

Research paper

Acidic polypeptides A_{1a}, A₃ and A₄ of Gly m 6 (glycinin) are allergenic for pigletsShugui Zheng^{a,*}, Guixin Qin^b, Jing Chen^a, Fei Zhang^a^a College of Animal Husbandry and Veterinary Medicine, Shenyang Agricultural University, 120 Dongling Road, Shenyang, Liaoning, 110866, PR China^b College of Animal Science and Technology, Jilin Agricultural University, 2888 Xincheng Street, Changchun, Jilin, 130118, PR China

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ABSTRACT

Gly m 6 (glycinin) is one of the major antigenic proteins in soybeans responsible for transient hypersensitivity to soybean meal in weaned piglets. The globulin is a hexamer consisting of subunits containing basic and acidic polypeptides. Multiple acidic polypeptides have long been demonstrated to be allergens for humans and play a key role in the overall allergenicity of Gly m 6. To date, knowledge on the allergenicity of the acidic polypeptides for piglets is very limited. The purpose of this study was to identify the acidic polypeptides that were allergenic for piglets and to characterize these acidic polypeptides by ELISA, western blot, skin prick and basophile histamine release test. The IgG and IgE antibody binding capacities of the acidic polypeptides of Gly m 6 were determined using ELISA and western blot analysis with sera from Gly m 6 sensitized piglets. Skin prick test and basophile histamine release test were conducted to measure the effector cell response to the polypeptides. Specific IgG and IgE antibodies against A_{1a}, A₃ and A₄ of Gly m 6 were identified in the sera of Gly m 6 sensitized piglets. Meanwhile, positive skin prick test and specific histamine release responses were also induced by the acidic polypeptide A_{1a}, A₃ and A₄ of Gly m 6 from the basophiles of Gly m 6 sensitized piglets. The results demonstrate that the acidic polypeptide A_{1a}, A₃ and A₄ of Gly m 6 are allergenic for piglets.

1. Introduction

Soybeans are valued nutritionally for their high seed protein content and their well-balanced amino acid profile (Friedman and Brandon, 2001). However, soybeans are also allergen sources for both humans (Gagnon et al., 2010) and animals (Liu et al., 2008; Sun et al., 2013). Among these species, piglets are particularly susceptible to soybean and often suffer a transient hypersensitivity when weaned onto a soybean-containing diet (Li et al., 1990; Hao et al., 2009; Sun et al., 2008a,b; Zheng et al., 2014). The hypersensitivity represents a significant health threat to weaned piglet, and has demonstrated to be mediated by IgE produced by Th2 lymphocytes (Sun et al., 2008b). It has also been hypothesized that the intestinal mucosal injury that happens in piglets during the immediate post-weaning period may be induced by antibody-mediated immune responses to soybean allergens (Hall and Byrne, 1989).

Gly m 6 (glycinin), a major storage globulin in soybean, is regarded as the primary allergen responsible for the hypersensitivity in the piglets abruptly weaned onto a soybean-containing diet (Li et al., 1990). Gly m 6 is a hexamer composed of subunits consisting of acidic polypeptides (A_{1a}, A_{1b}, A₂, A₃ and A₄) and basic polypeptides (B₁, B₂, B₃ and

B₄), which are joined by disulfide bridges (Moreira et al., 1979; Wang et al., 2014). The molecular weight of acidic polypeptides A_{1a}, A_{1b}, A₂ and A₄ are about 37 kDa, while A₃ has a molecular weight of approximately 42 kDa (Moreira et al., 1979). Multiple acidic polypeptides of Gly m 6 have long been documented to be allergens for humans. The IgE antibody from sera of soybean-sensitive patients exhibited strong bindings to acidic polypeptides, suggesting the acidic polypeptides played a significant role in the overall allergenicity of Gly m 6 (Pedersen and Djurtoft, 1989; Beardslee et al., 2000). So far, very limited studies have been conducted to characterize the allergenicity of the acidic polypeptides of Gly m 6 for piglets. The purposes of this study were to evaluate the allergenic potential of acidic polypeptides of Gly m 6 by ELISA, western blot analysis, skin prick test and basophile histamine release test.

