

Identification and Characterization of β -Lathyrin, an Abundant Glycoprotein of Grass Pea (*Lathyrus sativus* L.), as a Potential Allergen

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Supporting Information

ABSTRACT: Grass pea, a protein-rich, high-yielding, and drought-tolerant legume, is used as food and livestock feed in several tropical and subtropical regions of the world. The abundant seed proteins of grass pea are salt-soluble globulins, which can be separated into vicilins and legumins. In many other legumes, the members of vicilin seed proteins have been identified as major allergens. However, very little information is available on the allergens of grass pea. In this study, we have identified an abundant 47 kDa protein from grass pea, which was recognized by immunoglobulin E (IgE) antibodies from sera drawn from several peanut-allergic patients. The IgE-binding 47 kDa protein was partially purified by affinity chromatography on a Con-A sepharose column. Matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry analysis of the 47 kDa grass pea protein revealed sequence homology to 47 kDa vicilin from pea and Len c 1 from lentil. Interestingly the grass pea vicilin was found to be susceptible to pepsin digestion *in vitro*. We have also isolated a cDNA encoding the grass pea 47 kDa vicilin (β -lathyrin), and the deduced amino acid sequence revealed extensive homology to several known allergens, including those from peanut and soybean. A homology model structure of the grass pea β -lathyrin, generated using the X-ray crystal structure of the soybean β -conglycinin β subunit as a template, revealed potential IgE-binding epitopes located on the surface of the molecule. The similarity in the three-dimensional structure and the conservation of the antigenic epitopes on the molecular surface of vicilin allergens explains the IgE-binding cross-reactivity.

KEYWORDS: allergen, β -lathyrin, grass pea, storage protein

INTRODUCTION

Grass pea (*Lathyrus sativus* L.) is an important legume that is cultivated as a food and forage crop in North Africa, Near East, Western Asia, and the Indian subcontinent.^{1–3} This underutilized legume can withstand harsh environmental conditions, including drought and flooding. On account of these characteristics, grass pea is often grown in drought-prone areas.³ Like other legumes, grass pea seeds are relatively rich in protein (about 20% of the seed dry weight) and can serve as an economic source of a highly nutritious and balanced source of human dietary protein. However, its use as a dietary protein source is limited as a result of the presence of the neurotoxic non-protein amino acid β -N-oxalyl-L- α , β -diaminopropionic acid (β -ODAP). Prolonged consumption of grass pea as a sole protein source leads to paralysis of lower limbs, a condition referred to as lathyrism.^{4,5} The concerted efforts of international research organizations have resulted in the development of improved varieties that contain lower levels of β -ODAP.^{6,7} Such improved grass pea varieties are vital for reducing the incidence of lathyrism among subsistence poor

farmers in countries that regularly have harsh environmental conditions.

Unlike the major legumes, little is known about the seed storage proteins of grass pea. Although several early studies have been reported,^{8–11} it was only in the year 2000 that a detailed biochemical investigation of grass pea seed proteins was initiated.¹² It was reported that the protein content of grass pea was about 20% of the seed dry weight. Globulin and albumin fractions represented 60 and 30% of the total seed proteins, respectively. The globulins were grouped into α -lathyrin, β -lathyrin, and γ -lathyrin. α -Lathyrin, the most abundant globulin of grass pea seeds, is represented by three proteins that were further subjected to proteolytic processing, resulting in larger and small subunits, similar to the 11S globulins of other legumes.¹² β -Lathyrin (7S globulin) is made up of several polypeptides of different molecular weights (14–

Received: May 2, 2018

Revised: July 25, 2018

Accepted: July 27, 2018

Published: July 27, 2018

66 kDa). At least two of the subunits of β -lathyrin are glycosylated, and one of them contains a disulfide bond.¹² Thus, the seed storage protein composition of grass pea is similar to that of other well-characterized legumes, although slight changes in the sedimentation coefficients are evident.¹² Immunological studies further confirm the similarity between lupin conglutins and grass pea lathyrins in subunit and polypeptide composition.¹²

Allergic reactions to legume seed protein (especially peanut, soybeans, lentil, chickpea, and pea) have been widely reported in the literature.¹³ In Mediterranean and Asian countries, allergic reactions to the ingestion of lentil, chickpea, and pea are prevalent. For example, 10% of the Spanish pediatric population was reported to be allergic to lentil proteins.¹⁴ The major allergen of lentil has been identified as vicilin (Len c 1), a member of 7S globulin.¹⁵ Interestingly, the 7S globulin members are also the most dominant allergens in peanut and soybean.^{16–18} In contrast to other legumes, very little is known about the allergenic potential of grass pea seed proteins. In sensitized individuals, grass pea can induce symptoms of food allergy as well as occupational-induced allergies, such as bronchial asthma, rhinoconjunctivitis, facial edema, and generalized urticaria.^{19–23} Three polypeptides (sized 46, 28, and 21 kDa) were recognized by immunoglobulin E (IgE) antibodies present in the blood from grass-pea-allergic patients.²¹ Subsequently, a 24 kDa allergenic protein from grass pea was purified and crystallized.²³ This allergenic protein was identified as a member of 2S albumin and showed 85% sequence homology with the 2S albumin of *Pisum sativum*. Because grass pea is often the only crop that poor farmers are able to depend upon to survive in extremely drought-prone growing regions, it is critical to identify and characterize potential allergens present in seeds of grass pea. Here, we have identified and characterized a 47 kDa IgE-binding protein from grass pea.

