



Structural insight into protein T1, the non-allergenic member of the Bet v 1 allergen family—An *in silico* analysis

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Abstract

Background: Bet v 1 family is a large group of allergens present in pollen and plant-derived foods. They show similarity in sequence and 3D structure, properties often correlated to their cross-reactivity. protein T1 is a naturally occurring non-allergenic member of this group, which, in spite of having 35–85% sequence similarity, does not exhibit antibody-mediated cross-reactivity with other members of the Bet v 1 allergen family. This protein has been suggested to be a good model to study structural features critically needed for allergenicity and cross-reactivity.

Objective: Structural bioinformatic approach to analyze the properties of protein T1 in relation to allergenicity and cross-reactivity.

Methods: A 3D model of protein T1 was constructed by the homology modeling procedure and was compared to the structure of Bet v 1 with special reference to conservation, solvent accessibility and electrostatic properties of the residues across the antigenic surface.

Results: The over-all fold of the homology model of T1 resembles that of the Bet v 1 group. The change in the solvent-accessible surface of the IgG (BV16)-binding residues has been quantified to be 75%. Comparing the allergenically potent dimer structure of Bet v 1 (predicted structure available in MSD of European Bioinformatics Institute), with the non-allergenic monomer, the BV16-binding surface and the p-loop seem to be of less importance for Bet v 1-mediated *in vivo* cross-linking of IgE. Instead, we think that other surface-exposed conserved patches are more significant. Absence of cross-reactivity between T1 and Bet v 1 comes from the lack of surface conservation leading to changes in the conformational as well as electrostatic properties. On the other hand, certain changes in the sequence, that might hinder dimerization of this protein, may result in the loss of allergenicity. Lastly, our structural docking experiment shows that protein T1 can bind two molecules of Brassinolide ligands like the other members of the Bet v 1 family.

Conclusion: Lack of dimerization and surface conservation might explain the reason why T1 is neither allergenic, nor cross-reactive to Bet v 1 group, although this protein can be a potential carrier for brassinosteroids.

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Keywords: Bet v 1; Protein T1; Structural bioinformatics; Allergenicity; Cross-reactivity

1. Introduction

Allergic diseases affect up to 20% of population in the industrialized world. Symptoms of type I allergy occur from cross-linking of effector cell-bound IgE by multivalent allergens. In central Europe, one of nine patients with type I allergy shows specific IgE-mediated reactions towards birch pollen, 95% of these patients being sensitized to the major pollen allergen Bet

v 1 (Joralim et al., 1989). Thus, Bet v 1 is a clinically important inhalant allergen estimated to cause allergy in 5–10 million individuals worldwide (Gajhede et al., 1996). Moreover, allergenic proteins, homologous to Bet v 1, were identified from diverse sources. Antibodies elicited by an allergen from one of the sources show extensive cross-reactivity with other members of the Bet v 1 allergen family. That is why people sensitive to Bet v 1 often display adverse reactions after inhalation of other pollens (Alder, Hazel) or ingestion of certain fresh fruits (apple, pear, peach, cherry), vegetables (carrot, celery) and nuts (hazelnut, walnut). Proteins of Bet v 1 allergen family exhibit good over-all sequence identity and contain a conserved glycine rich loop (P-loop). Based on the structural studies, it has been proposed that the proteins of Bet v 1 family function as brassinosteroid carriers (Morković-Housely et al., 2003). This was later

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supported by the binding studies with phytosteroids (Neudecker et al., 2001; Mogensen et al., 2002).

Protein T1 was discovered from the periwinkle plant (*Catharanthus roseus*) as a cytokinin (a plant growth regulator)-inducible protein (Carpin et al., 1998). It shows significant sequence identities with the members of Bet v 1 allergen family (Carpin et al., 1998; Laffer et al., 2003) and it also contains a glycine rich loop. Moreover, two regions, each of at least nine residues and sequence identity of at least 54% were identified in T1, Bet v 1 and other proteins of the Bet v1 group. In spite of having these common features, protein T1 is (a) completely non-allergenic (b) not recognised by serum IgE from Bet v 1 allergic patients (c) does not show immediate skin reaction in Bet v 1—allergic patients, nor induce histamine release from basophils and (d) rabbit anti T1 IgG does not react to Bet v 1 family in general. It is known as the non-allergenic member of the Bet v 1 allergen family and considered as the negative reference in allergenicity prediction server Allermatch (Fiers et al., 2004). It has been suggested that this non-allergenic protein, when compared to the highly allergenic Bet v 1 molecule, may teach us about the structural requirements for allergenicity of the Bet v 1 allergen family (Laffer et al., 2003).

