The Effect of the Floury-2 Gene on the Distribution of Protein Fractions and Methionine in Maize Endosperm[1]

L. W. HANSEL, C-Y. TSAI, and O. E. NELSON[2], Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana 47907

ABSTRACT

The proteins of *floury-2* (fl_2) endosperm contain about 60% more methionine than normal on a weight basis and little or no difference on an endosperm basis. Two main factors contribute to the increased methionine in fl_2 endosperm: the increase in methionine content of the fl_2 glutelins, and the shift of the zein-glutelin protein ratio. Based on total protein, zein decreases from 57 to 32%, and glutelin increases from 31 to 44% in fl_2 relative to normal. The major sources of methionine in each genotype are the zeins and glutelins. In normal, the zeins and glutelins account for 55 and 40%, respectively, of the total methionine. In fl_2 the values are zeins, 22%, and glutelins, 74%. The remaining percentage in each genotype is distributed among the albumins, globulins, and the nonextractable residue. Twenty-four subfractions are obtained following the fractionation of the Osborne fractions and their components on Sephadex. The results show that quantitative shifts in proteins occur in each of the fractions and components. However, there are no new or missing subfractions in fl_2 compared to normal. The distribution of N-terminal methionine is found to coincide generally with the distribution of total methionine. The contribution of N-terminal methionine by each genotype is small, comprising about 1.5% of the total methionine. The fractionation results, in conjunction with the methionine results, show that the gene substitution at the fl_2 locus generally increases production of proteins with a higher methionine content and suppresses synthesis of the proteins with a lower methionine content, and that the fl_2 mutation does not increase methionine by increases in N-terminal methionine residues.

Mertz, Bates, and Nelson (1) discovered that the *opaque-2* (o_2) gene caused a change in the amino acid composition in the maize endosperm. Two of the amino acids that increased, lysine and tryptophan, are known to be the limiting amino acids in maize diets for many animals and man. Since that time, much work has been focused on determining the effects of these changes, and finding other genes that would alter amino acid composition.

Subsequently, Nelson, Mertz, and Bates (2) reported that the *floury-2* (fl_2) gene also caused a change in amino acid pattern in the maize endosperm. In addition to having an increased lysine and tryptophan content, $fl_2/fl_2/fl_2$ endosperms were found to contain 50 to 70% more methionine (mg. per 100 mg. of protein) than normal. On the other hand, $o_2/o_2/o_2$ contained slightly less methionine than normal.

Cromwell et al. (3) showed that diets containing fl_2 maize caused faster growth and a greater feed conversion efficiency in chickens than diets containing normal maize.

Since methionine is the limiting amino acid for growth of man and monogastric animals on cereal diets supplemented with legume protein, and the fl_2 gene is the only gene known in maize which causes a considerable increase of methionine, the primary purpose of this study was to determine the effect of the fl_2 gene on methionine content in the maize endosperm. Also, since Adams and Capecchi (4) as

[1]Journal Paper No. 4959, Purdue University Agricultural Experiment Station. Part of a thesis submitted by the senior author in partial fulfillment of the requirement for the M.S. degree. Supported by CSRS Grant No. 716-15-11.

[2]Present address: Department of Genetics, University of Wisconsin, Madison 53706.

well as others have demonstrated that *N*-formyl-methionyl-tRNA is the initiator of protein synthesis in *Escherichia coli*, the second purpose of this work was to ascertain whether the fl_2 gene might increase methionine content by increasing the proportion of N-terminal amino acids that were methionine. Such a result might be encountered if protein synthesis in maize endosperm were initiated by a codon for methionine but the terminal methionine residues were not subsequently cleaved from the nascent polypeptide chain. It has been claimed that in yeast a requirement for *N*-formyl-methionyl-tRNA in protein chain initiation is confined to 70 S ribosomes (Smith and Marcker, 5). However, Weeks et al. (6) demonstrated that with 80 S wheat embryo ribosomes, protein synthesis is initiated with a 40 S subunit–mRNA-Met-tRNA complex that subsequently combines with a 60 S subunit to form a functional 80 S monoribosome.

MATERIALS AND METHODS

Sample Preparation

Mature kernels of W64A normal (+/+) and the fl_2 mutant (fl_2/fl_2 backcrossed to W64A three times) were soaked in water 4 hr. The pericarps, embryos, and scutella were removed by dissection, and the air-dried endosperms ground in a Waring Blendor and defatted for 36 hr. with *n*-hexane in a Soxhlet apparatus. The dry, defatted endosperm was ground further in a Wig-L-Bug or a Wiley mill using an 80-mesh screen.

