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Original Article

Recombinant *Zea mays* profilin forms multimers with pan-allergenic potential

Telly Psaradellis,[1] Neil L Kao[2] and Michael Babich[1]

[1]Department of Biomedical Sciences, University of Illinois College of Medicine, Rockford, Illinois and [2]Allergy and Asthma Centre of Dayton, Dayton, Ohio, USA

Abstract

European studies have shown that approximately 20% of all pollen-allergic patients display IgE reactivity to various plant profilins. Profilins are ubiquitous intracellular proteins, with a role in cell signalling and morphology. Recently, functionally relevant human profilin tetramers were identified, but the characterization and allergenic roles of plant profilin multimers have not been reported. Because larger molecules are generally more antigenic, the present objectives were to: (i) determine if plant profilin forms multimers; (ii) use the allergenic property of profilin in the design of an immunoassay to detect type I allergies in the local population; and (iii) assess the allergenic potential of monomeric versus multimeric profilin. In agreement with other known profilin forms, silver-stained sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblot analyses revealed that a significant 14.8 kDa protein was purified from *Escherichia coli* transformed with the cDNA of a plant (*Zea mays*) profilin isoform (ZmPRO1). Higher molecular weight proteins (particularly 60 kDa and 30 kDa) were also observed, which became predominant and larger (> 90 kDa) in the absence of reducing agents. Human IgE reactivity to profilin was measured by enzyme-linked immunosorbent assay (ELISA) that was developed using patient serum samples classified as either negative (no type I allergies), positive (type I plant allergies) or miscellaneous (i.e. allergies other than classical type I plant allergies). The IgE-ZmPRO1 complexes were seen in three of nine patients with type I plant allergies, compared with one of eight negative controls and three of 14 from the miscellaneous category. Dot filtration immunoblots were subsequently developed to absorb profilin diluted in the presence or absence of reducing agent to yield mostly monomeric or multimeric profilin, respectively. Immunoglobulin E from positive patients displayed a greater intensity of binding to ZmPRO1 under conditions that favored profilin multimers. In summary, recombinant ZmPRO1 profilin forms multimers and is suitable for a developed ELISA. The data further suggest that profilin has pan-allergenic potential in the North American population and raise the possibility that profilin multimers have greater immunogenicity than monomers.

Key words: allergy, diagnostic, multimer, oligomerization, plant profilin, profilin.

Introduction

Profilins are cytoskeletal proteins that sequester G-actin and bind to membrane-associated phosphatidylinositol-4,5-bisphosphate,[1–7] thereby affecting both cell morphology and signal transduction. Recently, the existence of human profilin multimers has been reported, in which tetramers may be the high-affinity actin-binding form.[8] Profilins have also been identified and purified from tree, grass and weed pollens and have been produced recombinantly.[9–14] European studies have revealed that profilin isoforms isolated from various plant sources may act as pan-allergens[9,10,15,16] and that approximately 20% of all pollen-allergic patients display IgE reactivity to recombinant birch profilin. The recombinant birch profilin, as well as natural profilins from birch, timothy grass and

Correspondence: Dr Michael Babich, University of Illinois College of Medicine, 1601 Parkview Avenue, Rockford, IL 61107-1897, USA. Email: michaelb@uic.edu

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mugwort, are able to elicit IgE-mediated histamine release from basophils of pollen-allergic patients. Thus, profilin may be a pan-allergen as well as being responsible for the sensitization and maintenance of a significant number of type I allergic patients.

Structural analysis has revealed remarkable homology between profilins from different plant species.[9–11] Plant profilins appear to share common IgE epitopes and IgE from all sensitive patients cross-reacts with different profilins. In addition, rabbit polyclonal antibodies raised against recombinant birch profilin[16] cross-react with virtually all plant profilins studied thus far. The available data indicate that profilin from one plant source can cross-sensitize an individual to several plant species and may explain why some patients with type I hypersensitivities display reactions to a wide range of distantly related pollens and foods.

