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PROPERTIES OF GLUTELIN FROM MATURE AND DEVELOPING RICE GRAIN*

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Key Word Index—*Oryza sativa*; Gramineae; rice; glutelin; amino acid analysis; SDS-polyacrylamide gel electrophoresis.

Abstract—Aminograms and SDS-polyacrylamide electrophoresis of milled rice glutelin of 12 *Oryza sativa* samples showed similar composition and ratio of 1:1:1 for subunits with MW 38000:25000:16000, indicating little possibility of finding variants of rice glutelins. Fractionation of *S*-cyanoethyl glutelin of 3 rices on polyacrylamide–agarose gels gave MW subunits differing in amino acid analysis of which the subunits with MW >38000 had the highest lysine content. Of the solubility fractions of endosperm glutelin, the fraction extracted by 0.5 M NaCl–0.6% β-mercaptoethanol–0.5% SDS was closest to glutelin in properties. In the developing grain of two varieties, appearance of protein bodies and rapid synthesis of glutelin from 7 days after flowering onward coincided with a drop in lysine content and appearance of MW 38000 and 25000 of crude glutelin. The MW 38000 subunit is thus unique to endosperm glutelin.

INTRODUCTION

Rice glutelin constitutes at least 80% of endosperm protein [1] and has an amino acid composition similar to that of whole milled-rice protein [2]. Increase of glutelin in the developing rice grain coincides with the appearance of protein bodies in the endosperm 7–8 days after anthesis [3]. Three principal subunits are obtained from reduced and alkylated rice glutelin [4, 5] and preliminary results indicate that rice samples may vary in the ratio of these 3 principal subunits [4]. As part of our study of rice endosperm protein [4, 6], we checked varietal differences in electrophoretic and amino acid composition of glutelins from various types of rices, the nature of the solubility of subfractions of rice glutelin, and the changes in properties of brown-rice glutelin during grain development.

RESULTS

Varietal differences in glutelin of milled rice

Glutelin prepared by extracting albumin–globulin and prolamin from 14 milled rices with protein content ranging from 5.2 to 11.9% constituted 77 to 94% of total protein. Milled rice glutelin and protein contents of milled rice had a highly significant positive correlation (Table 1). However, no significant correlation was shown by the lysine content of milled rice with that of their corresponding glutelins. The *japonica* samples (Fujisaka 5 and Jinheung) and the *javanica* samples (Do Khao and Dokyah Hom) tended to have lower glutelin fractions than the 6 *indica* (IR28, IR29, IR4432-103-6, IR4432-32-4, IR480-5-9 and Kolamba 540), two *indica* × *japonica* (Tongil and Mahsuri), and the *O. nivara* and *O. glaberrima* rices.

The lysine content of the 14 milled rices ranged from 3.01 to 4.45% whereas that of crude glutelin ranged from 3.67 to 4.55% (Table 1). Amino acid analysis of the crude glutelins, including lysine and threonine, showed only slight varietal differences. Kolamba 540, which was earlier identified as higher lysine [7], had normal lysine content in both milled rice and glutelin. Glutelin has a similar aminogram to whole milled rice protein [2]. Among the amino acids, only the lysine content was negatively correlated with protein and glutelin contents of milled rice (Table 1).

SDS-polyacrylamide gel electrophoregram of the glutelins showed essentially the same ratio of 1:1:1 for the major subunits with MW 38000:25000:16000 based on densitometric tracings of gels stained with Amido Black, regardless of protein and glutelin content, or endosperm type—waxy (IR29) or nonwaxy (IR28). An exception was the *O. nivara* (Acc. 101508) sample, which gave a ratio of 1:1:2 (Fig. 1). Three additional samples of *O. nivara* (Acc. No. 101508, 101524, and 102185) also gave the subunit ratio 1:1:2, indicating a higher proportion of the total MW 16000 subunit than the 12 *O. sativa* samples and the *O. glaberrima* sample. However, *O. nivara*'s glutelin had a similar amino acid pattern to the 13 other glutelins. Mean MW values for the major subunits of rice glutelin were 34500, 20700 and 16000.

The MW 25000 and 38000 subunits were more diffused on staining with Amido Black in HOAc than with Coomassie Brilliant Blue in TCA, indicating that they are composed of two or more proteins. 2-Vinyl pyridine had no advantage over acrylonitrile as an alkylating agent based on electrophoretic separation in the presence of SDS. In addition, alkylating time is longer and excess vinyl pyridine is harder to remove. SDS from BDH was needed to obtain all 3 subunits of glutelin on SDS-electrophoresis.

