

Short communication

Purification and IgE-binding properties of soybean β -conglycinin subunitsShugui Zheng^{a,b}, He Tian^b, Na Ma^c, Guixin Qin^{a,*}, Zewei Sun^a, Chunyan Yu^b^a College of Animal Science and Technology, Jilin Agricultural University, 2888 Xincheng Street, Changchun, Jilin 130118, PR China^b College of Animal Husbandry and Veterinary Medicine, Shenyang Agricultural University, 120 Dongling Road, Shenyang, Liaoning 110866, PR China^c Shengyang Fujinmen Hospital of Traditional Chinese Medicine, 70 Dadong Road, Shenyang, Liaoning 110042, PR China

ARTICLE INFO

Article history:

Received 22 October 2011

Received in revised form 16 June 2012

Accepted 3 July 2012

Available online 9 July 2012

Keywords:

 β -Conglycinin subunits

Purification

IgE immunoblotting

Basophil histamine release test

ABSTRACT

The purpose of this study was to purify soybean β -conglycinin subunits and analyze their IgE-binding properties with IgE immunoblotting and their response in a basophil histamine release test. The method presented in this paper offers an effective procedure to purify β -conglycinin subunits with higher yield and purity compared with previous methods by using a combination of ion exchange and metal chelate affinity chromatography. Beginning with a column load of 600 mg β -conglycinin, the new method yielded 100.4 mg of protein containing 96.7% α subunit, 97.0 mg of protein containing 91.6% α' subunit and 89.4 mg of protein containing 96.1% β subunit. All of the purified subunits of β -conglycinin reacted with IgE from sera of soybean-allergic patients and induced dose-dependent histamine release from basophils from these patients, suggesting that purified α , α' , and β subunits of β -conglycinin retained allergenic activity and could be used for in vitro and in vivo diagnosis of soybean-induced food allergy.

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1. Introduction

Soybean is an important source of high-quality protein due to its relatively well-balanced amino acid composition [1,2]. It is very popular in the food industry. However, soybean is also a dietary allergen source for humans [3] and has been identified as one of the top eight allergenic foods [4]. The incidence of soybean allergies in humans is high (approximately 0.5% of the American adult population [5,6]), especially in children nourished with soy protein-based formulas [7,8]. Some soybean proteins have long been recognized as allergens, with molecular weights ranging from 7 to 71 kDa [9]. Prominent among those are Gly m Bd 30K, Gly m Bd 28K, Gly m Bd 60K, and G1 and G2 glycinin proteins [10–15].

β -Conglycinin, also called 7S protein, accounts for more than 30% of the total soybean protein [16]. It is a heterotrimer with a molecular mass of approximately 180 kDa and is mainly composed of three subunits: α' (72 kDa), α (68 kDa), and β (52 kDa) [17]. The relative percentages of α , α' , and β subunits in the trimer are approximately 45%, 35%, and 20%, respectively [18]. All three subunits are completely N-glycosylated and share extensive amino acid sequence homology. The amino acid sequences deduced from the nucleotide sequences reveal that the α and α' subunits

contain extension regions in addition to the core regions, which are common to all three subunits. The absolute similarities in the core regions between α and α' , α and β , and α' and β are 86.8%, 75.5% and 71.4%, respectively [19]. Of the three subunits of β -conglycinin, the α subunit has been unambiguously demonstrated as a major allergen in soybean [20–23] and is known as Gly m Bd 60K [12] and designated Gly m 5.0101 according to International Union of Immunological Societies allergen nomenclature [23]. Although the α' and β subunits of β -conglycinin failed to be recognized as allergenic in earlier reports [20], they were also demonstrated to be soybean allergens in more recent publications [22,23] and designated Gly m 5.0201 and Gly m 5.0301, respectively [23].

Purification of allergen molecules is an essential requirement for experimental investigations and clinical applications. Purified allergen molecules can be used in the quantification of individual allergens in the standardization of allergen extracts [24]. Highly purified allergen can also be directly used as an accurate diagnostic tool for food allergy [25,26]. Because recombinant allergens lack posttranslational modifications, such as glycosylation, the use of natural allergens is preferable for glycoprotein allergens, such as soybean β -conglycinin subunits. All of the applications require not only highly purified allergens but also an adequate amount of protein. Moreover, the allergenic activity of the allergen should be retained. Because β -conglycinin subunits have been recognized as the major allergens for soybean-allergic patients in more recent publications [22,23], highly purified soybean allergen molecules may be more useful in predicting the presence and severity of clinical soybean allergy than currently used skin or blood tests, which are based on whole soybean extracts.

