proportion of the methionine-rich 10-kd and 14-kd zeins and a decrease in the 22-kd and 24-kd zeins.

MATERIALS AND METHODS

Preparation of Plant Material

The inbreds used in this study were BSSS-53, B37, A619, and W23. BSSS-53 is a random line isolate from the Iowa Stiff Stalk Synthetic population; our line descended from material provided by J. L. Geadelmann, University of Minnesota. A619 and W23 were available from the backcross program of R. L. Phillips. Seed from all four lines was produced in 1980.

Bulk samples of the lines under study were prepared by removing an equal number of kernels from each of several ears. One- to two-hundred kernels of each inbred were ground to pass a 100-mesh (0.15 mm) screen in a cyclone sample mill (Udy Corp., Boulder, CO).

Protein Fractionation

The major Osborne fractions were obtained by a method similar to that used by Sodek and Wilson (1971). Three separate serial extractions were performed for each line; the entire procedure was always completed in one day. The zein-2 fraction was differentiated from zein-1 based on β-mercaptoethanol (βME) solubility.

Whole meal samples (250 mg) were placed in 15-ml Corex centrifuge tubes; a small magnetic stir bar was added, and extractions were carried out with continuous stirring. Before extraction the samples were defatted by two washes with cold acetone. The samples were dried before addition of the first solvent.

Table I outlines the sequential solvent system. Samples were extracted twice with 10 ml of each solvent for 30 min; supernatants were obtained by centrifuging at 20,000 $\times g$ for 4 min at the proper temperature. Appropriate supernatants were combined and treated as follows. The albumin/globulin and zein-1 fractions were stored until assayed or hydrolyzed. A 1-ml aliquot of the zein-2 fraction was removed and dried on a speed-vac evaporator; βME was completely driven off by resuspending the sample in 70% aqueous ethanol and again bringing to dryness. This aliquot was used for protein determination of the zein-2 fraction and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). To the remaining 19 ml of the zein-2 fraction, 10 mg dithiothreitol (DTT) was added, and the fraction brought to dryness on the speed-vac evaporator. The glutelin fractions were precipitated from 10% trichloroacetic acid (TCA) and stored as a pellet. All fractions were stored at $-20°$ C.

Protein Determination

Protein concentrations of the major Osborne fractions were determined against a bovine serum albumin (BSA) standard curve

by the method of Lowry et al (1951). The albumin/globulin and zein-1 fractions were assayed directly in the extraction solvent. The βME-free zein-2 sample was dissolved for assay in 0.25 ml of 70% ethanol. The TCA-precipitated glutelin fractions were redissolved in 4.0 ml of 0.1 *M* NaOH before assay.

An empirical relation between A_{280} and mg/ml BSA equivalent for the zein-2 fraction was determined. Zein-2 was obtained from BSSS-53 and dissolved in a solution of 1 m*M* Tris, 6*M* urea, and 1% SDS, pH 8. The protein concentrations of several zein-2 samples were determined by the Lowry test using the BSA standard curve. Corresponding A_{280} values of these zein-2 samples were measured and the following linear relation obtained:

$$\text{mg/ml BSA equivalent} = 1.11\ (A_{280}\ \text{zein-2}) + 0.02.$$

This relation was used to estimate protein concentration in buffers containing thiol-reducing agents.

SDS-PAGE

SDS-PAGE was performed as described by Laemmli (1970). Separating slab gels of 15% acrylamide were 0.75 × 130 × 150 mm.

The zein-2 gel was loaded with 12.5-μg zein-2 protein per lane in a sample buffer containing 5% βME. The samples were heated in a 100° C bath for 5 min before electrophoresis. Each lane represented zein-2 from a separate Osborne extraction. The gel was stained with Coomassie Blue. The stained gel was photographed and negatives were scanned with a Quick Scan densitometer (Helena Laboratories); integration was by peak weight. The percentage of each subfraction present in zein-2 was estimated by averaging the scans from two negatives.

