

Recombinant Soybean Protein β -Conglycinin α' -Subunit Expression and Induced Hypersensitivity Reaction in Rats

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Key Words

Recombinant protein • β -Conglycinin α' -subunit • Hypersensitivity reaction • IgE • Mast cells • Histamine

Abstract

Background: The major storage protein in soybean seed is β -conglycinin and this protein has been identified as being responsible for food-allergic reactions in several species. However, the mechanism through which β -conglycinin induces an allergic reaction has not yet been elucidated. In addition, assessing the antigenic activity of β -conglycinin by studying the activity of a subunit has rarely been conducted. Therefore, the objective of the present study was to characterize the antigenic specificity of the β -conglycinin α' -subunit. **Methods:** We established an *Escherichia coli* expression system to obtain β -conglycinin α' -subunit. The fusion proteins were then used in a rat model to induce a hypersensitive reaction. Immunoblotting, IgE and IgG1 level, histamine release, and passive cutaneous anaphylaxis reactions and intestinal histology were tested to assess the allergenic activity of the β -conglycinin α' -subunit. **Results:** Pure β -conglycinin α' -subunit was obtained by expression in *E. coli*. The recombinant proteins were shown to have the same biological activity as the natural β -conglycinin α' -subunit using immunoblotting analysis. Both the IgE and IgG1 level in serum and the histamine concentration in the intestine were increased while passive cutaneous anaphylactic reactions were induced in Brown Norway rats by intragastric gavage

with the α' -subunit. Histamine release of mast cells was also elevated in vitro. **Conclusions:** Our results indicate that the β -conglycinin α' -subunit possesses an intrinsic immune-stimulating capacity and that it can induce an allergic reaction. Moreover, this study showed that β -conglycinin α' -subunit-induced anaphylaxis is IgE mediated, and mast cell degranulation and histamine release are associated with anaphylactic symptoms.

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Introduction

Soybean protein is widely utilized by the food industry as a relatively inexpensive source of dietary protein [1]. However, allergies to soybean protein are common among infants [2] and newborn farm animals such as neonatal pigs [3, 4] and young cattle [5]. β -Conglycinin and glycinin are the two major components of soybean, which account for about 30 and 40% of the total seed protein, respectively [6, 7]. It is composed of three subunits: α (~67 kDa), α' (~71 kDa), and β (~50 kDa) [8, 9]. The α -subunit has been identified as an allergenic protein in the soybean β -conglycinin fraction [10].

Several methods have been developed for the isolation of the entire β -conglycinin oligomer [11, 12], but there are few reports available on the purification of workable amounts of a single β -conglycinin subunit. In addition, most research conducted on the allergenic effects of soy-

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bean β -conglycinin has been based on the use of antibodies raised against the entire β -conglycinin molecule [13–16] whereas the role of the individual subunits of β -conglycinin in allergic reactions has not been extensively studied.

Our research group has been active in studying the biological action of soybean protein both in validated rat and swine models [17–19]. In previous studies, we used a rat model to demonstrate that β -conglycinin-specific immunoglobulin E (IgE) and immediate hypersensitivity reactions can be induced by intragastric administration of purified soybean protein β -conglycinin. To further study the role of β -conglycinin in allergic reactions, a technique was developed to obtain large quantities of the pure α' -subunit of β -conglycinin using an *Escherichia coli* expression system. We then investigated the allergenic activity of the α' -subunit using a rat model.

Methods

Extraction of Soybean Protein Total RNA

Total RNA was extracted from the leaves of young soybean plants (*Glycine max* L. Merr.) following the procedures described by Gasic et al. [20]. The presence of RNA was verified by running samples on 1% agarose gels (Biowest, Miami, Fla., USA). The extracted RNA was then stored at -80°C until needed.