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Currently, no satisfactory method has been developed to prepare sufficient amounts of purified β -conglycinin subunits to meet the need of experimental investigations and clinical applications. Because the α and α' subunits share similar physicochemical parameters, such as molecular mass and isoelectric points, previous reports indicated that the two subunits were difficult to separate by single-ion exchange chromatography. Coates et al. attempted to resolve the subunits of β -conglycinin on DEAE-sephadex A50 at higher pH (pH 8.1) and lower pH (pH 7.0). Their results showed that the α and α' subunits of β -conglycinin were eluted as a single peak at pH 8.1 and overlapped at pH 7.0 [27]. Ogawa et al. and Thanh et al. also obtained a similar chromatographic profile by a single-ion exchange chromatographic procedure [17,20]. These reports indicated that it was difficult to obtain sufficient amounts of individual β -conglycinin subunits that were not contaminated by other protein components. In the current study, we report an improved separation procedure through which efficient purification of β -conglycinin subunits can be achieved. This new method is a significant advancement over past techniques because the new method cannot only significantly improve the purification efficiency but also retain the original allergenic activity. The purified allergen molecule might

be a promising molecular diagnostic tool for soybean-related allergy.

2. Materials and methods

2.1. Purification of soybean β -conglycinin

Crude soybean β -conglycinin was isolated from soybean cultivar fengjiao 7607 using a simplified purification method essentially according to Thanh and Shibasaki [28]. The resulting β -conglycinin fraction was further purified by Sepharose CL-6B gel chromatography as described previously [29]. After lyophilization, purified β -conglycinin was stored at -20°C .

2.2. Ion exchange chromatography

Purified β -conglycinin was dissolved in elution buffer A (20 mM potassium phosphate, pH 7.0, and 8 M urea), which was used as a denaturing agent to dissociate β -conglycinin subunits. After centrifugation ($9000 \times g$, 30 min, 4°C) and filtration through a $0.22 \mu\text{m}$ membrane filter to remove minor particulates, the resulting solution was loaded onto an XK-50 column packed with 185 mL of DEAE-Sepharose Fast Flow, which was pre-equilibrated with elution buffer A. The column was washed with elution buffer A until the absorbance of the effluent at 280 nm was <0.05 and then washed with a gradient of 0–50% elution buffer B (20 mM potassium phosphate, pH 7.0, 8 M urea and 1 M NaCl) using a gradient mixer. Fractions containing the β subunit of β -conglycinin, as identified by SDS-PAGE followed by