Available data regarding profilin as a pan-allergen have thus far been drawn from European patients, whereas sensitivity of the North American population to plant profilin is unknown. Furthermore, the ability of plant profilin to form multimers has not been directly studied. However, the existence of multimers should be considered when investigating the allergenic properties of profilin, because larger antigens would likely present additional epitopes to elicit a greater IgE-mediated histamine release. Therefore, recombinant plant profilin multimerization was studied and immunoassays were developed to assess IgE reactivity of individuals to plant profilin. The correlation between type I hypersensitivities and reactivity to plant profilin within the local population was subsequently examined.

METHODS

Reagents

The cDNA encoding an isoform of profilin derived from the pollen of *Zea mays* (ZmPRO1; courtesy of Dr Christopher Staiger, Purdue University, West Lafayette, IN, USA)[17] was provided in a transfection vector (pET-23a; Novagen, Madison, WI, USA) with an isopropyl beta-D-thiogalactopyranoside (IPTG)-inducible promoter; polyclonal rabbit IgG that recognizes the protein product encoded by ZmPRO1 cDNA was also provided. Cyanogen bromide (CNBr)-activated sepharose 4B was purchased from Pharmacia (Piscataway, NJ, USA) and poly(L-proline) (PLP; 10 000–30 000 MW) was purchased from Sigma Chemical Co. (St Louis, MO, USA). Horseradish peroxidase (HRP)-conjugated monoclonal antibodies (goat antirabbit IgG, goat antihuman IgE) and silver staining kits were purchased from Pierce Chemical Co. (Rockford, IL, USA).

Patient serum samples

Human serum samples from routine blood draws were kindly provided by the University of Illinois College of Medicine at Rockford, Office of Family Practice (Rockford, IL, USA) with appropriate patient consent; patient-declared allergies were annotated on each serum container. Serum isolated from whole blood by standard centrifugational methods was either stored at 4°C (and used within 1 week) or aliquoted and stored at – 20°C. The samples were categorized into one of three groups: (i) no declared allergies; (ii) miscellaneous reactions (i.e. non-plant allergens, such as dust, adhesive tape, synthetic materials etc.); or (iii) classical type I allergies to plant pollens.

Expression and purification of ZmPRO1 profilin

Pre-thawed competent BL21(DE3) *Escherichia coli* cells (Novagen Inc., Madison, WI, USA) were transformed with ZmPRO1/pET-23a by a modified protocol from the manufacturer and essentially as described for plant[11,13,18] and human profilins.[19] The DNA content and quality from lysates of various transformed *E. coli* clones were analyzed by standard spectrophotometric measurement (i.e. 260 nm, concentration; 260 nm/280 nm, relative nucleotide purity *vs* protein) and agarose (0.7%) gel electrophoresis. The *E. coli* clones expressing the highest concentrations of ZmPRO1 cDNA were selected for profilin production.

Transformed *E. coli* initially grown in 10 mL L-broth (in g/L: 10 tryptone, 5 bacto yeast extract, 10 NaCl + 0.15 ampicillin) at 37°C for 10 h were brought to a final 1 L volume of L-broth and incubated for an additional 2 h (37°C, gentle mixing at 100 r.p.m.) prior to the addition of either IPTG (0.4 mmol/L final concentration) or vehicle for an additional 6 h incubation. The cultures were centrifuged (1000 *g* for 30 min at 22°C) to yield pellets that were resuspended in 5 volumes of ice-cold lysis buffer (0.01% Triton X-100, 2 μmol/L leupeptin, 1 μmol/L aprotinin, 0.2 μmol/L pepstatin, 5 mmol/L This-HCl, pH 7.2) and sonicated (continuous output control setting 2 × 10 s, Sonifier cell disruptor, Branson

