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Profilin Is a Cross-Reactive Allergen in Pollen and Vegetable Foods

Key Words

Profilin
Allergy
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IgE

Abstract

Sera with IgE antibodies against grass pollen often contain IgE against vegetable foods. We investigated the role of the ubiquitous protein profilin in this cross-reactivity. Profilin was purified from *Lolium perenne* grass pollen by means of affinity purification with Sepharose-coupled poly(*L*-proline). This solid phase was also used as capturing agent for profilin from pollen and food extracts for application in a radioallergosorbent test. It was shown that profilin is an allergen in grass pollen and in a wide range of vegetable foods, like potato and celery. Within a grass-pollen-sensitive population, patients with IgE to vegetable foods have a high incidence of antibodies against profilin. IgE antibodies against grass pollen profilin were shown to be cross-reactive with respect to vegetable foods.

Introduction

Patients with IgE antibodies to pollen often have IgE to a wide range of vegetable food allergens: in a group of grass-pollen-positive sera, approximately 35% were found to be positive for vegetable foods [1]. IgE antibodies of these patients recognize similar structures in often botanically unrelated allergen sources. Well-known examples of such cross-reactive structures are: (1) mugwort pollen and vegetables like carrot and celery [2, 3]; (2) birch pollen and various fruits of the family of the *Rosaceae* (apple, pear, cherry, peach) [4, 5]; (3) grass pollen and potato [1, 6], and (4) ragweed and some fruits, like melon and banana [7, 8]. It is known that some of these cross-reactions are caused by carbohydrate structures on allergens [1, 5]. These structures are susceptible to periodate treatment. Other cross-reactions are not affected by this treatment. These are presumably caused by evolutionary conserved protein an-

tigens. In most allergen sources of plant origin, we find both types of cross-reactive groups, although the degree in which they are present can vary strongly.

Recently Valenta et al. [9] cloned an allergen from birch pollen and identified it as profilin. By making use of its affinity for poly(*L*-proline, PLP), they were able to purify the native allergen, not only from birch pollen but also from various other allergenic pollen and, remarkably, from human platelets. Profilin is a highly conserved protein, present in almost all eukaryotic organisms. In this paper, we describe the isolation of profilin from *Lolium perenne* (*Lol p*) grass pollen and its characterization as a cross-reactive allergen not only in different pollen but also in vegetable food extracts.

Materials and Methods

Allergen Extracts

Rye grass (*Lol p*), orchard grass [*Dactylis glomerata* (*Dac g*)], timothy grass (*Phleum pratense*), birch (*Betula verucosa*) and mugwort (*Artemisia vulgaris*) pollen were obtained from Diephuis Laboratories (Groningen, The Netherlands). The pollen was extracted as described previously [10]. In short, they were extracted with distilled water (5% wt/vol) for 2 h. The pH was maintained at 8.5 by readjustment with NaOH (0.1 mol/l) every 15 min. After centrifugation, the supernatant was defatted, dialyzed against distilled water and freeze-dried.

Extracts of foods were prepared from uncooked materials; the raw material was obtained from the local foodstore. The extracts from apple, avocado, red currant, strawberry, cherry, kiwi, melon, nectarine, papaya, pear, peach, prune, orange, tomato, potato, cucumber, spinach, carrot, celery and parsley were prepared at pH 7.5 in potassium phosphate buffer, according to Björkstén et al. [11] with the use of polyvinylpyrrolidone (PVPP; Fluka, Buchs, Switzerland) and diethyldithiocarbamate (DIECA), defatted and dialyzed against distilled water and then lyophilized. Almond, cashewnut, hazelnut, walnut, basil, rosemary, thyme, nutmeg, green pea, peanut, soy, barley, oat, maize, rye, rice and wheat flour, onion, mushroom, sesame and green coffee bean were extracted with water at pH 8, followed by defatting, dialysis and lyophilization. With the exception of the different flours, all foods were blended in a kitchen blender prior to extraction. All extracts were prepared in a 10% wt/vol ratio.

Protein Determination

Protein was determined with BCA Protein Assay Reagent (Pierce, Rockford, Ill., USA) according to the manufacturer's instructions [12]. BSA was used as protein standard.

