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Short sequence-paper

Sequence polymorphism and structural analysis of timothy grass pollen profilin allergen (Phl p 11) [1]

Juan A. Asturias *, M. Carmen Arilla, Borja Bartolomé, Jorge Martínez, Alberto Martínez, Ricardo Palacios

R&D Department, IFIDESA-ARISTEGUI, Bilbao, Spain

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Abstract

Three cDNA clones encoding timothy grass pollen profilin (Phl p 11) were newly isolated. Comparison of the sequences of four cDNA clones, including a previously isolated clone, showed a low level of polymorphism. Isoelectrofocusing of highly purified timothy grass profilin indicated the existence of at least five isoforms. One recombinant profilin showed similar immunological properties to natural timothy grass profilin. Tertiary structure of *Phleum pratense* profilin was obtained by homology-based molecular modeling. © 1997 Elsevier Science B.V.

Keywords: Phl p 11; Sequence polymorphism; Recombinant allergen; Gene expression; Timothy grass pollen; Variant

Profilin, a small cytoskeletal protein found in numerous eukaryotic cells, interacts specifically with at least two macromolecules in the cell: phosphatidylinositol-4,5-bisphosphate and actin. The dual binding capabilities of profilin suggest its implication in the signal transduction cascade to cytoskeletal rearrangements [1,2]. Profilin has also been described as an allergen present in pollen of trees, grasses and weeds [3,4]. Profilin polymorphism has been described in tabacum [5], maize [6] and wheat [7], but not in bean [8], birch [9], *Phleum* [10] or *Cynodon* (Asturias et al., submitted). Allergen polymorphism has been observed in numerous systems and, depending of the location of these sequence polymorphisms, recognition of IgE/IgG may be affected. The three polymorphic Der f 2 variants were equally recognized by IgE from 14 mite allergic patients [11]. On the other hand, nine Bet v 1 isoforms, sharing an average identity of 84–99%, displayed different allergenic properties both in vivo and in vitro [12].

Three full-length cDNA sequences, PpPRO2, PpPRO3, PpPRO4, were obtained by reverse-transcripted polymerase chain reaction amplification and subcloned in pBluescript. Primers were designed according to the nucleotide sequence of timothy grass profilin described previously [10]. Inserts were sequenced using fluorescence-labeled dideoxynucleotides and the sequencing reactions were analyzed on an ABI 373A DNA sequencer (Applied Biosystems Inc.). The inserts had a length of 396 bp, which encoded a polypeptide of 131 amino acids with a predicted average molecular mass of 14.1 kDa and a

* Corresponding author. Fax: +34 4 4438016; E-mail: im000001@sarenet.es

[1] The nucleotide sequences reported in this paper have been submitted to the GeneBank™, EMBL and DDBJ Nucleotide Sequences Databases under accession numbers: Y09456 (PpPRO2), Y09457 (PpPRO3), and Y09458 (PpPRO4).

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p*I* of 4.9. High amino acid identity (more than 97%) was found between translated sequences of the clones described here and the previously published timothy grass profilin [10] (Fig. 1). There were 10 nucleotide changes in the sequence, but only four of them resulted in two amino acid conversions. The remaining six nucleotide changes showed predominantly pyrimidine transitions (Fig. 1). One of the two amino acids changes ($Gly^{69} \rightarrow Ala$) is conservative, while the other is a conversion of an uncharged residue to a positively charged one ($Ala^{81} \rightarrow Arg$), which resulted in a change of the polypeptide's isoelectric point (p*I*) values from 4.92 to 5.07. This change affects the conserved motif $(A/V)^{81}$VIRGKKG(T/S/A)GGIT (V/I)KKT^{97}, that is found in all the plant profilins sequenced except PpPRO1, and is described to be involved in binding of phosphatidylinositol-4,5-bisphosphate [13]. Pairwise alignments, using the BESTFIT program included in the GCG Program Package (Genetics Computer Group Inc., Madison, WI), indicated an identity among plant profilins of 73–88%.

