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Original Article

Epitope mapping and identification on a 3D model built for the tartary buckwheat allergic protein TBb

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Allergic protein TBb, a major allergen in tartary buckwheat, was divided into four epitope-containing fragments and was named F1, F2, F3, and F4, respectively. Results of immunological assays revealed that F2 had the strongest IgE-binding activity to patient's sera, which indicated that it might contain the linear IgE-binding epitope of TBb. According to the results of sequence analysis and molecular modeling of tartary buckwheat allergen, three mutants of F2 gene (R139A, R141A, and D144A) were reconstructed using site-directed mutagenesis, and each mutant was expressed in *Escherichia coli* BL21 (DE3). Following purification by Ni^{2+} affinity chromatography, enzyme-linked immunosorbent assay and dot blot were performed for wild-type F2 and its mutants using sera from buckwheat-allergic patients and a negative control (non-allergic patient). Results showed that mutants R139A and D144A had weaker IgE-binding activity to patient's sera than wild-type F2, implying that Arg^{139} and Asp^{144} might be involved in the allergic activity of TBb. However, R141A had the weakest IgE-binding activity, suggesting that Arg^{141} may be the critical amino acid of TBb. This is the first report on the epitope mapping and identification of TBb. Our findings will contribute to the production of TBb hypoallergens and to allergen-specific immunotherapy for tartary buckwheat allergy.

Keywords epitope mapping; tartary buckwheat; allergen; 3D model

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Introduction

Buckwheat belongs to the family Polygonaceae and includes two cultivated species: common buckwheat (CB) (*Fagopyrum esculentum*) and tartary buckwheat (TB) (*Fagopyrum tataricum*) [1]. It is reported that the protein content of buckwheat flour is significantly higher than that of rice, wheat, millet, sorghum, and maize. In addition to high-quality protein, buckwheat seeds also contain many bioactive components such as dietary fiber, flavonoids, phytosterols, fagopyrins, and thiamine-binding proteins that have beneficial effects on the cardiovascular health [2,3]. Thus, it is an important health food, and has been widely studied in terms of planting, breeding, and estimation of nutritional qualities [4]. However, buckwheat can also cause allergy [5], and results in clinical symptoms including asthma, dermatitis, eczema, and even anaphylactic shock [6]. Since the first buckwheat-allergic patient was reported in 1909, buckwheat has been recognized as one of most important food allergens manifested by severe and dangerous symptoms induced by ingestion or inhalation of a small amount of the flour or food products [7]. The largest study on buckwheat allergy has been performed among 92,680 school children of Yokohama in the 1990s [8]. The prevalence of buckwheat allergy is determined to 0.22% and the incidence of anaphylactic shock due to buckwheat is higher than those due to egg and milk allergy [9]. A series of seven Chinese patients with buckwheat allergy also has been reported in 2010 [10]. These negative effects of buckwheat limit its broad use as an alternative food and popular additive. Therefore, it is urgent to identify allergen molecules for investigating the mechanism of buckwheat allergy.

In the past decades, scientists have reported the allergic composition in CB [11]. Some allergic proteins in CB with different molecular weights of 6, 9, 19, 22, 24, and 60–70 kDa have been identified [12,13]. With high nutritional and medical values, TB has attracted more attention in recent years. However, reports on allergic composition of TB are limited. Previously, we have obtained several allergic proteins from TB with molecular weight of 24, 34, and 56 kDa, designated TBa, TBb, and TBt, respectively. It has been identified that TBt (a full-length allergic protein in TB) contains cupin fold. This family represents the conserved barrel domain of the Cupin superfamily ('Cupa' is

the Latin term for a small barrel), containing 7S and 11S plant seed storage proteins [14]. Each TBt protomer consists of an N-terminal subunit TBb and a C-terminal subunit TBa, which is known to be separated into two peptide chains by post-translational peptidase cleavage. Both TBa and TBb have higher IgE-binding activity than TBt in the detection of immunological activity [15,16]. We have shown that an epitope called E1 is the major epitope in TBa [17], but we do not know that about TBb.