MATERIALS AND METHODS

Seed Materials. Dry seeds of *L. sativus* cv. LZ(2) were harvested in 2016 from an experimental farm of Northwest A&F University, China. Seed of soybean (*Glycine max* cv. Williams 82) were obtained from Missouri Crop Improvement Association, Columbia, MO, U.S.A. Seeds of Spanish peanut (*Arachis hypogaea*) was purchased from a local grocery store, Columbia, MO, U.S.A. All seeds were stored at 4 °C until used.

Sera of Patients. Sera from eight patients with documented allergy to peanut were obtained from IBT Laboratories (Lenexa, KS, U.S.A.). The ImmunoCAP fluorescent enzyme immunoassay (FEIA), which has been approved for diagnostic use by the U.S. Food and Drug Administration (FDA), revealed 175.0–50.9 kU/L peanut-specific IgE in the sera of these patients. Clinical symptoms of these patients ranged from impaired breathing, itchy skin, nausea, to runny nose. Four of eight peanut-allergic patients also contained IgE specific to soybean flour, as determined by the ImmunoCAP FEIA. Serum from a health individual with no known history of allergy was also acquired from IBT Laboratories.

Protein Isolation and Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE). Dry seeds were ground to a fine powder in a mortar and pestle. Then, 20 mg of seed powder was transferred to a 2 mL microcentrifuge tube, and 1 mL of sodium dodecyl sulfate (SDS) sample buffer [60 mM Tris–HCl at pH 6.8, 2% SDS (w/v), 10% glycerol (v/v), and 5% 2-mercaptoethanol (v/v)] was added. The contents of each tube were vigorously vortexed for 10 min at room temperature, followed by boiling for 5 min. Each sample was then subjected to centrifugation at 15800g for 5 min. The clear supernatant was collected and designated as the total seed protein fraction. Seed proteins were then resolved by SDS–PAGE gels using a

Hoeffer SE 250 mini-vertical electrophoresis apparatus (GE Healthcare, Pittsburgh, PA, U.S.A.), and ~50 μ g of seed proteins was loaded onto a 13.5% acrylamide gel. Separation of proteins was achieved with a constant 20 mA/gel until the tracking dye reached the bottom of the gel. Gels were then removed from the cassette and stained overnight with Coomassie Blue R-250.

Immunoblot Analysis. Seed proteins were first resolved by SDS–PAGE and then electrophoretically transferred to nitrocellulose membranes (Protran, Schleicher & Schuell, Inc., Keene, NH, U.S.A.) as previously described.²⁴ Membranes were blocked with 5% nonfat milk in Tris-buffered saline (TBS, pH 7.3) for 1 h at room temperature. Following this step, the nitrocellulose membranes were incubated in a 1:500 dilution of sera from peanut-allergic patients (IBT Laboratories, Lenexa, KS, U.S.A.) overnight at room temperature with gentle rocking. After washing [4 times with TBS containing 0.05% Tween 20 (TBST), 10 min/each], each membrane was incubated for 2 h in a 1:5000 dilution of goat anti-human IgE–horseradish peroxidase conjugate antibody (Biosource, Camarillo, CA, U.S.A.). IgE-binding polypeptides were then detected using an enhanced chemiluminescent substrate (Super Signal West Pico Trial Kit, Pierce Biotechnology, Rockford, IL, U.S.A.) according to the protocol of the manufacturer.

Concanavalin A (Con A)–Sephacrose Affinity Chromatography. A total of 5 g of dry grass pea seed was ground to a fine powder using a mortar and pestle. To the seed powder, 50 mL of 50 mM Tris–HCl at pH 7.5, 150 mM NaCl, and 0.1 mM phenylmethylsulfonyl fluoride were added. The mixture was then placed in an orbital shaker (160 rpm at 30 °C) for 30 min. The slurry was clarified by centrifugation at 13320g for 20 min. To the resulting clear supernatant solid, $(\text{NH}_4)_2\text{SO}_4$ was added gradually to achieve 70% saturation. The precipitated proteins were then recovered by centrifugation as described previously. The resulting protein pellet was resuspended in 20 mL of dialysis buffer [100 mM sodium phosphate (pH 6.5), 1 mM MnCl_2 , 1 mM CaCl_2 , 1 mM MgCl_2 , 1 mM dithiothreitol (DTT), and 0.5 M NaCl], and the concentrated protein solution was dialyzed overnight against the same buffer at room temperature. The dialyzed protein solution was then loaded onto a column (1 $\times$ 16 cm) of Con A–Sephacrose (Sigma Chemicals, St. Louis, MO, U.S.A.), and the column was washed extensively with dialysis buffer and then subjected to a step gradient of 0–250 mM α -methyl mannoside in dialysis buffer. Fractions of 5 mL were collected, and alternative fractions were subjected to SDS–PAGE analysis.