Solid-Phase Immobilization of Allergens and PLP

To 1 g (3.5 ml swollen gel) of CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) was coupled 5 mg of dialyzed, lyophilized *Lol p* or *Dac g* pollen extract, 25 mg of *Betula verucosa* or *Artemisia vulgaris* pollen extract and 40 mg of *Phleum pratense* pollen. Of the food extracts, 40 mg was coupled, except in the case of almond, basil, cashewnut, green pea, green coffee bean, maize, mushroom, nutmeg, oat, onion, papaya, peanut, rosemary, sesame, soy and thyme. In this case, 50 mg of extract was coupled to 1 g of Sepharose. Sepharose-coupled PLP (PLP-Sepharose) was used for adsorbing profilin from allergen extracts. PLP (molecular weight, MW, between 30.2 and 37 kD) was purchased from Sigma (St. Louis, Mo., USA). To 1 g of CNBr-activated Sepharose 4B was coupled the water-soluble part of 15 mg PLP. Not dissolved material (after overnight end-over-end incubation) was removed prior to coupling, by means of centrifugation.

Coupling of allergen extracts and of PLP was followed by inactivation of the Sepharose with 0.5 mol/l glycine pH 8.5.

Purification of Profilin

Profilin was purified from *Lol p* pollen extract by taking advantage of its affinity for PLP. To a column of PLP-Sepharose (2.5 ml), equilibrated in PBS, 95 mg of pollen extract (in 4.75 ml of PBS) was applied. Nonbound material was collected, followed by extensive washing with PBS. Elution of bound material was performed with PBS containing 6 mol/l urea. Absorbance at 280 nm revealed two elution peaks A and B. Both were pooled separately, dialyzed against

PBS and concentrated 5.5 and 8.5 times, respectively, by ultrafiltration over an Amicon YM10 (Amicon, Danvers, Mass., USA) filter. The presence of profilin in nonbound material and both elution peaks was monitored by means of the above-described indirect RAST with PLP-Sepharose and serum pf40. In peak A, the profilin eluted, and this material was used for radiolabeling. Purity of peak A was checked by SDS-PAGE and CBB staining. Peak B contained no detectable profilin. Profilin was also purified from *Lol p* according to the method of Lind et al. [13], used with human platelet extracts. In this case the grass pollen extract (80 mg) was dissolved in 20 ml of a buffer containing 20 mmol/l Tris 150 mmol/l NaCl and 0.5 mmol/l ATP, pH 7.6 (TBS-ATP). This was also the washing buffer for the affinity column: 2.5 ml of PLP-Sepharose layered upon a 50-ml Sephadex G-25 column. Elution of bound material was performed in two steps: (1) 50 ml of TBS-ATP containing 2 mol/l urea, and (2) 50 ml of TBS-ATP containing 6 mol/l urea. After each step in the procedure, the column was washed with TBS-ATP. Unbound material, and the two elution peaks were each pooled, dialyzed against PBS and analyzed on SDS-PAGE followed by silver staining. In both purification procedures, the yield was comparable: 350 µg of profilin from 95 mg of pollen extract and 275 µg from 80 mg, respectively. This corresponds to 3.5 mg profilin/g *Lol p* pollen.

Iodination

Purified profilin from *Lol p* was radiolabeled by the chloramine-T method with carrier-free ¹²⁵I (Amersham, Amersham, UK); 25 µg of protein was iodinated with 1 mCi of ¹²⁵I. Unbound ¹²⁵I was separated from the labeled profilin on a Sephadex G-75 column equilibrated in PBS with BSA (3 mg/ml). The resulting specific activity of the radio-labeled profilin was about 15,000 cpm/ng profilin. When the labeled profilin was analyzed on SDS-PAGE, followed by autoradiography, a single band of 12 kD was detected.

