

Crystal structure analysis of NP24-I: a thaumatin-like protein

Raka Ghosh · Chandana Chakrabarti

Received: 7 May 2008 / Accepted: 8 July 2008
© Springer-Verlag 2008

Abstract The crystal structure of NP24-I, an isoform of the thaumatin-like protein (TLP) NP24 from tomato, has been reported. A prominent acidic cleft is observed between domains I and II of the three-domain structure of this antifungal protein, a feature common to other antifungal TLPs. The defensive role of the TLPs has also been attributed to their β -1,3-glucanase activity and here too the acidic cleft is reported to play a vital role. NP24 is known to bind β -glucans and so a linear β -1,3-glucan molecule has been docked in the interdomain cleft of NP24-I. From the docked complex it is observed that the β -glucan chain is so positioned in the cleft that a Glu and Asp residue on either side of it may form a catalytic pair to cause the cleavage of a glycosidic bond. NP24 has been reported to be an allergenic protein and an allergenic motif could be identified on the surface of the helical domain II of NP24-I. In addition, some allergenic motifs bearing high similarity/identity with some predicted Ig-E binding motifs of closely related allergenic TLPs like Jun a 3 (*Juniperus ashei*, from mountain cedar pollen) and banana-TLP have been identified on the molecular surface of NP24-I.

Keywords Antifungal protein · Crystal structure · β -Glucanase activity · Thaumatin-like protein

Abbreviations

TLP	Thaumatin-like protein
PR	Pathogenesis related
OLP	Osmotin-like protein
CM-pachyman	Carboxymethyl pachyman
Ig-E	Immunoglobulin E
PEG	Polyethylene glycol

Introduction

The thaumatin-like proteins (TLPs) belong to the Group 5 of the pathogenesis related (PR) family. The TLPs constitute a homogeneous family, members of which are produced by plants in response to different kinds of stress. Osmotin and osmotin-like proteins (OLPs) are non-sweet, salt induced proteins belonging to this group. NP24 is one such salt-induced protein from tomatoes. Though NP24 primarily accumulates in the root, it was first discovered by Pressey (1997) that the tomato fruit is a rich source of the protein. Two isoforms of NP24 (I and II) were observed, among which NP24-I was found to increase significantly during ripening of the fruit, which indicates that this salt-induced protein is developmentally regulated as well.

When tobacco and tomato suspension cells are adapted for growth in the presence of NaCl or other water potential reducing agents, an abundant quantity of a 24 kDa protein accumulates (King et al. 1986). The cDNA clones of these proteins have been subsequently characterized (King et al. 1988; Singh et al. 1989) as NP24 and osmotin, respectively. In addition to their roles in cellular tolerance of dehydration, both these proteins were found to cause sporangial lysis of *Phytophthora infestans* (Woloshuk et al. 1991), which suggests their involvement in protecting plant

Electronic supplementary material The online version of this article (doi:10.1007/s00425-008-0790-5) contains supplementary material, which is available to authorized users.

R. Ghosh · C. Chakrabarti (✉)
Crystallography and Molecular Biology Division,
Saha Institute of Nuclear Physics, 1/AF Bidhannagar,
Kolkata 700 064, India
e-mail: chandana.chakrabarti@saha.ac.in

cells from pathogens. Antifungal properties of TLPs have been extensively studied against a number of fungal species (Vigers et al. 1992). Though the exact molecular mechanisms through which the PR5 proteins exert their antifungal activity is yet unclear, it has been suggested that the fungal cell walls could be involved in the interactions between TLPs and invading fungi (Abad et al. 1996; Yun et al. 1998). Some TLPs bind β -1,3-glucans of the type commonly found in fungal walls (Theis and Stahl 2004). Consequently, those elements which are able to interact or degrade β -1,3-glucans are of importance in the battle against fungal invasion/infection. TLPs can exhibit glucan binding or glucanase activity (Trudel et al. 1998; Grenier et al. 1999; Osmond et al. 2001; Van Damme et al. 2002). Grenier et al. (1999) demonstrated that six TLPs (including NP24), binding or not to water-insoluble β -1,3-D-glucans, act as endo- β -1,3-glucanases on polymeric β -1,3-D-glucans. These findings, illustrating the unusual enzymatic activity of some TLPs, bring new insights into the mechanisms attributed to the antifungal activity of TLPs.

The pathogenesis-related proteins are reported to be a rich source of allergens (Knox and Suphioglu 1996; Astwood et al. 1996; Breiteneder 2004). The PR-5 family, in particular, comprises a number of allergenic proteins. Several allergenic TLPs are present in fruits (Breiteneder 2004) such as apple (Mal d 2), cherry (Pru av 2), bell pepper (Cap a 1 w), kiwi fruit (Act c 2), tomatoes (Lyc e NP24), etc. Jun a 3 from mountain cedar was the first isolated pollen allergen of this group. As these PR-5 allergens in pollen and fruits have similar structures, the knowledge can be utilized to guide specific immunotherapeutic treatment of allergy. It is essential to have a structural knowledge of the epitopes, which are responsible for allergenicity of these proteins and this knowledge can be utilized to design therapeutic agents based on them. NP24 belongs to the food allergen category of the Structural Database of Allergenic Proteins (SDAP) (<http://fermi.utmb.edu/SDAP>) (Schein et al. 2007). An allergenic motif has been identified in the sequence of NP24-I.

