

Identification of a New IgE-Binding Epitope of Peanut Oleosin That Cross-React with Buckwheat

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Peanut and buckwheat induce a severe allergic reaction, anaphylaxis, which is considered to be mediated by immunoglobulin E (IgE). We identified in this study a new IgE-binding epitope of the peanut allergen that cross-reacted with buckwheat. The phosphate-buffered saline-soluble fraction of buckwheat inhibited the binding between IgE and the peanut allergen. A cross-reactive peptide was isolated from the α -chymotrypsin hydrolysate of peanut. Based on the amino acid sequence and mass spectrometric analysis data, the peptide was identified as Ser-Asp-Gln-Thr-Arg-Thr-Gly-Tyr (SDQTRTGY); this sequence is identical to amino acids 2–9 in the N-terminal hydrophilic domain of oleosin 3 which is located on the surface of the lipid storage body. Synthetic SDQTRTGY was found to bind with IgE in the sera of all eight peanut-allergic patients tested. Since many foods of plant origin contain oleosin, the possibility of an anaphylactic cross-reaction in allergic patients should always be considered.

Key words: peanut; buckwheat; allergen; cross-reaction; oleosin

Peanut (*Arachis hypogaea*) allergy is one of the most common food allergies in USA and in several European countries, with more than 3 million people being affected in USA alone.¹⁾ The symptoms of the allergic reaction to peanut are severe, and ingesting even a small amount of its allergens can induce a fatal or life-threatening anaphylactic reaction in sensitized patients.²⁾ Although most children outgrow allergies to milk and eggs, peanut allergies persist even in adulthood, and may last for the entire lifetime of an individual.³⁾ Avoidance is currently the only treatment in clinical medicine. It is unfortunate that the use of peanut as a protein supplement in processed foods complicates the avoidance of accidental consumption.

The allergenic components in peanuts have been extensively studied, and 11 of those, namely *Ara h* 1–11, have been registered to date by the Allergen Nomenclature Subcommittee of the International Union of Immunological Societies (IUIS). The three major allergens *Ara h* 1–3 are the respective seed storage proteins, vicilin, conglutin, and glycinin.⁴⁾ *Ara h* 1 exists as a stable trimer, and its epitopes are heat- and digestion-stable.⁵⁾ *Ara h* 1 and *Ara h* 2 are recognized by serum immunoglobulin E (IgE) in almost 90% of peanut-sensitive patients and are thus considered to be important allergens.^{3,5)} In the case of *Ara h* 2, IgE mostly binds to conformational (*i.e.*, 3-dimensional) rather than linear (*i.e.*, corresponding to consecutive amino acids) epitopes.⁶⁾ However, it is still unclear whether conformational or linear epitopes are clinically more important. *Ara h* 3 and *Ara h* 4 are almost identical isoforms associated with symptoms of hypersensitivity,⁷⁾ and *Ara h* 6 is very similar to *Ara h* 2 and *Ara h* 7.⁸⁾ *Ara h* 5 is peanut profilin⁹⁾ and *Ara h* 8 is a protein homolog of the major birch pollen allergen, *Bet v* 1.¹⁰⁾ *Ara h* 9 is a lipid transfer protein.¹¹⁾ *Ara h* 10 and 11 are both oleosins and have been reported as potential candidates for peanut allergens,^{12,13)} although their clinical relevance is not yet known.

Buckwheat (*Fagopyrum esculentum*) is grown in many Asian countries under poorer conditions than those for growing rice, and both the grain and leaves can be used for food; furthermore, its growth is rapid, and two crops can be harvested each year.¹⁴⁾ Buckwheat products are increasingly being used as health foods because of their high protein content, with an abundance of vitamins B₁ and P, dietary fiber and low fat content.¹⁵⁾ Buckwheat flour is used in soba noodles, dumplings, and cookies, especially in Japan, and also frequently as a gluten-free ingredient in bread, pasta, and cakes for patients with celiac disease.¹⁶⁾ Although buckwheat