Selection of Sera

In this study, two groups of sera were used. The first group (group A; n = 52) was selected from sera submitted for serologic allergy testing (RAST) by physicians throughout The Netherlands. Such sera are routinely tested on a panel of the most common inhalant allergens and/or the most common food allergens. The second group of sera (group B; n = 62) was selected from in-house allergic reference sera that were collected by means of plasmapheresis from allergic volunteers. Amongst these were serum pf40 and pf72. Specific IgE to grass pollen or to a combination of grass pollen and vegetable allergens was used as selection criterion for both groups. Whether they had specific IgE against other inhalant or food allergens was not taken into account. At present, no clinical data are available on the patients in this study, with the exception of pf40. This patient suffers from atopic dermatitis and allergic rhinitis related to vegetable foods and pollen.

Radioallergosorbent Test

RAST was performed as described previously [1]. In case of the food allergens, 1.5 mg of Sepharose was used per test, for the pollen allergens 0.5 mg/test. *Dac g* and *P. pratense* Sepharose were used as a 1:1 mixture (1 mg/test), for screening of sera for specific IgE to grass pollen. Antivegetable IgE was detected with a mixture (1:1:1:1) of Sepharose-coupled carrot, cucumber, potato and spinach (3 mg/test). In all cases, 50 µl of serum were added per test. ¹²⁵I-labeled sheep antibodies to human IgE were used for the detection of bound IgE. RAST results were expressed as percentage of radioactivity bound,

after correction for nonspecific binding ($< 1\%$). A percentage binding $> 2\%$ was regarded as positive. For detection of specific IgE antibodies to profilin from *Lol p*, 1 mg of PLP-Sepharose was incubated overnight (end over end) with *Lol p* extract (100 μ g of protein) and 50 μ l of serum in a final volume of 350 μ l. The amount of pollen extract used was just enough to saturate the PLP-Sepharose with profilin. Unbound allergen was not washed away, prior to addition of serum. When unbound allergen was washed away, some sera showed IgE directed to allergen(s) nonspecifically bound to the PLP-Sepharose: they gave the same binding (in some cases up to 45%) when PLP-Sepharose was substituted by gly-Sepharose. When unbound allergen was not washed away, the controls with gly-Sepharose were low, presumably because an excess of allergen(s) capable of binding nonspecifically to Sepharose remains in solution. In all cases, gly-Sepharose incubated with pollen extract and serum served as negative control. The rest of the procedure was the same as a normal RAST. The results obtained with the gly-Sepharose were subtracted from those obtained with PLP-Sepharose. PLP-Sepharose was also used for detecting profilin in other allergen extracts. For this purpose, serum pf40 was used. In this case, 30 μ g of protein was incubated with the Sepharose (minimally 4 h). With this serum, nonspecific binding to PLP-Sepharose or gly-Sepharose of IgE-binding activity from food extracts was not observed. It cannot be ruled out that other sera would detect such binding. It is therefore important to include the gly-Sepharose control at all times.

Two-Site RIA for Total Multivalent Antibody

For this assay, the same procedure was followed as in case of the RAST, only the ^{125}I -anti IgE was replaced by ^{125}I -profilin (15,000 cpm). In this way, multivalent antibodies to profilin, presumably mainly IgG but possibly also IgE, were detected.

Affinity Purification of Anti-Profilin IgE

Serum (1 ml) was incubated end over end for 6 h at room temperature with 200 mg of PLP-Sepharose preincubated with *Lol p* or potato extract (6 mg of protein). PBS-AT (PBS, 0.3% BSA, 0.1% Tween 20) was added to a total volume of 10 ml. After extensive washing with PBS, the IgE was eluted with 2×1 ml of 0.1 mol/l glycine/HCl, 100 mg/l BSA, pH 2.5. The eluted material was neutralized with 1 mol/l Tris. The efficiency of the elution was checked with a RAST for profilin from *Lol p* pollen or potato as described above (150 μ l/test). In general, approximately 25% of the original profilin-binding IgE activity were recovered in the eluate. The eluates were also tested with a *Lol p* blot. In this case, 500 μ l were added per strip.

