

Epitopes from two soybean glycinin subunits are antigenic in pigs

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Abstract

BACKGROUND: Glycinin is a seed storage protein in soybean (*Glycine max*) that is allergenic in pigs. Glycinin is a hexamer composed of subunits consisting of basic and acidic portions joined by disulfide bridges. There are five glycinin subunit isoforms designated Gy1–Gy5. The purpose of this study is to identify epitopes from selected glycinin subunits that are antigenic in pigs.

RESULTS: Twenty-seven out of 30 pigs had antibodies against glycinin in their sera. Ten of these sera had immunoglobulin G (IgG) against the Gy4 (A5A4B3) or Gy1 (A1aBx) subunit. Three sera recognised overlapping regions between the two subunits tested, though no serum stained both A5A4B3 and A1aBx. Two sera stained a highly conserved region between A5A4B3 and A1aBx, though again neither serum stained both peptides. The basic part of the A1aBx subunit was not recognised by any of the sera tested even though immunoblot data indicated that the basic and acidic subunits of glycinin are nearly equally antigenic.

CONCLUSION: Two antigenic regions of A5A4B3 and A1aBx were identified that bound antibodies in half of the sera that reacted with these two proteins. Half of the sera reacted with unique regions of A5A4B3 and A1aBx. The failure of the basic portion of A1aBx to bind pig antibodies may indicate that it is less antigenic than the basic portion of A5A4B3 and other glycinin subunits. Published 2013. This article is a U.S. Government work and is in the public domain in the USA.

Supporting information may be found in the online version of this article.

Keywords: A1aBx; A5A4B3; allergen; antibody; ELISA; seed storage protein

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) meal supplements the protein content of food for humans and feed for pets, poultry, swine and cattle. In 2010, over 90 million metric tons (valued at almost US\$39 billion) were harvested, with over 24 million metric tons going to the feed industry.¹ Although soybean meal is a valuable protein supplement, soybean proteins have also been shown to be allergens. Allergic responses to soy protein in mammals are associated with skin rash, gastrointestinal problems, rhinitis, asthma, hypotension and anaphylaxis.² Around 5–8% of infants and 2% of adults are allergic to soy protein.³ The incidence of soy allergy in adults varied among 12 countries, ranging from a low of 0% in Iceland to 4.7% in the USA.⁴ Inclusion of soybean proteins in the postweaning diet of pigs resulted in a growth lag and changes in intestinal villi associated with malabsorption.^{5–7} The presence of immunoglobulin G (IgG) against soybean proteins in the serum of pigs fed soybean meal indicated that the growth lag is associated with an allergy to soy proteins. In addition to allergies to soy, pigs and humans also have a similar gastrointestinal physiology and are born immunoincompetent.⁸ These observations have allowed pigs to be used as a model system to study allergies in humans.

At least 16 soybean proteins cause allergies in humans.⁹ Among the best characterised soy protein allergens are the seed storage proteins (SSPs), which constitute 40–60% of proteins present in soybean meal. The soybean SSPs include conglycinin and glycinin. Conglycinin is a 7S trimer composed of different combinations of β , α and α' subunits.¹⁰ Analyses of the antigenic properties of the α subunit have identified an immunogenic

epitope capable of causing antibody productions in pigs.¹¹ Our analyses have identified a second antigenic epitope in the α subunit of conglycinin in pigs (personal communication). Glycinin is an 11S hexamer in which each subunit is composed of an acidic and a basic region. Glycinin genes include acidic and basic subunits joined by disulfide bonds, which are post-translationally cleaved into separate peptides. There are five reported major glycinin isoforms.¹² The five isoforms are divided into two groups based on similarity: group 1 consists of Gy1, Gy2 and Gy3 and group 2 consists of Gy4 and Gy5.¹² The aim of this study is to identify epitopes in selected glycinin subunits that are antigenic when fed to pigs. Characterisation of these antigenic epitopes may allow identification of less immunogenic variants in the *Glycine* germplasm. We identify the antigenic epitopes of a glycinin subunit from group 1 (A1aBx) and group 2 (A5A4B3) using pig sera.^{12,13} We focus on epitopes that bind pig IgG, because the required labelled antibodies capable of robustly labelling pig IgE are not available. One of the antigenic regions may be conserved between different glycinin genes, and some appear to be unique. These antigenic epitopes are likely to contribute to the allergenicity of soy proteins. The distribution of the antigenic

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epitopes of glycinin suggests soybean-breeding strategies for reducing allergenic glycinin subunits in soybean.

