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All Three Subunits of Soybean β-Conglycinin Are Potential Food Allergens

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Soybeans are recognized as one of the "big 8" food allergens. IgE antibodies from soybean-sensitive patients recognize more than 15 soybean proteins. Among these proteins only the α-subunit of β-conglycinin, but not the highly homologous α′- and β-subunits, has been shown to be a major allergenic protein. The objective of this study was to examine if the α′- and β-subunits of β-conglycinin can also serve as potential allergens. Immunoblot analysis using sera collected from soybean-allergic patients revealed the presence of IgE antibodies that recognized several soy proteins including 72, 70, 52, 34, and 21 kDa proteins. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) analysis of trypsin-digested 72, 70, and 52 kDa proteins indicated that these proteins were the α′-, α-, and β-subunits of β-conglycinin, respectively. Additionally, purified α′-, α-, and β-subunits of β-conglycinin were recognized by IgE antibodies present in the soybean-allergic patients. The IgE reactivity to the β-subunit of β-conglycinin was not abolished when this glycoprotein was either deglycosylated using glycosidases or expressed as a recombinant protein in *Escherichia coli*. The results suggest that in addition to the previously recognized α-subunit of β-conglycinin, the α′- and β-subunits of β-conglycinin also are potential food allergens.

KEYWORDS: Allergen; β-conglycinin; IgE reactivity; glycoprotein; soybeans

INTRODUCTION

Soybeans are the major source of protein and vegetable oil in the world. The United States is the leading producer and exporter of soybean. It is estimated that the farm value of soybean produced in 2006/2007 was $20.4 billion, which is second only to corn (http://www.ers.usda.gov/Briefing/SoybeansOilCrops/). Soybean meal is extensively used for livestock because it contains high-quality protein with an amino acid composition complementary to corn protein. Furthermore, it is relatively inexpensive compared to other protein sources used for livestock. In addition to their extensive use in the animal industry, humans also are increasingly consuming soybeans and soy products. The use of soybeans in human food is widespread in the Orient and Southeast Asia. For several centuries, ancient populations have recognized the multiple health benefits of soybean. Epidemiological studies have shown populations that consume soybeans and soy products have lower incidences of cancer, heart diseases, and other chronic illness (*1, 2*). The use of soybeans in United States has steadily increased recently primarily due to these well-documented health benefits.

Soybean has been recognized by regulatory authorities as one of the "big 8" food allergens (*3, 4*). Soybean allergy is most common among children nourished with soybean-based infant formula (*5*). However, the incidence of soybean allergies, while lower in adults, is still estimated at about 0.5% of the U.S. population (*6, 7*). Allergic symptoms to soybean include skin, gastrointestinal, and respiratory reactions and in some cases anaphylaxis (*6, 7*). Several soybean proteins have been identified as allergens in the molecular mass range from 7 to 71 kDa (*8*). Prominent among them are the Gly m Bd 30 K, Gly m Bd 28 K, Gly m Bd 60 K, and G1 and G2 glycinin proteins (*9−13*). Of the 33 identified IgE-binding allergenic proteins in soybeans, only a limited number of proteins are responsible for a majority of adverse reactions to soybeans (*8*).

Soybean 7S storage protein, the β-conglycinin, has been identified as one of the most allergenic proteins (*14, 15*). Ogawa et al. (*12*) showed about 25% of soybean-sensitive Japanese patients with atopic dermatitis developed IgE antibodies against the α-subunit of β-conglycinin. β-Conglycinin is composed of three subunits, namely, α′ (76 kDa), α (72 kDa), and β (52 kDa) (*16*). These subunits share extensive amino acid sequence homology (*17*). Despite the close similarity among these subunits, the IgE antibodies from soybean-sensitive patients failed to cross-react against the α′- and β-subunits (*18*). Here,

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it was demonstrated that only the α-subunit of β-conglycinin, but not the other two subunits, could elicit IgE antibodies in soybean sensitive-patients with atopic dermatitis. In this study, we employed immunoblot analysis to detect soy proteins that elicited IgE antibodies in patients who were allergic to soybeans. Sera from soybean-allergic patients contained IgE antibodies that strongly reacted against several proteins including 72, 70, 52, 34, and 21 kDa proteins. The 72, 70, and 52 kDa proteins have been identified as the α′-, α-, and β-subunits of β-conglycinin. Our results demonstrate that all three subunits of β-conglycinin can elicit IgE antibody in soybean-sensitive patients.

