

Original article

Screening the allergenic repertoires of wheat and maize with sera from double-blind, placebo-controlled food challenge positive patients

Background: Food allergy to wheat and maize is an increasing factor of deterioration of life quality, especially childhood and can, in rare cases, even induce anaphylaxis. Although omega-5 gliadin from wheat and maize lipid transfer protein have been characterized as major cereal allergens on the molecular level, the list of food allergens is far to be complete.

Methods: To identify the IgE-binding repertoires of wheat and maize we screened respective cDNA libraries displayed on phage surface with sera from patients with a confirmed food allergy. The study included six patients with a positive double-blind, placebo-controlled food challenge (DBPCFC) to wheat, nine patients with a positive DBPCFC to maize, and six patients with anaphylactic reactions after ingestion of wheat.

Results: The enriched sequences encoding IgE-binding proteins showed heterogeneous repertoires for both, wheat and maize. The selected wheat repertoire yielded 12, the maize repertoire 11 open reading frames. Among these we identified allergens belonging to already characterized allergens families, such as gliadin, profilin and beta-expansin. Besides, we found novel proteins with high cross-reactive potential, such as thioredoxins, as well as sequences that had so far not been related to cereal allergy at all. The IgE-binding capacity of some selected proteins was evaluated *in vitro* and cross-reactivity was demonstrated by competition ELISA.

Conclusion: With regard to the heterogeneity of the characterized sequences as well as to the biochemical nature of the new allergens detected we conclude that wheat and maize-related food allergy is more complex than so far anticipated.

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Key words: cDNA phage surface display; IgE-binding repertoires; maize allergy; recombinant allergens; wheat allergy.

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In the past decade molecular allergology has greatly contributed to our understanding of IgE-mediated allergic reactions. Recombinant techniques facilitated the production of a large number of allergens in good yield and high purity with IgE-binding capacities comparable with those of their natural counterparts (1). The symptoms of food allergy are quite diverse and can range from mild itching affecting the oral mucosa to skin symptoms such as urticaria, angioedema or exacerbation of atopic eczema, respiratory reactions, and in rare cases even to anaphylaxis. Allergic conditions represent a multifactorial disease, not only with respect to the individuals' bias to atopy and the various symptoms along with it, but also with regard to the heterogeneity of allergenic sources. Thus, the diversity of allergens of a particular allergenic source can range from only few as in the case of allergy to cats (2), birch pollen (3) or insect venom allergy (4) to more than 80 as reported for mould allergy (5, 6). Additionally, cross-reactivity between homologous pro-

teins present in related or phylogenetically distant allergenic sources can exacerbate the progression of allergic diseases (7) and complicate the identification of the primary cause of sensitization (8).

For the development of food allergy two ways of primary sensitization may be distinguished. First, sensitization in particular to hen's egg and cow's milk through the gastrointestinal tract which affects mainly young children (9). However, these hypersensitivity reactions often regress during childhood, although sometimes replaced by other forms of allergy in the adults' life (10). Allergic reactions after ingestion of peanut or seafood represent exceptions as they persist in adults and may often manifest in very severe symptoms (11).

Secondly, sensitization leading to food allergy may occur through the respiratory tract upon inhalation of airborne allergens such as tree or grass pollen and has therefore been termed 'pollen-food allergy syndrome' (for a review see Ref. 12). A very common and prevalent

example thereof is the celery-birch-mugwort syndrome in Europe (13). The prominent characteristic of this type of allergy is that the food allergy symptoms often overlap with seasonal pollinosis or vice versa. Such complex clinical pictures derive from a high degree of cross-reactivity between homologous molecular structures present in different allergenic sources.

Thus, serological tests as well as skin prick tests performed with complex allergen extracts can in some cases lead to ambiguous diagnostic outcomes. An elaborate survey of the problematic nature of diagnosing food allergy using allergen extracts was recently summarized by Bohle and Vieths (14). In that context the authors emphasize the indispensability of recombinant proteins as diagnostic tools for food allergy.