EXPERIMENTAL

Animals

Thirty pigs (Camborough-22, PIC, Hendersonville, TN, USA) were used during pregnancy and subsequent lactation periods. Pigs were fed diets containing corn as a major energy source and soybean meal as a major protein source. The gestation diet contained 122 g kg⁻¹ crude protein (CP) and 3.1 Mcal metabolisable energy (ME) kg⁻¹ and the lactation diet contained 192 g kg⁻¹ CP and 3.3 Mcal ME kg⁻¹. Diets were formulated according to nutrient requirements of swine.¹⁴ Sows were limited throughout gestation, receiving 2 kg day⁻¹ (as-fed basis) of the gestation diet. On day 109 of gestation, sows were moved into farrowing crates. After farrowing, sows were given *ad libitum* access to the lactation diet. Fresh feed was provided three times, at 08:00, 12:00 and 17:00, and the amount given at each meal was based on how much feed was consumed.

Sampling of porcine serum

Blood samples (9 mL) were taken from 30 pigs during their pregnancy (days 30 and 110 of pregnancy) and subsequent lactation (day 21 of lactation). The average body weight of pigs at day 30 of pregnancy was 184.4 ± 7.2 kg and the average parity was 1.74 ± 0.94. Blood was collected from the jugular vein using 10 mL syringes and disposable 16-gauge × 0.1 mm hypodermic needles (Air-Tite Product Co. Inc., Virginia Beach, VA, USA). Blood was then centrifuged at 5000 × *g* for 15 min at room temperature, and serum was separated and stored at -80 °C for analysis. The protocol of this study was approved by North Carolina State University Animal Care and Use Committee.

Analysis of glycinin

Glycinin was purified by the method of Kwanyuen *et al.*¹⁵ The fraction enriched in glycinin was separated on 125 g L⁻¹ sodium dodecyl sulfate (SDS) polyacrylamide gel using a Mini-Protean system (Bio-Rad, Hercules, CA, USA) following the manufacturer's instructions. Each gel included one lane of broad-range prelabelled molecular weight standards and a single long well of the enriched glycinin in SDS buffer. The fractionated protein was transferred to nitrocellulose paper using a Bio-Rad Mini-Trans-Blot cell following the manufacturer's instructions. Transfer of the labelled molecular weight standard was confirmed visually. A Bio-Rad manifold allowed the use of up to 18 different sera to stain each protein blot. Briefly, the blots were incubated in 100 mL of blocking solution (TTBS (0.1 mol L⁻¹ Tris, pH 7.5–8, 9 g L⁻¹ NaCl, 0.5 mL L⁻¹ Tween 20), 10 g L⁻¹ non-fat dry milk) for 1 h with gentle agitation at room temperature. The blots were clamped into the manifold, and blocking solution was added to each channel to confirm that the apparatus did not leak. Each channel to be utilised was evacuated and the pig serum diluted 1:50 (v/v) in blocking solution was added and incubated overnight at 4 °C with gentle agitation. The channels were evacuated and rinsed three times for 15 min with blocking solution. To immunostain the proteins that reacted with pig IgG, alkaline phosphatase (AP)-labelled rabbit anti-swine IgG (Sigma, St Louis, MO, USA) was diluted (1:2000 v/v) in blocking solution and added to each channel. A control with no primary serum was included. Note that it was critical to confirm that each batch of secondary antibodies was free of antibodies to soybean proteins.

The blot was gently agitated for 1 h. The channels were evacuated and rinsed three times for 15 min with blocking solution and three times with TTBS. The blot was removed from the manifold and rinsed three times with 50 mL of TBS (0.1 mol L⁻¹ Tris, pH 7.5–8, 9 g L⁻¹ NaCl) for 30 min. The bands were visualised by staining with an AP conjugate substrate kit (Bio-Rad). Images were captured on a FOTO/Analyst Investigator (Fododyne, Hartland, WI, USA).

