Phytochemistry, Vol. 21, No. 8, pp. 1865–1869, 1982.
Printed in Great Britain.

0031-9422/82/081865-05$03.00/0

DISTRIBUTION AND AMINO ACID COMPOSITION OF PROTEIN FRACTIONS IN *OPAQUE*-2 MAIZE GRAINS

J. LANDRY* and T. MOUREAUX†

Laboratoire d'étude des Protéines, I.N.R.A. (Institut National de la Recherche Agronomique) C.N.R.A., 78000 Versailles, France

(*Revised received* 19 *February* 1982)

Key Word Index—*Zea Mays*; Gramineae; maize; development; protein; amino acids.

Abstract—Immature *opaque* 2 (*o*2) maize grains were compared with mature grains of *o*2 and normal maizes to determine the distribution and amino acid composition of protein fractions isolated by selective extraction. All the fractions are accumulated in *o*2 grains on development, except the albumins whose concentration decreases in the last stage of maturation. Each fraction has a nearly constant amino acid composition which is similar to that of the corresponding fractions present in normal grains. The data confirm that the main effect of the *o*2 gene is to alter the distribution of protein fractions by decreasing the proportion of accumulated zein and by increasing the proportions of salt-soluble proteins and G_3-glutelins.

INTRODUCTION

The quantitative accumulation of protein fractions in developing *opaque* 2 (*o*2) grains has been the subject of many investigations [1–8]. The results show that the *o*2 gene, as compared to normal, partially inhibits zein synthesis, and promotes the accumulation of glutelins, especially G_3-fractions, as defined by the procedure of Landry and Moureaux [9]. Concerning the qualitative evolution of the protein fractions, the available data on this are not in complete agreement. Indeed Murphy and Dalby [2] found some variations in amino acid composition of zein and of glutelins isolated at different stages of development, which were confirmed by Ahuja *et al.* [10]. On the contrary, Righetti *et al.* [11] showed by electrofocusing that all the components of zein and alcohol-soluble glutelins (zein-1 and zein-2) are present as soon as synthesis of these proteins begins. This observation agrees with our finding [12] relative to the constancy of amino acid composition of any protein fractions isolated from developing grain of normal maize.

The present paper demonstrates that the amino acid composition of the various protein groups isolated from immature and mature *o*2 grain remains constant during their accumulation. The amino acid composition of *o*2 protein fractions is also compared with their corresponding solubility homologues present in mature normal grain.

RESULTS

The distribution of the protein fractions in samples harvested at two stages of *o*2 grain development are given in Table 1. Data are expressed as the ratio of nitrogen in each fraction to the total protein nitrogen (represented by the difference between amounts of total and non-protein nitrogen). At immature stages, the accumulation of each protein fraction is also evaluated by the ratio of nitrogen amount present at time t to that present in mature grain $(N_i)_t/(N_i)_{94}$. At 34 days after pollination (DAP), this ratio is 0.70 for salt-soluble proteins and has an average value of 0.33 for zein and for G_1-, G_2- and G_3-glutelins. At 48 DAP it ranges from 0.67 (G_1-glutelins) to 0.92 (zein), which shows that nearly all zein present in mature grains is already synthesized at this stage. It is noteworthy that albumins are the only proteins to have a ratio greater than unity.

The amino acid composition of the basic and endosperm-specific proteins [12] are shown in Tables 2 and 3, respectively. For *o*2 grains each protein group has a nearly constant composition at any period of development. The most important differences noted between immature and mature stages are: (i) His, Arg, Asx, Glx, Pro and Leu for salt-soluble proteins; (ii) Tyr for G_3-glutelins; (iii) Gly, Val and Ile for zein; and (iv) Asx, Pro, Val, Ile and Phe for G_2-glutelins.

Regarding the mature stages, the composition of the protein fractions in the *o*2 line was quite similar to those in the normal hybrid. The most notable discrepancies concern: (i) Arg for salt-soluble proteins; (ii) Tyr for albumins; (iii) Lys for globulins; (iv) Gly and Val for zein; (v) Lys, His and Pro for G_1-glutelins; (vi) Lys and Asx for G_2-glutelins; (vii) Arg for G_3-glutelins; and (viii) His for residue.

