STUDIES ON CORN PROTEINS. IX. COMPARISON OF THE AMINO ACID COMPOSITION OF LANDRY-MOUREAUX AND PAULIS-WALL ENDOSPERM FRACTIONS[1]

P. S. MISRA, E. T. MERTZ, Department of Biochemistry, and D. V. GLOVER, Department of Agronomy, Purdue University, Lafayette, IN 47907

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ABSTRACT

Comparison of the average amino acid composition of the Landry-Moureaux (LM) endosperm fractions of two normal corn (*Zea mays* L.) inbreds and five high-lysine mutants with the amino acid composition of the Paulis-Wall (PW) endosperm fractions of a normal corn hybrid shows marked similarity between LM fraction II and PW alkylated-reduced zein, LM fraction III and PW guanidine and 70% ethanol-soluble alkylated-reduced glutelin, LM fraction IV and PW guanidine-insoluble alkylated-reduced glutelin, and LM fraction V and PW guanidine-soluble 70% ethanol-insoluble alkylated-reduced glutelin. Both the normal and mutant LM fractions I and V contain high levels of lysine; LM fractions II and III contain low levels of lysine; LM fraction III contains high levels of methionine; and LM fraction IV contains high levels of histidine. Although the glutelins are separated in a different manner, the corresponding PW fractions show these same differences. Since the mutant fractions resemble the normal fractions, this is further evidence that the high-lysine levels in the five mutants are due to the previously reported increase in LM fractions I and V, not to new proteins high in lysine.

The Landry-Moureaux (LM) (1) fractionation method has provided us with useful protein fractions from the endosperm of normal and mutant corn (*Zea mays* L.) genotypes. Marked changes in the distribution of proteins in mature normal and mature high-lysine mutants have been found (2,3). We have also reported major differences in protein patterns in the developing endosperms of normal and high-lysine mutants (4).

The LM method produces five soluble fractions: I (saline-soluble; albumin; globulin), II (alcohol-soluble; zein), III (mercaptoethanol, alcohol-soluble; zein-like), IV (mercaptoethanol, pH 10-soluble; glutelin-like) and V (mercaptoethanol, pH 10, detergent-soluble; true glutelin).

Paulis and Wall (PW) (5) isolated the saline-soluble (fraction I) and alcohol-soluble proteins (fraction II) in the same manner as Landry and Moureaux, but a different procedure was used for isolating the glutelins. The endosperm residue remaining after saline and alcohol extraction was freed of starch with α-amylase to give a crude glutelin protein residue. This protein was reduced with mercaptoethanol and alkylated with acrylonitrile. The reduced-alkylated protein was divided into two fractions based on solubility in $6M$ guanidine hydrochloride. The guanidine-insoluble fraction will be designated as IARG (guanidine-insoluble alkylated-reduced glutelin). The guanidine-soluble fraction was divided into two parts, one soluble in 70% ethanol, which we will designate ESARG (guanidine and ethanol-soluble alkylated-reduced glutelin), and the

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other insoluble in ethanol, EIARG (guanidine-soluble, ethanol-insoluble alkylated-reduced glutelin). Their zein fraction was also reduced and alkylated (RAZ).

In this report, we compare the amino acid composition of LM fractions II, III, IV, and V from inbred normal and mutant endosperms with PW fractions RAZ, ESARG, IARG, and EIARG from a normal hybrid endosperm.

MATERIALS AND METHODS

Near-isogenic lines of *opaque*-2 (o_2), *floury*-2 (fl_2), *brittle*-2 (bt_2), and the double mutant *brittle*-2, *opaque*-2 (bt_2o_2) in the Oh 43 inbred line and *opaque*-7 (o_7) in the W22 inbred line were recovered by backcrossing as described previously (2,3). Separation of the endosperms of these maize lines and fractionation of the nitrogen by the Landry-Moureaux method followed the procedures described by Misra *et al.* (3).

Amino Acid Determination

For each of the five fractions, an aliquot of aqueous extract containing 2.5 mg of nitrogen was hydrolyzed in a final volume of 100 ml of 6*N* HCl for 24 hr under reflux. The hydrochloric acid was evaporated *in vacuo* in a rotary evaporator and the residue dissolved in 10 ml diluter buffer (Beckman-Spinco) at pH 2.2. One-milliliter portions were applied to the short and long columns of a Beckman-Spinco Automatic amino acid analyzer.

The fractionation and amino acid determination methods used by Paulis and Wall on the endosperm of a single-cross hybrid field corn are described in the literature (5). Their data are discussed below.

