

# Ubiquitous structures responsible for IgE cross-reactivity between tomato fruit and grass pollen allergens

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*The simultaneous presence of IgE reactivity to tomato fruit and grass pollen allergens is evident in many patients with allergy and may be caused by cross-reactivity. Using sera from polysensitized patients with a positive enzyme allergosorbent test (EAST) result (score >2), we tested reactivity to both allergen sources. IgE reactivity against both extracts was demonstrated in eight serum samples, and cross-reactivity was confirmed by the EAST inhibition assay. The structures responsible for this cross-reactivity were identified by Western blotting: five of the eight sera demonstrated a 16 kd protein in both extracts, which was identified as profilin. Additionally, seven of the eight sera showed IgE binding to epitopes on carbohydrate moieties, which contained  $\alpha$ 1,3 fucosylations. To determine the allergens of tomato fruit extract, we performed two-dimensional polyacrylamide gel electrophoresis blotting. We were able to demonstrate one highly concentrated and about 20 weaker proteins possessing terminal fucose residues. These are similarly found in grass pollen extracts. It is therefore postulated that the cross-reactivity is affected by profilins and similar carbohydrate determinants. If carbohydrate structures can provoke IgE cross-reactivity between phylogenetically distant species, such structures may play an important role in sensitization and mediator release. The ubiquitous nature of the IgE-binding determinants was studied by additional EAST inhibition tests with tomato allergen disks and extract from birch pollen, mugwort pollen, apple, and celery, leading to significant inhibitions among all these allergen sources. Epitopes exclusive to grass pollen and tomato have not been detected. (J Allergy Clin Immunol 1996;98:805-15.)*

**Key words:** Carbohydrate structure, cross-reactivity, grass pollen allergens, IgE binding, immunoblotting, tomato allergens

In contrast to many other vegetable food allergens, no molecular studies of tomato allergens have been done in the past decade. Sensitization to tomato seems to be particularly prevalent in the Mediterranean area. In Italy, tomatoes elicited symptoms in 20.6% of patients with oral allergy syndrome, and the authors reported a coincidence of IgE reaction to grass pollen of 10%.<sup>1</sup> De Martino et al.<sup>2</sup> reported that 40 of 102 children who were sensitive to grass pollen but not to other

## Abbreviations used

|           |                                                          |
|-----------|----------------------------------------------------------|
| AAA:      | <i>Aleuria aurantia</i> agglutinin                       |
| EAST:     | Enzyme allergosorbent test                               |
| pI:       | isoelectric point                                        |
| SDS-PAGE: | sodium dodecylsulfate-polyacrylamide gel electrophoresis |
| TBS:      | Tris-buffered saline                                     |
| TTBS:     | TBS containing 0.1% (vol/vol) Tween-20                   |

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inhalant allergens also demonstrated a positive skin test response to tomato and confirmed cross-reactivity between these two allergen sources by RAST inhibition. In contrast, tomato allergy seems to be of minor importance in the more northern parts of Europe. Wüthrich<sup>3</sup> recently reported a frequency of 1.5% among 402 patients allergic to foodstuffs. However, there is a lack of molecular

information about tomato allergens, and the cross-reactive structures have not yet been identified.

Examples of IgE cross-reactivity between similar allergenic structures are manifold. Among the temperate grasses, a high cross-reactivity between defined components was found. This led to their classification into allergen grass groups.<sup>4</sup> Sequence data of the major allergens from different species show about 85% homology for grass group I and about 70% for group V.<sup>5,6</sup> Because of this, patients allergic to grass pollen will usually react to many species. Food allergens related to Bet v 1, the major birch pollen allergen, provide another example of high sequence homology being responsible for allergenic cross-reactions.<sup>7</sup> The profilins are very important cross-reactive allergens because they are detectable in nearly all organisms; however, their primary sequences are highly variable. Because they are responsible for a wide range of cross-reactivity among pollens and plant foods,<sup>8-12</sup> they have been termed *panallergens*.<sup>13</sup>

Other proteins, such as tropomyosin, are also widely distributed and can cause cross-reactions.<sup>14</sup> Carbohydrate determinants can provoke cross-reactivity in phylogenetically unrelated organisms such as vegetable foods, pollen, and bee venom.<sup>15</sup> The contribution of carbohydrate structures in the binding of IgE antibodies has often been detected by periodate oxidation, which preferentially destroys glycans.<sup>15</sup> More sophisticated methods to determine carbohydrate structures and their IgE-binding epitopes have only been performed in a few cases. The IgE-binding determinant is formed by  $\alpha$ 1,3 fucosylation in phospholipase A<sub>2</sub> of bee venom.<sup>16</sup> Recently, we were able to show that such a configuration might also exist in the group I allergens of the grasses.<sup>17</sup> Additional carbohydrate structures were identified on Art v 2 of mugwort<sup>18</sup> and in Cry j 1 of the Japanese cedar.<sup>19</sup> The carbohydrate moieties of both allergens were found to bear IgE-reactive epitopes.

Although tomato has been widely investigated for breeding purposes, its allergenicity has been poorly studied. Unexpectedly, we were able to identify eight German patients with grass pollen allergy who also had positive skin prick test responses and/or demonstrated a positive EAST response ( $\geq 2$ ) to tomato. These sera were isolated from 167 serum samples obtained from patients allergic to at least one of the foods being tested (apple, hazelnut, celery, and carrot).<sup>20</sup> The aims of this study were to prove the possible cross-reactive nature of these IgE reactions, to analyze the

cross-reactive structures, and to identify prominent IgE-binding components of tomato fruits.