Fractionation

A modified Osborne-Mendel method (7) was used to progressively extract the protein of the endosperm sample into four fractions (albumin, globulin, zein, and glutelin). These four fractions were subsequently separated into two components each as described in the following paragraphs.

Water treatment. Each 10-g. sample was stirred with 150 ml. of water 12 hr. at 5°C., followed by centrifugation at 700 × g for 40 min. The residue was washed twice by stirring for 1 hr. with 75 ml. of water. To the combined supernatant fluid, sodium tungstate solution (5.0 g. of sodium tungstate plus 6.0 g. of anhydrous monobasic sodium phosphate in 500 ml. of 0.88N HCl) was added until the pH was lowered to 2.7. The solution was cooled (5°C.) for 12 hr., and the mixture centrifuged at 700 × g for 40 min. The precipitate was regarded as the protein component. The supernatant was reduced in volume at 40°C. in a rotary evaporator under vacuum and finally lyophilized. The fraction obtained after evaporation was regarded as the nonprotein component.

Sodium chloride treatment. The residue remaining following the water treatment was treated with 5% NaCl. The same volumes and procedures were used in this treatment as in the water treatment with the exception of the second washing, in which water was used instead of NaCl. The combined supernatant fluid was dialyzed against several changes of water, evaporated, and lyophilized as in the water fraction.

Ethanol treatment. The residue remaining from the NaCl treatment was resuspended in 70% ethanol. The procedure was the same as in the water treatment, except the stirring was performed at room temperature for 5 hr. Water was added to the combined supernatants until a final alcohol concentration of 35% was attained; the solution was then cooled and centrifuged. The pellet was lyophilized and referred to as the 35% ethanol-insoluble component. The supernatant was

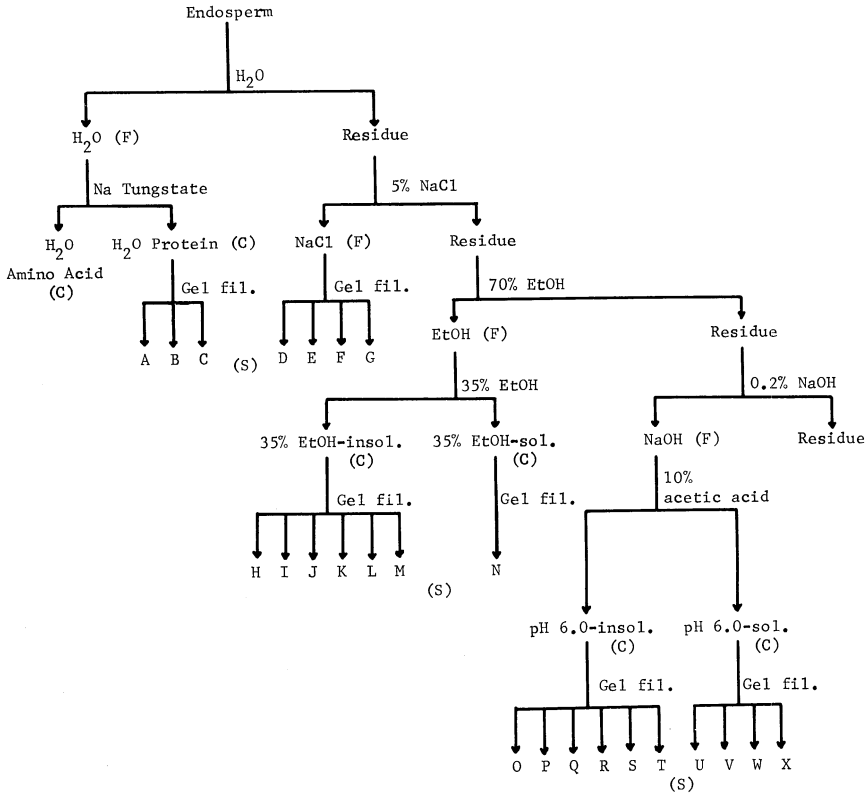

Fig. 1. Diagram of the protein fractionation of the maize endosperm. F, fraction; C, component; S, subfraction.

evaporated and lyophilized. This residue was referred to as the 35% ethanol-soluble component.

