



Identification of antigenic sites destructed by high hydrostatic pressure (HHP) of the β subunit of β -conglycinin

Jun Xi^{*}, MengXue He¹, JiangYi Pi¹

Engineering Technology Research Center for Grain & Oil Food, State Administration of Grain, School of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, Henan, China

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ABSTRACT

β -conglycinin is one of the most allergenic proteins, and its constituent subunits α' , α , and β are all potential allergens to humans. In the present study, we concentrated on the destructed antigenic sites of β subunit of β -conglycinin after high hydrostatic pressure (HHP) treatment. In this paper, the overlapping gene fragments of the β subunit of β -conglycinin were amplified by polymerase chain reaction (PCR) and cloned into T7 phage vectors. After being packaged *in vitro*, the recombinant T7 phage was constructed, and the overlapping fragments of the β subunit were displayed on the phage surface. The recombinant phages that expressed the overlapping fragments of the β subunit were used to react with specific anti-serum by indirect ELISA to identify the HHP destructed antigenic sites. After three rounds of expression and identification, we used synthetic peptide technology to identify that the obtained fragment was a conformational epitope. We further confirmed that HHP treatment changed the conformational structure of β -conglycinin, which reduced the antigenicity of the protein.

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1. Introduction

Epitopes, which are also known as antigenic determinants, are specific chemical groups that determine the specificity of an antigen. Based on the structural characteristics of the epitopes, they are divided into continuous/linear epitopes or discontinuous/conformational epitopes [1]. Linear epitopes consist of short-segment contiguous amino acid residues of antigenic sequence, and a conformational epitope is composed of residues that are distant in the primary sequence, but which are approximated by conformational folding of the native protein [2].

Allergies affect >25% of the European population, which increases the demand for allergens as diagnostic, therapeutic, and research reagents [3,4]. Soybean is a major source of high-quality protein and is also rich in vitamins and minerals; therefore, it is widely used in the food industry [5,6]. However, soybean also contains many antinutritional factors, and it is one of the eight most significant food allergens [7–10]. Approximately 25% of all food allergens are caused by soybean, which can cause many pathologies, such as stomach upset, intestinal damage, allergic dermatitis, and even anaphylactic shock that can be life-threatening

[11]. The 7S storage protein β -conglycinin and the 11S storage protein glycinin are the two primary proteins in soybean [12]. These major seed storage proteins in soybean account for 70–80% of the total seed globulin fraction [13,14]. β -conglycinin is one of the most allergenic proteins, and its constituent subunits α' , α , and β are all potential allergens to humans [15]. Under Regulation (EU) No. 1169/2011 [16] of the European Parliament and of the Council, soybean and soybean products have to be labelled. Investigations have shown that HHP can reduce the antigenicity of β -conglycinin effectively [17,18] by destroying the three-dimensional structure of β -conglycinin, which leads to depolymerization, cross-linkages, and conformational changes in protein macromolecules. However, it is unclear which sites on the β subunit of β -conglycinin are destructed. What are the characteristics of the structure and the amino acid composition of the destructed antigenic sites?

A large number of groups have recently presented data on predictions about food allergen epitopes [19] and different epitope mapping methodologies, such as protein fragments, peptide arrays, and phage displays [20–22]. Phage display technology (PDT), which is also known as epitope mapping, has been used to identify the antibody binding sites on antigens [22,23]. Li et al. [24,25] obtained six mimic epitopes by panning the phage display peptide library with specific rabbit serum against buffalo β -lactoglobulin, and seven conformational epitopes of buffalo β -lactoglobulin were

^{*} Corresponding author.

E-mail address: xijunhnu@163.com (J. Xi).

¹ These authors contributed equally to this work.

produced. Tordesillas et al. [26] panned the conformational mimotope of the allergen Pru p 3 from a phage display 12 mer peptide library and identified two conformational epitopes using electrostatic and solvent exclusion surface properties as parameters. However, using PDT to identify other allergens in soybeans has not been reported, especially in antigen epitopes that have been destructed. Therefore, here we investigated the identification of destructed antigenic sites of the β subunit of β -conglycinin by HHP treatment. Our research not only provides food companies with the theoretical basis of processing methods, but it also further develops the products to detect the desensitization effect of processed foods rapidly.

2. Materials and methods

2.1. Materials

The sequence of the β subunit of β -conglycinin was obtained from NCBI Genbank (accession number: LOC547465), which was synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and stored in a PUC57 plasmid vector. Ex-Taq DNA premix, *E. coli* JM109, pMD18-T vector, and the restriction enzymes *EcoRI* and *HindIII* were procured from Takara Bio Co., Ltd. (Dalian, China). X-Gal, and isopropyl β -D-1-thiogalactopyranoside (IPTG) were procured from Sangon Biotech Co., Ltd. (Shanghai, China). *E. coli* BLT5615 and the T7Select10-3b cloning kit were purchased from Novagen Company (USA). 3, 3', 5, 5'-tetramethylbenzidine (TMB), AEC, and goat anti-rabbit IgG-HRP were obtained from Sigma Chemical Co., St. Louis, MO.

