Changes in Peptide Subunit Composition of Albumins, Globulins, Prolamins, and Oryzenin in Maturing Rice Grains

Joseph Chrastil* and Zigrida Zarins

Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, Louisiana 70179

Peptide subunit albumin, globulin, prolamin, and oryzenin (rice glutelin) preparations from preharvest maturing rice grains of U.S. Lemont (long grain) rice variety have been studied by SDS–PAGE. Relative quantitative composition of peptide subunits in albumin, globulin, prolamin, and oryzenin (rice glutelin) fractions changed significantly during maturing of rice grains. The results revealed that the synthesis of peptide subunits during maturing was uneven. The lower molecular weight subunits increased and the higher molecular weight subunits decreased between 14 and 50 days after flowering. This was especially apparent in the rice storage protein (oryzenin) fraction and resulted in a significantly lower average molecular weight of this fraction. The synthesis of intramolecular –SS– bonds was preferable to intermolecular –SS– bonds during this maturing period.

Keywords: *Rice; maturing; proteins; peptides; subunits*

Rice protein differs significantly from that of other cereals. It is richest in oryzenin (rice glutelin) and poorest in prolamin (Juliano, 1985). The major protein in protein bodies of rice is oryzenin, but in other cereals it is prolamin.

Great effort has been concentrated on the extraction, separation, and purification of albumin, globulin, prolamin, and oryzenin from rice (Juliano, 1985). Rice proteins, albumins, globulins, prolamins, and glutelins, have been studied from the point of view of varietal differences, composition, nutritional values, and genetics (Sawai and Morita, 1970; Sawai et al., 1970; Tecson et al., 1971; Iwasaki et al., 1972, 1975, 1982; Cagampang et al., 1976; Juliano and Boulter, 1976; Mandac and Juliano, 1978; Perdon and Juliano, 1978; Park and Stegemann, 1979; Padhye and Salunkhe, 1979; Shadi and Djurtoft, 1979; Tanaka et al., 1980; Villareal and Juliano, 1981, Yamagata et al., 1982; Bietz, 1982; Luthe, 1983; Zhao et al., 1983; Robert et al., 1985; Lookhart et al., 1987; Huebner et al., 1991; Marshall and Chrastil, 1992; Chrastil, 1993).

Several studies have been also done on the changes of the rice protein fractions by environmental factors (Juliano, 1985), during storage (Chrastil, 1990a–c, 1992) and during rice maturing (Cagampang et al., 1976; Mandac and Juliano, 1978; Sajwan et al., 1989; Li and Okita, 1993).

During maturing of rice grains significant qualitative and quantitative changes occur. For example, from 12 days after flowering (DAF) up to 20 DAF oryzenin showed the most dramatic increase, up to 100% (Palmiano et al., 1968; Cagampang et al., 1976; Li and Okita, 1993). Prolamin also increased significantly, but albumin and globulin increased much less and after 35 DAF slightly decreased. Arginine, tyrosine, cysteine, cystine, and glutamic acid increased, but alanine, glycine, threonine, phenylalanine, leucine, and lysine decreased in both albumin and globulin fractions. Rice prolamin always had the highest cysteine plus cystine content (Wieser et al., 1980, 1981).

In this work a more detailed study is presented of the maturation changes of peptide subunits from different rice protein fractions in greenhouse-grown Lemont long-grain rice variety.

EXPERIMENTAL PROCEDURES

Materials. All chemicals and substrates were analytical reagents of the highest obtainable purity from Sigma Chemical Co. (St. Louis, MO) or Aldrich Chemical Co. (Milwaukee, WI). Gels for Phastgel automatic electrophoresis system were from Pharmacia-LKB (Piscataway, NJ).

Preharvest Rice. A typical U.S. long-grain rice variety (Lemont) was obtained from the Crowley Research Station, Louisiana. Rice was planted in the greenhouse in Hoagland-Snyder solution as a nutrient (Yoshida et al., 1976). At selected time intervals after flowering, rice grain samples were taken in random order. Grains that differed very much from the average grain size, color, or shape were excluded.

Grinding. Rice grains were ground in a water-cooled micromill (Technilab Instruments, Pequannock, NJ) to flour (10 g of grains, 3 min of grinding). The flour was used for extraction of protein fractions.