MATERIALS AND METHODS

Human Sera. Sera from five adult soybean-allergic patients with high soybean-specific IgE levels were obtained from PlasmaLab International (Everett, WA). Sera from two soy-sensitive patients that contained IgE antibodies against soy proteins were a generous gift from Dr. Michael Zeece at the University of Nebraska. Sera from individuals with no known history of soybean allergic reactions were used as negative controls.

Seed Protein Extraction and SDS-PAGE. Dry soybean cultivar Williams 82 seeds were ground to a fine powder by mortar and pestle. Ground seed powder (10 mg) was extracted with 1.0 mL of a solution containing 125 mM Tris-HCl buffer, pH 6.8, 4% sodium dodecyl sulfate (w/v), 20% glycerol (v/v), 50 μL of 2-mercaptoethanol, and 0.03 mM bromophenol blue. Samples were heated in a boiling water bath for 5 min and clarified by centrifugation (5000*g*, 15 min). The supernatant was transferred to a clean tube, and 10 μL was loaded onto a 13.5% SDS-PAGE gel (*19*) using the Hoefer SE260 minigel electrophoresis apparatus (GE Healthcare, Piscataway, NJ). After separation, the gels were stained with 0.1% Coomassie Blue R-250.

Two-Dimensional PAGE Analysis. The procedure employed to isolate soybean seed proteins and their fractionation by 2D PAGE analysis has been described earlier (*20*). Briefly, isoelectric focusing was performed using 13 cm IPG strips (pH 4−7) in the IPGphor System (GE Healthcare). Following this, the proteins were separated with a 13.5% SDS-PAGE (*19*). The gels were fixed overnight in a solution of 50% ethanol (v/v) and 3% phosphoric acid. After a distilled water wash, the gels were prestained for 1 h in 34% methanol, 17% ammonium sulfate, and 3% phosphoric acid and then stained in 0.066% Coomassie Blue G250 (w/v).

Immunoblot Analysis. Proteins separated by SDS-PAGE were electrophoretically transferred to a Protran nitrocellulose membrane (Schleicher & Schuell Inc., Keene, NH). Membranes were blocked with 5% milk in Tris-buffered saline (TBS, pH 7.3) for 2 h and then incubated in 1:500 dilution of plasma from individual adult soybean-allergic patients (PlasmaLab International) overnight at room temperature with gentle rocking. In some cases, sera from soybean-allergic patients reacting against the 72, 70, and 52 kDa proteins were pooled and used for immunoblot analyses. After three washings with TBS containing 0.05% Tween-20 (TBST, 10 min/each), the membrane was incubated for 2 h in a 1:5000 dilution of goat anti-human IgE−horseradish peroxidase conjugate secondary antibody (Biosource, Camarillo, CA). Following this the membranes were washed three times for 10 min with TBST and one time with TBS. Immunoreactive polypeptides were detected with the Super Signal West Pico enhanced chemiluminescent substrate (Pierce Biotechnology, Rockford, IL) according to the manufacturer's protocol.

Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) Analysis. Protein spots selected for mass spectrographic analysis were excised from the gels, washed in distilled water, and destained in a 50% solution of acetonitrile (v/v) containing 25 mM ammonium bicarbonate. Gel spots were subjected to digestion with 20 μL (10 μg/mL) modified porcine trypsin in 25 mM ammonium bicarbonate (Promega, Madison, WI). Resulting peptides were analyzed with a Voyager DE-STR MALDI-TOF mass spectrometer (Applied Biosystems, Framingham, MA). The peptides were cocrystallized with α-cyano-4-hydroxycinnamic acid matrix. A 337 nm nitrogen laser operating at 20 Hz was used in sample ionization. Trypsin autolysis peaks of charge mass ratios 842.51 and 2211.10 served as internal standards. For MALDI-TOF-MS data to qualify as a positive identification, a protein's molecular weight search (MOWSE) score had to equal or exceed the minimum significant score of 64.

