

Antigenic Properties of Albumin, Globulin, and Protein Concentrate Fractions from Rice Bran^{1,2}

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ABSTRACT

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Alkali-extracted rice bran protein was tested for antigenic properties by development of polyclonal antibodies in the rabbit. Animals were also inoculated with soy protein isolate and nonfat dry milk. Titers, determined by enzyme-linked immunosorbent assay, for the three groups were compared. Soy protein isolate evoked the highest class G immunoglobulin (IgG) antibody titer (21,845,000), followed by rice bran protein (655,000), and nonfat dry milk (27,000). Rice bran proteins consisted of 29% albumins, 23% globulins, and 20% glutelins. Albumins and glob-

ulins made up 69% of the extractable protein. Albumins, globulins, and two rice bran protein concentrates, produced by extraction in alkali, followed by isoelectric precipitation or spray-drying, were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and reacted against the antibody protein polyclonal antibodies in Western blot analysis. A small amount of highly antigenic protein (~16 kDa) was found in the alkali-extracted rice bran protein concentrates, but it was absent in the albumin and globulin protein fractions.

Rice has historically been perceived as hypoallergenic. It is the only grain allowed on an extensive elimination diet for allergy testing (Van Hooser and Crawford 1989). Rice is gluten-free and is often recommended as a replacement for wheat or corn. While rare in the United States and in populations consuming Western-style diets, allergies to rice are not uncommon in Asia where rice is a staple food. A substance is antigenic when it induces the formation of an antibody of any class. A substance is allergenic when it elicits a Type I hypersensitivity reaction that specifically involves class E immunoglobulins (IgE).

The literature contains little information concerning the antigenic and allergenic properties of rice bran, although there has been some research dealing primarily with endosperm proteins. Matsuda et al (1988) identified an allergenic protein of ~16 kDa that positively reacted against serum IgE from three rice-allergic individuals. When measured by single immunodiffusion, the allergenic protein was 5 and 0.2% of total extractable protein from endosperm and bran, respectively. In later work, Matsuda et al (1991) extracted three immunoreactive proteins from rice endosperm: 16, 15.5, and 14 kDa, with pI 6.3, 6.5, and 7.9, respectively. Matsuda et al (1988, 1991) demonstrated that, in the case of rice proteins, antigenicity was an indicator of allergenicity. Watanabe et al (1990) treated milled rice with a surfactant and enzyme mixture to prepare a hypoallergenic milled rice grain, but did not investigate bran.

Possible hypoallergenic properties make rice bran a potential protein source for an infant formula for babies who are allergic to milk or soy. The antigenic properties of rice bran protein concentrates (RBPC) produced in our laboratory were investigated. To evaluate the antigenicity of RBPC as compared to that of milk and soy proteins, polyclonal IgG antibodies were developed in rabbits, and serum titers were compared. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate proteins by molecular weight. The bands were transferred to a membrane for Western blot analysis against the polyclonal antibodies.

MATERIALS AND METHODS

Preparation of Rice Bran Protein Concentrates and Fractions

Full-fat rice bran was donated by Riceland Foods, Stuttgart, AR. Bran was defatted before fractionation or preparation of protein concentrates by mixing with petroleum ether using a 1:5 bran-to-solvent ratio. The procedure was repeated, the bran was air-dried for 24 hr under a vent hood, and then reground.

Two RBPC were prepared for SDS-PAGE and Western blot analysis by methodologies that might have commercial application. Defatted rice bran protein was extracted using a pH-stat type system where the water-bran suspension was kept at constant pH 9.0 for 1 hr by addition of NaOH. The mixture was centrifuged at 3,500 × g for 10 min, and the supernatant was strained through glass wool. The proteins were isoelectrically precipitated by adjusting the supernatant to pH 4.5 with 1N HCl; the mixture was centrifuged, neutralized, and freeze-dried. The alkali extract was also neutralized and spray-dried in an Anhydro spray dryer (Copenhagen, Denmark) (no isoelectric precipitation).

Albumin (water-soluble) and globulin (salt-soluble) protein fractions were prepared for SDS-PAGE and Western blotting by sequentially extracting defatted bran with water and 0.5M NaCl. The two fractions were then strained through glass wool, dialyzed against water, and lyophilized.

