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cDNA cloning and expression of timothy grass (*Phleum pratense*) pollen profilin in *Escherichia coli*: comparison with birch pollen profilin

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SUMMARY: Profilin, an actin-binding protein, was previously described as a ubiquitous allergen which is responsible for cross-reactivities in about 20% of pollen and food allergic patients. A complete cDNA clone coding for timothy grass (*Phleum pratense*) pollen profilin was isolated using allergic patients IgE. The deduced amino acid sequence of timothy grass profilin shares a sequence identity of 79% with birch profilin and other plant profilins and a lower average sequence identity of 35% with other eukaryotic profilins. The high degree of homology among different plant profilins at the DNA and protein level explains the extensive cross-reactivities observed in profilin allergic patients. Recombinant timothy grass pollen profilin was expressed in *Escherichia coli* as a ß-galactosidase fusion protein and shown to bind IgE from profilin allergic patients similar to recombinant birch profilin. Slight differences regarding the IgE-binding capacity of birch and timothy grass profilin indicate that not all

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IgE-epitopes of the two profilins are conserved. It is speculated that profilin allergic patients were initially sensitized against a certain profilin and then cross-react with the homologous proteins.

Birch pollen profilin was initially discovered as important pollen allergen (1, 2). More recently plant profilins were shown to bind plant and mammalian actin (3, 4, 5) and also to interact with polyphosphoinositides (Drobak et al., submitted). Profilin allergic patients showed extensive IgE cross-reactivities which are most pronounced with plant profilins derived from pollen and other tissues of monocot and dicot plants (2, 6, 7). In addition, plant profilins could be isolated by poly-(L-proline) affinity chromatography and their immunological properties were found to be similar to recombinant birch profilin (2). To attribute the immunological similarity of plant profilins to the primary structure, a cDNA coding for timothy grass (*Phleum pratense*) pollen profilin was isolated using IgE from a profilin allergic patient by expression screening. The cDNA and deduced amino acid sequence revealed a more than 75% identity of plant profilins (4) compared to a low sequence identity of 35% of plant profilins with other eukaryotic profilins (3). The high degree of sequence homology among the plant profilins provided an explaination for the IgE- cross-reactivities among profilin allergic patients. Using recombinant birch profilin which we had expressed as a non-fusion protein in *Escherichia coli* (8) and timothy grass pollen profilin expressed as a ß-galactosidase fusion protein, the IgE-binding capacity of both recombinant proteins was compared. Profilin allergic patients showed IgE-cross-reactivity with birch and timothy grass profilin. Differences in the IgE-binding capacity of the proteins indicate that some patients were initially

sensitized against one of the allergens and then displayed cross-reactivity with the homologous proteins.

MATERIALS AND METHODS

Materials. Pollen from birch (*Betula verrucosa*) and timothy grass (*Phleum pratense*) that were more than 95% pure as determined by light and electronmicroscopy were purchased from Allergon, AB (Välinge, Sweden). Recombinant birch profilin was expressed in pKK223-3, *E. coli* JM105 (8) and was purified as described (9, 10, 2).

Antibodies. Serum IgE specific for plant profilins was characterized as described (8, 11). Rabbit anti-profilin antibodies were used to characterize recombinant profilins. A rabbit antibody designated RP1 was raised against purified celery root profilin (6), two rabbit antibodies, RP2 and RP4, were raised against purified recombinant birch profilin that had been expressed in *E. coli* JM105/pKK223-3 and RP3 was raised against the 25 C-terminal amino acid peptide of birch profilin conjugated to keyhole limpet hemocyanin (3).

Construction and IgE-immuno-screening of the timothy grass pollen cDNA library. Total RNA was prepared from pollen of timothy grass and subjected to oligo-dT-cellulose chromatography (12). cDNA was synthesized using a cDNA-synthesis kit from Amersham, London, UK. Blunt-ended cDNA was methylated with EcoRI methylase (Promega, Madison, WI, USA), ligated to EcoRI linkers (Boehringer Mannheim, Germany) cut with EcoRI (Boehringer Mannheim, Germany). cDNA was separated from linkers with Nick columns (Pharmacia, Uppsala, Sweden), was ligated into the EcoRI-cut, dephosphorylated lambda gt11 arms (Pharmacia, Uppsala, Sweden) and then packaged *in vitro* (Amersham, London, UK). 200,000 recombinant phage were

screened with IgE from profilin-allergic patients (13, 14). Immunepositive clones were also tested for reactivity with rabbit anti-profilin antibodies RP1, RP2, RP3, and RP4.