Sodium hydroxide treatment. The residue remaining following the ethanol treatment was treated with 0.2% NaOH. The procedure was the same as in the ethanol treatment. Ten percent (w./v.) acetic acid was added to the NaOH extract until a pH of 6.0 was attained. The solution was cooled, centrifuged, and the pellet lyophilized. This pellet was referred to as the pH 6.0-insoluble component. The supernatant was dialyzed against water, evaporated, and lyophilized as before. This fraction was referred to as the pH 6.0-soluble component. The material which was not soluble in any of the four solvents (residue) was lyophilized and used for further analysis. Gel filtration with Sephadex was used to fractionate further the proteins contained in each of the Osborne fractions. The albumins were fractionated on G-100 with 0.025M sodium acetate-acetic acid buffer (pH 4.4). The globulins, zeins, and glutelins were fractionated on G-200 with 0.05M aluminum lactate-lactic acid buffer (pH 3.2) containing 8M urea. The pH of the buffer system after the addition of the urea was 3.5. Each of the Osborne fractions (30 to 100 mg. of protein, measured by the method of Lowry et al. (8))was mixed with 10 ml. of the appropriate buffer, shaken for 24 hr., and then centrifuged at 15,000 X g

for 20 min. The supernatant fluid was added to the Sephadex column (2.0 X 47 cm.) and eluted with the same buffer as used to dissolve the sample. Four-milliliter fractions were collected. Fractions representing a single peak were combined, dialyzed against cold distilled water, and lyophilized. Figure 1 shows a simplified schematic form of the method followed in fractionating the endosperm into its fractions, components, and subfractions.

Nitrogen Determination

Nitrogen content of each sample was determined with a Coleman Automatic Nitrogen Analyzer, which utilizes the Dumas method as described by Jacobs (9). Nitrogen content was multiplied by 6.25 to estimate protein content.

Protein Hydrolysis

To a 50-ml. round-bottom flask containing about 6 mg. of protein, 10 ml. of 6N HCl was added. The flask was flushed with nitrogen gas and sealed under vacuum. Hydrolysis was effected at 110°C. for 24 hr. After hydrolysis the solution was filtered, evaporated to dryness, and the residue dissolved in 1.0 ml. of 10% (v./v.) isopropyl alcohol. Continuous nitrogen flushing of the flask during open reflux hydrolysis was found to cause less destruction and oxidation of methionine than when no protection was used. However, the least destruction occurred when the protein was hydrolyzed in an evacuated sealed tube. The methionine ratios of the fl_2:normal ranged between 1.5 and 1.7 for the endosperm samples, regardless of the method of hydrolysis used. This indicated that approximately the same percentage of destruction was occurring in both genotypes. Thus, relative comparisons can be made between the normal and fl_2, if the same method of hydrolysis is employed for each genotype.

Methionine Determination

The total methionine content of the protein samples was determined quantitatively by the use of two-dimensional paper chromatography. The first dimension was run for 24 hr. in a descending direction using *n*-butanol:acetic acid:water (4:1:5). The chromatogram was air-dried, and then run in the second dimension using 92% (v./v.) ethanol in a descending direction for 24 hr. The chromatogram was again air-dried, dipped in 0.05% (w./v.) ninhydrin in acetone, and the color left to develop for 12 hr. at room temperature. The spot corresponding to methionine was cut out and eluted with 2.0 ml. of 75% (v./v.) acetone in water for 20 min. The absorbance at 570 nm. was read on a Spectronic 20 against an extract of blank paper.

To obtain a standard curve for methionine, DL-methionine (1 to 5 mg.) was added to a protein sample. The hydrolysis and chromatography were performed as described above. After subtracting the quantity of methionine contributed by the protein sample, a standard curve for methionine was obtained.

N-terminal methionine was determined by a modification of the method of Signor et al. (10). To a 50-ml. flask containing 15 to 100 mg. of protein, 6 ml. of 0.1M carbonate buffer (pH 9.0) and 10 ml. of absolute methanol containing 10 mg. of 2-chloro-3,5-dinitropyridine were added accordingly. After shaking the mixture for 4 hr., concentrated HCl was added to adjust the pH to 2.0 followed by centrifugation at 10,000 X g for 15 min. In order to remove excess reagent, the pellet was washed repeatedly with 5 ml. of 2N HCl plus 5 ml. of ethyl acetate. To the washed pellet 2 ml. of 6N HCl and 1 ml. of 30% (v./v.) formic acid were added. The hydrolysis flask was flushed with nitrogen gas, sealed under vacuum, and the

