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Cross-reactivity within the profilin panallergen family investigated by comparison of recombinant profilins from pear (Pyr c 4), cherry (Pru av 4) and celery (Api g 4) with birch pollen profilin Bet v 2

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Abstract

Profilin is a panallergen which is recognised by IgE from about 20% of birch pollen- and plant food-allergic patients. Little is known about epitope diversity among these homologous proteins, and about the correlation between IgE-cross-reactivity and allergenic reactivity. Plant food profilins from pear (Pyr c 4) and cherry (Pru av 4) were cloned by polymerase chain reaction and produced in *Escherichia coli* BL21. The profilins were purified as non-fusion proteins by affinity chromatography on poly-(L-proline)-Sepharose and characterized by immunoblotting, IgE-inhibition experiments and histamine release assays. The coding regions of the cDNA of pear and cherry profilin were identified as a 393 bp open reading frame. The deduced amino acid sequences showed high identities with birch pollen profilin Bet v 2 (76–83%) and other allergenic plant profilins. Pyr c 4 and Pru av 4 were investigated for their immunological properties in comparison with profilins from celery (Api g 4) and birch pollen (Bet v 2). Fourty-three of 49 patients (88%), preselected for an IgE-reactivity with Bet v 2 showed specific IgE-antibodies to the recombinant pear protein, 92% of the sera were positive with the recombinant cherry allergen and 80% of the sera were reactive with the celery protein. Inhibition experiments showed a strong cross-reactivity of IgE with profilins from plant food and birch pollen. However, IgE binding profiles also indicated the presence of epitope differences among related profilins. All investigated profilins, Pyr c 4, Pru av 4, Api g 4 and Bet v 2, presented almost identical allergenic properties in cellular mediator release tests. Therefore, cross-reactivities between related profilins may explain pollen-related allergy to food in a minority of patients. The nucleotide sequences reported have been submitted to the Genbank database under accession numbers AF129424 (Pyr c 4) and AF129425 (Pru av 4).

Keywords: Food allergy; Cross reactivity; Profilin; Pru av 4; Pyr c 4; Api g 4; Bet v 2

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1. Introduction

Food allergy in patients sensitized to birch pollen is a well-known phenomenon [1,2]. Birch pollen allergic patients with allergic reactions to cherry, pear, apple or native celery tuber mainly suffer from oral allergy syndrome and rarely report gastrointestinal symptoms, urticaria or bronchial asthma [3,4]. The cross-reactivity of IgE to birch pollen allergens and the related food proteins is due to structural similarities of homologous major allergens [5–7]. Moreover, carbohydrate determinants and profilins were identified as IgE-cross-reactive structures. Approximately 10–20% of pollen allergic patients are sensitized to profilins [8–10].

Profilins are an ubiquitous family of actin- and phosphatidylinositol 4,5-bisphosphate-binding proteins [11,12] involved in signal transduction from the outer cell membrane to the inner cell and regulating the actin-polymerisation in non-muscle cells [13]. Profilins are small proteins with molecular masses of approximately 14 kDa. Profilin was first identified as minor allergen in birch pollen, Bet v 2 [8]. Moreover, allergenic profilins were characterized in pollen from olive tree, Ole e 2 [14], timothy grass, Phl p 11 [15], bermuda grass, Cyn d 12 [16], sunflower, Hel a 2 [17], mercury, Mer a 1 [18], and various foods such as peanut, Ara h 5 [19], soybean, Gly m 3 [20] and celery, Api g 4 [21]. Because of their ubiquitous occurrence, profilins were termed panallergens [9]. The presence of profilin-specific IgE-antibodies is a reason for cross-reactivity to botanically unrelated allergen sources. IgE against grass pollen profilin was shown to cross-react with profilins from a wide range of vegetable foods [22]. Moreover, IgE against Bet v 2 cross-reacts with profilins from celery, pear and apple [23]. Recently, profilin from celery, Api g 4, was described as Bet v 2 homologous molecule with an overall identical immunoreactivity [21].

In the present study, the complete amino acid sequences of pear and cherry profilin were deduced from their cDNA sequence. To investigate their role in birch pollen associated food allergy at the molecular level, recombinant profilins from pear, cherry and from celery were produced in *Escherichia coli*, purified as non-fusion proteins and tested for their IgE-binding properties. Moreover, the cross-reactivity between recombinant plant food profilins and Bet v 2 was investigated by inhibition experiments. Lastly, their potential to induce type I reactions was shown by mediator release from passively sensitized umbilical cord blood cells.

2. Experimental

2.1. Patients' sera

Sera from 49 birch pollen-allergic patients with a positive (at least class 2) CAP or EAST (Enzyme Allergo Sorbent Test) to birch pollen profilin (Bet v 2) were selected for this study. Most of the sera ($n=35$) were selected from the PEI serum bank, other sera ($n=5$) were obtained from Allergopharma, Reinbek, Germany and from MAST Diagnostica, Reinfeld, Germany (3). A subgroup of sera (6) was obtained from patients who presented symptoms after double blind placebo-controlled food challenge (DBPCFC) with celery ($n=3$) and hazelnut ($n=3$). Allergy diagnosis (indicated in Fig. 3) was based on clinical history of birch pollen allergy and reports of oral allergy syndrome after ingestion of various plant foods, especially fruits. The patients' reports were confirmed by CAP test and EAST with the corresponding allergens.

