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Identification, Characterization, Epitope Mapping, and Three-Dimensional Modeling of the α-Subunit of β-Conglycinin of Soybean, a Potential Allergen for Young Pigs

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Soybean meal (SBM), the major byproduct of soybean oil extraction, is the main protein source for swine diets globally. In the United States, 8.6 million metric tons of SBM was used in swine rations in 2004. The pathological effect and immunological response of SBM feeding have been demonstrated in swine. In this study, we have utilized plasma collected from piglet feed with SBM in immunoblot analysis to detect proteins that elicited antigenic responses. We have identified soybean β-conglycinin α-subunit as being a potential allergen for young piglets. Characterization of this protein indicated that deglycosylation and pepsin digestion did not eliminate immunoreactivity of this protein. Epitope mapping utilizing planar cellulose supports technology (SPOT) showed that three peptides spanning amino acids S185−R231 were critical for the allergenicity. A computer-generated three-dimensional structure model of the α-subunit of β-conglycinin indicated that the antigenic epitopes were located on the surface of the protein. Information from this study may assist in the construction of recombinant nonallergenic soybean protein useable for both immunotherapy and the potential production of hypoallergenic soybean plants.

KEYWORDS: Allergens; epitope mapping; β-conglycinin; pig; soybean meal; three-dimensional structure

INTRODUCTION

Soybeans are an important source of edible vegetable oil and protein throughout the world. Most of the soybeans produced in the United States are crushed for oil and meal. In 2004, U.S. production of soybean meal (SBM) was 32.95 million metric tons. Approximately 97% of the SBM is used for animal feed, primarily in poultry, swine, and cattle diets. On a global basis, SBM accounts for approximately 63% of all protein sources used in animal feeds. In the United States, SBM accounts for 27% of the protein used in swine feeds (*1*).

Soybean ranks among the eight most significant food allergens (*2*, *3*). Nearly 2% of adults and 5−8% of infants in the United States and Europe are reported to be allergic to soybeans (*4*). Many soybean allergens for humans have been identified and characterized (*5*−*9*). The resistance to mammalian digestion and heat denaturation of the soybean allergens (*10*, *11*) make them bioactive to animals. A joint FAO/WHO consulting group recommended feed-grade soybean testing to reduce the allergen challenges that could have a far-reaching impact on commercial animal production operations (*10*, *11*). Animal sensitization to

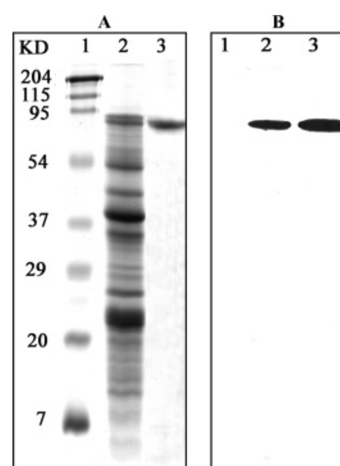

Figure 1. SDS-PAGE/immunoblot analysis of the soybean seed proteins. Total seed proteins (100 μg) and purified α-subunits of β-conglycinin (5 μg) from soybean were fractionated by SDS-PAGE on a 13.5% gel and stained with Coomassie Blue (**A**). Immunological detection of antigenic proteins (**B**). Proteins shown in panel **A** were transferred to a nitrocellulose membrane and probed with pig plasma. Immunoreactive proteins were identified using anti-pig IgG-horseradish peroxidase conjugate antibody followed by chemiluminescent detection. Lane 1, protein marker; lane 2, soybean total seed protein; and lane 3, purified α-subunit of β-conglycinin.