RESULTS AND DISCUSSION

The complete amino acid patterns of each of the five Landry-Moureaux fractions of normal Oh 43, and of the mutants o_2, fl_2, bt_2, and bt_2o_2, and of the normal W22 and W22 o_7 have been published (6). The average values and the range in amino acid composition for Landry-Moureaux fraction I of normal and high-lysine mutants are given in Table I. Fraction I consists mainly of albumins and globulins. The lysine level is high in normal and high-lysine mutants. The level appears to be slightly higher in the normal than it is in the mutants. This fraction is also characterized by high levels of arginine and low levels of glutamic acid, proline, and leucine. The cystine and methionine values are lower than normal because of oxidation losses during hydrolysis.

The average amino acid composition of endosperm fraction II obtained by the LM method was similar for the normals and mutants (Table II). The amino acid values obtained on the PW alkylated-reduced zein agree very well with the LM values, except for cystine. Since the cystine was reduced and alkylated before analysis in the PW method, this value is probably close to the true value. In the LM fractions, the cystine was probably lost due to oxidation during hydrolysis. The zein fraction is characterized by very low levels of lysine and very high levels of glutamic acid, proline, and leucine.

Table III compares the amino acid composition of LM endosperm fraction III and PW guanidine and ethanol-soluble alkylated-reduced glutelin. This is a

TABLE I
Amino Acid Composition of LM Endosperm Fraction I[a]

Amino Acid	Normal Inbreds[b]		High-Lysine Mutants[b]	
	Average	Range	Average	Range
Lysine	6.2	6.1 – 6.3	4.8	3.7 – 5.8
Histidine	2.8	2.5 – 3.2	2.5	2.2 – 3.3
Arginine	11.0	10.4 – 11.7	8.4	6.1 – 11.8
Aspartic Acid	9.5	7.9 – 11.1	8.6	7.4 – 9.6
Threonine	4.7	4.2 – 5.3	4.0	3.5 – 4.9
Serine	5.1	4.5 – 5.8	4.5	3.9 – 5.2
Glutamic Acid	14.7	10.9 – 18.5	13.3	9.2 – 14.8
Proline	5.6	4.8 – 6.4	5.7	4.5 – 7.4
Glycine	7.3	6.4 – 8.2	6.6	5.8 – 7.5
Alanine	7.9	7.0 – 8.8	7.4	5.8 – 8.5
Cystine	0.9	0.3 – 1.6	2.5	0.1 – 4.2
Valine	5.4	4.7 – 6.2	5.5	4.7 – 6.8
Methionine	2.1	1.7 – 2.5	1.6	0.8 – 3.0
Isoleucine	4.2	3.9 – 4.6	3.6	3.1 – 4.3
Leucine	5.9	5.4 – 6.4	5.0	4.4 – 6.1
Tyrosine	3.6	2.8 – 4.5	3.7	3.0 – 4.2
Phenylalanine	3.3	2.4 – 4.2	3.6	3.1 – 4.5

[a]Grams amino acid/100 g of protein (N × 6.25).
[b]Oh 43 +, W22 + (normals); Oh 43 o_2, fl_2, bt_2, and bt_2o_2; W22 o_7 (mutants).

TABLE II
Amino Acid Composition of LM Endosperm Fraction II and PW Alkylated-Reduced Zein (ARZ)[a]

Amino Acid	Paulis-Wall Normal	Landry-Moureaux			
		Normal Inbreds[b]		High-Lysine Mutants[b]	
		Average	Range	Average	Range
Lysine	0.0	0.1	0.1 – 0.2	0.2	0.1 – 0.5
Histidine	1.2	1.6	1.6 – 1.6	1.5	1.0 – 2.1
Arginine	1.7	1.7	1.6 – 1.9	1.6	1.6 – 1.7
Aspartic Acid	5.6	5.9	5.9 – 6.0	6.1	5.8 – 6.8
Threonine	3.1	3.4	3.4 – 3.4	3.7	3.3 – 4.2
Serine	5.9	5.9	5.9 – 5.9	5.9	5.7 – 6.2
Glutamic Acid	29.0	31.6	30.5 – 32.8	31.5	30.3 – 33.3
Proline	14.7	10.9	10.9 – 11.0	10.7	10.4 – 11.2
Glycine	1.9	1.5	1.5 – 1.6	1.7	1.5 – 1.9
Alanine	10.4	10.8	10.4 – 11.2	10.7	10.2 – 11.3
Cystine	1.7	0.4	0.1 – 0.7	0.3	0.1 – 0.6
Valine	3.7	4.1	4.1 – 4.2	4.4	3.7 – 4.9
Methionine	1.6	2.0	1.9 – 2.2	2.5	1.7 – 4.0
Isoleucine	4.4	4.4	4.4 – 4.4	4.6	4.3 – 4.9
Leucine	23.0	20.4	20.3 – 20.5	20.2	19.6 – 21.1
Tyrosine	6.5	5.8	5.5 – 6.1	5.8	5.4 – 6.0
Phenylalanine	7.1	7.8	7.7 – 8.0	7.8	6.9 – 8.3

[a]Grams amino acid/100 g of protein (N × 6.25).
[b]Oh 43+, W22 + (normals); Oh 43 o_2, fl_2, and bt_2; W22 o_7 (mutants). bt_2o_2 contains no true zein (fraction II).

zein-like fraction which is low in lysine and high in glutamic acid, proline, and leucine. It is also characterized by its unusually high levels of cystine and methionine. Here again, the cystine values obtained by Paulis and Wall are high because of the protection of the cystine with reduction and alkylation. The total sulfur amino acids in fraction III exceed 12%, based on the PW method.

