



IgE-binding epitopic peptide mapping on a three-dimensional model built for the 13S globulin allergen of buckwheat (*Fagopyrum esculentum*)

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ABSTRACT

The three-dimensional model built for the 13S globulin allergen of buckwheat (*Fagopyrum esculentum*) consists of three protomers exhibiting the cupin motif, arranged in a homotrimer around a three-fold symmetry axis. Using the SPOT technique, 11 continuous IgE-binding epitopic peptides were characterized on the molecular surface of the 13S globulin allergen of buckwheat. Except for one of them, they all correspond to well exposed regions containing electropositively and/or electronegatively charged residues, which cover up to 40% of the molecular surface of the allergen. Some of these epitopes come in close contact to probably create more extended discontinuous epitopes, especially those located on the edge of the 13S globulin homotrimer. Half of the identified epitope peptides remain unaltered in a core structure protected against hydrolysis by digestive proteases and are thus assumed to promote the allergenicity of the 13S globulin. In addition, a few of these epitopes coincide with sequential IgE-binding epitopes previously characterized in soybean 11S globulins, that could account for the IgE-binding cross-reactions observed between soybean and buckwheat in Western blot experiments.

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1. Introduction

Hypersensitivity to buckwheat (*Fagopyrum esculentum*) is a frequent cause of allergy in Japan, Korea and other Asian countries, triggering anaphylactic type reactions in sensitized individuals, especially in children [31]. Although less frequent in adults, both children and adults scarcely experienced severe anaphylactic shock requesting a medical emergency treatment after the ingestion of extremely low amounts of buckwheat [13]. In many European countries and USA, buckwheat allergy is now becoming a worrying concern of public health due to the development of buckwheat as a health food and the increasing use of buckwheat flour in pastries or as a substitute for wheat flour in gluten-allergic persons suffering from coeliac disease [28]. In addition, asthma on inhalation to buckwheat or nocturnal asthma from sleeping on buckwheat chaff-stuffed pillows (commonly used in Korea) have been reported, especially in children [8,17]. Allergenic seed proteins have been identified in this gluten-free knot-grass plant originating from Asia [35]. They essentially consist of a legumin-like storage protein of 24 kDa [23] and a 2S albumin of 10 kDa [20]

or 16 kDa [14]. The larger storage protein apparently corresponds to a methionine-rich 13S globulin of 453 amino acid residues [27]. In addition, some cross-sensitization between buckwheat and poppy seeds (*Papaver somniferum*) has been reported in a poppy seed allergic patient [22] that could result from the occurrence of closely related allergens in both seeds.

Our studies were focused on the mapping and structural analysis of IgE-binding epitopic peptides on the molecular surface of 13S globulin allergen (Fag13S) because of its abundance in buckwheat seeds and sequence similarity with other seed globulins. Some of these IgE-binding epitopes apparently correspond to conformational epitopes responsible for the anaphylactic response of previously sensitized individuals.

2. Materials and methods

2.1. Human sera and seed protein extracts

Blood samples were drawn after informed consent of three patients experiencing anaphylactic reactions to buckwheat: a generalized anaphylactic shock upon inhalation of buckwheat flour-containing dust for two of them (12.2 kU_A/L and 14.3 kU_A/L of specific IgE, respectively; skin-prick tests of 12 mm and 13 mm, respectively), a Qincke oedema upon consumption of a buckwheat-

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containing pastry for the third one (9.8 kU_A/L of specific IgE; skin-prick test of 10 mm).

A crude protein extract was prepared by suspending 2 g of buckwheat seed flour in 20 mL of 20 mM Tris buffered saline (pH 7.5) and stirring for 4 h at 4 °C. The slurry was centrifuged at 10,000 × g for 30 min and the resulting supernatant was dialyzed against several changes of the same buffer for 48 h. After a new centrifugation step, the supernatant was filtered through a 0.2 µm membrane and frozen at –80 °C until used. Protein extracts from other edible seeds (poppy, peanut, soybean, sesame) were similarly prepared.

2.2. Analytical methods

Proteins from a crude extract of buckwheat seeds were separated by SDS-PAGE in 15% polyacrylamide gels using Tris-taurine as trailing ion [32] and stained with Coomassie blue. Coomassie blue stained bands were digested with trypsin in the gel and mass mapped by MALDI-TOF analysis as previously described [29]. The software Protein Prospector was used for the identification of the protein using the NCBI non-redundant database. The protein content of the buckwheat sample was estimated using the bicinchoninic acid kit reagent (Pierce) [30] with bovine serum albumin as a standard.