Subcloning, DNA sequencing of profilin from timothy grass pollen and sequence comparison with other profilins. The recombinant lambda gt11 phage expressing profilin from timothy grass pollen were isolated with serum IgE from a profilin-allergic patient and tested for reactivity with rabbit anti-profilin antibodies. Lambda DNA was prepared, and the KpnI/SacI fragment containing the insert together with two flanking fragments of lambda gt11 DNA was subcloned into pUC18 and transformed into *E. coli* XL-1 blue (15). Plasmid DNA was prepared and both strands sequenced (16) using synthetic oligonucleotide primers. All DNA manipulations followed current methods (15, 12). DNA and amino acid sequences of timothy grass profilin are to be submitted to GenBank.

Expression of recombinant timothy grass and birch pollen profilin, immunoblotting. Recombinant birch profilin was expressed in *E. coli* JM105, pKK 223-3 as a non-fusion protein and purified as described (2). Timothy grass pollen profilin was expressed as a ß-galactosidase fusion protein upon infection of lysogenic *E. coli* Y1089 with recombinant lambda gt11 phage as described for *Phl p* II a major timothy grass pollen allergen (17). Proteins were separated by denaturing SDS-polyacrylamide gel electrophoresis (18) and blotted to nitrocellulose (19). IgE-immunodetection was done as described (2).

RESULTS

Isolation of a cDNA encoding profilin from timothy grass pollen. A cDNA clone encoding birch profilin had been isolated previously (1). To compare sequences from a dicot profilin with the profilin from a monocot, a lambda gt11 cDNA library comprising $2x10^7$

clones with >90% recombinants was constructed from pollen mRNA of timothy grass. One of the clones which was obtained by screening with serum IgE from a profilin-allergic individual reacted with serum IgE from several profilin-allergic patients (8, 2) and with rabbit anti-profilin antibodies (3). The cDNA was subcloned into plasmid pUC18, and both DNA strands were sequenced (16) using a forward sequencing primer (Pharmacia, Uppsala, Sweden), a M13/pUC reversed sequencing primer (Boehringer, Mannheim, Germany) and specific oligonucleotide primers (Schmidheini, Windisch, Switzerland). One open reading frame encoded a polypeptide of 131 amino acid residues with end to end homology with profilins.

Sequence comparison of plant profilins with profilins from other eukaryotes. Comparison of the cDNA and deduced amino acid sequences of profilin from timothy grass pollen with birch pollen profilin is displayed in Figures 1 and 2. Although dicots and