DISCUSSION

The two immature stages of *o*2 maize studied correspond to the phase of intense synthesis of proteins since the accumulation of their fractions in grain exhibits a two to three-fold increase between 34 and

*Present address: Laboratoire de Chimie Biologique, I.N.R.A., I.N.A.-P.G., 78850 Thiverval-Grignon, France.

†Present address: Laboratoire de Biologie Cellulaire, I.N.R.A., C.N.R.A., 78000 Versailles, France.

Table 1. Distribution of fractions $(N_i)_t$ in developing and immature *o2* and in ripe normal (+) maize grain

	o2					(+)
	34 DAP†		48 DAP		94 DAP	84 DAP
	$(N_i)_{34}/(N_p)_{34}$	$(N_i)_{34}/(N_i)_{94}$	$(N_i)_{48}/(N_p)_{48}$	$(N_i)_{48}/(N_i)_{94}$	$(N_i)_{94}/(N_p)_{94}$	$(N_i)_{84}/(N_p)_{84}$
Salt-soluble proteins	0.553	0.70	0.382	0.84	0.347	0.167
Albumins	0.509	1.49	0.282	1.44	0.174	0.089
Globulins	0.044	0.16	0.100	0.50	0.173	0.078
Zein	0.087	0.32	0.142	0.92	0.118	0.422
G_1-glutelins	0.061	0.31	0.075	0.67	0.086	0.100
G_2-glutelins	0.097	0.35	0.128	0.80	0.123	0.100
G_3-glutelins	0.158	0.30	0.222	0.73	0.232	0.144
Insoluble	0.043	0.22	0.053	0.43	0.094	0.067
Protein nitrogen/ total nitrogen	0.689	0.82	0.733	0.87	0.840	0.930

Results expressed as proportion of nitrogen of protein fraction $(N_i)_t$ to total protein nitrogen $(N_p)_t$ or to nitrogen of protein fraction present in mature grain $(N_i)_{94}$.

†DAP, days after pollination.

Table 2. Amino acid composition of basic proteins in developing and mature *o*2 at 34, 48 and 94 DAP† and mature normal (+) maize grain at 84 DAP*

	Salt-soluble proteins				Albumins		Globulins			G_3-glutelins			Residue	
		*o*2		+	*o*2	+	*o*2	+		*o*2		+	*o*2	+
Amino acid	34	48	94	84	94	84	94	84	34	48	94	84	94	84
Lys	66	64	61	62	65	73	51	60	61	57	55	54	41	42
His	20	23	31	26	26	23	29	31	24	24	27	25	22	36
Arg	51	60	79	67	56	56	84	87	50	47	57	50	37	38
Asx	101	91	79	82	97	94	76	79	88	92	88	83	63	66
Thr	51	51	47	49	60	56	42	42	53	52	48	49	68	61
Ser	60	60	66	66	56	60	70	69	67	67	61	64	58	56
Glx	114	120	132	122	105	119	140	135	120	131	118	118	117	111
Pro	58	76	57	52	61	55	60	57	72	69	65	64	136	128
Gly	93	100	102	105	98	96	103	101	94	94	89	89	126	114
Ala	97	97	95	102	93	104	83	87	103	105	94	105	87	86
Cys	(11)	(8)	(22)	(14)	(20)	(16)	(25)	(18)	nd‡	nd	nd	nd	nd	nd
Val	69	62	63	71	76	70	64	66	65	63	71	70	69	70
Met	(16)	(12)	(16)	(16)	(4)	(12)	(11)	(5)	(10)	(8)	(13)	(21)	(3)	(13)
Ile	45	39	36	36	42	38	32	34	42	40	43	43	28	31
Leu	88	79	68	71	80	74	65	66	97	100	105	98	79	82
Tyr	25	25	24	24	29	24	26	24	14	12	25	26	28	29
Phe	35	34	36	35	34	32	40	39	41	40	42	40	28	30

Results expressed as residues per 1000 residues.
*Data in parentheses are only estimations.
†DAP, days after pollination.
‡nd, Not determined.