TABLE I. PROTEIN CONTENT OF THE OSBORNE FRACTIONS AND THEIR COMPONENTS OF NORMAL AND fl_2 ENDOSPERMS

	Protein Content					
	Normal			fl_2		
Fraction	mg./g. endosperm	mg./endosperm	% of total	mg./g. endosperm	mg./endosperm	% of total
H_2O-protein	1.86	0.33	1.3	4.39	0.53	3.2
H_2O-nonprotein	1.44	0.26	1.0	3.35	0.41	2.4
H_2O total	3.30	0.59	2.3	7.74	0.94	5.6
Globulins	3.22	0.58	2.3	4.62	0.56	3.4
35% EtOH-insoluble	81.0	14.52	56.9	43.8	5.33	31.8
35% EtOH-soluble	0.91	0.16	0.6	0.69	0.08	0.5
Total (zeins)	81.9	14.68	57.5	44.5	5.41	32.3
pH 6.0-insoluble	34.7	6.22	24.4	54.1	6.57	39.2
pH 6.0-soluble	9.78	1.75	6.8	6.98	0.85	5.1
Total (glutelins)	44.5	7.97	31.2	61.1	7.42	44.3
Residue	9.57	1.72	6.8	20.1	2.44	14.5
Total protein recovered	142.5	25.54	97.5	138.0	16.79	98.6
Total protein	146.2			139.8		
No. kernels/10 g.	55.8			82.2		

mixture hydrolyzed for 12 hr. at 60°C. Upon completion of hydrolysis, water was added to make the solution 2N with respect to HCl. To the mixture was added three 5-ml. portions of ethyl acetate. Each time, the solution was shaken for 1 min. and the organic and aqueous phases separated. The three ethyl acetate fractions were combined and evaporated to dryness. Five milliliters of 5% sodium bicarbonate solution was added to the dry residue to dissolve the amino acid derivatives. Upon lowering the pH of the solution to 2.0 with concentrated HCl, the solution was again extracted with three 5-ml. portions of ethyl acetate. The ethyl acetate extracts were combined, shaken with 5 ml. of water, and centrifuged at 10,000 × g for 15 min. The ethyl acetate was decanted and evaporated to dryness, and the residue remaining was dissolved in a known volume of acetone.

Chromatography of the DNPyr-amino acids was similar to that described by Biserte and Osteux (11). In all analyses, DNPyr-amino acids other than DNPyr-methionine were present but were not identified. The spot corresponding to DNPyr-methionine was cut out and eluted with 1% sodium bicarbonate solution at 60°C. for 30 min. The solution was cooled and read at 340 nm. on a Spectronic 20 against an extract of blank paper.

A standard curve was made by adding DNPyr-methionine (1 to 5 mg.) (Nutritional Biochemical Corporation) to protein before hydrolysis and following the same hydrolytic and chromatographic procedures as with the protein samples alone. After substracting the methionine contributed by the protein sample, a linear DNPyr-methionine standard curve was obtained.

RESULTS AND DISCUSSION

Fractionation by the Modified Osborne-Mendel Method

Table I shows that the major difference in protein production of the normal and *fl*$_2$ occurs in the zein fraction. More specifically, the greatest change was in the 35%

ethanol-insoluble component. This component of the normal contains 85% more protein than the fl_2 on a weight basis, and approximately 2.7 times more on an endosperm basis. This component accounts for 57% of the total protein in normal, but only 32% in fl_2.

Another important difference in the normal and fl_2 is in the glutelin fraction. In the fl_2 the total glutelin fraction and the component insoluble at pH 6.0 contain 37 and 56% more protein, respectively, than their normal counterparts on a weight basis, but not on an endosperm basis. The glutelin fraction accounts for 44% of the protein in the fl_2, and 31% in the normal.

The water-soluble protein in fl_2 is 2.4 times greater on a weight basis than normal. Although this is the greatest difference found within any fraction, it has only a minor effect on the whole endosperm because it accounts for only 5.6% of the total protein in the fl_2 endosperms.

The sodium chloride-soluble fraction of the fl_2 contains 1.4 times more protein on a weight basis than the normal. On an endosperm basis the contribution was equal.

The protein fraction that was not extracted by the four solvents (residue) was found to be higher in fl_2 than normal by 2.2 times on a weight basis, and 1.4 times on an endosperm basis. In general, the results of the fractionation by the modified Osborne-Mendel method were found to be similar to those of Jimenez (12).