Allergen-Binding Assay

In the allergen-binding assay, Sepharose-coupled mAb-antihuman IgE (10 mg of mAb on 3 g of Sepharose) was used as antibody-capturing agent (mAb from our institute: CLB-MH 25-01). Sepharose was incubated overnight with 50 μ l of serum, in a final volume of 300 μ l (PBS-AT). After washing five times, 60,000 cpm of ^{125}I -profilin were added. To increase the sensitivity, four times more antigen was added than in the above-described sandwich assay. The final volume was 700 μ l (PBS-AT). After overnight incubation (end over end) at room temperature, the samples were washed four times and radioactivity was counted. Results are expressed as counts per minute. The mean background, obtained with 19 sera negative ($< 2\%$) in a RAST for grass pollen as well as vegetables, was 90 ± 6 cpm (SD), and > 150 cpm was regarded as positive. For serum pf40, the assay was also carried out as inhibition experiment. In this case, different allergen ex-

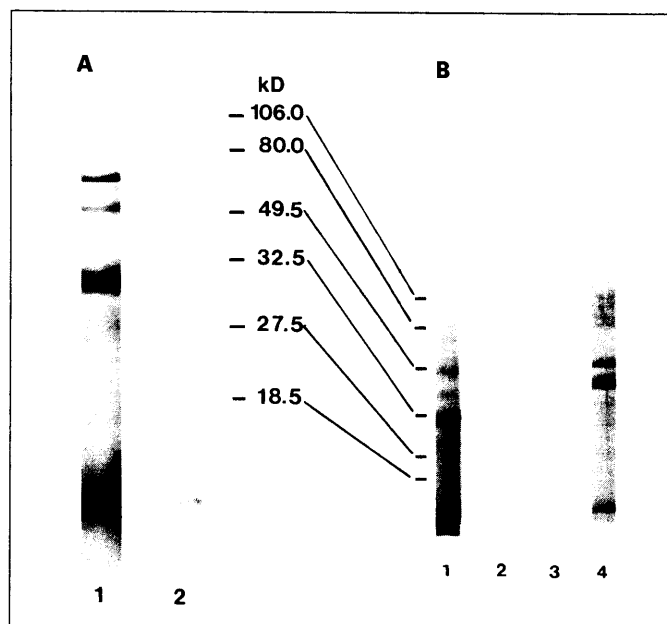


Fig. 1. **A** SDS-PAGE on a 15% gel, followed by Coomassie staining. Lane 1 is total *Lol p* extract (200 μ g of protein) and lane 2 is protein bound to 50 mg of PLP-Sepharose from 20 mg of *Lol p* extract. In this case, the Sepharose was directly boiled in sample buffer. **B** SDS-PAGE on a 8–18% gradient gel, followed by silver staining. Lane 1 is *Lol p* extract (25 μ g of protein). Lane 2 shows the material that is eluted from PLP-Sepharose at 2 mol/l urea, lane 3 at 6 mol/l urea. Lane 4 is celery extract (30 μ g of protein). It is clearly visible that celery extract contains a major protein band of a comparable MW as pollen profilin.

tracts were added in fivefold dilutions to the samples during the incubation with the ^{125}I -profilin. An allergen-binding inhibition assay was also performed with a polyclonal rabbit antiserum (anti-*Dac g* pollen). In this case, 0.5 mg of Sepharose-coupled protein A (Pharmacia Fine Chemicals) per test was used as antibody-capturing agent and 2.5 μ l of serum. All reagents were added at the same time, including ^{125}I -profilin (15,000 cpm) and the inhibitor. The total volume was 350 μ l (PBS-AT). After overnight incubation (shaking), the samples were washed four times and radioactivity was counted.

Results

Purification

PLP-Sepharose was used for affinity purification of profilin from *Lol p* pollen. The affinity-purified material was analyzed on SDS-PAGE. The main band had an apparent MW of 12 kD (fig. 1). Two minor impurities with MW > 50 kD copurified. Analysis by isoelectric focussing of our profilin preparation revealed two bands with pI of 4.7 and 4.8.