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Table 1. Identification of 72 kDa Protein as α-Subunit of β-Conglycinin by MALDI-TOF-MS

protein identified (*Glycine max*)	accession no. NCBInr	MOWSE score (25 ppm)	sequence coverage	peptides matched
α-subunit of β-conglycinin	gi|9967357	217	39%	QEEEHEQREEQEWPR GSEEEDEDEDEEQDERQFPFPRPPHQKEER NKNPFLFGSNRFETLFK SPQLQNLR LQSGDALRVPSGTTYYVVNPDNNENLRLI- TLAIPVNKPGRFESFFLSSTEAQQSYLQGFSR EEGQQQGEQRLQESVIVEISK KTISSEDKPFNLRSRDPIYSNK FFEITPEKNPQLR EQQQEQQQEEQPLEVR ESYFVDAQPK

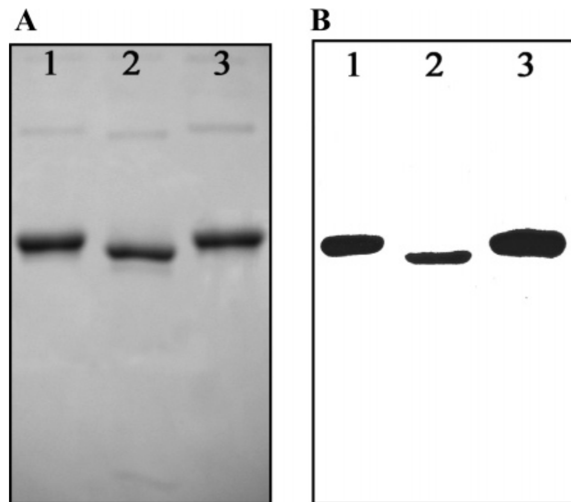

Figure 2. SDS-PAGE/immunoblot analysis of deglycosylated α-subunit of β-conglycinin. Purified native and deglycosylated α-subunits of β-conglycinin (7.5 μg each) were fractionated by SDS-PAGE on a 10% gel and stained with Coomassie Blue (**A**). Proteins shown in panel **A** were transferred to a nitrocellulose membrane and probed with pig plasma (**B**). Lane 1, glycosylated α-subunit of β-conglycinin; lane 2, deglycosylated α-subunit of β-conglycinin; and lane 3, glycosylated α-subunit of β-conglycinin.

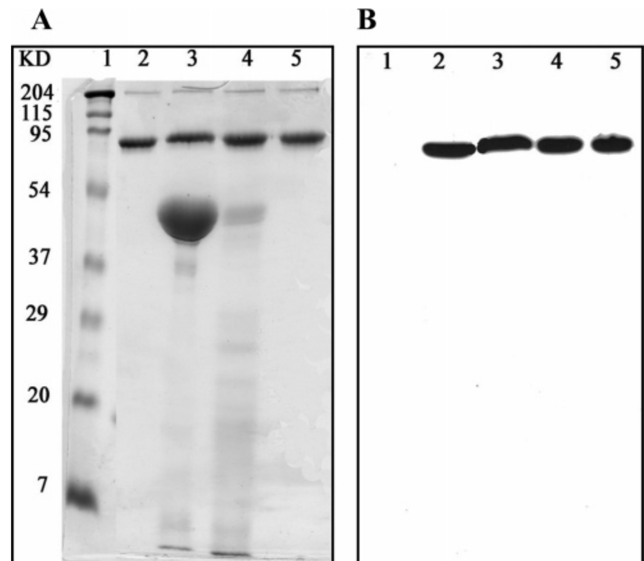

Figure 3. SDS-PAGE/immunoblot analysis of pepsin-digested purified β-conglycinin α-subunit protein. The purified α-subunit of the β-conglycinin was subjected to pepsin digestion and fractionated by SDS-PAGE on a 13.5% gel and stained with Coomassie Blue (**A**). Immunoblot analysis of the transblotted proteins (**B**). Lane 1, protein marker; lane 2, α-subunit; lane 3, α-subunit digested with pepsin; lane 4, α-subunit digested with deactivated pepsin; and lane 5, α-subunit treated with simulated gastric fluid without pepsin. Each lane contains about 7.5 μg of purified α-subunit of the β-conglycinin.

the dietary proteins has been attributed to a variety of factors, including the amino acid sequence of the allergens, their carbohydrate structure, and their stability with respect to digestive enzymes in the intestinal tract (*12*).