Partial Purification and Enzymatic Deglycosylation of β-Conglycinin. The 7S β-conglycinin globulin fraction from soybean seeds was partially purified essentially as described by Nagano et al. (*21*) and separated using a 10% preparative SDS-PAGE. Following electrophoresis, the gel was briefly stained with Coomassie Blue. The 52 kDa β-subunit of β-conglycinin was eluted from the gel as described (*22*) and deglycosylated utilizing the GlycoProfile II enzymatic N-deglycosylation kit (Sigma, St. Louis, MO). Ten micrograms of gel-purified β-conglycinin was deglycosylated following the denaturing protocol suggested. Deglycosylation was achieved with the use of PNGase F, which removes all Asn-linked oligosaccharides, and a combination of NANase II and *O*-glycosidase DS that releases all Ser/Thr-linked Gal (β1,3)GalNAc(α1) and all sialic acid substituted Gal(β1,3)LGalNAc(α1) from glycoproteins. The efficiency of the deglycosylation was verified by a shift in mobility of the protein using SDS-PAGE.

Expression of Recombinant β-Subunit of β-Conglycinin in *Escherchia coli*. The coding region of the β-subunit of β-conglycinin lacking the signal peptide was obtained following RT-PCR amplification of the total seed RNA using gene specific primer pairs. The N- and C-terminal specific primers were 5′-*CATATG*TTAAAGGTGAGAGAG-GATGAGAATAAC-3′ and 5′-*CTCGAG*TCAGTAGAGAGCACCTAA-GATTGAAG-3′, which included *Nde*I and *Xho*I restriction sites, respectively, to facilitate cloning. The PCR product was purified from an agarose gel, digested with *Nde*I and *Xho*I (Takara Mirus Bio, Inc., Madison, WI) and ligated into the *Nde*I/*Xho*I site of *E. coli* expression vector pET 28(a)+ (Calbiochem-Novabiochem, San Diego, CA) using the ExTaq ligase kit (Takara Mirus Bio, Inc.). The resultant plasmid, pBBCON, was introduced into ER2566 *E. coli* strain (New England Biolabs, Beverly, MA) and grown in 5 mL of Luria broth medium in the presence of 100 μg/mL kanamycin at 37 °C. This culture was used to inoculate 100 mL of Luria broth containing 100 μg/mL kanamycin and grown at 37 °C. When the culture reached an optical density of 0.5 (A_{600nm}), isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM, and growth was allowed to continue overnight at 37 °C. Recombinant β-subunit of β-conglycinin was purified under denaturing conditions following the manufacturer's suggested protocol (Calbiochem-Novabiochem). Protein concentration was determined spectrophotometrically utilizing the DC Standard Protein Assay Kit (Bio-Rad Laboratories, Richmond, CA).

RESULTS

Different Soybean Seed Proteins Elicit IgE Antibodies in Soybean-Sensitive Patients. Western blot analysis was performed to identify soy proteins that are recognized specifically by IgE antibodies from patients who are sensitive to soybeans. Sera from seven adult soybean-allergic patients were examined in this analysis. As shown in **Figure 1**, the sera from these individuals showed cross-reaction against several soy proteins. Prominent among them were 72, 70, 52, 34, and 21 kDa proteins. Of the seven sera tested, three of them showed cross-reactivity to the 72, 70, and 52 kDa protein. Sera from two patients showed very specific reaction to a 21 kDa protein. The 72, 70, and 52 kDa proteins were located at the same positions in the acrylamide gels that correspond to the α′-, α-, and β-subunits of β-conglycinin (**Figure 1**). A previous study has shown that the 70 kDa α-subunit of β-conglycinin is a major allergenic protein (*18*). The α′- (72 kDa) and β-subunits (52 kDa), which show extensive sequence homology to the α-subunit of β-conglycinin, were not reported as allergenic proteins. However, in our study both the 72 and 52 kDa proteins were recognized by sera from soybean-sensitive patients, leading to the hypothesis that all three subunits of β-conglycinin may be allergenic proteins.