Cross-reactivity is believed to be the result of conserved surface-exposed residues, forming cross-reactive epitopes. Attempts have been made to identify IgE-binding residues of Bet v 1 by (a) mapping conserved residues on cross-reactive allergen surfaces (Gajhede et al., 1996; Schirmer et al., 2005), (b) site directed mutagenesis (Ferreira et al., 1998), (c) phage-displayed allergen mimotope technology (Gangenberger et al., 2000) and (d) X-ray crystallography (Mirza et al., 2000). Recently, dimerization of this molecule has been found to be important for its *in vivo* cross-linking potential. But, although Bet v 1 family is a group of extremely important allergens, little is known about their B-cell epitopes (Schirmer et al., 2005).

Structural bio-informatics has recently been described as a potential tool to understand the molecular basis of allergenicity and cross-reactivity (Jenkins, 2005; Breiteneder and Mills, 2006). Using this approach, we previously identified conserved surface-exposed patches of the allergenic cyclophilins to explain the structural basis of their cross-reactivity (Roy et al., 2003). In the present study, we have constructed a 3D model of protein T1, and studied its relevant structural features. Our study will help design further experiments for better understanding of structural features responsible for allergenicity and cross-reactivity amongst Bet v 1 allergen family.

2. Materials and methods

The sequence of protein T1 was obtained from Laffer et al., 2003. The other protein sequences were picked up from the Swiss server (<http://www.expasy.org>). A 3D model of T1 has been built using the crystal structure of Bet v 1 molecule as the template. Its co-ordinates (1BV1.pdb) were taken from the Protein Data Bank (<http://www.rcsb.org/pdb>). The sequence of protein T1 was aligned with that of the allergens using CLUSTAL W (Thompson et al., 1994). Program Modeller (Sali and Bundell, 1993) was used for homology modeling. Energy

minimization was carried out using the program Discover with Consistent Valence Force Field (CVFF) (Hagler et al., 1979). The stereochemical quality of the model was checked using the program PROCHECK (Laskowski et al., 1993) and the Ramachandran plot (Ramachandran and Sashisekharan, 1968) was drawn. The ribbon diagram and the molecular surface showing the conserved patches (putative epitopes) were generated using program PyMol (DeLano Scientific LLC, 2006). The alpha carbon backbones of the template and the model were superimposed and the backbone RMSD was determined using the program SPDBV (Guex and Peitsch, 1997). The electrostatic surface of the protein was visualized by using the APBS (Baker et al., 2001) module of PMV (Sanner, 1999). For graphical representation, the electrostatic potential of the residues were calculated by using the DelPhi (Rocchia et al., 2001) module of Insight II (Molecular Simulations Inc.), and plotted against residue number. All calculations were done assuming dielectric constants of 2 and 80 for protein and solvent, respectively and an ionic strength of 0.3 M.

The co-ordinate structure of dimerized Bet v 1 has been obtained from macromolecular structure database of European Bioinformatics Institute (<http://www.ebi.ac.uk/msd-srv/msdlite/atlas/assembly/1bv1.html>). Values of Solvent Accessible Surface Area (SASA) were calculated using the program DSSP (Kabsch and Sander, 1983) and used throughout the study, unless specified. This program calculates the SASA values assuming a radius of 1.4 Å for the water molecule. The relative accessibility values were calculated as a percentage of solvent accessibility of the amino acid (X) in the tri-peptide gly–X–gly.

The co-ordinate structure of Brassinolide has been obtained from Cambridge Structural Database (CCD code: BRASSA01). The polycyclic ring structure and the stereochemistry are analogous for deoxycholate and brassinolide (Morković-Housely et al., 2003). Docking of brassinolide as the inner and outer ligand of protein T1 was performed by superimposition of C and D rings of the brassinolide on the inner and outer deoxycholate molecules of the crystal structure of Bet v 11 complexed with deoxycholate (PDB code 1FM4) (Mirza et al., 2000). Brassinolide molecules are easily fitted in the binding cavity of protein T1 without any sterical hindrance (Fig. 2d). All possible substituents at ring positions 2, 3, 6 and 7 of the brassinolide accommodated into the binding cavity without alteration of the binding mode observed with deoxycholate in the crystal structure. The interaction of the alkyl side-chain of the brassinolide molecules with residues of protein T1 were optimized by minimizing the energy of the T1-brassinolide complex. The diagram of the final structure was drawn using program PyMol (DeLano Scientific LLC, 2006).