Protein Content and Amino Acid Composition in the 35% Ethanol-Insoluble Component of Normal and fl_2 and Their Subcomponents

When the protein of the 35% ethanol-insoluble component was redissolved in 70% ethanol and water was added to reduce the ethanolic concentration to 35%, approximately 50% of the protein precipitated (425 mg. of protein was precipitated and 386 mg. remained) in the normal preparation, whereas all precipitated in the fl_2 (438 mg.). These findings indicate that possibly the zein, which did not precipitate after resolubilization in normal, was the subcomponent which was not produced in fl_2. This possibility was tested against the amino acid composition in the 35% ethanol-insoluble component of normal and fl_2, and the subcomponents of normal 35% ethanol-insoluble component. Table II shows that the amino acid concentrations of the subcomponents of the normal are approximately equal to each other and to the single subcomponent recovered in the fl_2. These results suggest that there are no major changes in the proteins of the two subcomponents of the normal, and the one subcomponent of fl_2, recovered following resolubilization and reprecipitation. Further studies using gel electrophoresis might help to explain the partial precipitation that occurs in normal but not in fl_2, and to determine whether qualitative changes do occur in the proteins of the zein fraction of the normal and fl_2.

Fractionation by Gel Filtration

The results of the gel-filtration patterns and the distribution of protein in the subfractions were based upon duplicate fractionations of the 35% ethanol-insoluble component and triplicate fractionations of the pH 6.0-insoluble component. The results for the other fractions and components were based upon single runs in each case. Gel filtration of each of the Osborne fractions, or their components, from normal endosperm results in three peaks (subfractions) for the water-soluble albumin fraction, four subfractions for NaCl-soluble fraction, six subfractions for

TABLE II. AMINO ACID COMPOSITION IN THE 35% ETHANOL-INSOLUBLE COMPONENT OF NORMAL AND fl_2 AND THE SUBCOMPONENTS OF NORMAL 35% ETHANOL-INSOLUBLE COMPONENT

	Composition[a]			
	Normal			fl_2
Amino Acid	35% EtOH-insol. component	Insol. subcomponent[b]	Sol. subcomponent[c]	35% EtOH-insol. component
Aspartic acid	5.7	6.0	6.0	5.6
Threonine	1.9	1.8	1.6	1.7
Serine	5.6	4.7	5.7	5.6
Glutamic acid	21.4	22.4	21.2	21.8
Glycine	1.3	1.5	1.6	1.3
Alanine	9.4	10.4	10.6	9.8
Valine	1.9	1.7	1.5	1.7
Methionine	2.2	2.3	1.8	1.5
Isoleucine	1.5	1.4	1.3	1.4
Leucine	15.5	15.8	14.9	15.3
Tyrosine	4.5	4.6	4.5	4.4
Phenylalanine	5.8	6.1	5.8	5.6

[a]Amino acid composition was determined on a Beckman automatic amino acid analyzer. Values expressed as mg. per 100 mg. of component protein.
[b]35% ethanol-insoluble subcomponent following resolubilization and reprecipitation of the 35% ethanol-insoluble component.
[c]35% ethanol-soluble subcomponent following resolubilization and reprecipitation of the 35% ethanol-insoluble component.

the 35% ethanol-insoluble component, one fraction for the 35% ethanol-soluble component, six subfractions for the pH 6.0-insoluble component, and four subfractions for the pH 6.0-soluble component. Similar results are obtained for the fl_2 endosperm (Table III). Therefore, it is assumed that there are no new or missing peaks in either genotype. However, these findings alone are not sufficient evidence that the fl_2 gene does not cause any qualitative protein changes in the endosperm.

Compared with each of the normal subfractions, fl_2 subfractions contribute as much or more protein on a weight basis, with the exception of those subfractions obtained from 35% ethanol-insoluble components (Table III). The normal subfractions H, I, J, and K of this component contain more protein than the corresponding fl_2 subfractions.

Total Methionine in the Osborne Fractions

The methionine concentrations (percent of protein) of the total endosperm are: normal, 1.8, and fl_2, 2.9. Since the protein contents of the normal and fl_2 are almost equal, there is about 60% more methionine in fl_2 than normal on a unit weight basis. This is in agreement with the findings of Nelson et al. (2). Because of the smaller endosperm of fl_2 (32% lighter) there is very little difference on an endosperm basis. The main contributors of methionine in each genotype are the zeins and glutelins. The combined zein and glutelin fractions of normal and fl_2 account for 95 and 96%, respectively, of the total methionine in the endosperm (Table IV). In normal the zeins account for the greatest percentage of methionine, 55%, while in the fl_2 the glutelins contribute the greatest percentage, 74%.