Specific IgE to Profilin

PLP-Sepharose in combination with *Lol p* extract was used in a RAST procedure as described. Figure 1A shows that under these conditions the 12-kD band (profilin) is almost exclusively bound to the solid phase. The above-

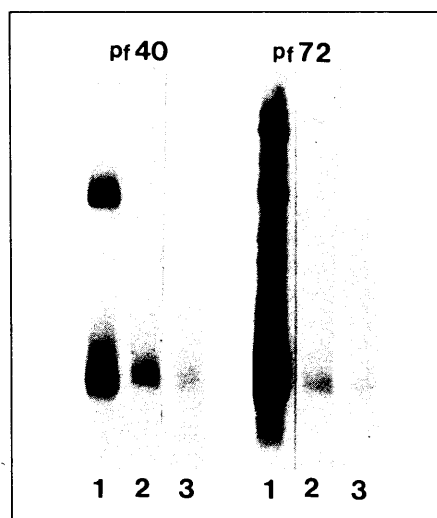


Fig. 2. Immunoblotting with *Lol p* blots and 2 sera used for affinity purification of IgE. Lane 1 represents the starting serum, lane 2 the IgE eluted from PLP-Sepharose that was preincubated with pollen extract and lane 3 the IgE that was eluted after preincubation with potato extract.

mentioned two minor bands with a MW > 50 kD were however also visible. With this solid phase, we tested all sera ($n = 114$) from groups A and B. Figure 2 illustrates for two sera that the IgE antibodies bound to this solid phase are indeed directed to profilin. Eluted antibodies were exclusively directed to profilin when analyzed on immunoblot. All sera were also tested in the allergen-binding assay with ^{125}I -labeled profilin and Sepharose-coupled anti-IgE. Both assays correlated quite well (linear correlation coefficient $r = 0.70$; $p < 0.001$), only in case of low responders some sera were only positive in one of both tests.

Association between IgE to Vegetable Foods and IgE to Grass Pollen Profilin

We analyzed whether in sera from patients with IgE antibodies to grass pollen, IgE to profilin occurs more frequently in patients with IgE to vegetable foods. For this purpose two subselections of the sera were made. (1) Twenty-seven sera from group A originated from patients that were tested in our diagnostic department for specific IgE to the most common inhalant allergens and were found to be highly positive (> 20% binding in a RAST) for grass pollen. Testing for specific IgE to food allergens was not requested. From this group of sera, 8 (30%) had a positive RAST (> 2%) for profilin from *Lol p*. (2) In groups A and B together ($n = 114$), 56 sera were found with > 10% binding in a grass pollen RAST and > 5% binding in a veg-

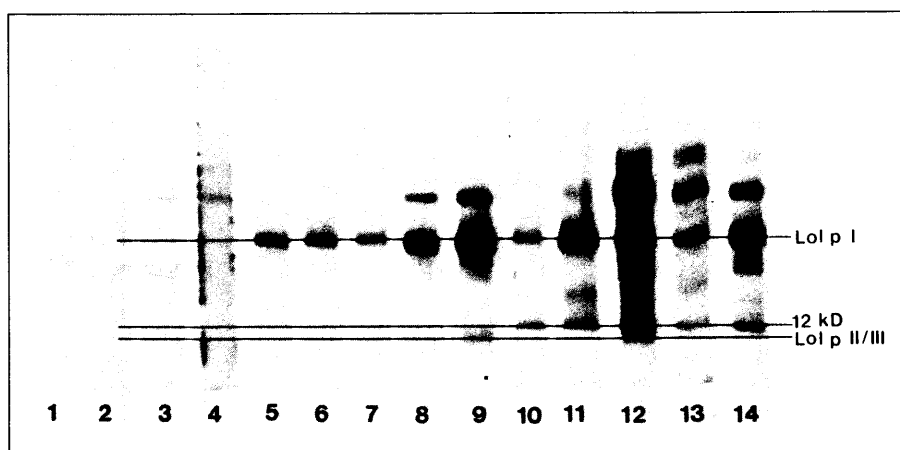


Fig. 3. *Lol p* immunoblot. Lane 1: negative control serum (negative for grass pollen, positive for cat allergen), lane 2: serum negative for grass pollen and profilin and weakly positive for vegetables, lanes 3 and 5-9: sera positive for grass pollen but negative for profilin, lane 4: serum with a very high total IgE titer (> 25,000 IU/ml) and negative for grass pollen, and lanes 10-14: sera positive for grass pollen and for profilin (lane 10: serum pf40). This figure illustrates that bands representing profilin can easily be mistaken for *Lol p* II and III. The distance between the middle of the *Lol p* I band on one hand and either the profilin or *Lol p* II/III band on the other hand is significantly different ($\Delta = 2$ mm).

etable RAST, of which 28 (50%) had IgE to profilin. For the resulting difference of 20% between the two frequencies the SE was 8% ($p < 0.01$).