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; IgE, immunoglobulin E; IUIS, International Union of Immunological Societies; LCMS-IT-TOF, high-performance liquid chromatography ion-trap time-of-flight mass spectrometer; NCBI, National Center for Biotechnology Information; nr, non-redundant; ODS, octadecylsilyl; PBS, phosphate-buffered saline; TFA, trifluoroacetic acid

allergy is not common, it is an immediate IgE-mediated reaction that can cause a severe allergic reaction similar to that caused by peanut allergy.¹⁷⁾ Many clinical cases of buckwheat allergy have been reported in Japan, where it was the fourth most frequent food allergen according to the Japanese Ministry of Health and Welfare survey in 1999.¹⁸⁾ Several buckwheat allergens have been identified, of which the 24 kDa (*Fag e 1*), 26 kDa, and 67–70 kDa proteins have been suggested to be of importance.¹⁹⁾ *Fag e 1*, which is homologous to 11S or 12S globulin, has reacted with the serum IgE of all buckwheat allergy patients. The 16 kDa protein (*Fag e 2*), which is resistant to digestion, has been identified as a major buckwheat allergen in Japanese and Korean patients with buckwheat allergy.²⁰⁾

The epitopes of peanut and buckwheat that are responsible for the anaphylactic reaction have not yet been identified. Ingesting only a small amount of both peanut and buckwheat proteins can induce a severe immediate hypersensitivity allergic reaction, anaphylaxis, which is considered to be mediated by IgE. There have been many reports on the IgE cross-reactivity between peanut and other closely related edible legumes.^{21–23)} Since peanuts (Fabaceae) and buckwheat (Polygonaceae) manifest similar clinical allergic features, new insight into peanut allergens could appear if the presence of putative cross-reactive IgE-binding epitopes in peanuts and buckwheat was identified. Identification of the putative cross-reactive IgE-binding epitope(s) of peanut and buckwheat is therefore of interest.

We identified in this study a novel cross-reactive IgE-binding epitope of peanut allergen oleosin. This finding should aid the design of improved diagnostic and therapeutic approaches to treat individuals with peanut hypersensitivity.

Materials and Methods

Samples. Peanut and buckwheat were purchased from a market. Egg white was purchased from Wako Pure Chemicals (Osaka, Japan). The SDQTRTGY peptide was synthesized according to the solid-phase method with a peptide synthesizer and purified by reversed-phase high-performance liquid chromatography (HPLC) by Takara Bio (Osaka, Japan).

Sera. Sera from eight patients with documented peanut allergy (25 years mean age) were subjected to an enzyme-linked immunosorbent assay (ELISA). Reference sera were purchased from International Diagnostic Resources (Menifee, CA, USA).

Enzyme-linked immunosorbent assay (ELISA). ELISA was performed as previously described.^{24,25)} Briefly, peanuts were crushed and defatted with *n*-hexane. The defatted peanut and buckwheat samples (10 mg) were suspended in phosphate-buffered saline (PBS, 1 mL). The suspension was stirred for 2 h, then centrifuged at $5,000 \times g$ for 20 min, and the supernatant was diluted. Extracted protein was determined by using a protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA), using bovine serum albumin (2%; Sigma-Aldrich, St. Louis, MO, USA) as a standard. The wells of an ELISA plate were coated with the PBS-soluble fraction of buckwheat (0.016–10 mg/mL) or peanut (0.016–10 mg/mL) as an antigen and incubated overnight at 4 °C. Unoccupied sites in the wells were saturated by incubating with 200 μ L of bovine serum albumin at 37 °C for 1 h. Ten-times diluted sera (100 μ L) from the patients were added to the wells, and the plate was incubated at room temperature for 1 h. This procedure was followed by the addition of biotinylated anti-human IgE (Kirkegaard & Perry Laboratory, Gaithersburg, MD, USA), streptavidin-peroxidase conju-

gate (Boehringer Mannheim, Ingelheim, Germany) and *o*-phenylenediamine (Wako Pure Chemicals). The wells were washed between the steps with PBS containing 0.05% Tween 20 (pH 7.4). After the reaction, the optical density value was read with a microplate reader (Bio-Rad Laboratories), using a 490-nm filter.