In order to address the question of timothy grass profilin variants at the protein level, highly purified profilin was obtained from timothy grass pollen after poly-(L-proline)-Sepharose affinity chromatography (PLP-Sepharose) [14] and gel filtration chromatography in Superdex 75 PC 3.2/30 column (SMART, Pharmacia Biotech). After PLP-Sepharose affinity chromatography, profilin preparation is almost pure, but due to the tendency of profilin to aggregate [15], a filtration chromatography step was performed to isolate timothy grass pollen profilin in the monomeric form. SDS-PAGE analysis [16] of the isolated protein revealed a unique band of 13.9 kDa (Fig. 2A), which

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     M   S   W   Q   T   Y   V   D   E   H   L   M   C   E   I   E   G   H   H   L    20
(1) ATG TCG TGG CAG ACG TAC GTG GAC GAG CAC CTG ATG TGC GAG ATC GAG GGC CAC CAC CTC   60
(2) ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
(3) ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ...
(4) ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

     A   S   A   A   I   L   G   H   D   G   T   V   W   A   Q   S   A   D   F   P    40
(1) GCC TCG GCG GCC ATC CTC GGC CAC GAC GGC ACC GTC TGG GCC CAG AGC GCC GAC TTC CCC   120
(2) ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
(3) ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
(4) ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

     Q   F   K   P   E   E   I   T   G   I   M   K   D   F   D   E   P   G   H   L    60
(1) CAG TTC AAG CCT GAG GAG ATC ACC GGC ATC ATG AAG GAT TTC GAC GAG CCG GGG CAC CTC   180
(2) ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
(3) ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
(4) ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

     A   P   T   G   M   F   V   A  G→A  A   K   Y   M   V   I   Q   G   E   P   G    80
(1) GCC CCC ACC GGC ATG TTC GTC GCA GGT GCC AAG TAC ATG GTC ATC CAG GGT GAA CCC GGT   240
(2) ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C
(3) ... ... ... ... ... ... ... ... .C. ... ... ... ... ... ... ... ... ... ... ..C
(4) ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C

    R→A  V   I   R   G   K   K   G   A   G   G   I   T   I   K   K   T   G   Q   A   100
(1) CGC GTC ATC CGT GGC AAG AAG GGA GCA GGA GGC ATC ACC ATA AAG AAG ACC GGG CAG GCG   300
(2) GCG ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
(3) GCG ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ...
(4) GCG ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

     L   V   V   G   I   Y   D   E   P   M   T   P   G   Q   C   N   M   V   V   E   120
(1) CTG GTC GTC GGC ATC TAT GAC GAG CCC ATG ACC CCT GGG CAG TGC AAC ATG GTG GTG GAG   360
(2) ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
(3) ... ..T ... ... ... ..C ..T ... ... ... ... ... ... ... ... ... ... ... ... ...
(4) ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ...

     R   L   G   D   Y   L   V   E   Q   G   M   *   132
(1) AGG CTT GGC GAC TAC CTC GTT GAA CAA GGC ATG TAG  396    PpPro1
(2) ... ... ... ... ... ... ... ... ... ... ... ...        PpPro2
(3) ... ... ... ... ... ... ... ... ... ... ... ...        PpPro3
(4) ... ... ... ... ... ... ... ... ... ... ... ...        PpPro4
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Fig. 1. Nucleotide and translated amino acid sequences of timothy grass profilin cDNA clones. Number on the right indicates the nucleotide or amino acid positions. Sequences are compared to the previously determined timothy grass profilin sequence (PpPRO1) [10].