Gel filtration column fractions were prepared for SDS-PAGE by mixing four parts of a denaturing buffer (described in the next section) with one part of a solution freshly prepared from 4.05 ml glycerol, 165 mg Tris base, 0.115 ml concentrated HCl, 0.1 mg bromophenol blue, water to 4.5 ml, and 95% ethanol to 6 ml. This sample buffer is not highly reducing, and samples were not heated before electrophoresis. The gels were loaded with about 1.5 μg of protein per lane and were silver stained (Merril et al 1981).

TABLE I
Sequential Solvent System

Protein Fraction	Solvent
Free amino acids Albumins Globulins	0.5 *M* NaCl + 1 m*M* phenylmethylsulfonylfluoride at 0° C
	Water rinse (discard)
Zein-1	70% EtOH + 1 m*M* phenylmethylsulfonylfluoride at 25° C
Zein-2	70% EtOH + 1% (v/v) mercaptoethanol + 1 m*M* phenylmethylsulfonylfluoride at 25° C
Glutelin	0.05 *M* NaOH + 1 m*M* phenylmethylsulfonylfluoride at 25° C

TABLE II
Total Nitrogen and Sulfur Concentrations of Four Inbred Maize Lines

Line	Nitrogen (%)[a]	Sulfur (%)[a]
BSSS-53	2.10	0.188
B37	2.02	0.171
A619	2.06	0.165
W23	1.64	0.106

[a] Determinations on whole kernel meal by micro-Kjeldahl and nitric/perchloric acid digestion.

Purification of Zein-2 Subfractions

Gel filtration through a column of Sephadex G-75 superfine was used to separate the zein-2 fraction into three subfractions of 22 + 24 kd, 14 kd, and 10 kd. A highly denaturing buffer (DB) system (1 m*M* Tris, 6*M* urea, 1% SDS, 0.1% DTT, pH 8) was required to obtain the desired degree of resolution of the three subfractions.

The zein-2 fractions from three separate Osborne extractions were combined and dissolved in 0.40 ml of DB. A portion of this solution (0.35 ml) containing 7–10 mg of zein-2 protein was loaded onto the G-75 column (0.8 × 118 cm); the column was eluted with DB under a pressure of 100 cm of water giving a flow rate of about 1 ml/hr. After a prerun of 8–10 ml, fractions (0.7 ml) were collected for about 40 hr. Protein contents were estimated by A_{280} (absorbance at 280 nm), and column fractions were analyzed by SDS-PAGE. Those column fractions which contained homogeneous zein-2 subfractions were pooled and stored at 4° C.

Amino Acid Analysis

Total zein-2 and zein-2 subfractions were prepared for hydrolysis by removal of SDS (Weber and Kuter 1971) and dialysis into ethanol.

Since methionine was of primary interest in this study, proteins were performate oxidized before acid hydrolysis and amino acid analysis. Most samples were oxidized and hydrolyzed in duplicate; exceptions were the 10-kd zein-2 subfraction, for which sufficient protein was not available for duplicate analysis, and one sample each of zein-2 and 10-kd zein-2 subfraction from BSSS-53 that were hydrolyzed without prior performate oxidation.

Performate oxidation and acid hydrolysis (110° C, 72 hr) were as described by Glazer et al (1975). After hydrolysis the samples were analyzed with an automated Dionex Amino Acid/Peptide Analyser. Data are reported as mole percent of amino acids present. Tryptophan and tyrosine are destroyed in this procedure, and proline, though often present in large amounts, was not determined.

Elemental Analysis

Nitrogen and total sulfur analyses were performed by micro-Kjeldahl (Bremner 1965) and nitric/perchloric acid digestion (Blanchar et al 1965), respectively, in the analytical laboratory of R. C. Munter, University of Minnesota.