Sonic Power Co., Danbury, CT, USA). The lysates were centrifuged (12 000 *g* for 30 min at 4°C) and supernatants poured ontoa poly(L-proline)-sepharose 4B (i.e. PLP bead) affinity column, as described previously.[8,20] Briefly, a step-wise elution gradient with urea was used to collect and purify profilin for overnight dialysis (at 4°C) against 2 mmol/L *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.2/0.1 mmol/L $CaCl_2$ and concentration by centrifugation (centriplus-3, Amicon Inc., Beverly, MA, USA) to a final concentration of approximately 1 mg/mL, which was stored at – 20°C. In some cases, ZmPRO1 profilin was isolated by a co-incubation of *E. coli* lysate:PLP bead slurry (1:4 vol; 4–16 h at 4°C, gentle shaking), followed by centrifugation to pellet and wash the profilin-PLP bead complexes (3 times with 100 mmol/L NaCl, 100 mmol/L glycine, 0.01 mmol/L DTT, 10 mmol/L Tris base, pH 7.8). The final pellet was suspended and boiled in sample buffer, with or without β-mercaptoethanol (BME).

Proteins isolated by either PLP bead slurry (e.g. Fig. 1; initial purification and validation that profilin was made in *E. coli*) or column chromatography were analyzed by standard silver-stained sodium dodecyl sulfate–polyacrylamide gel electropheresis (SDS-PAGE; 15% acrylamide) techniques. Profilin was further characterized by western immunoblotting, as previously described.[8] The immunoblot was developed by incubation with rabbit anti-ZmPRO1 primary antibody (1:1000) and goat antirabbit secondary antibody conjugated with horseradish peroxidase (1:500). Proteins were visualized with either a fluorescent substrate or enhanced metal substrate (Super Signal or metal diaminobenzidine tetrahydrochloride (DAB), Pierce Chemical Co., Rockford, IL, USA).

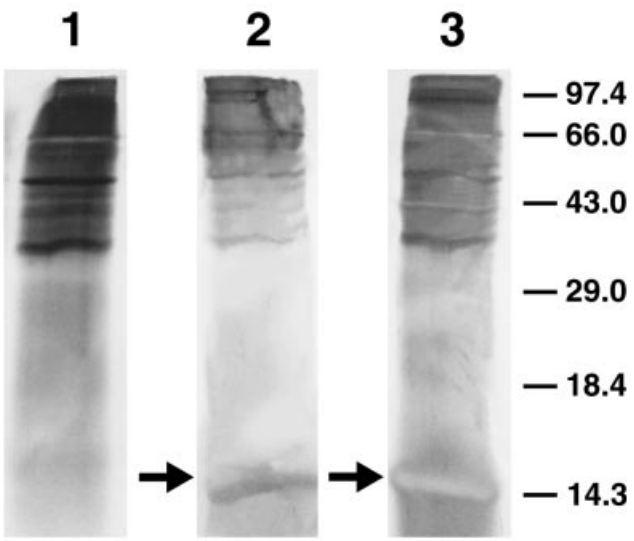

Fig. 1 Silver-stained sodium dodecyl sulfate polyacrylamide gel electrophoresis separation of *Escherichia coli* transformed with *Zea mays* (Zm) PRO1 cDNA. The protein encoded by ZmPRO1 cDNA was purified by affinity bead slurry separation as described in Methods. Lane 1, non-transformed *E. coli* (negative control); lane 2, *E. coli* containing pET23a/ZmPRO1 vector; lane 3, *E. coli* containing pET23a/ZmPRO1 vector + induced. Arrows show monomeric profilin. Protein molecular weight marker migrations are listed on the right (in kDa).