Preparation of the buckwheat, peanut, and egg white protein hydrolysate. Peanuts were ground with a pestle and mortar. The ground peanuts were defatted by twice the amount of hexane. The buckwheat and defatted peanut (20 g) were suspended in PBS (200 mL) and adjusted to pH 8.5 with 0.1 N NaOH; 0.1% α -chymotrypsin (Wako Pure Chemicals) was then added, and the mixture was stirred for 2 h at 37 °C. The hydrolysate was boiled for 5 min to inactivate the enzyme, and then centrifuged at $5,000 \times g$ for 20 min to obtain a supernatant. This supernatant was applied to an octadecylsilyl (ODS) column (Sep-Pak C18; Waters, Boston, MA, USA) which was washed with 0.1% trifluoroacetic acid (TFA) and eluted with 90% methanol in 0.1% TFA. The fractions of the peanut hydrolysate adsorbed to the Sep-Pak and eluted with 0%, 45%, and 90% methanol are respectively designated as Frs 1, 2, and 3. The eluate was evaporated under vacuum, and the resulting residue was dissolved in PBS or water, before being respectively subjected to an inhibition ELISA or isolation procedure. Hydrolysis of the egg white protein with α -chymotrypsin and the ODS column pretreatment were performed in the same manner.

Inhibition ELISA. The IgE-binding inhibitory activity of the buckwheat extract (0.1 mg/mL), and the hydrolysates of buckwheat (4 mg of protein/mL), egg white (4 mg of protein/mL) and peanut (1.5 mg of protein/mL) were evaluated by inhibition ELISA as previously reported.^{24,25)} Aliquots of serum (40 μ L) from the patients were pre-incubated with a test sample (80 μ L in PBS) at room temperature for 1 h (the inhibition reaction). Five-times-diluted serum (100 μ L each) of the treated sera were used as the antibody for ELISA, and untreated serum was used as a control. Other procedures were performed on similar aliquots. A test sample was judged to be allergenic if it inhibited antigen-antibody binding by more than 10%.

Isolation of cross-reactive IgE-binding epitope peptide. The Sep-Pak-adsorbed fractions were further fractionated by HPLC in a reversed-phase column (Inertsil ODS-3, 10 $\times$ 250 mm; GL Sciences, Tokyo, Japan). Linear gradient elution was performed at 24 °C with 20%–40% methanol in 0.1% TFA over 0–50 min at a flow rate of 3 mL/min. Peaks were detected at a wavelength of 220 nm. The eluates collected near to and at each peak were dried and then examined for inhibition ELISA. The peak fraction of inhibitory activity obtained in the first HPLC run was chromatographed again twice under the same conditions. The peak of inhibitory activity obtained in the third HPLC run was separated by a fourth HPLC run, using a Capcellpak™ ODS column (4.6 $\times$ 150 mm; Shiseido, Tokyo, Japan) eluted at room temperature with a linear gradient of 10%–40% methanol in 0.1% TFA over 0–15 min at a flow rate of 1 mL/min. The absorbance measured at 220 nm was used for detection.

Identification of the cross-reactive peptide. The mass spectrum of the peptide was recorded with a high-performance liquid chromatograph ion-trap time-of-flight mass spectrometer (LCMS-IT-TOF; Shimadzu, Kyoto, Japan) fitted with an electrospray ionization source. HPLC was performed with an LC-20A system (Shimadzu) equipped with a Capcellpak™ ODS column (2.0 $\times$ 150 mm; Shiseido), using linear gradient elution with 10%–40% methanol in 0.1% formic acid at 0.1 mL/min. The analytes were detected in the positive and negative modes. The N-terminal amino acid sequence of the inhibitory compound was obtained with an amino acid sequencer (G1005A; Hewlett Packard, Palo Alto, CA, USA). The degree of amino acid sequence similarity between the cross-reactive compound and the other proteins was determined by using the Fast Homology Search CDS (GenBank).

Database search. The non-redundant (nr) protein database for buckwheat (*Fagopyrum*) at National Center for Biotechnology Information (NCBI) was searched by using the BLASTP algorithm with a query for the “SDQTRTGY” sequence and an automatically adjusted parameter for the short input sequence option at the NCBI BLAST web site.

