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Endosperm Protein Synthesis in Maize Mutants with Increased Lysine Content

Abstract. *The endosperm proteins of the maize mutants,* opaque-*2*, opaque-*7*, floury-*2*, brittle-*2, and the double mutant of* opaque-*2 and* brittle-*2, were separated into five soluble fractions by the Landry-Moureaux method. As compared to their isogenic normal counterparts, the mutant endosperms had higher concentrations of albumins, globulins, and glutelin-3, and lower concentrations of prolamines. The combination of the* opaque-*2 and* brittle-*2 genes enhanced this difference. Although the four mutant genes are located on three different chromosomes, they exert a similar effect on endosperm protein composition. Five other starch-modifying mutants with high lysine content resemble the* brittle-*2 mutant in endosperm protein composition, when the gene is present either singly or combined with* opaque-*2. Therefore, the pattern of protein synthesis in all maize mutants with high lysine concentrations may be either identical or very similar. Because no synergistic effect on lysine concentration is obtained when* floury-*2 is combined with* opaque-*2, different pathways leading to reduced zein synthesis may exist in the floury and starch-modifying mutants with high lysine concentrations.*

In 1964 Mertz *et al.* (*1*) reported that the *opaque*-2 (o_2) gene (chromosome 7) changed the protein composition and increased the lysine content of maize endosperm. Using a copper fractionation method (*2*), they found that the zein concentration of o_2 endosperm was lower, and the glutelin concentration was higher, than in normal endosperm. A second maize mutant with high lysine concentrations, *floury*-2 (fl_2) (chromosome 4), was identified and reported in 1965 (*3*). Recently, McWhirter (*4*) identified a third maize mutant with high lysine content, designated *opaque*-7 (o_7). This appeared as a spontaneous mutation in the inbred line W22; linkage studies (*4*) show it is located on chromosome 10.

In studies of starch-modifying mutant genes and their combinations with the o_2 gene, we found that the *sugary*-1 (su_1), *shrunken*-1 (sh_1), *shrunken*-2 (sh_2), *shrunken*-4 (sh_4), *brittle*-1 (bt_1), and *brittle*-2 (bt_2) genes increased the lysine content of the endosperm substantially above the isogenic normal control, and each gene had an enhanced effect on lysine when the gene was combined with o_2 (*5*). In addition, we found that the distribution of endosperm proteins in these six mutants resembled that found in the three floury maize types with high lysine content (o_2, o_7, and fl_2).

We present data on the bt_2 mutant and its combination with o_2 as a typical example of the starch-modifying mutants that exhibit more or less comparable degrees of endosperm defectiveness in the mature kernel. We have added, for comparison, the data on o_2 and fl_2 mutants in the same isogenic background, and have included the o_7 mutant, whose complete amino acid and protein patterns have not been reported previously. The bt_2 gene is located on chromosome 4 at a locus distinct from that of fl_2. The endosperm of the bt_2 mutant is translucent and shrunken (*6*).

Near isogenic sublines of o_2, fl_2, and bt_2 of inbred Oh43 were recovered after six backcrosses to the recurrent parent. The double mutant bt_2o_2 was isolated by a system of backcrossing and selfing, which permitted the classification for the segregation of the bt_2 gene in the background of the o_2 gene. The double mutant endosperm is opaque and shrunken, and the mature kernels are comparable in defectiveness to those of the bt_2 mutant (*7*).

The dry corn kernels were soaked in distilled water for 30 minutes, and were then separated with a scalpel into pericarp, embryo, and endosperm. The endosperms were dried in air overnight, were defatted with hexane, and were ground to a fine powder in a ball mill. The concentration of nitrogen was determined on the powder by the micro-Kjeldahl technique; 25-mg samples were hydrolyzed with 100 ml of 6*N* HCl under reflux for 24 hours, the acid was removed in a vacuum on a rotary evaporator, and the residue was taken up in *p*H 2.2 citrate buffer. This solution was applied to the columns of an automatic amino acid analyzer (Beckman-Spinco). Data were obtained on single hydrolyzates because duplicate hydrolyzates on many similar maize mutant samples did not differ by more than 5 percent in their lysine content. The concentration of tryptophan was determined in 100-mg samples of defatted powder by a colorimetric method (*8*).