## METHODS

### Preparation of tomato and grass pollen extracts

Tomato extract was prepared by a low-temperature "acetone powder" method as described by Vieths et al.<sup>21</sup> Briefly, mature fresh tomatoes were homogenized in acetone and dry ice at  $-60$  to  $-65^{\circ}\text{C}$ , and the mixture was kept at this temperature overnight. Resulting precipitates were washed twice with acetone and finally with acetone/diethylether (1:1, vol/vol) at  $-60^{\circ}\text{C}$ . The sediment was filtered, lyophilized, and stored at  $-20^{\circ}\text{C}$ . To obtain protein extracts, 2 gm of acetone powder was diluted in 30 ml of phosphate-buffered saline (0.01 mol/L potassium phosphate, 0.15 mol/L NaCl; pH 7.4) and centrifuged at 20,000 g for 45 minutes ( $5^{\circ}\text{C}$ ). The supernatant was then passed through a 0.2  $\mu\text{m}$  filter, dialyzed against double-distilled water overnight, lyophilized, and stored until use at  $-20^{\circ}\text{C}$ .

One gram of timothy grass pollen (ARTU Biologicals, Lelystad, The Netherlands) was incubated in 50 ml of 0.1 mol/L  $\text{NH}_4\text{HCO}_3$  buffer (pH 8.0) for 30 minutes. The soluble fraction was then isolated by centrifugation at 17,000 g for 30 minutes and dialyzed against double-distilled water overnight. The extract was lyophilized and stored at  $4^{\circ}\text{C}$  until use.

### Patient sera, antisera, and monoclonal antibodies

Serum samples were drawn from a group of 167 patients with pollinosis, all of whom showed a clinically relevant food hypersensitivity to at least one of the following food extracts: apple, hazelnut, celery, and carrot. With regard to pollen sensitization, 162 of these patients were hypersensitive to birch, 107 were hypersensitive to mugwort, and 129 were hypersensitive to grass.<sup>20</sup> In a retrospective study eight of these patients, who were all allergic to grass pollen, also demonstrated a specific IgE to tomato. The patients' sera were obtained from Hospital Borkum Riff, Germany (Dr. H. Aulepp).

Antisera raised in rabbits against profilin of ragweed and celery were kindly provided by Dr. P. Deviller (Immuno-Virologie Moléculaire et Cellulaire, Lyon, France).<sup>10</sup> The monoclonal antibodies, Bo 1 and IG 12, were raised in BALB/c mice against crude timothy pollen extract and were specific for grass group V and I allergens, respectively.<sup>5,22</sup>

### Determination of specific IgE

Allergenic extracts were coupled to cyanogen bromide-activated filter paper disks at a protein concentration of 12  $\mu\text{g/ml}$ . Specific IgE was determined by a commercially available enzyme allergosorbent test (EAST) according to the manufacturer's instructions (Allergopharma, Reinbek, Germany). The results were expressed as EAST classes

(corresponding to the classes of Phadezym RAST [Pharmacia, Uppsala, Sweden]). Results were calculated to the nearest first decimal place.<sup>23</sup>

### EAST inhibition assay

Competitive EAST inhibition assays were performed as previously described.<sup>23</sup> Patient sera were diluted 1:2.5 (except for Bo 64, which was 1:25) with Tris-buffered saline (TBS) containing 0.1% (vol/vol) Tween-20 (TTBS) and were incubated with inhibitor solutions at a protein concentration of 250 µg/ml on tomato allergen disks overnight. Extracts from tomato fruit (positive control), timothy grass pollen, birch pollen, mugwort pollen, celery, apple, and ovalbumin (negative control) were used as the inhibitors. The EAST was subsequently performed according to the standard procedure.

### Western blotting

Proteins (14 µg/cm) were separated by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (T = 13%, C = 0.35%; gel size, 180 × 170 × 1 mm<sup>3</sup>) by using the Laemmli buffer system.<sup>24</sup> The proteins were subsequently transferred onto nitrocellulose membranes (Schleicher & Schüll, Dassel, Germany) by semi-dry blotting.<sup>25</sup>

### Two-dimensional PAGE blot

Two-dimensional PAGE was carried out according to the method of Görg et al.<sup>26</sup> In the first dimension isoelectric focusing was performed in polyacrylamide strips (T = 4%, C = 4%; size, 240 × 80 × 0.5 mm<sup>3</sup>) with an immobilized pH gradient of 3.6 to 9.3, containing 8 mol/L urea and 10 mmol/L dithiothreitol. Two hundred micrograms of timothy grass pollen extract was applied per lane and separated at 3000 V and 2 mA for 7 hours. After incubation for 30 minutes in an equilibration solution (6 mol/L urea, 65 mmol/L dithiothreitol, and 260 mmol/L iodoacetamide), in the second dimension SDS-PAGE was carried out in discontinuous gels (T = 8% to 13%, C = 4%; size, 240 × 80 × 0.5 mm<sup>3</sup>) at 200 V and 30 mA. The isoelectric point (pI) and molecular mass were determined by using marker proteins, test mix 9 (pH range, 3.5 to 10.5; Serva, Heidelberg, Germany), and low-range SDS-PAGE standards (14.4 to 97.4 kD; Bio-Rad, Richmond, Calif.), respectively.

