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Tomato (*Lycopersicon esculentum*) allergens in pollen-allergic patients

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Abstract This study was undertaken to investigate the relevance of tomato allergy in pollen-allergic patients, and to identify and characterize tomato allergens. Therefore, sera of a group of 32 pollen-allergic patients with a history of adverse reactions to tomato were investigated by means of immunoblotting and specific IgE measurements (CAP, EAST, EAST inhibition and CCD [cross-reactive carbohydrate determinants] – ELISA). IgE-reactive spots on 2D blots were analyzed by amino acid microsequencing.

Tomato allergy was found to occur with a prevalence of about 9% in our group of birch pollen-allergic patients. Of the patients with adverse reactions to tomato, 44% presented IgE to tomato profilin and 35.5% specific IgE to CCD. Two patients were sensitized to a lipid transfer protein in tomato. Microsequencing of other IgE-reactive proteins, separated by 2D-gel electrophoresis and transferred to PVDF membranes, revealed β -fructofuranosidase, polygalacturonase 2A, and pectinesterase as further tomato allergens. Our data show that tomato is a relevant allergenic food in pollen-allergic patients. It seems that the majority of pollen-associated allergies to tomato is caused by known ubiquitous allergenic structures such as profilin and cross-reactive IgE-binding *N*-glycans. Furthermore, lipid transfer protein, as well as polygalacturonase 2A, pectinesterase and β -fructofuranosidase may represent birch pollen-independent tomato allergens for certain patients.

Keywords Tomato · Food allergy · Profilin · Carbohydrate epitopes · IgE

Introduction

Although allergy to tomato is frequently dealt with in allergy-related publications, it seems to be a rare event and is only mentioned in connection with other allergies, or in case reports.

The prevalence of tomato allergy was estimated to range from 1.5% [1] to 16% [2] among food-allergic populations and reached up to 39.2% among grass pollen allergics [3]. Of latex allergics, 2.2% [4] to 4.3% [5] showed allergic reactions to tomatoes.

Until now only a few attempts have been made to identify and characterize tomato allergens. In 1966, Bleumink et al. [6] described the isolation of a glycoprotein fraction from tomatoes with enhanced skin prick test activity. In 1970, these authors also reported the molecular weights of these glycoproteins to be between 20 and 30 kDa [7]. The allergens appeared to be resistant to heat and digestion. In 1991, McCormick et al. [8] showed that the tomato pollen proteins, encoded by the genes LAT 56 and LAT 59, presented sequence similarities to tryptic peptides of ragweed and Japanese cedar pollen, respectively. The distribution of tomato profilin in tomato tissues was investigated by Darnowski et al. [9]. Moreover, the structure of free [10] and linked [11] *N*-glycans in tomatoes were analyzed and were shown to contain the plant-characteristic glycan core with xylose and fucose, which is known as IgE binding epitope. A further step towards the characterization of tomato allergens was the work of Petersen et al. [12]. A 52-kDa allergen was detected by two-dimensional (2D) gel electrophoresis, which was analyzed by microsequencing and found to be a β -fructofuranosidase. These authors also showed that *N*-glycans containing α -1,3-fucose and the panallergen profilin [13] are relevant IgE-binding components in tomato extract. Recently, an intracellular pathogenesis-related gene of tomato named TSI-1 (tomato stress in-

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duced) was published, which codes for a 20.4-kDa protein that shows homologies to potato-STH 2/21 and tree pollen allergens [14].

This study was aimed at the investigation of the relevance of tomato allergy in pollen-allergic patients, and at obtaining further information about tomato allergens. For this, we studied a group of 32 pollen-allergic patients with a history of adverse reactions to tomato. The results indicate that pollen-related tomato allergy occurs more frequently than anticipated, and that ubiquitous allergenic structures, as well as allergens from other protein families, are recognized by IgE from tomato-sensitive subjects.

Materials and methods

Patients, human sera, and antibodies. Sera from 32 adult patients with a history of adverse reactions to tomato were investigated in this study (Table 1). Serum from a non-allergic patient (PEI 19) was used as a control. After obtaining informed consent, sera were taken from all patients and stored at -20°C until used. Rabbit sera against celery profilin and ragweed pollen profilin were kindly provided by Dr. P. Deviller, Lyon, France.

Allergen extracts and allergens. Recombinant birch pollen allergens Bet v 1 and Bet v 2 were purchased from BIOMAY (Linz,

Austria). Recombinant celery profilin Api g 4 [15] and recombinant cherry lipid transfer protein (LTP) [16] were a kind gift of Dr. S. Scheurer (Paul-Ehrlich-Institut, Langen, Germany). Tomato extract was prepared from fresh and cooked (20 min) fruits as described in [17]. N-linked glycopeptides with the glycan structure $\text{Man}\alpha 1-6(\text{Xyl}\beta 1-2)\text{Man}\beta 1-4\text{GlcNAc}\beta 1-4(\text{Fuc}\alpha 1-3)\text{GlcNAc}$ (XF) coupled to 2–4 amino acid residues were prepared from pine-apple stem bromelain by pronase digestion and purified by a series of chromatographic steps as described in [18]. Glycopeptides with the common pentasaccharide core $\text{Man}\alpha 1-6(\text{Man}\alpha 1-3)\text{Man}\beta 1-4\text{GlcNAc}\beta 1-4\text{GlcNAc}$ (MM) were prepared from bovine fibrin as described in [18] and used a negative control. Amino acid and monosaccharide analyses of the glycopeptides were performed as described in [19, 20]. All antigens were stored freeze-dried at -20°C until used.