2. Materials and methods

2.1. Animals and experimental design

Three pregnant Landrace sows were fed on a non-soybean diet from day 108 of pregnancy until the end of lactation in order to reduce

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Table 1
Composition and nutritional levels of Gly m 6 sensitizing and control diets.

	Diets	
	Control	Sensitizing
<i>Composition(%)</i>		
Corn	66.0	66.0
Corn gluten meal	10.0	0.0
Fish meal	8.0	0.0
Spray dried porcine plasma	4.0	0.0
Gly m 6	0.0	18.0
Whey powder	6.0	6.0
Corn oil	2.0	2.0
Premix ^a	1.0	1.0
Choline chloride	0.1	0.1
Cornstarch	1.0	4.3
MDCP ^b	0.4	1.2
Limestone	0.8	1.0
Salt	0.0	0.3
L-Lysine HCl	0.60	0.08
DL-Methionine	0.00	0.00
L-Threonine	0.10	0.02
<i>Chemical analysis</i>		
Crude protein(%) ^c	20.10	20.69
Calcium(%) ^c	0.69	0.70
Phosphate(%) ^c	0.59	0.59
Lysine(%) ^d	1.36	1.35
Methionine(%) ^d	0.37	0.35
Threonine(%) ^d	0.86	0.86
Digestible energy(Mcal/kg) ^d	3.40	3.40

^a Premix provided per kilogram of complete diet: vitamin A 2200 IU; vitamin D₃ 220 IU; vitamin E 16 IU; vitamin K 0.5 mg; vitamin B₁ 1 mg; vitamin B₂ 3.5 mg; vitamin B₆ 1.5 mg; vitamin B₁₂ 0.175 mg; pantothenic acid 10 mg; niacin 15 mg; folic acid 0.3 mg; biotin 0.05 mg; copper 150 mg; iron 100 mg; zinc 100 mg; manganese 4 mg; selenium 0.3 mg; iodine 0.14 mg.

^b MDCP, monocalcium and dicalcium phosphate.

^c Analysed value.

^d Calculated value.

transfer of maternally-derived anti-soybean protein antibodies to their piglets via colostrum (Helm et al., 2002). The crossbred (Yorkshire × Landrace) piglets were weaned at 28 days old. One week after weaning, four piglets from each of the three litters were randomly divided into two groups. A total of 12 piglets (35 days old) were chosen from the three litters and each group consists of six piglets. All the piglets were housed in an environmentally controlled room and had ad libitum access to water and food.

Two corn-based diets were formulated. The control diet, entirely devoid of soybean, contained 8% fish meal, 4% spray-dried porcine plasma and 10% corn-gluten meal on an air-dry basis as protein sources. The purified Gly m 6, extracted from soybean cultivar Fengjiao 7607 using a simplified method (Thanh and Shibasaki, 1976), was used to replace all of the above protein sources in the sensitization diet. Both diets contained 66% corn on an air-dry basis as an energy source and were formulated to meet or exceed nutrient requirements according to the National Research Council (2012) (Table 1).

The six piglets in control group were fed on the non-soybean control diet during the whole period. The six piglets in the sensitized group, however, were fed on the sensitization diet from days 0–10 and days 18–20, then fed on the sensitization diet at one week intervals, each for three successive days, and these piglets were fed the control diet at other times.

All piglets in both groups were weighed on days 0 and 10 and feed consumption was individually recorded to calculate growth performance. Diarrhoea incidence was recorded daily throughout the experiment. Faecal consistency was graded at four levels (0, solid; 1, semi-solid; 2, semi-liquid; and 3, liquid) (Azevedo et al., 2006). The occurrence of diarrhoea was designated as grade 2 or 3 faecal consistency for over two successive days (Taras et al., 2006). Diarrhoea incidence was

defined as the percentage of the number of piglets with diarrhoea divided by the total number of animals (Taras et al., 2006). Blood sample was taken via precaval vein from each piglet at day 0, day 10, day 20, day 30 and day 40, respectively. The sera were separated and stored at 80°C for ELISA and western blot analysis. 2 mL of heparinised venous blood was also taken simultaneously from each piglet at day 40 for basophile histamine release test.