Thermoblotting was used to perform immunologic tests. For amino acid sequencing, the proteins were electroblotted onto Immobilon P membranes (Millipore, Eschborn, Germany) for 30 minutes at 200 mA by using 10 mmol/L 3-(cyclohexylamino)-1-propanesulfonic acid (pH 11.0) as blotting buffer.<sup>27</sup>

### Protein staining and immunologic detections

We used 0.1% (vol/vol) India ink (Pelikan AG, Hannover, Germany) for staining proteins on nitrocellulose membranes.<sup>28</sup> Immunodetection of specific IgE was accomplished either by a peroxidase-based tertiary anti-

body-amplified system with 3,3',5,5'-tetramethylbenzidine as the substrate, as described previously (Figs. 1 and 2)<sup>9</sup> or by means of alkaline phosphatase-labeled antibodies (Figs. 3, 4, and 5).<sup>29</sup>

### Immunoblot inhibition test

Preincubation was carried out for 1 hour at room temperature on 80 µl of patient serum Bo 64 with 500 µg inhibitor (tomato fruit extract, timothy grass pollen extract, or ovalbumin) in a total volume of 2 ml TBS with 0.1% Tween-20. The samples were then used for immunostaining according to the standard procedure.

### Carbohydrate detection and determination by immunoblotting

To study the carbohydrate moieties, blotted samples were incubated with nine digoxigenin-labeled lectins (Glycan Differentiation kit; Boehringer Mannheim, Mannheim, Germany).<sup>30</sup> Lectin binding was visualized by alkaline phosphatase-labeled digoxigenin and nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl phosphate substrate. A specific binding to the lectins was shown to decrease after addition of the corresponding monosaccharides.

Periodate oxidation of carbohydrate structures was performed after blotting onto nitrocellulose membranes by incubation in 10 mmol/L sodium acetate buffer (pH 5.0) containing 50 mmol/L NaIO<sub>4</sub>.<sup>31</sup> This was performed in the dark at room temperature for 2 hours. (These reaction conditions were determined from comparable Western blotting studies on grasses. The carbohydrate moieties were completely destroyed, leaving the protein backbone unaltered.<sup>17</sup>) The reaction was stopped by addition of sorbitol to a final concentration of 0.015 mol/L.

### Glycan determination by ELISA

The isolation of the glycopeptides and the glycan ELISA studies were kindly performed by Dr. F. Altmann (Institut für Chemie der Universität für Bodenkultur, Vienna, Austria). Glycopeptide preparation was described in detail by Tretter et al.<sup>32,33</sup> Briefly, bromelain (Sigma-Aldrich, Deisenhofen, Germany) was purified by ion-exchange chromatography and digested by pepsin. The glycopeptide was isolated by several chromatography procedures. For defucosylation, the bromelain glycopeptides were treated with trifluoroacetic acid and subsequently separated by gel filtration. Bovine fibrin glycopeptides were first desalted with sulfuric acid, and after desalting, were digested with β-galactosidase and β-N-acetylhexosaminidase. The peptide and the glycan structures were examined by amino acid analysis and methylation analysis, respectively. Afterwards, the glycopeptides were coupled to bovine serum albumin by using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in phosphate-buffered saline.

For the ELISA assay, 100 µl of bovine serum albumin-coupled glycopeptides or bovine fibrin (10 µl/ml) was coated to wells of a microtiter plate at 37° C for 90