Pathological effects and immunological responses of soybean or SBM feeding on swine performance have been investigated (*13*−*17*). It has been shown that SBM can affect pig performance in the first few weeks following weaning (*18*) and elicit specific antibodies in weaned piglets (*14*, *15*, *19*). However, these proteins have not been identified and characterized. Therefore, the objective of this study was to identify and characterize allergenic soybean proteins that elicit allergic responses in young pigs.

MATERIALS AND METHODS

Animals and Diets. Blood samples were taken from piglets (17.78 ± 0.56 kg body weight) fed corn/SBM diets for 9 days after weaning. After centrifugation at 1500*g* for 15 min at 4 °C, plasma was collected and kept at −20 °C until analysis. Plasma from ten piglets was individually assayed for IgG-binding response to soy proteins. Plasma from six piglets, which revealed IgG binding, was pooled for subsequent immunoblot analysis. The control plasma was from six piglets fed diets void of SBM and was used for nonspecific IgG binding to SBM protein. The corn/SBM diet was formulated to meet or exceed NRC requirements (*20*) and contained 40% corn, 20% SBM, and 40% supplement (protein, mineral, vitamins, amino acids, and fatty acids, etc.) on a dry matter basis. The non-SBM diet contained 45% corn, 15% HP-300 (a commercialized protein source), and 40% supplement. The experiment was carried out at the University of Missouri and approved by the Animal Use Advisory Committee.

Sodium Dedecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). The SBM used in the animal diets was ground to a fine powder in a mortar and pestle and extracted (1:30 wt/v) in 1× SDS−sample treatment buffer (62.5 mM TrisHCl, 2% SDS, 10% glycerol, and 30 mM bromphenol blue, pH 6.8). After centrifugation (15800*g*, 10 min), 5% (v/v) β-mercaptoethanol (β-ME) was added to the supernatant and boiled for 5 min. Ten microliter aliquots of the supernatant were loaded onto a 13.5% acrylamide gel and fractionated by SDS-PAGE (*21*). Gels were stained overnight with Coomassie Blue G-250. All buffers and reagents were prepared according to Amersham Pharmacia Biotech protocols.

Immunoblot Analysis. Proteins separated by SDS-PAGE were electrophoretically transferred to nitrocellulose membranes (Protran, Schleicher & Schuell Inc., Keene, NH). Membranes were blocked with 5% milk in Tris-buffer saline (TBS, pH 7.3) for 1 h and incubated in a 1:500 dilution of plasma overnight at room temperature with gentle