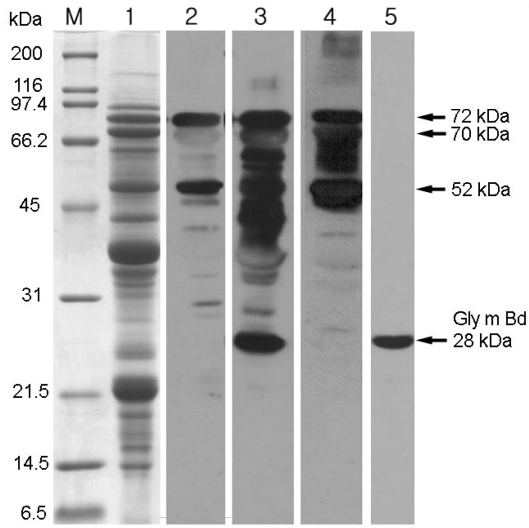

Figure 1. SDS-PAGE and reactivity analysis of soybean seed proteins probed with individual sera from soybean-sensitive patients. Lanes: M, molecular weight markers; 1, soybean total seed proteins stained with Coomassie Blue; 2−5, corresponding immunoblot probed with sera from individuals sensitive to soybean. IgE-binding proteins were detected by chemiluminescence using anti-human IgE horseradish peroxidase conjugate.

All Three Components of 7S Globulin Fractions Are Recognized by IgE Antibodies. To examine if the 72 and 52 kDa proteins correspond to the α′- and β-subunits of β-conglycinin, 2D gel electrophoresis was performed (**Figure 2**). The three subunits of β-conglycinin were well resolved by this procedure. The α′- and α-subunits separated into distinct spots with isoelectric points of 5.2 and 4.9, respectively (**Figure 2A**). The β-subunit was resolved into four distinct spots having isoelectric points ranging from 5.6 to 6.0 (**Figure 2A**). Proteins resolved by 2D gels were transferred to a nitrocellulose membrane and incubated with pooled sera from individuals reacting against the 72, 70, and 52 kDa soybean proteins. Western blot analysis showed strong reaction against the two spots corresponding to the β-subunit and a weaker reaction against the α′- and α-subunits (**Figure 2B**). From an identical 2D gel, protein spots showing cross-reaction were excised from the gels and subjected to MALDI-TOF-MS analysis. A comparative search of peptides of known protein listed in the National Center for Biotechnology Information nonredundant database with peptides generated by trypsin digestion showed significant homology to the α′-, α-, and β-subunits of β-conglycinin (**Table 1**).

Additional confirmation that the 72, 70, and 52 kDa proteins are the three subunits of β-conglycinin was obtained by Western blot analysis using gel-purified components of β-conglycinin. First, we obtained a partially purified 7S β-conglycinin globulin fraction from soybean seeds essentially as described (*21*). This fraction was further fractionated on SDS-PAGE, and the three subunits of β-conglycinin were purified from the gels (**Figure 3A**). The gel-purified subunits were tested by SDS-PAGE and immunoblot analyses with pooled sera from patients containing IgE antibodies against 72, 70, and 52 kDa soybean proteins. This analysis clearly demonstrated that the IgE antibodies reacted against the α′-, α-, and β-subunits of β-conglycinin (**Figure 3B**).

Removal of Oligosaccharides from the β-Subunit of β-Conglycinin Does Not Prevent IgE Binding. It is well-known that the carbohydrate moieties of a glycoprotein may be involved in IgE reactivity (*23*). Because the β-conglycinin proteins are glycoproteins, we examined the IgE reactivity against these proteins directly against the N-linked glycans. Gel-purified β-subunit of β-conglycinin was subjected to deglycosylation by incubating the protein sample with glycosidases PNGase F, NANase II, and *O*-glycosidase DS. Treatment with glycosidases resulted in faster migration of the β-subunit of β-conglycinin in comparison to unglycosylated protein (data not shown). IgE antibodies from patients sensitive to soy proteins recognized both the unglycosylated and deglycosylated forms of the β-subunit of β-conglycinin. Because it may be possible that under our experimental conditions enzymatic deglycosylation may be incomplete, we expressed an unglycosylated form of the β-subunit of β-conglycinin as a recombinant protein in *E. coli* (**Figure 4A**). Because *E. coli* does not possess the same type of cellular machinery used for glycosylation in plants, the β-subunit of β-conglycinin expressed in *E. coli* will not be glycosylated. Immunoblot analysis clearly showed that the IgE antibodies from patients sensitive to soy proteins were able to recognize the unglycosylated form of the β-subunit of β-conglycinin (**Figure 4B**).