2.2. Identification and construction of recombinant phages of the β subunit and its overlapping fragments

The β subunit was divided into three overlapping fragments (A: 73–600 bp; B: 78–930 bp; C: 808–1317 bp), and a series of primers (Table 1) were designed and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The overlapping fragments were amplified by PCR, and the products were ligated into a pMD18-T vector and transformed into *E. coli* JM109 to construct the cloning vector. Then, the β subunit and its overlapping fragments were digested by *EcoRI* and *HindIII*. After being separated by agarose gel and purified by a gel recovery kit, the β subunit gene and its overlapping fragments were ligated to T7 vector arms, packaged with T7 select packaging extract, and the T7 phage expression system of the β subunit and its overlapping fragments were constructed. The T7 phage expression system was transformed into *E. coli* BLT5615 and cultured in M9 TB medium at 37 °C, and the recombinant β subunit and its overlapping protein fragments were expressed in the presence of IPTG. Bacteriophage packaging products were diluted serially to determine the phage titers, and some plaques were chosen randomly. After being dissolved in TE buffer (Tris-EDTA buffer solution), heated at 65 °C for 10 min, and centrifuged at 12000 r/min, the supernatant was used as a template for PCR identification. Agarose gel (1%) electrophoresis was used to detect the size of inserts and the rate of phage recombination.

2.3. Amplification and purification of phage lysates

Recombinant phages were purified by liquid-phase lysis. BLT 5615 bacterial cells were cultured at 37 °C, induced by IPTG, and infected with the recombinant phage. After incubation for 2–3 h, the phage lysate was centrifuged, the precipitate was discarded, and the supernatant was stored at –80 °C with 10% glycerol added. DNase I and RNase A (1 mg/mL) were added to the phage lysate, digested at room temperature for 30 min, and PEG 8000 and NaCl

Table 1

Primers for β subunit and its overlapping fragments.

Primer	Sequence	Enzyme introduced
A up	5'-TACGAATTCAGGTGAGAGAGGATGAG-3'	<i>EcoRI</i>
A down	5'-CTCAAGCTTTTCCTCTCCAAACAAAAC-3'	<i>HindIII</i>
B up	5'-GCGGAATTCGATTTCTTCTATCTAGC-3'	<i>EcoRI</i>
B down	5'-CCTAAGCTTGTTCATGTTTGCATCT-3'	<i>HindIII</i>
C up	5'-ACCGAATTCCTGGATATCTTCCTCAGT-3'	<i>EcoRI</i>
C down	5'-ACTAAGCTTGTAGAGAGACCTAAGATTG-3'	<i>HindIII</i>
C1 up	5'-ACCGAATTCCTGGATATCTTCCTCAGT-3'	<i>EcoRI</i>
C1 down	5'-ATCAAGCTTGTCTGGAATTACAAATACA-3'	<i>HindIII</i>
C2 up	5'-ACCGAATTCACAGAGCTGAATTGTCT-3'	<i>EcoRI</i>
C2 down	5'-TACAAGCTTATCTTGTGCAGACCCAG-3'	<i>HindIII</i>
C3 up	5'-ACCGAATTCCTGGATATCTTCCTCAGT-3'	<i>EcoRI</i>
C3 down	5'-ATCAAGCTTGTCTGGAATTACAAATACA-3'	<i>HindIII</i>
C2-1 up	5'-ACCGAATTCACAGAGCTGAATTGTCT-3'	<i>EcoRI</i>
C2-1 down	5'-AATAAGCTTTTGTAGGTAGCGTTGAC-3'	<i>HindIII</i>
C2-2 up	5'-ATTGAATTCCTCATTTGTGTCAACG-3'	<i>EcoRI</i>
C2-2 down	5'-ATTAAGCTTGCCTTACCACATTGTCT-3'	<i>HindIII</i>
C2-3 up	5'-ACCGAATTCAGACAAATGTGTAAGG-3'	<i>EcoRI</i>
C2-3 down	5'-TACAAGCTTATCTTGTGCAGACCCAG-3'	<i>HindIII</i>

were added at concentrations of 10% (m/v) and 1 M, respectively. Phage particles were precipitated in an ice bath for >1 h, and the phage pellets were collected by centrifugation (4 °C, 11000 g, 10 min) and resuspended in sterile SM solution (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 6 mM MgSO₄). After 1 h at room temperature, an equal volume of chloroform was added for extraction. After mixing gently for 30 s, the phage was separated by centrifugation (4 °C, 3000 g, 15 min).

2.4. Preparation of site-specific antiserum

We had reported the effect of HHP treatment on the antigenicity and structural properties of β -conglycinin, and we found that after HHP treatment, the secondary structure of β -conglycinin changed. The content of the random coil increased considerably, which corresponded with a significant decrease in antigenicity. However, the helix1 and strand1 content decreased with the reduction in antigenicity. [18] The optimal process conditions for reduced antigenicity of β -conglycinin were determined as follows: with HHP treatment pressure of 455 MPa, HHP treatment time of 18 min, and β -conglycinin concentration of 15 mg/mL, the antigenicity inhibition rate of β -conglycinin was 49.59%. The site-specific antiserum was prepared by adding an excess of HHP-treated β -conglycinin antigen to rabbit antiserum against β -conglycinin, incubating for 1 h at 37 °C, and removing the precipitate by centrifugation (10,000 g, 30 min, 4 °C). The supernatant was the specific antiserum that recognized HHP-destructed sites.