2.3. Immunoblotting

IgE-containing sera from patients allergic to buckwheat were used in Western blotting as probes for Fag13S. Following 1D SDS-PAGE, buckwheat proteins were transferred onto a Protran nitrocellulose 0.2 mm membrane (Schleicher & Schuell) at 20 V during 45 min using a 48 mM Tris/39 mM glycine/20% (v/v) methanol mixture. After an overnight incubation in 10 mM PBS (pH 7.4) containing 0.2% (v/v) tween and 5% (v/v) skimmed milk, the membrane was soaked in the patient IgE-containing sera diluted 1:10 in the same buffer and incubated for 2 h in a moist chamber. After three washes of 10 min each with the same buffer, the membrane was soaked in rabbit HRP-labelled anti-human IgE diluted 1:5000 in the buffer and incubated for 1 h under gentle stirring. Following three washes of 10 min each with buffer, the immunolabelled spots were detected using the ECL Plus detection (Amersham) after 3 min exposure in cassette. All the handling was carried out at room temperature.

2.4. Peptide synthesis on SPOT membrane

Overlapping 15-mer peptides, frameshifted by three residues, corresponding to the entire amino acid sequence of Fag13S (accession no. AAQ56206) were prepared by using the SPOT technique [7]. The protocol previously described in details [16] was followed, with the exception of the utilization of the Multiprep automatic Spot synthesizer (Intavis). Briefly, peptides were assembled using the Fmoc chemistry on a cellulose membrane harboring an amino polyethylene glycol moiety. The activated C-terminal residue of each peptide was coupled to the membrane. After Fmoc deprotection, the following amino acids were sequentially added. At the end of the synthesis, side chain protecting groups were removed by a trifluoroacetic acid treatment while the linkage of the peptides to the membrane was maintained.

2.5. IgE-binding on SPOT membrane

The membrane was soaked overnight into 20 mL of Tris-buffered saline (TBS) containing 2 mL blocking buffer (Roche) and 1 g sucrose (pH 7.0), and then washed three times with TBS

containing 0.1% (v/v) tween (TBSTw). A 1:10 (v/v) diluted patient serum was added in the presence of an anti-protease cocktail (Roche) and the membrane was incubated in a moist chamber for 2 h. After three washes with TBSTw (pH 7.0) the membrane was stirred in a 1:4000 dilution of mouse monoclonal anti-human IgE labeled with alkaline phosphatase (Sigma) for 1 h. After three washes with TBSTw (pH 7.0), the interacting peptide spots were colored for 30 min by adding the 5-bromo-4-chloro-3-indolylphosphate (BCIP) substrate for alkaline phosphate (Promega). The membrane was washed three times with deionized water and dried for scanning. The membrane can be used repeatedly after a regeneration step consisting in one wash in dimethylformamide for 1 min, three washes in deionized water, three washes in 8 M urea containing 1% (w/v) SDS and 1% (w/v) β-mercapto-ethanol and three subsequent washes in a mixture of ethanol-acetic acid-water (50:10:40, v/v/v). As a negative control, the IgE-containing serum was omitted and the SPOT membrane was revealed in the presence of the 2nd labeled antibody (see Fig. 3). A similar negative result was obtained by using a serum from a non allergic patient as the 1st antibody (result not shown).

2.6. Bioinformatics

Multiple amino acid sequence alignments were carried out with CLUSTAL-X [33] and displayed with ESPrpt [11] using the Risler's structural matrix for homologous amino acid residues [26]. The amino acid sequences of food proteins were checked for putative trypsin- and chymotrypsin-cleavage sites by selecting trypsin and chymotrypsin-high specificity (any C-amino acid residue, except for Pro, at N-Trp, -Tyr or -Phe) of the web PeptideCutter program (<http://expasy/tools/peptidecutter>). MacVector (Kodak) was also used to check for the putative trypsin- and chymotrypsin-cleavage sites. The HCA (Hydrophobic Cluster Analysis) [9] was performed to delineate the conserved secondary structural features (strands of β-sheet and stretches of α-helix) along the amino acid sequence of Fag13S by comparison with the soybean (*Glycine max*) β-conglycinin [18] used as a model. HCA plots were generated using the HCA server (<http://www.lmcp.jussieu.fr>).