Portions of the powdered defatted endosperms (1 g each) were subjected to fractionation sequence D of the procedure described by Landry and Moureaux (*9*). Fraction I contains proteins soluble in saline (albumins, globulins); fraction II, zein (prolamine); fraction III, glutelin-1; fraction IV, glutelin-2; and fraction V, glutelin-3.

Other data (*5*) show that the embryos

Table 1. Amino acid composition of defatted maize endosperms.

Amino acid	Amino acid concentration (grams per 100 g of protein) in						
	Oh43					W22	
	+	fl_2	o_2	bt_2	o_2bt_2	+	o_7
Lysine	1.6	2.7	3.5	3.3	5.3	2.3	3.8
Tryptophan	0.3	0.5	0.8	0.7	1.3	0.4	0.7
Leucine	16.4	15.4	12.1	12.3	8.3	15.9	12.5
Isoleucine	4.3	4.3	4.3	4.1	4.1	4.3	4.3
Threonine	4.0	3.9	4.4	4.4	5.5	4.0	4.4
Methionine	2.4	3.7	2.7	3.4	2.4	3.2	3.2
Cystine	2.1	1.4	2.1	2.0	2.3	2.4	1.9
Phenylalanine	6.8	6.1	6.0	5.6	5.2	6.7	5.2
Tyrosine	5.9	5.1	5.2	5.1	4.9	5.8	4.9
Valine	5.2	5.2	5.9	5.6	7.0	5.7	6.7
Histidine	3.0	2.9	3.4	3.2	3.6	3.6	4.1
Arginine	3.4	5.8	5.1	5.0	7.2	3.7	5.2
Glycine	3.3	3.2	4.9	4.6	7.4	4.0	5.2
Alanine	10.1	8.9	8.2	8.3	7.5	8.5	7.9
Serine	6.0	5.3	5.4	5.6	5.6	5.4	5.6
Aspartic acid	7.5	7.2	9.5	8.1	10.7	6.7	9.4
Glutamic acid	30.0	26.4	23.6	23.6	19.0	27.4	25.5
Proline	11.3	9.5	9.8	9.6	8.9	11.5	11.0
Protein (percent)	11.8	12.3	10.1	13.4	12.9	8.5	7.3

Table 2. Nitrogen distribution in maize endosperms.

Fraction	Soluble nitrogen (percent of total) in						
	Oh43					W22	
	+	fl_2	o_2	bt_2	o_2bt_2	+	o_7
I (saline)	5.8	9.2	13.6	12.1	22.3	6.9	16.6
II (zein)	59.0	49.1	26.9	26.1	2.9	40.6	20.3
III (glutelin-1)	5.8	9.0	8.4	15.4	5.5	15.3	12.0
IV (glutelin-2)	12.7	7.6	14.0	8.7	12.2	12.8	18.8
V (glutelin-3)	13.8	22.0	29.2	27.9	48.0	21.0	29.5
(Total nitrogen extracted)	97.1	96.9	92.1	90.2	90.9	96.6	97.2

of all four mutants and the double mutant bt_2o_2, have similar amino acid patterns, with lysine content ranging from 5.4 to 6.1 percent of total protein.