The present work further develops the idea of using recombinant proteins for the characterization of food allergy related to wheat and maize. Wheat products regarded major sources responsible for atopic dermatitis in young children (15) have been reported to be able to induce IgE-mediated food-dependent exercise-induced anaphylaxis (16) often because of sensitization to gliadins (17, 18). Furthermore wheat flour is considered the major cause of baker's asthma (19), the most prevalent form of occupational asthma. Major allergens associated with this type of allergy belong to the alpha-amylase/trypsin-inhibitor family (20).

Although much less common, investigation of food allergy to maize appears attractive, as cross-reactivity to soy, peanut and rice has been demonstrated at least *in vitro* (21). Moreover, cases of anaphylactic reactions to maize have been reported in the literature (22–24).

However, to date only a 9-kDa lipid transfer protein (LTP) has been identified as a major allergen in terms of food allergy to maize (23, 24).

For further molecular characterization of wheat and maize allergens we screened phage surface display cDNA libraries with serum IgE from patients who reacted positive to double-blind, placebo-controlled food challenge (DBPCFC). Phage surface display technology allows the fast enrichment of IgE-binding proteins physically linked to their encoding cDNA and offers the chance of identifying whole repertoires of IgE-binding molecules (25) which may be helpful for the development of new diagnostic procedures based on recombinant proteins.

Materials and methods

Patients' sera

Sera were obtained from patients with a history of food allergy to wheat or maize on the basis of DBPCFC, according to the guidelines of the FAREDAT project QLRT-2001-00301 or from patients who had severe anaphylactic reactions after the ingestion of products containing wheat or maize flour. The serological data are summarized in Table 1. A full explanation of the procedure was given to all participants, and their written consent was obtained.

Construction of wheat and maize phage surface display cDNA libraries

The construction of a wheat endosperm cDNA library used for biopanning is described elsewhere (26). For the construction of a maize cDNA library displayed on phage surface 1×10^8 pfu of a Lambda ZAP[®] II (Stratagene, La Jolla, CA) endosperm cDNA

Table 1. Serological and clinical data of patients with positive double-blind, placebo-controlled food challenge (DBPCFC) to wheat (1–6) and maize (7–15). OAS: oral allergy syndrome

Patient	Age (years)	Sex	CAP-RAST (kU/l)	Symptoms under DBPCFC	History	Pollens causing symptoms
1	31	F	<0.35 (wheat flour)	Wheat: itching mouth/lip, pharynx atopic edema	OAS, atopic eczema, cough, wheeze	Birch
2	44	M	38.7 (wheat flour)	Wheat: neck erythema, pruritus of genitals and hands, cough	Abdominal pain, asthma	Birch, pellitory
3	60	F	3.57 (wheat flour)	Wheat: OAS, urticaria	OAS	Birch, grasses
4	42	M	17.2 (wheat flour)	Wheat: generalized erythema	Urticaria, atopic eczema	Pellitory
5	50	F	6.30 (wheat flour)	Wheat: pruritus, headache	Pruitus, OAS, rhinitis, asthma	Grasses, birch, pellitory, maize
6	54	F	1.45 (wheat flour)	Wheat: heartburn, abdominal swelling, nausea, vomiting	Atopic eczema	–
7	32	M	2.8 (maize flour)	Maize: erythema, itching genitals	Urticaria	Grasses, pellitory
8	50	F	68.3 (maize flour)	Maize: nausea, urticaria	Urticaria, oral itching, pharynx edema	Grasses, birch, pellitory
9	35	F	7.51 (maize flour)	Maize: OAS	Atopic eczema after face-mask with corn flour	Grasses, birch
10	24	F	63.4 (maize flour)	Maize: flushing, erythematous flare of atopic dermatitis, abdominal pain	Asthma upon inhaling maize flour, itching atopic dermatitis	Grasses, birch, pellitory
11	25	F	5.59 (maize flour)	OAS	OAS, erythema	Grasses, birch, pellitory
12	24	F	1.36 (maize flour)	Maize: throat itching, mucosal erythema, pharynx atopic edema	OAS, urticaria	–
13	12	F	4.34 (maize flour)	Maize: OAS, abdominal pain	Vomiting, abdominal pain	–
14	23	F	n.d.	Maize: OAS	OAS	Grasses, birch
15	33	F	n.d.	Maize: OAS, blisters of the oral mucosa	OAS	Grasses, birch

RAST, radioallergosorbent test; n.d., not determined.