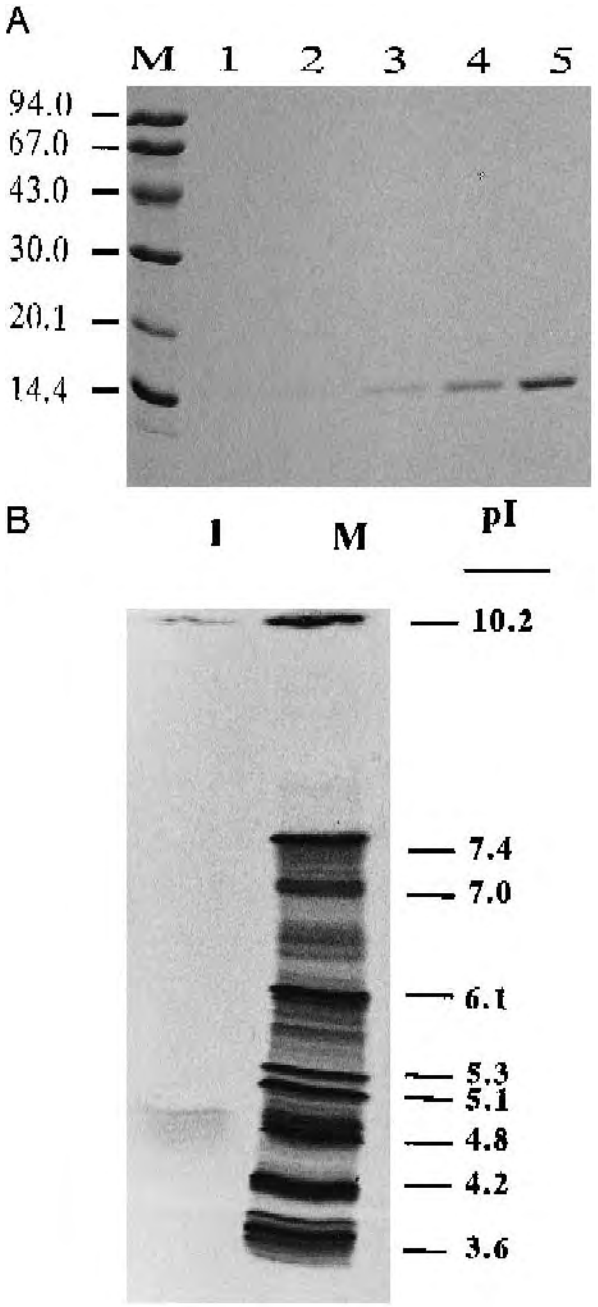

Fig. 2. (A) Coomassie-stained 12% SDS-PAGE of timothy grass pollen profilin after gel filtration in Superdex 75 PC 3.2/30 column (SMART). Lane M: molecular mass markers (kDa). Lanes 1–5, correspond to 0.125, 0.25, 0.5, 1, and 2 μg of purified profilin. (B) Isoelectrofocusing analysis of purified timothy grass pollen profilin. 20 μg of protein were applied on agarose plates (FMC BioProducts, Rockland, MN) over the range pH 3 to 10.

is in good agreement with the deduced molecular mass from the nucleotide sequence. Isoelectrofocusing analysis of this sample on agarose plates demonstrated that this band is composed of at least five isoforms of p*I* values ranging from 4.4 to 4.9 (Fig. 2B). This range of p*I* indicated that timothy grass pollen could contain more profilin sequence variants than the four described here. The high occurrence of profilin isoforms could be a cellular mechanism to assure functionality of this important protein involved in signal transduction between the outside of the cell and the actin cytoskeleton [17]. A non-fusion form of timothy grass profilin (PpPRO3) was expressed in *Escherichia coli* BL21 (DE3). After Western blotting [18], recombinant and natural profilin showed no differences in binding to IgG or IgE from rabbit anti-serum obtained against sunflower profilin or human sera from a allergic patient, respectively (data not shown).