Enzyme-linked immunosorbent assay for human IgE-ZmPRO1 profilin detection

Purified ZmPRO1 product (50 ng/well) or a control vehicle (tris-buffered saline (TBS), pH 7.4) was added to designated wells of a 96-well immunoassay plate (Immulon-2, Dynatech Laboratories Inc., Chantilly, VA, USA) that was stored overnight at 4°C. The general sequence to develop the appropriate wells for the ELISA was as follows: (i) block non-specific sites (4% non-fat powdered milk, 0.1% bovine serum albumin (BSA), 0.02% NaN_3 in TBS, 20% SuperBlock from Pierce Chemical Co. for 2 h at 4°C); (ii) 1 × TBS wash and incubate with either serum samples or a control vehicle (TBS or heat-inactivated fetal calf serum; overnight at 4°C); (iii) discard samples and add either TBS to sample wells or primary rabbit IgG antiplant profilin (1:1000 dilution in TBS, 0.01% Tween-20, 0.01% BSA for 1.5 h) into control wells to ensure ZmPRO1 profilin coating; and (iv) 1 × TBS wash of all wells, add appropriate secondary antibodies (either goat antihuman IgE-HRP in wells that contained serum or goat antirabbit IgG-HRP in control wells; 1:500 dilutions for 2 h at 4°C). The plate was extensively washed then developed using 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) as a colorimetric method substrate (although other established HRP substrates were also successful in preliminary work) and optical densities were measured with a microtiter platereader ($\lambda_{570\ nm}$). Serial dilution assay of profilin standards indicated that the assay was linear between 0.1 and 100 ng additions of profilin per well. Serum samples were considered reactive with profilin (i.e. positive) if there was at least one standard deviation difference between the optical densities obtained from the wells containing profilin versus those without profilin. Serum identified as positive gave a linear increase in signal intensity at concentrations between 10 and 100%.

Dot-filtration immunoblot

In some instances, a dot filtration immunoblot assay (Bio Rad, Hercules, CA, USA) using supported nitrocellulose (0.2 μm pore size) was a necessary alternative to the ELISA to determine the allergenic potential of ZmPRO1 monomers versus multimers. Profilin was either placed under reducing (4.5% BME at 95°C for 3 min) or non-reducing conditions (to favor monomeric or multimeric conditions, respectively) and subsequently allowed to adhere to the dot immunoblot (50 ng/well for 2 h) prior to vacuum filtration to remove the medium from the membrane. The BME was then removed by thorough washing with TBS and the dot immunoblot was developed with antibodies similar to the ELISA method, but with an enhanced metal DAB as the HRP substrate. Quantitative values for the intensity of immunorecognition (i.e. darkness) were obtained by computer scanning the dot-filtration immunoblot and using Adobe Photoshop (Adobe Systems, San Jose, CA, USA) software program (under histogram, black channel). The average brightness value was obtained from the fixed number of pixels (486) that covered each dot and the corresponding darkness value was calculated by 100 × the inverse of the brightness (i.e. increased relative value represents increased darkness or immunoreactivity). Comparisons between the means of different treatments were made by Student's *t*-test.[21]

RESULTS

Previous characterization of native human profilin has revealed the formation of functionally relevant tetramers.[8] Immunoblot and SDS-PAGE analyses have shown that approximately 14.8 kDa human profilin monomers form dimers and tetramers. Furthermore, functional significance has been inferred from preferential actin binding to the tetrameric form. In light of the human profilin findings, the ability of plant profilin to form multimers was examined. Purified recombinant protein encoded by the ZmPRO1 cDNA was visualized by silver-stained SDS-PAGE separation (Figs 1,2a). A predominant band at 14.8 kDa was identified in transformed *E. coli*, particularly after IPTG induction. Other extraneous bands (> 30 kDa) presumably include multimeric profilin and/or cellular proteins that remain with the more convenient PLP affinity bead slurry. It is apparent that ZmPRO1 profilin separated by column chromatography yields a cleaner preparation that was also studied under both reduced and non-reduced conditions (Fig. 2a). A predominant band appeared as expected for monomeric ZmPRO1 plant profilin (approximately 14.8 kDa), but, consistent with human profilin, higher molecular weight proteins remained that were resistant to reducing agents. The band at approximately 60 kDa (Fig. 2a, + BME) suggests the formation of a tetramer resistant to reducing