Lipid Extraction. Rice flour (50 g) was extracted overnight by shaking with 750 mL of methanol–ethyl ether mixture (1 + 1) at room temperature (25 °C). The mixture was filtered by medium fritted glass filter and the supernatant discarded. The extraction was repeated twice in the same manner. The extracted flour was dried on air and used for albumin extraction.

Albumin. After lipid extraction, the rice flour residue was extracted by 60 min of shaking with 750 mL of distilled water at 25 °C. The suspension was centrifuged at 10000*g* at 25 °C for 30 min and the supernatant decanted. This procedure was repeated twice. Combined supernatants were filtered by medium fritted glass filter and freeze-dried. These preparations usually contained over 30% starch. Thus, to obtain higher purity (over 90% protein), the albumin was redissolved and the precipitation was repeated several times.

Globulin. The rice flour residue after albumin extraction was extracted with 750 mL of 1 M NaCl for 1 h at 25 °C. The suspension was centrifuged at 10000*g* for 30 min and the supernatant decanted. This procedure was repeated twice. Combined supernatants were filtered by medium fritted glass filter, dialyzed extensively against distilled water, and lyophilized.

Prolamin. The rice flour residue after globulin extraction was extracted with 750 mL of 55% 1-propanol for 1 h at 25 °C. The suspension was centrifuged at 10000*g* for 30 min and the

supernatant decanted. The second extraction was effected with 55% 1-propanol in the same manner as before. Combined supernatants were filtered by medium fritted glass filter, dialyzed extensively against distilled water, and lyophilized.

Oryzenin. The rice flour residue after prolamin extraction was washed first with 750 mL of distilled water and centrifuged at 20000*g* for 30 min. The supernatant was discarded. The residue was extracted with 750 mL of 0.05 M NaOH for 1 h at 25 °C. The suspension was centrifuged at 20000*g* for 30 min and the supernatant decanted. The extraction was repeated twice. Combined supernatants were filtered by medium fritted glass filter, and the protein was precipitated by adding TCA to a final concentration of 5%. The precipitate was separated by centrifugation at 22000*g* for 1 h at 25 °C, resuspended in water, dialyzed extensively to remove TCA, and lyophilized.

Protein Content. Protein was determined in the diluted albumin, globulin, prolamin, or oryzenin solution (200 mg/L of 0.1 M NaOH) according to the method of Lowry et al. (1951) with 200 mg/L bovine albumin as a standard. The results were expressed as an average from triplicate samples. The standard deviation of the mean of the triplicates was less than ±0.2% protein.

Carbohydrate Content. Carbohydrate content was determined in the diluted albumin, globulin, prolamin, or oryzenin solution (0.5 g/L in 0.1 M NaOH) according to the method of Montgomery (1961). One milliliter of this solution was mixed with 1 mL of 5% phenol and 5 mL of H_2SO_4 (95%). After 15 min, the absorbance was read at 490 nm vs H_2O and compared with the standard curve of glucose (10–100 mg/L). The results were expressed as an average from triplicate samples. The standard deviation of the mean of the triplicates was less than ±0.3% carbohydrate.

Cysteine and Cystine Contents. Albumin, globulin, prolamin, or oryzenin was dissolved in 10% formic acid, and the free –SH and –SS– bonds were determined directly (Chrastil, 1989) without hydrolysis. The results were expressed as an average from triplicate samples. The standard deviation of the mean of the triplicates was less than ±0.002% S.

Electrophoresis. Albumin, globulin, prolamin, or oryzenin protein samples were dissolved in 1 mL of 0.055 M Tris buffer, pH 6.8, containing 4.3% 2-mercaptoethanol, 2% SDS, 5 M urea, and 0.002% bromophenol blue and heated for 10 min at 95 °C. All samples contained 4 mg/mL protein and 0.7 mg/mL β-galactosidase as a standard (MW = 116 000). One microliter of this solution was applied to 20% Phastgels and run on the Pharmacia Phastgel SDS Buffer and Phastgel Blue R Staining automatic system.

Densitometry. The electrophoretic spots of peptide subunits were measured on a CAMAG Scanner II in refractive mode at 550 nm. In the computerized densitometric analysis, very small spots (less than 0.5% total) were automatically ignored (some of them could be impurities from other than the analyzed protein fractions), and the quantity of the remaining peptide subunits was expressed in relative percent of the total. The R_f of the peaks was compared to the standard curve obtained from STD proteins (ribonuclease, MW = 12 640; cytochrome *c*, MW = 13 370; α-lactalbumin, MW = 14 400; myoglobin, MW = 16 890; trypsin inhibitor, MW = 20 100; carbonic anhydrase, MW = 30 000; ovalbumin, MW = 43 000; albumin, MW = 67 000; phosphorylase *b*, MW = 94 000; ferritin, MW = 220 000). From each rice sample two electrophoretic gels were measured, and the results were plotted with automatic baseline corrections by means of a computer program. The average molecular weights and the average relative intensities were calculated with the standard deviations of the mean.