Specific IgE. Diagnostic measurements of total and allergen-specific IgE were performed with the CAP System FEIA (Pharmacia & Upjohn, Uppsala, Sweden) according to the manufacturer's instructions.

Self-prepared solid phases were used together with a commercial enzyme allergosorbent test (EAST) according to the instructions of the manufacturer (Allergopharma, Reinbek, Germany). The antigens for EAST were coupled to cyanogen bromide activated filter paper discs (Hycor, Kassel, Germany) at a concentration of $2\text{ }\mu\text{g}$ tomato protein per disk.

Dose-related EAST inhibition studies were performed as described in [17].

IgE specific for carbohydrate structures was assayed by a glycan ELISA as described in [21]. The results of the glycan ELISA

Table 1 Results of specific IgE determinations (EAST/CAP/CCD-ELISA)

No.	Patient	Tomato-related symptoms ^a	Pollen sens. ^b	EAST class (U/ml) tomato	CAP class (kU/L)		CCD-ELISA ^c	
					Tomato	Bet v 2	XF	MM
1	Bo 3	OAS	b,g,m	2 (1.04)	3 (4.66)	0 (<0.35)	1.4	–
2	Bo 9	ND	b,g,w	3 (6.65)	4 (27.3)	0 (<0.35)	1.18	–
3	Bo 14	Skin itching	b,g,m	0 (0.2)	3 (6.01)	0 (<0.35)	–	–
4	Bo 15	OAS	b,g,m	2 (0.74)	4 (34.5)	1 (0.56)	–	–
5	Bo 32	OAS	b,g,m	1 (0.57)	2 (1.16)	2 (1.56)	–	–
6	Bo 33	OAS,Nausea,U	b,g,m	3 (4.12)	3 (17.1)	2 (0.7)	0.46	–
7	Bo 47	OAS	b,g,w	2 (1.1)	2 (2.48)	2 (1.96)	0.45	–
8	Bo 92	OAS	b,g	2 (0.98)	3 (6.44)	1 (0.39)	0.4	–
9	Bo 93	OAS,U	b,g,m	1 (0.36)	2 (1.53)	2 (1.68)	–	–
10	Bo 96	OAS	b,g,w	0 (0.12)	2 (1.48)	0 (<0.35)	–	–
11	Bo 99	Skin itching	b,g,m	1 (0.51)	3 (6.11)	2 (2.27)	nd	nd
12	Bo 134	OAS	b,g,m	0 (0.21)	2 (2.08)	0 (<0.35)	–	–
13	Bo 191	OAS	b,g,m	3 (4.65)	3 (15.1)	4 (35.7)	0.61	–
14	PEI 6	OAS	b,g,w	2 (1.47)	2 (2.93)	0 (<0.35)	–	–
15	PEI 7	OAS	b,g,m	2 (2.39)	3 (8.12)	3 (10.5)	–	–
16	PEI 8	OAS	b,g,w	2 (1.91)	0 (<0.35)	0 (<0.35)	–	–
17	PEI 14	U	b,g,m	3 (6.4)	3 (14.6)	0 (<0.35)	1.3	–
18	PEI 26	Spasm,ABP	b,g,m	3 (14.71)	4 (38.6)	2 (2.0)	2.7	–
19	PEI 27	OAS	b,g,m	2 (0.97)	2 (3.25)	3 (4.3)	–	–
20	PEI 50	U	b,g,m	0 (0.2)	2 (0.94)	0 (<0.35)	–	–
21	PEI 66	OAS	b,g,m	2 (0.86)	3 (9.64)	3 (9.63)	–	–
22	PEI 89	OAS	b,g,m	2 (3.11)	3 (6.68)	1 (0.38)	1.92	–
23	PEI 90	OAS	b,g	1 (0.37)	2 (1.12)	0 (<0.35)	–	–
24	PEI 92	OAS	b,g,w	1 (0.42)	2 (0.9)	0 (<0.35)	0.3	0.36
25	PEI 94	OAS	g,m	1 (0.39)	2 (0.85)	0 (<0.35)	–	–
26	PEI 99	OAS	b,g,m	2 (0.86)	2 (1.88)	3 (3.9)	–	–
27	PEI 101	Nausea	b,g,w	2 (1.07)	3 (3.83)	3 (5.7)	1.1	–
28	PEI 104	OAS	b,g,m	0 (0.12)	2 (2.0)	0 (<0.35)	–	–
29	PEI 115	OAS	b,g,m	1 (0.44)	4 (46.9)	0 (<0.35)	0.38	–
30	PEI 128	OAS	b,g,w	0 (0.02)	2 (1.27)	0 (<0.35)	–	–
31	PEI 131	OAS	b,g,m	2 (2.22)	3 (15.5)	3 (14.6)	–	–
32	PEI 144	OAS	b,g,m	0 (0.2)	2 (0.85)	2 (1.19)	–	–