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Table 1. Range and mean values of crude glutelin and whole protein of 14 milled rices

Property	*O. sativa* (n = 12) Range	Mean	*O. glaberrima*	*O. nivara* (Acc. 101580)	Correlation coefficients (n = 14) with protein content of Milled rice	Crude glutelin
Milled rice protein (% at 14% moisture)	5.2 –11.9	7 89	9.25	11.0	1 00**	0 96**
Lysine of milled rice (g/16.8 g N)	3.01– 4.45	3.84	3.01	4.22	–0.16	0.04
Milled rice glutelin (% at 14% moisture)	4.6 –10.2	6.80	8.0	10.2	0.96**	1 00**
Glutelin (% of total protein)	77.0 –94.0	8 64	86.0	89.0	–0.06	0 20
Amino acid content of glutelin						
Lys (g/16.8 g N)	3.67– 4.55	4.12	3.72	3.85	–0.62*	–0.55*
His	2.34– 2.74	2.54	2.50	2.34	–0.34	–0 44
Amm	2.25– 3.08	2.65	2.59	2.58	0 14	0.15
Arg	7.36– 9.22	7.99	7.26	7.50	–0.18	–0.13
Asp	9.48–12.0	10.88	9.86	10.8	–0.25	–0.22
Thr	4.04– 4.70	4.42	4.06	3.94	–0.23	–0.12
Ser	5.68– 6.45	6.15	6.09	6.22	0.26	0.19
Glu	19.3 –23.0	21.08	22.4	21.5	0.38	0.29
Pro	4.80– 5.36	5.22	5.11	4.98	–0 35	–0.17
Cys	0.60– 1.60	1.10	1.58	1.24	0.25	0.11
Gly	4.32– 4.86	4.71	4.26	4.38	–0.50	–0.52
Ala	5.77– 6.43	6.16	6.15	6.26	0.13	0.05
Val	6.14– 7.46	6.96	6.64	6.62	–0.15	–0.03
Met	1.04– 2.36	1.78	2.22	1 92	–0.08	–0.06
Ile	4.50– 5.32	5.03	4.78	4 87	–0.23	–0 18
Leu	8.20– 9.80	9.28	9.86	9.87	0.38	0.21
Tyr	3.87– 5 50	4.46	4.86	4.72	0.15	–0.03
Phe	5.26– 6.39	5 86	6.09	5.92	0.39	0.24

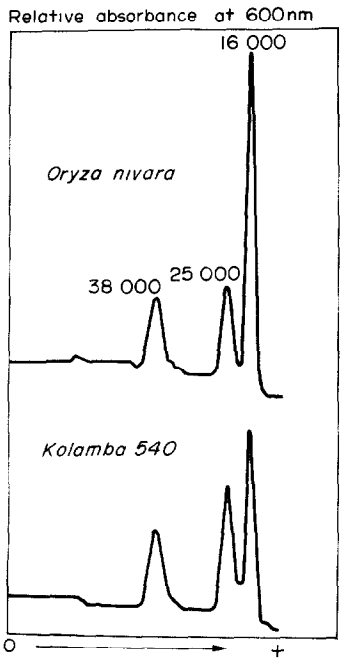

Fig. 1. Typical densitometric tracing of Ce-glutelins of Kolamba 540 and *O. nivara* on SDS-polyacrylamide disc (12%) gel stained with Amido Black.

Subunits of Ce-glutelin

Ce-glutelins of both IR480-5-9 and Kolamba 540 were partially separated by gel filtration on Ultrogel 44, as indexed by SDS-gel electrophoresis (Fig. 2). Poor separation was obtained with IR28. The first peak corresponded to subunits with MW > 38000; the second peak a mixture of subunits 38000 and 25000 and the last peak contained only the MW 16000 subunit. Fractionation of the second peak, obtained from Ultrogel 34 through rechromatography on Ultrogel 34, gave only one peak from which pure subunits were obtained (Fig. 2).

SDS-polyacrylamide gel electrophoresis of the subunits of Ce-glutelin showed mean MW of subunits for IR480-5-9 of 17500, 22500 and 35000 using Amido Black as the staining agent. Kolamba 540 Ce-glutelin showed further resolution of the higher MW subunits into two or three bands, particularly when Coomassie Brilliant Blue was used. The MW of the subunits of Ce-glutelin of Kolamba 540 were 17000, 24500 and 25500 (mean 25000), and 35500, 36500 and 40000 (mean 37300). Broad bands were generally obtained with Amido Black.

