Gel Filtration and Electrophoresis of Soluble Rice Proteins Extracted from Long, Medium, and Short Grain Varieties

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ABSTRACT

Proteins were extracted from milled rice of long, medium, and short grain varieties with 5% sodium chloride and were separated by ammonium sulfate precipitation into albumin and globulin fractions. These were then investigated by gel filtration, starch-gel electrophoresis, and amino acid analysis. Both albumins and globulins gave four fractions by gel filtration. Molecular weights of albumins ranged from 10,000 to 200,000; those of globulins ranged from 16,000 to 130,000 (the fourth globulin fraction was not determined). Starch-gel electrophoresis gave about 20 bands for both albumins and globulins. Some differences were noted that support the idea of classifying rice varieties into groups by differences in electrophoretic patterns. Amino acid analysis of albumins and globulins showed several differences among the three varieties.

Only a few papers on soluble rice proteins have been published within the past ten years. Houston and Mohammad (1970) reported on insoluble globulin of rice endosperm. They proved that the globulin is a single component, using gel filtration and ultracentrifugation. Morita and Yoshida (1968), Sawai and Morita (1970a, 1970b, 1970c) and Morita et al (1971) characterized three γ-globulin fractions of the rice embryo. Perdon and Juliano (1978) showed that α-globulin is the major component of rice endosperm globulins. Shadi and Djurtoft (1979) studied rice proteins by cross immunoelectrophoresis, gel electrophoresis, and isoelectric focusing. The authors (Iwasaki et al 1971, 1975) reported on soluble proteins of short grain rice varieties produced in Japan.

This article describes an investigation of albumins and globulins extracted from the rice of different grain types produced in different countries by gel filtration, starch-gel electrophoresis, and amino acid analysis.

MATERIALS AND METHODS

Materials

The rice varieties used were Raung Nakk 16, a long grain variety produced in Thailand; Calrose, a medium grain variety produced in California; and Nihonbare, a short grain variety produced in Japan. Milled rices of the three varieties were ground to pass an 80-mesh sieve, and the flours were defatted by percolation with ethyl ether.

Preparation of Protein Samples

Protein extraction was conducted by a modification (Iwasaki et al 1972) of the Jones and Gersdorff (1927) method. Proteins were extracted with 5% sodium chloride solution for 1 hr, while the mixture was stirred. Solids were centrifuged out, and supernatant was filtered through filter paper with Celite 545.

Ammonium sulfate was added to the filtrate to 35% of saturation, where some of the proteins precipitated. The supernatant was labeled Solution I. The precipitate, which contained most of the globulins and a small amount of contaminating albumins, was redissolved in 5% sodium chloride solution and dialyzed against distilled water. Globulins precipitated, and contaminating albumins remained in solution in the dialysis tubes. The precipitate was collected and redissolved in 5% sodium chloride solution and dialyzed again to yield pure precipitated globulins. To the solution in the dialysis tubes, which contained contaminating albumins, sodium chloride was added to 5% concentration; ammonium sulfate was then added to 35% saturation (Solution II). Solutions I and II were mixed in a beaker and stirred to give a thoroughly homogeneous solution. Ammonium sulfate was added to the mixed solution to 60%