Cloning the Gene of the α' -Subunit and Construction of Expression Plasmid

Primers were constructed on the basis of the conserved sequence for the soybean protein β -conglycinin α' -subunit gene according to the GenBank Nucleotide Sequence Database with the accession No. P11827. The primers synthesized were forward 5'-GGAATTCCATATGTTTGGCATTGCGTATTGGGA-AAAGCA-3' (underlining indicates *Eco*RI sites) and reverse 5'-CCGGAATTCTCAGTAAAAAGCCCTCAAAATTGAA-3' (underlining indicates *Nde*I sites). The entire target coding region was amplified by using a polymerase chain reaction (PCR). The resulting PCR products were digested using *Nde*I at 37°C for 4 h, and then further digested using *Eco*RI for 3 h. The digested PCR product was inserted into the *Nde*I and *Eco*RI sites of pET28a vector (Novagen, USA) under the control of the T7 promoter. The DNA sequencing was analyzed using the former T7 promoter primer and the posterior primer 5'-AAGAGTCCCTGCAG-GAACCA-3' (Applied Biosystems, Foster City, Calif., USA). DNAMAN (Lynnon Biosoft, version 5.2.2) was used to analyze the sequence data and to identify potential homologies.

*Expression and Purification of Recombinant α' -Subunit in *E. coli**

E. coli cells (BL21), harboring a corresponding expression plasmid, were cultured, diluted 100-fold with fresh prewarmed Luria-Bertani medium supplemented with 50 $\mu\text{g}/\text{ml}$ kanamycin, and cultured at 37°C until absorbance at 600 nm reached 0.6–1.0. Isopropyl-1-thio- β -D-galactopyranoside (Amresco, USA) was added

to a final concentration of 1 mM to induce the expression of the α' -subunit at 30°C for 5 h. Twelve percent sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was done. The concentration of protein in the gel was determined by the Syngene Gel Density Image Analysis System (GeneTools Analysis software, version 3.03.03). The method of purification followed the procedure described by Huang and Liu [21] with some modifications. Briefly, after sonication, the pellet was resuspended in lysis buffer containing 8 M urea. The supernatant was loaded on a nickel-nitrilotriacetic acid sepharose affinity column. Eluted fractions were dialyzed in lysis buffer containing step gradients of urea (6, 4, 2, 0 mM). Each step lasted 12 h at 4°C . The purified protein was characterized using 12% SDS-PAGE as described above.

Preparation of the Complete β -Conglycinin Extract

The complete β -conglycinin protein was extracted and purified from the defatted soybean flour as described elsewhere [11]. The sources of the extracted β -conglycinin and the soybean total RNA were identical.

Protein Measurement

The content of the recombinant protein and complete β -conglycinin extract were determined by a BCA Protein Assay Kit (Pierce, Rockford, Ill., USA) according to the manufacturer's instructions.

Production of Mice Polyclonal Antibodies

The method followed the procedures described by Petrucci et al. [14] with slight modifications. Briefly, 8 BALB/c female mice, 3 weeks of age, were immunized by intraperitoneal injections with 100 μg of the complete β -conglycinin extract in 100 μl of phosphate-buffered saline (PBS) mixed with the same volume of Freund's adjuvant (Sigma, St. Louis, Mo., USA).

Immunoblot Analysis

SDS-PAGE was performed as described above. Electrophoretic transfer and immunoblotting on nitrocellulose paper were performed by the procedures of Beardslee et al. [22] with some modifications. Briefly, the transfer to a polyvinylidene difluoride membrane (Amersham, Piscataway, Calif., USA) was performed for 1 h at 360 mA and 4°C . Membranes were incubated in pooled polyclonal antibody of β -conglycinin diluted 1:500 and goat anti-mouse IgG-HRP (Zymed, USA) diluted 1:5,000 in Tris-buffered saline at room temperature. The membranes were placed in diaminobenzidine visualization solution until the bands had adequate intensity following the manufacturer's instructions (Sigma).

Animals, Diets and Experimental Protocol

Eighteen female Brown Norway rats, 4–6 weeks of age, were individually housed in polycarbonate cages on soft wood granulate. The cages were located in a room with controlled lighting (12 h/day), temperature (23°C) and relative humidity (65%). Drinking water and feed were available ad libitum. Rats were fed soy-free, laboratory-produced diets based on casein as the only source of dietary protein [23]. The experiment was conducted at the Ministry of Agriculture Feed Safety and Bio-Availability Evaluation Center (Beijing, PR China) and all procedures were approved by the China Agricultural University Animal Care and Use Committee.

