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Resolution of the structure of the allergenic and antifungal banana fruit thaumatin-like protein at 1.7-Å

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Abstract

The structure of a thaumatin-like protein from banana (*Musa acuminata*) fruit, an allergen with antifungal properties, was solved at 1.7-Å-resolution, by X-ray crystallography. Though the banana protein exhibits a very similar overall fold as thaumatin it markedly differs from the sweet-tasting protein by the presence of a surface exposed electronegative cleft. Due to the presence of this electronegative cleft, the banana thaumatin-like protein (Ban-TLP) acquires a strong (local) electronegative character that eventually explains the observed antifungal activity. Our structural analysis also revealed the presence of conserved residues of exposed epitopic determinants that are presumably responsible for the allergenic properties of banana fruit towards susceptible individuals, and provided evidence that the Ban-TLP shares some structurally highly conserved IgE-binding epitopes with thaumatin-like proteins from fruits or pollen from other plants. In addition, some overlap was detected between the predicted IgE-binding epitopes of the Ban-TLP and IgE-binding epitopes previously identified in the mountain cedar Jun a 3 TLP aeroallergen. The presence of these common epitopes offers a molecular basis for the cross-reactivity between aeroallergens and fruit allergens.

Keywords: Thaumatin-like protein; PR-5 protein; Banana; Antifungal protein; Allergen; IgE-binding epitope

1. Introduction

Many plant species contain proteins that have been identified as very potent allergens capable of triggering more or less severe allergenic responses in susceptible individuals. Basically there are two major routes of sensitization to plant proteins. Class 2 allergy, which is typified by symptoms as rhinitis, conjuctivis or asthma occurs after the inhalation of aeroallergens (mainly pollen grains) [1]. In class 1 allergy, also referred as food allergy, the sensitization results from the recognition by the lymph nodes in the gastro-intestinal tract of allergenic plant proteins (that are in general extremely resistant to digestive enzymes) [2]. Various proteins from a wide range of edible fruits such as banana, apple, cherry, peach, apricot and kiwi fruit, have been identified as potent or even dangerous food allergens [3]. Besides class I chitinases, which contain an N-terminal hevein-like domain that can cause the so called "latex-fruit syndrome" [4], several other types of fruit proteins apparently act as allergens responsible for the "Oral Allergy Syndrome (OAS)" corresponding to a rapid itching of the lips, mouth and pharynx and a concomitant swelling of the lips, tongue and throat. Most of these fruit allergens are structurally and evolutionary related to the

Abbreviations: Ban-TLP, banana thaumatin-like protein; Mal d 2, *Malus domestica* (apple) thaumatin-like protein; MHC, major histocompatibility complex; OAS, oral allergy syndrome; PR, pathogenesis-related; PR-5 proteins, pathogenesis-related proteins of the family 5; Pru a 2, *Prunus avium* (cherry) thaumatin-like protein; TCR, T-cell receptor; TLP, thaumatin-like protein; Vitvi-TLP, *Vitis vinifera* (grape) thaumatin-like protein.

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"Pathogenesis-related proteins" (or PR-proteins). Though according to the original definition PR-proteins do not occur in unchallenged plants but are only synthesized in response to either biotic or abiotic stress, there is compelling evidence that identical or closely related proteins are also either constitutively expressed in healthy, unstressed plants or as part of a developmental program. Interestingly, in many plants fruit ripening is apparently accompanied by a developmentally regulated accumulation of reasonable to very large quantities of one or more fruit-specific proteins belonging to the classic families of PR-proteins [5]. Most of these fruit PR-like proteins belong to the PR-2 (glucanases), PR-5 (thaumatin-like proteins or TLP) or PR-10 family (lipid transfer proteins or LTP) [6].

During the last decade substantial efforts have been undertaken to identify and characterize ripening-associated proteins and allergens in banana fruits. Though only class I chitinases have been described as major allergens [7] it is evident that several other potential allergens such as an endo-β1,3-glucanase, a mannose-binding lectin and a TLP accumulate in large quantities in the pulp of ripening bananas [8–10]. The identification of Ban-TLP as one of the most abundant fruit proteins (representing up to approximately 50% of the total fruit protein) [8] is of particular interest because similar proteins from apple [11], cherry [12], bell pepper [13] and grape [14] have been recognized as major fruit allergens [15]. Moreover, the family of TLP apparently also comprises aeroallergens as is illustrated by Jun a 3, a TLP that is responsible for the potent allergenicity of the airborne mountain cedar pollen [16,17].