In this study, to understand the allergic mechanism of buckwheat foods, we focused on mapping the epitopes of the TB allergic protein TBb and identifying them on a 3D model of the protein.

Materials and Methods

Materials

Thermostable *Pfu* DNA polymerase was purchased from TIANGEN Biotech (Beijing, China). Taq DNA polymerase was obtained from TaKaRa (Dalian, China). Restriction endonucleases and other enzymes for molecular cloning were purchased from Sangon (Shanghai, China). Horseradish-peroxidase (HRP)-conjugated mouse anti-human IgE antibodies were purchased from Southern Biotech (Bimingham, USA).

Sera samples

The sera of three patients (**Table 1**) with a history of respiratory, dermatologic, or gastrointestinal symptoms occurring within 1 h following ingestion of buckwheat were obtained from the Blood Center of Taiyuan, China. Healthy sera from volunteers not allergic to buckwheat were used as a negative control.

Cloning of epitope-containing fragments in TBb

According to the method of segmental expression, the full-length allergic protein TBb (298 amino acid residues) was divided into four fragments (**Fig. 1**), which were named F1, F2, F3, and F4, respectively. The four epitope-containing fragments cDNA were polymerase chain reaction (PCR)-amplified from plasmid pET-32m-TBb using the primers listed in **Table 2**, and all of their PCR programs were 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. PCR products were subcloned into the pGEM-T Easy Vector for sequencing. After being confirmed, the vector was digested with *Bam*HI and *Hin*dIII. The fragments obtained were ligated into the expression vector pET-32m to generate four recombinant plasmids (pET-32m-*F1*, pET-32m-*F2*, pET-32m-*F3*, and pET-32m-*F4*) [18,19].

Sequence analysis and molecular modeling

Primary sequences were aligned using BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and displayed with the MegAlign program in the DNAStar software for homologous amino acid residues. Molecular modeling of the TB allergic protein TBt, which contains an N-terminal subunit TBb and a C-terminal subunit TBa, was carried out on a SWISS-MODEL workstation [20], and displayed with the Cn3D program [21]. The x-ray-solved Cupin structure, available at the Protein Data Bank (PDB) for the almond Pru1 protein (PDB code 3fz3) [22], shares ~60% homology with TBt. Furthermore, TBt had the same cleavage pattern as almond Pru1 protein. Therefore, the almond Pru1 protein could be used as a template to build relatively accurate 3D models of the allergen.

Site-directed mutagenesis of F2

Three mutants with one amino acid replaced by alanine were produced by PCR, using a pair of primers with AGA-to-GCA, AGA-to-GCA, and GAC-to-GCC mutations [23], respectively (**Table 2**). The PCR products were then digested and ligated between the *Bam*HI and *Hin*dIII multi-cloning sites into the pET-32m expression vector. The mutations in F2 were confirmed by sequence analysis (TaKaRa).

Expression and purification of epitope-containing fragments and mutant fusion proteins

The constructed vectors containing the gene of epitope-containing fragments or mutants were transformed into *Escherichia coli* strain BL21 (DE3), and expression of the fusion protein was induced by the addition of isopropyl-β-thiogalacto-pyranoside at a final concentration of 1 mM in LB liquid medium. After 3 h of continuous shaking at 37°C, the fusion proteins were purified using a HisTrap affinity purification kit (Amersham Pharmacia

Table 1 Characterization of sera from patients with positive IgE binding to buckwheat

Serum no.	Immediate hypersensitivity reaction	Sex	Age (years)	Buckwheat-specific IgE score	Buckwheat-specific IgE levels (kU/L)	Major diagnosis
Patient 1	Positive	F	22	3	18.5	Buckwheat allergy, severe atopic dermatitis
Patient 2	Positive	F	40	3	17.2	Buckwheat and elm pollen allergy
Patient 3	Positive	F	38	3	16.4	Buckwheat and acarid allergy

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Biotech, Uppsala, Sweden). Each recombinant epitope-containing fragment or mutant was analyzed by means of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 12.5% polyacrylamide gel [18,24].