The largest changes in methionine concentrations occur in both components of the glutelin fraction. The methionine concentration of the pH 6.0-insoluble

TABLE III. PROTEIN CONTENT OF NORMAL AND fl_2 GEL-FILTRATION SUBFRACTIONS

Component	Subfraction	Protein Content			
		Normal		fl_2	
		mg./g. endosperm	mg./ endosperm	mg./g. endosperm	mg./ endosperm
H_2O-protein	A	0.23	0.04	0.63	0.08
	B	0.71	0.13	2.40	0.29
	C	0.92	0.16	1.36	0.17
5% NaCl-soluble	D	1.13	0.20	1.94	0.24
	E	0.46	0.08	0.49	0.06
	F	0.88	0.16	1.68	0.20
	G	0.75	0.13	0.50	0.06
35% EtOH-insoluble	H	23.9	4.28	14.8	1.80
	I	29.3	5.25	9.46	1.15
	J	15.3	2.74	11.42	1.39
	K	11.6	2.08	6.45	0.78
	L	0.84	0.15	1.03	0.12
	M	0.01	0.002	0.66	0.08
35% EtOH-soluble	N	0.81	0.15	0.69	0.08
pH 6.0-insoluble	O	8.55	1.53	16.4	1.99
	P	9.27	1.66	15.6	1.90
	Q	6.79	1.22	9.0	1.09
	R	5.58	1.00	8.96	1.08
	S	3.51	0.63	3.54	0.43
	T	1.03	0.18	0.65	0.08
pH 6.0-soluble	U	0.80	0.15	1.05	0.13
	V	2.76	0.49	2.24	0.27
	W	5.47	0.98	3.10	0.38
	X	0.75	0.13	0.50	0.06

component of the *fl*$_2$ is 5.1%, compared to 2.7% in normal. The values for the pH 6.0-soluble component are: *fl*$_2$, 2.3%, and normal, 0.9% (Table IV).

The increase in methionine in the *fl*$_2$ endosperm is due mainly to two factors. One is the greater concentration of methionine present in the *fl*$_2$ glutelins than those of the normal. The *fl*$_2$ glutelins contain more than twice as much methionine on a percentage basis. The other is the shift of the zein-glutelin protein ratio. Based on total protein, zein decreases from 57 to 32% and glutelin increases from 31 to 44% in *fl*$_2$ relative to normal. In this respect the elevated methionine associated with *fl*$_2$, resulting from a lowering of zein and a concomitant increase of glutelin, is similar to that of the elevated lysine associated with *o*$_2$ reported by Jimenez (13), Dalby (14), and Murphy and Dalby (15).

Total Methionine in the Sephadex Subfractions

In general, the methionine concentration (percent of component protein) of each of the *fl*$_2$ subfractions is either greater or approximately equal to that of the corresponding normal subfractions (Table V). In the subfractions of the albumins, globulins, and zeins, differences between *fl*$_2$ and normal in the total methionine

TABLE IV. DISTRIBUTION OF TOTAL METHIONINE IN THE OSBORNE FRACTIONS AND THEIR COMPONENTS OF NORMAL AND fl_2 ENDOSPERMS

	Methionine Content							
	Normal				fl_2			
	% of component protein	mg./g. endosperm	mg./ endosperm	% of total	% of component protein	mg./g. endosperm	mg./ endosperm	% of total
H_2O-protein	0.8	15	2.76	0.6	0.5	23	2.80	0.6
H_2O-nonprotein	0.4	6	1.00	0.2	0.14	5	0.60	0.1
H_2O total	0.6	21	3.76	0.8	0.4	28	3.40	0.7
Globulins	1.0	34	6.11	1.3	1.2	57	6.99	1.5
35% EtOH-insoluble	1.8	1432	256.6	55.2	2.0	856	104.1	21.8
35% EtOH-soluble	0.2	2	0.30	0.06	0.13	1	0.12	0.02
Total (zeins)	1.8	1434	256.9	55.3	1.9	857	104.2	21.8
pH 6.0-insoluble	2.7	951	170.4	36.7	5.1	2760	335.7	69.4
pH 6.0-soluble	0.9	93	16.6	3.5	2.3	162	19.7	4.1
Total (glutelins)	2.3	1044	187.0	40.2	4.8	2922	355.4	73.5
Residue	0.6	59	10.50	2.4	0.5	100	12.1	2.5
Total methionine	1.8	2592	464.3	100	2.9	3964	482.0	100
Actual recovery (%)	109.2				107.8			