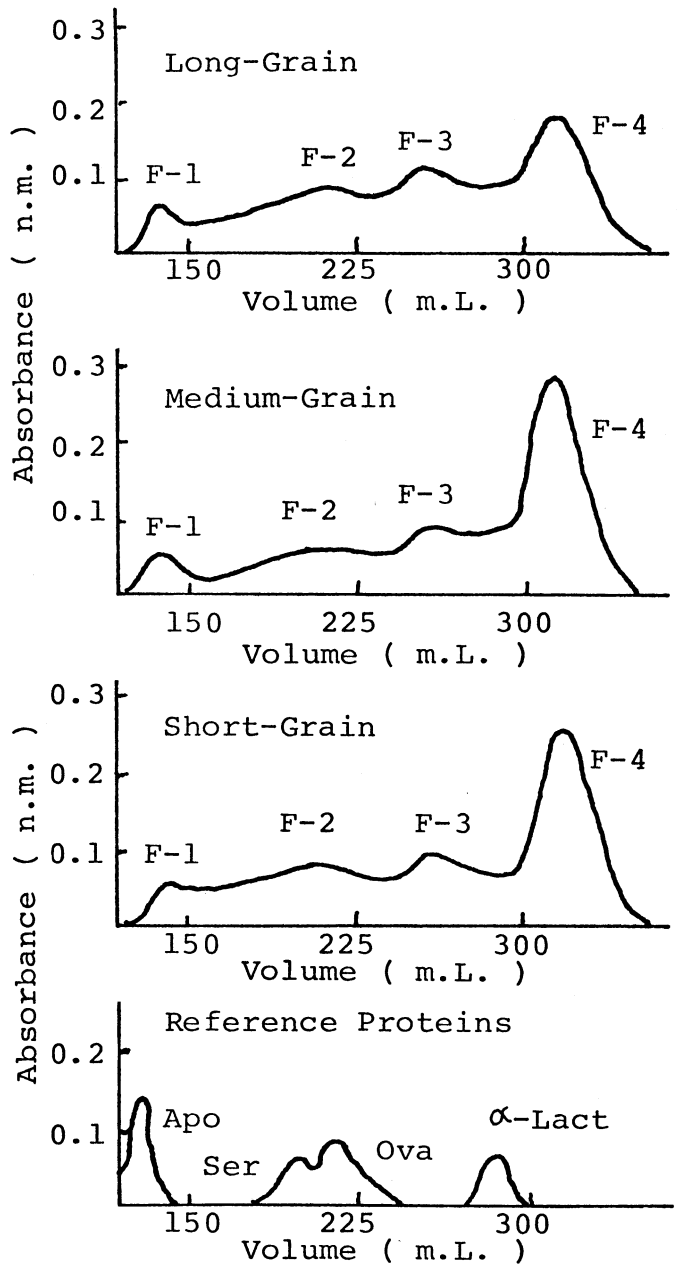

Fig. 1. Gel filtration patterns of rice albumins. Reference proteins. **Apo** = apo-ferritin (mol wt 480,000), **Ser** = serum albumin (mol wt 67,000), **Ova** = ovalbumin (mol wt 45,000), **α-lact** = α-lactalbumin (mol wt 15,500), **F** = fraction.

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saturation. The precipitated pure albumins were redissolved in distilled water and dialyzed. Solutions of pure albumins and pure globulins were lyophilized separately.

Gel Filtration

Albumins and globulins were fractionated on a 2.5 × 78-cm Sephadex G-100 column in 0.1 *M* tris buffer (pH 8.2) for albumins, and in the same buffer but with 5% sodium chloride for globulins.

Molecular Weight Estimation

Molecular weights of rice protein fractions were estimated by the method of Whitaker (1963). Gel filtration was conducted on several reference proteins, the molecular weights of which were known, under the same conditions as rice proteins. Calibration curves of the reference proteins were drawn by plotting log molecular weight against V_e/V_o. (V_o is the bed volume of a column; V_e is the elution volume of a reference protein.) Molecular weights of rice protein fractions were estimated from these calibration curves. Apoferritin (mol wt 480,000), serum albumin (mol wt 67,000), ovalbumin (mol wt 45,000), and α-lactalbumin (mol wt 15,500) were used as reference proteins for estimating molecular weights of rice albumin fractions. γ-Globulins (mol wt 160,000), serum albumin (mol wt 67,000), ovalbumin (mol wt 45,000), and cytochrome C (mol wt 12,400) were used as reference proteins for estimating molecular weights of rice globulin fractions.

Starch-Gel Electrophoresis

Starch-gel electrophoresis of acidic gels was performed by the method of Cole and Mecham (1966). Acidic gels were prepared in 0.017 *M* aluminum lactate-lactic acid buffer (pH 3.1) with 7.5 *M* urea. Starch-gel electrophoresis on alkaline gels was prepared by the method of Feillet and Bourdet (1968). Alkaline gels were prepared in 0.05 *M* tris-citric acid buffer (pH 8.9) with 7.5 *M* urea. After electrophoresis, gels were stained with amido black 10 B dye