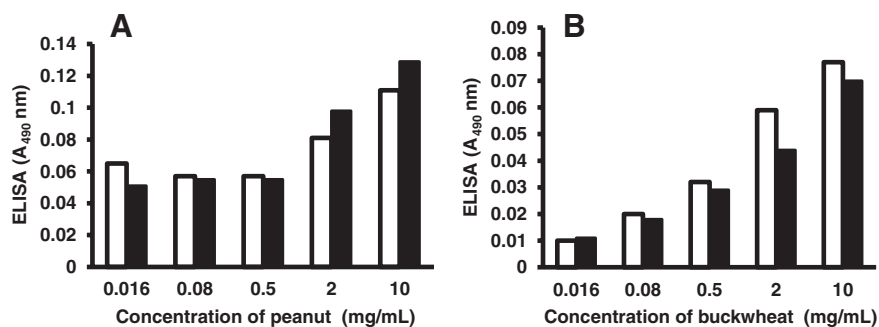


Fig. 1. IgE-Binding Ability of Extracts of Peanut and Buckwheat.

A, The wells of an ELISA plate were coated with the PBS-soluble fraction of peanut as an antigen. B, The PBS-soluble fraction of buckwheat was used as an antigen. □, patient 1; ■, patient 2.

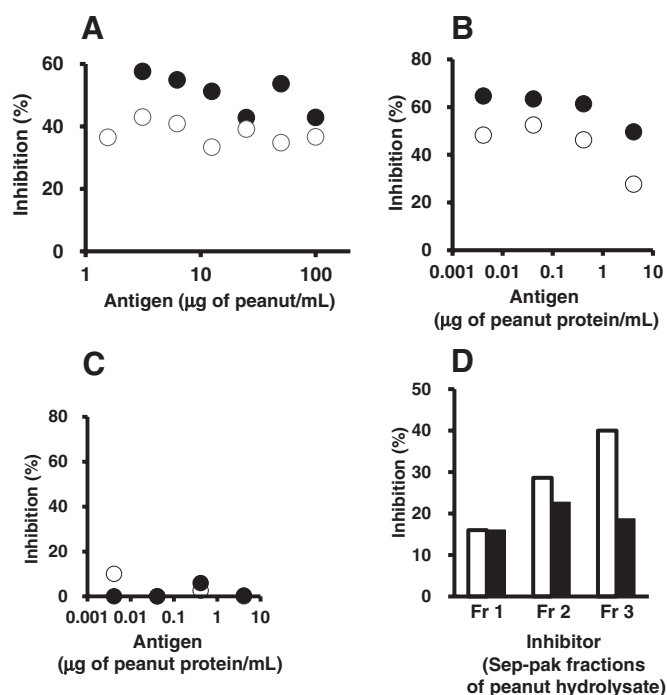


Fig. 2. Inhibition ELISA of Hydrolysates of Buckwheat, Egg White, and Peanut.

The wells of an ELISA plate were coated with the PBS-soluble fractions of peanut (A–C) or buckwheat (D) as an antigen. Individual sera from peanut-allergic patients were pre-incubated with the PBS-soluble fraction of buckwheat (A), and with hydrolysates of buckwheat (B), egg white (C), and peanut (D), aliquots being used as the antibody in the assay. The percentage inhibition was calculated by using intact serum at the same dilution with PBS as a control. ○ and □, patient 1; ● and ■, patient 2.

The results of searches for buckwheat floral transcriptome provided nucleotide sequences of 25,435 and 25,401 contigs and 56,874 and 42,913 singletons for *Fagopyrum esculentum* and *Fagopyrum tataricum* that has been published by Logacheva *et al.*²⁶⁾ Each dataset was searched with the TBLASTN algorithm and BioEdit software (<http://www.mbio.ncsu.edu/bioedit/bioedit.html>), using the PAM40 matrix, no filter for low-complexity regions and an E-value threshold of 100.

Results

Evaluation of the IgE-binding cross-reactivity between peanut and buckwheat

IgE from two peanut-allergic patients was bound to the PBS-soluble fraction of both peanut and buckwheat (Fig. 1). Furthermore, as shown in Fig. 2A, the PBS-soluble fraction of buckwheat inhibited the binding between specific IgE in the patient sera and the peanut allergen.