2.5. The identification of the destructed antigen sites

All recombinant phages that expressed the overlapping fragments of the β subunit were coated in a 96-well microtiter plate overnight at 4 °C. Plates were washed five times with PBST (0.05% Tween 20 in 0.01 M pH 7.2 phosphate-buffered saline (i.e., PBS)) and blocked with 200 μ L/well blocking buffer (5% skim milk in 0.01 M pH 7.2 PBS), followed by incubation for 1 h at 37 °C. The solution was then discarded, and the plates were washed five times with PBST. Site-specific antiserum was added at 100 μ L/well, and the plates were incubated for 30 min at 37 °C; rabbit antiserum against β -conglycinin was used as control. After washing, goat anti-rabbit IgG-HRP diluted 1:3000 with PBST was added at

100 μL /well followed by incubation for 30 min at 37 °C. The plates were washed, and freshly prepared substrate TMB was added at 100 μL /well. After incubating at room temperature for 10 min, the reaction was stopped using 50 μL /well of 2 mol/L H_2SO_4 . The absorbance in each well was measured at 450 nm, and all experiments were carried out in triplicate. The larger the optical density (OD) value was for HHP treated antigen-absorbing antiserum, the more significant the destruction was.

Then, the segment that was most destroyed was further divided into three fragments with 30 amino acid residue overlaps, amplified by PCR, ligated with T7 phage vector, and expressed on the surface of T7 phage; then, we measured its binding characteristics with site-specific antiserum. After 3 rounds of overlap, the precise site of the β subunit that was destroyed by HHP treatment was identified.

2.6. Confirmation of site type by peptide synthesis

The peptide that contained the precise site of the β subunit that was destroyed by HHP treatment was synthesized using solid-phase peptide synthesis, as described previously [27]. We added cysteine residue to the end of the N-terminal of the peptide. The purity of the peptide was 99%, as assessed by high-performance liquid chromatography (HPLC), and the molecular mass of the peptide was confirmed by mass spectrometry (MS). The peptide was conjugated to a carrier protein of IgG-free bovine serum albumin (BSA, Sigma) using a hetero-bifunctional cross-linker Sulfo-SMCC (Thermo, San Jose, CA, USA) [28]. First, a total of 5 mg IgG-free bovine serum albumin (BSA, Sigma) protein was dissolved in conjugation buffer (0.15 M NaCl, 0.1 M PB pH 7.2, 1 μM EDTA), and a total of 1 mg Sulfo-SMCC was dissolved in 50 μL of DMSO and then mixed with BSA solution (37 °C, 30 min). The reaction mixture was then dialyzed extensively, and the protein concentration was adjusted to 5 mg/mL using conjugation buffer. Next, a total of 2 mg synthetic peptide was dissolved in 50 μL of DMF, and 150 μL of PB buffer (pH 7.2) was added. Then, we diluted the peptide solution with an equal volume of PB buffer, and added two volumes of SMCC-BSA solution. After mixing, we allowed the mixture to react at room temperature for 30 min (or 4 °C for 2 h). Finally, we adjusted the peptide concentration to 1 mg/mL in PB buffer for epitope identification. The conjugated peptide was identified by SDS-PAGE to see whether it conjugated to BSA. A 20 μL aliquot of 1 mg/mL conjugated peptide and BSA solution were mixed with an equal volume of 2 $\times$ SDS-PAGE loading buffer, and the details were the same as described previously [29].

During synthesis, a cysteine residue was added to the N-terminal of all peptides, unless it was already present for later conjugation. Peptides were conjugated to a carrier protein of IgG-free bovine serum albumin (BSA, Sigma) using a hetero-bifunctional cross-linker Sulfo-SMCC (Thermo, San Jose, CA, USA), according to the manufacturer's instructions. The conjugated peptides were stored at –20 °C until used.

2.7. Dot-blot assay

The conjugated peptide (2 μL) was spotted on the nitrocellulose membrane, dried at room temperature for 30 min, and BSA and β -conglycinin were used as a blank and positive control, respectively. After washing with PBST, the membrane was incubated for 2 h in a blocking solution with 0.2% gelatin added to PBST at 37 °C. The membrane was washed and incubated with rabbit antiserum against β -conglycinin for 30 min at 37 °C, washed with PBST, and incubated with goat anti-rabbit HRP-IgG at 37 °C for 30 min. Then, we washed the membrane and incubated it with AEC.

2.8. Structural analysis

The three-dimensional crystal structure of the β subunit of β -conglycinin was analyzed by the PDB database to know the spatial position of the amino acid sequence, and a GOR IV online server was used to analyze the secondary structure of the damaged antigen epitope region. The server only required the amino acid sequence of the protein, and it provided the α -helix, β -turn, and random coil content.