The amino acid composition of LM endosperm fraction IV and PW guanidine-insoluble alkylated-reduced glutelin was similar for normals and mutants (Table IV). This fraction resembles true glutelin and contains levels of lysine intermediate between that of the zein fractions and the true glutelin fraction. The most striking characteristic of fraction IV is the unusually high level of histidine. The level is at least 7.6% or more in this fraction (Table IV). Fraction IV is high in glutamic acid and proline, and probably also high in cystine based on the more accurate PW value.

The amino acid composition of LM endosperm fraction V and PW guanidine-soluble, ethanol-insoluble alkylated-reduced glutelin was also similar (Table V). The outstanding feature of this fraction is the unusually high level of lysine, which equals or exceeds 5.7% in all cases studied. This fraction is also characterized by relatively low levels of glutamic acid and proline, and relatively high levels of isoleucine. Based on the PW analysis, the level of total sulfur amino acids is quite high, totaling approximately 6%.

It is obvious that LM fractions II–V resemble the corresponding PW fractions in amino acid composition. It is also interesting that glutelins prepared by two entirely different procedures (one in which the glutelin fractions are extracted, leaving a residue high in starch, and the other in which the starch is digested,

TABLE III
Amino Acid Composition of LM Endosperm Fraction III and PW Guanidine and Ethanol-Soluble Alkylated-Reduced Glutelin (ESARG)[a]

Amino Acid	Paulis-Wall Normal	Landry-Moureaux Normal Inbreds[b] Average	Normal Inbreds[b] Range	High-Lysine Mutants[b] Average	High-Lysine Mutants[b] Range
Lysine	0.1	0.5	0.5 – 0.6	0.5	0.4 – 0.8
Histidine	4.8	4.6	4.5 – 4.8	3.3	1.9 – 4.2
Arginine	3.4	4.0	3.2 – 4.8	3.1	2.2 – 4.3
Aspartic Acid	2.8	2.9	2.8 – 3.1	3.9	1.6 – 5.2
Threonine	3.7	4.1	4.0 – 4.3	4.1	3.5 – 4.5
Serine	4.6	5.0	4.8 – 5.2	5.7	5.1 – 6.3
Glutamic Acid	24.0	27.5	25.7 – 29.3	30.4	27.0 – 31.8
Proline	22.0	16.3	15.6 – 17.1	14.9	12.4 – 17.2
Glycine	3.4	4.6	4.5 – 4.7	4.3	2.8 – 6.6
Alanine	6.9	7.8	7.5 – 8.2	8.8	6.5 – 10.1
Cystine	5.0	0.3	0.2 – 0.4	0.5	0.3 – 0.6
Valine	4.6	5.1	4.7 – 5.6	4.5	3.8 – 5.1
Methionine	7.9	5.0	4.3 – 5.7	7.0	2.5 – 9.2
Isoleucine	2.5	2.4	2.3 – 2.5	2.8	1.1 – 3.5
Leucine	13.9	13.8	13.7 – 13.9	15.2	10.5 – 17.7
Tyrosine	6.3	4.5	4.5 – 4.6	5.1	4.0 – 6.9
Phenylalanine	3.6	4.3	4.2 – 4.5	6.1	5.1 – 6.8

[a]Grams amino acid/100 g of protein (N × 6.25).
[b]Oh 43 +, W22 + (normals); Oh 43 o_2, fl_2, bt_2, and bt_2o_2, W22 o_7 (mutants).