TABLE V. DISTRIBUTION OF METHIONINE IN NORMAL AND fl_2 GEL-FILTRATION SUBFRACTIONS

Component	Sub-fraction	Methionine Content					
		Normal			fl_2		
		% of component protein	mg./g. endo-sperm	mg./ endo-sperm	% of component protein	mg./g. endo-sperm	mg./ endo-sperm
H_2O-protein	A	1.1	2.4	0.43	1.2	7.1	0.86
	B	1.2	8.7	1.57	0.5	12.0	1.45
	C	0.5	4.3	0.76	0.3	4.4	0.53
5% NaCl-soluble	D	1.3	14.9	2.66	1.21	23.9	2.90
	E	0.9	4.1	0.74	1.1	5.6	0.68
	F	1.6	13.7	0.49	1.6	27.6	3.35
	G	0.16	1.25	0.22	0.1	0.52	0.06
35% EtOH-insoluble	H	2.7	647	115.8	2.5	372	45.3
	I	1.5	443	79.1	2.4	220	26.9
	J	1.9	294	52.4	2.0	226	27.7
	K	0.4	50	8.90	0.7	43.5	5.4
	L	...	...	...	0.06	0.0	0.0
	M	...	...	...	...	...	...
35% EtOH-soluble	N	0.2	2.0	0.30	0.13	1.0	0.10
pH 6.0-insoluble	O	2.6	220	39.4	3.8	615	74.9
	P	3.0	274	49.2	8.1	1262	153.6
	Q	1.5	102	18.4	2.7	246	30.0
	R	3.7	204	36.6	5.1	456	55.6
	S	4.3	151	27.0	5.1	179	21.9
	T	0.0	0.0	0.0	...	...	...
pH 6.0-soluble	U	1.1	9.6	1.71	0.8	8.4	1.02
	V	0.8	23.2	4.14	2.6	57.6	7.01
	W	1.0	58.1	10.0	3.0	92.8	11.2
	X	0.3	2.1	0.38	0.5	2.7	0.33

content (γ per unit weight) are accountable for in terms of protein content, since little or no difference in the percentage of methionine based on protein is apparent. However, in the case of the subfractions in the glutelins, elevated levels both of protein and the amount of methionine in the protein are seen in fl_2 relative to normal. The most marked increase in methionine of any subfraction is subfraction P of the pH 6.0-insoluble component of fl_2. The methionine concentration is 8.1%, which is about three times higher than the corresponding normal subfraction (Table V). This subfraction alone contributes about 50% as much methionine as the whole normal endosperm on a weight basis. These findings suggest that the gene substitution at the fl_2 locus causes the production of protein containing a higher content of methionine and/or prevents the production of protein containing a lower content of methionine.

N-Terminal Methionine in the Osborne Fractions

The data presented were based upon chromatographic results using duplicate hydrolysates. As shown in Table VI, the fl_2 endosperm contains a 57% higher N-terminal methionine concentration than the normal on a protein basis. On a weight basis (per g. endosperm) fl_2 was 52% higher. On an endosperm basis there is only slightly more in fl_2.

The distribution of N-terminal methionine in maize endosperm is found to coincide generally with the total methionine distribution. In the NaCl fraction, 35% ethanol-soluble component, and the fraction insoluble in the four solvents (residue), no N-terminal methionine can be detected. This might be because small quantities of protein were used for analysis. But even if minute quantities of N-terminal methionine were present in these fractions, they would have very little effect upon the total distribution. As has been found for total methionine, the

TABLE VI. DISTRIBUTION OF N-TERMINAL METHIONINE IN THE OSBORNE FRACTIONS AND THEIR COMPONENTS OF NORMAL AND fl_2 ENDOSPERMS

	N-Terminal Methionine							
	Normal				fl_2			
Component	mg./100 mg. component protein	mg./g. endo-sperm	mg./ endo-sperm	% of total	mg./100 mg. component protein	mg./g. endo-sperm	mg./ endo-sperm	% of total
H_2O-protein	12.9	0.19	0.03	0.5	7.4	0.32	0.04	0.6
Globulins	0.0	0.00	0.00	0.0	0.0	0.0	0.00	0.0
35% EtOH-insoluble	29.4	23.8	4.27	63.1	25.6	11.2	1.38	19.7
35% EtOH-soluble	0.0	0.00	0.00	0.0	0.0	0.0	0.0	0.0
Total (zeins)	29.4	23.8	4.27	63.1	25.6	11.2	1.38	19.7
pH 6.0-insoluble	34.4	11.8	2.12	31.3	82.2	44.4	5.41	77.1
pH 6.0-soluble	19.5	1.9	0.34	5.0	21.1	1.5	0.18	2.6
Total (glutelins)	31.1	13.7	2.46	36.3	75.4	45.9	5.59	79.7
Residue	0.0	0.00	0.00	0.0	0.0	0.00	0.00	0.0
Total N-terminal methionine	26.5	37.7	6.76	100	41.6	57.4	7.01	100