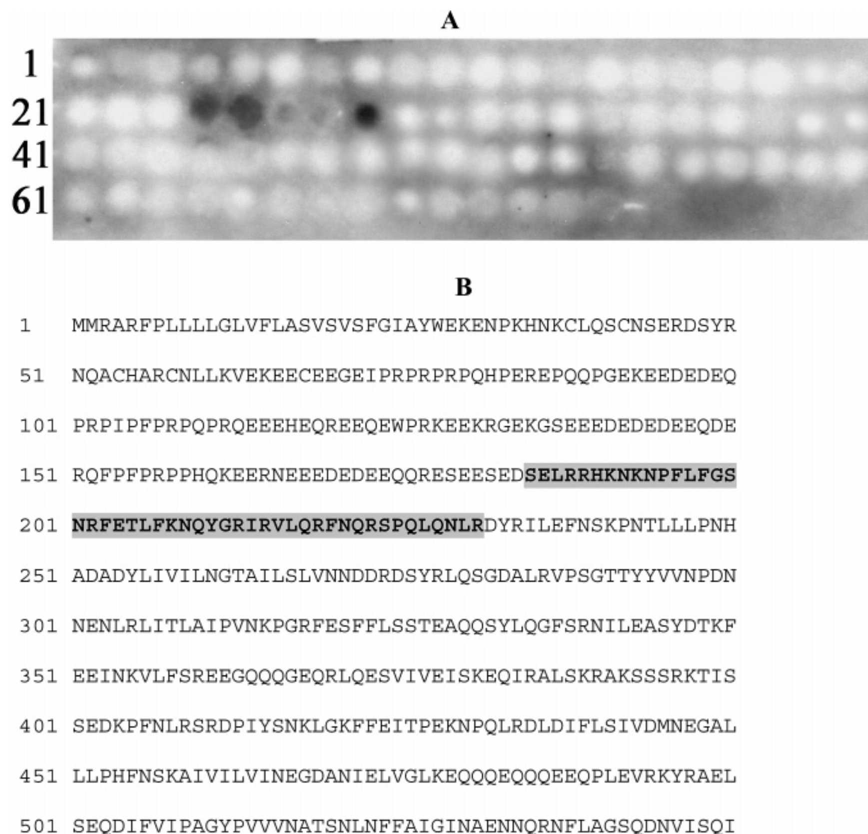

Figure 4. Multiple IgG-binding regions identified on the α-subunit allergen. (**A**) Epitope mapping of the IgG-binding region in β-conglycinin α-subunit protein on SPOT membrane was performed as described in the Materials and Methods. (**B**) Amino acid sequence of the β-conglycinin α-subunit. Antigenic epitopes are shown in hatched areas.

rocking. Preliminary experiments with different dilutions of plasma revealed that 1:500 dilution was optimal for antigen detection. After washing four times with TBS containing 0.05% Tween-20 (TBST, 10 min/each), the membrane was incubated for 2 h in a 1:5000 dilution of rabbit anti-pig IgG-horseradish peroxidase conjugate antibody (Sigma, St. Louis, MO). Immunoreactive polypeptides were detected with an enhanced chemiluminescent substrate (Super Signal West Pico trial kit; Pierce Biotechnology, Rockford, IL) according to the manufacturer's protocol.

Purification of Soybean Allergen by Preparative SDS-PAGE. Soybean total seed proteins were fractionated by preparative SDS-PAGE and briefly stained with Coomassie Brilliant Blue. Gel slices corresponding to the 72 kDa α-subunit of β-conglycinin were excised and rinsed extensively in distilled water. After the gel slices were washed twice in 250 mM Tris-HCl and 250 mM EDTA, pH 6.8, they were mashed into a fine powder with the help of a mortar and pestle and extracted with 20 mM Tris-HCl and 0.1% SDS. Eluted proteins were collected by precipitation with 3 volumes of acetone (overnight, −20 °C) followed by centrifugation at 15800*g* for 20 min. The protein pellets were air-dried and resuspended in a small volume of distilled water. The concentration of the gel-eluted protein was determined using a Bradford Protein Assay Kit (Pierce) following the manufacturer's instructions.

Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) Analysis of Trypsin-Digested Proteins. MALDI-TOF-MS analysis and data interpretation were performed essentially as described earlier (*22*). Briefly, the 72 kDa gel-purified protein was washed in distilled water and then destained in a 50% solution of acetonitrile (v/v) containing 25 mM ammonium bicarbonate. After a 100% acetonitrile wash, the proteins contained in the gel spots were subjected to digestion using 20 μL (10 μg/mL) of modified porcine trypsin in 25 mM ammonium bicarbonate (Promega, Madison, WI). Peptides resulting from tryptic digestion were analyzed by mass spectroscopy (Voyager DE-STR MALDI-TOF, Applied Biosystems, Framingham, MA). The peptides were cocrystallized with the matrix material *R*-cyano-4-hydroxycinnamic acid. 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.

Enzymatic Deglycosylation of Soybean Allergen. Deglycosylation of the soybean allergenic protein was carried out using an enzymatic N-deglycosylation kit (GlycoProfile II, Sigma) in accordance with the manufacturer's instructions. The kit includes 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. Fifteen micrograms of purified α-subunit of β-conglycinin was deglycosylated following the denaturing protocol suggested by the vender. The efficiency of the deglycosylation was verified by a shift in mobility of the protein on a SDS-PAGE gel.