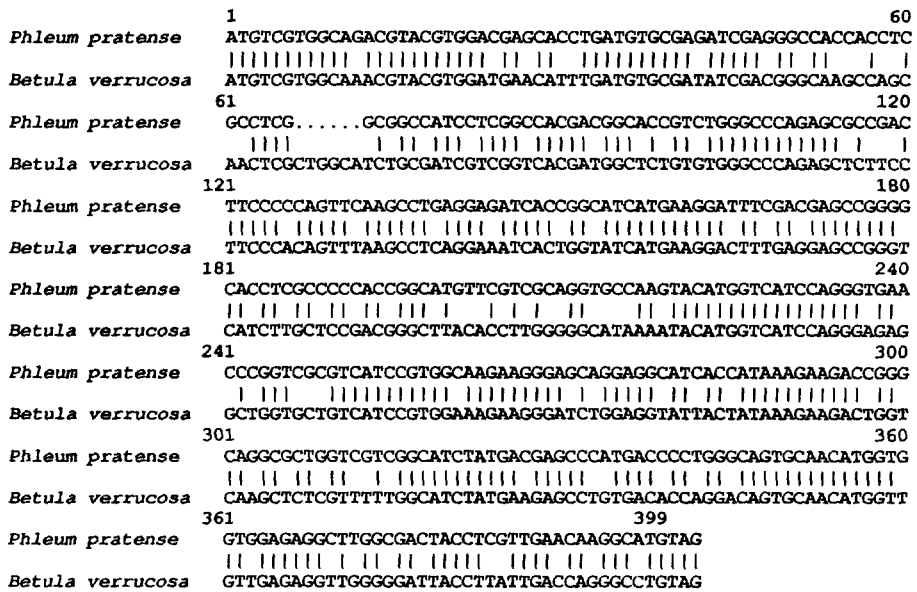

FIGURE 1. Comparison of the nucleotide sequences (open reading frames) from timothy grass pollen profilin and birch pollen profilin. Gaps are indicated by periods.

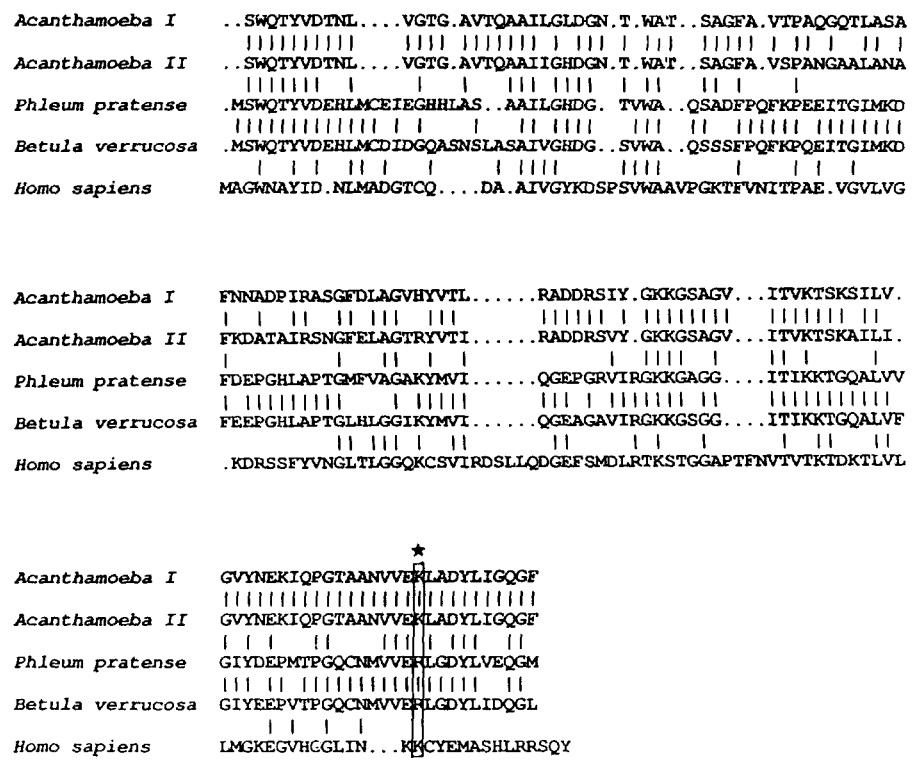

FIGURE 2. Comparison of the amino acid sequences of birch and timothy grass pollen profilin with *Acanthamoeba* profilin isoforms I/II, and human profilin. Identical amino acids are indicated by vertical bars. The arginine residues in the plant sequences which correspond to lysine 115 in the *Acanthamoeba* sequences are *boxed* and indicated with an *asterisk*.

monocots are distantly related in evolution, birch and timothy grass profilin cDNA sequences are closely related. The identities in the first, second and third bases of codons are 83%, 95% and 52% (average 77%). The amino acid sequence identity of timothy grass and birch profilin is 79% compared to a low degree of sequence identity in the range of 40% with *Acanthamoeba* I/II (20) and 30% with human profilin (21) (Fig. 2). The lysine residue at position 115 (Fig. 2, asterisk) in the *Acanthamoeba* sequences which was suggested to participate in actin-binding (22) is replaced by an arginine in the plant profilins. Figure 2 illustrates

the amino acid sequence identity of birch and timothy grass profilin with other eukaryotic profilins. Both plant profilins share a sequence identity of 79%, whereas the sequence identities observed with other profilins do not exceed 49%. Profilins from *Dictyostelium* profilin (23) and *Physarum* profilin (24) are most closely related to the plant profilins.