2.2. Recombinant allergens and protein extracts

Recombinant birch pollen profilin Bet v 2 and the major allergens from celery, rApi g 1, and birch pollen, Bet v 1 were obtained from BIOMAY, Linz, Austria. Allergen extracts from plant food and skimmed milk were prepared by a low temperature method as described elsewhere [24]. Birch pollen extract was obtained from MAST Diagnostica, Reinfeld, Germany. Recombinant celery profilin, Api g 4, was cloned and purified as described [21].

2.3. RNA purification and first-strand cDNA synthesis

Pear and sweet cherry were ground to a fine powder under liquid nitrogen. Total RNA was extracted from 100 mg powder using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). First strand cDNA was synthesized from approximately 1 μg

total RNA using a degenerated 5′-T_{16}NN-3′-primer (Eurogentec, Searing, Belgium) and the Moloney murine leukaemia virus reverse transcriptase (First-Strand cDNA synthesis Kit, Pharmacia, Freiburg, Germany) as recommended by the manufacturer. The reaction was performed at 37°C for 60 min followed by heat denaturation at 99°C for 5 min.

2.4. Polymerase chain reaction

For cDNA amplification, a degenerated oligonucleotide primer Prof5(deg) 5′-ATG TCG TGG CAR RCG TAC GT-3′ (ARK, Darmstadt, Germany) (R: A/G) was designed according to the known N-terminal amino acid sequence of different plant profilins. For the reverse direction the 3′-primer 5′-T_{16}NN-3′ was used. The first-strand cDNA amplification was performed in a final volume of 100 μl, containing 0.1 μmol of dNTPs (Pharmacia, Freiburg, Germany), 20 pmol of each primer and of 2 μl of first-strand cDNA synthesis product as template. After denaturation at 94°C for 1 min 2.5 U Taq DNA polymerase (Pharmacia, Freiburg, Germany) was added. The sample was subjected to 30 cycles (1 min denaturation at 94°C, 1 min annealing at 52°C and 1 min extension at 72°C, and a final extension for 5 min at 72°C).

2.5. 5′RACE (Rapid Amplification of 5′cDNA Ends)

For amplification of the unkown 5′-end of the food profilins the 5′RACE-method was used according to the manufacturer's instructions (5′-RACE System, GIBCO BRL, NY, USA). First-strand cDNA synthesis was primed with an antisense gene specific primer PE3(211–230) 5′-ACC ATG TAC TTT GTC CCA CC-3′ for pear and CH3(211–230) 5′-ACC ATA TAT TTT GTC CCA CC-3′ for cherry. A homopolymeric dC-tail was ligated to the 3′-end of the purified cDNA by a terminal desoxynucleotidyltransferase reaction. The dC-tailed cDNA was amplified by PCR using a nested second gene specific primer PE3(187–203) 5′-AAT CCA GTT GGG GCA AG-3′ for pear, CH3(187–203) 5′-AAC CCA GTT GGG GCA AG-3′ for cherry, and an abridged anchor primer (enclosed in the 5′-RACE system).

2.6. Cloning and sequencing of profilin cDNA

The purified profilin cDNAs were ligated into pGEM-T (pGEM-T Vector System I, Promega, Wi, USA) and transformed in the *E. coli* strain XL1-Blue (Stratagene, Heidelberg, Germany). Positive clones were selected by a blue/white-screening and PCR screening with the T7 promoter primer 5′-TAA TAC GAC TCA CTA TAG G-3′ and the SP6 primer 5′-GAT TTA GGT GAC ACT ATA GA-3′. DNA sequence analyses of the subsequently isolated plasmids were performed with an ABI 373 or 377 ''stretch'' automatic fluorescent sequencer (Perkin-Elmer, Weiterstadt, Germany).

2.7. Expression of recombinant profilins

For ligation into the pET-30a expression vector (Novagen, USA) Nde I and Hind III, restriction sites were introduced at the 5′- and 3′-ends of the profilin cDNAs by PCR using the universal primer PROF5Nde 5′-GGA ATT CCA T**AT G**TC GTG GCA GGC GTA CGG TG-3′ and the primers PE3Hind 5′-CCC AAG CTT GGG **TTA** TAG GCC TTG ATC AAT CAG G-3′ for pear and CH3Hind 5′-CCC AAG CTT GGG **TTA** CAG ACC CTG CTC GAT AAG G-3′ for cherry (restriction sites are underlined, start and stop codons are printed in bold). To obtain non-fusion proteins the N-terminal His-tag sequence of pET-30a was deleted by Nde I and Hind III restriction enzymes. Initial cloning was done in *E. coli* strain JS5 (BioRad, Munich, Germany). Positive clones were selected by analytical restriction cuts using the enzymes Nde I and Hind III, PCR screening with the vector-specific primer T7 promoter and the gene specific primer PROF3Hind, and by sequencing. For target gene expression the plasmid was transformed into *E. coli* BL21(DE3) cells (Novagen, USA). The cells were grown in LB-medium supplemented with 50 μg/ml kanamycin to an absorbance of 0.7 at a wavelength of 600 nm. Isopropyl-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 m*M*, and incubation was continued for 4 h at 30°C. Cells were harvested by centrifugation and stored at −70°C.