2.7. Molecular modeling

Molecular modeling of Fag13S was carried out on a Silicon Graphics O2 R10000 workstation, using the programs InsightII, Homology and Discover3 (Accelrys, San Diego CA, USA). The atomic coordinates of the soybean β-conglycinin [18] (RCSB Protein Data Bank code 1IPJ) were used to build the three-dimensional model of the allergen. The moderate percentages of homology (~60%) the vicilin dimer of Fag13S shares with β-conglycinin allowed us to build a rather accurate three-dimensional model using the X-ray coordinates of 1IPJ as a template. The extended loop regions corresponding to the two X-ray unsolved 178–180 and 288–303 sequence stretches of β-conglycinin were tentatively modeled. The best geometrical fitting with the core domain compatible with the homotrimeric organization (absence of steric clashes) was used as a criterion for choosing among the different loop conformations available at the PDB. Steric conflicts were corrected during the model building procedure using the rotamer library [25] and the search algorithm implemented in the Homology program [19] to maintain proper side-chain orientation. An energy minimization of the final model was carried out by 150 cycles of steepest descent using the cvff forcefield of Discover. PROCHECK [15] was used to assess the geometric quality of the three-dimensional model. In this respect, about 80% of the residues of Fag13S were correctly assigned to the best allowed regions of the Ramachandran plot, the remaining residues being located in the generously allowed regions of the plot except for two residues (Gln289 and Asn336)

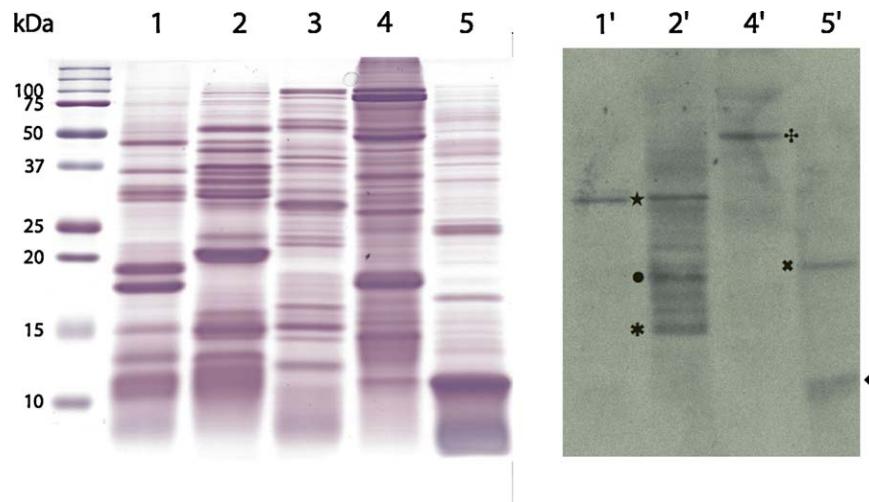


Fig. 1. Immunoblots. Coomassie blue stained protein fractions separated by SDS-PAGE of seed protein extracts from poppy (line 1), buckwheat (line 2), peanut (line 3), soybean (line 4) and sesame (line 5). Immunoblotting of seed protein extracts from poppy (line 1'), buckwheat (line 2'), soybean (line 4') and sesame (line 5') with a serum from buckwheat allergic patient. Markers of known molecular mass (kDa) are indicated. 13S globulin of buckwheat and poppy (1), 11S globulin or Fag e 1 of buckwheat (2) [32], β -conglycinin of soybean (3), 11S globulin of sesame (4), and 2S albumin of buckwheat (5) were identified by MALDI-TOF from Coomassie blue stained protein fractions after hydrolysis with trypsin. The identification of a IgE-reacting protein fraction of 10 kDa from sesame (arrow) was unsuccessful. A similar IgE-binding reactivity pattern was obtained with sera from all of the three checked buckwheat allergic patients. No IgE-reactive protein fraction could be revealed using a serum from a non allergic individual as a probe.

which occur in the non allowed region (result not shown). Ribbon diagrams were drawn with the UCSF Chimera package [24]. PyMol (W.L. DeLano (<http://pymol.sourceforge.net>)) was used to draw the IgE-binding epitopes on the molecular surface of the allergen. According to the location of the exposed trypsin and chymotrypsin cleavage sites on the three-dimensional models, both the preserved central core structure and the surface-exposed peeled off regions of the proteins upon the proteolytic attack were identified.

2.8. Mapping of electrostatic potentials

Electrostatic potentials were calculated and displayed with GRASP using the parse3 parameters [21]. The solvent probe radius used for molecular surfaces was 1.4 Å and a standard 2.0 Å-Stern layer was used to exclude ions from the molecular surface [10]. The inner and outer dielectric constants applied to the protein and the solvent were, respectively, fixed at 4.0 and 80.0 and the calculations were performed keeping a salt concentration of 0.145 M.