Comparison of the calculated hydrophilicity and antigenicity of birch and timothy grass pollen profilin. Analysis of the deduced amino acid sequences was done on a Micro VAX computer (Digital Equipment corp.) with the PEPTIDESTRUCTURE program of the University of Wisconsin Genetics Computer Group program package version 4. Comparing the hydrophilicity and surface probability of birch and timothy grass profilin no significant differences were observed (Figure 3). The prediction of antigenic sites according to Jameson-Wolf (25) showed however some differences between amino acid 65-80 which might acount for the slightly different IgE-antibody binding pattern observed in profilin allergic patients. Both proteins showed no glycosilation sites.

Comparison of the IgE-binding capacity of recombinant birch and timothy grass pollen profilin. Recombinant birch profilin was compared regarding the IgE-binding capacity with recombinant timothy grass profilin by immunoblotting with sera from profilin allergic patients (Figure 4, lanes 1-8). Sera from patients sensitized against other allergens and serum from a non-allergic patient were included as negative controls (Figure 4, lanes 9-11). All profilin allergic patients showed IgE-reactivity to nitrocellulose blotted recombinant birch and timothy grass profilin whereas the control individuals did not react. No reactivity was observed with ß-galactosidase. A different intensity of binding to recombinant birch and timothy grass profilin was

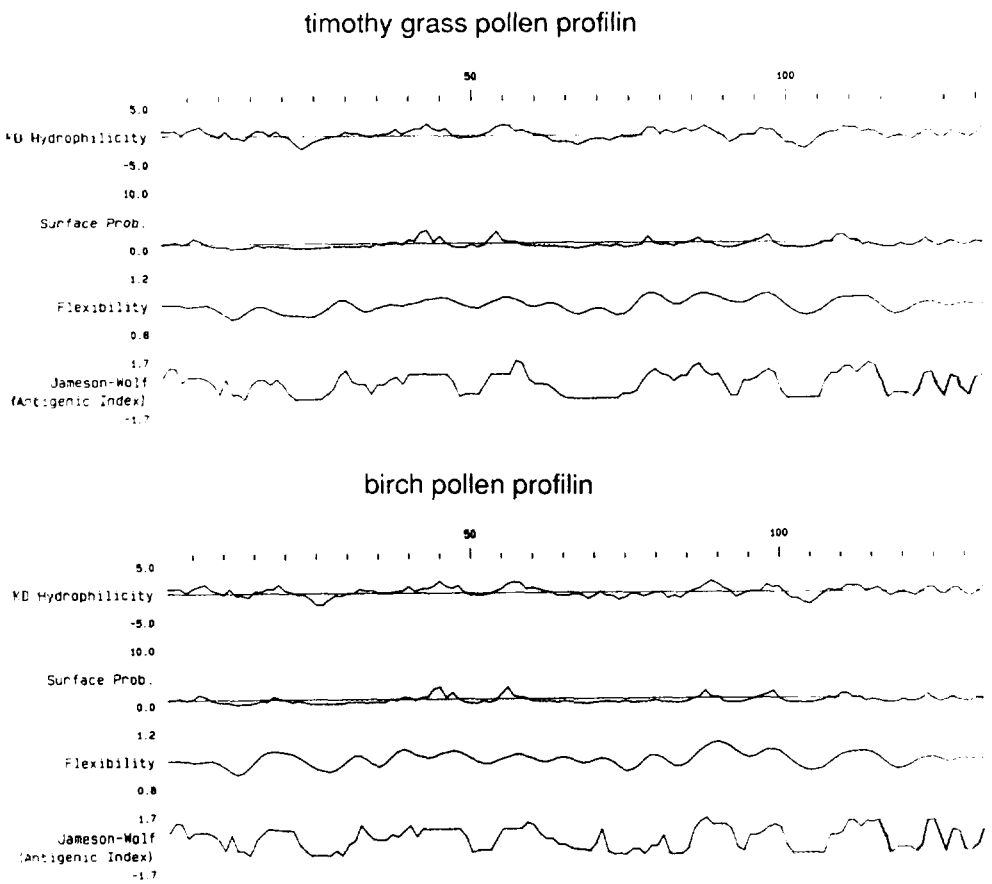

FIGURE 3. Comparison of predictions for hydrophilicity, antigenicity and surface probability of birch and timothy grass pollen profilin.

however noted in patients #3 and #6 although both allergens were present in excess to IgE on the nitrocellulose membrane. The result suggests that most of the IgE-epitopes are conserved between birch and timothy grass profilin whereas some epitopes are present exclusively on one of the two profilins.