TABLE III
Distribution of Protein in Whole Kernel Meal of Four Maize Inbred Lines

Protein Fraction	Line							
	BSSS-53		B37		A619		W23	
	(mg/g)[a]	(%)[b]	(mg/g)[a]	(%)[b]	(mg/g)[a]	(%)[b]	(mg/g)[a]	(%)[b]
Free amino acid + albumin + globulin	20.8 ± 0.8	19.5	23.8 ± 0	22.7	23.8 ± 0.6	22.7	15.7 ± 0.2	19.6
Zein-1	42.4 ± 0.5	39.8	37.9 ± 0.8	36.2	42.1 ± 1.2	40.2	31.6 ± 0.8	39.4
Zein-2	15.5 ± 1.2	14.5	13.9 ± 0.6	13.3	13.2 ± 0.5	12.6	12.0 ± 0.8	14.9
Zein-1 + Zein-2	57.9 ± 0.9	54.4	51.8 ± 0.6	49.5	55.3 ± 0.8	52.7	43.6 ± 0.4	54.3
Glutelin	27.7 ± 0.4	26.1	29.0 ± 0.4	27.7	25.8 ± 1.6	24.6	20.9 ± 0.4	26.1

[a] BSA equivalent/meal dry weight basis.
[b] Percent of extractable protein.

RESULTS AND DISCUSSION

The nitrogen and sulfur contents of kernels of the four inbred lines studied are presented in Table II. The distribution of protein among the major fractions showed only a small amount of variation among these inbreds (Table III). It is particularly notable that the amount of zein-2 fraction (see Table I) varies only from 12.6 to 14.9% of the extracted protein and that the inbred with the lowest overall methionine concentration, W23, contains the highest proportion of zein-2. (Previously published data from this laboratory showed the following total methionine concentrations: BSSS-53, 4.0 mol %; B37, 3.8 mol %; A619, 3.4 mol %; W23, 3.1 mol %. These data are converted from grams amino acid/100 g protein N listed in Phillips et al (1981). The earlier analysis included proline and tyrosine whereas the present analysis does not.) Calculations using data of other authors (Sodek and Wilson 1971) show that the zein-2 fraction contains over half the endosperm methionine. Therefore, we had expected a relationship between whole kernel methionine concentration and increasing proportion of zein-2. This was not the case and, thus, it appeared that the amino acid composition of the fractions must be altered.

Amino acid analysis of the major protein fractions from the four inbreds (Tables IV, V, VI, and VII) showed that, except for zein-2, their amino acid compositions were comparable in all respects. The amino acid composition of the zein-2 fractions (Table V), however, did show variation among inbreds. Significantly, the methionine concentration was much higher in zein-2 of BSSS-53 (8.5 mol %) than in the other inbreds tested (B37, 5.4 mol %; A619, 6.5 mol %; W23, 3.7 mol %).

Analysis of the zein-2 fractions by SDS-PAGE (Fig. 1) indicated that all four inbreds contained the peptides usually associated with this fraction, namely the 24-kd, 22-kd, 14-kd, and 10-kd zein peptides. We refer to the two bands in the 14-kd region as a single species because covalent modification of cysteine residues with iodoacetic acid resulted in a single band in the 14-kd region (gel not

TABLE IV
Amino Acid Composition of the Combined Free Amino Acid, Albumin, and Globulin Fractions[a]
(mol % of amino acids present at analysis)

	BSSS-53	B37	A619	W23
Cysteic acid	3.8	4.4	3.2	3.8
Aspartic acid	14.5	13.2	14.6	15.6
Methionine sulfone	1.3	1.3	1.4	1.3
Threonine	4.6	4.2	4.8	2.6
Serine	5.3	5.5	5.6	2.3
Glutamic acid	16.9	18.7	15.8	16.7
Glycine	11.7	12.5	13.0	10.7
Alanine	10.0	10.1	11.2	13.6
Valine	5.9	6.1	7.2	7.2
Cystine	0	0	0	0.3
Isoleucine	3.3	3.2	3.7	3.7
Leucine	5.6	5.8	6.8	6.8
Phenylalanine	0	0	0.2	0
Histidine	0.9	0.6	1.2	1.2
Lysine	8.4	6.0	5.8	7.1
Arginine	8.3	8.0	5.9	7.4

[a] Mean of two analyses of a single hydrolysate.