library 10 days after pollination (10-DAP) (27) were used for mass excision according to the manufacturer's instructions (Stratagene). For the preparation of the cDNA inserts 100 ml of an exponentially growing *Escherichia coli* XL1-Blue culture (Stratagene) were infected with the excised phagemids and isolated 3 h after infection. Ten micrograms of the purified phagemid DNA were restricted with *EcoRI* and *XhoI*, and the gel-purified cDNA inserts were used for ligation with 10 µg of likewise restricted pJuFoII vector (28). After ethanol precipitation the ligation mixture was used for transformation of electrocompetent XL1-Blue cells which were then spread on 25 LB/ampicillin + tetracyclin (Amp + Tc) plates (14 cm diameter) for positive selection. Transformants were harvested from the plates by resuspending in 150 ml 2 × YT/Amp + Tc medium and used for the generation of a phage display library upon superinfection with helper phage VCS-M13 (Stratagene).

Affinity selection of IgE-binding phagemids

The phage display cDNA libraries were subjected to three rounds of affinity selection against individual sera (Table 1). After three rounds sera which did not clearly yield specific enrichment of phage were pooled and used for further enrichment during two additional biopanning rounds. From the enriched repertoires a total of 78 clones (wheat) and 169 clones (maize) were randomly picked and the cDNA inserts analyzed by restriction analysis. Multiply occurring clones with cDNA inserts of the same size were sequenced and sequences analyzed by BLAST searches (WU BLAST version 2.0 MP-WashU [13-Dec-2004]).

Production of recombinant proteins and IgE Western blot analysis

The enriched cDNA inserts were cloned into the high level expression vector pQE30 (Qiagen, Hilden, Germany), used for electrotransformation of M15/pREP4 cells (Qiagen) and expressed according to the manufacturer's instructions in a small scale (50 ml) at 37°C. Additionally, the cDNA encoding wheat profilin was amplified from the wheat library using the following primers designed from the EMBL entry CK213243: 5'-primer: 5'-CG GGATCC ATG TCG TGG CAG ACG TAC G-3', 3'-primer: 5'-CCC AAGCTT A GAA ACC CTG CTC GAC C-3'. After *BamHI*/*HindIII* digestion the DNA fragment was subcloned into accordingly restricted vector pQE30 processed as mentioned as described above.

All proteins were purified over Ni-NTA under denaturing conditions in the presence of 8 M urea and eluted in a final volume of 100 µl.

For Western blot 10 µl of each eluate was subjected to SDS-PAGE (NuPAGE™, 12% Bis-Tris, Invitrogen™) and electrotransferred onto a Hybond™-P PVDF membrane (Amersham Pharmacia Biotech, Uppsala, Sweden). Free binding sites were blocked with 5% nonfat dried milk, 0.1 % (v/v) Tween-20 in TBS. After incubation with the pool of patients sera used for affinity selection (1 : 10 in blocking buffer) binding of specific IgE was detected by incubation with TN-142 mouse anti-human IgE mAb (1 mg/ml) at a dilution of 1 : 1000. Bound IgE was visualized after incubation with HRP-conjugated goat anti-mouse IgG (NXA931, 1 : 5000 in blocking buffer, Amersham Pharmacia Biotech) and enhanced chemiluminescence reagents (ECL Plus™, Amersham Pharmacia Biotech) as substrate.

Titration of allergen-specific serum IgE and competition ELISA

For allergen-specific IgE titrations Nunc-Immuno Plates (Maxisorp Surface) were coated overnight at 4°C with 50 µl of 5 µg/ml antigen

in PBS, washed and blocked with 2% (w/v) skimmed milk powder in PBS containing 0.5% Tween-20 (buffer A). After washing with PBS containing 0.1% Tween-20 (buffer B), patients' serum IgE was titrated starting with a serum dilution of 1 : 8 in buffer A, further serially diluted twofold throughout the plate and incubated for 2 h at 37°C. IgE binding was detected by incubation with mAb TN-142 mouse anti-human IgE mAb (1 mg/ml) diluted 1 : 6500 in buffer A and visualized with alkaline-phosphatase-conjugated goat-anti mouse IgG H + L chain (Pierce, #31320, 1 : 6500). Competition assays between the different antigens were performed on ELISA plates coated as described above. The antigens were titrated starting with a concentration of 50 µM keeping the serum concentration constant (1 : 10 in buffer A). Detection and visualization was performed as described above. Percentage of competition was calculated from the background-corrected absorbency of samples where serum was incubated in blocking buffer without antigen in the liquid phase (0%). For graphic display, the measured absorbencies were plotted against the antigen concentration in the fluid phase.