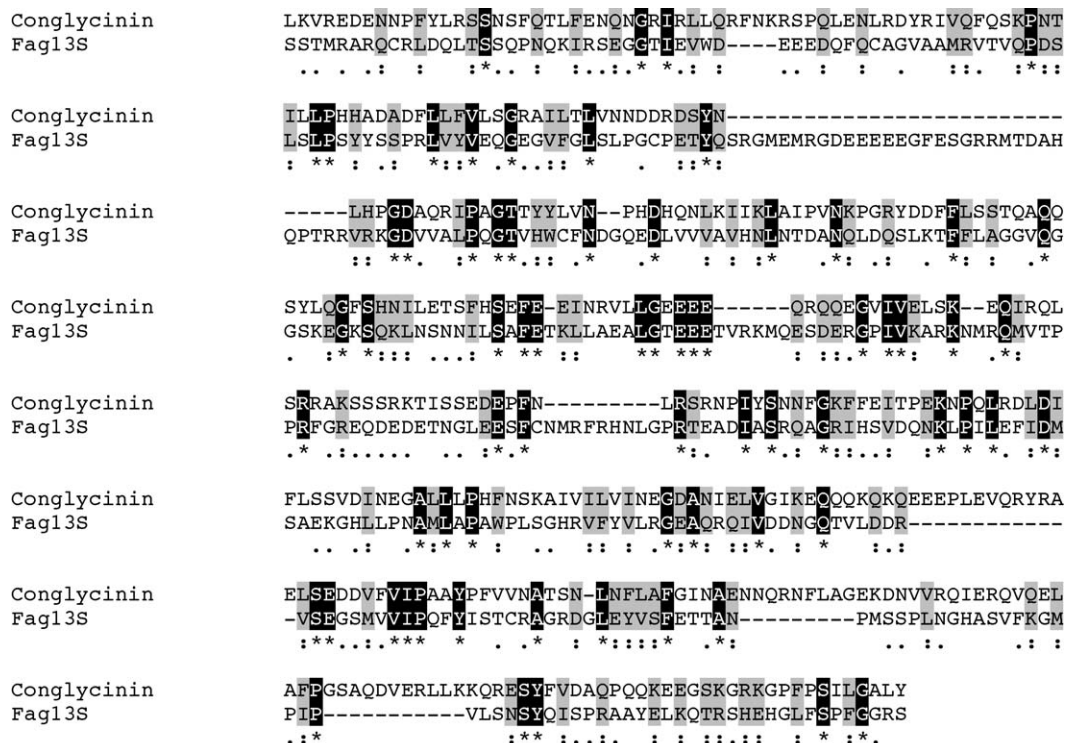


Fig. 2. Amino acid sequence alignment. Alignment of β -conglycinin from soybean with Fag13S performed with CLUSTAL-X [33]. Identical residues (*) are in black boxed white letters and strongly homologous residues (:) are gray shaded. Weakly homologous residues (.) and gaps (-) are indicated.

3. Results

3.1. IgE recognize 13S globulin allergen

The IgE-containing sera from buckwheat allergic patients essentially reacted with Fag13S (13S globulin) and Fag2S (2S albumin) in Western blot experiments (Fig. 1), suggesting that both allergens correspond to the main allergens of buckwheat. However, some faint reaction also occurred with other protein fractions of 20 kDa, 30 kDa, 35 kDa and 50 kDa that most probably correspond to other buckwheat allergens. In addition, an interaction also occurred with 13S globulin, 11S globulin and 2S albumin from soybean, poppy seeds and sesame, respectively, suggesting

some possible cross-reaction between structurally-related allergens of different origin.

3.2. 13S globulin of buckwheat contains the cupin motif

The five X-ray solved cupin structures available at the PDB, including phaseolin from kidney bean (code 2PHL), canavalin from Jack bean (code 1CAU), β -conglycinin from soybean (code 1IPJ), germin from barley (code 1FI2), and a seed globulin from pumpkin (code 1EVX), share moderate percentages of both identity ($\sim 18\%$) and homology ($\sim 60\%$) with Fag13S. In spite of this low degree of amino acid sequence conservation, the HCA profiles indicate a very similar fold for all these globulins (result not shown). Since the best