2.8. Purification of recombinant profilins

The pellet was resuspended in 20 ml of lysis buffer (50 m*M* Tris–HCl, 100 m*M* NaCl, pH 7.5) and disrupted by freezing in liquid nitrogen. Insoluble material was removed by centrifugation at 15 900 *g* for 45 min. Proteins from 1 litre of supernatant were applied overnight at 4°C to a poly-L-proline (PLP) -column according a modified method described by Lindberg [25]. Briefly, the column was prepared by coupling 34 mg PLP (Sigma, Deisenhofen, Germany) to 1 g freeze dried CNBr-activated Sepharose 4B (Pharmacia, Freiburg, Germany). Coupling of PLP was performed at 4°C overnight in 0.1 *M* $NaHCO_3$, 0.5 *M* NaCl, pH 8.3. The supernatant was incubated overnight at 4°C with the prepared PLP-Sepharose. The column was washed with 50 ml of lysis buffer. Finally, the profilin was fractionally eluted with 6 *M* urea in lysis buffer, pooled, dialyzed three times within 30 h against decreasing amounts of urea (4 *M*, 2 *M*, water) and lyophilized. After reconstitution, determination of the protein concentration was performed with the Coomassie Plus Protein Assay Reagent (Pierce, Rockford, IL, USA) according to the Bradford method with bovine serum albumin as standard.

2.9. SDS–PAGE and immunoblotting

Purified recombinant profilins (2 μg/cm slot) were analysed by SDS–PAGE under reducing conditions according to Laemmli [26]. Proteins were visualized by Coomassie Brilliant Blue R250 staining or immunoblotting. For immunoblot analysis, recombinant profilins (0.5 μg/cm slot) were electrophoretically transferred onto nitrocellulose membranes (0.45 μm, Schleicher and Schuell, Dassel, Germany) by tank blotting. IgE-binding of patients' sera (1:6.7 diluted, overnight incubation) was detected with an alkaline phosphatase (AP)-labelled mouse anti-human IgE monoclonal antibody (1:1000, 4 h) (Pharmingen, Hamburg, Germany). Antibody binding was visualized by the ''AP Conjugate Substrate Kit'' (BioRad, Munich, Germany).

2.10. Immunoblot inhibition

For immunoblot inhibition of IgE-binding to rBet v 2, 50 μl of patient serum was preincubated for 2 h with 1 to 10^{-4} μg rBet v 2, rApi g 4, rPru av 4 and rPyr c 4, respectively, and 10 μg rApi g 1 as control. Thereafter, samples were diluted to 1 ml PBS containing 0.05% Tween 20 and 0.1% BSA and incubated with 3 mm wide blot strips. Bound IgE was detected with a rabbit anti-human IgE antiserum (1:4000, 1 h) (DAKO, Glostrup, Denmark), followed by a biotin-labeled goat anti-rabbit immunoglobulin antibody (1:6000, 1 h) (DAKO, Glostrup, Denmark) as the secondary antibody and streptavidin labeled with horseradish peroxidase (1:8000, 1 h) (Medac, Hamburg, Germany). Visualization was accomplished by enhanced chemiluminescence (''ECL™ Western blotting detection reagents'', Amersham, Buckinghamshire, UK). Quantification of the stained bands was performed with the program GELSCAN 3D (Bioscitec, Marburg, Germany).

2.11. Histamine release from umbilical cord blood

Fresh human heparinized umbilical cord blood (3 ml) was washed with PIPES buffer (10 m*M* Pipes, 137 m*M* CH_3COONa, 5 m*M* CH_3COOK, 0.6 m*M* $CaCl_2$, 1.1 m*M* $MgCl_2$, pH 7.4 adjusted with 1 *M* Tris) and passively sensitized with 1 ml of patient serum for 2 h at 37°C. After washing, cells were incubated with 10 ng rIL-3/ml blood (R&D, Wiesbaden, Germany) for 30 min at 37°C, aliquoted (25 μl) into each well of a fibre plate (RefLab, Copenhagen, Denmark). Cross-linking of sensitized cells was performed with 25 μl of recombinant allergen or allergen extract in a dose-dependent manner (1:3.5 dilutions of recombinant allergen with a maximal concentration of 1 μg/ml and allergen extract with a maximal protein concentration of 10 μg/ml) for 1 to 2 h at 37°C. Serum from a non-allergic patient, milk extract and rBet v 1 were used as controls. Histamine release was measured as described [27].