As compared to their normal isogenic counterparts (Table 1), the four endosperm mutants can be classified as maize mutants with high lysine content. The combination of the o_2 and bt_2 genes increases the difference in amino acid content: lysine and tryptophan increase to 5.3 and 1.3 percent, respectively, and leucine decreases to 8.3 percent. When whole ground kernels of o_2, bt_2, and the double mutant were each fed to two inbred Wistar rats at an amount that was 95 percent of the ration, for 10 days, the animals fed the double mutant gained an average of 4.8 g/day, which is 1.6 times that (3.0 g/day) of the animals on the other two diets (*10*). These initial tests suggest that the extra lysine and tryptophan of the double mutant are available for growth purposes. However, because of the shrunken endosperm of the double mutant, one would expect reduced yields for mature grains, making this nutritious kernel economically impractical for animal feeds. Nevertheless, even at twice the current production cost of normal corn (2¢ per pound), the double mutant could have value as a special food for infants, or as an edible corn in the green stage of development.

Table 2 shows the protein distribution in the fractions extracted from each sample. All mutants show a substantial increase in proteins soluble in saline; again, there is an enhanced effect with the double mutant. The zein concentration (fraction II) drops with introduction of any one of the mutant genes; the enhanced effect is pronounced in the double mutant, where zein content drops to 2.9 percent. The major glutelin fraction (glutelin-3, fraction V) increases with the introduction of each one of the mutant genes. The enhanced effect of the o_2 and bt_2 genes is evident, with this fraction comprising 48 percent of the soluble nitrogen (Table 2, column 6). The endosperm proteins of near isogenic (Oh43), su_1, sh_1, sh_2, sh_4, and bt_1 also have increases in lysine, albumins, globulins, and glutelin-3, and a reduction in zein, when the gene is present either singly or combined with o_2 (*5*).

Complete amino acid patterns (with the exception of tryptophan) have been obtained on the protein fractions listed in Table 2 (*5*). The range of lysine values in these fractions was as follows (grams of lysine per 100 grams of protein): I, 3.7 to 6.3; II, 0.1 to 0.5; III, 0.4 to 0.8; IV, 1.4 to 2.8; and V, 6.4 to 7.1. The increased lysine content (Table 1) for fl_2, bt_2, o_2, o_2bt_2, and o_7 is due primarily to increases in fractions I and V (proteins high in lysine content), and to decreases in fraction II (proteins low in lysine content) (Table 2).

These studies show that the mutant genes fl_2, o_2, bt_2, and o_7 exert a similar effect, namely, a repression of zein synthesis, and a stimulation of albumin, globulin, and glutelin synthesis. Thus, similar effects are observed with mutant genes from three different chromosomes, and a synergistic effect is obtained when o_2 (chromosome 7) and bt_2 (chromosome 4) are combined to form the double mutant. Since five other starch-modifying mutants high in lysine content resemble the bt_2 mutant in protein composition, either singly or when combined with o_2, the pattern of protein synthesis in all maize types high in lysine content may be either identical or very similar.

In contrast to the starch-modifying mutant genes, no enhanced effect is observed when fl_2 is combined with o_2 in the Oh43 background. The double mutant o_2fl_2 has a lysine concentration that is intermediate between that of o_2 and fl_2 (*5*). Therefore, while the pattern of protein synthesis appears to be the same, the pathway leading to reduced zein synthesis in the fl_2 mutant may be different from that in the starch-modifying mutants.

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Biosynthesis of Hemoglobin Ann Arbor: Evidence for Catabolic and Feedback Regulation

Abstract. *Hemoglobin Ann Arbor, in which arginine replaces leucine in position 80 of the α chain, occurs in affected individuals in low proportion to hemoglobin A. Biosynthetic studies were performed on reticulocytes of a patient heterozygous for this hemoglobin. These studies suggested that the low percentage of hemoglobin Ann Arbor is primarily due to preferential destruction of the abnormal component. The reduced concentration of α Ann Arbor chains was also reflected in a decreased synthesis of normal β chains.*

Hemoglobin Ann Arbor is an α chain variant in which the substitution has been shown to be leucine to arginine at position 80 (*1*). The individual in which this variant was originally detected is a Caucasian male who was splenectomized at age 30 because of hemolytic anemia. He now has 12 g of hemoglobin per 100 ml of blood, with 10 percent of red cells as reticulocytes. His erythrocytes are slightly microcytic and moderately hypochromic with moderate anisocytosis and poikilocytosis. Target cells are abundant. Siderocytes are present in the peripheral blood, and the bone marrow is laden with iron. The patient's hemoglobin Ann Arbor component migrates slightly slower than hemoglobin S on starch gel electrophoresis at *p*H 8.6 and comprises about 14 percent of the total hemoglobin.