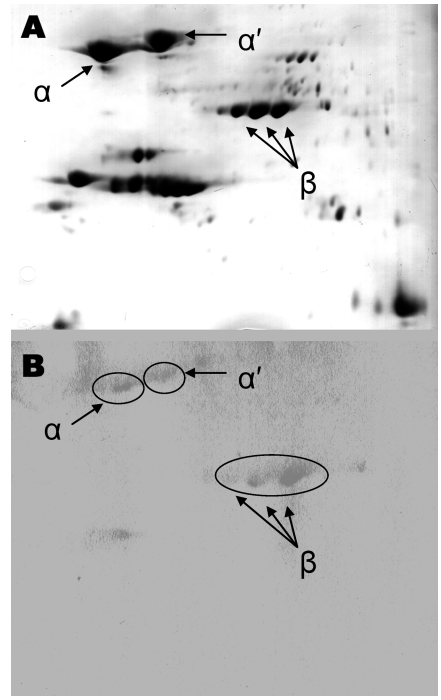

Figure 2. Two-dimensional gel electrophoresis of soybean allergenic proteins. (**A**) Soybean seed proteins were first separated by isoelectric focusing using a pH gradient from 4 to 7 and then by SDS-PAGE on a 12% gel. Proteins were transferred to a nitrocellulose membrane and stained with Ponceau-S. The abundant seed storage proteins are identified. (**B**) Nitrocellulose membrane was probed with pooled sera from individuals reacting against the 72, 70, and 52 kDa soybean proteins, and bound IgE was detected by chemiluminescence using anti-human IgE horseradish peroxidase conjugate. Protein spots (indicated with arrows) were excised and analyzed by MALDI-TOF-MS.

DISCUSSION

Several soybean proteins have been recognized as allergens primarily on the basis of their reactivity to IgE antibodies from soybean-sensitive patients (*24, 25*). Some of these proteins can bring about food allergies, whereas others can elicit respiratory allergies. Three soybean seed coat allergens, Gly m 1A, Gly m 1B, and Gly m 2, are responsible for asthma outbreaks in Barcelona, Spain (*26, 27*). Soybean 11S, 7S, and 2S globulin

Table 1. Identification of Proteins Reactive to Human Serum IgE as Subunits of β-Conglycinin by MALDI-TOF-MS

protein spot	protein identified	accession no. NCBI	MOWSE score (100 ppm)	sequence coverage (%)	theor mol mass (Da)	peptides matched[a]
1	α′-subunit of β-conglycinin (*Glycine max*)	AB008680.2 BAA74452 gil9967361	646	25	65160	DSYNLQSGDALR VPAGTTYYVVNPDNDENLR MITLAIPVNKPGR NILEASYDTK(FEEINK) LQESVIVEISK TISSEDKPFNLR LFEITPEKNPQLR QQQEEQPLEVR DNVISQIPSQVQELAFPGSAK SQSESYFVDAQPQQK
2	α-subunit of β-conglycinin (*Glycine max*)	AB008678.2 BAA23360 gil9967357	505	17	63184	(NK)NPFLFGSNR FETLFK SPQLQNNLR VPSGTTYYVVNPDNNENLR LITLAIPVNKPGR NILEASYDTK(FEEINK) LQESVIVEISK (K)TISSEDKPFNLR
3	β-subunit of β-conglycinin (*Glycine max*)	AB213029.1 BAD98463 gil63852207	256	49	48358	(KV)REDENNPFYLR RSSNSFQTLFENQNGR (K)RSPQLENLR AILTLVNNDDR DSYNLHPGDAQR IPAGTTYYLVNPHDHQNLK LAIPVNKPGR VLFGEEEQR(QQEGVIVELSK) KTISSEDEPFNLR SRNPIYSNNFGK FFEITPEKNPQLR QKQEEEPLEVQR NFLAGEKDNVVR QVQELAFPGSAQDVER (QR)RESYFVDAQPQQK(EEGSK)

[a] Parentheses denote additional residues found on additional matched peptide.

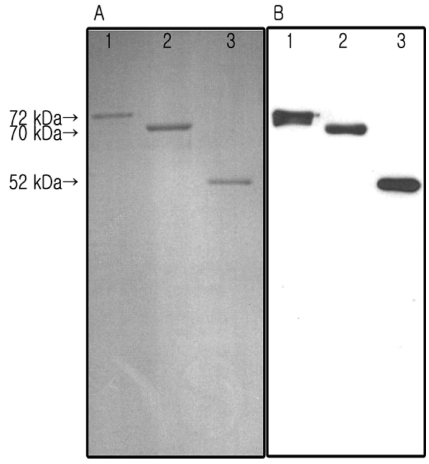

Figure 3. IgE binding to purified α′-, α-, and β-subunits of β-conglycinin. (**A**) Purified subunits of β-conglycinin were separated on a 10% SDS-PAGE and stained with Coomassie Blue. (**B**) Proteins shown in panel **A** were transferred to a nitrocellulose membrane and probed with pooled sera from individuals reacting against the 72, 70, and 52 kDa soybean proteins. IgE-binding proteins were detected by chemiluminescence using anti-human IgE horseradish peroxidase conjugate.