In order to understand its function from a structural point of view, the crystal structure of NP24-I has been determined at 2.45 Å resolution and docking studies were carried out with a β -1,3-glucan molecule to explain the glucan binding and glucanase activity of the protein.

Materials and methods

Purification, crystallization, data collection, structure determination and refinement

NP24-I was purified from locally available ripe tomatoes (*Lycopersicon esculentum* L.) as previously described

(Ghosh and Chakrabarti 2005). Needle-shaped crystals were obtained by the hanging-drop vapor-diffusion method using the optimized condition No.4 of the PEG/Ion screen (Hampton Research Inc., Aliso Viejo, CA, USA). The crystals belonging to the space group P43 with unit cell parameters $a = b = 61.01$, $c = 62.90$ Å were characterized and preliminary X-ray studies were reported (Ghosh and Chakrabarti 2005) at 2.45 Å. Diffraction data were collected in-house at room temperature with a MAR345dtb imaging-plate (Marresearch, Hamburg, Germany) system and a BRUKER FR591 rotating anode generator (Bruker AXS, Madison, WI, USA) equipped with the Osmic Confocal Max-Flux optic system (Rigaku/MSO, The Woodlands, TX, USA). The phase problem was solved by molecular replacement using the program AMoRe (Navaza 1994) implemented in the CCP4 suite (Collaborative Computational Project, Number 4 1994) using the crystal structure of osmotin (PDB ID:1PCV) as the template. Rigid body refinement with AMoRe (Navaza 1994) yielded an R factor of 32.6% and a correlation factor of 71.1%. Molecular replacement was followed by one run of rigid-body refinement using CNS (Brünger et al. 1998), which resulted in R and R_{free} values of 30.5 and 29.0%, respectively, with 5% data in the test set. The resolution range was 20–3.0 Å. Subsequent positional refinement with manual model building using QUANTA (Accelrys Inc., San Diego, CA, USA) according to $F_o - F_c$ and $2F_o - F_c$ electron density maps yielded R of 23.0% and R_{free} of 27.1%. Following simulated annealing refinement (3,000–300 K) the R factor was reduced to 22.4% ($R_{\text{free}} = 26.1\%$). Water molecules were incorporated into the model conservatively, i.e., only if they appeared as discrete spherical peaks in both $2F_o - F_c$ (1.0σ) and $F_o - F_c$ (3.5σ) electron density maps, satisfying the hydrogen-bonding criteria as incorporated in CNS (Brünger et al. 1998). Finally, a few cycles of positional B-group refinement of the protein structure with 67 water molecules and a Cl^- ion yielded a final R -factor of 17.1% and an R_{free} factor of 22.4%. The refinement statistics are given in Table 1. The final model was validated by PROCHECK (Laskowski et al. 1993). Molecular images were created by PyMOL (<http://pymol.sourceforge.net>) and GRASP (Nicholls et al. 1991). The structure has been deposited in the Protein Data Bank (PDB ID:2I0W).

Docking of a 1,3- β -D-glucan

Three-dimensional molecular modeling studies were performed on an Insight II (MSI, San Diego, CA, USA) molecular modeling platform run on a Silicon Graphics Octane2 workstation (Silicon Graphics, Sunnyvale, CA, USA). The ligand protein complex was generated based on the coordinates of NP24-I and curdlan (Deslandes

Table 1 Summary of data collection and final refinement statistics

<i>Crystal data</i> ^a	
Space group	P4 ₃
Unit cell params (Å)	a = b = 61.01, c = 62.90
Resolution (Å)	100–2.45 (2.51–2.45)
Completeness (%)	99.9 (99.8)
Multiplicity	4.06 (4.04)
I/σ (I)	3.9 (1.5)
R _{merge} ^b (%)	9.10 (33.69)
<i>Refinement statistics</i>	
Resolution range (Å)	20–2.45
No. of protein atoms	1553
No. of solvent molecules	67 (water), (1 Cl [−])
R factor ^c (%)	17.1
R _{free} ^c (%)	22.3
<i>R.m.s. deviation from ideality</i>	
Bond lengths (Å)	0.006
Bond angles (°)	1.40
Average B values (Å ²)	33.2
<i>Ramachandran plot, residues in (%)</i>	
Most favored regions (%)	88.2
Additionally allowed regions (%)	11.8

^a Values in parentheses are for the outer resolution shell

^b $R_{\text{merge}} = [\sum (|Ih| - \langle Ih \rangle)^2 / \sum (Ih)^2]^{1/2}$, where $\langle Ih \rangle$ is the average intensity of reflection h and symmetry-related reflections

^c $R = R_{\text{free}} = \sum |F_o| - |F_c| / \sum |F_o|$ calculated for reflections of the working set and test (5%) sets, respectively

et al. 1980) determined by X-ray diffraction studies. For thaumatin-like proteins, the polysaccharide-binding site is likely to be located in the surface cleft region (Osmond et al. 2001; Menu-Bouaouiche et al. 2003). The ligand was therefore introduced manually into the cavity in a position suitable to get a minimum number of clashes. In order to avoid steric hindrance, five crystallographic waters were removed from the cavity (22, 48, 50, 54 and 66) of NP24-I. Hydrogen atoms were added to the complex structure using the BUILDER module of Insight II (MSI Inc.). The force-field potentials and partial charges were assigned subsequently. The consistent valence force field (CVFF) was used for simulation studies. The resulting complex structure was then subjected to energy minimization using the DISCOVER module of Insight II (MSI Inc.). A dielectric constant 1.0 was used throughout the energy minimization. The complex was subjected to 500 steps of conjugate-gradient minimization. The final rms derivatives of energies were below 1.0. The aromatic ring of Phe95 of NP24-I was kept at a distance 6 Å from the hydrophobic face of one glucosyl residue and this restraint was used throughout the entire docking procedure.