Results

Phage surface displayed cDNA libraries can be enriched for IgE-binding proteins within three rounds of affinity selection. The hand-picked repertoires of selected IgE-binding clones from wheat and maize are listed in Table 2A,B. The wheat repertoire contains members of the alpha-amylase/trypsin-inhibitor family, alpha/beta- and gamma-gliadins, thioredoxin, a putative high mobility group protein, a serine carboxypeptidase, a putative leucine-rich repeat protein, beta-purthionin, a protein with unknown function and a wheat homolog of the major allergen of *Phleum pratense*, Phl p 1. Five of the 12 wheat clones (clones W1, W2, W6, W10, W12) represent complete open reading frames, with the beta-expansin 11, a Phl p 1 homologue (clone W12), being the largest among them and demonstrating that full length cDNAs coding for proteins up to 30 kDa can be enriched by the pJuFo surface expression technology. The remaining seven clones (clones W3, W4, W5, W7, W8, W9, W11) represent partial coding sequences with clone W8, encoding a 29.1-kDa fragment of the serine carboxypeptidase II, being the largest among those.

After subcloning the cDNA inserts into expression vector pQE30 the respective wheat proteins were produced in *E. coli* M15/pREP4 in a 50 ml scale and purification of the His₆-tagged proteins was performed under denaturing conditions. With exception of beta-purthionin, which caused strong lysis of the bacterial host, all proteins could be produced in reasonable amounts. Testing of the IgE binding capacity of the recombinant proteins by Western blot analysis showed that all proteins were in fact IgE binders (Fig. 1). However, the differences observed between the signal intensities could be due to both, amount of protein blotted or concentration of antigen-specific IgE in the serum pool used for detection.

In the case of maize we identified two *h*-type thioredoxins, profilin, a putative transport protein particle

Table 2. Enriched repertoire of IgE-binding sequences of wheat. Molecular weight estimates ^(a) were made on the basis of BLAST searches (WU BLAST version 2.0 MP-WashU [13-Dec-2004]). Sequences generated during this work are shown in bold

Protein name/function (Allergen name)		EMBL acc. no.	M _r of displayed cDNA product (kDa)	M _r of full length protein (kDa)	Reference
(A) wheat cDNA clone					
W1	Alpha-amylase inhibitor CM 17	X59791	13.9	13.4	(20)
W2	Alpha-amylase inhibitor 0.19	AY729672	12.8	13.3	(20)
W3	Alpha/beta-gliadin A-IV	M11075	19.6	32.1	
W4	Alpha/beta-gliadin MM1	X17361	23.0	33.3	
W5	Gamma gliadin	M36999	17.4	32.3	
W6	Thioredoxin <i>hB</i> (Tri a 25)	AJ404845	13.4	13.4	
W7	Putative high mobility group (HMG) protein	AJ890015	11.9	≈14 ^a	
W8	Serine carboxypeptidase II	AJ890016	29.1	47.1 (29.1 + 18.0)	
W9	Putative leucine-rich repeat protein	AJ890017	15.2	≈23 ^a	
W10	Beta-purothionin precursor	X96445	13.0	11.9	
W11	Unknown	AJ890018	10.4		
W12	EXPB11, Phl p 1 homolog	AJ890019	29.8	27.3	
So far characterized wheat allergens (IUIS):					
	Agglutinin (Tri a 18)				http://www.allergome.org (18, 30)
	Omega-5 gliadin (Tri a 19)	PIR: A59156		65	
(B) maize cDNA clone					
M1	Thioredoxin h, ZmTRX <i>h1</i> (Zea m 25)	AJ890020	13.9	13.9	
M2	Thioredoxin h, ZmTRX <i>h2</i>	AJ890021	13.0	13.0	
M3	Profilin 5 (Zea m 12)	AF201459	15.5	15.5	(31)
M4	Putative transport protein particle component	AJ890022	4.4	≈21 ^a	
M5	Putative KED-like protein	AJ890023	11.5	unknown	
M6	Putative translation initiation factor IF-2	AJ890024	19.8	≈77 ^a	
M7	Putative aldose reductase	AJ890025	4.9	≈35 ^a	
M8	Putative cytosolic 6-phosphogluconate dehydrogenase	AF061838	15.3	53.0	
M9	Basal layer antifungal peptide precursor	AJ297903	11.1	7.9	
M10	MEG3 protein	AY536123	9.6	9.6	
M11	Unknown	AJ890026	20.9		
So far characterized maize allergens (IUIS)					
	Lipid transfer protein (Zea m 14)	J04176		9.0	(23)