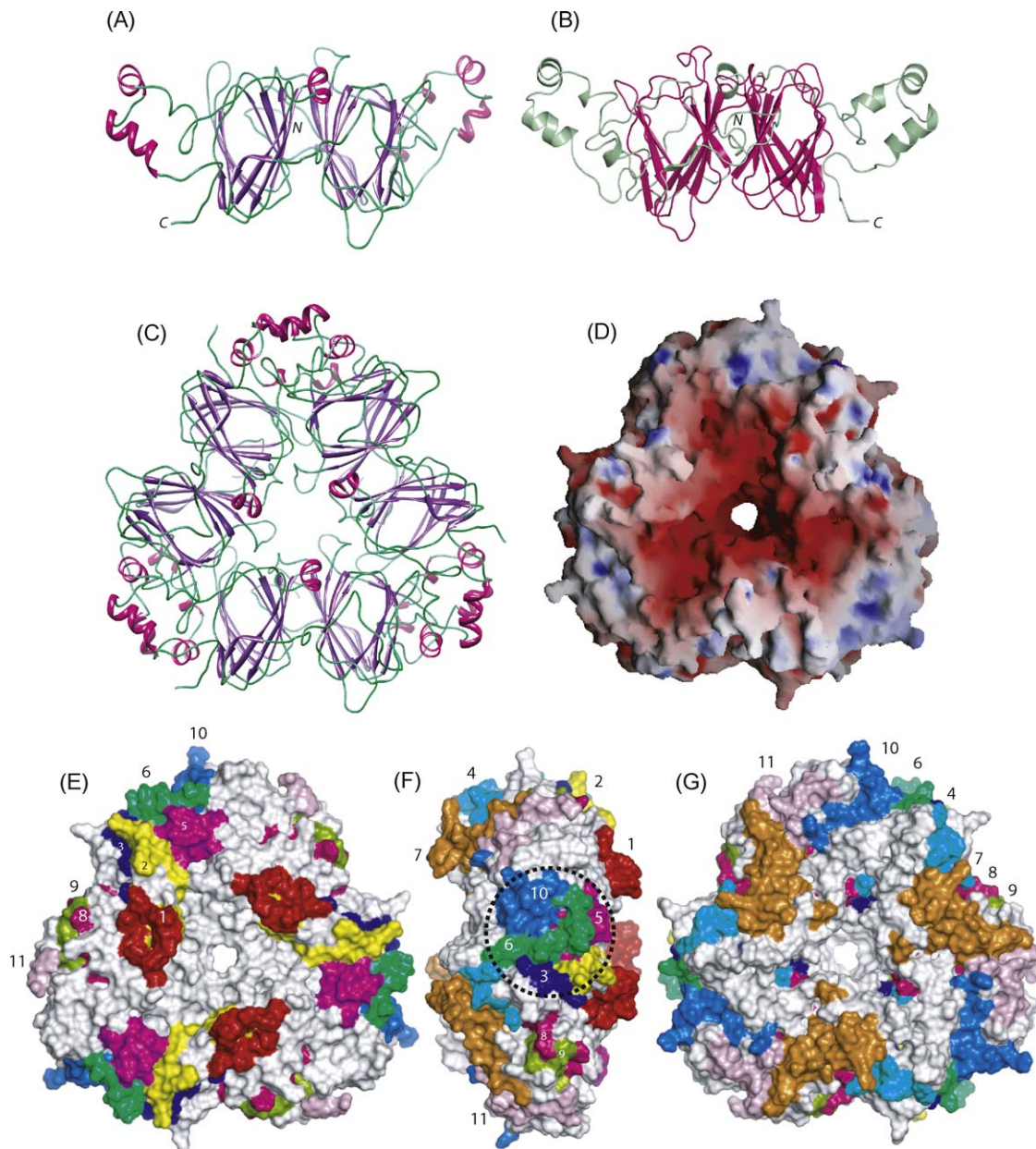


Fig. 3. Three-dimensional model of Fag 13S. Ribbon diagrams of the 13S globulin dimer (A) and homotrimer (C) of buckwheat. N and C correspond to the N- and C-terminal ends of the monomer, respectively. Ribbon diagram showing the core structure corresponding to the cupin motifs (colored pink) remaining unattacked upon the trypsin/chymotrypsin hydrolysis of Fag13S monomer (B). Distribution of the electrostatic potentials on the molecular surface of Fag13S homotrimer (D: front face). The negative potential is colored red and displayed at $-10kT$ level, the positive potential is colored blue and displayed at $+10kT$ level ($1kT = 0.6$ kcal). Neutral surfaces are white. Surface occupied by the continuous IgE-binding epitopic stretches identified in Spot experiments on Fag13S monomer homotrimer (E: front face, F: edge, G: back face). Surface epitopes are colored red (#1), yellow (#2), blue (#3), cyan (#4), magenta (#5), green (#6), orange (#7), pink (#8), olive green (#9), marine (#10) and light pink (#11), respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

sequence identity and structural homologies as shown from both the amino acid sequence alignments and HCA plots occurred with β -conglycinin (Fig. 2), we used the atomic coordinates of the soybean β -conglycinin (code 1IPJ) to build the three-dimensional model of the allergen. In addition, the missing parts occurring in the different X-ray solved proteins are not superposed from one protein to another and, especially, an apparently conserved region among the cupins was shown to lack in both the phaseolin (residues 211–219) and canavalin (residues 224–245) three-dimensional structures, that strongly diminished the advantage of using all the cupin structures as a common template. However, the extended loop region corresponding to a X-ray unsolved 288–303 sequence stretch of β -conglycinin was omitted in the model built for Fag13S. Of course, this model should still be subject to further confirmation by X-ray crystallography, especially the arrangement of protomers in a symmetric homotrimer.