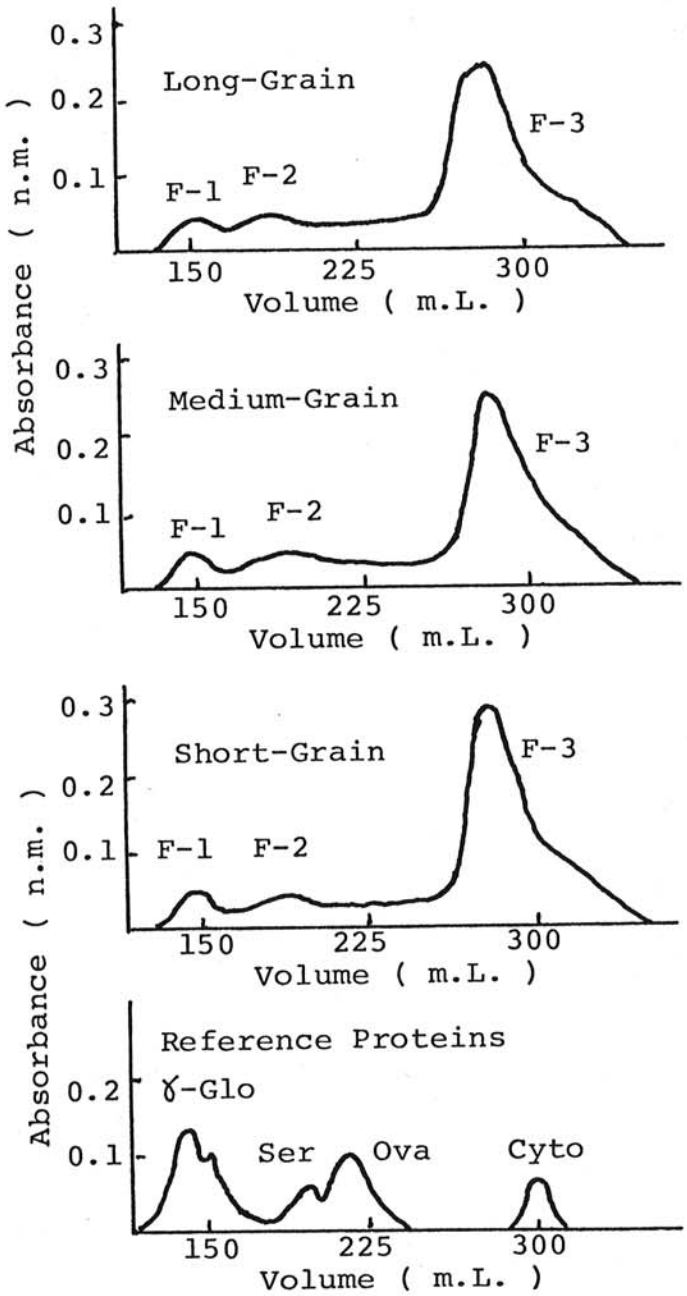

Fig. 2. Gel filtration patterns of rice globulins. Reference proteins. **γ-Glo** = γ-globulins (mol wt 160,000), **Ser** = serum albumin (mol wt 67,000), **Ova** = ovalbumin (mol wt 45,000), **Cyto** = cytochrome C (mol wt 12,400), **F** = fraction.

TABLE I
Molecular Weight of Proteins Fractionated by Gel Filtration

	Long	Medium	Short
Albumins			
F-1	195,000	191,000	166,000
F-2	49,000	51,000	48,000
F-3	26,000	24,000	24,000
F-4	10,000	10,000	11,000
Globulins			
F-1	129,000	135,000	135,000
F-2	76,000	69,000	73,000
F-3	17,000	16,000	16,000