Matrix-Assisted Laser Desorption/Ionization–Time-of-Flight Mass Spectrometry (MALDI–TOF–MS) Analysis. Partially purified grass pea vicilin fractions obtained from Con A–Sephacrose chromatography were subjected to SDS–PAGE. The 47 kDa IgE-binding protein was excised from the acrylamide gel, washed in distilled water, and then destained in a 50% solution of acetonitrile (v/v) containing 25 mM ammonium bicarbonate. After destaining, a 100% acetonitrile wash was performed and the 47 kDa protein was digested with 20 μ L (10 μ g/mL) of modified porcine trypsin in 25 mM ammonium bicarbonate (Promega, Madison, WI, U.S.A.). The peptides resulting from tryptic digestion were then analyzed by mass spectroscopy as previously described.²⁴

Enzymatic Deglycosylation of Grass Pea Allergen. Deglycosylation of grass pea 47 kDa vicilin was carried out using GlycoPro Enzymatic Deglycosylation Kit (Prozyme, Hayward, CA, U.S.A.) per the instructions of the manufacturer. The kit includes N-glycanase, sialidase A, O-glycanase, β (1–4)-galactosidase, and β -N-acetylglucosaminidase, which removes all N-linked glycans and simple O-linked glycans from glycoproteins. About 100 μ g of the Con A–Sephacrose-purified vicilin fraction was denatured and deglycosylated following the protocol of the manufacturer. The efficiency of the deglycosylation was verified by a shift in protein mobility on a 10% SDS–PAGE gel.

Protease Digestion of Grass Pea Allergen. The stability of grass pea 47 kDa vicilin to pepsin digestion was carried out as described previously.^{25,26} About 20 μ g of the Con A–Sephacrose-purified vicilin fraction was digested with pepsin (Sigma), at one of several time intervals at 37 °C in simulated gastric fluid (SGF).

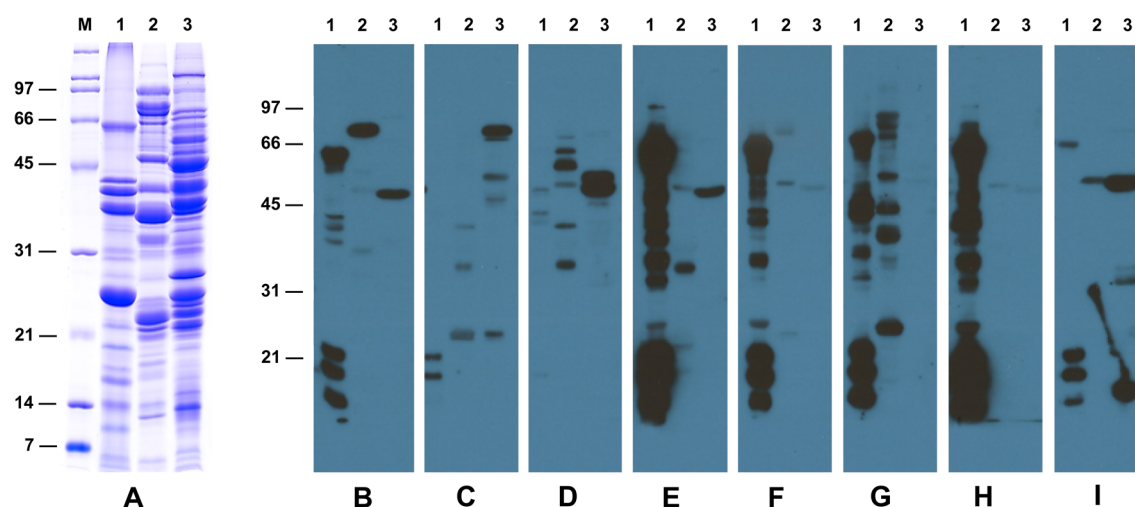


Figure 1. SDS-PAGE and IgE cross-reactivity of grass pea proteins. (A) Total seed proteins extracted from seeds of peanut (lane 1), soybean (lane 2), and grass pea (lane 3) were separated by 13.5% SDS-PAGE gel. Resolved proteins were detected by staining the gel with Coomassie Blue. (B–I) Western blot analysis showing cross-reactivity of grass pea proteins with sera from patients with peanut allergy. Each panel was incubated with sera from individual peanut-allergic patients. Cross-reacting proteins were identified using goat anti-human IgE–horseradish peroxidase conjugate antibody, followed by chemiluminescent detection. Lane M contains the molecular weight markers whose sizes in kilodaltons are shown on the left side of the figure.