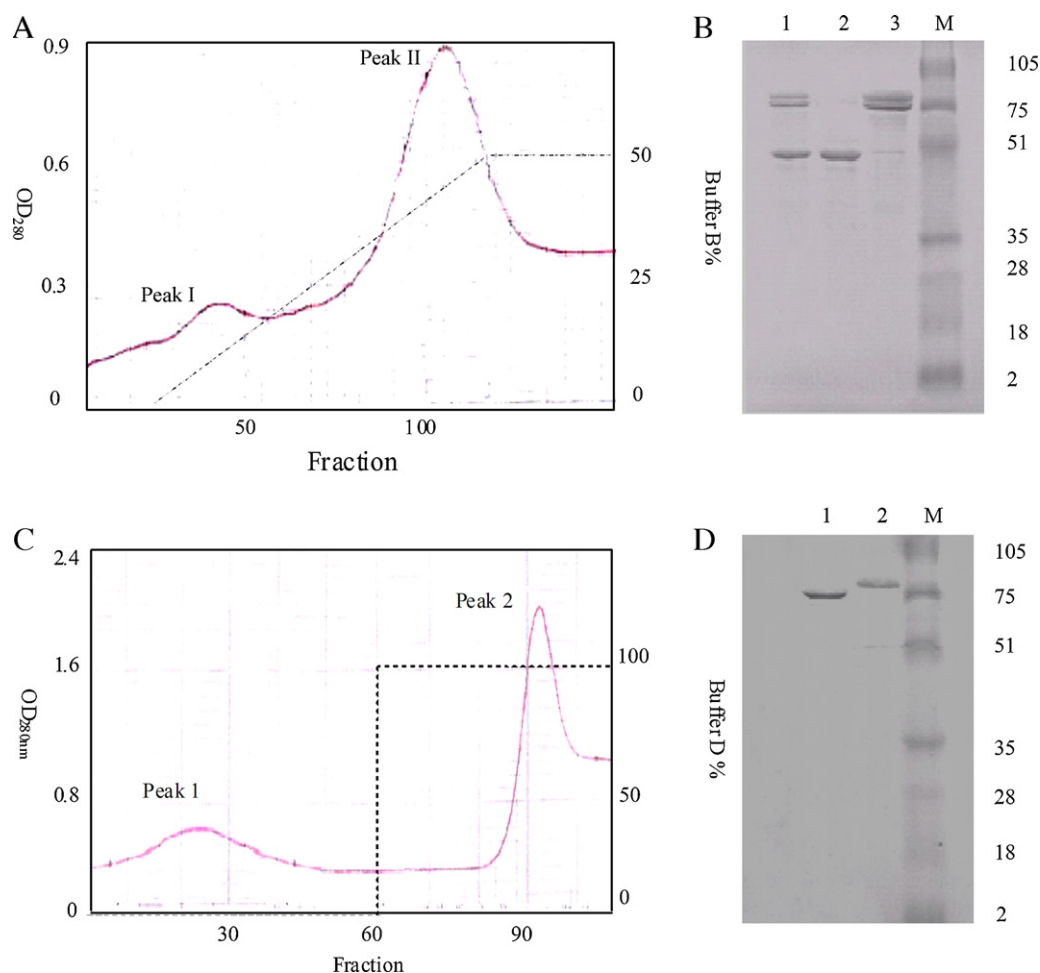


Fig. 1. Purification of β -conglycinin subunits. (A) A sample of β -conglycinin dissolved in elution buffer containing 8 M urea was applied to an ion exchange column and eluted by a NaCl gradient. (B) The protein components separated by the ion exchange column were fractionated by SDS-PAGE and stained with Coomassie brilliant blue. Lane M, protein marker; lane 1, purified β -conglycinin; lane 2, protein component eluted from the ion exchange column in peak I; lane 3, protein component from peak II. (C) Peak II from the ion exchange column was loaded onto a zinc chelate column. The unbound protein was eluted with buffer C, and the bound protein was washed with buffer D, which contained 0.1 M imidazole. (D) The eluted protein samples from the zinc chelate column were subjected to SDS-PAGE followed by Coomassie brilliant blue staining. Lane M, protein marker; lane 1, protein component eluted from zinc chelate column in peak 1; lane 2, protein component from peak 2.

Coomassie brilliant blue staining, were successively dialyzed against buffer B containing 4 M, 2 M, 1 M, and 0 urea at 4 °C for at least 12 h each. After being lyophilized, the purified subunit was stored at −20 °C. Fractions containing the α and α' subunits of β -conglycinin were pooled for subsequent use. All of the procedures of DEAE-Sephacel chromatography were conducted in a MiniColdLab (LKB 2023, GE Healthcare Life Science) at 4 °C.

2.3. Metal chelate affinity chromatography

An XK-26 column was packed with 50 mL of Chelating Sepharose Fast Flow and washed with at least two column volumes (100 mL) of 200 mM zinc sulfate solution (pH 4.0) to charge the column with zinc ions. After washing the column with 250 mL of ultrapure water and the same volume of acidic buffer (20 mM sodium acetate and 0.5 M sodium chloride, pH 4.0) to remove excess and loosely bound metal ions, the column was thoroughly equilibrated with five column volumes (250 mL) of elution buffer C (50 mM Tris–HCl, pH 7.4, 8 M urea and 0.5 M NaCl).

Pooled fractions containing the α and α' subunits of β -conglycinin from ion exchange chromatography were dialyzed against elution buffer C with 14 kDa dialysis bags: each dialysis step lasted for two hours, and the procedure was repeated five times. The obtained solution was applied to the zinc chelate column. The column was washed with the buffer C to elute unbound fraction until the absorbance of the effluent was <0.05. Subsequently, the bound fraction was eluted with elution buffer D (50 mM Tris–HCl, pH 7.4, 8 M urea, 0.5 M NaCl, and 0.1 M imidazole). All of the metal chelate affinity chromatography procedures were performed at 4 °C in a

MiniColdLab (LKB 2023, GE Healthcare Life Science). After being identified by SDS-PAGE followed by Coomassie brilliant blue staining, the bound and unbound fractions were successively dialyzed against buffer C containing 4 M, 2 M, 1 M, and 0 urea at 4 °C for at least 12 h each. The two purified protein was then lyophilized and stored at −20 °C.

2.4. The calculation of protein output in the purification

The concentrations of the protein solutions were determined by measuring absorbance at 260 and 280 nm as described by Layne [30]. The protein output at each step in the purification process was calculated according to pooled volumes and the concentrations of purified protein at each step in the purification.