Results

Crystal structure of NP24-I

The three-dimensional structure of NP24-I (Fig. 1) comprises the characteristic thaumatin-like fold. It consists of three domains. Domain I forms the central core of the molecule. It is made up of 11 β-strands forming a β-sandwich. Six antiparallel β-strands form the front sheet and the other five form another β-sheet, which together constitute the β-sandwich. Three of the eight disulfide bonds in the structure are in this domain (residues 9–104, 120–192 and 125–175). Domain II, on one side of the core domain I, consists of an α-helix, three α-helical segments and a β-sheet and is stabilized by three disulfide bonds (residues 133–143, 147–156, 157–162). The short domain III, on the other side of domain I is made up of a stretch of 32 amino acid residues comprising a long loop and a β-sheet. This domain has two disulfide bonds (residues 51–61, 66–72). All the eight disulfide bonds, marked in yellow in Fig. 1, are highly conserved in thaumatin (Ogata et al. 1992) and the TLPs (Batalia et al. 1996; Koiwa et al. 1999; Min et al. 2004; Leone et al. 2006).

The cleft region

In the structure of NP24-I, a prominent cleft is observed between domains I and II, a characteristic feature observed in thaumatin as well as in the TLPs. Electrostatic modeling of NP24-I reveals a highly acidic cleft (Fig. 2a) as also

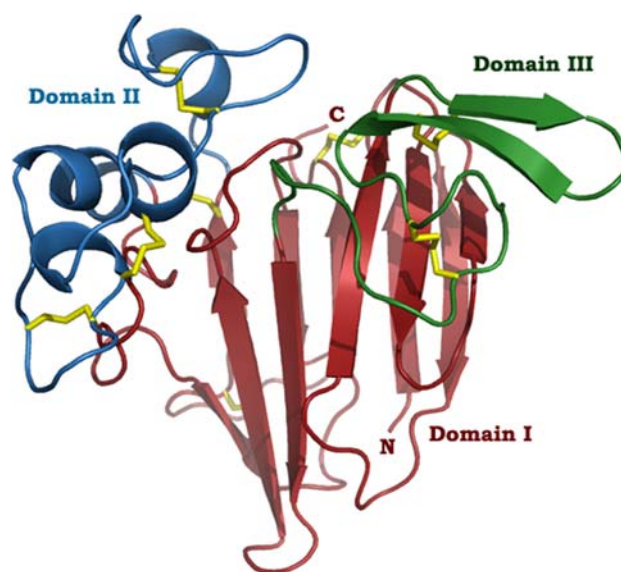


Fig. 1 A ribbon representation of NP24-I, showing the three domains in pink (I), blue (II) and green (III). The eight disulfide bonds in the structure are indicated in yellow. The cleft region lies at the interface of domains I and II

observed in the three-dimensional structures (Batalia et al. 1996; Koiwa et al. 1999; Min et al. 2004; Leone et al. 2006) of other antifungal TLPs. The side-chains of four conserved acidic residues, Glu84, Asp97, Asp102 and Asp 185 in NP24-I, intrude inside the cleft from domain II. Two of these, Glu89 and Asp101, however, are also present in thaumatin, which has a predominantly basic cleft. An important residue which makes an important contribution towards the basicity of the interdomain cleft in thaumatin is Lys49. In NP24-I, though the corresponding residue is also basic (Arg44), it is masked by Trp75, one of the four aromatic residues in the vicinity (the others being Phe90, Phe95 and Tyr179) surrounding its acidic cleft. In addition, a number of lysine residues, absent in the TLPs, surround the cleft region in thaumatin, which are thought to account for its sweet property (Kaneko and Kitabatake 2001). In NP24-I, residues Thr73, Leu93, Asp185 and Ala136 replace these lysine residues. Moreover, a stretch of amino acid residues (19KGDAALDAGGR29) observed in thaumatin, called the 'thaumatin loop', is also thought to be responsible for its sweet taste (Slootstra et al. 1995). This stretch in NP24-I is much shorter and exhibits a deletion of six residues, 20IG...GGR24. It is interesting to note, however, that the back surface of NP24-I, like thaumatin, is basic in nature (Fig. 2b).