Whole Ce-glutelin of the 3 rices were of higher lysine content than crude glutelin (Table 2). The aminogram of the 3 major subunits of glutelin showed variation among subunits for both varieties, but the amino acid composition of whole glutelin was not always within the range of the 3 subunits such as for lysine, proline and valine. The IR480-5-9 glutelin again showed the highest lysine content for its MW 25000 subunit as reported for another

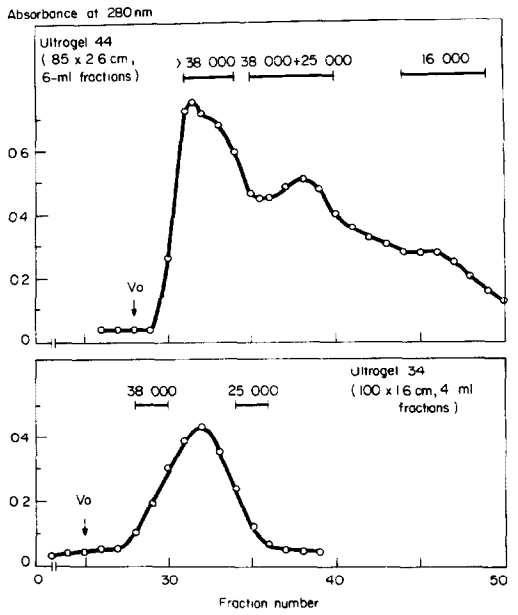

Fig. 2. Typical gel filtration profile of Ce-glutelin of IR480-5-9 milled rice on polyacrylamide-agarose columns in 0.05 M Tris–HCl pH 8.6 buffer–0.5% SDS–0.2% Na azide.

sample of IR480-5-9 [4]. Analysis of the Ce-glutelin fraction of IR28 with MW >38000 verified that this fraction had higher lysine content than the lower MW subunits of glutelin (Table 2).

Subfractions of glutelin of IR28 milled rice

Solubility fractionation of IR28 rice protein according to Landry and Moureaux [8] gave typical fractions of albumin–globulin, prolamin and glutelin (Table 3), similar to those previously reported for IR8 [9]. The protein extracted with 70% *iso*-propanol with 0.6% β-mercaptoethanol, and with 0.5 M NaCl in 0.1 M borate buffer pH 10 containing 0.6% β-mercaptoethanol, were only a small fraction of the total glutelin (4 and 3%, respectively). Addition of 0.5% SDS to the buffer–NaCl–β-mercaptoethanol solvent improved its extraction efficiency but about 19% of the glutelin was still insoluble. A value of 83% solubility was previously reported by direct extraction of IR8 crude glutelin with this solvent [9].

Amino acid analysis showed that this major fraction (true glutelin) has the closest pattern to crude glutelin among the 3 subfractions (Table 3). In addition, SDS-polyacrylamide electrophoresis showed this fraction to have an identical pattern to whole glutelin. The fraction extracted with *iso*-propanol-β-mercaptoethanol had an identical electrophoretic pattern to prolamin (*iso*-propanol extract) with one subunit of MW 17000. It was also similar in aminogram to prolamin, but higher in lysine and glutamic acid and lower in tyrosine. The borate–NaCl–β-mercaptoethanol extract also showed MW 17000 subunit as the major band on SDS-polyacrylamide gel, but with traces of some very high MW subunits. Only this fraction showed a high carbohydrate content of 37.5%, while the others had 4 to 6% carbohydrate only. Its aminogram is also similar to that of albumin–globulin [9]. During dialysis of this fraction, a fluffy precipitate formed from the extract, which was 96% carbohydrate. These results indicate that the final subfraction obtained after removal of 'bound' prolamin and albumin–globulin is probably true glutelin.

Glutelin in IR8 grain parts

Crude glutelin prepared from IR8 grain parts [6] showed differences in SDS-polyacrylamide gel electrophoretic pattern (Fig. 3). Embryo glutelin gave a very complex subunit pattern of which the MW 16000 subunit was the major band. Pericarp glutelin gave mainly the MW 16000 subunit, while aleurone glutelin was similar in subunit composition to milled rice glutelin.