Aliquots of samples were removed after 0.5, 1, 5, 30, and 60 min. To these aliquots, 30 μ L of Na_2CO_3 (200 mM), 34 μ L of 6 $\times$ SDS sample treatment buffer (350 mM Tris–HCl, 10% SDS, 30% glycerol, and 175 mM bromophenol blue at pH 6.8), and 10 μ L of β -mercaptoethanol (β -ME) were added, mixed, and then boiled for 5 min. Controls included pepsin in SGF without added protein or protein samples in SGF that did not contain pepsin. The generation of proteolytic fragments as a result of pepsin digestion was examined by SDS-PAGE and immunoblot analysis.

Homology Model of 47 kDa Vicilin from Grass Pea. Using the three-dimensional structural model of the homotrimeric soybean β -conglycinin β subunit²⁷ as a template, a homology model of grass pea 47 kDa vicilin was built. The homology model was generated as a homotrimer and energy-minimized using the SWISS-MODEL website (<http://swissmodel.expasy.org>).²⁸ The stereochemistry and overall quality of the homology model were examined via the program Procheck.²⁹

RESULTS AND DISCUSSION

IgE Cross-Reactivity of Grass Pea Seed Proteins with Sera from Peanut-Allergic Subjects. Allergy to peanuts is a major public health concern in the United States. It has been estimated that about 2% of U.S. children have peanut allergy. The official allergen database (www.allergen.org) has recognized 12 peanut allergens, with two belonging to the cupin (Ara h 1 and Ara h 3), four belonging to the prolamin (Ara h 2, Ara h 6, Ara h 7, and Ara h 9), one belonging to the profilin (Ara h 5), one belonging to the Bet v 1 (Ara h 8), two belonging to the glycosyl transferase GT-C (Ara h 10 and Ara h 11), and two belonging to the scorpion-like knottin (Ara h 12 and Ara h 13) superfamilies.³⁰ Interestingly, peanut-allergic patients also develop clinically relevant sensitization to several different legumes, such as soybean, pea, lupin, and lentil.³⁰ Allergic reactions to consumption of legumes, such as lupin, lentil, chickpea, and pea, are commonly reported in Mediterranean and Asian countries. Sera from subjects with peanut and/or soybean allergy have been used to demonstrate IgE cross-reactivity with seed proteins from some of these legumes.^{30–33} To verify if the sera from peanut allergy patients can cross-react with seed proteins of grass pea, we performed

immunoblot analysis using sera from eight different patients with peanut allergies (Figure 1). As expected, peanut allergy sera from all eight patients recognized several allergens from the peanut protein extract. The sera also cross-reacted with both soybean and grass pea proteins. Sera from 6/8 peanut-allergic subjects strongly reacted against grass pea proteins, while no cross-reactivity was detected from the sera from another two patients. In the case of grass pea, a protein with an apparent molecular weight of 47 kDa cross-reacted with sera from most of the peanut-allergic subjects. In addition to 47 kDa immunodominant protein, IgE cross-reactivity against a few other proteins was also detected from grass pea protein extract (Figure 1).

Identification of Grass Pea 47 kDa IgE Binding Protein as β -Lathyrin. The molecular weight and relatively high abundance of grass pea 47 kDa IgE binding protein suggested that this protein may belong to the 7S vicilin family. Native vicilins are present as trimers with a molecular weight ranging from 150 to 210 kDa and are encoded by multigene families.³⁴ Vicilins fall into two groups: the first made of subunits with molecular weights of approximately 50 kDa, while the second group includes 60–70 kDa proteins.³⁴ These two groups of proteins are largely homologous, except that the first group bears a N-terminal extension of variable length, which results in higher molecular weights.³⁴ Vicilins are subjected to post-translational modifications (glycosylation and proteolysis), leading to variation in their subunit molecular weights. Most of these glycosylated proteins are potential allergens and are resistant to digestion and food processing.³⁵ In the case of grass pea, it has been previously shown that some grass pea 7S globulins are glycoproteins.¹² Therefore, we used Con A–Sepharose affinity chromatography to purify the 47 kDa IgE-binding protein from grass pea. When TBS-extracted grass pea proteins were loaded on a Con A column, several of the abundant seed proteins did not bind to the Con A column (Figure 2).

A 47 kDa protein, which was initially retained on the Con A column, was eluted with approximately 50 mM α -methylmannoside. SDS-PAGE analysis of the 50 mM α -methylmannoside-