2.2. Purification of Gly m 6 and multiple acidic polypeptides

Crude Gly m 6 was isolated from soybean cultivar fengjiao 7607 using a method of isoelectric point precipitation (Liu et al., 2007). The allergen was further purified by gel filtration chromatography on a Sepharose CL-6B column (Iwabuchi and Yamauchi, 1987). Multiple acidic polypeptides of Gly m 6 were purified by anion exchange chromatography according to our previously published method (Zheng et al., 2009). Briefly, purified Gly m 6 was dissolved in a phosphate buffer (pH6.4) containing 6 M urea and 0.2 M β-mercaptoethanol to dissociate the protein into free polypeptides and then loaded onto a DEAE-Sepharose Fast Flow column, which was pre-equilibrated with the phosphate buffer. The column was washed with the same phosphate buffer until the absorbance of the effluent at 280 nm was < 0.05 and then washed with the phosphate buffer containing 0–0.5 M NaCl. As a result, a total of five fractions that consisted of a mixture of basic polypeptides, acidic polypeptides A_{1a}, A₂, A₃ and A₄, respectively, were eluted with a gradient from 0 to 0.5 M sodium chloride (Zheng et al., 2009). The resulting polypeptides were dialyzed against phosphate buffers containing 4 M, 2 M, and 0 M urea successively, then freeze-dried and stored at –20°C.

2.3. ELISA

An indirect ELISA was performed to measure IgG antibody against Gly m 6 and acidic polypeptides in sera (Helm et al., 2002). A 96-well microtiter plate was coated with 100 μL per well of solutions (5 μg/mL protein in 100 mmol/L sodium carbonate buffer, pH 9.6). After incubation for 4 h at 37°C, the plate was washed with PBST for five times. Thereafter, 20-fold diluted serum from each piglet was added and incubated for 1 h at 37°C. After incubation with HRP-conjugated rabbit anti-pig IgG (Beijing Biosynthesis Biotechnology) for 1 h at 37°C, phenylenediamine was added to visualize antibody binding. The plate was read at 492 nm and results were expressed in optical density units.

2.4. SDS-PAGE and Western blot

Electrophoresis was conducted on slab gels composed of a 5% stacking gel and a 12% resolving gel. Purified protein samples were dissolved in SDS-PAGE loading buffer (50 mmol/L Tris-HCl, 10% glycerol, 1% β-mercaptoethanol, 2% sodium dodecyl sulphate, 0.1% bromophenol blue, pH 6.8). After being heated in a boiling water bath for 10 min, an aliquot of 10 μL of solution was loaded onto the gel and separated by SDS-PAGE. Then gels were stained with 0.1% Coomassie brilliant blue R250.

Western blot analysis was performed essentially as previously described (Zheng et al., 2014). For detection of IgG antibodies against polypeptides of Gly m 6, proteins separated by SDS-PAGE were blotted onto a polyvinylidene difluoride (PVDF) membrane (Millipore). The PVDF membrane was blocked with 3% bovine serum albumin (BSA) in Tris-buffered saline (TBS, pH7.3) and then incubated with appropriately diluted pooled serum from six sensitized or six control piglets, respectively. After three washes with TBS containing 0.05% Tween-20 (TBST), the membrane was incubated with a 1:3000 dilution of horseradish peroxidase (HRP)-conjugated rabbit anti-pig IgG antibody (Beijing Biosynthesis Biotechnology). Following three washes, immunoreactive proteins were visualized with 3,3'-diaminobenzidine (DAB) substrate (Tiangen Biotech).

For detection of IgE antibodies against polypeptides, the PVDF membrane with electroblotted proteins was blocked with 3% BSA in PBS and then incubated by properly diluted pooled serum from sensitized or control piglets for 18 h at 4°C, respectively. After four washes with PBST, the membrane was incubated with biotinylated anti-pig IgE (RapidBio) and then HRP-conjugated streptavidin (Beijing Biosynthesis Biotechnology). Following four washes, the membrane was detected with chemiluminescence (Super Enhanced Chemiluminescence Detection Kit, Applygen Technologies).

2.5. Skin prick test

To measure the capacities of acidic polypeptides to activate dermal effector cell, skin prick test was conducted on day 30 by intradermal injection of 0.5 mg of acidic polypeptide in sterilized physiological saline solution into shaved flank regions of individual piglets according to the method of Sun et al. (2008). The diameters of erythematous areas were gauged with vernier calipers after thirty minutes of intradermal injection.