2.9. Statistical analysis

Each experiment was performed three times. An analysis of variance (ANOVA) was performed using SPSS 16.0 (IBM Corporation, NY, USA), and a significance level of $p \leq 0.05$ was adopted. Duncan's multiple range tests were used to detect for discrepancies between means.

3. Results and discussion

3.1. Identification and construction of recombinant phages of the β subunit and its overlapping fragments

The synthetic plasmid vector pUC57 from Sangon Biotech Co., Ltd. was used as a template. The β subunit and its overlapping gene fragments were obtained by PCR and agarose gel electrophoresis (Fig. 1a1). Strips that were consistent with the size of the expected fragment were observed, and no nonspecific bands were observed. PCR and double-enzyme digestion of the β subunit and its overlapping segments showed the same size bands (Fig. 1a2, Fig. 1a3), which indicated that the β subunit and its overlapping fragments were inserted successfully into the pMD18-T vector, and the sequence of the inserted fragment was consistent with its gene sequence. The β subunit gene and its overlapping fragments were digested with *EcoRI* and *HindIII* and ligated with a T7 phage vector arm. Phage packaging extract was added and transformed into BLT5615 for 2–3 h to form a plaque. Multiple plaques were chosen at random and amplified by PCR. Agarose gel electrophoresis showed that the recombination rate was >90% (Fig. 1a4). DNA sequencing results showed that the exogenous DNA fragment was correct.

3.2. The identification of the destroyed antigen sites

The reaction between phage-expressed proteins and antiserum against β -conglycinin showed that β subunit, recombinant phage-expressed proteins were antigenic, which indicated that the β subunit and its overlapping fragments were expressed successfully. The reaction of phage-expressed proteins to HHP treated, antigen-absorbing antiserum was lower than that of antiserum against β -conglycinin, which indicated that HHP destroyed all of A, B, and C fragments, and among these three fragments, the C fragment showed relatively stronger antigenicity (Fig. 2a). The reaction between phage-expressed proteins and site-specific antiserum showed that the OD of the C fragment was higher than that of the A and B fragments, which indicated that the antigenic site for the C fragment was destroyed more obviously (Fig. 2a).

After further segmented expression of the C fragment (Fig. 1b), the reaction of the phage-expressed proteins and site-specific antiserum (Fig. 2b) showed that the most destroyed fragment of HHP was in the C2 fragment. After further segmented expression of the C2 fragment (Fig. 1c), the reaction of the phage-expressed proteins and site-specific antiserum (Fig. 2c) showed that the most destroyed fragment of HHP was in the C2–3 fragment. Taliercio et al. [8] studied the antigenic epitopes of the β subunit of β -conglycinin in