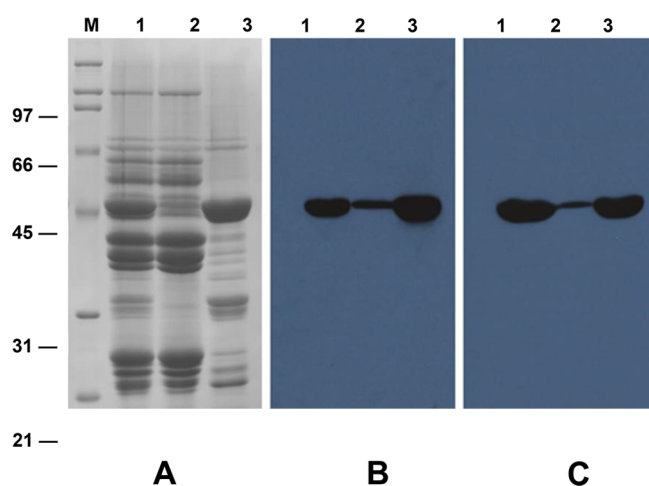


Figure 2. Purification of 47 kDa IgE-binding grass pea protein. (A) Grass pea protein were separated by 10% SDS–PAGE and visualized by staining with Coomassie Blue or electrophoretically transferred to nitrocellulose membrane and incubated with (B) pooled sera from several peanut-allergic patients or (C) soybean β -conglycinin antisera. Immunoreactive proteins were identified using goat anti-human IgE–horseradish peroxidase conjugate or anti-rabbit IgG–horseradish peroxidase conjugate, followed by chemiluminescent detection. Lane 1, TBS-extracted proteins from grass pea; lane 2, Con A–Sepharose unbound proteins; and lane 3, 50 mM α -methylmannoside-eluted proteins. The numbers on the left side of the figure indicate the sizes of protein standards in kilodaltons.

eluted protein fraction revealed an abundant protein with an estimated molecular weight of 47 kDa and several other less abundant proteins (Figure 2). Antibodies raised against the soybean β subunit of β -conglycinin, a glycoprotein, strongly reacted against the 47 kDa protein (Figure 2). Similarly, western blot analysis using pooled sera from peanut allergy patients also reacted strongly against the 47 kDa protein. We excised the IgE-binding SDS–PAGE-separated 47 kDa protein, digested it with trypsin, and subjected it to MALDI–TOF–MS (Table 1). Using Mascot, the empirically determined mass-to-charge ratios of the peptides were then compared to known peptides present in the National Center for Biotechnology Information non-redundant database. Three peptides from the 47 kDa protein gave statistically significant protein scores for matches with Len c 1, a major allergen from lentil seeds (Table 1). Similarly, the same three peptides gave significant molecular weight search (MOWSE) score matches against a hypothetical protein from clover (*Trifolium subterraneum*) and pea vicilin (Table 1). Blast analysis of this hypothetical protein

revealed significant amino acid sequence homology to vicilins from several legumes, *Medicago truncatula*, *Cicer arietinum*, *P. sativum*, and *G. max*, as well as convicilins from *Vicia narbonensis*, *P. sativum*, and *Lotus japonicas*.

Our laboratory is also analyzing the expression profiles of grass pea genes by RNA sequencing and have generated an in-house grass pea transcriptome database. A search of this database with *M. truncatula* vicilin sequences resulted in the identification of a transcript encoding 47 kDa β -lathyrin. The deduced amino acid sequence of identified cDNA encodes a truncated protein with an apparent molecular weight of 42 kDa. Bioinformatic analysis indicated that the grass pea β -lathyrin belongs to the cupin superfamily (Figure 3). Blast analysis revealed that grass pea β -lathyrin shares extensive amino acid sequence homology with 47 kDa vicilin from *P. sativum* and *M. truncatula*, a hypothetical protein from *M. truncatula*, allergen Len c 1 from *Lens culinaris*, and several other legume 7S globulin family members (Figure S1 of the Supporting Information). A search of the amino acid sequence of 47 kDa grass pea β -lathyrin revealed exact matches to several trypsin-digested peptide sequences generated from the 47 kDa IgE-binding peptide (Figure 3). On the basis of these analyses, the 47 kDa IgE binding protein from grass pea has been identified as β -lathyrin.

Carbohydrate Moiety of Grass Pea β -Lathyrin Is Not Responsible for the Immunoreactivity. Glycosylation is a common feature to many food allergens.³⁵ It is believed that glycan moieties may exert a stabilizing effect on the protein structure and enhance immunogenicity.^{36,37} Even though antibodies specific to carbohydrate determinants are commonly found, they appear not to have clinical relevance.³⁵ However, anaphylaxis induced by carbohydrate epitopes has been reported.³⁸ To evaluate the role of the carbohydrate moiety in IgE binding, partially purified grass pea β -lathyrin was subjected to deglycosylation (Figure 4).

The product of enzymatic deglycosylation was then analyzed by SDS–PAGE, which revealed two prominent proteins of 47 and 45 kDa proteins. The 47 and 45 kDa proteins presumably represent the glycosylated and deglycosylated forms of β -lathyrin, respectively (Figure 4). The removal of the carbohydrate moiety caused a decrease in molecular weight of β -lathyrin; protein corresponding to the same size was not detected in the control lane (Figure 4). Western blot analysis, using pooled sera from peanut allergy patients, revealed IgE binding to both native and deglycosylated proteins (Figure 4). This observation indicates that the carbohydrate moiety of β -lathyrin may not be critical for IgE binding. Interestingly, antibodies raised against the soybean β subunit of β -

