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PROPERTIES OF A MAJOR α-GLOBULIN OF RICE ENDOSPERM*

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Abstract—Globulin was isolated from milled rice (*Oryza sativa*, line IR480-5-9) by 5% NaCl extraction and was precipitated by $(NH_4)_2SO_4$ or by dialysis against water. The extract was purified by repeated isoelectric precipitation at pH 4.5. The major globulin fraction (40%) exhibited one band by electrophoresis at pH 4.5 but two bands at pH 8.3. Similarly, one sharp peak was shown by sedimentation corresponding to 1.41S (α-globulin) in acetic acid (pH 2) and NaOH (pH 11.7) but a broad asymmetric peak was obtained at pH 6.7, 8.3 and 8.9. Gel filtration of the α-globulin at pH 6.5 exhibited 2 proteins with MW 20000 and 98000. The results suggest a pH dependent aggregation phenomenon. The two proteins could not be separated by DEAE-cellulose chromatography. SDS-polyacrylamide electrophoresis of α-globulin revealed one subunit with MW 18000. This α-globulin is poorer in lysine and histidine but richer in cystine, methionine, arginine, tyrosine and glutamic acid than whole milled rice protein.

INTRODUCTION

There has been little research on the globulins of milled rice by comparison with the work on rice bran. Morita *et al.* [1] reported the presence of α, β-, γ- and δ-globulins in rice endosperm. A major globulin fraction of rice endosperm was purified and characterized by Houston and Mohammad [2], which was distinct from the high-sulphur globulin they reported earlier [3]. All other studies were on the electrophoretic and amino acid characterization of crude extracts from milled rice [4, 5]. As part of our studies of proteins of developing and mature rice grain [6–8], we studied the globulin proteins of milled rice.

RESULTS

Globulins were precipitated from NaCl extracts of milled high-protein IR480-5-9 rice by dialysis and by $(NH_4)_2SO_4$ addition to 30% saturation. Recovery by dialysis was 4% of total N and only 2.5% by $(NH_4)_2SO_4$ addition. The two preparations had identical disc electrophoretic patterns at pH 8.3 with two major slow migrating bands (band 1 and 2) and 3 minor fast migrating bands (band 3 to 5) (Fig. 1). A similar pattern was obtained for a sample of low-protein IR32 globulin, except that band 3 was relatively more intense. An electrophoregram of IR480-5-9 bran globulin indicated that band 3 was the major globulin, in addition to 6 other minor bands. IR480-5-9 was relatively overmilled as compared to IR32, which may explain its lower content of band 3 globulin. Globulin prepared by $(NH_4)_2SO_4$ precipitation was used in a subsequent purification studies because of its lower content of nucleic acid as indicated by 260 nm/280 nm ratios of the preparation in 0.3 N NaOH solution. Drying the harvested grain at 40° had little effect on the electrophoretic pattern of globulin as compared to air drying at 25°.

Isoelectric precipitation at pH 4.5 by the method of Houston and Mohammad [2] resulted in the separation of band 1 and 2 from the other globulins (Fig. 1). Gel filtration on Sephadex G-100 of this purified globulin resulted in a major peak with MW 20000 and a minor peak with MW 98000. Both fractions, however, gave electrophoretic patterns identical to the starting globulin preparation. DEAE-cellulose column chromatography gave essentially only one protein peak at 0.22 M NaCl, which corresponded to a mixture of band 1 and 2 as seen on electrophoresis at pH 8.3.

The ultracentrifugation pattern of this globulin at pH 2 showed only one protein peak corresponding to 1.41 Svedbergs (S). Thus the globulin is an α-globulin based on its S value of 2.1 or less [9]. The disparity between the results of one fraction by ultracentrifugation at pH 2 and of two fractions by electrophoresis at pH 8.3 and gel filtration at pH 6.5 suggested the need to repeat the tests at the same pH. At pH 6.7, 8.3 and 8.9, the sedimentation pattern of α-globulin exhibited a broad asymmetric peak comparable to the pattern of rice endosperm β-globulin in M NaCl [1]. However, at pH 11.7, α-globulin gave the same pattern of a single symmetrical peak as it did at pH 2. Electrophoretic pattern of the α-globulin at pH 4.5 showed only one protein band, in contrast to two at pH 8.3 (Fig. 1). Extracts of band 1 from electrophoresis at pH 8.9 and rerun at the same pH gave both band 1 and 2. SDS-polyacrylamide gel electrophoresis of α-globulin gave only one band corresponding to MW 18000, with or without prior cyanoethylation.

Globulin of milled rice was verified to be slower migrating on electrophoresis at pH 8.3 than albumin as previously reported for brown rice of developing grain [7] (Fig. 1). There were 5 major bands and 6 minor albumin bands for both IR480-5-9 and IR32.