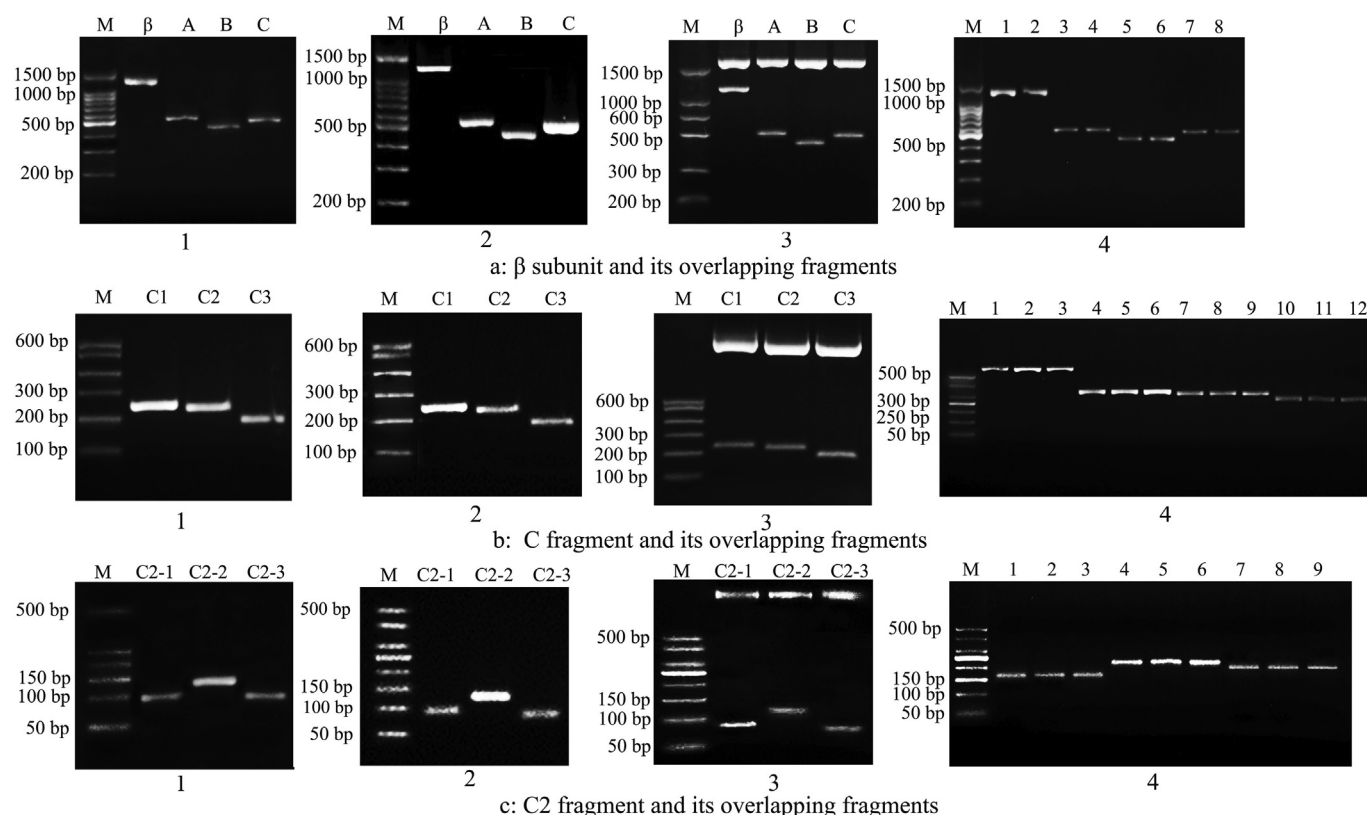


Fig. 1. Construction of recombinant phages of the β subunit and its overlapping fragments (a: β subunit and its overlapping fragments; b: C fragment and its overlapping fragments; c: C2 fragment and its overlapping fragments) (a1: PCR amplification of β subunit and its overlapping fragments; a2: PCR identification of recombinant plasmid; a3: double-enzyme digestion identification of recombinant plasmid; a4: PCR identification of recombinant phages) (M: DNA Marker; 1–8: recombinant phages) (b1: PCR amplification of C fragment and its overlapping fragments; b2: PCR identification of recombinant plasmid; b3: double-enzyme digestion identification of recombinant plasmid; b4: PCR identification of recombinant phages) (M: DNA Marker; 1–12: recombinant phages) (c1: PCR amplification of C2 fragment and its overlapping fragments; c2: PCR identification of recombinant plasmid; c3: double-enzyme digestion identification of recombinant plasmid; c4: PCR identification of recombinant phages) (M: DNA Marker; 1–9: recombinant phages).

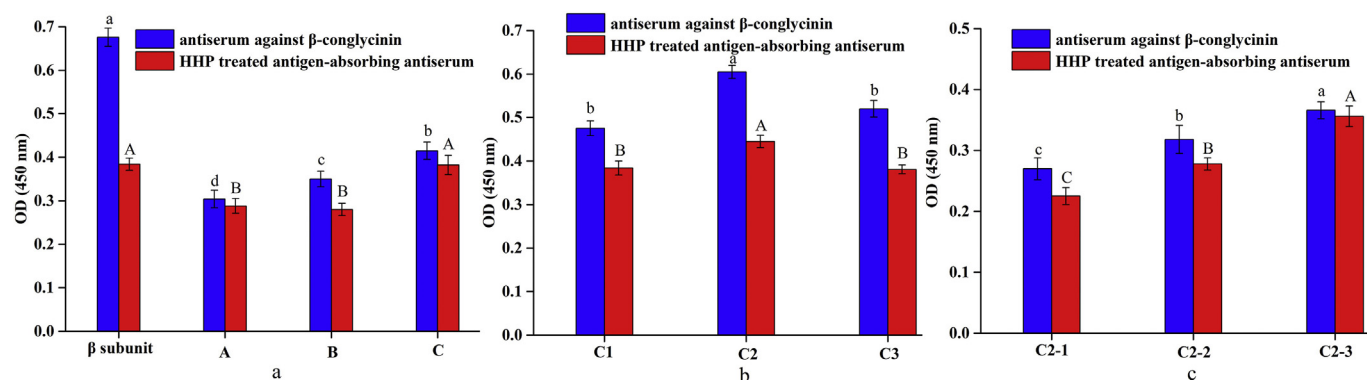


Fig. 2. The reaction of the recombinant phages and epitope-specific antiserum (a: β subunit and its overlapping fragments; b: C fragment and its overlapping fragments; c: C2 fragment and its overlapping fragments) (The reaction with antiserum against β -conglycinin indicates the antigenicity of recombinant phages, and the reaction with HHP treated, antigen-adsorbing antiserum indicates the degree of destruction. The larger the optical density (OD) value of processed HHP treated, antigen-adsorbing antiserum, the more significant the destruction was.) (All values were mean $\pm$ standard deviation of three values; different superscripts a-d/A-C in the same color indicate they are significantly different at the $p < 0.05$ level).

multiple species of animals, and they found that one of the most important regions (250 N–293I) across species was located in the B fragment. At the same time, a particularly antigenic region was found for both pigs and rabbits, and the amino acid was $^{49}\text{QNGRIRLLQRFNKRSP}^{64}$ [8]. Compared to their study, we found that the C2–3 fragment did not react with any of the animal species, probably because we used phage display technology to express the β subunit.