Protease Digestion of Soybean Allergen. Eighteen micrograms of the gel-purified α-subunit of β-conglycinin was digested with pepsin (Sigma) for 60 min at 37 °C in simulated gastric fluid (SGF) according to Helm (*10*) and Astwood et al. (*23*). Briefly, 500 ng/μl of protein was digested in 100 μL of prewarmed SGF containing 0.32% pepsin (pepsin/protein: 320/18). After 60 min, 30 μL of Na_2CO_3 (200 mM), 34 μL of 6× SDS−sample treatment buffer (350 mM Tris-HCl, 10% SDS, 30% glycerol, and 175 mM bromophenol blue, pH 6.8), and 10 μL of β-ME were added, mixed, and boiled for 5 min. Controls included protein samples in SGF that did not contain pepsin or contained deactivated pepsin. The generation of proteolytic fragments due to pepsin digestion was examined by SDS-PAGE and Western blot analysis.

Peptide Synthesis on SPOT Membrane and Epitope Mapping. The α-subunit of β-conglycinin is a 72 kDa protein and is made up of 605 amino acids. A total of 74 peptides covering the entire region of the protein were synthesized on a modified SPOT cellulose membrane (Genosys Biotechnologies, Woodlands, TX). Each peptide was 15 amino acids long and overlapped by seven amino acids (eight amino acids offset) between two adjacent peptides. Epitope mapping was

conducted with pooled pig plasma diluted 1:250 (in accordance with the manufacturer's recommendations). Briefly, the membrane was rinsed with a small volume of methanol followed by three washes in TBS. To prevent nonspecific binding, the membrane was incubated with 5% milk in TBST for 2 h. Following incubation with diluted pooled pig plasma for 3 h, the membrane was washed once in TBST for 10 min and incubated for 2 h in a 1:5000 dilution of rabbit anti-pig IgG-horseradish peroxidase conjugate antibody. Immunoreactive peptides were detected with an enhanced chemiluminescent substrate (Super Signal West Pico trial kit; Pierce Biotechnology) according to the manufacturer's protocol.

Homology-Model of the α-Subunit of *β*-Conglycinin from Soybean. The three-dimensional structure of the homotrimeric soybean *β*-conglycinin α′-subunit (*24*) was used as a template to build a homology model of the soybean *β*-conglycinin α-subunit. The homology model was generated as a homotrimer and energy minimized using the Swiss-Model website (http://swissmodel.expasy.org//SWISS-MODEL.html) (*25*). The stereochemistry and overall quality of the homology model were checked with the program Procheck (*26*). The final model of the soybean *β*-conglycinin α-subunit included coordinates for residues 188−605 of each monomer in the model.

RESULTS

Identification of Soybean Allergen. Western blot analysis was performed to identify soybean allergens by utilizing pooled plasma collected from piglets fed with corn/SBM diets. A representative picture of the Coomassie Blue-stained gel and immunoblot membrane is shown in **Figure 1A**,**B**. Immunoblot analysis revealed that the IgG in the pig plasma reacted specifically against a 72 kDa protein (**Figure 1B**). The IgG from piglets fed a diet lacking SBM did not react with any soybean seed proteins (data not shown). This indicated that the diet without SBM did not provoke specific soy IgG antibodies. The *β*-conglycinin of soybean is made up of 76 kDa α′-, 72 kDa α-, and 53 kDa *β*-subunits (*27*). On the basis of the size of the protein, we suspected that the immunoreactive protein could be the α-subunit of *β*-conglycinin. To confirm this observation, the α-subunit was purified from soybean seeds by preparative SDS-PAGE. When the purified α-subunit of *β*-conglycinin (**Figure 1**) was used in immunoblot analysis, strong reactivity with pig plasma was seen (**Figure 1B**). This immunoractive protein was subjected to MALDI-TOF-MS analysis. Using Mascot, the empirically determined mass-to-charge ratios of peptides were compared with peptides of known proteins listed in the National Center for Biotechnology Information non-redundant database. The result of this analysis is shown in **Table 1**.

Out of the 46 peptides submitted to Mascot search, 25 showed significant sequence homology to the α-subunit of *β*-conglycinin. The top four scores, all above the cutoff confidence limit of 95% ($P < 0.05$), belonged to the α-subunit of *β*-conglycinin, indicating that the unique 72 kDa pig IgG-binding protein is the α-subunit of *β*-conglycinin.