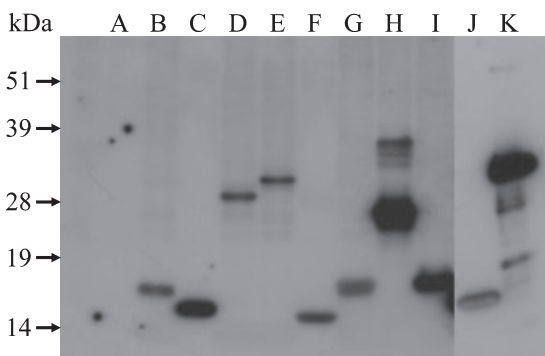


Figure 1. Western Blot analysis of enriched wheat proteins. (A) negative control, bee venom PLA₂ (Sigma-Aldrich, St Louis, MO, USA); (B) alpha-amylase inhibitor CM 17 (clone W2, Table 2A); (C) alpha-amylase inhibitor 0.19 (clone W2, Table 2A); (D) gamma gliadin (clone W5, Table 2A); (E) alpha/beta-gliadin MM1 (clone W4, Table 2A); (F) thioredoxin *hB* (Tri a 25; clone W6, Table 2A); (G) putative high mobility group (HMG) protein (clone W7, Table 2A); (H) serine carboxypeptidase II (clone W8, Table 2A); (I) putative leucine-rich repeat protein (clone W9, Table 2A); (J) unknown protein (clone W11, Table 2A); (K) EXPB11 (clone W12, Table 2A). Signals in lines A-I were obtained after 1-min film exposure, signals in lines J and K were obtained after exposure of 5 s.

component, KED-like protein, translation initiation factor, aldose reductase, cytosolic 6-phosphogluconate dehydrogenase, a basal layer antifungal peptide, a MEG3 protein as well as a protein with unknown function.

Figure 2A shows that the sequence identity between the two maize thioredoxins is only 48% in contrast to 74% found between maize thioredoxin and the wheat thioredoxin formally termed Zea m 25 (EMBL acc. no. AJ890020) and Tri a 25 (EMBL acc. no. AJ404845), respectively, according to the recommendations of the allergen nomenclature committee (<http://www.allergen.org>; accessed 10 November 2005). Additionally, maize profilin 5 (ZmPRO5, Zea m 12) which constituted more than half of the eluted clones after the third round of biopanning shares 83% sequence identity with its wheat homolog Tri a 12 (Fig. 2B).

Thus, both protein families, the thioredoxins and the profilins, respectively, bear cross-reactive potential. Further investigations by Western blot analysis clearly demonstrate that the thioredoxins as well as the profilins bind serum IgE of patients with food allergy to wheat and maize, respectively (Fig. 3A,B). Furthermore, we observed sensitization against human thioredoxin in both, wheat and maize food allergic patients (Fig. 3A).