3.3. Confirmation of site type by peptide synthesis

To study whether the obtained antigenic fragment had a linear antigenic site, we used synthetic peptide technology. The identification of the synthesized peptide using HPLC (Fig. 3a) and MS (Fig. 3b) showed that the synthesized peptide was correct, and its purity reached 99.27%. The analysis from SDS-PAGE gel electrophoresis showed that the conjugated peptide exhibited larger

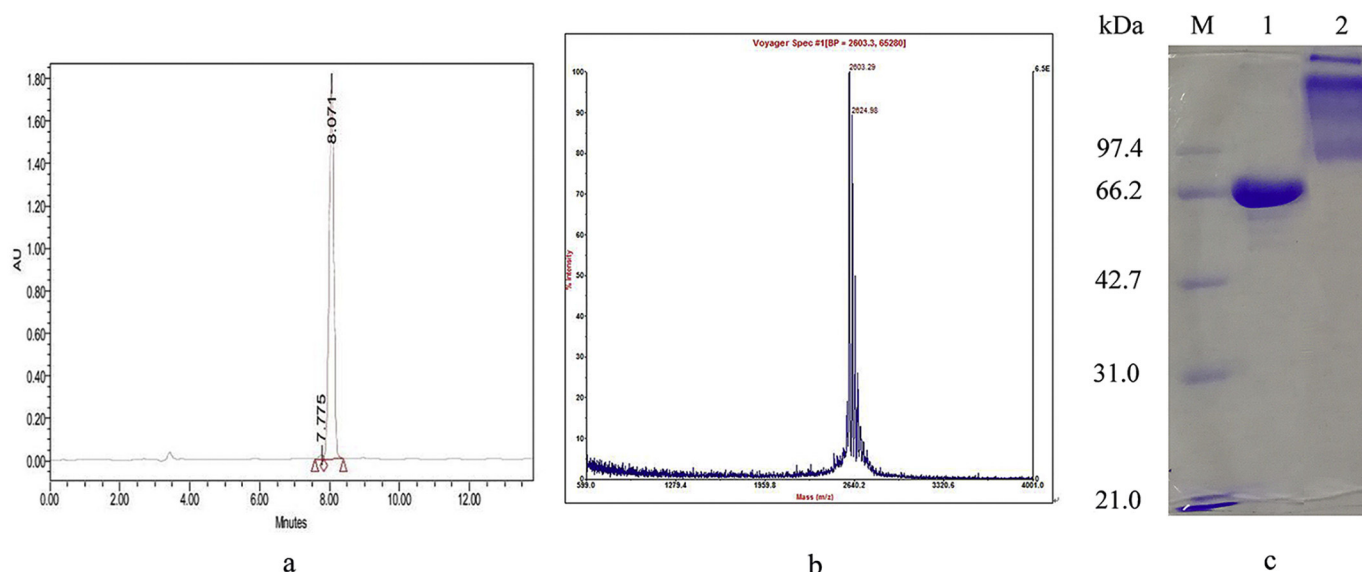


Fig. 3. Identification of synthesized and conjugated peptides (1: HPLC; 2: MS; 3: SDS-PAGE) (3: M: marker; 1: BSA; 2: BSA-peptide).

molecular weights than BSA (Fig. 3c, the bands of molecular marker, BSA, and BSA-peptide), which indicated that the peptide was conjugated successfully to the BSA protein. Furthermore, the number of BSA-linked peptides was not constant and, under normal conditions, a BSA molecule can bind to 30–35 peptides [30]. The molecular weight of the peptide was identified as 2603 D by MS, and the molecular weight of BSA was 66.2 kD. The molecular weight of the heavy conjugate peptide was approximately 148 kD, and the number of linked peptides was estimated to be 31. However, the molecular weight of the light conjugate peptide was approximately 75 kD, and it only combined with three or four peptides, which suggested that it still had the ability to conjugate with more peptides.

3.4. Dot-blot assay

The reaction color of β -conglycinin and antiserum against β -conglycinin was most intense, and the antigenicity was stronger than recombinant phages (Fig. 4). The results showed that recombinant phages can react with rabbit antiserum against β -conglycinin, and the antigenicity of the C fragment phage was greater than that of the C2 and C2–3 fragment phages. However, the peptide of the C2–3 fragment that we synthesized did not react with the antiserum, which indicated that there was no linear epitope in this fragment and that the identified HHP-destroyed antigenic region of the C2–3 fragment was conformational. As we know, HHP treatment did not lead to the destruction of the linear epitope of the protein, and what we found in this research confirmed this further.

3.5. Structural assay

The position of the C2–3 fragment in the spatial structure showed that the middle part of C2–3 fragment was α -helix, and the end of this fragment was random coil (Fig. 5). It confirmed that the β -turn and random coil were mostly located on the surface of the protein and were easily bound to the antibody, which made them more likely to become antigenic epitopes [31]. The secondary structure of the C2–3 fragment was analyzed using the GOR IV online server, which showed that the content of the α -helix, β -sheet, and random coil were 31.82%, 13.64%, and 54.55%, respectively. The main structure at the end of this fragment was random

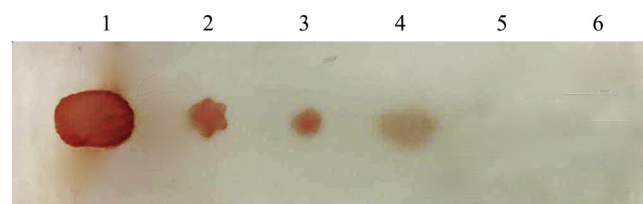


Fig. 4. Binding of antiserum to β -conglycinin, recombinant phages, and conjugated peptide (1: β -conglycinin; 2: C fragment recombinant phage; 3: C2 fragment recombinant phage; 4: C2–3 fragment recombinant phage; 5: BSA–C2–3 peptide; 6: BSA) (the images shown were original and not previously cropped).