Antifungal and β -glucanase activity

It has been suggested that the electronegative character of the interdomain cleft of the TLPs makes an important contribution towards their antifungal and β -glucanase activities (Grenier et al. 1999; Leone et al. 2006). The endo- β -1,3-glucanase activity of NP24 on a polymeric β -1,3-D-glucan has been demonstrated by Grenier et al. (1999). So, docking studies have been done to investigate the structural basis for the interaction of NP24-I with β -1,3-D-glucans and to understand its glucan binding properties and glucanase activity. For this purpose, curdlan, a water

insoluble β -1,3-D-glucan, was manually docked into the surface cleft of NP24-I. The clefts of TLPs are not sufficient to accommodate insoluble glucans like curdlan in untreated conditions owing to their large size and rigidity of the triple-helical structure, and interactions, as corroborated from experiments on the barley TLP (Osmond et al. 2001), are possible only in an extended conformation. Therefore, a single curdlan chain was used for docking and the shape complementarity between the protein and the ligand was good (Fig. S1). The model predicts that the residues Arg44, Gly59, Thr60, Asp65, Gly67, Trp75, Lys77, Pro78, Glu84, Asp102, Thr148, Cys156, Pro182, Asp185 are present within a distance of 3.5 Å from the docked glucan molecule (Fig. 3). Among these residues, Arg44, Gly59, Thr60, Asp65, Trp75, Lys77, Glu84, Asp102, Thr148, Cys156, Asp185 are involved in hydrogen bonding with the bound polysaccharide. In the docked complex, the β -1,3-D-glucan residue passes through the central cleft region with Glu84 on one side and Asp102, Asp97 on the other. This kind of orientation of a Glu-Asp pair, which is found in glucanases, is a pre-requisite for the cleavage of a glycosidic bond. In glucan hydrolysis, the average distance between the carboxylic oxygen atoms of the two catalytic residues is about 5 Å for the retaining mechanism (Zechel and Withers 2001). In NP24-I, the distance between O ϵ of Glu84 with O δ of Asp97 is 2.78 Å, which is too short to act as a catalytic pair. However, for the Glu84-Asp102 pair, this distance is greater (6.31 Å) and it perhaps functions as the proton donor and nucleophile pair that acts as the plausible catalytic center required for endo-glucanase activity.

Prediction of allergenicity

On analysis of the NP24-I sequence, using the WebAllergen server (<http://weballergen.bii.a-star.edu.sg/>), a 35-residue allergenic motif 152GQQYCCTQGPGPTLSKFFKKRCPDAYSYQDDP186 was identified, by comparing with a

Fig. 2 a, b GRASP (Nicholls et al. 1991) representation of the electrostatic surface potentials of NP24-I showing the highly acidic cleft (a) and a view of the same molecule turned 180 about the x axis (b). The negative potential (colored red) and the positive potential (colored blue) are displayed at -5 kT level and $+5$ kT level (1 kT = 0.6 kcal), respectively

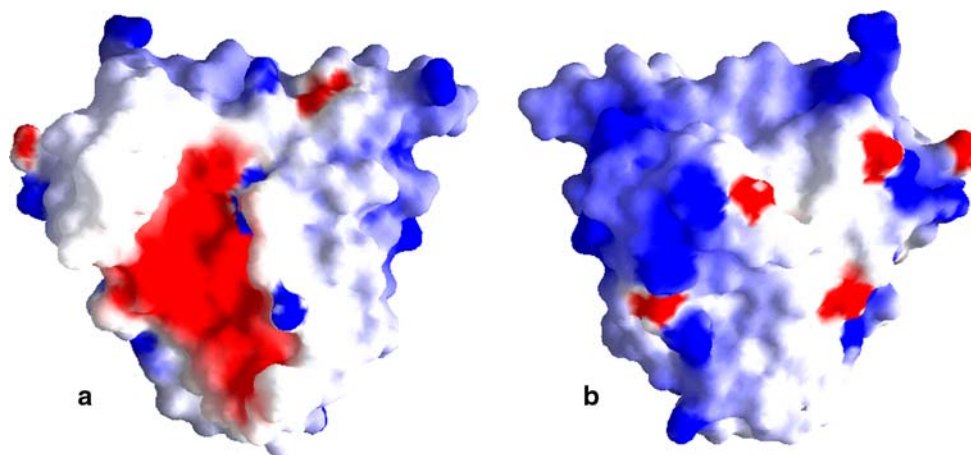
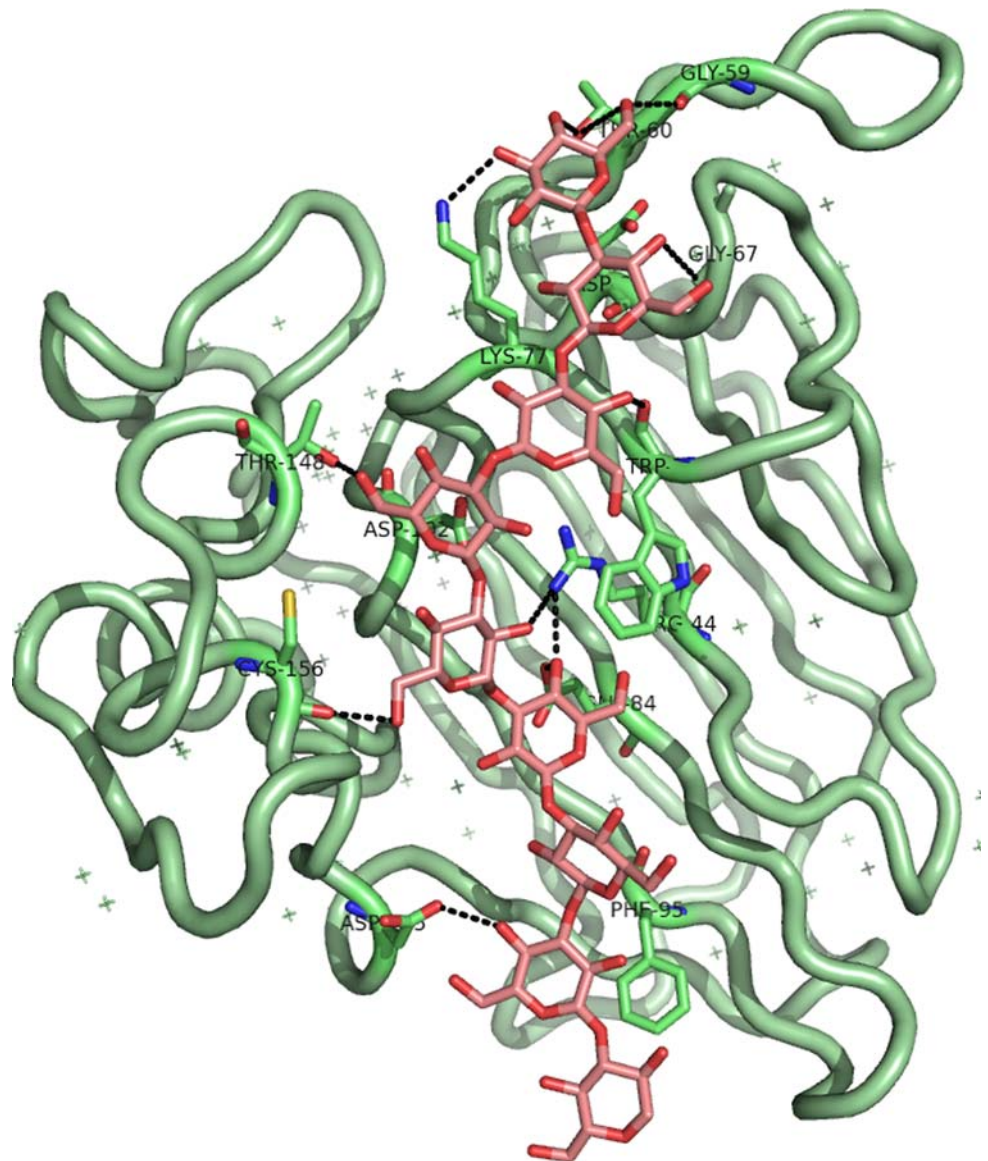