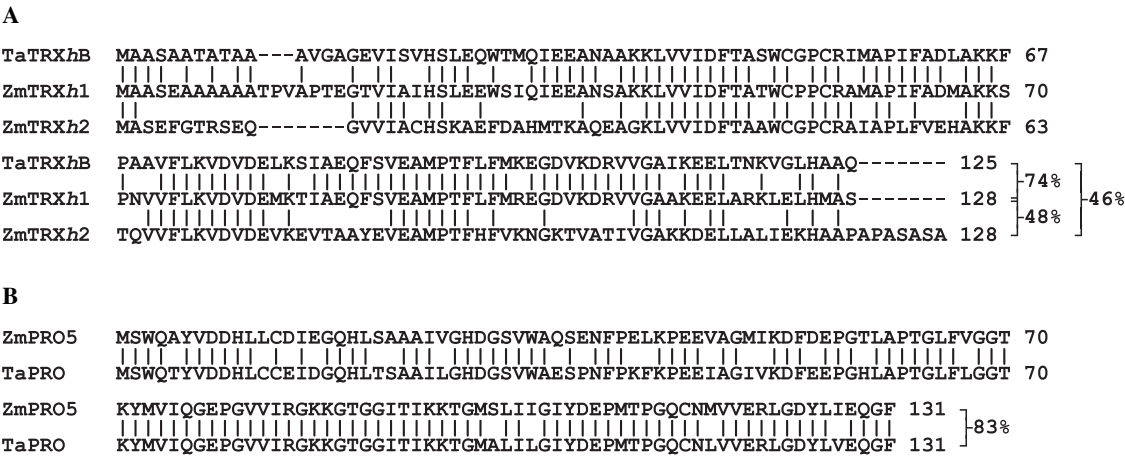


Figure 2. Sequence alignments of cereal thioredoxins (A) and cereal profilins (B). Amino acid identities are indicated with bars.

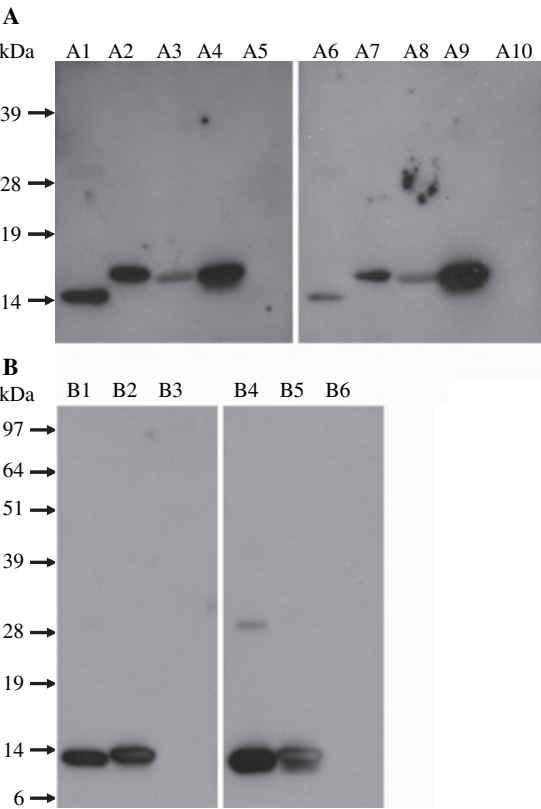


Figure 3. Western Blot analyses of IgE binding of thioredoxins (A) and profilins (B): TaTRXhB (A1, A6), ZmTRXh1 (A2, A7), ZmTRXh2 (A3, A8), human TRX (A4, A9); profilins of wheat (B1, B4) and maize (B2, B5). After electrotransfer the blots were incubated with a serum pool of individuals with food allergy to wheat (left) and food allergy to maize (right), respectively, and developed as described in *Materials and methods*. PLA₂ from bee venom (A5, A10, B3, B6) was used as a structurally unrelated control allergen.

Direct evidence for IgE cross-reactivity of the profilins is given by competition ELISA (Fig. 4A,B).

For the expansin EXPB11 we determined the IgE-binding capacity by ELISA. This protein reacted strongest against the serum pool of wheat allergic patients (Fig. 1) and shows 52% identity to the major allergen of timothy grass pollen, Phl p 1. IgE binding to EXPB11 was found in two sera of the wheat allergic patients (one with oral allergy syndrome, one with exercise-induced anaphylaxis), two sera of maize allergic patients and in four patients with grass pollen allergy, without history of wheat or maize allergy (Fig. 5).

Discussion

In this work we present repertoires of IgE-binding proteins from wheat and maize. It has recently been shown that the pJuFo phage surface technology in combination with high throughput screening allows a fast enrichment of repertoires from various allergenic sources (5, 25). As the repertoires presented in this work were hand picked we do not claim completeness, which may also explain that LTP (Zea m 14), the major food allergen in maize (23), has not been detected among the enriched cDNAs.