Protein Classification

Proteins were fractionated into four classes (albumin, globulin, prolamin, and glutelin) using a modified Osborne method of stepwise extraction based on protein solubility in different solvents. Albumins were extracted with water, globulins with 0.5M NaCl, prolamins with 60% ethanol, and glutelins with 0.5% SDS + 0.6% 2-mercaptoethanol in a 0.1M borate buffer (pH 10). Flour to solvent ratio was 1:20, and each fraction was obtained from three washes at 60, 30, and 15 min. Nitrogen in each fraction, and in the defatted bran, was determined by the micro Kjeldahl method.

Polyclonal Antibodies

Polyclonal antibodies against nonfat dry milk (NFDM) (Carnation, Los Angeles, CA) proteins, soy protein isolate (SPI) PP710 (donated by Protein Technologies International, St. Louis, MO), and alkali-extracted rice bran proteins were developed in nine New Zealand white rabbits of the same age, obtained from the University of Arkansas rabbitry. Each animal acted as its own control, and preinoculum serum was drawn from the marginal ear vein. The rabbits were randomly assigned to an RBPC, NFDM, or SPI test group.

The rice bran proteins used for polyclonal antibody production were obtained from rice bran that had been defatted by shaking

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with petroleum ether for 1 hr at room temperature. The defatted bran was stirred in 20 volumes of 0.1N NaOH for 1 hr at room temperature, followed by centrifugation for 10 min at 10,000 × g. The supernatant, which preliminary work showed to contain ~70% of the total protein (including albumin, globulin, and glutelin proteins), was dialyzed (Spectra por membrane-1, Fisher Scientific, Atlanta, GA) against four changes of water at 4°C for 48 hr and lyophilized. Protein content of the alkali-extracted proteins, SPI, and NFDM was determined by micro Kjeldahl nitrogen assay (method 46-13, AACC 1983).

The animals were subcutaneously injected with 10 mg of protein in Freund's complete adjuvant (Sigma Chemical Co., St. Louis, MO) followed by four inoculations of the same protein in Freund's incomplete adjuvant (Sigma) at seven-day intervals. Two weeks after the last injection, they were bled from the marginal ear vein, and the serum was collected.

Enzyme-Linked Immunosorbent Assay Protocol

The serum collected from the rabbits was diluted in twofold increments and tested for IgG titer by enzyme-linked immunosorbent assay technique as described by Bhunia et al (1991). Bound antibodies were detected using horseradish peroxidase-conjugated goat antirabbit IgG (Sigma). Absorbance at 492 nm was determined using a microplate reader (Dynatech MN 600, Alexandria, VA) with the last positive reading indicative of titer (machine was zeroed on highest absorbance for blank control).

SDS-PAGE

After solubilization to a concentration of 150 µg per 20 µl of sample buffer, the isoelectrically precipitated alkali-extracted RBPC, spray-dried alkali-extracted RBPC, and the dialyzed albumins and globulins were separated by SDS-PAGE using a 10–20% acrylamide gradient gel with a 4% stacking gel. The gel was electrophoresed at 13 mamp for 1 hr and then at 26 mamp for ~5 hr. After electrophoresis, the gel was cut in half. The side with the molecular weight markers was stained with Coomassie Blue dye, while the mirror image side was used for Western blot analysis.

Western Blot Analysis

The protein bands were transferred to Immobilon PVDF transfer membrane in a Bio-Rad mini transblot Protein II apparatus (Richmond, CA) at 200 mamp for 1 hr according to the instruction manual (Bio-Rad 1991). After transfer, the membrane was washed with 10 mM phosphate-buffered saline-Tween 20 (PBS-T20) for 10 min and blocked with a 1% bovine serum albumin solution at 37°C for 1 hr. The washing step was repeated twice, and rabbit

serum diluted 1:250 in 10 mM PBS was added. After incubation for 1 hr at 37°C and a repetition of the washing step, goat anti-rabbit IgG conjugated to alkaline phosphatase diluted 1:1,000 in 10 mM PBS was added. The membrane was incubated again for 1 hr and washed three more times. Color was developed by adding substrate of 33 µl 5-bromo-4-chloro-3-indoylphosphate and 66 µl nitro blue tetrazolium in 10 ml of alkaline phosphate buffer (pH 9.5).

