Heterogeneity of Rice Glutelin

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Palminao *et al.*¹⁾ have reported the Sephadex G-100 chromatography of albumin, globulin, prolamin and glutelin eluted from defatted rice powder. Glutelin fraction showed a single peak being passed through the column in the void volume. Sawai and Morita²⁾ have reported an advanced method to obtain chemically pure glutelin with very little contamination of nucleic acid and carbohydrate from rice endosperm and investigated chemical and physicochemical properties of S-cyanoehtyl glutelin in order to obtain information on the gross-structure of glutelin. They obtained only one peak in Sephadex G-200 gel filtration. As the average molecular weight of glutelin has been considered to be about two to three millions, it is quite possible that the purified glutelin is still separated into several components of macromolecules. The present communication concerns with the separation of three components from rice glutelin by Sepharose 2B gel filtration, which can separate molecules and particles up to molecular (particle) weight of several millions.

To prepare crude glutelin fraction, powdered milled rice was extracted first with water (albumin) five times, then with 1 M sodium chloride (globulin) five times and finally with 70% ethanol (prolamin) five times successively by means of centrifugation, $3000\times g$, for 30 min. The residue was shaken for 30 min with a volume of 0.1N sodium hydroxide solution five times its weight, and centrifuged

at $46,000\times g$, for 30 min. The combined supernatant was adjusted to pH 5.6 with 0.1 M acetic acid and the precipitate was collected by centrifugation, $400\times g$, for 5 min. It was repeated three times.

The crude glutelin was dissolved in a small volume of 0.1N sodium hydroxide solution and dialyzed overnight against 0.05 M tris (hydroxymethyl) aminomethane (Tris) buffer, pH 9.8, containing 4 M urea. The insoluble substances produced were removed by means of centrifugation, $46,000\times g$, for 30 min. The dialyzed preparation gave a single peak in Sephadex G-200 gel filtration. This was submitted to the gel filtration on a Sepharose 2B column in 0.01 M Tris buffer, pH 9.7 containing 4 M urea as the solvent. As shown in Fig. 1, the gel filtration gave three distinct peaks, P-1, P-2 and P-3, which were tentatively designated according to their elution order. Molecular weights of each fractions are presumed to be more than $20\sim30\times10^6$ for P-1, and less than 2×10^6 for P-3. P-2 must be an intermediate of both fractions.^{3,4)} Fractions under each peaks were individually combined and their protein contents were measured. Relative amounts of protein in these three components was 27.3% in P-1, 59.7% in P-2 and 13.6% in P-3.

The existence of three components was confirmed by rechromatography; each component was concentrated by means of isoelectric precipitation and dialyzed against distilled water. It was redissolved in small

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FIG. 1. Elution Curves from Sepharose 2B (5×23 cm) of Rice Glutelin.

Solvent: 0.01 M Tris buffer, pH 9.7, containing 4 M urea.

Flow rate: 1 ml/min.

FIG. 2. Elution Curves from Sepharose 2B of Each Separated Component of Rice Glutelin.

Conditions were the same as in Fig. 1.

amounts of 0.1 N sodium hydroxide and dialyzed against the same Tris buffer. Figure 2 shows the rechromatographic patterns of each component. Each component showed a single main peak, though some contaminations from the other components could not be avoided.

Using the fractions eluted at around the main peak, the chemical analysis of each component was performed (Tables I and II). These purified components contained more phosphorous and carbohydrate than the purified preparation prepared by Sawai and Morita.²¹ P-1 component seemed to have different constituents from P-2 and P-3. Amino acid compositions were also different in several amino acids among the fractions. The ultraviolet absorption spectra of each component in alkaline solution are shown in Fig. 3. P-2 and P-3, similar to original glutelin, exhibited a maximum at 291 mμ and a minimum at 274 mμ; P-1, however, exhibited a quite different spectrum.

Solubility of each component into the buffer solutions of various pH values ranging from 1.84 to 10.28 (sodium citrate-HCl buffer for pH 1.84~3.50, McIlvaine buffer for pH 3.50~8.78 and Na₂CO₃-H₃BO₃-KCl buffer for pH

TABLE I. PHOSPHORUS AND CARBOHYDRATE CONTENTS OF EACH COMPONENT SEPARATED FROM RICE GLUTELIN

	Phosphorus	Pentose	Hexose
P-1	36 μg	638 μg	1290 μg
P-2	74	173	660
P-3	75	270	670

The values were calculated to 100 mg of protein. Phosphorus was determined by Allen's method.⁵¹ Pentose and hexose were determined following to the combination of the Bial and the Tillman reactions.^{6,71}

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TABLE II. AMINO ACID COMPOSITION OF EACH COMPONENT OF RICE GLUTELIN

Amino acids	Original glutelin	P-1 fraction	P-2 fraction	P-3 fraction
Ala	4.5	4.1	4.3	4.5
Asp	9.7	9.0	10.0	10.6
Arg	8.2	9.7	11.6	11.6
Cys	0	0	0	0
Glu	17.0	15.3	17.3	15.6
Gly	4.3	4.1	4.4	4.2
His	3.8	4.9	3.5	3.6
Ileu	4.1	4.2	4.6	4.7
Leu	9.4	12.4	8.3	9.0
Lys	5.3	8.0	4.3	4.4
Met	1.0	0.8	1.4	0.5
Phe	5.4	4.4	6.2	4.3
Pro	7.8	9.3	5.7	7.6
Ser	5.7	3.3	5.5	5.5
Thr	3.1	2.3	3.5	3.4
Try	trace	trace	trace	trace
Tyr	2.7	1.0	2.6	1.8
Val	5.6	4.3	5.1	7.2
NH ₃	2.4	2.9	1.7	1.5

Each component was hydrolyzed with 6N HCl in a sealed glass tube for 24 hr at 110°C. The hydrolyzate was analyzed its amino acid contents by a amino acid analyzer, Hitachi Model KLA-3B. The values were calculated as per cent of total amino acids found.

8.78~10.28 were used) having ionic strength of 0.24 differed from one another; P-1 did not dissolve at any pH, P-2 did not dissolve at pH 4~6 and P-3 did not dissolve at pH 2~4. Urea was added to each solution increasing its concentration from 2M to 8M. Solubility of P-1 did not increase with increasing urea concentrations, P-2 became soluble at pH 4~6 when urea concentration was over 6M, and P-3 component became soluble covering whole pH range when urea concentration was over 4M. Changes in solubility and viscosity of each component in

FIG. 3. Ultraviolet Absorption Spectra of Each Component of Rice Glutelin.

alkaline solution with increasing alkaline concentration were also examined. P-1 component was somewhat different from the others; it seemed to be quite labile and easy to become insoluble in alkaline solution.

It is quite probable that P-1 is a denatured component occurred in the separation procedures. As P-2 and P-3 components does not give rise the P-1 component in the elution patterns of their rechromatographies, there must exist initially a component that gives P-1 in the gel filtration pattern on Sepharose 2B, becomes insoluble and to exhibit different properties during or after separating and purifying procedures. Further investigation is now in progress.

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