fractions have also been identified as food allergens. Kunitz trypsin inhibitor is the prominent allergen in the 2S fraction (*14*). The 11S glycinins, the most abundant storage protein of soybean, are synthesized as precursor proteins and posttranslationally processed into 40 kDa acidic and 20 kDa basic subunits. Both the acidic (*28, 29*) and basic (*10*) subunits of glycinins have been identified as major allergens. IgE immunoblot and amino acid sequence analysis confirmed that the basic glycinin subunits from all five members of the glycinin gene family are allergens (*10*). A previous study also reported positive tests of IgE antibodies to the acidic subunit (40 kDa) but not to the basic subunit (20 kDa) glycinin (*29*). In the current study we did not detect IgE binding to proteins corresponding to the 40 and 20 kDa glycinin subunits. This may be due to differences among serum sources employed in this investigation. Another possibility is that soybean protein that is allergenic to a certain group of individuals may not necessarily elicit the same antigenic response in other groups of people.

The 7S globulin fraction of soybean, the β-conglycinin, consists of α′-, α-, and β-subunits of approximately 72, 70, and 52 kDa, respectively, and shares extensive sequence homology (*17*). Despite this homology, it was reported that only the 70 kDa α-subunit was recognized by IgE antibodies from soybean-sensitive patients with atopic dermatitis (*18*). Cleavage of the peptide bonds with CNBr and chymotrypsin and subsequent N-terminal amino acid sequence determination of the peptides indicated that IgE-binding sites was located between amino acid residues 232 and 383 of the α-subunit of β-conglycinin (*18*). A comparison of the IgE-binding peptide region of α-subunit to the corresponding regions of the α′- and β-subunits reveals some amino acid differences in this region. These differences were speculated to be one possible reason for the absence of the IgE binding to α′- and β-subunits of β-conglycinin (*18*). In contrast

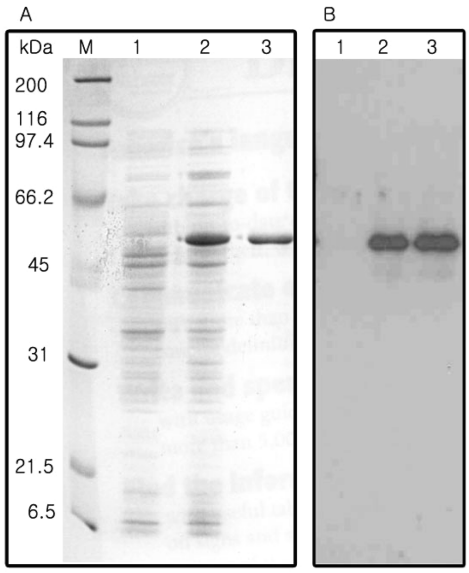

Figure 4. Expression of β-subunit of β-conglycinin in *E. coli* and its reactivity to human serum IgE. (**A**) The coding region of β-subunit of β-conglycinin was cloned in expression vector pET28a(+) and introduced into *E. coli* BL21 (DE3). Recombinant protein was purified on a Ni-affinity column. Proteins were resolved by SDS-PAGE on a 12% gel and stained with Coomassie Blue. (**B**) Proteins shown in panel **A** were transferred to a nitrocellulose membrane and probed with pooled sera from individuals reacting against the 72, 70, and 52 kDa soybean proteins. Immunoreactive proteins were detected by chemiluminescence using anti-human IgE horseradish peroxidase conjugate. Lanes: M, molecular weight markers; 1, total proteins from cells grown in the absence of IPTG; 2, total protein from cells grown in presence of IPTG; 3, purified β-subunit of β-conglycinin.