2.5. SDS-PAGE and the quantitation of the homogeneity of purified β -conglycinin subunits

The protein fraction from each step of purification was analyzed by SDS-PAGE under reducing conditions. Electrophoresis was performed essentially according to the method of Laemmli [31] on slab gels consisting of a 12% acrylamide resolving gel and a 5% stacking gel. The gel was then stained with Coomassie brilliant blue. A quantitative assessment of the homogeneity of purified β -conglycinin subunits was carried out by scanning densitometry of SDS-PAGE gels, according to the method of Mujoo et al. [32].

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 $\alpha'$  VEEEECEEGQIPRPRP—QHPERERQQHGEKEEDEGEQPRPFPPRPRQP HQEEEHEQK
 $\alpha$  VEKEE—CEEGEIPRPRPRPQHPEREPQPGKEKEEDEGEQPRPIPFPRP—QPRQEEHEQR

 $\alpha'$  EEEHEWHRKEEKHGGKGS EEE-----QDERE HPRPHQPHQKEEEKHEWQH KQEK HQGKE
 $\alpha$  EEQEWPRKEEKRGEKGS EEEDEDEDEE QDERQFPFPRPP HQKEERN-----

 $\alpha'$  S EEEEEEDQDEDEEQDKE SQESEGSESQREPRRHKNKNPFHF—NSKRFQTLFKNQYGHVRV
 $\alpha$  -----EEDEDEEQQRE SE ESEDESEL—---RRHKNKNPFLF—GSNRFETLFKNQYGRIRV

 $\alpha'$  LQRFNKRSQLQNLRDYRI LEFN SKPNTLLLP HHAADADYLIVILNGTAILTLVNDDRDS
 $\alpha$  LQRFNQ RSPQLQNLRDYRI LEFN SKPNTLLLP HHAADADYLIVILNGTAILSLVNDDRDS

 $\alpha'$  YNLQSGDALRVPAGTTFYVVPNDNENLRMIAGTTFYVVPNDNENLRMITLAIPVNKPG
 $\alpha$  YRLQSGDALRVPAGTTFYVVPNDNENLRMI-----TLAIPVNKPG

 $\alpha'$  RFESFFLSS TQAQQSYLQGF SKNILEAS YDTKFEEINKVLFGR EEGQQQEERLQESVIV
 $\alpha$  RFESFFLSS TEAQQSYLQGF SRNILEAS YDTKFEEINKVLF SREEGQQQEQLQESVIV

 $\alpha'$  EISKQIRELSKHAKSSSRKTISSEDKPFNLGSRDPIYSNKLGLFEIT—QRNPQLRDLD
 $\alpha$  EISKEQIRALSKRAKSSSRKTISSEDKPFNLRSRDPIYSNKLGLFEITPEKNPQLRDLD

 $\alpha'$  VF LSVDMNEGALFLPHFN SKAIVVLV INEGANIELVGIKEQQQRQQQEQLPVRKYR
 $\alpha$  IFLSIVDMNEGALLLP HFN SKAIVILV INEGDANIELVGLKEQQQEQLPVRKYR

 $\alpha'$  AELSEQDIFVIPAGYPVMVNATSDLNFFA FGINAENNQRNFLAGSKDNVISQIPSVQVEL
 $\alpha$  AELSEQDIFVIPAGYPVVMVNATSNLNF FA FGINAENNQRNFLAGSKDNVISQIPSVQVEL

 $\alpha'$  AFPRSAKDIENLIKSSQESYFVDAQPQQKEEGNKGRKGPLSSILAFY
 $\alpha$  AFGSAQAVEKLLKNQRESYFVDAQPKKEEGNKGRKGPLSSILAFY

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Fig. 2. Multiple sequence alignments of α and α' subunits. The amino acid sequences of α and α' subunits were obtained from Swiss-Prot protein knowledgebase (accession number P13916, P11827) [45]. The signal and the presequence have been deleted. Dashes (–) are introduced for maximal alignment of the sequences. Histidines (H) are in bold, italic, and underlined. The α' subunit contains more histidine residues in its amino acid sequence (especially in the N-terminal extension) compared with the α subunit. The structural characteristics can be used for separation of the two subunits by metal chelate chromatography.

Table 1
Summary of the results of each step in the purification of the α, α', and β subunits from β-conglycinin.

