AMINO ACID COMPOSITIONS OF THE GRAIN HUSKS OF ORDINARY AND HIGH-LYSINE MAIZE

N. I. Shtemenko, A. N. Vinnichenko,
L. F. Zamorueva, and I. A. Filonik

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A number of physiological-biochemical features of grain husks of ordinary and high-lysine maize have been revealed that may be used to determine disease-resistant and nonresistant forms of maize.

It has been shown previously that the surface lipids of the grains of ordinary (+/+) and "high-lysine" (lysine-rich) (o2/o2) forms of maize have different fatty-acid compositions [1]. It was assumed that these differences are responsible for different susceptibilities of the grain to diseases. Since the compositions of the lipids of the endosperms of the same forms had no particular differences [2], it is logical to assume that the surface lipids extractable by brief treatment with hot chloroform are derived mainly from the husk of the grain. In spite of the considerable number of investigations of the physiological-biochemical features of the manifestation of the mutation opaque-2 (o2), bearing an important agricultural characteristic — increased food value — the question of differences in the compositions of the grain husks of ordinary and high-lysine forms has remained open. At the same time, it is known that the surface of the seeds is the most important component in their resistance to any damage [3], while proteins and peptides play an important role in the protection of plants from foreign agents [4, 5].

The aim of the present work was an investigation of the amino acid compositions of the grain husks of ordinary and high-lysine maize.

On comparing the amino acid compositions of the husks (Table 1) with those of the protein fractions of maize grains isolated by Osborne's method [6, 7] (classification according to solubility) the following may be noted: the amino acid composition of the proteins of the grain husks is close to that of the albumins in relation to the high lysine content and is close to that of the scleroproteins in relation to the threonine and proline contents. If we compare from this point of view the husk proteins of the +/+ and o2/o2 forms with one another, the most pronounced difference is the high content in the o2/o2 forms of glutamic acid (considerable, almost twofold, in the W155 and W64 A lines) and of aspartic acid and lysine, which may indicate a high physiological activity of the husk proteins of the o2/o2 forms. It is known that a high proteolytic activity has been found in the grain husks that is particularly important in the ripening period and in the growth process [8]. The above-mentioned hypothesis on the increased activity of the readily soluble proteins in the husks of the o2/o2 forms is confirmed by the discovery in the grain husk of A 204 o2/o2 of a considerable activity of proteinase inhibitors such as trypsin inhibitor and subtilisin inhibitor, in contrast to a sample of the initial A 204 +/+ maize (Table 2). At the same time, the converse relationship is characteristic for the chymotrypsin inhibitor. The activity of the proteinase inhibitors in the husks is an order of magnitude less than in the endosperm of the same forms; however, the nature of the influence of the o2 gene is the same for the caryopsis and for the husk [4].

The differences in the stability of the enzyme-inhibitor complexes in the forms of maize that we have compared are probably the reason for the formation of husk proteins with different amino acid compositions. In higher plants, proteinase-inhibiting proteins may fulfill protective functions by suppressing the activities of the proteinases of insects and of pathogenic microorganisms. In a study of the action of serine proteinase inhibitors from maize on a number of enzymes of microbial origin it was shown that the chymotrypsin inhibitor suppressed the proteolytic activities of the enzymes studied to a greater extent than the trypsin inhibitor

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TABLE 1. Amino Acid Compositions of the Proteins of the Grain Husks of Ordinary and High-Lysine Maize, %

Amino acid	Form of maize							
	A 204		W 155		Wf 9		W 64 A	
	+/+	o2/o2	+/+	o2/o2	+/+	o2/o2	+/+	o2/o2
Lysine	9,42	10,48	8 17	9,85	8,36	9,73	8,21	8,57
Histidine	3,08	2,59	3,64	3,28	2,71	2,65	5,22	4,29
Arginine	3,93	3,07	4,52	2,92	3,92	6,64	1,49	4,76
Asp. acid	6,70	9,36	10,17	10,95	10,96	11,95	9,70	10.95
Threonine	10,92	9,41	9,06	5.47	8,96	6,64	10.45	5.24
Serine	4,69	3,49	6,21	2,19	5,98	5,31	6,72	7,14
Glut. acid	7,54	10,97	5,39	10,58	8,41	10,18	6,72	10,48
Proline	12,50	13.49	6,95	10,95	5,74	5,31	8,96	6,67
Glycine	7,58	6,86	7,91	6,57	6,80	5,75	7,46	5,71
Alanine	6,41	5,97	7,34	6,20	7,61	7,08	7,46	7,62
Valine	4,27	4,97	4,08	7,32	5,97	7,52	5,22	5,71
Methionine	1,81	1,04	1,10	0,36	1,64	0,88	0,75	0,95
Isoleucine	4,24	3,19	5,00	4,74	4,35	3,98	4,48	4,29
Leucine	6,91	6,05	8,04	7,66	7,88	7,09	7.46	7,14
Turosine	5,74	4,13	5,08	4,74	4,69	3,54	5,22	4,29
Phenylalanine	4,26	4 93	7,34	6,22	6,02	5,75	4,48	6,19

TABLE 2. Activity of the Proteinases in the Grain Husks of the Ordinary and the High-Lysine Maizes A 204 +/+ and A 204 o2/o2 (mg/g of protein)

Trypsin inhibitor		Chymotrypsin inhibitor		Substitution inhibitor	
+/+	o2/o2	+/+	o2/o2	+/+	o2/o2
—	0,76	0,33	0,18	—	2,53

[9]. At the same time, the increased activity of the trypsin inhibitor in the grain of the o2/o2 forms is associated with their greater susceptibility to diseases. This may be explained by the assumption that the protective functions of plants are due to a complex of physiological-biochemical factors, with a predominance of the significance of individual ones in concrete cereal phenotypes.