However, biopanning of the wheat library yielded alpha/beta- and gamma-gliadins, two alpha-amylase inhibitors as well as seven new sequences that have not yet been related to wheat allergy at all (Table 2A). Alpha-amylase inhibitors have been shown for a long time to play an important role as inhalant and food allergens, involved in baker's asthma and food allergy to wheat (20, 29), while gliadins are rather considered to act exclusively as food allergens related to hypersensitivity reactions in children or exercised-induced anaphylaxis (EIA) (18, 30).

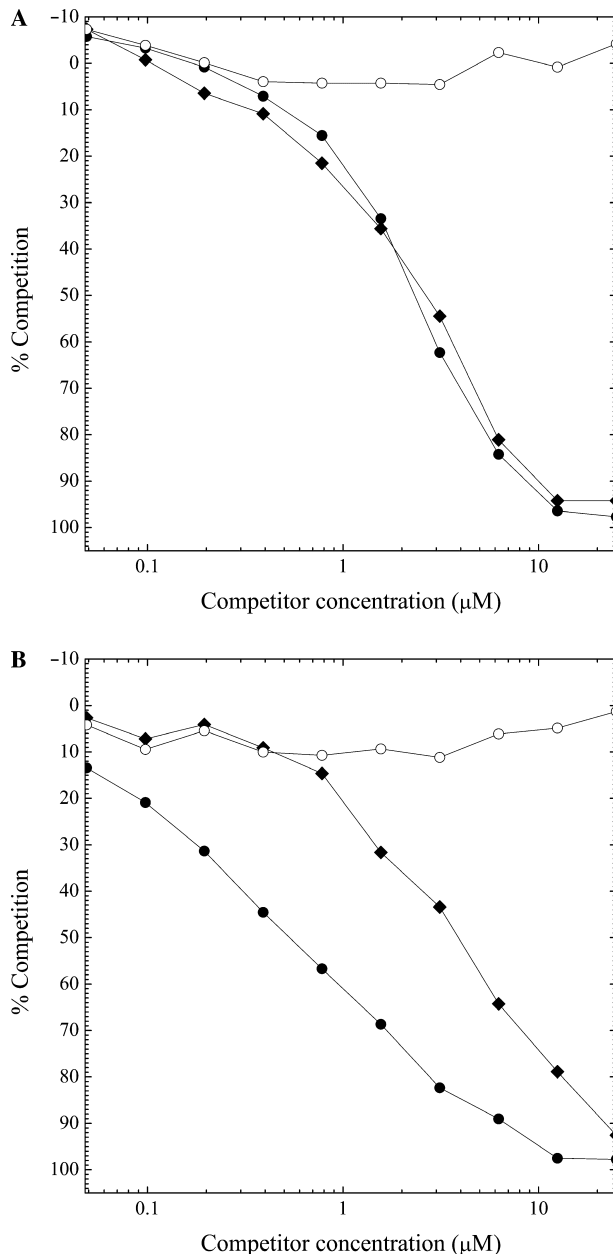


Figure 4. Inhibition of IgE binding to wheat profilin coated on solid surface by wheat (●) and maize (◆) profilins in the fluid phase. PLA₂ from bee venom (○) was used as negative control. The inhibitions were performed using serum from a wheat allergic (5A) and a maize allergic (5B) individual.

All other sequences listed in Table 2A represent novel IgE-binding proteins. Among those, EXPB11 (clone W12) which shares 52% sequence identity with the major allergen of timothy grass pollen, Phl p 1, may bear a high cross-reactive potential in patients who suffer from both, food allergy and pollinosis.