The three-dimensional model built for the Fag13S protomer consists of a typical cupin motif made of two tandemly arrayed modules related by a pseudo-dyad axis. Each module contains a β -barrel core domain of two walls of antiparallel β -sheet (cupin) associated to a loop domain which predominantly contains α -helices. A α -helix-containing linker region connects the two modules (Fig. 3A). Three cupin motifs are arranged in a homotrimeric structure around a three-fold symmetry axis in such a way that the three cupin motifs occupy the faces of a flattened prism (Fig. 3C). Mapping of electrostatic potentials on the molecular surface showed that both allergen structures exhibit exposed electronegatively (Asp and Glu residues) and electropositively (Arg and Lys residues) charged areas susceptible to belong to IgE-binding epitope regions (Fig. 3D).

3.3. Scanning of continuous IgE-binding epitopes in 13S globulin

Eleven continuous sets of IgE-binding synthetic peptides were identified in SPOT experiments corresponding respectively to stretches 7SSTMRAQCRLDQLTSSQ24 (#1), 28KIRSEGGTIEVWDEE42 (#2), 55RVTVPQDSLPSYYSSPRLV75 (#3), 120DAHQPTRVRKGDVVALP137 (#4), 181QGGSKEGKSQKLN194 (#5), 222ESDERGPVVKARKNMRQMT241 (#6), 263NMRFRHNLGPRTEADIASRQAGRIHSVD290 (#7), 312NAMLAPAWPLSGHRVYFVLRGEAQ338 (#8), 360QFYISTCRAGRD372 (#9), 393HASFVKGMPIPVLSNSYQISPR414 (#10) and 421QTRSHEHGLFSPFGGRS437 (#11) along the amino acid sequence of Fag13S (Fig. 4). All of these peptides contain both electropositively (K,R) and electronegatively (D,E) charged residues (underlined residues). Some of the epitopes strongly reacted with IgE, especially epitopes #1, #3, #4, #7, #10 and #11, whereas other epitopes #2, #5, #8 and #9 weakly interacted with IgE from allergic patients (Fig. 4). All of the three checked sera from buckwheat allergic patients gave identical results in SPOT experiments.

3.4. Mapping of IgE-binding epitopes on the molecular surface of 13S globulin

Continuous epitopic peptides mostly coincide with nicely exposed regions at the surface of Fag13, with the exception of epitope #8 which is much more less exposed on the molecular surface. In fact, 14 peptide stretches of 5–13 residues occurring along the amino acid sequence stretches previously identified as continuous epitopes are readily exposed on the molecular surface of the Fag13S monomer to act as IgE-binding epitopes (Table 1). In addition, mapping of IgE-binding epitopes on the molecular surface of the Fag13 homotrimer revealed that many of them essentially locate on the edge of the trimer as nicely exposed epitopes, except for epitopes #1 and #7–8–9 which protrude on the back and front face of the Fag13S trimer, respectively (Fig. 3E–

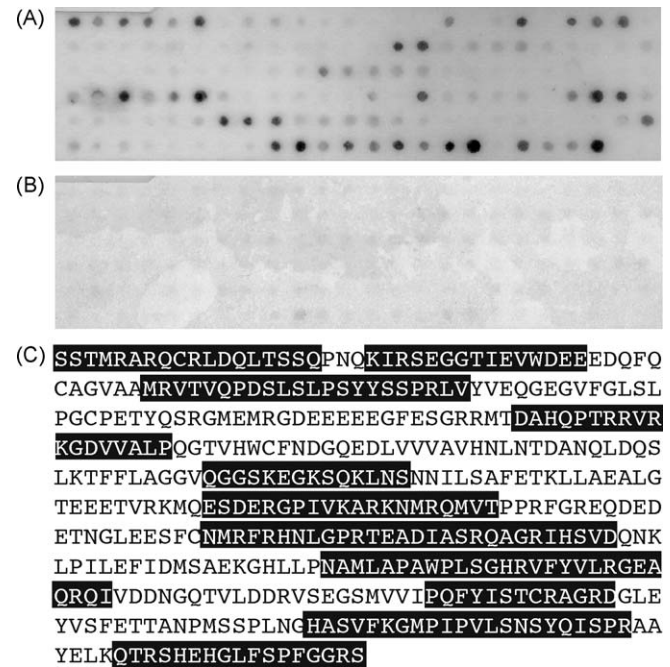


Fig. 4. SPOT results. (A) Example of IgE-binding peptides (dark spots) revealed on the SPOT membrane using a serum of patient who experienced a severe anaphylactic reaction upon inhaling the buckwheat dust-containing air in a bakery. (B) Pattern obtained by omitting the 1st patient serum antibody (a similar negative pattern was obtained with a serum from a non allergic individual). (C) Mapping of the corresponding continuous IgE-binding epitopic peptides (black boxed white letters) along the amino acid sequence of Fag13S.