IgE antibodies to a 12-kD allergen were also demonstrated by means of immunoblotting in 5 sera that were shown to have IgE to profilin (fig. 3). Although they can easily be mistaken for IgE antibodies against *Lol p* II or III (both 11 kD) [16], IgE antibodies against profilin bound consistently approximately 2 mm higher on the grass pollen blots.

Profilin in Food Extracts

A panel of vegetable food allergens was tested in the RAST with PLP-Sepharose and serum pf40. On immunoblot this serum detected in four out of five tested food extracts (celery, peanut, wheat and apple) only one allergen with an MW between 12 and 15 kD. In potato extract, some weakly staining additional bands with higher MW were demonstrated (data not shown). Table 1 (profilin RAST) illustrates that PLP-Sepharose was able to bind an IgE-binding component in a large number of food extracts. Among the allergen sources with high IgE-binding capacity and affinity for PLP are celery and potato. When the same procedure was carried out with gly-Sepharose, no IgE binding was demonstrated ($< 1\%$ binding). These results show that profilin is present in a large number of food extracts in quantities large enough to bind IgE.

Cross-Reactive Nature of Profilin

To establish the cross-reactive nature of specific IgE against profilin, serum pf40 was used in an allergen-binding inhibition assay with Sepharose-coupled mAb-anti-IgE and ^{125}I -labeled profilin. The binding of profilin to IgE was inhibited with some vegetable food and pollen extracts. With all extracts, the binding of grass pollen profilin was inhibited with $> 90\%$ (fig. 4A). The same assay was carried out with polyclonal rabbit antiserum against *Dac g* pollen extract. Binding of profilin was inhibited by all extracts (fig. 4B). Another confirmation of the cross-reactive nature of antipprofilin antibodies was obtained by immunoblotting. Affinity purified IgE against potato profilin (purified with PLP-Sepharose preincubated with potato extract) bound to profilin on a grass pollen blot (fig. 2). Celery extract was also separated on SDS-PAGE and blotted. When this blot was then incubated with serum pf40, followed by detection of bound antibodies with ^{125}I -profilin instead of ^{125}I -anti-IgE, a band appeared with a MW of 12 kD (not shown). By means of a two-site RIA with Sepharose-coupled allergen extracts and ^{125}I -labeled profilin from grass pollen, it was demonstrated that serum pf40

Table 1. Profilin in different vegetable food extracts detected with two different assays

Profilin RAST	105 > 27	Two-site RIA
Allergen source	binding, %	binding, %
Onion ✓	30.8	12.5
Avocado ✓	30.8	2.8
Carrot ✓	30.6	11.8
Tomato ✓	30.3	22.8
Celery ✓	27.3	30.2
Orange ✓	27.0	27.2
Cucumber ✓	27.0	21.9
Peach ✓	26.6	16.1
Cherry ✓	26.6	15.5
Pear ✓	25.8	18.1
Strawberry ✓	25.7	11.0
Nectarine	25.2	17.9
Potato	25.2	11.2
Peanut	24.2	23.7
Almond ✓	24.2	16.8
Pea, green ✓	23.8	19.4
Parsley ✓	23.6	27.8
Kiwi ✓	22.7	16.1
Walnut ✓	22.7	14.6
Apple ✓	22.5	9.8
Maize ✓	20.6	10.5
Soy ✓	20.2	17.8
Spinach ✓	15.1	11.3
Hazelnut ✓	9.5	7.5
Sesame ✓	8.8	1.6
Rice ✓	7.1	30.7
Coffee bean, green ✓	4.9	4.3
Barley ✓	4.6	8.0
Prune ✓	3.2	2.6
Rye	2.9	10.3
Cashewnut	1.1	1.3
Oat	0.9	0.9
Thyme	0.9	0.7
Melon	0.8	0.8
Basil	0.7	1.3
Mushroom	0.7	0.7
Currant, red	0.7	0.5
Papaya	0.7	0.2
Nutmeg	0.6	0.8

