

Allergenicity of Major Component Proteins of Soybean

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Abstract. Allergenic activities of major component proteins of soybean 11S-, 7S- and 2S-globulins were examined by the radioallergosorbent test (RAST) and by RAST inhibition experiments with the sera from soybean-sensitive patients. The reactivity with specific IgE antibody was found in all fractions. There was considerable cross-reactivity among three fractions, and 2S-globulin had the highest allergen potency to inhibit the RAST against 11S-, 7S- and 2S-globulin. When the fractions were heat-treated at 80, 100 and 120 °C for 30 min, the RAST reactivity of 2S-globulin heated at 80 °C for 30 min was slightly enhanced, while that of others was reduced to 39-75% of that of the native globulin. IgE antibody could be produced in BALB/c mice only with one of the three fractions, i. e. 2S-globulin, and the produced IgE antibody lacked reactivity with 11S- and 7S-globulin.

Introduction

A variety of adverse reactions to soybeans, which are widely used in food products including flavoring agents, has been reported [4, 5, 12, 14]. However, there are few reports on the allergenicity of component proteins [17]. Soybean proteins are broadly divided into two fractions, globulin and whey fractions, by acid precipitation at pH 4.5. The globulin fraction is a major component, comprising 85% of the total protein, and consists of four subcomponents with approximate sedimentation constants of 15, 11, 7 and 2S [20]. On the other hand, the whey fraction is minor in quantity (10%), containing different kinds of biologi-

cally active substances such as hemagglutinin, a trypsin inhibitor, urease etc. [20]. In this paper, we report on the allergenicity of the component proteins of the soybean globulin fraction by the radioallergosorbent test (RAST) and IgE antibody production in BALB/c mice.

Materials and Methods

Subjects

Sera were obtained from 5 atopic patients, 4-12 years of age, who showed immediate skin reaction (wheal diameter > 5 mm) to intradermal injection of 1 µg of soluble soybean extract. 2 cases (case S. O. and case F. F.) had repeated episodes of asthma when exposed to soybean flour

and the other 3 cases had exacerbation of eczema by ingestion of soybean products. The presence of specific IgE antibody in the sera was confirmed by the passive transfer test with diluted serum of 1:20. The sera from 5 nonatopic subjects without hypersensitivity to soybeans were pooled to serve as a control for background-binding in the RAST assays. To exclude the possibility of nonspecific adsorption of IgE antibody by soybean protein, the serum was obtained from an asthmatic patient with high levels of IgE antibodies against ovalbumin (OA) and mite allergen (*Dermatophagoides farinae*).

Preparation of Soybean Globulins

Turunoko variety soybeans grown in Japan were crushed and defatted with ethyl ether at 35 °C for 24 h. The defatted flakes were extracted with water (1:5 ratio of flakes to water) for 1 h at room temperature, and the extract was centrifuged at 20,000 rpm for 20 min. Then the supernatant was acidified to pH 4.5 with 1 N CH₃COOH, the resultant precipitate dialyzed against phosphate buffer (0.0325 M K₂HPO₄, 0.0026 M KH₂PO₄, 0.4 M NaCl and 0.01 M 2-mercaptoethanol, pH 7.6) for 2 days, and this was employed as the whole globulin fraction. The 11S-globulin was prepared from cold insoluble fraction which was precipitated at 4 °C according to the method described by Koshiyama [9]. The 7S- and 2S-globulins were isolated from the whole globulin fraction by the methods described by Koshiyama [10] and Catsimpoalas and Ekenstam [1], respectively.

Ultracentrifugal Analysis

Sedimentation analysis was carried out at room temperature with a Hitachi 880 centrifuge using phosphate buffer containing 0.4 M NaCl and 0.01 M 2-mercaptoethanol (2-ME), pH 7.6, as the solvent.

Heat Treatment

10 mg of each sample solution were incubated at 80 °C, boiled at 100 °C or autoclaved at 120 °C each for 30 min.