^a OAS=Oral allergy syndrome, U=Urticaria, ABP=abdominal pain, ND=Neurodermatitis

^b b=birch, g=grass, m=mugwort, w=other weeds

^c XF=bromelain gp, MM=fibrin gp ($A_{405}>0.26$)

were judged as positive for a specific glycopeptide if the absorbance of a tomato-allergic patient was at least twice that of the non-allergic control ($A_p \geq 2A_{NA}$) and were expressed as differences of absorbances between allergic patient and nonallergic control.

Electrophoresis and immunoblotting. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) of tomato extract was performed as described in [22, 23]. After transferring the separated glycoproteins to nitrocellulose membranes by semi-dry blotting, the membranes were cut into 3–4 mm wide strips and probed with 1:10 dilutions of sera from the allergic patients or from a nonallergic control. Immunostaining of bound IgE antibodies was performed with alkaline phosphatase coupled to mouse anti-human IgE (Pharmingen, Hamburg, Germany; 1:1000, 3 h) and 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate as the substrate.

Inhibition of IgE binding to blotted tomato extract was carried out by pre-incubation of selected patient sera (PEI 101 and Bo 191, both diluted 1:10; PEI 26, diluted 1:50) with various inhibitors as described in the results section.

Two-dimensional PAGE blot. Two-dimensional PAGE was performed according to the method of Görg et al. [24]. In the first dimension, isoelectric focusing was carried out in polyacrylamide strips (Immobiline Dry Strip – 11 cm, Amersham Pharmacia Biotech AB, Uppsala, Sweden) with an immobilized pH gradient of 3 to 10. For each strip, 200 µg of tomato extract protein were applied and separated as described [24]. The second dimension was performed with the strip polymerized in the stacking gel of a vertical electrophoresis unit. The isoelectric point (pI) and molecular weights were determined by using marker proteins, IEF calibration kit (pH 3–10; Pharmacia Biotech, Uppsala, Sweden), and low range SDS-PAGE standards (14.4–94.0 kDa; [Bio-Rad, Munich, Germany]), respectively. Subsequently, the proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad, Munich, Germany) or to nitrocellulose as previously described for apple proteins in [25].

N-terminal amino acid sequencing. Microsequencing was performed according to the method of Matsudaira [26] by using a Procise 492 protein sequencer in pulsed liquid mode (Applied Biosystems, Foster City, CA, USA). All chemicals used were from Applied Biosystems.

Results

Case history and serological evaluation of patients

In a panel of 357 pollen-allergic patients, 32 subjects (9%) were identified who reported adverse reactions to tomato and presented IgE antibodies specific for tomato extract. Pollen sensitization was confirmed by either EAST or CAP or skin prick tests (specific results not shown). Thirty one patients (97%) presented serum IgE to birch pollen, 32 (100%) to grass pollen, and 30 (94%) to pollen of mugwort or other weeds, respectively. According to reports of the patients, 23 (72%) had relatively mild oral allergy syndrome (OAS) after ingestion of fresh tomatoes (itching of the lips, tongue, and throat). Itching of the skin was reported by two patients, four patients had urticaria, two presented nausea, and one abdominal pain and spasm. Worsening of atopic dermatitis was reported by one patient (Table 1).

Besides the commercially available CAP tests for tomato, we used self-prepared solid phases in an enzyme allergosorbent test (EAST) for measuring the specific

IgE against tomato (2 µg per disc) in the patients' sera. The results of the determinations of specific IgE to tomato are shown in Table 1. CAP testing revealed 31 sera (97%) reacting with tomato (CAP classes of 2 and above), whereas only 25 patients (78%) were positive in EAST. One patient (PEI 8) who was negative in the CAP assay, was positive in the EAST. Seventeen patients (53%) presented IgE specific for Bet v 2 (Table 1).

To investigate the sensitization to cross-reactive carbohydrate epitopes, the patients' sera were subjected to a glycan ELISA with glycopeptides isolated from bromelain and bovine fibrin covalently attached to the solid phase (Table 1). Sera of twelve patients (39%) showed binding to the bromelain glycopeptide XF that contains α -1,3-fucose and β -1,2-xylose that are known to be important constituents of CCD occurring in many plant extracts. The IgE of one patient (PEI 92) also reacted with the fibrin glycopeptide which was used as a negative control lacking the fucose and xylose residues.