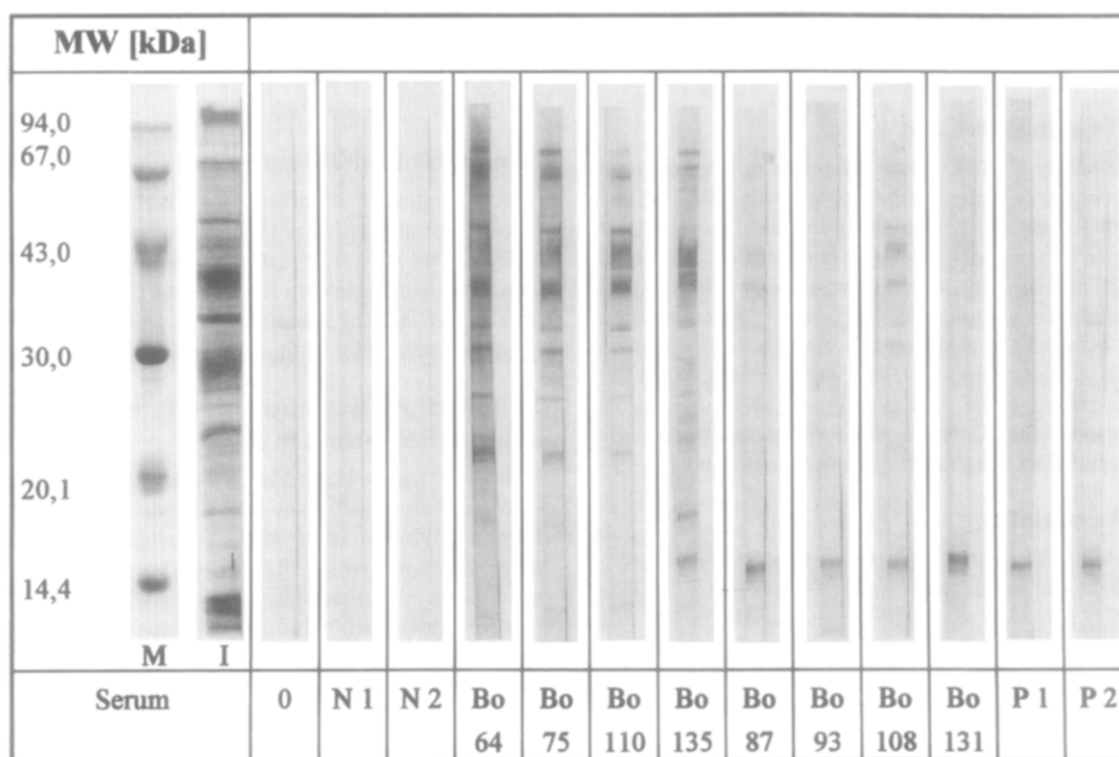


**FIG. 1.** Binding patterns of patients' IgE to tomato fruit extract after Western blotting. *MW*, Molecular weight; *M*, molecular weight marker; *I*, protein staining by India ink; *0*, buffer control; *N*, healthy control serum; *Bo 64* - *Bo 131*, individual patient sera; *P1*, rabbit antiserum raised against celery profilin; *P2*, rabbit antiserum raised against ragweed profilin.

minutes. Glycopeptides of bromelain from pineapple (Man $\alpha$ 1-6(Xyl $\beta$ 1-2)Man $\beta$ 1-4GlcNAc $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc-...) and chemically defucosylated bromelain (Man $\alpha$ 1-6(Xyl $\beta$ 1-2)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-...) containing about two to four amino acids of bovine fibrin (Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-...) were used as antigens (see above). Blocking was then performed with 3% (wt/vol) bovine serum albumin in TBS. Patient serum was diluted stepwise (1:3) and incubated overnight at room temperature. After washing, the plates were incubated for 90 minutes with 100  $\mu$ l of mouse anti-human IgE. The plates were then washed and incubated with 100  $\mu$ l of alkaline phosphatase-labeled goat anti-mouse IgG for an additional 90 minutes. For visualization, 100  $\mu$ l of substrate solution (1 mg/ml p-nitrophenyl phosphate in TBS, pH 9.6) was used. After an additional 90 minutes, the reaction was stopped by addition of 50  $\mu$ l of 5 mol/L NaOH, and the absorbance at 405 nm was measured.<sup>33</sup>

#### Amino acid sequencing

After blotting, the polyvinylidene difluoride membrane was washed with double-distilled water, stained by 0.1% (wt/vol) Coomassie R-250 (Serva, Heidelberg, Germany) in 50% methanol, de-stained in 50% methanol, and air-dried. Protein spots were excised, and microsequencing was performed by using a 473 A pro-

tein sequencer with on-line PTH amino acid analyzer (Applied Biosystems, Weiterstadt, Germany).

#### Computer analyses

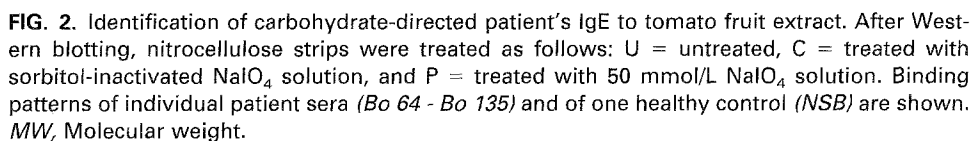
Sequence data were analyzed on GeneWorks and PCGENE software (Intelligenetics, Geel, Belgium). To screen for sequence homology we used the Swiss Prot Data Library (EMBL [European Molecular Biology Laboratory]).

## RESULTS

### Characterization of sera from patients demonstrating IgE reactivity to tomato fruit and grass pollen allergens

Sera from eight patients allergic to grass pollen with positive skin prick test and/or positive EAST (score  $\geq 2$ ) responses to tomato were tested in an EAST and EAST inhibition assay.

As shown in Table I, all sera showed IgE reactivity to tomato and timothy grass pollen with scores greater than 2.2 and greater than 3.2, respectively. These sera additionally showed a positive EAST response to birch and mugwort pollen (data not shown). EAST inhibition was performed with tomato extract-coupled RAST disks. When



### Determination of cross-reactivity by Western blotting

As indicated in Fig. 1, the tomato fruit shows several protein bands in the molecular weight range of 18 to 70 kd. The first three patients demonstrated similar IgE-binding patterns, differ-

To detect cross-reactive carbohydrate determinants, we treated nitrocellulose strips with sodium periodate after SDS-PAGE blotting and compared the resulting IgE-binding patterns with those obtained from untreated and control samples. Optimal incubation conditions were determined as 2 hours with 50 mmol/L sodium periodate.

Fig. 2 shows the reactivity of five patient sera and one control serum. The strips indicated by *P* had been treated by periodate, whereas strips *U* and *C* are controls (*U* is untreated, and *C* denotes those to which sorbitol had been immediately added to prevent periodate oxidation). Periodate treatment resulted in a dramatic decrease in IgE reactivity. Only the 16 kd band was demonstrable by patient sera Bo 87, Bo 108, and Bo 135; this band was previously identified as profilin (Fig. 1).