Table 1. MALDI–TOF–MS Identification of the Grass Pea 47 kDa Protein

protein ID	peptides matched (sequences)		accession	(position) peptide matched	peptide score
	MOWSE	percent coverage			
allergen Len c	249	3 (3)	CAD87730.1	(16–28) R.FQTLFENENGHIR.L	71
1.0101, partial		10%		(219–230) K.SVSSSESEPFNLR.N	80
(<i>Lens culinaris</i>)				(368–386) R.NFLAGEEDNVISQIQRPVK.E	98
hypothetical protein	255	3 (3)	GAU31893.1	(41–53) R.FQTLFENENGHIR.L	78
TSUD_270780		9%		(245–256) K.SVSSSESEPFNLR.N	80
(<i>Trifolium subterraneum</i>)				(394–412) R.NFLAGEEDNVISQIQRPVK.E	98
vicilin OS	205	3 (3)	VCLC_PEA	(43–55) K.FQTLFENENGHIR.L	78
(<i>Pisum sativum</i>)		7%		(66–74) K.IFENLQNYRL	48
				(246–257) K.SVSSSESEPFNLR.S	80



Figure 3. (A) Schematic representation of grass pea β -lathyrin coding sequences showing cupin 1 domains and (B) amino acid regions that match with the trypsin-digested peptide sequences from the 47 kDa IgE-binding protein.

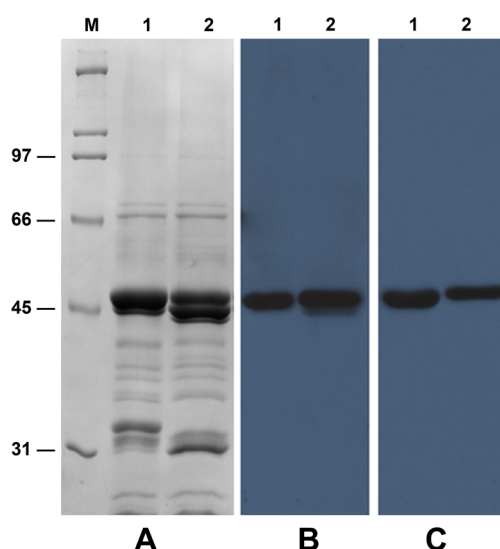


Figure 4. SDS-PAGE/immunoblot analysis of enzymatically deglycosylated grass pea β -lathyrin. (A) Partially purified native (lane 1) and deglycosylated (lane 2) grass pea β -lathyrins were separated by SDS-PAGE on a 10% gel and stained with Coomassie Blue or electrophoretically transferred to nitrocellulose membrane and challenged with (B) pooled sera from peanut-allergic patients or (C) soybean β -conglycinin antisera. Immunoreactive proteins were identified using goat anti-human IgE-horseradish peroxidase conjugate or anti-rabbit IgG-horseradish peroxidase conjugate, followed by chemiluminescent detection. The numbers on the left side of the figure indicate the sizes of protein standards in kilodaltons.

conglycinin (which is also a glycoprotein) recognized native but not deglycosylated β -lathyrin (Figure 4). The reason why soybean β -conglycinin-specific antibodies did not react against deglycosylated β -lathyrin is not clear. It is likely that soybean β -conglycinin antibodies are mainly targeted to the core oligosaccharides covalently attached to β -conglycinin and β -lathyrin. Soybean β -conglycinin contains N-linked mannose-containing core oligosaccharides,³⁹ and the strong cross-reactivity of the soybean β -conglycinin antibodies against β -

lathyrin indicates the presence of similar core oligosaccharides bound to grass pea β -lathyrin.

Grass Pea β -Lathyrin Is Susceptible to Pepsin Digestion. It is generally believed that food allergens can survive the acidic gastric environment because they are resistant to pepsin digestion.⁴⁰ On account of this, digestion assays in SGF are routinely employed to predict the allergenic potential of food proteins. An examination of the stability of 25 different food proteins to pepsin digestion demonstrated that allergens have greater stability to protease digestion.²⁶ To examine if grass pea β -lathyrin is also stable to digestion, partially purified proteins were subjected to pepsin digestion *in vitro* (Figure 5).

Incubation of β -lathyrin with pepsin resulted in rapid breakdown of mature β -lathyrin and the appearance of proteolytic products within 30 s. By the end of 60 min, β -lathyrin was completely digested (Figure 5). Western blot analyses using antibodies raised against soybean β -conglycinin antibodies or pooled sera from peanut allergy patients failed to detect either mature convicilin or the breakdown products after 1 min of pepsin digestion (Figure 5). Earlier studies have shown that soybean Kuntiz trypsin inhibitor (KTI), a potent allergen capable of inducing food anaphylaxis,⁴¹ has demonstrated remarkable stability to both thermal and acid denaturation.⁴² For comparison, we examined the stability of KTI to pepsin digestion under the experimental conditions used to test the stability of β -lathyrin. Unlike grass pea β -lathyrin, soybean KTI was stable even up to 60 min of digestion using pepsin (Figure 5). However, resistance to pepsin digestion alone cannot be used as criteria for predicting the allergenicity of a protein. Previous studies have established that some well-known allergens are also digested by pepsin within a few seconds.^{43,44}