The molecular structure of several profilins had been previously determined at high resolution by X-ray crystallography [19–21]. A structural model of PpPRO4 (Fig. 3) was constructed from the X-ray coordinates of profilin II from *A. castellanii* [21] using the WhatIf program [22]. Plant profilin is built around a central six-stranded antiparallel β-sheet. Both termini are α-helical and pack against the same side of the central sheet, connected by two short loops. Residues on the opposing face of the central sheet form two α-helices and a small two-stranded β-sheet. The model obtained is very similar to the three-dimensional model for human profilin [20,23], supporting the role of human profilin in prolonging allergic symptoms caused by pollen profilin in profilin-allergic patients [9]. Using the PHD Predict Protein Server from EMBL (http://www.embl-heidelberg.de/predictprotein/), three antigenic regions from *Phleum* profilin (Fig. 3) were predicted according to: (1) solvent accessibility and secondary structure; (2) degree of mobility based on the temperature factor of the crystallographic structure; (3) characteristics of the amino acids involved; and (4) regions with sequence differences to human profilins. The presence of antigenic determinants on loops which protruded from the surface of the protein is well-documented [24]. Molecular modeling and sequence polymorphisms characterization would help the identification of conserved regions, which could be candi-

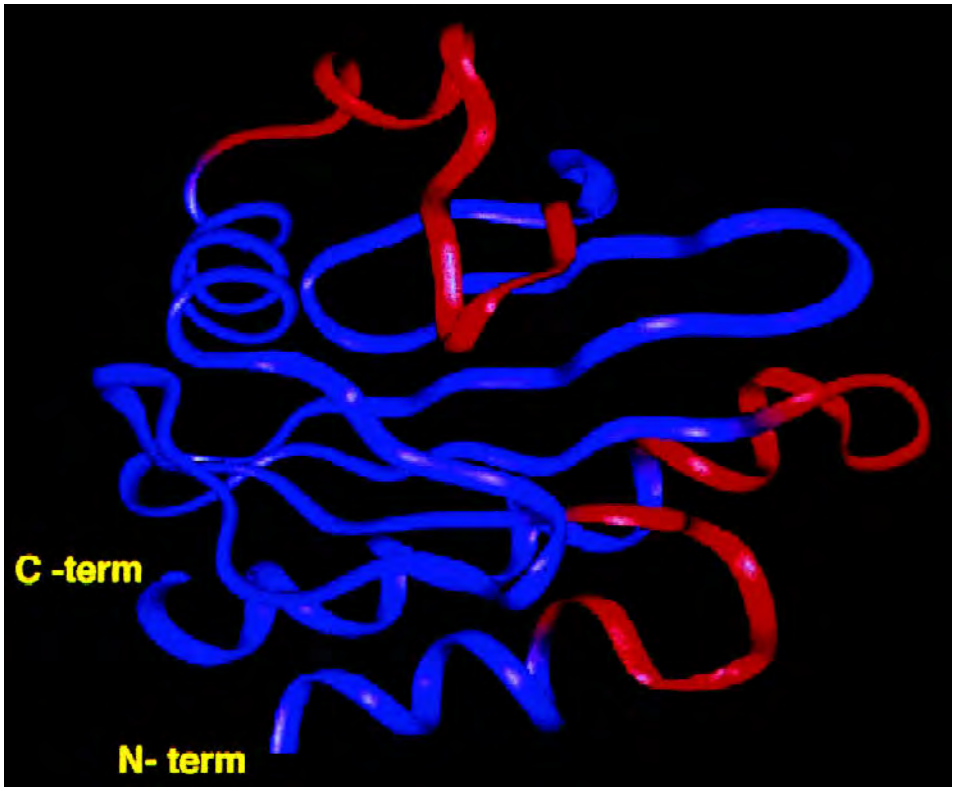

Fig. 3. Molecular model of the *Phleum* profilin (Phl p 11 allergen). The Insight II program (Molecular Simulations Inc., San Diego, CA) was used for graphic visualizations and manipulations. Predicted antigenic regions are shown in red.

dates for the development of peptide-based immunotherapeutic reagents for pollen allergy, as has already been described for other allergens [25].

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