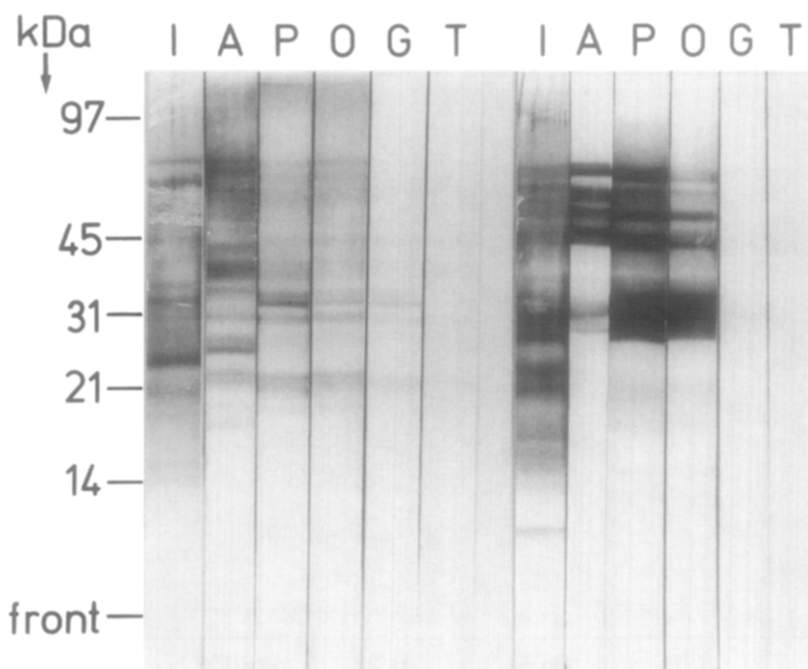


**FIG. 3.** Immunoblot inhibition test of tomato fruit (left) and timothy grass pollen extract (right). Inhibitors (ovalbumin, timothy grass pollen, and tomato fruit extract) were preincubated at concentrations of 250  $\mu$ g/ml of serum Bo 64 (diluted 1:25). O, Ovalbumin; G, timothy grass pollen; T, tomato fruit extract; I, protein staining by India ink; P, serum Bo 64 without inhibitor; A, *Aleuria aurantia* agglutinin.

Serum Bo 64 showed proteins of 94 kd and 42 to 45 kd; the binding patterns decreased under harsh conditions. Thus we were able to demonstrate IgE reactivity against carbohydrate structures in sera from seven of the eight patients.

With regard to the binding patterns to timothy grass pollen (data not shown), all patient sera recognized the grass group I and V allergens in the molecular weight range of 30 to 40 kd, as do the monoclonal antibodies IG 12 and Bo 1. It was also noted that, with the exception of Bo 131, all sera reacted strongly to many components in the 55 kd region (grass group IV). These seven sera show IgE binding to the allergen grass groups I and IV, which are known to be glycoproteins. Bo 131 bound to a 16 kd band, which was also detected by the sera Bo 135, 87, 93, and 108. This is consistent with timothy profilin as identified by the two antisera P1 and P2.

The cross-reactive components of tomato fruit and timothy grass pollen extract were identified by immunoblot inhibition tests. For this, we used patient serum Bo 64, because it strongly identified all the allergenic components (also detected by the other patient sera), except the 16 kd profilin band.

Fig. 3 clearly shows that IgE binding to tomato

fruit and timothy grass pollen can be inhibited by each other. The decrease in IgE reactivity is not related to specific components but to the concentration of these proteins in the extract. All these proteins, except the 35 kd component of tomato extract, intensely bind lectin *Aleuria aurantia* agglutinin (AAA) (directed against  $\alpha$ -fucose residues). The 35 kd band appears unchanged after inhibition with timothy grass pollen but disappears after tomato fruit preabsorption. This clearly shows the specific binding between cross-reactive structures. Equivalent results were obtained in the reverse experiment when timothy grass pollen extract was separated and examined by Western blotting. No significant decrease in binding intensity was observed when ovalbumin was used as an inhibitor (negative control), as compared with using no inhibitor.

#### Characterization of the IgE-binding structures

Two-dimensional PAGE immunoblotting of tomato fruit extract was performed to identify and further characterize the IgE-reactive components. Prior testing had shown that the molecular masses of the extract components ranged from 25 to 50 kd