2.6. Histamine release test

Basophile histamine release test was performed to determine the histamine-release capability of acidic polypeptides as previously described (Zheng et al., 2012). Briefly, heparinized blood was diluted 1:7 in a buffer (25 mmol/L PIPES, 110 mmol/L NaCl, 5 mmol/L KCl, and 2.0 mmol/L CaCl₂, pH 7.35), then 100 µL of diluted blood was incubated with 50 µL of acidic polypeptide (3 µg/mL) at 37°C for 45 min in microtitre plates. The plates was placed on ice to stop the reaction, then blood cells were immediately centrifuged at 300g at 4°C. Histamine in the supernatant was determined by using a pig histamine ELISA kit (RapidBio). Spontaneous release was determined by incubation of the diluted blood in the buffer devoid of purified proteins under the same conditions. The total amount of histamine release was measured after cell lysis in distilled water and freeze-thawing of the blood cells. Basophile histamine release rate was calculated as follows:

Histamine release ratio (%) = [(allergen-stimulated histamine release – spontaneous release)/(total histamine release – spontaneous release)] × 100%.

2.7. Statistical analysis

Differences in growth performance, antibody levels, skin prick responses and histamine release responses between the groups were analyzed by Student's *t*-test using SAS version 9.2 statistical software (SAS, 2008), whereas the data amongst group were analyzed using the analysis of variance (ANOVA) procedure of SAS version 9.2 statistical software (SAS, 2008). Differences in the occurrence of diarrhoea between the groups were analyzed by the χ^2 contingency test. *P* values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Growth performance and diarrhoea

Although the average daily feed intake (ADFI) was not significantly different (*P* = 0.553), sensitized piglets had lower ADG (*P* = 0.019), but higher feed to gain ratio (F/G) (*P* = 0.005), relative to control piglets (Table 2). Two piglets in the sensitized group presented semi-liquid faeces for over two successive days, but none of piglets in the controls had diarrhea. The difference of diarrhoea between the two groups was not statistically significant (*P* > 0.05).

Table 2

Effects of Gly m 6 supplementation on growth performance and diarrhoea incidence in sensitized and control piglets.

	Controls	Sensitized group	<i>P</i> value
ADG(g/d)	550.4 ± 106.9	421.3 ± 40.1	0.019
ADFI(g/d)	991.4 ± 149.0	963.3 ± 121.8	0.553
F/G	1.80 ± 0.16	2.29 ± 0.15	0.005
Diarrhea incidence (%)	0 (0/6)	33.3 (2/6)	> 0.05 ^a

ADG, average daily gain; ADFI, average daily feed intake; F/G, feed to gain ratio.

Data are presented as group means ± standard deviations of six piglets per group.

^a χ^2 contingency test.

Table 3

Gly m 6-specific IgG levels in sera of piglets in sensitized and control group during different sensitization periods (OD_{492nm}).

Groups	Sensitization periods					SEM ²	<i>P</i> value
	Day 0	Day 10	Day 20	Day 30	Day 40		
Sensitized group	0.31 ^c	0.38 ^{bc}	0.58 ^b	0.87 ^a	0.91 ^a	0.060	< 0.001
Controls	0.30	0.27	0.31	0.31	0.33	0.017	0.909
<i>P</i> value ¹	0.910	0.109	0.035	0.006	0.002	–	–

Each value is the mean of data from six piglets.

¹ *P* values for two-group Student's *t* test.

² SEM, standard error of the mean.

3.2. Gly m 6-specific antibody production

Using an indirect ELISA to quantify Gly m 6-specific IgG, serum from sensitized piglets exhibited increased IgG levels with sensitization periods, whereas serum from control group exhibited similar IgG levels during different sensitization period (Table 3).

3.3. Acidic polypeptide-specific IgG and IgE antibody

Using an indirect ELISA, levels of IgG against acidic polypeptide A_{1a}, A₂, A₃ and A₄ were significantly higher than in control piglets (*P* = 0.008, 0.023, 0.004 and 0.029, respectively) (Table 4). Using western blot analysis, serum IgG (Fig. 1) and IgE (Fig. 2) from sensitized piglets reacted specifically with acidic polypeptide A_{1a}, A₃ and A₄ but did not react with A₂, and serum from control pigs did not react with any of the polypeptides.