According to the available data, TLP are most probably ubiquitous proteins [18]. The finding that both fruit and pollen TLP act as allergens raises the question whether the same applies to other TLP. In addition, taking into account the structural conservation [19], the question has to be addressed whether TLP can be responsible for cross-sensitization in susceptible individuals.

To fully characterize and understand the structure and biological activities of potentially allergenic proteins present in banana fruits we resolved the structure of the banana TLP (Ban-TLP) at 1.7-Å-resolution by X-ray crystallography. Evidence is presented that the banana protein possesses similar IgE-binding epitopes as other allergenic fruit TLP. In addition, the resolution of the structure provides a structural basis for the previously reported antifungal properties [20] of Ban-TLP.

2. Materials and methods

2.1. Purification, crystallization and data collection

Ban-TLP was purified from the pulp of ripe bananas as previously described [10].

Ban-TLP crystals were obtained by vapor diffusion in hanging drops at 20 °C. The hanging drops consisted of 2 μl of Ban-TLP solution mixed with 2 μl of the reservoir solution containing 0.1 M sodium acetate (pH 4.6), 24% PEG 4000 [poly(ethylene glycol) of molecular mass 4000] and 2 M ammonium sulfate. Crystals with a maximum size of 0.6 × 0.5 × 0.4 mm grew within 5 days. These crystals, which belonged to the $P2_12_12_1$ space group, had the following cell dimensions: $a = 42.8$ Å, $b = 52.8$ Å, $c = 50.6$ Å and diffracted to 1.7 Å resolution. Ethylene glycol was used at a concentration of 20% as the cryoprotectant in the subsequent data collection stage.

Table 1
Data collection (a) and final refinement (b) statistics

(*a*)	
Data collection	*Value*
Resolution limit (Å)	15–1.7
Data > 1σ (%)	91.4 (89.7)
Completion (%)	96.9 (94.9)
Redundancy	4.6 (4.3)
Rsym[a]	4.2 (13.1)
(*b*)	
Refinement parameters	*Value*
Resolution limits (Å)	15–1.7
Number of reflections (test set)	20067 (1027)
Number of protein atoms	1484
Number of water molecules	248
Final *R*-factor[b]/*R*-free[c] (%)	14.2/17.7
B-factors (Å^2)	
Protein	14.2
Water molecules	28.5
R.m.s. deviations	
Bond (Å)	0.010
Angles (°)	1.35

Data statistics for the last shell (1.76–1.70 Å) are given in parentheses.

[a] $R\text{sym} = \sum_h \sum_i |I_{hi} - < I_h >| / \sum_h \sum_i I_{hi}$.

[b] $R_{\text{factor}} = \sum_h ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum_h |F_{\text{obs}}|$ where F_{obs} and F_{calc} are the observed and calculated structure factor amplitudes, respectively.

[c] R_{free} is calculated with 5% of the diffraction data, which were not used during the refinement.

X-ray diffraction data were collected at 100 K on a Mar-Research plate scanner developed by Hendrix and Lentfer (Hamburg, Germany) with CuKα radiation. The diffraction data were processed with DENZO and SCALEPACK programs [21]. Data collection statistics are summarized in Table 1.

2.2. Structure determination and refinement

The structure of Ban-TLP was solved by molecular replacement using the AMoRe program [22] and the structure of thaumatin from *Thaumatococcus daniellii* (PDB code 1THV) as a search model. The rotation function yielded one solution and the translation function a unique solution, with a correlation coefficient and an *R*-factor of 60% and 41%, respectively, with data between 10 and 3 Å.

Refinement procedures were performed using the REFMAC program [23]. After manual corrections of the model using the TURBO-FRODO program [24], interspersed with cycles of slow cooling protocol refinement with all data up to