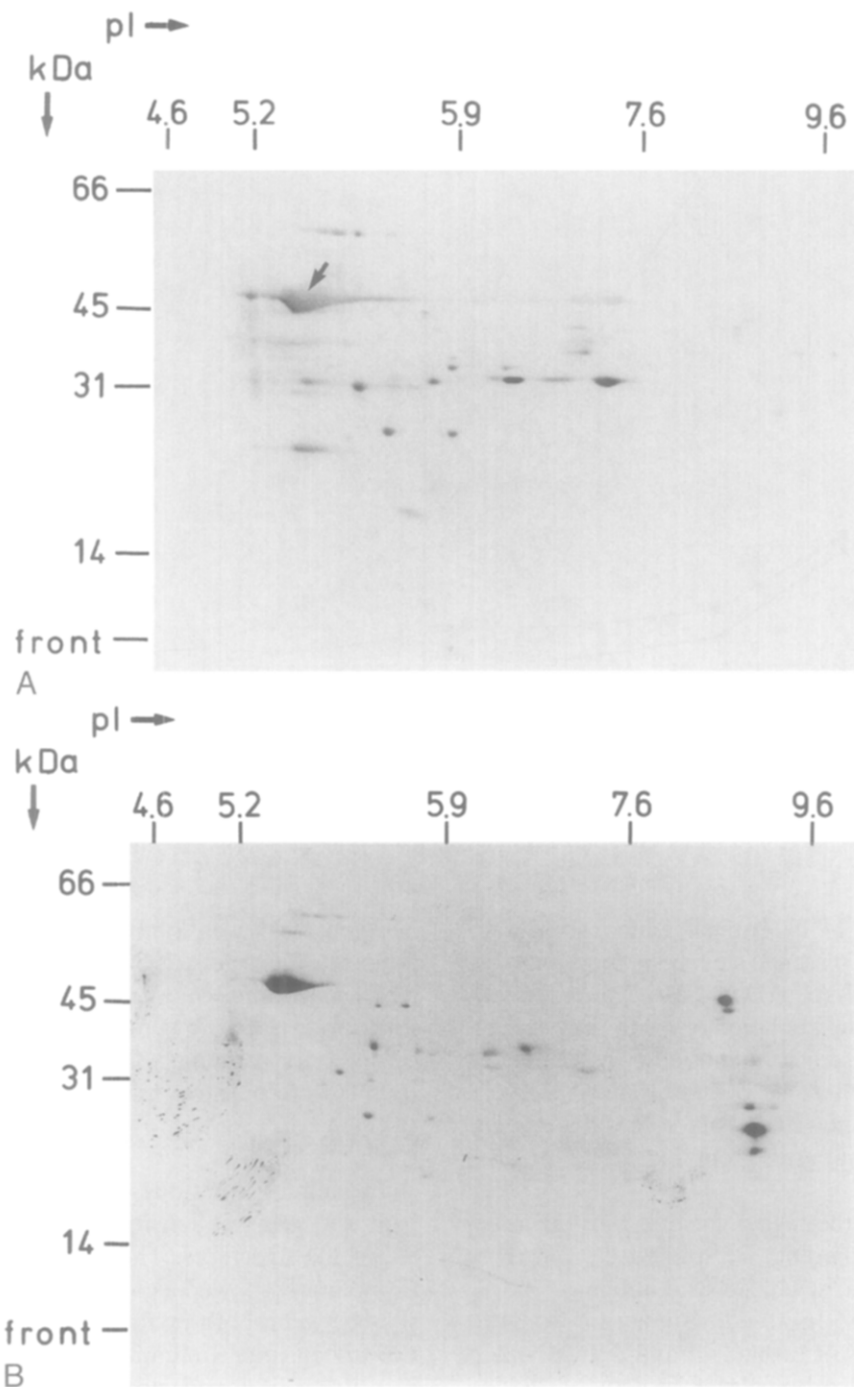


**FIG. 4.** Two-dimensional PAGE blotting for the identification and characterization of single components of tomato fruit extract. **A**, IgE reactivity of a patient's serum. **B**, Detection by AAA. (Protein marked in **A** was investigated by N-terminal microsequencing.)

with pIs between 5.2 and 9.6, with the most intense spots present at acidic and basic pIs.

The IgE-binding pattern (Fig. 4, *A*) is quite complex: one intense component of 52 kd with a pI of 5.3 and about 20 smaller and weaker products with pIs between 5.2 and 7.5 were observed. A good correlation

exists between IgE- and AAA-reactive components, with the exception of a few strong bands observed in the basic pI range of 8 to 9.5 (Fig. 4, *B*).

The highly concentrated 52 kd protein was recognized by five of the eight sera (Bo 64, 75, 87, 108, and 110) and was therefore further investigated by micro-