	Total protein output (mg) ^a			Purity (%) ^b		
	α	α'	β	α	α'	β
DEAE-sepharose Fast Flow	243.5 ± 25.1	–	89.4 ± 18.1	51.2 ± 3.3	36.9 ± 2.6	96.1 ± 0.6
Chelating sepharose Fast Flow	100.4 ± 15.0	97.0 ± 22.1	–	96.7 ± 1.4	91.6 ± 7.0	–

^a In total, 600 mg of β-conglycinin was dissolved in elution buffer A. After centrifugation and filtration, the resulting solution was used for subsequent chromatographic purification. Total protein output was calculated according to the pooled volumes and the concentrations of protein solution at each purification step. Protein concentrations were estimated by measuring absorbance at 280 nm and 260 nm [30]. The values are presented as the mean ± SD (standard deviation) of triplicate determinations. The total amount of the mixture of the α and α' subunits from ion exchange chromatography is 243.5 ± 25.1 mg.
^b The purity of the α, α', and β subunits was estimated by densitometry, as described by Mujoo et al. [32].

2.6. Amino-terminal sequencing

For amino-terminal sequencing, aliquots of the final purified proteins separated by SDS-PAGE were blotted onto a polyvinylidene difluoride membrane (Immobilon PVDF, Millipore Inc., USA) in CAPS transfer buffer (pH 11.0). The protein bands were excised and sequenced by automated Edman degradation using a PROCISE™ 492 cLC Protein Sequencer (Applied Biosystems, USA).

2.7. IgE Immunoblotting

Sera from eight patients with a positive case history of type I allergy to soybean were selected. The patients were all allergic to soybean and experienced at least one of the typical allergy symptoms, such as skin, gastrointestinal or respiratory reactions and anaphylaxis, after being orally exposed to soybean proteins. Their specific IgE levels to soy extract were higher than 3.5 kU/L, as measured with the CAP system (Phadia, Uppsala, Sweden). Sera from four healthy persons with a negative case history of type I allergy to soybean allergens were included as controls. The study was approved by the Human Use Advisory Committee at Shengyang Fujinmen Hospital of Traditional Chinese Medicine. All of the subjects provided informed consent.

IgE immunoblotting was performed essentially as previously described [33]. Briefly, proteins separated by SDS-PAGE were blotted onto a polyvinylidene difluoride membrane (Immobilon PVDF, Millipore Inc., USA). Then, the PVDF membrane containing blotted proteins was blocked using 3% bovine serum albumin (BSA) in 0.05% PBS-Tween (PBST) for 2 h at room temperature and incubated with the sera from soybean-sensitive patients and controls (2 × dilution with PBST plus 1% BSA) for 18 h at 4 °C. After four washes with PBST, the membrane was incubated with biotin-labeled anti-IgE for 2 h at room temperature, washed four times again with TBST, and subsequently incubated with horseradish peroxidase (HRP)-conjugated streptavidin for 2 h at room temperature. The membrane was washed four times and visualized with a Super ECL detection kit (Enhanced chemiluminescence detection kit, Applygen Technologies Inc., Beijing, China).

2.8. Basophil histamine release test (BHRT)

Heparinized blood (3 mL) was collected from eight patients with documented soybean hypersensitivity reactions who recognized β-conglycinin subunits with

IgE immunoblotting and four healthy volunteers with a negative case history of type I allergy to soybean allergens as controls. The histamine-releasing capabilities of purified β-conglycinin subunits were determined essentially according to a previously published method [34]. Heparinized whole blood was diluted 1:7 in buffer (25 mmol/L PIPES, 5 mmol/L KCl, 2.0 mmol/L CaCl₂, and 110 mmol/L NaCl, pH 7.35), and 100 μL of diluted blood was incubated with 50 μL of individual purified subunits at different dilutions at 37 °C for 45 minutes in 96-well microplates. The reaction was stopped by placing the plates on ice. Then, blood cells were immediately centrifuged (300 g, 4 °C), and histamine was measured in the cell-free supernatants by competitive enzyme immunoassay using a histamine kit. Spontaneous release was determined by incubation with a buffer control under the same conditions. Total histamine released into the supernatant was measured with the histamine kit after cell lysis in ultrapure water and freeze–thawing of the blood cells. The basophil histamine release ratio was calculated as follows: histamine release ratio (%) = [(allergen-stimulated histamine release – spontaneous release)/(total histamine release – spontaneous release)] × 100%.