to the earlier study (*18*), the results presented in this investigation clearly demonstrate that all three subunits of β-conglycinin are potential food allergens. The different conclusions on the apparent allergenicity of the three subunits of β-conglycinin may be attributed to differences among serum sources and if the source is from an adult or infant. Our conclusion that all three subunits of β-conglycinin are potential allergens is further supported by recent studies conducted in rats. In this study, in which rats were fed purified β-conglycinin, it was concluded that that dietary soybean β-conglycinin has negative effects on growth and immune function in rats (*30*). Furthermore, it was demonstrated that the recombinant α′-subunit of soybean β-conglycinin possesses an intrinsic immune-stimulating capacity and can induce allergic reaction in Brown Norway rats (*31*). These results in combination with our current study using immunoblot analyses indicate that all three subunits of β-conglycinin can be potential allergens. However, in vitro immunoblotting studies with commercially obtained sera may sometimes lead to ambiguous results, and therefore the potential biological significance of IgE binding to soybean β-conglycinin for human soy-based allergies in vivo warrants further investigation.

ACKNOWLEDGMENT

We thank Nathan Oehrle for critically reading the manuscript. Names are necessary to report factually on available data: however, the USDA neither guarantees nor warrants the standard of product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may be suitable.

LITERATURE CITED

(1) Messina, M.; Barnes, S. The role of soy products in reducing risk of cancer. *J. Natl. Cancer Inst.* **1991**, *83*, 541–546.
(2) Montgomery, K. S. Soy protein. *J. Perinat. Educ.* **2003**, *12*, 42–45.
(3) Food and Drug Administration (FDA). Food Allergen Labeling and Consumer Protection (FALCP) Act of 2004; http://www.cfsan.fda.gov/~acrobat/alrgact.pdf.
(4) Cordle, C. T. Soy protein allergy: Incidence and relative severity. *Am. Soc. Nutr. Sci.* **2004**, *134*, 1213S–1219S.
(5) Zeiger, R. S.; Sampson, H. A.; Bock, S. A.; Burks, A. W.; Harden, K.; Noone, S.; Martin, D.; Leung, S.; Wilson, G. Soy allergy in infants and children with IgE-associated cow's milk allergy. *J. Pediatr.* **1999**, *134*, 614–622.
(6) Sampson, H. Update on food allergy. Current reviews of allergy and clinical immunology. *J. Allergy Clin. Immunol.* **2002**, *113*, 805–819.
(7) Sicherer, S. H.; Sampson, H. A. Food allergy. *J. Allergy Clin. Immunol.* **2006**, *117*, S470−S475.
(8) Wilson, S.; Blaschek, K.; González de Mejia, E. Allergenic proteins in soybean: processing and reduction of P34 allergenicity. *Nutr. Rev.* **2005**, *63*, 47–58.
(9) Beardslee, T. A.; Zeece, M. G.; Sarath, G.; Markwell, J. P. Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. *Int. Arch. Allergy Immunol.* **2000**, *123*, 299–307.
(10) Helm, R. M.; Cockrell, G.; Connaughton, C.; Sampson, H. A.; Bannon, G. A.; Beilinson, V.; Livingstone, D.; Nielsen, N. C.; Burks, A. W. A soybean G2 glycinin allergen 1. Identification and characterization. *Int. Arch. Allergy Immunol.* **2000**, *123*, 205–212.
(11) Ogawa, T.; Tsuji, H.; Bando, N.; Kitamura, K.; Zhu, Y. L.; Hirano, H.; Nishikawa, K. Identification of the soybean allergenic protein, Gly m Bd 30K, with the soybean seed 34-kDa oil-body-associated protein. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 1030–1033.
(12) Ogawa, A.; Samoto, M.; Takahashi, K. Soybean allergens and hypoallergenic soybean products. *J. Nutr. Sci. Vitaminol.* **2000**, *46*, 271–279.
(13) Xiang, P.; Beardslee, T. A.; Zeece, M. G.; Markwell, J.; Sarath, G. Identification and analysis of a conserved immunoglobulin E binding epitope in soybean G1a and G2a and peanut Ara h 3 glycinins. *Arch. Biochem. Biophys.* **2002**, *408*, 51–57.
(14) Shibasaki, M.; Suzuki, S.; Tajima, S.; Nemoto, H.; Kuroume, T. Allergenicity of major component proteins of soybean. *Int. Arch. Allergy Appl. Immunol.* **1980**, *61*, 441–448.
(15) Bush, R. K.; Schroeckenstein, D.; Meiner-Davis, S.; Balmes, J.; Rempel, D. Soybean flour asthma: detection of allergens by immunoblotting. *J. Allergy Clin. Immunol.* **1988**, *82*, 251–255.
(16) Thanh, V. H.; Shibasaki, K. β-Conglycinin from soybean proteins. Isolation and immunological and physicochemical properties of the monomeric forms. *Biochim. Biophys. Acta* **1977**, *490*, 370–384.
(17) Harada, J. J.; Barker, S. J.; Goldberg, R. B. Soybean β-conglycinin genes are clustered in several DNA regions and regulated by transcriptional and posttranscriptional processes. *Plant Cell* **1989**, *1*, 415–425.
(18) Ogawa, T.; Bando, N.; Tsuji, T.; Nishikawa, K.; Kitamura, K. α-Subunit of β-conglycinin, an allergenic protein recognized by IgE antibodies of soybean-sensitive patients with atopic dermatitis. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 831–833.
(19) Laemmli, U. K. Cleavage of structure proteins during assembly of the head of bacteriophage. *Nature* **1970**, *227*, 680–685.
(20) Krishnan, H. B.; Natarajan, S. S.; Mahmoud, A. A.; Bennett, J. O; Krishnan, A, H.; Prasad, B. N. Assessment of indigenous Nepalese soybean as a potential germplasms resource for improvement of protein in North American cultivars. *J. Agric. Food Chem.* **2006**, *54*, 5489–5497.
(21) Nagano, T.; Hirotsuka, M.; Mori, H.; Kohyama, K.; Nishinari, K. Dynamic viscoelastic study on the gelation of 7S globulin from soybeans. *J. Agric. Food Chem.* **1992**, *40*, 941–944.