Rats were randomly divided into three groups ($n = 6$) according to body weight. Rats were sensitized by means of intragastric gavage with purified β -conglycinin α' -subunit [5 or 25 mg protein/ml PBS (pH 7.4)] on day 0, 7 and 14 (1 ml/animal). Rats were challenged by intragastric gavage with β -conglycinin α' -subunit (5 or 25 mg) on day 21 and 28. The control group was gavaged with 1 ml PBS using the same schedule [24, 25]. Blood was obtained at weekly intervals from the tail vein before the challenge. Serum was collected and stored at -80°C . On day 28, after 1 h of the second challenge, all rats were sacrificed by cervical dislocation. Segments of the intestine were removed from the duodenum (5 cm from the pylorus), jejunum (5 cm posterior to the yolk stalk), and ileum (2 cm anterior to the ileocecal valve). The intestinal segments were thoroughly flushed with saline to remove the intestinal contents and immediately immersed in liquid nitrogen and preserved at -80°C for later histamine analysis.

Light Microscopy

A 2-cm segment middle duodenum was removed immediately after slaughter, rinsed with saline, fixed by immersion in cold 10% formalin and processed by standard histological techniques. Samples were sectioned at 6- μm thickness and stained with hematoxylin and eosin. The morphological structure of duodenum was observed by using light microscopy at $400\times$.

Development of Diarrhea

In this study, to assess the development of diarrhea, the consistency of rat feces was classified as described by Buenau et al. [26]. Briefly, rat feces were classified as follows: 1 = normal; 2 = soft but retaining their form; 3 = loose, not retaining their form, or 4 = watery. Rats with feces classified as 3 or 4 on ≥ 2 consecutive days were regarded as cases of diarrhea. Diarrhea occurrence (%) = diarrhea of rats with each treatment/6 $\times$ 100.

Measurement of Serum Antibodies

The total serum IgE antibodies were measured by sandwich enzyme-linked immunosorbent assay kits (ELISA kit; Bethyl, USA) according to the instructions of the manufacturer. Levels of β -conglycinin α' -subunit-specific IgE and IgG1 antibodies were measured by indirect sandwich ELISA following the procedures described by Brandt et al. [27] with slight modifications. Briefly, 96-well microplates (BioFil, Canada) were coated with 20 $\mu\text{g}/\text{ml}$ β -conglycinin α' -subunit in carbonate buffer (0.05 M, pH 9.6), and then serial dilutions of serum obtained from experimental rats (100 $\mu\text{l}/\text{well}$) were added and incubated. Thereafter, the mouse anti-mouse IgE and IgG1 antibodies (Sigma) and a tetramethylbenzidine liquid substrate (100 $\mu\text{l}/\text{well}$) containing H_2O_2 (Sigma) were used to measure the absorbance at 450 nm using an ELISA reader (Tecan, Austria).

Preparation and Sensitization of Rat Peritoneal Mast Cells

Eight normal Brown Norway rats, 3 weeks of age, were obtained from the same source as those used for the *in vivo* study. Rat peritoneal mast cells were isolated following the procedures described by Hong et al. [28]. Purified mast cells were resuspended in Tyrode's buffer A (containing calcium), and mast cell suspensions (1×10^6 cells/ml) were divided into three treatments supplemented with sensitized rat serum (5 or 25 mg α' -subunit groups) or normal rat serum (control group) in 12-well microplates (BioFil, Canada). The mast cells were preincubated with rat

serum in 5% CO_2 at 37°C for 2 h, and then incubated for 1 h with 100 $\mu\text{g}/\text{ml}$ β -conglycinin α' -subunit. The reaction was stopped by cooling the plates on ice. The histamine content of the cell pellet and the supernatant was measured. Residual histamine in the cell pellet was released by disrupting the cells in perchloric acid and centrifugation at 400 g for 5 min at 4°C . Histamine release ratios were calculated using the following equation: histamine release ratios (%) = [histamine content in the supernatant/(histamine content in the supernatant + histamine content in the cell pellet)] $\times$ 100.