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was performed to detect the IgE-binding activity of epitope-containing fragments and F2 mutants. After coating 96-well plates with each protein (1.0 µg/50 µl of 50 mM sodium carbonate buffer/well, pH 9.6) and incubating at 4°C overnight, the plates were washed with phosphate-buffered saline (PBS, pH 7.5) containing 0.05% Tween 20 (PBS-T), followed by blocking with 1% bovine serum albumin for 1 h at 37°C. The plates were then washed with PBS-T, and incubated at 4°C overnight with diluted serum samples (1:20). After further washing using PBS-T, HRP-conjugated mouse anti-human IgE antibodies (Southern Biotech), diluted in PBS-T, were added in wells and incubated for 1 h at 37°C, and reacted with chromogen and orthophenylene diamine [23]. The optical density values of the mixture were measured at 490 nm with a microtiter plate reader (Bio-Rad, Hercules, USA). All ELISAs were performed in triplicate and the data were expressed in mean ± SD.

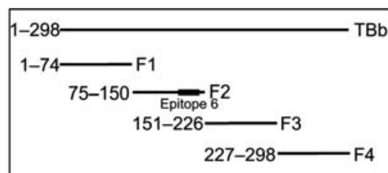

Figure 1 Mapping of IgE-binding regions in TBb Schematic location of each epitope-containing fragment and epitope 6 are shown.

Human IgE dot blot analysis

To test the specific IgE-binding capacity of the recombinant epitope-containing fragments and mutants, an immuno dot-blot assay was performed. Purified proteins were applied to a nitrocellulose membrane and blocked with PBS (pH 7.5) containing 5% skim milk. Both pooled and individual allergic sera were diluted 1:5 in PBS-T, and incubated with the dot-blots for 1 h at room temperature. Incubation of the membranes with the secondary antibody was performed with a 1:500 dilution of HRP-conjugated mouse anti-human IgE for 1 h. The bound human IgE was detected by incubating with the colorimetric substrate, 3,3′-diaminobenzidine (Boster Biotech), and allowing the color signal on the membranes to develop. The membranes were washed extensively with PBS containing 0.1% Tween 20 after each step throughout the protocol.

Statistical analysis

All experiments were conducted in triplicate and the data were expressed in mean ± SD. The statistics analysis was performed using SPSS 13.0 software (Chicago, USA) and Microsoft Excel version 5.0 for comparing differences.

Results

Expression, purification, and IgE binding of epitope-containing fragments in TBb

Using the method of segmental expression, the vectors containing the genes of four epitope-containing fragments

Table 2 Primers designed for cloning epitope-containing fragments of TBb and site-directed mutagenesis of F2

Name	Primers	Sequences (5′→3′)
F1	P1	5′ ATGGATCCGCGCAGCTATGGCCATGG 3′
	P2	5′ ATAAGCTTTTACAAGAGGCCTCCTGGCTG 3′
F2	P3	5′ ATGGATCCCTTCCTTCCTACTCCAACG 3′
	P4	5′ ATAAGCTTTTAGGCGGGAGATGGGATGAC 3′
F3	P5	5′ ATGGATCCGGTGTCGTGCAGTGGACT 3′
	P6	5′ ATAAGCTTTTATGCGCCGATAAGTGCTTC 3′
F4	P7	5′ ATGGATCCAACATCTTGAGTGGATTCCA 3′
	P8	5′ ATAAGCTTTTAATTGCTCCTTCCGCTTCCTC 3′
R139A	P3	5′ ATGGATCCCTTCCTTCCTACTCCAACG 3′
	F1	5′ ATAAGCTTTTAGGCGGGAGATGGGATGACGTCACCTTCTCTGACTGCGAAAAT-3′
R141A	P3	5′ ATGGATCCCTTCCTTCCTACTCCAACG 3′
	F2	5′ ATAAGCTTTTAGGCGGGAGATGGGATGACGTCACCTTCTGCGACTCT- 3′
D144A	P3	5′ ATGGATCCCTTCCTTCCTACTCCAACG 3′
	F3	5′ ATAAGCTTTTAGGCGGGAGATGGGATGACGGCACCTTC-3′

The mutated sites are underlined.