To evaluate the cross-reactivity between buckwheat and peanut, buckwheat was hydrolyzed with α -chymo-

trypsin in PBS, the hydrolysate was subjected to solid-phase extraction in a C18 Sep-Pak cartridge to remove the proteins and such other constituents as carbohydrate and minerals, and the buckwheat polypeptides adsorbed to the Sep-Pak material were recovered by eluting with 90% methanol. Inhibition ELISA showed that the buckwheat hydrolysate inhibited binding between IgE and the peanut allergen (Fig. 2B), while fractions of the similarly treated egg white protein hydrolysate did not (Fig. 2C).

Isolation and identification of the cross-reactive peptide from peanut

A peanut total protein extract was hydrolyzed and fractionated with the C18 Sep-Pak cartridge into Frs 1, 2 and 3. Since the inhibitory activity of Fr 3 was the highest (Fig. 2D), it was then subjected to reversed-phase HPLC to isolate the cross-reactive peptide (Fig. 3). Inhibition ELISA showed that the asterisked

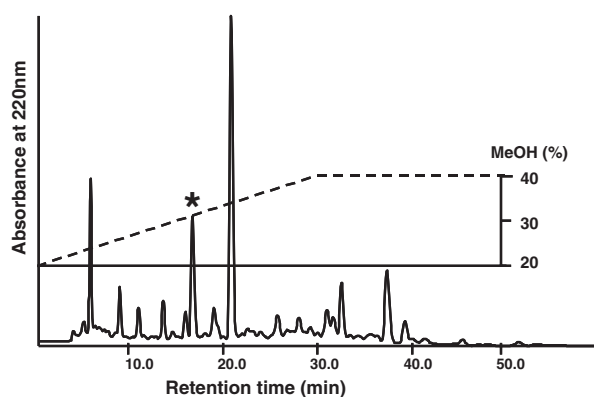


Fig. 3. Reversed-Phase HPLC Profile of the α -Chymotryptic Hydrolysate of Peanut.

Inhibition ELISA showed that the asterisked peak exerted the most potent inhibitory activity.

peak contained the most potent inhibitory activity. This peak was again purified twice under the same HPLC conditions, then subjected to a fourth HPLC run in a different column to produce a highly purified peptide.

The positive and negative IT-TOF-MS spectrum of the cross-reactive peptide gave an $[M + H]^+$ ion at m/z 969.3292 and $[M - H]^+$ ion at m/z 967.3674. Determination with a protein sequencer yielded the sequence X_1 - X_2 -Gln-Thr-Arg-Thr-Gly-Tyr (X_1 and X_2 , unidentified). Gln-Thr-Arg-Thr-Gly-Tyr coincides with the sequence of amino acids 4–9 of peanut oleosin 3 (NCBI accession No., GenBank ID, AAU21501). Based on the reported oleosin 3 sequence, X_1 and X_2 were respectively expected to be Ser and Asp. The calculated molecular weight of octapeptide Ser-Asp-Gln-Thr-Arg-Thr-Gly-Tyr (SDQTRTGY) is 926, which is 42 mass units smaller than the measured value (968). This difference in molecular weight is consistent with the possibility that N-terminal serine was blocked by an acetyl group.

Evaluation of the cross-reactivity between epitope peptide SDQTRTGY and buckwheat

Octapeptide SDQTRTGY was synthesized and subjected to inhibition ELISA with the sera of eight patients who were allergic to peanuts; we confirmed that it inhibited binding between buckwheat and IgE from all the patient sera (Fig. 4).

Analogous sequence of SDQTRTGY

Oleosin 3 in buckwheat has not been identified and could not be determined, because no genome database for buckwheat has been reported. We searched for buckwheat proteins with sequences homologous to SDQTRTGY by using a BLASTP search of the NCBI nr database. However, we only found proteins with a maximum of 4 of the 8 amino acid residues identical to SDQTRTGY.