An enzyme-linked immunosorbent assay (ELISA) was performed by drying 10 µg of the purified glycinin overnight in a 96-well plate. The wells were blocked three times for 30 min with blocking solution. A 100 µL aliquot of pig serum diluted 1:50 (v/v) in blocking solution was added to each well and incubated overnight. Each serum was represented in triplicate on the plate. The wells were rinsed with blocking solution, then a 1:1000 (v/v) dilution of AP-labelled rabbit anti-pig IgG was added to each well. The plate was incubated at room temperature for 1 h and rinsed three times for 15 min with blocking solution, TTBS and TBS. A control with no primary serum was included to assess the background due to the labelled antibody. A Bio-Rad colorimetric kit was used to quantify the pig IgG bound to the samples by reading the absorbance on a Bio-Rad 680 microplate reader following the manufacturer's instructions.

Analysis of peptide library

A set of peptides was designed to represent the A5A4B3 (gi:56201482) and A1aBx (gi:225651) glycinin proteins. The peptides represent 16 amino acids with an 11-amino-acid overlap (Mimtopes, Raleigh, NC, USA). Detailed information about the peptides used in this study is shown as supplementary data 1 (see 'Supporting information'). The first peptide from the amino end of the protein was biotinylated at the carboxyl end. The remaining peptides had a biotinylated amino end extension of GSGS. The peptides were dissolved in PBST-azide (phosphate-buffered saline (PBS), 0.1 g L⁻¹ Tween 20, 1 mL L⁻¹ sodium azide). A 1 ng portion of each peptide was bound for 1 h to a streptavidin-coated 96-well plate that was blocked for 1 h with 200 µL of blocking solution. Each plate included a positive and a negative control peptide provided by the manufacturer of the peptide library. Each well was rinsed four times with 250 µL of PBS and dried at 37 °C for 1 h. Plates were stored in a refrigerated desiccator for up to 2 weeks. Peptide plates were immunostained as described for protein plates. The positive and negative control peptides were stained with serum provided by Mimtopes and stained as expected. Initial screening was unreplicated. Putative positives were rechecked in triplicate and a contiguous non-reacting peptide was included in triplicate on the same plate. Averages and standard deviations were calculated with Microsoft EXCEL (Redmond, WA, USA).

Competition of peptide with total protein

An ELISA was performed as described previously, but the serum was preincubated for 1 h with the addition of 25–0 ng of the active peptide for that particular serum. A mixture of ten non-reactive peptides at the same concentrations was used as a negative control.

RESULTS

Identification of swine sera with antibodies against glycinin

The ELISA identified sera from swine that contained IgG against glycinin (Table 1). Sixty-eight sera from 30 animals were collected 21 days after parturition, 30 days into pregnancy and 110 days into

Table 1. ELISA to identify pig sera harvested during pregnancy (30 and 110 days) or lactation (21 days) that contain IgG against glycinin

Serum	Day	Ave ^a	SD ^a
R101 30	30	0.08	0.00
R160 30	30	0.39	0.00
R186 30	30	0.51	0.00
R112 110	110	0.14	0.00
R117 21	21	0.44	0.02
R142 110	110	0.15	0.00
R157 30	30	0.05	0.01
R158 30	30	0.09	0.02
R164 110	110	0.16	0.03
R165 21	21	0.15	0.00
R169 110	110	0.17	0.00
R172 110	110	0.40	0.03
R173 30	30	0.28	0.02
R177 30	30	0.31	0.01
R181 30	30	0.33	0.01
R188 30	30	0.28	0.02
R29 21	21	0.17	0.01
R600 21	21	0.40	0.00
R65 30	30	0.38	0.00
R63 110	110	0.35	0.02
R76 21	21	0.22	0.00
R28 110	110	0.19	0.01
R95 21	21	0.11	0.01
R97 110	110	0.10	0.01
Y187 110	110	0.10	0.00
Y199 30	30	0.25	0.01
Y201 30	30	0.33	0.00
R179 30	30	0.45	0.03
R163 30	30	0.24	0.00
R187 110	110	0.12	0.01

^a Values are average (Ave) and standard deviation (SD) of three samples.

pregnancy unless noted otherwise. ELISA values were consistent in an individual animal regardless of the time point, so only one time is shown in Table 1. Sera from 27 pigs were determined to have antibodies that bind glycinin. Pigs naïve to soybean meal were not available, so it was also important to identify sera that did not react against the 11S SSP. Two sets of sera (R101 and R157) with the lowest ELISA values were not significantly above the negative control (no primary serum) at all time points tested.