Carbohydrate Moiety of the α-Subunit of *β*-Conglycinin Is not Responsible for the Immunoreactivity. An allergen is a substance that provokes the immune response mediated by the production of IgE. Several studies have shown that *N*- and *O*-linked oligosaccharides of glycoproteins are crucial epitopes for human IgE (*27*). The 11S storage protein of soybean, the *β*-conglycinin, is a glycoprotein (*27*). It is synthesized on rough endoplasmic reticulum and cotranslationally glycosylated by the addition of mannose-containing core oligosaccharide (*29*). To examine the contribution of the carbohydrate moiety in allergenicity, the purified α-subunit of *β*-conglycinin was deglycosylated using an enzymatic deglycosylation kit. As expected, the removal of the carbohydrate moiety resulted in a decrease

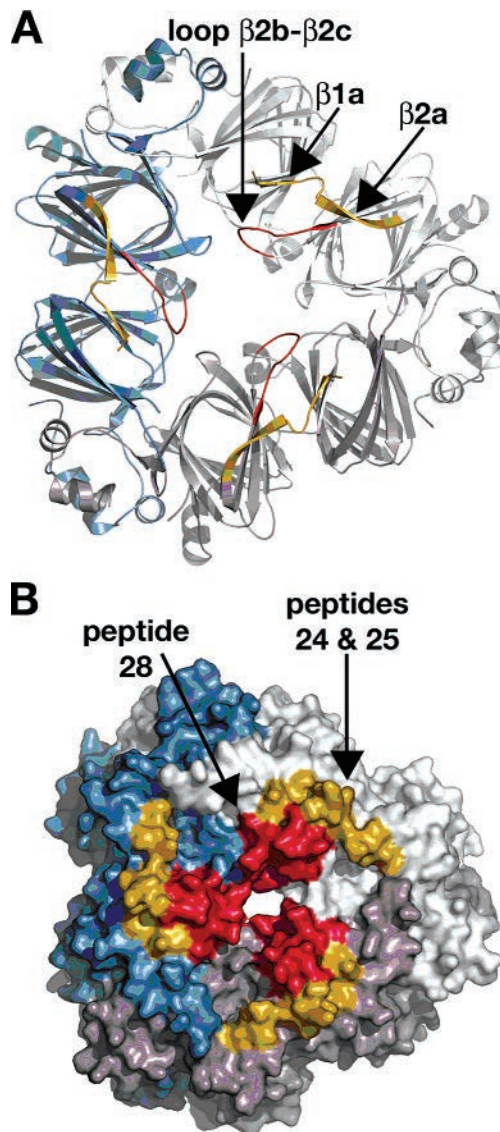

Figure 5. Mapping of immunogenic epitopes on the homology-modeled structure of the soybean *β*-conglycinin α-subunit. (**A**) The ribbon diagram shows the overall secondary structure of the *β*-conglycinin α-subunit homotrimer with each monomer colored in white, blue, and purple. *β*-Strands are shown as arrows, and α-helices are shown as coils. The structural features corresponding to peptides 24 and 25 (strands *β*1a and *β*2a) and peptide 28 (loop *β*2b–*β*2c) are colored gold and red, respectively. (**B**) The molecular surface of the homology-modeled structure with the surfaces corresponding to each monomer colored as in **A**. The molecular surfaces of peptides 24 and 25 and peptide 28 are colored gold and red, respectively. The figure was made using PyMOL (DeLano, 2002).

in the molecular weight of the protein (**Figure 2**). Western blot analysis indicated that both the native and the deglycosylated proteins were recognized by the pig plasma indicating that the carbohydrate moiety does not serve as an antigenic epitope of the α-subunit of *β*-conglycinin.