3. Results and discussion

3.1. Sequence comparison

The sequence alignment (Fig. 1) of Protein T1 (designated as 'Pro' in the figure) with Bet v 1 and five important cross-reactive allergens (Mal d 1, Pru av 1, Dau c 1, Api g 1 and Car b

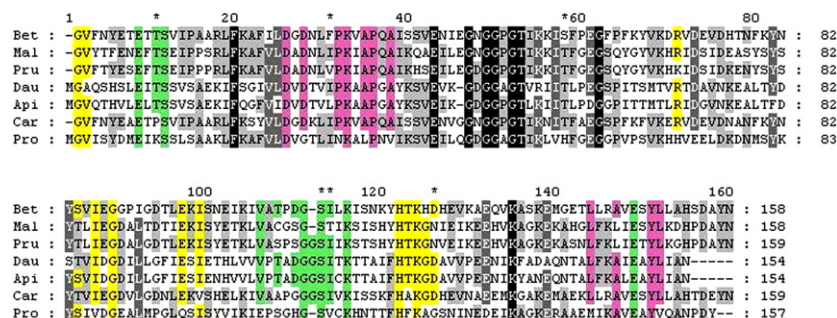


Fig. 1. The sequence of protein T1 (designated as 'Pro') was aligned with Bet v 1 and related allergens from Apple (Mal d 1, P43211), Cherry (Pru av 1, O0429), Carrot (Dau c 1, O24248), celery (Api g 1, P49372) and hornbeam (Car b 1, P38949). Amino acid numbers have been printed on top. Residues important for IgE-binding, as identified by site directed mutagenesis, are marked by asterisk on top. Conserved residues of the three cross-reactive epitopes proposed from structure-based prediction, are shown in yellow, green and magenta. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

1) shows two dominant features. First, Bet v 1, Mal d 1, Pru av 1 and Car b 1 have a 5-residue extension at their C-termini, which is absent in Carrot and Celery allergens (Dau c 1 and Api g 1). In case of Protein T1, there is an extension of three residues. These residues, at the alpha-helical C-termini, which contain the T-Cell epitopes, should not affect the allergenicity, as shown by mutagenesis studies (Neudecker et al., 2003). Secondly, the negatively charged glutamic acid residue, at the 45 position (E₄₅, here, and through out the Bet v 1 numbering is used), is absent in T1. This residue is critically important for IgG and IgE binding (Mirza et al., 2000). Lastly, Ferreira et al. (1998), by site-directed mutagenesis, identified six residues critical for the IgE-binding. Four out these six residues have been changed in case of protein T1 (marked by asterisk on the protein sequences

in Fig. 1). These changes might confer the loss of allergenicity to protein T1.

There are two regions (henceforth designated as region I and region II) with lengths of at least nine amino acids and sequence identity of at least 54%, present in T1, Bet v 1 and other cross-reactive allergens (Laffer et al., 2003). Protein T1 contains two amino acid changes in each of these regions. To quantify the changes, we found that the solvent-accessible surface of region I (A₁₆ to D₂₆) measures about 405.13 Å² and the changed residues (R16 K and I24 V replacements) occupy 18% of this surface. Similarly, the unmatched residues (N47D and P50A) spans 219.29 Å² out of a total 428.65 Å² surface of region II (G₄₇ to K₅₅), which accounts for a change of more than 52% in this region.