3. Results

3.1. Cloning of profilins, cDNA and sequence analysis

PCR amplification of single strand cDNA using the degenerate 5′-primer Prof5(deg) and the 3′-primer T_{16}NN-3′ resulted in a dominant fragment of

approximately 700 bp. Sequencing of cDNA from pear and cherry revealed a 393 bp open reading frame coding for a protein of 131 amino acids, respectively. The unknown 5′-end of the cDNAs, obtained by PCR with degenerate primer was analyzed by 5′RACE. In all cases, the PCR resulted in a fragment of 300 bp length with an overlap of approximately 200 bp with the coding region of the respective profilin. The complete deduced amino acid sequences comprised proteins with calculated molecular masses of 14.0 kDa and calculated p*I*'s of 4.47 for cherry and 5.05 for pear. The sequence analysis by PROSITE search [28] revealed two potential phosphorylation sites K_{86}KGS for cAMP- or cGMP-dependent protein kinases and T_{93}IK for protein kinase C, a profilin consensus sequence S_2WQAYVD and no putative N-glycosylation sites (NXT/S) for the investigated profilins. Comparison of the profilin amino acid sequences with the SWALL Protein Database showed high identities (82.7% for Pyr c 4 and 76.1% for Pru av 4) with profilin from birch pollen, Bet v 2 (Acc. No.: M65179). Moreover, amino acid identities of 82.1% for Pyr c 4 and 76.9% for Pru av 4 were observed with profilin from celery, Api g 4 (Acc. No.: AF129423) (Fig. 1). High sequence identities of 68–86% were obtained with other plant profilins recently described as allergens according to the official allergen list of the WHO/IUIS allergen nomenclature subcommittee (ftp://biobase.dk/pub/who-iuis/allergen.list). The size of all analyzed profilins ranged from 131 to 134 amino acid residues.

3.2. Expression and purification of rPyr c 4 and rPru av 4

Profilins were expressed as non-fusion proteins in *E. coli* BL21 (DE3) after induction with IPTG for 4 h at 30°C using a modified pET-30a vector. Recombinant Pyr c 4 and Pru av 4 were recovered as inclusion bodies in the pellet and as soluble material in the supernatant. The soluble cherry and pear proteins were further purified on PLP-coupled sepharose. After elution with 6 *M* urea, highly pure recombinant proteins with apparent molecular masses of approximately 14 kDa were isolated (Fig. 2). After dialysis 1–3 mg of recombinant proteins were obtained from 1 litre of bacterial culture.

3.3. Immunological characterization of recombinant profilins

IgE-reactivity to rPyr c 4, rPru av 4 and rApi g 4 was investigated by immunoblotting experiments (Fig. 3). Sera from 49 patients with IgE-reactivity to

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                1         10         20         30         40         50         60
                .         .          .          .          .          .          .
Bet v 2         MSWQTYVDEHLMCDIDGQASNSL-ASAIVGHDGSVWAQSSSFPQFKPQEITGIMKDFEEPGHLAPTGLHL
Api g 4         ....A...D....EVE.NPGQT.T.A..I..........T...I..E..A......D.........Y.
Pyr c 4         ....A...D........H--HLT-.A..L..........T..K...E...A.....D...S........
Pru av 4        ....A...D.........---.R.T.A..L.Q.....S..AT..A...E..AA.L..LDQ..T......F.