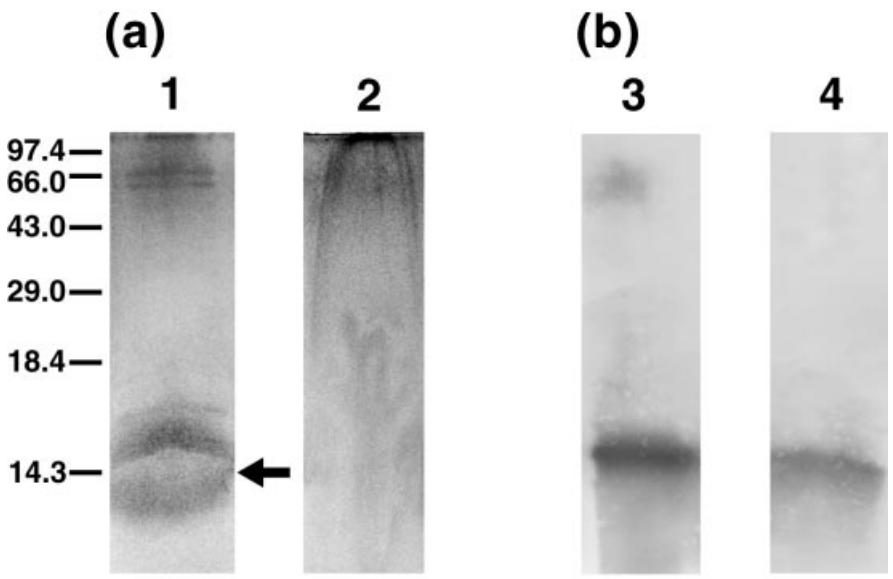

Fig. 2 Affinity column purified *Zea mays* (Zm) PRO1 from transformed *Escherichia coli*. (a) Similar to silver-stained sodium dodecyl sulfate polyacrylamide gel electrophoresis in Fig. 1, but profilin separated by affinity column chromatography as described in Methods. Lane 1, reducing conditions (+β-mercaptoethanol (BME)). Arrow depicts location of monomeric profilin; lane 2, non-reducing conditions (– BME). (b) Corresponding immunoblot of samples run in parallel with (a). Lane 3, + BME; lane 4, – BME. Rabbit anti-ZmPRO1 and horseradish peroxidase-conjugated secondary goat antirabbit IgG method was used to visualize immunologically distinct profilin proteins. Protein molecular weight marker migrations are listed on the left (in kDa).

agents, in addition to the distinct aggregation of proteins near the top of the gel (> 97 kDa). The larger proteins become more pronounced under non-reducing conditions (Fig. 2a, – BME) and are associated with a corresponding loss of monomeric profilin. The presence of stained proteins that remained in the stacking gel (not shown) further support the idea of natural protein (i.e. profilin) aggregation/multimerization. Any faint protein represented at 14.8 kDa in the absence of reducing agent becomes more evident on development of corresponding immunoblots with sensitive substrates (Fig. 2b). However, western immunoblotting gave inconsistent positive identification for the higher molecular weight profilin multimers; presumably this is due to the different efficiencies in transfer of various protein sizes, alterations in net charge that may occur on protein–protein interactions (e.g. ionic bonds as discussed by Babich *et al*.[8]), difficulty for > 90 kDa profilin multimers in migration from the stacking gel to the separating gel or a lack of antibody recognition due to epitope masking when profilin aggregates/multimerization occurs. Indeed, differences in the observed western immunoblot band intensities (monomer > tetramer >> higher orders) support these explanations. Collectively, the results suggest that, similar to native human profilin, immunologically distinct *Zea mays* profilin was produced and purified and preferentially forms multimers.

An ELISA was developed to further immunologically identify the purified recombinant protein and to provide a means to study whether human serum from allergic individuals recognizes ZmPRO1 profilin. Six representative control wells are pictured (Fig. 3), of which only those coated with the purified protein elicited a significant colorimetric response. In addition, rabbit antihuman profilin IgG did not recognize ZmPRO1 profilin (not shown), which is similar to the inability of rabbit antiplant profilin IgG to recognize human profilin.[18] Thus, a method was established with a clear signal-to-noise ratio that was selective for ZmPRO1 profilin and further verified the production of immunologically distinct plant profilin.