3.4. Skin prick and histamine release responses induced by acidic polypeptides

The diameters of erythematous areas measured 30 min after injection of acidic polypeptides A_{1a}, A₃ and A₄ of Gly m 6 were significantly larger in sensitized piglets compared with control piglets (*P* = 0.017, 0.028 and 0.007 for the A_{1a}, A₃ and A₄, respectively), whereas the

Table 4

Specific IgG levels in the sera of sensitized and control piglets(OD_{492nm}).

Groups	Gly m 6 and acidic polypeptides					SEM ²	<i>P</i> value
	Gly m 6	A _{1a}	A ₂	A ₃	A ₄		
Sensitized group	0.91 ^a	0.68 ^{ab}	0.48 ^b	0.62 ^b	0.57 ^b	0.044	0.015
Controls	0.33	0.31	0.28	0.32	0.32	0.012	0.781
<i>P</i> value ¹	0.002	0.008	0.023	0.004	0.029	–	–

Each value is the mean of data from six piglets.

¹ *P* values for two-group Student's *t*-test.

² SEM, standard error of the mean.

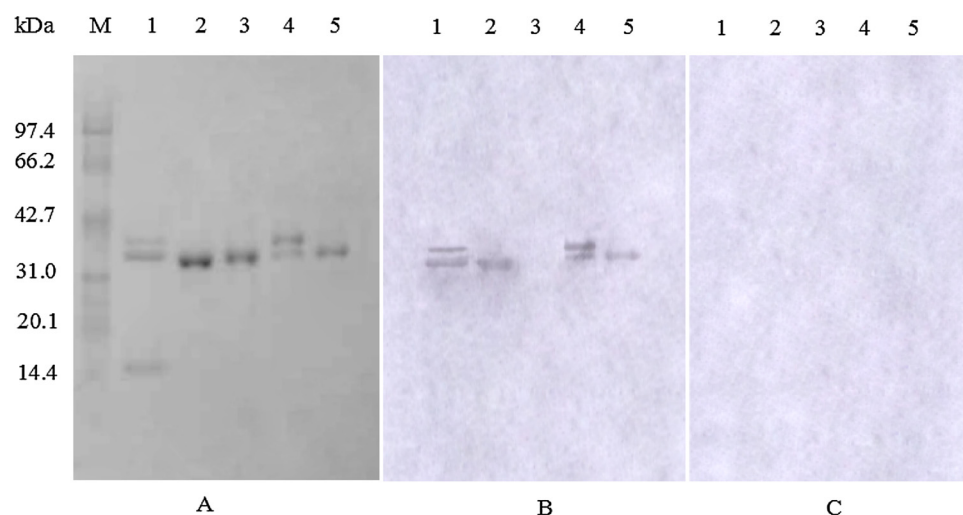


Fig. 1. SDS-PAGE and IgG immunoblotting of acidic polypeptides of Gly m 6. (A) Purified Gly m 6, acidic polypeptides resolved on a 12% SDS-PAGE gel and stained with Coomassie Brilliant Blue R250. (B) Proteins in panel A were transferred to PVDF membranes and probed with pooled serum from sensitized piglets. (C) Representative immunoblot probed with pooled serum from control piglets. IgG-binding proteins were identified using HRP-conjugated anti-pig IgG followed by DAB detection. Lane M, protein marker; lanes 1–5, purified Gly m 6, A_{1a}, A₂, A₃ and A₄, respectively. Purified Gly m 6 were shown to contain acidic polypeptide A₃(the upper band), a mixture of acidic polypeptides (the middle band), and a mixture of basic polypeptides(the lower band) on SDS electrophoresis (panel A). Serum IgG from sensitized piglets reacted specifically with the acidic polypeptides of purified Gly m 6, A_{1a}, A₃ and A₄, but did not react with A₂

(panel B), and serum from control pigs did not react with any of the polypeptides (panel C).

diameters of erythematous areas following intradermal injection of acidic polypeptides A₂ was similar ($P = 0.242$) (Table 5).

Histamine release rates from basophile induced by acidic polypeptides A_{1a}, A₃ and A₄ of Gly m 6 were significantly higher in sensitized piglets compared to the control group ($P = 0.008$, 0.009 and 0.007 for A_{1a}, A₃ and A₄, respectively), whereas histamine release rates from basophile induced by acidic polypeptides A₂ was similar ($P = 0.210$) (Table 6).