                70         80         90         100        110        120        130    AA  AA-ID(%)
                .          .          .          .          .          .          .
Bet v 2         GGIKYMVIQGEAGAVIRGKKGSGGITIKKTGQALVFGIYEEPVTPGQCNMVVERLGDYLIDQGL  133
Api g 4         ..A........PN...........V............V.D.........VI.............  134  79.9
Pyr c 4         ..T........G............V.V...S...........L.......I.............  131  82.7
Pru av 4        ..T.......................V...N...II...D..L.......I.........E...  131  76.1
```

Fig. 1. Amino acid sequence comparison of food profilins Pyr c 4 (Acc. No.: AF129424) from pear, Pru av 4 (Acc. No.: AF129425) from cherry and Api g 4 (Acc. No.: AF129423) from celery with the birch pollen profilin Bet v 2 (Acc. No.: M65179). Dots indicate identical amino acids.

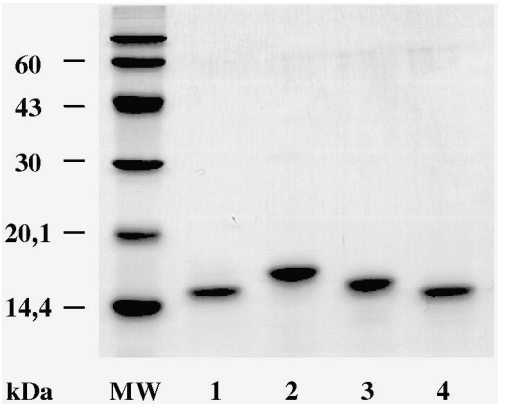

Fig. 2. SDS–PAGE and Coomassie Brillant Blue staining of purified rBet v 2 (1), rApi g 4 (2), rPyr c 4 (3) and rPru av 4 (4).

rBet v 2 were selected for immunoblotting studies. All of the patients were suffering from pollinosis, and most of them reported oral symptoms after ingestion of various fruits. Histories of allergy to pear, cherry and celery are indicated in Fig. 3. Serum from a non-allergic patient, PEI22, was used as control. Fourty-three of 49 (87.8%) sera exhibited IgE to recombinant pear profilin. Oral allergy to fresh pear was known for 15/49 patients. Within this group of allergic patients IgE-binding to rPyr c 4 was shown in all cases. Moreover, IgE-reactivity to rPyr c 4 was obtained for 15 of 16 sera without history of allergy to pear. IgE from 45 sera (91.8%) tested bound to rPru av 4. All patients with allergy to cherry (13/49) showed IgE-reactivity to the cherry profilin. Additionally, antibody-binding to Pru av 4 was obtained with sera from 17 patients not allergic to cherry. Moreover, 39 of 49 sera preselected for IgE binding to Bet v 2 were reactive with Api g 4. Within this group 9/10 celery allergic patients had IgE to the celery profilin. The serum of the non-allergic volunteer presented no specific IgE to any of the recombinant molecules.

3.4. Inhibition experiments

Dose-dependent IgE-immunoblot inhibition experiments were performed to investigate the cross-reactivity of IgE with rPyc c 4, rPru av 4, rApi g 4 and rBet v 2 (Fig. 4). Three sera from patients with pollinosis and additional food allergy to pear, cherry and celery were selected for inhibition of IgE-binding to rBet v 2 blotted onto NC. All sera showed IgE-reactivity to the three recombinant food profilins when tested by EAST (not shown) and immunoblotting (Fig. 3, lane 6: PEI61; lane 12: PEI131; lane 13: PEI133). Pre-incubation of the sera was performed with 10^{-4} to 1 μg of rPyc c 4, rPru av 4, rApi g 4 and rBet v 2, respectively. Complete inhibition of IgE-binding of all serum samples to the recombinant birch profilin was obtained with 0.1 μg rBet v 2 used as positive control. For serum PEI61, inhibition curves identical to rBet v 2 were obtained for the inhibitors rApi g 4 and rPru av 4. Only 60% inhibition of IgE-binding was observed with rPyr c 4 at the maximal concentration. Moreover, preincubation of serum PEI133 with the highest amount of 1 μg recombinant food profilins showed a maximal inhibition of antibody binding of approximately 77% for rPru av 4, 63% for rPyr c 4 and 52% for rApi g 4, respectively. For serum PEI131 only a weak inhibition of IgE-binding (ranging from 10 to 28%) to rBet v 2 was measured with the investigated plant food profilins. No inhibition was observed with the non-related major celery allergen rApi g 1 [6].