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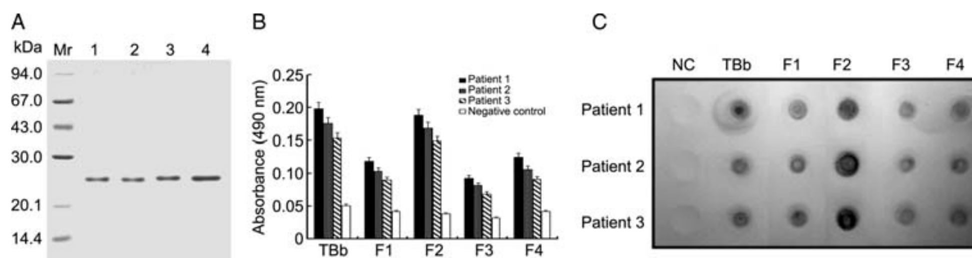

Figure 2 SDS-PAGE, ELISA, and Dot blot analysis of purified epitope-containing fragments proteins of TBb (A) SDS-PAGE analysis of epitope-containing fragments. Mr, low molecular weight protein marker; lane 1, F1-Trx; lane 2, F2-Trx; lane 3, F3-Trx; lane 4, F4-Trx. (B) Statistically significant differences between buckwheat-allergic patients and a negative control (non-allergic patient), and between the four epitope-containing fragments and TBb in their IgE-reactivity were detected by ELISA. Data were expressed in mean ± SD ($n = 3$). (C) Dot blot analysis of epitope-containing fragments. Purified recombinant fusion proteins were applied onto a nitrocellulose membrane. The dot blot was probed with sera from three buckwheat-allergic patients.

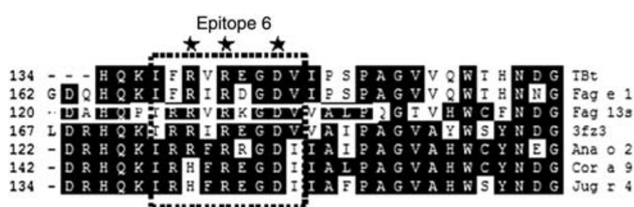

Figure 3 Alignment of the amino acid sequences of TBt and other plant seed storage proteins The black portion indicates the conserved amino acid residues. The linear IgE-binding epitope 6 of TBb (which is the N-terminal subunit of TBt) is dash-line boxed and the epitope 4 of Fag13S is white boxed. The mutation residues are indicated with black stars. Abbreviations: Fag e 1, buckwheat (*F. esculentum*, Swiss-Prot Q9XFM4); Fag 13S, buckwheat (*F. esculentum*, GenBank AAQ56206); 3fz3, almond (*Prunus dulcis*, GI 266618563); Ana o 2, cashew (*Anacardium occidentale*, GenBank AAN76862); Cor a 9, hazelnut (*Corylus avellana*, GenBank AAL73404); Jug r 4, English walnut (*Juglans regia*, GenBank AAW29810).

(**Fig. 1**) of TBb were constructed successfully. These proteins with 6×His-tagged were expressed and purified according to the manufacturer's instructions (Amersham Pharmacia Biotech). Result of SDS-PAGE showed that the molecular weight was in good agreement with the theoretical prediction [**Fig. 2(A)**], and Ni^{2+} affinity chromatography analysis showed that the purity was >98% without significant peaks of impurities.

To determine the IgE reactivity of these epitope-containing fragments and to identify the immunodominant region in TBb, ELISA was performed with each recombinant protein using three IgE-positive sera and a healthy control. As shown in [**Fig. 2(B)**], F2 exhibited the strongest IgE reactivity among all tested epitope-containing fragments ($P < 0.05$). Additional evaluation of the IgE-binding activity was performed by the dot blot method. As expected, results showed that F2 exhibited the strongest reaction with serum IgE from patients, comparing with other epitope-containing fragments [**Fig. 2(C)**]. Similar results were obtained by using three sera from buckwheat-allergic patients.