Identification of sera that stain acidic and/or basic subunit of glycinin

The ELISA measures antibody IgG binding to either the basic or the acidic subunit of the 11S SSP. Protein blots of the glycinin-enriched protein fraction were stained with the pig sera to determine which subunit was immunoreactive (Fig. 1). R177 was the only serum that clearly stained both the acidic and basic peptides. Five sera (R182, R142, R188, R157, R183) only stained an acidic portion of glycinin and five sera (R174, R20, R65, R186, R76) only stained a basic portion of glycinin (Fig. 1). Staining of the acidic subunit by R173 was ambiguous. Overall, the acidic and basic regions of glycinin are equally antigenic. The failure of sera positive for glycinin staining in the ELISA to stain proteins on the blots may reflect the greater sensitivity of the ELISA or indicate that some epitopes

are destroyed by denaturation with SDS. R157 is an exception to the suggested greater sensitivity of the ELISA, because, while it did not immunostain a protein in the ELISA, it did stain a band on the protein blot. Therefore R157 is not suitable as a negative control for these analyses. However, serum R101 failed to stain any protein, consistent with the absence of antibodies against total glycinin observed in the ELISA (Fig. 1), and is a suitable negative control for subsequent experiments.

Identification of antigenic epitopes of A5A4B3 and A1aBx glycinin subunits

The tiled peptide array representing the mature A5A4B3 and A1aBx glycinin proteins was used to identify the antigenic epitopes that bind pig IgG from individual animals. Included on the plate were positive and negative control peptides provided by the array manufacture. In all cases, these controls gave expected results (data not shown). Initial screens were unreplicated to identify sera likely to have IgG against a peptide(s) in the array (data not shown). Serum R101 failed to immunostain any peptide on the array, as expected. Putative positives were tested in triplicate with a contiguous non-reactive peptide included as a negative control. The replicated data are reported in Table 2. Serum was pooled from multiple time points from individual pigs for the epitope mapping, because the ELISA of total glycinin protein, the protein blot analyses and the initial epitope mapping indicated that all time points collected from the same individual gave similar results. Ten sera contained IgG that bound specific peptides present in the A5A4B3 and A1aBx glycinin proteins. Six sera stained A5A4B3 and five sera stained A1aBx. One serum, Y199, bound epitopes in both A5A4B3 and A1aBx. No serum, including Y199, stained conserved regions shared between the two proteins, indicating that antigenic sites are not well enough conserved to cross-react. Remaining sera that stained glycinin in the ELISA and/or on the protein blots but failed to stain peptides on the array may stain nonlinear epitopes or other glycinin proteins that do not share conserved antigenic epitopes with A5A4B3 or A1aBx.

An alignment of A5A4B3 and A1aBx with the regions that bound pig IgG highlighted is shown in Fig. 2. Three sera stained an overlapping region of the mature proteins in the acidic regions. Two sera stained a common region in both A5A4B3 and A1aBx. The remaining sera stained regions unrecognised by other sera. The basic subunit of A1aBx was not antigenic for these pigs, while two pigs produced IgG against the basic subunit of A5A4B3.

We wanted to determine if the linear peptides could compete with total protein for binding IgG. For these experiments we used a 1:400 (v/v) dilution of selected sera, because total signals of the ELISA were very dependent on serum concentrations in this range of dilutions (data not shown). We selected sera for the inhibition assays that stained only one epitope on the ELISA of the peptide array. Up to 25 ng of the reactive peptide was incubated with the serum for 1 h prior to being added to the well for the ELISA of the 11S SSP. Higher concentrations of the reactive peptide and higher concentration of the mix of ten non-reactive peptides that served as the negative control often produced unacceptable backgrounds. Preincubation of sera R600 and R181 with peptides 140 and 146 respectively resulted in a reduction in IgG binding to glycinin relative to mock incubation (no peptide included in the preincubation) and preincubation with non-reactive peptides (Table 3). It is unclear why preincubation of R600 with the non-reactive peptides resulted in a higher signal than the mock incubation control. Preincubation of R172, R82 and

