ORIGIN OF HIGH METHIONINE CONTENT IN SUGARY-1 CORN ENDOSPERM

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ABSTRACT

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The endosperm proteins of two *sugary-1* (su_1) corn inbreds (B37 and Oh43) contain 21 and 36%, respectively, more methionine than does the protein of their normal counterparts. Increases in amount and methionine content of alcohol-soluble reduced glutelin (ASG) proteins contribute to the increased methionine in su_1 endosperm. The salt-soluble and alcohol-insoluble reduced glutelin proteins constitute a greater proportion and zein a smaller proportion of the su_1 endosperm protein compared with the relative amounts of those proteins in the endosperms of normal counterparts. These changes are accompanied by 32 and 56% increases in the su_1 endosperm content of lysine over that of normal endosperms. The changes in protein distribution seem to be affected by the background into which the su_1 is introduced, because a commercial sweet corn containing su_1 gene did not exhibit an increased content of ASG protein and less zein than did the dent inbreds.

Although lysine and tryptophan are the first limiting amino acids of corn, corn-based feeds supplemented with soybean meal to 20% protein to ensure optimum growth generally have ample amounts of those amino acids. In such diets, methionine is the limiting amino acid, and synthetic methionine must be added to obtain maximum feed performance. Alternative economical natural sources of methionine, especially higher levels of methionine in corn, would be desirable to counteract the deficiency of sulfur-containing amino acids in soybean meal supplements.

Nelson et al (1) reported that the *floury-2* (fl_2) gene caused a change in amino acid pattern in corn endosperm. In addition to having an increased lysine and tryptophan content, $fl_2/fl_2/fl_2$ endosperms were found to contain 50–70% more methionine than normal. Two main factors contribute to the increased methionine in fl_2 endosperm: the increase in methionine content of the fl_2 glutelins and a decrease in the zein/glutelin protein ratio. In fl_2, the zeins and glutelins account for 22 and 74%, respectively, of the total methionine (2). Cromwell et al (3) showed that diets containing fl_2 corn caused faster growth and a greater feed conversion efficiency in chickens than did diets containing normal corn.

Nordstrom and Meade (4) reported that a variety of sweet corn (Northrup King Golden Delight) contained 49% more lysine, 31% more tryptophan, and 79% more total sulfur-containing amino acids (methionine plus cystine) than did normal hybrid corn. This variety generally produced more rapid growth in rats than did normal corn. At suboptimal protein levels, performances were similar for chicks fed high lysine or sweet corn diets (5). For chicks fed this sweet corn at the 22% protein level, growth and feed conversion were significantly improved over that for chicks fed normal or high-lysine corn.

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Misra et al (6) fractionated the proteins from single and double endosperm mutants of corn by the Landry-Moureaux method and compared the proteins. A near-isogenic *sugary-1* (su_1) genotype was shown to contain almost four times more alcohol-soluble reduced glutelin (ASG) protein than did its normal endosperm genotype counterpart. We and others (7–10) have shown this fraction to be high in sulfur-containing amino acids. Since the su_1 gene is present in sweet corn and is possibly responsible for an increase in certain essential amino acids that Nordstrom and Meade (4) observed in a sweet corn variety, we sought to determine the effect of su_1 on the protein distribution when that mutant gene is introduced in normal dent inbred lines of corn. Of special interest was the effect of variation in amount of ASG protein on the methionine content of corn endosperm.

MATERIALS AND METHODS

Treatment of Grain

Grain from two inbred corn varieties, B37 and Oh43, into which the su_1 gene was introduced and grain from their normal counterparts were analyzed in this study. Also, seed grain (f_1 generation) and mature crop grain (f_2 generation) from Commander sweet corn were investigated. Defatted endosperm meals from these corns were prepared as previously described (11).

Fractionation of Protein

Extraction of the protein fractions from the defatted endosperm meals followed an earlier procedure (11). The saline and zein proteins were examined only for extraction yield. The composition of the ASG protein was further investigated by amino acid analysis. These fractions were prepared by dialyzing extracts against cold water in Spectropor membrane tubing with the molecular weight cut off at 6,000–8,000 daltons and lyophilizing the retentate to dryness.

Analytic Methods

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

Samples for amino acid analysis were hydrolyzed in duplicate by refluxing in 6*N* HCl (2 ml/mg of sample) for 24 hr and analyzed with a Beckman amino acid analyzer following a previously described procedure for quantitation (12). The levels of methionine and cystine in the samples were determined in duplicate by converting these amino acids to methionine sulfone and cysteic acid by performic acid oxidation (13). All amino acid determinations were corrected to 97% recovery of nitrogen for comparison between samples.