Under these circumstances, it is notable that Logacheva *et al.*²⁶⁾ have recently reported a buckwheat transcriptome analyzed by *de novo* sequencing for two close species. Using their data, we searched for novel buckwheat proteins with sequences homologous to SDQTRTGY by TBLASTN (Table 1). We found 27 transcripts that putatively coded for proteins in which more than 6 of the 8 amino acid residues were identical to SDQTRTGY. We

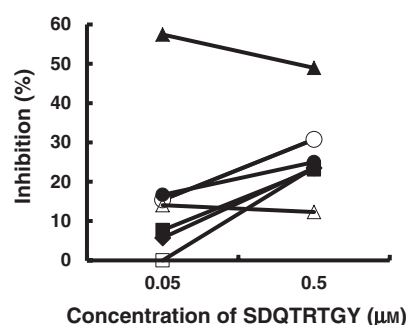


Fig. 4. Inhibition ELISA of Peptide SDQTRTGY.

The wells of an ELISA plate were coated with the PBS-soluble fraction of buckwheat as an antigen. Individual sera ($n = 8$) from peanut-allergic patients were pre-incubated with an SDQTRTGY solution, and aliquots were used as the antibody in the assay. The percentage inhibition was calculated by using intact serum at the same dilution with PBS as a control. The percentage inhibition figures were as follow: 0.05 μ M, 7.7; 0.5 μ M, 23.1 (patient 1), 0.05 μ M, 5.7; 0.5 μ M, 23.5 (patient 2), 0.05 μ M, 15.4; 0.5 μ M, 30.8 (patient 3), 0.05 μ M, 16.7; 0.5 μ M, 25.0 (patient 4), 0.05 μ M, 0.0; 0.5 μ M, 23.6 (patient 5), 0.05 μ M, 57.4; 0.5 μ M, 48.9 (patient 6), 0.05 μ M, 14.0; 0.5 μ M, 12.3 (patient 7), 0.05 μ M, 15.4; 0.5 μ M, 30.8 (patient 8).

excluded from these 6 transcripts that coded for known proteins on the opposite strand to the putative proteins with SDQTRTGY-like sequences; consequently, 21 transcripts remained as candidates. Among the remaining 21 sequences, the putative protein encoded by GAA4HQR01AQ413 had the highest similarity (7 of the 8 amino acid residues were identical). Although they have shown no similarity to anything known to date, the proteins encoded by GAA4HQR01AQ413 and/or the transcripts listed in Table 1 would be allergens.

Discussion

Since peanut and buckwheat allergies present similar features, we examined whether or not the sera from peanut-allergic patients would cross-react with buckwheat. Inhibition ELISA showed that the buckwheat peptide inhibited binding between IgE and the peanut allergen (Fig. 2B), whereas egg white peptide did not (Fig. 2C), suggesting that the cross-reaction was specific to peanut and buckwheat. Cross-reactivity between peanut and such other closely related edible legumes as lupin (*Lupinus perennis*), lentil (*Lens culinaris*), pea (*Pisum sativum*), kidney bean (*Phaseolus vulgaris*), and soybean (*Glycine max*) has been studied.²¹⁾ Dooper *et al.*²²⁾ have reported an IgE cross-reaction between peanut allergens and lupine conglutins, of which *Ara h 2* was the most potent inhibitor. Beardslee *et al.*²³⁾ have reported that soybean glycinin G1 shared IgE-binding epitopes with the peanut allergen, *Ara h 3*. Our study revealed a new putative cross-reactive IgE-binding epitope common to buckwheat (Polygonaceae) and peanut (Fabaceae) which belong to different plant families.

In this study, despite the N-terminal of the octapeptide being blocked by an acetyl group, we could determine the sequence of amino acids 3–8 by a proteinous sequencer. Gheorghe *et al.*²⁷⁾ have reported that cleavage of protein N-terminal acetyl groups by incubation with TFA in methanol resulted in efficient deacetylation,

Table 1. Analogous Sequence of “SDQTRTGY” Involved in Floral Transcriptome of Buckwheat