G). Additionally, most of these exposed peptides coincide with the electropositively and/or electronegatively charged areas as shown from the mapping of electrostatic potentials on the molecular surface of Fag13S (see Fig. 3D).

3.5. Some IgE-binding epitopes are preserved from proteolysis in the core structure

Fag13S globulin contain putative trypsin (39) and chymotrypsin (24) cleavage sites that are almost randomly distributed along

Table 1

Surface-exposed regions (black boxed) of the continuous IgE-binding epitopes identified along the amino acid sequence of Fag13S.

Continuous epitopes	Regions exposed on the molecular surface
#1	7 SSTMRAQCRLDQLTSSQ 24 1
#2	28 KIRSEGGTIEVWDEE 42 2
#3	55 MRVTVPQDSLPSYYSSPRLV 75 3
#4	120 DAHQPTRVRKGDVVALP 137 4
#5	181 QGGSKEGKSQKLN 194 5
#6	222 ESDERGPVVKARKNMRQMT 241 6
#7	263 NMRFRHNLGPRTEADIASRQAGRIHSVD 290 7 8 9
#8	312 NAMLAPAWPLSGHRVYFVLRGEAQRFY 338 10
#9	360 QFYISTCRAGRD 372 11 12
#10	393 HASVFKGMP IPVLSNSYQISPR 414 13 14
#11	420 QTRSHEHGLFSPFGGRS 437

the amino acid sequence. However, the number of sites actually accessible for a proteolytic attack is drastically lower depending on their exposition on the molecular surface of the protein. According to the distribution of the accessible sites, their proteolytic attack is assumed to consist of a peeling off which essentially eliminates the most exposed regions to leave intact an unattacked core structure which amounts ~50% of the polypeptide chain. The core structure consists of a tightly packed arrangement of β -sheets building the two cupin modules of the Fag13S dimer (Fig. 3B). Owing to its extended surface this core is assumed to contain a rather good proportion of unaltered B-cell epitopes. Four (#2, #3, #8, #9) out of the 11 IgE-binding epitopes identified on the Fag13S model remain in the core structure whereas 5 of them (#1, #5, #6, #10, #11) occur in the peeled off regions of the allergen upon the trypsin/chymotrypsin attack. The two other remaining IgE-binding epitopes (#4, #7) overlap the core and extra-core regions.

4. Discussion and conclusion

Fag13S, the 13S globulin of buckwheat, exhibits a canonical cupin motif structure as many other legume seed storage proteins even though it belongs to the distinct Polygonaceae family of higher plants. It readily interacts with all of the three IgE-containing sera from buckwheat allergic patients tested in Western blotting experiments, in addition to a 11S globulin (Fag e 1) and a 2S albumin allergens (Fig. 1).

Eleven continuous IgE-binding epitopic peptides have been identified along the amino acid sequences of the Fag13S monomer by systematically probing the antigenic activity of sequence-derived SPOT peptides with patient sera. Checking their surface exposure on the three-dimensional model built for Fag13S showed they correspond, in fact, to 14 exposed IgE-binding regions. This combined immunochemical/structural approach allowed us to overcome some limitation the SPOT technique introduces in delineating the IgE-binding epitopic regions of allergens. This is illustrated by the weak surface-exposed character of the predicted epitopic peptide stretch #8 (see Table 1), which is thereby unlikely to be strongly recognized by IgE on the molecular surface of Fag13S. These exposed IgE-binding epitopes roughly cover about 40% of the total accessible area of the allergen and a so large exposition on the molecular surface most probably accounts for the enhanced allergenic character of this protein. Some of these epitopes strongly reacted with sera from patients allergic to buckwheat in SPOT experiments and are therefore considered as possible major IgE-binding epitopes. They correspond to epitopes #1, #3, #4, #6, #8, #10 and #11, respectively (Fig. 3E,F,G). Due to the spatial arrangement of the Fag13S protomers in the Fag13S homotrimer, the epitopes are distributed on the edge of the trimer in a nicely exposed position, except for epitopes #1, #4 and #7 (Fig. 3E–G). Moreover, continuous epitopes #2, #3, 5#, #6 and #10 are close enough to possibly create a much more extended discontinuous epitope suitable for promoting a strong interaction with the corresponding IgE. Other coalescent epitopes susceptible to create more extended conformational epitopes also occur on the back face (epitopes #7, #10 and #11) of the Fag13S homotrimer (Fig. 3F). Some of these epitopic regions partly overlap with a few IgE-binding epitopes that have been previously characterized on the basic chain of Fag e 1, another 13S globulin identified as a major allergen of buckwheat [36]. However, the IgE-binding epitopic peptide stretches of Fag e 1 are moderately homologous to the corresponding amino acid sequence stretches of Fag13S (result not shown).