Radioallergosorbent Test

10 mg of whole, 11S-, 7S- or 2S-globulin and heat-treated globulins were separately dissolved in 1 ml of 0.1 M NaHCO₃ buffer containing 0.5 M NaCl. Each sample solution was incubated with

20 pieces of CNBr-activated paper discs overnight with occasional agitations according to the method described by Ceska *et al.* [3]. After three washing with the buffer, the native radicals were blocked with 0.1 M β -ethanolamine. RAST was performed as described by Wide *et al.* [24] with some modification, i.e., each paper disc was incubated with 50 μ l of test serum on the horizontal shaker overnight. After thorough washing, the discs were incubated overnight with 50 μ l ¹²⁵I-labeled anti-IgE obtained from Phadebas RAST kits (Pharmacia, Uppsala, Sweden). After washing to remove the excess anti-IgE, the radioactivity bound to the disc was measured by a gammacounter. RAST d-terminations were done in duplicate and the result was expressed as mean percent of binding $\pm$ standard deviation (SD) calculated by dividing the counts bound to the disc by total counts added to the tube $\times$ 100.

RAST Inhibition

100 μ l of serum were incubated overnight with 100 μ l of various concentrations of the inhibitors in buffer. The appropriate antigen disc was added to 50 μ l of the serum-inhibitor mixture and then subjected to the subsequent RAST. Percent inhibition was calculated by dividing the radioactivity bound to the disc in RAST with inhibitor by that without inhibitor. A semilogarithmic paper was used for plotting percentage inhibition against the dose of inhibitor added to 100 μ l of serum.

Allergization and Measurement of IgE Antibody of Mice

The inbred strain of BALB/c female mice were obtained from the laboratory animal center of Gunma University (Maebashi, Japan). All mice were allergized at 8–12 weeks of age. 5 mice per group were intraperitoneally injected with either 1 or 10 μ g of antigen mixed with 4 mg of Al(OH)₃ gel, which was prepared according to the method described by Levine and Vaz [11]. 4 weeks later the mice were boosted using the same dose of antigen-alum mixture. The mice were bled from orbital venous plexus 7 days after the second injection. The levels of IgE antibodies in pools of sera from 5 mice per group were determined by passive cutaneous anaphylaxis (PCA) reactions using Sprague-Dawley rats [13]. Briefly, 25- μ l aliquots of serum from each mouse in a given group were

pooled and serially twofold diluted in saline. 50 μ l of each serum dilution were injected intradermally into the dorsal skin surface of the test rats. Reactions were elicited 2–4 h after sensitization by intravenous injection of 1.0 mg of antigen in 1.0 ml of 1.5% Evans blue dye. Reactions were read and recorded as the reciprocal of the highest dilution of the serum evoking threshold PCA reactivity (5 mm in diameter). Sera which failed to elicit detectable PCA reactions were arbitrarily assigned a value of < 5 .

Results

Criteria of Purities of the Isolated Samples

The globulin fraction precipitated at pH 4.5 consisted of four components with approximate sedimentation values of 15, 11, 7 and 2S when analyzed in the ultracentrifuge at 58,200 rpm in phosphate buffer containing 0.4 M NaCl and 0.01 M 2-ME (fig. 1). Each of the purified preparations of 11S-, 7S- and 2S-globulins gave a single peak (fig. 2). The sedimentation coefficients of the respective components were 11.45, 7.09 and 1.88. Nothing is known about the physicochemical property of 15S-globulin except that it contains 11S-dimer [25]. Therefore, we excluded it from the present experiment.

RAST and RAST Inhibition

The results of RAST using whole globulin and its components are shown in table I.

The sera with specific IgE antibodies against the whole globulin fraction exhibited also specificity to 11S-, 7S- and 2S-components. In order to evaluate the allergenic cross-reactivities among 11S-, 7S- and 2S-globulins and the allergen potencies of them, a RAST inhibition experiment was performed with the pooled serum. Figures 3a–c show the actual inhibition patterns of three inhibitors to 11S-, 7S- and 2S-coupled RAST, respectively. The amount of inhibitor required for 50% inhibition of 11S RAST was about 1 μ g in 2S-globulin,

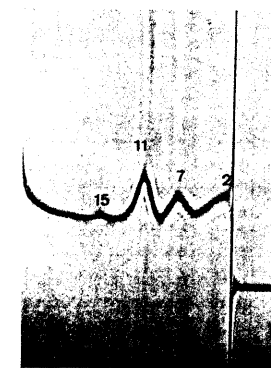


Fig. 1. Ultracentrifugal pattern of the whole globulin fraction in phosphate buffer containing 0.4 M NaCl and 0.01 M 2-ME (numbers = sedimentation values). Photograph was taken after 48 min of sedimentation at 58,200 rpm.



Fig. 2. Ultracentrifugal patterns of 11S-globulin (a), 7S-globulin (b) and 2S-globulin (c): Photographs were taken after 25 min of sedimentation at 58,700 rpm for a and b, and after 20 min for c.