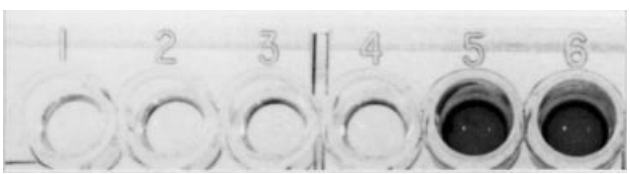

Fig. 3 Photograph of wells from enzyme-linked immunosorbent assay developed for plant profilin. Wells 1–2, secondary antibody alone; wells 3–4, primary antibody + secondary antibody; wells 5–6, primary antibody + secondary antibody + *Zea mays* (Zm) PRO1 profilin. Tris-buffered saline + ZmPRO1 profilin (i.e. negative control) gave no measurable optical density (not shown).

The developed ELISA was subsequently used to measure type I allergy patient IgE recognition of ZmPRO1 profilin. Sera from more than 30 individuals were used in preliminary work to establish conditions for the ELISA and other methods. Due to depletion of many of the sample volumes during the course of assay development and the lack of signal from the negative patient pool, sera selected for assay in the final study included a greater number of miscellaneous and positive samples (eight negative, 14 miscellaneous, nine positive, shown in Table 1). There was one positive reactivity to profilin among patients with no known allergies (one of eight; but no response from > 20 negative samples in the preliminary work to establish the assay), minimal reactivity in those with miscellaneous allergies (three of 14) and significant reactivity among those declaring type I allergies to pollen (three of six). The raw data from the three samples that gave a positive reaction to profilin (Table 2) show a strong IgE reactivity to profilin with minimal background (i.e. a relatively high ratio of the optical densities when serum was added to profilin-coated wells *vs* non-profilin coated wells). The results agree with previous work that type I allergy patients are immunoreactive to plant profilin and indicate that the current approach may be useful to screen for patients with type I allergies.

The allergenic potential of profilin monomers and multimers was tested by dot-filtration immunoblot analysis (Fig. 4). Although the ELISA is more sensitive and quantitative, the presence of reducing agents used to favor a monomeric profilin state would hinder the adsorption of profilin to plastic wells. Thus, a dot blot filtration apparatus was used to remove the reducing agents. In all instances, a greater response was measured from IgE recognition of + BME/profilin (i.e. profilin as a predominantly multimeric form) compared with – BME/profilin. In

Table 1 Enzyme-linked immunosorbent assay for human IgE reactivity with ZmPRO1 profilin

Category	+ Reaction	– Reaction
Negative	1	7
Miscellaneous	3	11
Positive	3	6

Positive (+) or negative (–) reaction of profilin with serum samples from the three patient categories ($N = 30$ samples; quadruplicate determinations for each point). Zm, *Zea mays*.

Table 2 Raw data from the positive samples

Patient sample no.	OD ($\times 10^{-3}$) of ± profilin coated wells	Declared allergies
25	940/80	Hay fever
74	630/340	Trees, pollen
90	214/12	Trees, grasses, strawberries

The ratio of optical densities (OD) from profilin coated versus non-coated wells for the three positive samples identified in Table 1 (mean OD shown; standard errors were within 10% of the mean).