Table 3. Amino acid composition of endosperm-specific proteins in developing and ripe *o*2 at 34, 48 and 94 DAP† and in ripe normal (+) maize grain at 84 DAP*†

	Zein				G_1-glutelins		G_2-glutelins			
Amino acid	*o*2 34	*o*2 48	*o*2 94	+ 84	*o*2 94	+ 84	*o*2 34	*o*2 48	*o*2 94	+ 84
Lys	0	2	1	1	2	1	14	14	17	11
His	10	9	8	9	16	11	61	56	66	66
Arg	11	11	13	11	14	15	24	23	29	30
Asx	51	51	47	50	34	38	19	24	30	22
Thr	32	32	28	31	39	33	49	45	48	47
Ser	68	67	66	67	62	61	54	57	51	54
Glx	212	207	204	209	192	193	219	224	193	196
Pro	119	119	102	96	145	107	237	211	194	188
Gly	29	26	34	20	59	48	81	86	80	80
Ala	135	136	123	140	111	126	62	68	62	66
Cys	(2)	(1)	(17)	(3)	nd‡	nd	nd	(2)	nd	nd
Val	25	24	36	42	39	38	42	40	69	67
Met	(2)	(4)	(13)	(10)	(28)	(58)	(5)	(4)	(5)	(7)
Ile	25	29	34	37	24	26	13	13	25	23
Leu	189	198	180	188	142	152	88	93	88	93
Tyr	38	37	39	37	41	47	22	24	20	24
Phe	51	52	54	50	49	43	12	16	22	23

Results expressed as residues per thousand residues.
*Data in parentheses are only estimations.
†DAP, days after pollination.
‡nd, Not determined.

48 DAP. After 48 DAP, the accumulation of zein ceases whereas that of globulins and glutelins continues. In contrast, the concentration of albumins in the grain reaches a maximum between 34 and 48 DAP and then decreases, probably through a partial insolubilization into G_3-glutelins, as was observed for the salt-soluble proteins of normal grain [6, 12].

To quickly identify proteins of each solubility group, amino acid compositions were determined from a single 24 hr hydrolysis of each crude extract. It is likely that crude extracts contain impurities which interfere with amino acid analyses despite the use of a large volume of hydrolysis media. The low content of tyrosine found for G_3-glutelins present in immature *o*2 grains would be the result of such interference, which has been also observed in other investigations on maize proteins [12, 13]. However, any purification could likewise fractionate proteins, especially glutelin fractions, and lead to isolation of samples not representative of polypeptides represent in the crude extracts. Similarly, different hydrolysis times would have improved the estimation of labile residues and those released slowly upon hydrolysis, e.g. Val and Ileu. Therefore, some variations of amino acid composition of a given protein fraction can be attributed to these imperfect experimental conditions.

However, the variable amino acid composition of salt-soluble proteins of *o*2 maize, characterized by progressive changes of the levels of His, Arg, Asx, Glx and Leu between earliest and mature stages, can be related to changes in the albumin–globulin ratio which, as shown in Table 1, decreases from 11 to 1 after 38 DAP. Since this ratio has different values according to the histological source in the grain, and since germ contains some proteins which are like zein and G_1- and G_2-glutelins [14, 15], the differences in composition between protein groups of mature normal and *o*2 grains may be attributed to the dissimilar proportions of germ and endosperm in the two varieties, as was shown specifically by Landry and Moureaux [14] and more generally by Bjarnason and Pollmer [16]. Some discrepancies in composition may be also interpreted by small changes in selectivity of the protein fraction extraction with varieties. Thus, *o*2 zein could be contaminated by some G_1-glutelins, since it contains more Gly and Pro than normal zeins [17]. Likewise, *o*2 G_1-glutelins would include some G_2-glutelins, able to be extracted by alcoholic medium [18], since they are richer in His and in Pro.

These results extend our previous observations on proteins of developing normal maize grain to *o*2 proteins. Therefore, the amino acid composition of *o*2 salt-soluble protein groups remains virtually constant. The similarity of 48 and 94 DAP G_3-glutelins is particularly interesting; it shows that, despite an alteration in their rate of accumulation during the last stage of maturation [6], probably through a partial insolubilization of albumins, they are a well-defined fraction, not contaminated by other protein groups which would have not been exhaustively extracted. Likewise, G_2-glutelins have a fairly well-defined amino acid composition, so it must be considered as a protein group distinct from G_3-glutelins, which is consistent with the observations about the histological localization of protein groups [14].