α-Subunit of *β*-Conglycinin Is Resistant to Pepsin Digestion. One of the known properties of food allergens is that they exhibit remarkable digestive stability. To examine if the α-subunit of *β*-conglycinin is stable to digestion, the purified protein was subjected to pepsin digestion in vitro (**Figure 3**). The α-subunit remained intact even after 60 min of digestion with pepsin (**Figure 3A**). The stability of the α-subunit of *β*-conglycinin to pepsin digestion was verified by Western blot

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α′   197   SESQREPRRHKNKNPFHF-NSKRFQTLFKNQYGHVRVLQRFNKRSQQLQNLR   248
α    185   SEL----RRHKNKNPFLF-GSNRFETLFKNQYGRIRVLQRFNQRSPQLQNLR   231
β      1   -----LKVREDENNPFYFRSSNSFQTLFENQNGRIRLLQRFNKRSPQLENLR    47
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B

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α        185   SELRRHKNKNPFLFGSNRFETLFKNQYGRIRVLQRFNQRSPQLQNLR   231
Ara h 1  166   RE--ETSRNNPFYFPSRRFSTRYGNQNGRIRVLQRFDQRSPQFQNLQ   211
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Figure 6. Amino acid sequence alignment of the antigenic region of α-subunit with the α′- and β-subunit of β-conglycinin (**A**). Amino acid sequence homology between the immunogenic region of α-subunit of β-conglycinin and the Ara h 1 allergen of peanut (**B**).

analysis (**Figure 3B**). Antibodies in the pig plasma recognized only the 72 kDa protein demonstrating that the α-subunit was resistant to pepsin digestion. Under similar conditions, the acidic subunit of glycinin (a nonallergenic protein) was completely digested by pepsin (data not shown).

Localization of IgG-Binding Epitopes in the α-Subunit of β-Conglycinin. To identify the region of the protein that could serve as the antigenic epitope, we tested the IgG-binding ability of peptides that were synthesized on a modified SPOT cellulose membrane (Sigma-Genosys). Peptides corresponding to the entire amino acid sequence of the α-subunit were prepared as 15-mer chains that were offset by eight amino acids. Using this approach, a 47 amino acid spanning region (S185−R231) that bound IgG was identified near the N terminus of the α-subunit that corresponded to peptides 24−28 (**Figure 4**). Within the region, the strongest reaction was detected with peptide 28, and peptides 24 and 25 showed relatively weak reactions (**Figure 4A**). On the basis of this study, the major IgG-binding region of the α-subunit molecule was identified as peptides 28, 24, and 25 (**Figure 4B**).

Location of Immunogenic Epitopes on the Modeled Structure of the Soybean β-Conglycinin α-Subunit. A homology model structure of the soybean β-conglycinin α-subunit was generated using the X-ray crystal structure of the soybean β-conglycinin α′-subunit core domain as a template (*24*). The two subunits share 89.8% amino acid sequence identity over the region included in the X-ray structure, which corresponds to residues 188−605 of the α-subunit. The immunogenic epitopes identified from the peptide library can be mapped to the N-terminal face of the homotrimer (**Figure 5**). Residues found in peptides 24 and 25 comprise portions of two β-strands (β1a and β2a) and the loop connecting these secondary structure features (**Figure 5A**). The sequence of peptide 28 maps to a loop region that connects β-strands β2b and β2c (**Figure 5A**). Both regions are located on the surface of the protein and are predicted to be exposed to solvent and presumably IgG (**Figure 5B**).

DISCUSSION

Soybeans are the best protein source for swine due to their high protein concentration and balanced amino acid profile. However, soybeans contain some antinutritional factors such as the trypsin inhibitor, phytic acid, oligosaccharides, and lectins that may interfere with the livestock performance and yield. In addition, some soybean storage proteins cause an inflammatory response in the intestines of early weaned pigs. For example, pigs that fed on SBM had decreased growth performance, lowered villus height, and increased plasma anti-soy titers (*14*, *15*). We have detected antibodies (IgG) in the plasma of young pigs that react with an abundant 72 kDa α-subunit of β-conglycinin. Our observation that the young piglets respond to SBM diets by generating IgG directed against the α-subunit of β-conglycinin is consistent with an earlier observation that claims legume proteins of the vicilin family are more immunogenic than those of the legumin family (*31*). In this current study, IgG binding was used because anti-pig IgE antibody was not available (*32*). The lack of antibodies specific for swine IgE has impeded the unequivocal identification of allergens in pigs. IgG binding has been successfully employed by many researchers in allergenicity studies using animal models (*31*, *33*−*36*). However, the results based on IgG binding should be treated with caution. IgG binding demonstrates that soy proteins such as the α-subunit of β-conglycinin are potent antigens but not necessarily allergens. Because it has been shown that humans produce α-subunit of β-conglycinin-specific IgE, it is likely that a similar response is also triggered in pigs because they resemble humans in gastrointestinal physiology and in the development of mucosal immunity.