Table I. RAST with soybean globulin and its components

Patient	Binding (%) of IgE antibody to soybean			
	whole globulin	11S-globulin	7S-globulin	2S-globulin
K.O.	13.9 ± 0.1	8.6 ± 0.2	9.8 ± 0	12.2 ± 0
H.F.	6.4 ± 0.1	2.7 ± 0	2.1 ± 0.1	3.3 ± 0.1
O.O.	4.9 ± 0.2	3.6 ± 0.1	3.4 ± 0.1	3.1 ± 0
K.S.	8.3 ± 0.1	7.6 ± 0.2	6.9 ± 0.1	6.4 ± 0.1
M.S.	6.7 ± 0.2	5.7 ± 0	4.6 ± 0	4.8 ± 0.1
Control	0.8 ± 0	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0

The results were expressed as average binding (%) ± SD of duplicate determinations. As a control, pooled serum from 5 normal individuals without hypersensitivity to soybean was simultaneously examined.

Table II. Inability of 2S-globulin to adsorb IgE antibody against OA and mite allergen

RAST disc	binding of RAST ¹ , %	
	without inhibitor	with inhibitor
Ovalbumin	6.2 ± 0.1	6.6 ± 0.3
Mite (<i>D. farinae</i>)	4.3 ± 0.2	4.4 ± 0.1

¹ The serum was obtained from an asthmatic patient with high levels of IgE antibody against OA and mite allergen.

10 µg in 7S-globulin and 50 µg in 11S-globulin (fig. 3a). A similar pattern of inhibition was found in 7S- and 11S-coupled RAST (fig. 3b, c). In addition, 50 µg of 2S-globulin could inhibit 90% of the RAST against 11S-, 7S- and 2S-globulin. This indicates that allergenic cross-reactivities exist among the three components and that 2S-globulin is the most potent allergen of soybean globulins. To exclude the possibility of nonspecific adsorption of IgE antibody by 2S-globulin, the serum with high levels of IgE antibodies against OA and mite allergen was previously incubated with 2S-globulin and assayed by RAST. As

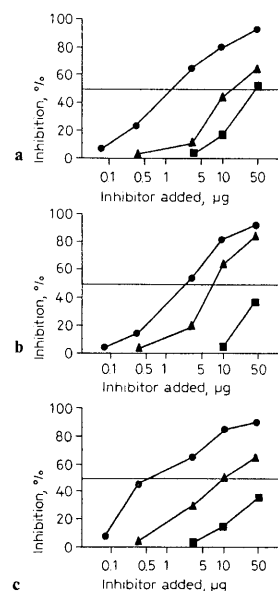


Fig. 3. Inhibition of RAST for serum H.O. Inhibitors added: ■ = 11S-globulin; ▲ = 7S-globulin; ● = 2S-globulin. **a** Inhibition of binding to 11S-globulin. **b** Inhibition of binding to 7S-globulin. **c** Inhibition of binding to 2S-globulin.

Table III. RAST with heat-treated allergens

Allergen disc	Patient	Reduction of RAST-binding (%) by heating at		
		80 °C, 30 min	100 °C, 30 min	120 °C, 30 min
Whole globulin	K.O.	76 ¹	82	82
	M.S.	66	67	77
11S-globulin	K.O.	42	41	39
	M.S.	66	48	60
7S-globulin	K.O.	67	59	41
	M.S.	75	69	71
2S-globulin	K.O.	116	85	83
	M.S.	133	109	73

¹ %: RAST-binding of heat-treated allergen × 100/divided by RAST-binding of native allergen.

Table IV. IgE antibody response to soybean globulins

Antigen	Doses, µg	PCA titer
Whole globulin	10	< 5 ¹
	1	< 5
11S-globulin	10	< 5
	1	< 5
7S-globulin	10	< 5
	1	< 5
2S-globulin	10	160
	1	160

¹ Reciprocal of titer.

shown in table II, the 2S-globulin could not inhibit the OA- and mite-coupled RAST.

Heating Experiment

To investigate the effect of heating or boiling on allergenicity of whole globulin and three components, the samples were in-

cubated under various conditions, i.e., at 80, 100 and 120 °C for 30 min. The results of RAST are shown in table III. RAST-binding of the 2S-globulin treated at 80 °C for 30 min was slightly enhanced up to 116–133% of that of the native one, while that of others was reduced to 49–85% of that of native ones following the treatment under these conditions.