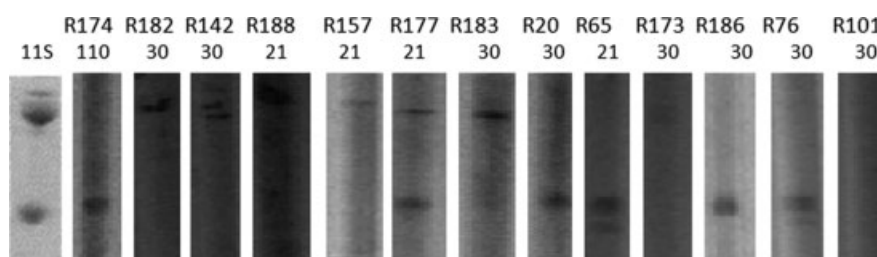


Figure 1. Selected sera from the indicated time points (days) were used to stain a glycinin protein blot. The lane labelled 11S represents the proteins gel stained with Coomassie blue to show the positions of the acidic (slower migrating band) and basic (faster migrating band) subunits.

Table 2. ELISA of selected pig sera that bind peptides from A5A4B3 and A1aBx subunits of glycinin

Serum	Positive peptide	Ave (SD) ^a	Negative peptide ^b	Ave (SD) ^a	Difference ^c
<i>A5A4B3</i>					
R28	99	0.26 (0.04)	98	0.14 (0.00)	0.12
R172	118	0.58 ((0.06)	117	0.21 ((0.03)	0.37
R600	140	0.34 (0.05)	141	0.20 (0.00)	0.14
R181	146	0.49 (0.07)	147	0.29 (0.03)	0.20
R76	165	0.26 (0.02)	164	0.15 (0.01)	0.11
R76	166	0.24 (0.01)	164	0.15 (0.01)	0.09
Y199	169	0.34 (0.03)	170	0.10 ((0.02)	0.24
<i>A1aBx</i>					
R29	6	0.33 (0.02)	8	0.18 (0.01)	0.15
R29	7	0.41 (0.01)	8	0.18 (0.01)	0.23
Y199	41	0.48 (0.09)	42	0.14 (0.01)	0.34
R179	41	0.23 (0.03)	42	0.12 (0.01)	0.11
R163	47	0.24 (0.02)	48	0.12 (0.00)	0.12
R173	6	0.24 (0.00)	8	0.11 (0.01)	0.13
R173	7	0.25 (0.01)	8	0.11 (0.01)	0.14

^a Average (Ave) of three samples is reported with standard deviation (SD) in parentheses.

^b A contiguous unreactive peptide is included for each positive peptide.

^c Difference between ELISA values of positive and negative peptides.

R76 with either the reactive or the non-reactive peptides failed to reduce binding of IgG in these sera to glycinin.

DISCUSSION AND CONCLUSION

The ELISA of 30 pig sera identified 27 that contained IgG against glycinin, confirming that glycinin is antigenic in pigs. Only 18 of these sera stained glycinin on a protein blot, probably reflecting the greater sensitivity of the ELISA. Screening a tiled peptide array representing the A5A4B3 and A1aBx glycinin subunit proteins identified ten sera that stained epitopes in these two glycinin proteins. Three sera stained two consecutive peptides on the array to identify two different epitopes. Assuming that these epitopes bind a single IgG in a given serum, we calculate that they are between seven and 11 amino acids long. The remaining epitopes are between 12 and 16 amino acids long. Three sera stained a region conserved between A5A4B3 (LIQTWNSQHPELKAGVTISK) and A1aBx (LIETWNPNNKPFQCGVALSR), though no serum stained the same region in both proteins. Two sera stained an overlapping core region conserved between A5A4B3 (IVTEGGLSVI) and A1aBx (IVTKGGLSVI). It is unknown if these regions are inherently more antigenic than other peptides or if some unknown factor (such as resistance to degradation in the gastrointestinal tract) causes an apparent increase in antigenicity.