TABLE V
Zein-2 Amino Acid Composition
(mol % of amino acids present at analysis)

	BSSS-53[a]	B37[b]	A619[c]	W23[c]
Cysteic acid	2.4 ± 0.1	1.9 ± 0.1	2.5 ± 0.1	2.0 ± 0.1
Aspartic acid	5.5 ± 0.1	5.6 ± 0.2	5.1 ± 0.1	5.6 ± 0.2
Methionine sulfone	8.5 ± 0.2	5.4 ± 0.1	6.5 ± 0.2	3.7 ± 0.9
Threonine	3.4 ± 0.1	3.4 ± 0.1	3.3 ± 0.2	3.4 ± 0.2
Serine	5.8 ± 0.2	5.8 ± 0.1	5.4 ± 0.1	5.8 ± 0.1
Glutamic acid	22.7 ± 0.4	24.1 ± 0.7	23.5 ± 0.6	24.6 ± 0.9
Glycine	4.4 ± 0.1	4.3 ± 0.1	4.4 ± 0.2	4.4 ± 0.2
Alanine	13.9 ± 0.7	14.8 ± 0.4	14.9 ± 0.7	15.5 ± 0.9
Valine	4.8 ± 0.7	5.7 ± 0.5	4.3 ± 1.0	4.0 ± 10.0
Isoleucine	3.5 ± 0.3	3.8 ± 0.4	3.7 ± 0.2	3.9 ± 0.3
Leucine	17.9 ± 0.4	17.8 ± 1.1	17.7 ± 0.5	19.3 ± 0.5
Phenylalanine	5.0 ± 0.2	4.6 ± 0.2	5.1 ± 0.3	5.4 ± 0.2
Histidine	1.0 ± 0.3	1.2 ± 0.1	1.9 ± 0.2	1.4 ± 0.5
Lysine	0.3 ± 0.2	0.4 ± 0.1	0.3 ± 0.3	0.2 ± 0.2
Arginine	1.2 ± 0.3	1.2 ± 0.1	1.4 ± 0.3	1.6 ± 0.4

[a] Two hydrolysates, two analyses each.
[b] One hydrolysate, three analyses.
[c] One hydrolysate, four analyses.

TABLE VI
Amino Acid Composition of Zein-1 Fraction
(mol % of amino acids present at analysis)

	BSSS-53[a]	B37[b]	A619[b]	W23[b]
Cysteic acid	1.0 ± 0.1	1.3 ± 0.3	1.1 ± 0.1	0.9 ± 0.1
Aspartic acid	6.3 ± 0.1	6.3 ± 0.1	6.1 ± 0.1	6.1 ± 0.1
Methionine sulfone	1.4 ± 0.1	1.4 ± 0	1.2 ± 0.1	0.9 ± 0.1
Threonine	3.3 ± 0.2	3.2 ± 0.1	3.1 ± 0.1	3.0 ± 0.1
Serine	6.0 ± 0.2	5.6 ± 0.3	5.8 ± 0.1	5.9 ± 0.2
Glutamic acid	25.2 ± 0.4	24.8 ± 0.2	24.9 ± 0.1	25.3 ± 0.7
Glycine	2.3 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.4 ± 0.1
Alanine	15.6 ± 0.8	16.4 ± 0.1	16.2 ± 0.3	16.0 ± 0.3
Valine	5.4 ± 0.1	5.9 ± 0.2	5.2 ± 0.1	4.7 ± 0.1
Isoleucine	4.3 ± 0.2	4.7 ± 0.2	4.7 ± 0.1	4.8 ± 0.1
Leucine	21.7 ± 0.7	21.3 ± 0.2	21.4 ± 0.3	21.8 ± 0.4
Phenylalanine	5.4 ± 0.1	4.9 ± 0.1	5.6 ± 0.3	5.8 ± 0.2
Histidine	1.2 ± 0.1	1.0 ± 0.2	1.1 ± 0.1	1.5 ± 0.3
Lysine	0	0	0	0
Arginine	1.3 ± 0.2	1.3 ± 0.2	1.4 ± 0.4	1.3 ± 0.1

[a] Four analyses on a single hydrolysate.
[b] Two analyses on each of two hydrolysates.