Amino acid sequence homology alignment and 3D modeling

The results of amino acid sequence homology alignment showed that the predicted epitope 6 (IFRVREGDV) in fragment 2 (F2) contain identical or structurally homologous amino acids. Furthermore, the surface-exposed regions of the linear IgE-binding epitope 4 (PTRRVRKGD) in Fag13S [25] coincided well with epitope 6 (IFRVREGDV) in TBb (**Fig. 3**). The 3D model built for TBt contained Cupin fold. Each protomer consisted of an N-terminal subunit TBb and a C-terminal subunit TBa that are known to be separated into two peptide chains by post-translational peptidase cleavage [**Fig. 4(A,B)**]. Both epitope 6 in TBb and epitope 4 in Fag13S formed a strongly charged area on the molecular surface [**Fig. 4(C,D)**] of the corresponding allergens and exhibited a rather conserved overall conformation characterized by some protruding/charged residues, especially Arg139, Arg141, and Asp144 [**Fig. 4(E,F)**].

Expression and purification of F2 and its mutants

To identify the amino acids in this epitope-containing fragment that is critical for IgE binding, we generated three different wild-type F2 mutants, each with an Arg or Asp replaced by an Ala residue, and referred to them as R139A, R141A, and D144A. These mutants were expressed as 6×His-tagged proteins according to the manufacturer's instructions. The purity was >98% without significant peaks of impurities after affinity chromatography. SDS-PAGE analysis showed that the molecular weight of each mutant was the same as that of wild type F2 [**Fig. 5(A)**].

ELISA assay of binding of WT F2 and its mutants to patient sera

F2 and its three mutants were compared for their IgE-binding activity by ELISA, using three serum samples from buckwheat-allergic patients, and one serum sample

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from a healthy donor as a negative control. As shown in **Fig. 5(B)**, all three mutants exhibited decreased IgE-binding activity in comparison with WT F2 ($P < 0.05$), implying that the three charged residues have significant contributions to the IgE binding of the allergen TBb. Among these mutants, R139A and D144A had weaker IgE binding activity (a decrease of nearly 30% from that of the wild type) to patient's sera ($P < 0.05$), indicating that Arg^{139} and Asp^{144} might be involved in the allergic activity of TBb. R141A, however, had the weakest IgE-binding activity (a decrease of nearly 50%), suggesting that Arg^{141} is more important than Arg^{139} and Asp^{144} for IgE-binding activity of TBb, and it is the critical amino acid in TBb.