Another protein family bearing a high cross-reactive potential related to food allergy is the profilin family. The profilin of peanut (Ara h 5) (31, 32) displays 74% and

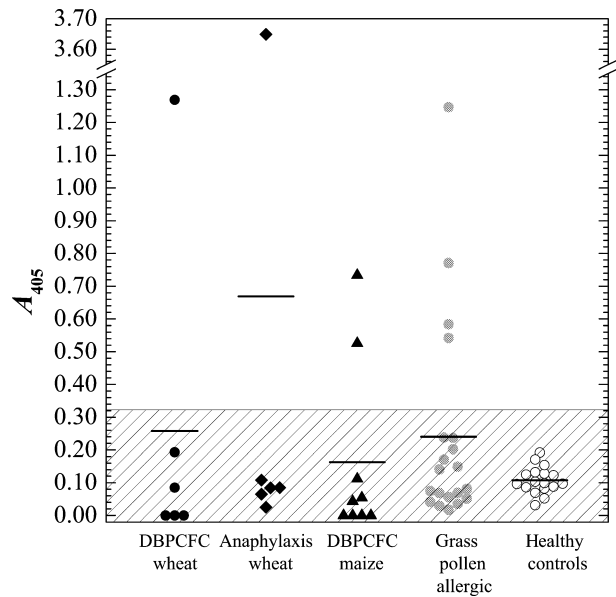


Figure 5. Immunoglobulin E-ELISA performed with EXPB11, a wheat homologue of the major grass pollen allergen Phl p 1, using sera from patients with allergic symptoms after wheat (●, ◆) or maize (▲) ingestion, individuals with grass pollen allergy, but without a history of wheat or maize allergy (○), as well as healthy individuals (○). The background was determined on the basis of the healthy control group and was arbitrarily assigned as the threefold average signal.

72% to the profilins from wheat and maize, respectively, and could partially explain the reported *in vitro* cross-reactivity between corn and peanut (21). Our data support the finding that wheat and maize contain cross-reactive structures, as shown by Western blot and competition ELISA (Figs 3 and 4).

Additionally, we identified two new thioredoxins of maize, ZmTRXh1 (Zea m 25) and ZmTRXh2, which share 74% and 46% sequence identity, respectively, with Tri a 25, a wheat thioredoxin. Recently, thioredoxin of the basidiomycete *Coprinus comatus* (Cop c 2, EMBL acc. no. AJ242791) was considered an airborne fungal allergen associated to allergic asthma. Among individuals suffering from atopic dermatitis between 20% and 40% are sensitized against thioredoxin of the yeast *Malassezia sympodialis* endemic on the human skin (A. Limacher, personal communication). However, no member of the thioredoxin family has so far been described in relation to allergy against cereals. Preliminary inhibition assays on IgE binding indicate that Tri a 25, Zea m 25, and ZmTRXh2 also cross-react *in vitro* (data not shown). Furthermore, the sensitization observed against the human thioredoxin could be the result of extended cross-reactivity among the thioredoxins, although the amino acid identity between cereal and human thioredoxins is only in the range of 30%. However, as serum pools were used in this study, the role of sensitization against human thioredoxin remains to be elucidated.

Except for the Phl p 1 homologous allergen of wheat, the gliadins, the alpha-amylase inhibitors and the profilins, all other sequences listed in Table 2A,B represent novel IgE-binding proteins, none of them belonging to any of the protein families which have so far been described as food-related allergens (33). However, the sensitization rate in patients with wheat or maize food allergy has not yet been investigated for the individual proteins and should be subject of future investigation. A recent study based on Western blot analysis of the different protein fractions of wheat also reported that the allergen repertoire recognized by food-allergic patients was quite heterogeneous and, as for other allergenic sources, patient-dependent (34). With regard to the biochemical nature of the identified cDNAs, and the clinical observation that most patients included in this study are also sensitized against grasses or birch (Table 1), it appears that cereal food allergy has a strong overlap with pollen-related allergy. Therefore, the identified sequences not merely represent indicators for wheat and maize related food allergy, but also include IgE-binding structures of airborne cereal derived pollens as

well as their potential cross-reactive homologues of grass or tree pollens. In fact, cross-reactivity between cereals and grasses is often observed in clinical practice (35). Taken together, the results imply that the development of diagnostic tools for wheat or maize food allergies based on recombinant proteins will be quite challenging and cost-intensive. However, a survey including only patients with a history of food-dependent EIA after wheat or maize ingestion might probably yield more restricted and relevant IgE-binding repertoires for both foods, besides the major food allergens of wheat and maize, omega-5 gliadin (30) and LTP (23).

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