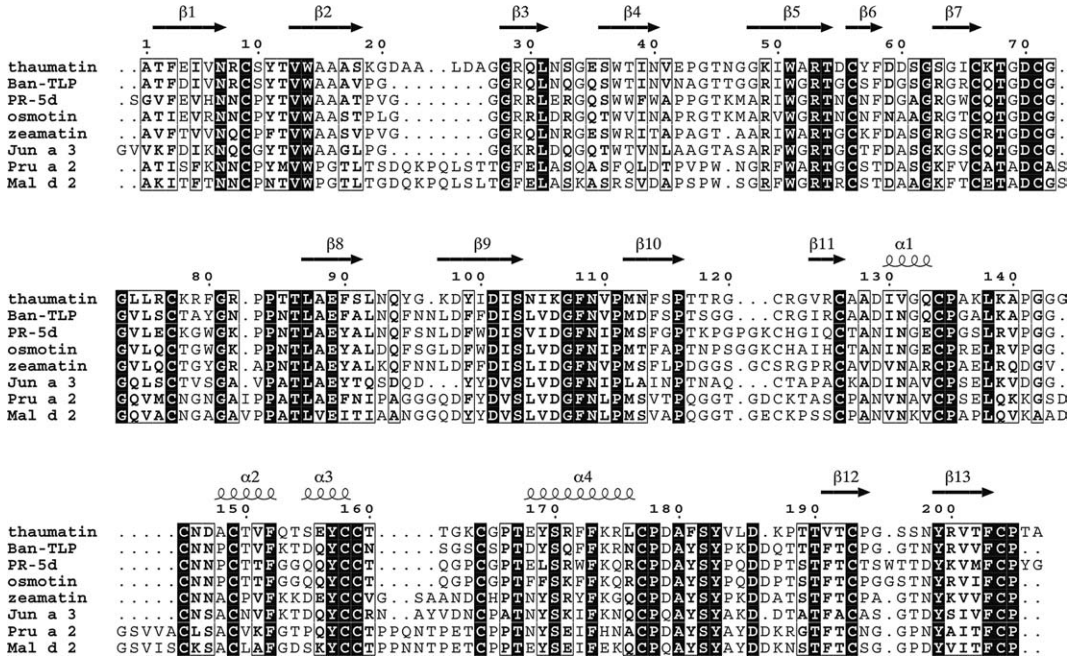

Fig. 1. Alignment of the amino acid sequences of thaumatin, Ban-TLP and some other thaumatin-like proteins. The multiple alignment was performed with CLUSTAL X [52]. Invariant residues are displayed as black boxed white letters. Strands of β-sheet (arrows) and stretches of α-helix occurring in thaumatin are indicated. Codes of the proteins are as follows: thaumatin: sweet-tasting protein from *T. daniellii* , Ban-TLP: TLP from banana (*Musa acuminata*), PR-5d and osmotin: tobacco-TLP (*Nicotiana tabacum*), zeamatin: TLP from maize (*Z. mays*), Jun a 3: TLP from the moutain Cedar (*J. ashei*), Pru a 2: TLP from cherry (*P. avium*), Mal d 2: TLP from apple (*M. domestica*).

1.7 Å-resolution, a final *R*-factor of 0.172 (R_{free} = 0.20) was obtained. Water molecules were added and inspected manually. The coordinates have been deposited in the Brookhaven Protein Data Bank (code 1Z3Q).

The final structure of Ban-TLP discussed below comprises all 200 amino acid residues present in the mature protein (Fig. 1). Apart from residue Phe89 (which is well defined in the density) all residues have conformational angles situated in permitted regions of the Ramachandran plot [25]. The final ($2F_o - F_c$) electron density map (1 σ contoured) showed that all residues of the model were well fitted. Further indicators of the quality of the structure presented in Fig. 2A are shown in Table 1. A total of 204 ordered water molecules were identified. Two *cis*-peptide bonds located between Val18 and Pro19 and between Pro77 and Pro78 were identified in the structure of Ban-TLP.

Cartoons of the three-dimensional structures were drawn with Molscript [26] and displayed with Bobscript [27] and Raster3D [28].

The partial charges of the atoms have been assigned with GRID [29]. The program MEAD [30] was used to calculate the term W_{ij} (ionizable group interaction in the protein), $\Delta\Delta G_{Born}$ (Born solvatation energy) and $\Delta\Delta G_{Back}$ (background charge interactions) corresponding to the electrostatic contribution of the ionized groups. The inner and outer dielectric constants applied to the protein and the solvent, were fixed at 4.0 and 80.0, respectively, and the calculations were performed keeping an ionic strength of 0.15 M. From the $pK_{1/2}$ values and the ionization state energies calculated for each ionizable site, a new atomic coordinates file corresponding to pH 7.0 was generated. Electrostatic potentials were calculated with DelPhi [31] and displayed on the molecular surface at –5 kT level and +5 kT level, respectively, using GRASP [32]. The surfaces occupied by hydrophobic (Leu, Ile, Val, Met, Phe, Trp, Tyr included) and negatively charged (Asp, Glu) residues on the molecule surface of Ban-TLP were displayed with PyMOL ("The PyMOL Molecular Graphics System" DeLano Scientific LLC, San Carlos, CA, USA, http://www.pymol.org).