TABLE VII
Amino Acid Composition of the Glutelin Fraction[a]
(mol % of amino acids present at analysis)

	BSSS-53	B37	A619	W23
Cysteic acid	2.6	2.6	2.5	2.9
Aspartic acid	7.6	7.3	8.0	7.3
Methionine sulfone	1.8	1.5	1.6	1.5
Threonine	5.1	4.0	4.9	5.1
Serine	5.4	1.7	5.5	5.3
Glutamic acid	19.7	20.5	19.3	19.6
Glycine	10.0	9.1	9.9	10.2
Alanine	10.6	14.8	10.6	10.4
Valine	8.4	8.9	8.3	8.9
Isoleucine	4.0	4.9	4.2	4.1
Leucine	11.0	10.7	11.0	10.9
Phenylalanine	3.1	3.0	3.1	2.8
Histidine	1.8	2.0	1.9	2.0
Lysine	4.3	4.4	4.6	4.2
Arginine	5.0	4.9	5.0	5.1

[a] Mean of two analyses of a single hydrolysate.

TABLE VIII
Percentage of Subfractions in Zein-2[a]

Zein-2 Subfraction	Line BSSS-53 (%)	B37 (%)	A619 (%)	W23 (%)
22 + 24 kd	35 ± 2.3	51 ± 1.8	42 ± 0.6	43 ± 1.5
14 kd	43 ± 1.0	33 ± 1.0	42 ± 0.8	44 ± 0.8
10 kd	22 ± 2.5	17 ± 1.0	16 ± 0.8	13 ± 0.8

[a] Mean and standard deviation of six SDS gel scans by densitometry.

TABLE IX
Amino Acid Composition of 21-kd + 24-kd Zein-2 Subfraction[a]
(mol % of amino acids present at analysis)

	BSSS-53	B37	A619	W23
Cysteic acid	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0	1.0 ± 0.1
Aspartic acid	6.4 ± 0.2	6.3 ± 0.1	6.2 ± 0.1	5.8 ± 0.6
Methionine sulfone	1.0 ± 0	1.7 ± 0.1	1.4 ± 0.1	0.5 ± 0.1
Threonine	2.9 ± 0.1	3.2 ± 0.1	3.0 ± 0.1	2.5 ± 0.5
Serine	6.0 ± 0.1	5.8 ± 0.2	6.0 ± 0.1	6.0 ± 0.3
Glutamic acid	25.4 ± 0.4	24.9 ± 0.4	24.5 ± 0.6	24.6 ± 0.2
Glycine	2.7 ± 0.1	2.2 ± 0.1	2.6 ± 0.1	2.7 ± 0.1
Alanine	16.5 ± 0.2	16.6 ± 0.2	16.5 ± 0.1	16.7 ± 0.3
Valine	5.1 ± 0.2	5.8 ± 0.4	4.3 ± 0.1	4.4 ± 0.3
Isoleucine	4.5 ± 0.1	4.5 ± 0.4	4.7 ± 0.2	4.6 ± 0.2
Leucine	21.0 ± 0.1	20.9 ± 0.4	21.3 ± 0.4	20.6 ± 1.1
Phenylalanine	5.0 ± 0.1	4.9 ± 0.3	6.0 ± 0.2	6.3 ± 0.2
Histidine	1.4 ± 0.3	1.3 ± 0.1	1.5 ± 0.2	1.7 ± 0.3
Lysine	0.3 ± 0.2	0	0.4 ± 0.2	0.3 ± 0.1
Arginine	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.1

[a]Two analyses on each of two hydrolysates.