The epitopes that bind pig IgG identified in this study overlap or are near epitopes that bind human IgE from individuals allergic to soy.^{16–18} There is also substantial alignment between the epitopes that bind pig IgG in these studies and the antigenic epitopes of the peanut Arah3 allergen. Figure 2 includes alignments of A5A4B3 and A1aBx with three of four of the allergenic epitopes from the peanut allergen Arah3 that are associated with peanut allergies in humans.¹⁹ These three epitopes overlap regions that are antigenic in pigs, indirectly supporting a role for these epitopes in allergic responses. Eight of the ten amino acids demonstrated to be important in binding IgE from allergic individuals are conserved in one or both soybean glycinin subunits. The antigenic epitope (IVTEGGLSVISPKWQE) is shared with the A3B4 glycinin isoform (BAB15802). The remaining antigenic regions of A5A4B3 and A1aBx that overlap the peanut allergenic epitopes are 87–67% conserved in other glycinin isoforms (NP_001236840.1 and NP_001235810, data not shown); therefore it is unknown if these epitopes are conserved enough to be similarly antigenic.

Protein blot analyses indicated that the acidic and basic portions of glycinin subunits were equally stained by the pig sera. One serum, R76, that stained the basic glycinin subunit on the protein blot also recognised a peptide in the basic region of A5A4B3 on the peptide array, as expected. Serum Y199 also stained a peptide in the basic portion of A5A4B3. However, we note that the basic portion of A1aBx did not bind IgG from any of the pig sera.

Acidic subunits

A1aBx FSSREQPQNECQIQKLNALKPDNRIESEGGLIETWNPNNKPFQCAGVAL
 A5A4B3 FAISSS-KLNECQLNNLNAL~~EP~~DHRVESEGGLIQTWNSQHPELKCAGTV
 AraH3 IETWNPNNQEFECAG

 A1aBx SRCTLNRRNALRRPSYTNGPQEIYIQQKGIFGMIYPGCPSTFEPEPQ-QPQ
 A5A4B3 SKLTLNRRGLHLPSPSPYPRMIIIAQKGKALGVAIPGCPETFEPEPQESN

 A1aBx QRGQSSRPQ---DRHQKIYNFREGDLIAVPTGVAWWMYNNEDTPVVAVSI
 A5A4B3 RRGSRSQKQLQDSHQKIRHENE~~GD~~VLVIPPVGPYWTYNTGDEPVVAISL

 A1aBx IDTNSLENQLDQMPRRFYLAGN---QE~~Q~~EFLKYQ~~Q~~EGGHQSOK-GKHQQ
 A5A4B3 LDTSNFNQLDQTPRVFYLAGNPDI~~E~~YPTMQQQQQKSHGGRKQGGHQQ

 A1aBx EEENE~~G~~GSILSGFTLEFLEHAFSV~~DKQIAKN~~LOGENEGEDKGAIVTVKGG
 A5A4B3 EEEEE~~G~~GSVLSGFSKHFLAQSE~~NTN~~EDIAEKLS~~PDDE~~--RKQIVTVEGG
 AraH3 GNIFSGFTPEFLEQA VTVRGG

 A1aBx LSVIKPPTDEQQQRPQE~~EEEEDE~~KP-----QCLGKDKHC
 A5A4B3 LSVISPKWQEQQDEDEDEDEDEDEQIPSHPPRRPSHGKREQDEDEDEDE
 LRLSPDRK

 A1aBx QRPRGSQ-SKSRRN-----
 A5A4B3 DKPRPSRPSQKREQDQDQDEDEDEDEQPRKSREWRSKKTQPRRPRQEE

 A1aBx -----
 A5A4B3 PRERGCETRN

Basic subunits

A1aBx GIDETICTMRIRHNIGQTSSPDIIYNPQAGSVTTATSLDFPALS~~WRL~~LSAE
 A5A4B3 GVEENICTLKLHENIARPSRADFYNP~~KAGRIST~~LN~~SLTLPAL~~RQFOLSAQ

 A1aBx, FGS~~LR~~KNAMEFVPHYNLNANSIIYALNGRALIQV~~VNC~~NGERVF~~DGEL~~QEGR
 A5A4B3 YV~~VLY~~KNGIYSPHWNLNANSVIYVTRGQGV~~VNC~~QGN~~AVD~~GELRRGQ

 A1aBx, VLI~~VP~~QNFVVAARSQSDNFEYVSFKTNDTPMIGTLAGANSLLNALPEEVI
 A5A4B3 LLV~~VP~~QNFVVAEQAGEQGF~~EY~~IVFKTHHNAVTSYLK---DVFR~~AIP~~SEVL

 A1aBx, QHTFNLKSQARQIKNNNP~~FK~~FLVPPQESQ---KRAVA
 A5A4B3 AHSYNLRQSQVSELKYEGNWG~~PLVN~~PESQ~~Q~~SPRVKVA

Figure 2. Alignment of A1aBx and A5A4B3 from soybean and allergenic regions of AraH3 from peanut. Identical regions of A1aBx and A5A4B3 are highlighted. Underlined regions were recognised by one serum. Underlined and bold regions were recognised by two sera. Sera that recognised consecutive peptides were assumed to bind the overlapping region. The amino acids double-underlined in AraH3 are amino acids important in binding IgE from allergic individuals.