Ubiquitous cross-reactive structures are important IgE-binding components in tomato extract

All sera from tomato-sensitive patients were investigated by IgE immunoblotting (Fig. 1). The IgE antibodies of 14 (44%) patients bound to the putative tomato profilin at about 16 kDa. The confirmation that this protein was tomato profilin was carried out by immunodetection with rabbit anti-celery (lane P1) or rabbit anti-ragweed profilin (lane P2), and by immunoblot inhibition with rBet v 2 and celery profilin rApi g 4 (not shown). Sera of 11 patients (34%) recognized several bands above 30 kDa that are believed to be glycoproteins with ubiquitous carbohydrate epitopes. IgE-binding to carbohydrates was confirmed by blot inhibition with serum PEI 26. No inhibition was observed with 15 µg of the fibrin glycopeptide as inhibitor, but almost all bands disappeared when 15 µg of the bromelain glycopeptide XF were used as inhibitor, except for proteins at 23 kDa, 40 kDa, and 43 kDa, respectively (Fig. 2).

In addition to the bands not inhibited by the XF glycan, the IgE of some patients also detected bands in the region of 20 kDa. One patient (PEI 115) showed strong binding to a protein at about 9 kDa. Five patients (16%) presented very weak or no binding to tomato extract immunoblots.

CCD, profilin, and lipid transfer protein bind a significant amount of tomato-specific IgE

To test the contribution of the different tomato components to the allergenicity of the whole extract, dose-dependent EAST inhibitions were carried out with a series of tenfold dilutions of tomato extract (raw or cooked), of the bromelain or fibrin glycopeptides, and of the recombinant allergens Bet v 1 or Bet v 2 as inhibitors, respectively. Tomato extract disks were simulta-

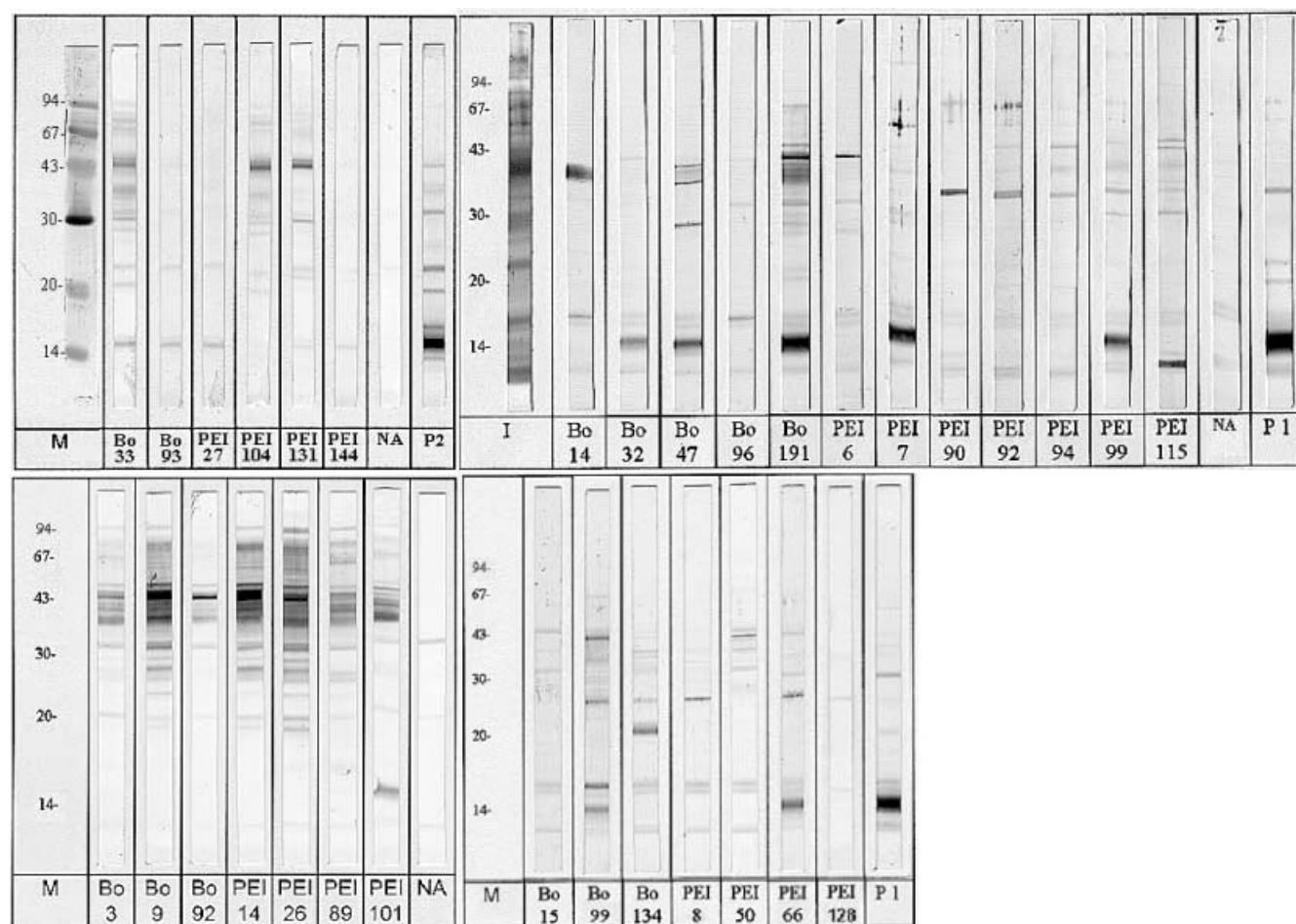


Fig. 1 Binding of IgE from human sera to tomato extract proteins separated by SDS-PAGE and transferred to NC. The molecular weight corresponds to marker proteins that were stained by India

ink. Sera numbers are indicated at the respective lanes. P1: rabbit anti-celery; P2: rabbit anti-ragweed profilin; NA: non-allergic control serum; I: India ink stain of tomato extract; M: Marker