RESULTS AND DISCUSSION

Rice Bran Protein Solubility Classes

Of the extractable protein in rice bran, the albumins made up the largest proportion, followed by globulins and glutelins (Table I). There was nearly no prolamins in the bran. This general trend was previously reported by Betschart et al (1977), though proportionally more globulin protein was found in the present study. The amount (74%) of the total bran protein extracted by the various solvents was close to the amount extracted using alkali (about 70%). The albumins and globulins made up 69% of the extractable protein in rice bran. Due to the ease of extraction of these proteins and their good nutritional quality, they may have potential as a usable protein concentrate. Antigenic properties of albumins and globulins were therefore further examined.

Polyclonal Antibody Titers

All nine rabbits had preinoculum IgG titers of <2,560. Mean postinoculum titers and ranges are listed in Table II. The SPI was highly antigenic and had the greatest variability in titer. Rice bran protein elicited a much lower titer than did SPI, though it elicited a higher titer than did NFDM. Polyclonal antibody production against the rice bran protein antigens was sufficient for further identification of antigenic proteins by immunoblotting. The relatively low titer for the milk was unexpected because milk is known to be allergenic in some humans. Perhaps this can be explained in that NFDM was readily dispersed in water, whereas the RBPC and SPI were held in suspension. The larger particles of the RBPC and SPI may have been better engulfed by antigen-presenting cells and carried to lymphocytes for IgG production. Low antibody titer in New Zealand white rabbits inoculated with milk protein was found previously (R. Knight, *personal communication*).

SDS-PAGE and Western Blot Analysis

The albumins, globulins, and isoelectrically precipitated and spray-dried alkali-extracted RBPC were separated by SDS-PAGE and reacted against the rabbit serum containing IgG antialkali-extracted rice bran protein polyclonal antibodies. The SDS-PAGE gel and Western blot are illustrated in Figures 1 and 2, respectively. A highly antigenic protein of ~16 kDa (see arrows) was a very minor protein band on the stained gel (Fig. 1). The antigenic protein was not found in either the albumin or globulin protein fractions.

Other antigenic protein bands were identified in the Western blot (Fig. 2). A protein of ~25 kDa in the alkali-extracted RBPC was antigenic to a much lesser degree than was the 16-kDa protein. Slightly antigenic bands of 40 and 50 kDa were found in all rice bran protein preparations. However, the immunoresponse was quite low relative to the high concentration of these proteins in the fractions. The presence of an antigenic protein does not necessarily mean that protein will elicit an allergic response involving IgE. Allergenicity of the antigenic proteins was not tested in this study because serum from rice-allergic individuals was unavailable.

The highly antigenic 16-kDa protein found in the RBPC is presumably the same protein that Matsuda et al (1988) identified as allergenic in rice-allergic Japanese patients. They found it to be primarily a rice endosperm protein. Matsuda et al (1991) indicated that the 16-kDa protein was either an albumin or globulin protein. However, in our study it was not soluble in either water or 0.5M NaCl.

TABLE I
Solubility Classes of Protein^a from Defatted Rice Bran

Protein Class	% of Flour (dwb) ^b	% of Total Protein
Albumin	5.4 (0.3)	28.8
Globulin	4.2 (0.2)	22.5
Prolamin	0.4 (0.4)	0.0
Glutelin	3.8 (0.1)	20.3
Nonextractable	5.0 (0.1)	26.7
Recovery	100.5	

^aN × 5.95.

^bRice bran contained 18.7% protein (dwb). Mean (standard deviation in parentheses).

TABLE II
Serum Class G Immunoglobulin Titers as Measured by Enzyme-Linked Immunosorbent Assay of Rabbits Inoculated with Alkali-Extracted Rice Bran Protein, Nonfat Dry Milk Protein, and Soy Protein Isolate

Rabbits	Inoculum	Mean Titer	Range
1–3	Rice bran protein	655,000	None ^a
4–6	Milk protein	27,000	2.0–4.1 (× 10 ⁴)
7–9	Soy protein isolate	21,845,000	2.6–41.9 (× 10 ⁶)

^aTiters from the three rabbits were the same.