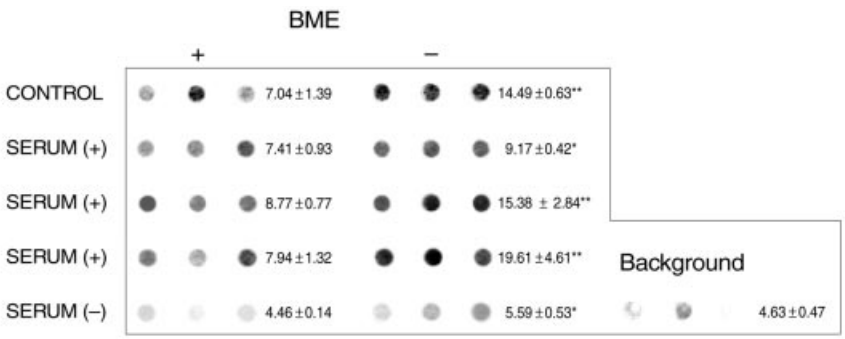

Fig. 4 Dot-filtration immunoblot of *Zea mays* (Zm) PRO1 profilin and human IgE. Profilin was adsorbed and filtered onto a dot-filtration apparatus under conditions that favor either monomers (+β-mercaptoethanol (BME)) or multimers (– BME), prior to addition of serum from patients declaring allergies (serum(+)) or without allergies (serum(–)). Control, rabbit anti-ZmPRO1 antibody (positive control). Triplicate well determinations of the colorimetric assay are shown for all samples (background, – BME + profilin + secondary antibody + metal diaminobenzidine substrate; the background was no different when + BME was included). Quantitative values (mean ± SEM) for the intensity of darkness were calculated as described in Methods and are presented next to each row. Student's *t*-test revealed significance levels of $*P < 0.05$ or $**P < 0.01$ for – BME versus corresponding + BME rows.

contrast to the relatively weak signal from negative control patients (i.e. serum(–)), IgE from the positive serum category displayed a significantly greater recognition of profilin (regardless of ± BME), thereby implicating higher profilin orders as allergens.

Discussion

The present work describes the production and purification of recombinant ZmPRO1 profilin, which yields multimeric forms. The protein was subsequently identified to be immunologically distinct through western immunoblot analysis and an ELISA that was developed to assess allergy patient IgE reactivity. An apparent tetrameric 60 kDa profilin was found in addition to higher multimeric orders (> 97 kDa) that become amplified under non-reducing conditions. Although relatively high percentage acrylamide gels were used to visualize and study monomeric or lower multimeric orders of ZmPRO1 profilin, it appears that fastidious high profilin multimers persist, despite exposure to heat and reducing agents. The findings are remarkably similar to the results obtained on characterization of native human profilin,[8] which has demonstrated tetramers to be the predominant functional multimeric form under identical non-reducing conditions. The recent identification of various plant profilin homopolymers and heteropolymers *in vitro*[22] is also consistent with the present proposal that plant profilin multimers exist and are the relevant allergenic form. The combination of near-capacity protein loading and a relatively more sensitive SDS-PAGE staining procedure appears requisite to identify the additional protein bands, compared with typical reports with Coomassie blue protein staining (e.g. Babich *et al.*[8]). In addition, plant and human profilins may be similar in their ability to resist chemical reduction. Computer-based molecular modeling of human profilin suggests a profilin–profilin interaction may occur that protects some of the disulfide bonds from harsh reducing agents (Babich *et al.*, unpubl. data, 1998; see also Mitterman *et al.*[22]). Modeling studies with plant profilin are needed to further the molecular comparison between plant and human profilin multimers and to identify relevant epitopes.

The existence of plant profilin multimers is supported by studies on birch pollen allergens. Induction of mouse and monkey IgE antibodies against recombinant Betv2 (a profilin form) has been demonstrated *in vivo*,[12] albeit to a lesser extent than another pollen, Betv1. Immunoblot and SDS-PAGE analyses have revealed that Betv2 self-associates through disulfide bonds, which have been suggested to decrease the type I immune response to the protein. In contrast, the present work is consistent withthe idea that larger molecules may elicit an immune response, such as by increased epitope presentation. Perhaps the ZmPRO1 structure differs from Betv2, in that epitopes are not blocked by multimerization and/orthe quaternary structure presents novel epitopes to the immune system. Further comparisons of the allergenic potential of various plant profilins are complicated, because it is also unknown what conformation is assumed when acquired or introduced *in vivo* (i.e. whether they form multimers *in vivo* or in solution[8]). In support of allergenic profilin multimers, recent enzyme allergosorbent tests and immunoblot inhibition studies have revealed that IgE from birch pollen allergic patients is very reactive with 34 and 35 kDa allergens,[23] of which one was thought to be a Betv1 dimer. Future analyses of known[24] and perhaps alternative epitopes, combined with computer-based molecular modeling, may shed light on optimum conformations for the allergenic potential of profilin.