Fig. 3 Interactions between NP24-I and the curdlan molecule in the cleft region. The curdlan molecule and interacting residues of NP24-I are shown as stick models with carbon atoms colored *pink* and *green*, respectively. Hydrogen bonds with a donor–acceptor distance below 3.5 Å are shown as *black dotted lines*. Hydrogen bond forming residues of NP24-I are labeled



set of pre-built allergenic motifs in this database. The identified epitope (152–186) of NP24-I is mainly distributed on the helical domain II (Fig. 4a). This epitopic region of NP24-I shares both sequence and structural similarity with banana-TLP (ban-TLP) (Fig. 4b), the only allergenic PR-5 family TLP whose three-dimensional structure is known (PDB ID: 1Z3Q). When this epitopic region of NP24-I is projected on to the three-dimensional structure of the molecule, it is found to lie on the surface of the molecule (Fig. 5a). In addition to this motif, some parts of NP24-I exhibit high similarity/identity with other predicted Ig-E binding motifs of closely related allergenic TLPs. These motifs (Table 2) have been identified from a search of the Structural Database of Allergenic Proteins (SDAP) (<http://fermi.utmb.edu/SDAP>) and are also located on the molecular surface of the protein (Fig. 5b). There is,

however, no experimental confirmation of the allergenicity of NP24-I.

Discussion

The crystal structure of NP24-I, an isoform of a thaumatin-like protein (TLP), with antifungal properties has been reported at 2.45 Å resolution. The structure comprises the characteristic thaumatin-like fold with a prominent acidic cleft between domains I and II of the three-domain structure. An interesting observation from structures of all the reported antifungal TLPs till date (Batalia et al. 1996; Koiwa et al. 1999; Min et al. 2004; Leone et al. 2006) is that each of them has an acidic interdomain cleft. In contrast, thaumatin, with its predominantly basic cleft region,

Fig. 4 Comparison of the allergenic motif identified in NP24-I (**a**) using the WebAllergen (<http://weballergen.bii.a-star.edu.sg/>) server with the corresponding region in banana-TLP (**b**)