TABLE X
Amino Acid Composition of 14-kd Zein-2 Subfraction
(mol % of amino acids present at analysis)

	BSSS-53[a]	B37[b]	A619[a]	W23[a]
Cysteic acid	6.3 ± 0.3	6.2 ± 0.4	5.7 ± 0.4	5.6 ± 0.8
Aspartic acid	2.0 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	1.6 ± 0.1
Methionine sulfone	7.1 ± 0.4	5.7 ± 0.3	9.6 ± 0.8	6.3 ± 0.3
Threonine	3.6 ± 0.1	3.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.9
Serine	4.4 ± 0.1	5.5 ± 0.2	5.0 ± 0.1	5.2 ± 0
Glutamic acid	26.6 ± 0.7	26.3 ± 0.5	24.4 ± 0.5	27.8 ± 2.4
Glycine	11.4 ± 0.1	11.9 ± 0.4	11.3 ± 0.1	12.1 ± 0.1
Alanine	13.8 ± 0.4	12.8 ± 0.5	14.2 ± 0.4	16.3 ± 2.5
Valine	4.0 ± 0.2	4.3 ± 0.2	4.0 ± 0.1	4.6 ± 0.1
Isoleucine + leucine[c]	11.8 ± 0.4	12.2 ± 0.3	12.3 ± 0.1	11.5 ± 0.2
Phenylalanine	2.8 ± 0.3	3.5 ± 0.3	2.7 ± 0.2	2.8 ± 0.3
Histidine	0.7 ± 0.3	2.0 ± 0.8	2.0 ± 0.4	1.0 ± 0.4
Lysine	3.0 ± 0.6	0.8 ± 0.2	0.9 ± 0.5	2.1 ± 0.6
Arginine	2.8 ± 0.2	2.4 ± 0.2	2.7 ± 0.2	2.7 ± 0.2

[a]Two analyses on each of two hydrolysates.
[b]Four analyses on a single hydrolysate.
[c]Isoleucine and leucine were not always resolved in these hydrolysates; for uniformity the sum is presented in all cases.

TABLE XI
Amino Acid Composition of 10-kd Zein-2 Subfraction[a]
(mol % of amino acids present at analysis)

	BSSS-53	B37	A619	W23
Cysteic acid	3.1	2.4	3.9	3.2
Aspartic acid	4.3	7.4	4.9	4.8
Methionine sulfone[b]	25.2[b]	9.1	16.5[b]	22.8[b]
Threonine		3.7		
Serine	1.3	7.3	6.9	6.1
Glutamic acid	19.7	17.4	17.8	17.8
Glycine	6.5	13.0	10.1	7.5
Alanine	13.1	14.3	11.7	9.3
Valine	5.2	4.4	5.3	5.4
Cystine	0.4			
Isoleucine	2.5	4.0	4.4	2.9
Leucine	13.6	10.1	12.4	13.4
Phenylalanine	3.4	3.2	4.0	3.8
Histidine	0.7	0.7	0.3	0.8
Lysine	0.7	2.2	1.4	1.2
Arginine	0.7	1.0	0.6	1.2

[a]Mean of two analyses of a single hydrolysate.
[b]Threonine and methionine sulfone were not resolved in these hydrolysates because of the large amounts of methionine sulfone present (see text).

shown). The inbred A619 appears to have an electrophoretic variant of the 14-kd zein because the variation occurred with procedures giving either one or two bands in the 14-kd region. Examination of Figure 1 and other SDS gels (not shown) suggested that zein-2 from the four inbreds showed considerable variation in the proportions of the various peptides present in the fraction. Densitometry tracing of the gel in Figure 1 (Table VIII) confirmed this observation and suggested that the percentage of 10-kd zein is most variable, ranging from 13% (W23) to 22% (BSSS-53) of the stained protein. This method of estimating the composition of the zein-2 fraction is dependent on the staining properties of the peptides and is subject to limitations (Bertolini et al 1976). We use Table VIII only for comparison, but note that the data must be close to the actual values because the zein-2 methionine content