Determination of Histamine

High-performance liquid chromatography was used to determine histamine contents in intestine, cell pellet, and supernatant as described by Veciana-Nogues et al. [29]. Briefly, the samples passed through a cation exchange column (Discovery, C18 5 μm , 4.6×250 mm, Sigma) maintained at 40°C and were eluted by a gradient elution program at a flow rate of 1 ml/min. Postcolumn derivatization with *o*-phthaldialdehyde (Sigma) was carried out and a fluorescence detector (Waters 2475 Multi λ Fluorescence Detector; Waters, Ireland) was used at 340 nm excitation and 450 nm emission. The data were obtained using a data integrator (Waters TM 600, Waters, Ireland). The amounts of histamine in the samples were quantified using known amounts of standards (Sigma).

Passive Cutaneous Anaphylaxis Testing

IgE-dependent cutaneous reactions were performed following the procedures described by Li et al. [30]. Briefly, 8 Brown Norway rats, 3 weeks of age, were used. The immune serum was obtained from the rats treated with 5 or 25 mg/ml β -conglycinin α' -subunit on day 28. The rats were intradermally injected with 50 μl of heated (56°C for 3 h) or unheated sera (1:5 dilution) into their abdomens. Control rats were injected an equal amount of diluted naive serum. Twenty-four hours later, rats were injected intravenously with 100 μl of 0.5% Evans blue dye, immediately followed by an intradermal injection of 50 μl of β -conglycinin α' -subunit (10 mg/ml). A reaction was scored as positive if the bluing of the skin at the injection sites was greater than 5 mm in diameter in any direction.

Statistical Analysis

The results are reported as the mean $\pm$ SEM. Data from the passive cutaneous anaphylaxis (PCA) reaction were analyzed statistically with an unpaired *t* test, using the SPSS statistical software package for Windows (Version 13.0, SPSS, Chicago, Ill., USA). Data on the concentrations of IgE and the histamine release ratios and histamine concentrations in the intestine were analyzed statistically using a one-way ANOVA. *p* values < 0.05 were considered to be significant.

Results

Construction, Expression and Purification of Recombinant β -Conglycinin α' -Subunit

In this study, high-quality soybean RNA was obtained. The extracted RNA was used for synthesizing cDNA and

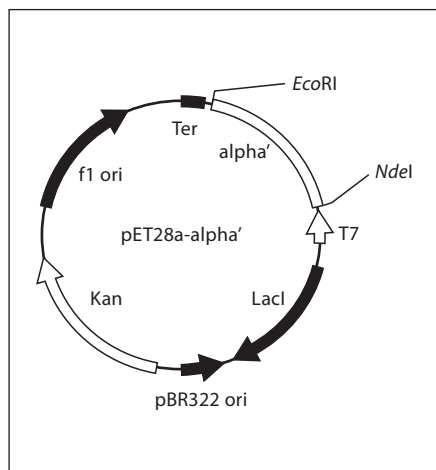


Fig. 1. Map of recombinant expression plasmid pET28a- α' . The construction of the plasmid is detailed in the Methods section. α' = β -Conglycinin α' -subunit.

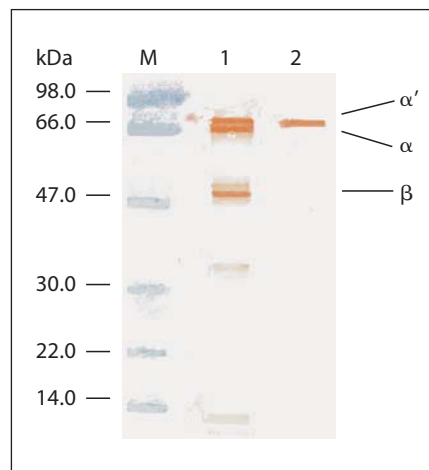


Fig. 3. Identification of α' -subunit by immunoblotting. Western blotting of lane M = Prestained molecular weight markers; lane 1 = extracted β -conglycinin; lane 2 = purified recombinant α' -subunit by Ni-NTA affinity column.