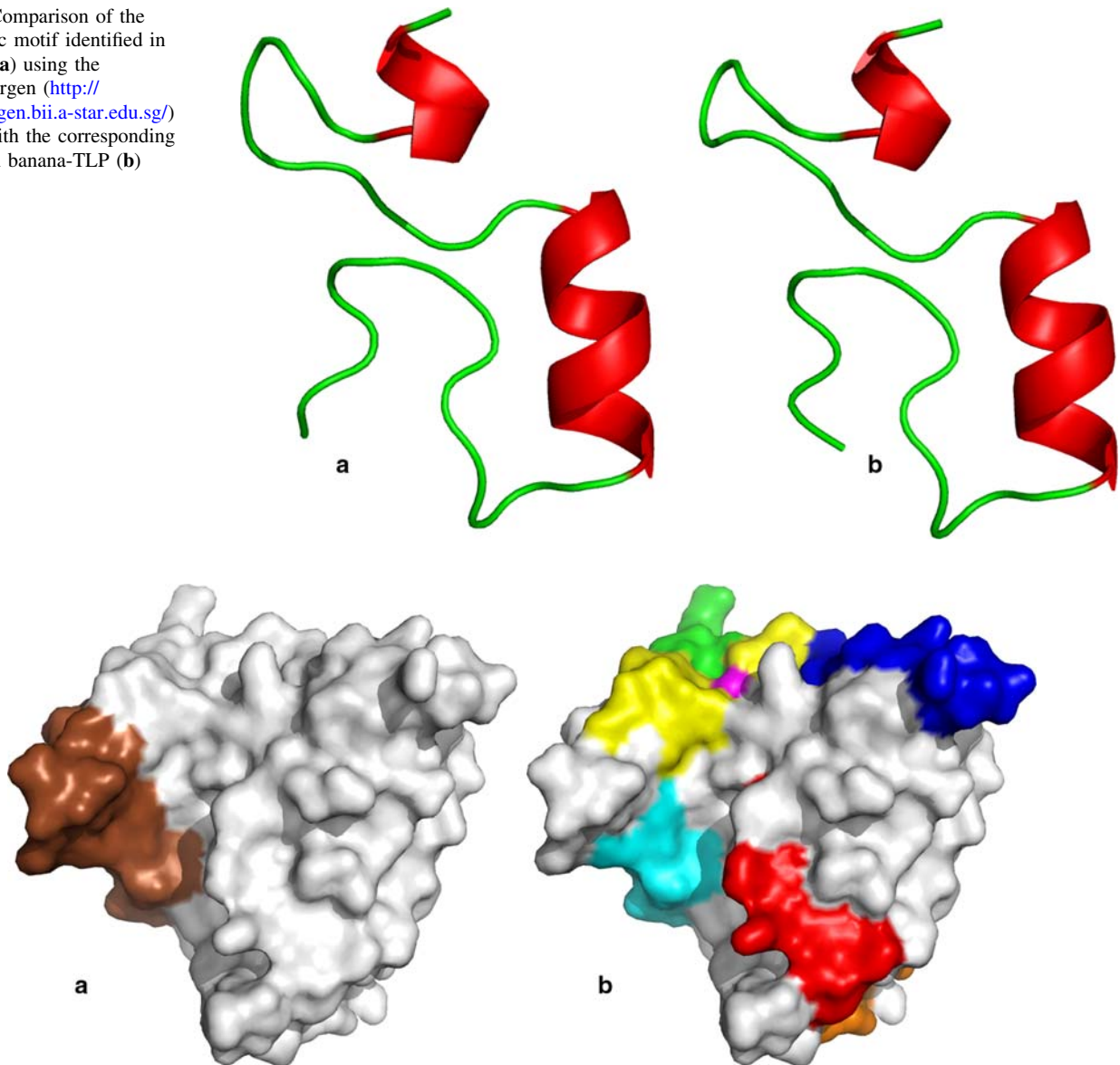


Fig. 5 **a, b** Predicted Ig-E binding epitopes located on the molecular surface of NP24-I. **a** The motif (amino acid residues 152–186) identified by the WebAllergen server. **b** The seven motifs identified by the Structural Database of Allergenic Proteins (SDAP) having

similarity with predicted epitopes in other allergenic TLPs. These epitopes, listed as 1–7 in Table 2, are colored *red, blue, green, yellow, magenta, cyan and orange*, respectively

Table 2 Some predicted allergenic motifs in NP24-I

Sequence of predicted epitopes in NP24-I	NP24-I residue no.	Sequence identity with
PRGTMARIWGRT	37–49	Jun a 3
NFNAAGRGTCQTG	52–64	Jun a 3
ANINGECPRALK	127–138	Jun a 3
VPGGCNPCTTFGG	139–152	Jun a 3
GGCNP	141–146	Ban-TLP
YSYPQDDPT	179–187	Ban-TLP
PGGSTNY	193–199	Ban-TLP

has been reported to have only a weak antifungal activity in synergism with nikkomycin (Vigers et al. 1991). The antifungal activity of the two isoforms of NP24 was experimentally determined by Pressey (1997) against some phytopathogenic fungi and NP24-I was found to be highly active against two such fungi.

Many antifungal TLPs can also either bind or cleave polymeric glucans, i.e. they can exhibit glucanase activity (Trudel et al. 1998; Grenier et al. 1999; Osmond et al. 2001; Van Damme et al. 2002; Menu-Bouaouiche et al. 2003). Efforts have been made to correlate the antifungal and β -1,3-glucanase activities of TLPs, mainly because of

their ability to bind and degrade β -1,3-D-glucans which are one of the major constituents of fungal cell walls. A low endo-glucanase activity NP24 was reported by Grenier et al. (1999) which was evident from its ability to hydrolyze water soluble β -1,3-D-glucans like CM-pachyman after prolonged incubation. From our docking studies of a β -1,3-D-glucan chain in the interdomain cleft of NP24-I a suitable orientation of a Glu-Asp pair required for glucanase activity could be located in the cleft and the lower endo-glucanase activity may be explained by the higher distance between the components of the catalytic pair (>5 Å) and a lack of proper environment.

NP24, belonging to the food allergen category of the Structural Database of Allergenic Proteins (SDAP), exhibits 55% sequence identity with Jun a 3, a major allergenic TLP from pollen of mountain cedar (*Juniperus ashei*), and 44% sequence identity with Pru av 2 and Mal d 2, allergens from cherry and apple, respectively. All these three are included in the list of the International Union of Immunological Societies (IUIS) (<http://www.allergen.org/Allergen.aspx>) accepted allergens and they display the ability to bind Ig-E from the respective allergic individuals (Inschlag et al. 1998; Soman et al. 2000; Krebitz et al. 2003). Sequence homology of NP24-I with these potent allergens, specifically with those Ig-E binding epitopes characterized from Jun a 3 (Table 2), facilitate to identify the epitopic regions in NP24-I.