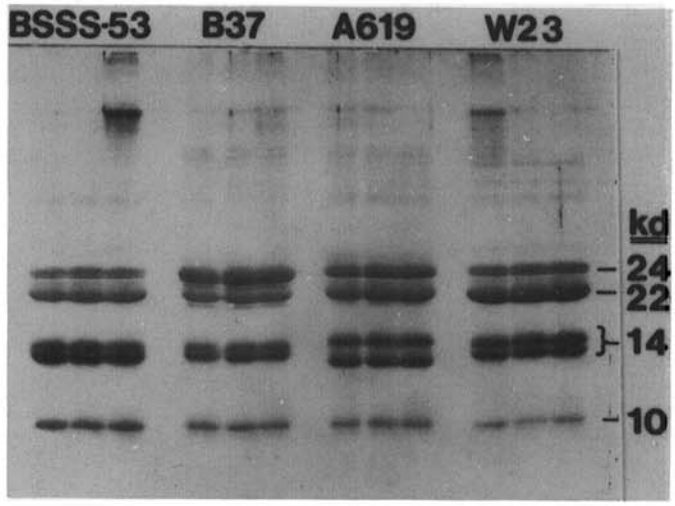

Fig. 1. SDS-PAGE analysis of zein-2 from four inbreds showing the 24-kd, 22-kd, 14-kd, and 10-kd zein peptides. High molecular weight species present in some isolates from BSSS-53 and W23 are complex aggregates of the smaller zein-2 peptides.

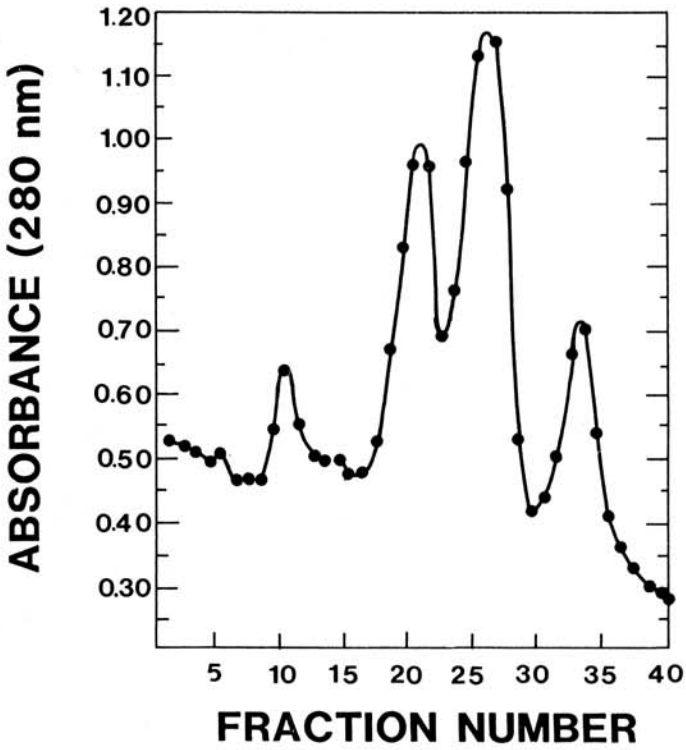

Fig. 2. Sephadex G-75 gel filtration of BSSS-53 zein-2 fraction separating subfractions.

listed in Table V can be approximated by summing the methionine contribution from each subfraction (i.e., by multiplying the percentages in Table VIII by the individual methionine contents listed in Tables IX, X, and XI).

Because the low molecular weight zeins are rich in sulfur-containing amino acids (Gianazza et al 1977), we used gel filtration to subfractionate zein-2 and determine the distribution of methionine among its constituent size classes in these four inbreds. As shown in Figures 2 and 3, the zein-2 was resolved into three main subfractions corresponding to 22 kd + 24 kd, 14 kd, and 10 kd.