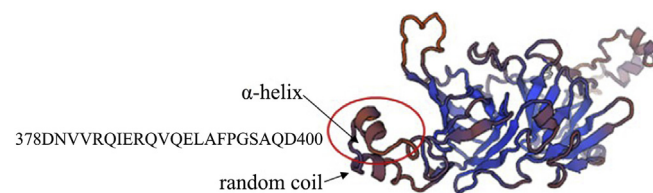


Fig. 5. The structure of the C2–3 fragment (the PDB database was used to analyze the spatial structure of C2–3 fragment).

coil, which was consistent with the results from the analysis of tertiary structure.

4. Conclusions

In our research, the β subunit was segmented, and after phage display, indirect ELISA assay was used to identify the destroyed antigenic sites. After several rounds of segmenting and displaying, we eventually found the main antigenic sites of HHP treatment destroyed. When this antigenic region was subjected to synthetic of the peptide, we found that the peptide did not react with the antiserum against β -conglycinin; that is, this region did not contain a linear epitope, which further confirmed that HHP treatment changed the conformational structure of β -conglycinin and, thus, reduced the antigenicity of the protein. The position of the C2–3 fragment in the spatial structure showed that the middle part of the C2–3 fragment was α -helix, and the end of this fragment was random coil (Fig. 5). It confirmed that the β -turn and random coil

were mostly located on the surface of the protein and were easily bound to the antibody, which made them more likely to become antigenic sites. The contents of the α -helix, β -sheet, and random coil of the C2–3 fragment were 31.82%, 13.64%, and 54.55%, respectively, and the main structure at the end of this fragment was random coil.

The phage display technique can not only identify antigen epitopes of soybean, but also identify destructed antigenic epitopes of soybean, which can be used to provide a new method for allergen research. Research on the identification of HHP-destructed antigenic sites of the β subunit of β -conglycinin is relevant for the identification of the molecular mechanism that causes food allergies and for the development of application products for rapid detection of the desensitization effect of processed foods.

Declaration of competing interest

The authors declare that there are no conflicts of interest. This article does not contain any studies with human subjects.