3.5. Histamine release

Histamine release from umbilical cord blood (Fig. 5) was performed with sera from patients PEI61, PEI131 and PEI133 who were allergic to pollen and suffered from multiple food allergies to pear, cherry and celery. Two additional sera from patients allergic to pollen and without additional allergy to any of the investigated foods (serum Ken), and with an associated allergy to cherry without symptoms to pear and celery (serum Bo52) were selected. IgE-reactivity to the recombinant profilins was confirmed by positve EAST (not shown) and immunoblotting (Fig. 3; lane 16: Ken; lane 34: Bo52). Cross-linking of passively sensitized umbilical cord blood cells was performed with recombinant profilins (rPyc c 4, rPru av 4, rApi g 4 and rBet v 2) and rBet v 1, the major birch pollen allergen [29] (0.01–10 μg/ml) and the corresponding allergen extracts (1–100 μg/ml). Skimmed milk protein and serum from a non-allergic subject were used as controls. With all recombinant profilins a maximal histamine release of 20–45 ng/ml was measured. For most sera the mediator release curves

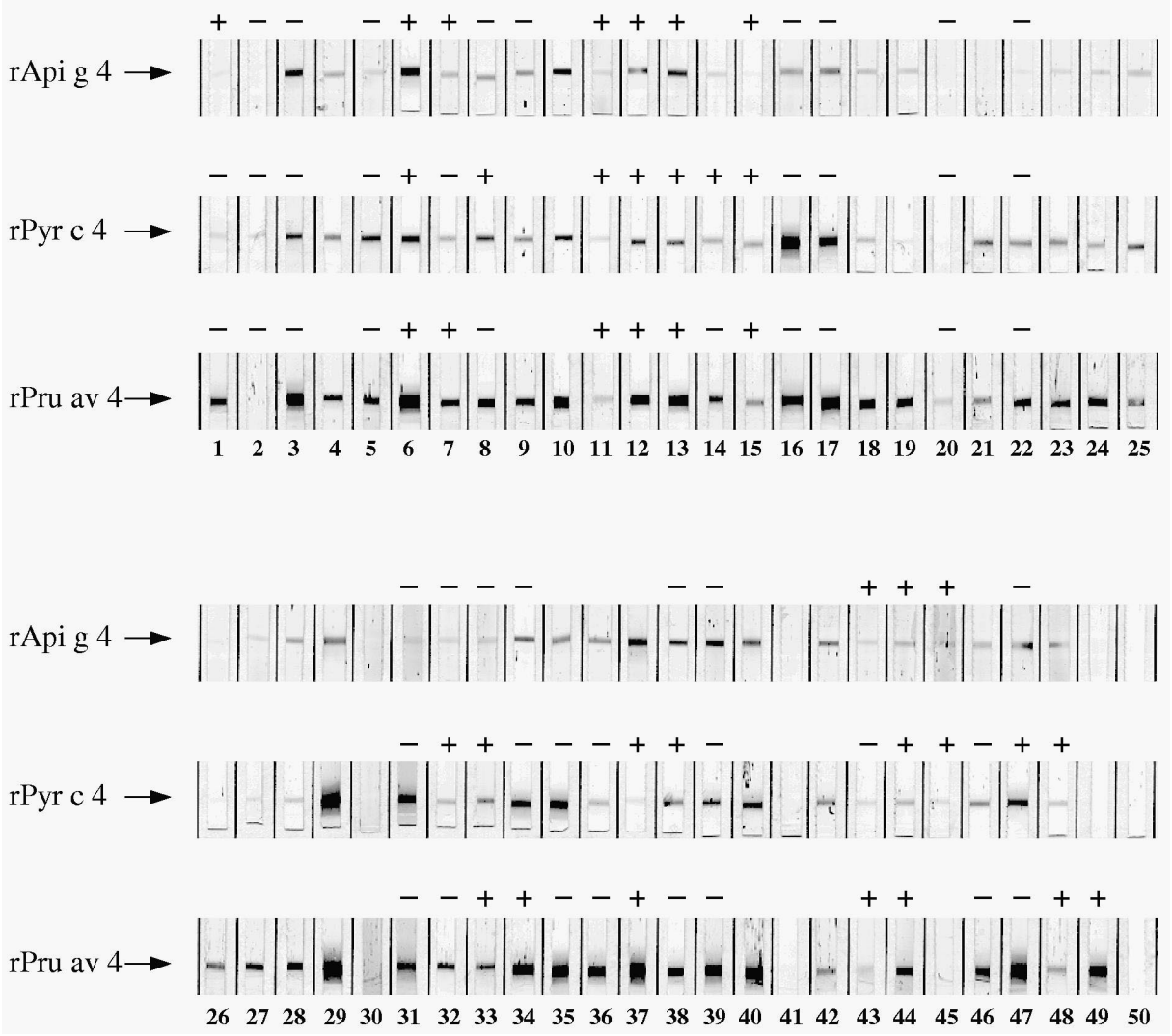

Fig. 3. IgE-immunoblot of purified rApi g 4, rPyr c 4 and rPru av 4 with 49 sera from birch pollen-allergic patients, preselected by positive IgE to Bet v 2 (lane 1–49) and a serum from a non-allergic control (lane 50). Patients with positive case history of food allergy are indicated by "+", patients without a corresponding allergy are marked with "−". Blanks indicate an unkown clinical history. Sera PEI61 (lane 6); PEI131 (lane 12); PEI133 (lane 13) were selected for inhibition experiments and histamine release. In addition, sera Ken (lane 16) and Bo52 (lane 34) were used for mediator release assays.

obtained with the profilins showed an overall identical pattern. In comparison to the food profilins only with serum Ken a significantly higher mediator release was triggered with the birch pollen profilin. In contrast to all investigated recombinant profilins a clearly higher histamine release was obtained with rBet v 1, except for serum Ken where Bet v 1 did not release mediators from the cells. For example, with patient PEI131, histamine concentrations up to 35 ng/ml were obtained in the supernatant after cross-linking of IgE with Bet v 1 whereas a maximal concentration of approximately 20 to 25 ng/ml was obtained with the minor allergens. All patients clearly demonstrated a mediator release in response to the allergen extracts from the correspnding foods. For all sera the strongest mediator release with

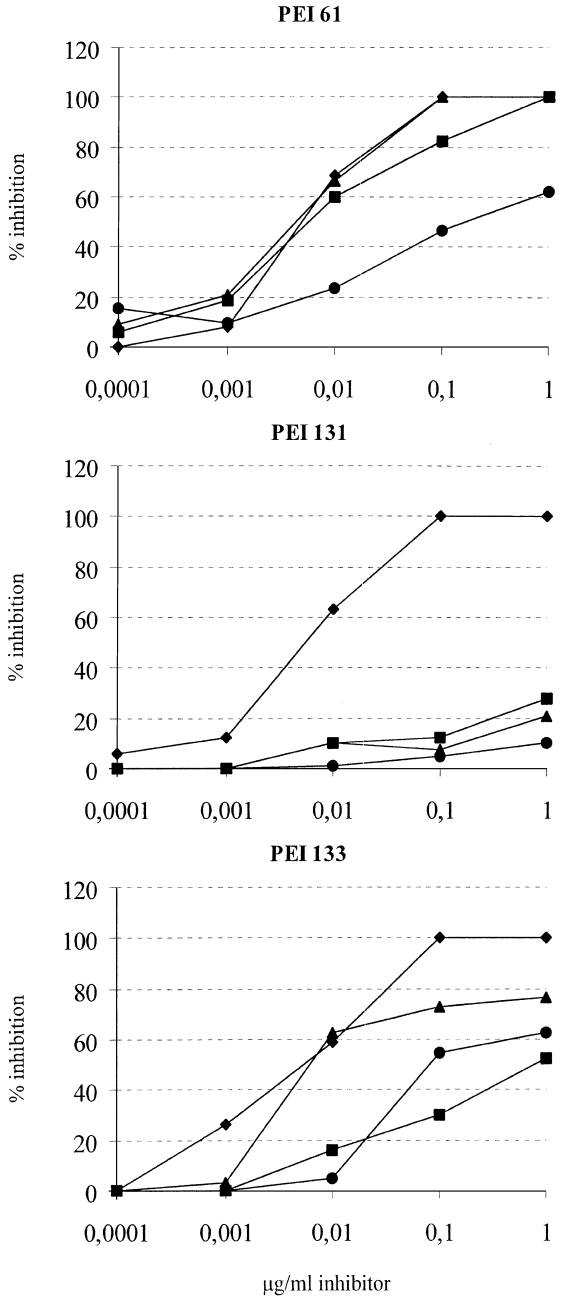

Fig. 4. Dose-related IgE-immunoblot-inhibition of IgE-binding to rBet v 2 on the solid-phase after preincubation of the patient sera PEI61, PEI131 and PEI133 with rBet v 2 (♦), rApi g 4 (■), rPyr c 4 (●) and rPru av 4 (▲) as inhibitors. Quantification of the stained bands was performed with the program GELSCAN 3D in relation to probes without inhibitor. Inhibition with an irrelevant allergen (10 µg Api g 1) and serum from a non-allergic subject were used as controls (not shown).