Identification of IgE-Binding Epitopes on the Modeled Structure of Grass Pea β -Lathyrin. A homology model structure of grass pea β -lathyrin was generated using the X-ray crystal structure of the soybean β -conglycinin β -subunit (SWISS-MODEL Template Library ID 1pk.1.A) core domain (residues 49–397) as a template.⁴⁵ The core domain of the

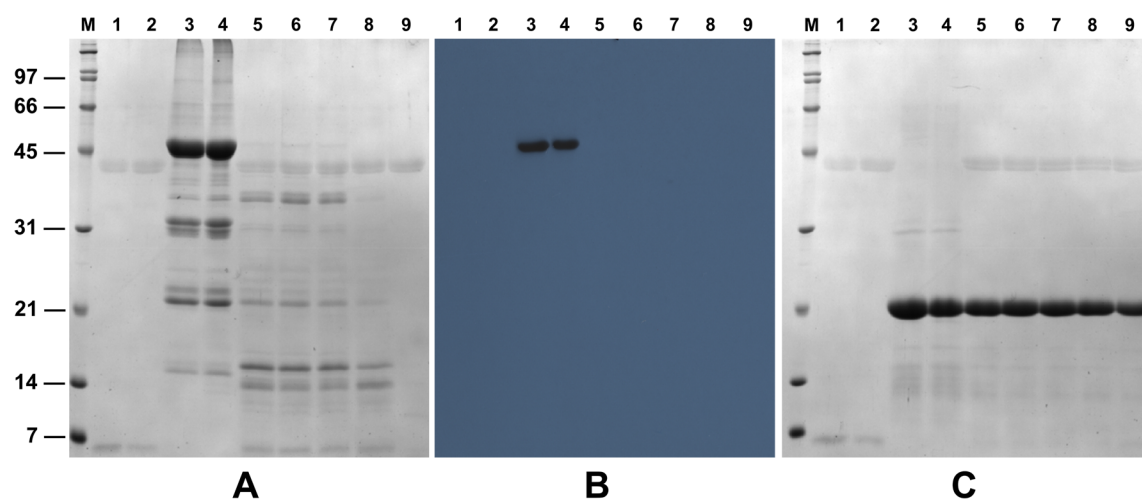


Figure 5. SDS–PAGE analysis of pepsin-digested grass pea β -lathyrin and soybean Kunitz trypsin inhibitor. (A) Partially purified grass pea β -lathyrin and (C) commercially purchased Kunitz trypsin inhibitor were digested with pepsin for 0.5, 1, 5, 30, and 60 min, and the products were fractionated by SDS–PAGE on a 15% gel and stained with Coomassie Blue. (B) Grass pea pepsin-digested β -lathyrin was electrophoretically transferred to nitrocellulose membrane and challenged with pooled sera from peanut-allergic patients. Immunoreactive proteins were identified using goat anti-human IgE–horseradish peroxidase conjugate or anti-rabbit IgG–horseradish peroxidase conjugate, followed by chemiluminescent detection. The numbers on the left side of the figure indicate the sizes of protein standards in kilodaltons. Lane M, protein markers; lane 1, pepsin in the absence of β -lathyrin at 0 time point; lane 2, pepsin in the absence of β -lathyrin at 60 min time point; lane 3, β -lathyrin in the absence pepsin at 0 time point; lane 4, β -lathyrin in the absence pepsin at 60 min time point; and lanes 5, 6, 7, 8, and 9, pepsin-digested protein samples that were removed at 0.5, 1, 5, 30, and 60 min, respectively.