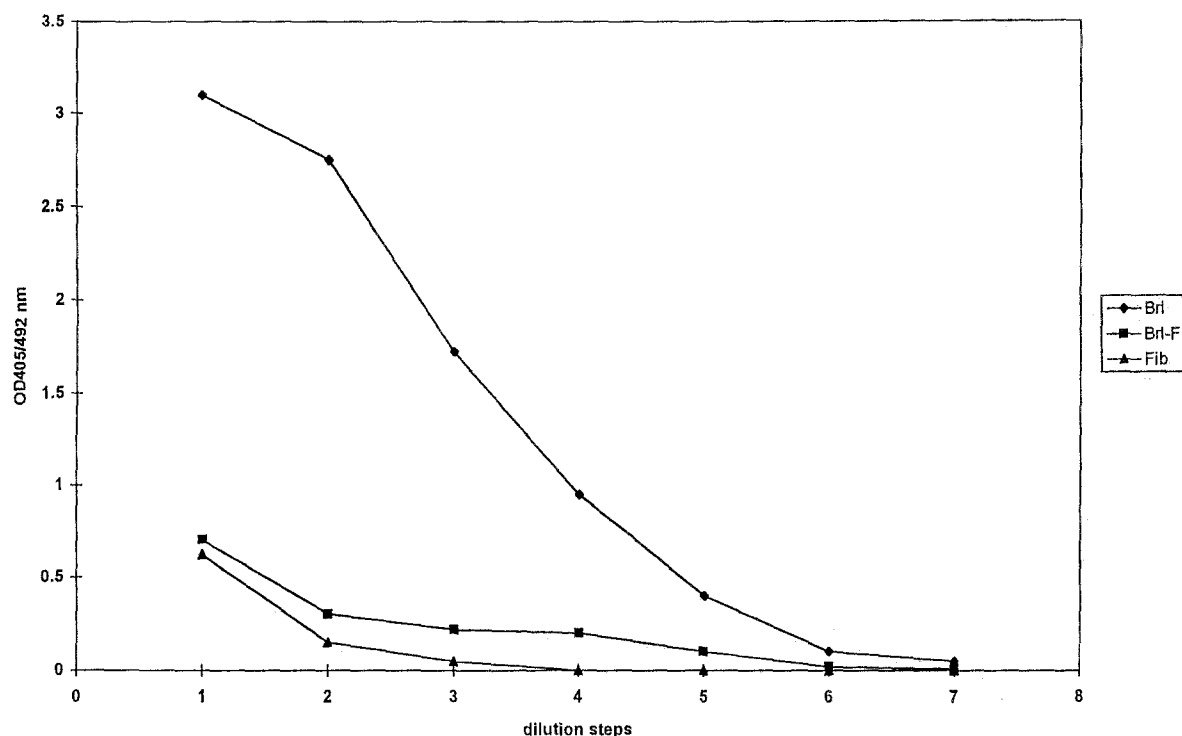


**FIG. 5.** Determination of glycan binding sites by ELISA with glycopeptides. Bromelain from pineapple (*Bri*), chemically defucosylated bromelain (*Bri-F*), and bovine fibrin (*Fib*) served as antigens. Serum of patient Bo 64 was diluted stepwise (1:3).

sequencing. The 14 N-terminal amino acids were determined and screened for homology with sequences in the Swiss Prot Data Base. This region was found to have complete homology with the N-terminal sequence of  $\beta$ -fructofuranosidase. Alignment of this sequence with those of grass pollen allergens (groups I and V) showed no homology with sequence identities of 20% for grass group I and 29% for grass group V.

To investigate the high coincidence of IgE reactivity and AAA binding, we studied the reactivity of serum Bo 64 to several glycan antigens.

Fig. 5 shows the IgE binding of serum Bo 64 to the glycopeptides of bromelain and defucosylated bromelain. The carbohydrate moiety of bovine fibrin, which consists of the N-glycan core structure, shows no IgE binding. These data clearly indicate that the loss of the  $\alpha$ 1,3 fucosylation provokes a significant decrease in IgE reactivity.

#### Extended testing for cross-reactivity

Profilins and carbohydrate determinants are known to be ubiquitous cross-reactive structures. Additional EAST inhibition assays should therefore highlight extended cross-reactivity of our patients' sera (Table II). IgE binding to tomato

allergen disks was inhibited with the sera of six patients by extracts of birch pollen, mugwort pollen, apple, and celery. In all cases, significant inhibitions (>30%) were obtained with 250  $\mu$ g/ml of inhibitor proteins. Ovalbumin controls (250  $\mu$ g/ml) were negative (<10%).

#### DISCUSSION

The aim of this study was to investigate tomato fruit and grass pollen for probable cross-reactive allergenic structures. The initial EAST inhibition assay clearly showed a competition for IgE binding sites between both extracts. Western blotting and two-dimensional immunoblotting were used to identify profilins and carbohydrate determinants as the important cross-reactive structures.

Profilins are detectable in all organisms and play a fundamental role in the formation of the cytoskeleton.<sup>34</sup> Valenta et al.<sup>13</sup> have reported that although the intensity of IgE binding is only moderate, IgE reactions to profilin are quite frequent. About 20% of patients allergic to pollen are sensitized to profilins. However, in the case of specific food allergies, profilin seems to be more important. Sensitization rates of 35% have been reported for patients sensitive to celery<sup>9</sup> and 75% for

**TABLE I.** Determination of patient sera by EAST and EAST inhibition assay

| Patient serum | EAST (class) |         | EAST inhibition on tomato coated RAST disks (% inhibition) |         |
|---------------|--------------|---------|------------------------------------------------------------|---------|
|               |              |         |                                                            |         |
|               | Tomato       | Timothy | Tomato                                                     | Timothy |
| Bo 64*        | 4.3          | 3.7     | 84                                                         | 49      |
| Bo 75         | 3.3          | 4.0     | 93                                                         | 63      |
| Bo 87         | 2.5          | 3.9     | 90                                                         | 77      |
| Bo 93         | 2.2          | 3.9     | 80                                                         | 70      |
| Bo 108        | 2.5          | 3.2     | 89                                                         | 73      |
| Bo 110        | 2.7          | 3.9     | 85                                                         | 85      |
| Bo 131        | 3.4          | 4.2     | 72                                                         | 87      |
| Bo 135        | 2.8          | 3.6     | 83                                                         | 92      |

For EAST inhibition, disks coated with tomato fruit extract were used. For inhibitors, tomato extract and timothy grass pollen extract were used.

\*Serum was diluted 1:25.