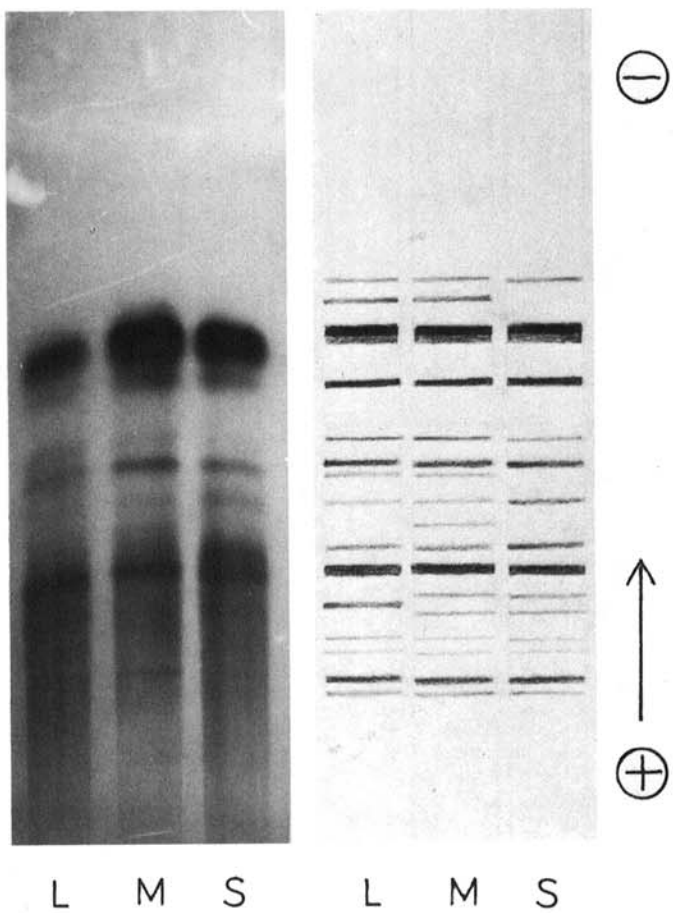

Fig. 3. Photograph and sketched pattern of acidic starch-gel electrophoresis of rice albumins. Aluminum lactate/lactic acid buffer with 7.5 *M* urea, pH 3.1. **L** = long grain, **M** = medium grain, **S** = short grain.

and then destained in water, glycerol, and acetic acid (85:10:5). Conditions of electrophoresis were 14 mA per plate at 270 V for 16 hr for acidic gels and 8 mA per plate at 255–310 V for 39–45 hr for alkaline gels.

TABLE II
Amino Acid Composition[a]

	Albumins			Globulins		
	Long	Medium	Short	Long	Medium	Short
Alanine	70.2	66.2	71.9	46.4	44.0	49.9
Arginine	77.4	86.9	84.0	150.0	160.5	159.9
Aspartic acid	83.6	81.8	90.4	42.9	43.1	43.2
Cystine	29.1	34.9	26.4	35.0	35.5	36.2
Glutamic acid	101.4	97.0	105.0	211.1	224.7	199.1
Glycine	58.3	58.9	61.0	54.3	52.3	56.6
Histidine	26.4	20.9	27.2	5.9	3.9	14.0
Isoleucine	29.7	26.1	31.7	20.9	19.4	22.6
Leucine	69.6	63.7	72.5	65.2	64.0	71.4
Lysine	44.0	32.7	43.3	11.0	7.0	14.2
Methionine	22.9	24.7	16.8	41.1	41.9	45.5
Phenylalanine	34.6	31.3	35.8	33.7	32.5	35.1
Proline	54.6	57.4	58.3	46.8	51.7	56.9
Serine	38.1	33.9	38.6	64.8	67.1	47.5
Threonine	37.8	32.5	38.6	22.0	20.5	19.1
Tyrosine	43.2	43.7	43.4	62.7	64.6	62.0
Valine	66.7	63.5	68.7	43.8	40.0	48.5
Ammonia	10.6	10.6	9.9	10.4	15.6	17.6
Total	898.2	866.7	924.4	968.5	988.3	999.3

[a] Milligrams per gram.

Amino Acid Composition

Approximately 10 mg of protein was hydrolyzed with hydrochloric acid solution. After hydrolysis, the hydrochloric acid was removed, and the hydrolysate was dissolved in 10 ml of citric acid (pH 2.2). Aliquots of the solution were applied to the amino acid autoanalyzer, a Hitachi model KLA-3B.

RESULTS

Gel Filtration

Results of gel filtration on Sephadex G-100 are shown in Figs. 1 and 2. Albumins of the three rice varieties showed similar patterns, with each giving four fractions. However, quantitative ratios of the fractions were slightly different. Globulins of the three rice varieties also gave four similar fractions, although once again the quantitative ratios were not the same. The shoulder on the third peak (Fig. 2) was considered to be the fourth globulin fraction.

Molecular Weight Estimation

Molecular weights of albumin and globulin fractions are shown in Table I. Elution volumes of the gel filtration of rice protein fractions were plotted on the calibration curves of the reference proteins. Molecular weights of albumin fractions gave values from 10,000 to 200,000. Molecular weights of globulin fractions gave values from 16,000 to 130,000. Because the fourth globulin fraction was not well separated, its molecular weight could not be established.