Table 2 Results of EAST inhibition experiments using tomato extract as solid phase antigen

Patient	Sensitization to	Maximal inhibition (%) with						
		Tomato extract		Glycopeptide from		Recombinant		
		Raw	Cooked	Bromelain	Fibrin	Betv1	Betv2	Ch LTP ^a
Bo 9	CCD	86.3	84.8	67.0	2.1	0.0	1.6	0.7
Bo 32	Profilin	82.8	24.0	17.0	17.4	0.0	70.1	6.6
Bo 47	CCD/Profilin	93.7	45.1	27.4	0.0	0.0	61.1	0.2
Bo 191	CCD/Profilin	90.8	66.6	28.3	6.8	0.0	32.5	0.0
PEI 7	Profilin	92.2	20.1	6.1	5.2	0.0	76.8	0.0
PEI 14	CCD	81.6	81.9	83.3	3.3	0.0	0.0	9.5
PEI 89	CCD	81.0	82.5	53.5	35.4	7.4	4.8	7.6
PEI 99	Profilin	86.5	28.1	6.2	8.6	3.1	65.5	0.0
PEI 101	CCD/Profilin	92.6	77.5	67.2	32.6	1.4	25.2	0.7
PEI 115	CCD/LTP	83.1	44.8	22.8	13.6	0.0	15.4	33.2
PEI 131	Profilin/LTP	95.0	74.5	3.4	2.2	0.0	21.0	45.7

^a Ch LTP=cherry lipid transfer protein

neously incubated with pre-diluted patients' sera and the different inhibitor solutions (maximal concentration 50 µg/ml of recombinant allergens or glycopeptides). Allergen extracts were applied at protein concentrations up

to 500 µg/ml. Table 2 lists the results with the highest inhibitor concentration of three different types of sera. The IgE reactivity of sera with sensitization to cross-reactive carbohydrates (Bo 9, PEI 14, PEI 89) was strongly inhib-

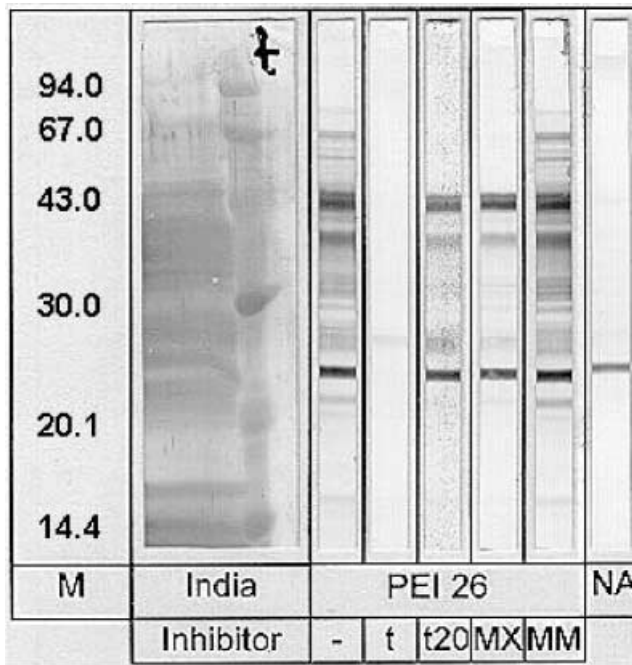


Fig. 2 Blot inhibitions on strips coated with tomato extract with human serum PEI26 (diluted 1:50). The molecular weight corresponds to marker proteins stained by India ink (right side of India ink stain; left side: tomato extract). Inhibitors: lane -: without inhibitor; lane t: 100 μ g tomato extract; lane t20: 100 μ g tomato extract, 20 min cooked; lane MX: 15 μ g bromelain glycopeptide; lane MM: 15 μ g fibrin glycopeptide; lane NA: non-allergic control

ited by the bromelain glycopeptide and by an extract of cooked tomato, but not with recombinant Bet v 2, used as a tomato profilin analogue. On the other hand, sera with predominant sensitization to tomato profilin (Bo 32, PEI 7, PEI 99) showed only very weak inhibitions with the glycopeptides and with extract of cooked tomato, but strong inhibitions with recombinant birch profilin (Bet v 2). The third kind of sera, representing those sera with sensitizations to both CCD and profilin (Bo 47, Bo 191, PEI 101), showed inhibitions (27.4–67.2%) with glycopeptides as well as with recombinant profilin. The sum of these inhibitions roughly accounted for the total inhibition potency of tomato extract. Recombinant Bet v 1 induced a negligible inhibition activity, indicating the absence of a Bet v 1-related allergen in tomato.