largest contributors of N-terminal methionine are the 35% ethanol-insoluble and pH 6.0-insoluble components of both genotypes. The analyses of the 35% ethanol-insoluble component and the pH 6.0-insoluble component of each genotype seem to show a fairly constant relationship between total methionine content and N-terminal methionine content. On the other hand, no constant relationship could be found in the pH 6.0-insoluble components.

Although the fl_2 endosperm contains a 57% higher N-terminal methionine concentration than the normal on a protein basis, the increased N-terminal methionine could not account for the total methionine increase seen in the protein of the mutant. The contribution of N-terminal methionine by each genotype was small, comprising about 1.5% of the total methionine.

The distribution of N-terminal methionine and the detection of N-terminal amino acids (not identified) other than N-terminal methionine indicate that the fl_2 gene does not condition an increased accumulation of N-terminal methionine in fl_2 endosperm protein.

Acknowledgment

We wish to thank E. T. Mertz in whose laboratory the column chromatographic (Beckman automatic amino acid analyzer) assays of the amino acids were made.

Literature Cited

1. MERTZ, E. T., BATES, L. S., and NELSON, O. E. Mutant gene that changes protein composition and increases lysine content in maize endosperm. Science 145: 279 (1964).
2. NELSON, O. E., MERTZ, E. T., and BATES, L. S. Second mutant gene affecting the amino acid pattern of maize endosperm proteins. Science 150: 1469 (1965).
3. CROMWELL, G. L., ROGLER, J. C., FEATHERSTONE, W. R., and CLINE, T. R. A comparison of the nutritive value of *opaque-2, floury-2*, and normal corn for the chick. Poultry Sci. 47: 840 (1968).
4. ADAMS, J. M., and CAPECCHI, M. R. N-formylmethionyl-sRNA as the initiator of protein synthesis. Proc. Nat. Acad. Sci. U.S. 55: 147 (1966).
5. SMITH, A. E., and MARCKER, K. A. N-formylmethionyl transfer RNA in mitochondria from yeast and rat liver. J. Mol. Biol. 38: 241 (1968).
6. WEEKS, D. P., VERMA, D. P. S., SEAL, S. N., and MARCUS, A. Role of ribosomal subunits in eukaryotic protein chain initiation. Nature 236: 167 (1972).
7. OSBORNE, T. B., and MENDEL, B. L. Nutritive properties of the maize kernel. J. Biol. Chem. 18: 1 (1914).
8. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265 (1951).
9. JACOBS, S. The determination of nitrogen in biological material. In: Methods of biochemical analysis, ed. by D. Glick; Vol. XIII, p. 241. Interscience: New York (1965).
10. SIGNOR, A., BIONDI, L., TERBOJEVICH, M., and PAJETTA, P. Nuovo methodo per la determiazione dell aminoacido N-terminale in polypeptidide proteine. Impiego del reattivo 2-Cl-3,5-dinitropyridina. Gazz. Chim. Ital. 94: 619 (1964).
11. BISERTE, G., and OSTEUX, R. La chromatographie de partage sur papier dis dinitrophenyl-aminoacids. Bull. Soc. Chim. Biol. 33: 50 (1951).
12. JIMENEZ, J. R. The effect of the *opaque-2* and *floury-2* genes on the production of protein in maize endosperm. Ph.D. thesis, Purdue University (1968).
13. JIMENEZ, J. R. Protein fractionation studies of high lysine corn. In: Proceedings of the high lysine corn conference, ed. by E. T. Mertz and O. E. Nelson. Corn Ind. Res. Found.: Washington, D.C. (1966).
14. DALBY, A. Protein synthesis in maize endosperm. In: Proceedings of the high lysine corn conference, ed. by E. T. Mertz and O. E. Nelson. Corn Ind. Res. Found.: Washington, D.C. (1966).
15. MURPHY, J. J., and DALBY, A. Changes in the protein fractions of developing normal and *opaque-2* maize endosperm. Cereal Chem. 48: 336 (1971).

[Received December 12, 1972. Accepted March 19, 1973]