2.3. Prediction of linear IgE-binding epitopes

IgE-binding epitopes were predicted from the hydropathic profiles as being the most hydrophilic, flexible and surface exposed regions of Ban-TLP. Different scales of hydrophilicity [33], flexibility [34], exposition to the solvent [35] and antigenicity [36], were used to build the hydropathic profiles with the MacVector (Kodak) software. The surface occupied by the predicted sequential epitopes along the amino acid sequence of Ban-TLP was calculated and displayed on the molecular surface of the proteins with PyMOL. The overall conformation of the sequential IgE-binding epitopes on the molecular surface was similarly displayed with PyMOL.

3. Results

3.1. Crystal structure of Ban-TLP

Ban-TLP exhibits the canonical thaumatin-fold, which is a composite of three distinct domains (Fig. 2A). The core

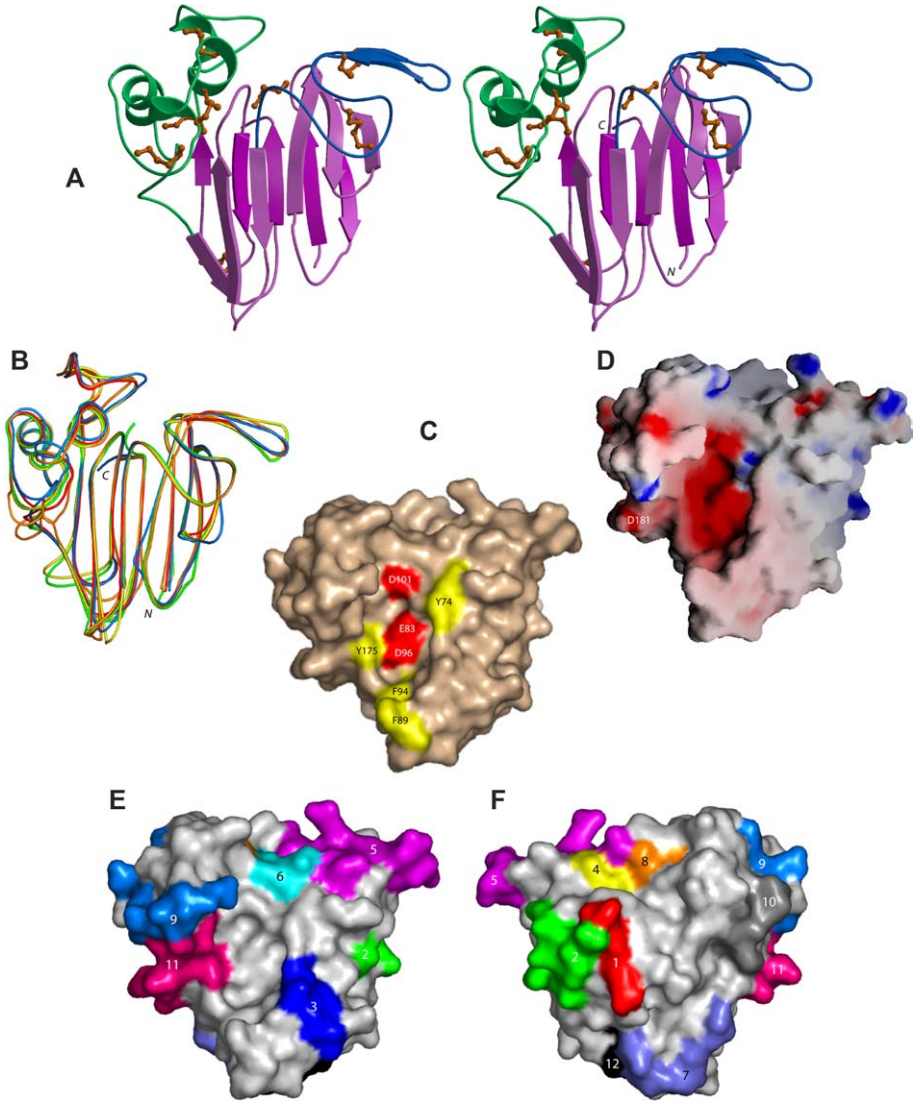

Fig. 2. A. Stereo ribbon diagram of Ban-TLP showing the overall three-dimensional structure of the protein. Ban-TLP consists of three structural domains: a central core domain I built from a β-sandwich of two β-sheets (light magenta for the six-stranded front β-sheet, magenta for the five-stranded back β-sheet), flanked by two shorter domains II (green) and III (blue). The disulfide bridges are shown as orange ball-and-sticks.
B. Superimposition of the backbone representations of Ban-TLP (red), thaumatin (blue), osmotin (yellow), PR-5d (green), and zeamatin (orange). PDB entries and codes of the proteins are as follows: Ban-TLP (1Z3Q): TLP from banana (*Musa acuminata*), thaumatin (1THV): sweet-tasting protein from *T. daniellii*, osmotin (1PCV) and PR-5d (1AUN): tabacco TLP (*N. tabacum*), zeamatin (1DU5): TLP from maize (*Z. mays*).
C. Molecular surface of Ban-TLP indicating the location of the central cleft delineated between the central core domains I and II built from the extended α4-helix. The acidic residues (in red) and the aromatic residues (in yellow) located inside and around the central cleft, respectively, are indicated and labeled.
D. Mapping of the electrostatic potentials on the molecular surface of Ban-TLP. The negative potentials and positive potentials are colored red and blue, respectively. Neutral surfaces are white. Note the pronounced electonegative character of the central cleft.
E, F. Mapping of the predicted epitopes on the molecular surface of the front (containing the central acidic cleft) (E) and back (face opposite to that containing the acidic cleft) (F) faces of Ban-TLP. The twelve surface exposed epitopes are differently colored.