RESULTS AND DISCUSSION

Purity of Protein Fractions. Lyophilized albumin, globulin, prolamin, or oryzenin fractions were analyzed for protein and carbohydrate content which resulted in 90–98% protein and 2–10% carbohydrate (mostly glucose or starch).

Table 1. Cysteine and Cystine in Protein Fractions[a]

protein fraction	% S					
	14 DAF[b]		30 DAF		50 DAF	
	CYS	CSS	CYS	CSS	CYS	CSS
albumin	0.39	0.04	0.40	0.04	0.40	0.04
globulin	0.10	0.10	0.10	0.10	0.10	0.10
prolamin	0.60	0.07	0.61	0.07	0.61	0.07
oryzenin	0.21	0.12	0.19	0.14	0.19	0.14

[a] Cysteine and cystine contents are expressed in percent S per gram of albumin, globulin, prolamin, or oryzenin protein. Values are averages from duplicates. The difference between duplicates was less than ±1%. [b] DAF, days after flowering.

Table 2. Albumins in Maturing Rice Grains[a]

peak		relative %			
R_f	kDa	14 DAF[b]	30 DAF	50 DAF	trend
0.28	102	20.1 ± 0.15	20.4 ± 0.17	20.5 ± 0.20	none
0.39	73	15.0 ± 0.11	11.5 ± 0.10	9.7 ± 0.08	decrease
0.43	66	10.3 ± 0.09	10.8 ± 0.08	11.5 ± 0.09	increase
0.61	37	19.6 ± 0.12	22.8 ± 0.17	23.9 ± 0.19	increase
0.71	27	7.2 ± 0.05	7.8 ± 0.06	8.1 ± 0.05	increase
0.87	17	27.8 ± 0.21	26.7 ± 0.19	26.3 ± 0.22	decrease

[a] Values are averages from duplicates and variations of the means. [b] DAF, days after flowering.

Table 3. Globulins in Maturing Rice Grains[a]

peak		relative %			
R_f	kDa	14 DAF[b]	30 DAF	50 DAF	trend
0.49	53	30.0 ± 0.25	30.2 ± 0.21	30.2 ± 0.22	none
0.59	39	15.4 ± 0.11	15.6 ± 0.11	15.8 ± 0.12	none
0.74	25	35.4 ± 0.30	32.5 ± 0.25	30.7 ± 0.30	decrease
0.89	15	9.6 ± 0.06	10.8 ± 0.08	11.6 ± 0.07	increase
0.93	14	9.6 ± 0.08	10.9 ± 0.07	11.7 ± 0.10	increase

[a] Values are averages from duplicates and variations of the means. [b] DAF, days after flowering.

Albumin. Over 90% of the cysteine plus cystine content in albumin was in the form of cysteine (Table 1). The total cysteine plus cystine sulfur content and the cystine:cysteine ratio did not change significantly during maturing (between 14 and 50 DAF). On the other hand, the relative content of peptide subunits changed significantly during maturing (Table 2). The 73 and 17 kDa peptides decreased during maturing (14–50 DAF) from 15.0 to 9.7% and from 27.8 to 26.3%, respectively. The 66, 37, and 27 kDa peptides increased from 10.3 to 11.5%, from 19.6 to 23.9%, and from 7.2 to 8.1%, respectively. The average molecular weight of albumin peptides (calculated from relative percent) decreased only by 2.1% (from 52 200 to 51 100) in the same maturing period.

Globulin. Globulin contained about equal amounts of cysteine and cystine. The total cysteine plus cystine sulfur content and the cystine:cysteine ratio changed only little during maturing (between 14 and 50 DAF) (Table 1). On the other hand, the relative content of peptide subunits changed significantly during maturing (Table 3). The 25 kDa peptide decreased during maturing (14–50 DAF) from 35.4 to 30.7%. The 15 and 14 kDa peptides increased from 9.6 to 11.6% and from 9.6 to 11.7%, respectively. The average molecular weight of globulin peptides (calculated from relative percent) decreased only by 0.9% (from 33 500 to 33 200) in the same maturing period.