3. Results and discussion

3.1. Purification of soybean β-conglycinin subunits

Food allergy is an abnormal immunologic response to food protein. Based on the immunological mechanism involved, most food allergy reactions are mediated by IgE antibodies, which pose a particular threat to susceptible individuals [35]. The diagnosis of food allergy, such as soybean allergy, which is a predominantly Th2-type immune response mediated by IgE [36], includes provocation testing and IgE serology using allergen extracts or purified allergen [37,38]. However, because allergen extracts contain impurities, highly purified allergens are needed for exact in vitro diagnosis [26]. The α, α' and β subunits of β-conglycinin, also known as Gly m 5.0101, Gly m 5.0201, and Gly m 5.0301, respectively, are three

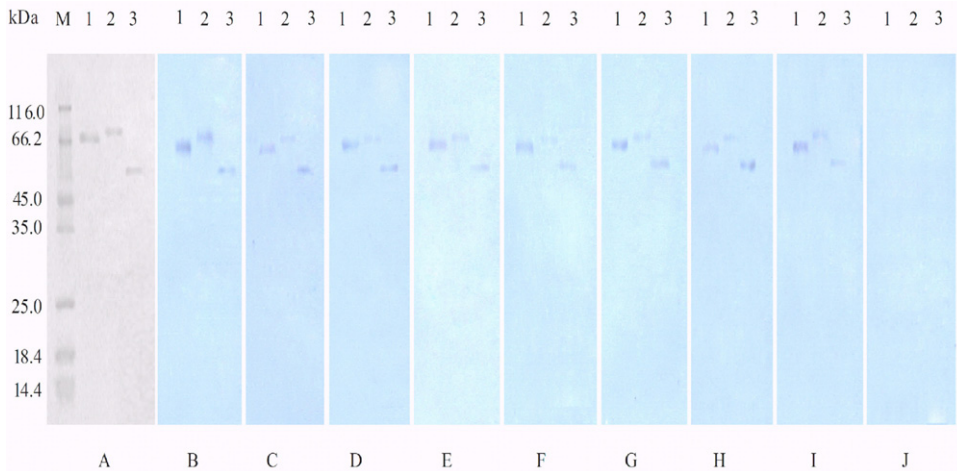


Fig. 3. SDS-PAGE and IgE immunoblotting of purified β-conglycinin subunits. Purified β-conglycinin subunits were subjected to SDS-PAGE, transferred to PVDF membranes and probed with individual sera from eight soybean-sensitive patients and four individuals non-allergic to soybean. The membranes were visualized with a super ECL detection kit after being incubated with biotin-labeled anti-IgE and HRP-conjugated streptavidin. (A) SDS-PAGE of purified β-conglycinin subunits. Lane M, molecular weight standards; lane 1, purified α subunit; lane 2, purified α' subunit; lane 3, purified β subunit. (B–I) Corresponding immunoblots probed with sera from eight soybean-sensitive patients (patients 1–8). (J) A representative immunoblot probed with sera from four individuals non-allergic to soybean.

major components of seed storage proteins in soybean. Nonetheless, full agreement is lacking as to whether all three subunits of β -conglycinin are truly allergenic as reports in the literature have been contradictory [20,22,23]. The purpose of this study, therefore, was to purify β -conglycinin subunits and perform allergenicity checks for the individual subunits that might be suitable candidates for further clinical applications.

A relatively simple method for the efficient separation and purification of β -conglycinin subunits was developed using a combination of ion exchange chromatography and metal chelate affinity chromatography. A sample of β -conglycinin obtained by a previous method from soybean cultivar fengjiao7607 was mainly

composed of the α , α' and β subunits. The sample was dissolved in a buffer containing 8 M urea to dissociate β -conglycinin into free subunits and was then separated by ion exchange chromatography on a DEAE-Sepharose Fast Flow column. The procedure was adopted based on the charge density differences among the three subunits of β -conglycinin. Due to the difference in the isoelectric points (pI), the β -conglycinin subunits carried different amounts of negative charge in elution buffer A (pH 7.0). The isoelectric point of the β subunit (pI = 5.66–6.00) was higher than the others (α , pI = 4.90; α' , pI = 5.18). The β subunit, which carried less negative charges, was eluted from the column first and formed peak I by sodium chloride gradient elution, as demonstrated by SDS-PAGE