domain I corresponds to a β-sandwich built up of two β-sheets comprising six (β2, β3, β5, β6, β7 and β10) and five (β1, β4, β8, β9 and β11) antiparallel strands forming the front and back sheet, respectively. This core domain I, which forms the central flattened part of the protein is flanked on both sides by the two shorter domains II and III. Domain II contains an extended α-helix (α4) associated with three shorter α-helices (α1, α2, α3), whereas domain III consists of a hairpin segment of two short strands of β-sheet linked to an extended loop. This three-domain fold is stabilized by eight disulphide bridges Cys9–Cys199, Cys50–Cys60, Cys65–Cys71, Cys116–Cys188, Cys121–Cys171, Cys129–Cys139, Cys143–Cys152, Cys153–Cys158, which are extremely well conserved in both thaumatin [37] and thaumatin-like proteins [38–40] (Fig. 1). Accordingly, Ban-TLP nicely superimposes with other TLP of known three-dimensional structures (Fig. 2B). Domains I and II create a central cleft, which comprises mainly hydrophilic residues but is surrounded by aromatic residues Tyr74, Phe89, Phe94 and Tyr175 (Fig. 2C). This cleft is particularly rich in acidic residues (Glu83, Asp96, Asp101, Asp181) and accordingly is a region with a strong electronegative character (Fig. 2D). It should be noted here

that a similar electronegative central cleft was previously identified in the crystal structure of zeamatin from *Zea mays* [38], PR-5d [39] and osmotin [40] from tobacco, and predicted by modeling studies in the fruit-specific TLP from apple [11] and cherry [12].

3.2. Structural features responsible for the antifungal activity

As was reported previously Ban-TLP possesses a weak but reproducible antifungal activity against some phytopathogenic fungi (e.g. *Verticillium albo-atrum*) [20]. In this respect Ban-TLP definitely differs from other fruit-specific TLP because none of these TLP exhibited any antifungal activity when tested under similar conditions [20]. No antifungal activity could be observed for thaumatin itself even though it was reported that this sweet-tasting protein inhibits the in vitro growth of *Candida albicans* in the presence of nikkomycin in the culture medium [18]. In contrast to thaumatin, zeamatin from maize (*Z. mays*) is considered a genuine antifungal protein because of its obvious strong antifungal activity. It has been proposed that this antifungal activity relies on the strong electronegative character of the cleft occurring between domains I and II [38]. Most probably, the occurrence of a similar electronegatively charged cleft explains why Ban-TLP possesses—in contrast to other fruit-specific TLP—antifungal activity. The presence of a negatively charged cleft as a structural requirement for antifungal activity is in good accordance with the lack of antifungal activity of thaumatin because in this sweet protein the corresponding cleft is electropositively charged. Interestingly, the presence of Lys residues located in the vicinity of the cleft is believed to be an essential feature for the sweet-tasting properties of the protein [41].