Crude globulin contained 16.8% carbohydrate and

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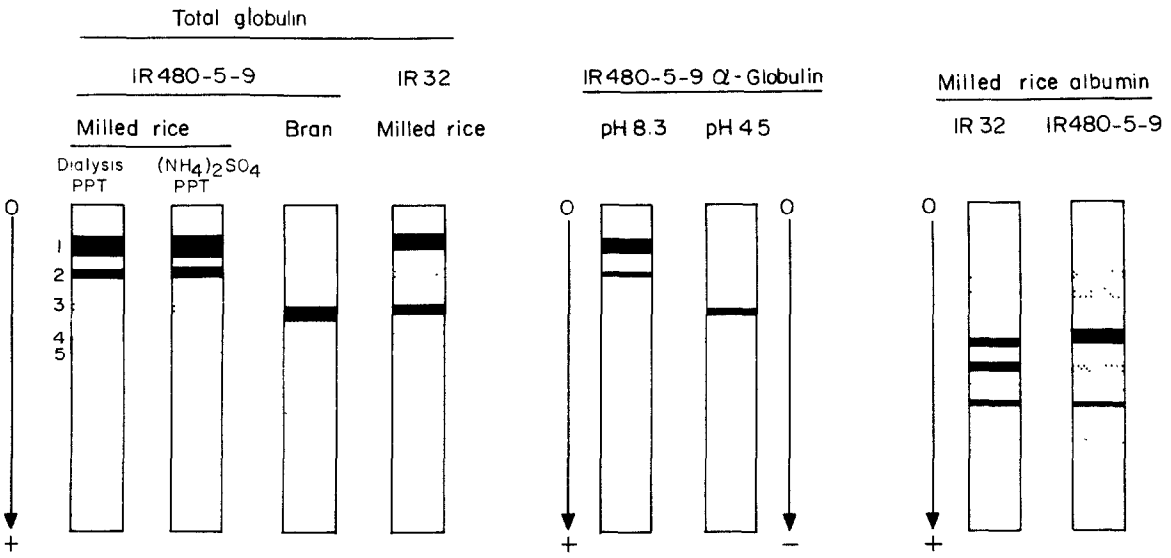

Fig. 1. Polyacrylamide gel electrophoregram of total globulin from IR480-5-9 milled rice and bran and IR32 milled rice, α-globulin from IR480-5-9 milled rice and milled rice albumin. The gels were cut at the position of the tracking dye. Stain: Amido Black 10B.

α-globulin had 8% carbohydrate (Table 1). Amino acid analysis showed high contents of arginine, glutamic acid, serine, cystine and methionine and tyrosine and lower contents of lysine, histidine, aspartic acid, threonine, isoleucine, phenylalanine and tryptophan for crude globulin than for whole protein of milled rice (Table 1). Purification of α-globulin resulted in further changes in the 13 amino acids mentioned above. Crude globulin from IR32 milled rice showed a similar aminogram to that of IR480-5-9 despite the slight mobility difference on electrophoresis (Fig. 1).

DISCUSSION

A convenient method of preparing α-globulin from milled rice is by $(NH_4)_2SO_4$ precipitation at 30% saturation from 5% NaCl extraction followed by repeated isoelectric precipitation at pH 4.5. Anomalous

Table 1. Composition and aminogram (g/16 g N) of globulins and albumins of IR480-5-9 and IR-32 milled rices and IR480-5-9 bran

Constituent	Globulin				Crude albumin						
	Milled rice			Bran	Milled rice		Bran	Milled rice		α-Globulin	LSD
	IR480-5-9		IR32	IR480-5-9	IR480-5-9	IR32	IR480-5-9	IR480-5-9	IR32	[2]	(5%)
	Total	α-Globulin	Total	Total							
%N × 6.25 (dry basis)	84.4	87.1	89.4	63.1	29.2	22.3	71.5	12.1	8.2	113.1	
% Carbohydrate (dry basis)	16.8	8.0	8.9	2.0	1.6	44.2	7.0	87.0	—	—	
Lys	1.81	1.06	1.50	5.66	4.92	6.04	7.46	3.34	3.72	0.13	0.46
His	1.56	0.42	1.42	2.84	3.22	2.06	2.64	2.34	2.38	trace	0.38
Amm	1.69	2.28	1.78	1.58	1.53	7.28	1.60	2.36	2.50	1.44	1.64
Arg	12.9	15.2	12.6	7.21	9.53	5.42	7.55	8.11	7.82	17.9	0.44
Asp	6.88	4.94	8.92	9.62	9.66	9.00	10.4	9.72	9.23	3.23	1.24
Thr	2.36	2.19	2.17	4.87	4.00	4.04	5.78	3.67	3.78	1.69	0.26
Ser	6.15	8.84	5.71	5.92	4.73	4.70	6.94	5.78	5.74	8.51	0.32
Glu	17.4	27.8	17.0	12.4	12.1	10.7	9.42	19.8	19.6	25.1	0.77
Pro	7.08	4.96	6.67	6.71	6.84	5.08	5.58	4.42	4.43	4.18	1.00
Cys	3.09	3.00	4.43	1.08	1.89	1.50	3.37	1.75	1.42	4.32	1.08
Gly	6.06	5.06	5.84	6.37	6.60	4.88	6.77	4.38	4.38	4.78	0.18
Ala	6.04	4.20	5.62	7.72	7.82	6.99	9.02	5.57	5.72	3.71	0.25
Val	5.38	4.04	5.29	7.32	7.38	6.04	6.66	5.80	6.10	3.28	0.26
Met	3.23	4.03	3.94	1.62	1.80	1.58	0.41	1.51	2.14	5.79	1.40
Ile	2.34	1.82	2.23	4.12	3.26	3.26	4.43	4.00	4.22	1.31	0.16
Leu	6.52	6.58	6.35	9.02	7.58	6.20	5.93	6.44	8.28	5.91	0.27
Tyr	6.00	6.72	5.99	4.20	4.90	3.05	3.56	4.70	3.60	7.28	0.59
Phe	3.10	3.39	3.04	4.81	3.21	3.01	2.72	5.46	5.34	3.00	0.22
Trp	1.44	0.62	1.35	1.51	1.44	0.63	1.97	1.17	1.30	0.95	NS
Total	101.0	107.1	101.8	105.3	102.4	91.4	102.3	100.3	101.7	102.5	
N recovery (%)	99.5	98.8	99.3	99.4	99.3	98.6	99.7	99.1	99.2	100.0	