The predominant storage proteins of soybeans are the glycinin (11S) and β-conglycinins (7S). This group of proteins accounts for 80% of the total seed protein of soybean (*27*). The β-conglycinins are synthesized as trimers with a molecular mass of 150−200 kDa and are composed of three subunits, α′ (76 kDa), α (72 kDa), and β (53 kDa). The genes encoding these subunits have been cloned and reveal extensive sequence homology (*29*, *37*). In spite of this sequence homology, the pig plasma used in this study reacted specifically with the 72 kDa α-subunit but not with α′- and β-subunits of β-conglycinin. However, alignment of the amino acid sequence covering the antigenic epitopes among the three subunits of β-conglycinin revealed significant differences (**Figure 6A**). These differences could explain the lack of reactivity of pig plasma with the α′- and β-subunits of β-conglycinin. Structural differences among closely related protein families likely impart different biochemical, rheological, and immunological properties (*31*).

The SPOT technology has been applied in many studies to map antigenic epitopes (*7*, *9*, *33*, *38*). Using this method, we identified potential antigenic sites (peptide sequences S185−F207 and L217−R231) within the α-subunit of β-conglycinin that are critical for the allergenic reaction. Our results are in agreement with previous studies, which demonstrate that short peptide regions are key for eliciting an immunological reaction (*7*, *9*). A major peanut allergen, Ara h 1, belongs to the vicilin family of legume storage proteins (*39*). Epitope mapping resulted in the identification of at least 23 different IgE-binding sites located throughout the length of the Ara h 1. Among them, four peptides were designated as immunodominant because they were recognized by IgE from 80% of peanut sensitive patients (*39*).

Similarity searches by BLAST analysis (http://www.ncbi.nlm-.nih.gov/) revealed that antigenic epitopes of Ara h 1 and soybean α-subunit of β-conglycinin shared 60% amino acid sequence identity (**Figure 6B**). This observation suggests conservation of amino acid sequences within IgG- and IgE-binding epitopes contained in legume allergens.

Previous studies have identified several soybean proteins as potential human food allergens based on their ability to bind IgE. These allergens include glycinin (11S), β-conglycinin (7S), Kunitz trypsin inhibitor, Gly m Bd 30 K, and Gly m Bd 28 K (*6*, *7*, *9*, *38*, *40*, *41*). Because soybean is widely used in food products and the livestock industry, efforts are underway to remove these allergens from soybeans. Progress in eliminating some of these allergens has been made utilizing genetic engineering (*42*), food processing (*41*), and mutation breeding (*41*, *43*−*45*). Takahashi et al. (*46*) have established a mutant line, Tohoku 124, lacking the α- and α′-subunit of β-conglycinin and suggested that it would be a promising material for less-allergenic soybean products (*43*). However, there is currently no data to show the characteristic and feed value of a nonallergenic soybean line.

The treatment of allergenic diseases is based mainly on allergen avoidance and the use of antihistamines, steroids, or immune suppressants (*47*). Recently, Kobayashi et al. (*48*) found a peptide by a novel procedure for producing a compound from sesame that could inhibit allergen absorption. In the food animal industry, replacing the SBM in diets is not probable. Therefore, the development of methods to reduce the allergenicity of soybean protein is urgently required. Other research indicated that allergen homologues could potentially be used for immunotherapy (*49*, *50*). The long-term and efficient strategies are to mutate the immunological response epitopes. Recent studies have shown that hypoallergenic Ara h 1 produced by altering the IgE-binding epitopes could be utilized as an immunotherapeutic option against allergic response in animal models (*51*). It will be interesting to examine the effect of the modification of the IgG-binding epitopes identified in this current study in lowering the allergic response in piglets.

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