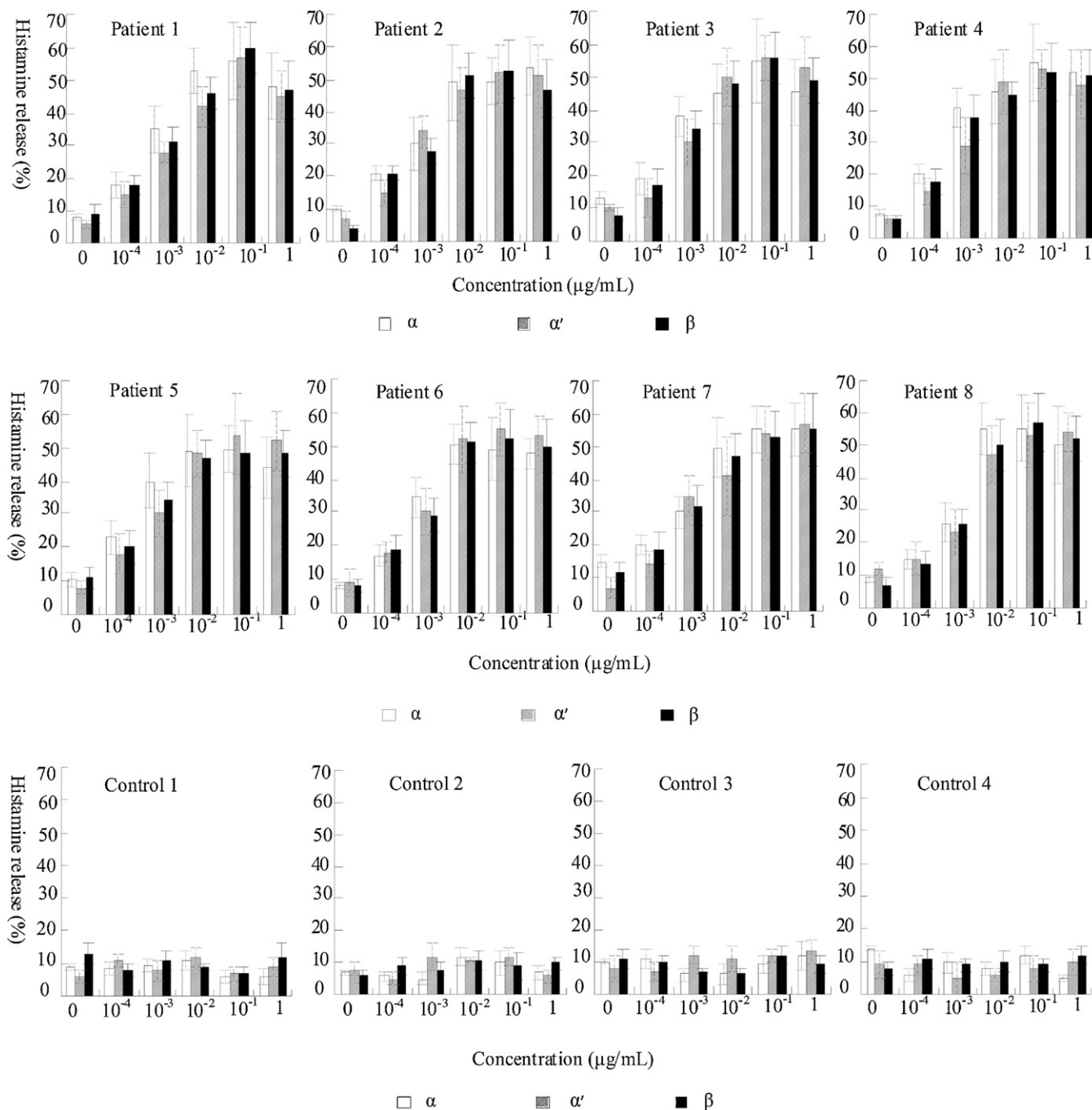


Fig. 4. Induction of basophil histamine release by purified β -conglycinin subunits. Basophils from eight patients allergic to soybean (patients 1–8) and four non-allergic subjects (controls 1–4) were incubated with different concentrations of individual β -conglycinin subunits. Histamine release is shown as a percentage of total histamine. The values are means of data from three different determinations at each concentration of the inducer.

(Fig. 1A and B) and the analysis of N-terminal amino acids, which showed LKVVRE – for the protein. The α and α' subunits, however, were eluted as a single peak (peak II) from the DEAE-Sepharose Fast Flow column in the elution buffer, as subsequently identified by SDS-PAGE, because they carry nearly the same amount of negative charges in the elution buffer (Fig. 1A and B).