A considerable amount of free acids was detected in the husks of the forms of maize that we studied (Table 3). As compared with the endosperm [7], there was 5 times less free acids per unit weight here; nevertheless, the nature of the influence of the o2 gene on their quantitative composition was the same as in the endosperm and in the whole caryopsis, namely: a considerable increase in the total amount of amino acids (2- to 2.5-fold) in specimens of the o2/o2 forms. With respect to their qualitative composition, the free amino acids of the endosperm differed considerably from those of the husks. While in the endosperm glutamine amounted to 10% (+/+) and 30% (o2/o2) of the amino acid pool [10], in the husk of an A 204 +/+ grain it was present in trace amounts and in A 204 o2/o2 it made up only 0.43% of the pool, and in the husk a large part consisted of neutral and low-molecular-mass amino acids (glycine, proline, valine, isoleucine, and leucine). The presence of a considerable amount of free amino acids — a readily assimilable form of nitrogen-containing compounds — in the grain husks of the o2/o2 form may be the reason for the increased susceptibility of the grain of high-lysine maize to fungal diseases, and it also, probably, indicates a penetrability of the membranes of the surface tissues of the grains of these forms and has been used by us in subsequent work to reveal disease-resistant and nonresistant forms of maize.

EXPERIMENTAL

The acquisition and the physiological and biochemical features of the +/+ and o2/o2 forms of maize that we studied have been described in [4]. To separate it into its anatomical parts, the ripe grain was soaked in distilled water for 0.5 h. The husk (pericarp) was separated with a scalpel from the moist grain, dried, and ground to a powder in a porcelain mortar. The preparation of the samples and amino acid analysis were performed by generally adopted

TABLE 3. Amounts of Free Amino Acids in the Grain Husks of Ordinary (+/+) and Mutational (o2/o2) Forms of Maize, mg%*

Amino acid	Form of maize							
	A 204		W 64 A		W 155		Wf 9	
	+/+	o2/o2	+/+	o2/o2	+/+	o2/o2	+/+	o2/o2
Aspartic acid	4,11	9,15	3,99	16,66	6,44	20,69	7,04	18,99
Threonine	2,53	6,78	2,46	3,54	3,53	9,05	3,98	3,86
Serine	12,10	29,06	11,09	23,83	14,80	28,63	19,11	20,74
Asparagine	6,31	15,26	7,32	53,78	11,51	61,23	0,33	40,26
Glutamic acid	1,92	4,27	0,98	6.17	3,59	17,44	3,05	7,57
Glutamine	Tr.	1,08	Tr.	6,31	Tr.	1,98	0,59	0,95
Proline	5,51	7,93	6.08	15,95	7,52	22,72	6,28	10,04
Glycine	5,80	15,57	4,13	7,02	6,49	11,38	8,71	8,06
Alanine	4,70	10,49	10,53	28,56	10,53	27,02	12,07	23.98
Valine	1,05	4,99	1,02	3.30	4.73	5,04	3,55	6,87
Cysteine	0,46	0,61	0,63	1,36	0,90	4,87	0,30	1,41
Methionine		0,43	0,04	0,42	0,42	0,45		0,41
Isoleucine	1,48	4,23	1,36	2,60	1,58	3,71	3.76	2,01
Leucine	1,48	4,23	1,11	2,01	1,45	3,58	3,75	1,05
Tyrosine	1,55	7,72	1,63	7,72	1,92	4,04	4,43	2,30
Phenylalanine	4,39	5,97	3,96	6,98	4,82	6,26	2,51	5,29
β-alanine	2,80	1,[illegible]6	3,48	3,56	3,27	6,25	Tr.	4,95
γ-Aminobutyric acid	1,28	1,32	1,96	7,97	6,78	17,34	6,20	11,58
Ornithine	3,75	11,77	3,64	3,69	5,56	7,49	5,28	6,44
Lysine	0,61	2,88	0,94	1,02	0,92	2,22	1,17	1,86
Histidine	1,93	4.91	2,06	8.47	2.47	3,77	2,69	5,55
Tryptophan	Tr.	2,90	Tr.	3,62	Tr.	1,59	0,56	1,91
Arginine	Tr.	1,57	Tr.	1,21	0,76	1,64	0,74	1,00
Total	63,91	154,78	68,41	215,75	99,99	268,39	96,10	187,08

*mg% – amount of amino acid in mg in 100 g dry weight of the sample.

methods [6] on a Biotronik LC 5001 automatic amino acid analyzer (FRG). The activities of the proteinase inhibitors were determined as in [4]. The amino acid compositions of the total protein from the husks of four initial forms of maize and four forms transformed on an o2 base are given in Table 1. The +/+ and o2/o2 forms showed no differences with respect to the total amount of bound amino acids, which was 2-5 g per 100 g sample and corresponded to the amount of protein in this anatomical part of maize grain determined previously [6].

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