IgE Antibody Production in BALB/c Mice

The allergenicity of whole globulin, 11S-, 7S- and 2S-components was investigated by IgE antibody production in BALB/c mice. As shown in table IV, allergization with either 1 or 10 µg of 2S-globulin resulted in the formation of IgE antibody (1:160), whereas the animals injected with whole globulin, 11S- or 7S-components failed to form any detectable amount of IgE antibody. The specificities of the IgE antibody formed were investigated by the PCA system. As shown in table V, whole globulin

Table V. Specificities of mice IgE antibody against 2S-globulin

Sensitizing serum	Challenged antigen			
	whole globulin	11S-globulin	7S-globulin	2S-globulin
Anti-2S reagenic serum	160 ¹	<5	<5	160

¹ Reciprocal of PCA titer.

and 2S-globulin were able to elicit the PCA reaction in rats sensitized with anti-2S-reagenic sera, while the 11S- and 7S-component failed to do so.

Discussion

The present paper demonstrated that a considerable cross-reactivity exists among the three component proteins of the soybean globulin fraction and that the 2S-component has the highest allergen potency to inhibit the RAST to three component proteins. Nonspecific adsorption of IgE antibody by 2S-globulin was excluded by the fact that 2S-globulin cannot adsorb the specific IgE antibodies against OA or mite allergen. *Vaintraub* [22] reported that 2S-globulin consists of two distinctive molecules with sedimentation constants of 2.3 and 2.8 S and with molecular weights of 18,200 and 32,600 daltons, respectively [19, 20], but their amino acid compositions are quite similar [19]. *Catsimpoolas* [2] reported that denatured 2S-globulin in the solvent system of phenol-acetic acid-0.2 M ME (2:1:1, w/v/v) containing urea of a final concentration of 5 M showed a disc electrophoretically single band as well as a native one, but the band was distinct from the dissociated subunits corresponding to 11S- and 7S-globulins in the same solvent system. Another investiga-

tor reported that the three component proteins of the soybean had quite similar amino acid compositions [20]. These findings suggest the possibility that the allergenic determinants of soybean globulin may be independent of subunits of quaternary structure, and depend on the linear sequence of amino acid residues.

2S-globulin, which is the most active allergenic component of the soybean globulin fraction, was relatively more heat-stable than other fractions. *Virture and Wittig* [23] reported that the most active allergen of egg white was ovomucoid with a molecular weight of about 27,000 daltons and that the exposure to heat of 75–100 °C resulted in no obvious allergenic change of ovomucoid. Heat stability may be of importance for the relevance of higher allergenicity of food proteins.

Allergization with 2S-globulin resulted in IgE antibody response in mice, but allergization with either 7S-, 11S- or the whole globulin fraction failed to do so. It is known that an inbred strain of mice which is a good responder to one antigen may be a poor responder to another antigen [6]. *Levine and Vaz* [11] reported that DBA/1 and C57BL/6 strains of mice were good responders to OA, but poor responders to ovomucoid. Thus, the reagenic antibody response to 2S-globulin and unresponsiveness to 11S- and 7S-globulin may depend on the geneti-

cal factors of BALB/c mice controlling their immune response. In addition, our observation that the whole globulin fraction containing the 2S-component cannot induce IgE antibody response might be explained by antigenic competition [15] or the presence of inhibitory factor(s) in the whole globulin fraction.

The IgE antibodies induced by 2S-globulin in mice lacked reactivity with 7S- and 11S-globulins, whereas our RAST result demonstrated that 2S-globulin has the same allergenic determinants expressed in the 7S- and 11S-globulin. It is well known that most antigens are thymus-dependent and require two determinants: haptenic (allergenic) determinants react with B cells, carrier determinants react with T cells [8, 16, 18]. Evidence suggesting that atopic patients had an increased number of IgE B cells in affected tissues or organs was reported [21]. Based on these considerations, it is conceivable that an increased number of IgE B cells in atopic individuals can react with a variety of allergenic determinants of allergen molecule(s), while IgE B cells of mice which lack atopic predisposition react with a limited number of allergenic determinants of allergen. It was also reported that infection of rats with *Nippostrongylus brasiliensis* caused nonspecific potentiation of unrelated IgE antibody responses to antigens such as OA and keyhole limpet hemocyanin [7]. It may be of interest to investigate whether allergenic cross-reactivity among soybean component proteins is also manifest in these helminth-infected animals.

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