Several points in support of plant profilin multimerizaton may also be raised from the recent finding of a 60 kDa cross-reactive allergen from mugwort.[25] Similar to human profilin tetramers:[8] (i) the size is alike; (ii) it is resistant to chemical breakdown; (iii) a protein 'ladder' has been detected (e.g. approximately 15, 30, 60 kDa) that is also recognized by pollen allergic patient IgE; and (iv) the N-terminus is blocked (also supported by Babich *et al.*, unpubl. data, 1998). Furthermore, (v) trypsin treatment abolishes IgE reactivity against profilin (monomer) and the 28–30 kDa allergen (dimer?), but not against the60 kDa protein, which, if the protein were to be a profilin tetramer, is consistent with the notion of a larger protein being more allergenic; (vi) consistent with the idea that the larger 60 kDa protein represents profilin multimers, monoclonal antibodies raised against the 60 kDa allergen also recognize smaller, but not larger, proteins from three different plant extracts; (vii) preincubation of patient IgE with the 60 kDa allergen (IgE-inhibition experiments) reduces subsequent IgE binding to $>$ 30 kDa proteins from plant extracts, but has little effect on IgE binding to the smaller MW species (e.g. 14–15 kDa or profilin): the latter point would also be consistent with the present notion that the 60 kDa protein is comprised of smaller proteins (e.g. 2 $\times$ 30 kDa dimers) and is more allergenic; and (viii) complementary DNA were not obtainable, which would be expected if the protein were truly a multimer(a point recognized by the investigators). Collectively, these points are consistent with the present conclusions; however, they may only represent a strong coincidence. Future studies should elucidate this issue.

The results support and extend the literature, which indicates that approximately 20% of pollen allergic individuals will show IgE reactivity to plant profilin.[9,10] Of the nine patients who declared type I allergies to plant pollens, three showed significant reactivity to ZmPRO1 profilin with the developed ELISA. Three additional serum samples from the miscellaneous category (e.g. dust) also yielded a positive response. This might be expected, considering that many type I allergy patients often include dust as an allergen; furthermore, the identification of profilin as an IgE-binding component in latex[26] raises the issue that allergens considered outside the conventional spectrum of type I candidates may indeed be recognized by type I allergy patient IgE.

The relatively greater recognition of ZmPRO1 profilin multimers by IgE reveals novel aspects of plant profilin as a proposed pan-allergen. Greater recognition of profilin multimers is not due to simple additive effects, because the same amount of total profilin was added to each well used in the dot-immunoblot experiments. Thus, it appears that profilin multimers act in synergy to either sterically facilitate access to binding sites or to present unique epitopes. In the absence of a larger sample size and defined conditions to assay specifically for profilin multimer–IgE interactions, the data thus far also suggest that: (i) more type I allergy patients than previously estimated have IgE that recognize profilin; and (ii) profilin multimers are causative agents for type I allergies. However, conditions to produce only monomers versus multimers first need to be defined to pursue these hypotheses. In addition, the current studies (including preliminary work) and those of others (e.g. Vrtala et al.,[11,12] Pauli *et al.*,[14] Astwood *et al.*[27]) indicate that other methods of detection are feasible and should be considered in future basic and clinical applications. Finally, it is recognized that, in order to truly establish a correlation between type I allergies and reactivity to profilin (particularly multimers or the plant profilin source), studies with a large number of patients, carefully screened for allergies by objective methods, are required.

The current work provides a framework for future research in the areas of allergy diagnosis and treatment (e.g. Valenta and Kraft[16]). Further verification of profilin as a pan-allergen would raise the issue of whether profilin is the causative agent in a proportion of type I allergies, as opposed to being only a correlative agent. Conceivably, therapeutic strategies may be developed to block profilin epitopes and to prevent or reverse *in vivo* self-association, thereby targeting the cause of the disease.

Acknowledgements

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