Figure 3 shows the inhibition curves of three sera representative for each of the three groups mentioned above. The very strong inhibitions by profilin in case of serum PEI 7 and the bromelain glycopeptide in case of the other two sera reflect the fact that a pure allergenic protein and a pure epitope (CCD) were used instead of a crude protein extract that contains many nonallergenic proteins.

Lipid-transfer proteins (LTP) have recently been described as pollen-independent allergens in fruits such as peach [27] or apple [28]. Cherry LTP that has recently been cloned in our laboratory [16] was used as inhibitor in initial experiments to test whether LTP contribute to

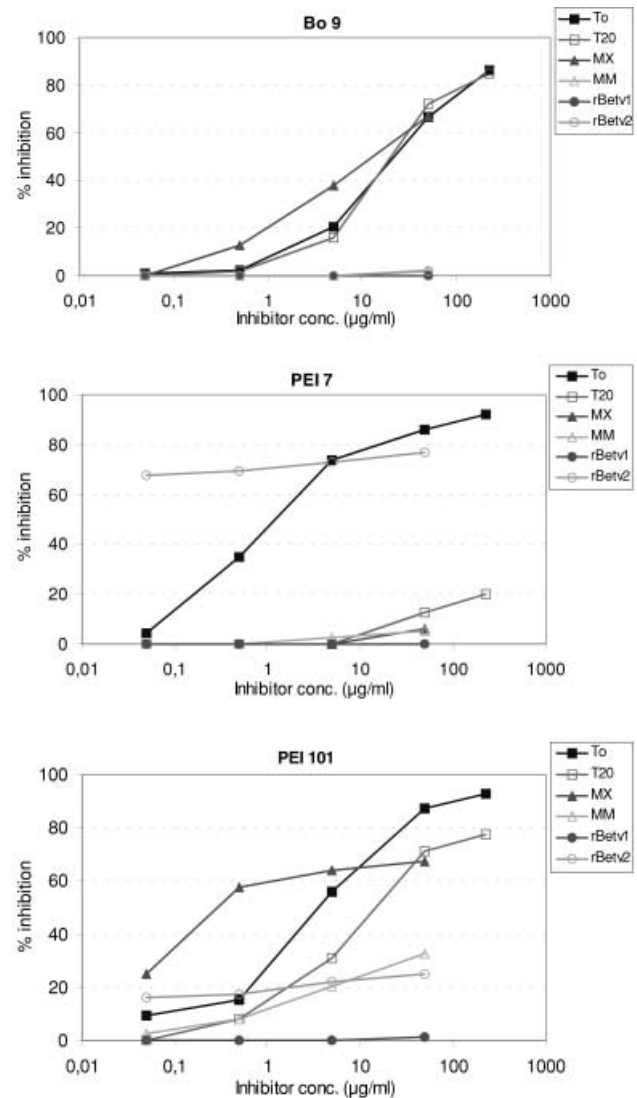


Fig. 3 Dose-related EAST inhibitions with Bo 9 (diluted 1:20) and with PEI 7 and PEI 101 (both diluted 1:5). Solid phase: tomato extract. Inhibitors: tomato extract (*filled squares*), cooked tomato extract (*open squares*), glycopeptides from bromelain (*filled triangles*) or fibrin (*open triangles*), recombinant Bet v 1 (*filled circles*) or Bet v 2 (*open circles*)

the allergenicity of tomato. In two patients, PEI 115 and PEI 131, significant inhibitions of 33.2% and 45.7%, respectively were obtained with 50 μ g/ml of cherry LTP (Table 2).

Two-dimensional separation of tomato extract

Figure 4 represents the results of the two-dimensional PAGE of tomato extract. In the first dimension, 200 μ g tomato protein per strip was separated by isoelectric focusing (IEF) followed by SDS-PAGE in the second dimension. Figure 4a shows the silver staining of one 2D-gel-electrophoresis of tomato extract. Further gels were blotted onto nitrocellulose and incubated with