RESULTS AND DISCUSSION

The introduction of the su_1 gene into the B37 and Oh43 inbred backgrounds resulted in an appreciable decrease in kernel weight (Table I). In the B37 background, the decrease in kernel weight occurred in both endosperm and germ, but in Oh43, the weight decline on introduction of the su_1 gene occurred primarily in the endosperm. The percentage of protein increased slightly in the endosperm of the B37 su_1 mutant strain compared with the protein content in the

normal B37 endosperm. In Oh43, however, the protein content of the endosperm was not changed. In both inbreds, introduction of the su_1 gene lowered the total protein per 100 endosperms (Table I). The Commander sweet corn seed and 60-day grain were not as greatly reduced in size compared with the dent inbreds as were the inbreds containing the su_1 mutant gene. Probably the long breeding program of sweet corn hybrids has selected a background that maintains grain

TABLE I
Amount and Composition of Endosperms of Sugary-1 and Normal Corns (As Is)

Variety	g/100 Kernels	g Endosperm/ 100 Kernels	Endosperm (%)	Protein in Endosperm (%)	mg Protein per 100 Endosperms	mg Methionine per 100 Endosperms	mg ASG per 100 Endosperms
B37							
+	25.5	76.8	19.6	11.8	2,310	8.3	310
su_1	14.0	85.0	11.9	13.3	1,587	7.6	376
Oh43							
+	23.8	85.6	20.4	10.3	2,100	6.9	290
su_1	18.2	67.2	12.2	10.4	1,271	5.1	351
Commander							
Seed	22.0	85.9	18.9	11.0	2,075	6.9	334
60 Day	19.9	76.7	15.3	11.2	1,709	5.8	280

TABLE II
Amino Acid Composition of Defatted Corn Endosperms[a]
(g/100 g Protein)

Amino Acid	B37		Oh43		Commander	
	+	su_1	+	su_1	Seed	60 Day
Lysine	1.9	2.5	1.8	2.8	2.4	2.6
Histidine	2.9	3.4	2.9	3.2	3.0	3.2
Ammonia	3.2	2.7	3.3	2.9	3.0	3.0
Arginine	3.8	4.6	3.8	4.8	4.4	4.5
Aspartic acid	5.7	6.0	5.8	6.1	6.0	6.1
Threonine	3.3	3.6	3.4	3.7	3.5	3.6
Serine	4.8	4.8	5.0	5.0	4.9	4.8
Glutamic acid	21.5	19.3	21.3	18.7	20.0	19.6
Proline	9.6	10.1	9.6	9.4	9.5	9.7
Glycine	2.8	3.6	3.2	4.0	3.5	3.4
Alanine	8.5	7.9	8.5	8.0	8.2	8.1
Cystine[b]	1.9	2.3	2.4	2.1	2.2	2.2
Valine	4.8	5.1	4.3	4.7	5.0	5.0
Methionine[b]	3.6	4.8	3.3	4.0	3.3	3.4
Isoleucine	3.8	3.8	3.8	3.8	3.8	3.8
Leucine	15.1	13.0	14.8	13.0	14.0	13.8
Tyrosine	5.0	5.4	5.1	4.9	5.0	4.8
Phenylalanine	5.3	5.2	5.7	5.2	5.2	5.0

[a]Average of duplicate analysis.
[b]Determined as performic acid oxidized product. LSD (0.05 level) is 0.55 for methionine.

size in the presence of su_1 gene. The endosperm size and protein content of the Commander sweet corn was closer to that of the dent inbreds than of the su_1 inbreds.

Marked changes occurred in amino acid composition of endosperm proteins when the su_1 gene was introduced into B37 and Oh43 inbreds (Table II). Lysine, glycine, and methionine were higher in concentration in both of the su_1 corn endosperms than in the normals. The endosperm protein of B37 inbred had 36% more methionine in the su_1 strain than did the normal, whereas the Oh43 su_1 had 21% more methionine than did its normal allelic sib. The su_1 endosperm proteins contained 32 and 56% more lysine than did their normal counterparts in B37 and Oh43 inbreds, respectively. Thus, proteins in these su_1 strains appeared to be superior in content of both methonine and lysine to normal corns.