Hemoglobin Ann Arbor is unstable to heat, a property that frequently disposes hemoglobin to form intraerythrocytic inclusions (*2*). When the erythrocytes of the patient were incubated with crystal violet, a classical pattern of inclusions could not be seen with light microscopy. However, some inclusions suggestive of precipitated hemoglobin have been seen by transmission electron microscopy (*3*).

Abnormal hemoglobins that occur in reduced proportion are not uncommon (*4*). Some hemoglobins are unstable and are known to precipitate readily (*2*). These hemoglobins may be lost from the circulating erythrocytes by the removal of such aggregates of precipitated hemoglobin in the spleen (*5*). A second mechanism, which is supported by experimental evidence, invokes a diminished rate of synthesis (*6*). The patient we used afforded a uniquely desirable combination of circumstances for studying the control of biosynthesis of an abnormal hemoglobin component. Because he lacked a spleen and obvious erythrocyte inclusions, the mechanism of precipitation seemed unlikely. In addition, with 10 percent reticulocytes the patient's blood was expected to be fairly active in the biosynthesis of new hemoglobin. We believed that incubation of samples of this patient's blood in the presence of an isotopically labeled amino acid would indicate whether a diminished rate of synthesis is responsible for the reduced proportion of this abnormal hemoglobin in the peripheral blood or whether some other form of control is manifested.

Venous blood was collected in tubes containing heparin and immediately chilled in ice. Cells were washed four times in NKM (*7*) and suspended in a complete medium, which contained all amino acids required for protein synthesis except leucine, which was added as the ^3H- or ^{14}C-labeled form. Incubation was carried out at 25°C in a Dubnoff metabolic shaking bath. Samples of the incubation were removed at the times shown in Table 1, and further synthesis was stopped by lysis of the cells with four volumes of 1.5 m*M* $MgCl_2$ at 0°C. The ionic strength was rapidly returned to the initial value by addition of a normalizing buffer. The lysates were kept chilled, and the stroma and undisrupted cells were removed by centrifugation at 30,000*g*. Polyribosomes were removed by layering the lysates over cushions of 30 percent sucrose in TKM (*7*) and centrifuging at 100,000*g*. The

Table 1. Specific radioactivities of the purified hemoglobins and their component chains at the time periods indicated. Specific activities (disintegrations per minute per micromole of leucine) and their ratios are given; A and AA refer to hemoglobin A and hemoglobin Ann Arbor, respectively.

Time (min)	Chains							Hemoglobin		
	αA	βA	αA/βA	αAA	βAA	αAA/βAA	αAA/αA	A	AA	AA/A
4	14.2	4.31	3.3	44.1	20.7	2.1	3.1	12.4	32.1	2.6
7	74.2	22.9	3.2	217	103	2.1	2.9	66.5	172	2.6
13	974	286	3.4	2,680	1,220	2.2	2.8	914	2,390	2.5
30	2,740	1,120	2.5	7,420	3,410	2.2	2.7	2,040	5,250	2.6
60	4,840	1,460	3.3	12,600	6,300	2.0	2.6	4,510	11,300	2.5
1,320	38,600	16,300	2.4	100,000	53,800	1.9	2.6	42,700	107,000	2.5
1,320*	1,210	509	2.4	3,150	1,700	1.8	2.6	1,420	3,600	2.5

* Experiment with [^{14}C]leucine.