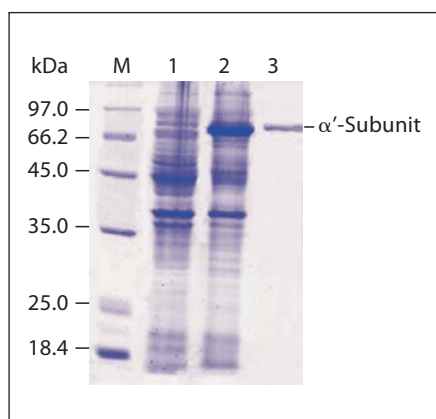


Fig. 2. SDS-PAGE analysis of the recombinant β -conglycinin α' -subunit. The protein was analyzed on a 12% SDS-PAGE. 0.5 μ g of protein sample was applied. Lane M = Low-molecular-weight standard; lanes 1 and 2 = lysates of *E. coli* cells harboring pET28a- α' -subunit before and after IPTG induction; lane 3 = purified recombinant α' -subunit by Ni-NTA affinity column.

the cDNA-coding DNA for the β -conglycinin α' -subunit was produced by RT-PCR. The entire coding region was inserted into the *EcoRI* and *NdeI* sites of expression vector pET28a to produce the recombinant protein in the *E. coli* strain (fig. 1).

DNA sequencing of this constructed plasmid confirmed that the desired inserts were successfully cloned into a pET28a expression vector and also that the con-

structs were in the correct reading frame. The nucleotide sequence data of pET28a- α' -subunit appeared in the EMBL/GenBank Nucleotide Sequence Database with the accession No. DQ845137. The nucleotide sequence length was 1,803-bp and encoded a $\sim$ 70 kDa protein. Homology comparison indicated that the sequence of recombinant α' -subunit and the sequence reported for β -conglycinin α' -subunit (P11827) were similar. The amino acid sequence showed 95.9% similarity.

The recombinant plasmid pET28a- α' -subunit was transformed into *E. coli* BL21 and induced by adding isopropyl-1-thio- β -D-galactopyranoside. After being lysed, the fusion protein was exhibited by SDS-PAGE and found to be identical to the reported protein (fig. 2), which had a molecular weight of $\sim$ 70 kDa. The SDS-PAGE results indicated that the α' -subunit expressed in *E. coli* was at a level of around 20% of the total bacterial protein (fig. 2, lane 2). Recombinant protein was rapidly purified by a Ni-NTA affinity chromatography. The purity of the final β -conglycinin α' -subunit fusion protein was more than 99% (fig. 2, lane 3) (GeneTools Analysis Software, version 3.03.03). The purified recombinant protein was produced at a level of 100 mg/l of culture.

Identification of α' -Subunit by Immunoblotting

Immunoblotting analysis was initially performed using the polyclonal antibody against the complete β -conglycinin molecule to identify the specificity of the anti-

Fig. 4. Effects of different levels of β -conglycinin α' -subunit (5 and 25 mg) on the morphological structure of duodenum in rat. HE, 10 $\times$ 40. **a** Control group rat. **b** 5 mg α' -subunit rat. **c** 25 mg α' -subunit rat.

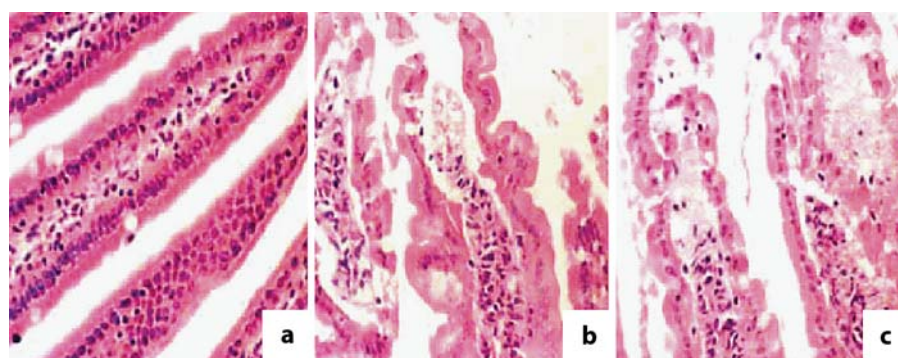


Fig. 5. Total serum IgE levels (**a**) and α' -subunit-specific IgE and IgG1 antibody (Ab) levels (**b**) were determined on day 28. The data of α' -subunit-specific serum IgE levels are presented as the mean 2 log IgE titer of 6 rats per group. Letters above the columns indicate significant difference ($p < 0.001$) between the treatments.