4. Discussion

Soybean allergy represents a significant animal health problem, frequently observed in several species of animals including preruminant calves and piglets (Dréau and Lallès, 1999; Hao et al., 2009). Weaned piglets exhibit a transient hypersensitivity after they are abruptly exposed to the diets containing soybean protein (Li et al., 1990). Gly m 6 (glycinin), a major storage globulin in soybean, is considered to be one of the primary allergenic proteins responsible for soybean allergen hypersensitivity, leading to growth retardation and diarrhoea in weaned piglets (Sun et al., 2008a,b). The degree of piglet sensitization can be evaluated by the reduced performance, the severity of the diarrhoea and specific antibody production (Helm et al., 2002; Huang et al., 2010). To focus on the allergenicity of the particular soybean allergen, it is preferable that purified allergen is used to avoid the influence of other allergenic components in soybean because soybean

Table 5

Skin prick test response of sensitized and control piglets to acidic polypeptides.

Groups	Gly m6 and acidic polypeptides					SEM ²	P value
	Gly m 6	A _{1a}	A ₂	A ₃	A ₄		
Sensitized group	7.2 ^a	5.5 ^{ab}	2.7 ^b	5.2 ^{ab}	5.0 ^{ab}	0.480	0.046
Controls	1.6	1.7	1.9	1.6	1.5	0.180	0.985
P value ¹	0.002	0.017	0.242	0.028	0.007	–	–

Diameter of erythematous areas (mm) induced by intradermal injection of acidic polypeptides.

Each value is the mean of data from six piglets.

¹ P values for two-group Student's *t*-test.

² SEM, standard error of the mean.

contains multiple allergens (Zheng et al., 2014, 2017). In the present study, the hypersensitivity response was reproduced by orally sensitizing piglets with the purified Gly m 6. Specific IgG antibody production at different sensitization times demonstrated that the hypersensitivity reactions to Gly m 6 gradually occurred in piglets. In good agreement with previous studies, reduced weight gain and lowered feed efficiency were observed in the piglets orally sensitized and challenged with 18% Gly m 6. However, there were no statistically significant differences in diarrhoea incidence between the piglets fed Gly m 6 or not, probably because of insufficient amounts of purified Gly

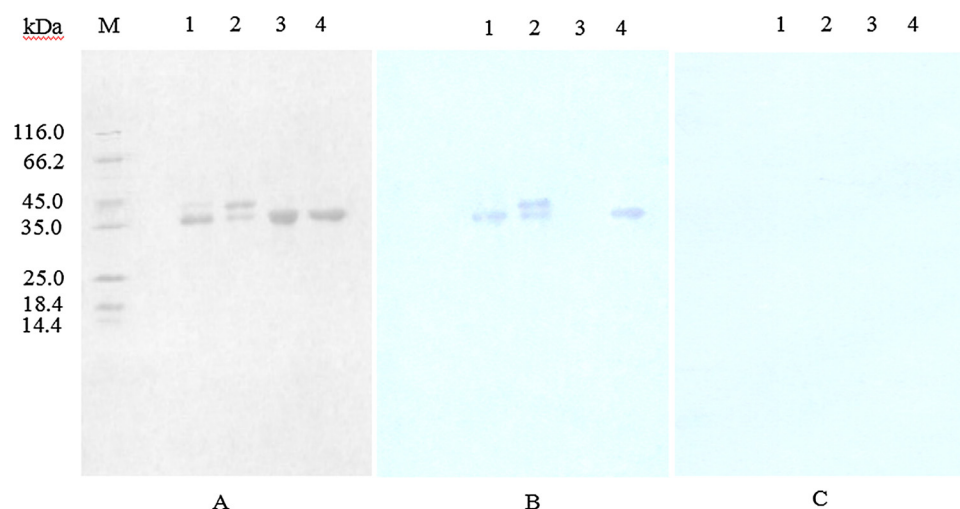


Fig. 2. SDS-PAGE and IgE immunoblotting of acidic polypeptides of Gly m 6. (A) Purified acidic polypeptides resolved on a 12% SDS-PAGE gel and stained with Coomassie Brilliant Blue R250. (B) Proteins in panel A were transferred to PVDF membranes and probed with pooled serum from sensitized piglets. (C) Representative immunoblot probed with pooled serum from control piglets. IgE-binding proteins were identified using HRP-conjugated anti-pig IgE and detected by chemiluminescence. Lane M, protein marker; lanes 1–4, acidic polypeptide A₄, A₃, A₂ and A_{1a}, respectively. Serum IgE from sensitized piglets reacted specifically with acidic polypeptide A_{1a}, A₃ and A₄ but did not react with A₂ (panel B), and serum from control pigs did not react with any of the polypeptides (panel C).