The data from this investigation also demonstrates that the overall amino acid composition of any protein groups from maize grain is little affected by the

presence of the *o*2 gene as first concluded by Mossé *et al.* [19] and subsequently by Sodek and Wilson [20] and Misra *et al.* [21]. It remains necessary to isolate subunits present in these protein groups to determine the effect of the *o*2 gene at the primary structure level.

EXPERIMENTAL

Two maize varieties studied, a three-ways hybrid (Inra (260) and its *o*2 version were grown under field conditions. Grain harvesting, isolation and characteristics have been described previously [3, 12].

Salt-soluble proteins, zein, G_1-, G_2-, G_3- and insoluble glutelins were separated from whole grains by the procedure of refs. [9] and [14]. The non-protein fraction and salt-soluble proteins were defined from saline extract on the basis of solubility and insolubility, respectively, in 10% TCA. Likewise albumins and globulins were estimated from H_2O and saline extracts on the basis of their insolubility in 10% TCA [14].

Crude extracts or protein pellets (in the case of salt-soluble proteins and their subfractions) were hydrolysed with an excess of 6 M HCl at 110° for 24 hr for amino acid analyses (Phoenix Precision Instruments). No corrections were made for losses of labile amino acids or incomplete hydrolysis. Results are expressed as no. per thousand recovered residues.

Acknowledgements—We gratefully acknowledge the cooperation of our colleagues J. C. Huet, S. Delhaye, M. C. Defrance (for amino acid analyses), M. Domin and C. Demarteau.

REFERENCES

1. Dalby, A. (1966) *Proceedings of the High Lysine Corn Conference* p. 80. Corn Industries Research Foundation.
2. Murphy, J. J. and Dalby, A. (1971) *Cereal Chem.* **48**, 336.
3. Moureaux, T. and Landry, J. (1972) *C. R. Acad. Sci.* **274**, 3309.
4. Tsai, C. Y. and Dalby, A. (1974) *Cereal Chem.* **51**, 825.
5. Soave, C., Pioli, F., Viotti, A., Salamini, F. and Righetti, P. G. (1975) *Maydica* **20**, 83.
6. Landry, J. and Moureaux, T. (1976) *Qual. Plant. Plait Foods Hum. Nutr.* **25**, 343.
7. Di Fonzo, N., Fornasari, E., Salamini, F. and Soave, C. (1977) *Maydica* **22**, 77.
8. Tsai, C. Y., Huber, D. M. and Warren, H. L. (1978) *Crop Sci.* **17**, 399.
9. Landry, J. and Moureaux, T. (1970) *Bull. Soc. Chim. Biol.* **52**, 1021.
10. Ahuja, V. P., Srivastana, K. N., Austin, A. and Nack, M. S. (1973) *Indian J. Biochem. Biophys.* **10**, 48.
11. Righetti, P. G., Gianazza, E., Viotti, A. and Soave, C. (1977) *Planta* **136**, 115.
12. Moureaux, T. and Landry, J. (1972) *Physiol. Veg.* **10**, 1.
13. Gianazza, E., Viglienghi, V., Righetti, P. G., Salamini, F. and Soave, C. (1977) *Phytochemistry* **16**, 315.
14. Landry, J. and Moureaux, T. (1980) *J. Agric. Food. Chem.* **28**, 1186.
15. Tsai, C. Y. (1979) *Biochem. Genet.* **17**, 1109.
16. Bjarnason, M. and Pollmer, W. G. (1972) *Z. Pflanzenkr. Pflanzenshutz* **68**, 83.
17. Landry, J. (1070) *Biochimie* **61**, 549.
18. Landry, J. and Moureaux, T. (1981) *J. Agric. Food. Chem.* **29**, 1205.
19. Mossé, J., Baudet, J., Landry, J. and Moureaux, T. (1966) *Ann. Physiol. Veg.* **8**, 331.
20. Sodek, L. and Wilson, C. M. (1971) *J. Agric. Food Chem.* **19**, 1144.
21. Misra, P. S., Mertz, E. T. and Glover, D. V. (1975) *Cereal Chem.* 52, 734.