allergen extracts was obtained with extract from birch pollen. The non-allergic control (not shown) and milk extract triggered negligible histamine release.

4. Discussion

The coding regions of the identified profilins from pear (Pyr c 4) and cherry (Pru av 4) comprise 393 bp coding for proteins with a length of 131 amino acids and a calculated molecular mass of approximately 14 kDa. These allergens belong to the conserved family of plant profilins, as confirmed by high sequence identities of 68–86% to known allergenic plant profilins. The profilins were expressed and isolated as highly pure non-fusion proteins from the soluble fraction of the bacterial lysate. However, differences in the apparent molecular mass of the recombinant molecules were observed in SDS–PAGE. This phenomenon can not be explained by post-translational modifications sites, such as phosphorylation, which may be the reason for the detection of profilins with different molecular masses in the same plant source [30,31].

Immunological characterization was performed with Pyr c 4 from Maloideae, Pru av 4 from Prunoideae and extended to the celery profilin Api g 4 belonging to the family of Apiaceae. For screening of IgE-binding sera from 49 patients were preselected for IgE-reactivity to Bet v 2. Fourty-three sera (87.8%) showed IgE-binding to recombinant Pyr c 4, 45 sera (91.8%) were reactive with rPru av 4 and 39 of the sera showed a specific IgE-binding to Api g 4. All preselected sera from patients with oral allergy to pear (15), cherry (13) and 9/10 sera from celery allergic patients contained IgE specific for the corresponding profilin. This prevalence of IgE against profilins is not representative for patients with pollen-related food allergy. In concordance with the known IgE-prevalence of approximately 10–20% against profilin in pollen allergic patients [4,9,22] the molecules from pear and cherry were classified as new minor allergens and named Pyr c 4 and Pru av 4 according to the revised allergen nomenclature [32]. The results showed a very good positive predictive value for the presence of profilin-specific IgE which means that all of the allergic subjects were picked

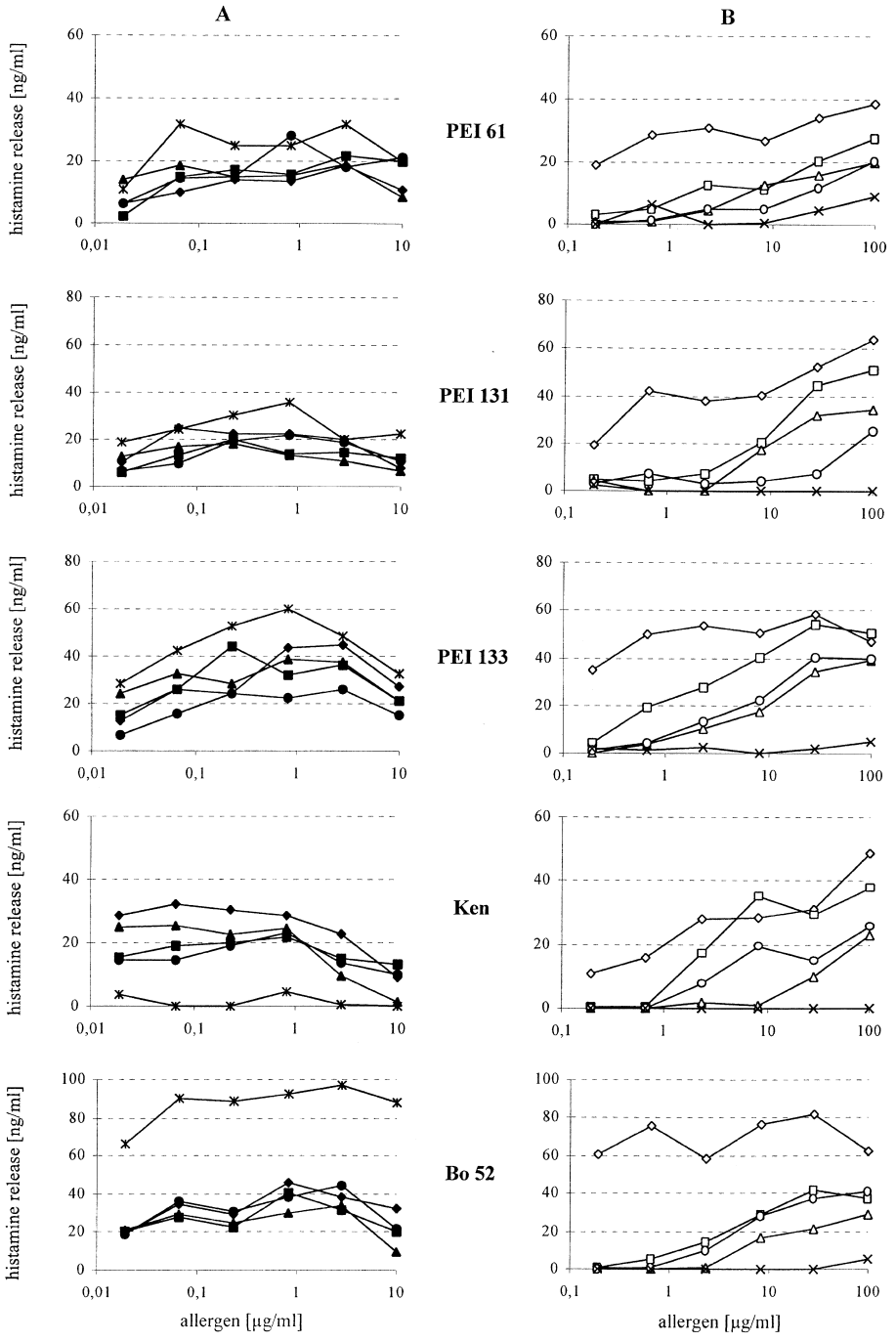

Fig. 5. Histamine release from umbilical cord blood. Cells were passively sensitzed with sera from patients with pollen associated food allergy to pear, cherry and celery (PEI61, PEI131, PEI133), with a serum from a patient allergic to pollen without additional allergy to any of the investigated foods (Ken), and with a serum from a patient with an associated allergy to cherry and no symptoms to pear and celery (Bo52). Cross-linking was performed with rBet v 2 (♦), rApi g 4 (■), rPyr c 4 (●), rPru av 4 (▲) and rBet v 1 (asterisk) as positive control (A), and with extracts from celery (□), pear (○), cherry (△), birch pollen (◇) and milk (×) as negative control (B). Additionally, serum from a non-allergic subject was applied (not shown).

up, but also very poor negative predictive values which means that most of the Bet v 2-positive subjects who did not suffer from a particular food allergy had positive IgE-reactivity to the allergens. Regarding to diagnosis of food-allergic reactions, IgE reactivities to profilins are not very specific markers.