TABLE IV
Amino Acid Composition of LM Endosperm Fraction IV and PW Guanidine-Insoluble Aklylated-Reduced Glutelin (IARG)[a]

Amino Acid	Paulis-Wall Normal	Landry-Moureaux Normal Inbreds[b] Average	Normal Inbreds[b] Range	High-Lysine Mutants[b] Average	High-Lysine Mutants[b] Range
Lysine	1.0	1.6	1.4 – 1.8	2.1	1.7 – 2.9
Histidine	8.2	8.9	8.2 – 9.6	8.4	7.6 – 9.0
Arginine	4.7	3.2	3.0 – 3.3	4.5	3.3 – 6.2
Aspartic Acid	3.1	2.4	2.2 – 7.7	3.0	2.3 – 4.0
Threonine	4.6	4.2	3.3 – 5.2	4.1	3.7 – 4.8
Serine	4.4	3.6	3.6 – 3.7	3.9	3.5 – 4.4
Glutamic Acid	23.4	24.4	23.7 – 25.1	25.1	21.6 – 30.0
Proline	29.0	15.2	13.0 – 17.4	16.0	13.9 – 17.3
Glycine	5.3	4.1	4.1 – 4.2	5.0	4.1 – 5.5
Alanine	4.9	4.0	3.9 – 4.1	4.2	3.8 – 4.5
Cystine	5.3	<0.1	0.0 – 0.1	0.1	0.1 – 0.1
Valine	7.2	5.6	5.2 – 6.0	5.9	5.2 – 6.5
Methionine	1.0	1.2	1.1 – 1.4	1.3	1.0 – 1.5
Isoleucine	2.4	2.3	2.3 – 2.4	2.5	2.4 – 2.8
Leucine	11.0	8.1	7.9 – 8.3	8.3	7.1 – 9.1
Tyrosine	3.3	1.8	1.5 – 2.2	1.9	1.5 – 2.7
Phenylalanine	2.5	2.2	1.9 – 2.5	2.3	1.8 – 2.6

[a]Grams amino acid/100 g of protein (N × 6.25).
[b]Oh 43+, W22 + (normals); Oh 43 o_2, fl_2, bt_2, bt_2o_2, and W22 o_7 (mutants).

TABLE V
Amino Acid Composition of LM Endosperm Fraction V and PW Guanidine-Soluble, Ethanol-Insoluble Alkylated-Reduced Glutelin (EIARG)[a]

Amino Acid	Paulis-Wall Normal	Landry-Moureaux Normal Inbreds[b] Average	Normal Inbreds[b] Range	High-Lysine Mutants[b] Average	High-Lysine Mutants[b] Range
Lysine	6.1	6.7	6.4 – 7.0	6.6	5.7 – 7.0
Histidine	4.0	3.7	3.6 – 3.8	3.7	2.8 – 4.8
Arginine	7.6	7.0	7.0 – 7.1	8.5	6.5 – 11.0
Aspartic Acid	9.8	9.1	8.8 – 9.4	10.0	8.9 – 11.2
Threonine	4.9	4.7	4.2 – 5.2	5.3	4.6 – 5.7
Serine	6.1	4.1	3.5 – 4.8	5.3	4.8 – 5.8
Glutamic Acid	18.8	14.1	13.0 – 15.2	16.4	15.7 – 19.5
Proline	8.6	5.3	5.3 – 5.3	6.1	5.4 – 6.7
Glycine	6.2	5.0	5.0 – 5.1	5.4	4.9 – 5.8
Alanine	7.0	6.9	6.8 – 7.0	7.1	6.4 – 8.0
Cystine	2.7	0.3	0.2 – 0.5	0.2	0 – 0.8
Valine	6.8	7.0	7.0 – 7.1	7.3	6.8 – 7.7
Methionine	3.9	3.2	2.4 – 4.0	2.7	2.4 – 3.2
Isoleucine	5.1	4.9	4.9 – 5.0	5.1	4.9 – 5.5
Leucine	11.0	9.7	9.4 – 10.1	10.0	9.1 – 10.4
Tyrosine	5.8	2.5	2.4 – 2.7	4.3	2.9 – 5.3
Phenylalanine	5.8	5.4	5.3 – 5.6	5.7	5.2 – 6.1

[a]Grams amino acid/100 g of protein (N × 6.25).
[b]Oh 43+, W22 + (normals), and Oh 43 o_2, fl_2, bt_2, bt_2o_2, and W22 o_7 (mutants).

leaving a residue high in protein) could give such similar amino acid values. Therefore, we have at our disposal well-defined fractions that can be prepared by either of these two methods for further use in studying the nature of the proteins in corn endosperm.

In the high-lysine mutants that we have described in this paper, which were fractionated by the LM method, endosperm fractions I and V were always higher, and fractions II, and II plus III always lower as percentage of total nitrogen than in the normal counterpart (2–4). This was especially evident in bt_2o_2, where fractions I and V increased more than threefold and fractions II plus III decreased to less than one-seventh of the normal values in the mutant endosperm (3). SDS-polyacrylamide gel electrophoresis analysis of fractions I–IV revealed no unusual polypeptide bands that could be responsible for the increased levels of lysine in the endosperm (7). Therefore, the increased amounts of fractions I and V, both of which are high in lysine, must be primarily responsible for the higher levels of lysine found in the mutants studied.

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