Table 6

Basophilic histamine release response of sensitized and control piglets to acidic polypeptides (%).

Groups	Gly m 6 and acidic polypeptides					SEM ²	P value
	Gly m 6	A _{1a}	A ₂	A ₃	A ₄		
Sensitized group	39.9 ^a	34.5 ^a	12.2 ^b	30.2 ^{ab}	28.9 ^{ab}	3.10	0.046
Controls	10.2	9.8	11.5	9.6	10.9	0.59	0.865
P value ¹	0.007	0.008	0.210	0.009	0.007	–	–

Each value is the mean of data from six piglets.

¹ P values for two-group Student's *t* test.

² SEM, standard error of the mean.

m 6 used in this experiment.

As one of primary storage globulins in soybean, Gly m 6 is a complex macromolecule with a molecular mass of approximately 320–360 kDa, composed of five kinds of subunits containing acidic and basic polypeptides linked by disulfide bond (Wang et al., 2014). Allergenicity of Gly m 6 derived from allergenicity of constituent polypeptide (Pedersen and Djurtoft, 1989; Beardslee et al., 2000). It is significant to reveal which polypeptides of Gly m 6 serve as allergens for the piglet if we want to have a deep understanding of the transient hypersensitivity to Gly m 6 that occurs in piglets. The acidic polypeptides of Gly m 6 have long been demonstrated to be allergenic to humans. The IgE antibody from sera of soybean-sensitive patients react strongly with the acidic polypeptides, suggesting the acidic polypeptides play an important role in the overall allergenicity of Gly m 6 (Pedersen and Djurtoft, 1989; Zece et al., 1999; Beardslee et al., 2000). However, allergenicity of acidic polypeptides to piglet is not thoroughly characterized. In the present study, by using an anion exchange chromatography under reduced condition, acidic polypeptide A_{1a}, A₂, A₃ and A₄ of Gly m 6 were successfully purified. Specific IgG and IgE antibodies were identified that recognized acidic polypeptides A_{1a}, A₃ and A₄ of Gly m 6 in the sera of Gly m 6 sensitized piglets.

The presence of allergen-specific antibodies is not enough to demonstrate a protein to be an allergen because the ELISA and western blot data reflect the humoral immune response alone. However, the skin prick test and the basophile histamine release test, providing information relating to the direct activation of specific effector dermal mast cells and serum basophile response, would be in a good agreement with the clinical signs (Atkins et al., 1985; Du Buske, 1993). Our results of the skin prick and basophile histamine release test confirmed the serological findings that acidic polypeptides A_{1a}, A₃ and A₄ was allergenic for piglets.

Allergen avoidance is thought to be an effective method to prevent hypersensitivity to allergen for humans (Zeiger, 2003). But it is impossible to remove soybean meal from piglet rations totally in consideration of its relatively low cost and its relatively well-balanced composition of amino acids. The identification and characterization of the allergenic components of soybeans may provide a basis for the production and application of hypoallergenic soybean products through the application of food processing (Ogawa et al., 2000; Verhoeckx et al., 2015), genetic engineering (Herman et al., 2003) and selection of mutant strains (Samoto et al., 1996; Joseph et al., 2006). Further investigations concentrated on the characterizing the basic polypeptides of Gly m 6 may contribute to a clearer understanding of this allergen for piglets.

5. Conclusions

The present study demonstrated that acidic polypeptides A_{1a}, A₃, and A₄ reacted with both serum IgG and IgE, and induced positive skin prick and histamine release responses in piglets experimentally sensitized to Gly m 6. These findings suggest that A_{1a}, A₃, and A₄ of Gly m 6 are potentially allergenic for piglets.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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