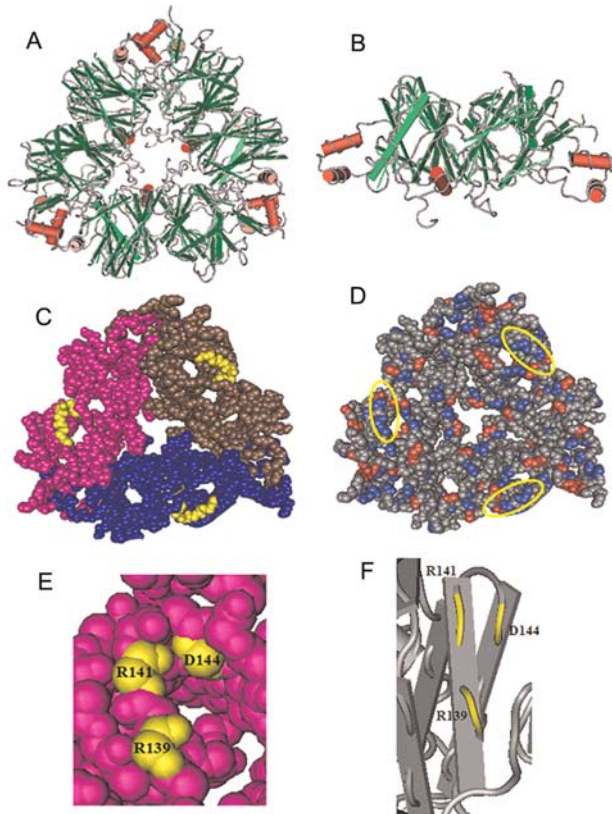

Figure 4 3D model of tartary buckwheat allergic protein TBt The homotrimer (A) and monomer (B) of tartary buckwheat-allergic protein TBt are shown by ribbon diagrams, and the core structure corresponding to the Cupin motifs (colored green). Distribution of the electrostatic potentials on the molecular surface of the homotrimer is shown in (D). The negative potential is colored red and the positive potential is colored blue. Neutral surfaces are gray. The position and electrostatic potentials of epitope 6 are shown in (C) and (D) (circled with yellow). The single amino acids substituted with alanine by site-directed mutagenesis are displayed by globular and ribbon diagrams in (E) and (F), respectively.

Dot blot analysis of WT F2 and its mutants

To evaluate the IgE reactivity of TBb, epitope-containing fragment F2 and its mutants, purified proteins were applied onto nitrocellulose membranes, and probed with serum samples collected from three allergic individuals. The dot blot results of IgE binding comparing the WT F2 with its three mutants were shown in **Fig. 5(C)**. It is clear that the substitute mutant R141A showed a weaker signal than R139A and D144A.

Discussion

In this study, the N-terminal subunit TBb was divided into four fragments using the segmental expression method, and the four recombinant epitope-containing fragments were expressed and purified to analyze their immuno-reactivity. The IgE-binding determinant(s) of TBb was mapped with four epitope-containing fragments, and named F1, F2, F3, and F4, respectively. The results of both ELISA and dot blot assays suggested that F2 is likely the immunodominant IgE-binding epitope-containing fragment, and it may contain the linear IgE-binding epitope of TBb. The 3D model built for TB allergen contained three protomers [**Fig. 4(A)**]. Each protomer consisted of an N-terminal subunit TBb and a C-terminal subunit TBa that are known to be separated into two peptide chains by post-translational peptidase cleavage. Both the TBb and the TBa contained a Cupin fold, and each Cupin structure was connected with a helical region containing three short helixes [**Fig. 4(B)**].

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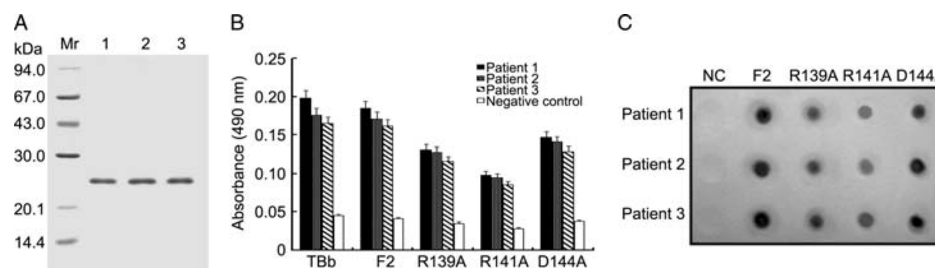

Figure 5 SDS-PAGE, ELISA and Dot blot analysis of purified F2 mutants (A) SDS-PAGE analysis of the purified F2 mutants. Mr: low molecular weight protein marker; lane 1: R139A-Trx; lane 2: R141A-Trx; lane 3: D144A-Trx. (B) Statistically significant differences between buckwheat-allergic patients and a negative control (non-allergic patient), and between the mutants and the wild-type F2 in their IgE-reactivity were detected by ELISA. Data were expressed in mean ± SD ($n = 3$). (C) Dot blot analysis of F2 and its mutants.