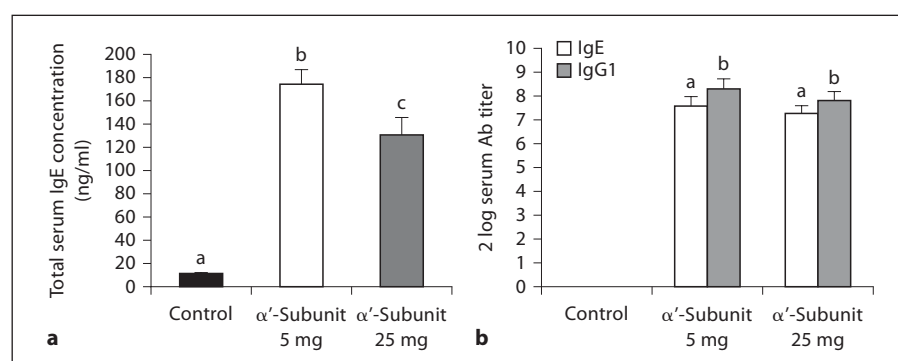


Table 1. Effects of different levels of β -conglycinin α' -subunit (5 and 25 mg) on diarrhea and histamine release in Brown Norway rats

	Treatments			SEM	p value
	control	α' -subunit, 5 mg	α' -subunit, 25 mg		
Diarrhea occurrence, %	0	66.67	50.00	–	–
Histamine release ratio, %	33.51 ^a	68.83 ^b	61.10 ^c	1.27	<0.001

Diarrhea occurrence was assessed during the whole experimental phase. Superscripts indicate a significant difference between the treatments ($p < 0.001$).

body recognition reaction of the recombinant protein. Figure 3 shows that the recombinant α' -subunit protein (lane 2) was specifically bound to the antibody. Recognizing bands of α' -subunit were located at ~ 70 kDa in the blotting membrane. This shows that the recombinant α' -subunit has the same immunochemical activity as the native α' -subunit of β -conglycinin.

Morphological Structure of Duodenum and Diarrhea Occurrence

To investigate the effect of β -conglycinin α' -subunit on the small intestine after intragastric sensitization, the

morphological structure of the duodenum was observed. Figure 1 shows that the intestines were damaged in the 5-mg (fig. 4b) and 25-mg (fig. 4c) α' -subunit groups compared with the control group (fig. 4a). Focal necrosis and exfoliation appeared in the intestinal villus. Intragastric gavage with β -conglycinin α' -subunit induced ($p < 0.01$) diarrhea in rats (table 1).

Total Serum IgE and α' -Subunit-Specific IgE and IgG1 Antibody Levels

To further explore the role of β -conglycinin α' -subunit in allergic reactions, the total serum IgE and α' -sub-

Table 2. Histamine concentrations in the intestine of rats fed diets containing β -conglycinin α' -subunit

	Treatments			SEM	p value
	control	α' -subunit 5 mg	α' -subunit 25 mg		
Histamine content, $\mu\text{g/g}$					
Duodenum	5.02 ^a	7.01 ^b	7.48 ^b	0.33	0.026
Jejunum	5.25 ^a	7.68 ^b	7.16 ^b	0.40	0.021
Ileum	5.11 ^a	7.77 ^b	6.79 ^b	0.33	0.048

Superscripts indicate a significant difference between the treatments ($p < 0.05$).

Table 3. PCA reactions after injection of heated or unheated immune serum

Immunization	Heat (56°C for 3 h)	Diameter (mean $\pm$ SD) mm	Positive reaction	
			n/total	%
Naive	–	1.25 $\pm$ 0.16	0/8	0
α' -Subunit 5 mg/ml	–	6.23 $\pm$ 0.76 ^a	8/8	100
	+	1.18 $\pm$ 0.15	0/8	0
α' -Subunit 25 mg/ml	–	6.04 $\pm$ 0.71 ^a	7/8	87.5
	+	1.22 $\pm$ 0.16	0/8	0