TBLASTN Hits with 6 identical amino acids to SDQTRTGY									
BLASTX search against NCBI nr database by Logacheva <i>et al.</i> (E-value < 10 ⁻⁶)									
Database	Sequence name	Hit sequence	Position	E-value	Identities	Sequence description	Accession No.	E-value	Direction from SDQTRTGY-like sequence
Fagopyrum_esculentum contigs	Fagopyrum_esculentum_MIRA_VER3_c20402	SDQTRTSY	178–155	6.3	6/8 (75%)	hydrolyzing o-glycosyl	CBI33116	6.67E–40	opposite
	Fagopyrum_esculentum_MIRA_VER3_c2829	SDQTRT	252–269	6.3	6/6 (100%)	af439853.1pathogen-induced calmodulin-binding protein	XP_002272395	5.39E–32	opposite
Fagopyrum_esculentum singlets	GAA4HQR01AQ413	DQTRTGY	241–221	0.31	7/7 (100%)	no hit			
	GAA4HQR01D5CO8	DQTRTG	395–412	9.1	6/6 (100%)	no hit			
	GAA4HQR01EU3HL	SDQTRT	222–239	9.1	6/6 (100%)	no hit			
	GAA4HQR01EXA49	DQTRTG	385–402	9.1	6/6 (100%)	no hit			
	GAA4HQR01IBDZ3I	DQTRTG	396–413	9.1	6/6 (100%)	inhibition protein homolog	AAT77910	6.71E–48	same
	GAA4HQR01C9057	DQTRTG	385–368	9.1	6/6 (100%)	no hit			
	GAA4HQR01CEG4K	SDQTRT	37–20	9.1	6/6 (100%)	no hit			
	GAA4HQR01AXD7O	SDQARKGY	87–64	9.1	6/8 (75%)	no hit			
	Fesculentum.AL.TLSC.s25539	DQTRTG	55–72	9.1	6/6 (100%)	no hit			
	GAA4HQR01D551J	SEQTTTGY	239–262	13	6/8 (75%)	no hit			
	GAA4HQR01BD3PK	SDQTRVG	138–158	25	6/7 (85%)	no hit			
	GAA4HQR01BCP1P	SDQTKTMY	83–60	35	6/8 (75%)	no hit			
Fagopyrum_tataricum contigs	Fagopyrum_tataricum_MIRA_VER3_c23668	QTRTGY	202–219	2.3	6/6 (100%)	s-adenosyl-L-methionine: beta-alanine n-methyltransferase	XP_002264566	4.61E–14	same
	Fagopyrum_tataricum_MIRA_VER3_c3062	QTRTGY	415–432	2.3	6/6 (100%)	s-adenosyl-L-methionine: beta-alanine n-methyltransferase	XP_002264566	3.69E–148	same
	Fagopyrum_tataricum_MIRA_VER3_c11174	SDQTRKG	888–868	17	6/7 (85%)	hypothetical protein	XP_002267199	0.00E+00	opposite
	Fagopyrum_tataricum_MIRA_VER3_c16803	SDQLRAGY	426–449	24	6/8 (75%)	ankyrin repeat-containing	XP_002284522	6.11E–47	opposite
Fagopyrum_tataricum singlets	GAA4HQR02HXC3M	QTRTGY	445–462	2.4	6/6 (100%)	no hit			
	GAA4HQR02I5CPV	QTRTGY	506–523	2.4	6/6 (100%)	conserved hypothetical protein	XP_002520082	8.16E–35	same
	GAA4HQR02FQ643	TDTRTGY	416–439	3.3	6/8 (75%)	no hit			
	GAA4HQR02IQ1FM	SDQTRT	312–295	6.5	6/6 (100%)	kelch repeat-containing protein serine threonine phosphoesterase family protein	CBI24435	4.65E–36	opposite
	GAA4HQR02GTPXN	DQTRTG	592–609	6.5	6/6 (100%)	no hit			
	GAA4HQR02J2SUK	SDQTRT	141–158	6.5	6/6 (100%)	aldehyde dehydrogenase	XP_002263479	6.62E–28	opposite
	GAA4HQR02JVH1X	DQTRTG	327–344	6.5	6/6 (100%)	no hit			
	GAA4HQR02I582Q	SDQTRIG	70–50	18	6/7 (85%)	predicted protein	XP_002274931	6.87E–51	same
	GAA4HQR02IBK1G	SSRTRTGY	283–260	18	6/8 (75%)	no hit			

thus allowing direct application to a protein sequencer analysis. We used TFA and methanol as an elution solvent in preparative HPLC which was used in the isolation, purification and concentration procedures for the peak (the octapeptide). We thus assumed that deacetylated octapeptide was produced during these procedures.