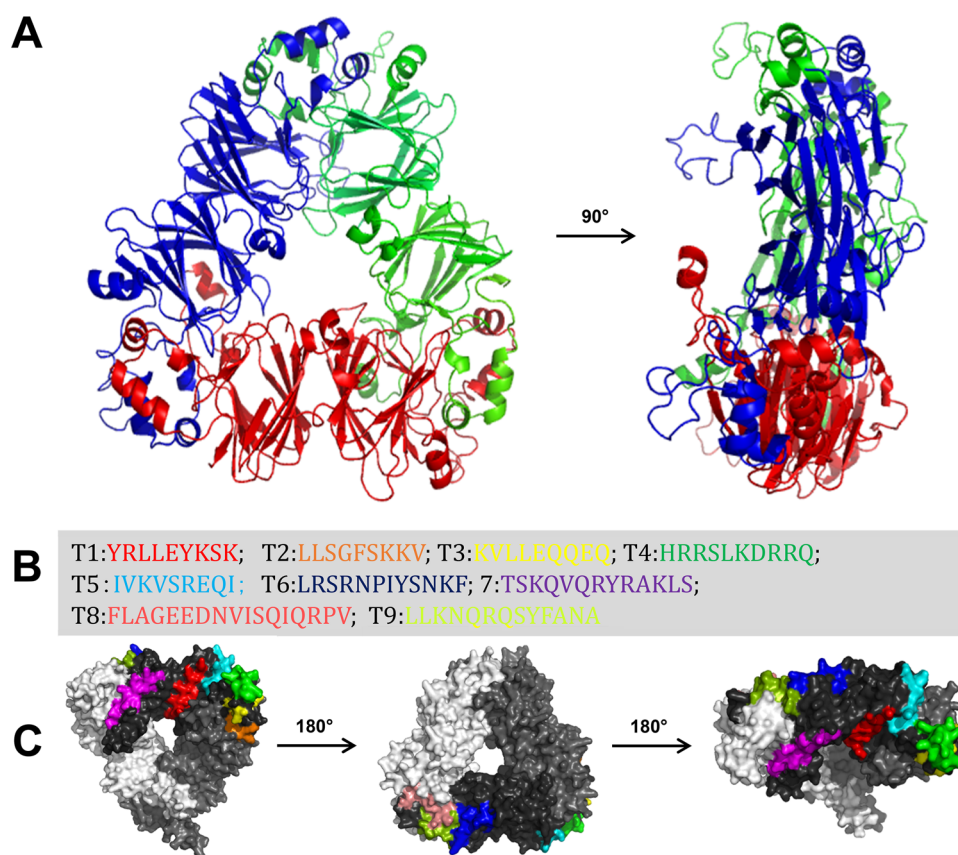


Figure 6. Homology-modeled structure of grass pea β -lathyrin. (A) Trimeric grass pea β -lathyrin is shown in a ribbon diagram with each monomer colored in red, blue, and green. (B) Potential IgE-binding epitopes of β -lathyrin. (C) Structural features corresponding to the nine predicted antigenic epitopes, which are colored following the color code shown in panel B.

soybean β -conglycinin β subunit and grass pea β -lathyrin share 59.4% amino acid sequence identity over the region included in the X-ray structure. The global model quality estimation

(GMQE) and qualitative model energy analysis (QMEAN) scores were 0.75 and -1.38 , respectively, which suggested good quality of the homology model structure. Figure 6 shows

the ribbon diagrams of grass pea β -lathyrin. The modeled structure of grass pea β -lathyrin is consistent with those of vicilin family members.⁴⁵ The grass pea β -lathyrin model structure is very similar to the structures of the 7S seed storage proteins from soybean,⁴⁵ peanut,^{46,47} adzuki bean,⁴⁸ mung bean,⁴⁹ kidney bean,⁵⁰ jack bean,⁵¹ and pecan.⁵² IgE-binding epitopes within grass pea β -lathyrin was also identified using the AlgPred server.⁵³ This computational method identified a single IgE-binding epitope (ELHLLGFGIN) and predicted grass pea β -lathyrin as an allergen. Similarly, a BLAST search of β -lathyrin against 2890 allergen-representative peptides also confirmed the existence of an allergenic representative peptide (PHFNSKAMVIVVVNKG TGNLELVA) in β -lathyrin. The NetMHCII 2.2 server was used to measure the binding ability between β -lathyrin and MHC II, and results suggested that β -lathyrin contains nine potential IgE-binding epitopes. These potential epitopes, named T1–T9, are highlighted by different colors in the monomers (Figure 6). Epitopes T2, T6, T7, and T8 were located in the conserved domain of cupin superfamily proteins (Figure 3). Epitope T8 is common in other legume allergens, such as Ara h 1, Gly m 5, Len c 1, Lup an 1, Pis s 2, and Vig r 2. In contrast, T4 is only shared with Len c1. Epitope T2 was specific for β -lathyrin and was not found in other species. The conservation of highly similar IgE-binding epitopes among different legume allergens may explain the IgE cross-reactivity of sera from peanut-sensitive patients to grass pea β -lathyrin.

In conclusion, we have identified grass pea β -lathyrin as a potential allergenic protein. Most of the IgE-binding epitopes of β -lathyrin identified in this study were found to be in common with other legume allergens and located on the predicted protein surface. The conservation of IgE-binding epitopes among several distinct legume allergens may be responsible for the observed phenomenon of cross-reactivity. Additional studies, leading to a better understanding of allergenicity potential of grass pea β -lathyrin, will aid in the design and development of effective immunotherapy strategies for the diagnosis and treatment of grass-pea-related food allergy.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b02314.

Amino acid sequence alignment of grass pea β -lathyrin with several other legume 7S globulin family members (Figure S1) (PDF)

Phylogenetic analysis of known food allergens (Figure S2) (PDF)

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Funding

This research was supported by the Agricultural Research Service (ARS), United States Department of Agriculture (USDA), the Chinese Universities Scientific Fund (2014YB040), the China Postdoctoral Science Foundation (2016M590975), and the Postdoctoral Science Foundation of

Shaanxi province (2016BSHEDZZ119), People's Republic of China.

Notes

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The authors declare no competing financial interest.

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