For further separation of the α and α' subunits, metal chelate affinity chromatography was introduced. When the eluates from an ion exchange column containing the α and α' subunits were loaded onto a zinc chelate column, a peak of unbound fractions was first eluted with buffer C, and a peak of bound fractions was eluted with buffer D (Fig. 1C). The substance that flowed through the zinc chelate column was a single 68 kDa protein, and the bound substance was predominantly a 72 kDa protein, as identified by SDS-PAGE (Fig. 1D). The analysis of N-terminal amino acids showed VEKEE- for the unbound protein and VEEEE- for the bound protein, which indicated that the unbound and bound proteins were the α and α' subunits, respectively. The above procedure was adopted based on the difference in structural characteristics of the α and α' subunits of β -conglycinin. When the amino acid sequence of the α' subunit was compared with the α subunit, it was found that the α' subunit contained more histidine residues than the α subunit in the N-terminal extension (Fig. 2). Histidine residues in proteins can form complexes with transition metals, such as zinc, nickel and copper ion. They have been widely used as a potent molecular tool for the purification of histidine-rich proteins from other proteins [39–41]. Chelating Sepharose Fast Flow with a suitable immobilized metal ion would selectively bind to exposed histidine residues in proteins. Indeed, when the mixture of α and α' subunits was applied to a zinc chelate column, the α' subunit was retained by the zinc-coupled matrix and the α subunit was not. Therefore, by taking advantage of the different histidine contents of the two subunits through chelate affinity chromatography, the α and α' subunits can be successfully purified to homogeneity in a creative way.

To obtain sufficient amounts of purified subunits, a two-step separation was performed at maximum column load. Beginning with a column load of 600 mg β -conglycinin, the procedure yielded 100.4 mg of protein containing 96.7% α subunit, 97.0 mg of protein containing 91.6% α' subunit and 89.4 mg of protein containing 96.1% β subunit (Table 1). In previous reports, when β -conglycinin subunits were purified by a single step of ion exchange chromatography, the α and α' subunits of β -conglycinin either overlapped or formed one elution peak. Therefore, the purification yield remained low [17,20,27]. Using a combination of ion exchange chromatography and metal chelate affinity chromatography in the current method, β -conglycinin subunits were purified to apparent homogeneity, and reasonable amounts of β -conglycinin subunits were obtained at the maximum column load of β -conglycinin.

3.2. IgE-binding properties of purified β -conglycinin subunits

Immunoblotting analysis was performed to evaluate the IgE-binding properties of purified β -conglycinin subunits. Human sera from eight patients who were allergic to soybean were selected. For control purposes, the sera from four non-allergic individuals to soybean were also included. Images of the Coomassie brilliant blue-stained gel and immunoblot membranes are shown in Fig. 3. All three subunits of β -conglycinin revealed strong IgE-binding capacities to all of the sera from the eight patients who were allergic to soybean. However, no allergen-specific IgE binding was detected using the sera samples from four non-allergic controls. This observation suggested that all of the purified β -conglycinin subunits contained intact IgE-binding epitopes and retained their allergenic activities.

The diagnosis of food allergy is sometimes hampered by the presence of specific IgE antibodies not always correlating with

clinical allergy symptoms [42,43]. In contrast with serological determination of specific IgE antibodies, biological tests, such as the basophil histamine release test, evaluate the IgE-dependent effector cell response and thus accurately reflect the patient's clinical symptoms. A good agreement between results obtained from the basophil histamine release test and provocation testing has also been reported [44]. In the present study, the allergenic activities of purified β -conglycinin subunits were further measured by the basophil histamine release test on the eight patients who were allergic to soybean. Basophils from four individuals non-allergic to soybean were used as negative controls. The data are shown in Fig. 4. As shown, all three subunits of β -conglycinin elicited dose-dependent histamine release from basophils from the patients allergic to soybean but not from the non-allergic healthy individuals. Data from the histamine release test support the results of immunoblotting analysis. Our results showed that the three purified β -conglycinin subunits were capable of inducing dose-dependent basophil histamine release and suggested that the purified allergens could be applied in a cellular in vitro test for the diagnosis of soybean allergy.

Published data on the allergenicity of β -conglycinin subunits are controversial. Among the three subunits, only the α subunit of β -conglycinin but not the highly homologous α' and β subunits was shown to be allergenic in earlier reports [20]. However, in more recent publications, all three subunits of β -conglycinin, including the α' and β subunits, were demonstrated to be major allergens in soybean [22,23]. In agreement with these results, our study suggested that all of the purified subunits of β -conglycinin possess good IgE-binding properties, as evidenced by IgE immunoblotting and the basophil histamine release test, which further demonstrated that all three subunits of β -conglycinin serve as major allergens for soybean-sensitive patients.

4. Conclusions

In the present study, an effective method to purify β -conglycinin subunits was developed by a combination of ion exchange and metal affinity chromatography. The new procedure was capable of providing highly purified β -conglycinin subunits with reasonable yields. The purified subunits retained allergenic activity, as evidenced by their ability to bind to IgE antibodies in the sera and induce histamine release from basophils from patients who were allergic to soybean, suggesting that purified allergens might be promising candidates for in vitro and in vivo diagnosis of soybean-related food allergy in humans.

Acknowledgement

This work was funded by the National Natural Science Foundation of PR China (No. 30430520).

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