References

- Abad LR, D'Urzo MP, Liu D, Narasimhan ML, Reuveni M, Zhu JK, Niu X, Singh NK, Hasegawa PM, Bressan RA (1996) Antifungal activity of tobacco osmotin has specificity and involves plasma membrane permeabilization. *Plant Sci* 118:11–23
- Astwood JD, Leach JN, Fuchs RL (1996) Stability of food allergens to digestion in vitro. *Nat Biotechnol* 14:1269–1273
- Batalia MA, Monzingo AF, Ernst S, Robertus JD (1996) The crystal structure of the antifungal protein zeamatin, a member of the thaumatin-like, PR-5 protein family. *Nat Struct Biol* 3:19–23
- Breiteneder H (2004) Thaumatin-like proteins a new family of pollen and fruit allergens. *Allergy* 59:479–481
- Brünger AT, Adams PD, Clore GM, DeLano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges M, Pannu NS, Read RJ, Rice LM, Simonson T, Warren GL (1998) Crystallography and NMR system: a new software suite for macromolecular structure determination. *Acta Crystallogr D* 54:905–921
- Collaborative Computational Project, Number 4 (1994) *Acta Crystallogr D* 50:760–763
- Deslandes Y, Marchessault RH, Sarko A (1980) Triple-helical structure of 1(3)- β -glucan. *Macromolecules* 1:1466–1471
- Ghosh R, Chakrabarti C (2005) Crystallization and preliminary X-ray diffraction studies of NP24-I, an isoform of a thaumatin-like protein from ripe tomato fruits. *Acta Crystallogr F* 61:806–807
- Grenier J, Potvin C, Trudel J, Asselin A (1999) Some thaumatin-like proteins hydrolyse polymeric β -1, 3-glucans. *Plant J* 19:473–480
- Inschlag C, Hoffmann-Sommergruber K, O'Riordan G, Ahorn H, Ebner C, Scheiner O, Breiteneder H (1998) Biochemical characterization of Pru a 2, a 23-kD thaumatin-like protein representing a potential major allergen in cherry (*Prunus avium*). *Int Arch Allergy Immunol* 116:22–28
- Kaneko R, Kitabatake N (2001) Structure-sweetness relationship in thaumatin: importance of lysine residues. *Chem Senses* 26:167–177
- King GJ, Hussey CE, Turner VA (1986) A protein induced by NaCl in suspension cultures of *Nicotiana tabacum* accumulates in whole plant roots. *Plant Mol Biol* 7:441–449
- King GJ, Turner VA, Hussey CE, Wurtele ES, Lee SM (1988) Isolation and characterization of a tomato cDNA clone which codes for a salt-induced protein. *Plant Mol Biol* 10:401–412
- Knox B, Suphioglu C (1996) Environmental and molecular biology of pollen allergens. *Trends Plant Sci* 1:156–164
- Koiwa H, Kato H, Nakatsu T, Oda J, Yamada Y, Sato F (1999) Crystal structure of tobacco PR-5d protein at 1.8 Å resolution reveals a conserved acidic cleft structure in antifungal thaumatin-like proteins. *J Mol Biol* 286:1137–1145
- Krebitz M, Wagner B, Ferreira F, Peterbauer C, Campillo N, Witty M, Kolarich D, Steinkellner H, Scheiner O, Breiteneder H (2003) Plant-based heterologous expression of Mal d 2, a thaumatin-like protein and allergen of apple (*Malus domestica*), and its characterization as an antifungal protein. *J Mol Biol* 329:721–730
- Laskowski RA, MacArthur MW, Moss DS, Thornton JM (1993) PROCHECK: a program to check the stereochemistry of protein structures. *J Appl Crystallogr* 26:283–291
- Leone P, Menu-Bouaouiche L, Peumans WJ, Payan F, Barre A, Roussel A, Van Damme EJ, Rougé P (2006) Resolution of the structure of the allergenic and antifungal banana fruit thaumatin-like protein at 1.7-Å. *Biochimie* 88:45–52
- Menu-Bouaouiche L, Vriet C, Peumans WJ, Barre A, Van Damme EJ, Rougé P (2003) A molecular basis for the endo-beta 1, 3-glucanase activity of the thaumatin-like proteins from edible fruits. *Biochimie* 85:123–131
- Min K, Ha SC, Hasegawa PM, Bressan RA, Yun D-J, Kim KK (2004) Crystal structure of osmotin, a plant antifungal protein. *Proteins Struct Funct Bioinform* 54:170–173
- Navaza J (1994) AMoRe: an automated package for molecular replacement. *Acta Crystallogr A* 50:157–163
- Nicholls A, Sharp KA, Honig B (1991) Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins Struct Funct Genet* 11:281–296
- Ogata CM, Gordon PF, de Vos AM, Kim SH (1992) Crystal structure of a sweet tasting protein thaumatin I, at 1.65 Å resolution. *J Mol Biol* 228:893–908
- Osmond RIW, Hrmova M, Fontaine F, Imbert A, Fincher GB (2001) Binding interactions between barley thaumatin-like proteins and (1, 3)- β -D-glucans. *Eur J Biochem* 268:4190–4199
- Pressey R (1997) Two isoforms of NP24: a thaumatin-like protein in tomato fruit. *Phytochemistry* 44:1241–1245
- Schein CH, Ivanciuc O, Braun W (2007) Bioinformatics approaches to classifying allergens and predicting cross-reactivity. *Immunol Allergy Clin North Am* 27:1–27
- Singh NK, Nelson DE, Kuhn D, Hasegawa PM, Bressan RA (1989) Molecular cloning of osmotin and regulation of its expression by ABA and adaptation to low water potential. *Plant Physiol* 90:1096–1101
- Slootstra JW, De Geus P, Haas H, Verrips CT, Melen RH (1995) Possible active site of the sweet-tasting protein thaumatin. *Chem Senses* 20:535–543
- Soman KV, Midoro-Horiuti T, Ferreón JC, Goldblum RM, Brooks EG, Kurosky A, Braun W, Schein CH (2000) Homology modeling and characterization of Ig-E binding epitopes of mountain cedar allergen Jun a 3. *Biophys J* 79:1601–1609