Table 2. Aminogram of *S*-cyanoethyl glutelin and MW fractions from milled rice* (g/16.8 g N)

	IR 28				IR480-5-9				Kolamba 540			
Amino acid	Whole	16000	25000/ 38000	>38000	Whole	16000	25000	38000	Whole	16000	25000	38000
Lys	4.62	3.23	4.78	6.28	4.54	2.0	4.3	2.9	4.80	2.5	3.1	3.2
His	2.66	2.42	2.78	2.89	2.65	2.1	2.8	2.6	2.88	2.6	2.7	2.6
Amm	2.22	2.98	2.32	1.98	2.20	3.2	3.4	2.9	2.12	3.0	2.6	2.6
Arg	8.39	8.46	9.60	8.36	9.52	7.8	9.6	9.5	9.41	8.5	9.3	10.1
Asp	10.7	9.67	11.62	12.74	10.7	7.7	12.8	9.5	10.9	8.2	9.9	9.8
Thr	4.18	3.83	3.96	4.56	4.06	3.6	3.7	3.9	3.99	3.8	3.9	3.9
Ser	6.04	5.14	4.96	5.04	5.90	6.4	5.4	6.8	5.82	6.6	6.6	7.1
Glu	20.2	23.62	19.74	17.46	20.8	25.6	13.9	25.0	19.8	23.5	21.2	23.0
Pro	5.66	5.11	4.64	4.72	6.18	5.8	4.8	4.8	5.90	5 8	5.7	4.9
Cys	0	0	0	0	0.32	0.5	0	0	0.10	0.3	0.4	0
Gly	4.47	4.18	4.70	5.16	4.28	4.1	3.6	5.0	4.52	4.1	4.3	5.2
Ala	5.98	6.06	6.12	6.46	5.45	6.2	5.8	4.5	5.60	5.9	6.0	4.8
Val	7.22	6.01	6.21	6.60	6.92	6.5	6.7	6.0	7.06	6.0	6.0	5.4
Met	1.78	3.41	1.50	1.65	1.62	2.5	0.1	0.2	1.44	2.5	2.1	0.9
Ile	5.08	4.34	4.52	4.98	4.68	4.3	5.1	3.5	4.84	4.3	4.3	3.4
Leu	8.44	8.87	8.54	9.77	8.09	9.0	7.4	7.4	8.08	8.2	7.9	7.9
Tyr	5.38	6.12	5.54	5.66	5.22	6.4	5.5	4.8	5.13	6.2	6.0	4.9
Phe	5.70	6.01	6.15	6.32	5.44	5.4	5.1	6.1	5.49	5.2	5.2	6.1

* Values with two significant figures are unreplicated.

Table 3. Composition of albumin–globulin, prolamin, glutelin and glutelin subfractions of IR28 milled rice

Property	Albumin–globulin	Prolamin	Glutelin subfractions*				
			Whole	'Prolamin'	'Albumin–globulin'	True glutelin	LSD (5%)
Solvent	0.5 M NaCl	70% *i*-PrOH	—	*i*-PrOH *β*-ME	NaCl *β*-ME	NaCl *β*-ME SDS	
Percent of total protein (%)	13 1	3.2	83.7	3.5	2 6	61 5	0.49
glutelin (%)	—	—	100.0	4.2	3.2	73.7	0.34
Amino acid (g/16.8 g N)							
Lys	4.52	0.21	4.39	1.25	3 73	4 17	0.49
His	2.76	1.86	2.46	1.66	2.94	2 53	0 34
Amm	2.39	4.08	2.53	4.36	4.48	2 71	1.07
Arg	8.52	5.58	7.91	3.97	8.74	8.02	1.29
Asp	10 7	7 28	11.0	5.34	8.78	9.93	1 37
Thr	4.12	2 66	4.60	4.28	3.12	4 05	0.49
Ser	6.08	5.59	6.12	5.83	5.04	5 66	0.85
Glu	21 8	27.5	20.4	31.4	15.2	20 4	2.78
Pro	5.98	5 04	5.39	6.27	5.78	5.22	0.75
Cys	0 89	0 32	0.60	0.98	0.54	2.02	0.70
Gly	4.27	2.98	4.82	2.49	6.01	4.30	0.38
Ala	5.82	6.86	6.19	6.16	5.16	5.71	0.47
Val	6.95	6.98	7 18	6.70	5.92	6.82	0.60
Met	1.60	0 26	2.08	3.96	0.71	2.89	1.52
Ile	4.71	5.03	5.02	5.02	3.22	4 84	0.41
Leu	8 34	12.7	8.66	9.74	6 00	8 44	1 27
Tyr	5.10	9.90	4 01	6.38	4.30	5.52	0.78
Phe	5.36	6.68	5.70	6.52	3.74	5 60	0 71
Carbohydrate (% anhydro-glucose)	—	5 4	n d.	4.2	37.5	6 1	

* Insoluble residue was 15 4% of total protein or 19.0% of glutelin.