PCA reactions were assessed as described in the Methods section. The immune serum was obtained from rats sensitized by 5 or 25 mg/ml α' -subunit. Superscripts indicate a significant difference between the heated immune serum and naive serum.

unit-specific antibodies levels were determined after intragastric β -conglycinin α' -subunit sensitization and challenge. Total IgE (fig. 5) and α' -subunit-specific IgE and IgG1 antibody (fig. 5) levels were significantly higher ($p < 0.001$) in the two groups treated with the α' -subunit compared with the control group. Also, there was a difference ($p < 0.01$) between the low-dose α' -subunit (5 mg/rat) and the high-dose α' -subunit (25 mg/rat) in the total IgE level (fig. 5). The control treatment did not induce a specific IgE antibody response.

Histamine Release Ratio in Cultured Mast Cells *in vitro*

Treatment with β -conglycinin α' -subunit levels of 5 and 25 mg significantly induced ($p < 0.01$) the peritoneal mast cells to release histamine at higher levels than in the control group (table 1). There was a difference ($p < 0.05$)

between the high-dose α' -subunit (25 mg/rat) and the low-dose α' -subunit (5 mg/rat).

Histamine Contents in the Small Intestine of Rats

The effects of dietary supplementation with β -conglycinin α' -subunit on histamine contents are presented in table 2. Histamine concentrations in the duodenum, jejunum and ileal of rats fed the α' -subunits (5 and 25 mg) were higher ($p < 0.05$) than in the control group.

PCA Reactions

PCA reaction was used to follow the induction of β -conglycinin α' -subunit hypersensitivity which was responsible for IgE. The PCA reactions were induced ($p < 0.01$) by the injection of serum from rats fed β -conglycinin α' -subunit, but not in normal rats. The reactions were eliminated by heated (56°C for 3 h) immune serum (table 3).

Discussion

The major soybean seed storage proteins 7S β -conglycinin and 11S glycinin have been investigated as a source of allergy in humans and animals for many years [2, 4, 15]. Sensitive human and animals may experience allergic symptoms including asthma, atopic response, and gastric distress [31]. β -Conglycinin elicits stronger gut hypersensitivity reactions than glycinin [5]. In a previous study, we demonstrated that purified β -conglycinin can induce allergic reactions in Brown Norway rats in a similar manner as that reported for mice, piglets and calves by other investigators [2, 14, 16].

Until recently it has been difficult to obtain large amounts of pure individual subunits. This limited research into the action of individual β -conglycinin subunits in causing allergic reactions. Major β -conglycinin subunits have not been studied further while the sequences of these subunits have been revealed in recent years [9].

In this study, the β -conglycinin α' -subunit was expressed in *E. coli*. The result of SDS-PAGE showed that the purified recombinant had a molecular weight of ~ 70 kDa. This agrees with the report of Maruyama et al. [9]. The preparation of abundant amounts of pure β -conglycinin α' -subunit allowed us to accurately study its allergenic activity *in vivo*. In addition, the immunoblot analysis showed that the recombinant protein could be recognized by β -conglycinin-sensitive serum. The data showed that the recombinant α' -subunit has the same immuno-

chemical activity as naive β -conglycinin α' -subunit. Thereby, the recombinant α' -subunit was considered suitable for assessing the allergenicity of β -conglycinin α' -subunit in a rat model. In previous studies, assessing the allergenic activity of individual β -conglycinin subunits was limited in immunoreactivity by using β -conglycinin-sensitive serum [15, 24, 32]. The α -subunit of β -conglycinin was reported as an allergenic protein recognized by IgE antibodies of soybean-sensitive patients [10]. By using monoclonal antibodies raised against the complete β -conglycinin molecule, the extension region of α - and α' -subunits was identified as the main immunogenic region [32]. In our study, the rat model was used to further verify that α' -subunit of β -conglycinin was an allergenic protein [33]. Supplementation with α' -subunit in rat diets significantly induced the allergic reactions in Brown Norway rats. These results represent the first in vivo confirmation that the α -subunit is not solely responsible for the allergic reaction.