Binding of IgG from three sera to glycinin was reduced by preincubation with the reactive peptide, consistent with the antibody recognising a linear epitope. Binding of the IgG from the three sera was not inhibited by the reactive antibody. While this result is inconsistent with the expectations of a linear antigenic epitope, it is possible that the sera in question bound other glycinin peptides. It is also possible that the glycinin bound to the plate was able to out-compete the synthetic peptide because it could fold into its native conformation. Differences between the ability of antigenic epitopes of glycinin to bind human IgE in ELISA and immunoblotting assays have been attributed to protein folding, though trivial causes such as buffer conditions could not be eliminated.¹⁶ The linearity of all these antigenic epitopes cannot be resolved by these experiments.

Soybean proteins were acknowledged as important food allergens in the Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108–282, Title II), which requires that

the content of eight common food allergens, including soy, be listed on food labels to warn allergic individuals. Identification and characterisation of common allergenic epitopes will allow natural and synthetic variants that alter or reduce these allergenic sites to be used in soybean breeding. For example, genetic variants of SSPs that alter immunogenic epitopes identified using tiled peptide arrays may prove less allergenic. Additionally, knowledge of the relative antigenicity of glycinin proteins and subunits may allow breeding for varieties lower in the most allergenic glycinin proteins. For example, soybean accessions with higher levels of A1aBx in which the basic subunit appears less antigenic may prove less allergenic. These experiments represent a step in the continuing effort to characterise soybean allergens. Identification of these antigenic epitopes of glycinin provides valuable targets for plant scientists to reduce allergic responses to soybean proteins in pigs and other animals as well if the same epitopes are antigenic in other species.

Table 3. Ability of reactive peptide or mixture of non-reactive peptides (0–25 ng) to reduce binding of IgG in selected sera to glycinin in an ELISA^a

Serum	Peptide	25 ng	12.5 ng	6.25 ng	3.13 ng	1.56 ng	0 ng
R82	99	1.06 (0.03)	0.96 (0.07)	1.00 (0.03)	1.00 (0.08)	1.00 (0.07)	1.00 (0.06)
R82	Neg	0.99 (0.03)	0.98 (0.07)	0.96 (0.07)	1.01 (0.05)	1.02 (0.04)	1.00 (0.06)
R172	118	1.06 (0.09)	1.02 (0.03)	1.01 (0.07)	1.02 (0.03)	1.03 (0.01)	1.00 (0.05)
R172	Neg	0.96 (0.02)	0.95 (0.04)	1.00 (0.03)	0.96 (0.01)	0.95 (0.02)	1.00 (0.03)
R600	140	0.73 (0.06)	0.61 (0.01)	0.70 (0.02)	0.92 (0.04)	0.81 (0.02)	1.00 (0.04)
R600	Neg	1.21 (0.09)	1.24 (0.06)	1.12 (0.07)	1.07 (0.07)	0.99 (0.11)	1.00 (0.05)
R181	146	0.46 (0.04)	0.53 (0.02)	0.60 (0.03)	0.77 (0.04)	0.75 (0.08)	1.00 (0.02)
R181	Neg	1.11 (0.07)	1.09 (0.09)	1.05 (0.03)	0.85 (0.08)	0.89 (0.05)	1.00 (0.05)
R76	165	0.94 (0.04)	0.97 (0.03)	0.87 (0.09)	1.00 (0.04)	0.90 (0.07)	1.00 (0.03)
R76	Neg	1.09 (0.08)	1.10 (0.05)	1.02 (0.18)	0.99 (0.17)	0.92 (0.15)	1.00 (0.02)

^a Values are presented as average (standard deviation) of three replications.

ACKNOWLEDGEMENTS

This work was supported by CRIS #6645-21000-028-00D. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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