Starch-Gel Electrophoresis

Starch-gel electrophoresis patterns are shown in Figs. 3–6. Both albumins and globulins gave many bands on acidic or alkaline starch gels. Although patterns of the three rice varieties were

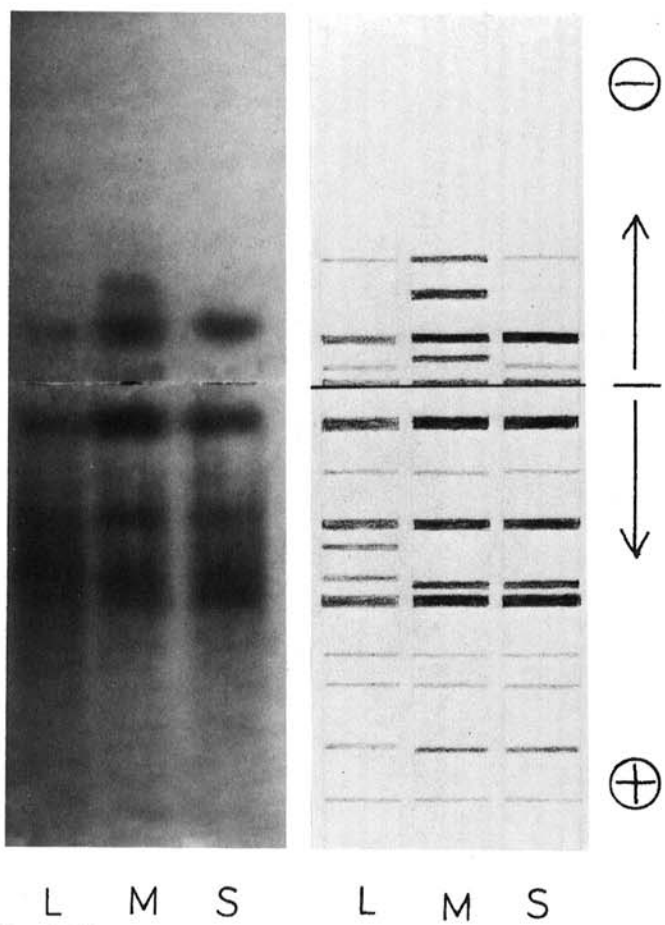

Fig. 4. Photograph and sketched pattern of alkaline starch-gel electrophoresis of rice albumins. Tris-citric acid buffer with 7.5*M* urea, pH 8.9. **L** = long grain, **M** = medium grain, **S** = short grain.

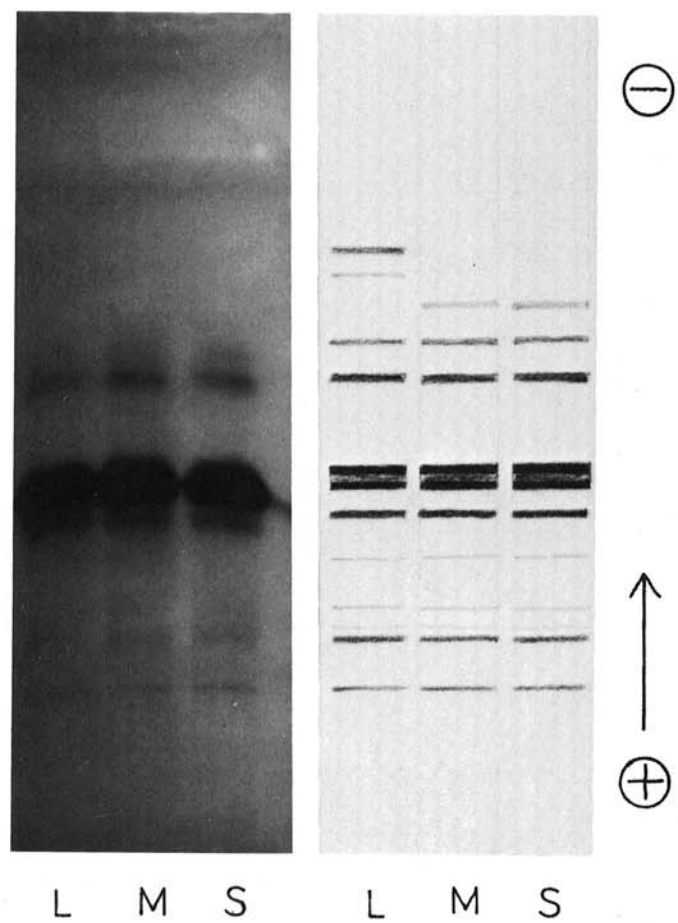

Fig. 5. Photograph and sketched pattern of acidic starch-gel electrophoresis of rice globulins. Aluminum lactate/lactic acid buffer with 7.5*M* urea, pH 3.1. **L** = long grain, **M** = medium grain, **S** = short grain.