DISCUSSION

Profilins were originally described as actin binding proteins in eukaryotes (26, 27). Recently birch profilin was identified as important pollen allergen (1) and it was demonstrated that

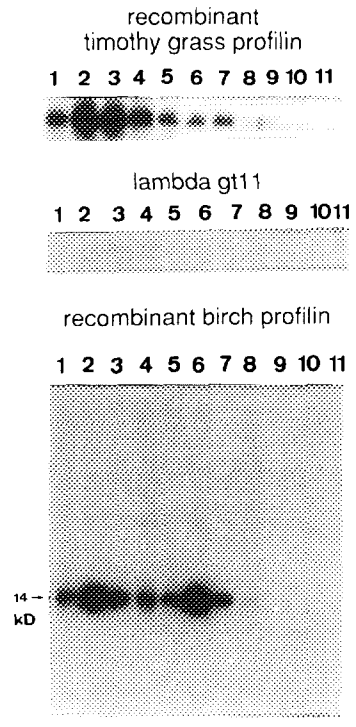

FIGURE 4. Comparison of the IgE-binding capacity of birch and timothy grass pollen profilin.
Purified recombinant birch profilin, recombinant timothy grass profilin and ß-galactosidase (negative control) were tested with serum IgE from profilin allergic patients (lanes 1-8), with serum IgE from two patients sensitized against other grass pollen allergens (lanes 9-10) and without addition of serum (buffer control) (lane 11).

profilin functions as actin-binding protein in higher plants (3, 4). Using proteinchemical and immunological techniques, plant profilins were characterized as cross-reactive allergens in pollen, vegetables and fruits (2, 11, 6, 7, 28). Using allergic patients serum IgE a cDNA coding for timothy grass (*Phleum pratense*) profilin was isolated and expressed in *E. coli* as a ß-galactosidase fusion protein. The cDNA and deduced amino acid sequences of birch profilin were compared with the timothy grass profilin sequences. 79% sequence identity was found between monocot

and dicot profilins whereas the sequence identity between plant and other eukaryotic profilins was rather low (<40%).

Birch profilin and timothy grass profilin were expressed in *E. coli* and tested for IgE-binding with sera from profilin allergic patients. Although both recombinant proteins bound serum IgE from all profilin allergic individuals tested, some patients bound more intensively to birch or timothy grass profilin indicating that most patients react with epitopes which are well conserved between birch and timothy grass profilin whereas some patients are sensitized against epitopes which are present on only one of the proteins. This finding suggests that profilin allergic patients might be sensitized initially against a certain profilin which then leads to a cross-reactivity with related epitopes on other profilins. The present data fit to the observation that more profilin allergic patients can be found in a population of grass pollen allergic patients (2, 11) than in a population of birch pollen allergic individuals (8) which would correlate with the faster elution of profilin from grass pollen (29). The slightly different pronounced reactivity of profilin allergic patients with birch and timothy grass profilin would indicate that profilin allergic individuals are sensitized initially against a certain profilin and due to structural similarities with other profilins show cross-reactivities. Since there were observed only small differences regarding the IgE-binding capacity of birch and timothy grass profilin, both molecules seem to be equally suited for diagnostic or therapeutic approaches. The structural similarities among different profilins and other important allergens such as the major tree (30, 31, 32, 33) and grass pollen allergens (13, 17, Laffer et al., submitted) provide the molecular basis for clinical relevant cross-reactivities observed in allergic patients which may finally lead to the concept of using a rather small number of structurally and

immunologically related allergens for the diagnosis and therapy of allergic diseases (34).

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