(22) Krishnan, H. B.; Jiang, G.; Krishnan, A. H.; Wiebold, W. J. Seed storage protein composition of non-nodulating soybean (*Glycine max* (L.) Merr.) and its influence on protein quality. *Plant Sci.* **2000**, *157*, 191–199.

(23) Fotisch, K.; Vieths, S. N- and O-linked oligosaccharides of allergenic glycoproteins. *Glycoconjugate J.* **2001**, *18*, 373–390.

(24) Herian, A. M.; Taylor, S. L.; Bush, R. K. Identification of soybean allergens by immunoblotting with sera from soy-allergic adults. *Int. Arch. Allergy Appl. Immunol.* **1990**, *92*, 193–198.

(25) Ogawa, T.; Bando, N.; Tsuji, H.; Okajima, H.; Nishikawa, K.; Sasaoka, K. Investigation of the IgE-binding proteins in soybeans by immunoblotting with the sera of the soybean-sensitive patients with atopic dermatitis. *J. Nutr. Sci. Vitaminol.* **1991**, *37*, 555–565.

(26) Gonzalez, R.; Zapatero, L.; Caravaca, F.; Carriera, J. Identification of soybean proteins responsible for respiratory allergies. *Int. Arch. Allergy Appl. Immunol.* **1991**, *95*, 53–57.

(27) Codina, R.; Lockey, R. F.; Fernandez-Caldas, E.; Rama, R. Identification of the soybean hull allergens responsible for the Barcelona asthma outbreak. *Int. Arch. Allergy Immunol.* **1999**, *119*, 69–71.

(28) Djurtoft, R.; Pedersen, H. S.; Aabin, B.; Bartholt, V. Studies of food allergens: soybean and egg proteins. *Adv. Exp. Med. Biol.* **1991**, *289*, 281–293.

(29) Zeece, M. G.; Beardslee, T. A.; Markwell, J.; Sarath, G. Identification of an IgE-binding region in soybean acidic glycinin G1. *Food Agric. Immunol.* **1999**, *11*, 83–90.

(30) Guo, P.; Piao, X.; Ou, D.; Li, D.; Hao, Y. Characterization of the antigenic specificity of soybean protein beta-conglycinin and its effects on growth and immune functions in rats. *Arch. Anim. Nutr.* **2007**, *61*, 189–200.

(31) Guo, P.; Piao, X.; Cao, Y.; Ou, D.; Li, D. Recombinant soybean protein β-conglycinin α'-subunit expression and induced hypersensitivity reaction in rats. *Int. Arch. Allergy Immunol.* **2008**, *145*, 102–110.

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