Prolamin. Almost 90% of the cysteine plus cystine content in prolamin was in the form of cysteine (Table 1). Prolamin contained high amounts of cysteine and cystine. Cysteine content was much higher than that

Table 4. Prolamins in Maturing Rice Grains[a]

peak		relative %			
R_f	kDa	14 DAF[b]	30 DAF	50 DAF	trend
0.72	27	28.3 ± 0.21	26.4 ± 0.22	25.1 ± 0.24	decrease
0.93	14	71.7 ± 0.52	73.6 ± 0.58	74.9 ± 0.68	increase

[a] Values are averages from duplicates and variations of the means. [b] DAF, days after flowering.

Table 5. Oryzenin in Maturing Rice Grains[a]

peak		relative %			
R_f	kDa	14 DAF[b]	30 DAF	50 DAF	trend
0.33	92	6.9 ± 0.06	5.4 ± 0.04	4.9 ± 0.04	decrease
0.48	55	31.7 ± 0.26	29.3 ± 0.28	27.8 ± 0.24	decrease
0.66	33	33.2 ± 0.26	31.3 ± 0.28	27.6 ± 0.22	decrease
0.77	22	18.3 ± 0.11	23.0 ± 0.15	26.0 ± 0.20	increase
0.93	14	9.9 ± 0.06	10.9 ± 0.10	13.8 ± 0.11	increase

[a] Values are averages from duplicates and variations of the means. [b] DAF, days after flowering.

of cystine. The total cysteine plus cystine sulfur content and the cystine:cysteine ratio did not change during maturation (between 14 and 50 DAF). On the other hand, the relative content of peptide subunits changed significantly during maturing (Table 4). The 27 kDa peptide in the prolamin fraction decreased during maturing (14–50 DAF) from 28.3 to 25.1%. The 14 kDa peptide increased from 71.7 to 74.9%. The average molecular weight of prolamin peptides (calculated from relative percent) decreased only by 2.3% (from 17 700 to 17 300) in the same maturation period.

Oryzenin. Oryzenin contained predominantly cysteine (over 60%). The total cysteine plus cystine sulfur content did not change significantly (between 14 and 50 DAF) (Table 1), but cysteine became less predominant during maturation. The relative content of peptide subunits changed significantly during maturing (Table 5). The higher molecular weight 92 000, 55 000, and 33 000 peptide subunits decreased during maturing (14–50 DAF) from 6.9 to 4.9%, from 31.7 to 27.8%, and from 33.2 to 27.6%, respectively.

On the other hand, the lower molecular weight peptide subunits 22 000 and 14 000 increased from 18.3 to 26.0%, and from 9.9 to 13.8%, respectively. This resulted in a significant decrease of the average molecular weight of the oryzenin peptides (calculated from relative percent) by 9.2% (from 40 200 to 36 500) in the same period.

Thus, intensive synthesis of oryzenin during maturation (see above) was not even. Synthesis of the lower molecular weight subunits, which contained the important 14 000 prolamin peptide subunit, was much faster than the synthesis of the higher molecular weight subunits. This agrees with the recent finding of Li and Okita (1993), who found increased synthesis of prolamins in rice grains than previously assumed.

These results seem to disagree with the simultaneous increase of the cystine:cysteine ratio in oryzenin (see above), but we have a simple explanation: the relative increase of –SS– bonds during maturation was predominantly intramolecular and not intermolecular.

The overall trend of the subunit distribution during maturation was different from the overall trend during storage of postharvest rice grains of the same variety (Chrastil and Zarins, 1992). The molecular weight of oryzenin during storage greatly increased and the relative amount of lower molecular weight peptide subunits decreased.

Conclusion. The results indicate that the synthesis of peptide subunits of albumin, globulin, prolamin, and oryzenin protein fractions in rice grains during maturing is uneven. Some subunits are synthesized at different rates, either more rapidly or more slowly than the whole protein fraction. The lower molecular weight peptide subunits of albumin, globulin, prolamin, and oryzenin are synthesized more quickly than the higher molecular weight peptides. This was especially apparent with oryzenin, where it caused a significant decrease in the average molecular weight of this fraction. The relative increase of –SS– bonds during maturing of oryzenin was predominantly intramolecular and not intermolecular.

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