3.3. Prediction of exposed epitopes

A detailed investigation of the location of epitope regions interacting with pooled IgE from patients suffering allergenic response to the mountain Cedar (*Juniperus ashei*) allergen Jun a 3 revealed that IgE-binding epitopes are mainly distributed on the helical domain II of this TLP [17]. They correspond to four stretches of amino acid residues exhibiting a C-terminal Lys residue: (1) 120ADINAVCPSELK 131 (2) 132VDGGCNSACNVFK 144 (3) 152NAYVDNCPATNYSK 165 and (4) 169NQCPQAYSYAK179. IgE-binding epitopes 3 and 4 partly coincide with the well-exposed α4-helix of domain II (Fig. 3D). Twelve highly exposed/accessible/flexible amino acid sequence stretches predicted as possible linear IgE-binding epitopes occur along the amino acid sequence of Ban-TLP: (1) 8R CS10 (2) 20GGGR QLNQGQS30 (3) 37AGTTG41 (4) 46GRTG49 (5) 53DGSGRGRCQTGDCGG67 (6) 75GNPPNT80 (7) 112TSGGCR117 (8) 137GGCNNP142 (9) 154NSGSCSPTDY163 (10) 169RNCP172 (11) 175YSYPKDDQT183 and (12) 189PGGTNY194. The twelve epitopes are distributed on both the front and back faces of the molecule (Fig. 2D, E). Some of these epitopes and, especially epitopes 9, 10 and 11, are also predicted to occur in osmotin, zeamatin and thaumatin, and fully or partly coincide with IgE-binding epitopes 2, 3 and 4 of Jun a 3 (Fig. 3A). All these predicted regions include the main α4-helix of domain II, which appears as an extremely conserved secondary feature in Jun a 3 and other structurally related TLP (Fig. 3B–E). Moreover, four conserved disulfide bridges Cys129–Cys139, Cys143–Cys152, Cys153–Cys158 and Cys121–Cys171, highly stabilize the conformation of this epitopic region in Ban-TLP and other closely related TLP. As shown on the molecular surface, the overall conformation of epitope 10–11 is particularly well conserved in other X-ray structures (Fig. 3F–M). This could account for some cross-reactivity with IgE antibodies.

4. Discussion

The present report deals with the resolution of the structure of a fruit-specific TLP from banana. Though in the past the structure of thaumatin itself and the TLP from maize (osmotin) and tobacco have been determined, the novel structure described here is important because it provides for the first time detailed information about the 3D-structure of a fruit-specific TLP and, in addition, contributes to a further understanding of the structural requirements for the different biological activities and allergenicity of TLP.

One of the major issues in the search for the biological activity and physiological role of TLP concerns their antifungal properties. Though there is compelling evidence that the antifungal properties of some TLP are somehow intimately linked to the occurrence of an electonegative cleft, the exact mode of action is still controversial. It has been suggested that polarized negative charges occurring in the cleft of PR-5 proteins enable them to interact with positively charged membrane proteins like ion or water channels, which eventually results in a dramatical increase in the water flow across the plasma membrane [38]. However, many TLP and, especially, fruit-derived TLP definitely comprise a strongly electronegatively charged cleft in their structure but nevertheless are either weakly or completely inactive against fungi when assayed in classical agar-plate tests [19,20]. For example, Mal d 2, an allergenic TLP from apple, was recently shown to display some antifungal activity in vitro against *Fusarium oxysporun* and *Penicillium expansum* [11]. However, this activity is extremely weak as compared to that of the genuine antifungal TLP (like osmotin). Moreover, in another set of experiments, Mal d 2 was completely devoid of antifungal activity [20]. In contrast, Ban-TLP definitely inhibited hyphal growth of *V. albo-atrum*, which confirms the proposed required presence of an acidic cleft for antifungal activity but at the same time indicates that other structural features may be more important because the antifungal activity of Ban-TLP is still far lower than that of, e.g. osmotin.