Previous studies have shown that IgE and IgG1 are involved in the immediate allergic reaction in murines [25, 34]. In the present study, the total serum IgE and α' -subunit-specific IgE and IgG1 levels increased after oral sensitization. The intestinal damage and diarrhea could have been caused by antibody-mediated immune reaction to antigen [18, 27]. Damaged villus and the occurrence of diarrhea in our study coincided with a rise in α' -subunit-specific IgE and IgG1 antibody levels. The PCA reactions were generated by the immune serum. The PCA reactions were eliminated by heat inactivation of immune serum, thereby strongly implicating IgE as a reagenic antibody in this model. Our data demonstrated that IgE played a crucial role in regulating the hypersensitivity reactions in our rat sensitization model.

Most food allergies are mediated by antigen-specific IgE and are characteristic of type I allergic reactions. IgE plays a critical role in this allergic reaction [35]. Individuals with hypersensitivity reactions to food antigens generate a specific humoral immune response which produces a large number of IgE antibodies [36, 37]. These IgE are quickly bound by high-affinity IgE receptors mainly on the surface of mast cells in the gastrointestinal tract and other tissues, and prime them to release histamine and other inflammatory mediators, leading to the wide variety of allergic symptoms [38]. Consistent with these reports, the results of our present study showed that histamine release and the histamine concentration in the intestine increased with increasing levels of IgE. These data are also in accordance with our previous finding of allergic reactions to whole β -conglycinin in the Brown

Norway rat model. Moreover, we found that the low dose of α' -subunits (5 mg) induced higher IgE levels. This phenomenon may be due to the fact that the high antigen dose (25 mg) induced tolerance. Similar results have been reported in other food antigen studies [30, 39].

As described above, mast cells play an important role in immediate hypersensitivity reactions [38]. Mast cells can be activated by antigen-specific IgE, resulting in a release of histamine, leukotriene, 5-hydroxytryptamine, tumor necrosis factor- α and lipid mediators upon antigenic stimulation [40, 41]. These active mediators induce the shrinkage of smooth muscle and the change of intestinal motility mediated by allergen [42]. In addition, mast cells are the major source of histamine in the gastrointestinal tract and other tissues, and the most frequently assayed mast cell product following exposure to antigen is histamine [37]. In this study, with the supplementation of different levels of the α' -subunit, histamine release from mast cells was greatly increased. The data indicated that the mast cells were activated by antigen-specific IgE after α' -subunit sensitization.

Histamine has important pathological and physiological functions, and thus is considered as a cellular messenger in the gastrointestinal tract [43]. Histamine may be one important mediator, as humans and animals with food allergy show elevated histamine release from the gastric and intestinal mucosa following antigen challenge [44]. An increase in histamine levels is widely known as one symptom of mast cell degranulation [27]. The occurrence of a food-allergic reaction depends on the release of histamine and other inflammatory mediators after IgE antibodies on the surface of the mast cells bind to the allergen. Thereby, the status of allergic reactions can be better reflected by determining the histamine release rather than merely determining IgE levels. In this study, to further assess the allergenic activity of β -conglycinin α' -subunit, we used the peritoneal mast cells sensitized by the α' -subunit to determine the histamine release in vitro. We found that dietary supplementation with β -conglycinin α' -subunit increased histamine release from mast cells in vitro. The histamine release was related to the level of α' -subunit-specific IgE in serum. As the serum IgE increased, histamine release also increased. These results are consistent with those reported by other researchers [36, 38]. In addition, the histamine concentrations were increased in the small intestinal mucosa of Brown Norway rats with the supplementation of α' -subunit in diets. Histamine brings into play secretory, contractile and immune effects on the intestine. Brandt et al. [27] reported that mast cells and histamine were respon-

sible for allergen-induced diarrhea. Consistent with this report, we found that dietary supplementation with α' -subunit induced diarrhea in some rats.

In summary, this study has established an *E. coli* expression system to obtain abundant amounts of pure β -conglycinin α' -subunit. We provide for the first time direct in vivo evidence of its allergenic activity in a rat model by α' -subunit-induced hypersensitive reaction using intragastric sensitization and challenge. β -Conglycinin

α' -subunit-induced anaphylaxis is IgE-mediated and mast cell degranulation and histamine release are associated with anaphylactic symptoms.

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