- Theis T, Stahl U (2004) Antifungal proteins: targets, mechanisms and prospective applications. *Cell Mol Life Sci* 61:437–455
- Trudel J, Grenier J, Potvin C, Asselin A (1998) Several thaumatin-like proteins bind to β -1, 3-glucans. *Plant Physiol* 118:1431–1438
- Van Damme EJM, Charels D, Menu-Bouaouiche L, Proost P, Barre A, Rougé P, Peumans WJ (2002) Biochemical, molecular and structural analysis of multiple thaumatin-like proteins from the elderberry tree (*Sambucus nigra* L.). *Planta* 214:853–862
- Vigers AJ, Wiedemann S, Roberts WK, Legrand M, Selitrennikoff CP, Fritig B (1991) A new family of plant antifungal proteins. *Mol Plant Microbe Interact* 4:315–323
- Vigers AJ, Wiedemann S, Roberts WK, Legrand M, Selitrennikoff CP, Fritig B (1992) Thaumatin-like pathogenesis-related proteins are antifungal. *Plant Sci* 83:155–161
- Woloshuk CP, Meulenhoff JS, Sela-Buurlage M, van den Elzen PJ, Cornelissen BJ (1991) Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*. *Plant Cell* 3:619–628
- Yun D, Ibeas JJ, Lee H, Coca MA, Narasimhan ML, Uesonop Y, Hasegawa PM, Pardo JM, Bressan RA (1998) Osmotin, a plant antifungal protein subverts signal transduction to enhance fungal cell susceptibility. *Mol Cell* 1:807–817
- Zechel DL, Withers SG (2001) Dissection of nucleophilic and acid-base catalysis in glycosidases. *Curr Opin Chem Biol* 5:643–649

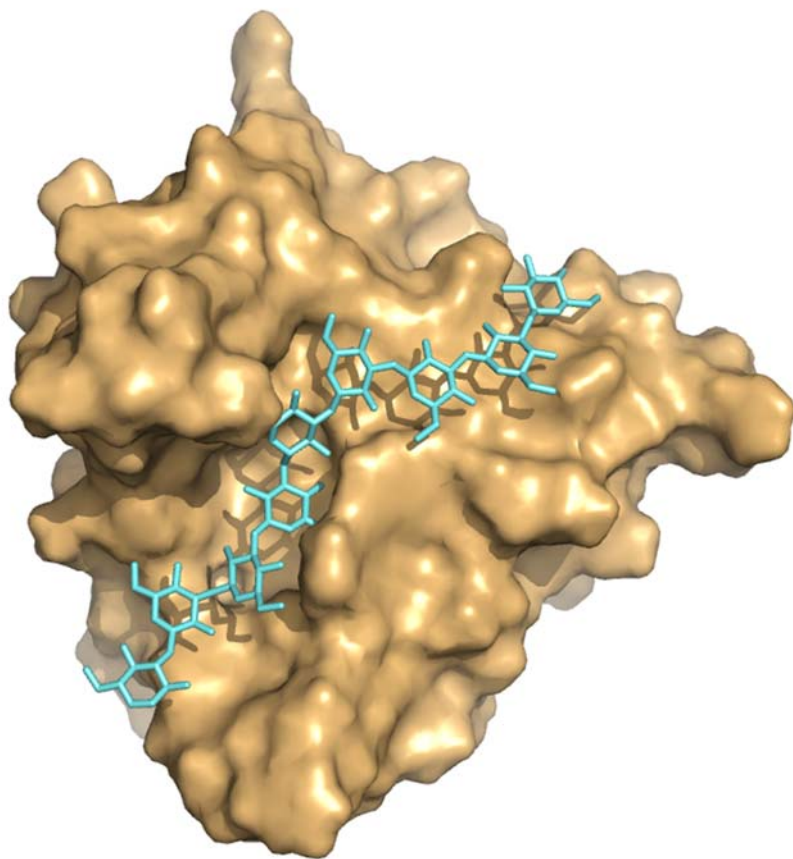


Fig. S1 Docking of a β -1,3-glucan (curdlan) in the acidic cleft of NP24-I, where the curdlan molecule is shown as a stick model (sky blue)