In the profilin RAST IgE and in the two-site RIA total multivalent antibody against profilin are measured. In both cases serum pf40 was applied. Food extracts are listed in descending order of reactivity in the profilin RAST.

contains cross-reactive antibodies against profilin from the same allergen extracts that were positive in the RAST with PLP-Sepharose (table 1). Both assays for antibodies against profilin gave similar results (linear correlation coefficient $r = 0.71$; $p < 0.001$), although in some cases the

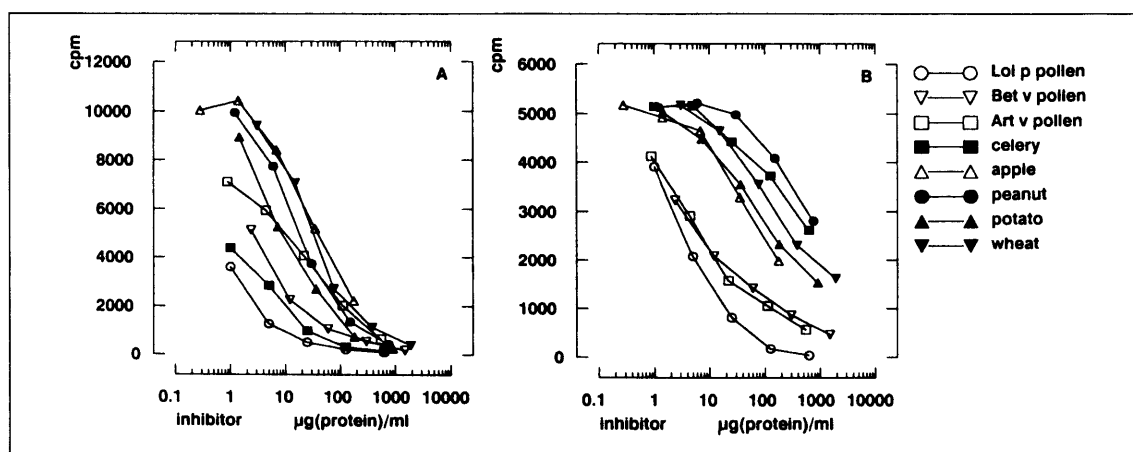


Fig. 4. Allergen-binding inhibition assay. *Bet v* = *B. verucosa*; *Art v* = *A. vulgaris*. **A** Inhibition of the binding of ^{125}I -labeled profilin to IgE (serum pf40). **B** Inhibition of the binding to rabbit anti-*Dac g* pollen.

Table 2. Two-site RIA for total multivalent antibody

	Serum					
	1	2	3	4	5	6
Pollen						
<i>Lol p</i>	0.6	0.1	21.1	17.4	40.5	35.3
<i>Bet v</i>	0.7	0.2	16.4	12.7	40.9	33.3
<i>Art v</i>	0.4	0.2	9.2	8.7	25.4	25.6
Foods						
celery	0.8	0.5	22.8	25.2	45.8	38.9
potato	1.3	0.3	5.3	16.1	42.8	36.8
peanut	1.3	0.5	15.1	16.9	44.5	38.2
wheat	1.1	0.7	8.7	6.6	22.0	11.4
apple	0.5	0.1	6.7	20.9	37.1	26.8
RAST for profilin	0.4	1.1	34.1	3.8	46.7	12.5

Bet v = *B. verucosa*; *Art v* = *A. vulgaris*. Two-site RIA with three pollen and five food extracts coupled to Sepharose and ^{125}I -labeled pollen profilin. Serum 1 and serum 2 were negative controls. Serum 1 was from a patient who received hyposensitization therapy for yellow jacket venom. Serum 2 had a high total IgE ($> 25,000$ IU/ml). Sera 3–6 were shown to have IgE against *Lol p* profilin. Sera 5 and 6 were from patients receiving hyposensitization therapy for grass pollen. Serum 3 is serum pf40.

results were different (e.g. avocado and rice). While the RAST measured IgE against profilin, the sandwich assay measured total multivalent antibody against profilin. In table 2, the results of the sandwich assay are shown for three pollen and five food extracts coupled to Sepharose and 6 sera. These results confirm that profilin is a cross-reactive allergen.