Microscopic examination, however, revealed some contamination of the embryo, pericarp and aleurone fractions with other grain parts. Glutelin is a relatively minor fraction in the nonendosperm components of rice grain [2].

Glutelin in developing rice caryopsis

Protein of dehulled grain (caryopsis) 4 to 7 days after flowering (DAF) was higher than at later stages in both IR26 and IR480-5-9, in agreement with previous results [6] and was higher in the high-protein rice, IR480-5-9 (Table 4). However, glutelin-type protein was lower in the younger grains than in the older developing grains of both rice samples. Amino acid analysis of the crude glutelin showed differences in composition between the 7 DAF grain and the 14 and 21 DAF grains, particularly in the higher lysine and glycine and lower glutamic acid and tyrosine of the younger samples for both IR26 and IR480-5-9. Similarly, SDS-gel electrophoresis showed that the MW 16000 subunit of glutelin was the major subunit 4-7 DAF in both rices (Fig. 4). Only traces of the MW 25000 and the MW 38000 subunits of glutelin of milled rice were present, in contrast to the electrophoregram of glutelins 14 and 21 DAF, which was identical to that of milled rice, showing a 1:1:1 ratio of the three major subunits of glutelin with MW 16000, 25000 and 38000. In addition, subunits with MW >38000 were absent in glutelin from the grain 4-7 DAF.

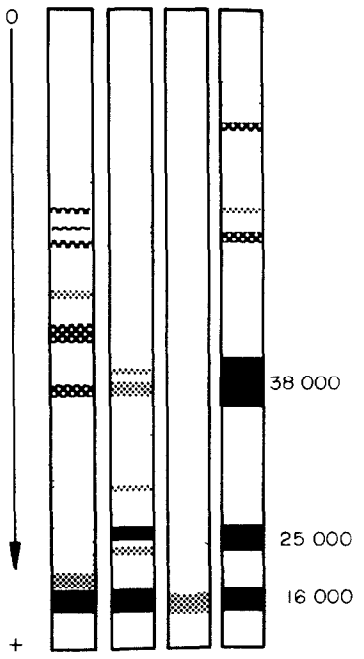

Fig. 3. SDS-polyacrylamide disc gel electrophoregram of Ce-glutelin from embryo, aleurone layer, pericarp and milled rice (endosperm) of IR8

Table 4. Composition of crude glutelin in dehulled developing IR26 and IR480-5-9 rice grain

Property	IR26			IR480-5-9			LSD (5%)
	7 DAF	14 DAF	21 DAF	7 DAF	14 DAF	21 DAF	
Brown rice protein (%)	10.3	9.6	9.4	13.9	12.4	12.6	
Brown rice glutelin (%)	5.1	7.3	8.0	9.8	10.2	11.0	
Glutelin content of protein (%)	49.5	76.0	85.1	70.5	82.3	87.3	
Amino acid content (g/16.8 g N)							
Lys	5.18	3.98	4.02	6.64	4.00	3.82	0.49
His	2.50	2.73	2.70	2.56	2.64	2.80	NS
Amm	2.60	3.01	3.70	2.80	3.12	3.87	1.07
Arg	7.81	8.54	9.09	8.35	8.92	9.23	NS
Asp	11.2	10.5	10.8	12.5	10.3	10.1	1.37
Thr	4.87	4.19	4.52	5.10	4.27	4.06	0.49
Ser	5.90	5.64	5.77	5.55	5.68	5.32	0.85
Glu	17.6	19.0	20.6	13.8	19.5	19.3	2.78
Pro	5.30	5.06	5.11	4.56	4.95	4.84	NS
Cys	0.54	0.87	0.88	0.82	0.61	0.71	NS
Gly	5.29	4.44	4.67	5.30	4.42	4.28	0.38
Ala	6.41	5.66	6.09	6.32	5.50	5.53	0.47
Val	7.36	7.14	7.44	7.31	6.85	6.82	NS
Met	1.36	1.92	2.25	2.76	1.70	1.64	NS
Ile	5.30	4.92	5.20	5.50	4.85	4.74	0.41
Leu	9.21	8.32	8.90	9.14	8.28	8.53	NS
Tyr	3.02	4.56	4.21	3.66	4.84	4.86	0.78
Phe	5.97	5.57	6.08	5.96	5.94	5.56	NS
Trp	1.61	1.32	1.24	1.61	1.34	1.11	NS