Epitope analysis of allergens is a key method to reveal the reason why different allergens cause hypersensitive reactions. Positively charged residues may be needed at these positions for antigen–antibody interaction [26]. Similarly, the four single mutants of Der f 13 allergen (E41A, K3A, K91A, and K103A) have significantly lower IgE-binding reactivity than that of the wild-type Der f 13, and all four residues are charged and exposed on the surface of the protein [27]. Even though some allergens of peanuts and other tree nuts possess a similar Cupin fold, they are different from their IgE-binding epitopes. The different distribution of charged residues (Asp, Glu, Arg, and Lys) on the molecular surface of the allergens is apparently responsible for a rather distinct amino acid sequence, and their overall 3D conformation [28]. Our results of sequence analysis and homology modeling showed that the predicted epitope 6 (IFRVREGDV) in fragment 2 (F2) contains identical or structurally homologous amino acids. Furthermore, the surface-exposed regions of the linear IgE-binding epitope 4 (PTRRVRKGD), which strongly reacted with IgE in Fag13S [25], coincides well with epitope 6 (IFRVREGDV) in TBb (**Fig. 3**). Furthermore, Arg^{139}, Arg^{141}, and Asp^{144} exhibit a rather conserved conformation characterized residues, and form a strongly charged area on the molecular surface [**Fig. 4(E, F)**]. Therefore, we chose Arg^{139}, Arg^{141}, and Asp^{144} to be substituted singly with alanine by site-directed mutagenesis.

Site-directed mutagenesis has been used successfully to produce recombinant forms with low IgE reactivity for some allergens. In this study, the results of ELISA and dot blot demonstrated that, compared with WT F2, all three mutants exhibited decreased IgE-binding activity, implying that the three charged residues have significant contributions to the IgE binding of TBb. Among these mutants, R139A and D144A had weaker IgE-binding activity to patient sera, indicating that Arg^{139} and Asp^{144} might be involved in the allergic activity of TBb. However, R141A had the weakest IgE-binding activity, suggesting that Arg^{141} is more important than Arg^{139} and Asp^{144} for IgE binding of TBb, and it is likely the critical amino acid in TBb.

In conclusion, a dominant linear IgE epitope-containing fragment of TBb was characterized in this study. We identified fragment 2 (F2) as an immunodominant epitope-containing fragment. Arg^{139}, Arg^{141}, and Asp^{144} are among the critical core amino acid residues of TBb recognized by human IgE antibodies. Based on these results, we believe that modified allergens displaying decreased allergenicity seem to reduce the risk of allergy symptoms, and that our research may provide a clue to treat TB allergy. Furthermore, application of our experiments may contribute to the development of a transgenic hypoallergenic crop.

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References

1 Bonafaccia G, Marocchini M and Kreft I. Composition and technological properties of the flour and bran from common and tatary buckwheat. Food Chem 2003, 80: 9–15.

2 Hong JH, Ikeda K, Kreft I and Yasumoto K. Near-infrared diffuse reflectance spectroscopic analysis of the amounts of moisture, protein, starch, amylose, and tannin in buckwheat flours. J Nutr Sci Vitaminol (Tokyo) 1996, 42: 359–366.

3 Rapala KM, Ostrowska K, Bednarczyk K, Dulinski R and Kozik A. Polypeptide components of oligomeric legumin-like thiamin-binding protein from buckwheat seeds characterized by partial amino acid sequencing and photoaffinity labeling. J Protein Chem 2003, 22: 167–175.

4 Li S and Zhang QH. Advances in the development of functional foods from buckwheat. Crit Food Sci Nutr 2001, 41: 451–464.

5 Wieslander G. Review on buckwheat allergy. Allergy 1996, 51: 661–665.

6 Smith HL. Buckwheat poisoning with report of a case in man. Arch Intern Med 1909, 3: 350–359.

7 Satoh R, Koyano S, Takagi K, Nakamura R, Teshima R and Sawada J. Immunological characterization and mutational analysis of the recombinant protein BWp16, a major allergen in buckwheat. Biol Pharm Bull 2008, 31: 1079–1085.

8 Wieslander G and Norback D. Buckwheat allergy. Allergy 2001, 56: 703–704.

9 Takahashi Y, Ichikawa S, Aihara Y and Yokota S. Buckwheat allergy in 90,000 school children in Yokohama. Arerugi 1998, 47: 26–33.

10 Tang R, Zhang HY and Wang RQ. Seven Chinese patients with buckwheat allergy. Am J Med Sci 2010, 339: 22–24.

11 Yoshimasu MA, Zhang JW, Hayakawa S and Mine Y. Electrophoretic and immunochemical characterization of allergenic proteins in buckwheat. Int Arch Allergy Immunol 2000, 123: 130–136.