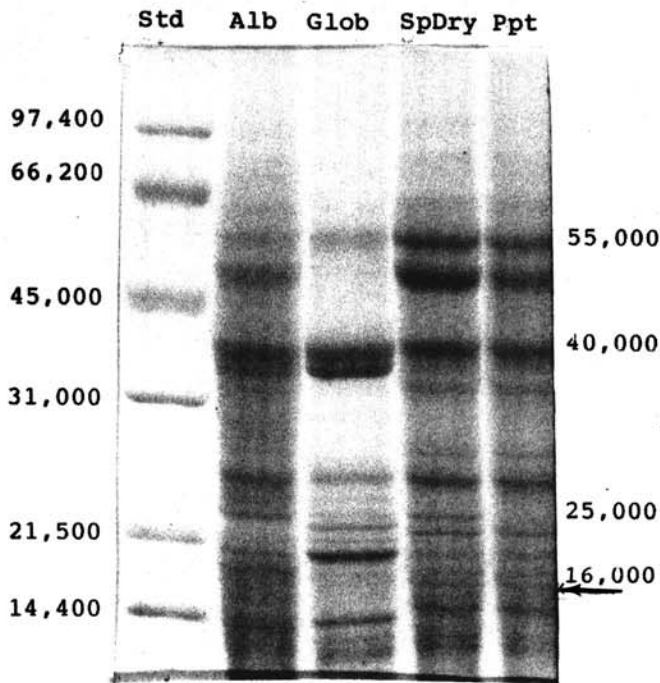


Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns of albumins (Alb), globulins (Glob), and spray-dried (SpDry) and isoelectric precipitated (Ppt) alkali-extracted rice bran protein concentrates. Std = molecular weight markers.

CONCLUSIONS

Rice bran protein was tested for antigenic properties by development of polyclonal antibodies in the rabbit. Titers were compared with serum from animals inoculated against SPI and NFD. SPI evoked the highest IgG antibody titer, followed by RBPC, then NFD. A highly antigenic protein of ~16 kDa was found in small amounts in RBPC obtained by alkali extraction. It appears possible to develop a process that excludes the antigenic protein by extraction in water at neutral pH. Further research is needed to determine protein functionality characteristics and validate the usefulness of RBPC for infant formula.

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Ppt SpDry Glob Alb

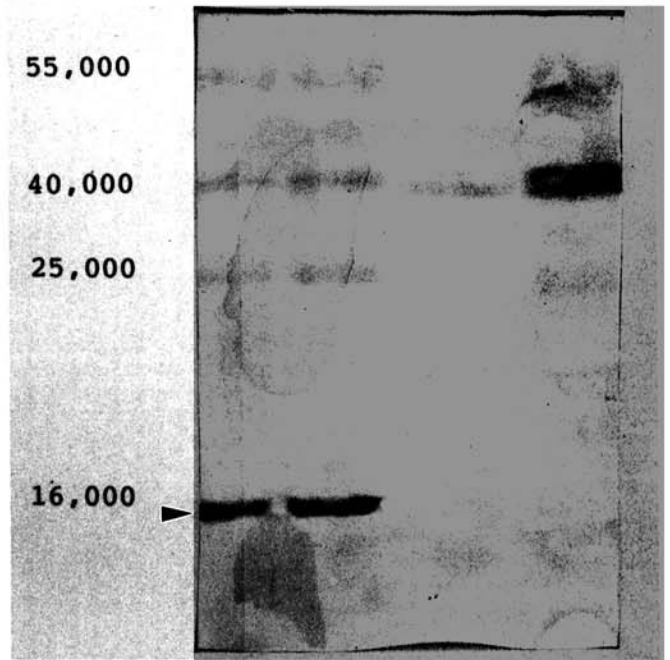


Fig. 2. Western blot analysis using polyclonal antibodies to rice bran proteins of albumins (Alb), globulins (Glob), and spray-dried (SpDry) and isoelectric precipitated (Ppt) alkali-extracted rice bran protein concentrates.

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