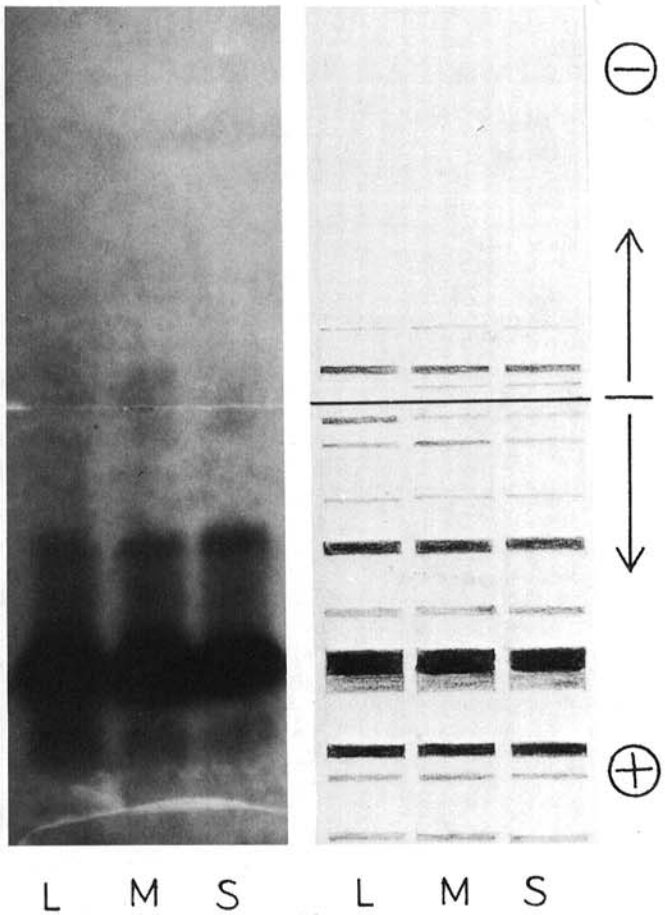

Fig 6. Photograph and sketched pattern of alkaline starch-gel electrophoresis of rice globulins. Tris-citric acid buffer with 7.5 *M* urea, pH 8.9, **L** = long grain, **M** = medium grain, **S** = short grain.

similar for both albumins and globulins, clear differences existed. Sometimes one band was found in only one variety, and sometimes one band was absent only in one variety. Even if the positions of bands were the same for the three varieties, darkness of the bands (concentration of proteins) was very often different.

Amino Acid Composition

Amino acid compositions are shown in Table II. Amino acid values agreed with the results of our previous research (Iwasaki et al 1975) and with the results of Houston et al (1969), Cagampang et al (1976), and Perdon and Juliano (1978). The results show that in comparison with other proteins of milled rice, albumins contained more lysine and aspartic acid, and globulins contained more arginine, glutamic acid, cystine, and methionine and less lysine, threonine, and isoleucine.

The amino acids patterns among the three varieties were quite similar for both albumins and globulins except for the following: long-grain albumin showed a low arginine content; medium-grain albumin showed a high content of cystine; short-grain albumin, a low content of methionine; medium-grain globulin, a low content of lysine; and short-grain globulin, a high content of histidine and low content of serine.

DISCUSSION

Both albumins and globulins were fractionated into four fractions by gel filtration. By starch-gel electrophoresis with urea, they were separated into about 20 bands. Albumins and globulins of rice endosperm are highly heterogeneous and have complicated structures. They appear to be composed of many subunits, as described in our previous articles (Iwasaki et al 1972, 1975).

Summarizing the results of gel filtration, starch-gel electrophoresis, and amino acid analysis, albumin and globulin proteins of the three types of rice showed many similarities and some clear differences.

Doekes (1968) tried to classify wheat varieties into several groups by examining characteristics of starch-gel electrophoretic patterns of wheat gliadin. Feillet and Monod (1972) also mentioned the differences in starch-gel electrophoretic patterns of rice proteins among different varieties. Our experiments support the idea of classifying rice varieties into groups by differences in electrophoretic patterns of proteins.

Although molecular weight estimation by gel filtration showed fairly good agreement with that of our previous paper (Iwasaki et al 1975), there were some differences. Molecular weight estimation by gel filtration does not always give good reproducibility. A combination of more than two methods will be necessary for obtaining more precise values.

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