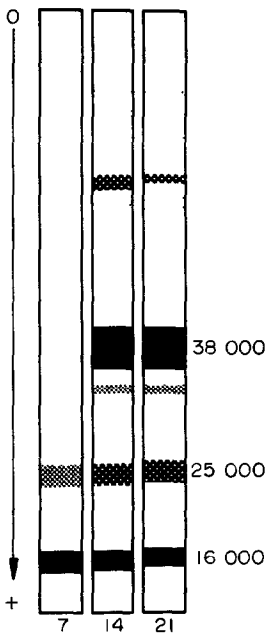

Fig. 4 SDS-polyacrylamide disc gel electrophoregram of Ce-glutelin from dehulled, developing IR480-5-9 grains 7, 14 and 21 days after flowering.

DISCUSSION

The survey of the aminogram and SDS-polyacrylamide gel electrophoregram of glutelins of milled rice showed little variation in these properties for the major fraction of endosperm protein. The results agree with the constancy in the amino acid pattern (lysine) of protein of *O. sativa*, previously obtained from 10500 brown rice samples [10]. Wild *Oryza* species have also been surveyed for amino acid analysis and were shown to be similar to that of *O. sativa* [11]. The different ratio of the 3 subunits of glutelin in *O. nivara* may not have nutritional significance since the aminogram of whole glutelin was similar to *O. glaberrima* and *O. sativa* (Table 1). Thus, the slim prospect of finding variants differing in amino acid composition was verified.

No definite trend was noted in the aminogram of the MW 16000, 25000 and 38000 subunits of milled rice glutelin for the 3 rices tested (Table 2). Juliano and Boulter [4] found the MW 25000 subunit of IR480-5-9 to have the highest lysine content and this was verified in the IR480-5-9 sample in this study. Varietal differences in the aminogram of subunits of similar MW are suggested. It was of interest that the glutelin fraction with MW > 38000 had a higher lysine content than that of the major subunits. In the preparation of concentrated rice protein by treating milled-rice flour with commercial amylases, a decrease in this higher MW subunit fraction of milled-rice protein coincided with a drop in lysine content of residual protein (A.P. Resurreccion, unpublished data).

The major fraction of milled rice glutelin was that soluble in SDS plus β-mercaptoethanol based on the solubility fractions of IR28 glutelin (Table 3). Since our extraction solvent for prolamin already contained 0.6% β-mercaptoethanol in 70% EtOH, 'bound' prolamin

would already have been removed. Preliminary extraction of crude glutelin with 0.5 M NaCl–β-mercaptoethanol may reduce the contamination with albumin–globulin of subunit MW 16000.

The glutelin of IR8 milled rice was shown to be electrophoretically distinct from that in the embryo and pericarp. Aleurone glutelin was similar on SDS-gel electrophoresis to milled rice glutelin, but the former was shown microscopically to be contaminated with endosperm cell particles. Since protein bodies in the aleurone layer are different morphologically from those of endosperm [12], differences in the properties of their glutelin may be expected as discussed below for the developing grain.

The small proportion of the MW 38000 and 25000 subunits of glutelin in 4- to 7-day-old developing grains and their presence in 14- and 21-day-old grains are in accordance with the suggestion that the MW 38000 is characteristic of rice glutelin [4]. The difference in amino acid pattern of glutelin in 7-day-old and 14- or 21-day-old grain is in accord with previous data on whole protein of dehulled developing rice grain [10, 13]. Since the pericarp, aleurone, and embryo are developed earlier than the endosperm during grain development [14], the glutelin in these outer layers probably differs in composition and SDS-electrophoregram from that of endosperm glutelin. The aleurone is already fully developed by 7 DAF.