Although oleosin is an oil body protein, it was isolated from the defatted peanut. Since ground peanut was defatted only once by hexane, a small amount of oleosin might have remained in the defatted fraction. To the best of our knowledge, information on the allergenicity of peanut oleosin is limited. Oleosins are amphipathic proteins of low molecular weight (15–26 kDa) which are located on the surface of lipid storage bodies.²⁸⁾ Oleosins consist of several isoforms; among these, two oleosins have been registered as *Ara h 10* and *Ara h 11* according to the IUIS nomenclature (www.allergen.org/Allergen.aspx). Pons *et al.*¹³⁾ have reported that peanut oleosin with a molecular weight of 18 kDa was a possible allergen for the IgE reaction to peanut, and may also be responsible for allergic reactions to peanut oil. According to Pons *et al.*¹³⁾ IgE binding with oleosin was detected in only 3 of 14 samples of sera from patients who had peanut allergy. They did not identify the IgE-binding epitope(s). In contrast, we found that SDQTRTGY in oleosin 3 was recognized by the serum IgE of all the eight peanut-allergic patients tested. This study is the first to report oleosin 3 as an IgE-binding allergen that was recognized by patients with a much higher frequency than other oleosins.

Oleosins consist of three distinct structural domains, *i.e.*, an amphipathic N-terminal domain, a highly conserved central hydrophobic domain, and an amphipathic α -helical domain at or near the C-terminus, this structure being highly conserved. Of the three domains of oleosin, the central core is buried in lipid bodies and therefore is not considered to be available as an epitope. Instead, it is reasonable that the N-terminal domain, which is exposed at the hydrophilic surface, might be readily recognized as an IgE-binding epitope. This speculation is in agreement with the previous report²⁹⁾ that epitopes of apolipoproteins in the seeds of the Cruciferae family were predominantly localized at the C- and N-terminal domains at the hydrophilic surface.

Only a few oleosins such as those from peanut, sesame,³⁰⁾ and hazelnut³¹⁾ have been reported to be allergens, and their IgE-binding epitope(s) has not yet been identified. Further investigation seems to be warranted, because oleosins are found in many foods of plant origin. In addition, diagnostic protocols such as the skin prick test should be reconsidered. For example, despite reports showing that sesame caused anaphylaxis, it has often been misdiagnosed as negative, because an inappropriate sesame extract prepared with saline was used; this has been pointed out by Leduc *et al.*³⁰⁾ who suggested that such hydrophobic allergens as oleosin that are insoluble in saline may be present in sesame.

Compared to peanuts, information on buckwheat allergens is limited. Since limited data are available on the genome and allergens of buckwheat, the outstanding result of the present study is that we could clearly demonstrate the cross-reactive epitope sequence that is common to buckwheat and peanut. Moreover, informa-

tion on cross-reactive epitopes would contribute to understanding the pathogenesis of severe allergies to both foods. We plan to prove the existence of protein(s) that possesses an SDQTRTGY homolog sequence in buckwheat.

We isolated SDQTRTGY from the α -chymotryptic hydrolysate of peanut. This indicates that the SDQTRTGY structure was highly resistant to digestive enzymes which would presumably be one characteristic of a cross-reactive epitope for anaphylaxis. The IgE-binding ability of SDQTRTGY was also relatively high (Fig. 4) by inhibition ELISA; the average and maximum inhibitory ratios of SDQTRTGY were 23% and 57%, respectively. However, more detailed analyses are necessary to clarify the common features of the epitope structure responsible for anaphylaxis such as the essential amino acid sequences, if indeed existing.

In conclusion, we have identified a novel IgE-binding epitope (SDQTRTGY) of peanut oleosin 3, and shown that it inhibited binding between buckwheat proteins and IgE of patients with peanut allergy. As many foods contain oleosin, the possibility of cross-reactions with other foods should also be investigated.

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