Discussion

Profilin was purified from *Lol p* grass pollen by means of PLP-Sepharose as was earlier described for profilin from various other sources [9, 13–15]. The MW of the minor impurities that copurified (> 50 kD on SDS-PAGE) agree with the ones described for the complex of profilin and actin, profilactin [14], and prolyl hydroxylase [15]. Besides profilin, these are the only proteins for which affinity for PLP was described.

We have confirmed the claim of Valenta et al. [9] that profilin from pollen is an important allergen. Their emphasis was based on birch pollen, while we focused on *Lol p* grass pollen. Lack of correlation between total IgE titers and binding of profilin (data not shown) indicated that profilin is a true allergen and is not binding to IgE in a nonimmunological manner. We have no evidence that the actin in profilactin or prolyl hydroxylase has IgE-binding properties. Affinity-purified IgE exclusively bound to profilin (12 kD) on a grass pollen blot (fig. 2). The good correlation of the RAST for profilin with the allergen-binding assay with ^{125}I -profilin (which was purified by affinity chro-

matography and gel filtration) also did not point towards involvement of actin or prolyl hydroxylase in IgE binding. The identification of profilin as a grass pollen allergen stresses the need of re-evaluation of the low-MW fraction (10–20 kD) of grass pollen allergens. So far, only three allergens were identified in this range: *Lol p* II, *Lol p* III [16] and cytochrome *c* (*Lol p* X) [17]. Recent investigations in our laboratory have shown that besides profilin, at least two additional allergens exist in this range: an 11-kD allergen (a breakdown peptide of *Lol p* V) and a 17-kD allergen [18]. For the study of grass pollen allergy, it is important to realize that the low-MW fraction is more complex than was thus far assumed.

In a group of grass-pollen-sensitive patients, we found that antiprolin IgE had a higher incidence amongst those patients that also had IgE against vegetable foods. From our data we cannot exclude that this association between IgE to vegetable foods and IgE to profilin is indirect: both may possibly occur more frequently in sera with very high IgE to grass pollen in general. All sera with IgE to grass profilin found so far ($n = 28$) were reactive with celery and potato. The involvement of profilin in pollen-food cross-reactivity that was suggested by these results was confirmed by the allergen-binding inhibition assay (IgE) and the two-site RIA (total cross-reactive antibody). Whether the two-site RIA detects both cross-reactive IgE and IgG was not investigated, however the lack of correlation between the profilin RAST and the two-site RIA and the finding that the highest responders in the two-site assay are receiving pollen immunotherapy suggest an important

influence of IgG in the sandwich. More sera with IgE to pollen profilin will have to be tested in these assays to confirm that these antibodies are highly cross-reactive. The observation that IgE to grass profilin seems to be so often cross-reactive does not indicate how much of the cross-reactivity between grass pollen and vegetable foods is due to profilin. This can be investigated by depleting sera for antiprolin IgE. First preliminary depletion experiments carried out with serum pf40 have shown that the RAST for celery and potato is decreased to about 25% of the control value. Whether the contribution of profilin is equally high with other cross-reactive sera is currently investigated.

Besides the importance of profilin for pollen-food cross-reactivity in vitro, its clinical relevance should be investigated. First preliminary results have shown that purified profilin from grass pollen can induce histamine release from purified basophils in vitro. If the presumption is correct that carbohydrate determinants play clinically a role of minor importance [1], a nonglycosylated allergen like profilin could be of even more interest than is indicated by serological investigations. This clearly could have implications for diagnosis and immunotherapy of this type of allergy.

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