Profilins were described as panallergens in botanically closely related [33] and distantly related plants [22,23,30]. Cross-reactivity of Bet v 2-specific IgE with these homologous molecules are one explanation for birch pollen associated food allergies and the birch-mugwort-celery syndrome [33–35]. In this study, the cross-reaction of purified rPyr c 4, rPru av 4 and rApi g 4 with rBet v 2 was investigated by IgE-inhibition experiments. Differences in the inhibition potency of the food profilins in comparison to Bet v 2 were observed depending on the individual serum applied. The results are in accordance with the observation of not overall identical IgE-epitopes of rApi g 4 and rBet v 2 [21]. The occurrence of differences in the IgE-epitopes of plant profilins from different species [29] and from the same taxonomical family was reported recently [32]. The fact that a small set of 10 patients' sera were not reactive with one or two of the three investigated food profilins may also be explained by differences in the IgE-epitopes resulting in differences of the IgE-affinity. We also have to consider that the recombinant profilins represent only one isoform of a set of isoforms in the natural extract [30]. However, the overall observed strong cross-reactivity between the birch and plant profilins [21,23,36,37] is due to homologue IgE-epitopes and most likely based on the common tertiary structure [38,39].

Birch pollen associated food allergies are based on primary sensitization by Bet v 1, the major birch pollen allergen [40]. Moreover, Bet v 1 showed the strongest IgE-reactivity among the major allergens from the Bet v 1-family, and about 100–1000 fold higher IgE-reactivity when compared to related food allergens. Bet v 1 comprises all IgE-binding epitopes of the major allergens from associated plant foods [7]. In comparison to the profilins from plant foods, Bet v 1 showed the highest allergenic potency when tested in umbilical cord blood mediator release, except for patient Ken who is monosensitized against profilin. By contrast, rBet v 2 and recombinant profilins from celery, pear and cherry induced histamine release in the same order of magnitude from passively sensitized umbilical cord blood cells, indicating no or little differences of allergenic potency between food and pollen profilins. It is worth mentioning the discrepancy in the correlation between diagnosis of type I allergy and the tested IgE-reactivity and allergenic potency of the profilins, a well known phenomenon reported earlier for Bet v 2 [4,8]. Since most of our patients presented IgE specific for Bet v 1, it is difficult to draw conclusions regarding the relevance of profilin-specific IgE for the food allergy of the patients. On the one hand, the data of patient Ken who had no IgE against Bet v 1 showed that a strong IgE response to profilin and even remarkable histamine release can be without clinical relevance. On the other hand, three sera obtained from patients with a positive DBPCFC to celery [40] contained IgE against Api g 4, indicating that profilin can be a clinically relevant allergen in celery-allergic patients.

In conclusion, the recombinant profilins may serve as a tool for more accurate diagnostic measures to verify the sensitization pattern of allergic patients and can be used for further immunological characterisation of the cross-reactivity between plant profilins. Investigation of the tertiary structure may be helpful to identify cross-reactive epitopes of this protein family.

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