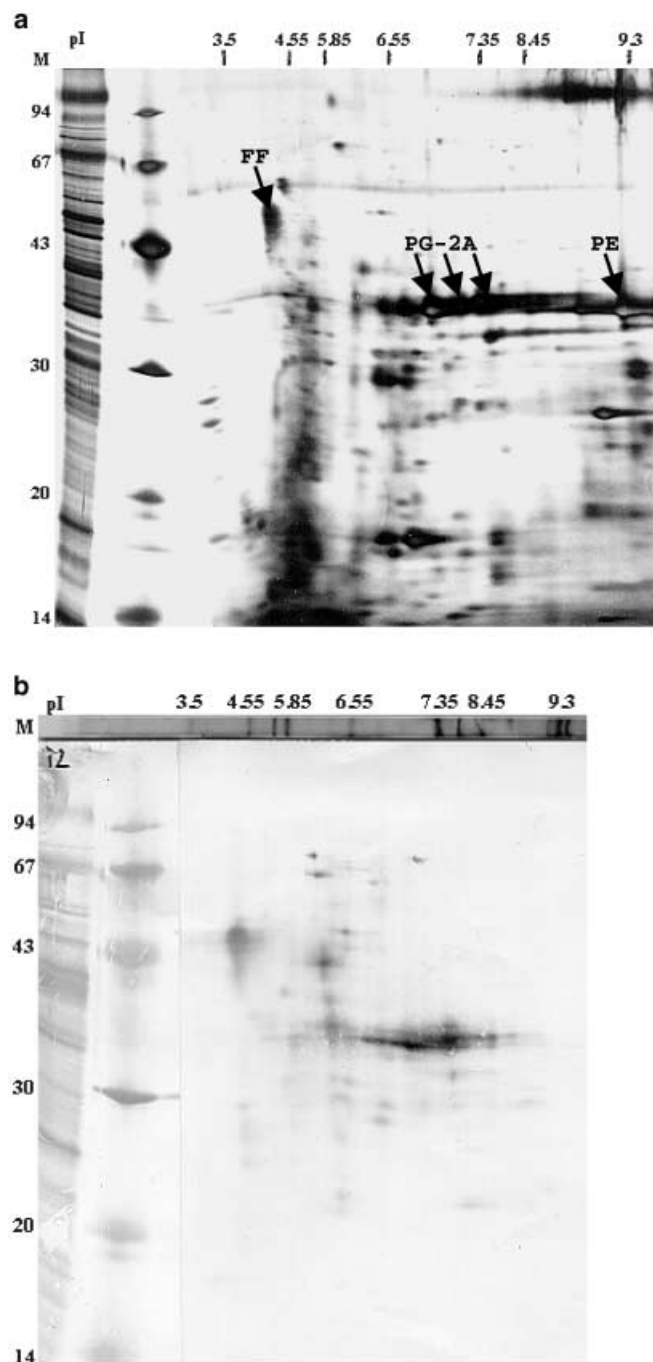


Fig. 4a,b Two-dimensional separation of tomato extract (200 µg). The pI values of marker proteins are given horizontally. The molecular weights (M) are shown vertically: **a** silver staining – the arrows indicate the positions of the sequenced proteins: FF=β-fructofuranosidase; PG-2A=polygalacturonase 2A; PE=pectinesterase; **b** IgE reactivity of serum PEI 26 (diluted 1:25) recognizing FF and PG-2A, but not PE

four different sera (Bo 191, PEI 26, PEI 50, PEI 101). Figure 4b demonstrates one example with serum PEI 26. Besides many weakly stained spots, one characteristic IgE-reactive protein of about 50 kDa and a pI value of 4–4.5 was recognized by all four sera. Three proteins of about 36 kDa and pIs between 6.8 and 7.5 were IgE-

reactive to sera Bo 191, PEI 26, and PEI 50. The serum PEI 101 recognized a further protein of 36 kDa and a pI of 9.3 (not shown). After blotting to a PVDF membrane and Coomassie staining, these five IgE-reactive proteins were excised and analyzed by microsequencing. The three proteins between pI 6.8 and 7.5 had the identical sequence “SKNAQQIHIKFE”, representing complete identity with the sequence of polygalacturonase 2A (Swiss-Prot No. P05117) starting at amino acid Ser 216. Furthermore, the eight N-terminal amino acids of the protein with pI 9.3 were determined and screened for homology with sequences in the Swiss Prot Data Base. This region (“IIANAVVA”) was found to have complete identity with the N-terminal sequence of the mature pectinesterase (Swiss-Prot P14280). Despite several attempts, no sequence could be obtained from the 50-kDa protein. When the corresponding lane from a 1D-gel was excised and subjected to Edman degradation, the sequence “YAWSNAM”, equivalent to the first seven amino acids of mature β-fructosidase (Swiss-Prot D11350), was obtained. This is also in accordance with the results of Petersen et al. [12].

Discussion

In a panel of 357 pollen-allergic patients, 32 subjects were identified who reported adverse reactions to tomato and presented IgE antibodies specific for tomato proteins. This corresponds to a prevalence of about 9% in patients with pollen allergy, which is comparable to the result of Hannuksela and Lahti [29] with 11% (16/148) birch pollinotics allergic to tomatoes.

Concerning the symptoms, 78% (25/32) of the patients had a relatively mild oral allergy syndrome. The most frequent systemic reaction was urticaria that affected four patients (Table 1). Potentially life-threatening reactions did not occur in the patient group.

The detection of specific IgE to tomato is shown in Fig. 1. In the serum of 44% (14/32) of the patients IgE against the 16-kDa allergen tomato profilin was detected by immunoblotting. All of these patients were also positive in the CAP test for birch pollen profilin, Bet v 2. Profilin is known as an ubiquitous minor allergen in pollen and foods [30]. The identification of the tomato profilin was confirmed by immunodetection with rabbit anti-celery profilin or rabbit anti-ragweed profilin and by inhibition of the IgE-binding to tomato extract with recombinant birch pollen profilin, Bet v 2, and celery profilin, Api g 4. Three sera (Bo 15, Bo 92, PEI 89) with low IgE titers for Bet v 2 (0.38–0.56 U/ml) did not react with tomato profilin in immunoblotting. This could be either due to a lower sensitivity of the blot compared to the CAP assay or to different epitopes on Bet v 2 and tomato profilin.