results of electrophoregrams and sedimentation constants may be explained by a pH-dependent partial aggregation of α-globulin to a pentamer between pH 6 and 9. This has also been reported for *Phaseolus vulgaris* globulin Gl [10]. The pH-dependent aggregation may explain the single protein peak on ultracentrifugation at pH 2 and 12, but an asymmetric peak at pH 6.7, 8.3 and 8.9. Likewise, α-globulin yields two protein bands on disc electrophoresis at pH 8.3 and only one at pH 4.5. The aggregate form was tentatively estimated as a pentamer based on the ratio of Sephadex G-100 chromatography fractions with MW 20000 and 98000. SDS-polyacrylamide disc gel electrophoresis also gave only one major α-globulin subunit with MW 18000.

Our α-globulin has similar properties to the α-globulin obtained by Houston and Mohammad [2], but showed some differences in electrophoretic behavior and amino acid composition. They reported a MW of 25400, a N content of 18.1% and the absence of any carbohydrate content. Their α-globulin preparation also gave one protein band on starch gel electrophoresis at pH 3, and was lower in lysine, histidine, ammonia, aspartic acid, threonine, glutamic acid, valine, isoleucine, phenylalanine but higher in arginine, serine, cystine and methionine, tyrosine and tryptophan than our α-globulin (Table 1).

EXPERIMENTAL

Materials. Mature rice grains of high-protein IR480-5-9 and low-protein IR32 were taken from the 1976 dry-season crop at the International Rice Research Institute. The grains were artificially dried below 40° and stored at 0–5° before use. Rough rice was dehulled in a Satake Dehusker Type THU 35A and milled in a Satake Grain Testing Mill TM-05 at 1450 rpm using a mesh no. 36 disc. The brown rice was first milled for 11 sec to obtain true bran (the other 4% by wt), and further milled for 33 sec to give rice milled to 12% wt removal. Milled rice was wiped with cheesecloth to remove the adhering bran and the samples were ground in a Udy cyclone mill with a 35 mesh sieve.

Extraction of proteins. Milled rice flour (1 part) was stirred with petrol (5 parts) in the cold for 2 hr and centrifuged at 1000 *g* for 10 min and the defatted flour air dried for 12 hr. Globulin was extracted with 0.7 M NaCl and separated from albumin by pptn during dialysis as in ref. [7]. A second method employed was extraction with 5% NaCl and globulin pptn by addition of $(NH_4)_2SO_4$ to 30% satn [2]. The $(NH_4)_2SO_4$ pptn was repeated twice. The globulin was then dialyzed against H_2O at 0–4° and freeze dried

Purification and fractionation of α-globulin. Purification of α-globulin by isoelectric pptn was carried out according to ref. [2]. α-Globulin was dissolved in 1% HOAc and pptd by adjusting the pH to 4.5 with N NaOH 3×. The final ppt. was then suspended in H_2O, dialyzed against H_2O at 4° and freeze-dried.

The α-globulin (100 mg) was fractionated by gel filtration chromatography on Sephadex G-100 column (2.6 × 63 cm) in 0.04 M Pi buffer pH 6.5 and 6-ml effluent fractions collected. Calibration of the column used BSA fraction V, ovalbumin, myoglobin and cytochrome *c*. The α-globulin was also subjected to DEAE-cellulose column (1.6 × 66 cm) chromatography equilibrated with 0.03 M Tris–HCl buffer pH 8.7 and employing a 0 to 1 M NaCl linear gradient. Effluent fractions of 6 ml vol. were collected. Methods of analyses were as refs [7, 8] including polyacrylamide disc gel electrophoresis [7] and SDS-polyacrylamide gel electrophoresis [8]. Sedimentation coefficients were determined for α-globulin (0.15, 0.23 and 0.50%) in 1% HOAc, 0.025 M Tris–glycine buffer (pH 6.7, 8.3 and 8.9) and 0.1 N NaOH (pH 11.7) at 20° at 200000 *g* in a single sector Al cell in an AnD rotor of a Spinco Model E Ultracentrifuge according to ref. [2].

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