A comparison of the IgE-binding epitopic peptides identified on the molecular surface of Fag13S with other IgE-binding epitopes previously characterized on other globulin allergens, e.g. Ara h 1 and Ara h 3 from peanut, revealed no striking structural similarity (result not shown). In agreement with this apparent lack of epitopic

similarity with other globulin allergens, allergens from poppy seeds (*P. somniferum*, Papaveraceae) are the sole food allergens susceptible to cross-react with buckwheat allergens reported to date [22]. In this respect, IgE-containing sera from buckwheat allergic patients strongly reacted with a protein fraction from a poppy seed extract identified as a 13S globulin by mass spectrometry (Fig. 1). However, another strongly reacting protein fraction of a soybean seed extract was identified as the 11S globulin glycinin (Fig. 1). A 11S globulin fraction of sesame seed extract also reacted with IgE from buckwheat allergic patients in Western blotting experiments (Fig. 1). This cross-reactivity suggests the occurrence of IgE-binding epitopes structurally related to Fag13 IgE-binding epitopes in globulins from other edible seeds. As shown from amino acid sequence comparison, epitope #6 and epitope #10 of Fag13S roughly coincide with epitopes #2 and epitope #11 characterized along the amino acid sequences of the soybean G1 and G2 glycinins, respectively [2,12]. Even though these epitopic peptides share moderate percentages of both identity and similarity, they could account for the reported cross-reactions observed in Western blots.

Upon trypsin/chymotrypsin attack, the Fag13S monomer should retain a core structure resistant to proteolysis which is predicted to amount for 50% of the polypeptide sequence. This preserved core structure, which contains half of the IgE-binding epitopic peptides identified on the molecular surface of Fag13S, accounts for the recognition of the allergen by the local immune system of the gastro-intestinal tract. In this respect, the cupin motif built up from two tightly packed β -sheet walls highly resistant to the proteolytic attack, consists of a structural feature nicely adapted for promoting the allergenicity of food proteins. Accordingly, the cupin motif is widely distributed as seed storage proteins [5] in many other major food allergens like peanut (Ara h 1, Ara h 3), tree-nut (Jug r 2 from walnut, Cor a 9 from hazelnut, Ana o 3 from cashew nut, Car i from Brazil nut, etc.), legume (Pis s 1 from pea, Len c 1 from lentil) and sesame (Ses i 3, Ses i 6) globulins. As a general rule, major food allergens exhibit structural patterns made of tightly packed secondary structures, e.g. 2S albumin allergens (Ara h 2 from peanut, Jug r 1 from walnut, Car i 2 from Pecan nut, etc.) or lipid transfer proteins LTP (Pru p 3 from peach, Mal d 3 from apple, etc.) built up from a tight packing of α -helices [1], susceptible to similarly preserve IgE-binding epitopes in core structures resistant to the digestive protease attack [3,6]. Most of the extra-core epitopic regions that are peeled off upon trypsin/chymotrypsin attack are predicted to be subsequently cleaved in small peptides of 3–6 amino acid residues that, most probably, do not retain any IgE-binding potency. However, three peptides of 8, 15 and 11 amino acid residues, corresponding to the SPOT epitopic stretches #1, #10 and #11, respectively, are sufficiently extended to retain their IgE-binding capacity and trigger some allergic response from the local immune system.

The identification of several IgE-binding epitopes of Fag13S that strongly and specifically interact with IgE from allergic patients is a first step towards the production of recombinant allergen suitable to improve the diagnosis of buckwheat allergy [4]. A further step will consist in the alteration of the primary sequence of the recognized IgE-binding epitopes to produce modified recombinant allergens with reduced IgE-binding capacity but still retaining functional T-cell epitopes, eventually suitable for immunotherapy [34]. Along this line, Ala scanning of the recognized continuous IgE-binding epitopic peptides is in progress to identify the immunodominant residues that have to be mutated in order to reduce the IgE-binding propensity of Fag13S.

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