Furthermore, 35.5% (11/31) of the tested patients showed specific IgE to carbohydrate epitopes with fucose and xylose structures in the glycan ELISA and in immunoblotting. This epitope specificity was proven by

inhibition of the IgE-binding to tomato extract with purified glycopeptides of defined structures that were prepared from bromelain. IgE antibodies of one patient (PEI 92) that recognized the fibrin glycoprotein stronger than the bromelain glycoprotein in the glycan ELISA bound only to distinct bands of the tomato extract and presented a different binding pattern than the other CCD-positive sera (Fig. 1). The immunoblotting pattern of the majority of CCD-positive sera suggests that the important carbohydrate determinant XF occurs on many high-molecular weight glycoproteins in tomatoes. The similar inhibitory effect of extract from cooked tomato as compared to the native food (Table 2) demonstrates that CCD are still present and thus highly thermostable. These results correspond to our observation that CCD are heat-resistant IgE-binding structures in plant foods such as celery [21, 31]. Although the clinical significance of CCD-specific IgE antibodies has been doubted [32, 33, 34], we anticipate that anti-CCD IgE plays a role in tomato-allergic-patients. First, this was the unique IgE reactivity to components in tomato extract in four of the patients (Bo 9, PEI 14, PEI 26, PEI 89). In addition, the sera of a high number of patients contain CCD-specific IgE. Hence, we believe that these IgE antibodies could be clinically relevant in some tomato-allergic patients. The occurrence of IgE-binding glycoproteins in tomato is in accordance with data of other authors [6, 12, 35].

The results of EAST inhibition indicated that the tomato LTP is an IgE-binding component for patients PEI 115 and PEI 131. The molecular mass of LTP is about 9 kDa [16, 27, 28]. A protein of this mass was strongly recognized by IgE from serum PEI 115 which could thus represent tomato LTP. However, despite a tomato CAP of 15.5 U/ml, IgE from PEI 131 showed only weak reactivity on tomato immunoblots and did not bind to a 9-kDa band. Since LTP contain disulfide bonds, a conformational epitope recognized by IgE of this serum may have been destroyed under the reducing conditions of SDS-PAGE.

Immunoblotting of 2D gels showed five characteristic IgE-reactive spots. These were analyzed by microsequencing and shown to be the already known β -fructofuranosidase [12] (reactive with all four sera), polygalacturonase 2A (recognized by sera Bo 191, PEI 26, and PEI 50), and pectinesterase (reactive with PEI 101). Polygalacturonase 2A acts in concert with pectinesterase in the ripening process of tomatoes and is involved in cell wall metabolism, specifically in polyuronide degradation.

In case of polygalacturonase, the different pIs of the three spots may be explained by the presence of isoenzymes or by different glycosylation of the protein since polygalacturonase possesses four potential *N*-glycosylation sites. Polygalacturonase 2A shares maximum (55.6%) identity with the polygalacturonase precursor of Kiwi (P35336), but also 41.2% identity with the Ozark white cedar pollen allergen Jun a 2 (CAC05582), 41.1% identity with the Japanese cypress pollen allergen Cha o 2 [36], 40.8% identity with the *Cryptomeria japonica* allergen Cry j 2 (P43212), and 37.2% identity

with the Timothy pollen allergen Phl p 13 (Q9XG86), respectively. These proteins are known to be major allergens in mountain cedar [37], in Japanese cypress [36], in *Cryptomeria* [38], and in timothy grass pollen [39], and the latter two have already been shown to belong to the family of polygalacturonases. The Swiss Prot Data Base contains polygalacturonases from at least 18 different fruits, vegetables, and pollens. Furthermore, recent investigations [40] showed that group XIII allergens exist in all temperate grasses and that they reveal pectinase activity. Therefore, polygalacturonases may represent a new class of panallergens, and may explain a clinical association between grass pollen and tomato allergy [3]. These proteins could be a major cause of strong IgE-reactivity in the high molecular weight range of these extracts. It remains unclear whether the IgE-reactivity is restricted to the *N*-glycans of these proteins. Our ongoing investigations will address this question. The recognition of both fructofuranosidase and polygalacturonase by serum PEI 50, which is negative in CCD-ELISA, indicates that protein epitopes also exist on these proteins.

In conclusion, our data show that tomatoes are a relevant allergenic food for pollen-allergic patients. It seems that the majority of pollen-associated allergies to tomato is caused by ubiquitous allergenic structures such as profilin and cross-reactive IgE binding *N*-glycans. In addition, the lipid transfer protein, polygalacturonase 2A, β -fructofuranosidase, and pectinesterase may represent birch pollen-independent tomato allergens for certain patients.

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