Neither the endosperm protein of Commander sweet corn seed nor of the mature grain showed any significant increase in methionine content over normal dent corn, although this grain does contain the su_1 gene (Table II). The level of lysine in the endosperm protein did approximate that of su_1 inbreds and was higher than that of the dent corn inbreds.

An explanation for the shift in amino acid analysis of the proteins of these endosperms can be found in the changed protein composition of the mutant grains. As shown in Table III, the fraction of total nitrogen contained in

TABLE III
Solvent Extraction of Endosperms of Normal, Sugary-1, and Sweet Corns

		B37[a]		Oh43[a]		Commander	
		+[b]	su_1[b]	+[b]	su_1[b]	Seed	60 Day
		Protein in Meal (As Is) (%)					
		11.8	13.3	10.3	10.4	11.0	11.2
Fraction	Proteins	Total Nitrogen Extracted (%)					
0.5*M* NaCl 1 × 5:1 (v/w) + 1 × 5:2 (v/w) 1 hr, 4°C	Albumins, globulins, nonprotein nitrogen	8.9	14.6	8.2	16.1	17.5	15.5
70% EtOH-0.5% NaOAc 3 × 10:1 (v/w) 1 hr, 25°C	Zeins	51.6	28.8	48.6	23.3	41.6	40.5
70% EtOH-0.5% NaOAc-0.1*M* ME[c] 2 × 10:1 (v/w) 0.5 hr, 25°C	Alcohol-soluble reduced glutelins	13.4	23.7	13.8	27.6	16.1	16.4
Residue	Mainly alcohol-insoluble reduced glutelin	26.1	32.9	29.4	33.0	24.8	27.6

[a]Inbred corn.
[b]Genotype.
[c]2-Mercaptoethanol.

albumins, globulins, and nonprotein nitrogen was almost doubled in both su_1 inbreds compared with the normal. The proportion of zein decreased in the su_1 endosperms, but that of ASG protein greatly increased in the mutants of both backgrounds. The su_1 corns also showed a small increase in proportion of residue protein that consists mainly of alcohol-insoluble reduced glutelin. The decrease in zeins was greater than the rise in ASG protein. Because the saline-soluble proteins and alcohol-insoluble reduced glutelins have a high content of lysine and the alcohol-soluble proteins (zein plus ASG protein) are low in this amino acid (2,9,14), the relative changes in the four protein fractions accounted for the improved lysine content. The rise in ASG protein was responsible for the higher methionine content in both su_1 inbreds, because this protein fraction was higher in methionine (7–10).

Both Commander sweet corn seed and 60-day endosperm proteins contained more albumins and globulins but slightly less zein than did those of normal inbred grains. The sweet corn endosperms showed only a small increase in content of ASG protein over the level found in the normal grains. These results were in agreement with the increase in lysine content and absence of increases of methionine in this sweet corn's endosperm. The Commander sweet corn methionine data (Table II) contrasted with the elevated amount of methionine that Nordstrom and Meade (4) and Chi and Speers (5) reported in Northrup King Golden Delight sweet corn. This difference may reflect the fact that our analysis was restricted to endosperm whereas they examined the whole kernels, or more probably indicates that marked varietal differences occur among sweet

TABLE IV
Amino Acid Composition of Alcohol-Soluble Reduced Glutelins[a]
(g/100 g Protein)

	B37		Oh43		Commander	
Amino Acid	+	su_1	+	su_1	**Seed**	**60 Day**
Lysine	0.2	0.2	0.2	0.3	0.2	0.2
Histidine	4.1	2.5	3.4	2.6	4.2	4.2
Ammonia	2.9	2.8	2.9	3.2	2.9	3.1
Arginine	2.8	2.2	2.3	2.5	2.8	2.8
Aspartic acid	2.8	3.4	2.9	3.4	2.3	2.1
Threonine	3.4	3.6	3.5	3.2	3.4	3.4
Serine	4.4	5.0	4.7	4.9	3.9	4.0
Glutamic acid	22.9	24.7	23.7	23.1	20.4	21.5
Proline	15.9	14.9	15.5	14.5	18.8	18.8
Glycine	3.6	3.6	3.8	3.3	3.9	3.8
Alanine	7.5	8.4	7.8	8.4	7.0	6.4
Cystine[b]	3.4	3.3	3.2	3.6	3.6	3.8
Valine	4.6	4.1	4.4	4.0	4.3	4.2
Methionine[b]	6.4	8.2	5.1	8.3	6.7	5.9
Isoleucine	2.6	2.7	2.4	2.8	2.4	2.1
Leucine	13.1	14.5	14.5	14.6	12.9	11.9
Tyrosine	5.5	6.5	5.8	5.6	5.8	5.5
Phenylalanine	3.3	4.6	4.2	4.9	3.0	2.9

[a]Average of duplicate analysis.
[b]Determined as performic acid oxidized product.

corns. Although all sweet corns contain the *su*$_1$ gene, they may have different backgrounds.