12 Tanaka K, Matsumoto K, Akasaka A, Nakajima T, Nagasu T, Iikura Y and Saito H. Pepsinresistant 16-kD buckwheat protein is associated with immediate hypersensitivity reaction in patients with buckwheat allergy. Int Arch Allergy Immunol 2002, 129: 49–56.

13 Nair A, Ohmoto T, Woo SH and Adachi T. A molecular-genetic approach for hypoallergenic buckwheat. Fagopyrum 1999, 16: 29–36.

14 Dunwell JM, Purvis A and Khuri S. Cupins: the most functionally diverse protein superfamily? Phytochemistry 2004, 65: 7–17.

15 Zhang X, Yuan JM, Cui XD and Wang ZH. Molecular cloning, recombinant expression, and immunological characterization of a novel allergen from tartary buckwheat. J Agric Food Chem 2008, 56: 10947–10953.

16 Wang Z, Zhang Z, Zhao Z, Wieslander G, Norbäck D and Kreft I. Purification and characterization of a 24 kDa protein from tartary buckwheat seeds. Biosci Biotechnol Biochem 2004, 68: 1409–1413.

17 Ren X, Zhang X, Li Y and Wang Z. Epitope mapping and immunological characterization of a major allergen TBa in tartary buckwheat. Biotechnol Lett 2010, 32: 1317–1324.

18 Fang L, Sun QM and Hua ZC. Expression of recombinant Chinese bovine enterokinase catalytic subunit in *P. pastoris* and its purification and characterization. Acta Biochim Biophys Sin 2004, 36: 513–517.

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19 Marikar FM, Sun QM and Hua ZC. Production of the polyclonal anti-human metallothionein 2A antibody with recombinant protein technology. Acta Biochim Biophys Sin 2006, 38: 305–309.
20 Arnold K, Bordoli L, Kopp J and Schwede T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics 2006, 22: 195–201.
21 Robotham JM, Hoffman GG, Teuber SS, Beyer K, Sampson HA, Sathe SK and Roux KH. Linear IgE-epitope mapping and comparative structural homology modeling of hazelnut and English walnut 11S globulins. Mol Immunol 2009, 46: 2975–2984.
22 Jin T, Albillos SM, Guo F, Howard A, Fu TJ, Kothary MH and Zhang YZ. Crystal structure of prunin-1, a major component of the almond (*Prunus dulcis*) allergen amandin. J Agric Food Chem 2009, 57: 8643–8651.
23 He J, Xu R, Chen X, Jia K, Zhou X and Zhu K. Simultaneous elimination of T- and B-cell epitope by structure-based mutagenesis of single Glu80 residue within recombinant staphylokinase. Acta Biochim Biophys Sin 2010, 42: 209–215.
24 Qian J, Guo ZY and Chi CW. Cloning and isolation of a conus cysteine-rich protein homologous to Tex31 but without proteolytic activity. Acta Biochim Biophys Sin 2008, 40: 174–181.
25 Sordet C, Culerrier R, Granier C, Didier A and Rougé P. IgE-binding epitopic peptide mapping on a three-dimensional model built for the 13S globulin allergen of buckwheat (*Fagopyrum esculentum*). Peptides 2009, 30: 1021–1027.
26 Cheng TT, Tam MF, Chou H, Tai HY and Shen HD. Lys89, Lys90, and Phe91 are critical core amino acid residues of the Pen ch 18 major fungal allergen recognized by human IgE antibodies. Biochem Biophys Res Commun 2008, 375: 671–674.
27 Chan SL, Ong ST, Ong SY, Chew FT and Mok YK. Nuclear magnetic resonance structure-based epitope mapping and modulation of dust mite group 13 allergen as a hypoallergen. J Immunol 2006, 176: 4852–4860.
28 Barre A, Jacquet G, Sordet C, Culerrier R and Rougé P. Homology modelling and conformational analysis of IgE-binding epitopes of Ara h 3 and other legumin allergens with a cupin fold from tree nuts. Mol Immunol 2007, 44: 3243–3255.

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