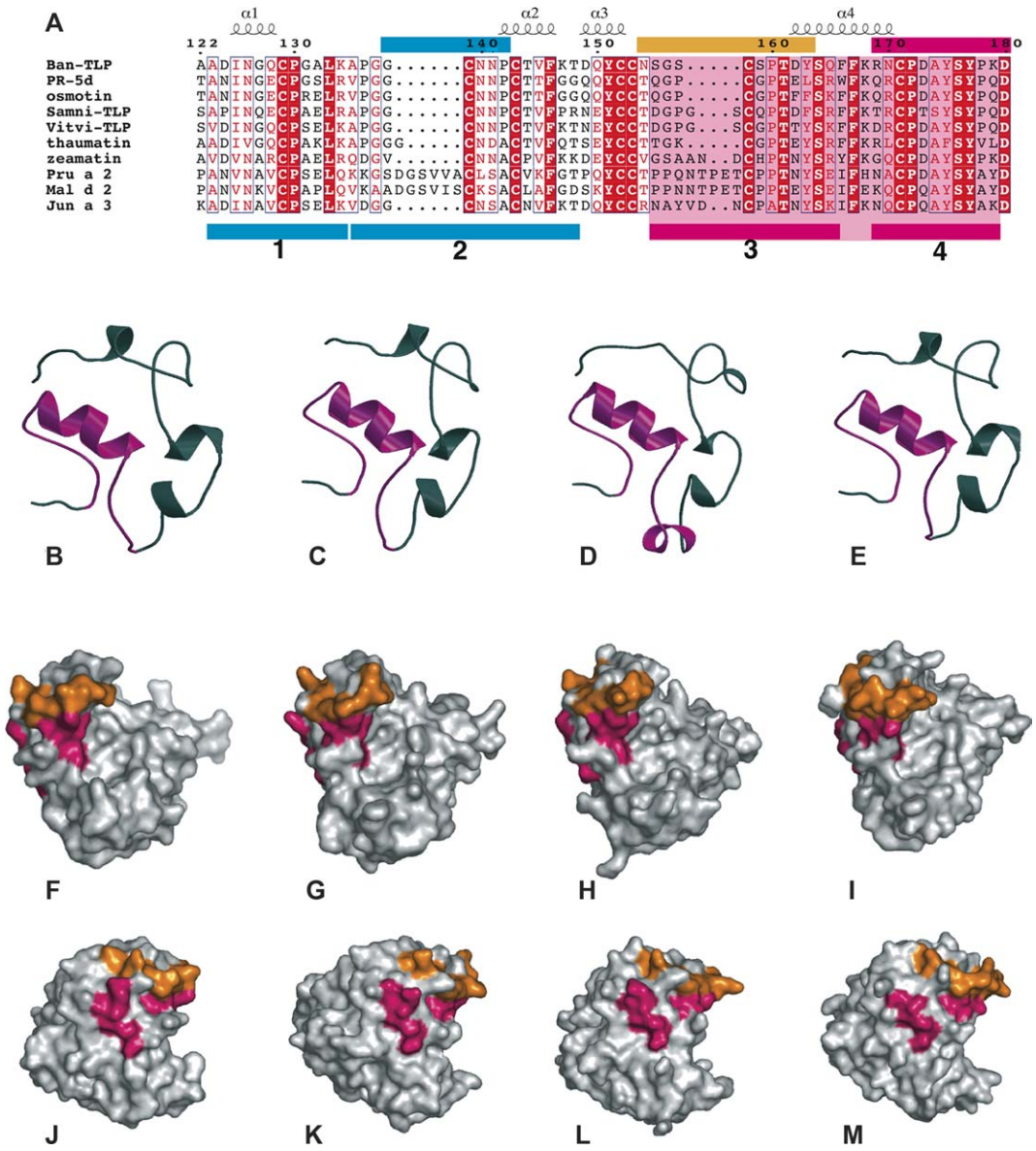

Fig. 3. (A) Multiple amino acid sequence alignments of different TLP in the region comprising the major IgE-binding epitopes 1 and 2 (blue boxes), and 3 and 4 (magenta boxes) of Jun a 3. Proteins are indicated as follows: Ban-TLP: TLP from banana (*M. acuminata*), PR-5d: tobacco-TLP (*N. tabacum*), osmotin: TLP from tobacco (*N. tabacum*), Samni-TLP: TLP from elderberry (*Sambucus nigra*), thaumatin: sweet-tasting protein from *T. daniellii,* zeamatin: TLP from maize (*Z. mays*), Pru a 2: TLP from cherry (*P. avium*), Mal d 2: TLP from apple (*M. domestica*), Jun a 3: TLP from the moutain Cedar (*J. ashei*). The best-conserved IgE-binding epitope predicted in all the TLP (pink box) corresponds to epitopes 3 and 4 (pink) of Jun a 3 and epitopes 9 (orange) and 10–11 (pink) of Ban-TLP. The multiple alignment was performed with CLUSTAL X [52] and represented with ESPript [53]. Invariant residues are displayed as white characters. Similar residues according to the Risler similarity matrix [54] are rendered as red letters.
(B–E) Ribbon diagram representation of the main IgE-binding epitope region (magenta) predicted in domain II of Ban-TLP (B), tobacco-TLP (PDB1AUN)(C), zeamatin (PDB1DU5)(D) and thaumatin (PDB1THW)(E). The main helix-α4 of domain II fully belongs to the predicted IgE-binding epitope region.
(F–M) Overall conformation of epitopes 9 (orange) and 10–11 (pink) on the molecular surface of Ban-TLP (F,J), tobacco-TLP (PDB1AUN)(G,K), zeamatin (PDB1DU5)(H,L) and thaumatin (PDB1THW)(I,M). Proteins are represented in two different orientations for a better delineation of the surface exposed epitopes.