The increase in methionine in endosperm protein of the two *su*$_1$ inbred corn lines was greater than could be accounted for solely on the basis of the increase in amount of ASG protein in their endosperms. Therefore, we examined the amino acid composition of the ASG protein of the endosperms of these corns and compared the endosperms to those of the normal inbreds and Commander sweet corn (Table IV). As reported earlier, all of the ASG proteins are practically devoid of lysine (7–10). The ASG protein of the *su*$_1$ inbreds contain more aspartic acid, alanine, and methionine than does the ASG protein of the normal counterparts. This increased content of methionine contributes to the elevated methionine content of the endosperm, as does the greater amount of ASG protein. The methionine of ASG protein in *su*$_1$ inbreds of B37 and Oh43 represents a considerable share of the endosperm methionine—38 and 54%, respectively—whereas ASG proteins in their normal counterparts account for only 22% of the endosperm methionine.

The increase in methionine in ASG protein of the *su*$_1$ inbreds probably can be explained by an increase in only certain proteins comprising this fraction. Paulis and Wall (7,15) have found that the ASG protein is heterogeneous and can be fractionated either by solubility in distilled water or by gel filtration chromatography into components that differ in molecular weight and electrophoretic mobilities. The water-insoluble and low molecular weight fraction of ASG protein is the one that contains high levels of methionine and may be the one that is increased in the *su*$_1$ inbreds.

The ASG protein fraction obtained from either Commander sweet corn seed or 60-day mature grain has only slightly more methionine than does the ASG protein from the normal inbred corns. In contrast, the sweet corn ASG protein has considerably more proline but less aspartic acid, serine, and glutamic acid than do the normals. In the Commander background, the *su*$_1$ gene does not alter the composition of the ASG protein and its amount and therefore does not elevate methionine levels in the grain.

Despite the larger percentage of methionine in the protein of the *su*$_1$ inbred lines compared with that of the normal dents, the total methionine per 100 endosperms was reduced in the *su*$_1$ versions due to their smaller endosperm size and lower amount of protein per 100 kernels (Table I). Even with their lower protein contents, however, the *su*$_1$ inbred lines contained more ASG protein per 100 kernels than did the normal grains (Table I). Thus, the *su*$_1$ mutant gene in these inbred backgrounds appears specifically to increase ASG protein production or to reduce the relative amount of other proteins. In the Commander sweet corn, the methionine content per 100 kernels was intermediate between that for the dent and the *su*$_1$ inbreds (Table I). The ASG protein content for 100 Commander seed endosperms was intermediate between that for the endosperms of normal dent and *su*$_1$ inbred lines, but 100 endosperms from 60-day Commander grain were lower in ASG protein than in 100 endosperms from the other strains.

Whether an elevation in the amount of ASG protein and its methionine content is responsible for the elevated level of methionine in *fl*$_2$ corn is debatable, based on data already in the literature. Hansel et al (2) reported that the increase in amount of glutelin in *fl*$_2$ and its increase in methionine relative to normal

accounted for the increase in methionine in the endosperm of this corn. Sodek and Wilson (9) have shown that ASG protein, also known as zein-2, does not increase greatly in *fl*$_2$ realtive to normal and that this fraction in *fl*$_2$ contains about as much methionine as does normal ASG protein. In contrast, Misra et al (6,14) reported an increase in ASG protein (fraction III) in *fl*$_2$ protein and a higher methionine in this ASG protein than in ASG protein from normal dent endosperms.

The results of this study demonstrate that increases in methionine content of corn protein can be obtained by mutationally induced changes that result in formation of more alcohol-soluble fraction of corn glutelin protein that is rich in methionine. The *su*$_1$ gene appears to induce this elevation in methionine in protein in inbred lines with certain backgrounds. High methionine content was not found in one sweet corn variety tested, and this was attributed to the background in which it occurred. Breeding corn with even greater methionine contents might be economically beneficial, based on use of the *su*$_1$ gene, but introduction of this gene must be accompanied by extensive breeding to improve grain yield, protein content, and energy value of the corn.

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