The period from 7 DAF onward represents the appearance and synthesis of endosperm protein bodies [3], and the increase in glutelin content of dehulled grain [6, 13]. Our results verify the parallel appearance of endosperm protein bodies and peak glutelin synthesis with the changes in aminogram and SDS-electrophoregram of dehulled rice glutelin. However, varietal differences were also evident in the aminogram of glutelin of IR26 and IR480-5-9.

EXPERIMENTAL

Samples of rough rice were obtained from crops of the International Rice Research Institute experimental farm. They were dehulled with a Satake dehuller, milled to 10% bran removal in Satake or test tube mills, and the milled rice ground to a 60-mesh flour with a Udy cyclone mill. The flours were defatted with petrol at 25° and air dried. Developing grains of IR26 and IR480-5-9 were from the 1976 dry season crop. They were sorted, hand dehulled, freeze-dried and ground in the Udy mill.

Preparation of glutelin Crude glutelin was prepared after ref. [4] employing a sequential extraction of albumin–globulin with 0.5 M NaCl and of prolamin with 70% EtOH containing 0.6% β-mercaptoethanol. The residue was washed 3× with H_2O, air dried, ground to a fine powder with a mortar and pestle, and subjected to protein and amino acid analysis. Crude glutelins were also prepared from grain parts of IR8 brown rice. Glutelin was extracted from the crude preparation by stirring twice in 0.1 M Pi buffer, pH 7 with 0.5% SDS (BDH) and 0.6% β-mercaptoethanol for 1 hr each. The extract was recovered by centrifuging at 28000 *g* for 15 min. The combined extract was placed in an amber coloured flask, made 3 M with urea and deaerated by flushing with N_2. Acrylonitrile was added to 1.5% (v/v) and the mixture stirred for 1 hr. The *S*-cyanoethylated glutelins were pptd with 3 vol. of Me_2CO. After standing at 0–4° for 1 hr, the supernatant was decanted and the fluffy ppt. washed twice with 90% Me_2CO, and then 4× with H_2O before storing under N_2 at 0–4°. In preliminary expts, 2-vinylpyridine showed no advantage over acrylonitrile as alkylating agent.

Fractionation of S-Ce glutelins. MW fractionation of *S*-cyanoethyl glutelins of 3 samples—IR480-5-9, IR28 and Kolamba 540—was attempted using gel filtration in Ultrogel polyacrylamide–agarose gel columns. The glutelins were dissolved in 0.05 M Tris–HCl pH 8.6 with 0.5% SDS and 0.2% Na azide and separated in an Ultrogel 44 column (2.6 × 90 cm) with an upward flow rate of 4.6 ml/cm²/hr. Efficiency of fractionation was assessed by SDS-polyacrylamide gel electrophoresis of the eluted fractions. The pooled fractions corresponding to the mixture of MW 38000 and 25000 subunits was refractionated through an Ultrogel 34 column (1.6 × 100 cm). The fractions were then dialyzed against H_2O and the protein ppt. collected.

Preparation of glutelin subfractions. Fractions of crude glutelin were prepared according to ref. [8] from IR28 defatted rice flour. Albumin–globulin was removed from the defatted flour by 3× extraction with 6 parts of 0.5 M NaCl at 25° for 1 hr, 0.5 hr, and 0.5 hr periods followed by centrifuging for 10 min at 28000 *g*. The residue was then washed twice with H_2O and extracted 3× with 6 parts 70% *iso*-PrOH for 0.5 hr periods. The resulting crude glutelin was air dried and subjected to subfractionation. The crude glutelin was extracted twice for 0.5 hr each with 70% *iso*-PrOH 0.6% β-mercaptoethanol and 3× for 1 hr, 0.5 hr and 0.25 hr periods in succession with 0.5 M NaCl in 0.1 M Na_3BO_3 buffer pH 10 with 0.6% β-mercaptoethanol, and then with that extractant containing 0.5% SDS. Extracts were pooled, dialyzed against H_2O, cyanoethylated and analyzed.

Analytical procedures. The methods used are as previously described [4, 6]. Protein was determined by micro-Kjeldahl analysis using the factor 5.95 [10] and by the Folin-phenol reagent [15]. Amino acid analysis was as described previously [9]. Carbohydrate content was assayed by the anthrone method [16]. SDS-polyacrylamide gel electrophoresis was done in 12% gels using BDH SDS [17]. The stain used was either 0.5% Amido Black in 7% HOAc or 0.0125% Coomassie Brilliant Blue G-250 in 12% TCA [18].

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