After the discovery of the binding of TLP to β1,3-glucanases and the hydrolysis of these glycans by some TLP [42,43], intensive efforts have been undertaken to unravel the molecular mechanism and structural requirements for this enzymatic activity. At present, it is generally accepted that the acidic character of the cleft located between domains I and II is a structural prerequisite for the relatively low but definite endo-β1,3-glucanase activity of some TLP. Even though Ban-TLP exhibits an acidic cleft, its endo-β1,3-glucanase activity remains extremely weak and, most probably, has no biological relevance [10].

Besides their antifungal and endo-β1,3-glucanase activity, TLP receive an increasing interest because evidence is accumulating that many plant food allergens belong to the family of thaumatin-like proteins [15,44]. Dietary TLP can cause class 1 food allergy, indeed, because due to the extreme

resistance to denaturation and, especially, to proteolytic digestion [2], TLP survive passage through the gastro-intestinal tract and hence can be presented to the lymph nodes and eventually lead to sensitization. Members of the ubiquitous TLP-family not only elicit class 1 food allergy (e.g. fruit TLP from cherry [12]) but also class 2-allergy (e.g. the aeroallergen Jun a 3 from the mountain Cedar [16]). This causes a huge problem because the presence of structurally conserved IgE-binding epitopes can at least in principle lead to cross-reactivity between food and airborne allergenic TLP. Such cross-reactivity between aeroallergens and food allergens often occurs and is well documented [45]. Since the determination of the structure of Ban-TLP clearly demonstrates that the banana protein shares common conserved IgE-binding epitopes not only with other fruit-specific TLP (e.g. Pru a 2 from cherry, Vitvi-TLP from grape and thaumatin from *T. daniellii*) but also with Jun a 3 from mountain cedar pollen, there is certainly a molecular basis for a cross-reactivity between aeroallergens and food allergens from the TLP family. Although hitherto no major adverse reaction has been reported for the sweetener thaumatin, previous studies have shown that individuals, which were intermittently exposed to inhalation of thaumatin over a long period, responded positively to commercial thaumatin (~ 10%) and to the *Dermatophagoides pteronyssinus* house dust mite (~ 30%) in skin prick tests [46]. However, until now, no oral sensitization to thaumatin has been demonstrated.

As already mentioned above, several fruit-specific TLP are major allergens. Mal d 2, a 31 kDa TLP from apple (*M. domestica*), has been recently identified as an important allergen from apple [11] (the main apple allergen being an ortholog of the major PR-10 allergen from birch (*Betula verrucosa*) pollen) [47]. A very closely related TLP, Pru a 2, was also identified as a major allergen of cherry [12]. Although no three-dimensional structure is available for these fruit TLP, three-dimensional models built from the coordinates of thaumatin (1THW) and Ban-TLP (this work), showed that domain II of these proteins exhibits a three-dimensional fold very similar to that of Jun a 3, Ban-TLP or thaumatin (Fig. 3B–E). Moreover, the amino acid sequence of this α-helical region is highly identical/similar to Jun a 3 and Ban-TLP (Fig. 3D) and is predicted as a main IgE-binding epitope. Ban-TLP and other plant TLP are interesting examples, of an ubiquitous family of proteins, that harbor very structurally conserved IgE-binding epitopes (Fig. 3F–M) and hence are suspected to elicit cross-reactive allergenic responses in individuals primary sensitized to aeroallergens containing similar TLP [48,49].

Even though a predictive bioinformatics approach was used, our results also confirm that some overlapping exists between IgE-binding epitopes predicted on the surface of different TLP, suggesting that the exposed regions of IgE-binding epitopes can also create more extended conformational IgE-binding epitopes (Fig. 2D–E). Since IgE cross-reactivity between pollen and plant-derived food allergens represents the molecular basis responsible for the OAS, the identification and distribution of IgE-binding epitopes on the molecular surface of food allergens and, especially, fruit allergens, should be of particular importance for both the diagnosis [50] and treatment [51] of this syndrome.

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