Results of amino acid analysis of these zein-2 subfractions are presented in Tables IX, X, and XI. These data show zein-2 subfraction amino acid compositions that are similar, but not identical, to those observed by Gianazza et al (1977). The differences are due to the improved recovery of sulfur-containing amino acids with peroxidation before hydrolysis and the elimination of proline from our analysis. Comparison of Tables VI and IX confirms the similarity between the 22-kd + 24-kd zein-2 subfraction and the zein-1 fraction, the main difference being the appearance of traces of lysine in the 22-kd + 24-kd subfraction of zein-2. The 14-kd zein peptides are present in zein-2 in amounts comparable to the 22-kd + 24-kd subfractions (Table VIII). These 14-kd peptides quantitatively differ in almost all amino acids from the 22-kd + 24-kd subfraction but most significantly in the sulfur-containing amino acids. Cysteic acid is increased from about 1 mol % in the 22-kd + 24-kd subfraction to about 6 mol % in the 14-kd subfraction. The range in methionine concentration increases from 0.5 mol % to 1.7 mol % in the 22-kd + 24-kd subfraction up to 5.7 mol % to 9.6 mol % in the 14-kd subfraction. The 10-kd subfraction is different from the other subfractions and is most notable for its extremely high methionine concentration. In this subfraction the methionine concentration is so high that threonine appears only as an unresolved shoulder on the methionine sulfone peak. That it is in fact a high methionine concentration that results in this large peak was confirmed by hydrolyzing the 10-kd subfraction (from BSSS-53) without prior peroxidation (complete data not presented). The nonperoxidized sample had a threonine content of 4.2 mol %, consistent with published values (4.1 mol %, Gianazza et al 1977) and with the value from B37, which has a sufficiently low methionine concentration to resolve methionine sulfone and threonine (9.1 mol % and 3.7 mol %, respectively). If the threonine concentration of the 10-kd subfraction is assumed to be about 4 mol %, the methionine concentrations of the three inbreds in which resolution is poor would be about 21 mol % (BSSS-53), 13 mol % (A619), and 19 mol % (W23). Discounting the presence of tyrosine and tryptophan and the probable major amino acid, proline, our results indicate that methionine could represent one residue in five in the 10-kd subfraction. Another interesting feature (Table XI) is that the methionine concentration varied from 9.1 mol % to 21 mol % in the two related inbreds B37 and BSSS-53. The value for B37 may not be accurate because of the difficulty in obtaining a pure 10-kd subfraction from this line (less than 20 μg of protein was available for analysis).

These data indicate that the increase in methionine in BSSS-53 is due primarily to an increase in the methionine concentration of its zein-2. The methionine concentration of the nonextractable protein could conceivably be altered also. This increase is accomplished in zein-2 by an increase in the methionine concentration of the sulfur-rich, low molecular weight zeins and by an increase in the proportion of these peptides relative to the 22-kd + 24-kd zeins. Because BSSS-53 was selected by virtue of its resistance to LT inhibition, it is of interest that the heritability of this resistance has been estimated to be 72% (Thompson 1980). It is not known whether the transmission of LT resistance from BSSS-53 is accompanied by either an increasing proportion of low molecular weight components in zein-2 or by an increase in the methionine concentration of these components. Preliminary results of feeding trials with young chickens indicate that the methionine in BSSS-53 is nutritionally available (N. Allen, Department of Animal Science, University of Minnesota, *personal communication*).

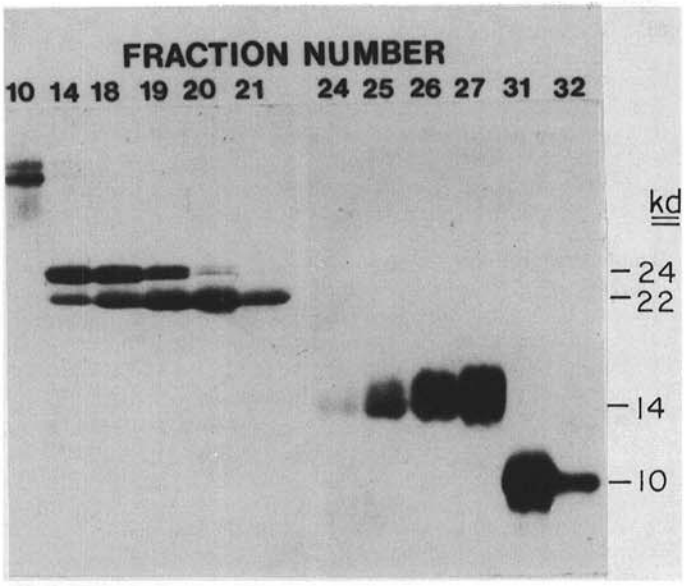

Fig. 3. SDS-PAGE analysis of BSSS-53 zein-2 subfractionated by gel filtration showing the 24-kd + 22-kd, 14-kd, and 10-kd zeins.

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