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References

- [1] X. Li, S. Yuan, S. He, J. Gao, H. Chen, Identification and characterization of the antigenic site (epitope) on bovine β -lactoglobulin: common residues in linear and conformational epitopes, *J Sci Food Agr* 95 (2014) 2916–2923.
- [2] M.J. Rowley, K. O'Connor, L. Wijeyewickrema, Phage display for epitope determination: a paradigm for identifying receptor-ligand interactions, *Biotech Annu Rev* 10 (2004) 151–188.
- [3] G. Schmidt, G. Gadermaier, H. Pertl, M. Siegert, K.M. Oksman-Caldentey, A. Ritala, M. Himly, G. Obermeyer, F. Ferreira, Production of recombinant allergens in plants, *Phytochem. Rev.* 7 (2008) 539–552.
- [4] H. Havenith, K. Kern, P. Rautenberger, H. Spiegel, M. Szardenings, E. Ueberham, Combination of two epitope identification techniques enables the rational design of soy allergen Gly m 4 mutants, *Biotechnol. J.* 12 (2016) 1600441.
- [5] A.E. De, R. Pilolli, S.L. Bavaro, L. Monaci, Insight into the gastro-duodenal digestion resistance of soybean proteins and potential implications for residual immunogenicity, *Food and Funct* 8 (2017) 1599–1610.
- [6] S.G. Zheng, G.X. Qin, J. Chen, F. Zhang, Acidic polypeptides A1a, A3 and A4 of Gly m 6 (glycinin) are allergenic for piglets, *Vet Immunol Immun* 202 (2018) 147–152.
- [7] A.W. Burks, M. Tang, S. Sicherer, A. Muraro, P.A. Eigenmann, M. Ebisawa, ICON: food allergy, *J Allergy Clin Immun* 129 (2012) 906–920.
- [8] E. Taliercio, T.M. Loveless, M.J. Turano, S.W. Kim, Identification of epitopes of the β subunit of soybean β -conglycinin that are antigenic in pigs, dogs, rabbits and fish, *J Sci Food Agr* 94 (2014) 2289–2294.
- [9] T. Wang, G.X. Qin, Z.W. Sun, Y. Zhao, Advances of research on glycinin and β -conglycinin: a review of two major soybean allergenic proteins, *Crit Rev Food Sci* 54 (2014) 850–862.
- [10] H. Saeed, C. Gagnon, E. Cober, S. Gleddie, Using patient serum to epitope map soybean glycinins reveals common epitopes shared with many legumes and tree nuts, *Mol. Immunol.* 70 (2016) 125–133.
- [11] X.L. Sun, X.H. Shan, Z. Yan, Y. Zhang, L. Guan, Prediction and characterization of the linear IgE epitopes for the major soybean allergen β -conglycinin using immunoinformatics tools, *Food and Chem Toxicol* 56 (2013) 254–260.
- [12] V.H. Thanh, K. Shibasaki, Major proteins of soybean seeds, subunit structure of β -conglycinin, *J Agr Food Chem* 26 (1978) 692–695.
- [13] Q. Huang, H. Xu, Z. Yu, P. Gao, S. Liu, Inbred Chinese Wuzhishan (wzs) minipig model for soybean glycinin and β -conglycinin allergy, *J Agr Food Chem* 58 (2010) 5194–5198.
- [14] C.L. Peng, C.M. Cao, M.L. He, Y.S. Shu, X.B. Tang, Y.H. Wang, Y. Zhang, X.D. Xia, Y. Li, J.J. Wu, Soybean glycinin and β -conglycinin-induced intestinal damage in piglets via the p38/JNK/NF- κ B signaling pathway, *J Agr Food Chem* 66 (2018) 9534–9541.
- [15] H.B. Krishnan, W.S. Kim, S. Jang, M.S. Kerley, All three subunits of soybean β -conglycinin are potential food allergens, *J Agr Food Chem* 57 (2009) 938–943.
- [16] Regulation (EU) no. 1169/2011 of the European Parliament and of the council of 25 October 2011 on the provision of food information to consumers, *Official Journal of the European Union. L* 304 (2011) 18–63.
- [17] H.J. Li, K.X. Zhu, H.M. Zhou, W. Peng, X.N. Guo, Comparative study of four physical approaches about allergenicity of soybean protein isolate for infant formula, *Food Agr Immunol* 27 (2016) 604–623.
- [18] J. Xi, M.X. He, High hydrostatic pressure (HHP) effects on antigenicity and structural properties of soybean β -conglycinin, *J Food Sci Tech* 55 (2018) 630–637.
- [19] A. Mishra, A. Jain, N. Arora, Mapping B-cell epitopes of major and minor peanut allergens and identifying residues contributing to IgE binding, *J Sci Food Agr* 96 (2016) 539–547.
- [20] V. Longo, M.A. Costa, F. Cibella, G. Cuttitta, G.S. La, P. Colombo, Multiple IgE recognition on the major allergen of the *Parietaria pollen* par j 2, *Mol. Immunol.* 63 (2015) 412–419.
- [21] C.S. Hansen, M. Dufva, K.L. Bøgh, E. Sullivan, J. Patel, T. Eiwegger, Linear epitope mapping of peanut allergens demonstrates individualized and persistent antibody-binding patterns, *J Allergy Clin Immun* 138 (2016) 1728–1730.
- [22] A. Christiansen, J.V. Kringelum, C.S. Hansen, K.L. Bøgh, E. Sullivan, J. Patel, High-throughput sequencing enhanced phage display enables the identification of patient-specific epitope motifs in serum, *Sci. Rep.* 5 (2015) 12913.
- [23] J. Pande, M.M. Szweczyk, A.K. Grover, Phage display: concept, innovations, applications and future, *Biotechnol. Adv.* 28 (2010) 849–858.
- [24] X. Li, H.B. Chen, P. Tong, X. Wen, Epitope mapping of buffalo β -lactoglobulin against rabbit polyclonal antibody following phage display technique, *J. Food Biochem.* 36 (2012) 56–65.
- [25] X. Li, S. Yuan, S. He, J.Y. Gao, H.B. Chen, Identification and characterization of antigenic site (epitope) on bovine β -lactoglobulin: common residues in linear and conformational epitopes, *J Sci Food Agr* 95 (2015) 2916–2923.
- [26] L. Tordesillas, L.F. Pacios, A. Palacin, J. Cuestaherranz, M. Madero, A. Diazperales, Characterization of IgE epitopes of Cuc m 2, the major melon allergen, and their role in cross-reactivity with pollen profilins, *Clin. Exp. Allergy* 40 (2010) 174–181.
- [27] W.C. Chan, P.D. White, *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*, vol. 222, 2000.
- [28] J. Xi, H.L. Yan, Epitope mapping and identification of amino acids critical for mouse IgG-binding to linear epitopes on Gly m Bd 28K, *Biosci. Biotechnol. Biochem.* 80 (2016) 1973–1979.
- [29] E. Penas, R. Gomez, J. Frias, M.L. Baeza, C. Vidal-Valverde, High hydrostatic pressure effects on immunoreactivity and nutritional quality of soybean products, *Food Chem.* 125 (2011) 423–429.
- [30] G.T. Hermanson, *Bioconjugate Techniques*, Academic Press, 2008.
- [31] V. Apostolopoulos, M. Yu, A.L. Corper, L. Teyton, G.A. Pietersz, I.F. McKenzie, I.A. Wilson, Crystal structure of a non-canonical low-affinity peptide complexed with mhc class I: a new approach for vaccine design, *J. Mol. Biol.* 318 (2002) 1293–1305.