Spanish patients allergic to grass pollen, apple, and peach.<sup>11</sup> The primary structures of the profilins in different organisms differ considerably. Valenta et al.<sup>35</sup> determined a 79% sequence homology between timothy and birch pollen profilin. Distantly related species have much lower degrees of homology. For example, *Acanthamoeba* I/II is 40%, and human profilin is only 30%. However, x-ray analysis of the structure and nuclear magnetic resonance have shown that the tertiary structures are very similar even among bovine and amoeba profilins.<sup>36, 37</sup> Thus the observation that profilins from distantly related organisms are highly allergenic and cross-reactive may be due to the highly conserved conformation. We used two antisera raised against ragweed and celery profilin to identify the profilins in timothy grass and tomato fruit. The strong binding of the antisera to tomato profilin might be due to high concentrations of this protein and the avidity of the polyclonal antibodies. The binding to timothy grass pollen was weak; however, sera from five of the eight patients recognized profilin. After destroying the carbohydrate structures by periodate treatment, the only remaining IgE-reactive component was profilin. This confirms that profilin contributes considerably to the cross-reactivity between tomato fruit and grass pollen. We have found that periodate oxidation decreased the IgE reactivity for seven of eight patients and many of the components in tomato fruit and timothy grass pollen extract. Besides oxidizing carbohydrate structures, periodate treatment may also alter the amino acids in the peptide chain.<sup>38</sup> It was therefore necessary to include suitable controls when carrying out this technique. A good correlation

**TABLE II.** Determination of patient sera by EAST inhibition assay

| Serum   | % Inhibition by |                |       |        |           |
|---------|-----------------|----------------|-------|--------|-----------|
|         | Birch pollen    | Mugwort pollen | Apple | Celery | Ovalbumin |
|         |                 |                |       |        |           |
| Bo 64*) | 55              | 31             | 60    | 51     | 2         |
| Bo 87   | 90              | 64             | 89    | 78     | 0         |
| Bo 108  | 77              | 55             | 81    | 75     | 0         |
| Bo 110  | 60              | 69             | 71    | 54     | 0         |
| Bo 131  | 93              | 85             | 91    | 85     | 0         |
| Bo 135  | 74              | 83             | 48    | 73     | 8         |

Allergen disks coated with tomato extract were used. The test was performed with four allergen extracts as inhibitors and ovalbumin as negative control.

\*Serum was diluted 1:25.

was found between the binding patterns of periodate-sensitive and lectin AAA-binding protein bands. AAA is directed against terminal  $\alpha$ -fucose residues.<sup>39</sup> Although  $\alpha$ 1,6 fucosylation is typical of mammalian glycoproteins,  $\alpha$ 1,3 fucosylation is only observed in plants and invertebrates. Therefore the latter can serve as a powerful antigenic structure (e.g., in horseradish peroxidase). Tretter et al.<sup>32</sup> showed that this configuration in phospholipase A<sub>2</sub> of bee venom contributes to the IgE-binding epitope. The influence of the fucosylation was further confirmed by our results obtained from the glycopeptide ELISA, which showed a high IgE reactivity to the  $\alpha$ 1,3 fucosylated bromelain in contrast to the defucosylated glycopeptide. The existence of IgE antibodies directed against  $\alpha$ 1,6 fucosylated structures was not tested, but appears unlikely because of potential autoimmune reactions.

The most prominent IgE-reactive component in tomato extract is  $\beta$ -fructofuranosidase, which shows no sequence homology to any of the grass pollen allergens.  $\beta$ -Fructofuranosidase possesses four N-glycosylation sites<sup>40</sup> and was shown to be AAA-reactive. Thus we assume that the cross-reactivity is due to the  $\alpha$ 1,3 fucosylation.

Recently, van Ree et al.<sup>41</sup> described a major grass pollen allergen detected in ryegrass (Lol p 11). This allergen shows a 40% sequence identity to an anther-specific protein of tomato termed LAT 52.<sup>42</sup> The structural similarities between these proteins are confirmed by the fact that the amino acid positions of the six cysteine residues are identical. The formation of disulfide bonds seems to be important for the conformation and allergenicity. Furthermore, it was reported that about 30% of the patients' sera that detect Lol p 11

recognize the carbohydrate structure and that fucose residues were demonstrated.<sup>41</sup> Because we performed the SDS-PAGE under reducing conditions, cystine-fixed conformational epitopes would have probably been destroyed. But even under reducing conditions, IgE-binding epitopes on the carbohydrate chains should be detectable.

Cross-reactivity caused by similar carbohydrate structures and by profilins can explain the IgE reactions to phylogenetically distant allergen sources.<sup>15</sup> In contrast to profilins, histamine release by IgE-reactive glycans has not been demonstrated. Because of this our results may be considered controversial. We have demonstrated cross-reactivity to other allergens, which are unrelated to grass pollen, in six of eight patients in our study. This supports the highly cross-reactive nature of the allergenic structures responsible for sensitization.<sup>1</sup> We were unable to identify structures that specifically mediate cross-reactivity between grass pollen and tomato. Thus it is unlikely that the same structures were responsible for the cross-reactivity determined by De Martino et al.<sup>2</sup> in patients monosensitized to grass pollen. However, all participants in our study had severe allergies, and four of them had neurodermatitis. In our opinion, it is unreasonable to assume that IgE reacting to carbohydrate structures does not elicit a mediator release from mast cells and basophils. Perhaps this could be clarified by further studies with neoglycopeptides. Further work is also required to identify tomato allergens that are exclusively shared with grass pollen but not with birch and mugwort pollen.

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