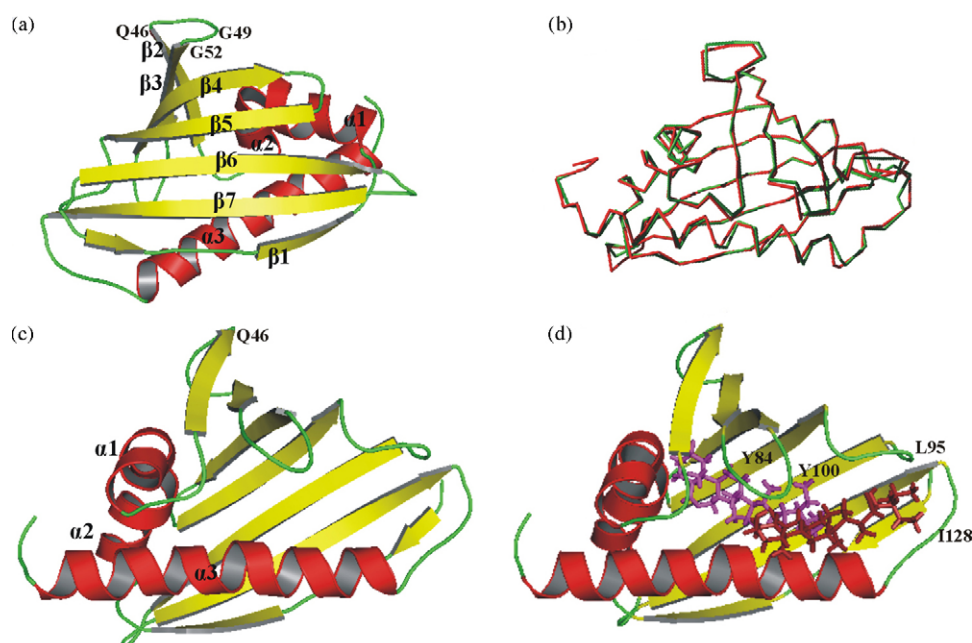


Fig. 2. (a) and (b) Ribbon diagram of protein T1: the protein structure consists of seven anti-parallel β-sheets (β1–β7), wrapped around a 23-residue long C-terminal α-helix (α3). There are two other α helices connecting β1 and β2. (c) α-Carbon backbone superimposition: the model structure of protein T1 and the crystallographically deduced structure of birch pollen allergen Bet v 1 (1BV1.pdb) have been shown in green and red respectively. (d) Result of structural docking showing the inner and outer ligand bound to protein T1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

3.2. Structure comparison

Fig. 2a is a cartoon representation of the structural model of T1. It is composed of seven anti-parallel β -sheets (β 1–7) folded into a compact cylinder that contains a large hydrophobic Y-shaped forked cavity. There are two short alpha helices (between β 1 and β 2) and a long c-terminal alpha helix, the latter containing 23 residues. The alpha carbon backbone of T1 can be superimposed (Fig. 2b) on that of Bet v 1 with an RMSD of 0.66 Å. The main structural difference is present in the residues 107–110 (RMSD more than 3 Å for four C α atoms), which lies in the loop region between β 6 and β 7 and corresponds to the green-colored residues (in Fig. 1). The over-all fold of the model strongly resembles that of Bet v 1a.

3.3. BV16-binding amino acids and the p loop

From the crystallographic studies of Bet v 1a, bound to its monoclonal antibody BV16, it was found that the relevant antigenic surface consists of 16 residues- a continuous stretch (E₄₂-T₅₂) along with a few others (R₇₀, D₇₂, H₇₆, I₈₆ and K₉₇). The sum-total of SASA values of these residues is 1123 Å² in Bet v 1. But, nine (871 Å²) out of these 16 residues have been replaced in T1, which accounts for a change of 75% in this surface.

A perfect fit in the topographies of the complementary surfaces of the epitope/paratope interaction has been described as a major factor in determining the binding affinity (Davies et al., 1990). A closer look at the experimentally deduced antigenic surface of Bet v 1, as evident from the crystal structure of Bet v 1-BV16 complex, shows that E₄₅ is located centrally in the binding pocket of Bet v 1a and fits nicely into the groove on the antibody surface and its carbonyl oxygen atoms form two hydrogen bonds with antibody backbone nitrogen atoms (Mirza et al.,

2000). The stretch E₄₂-T₅₂ of T1 was superimposed on the corresponding region of Bet v 1 (figure not shown) to understand the structural diversity of this region which shows an RMSD of 1.2 Å. Although, in this context, it might be mentioned that the loss of charge complementarity between the allergen (due to E45Q substitution) and the positively charged paratope at the very center of the Fab binding pocket of the antibody alone can very well explain the inability of protein T1 to bind the BV16 antibody. In fact, an E45S mutation has actually been made in Bet v 1, which showed no effect on either local or global conformation of the protein (Spangfort et al., 2003), but abolished the binding of BV16 and substantially reduced the binding of polyclonal human IgE.

In our structural model of protein T1, the p-loop exists between β 2 and β 3 and composed of seven residues (Q₄₆ to G₅₂, as defined by SPDBV). Although of similar length, this region of T1 differs from that of Bet v 1 in lacking the negative charge due to E45Q replacement, evidently changing the electrostatic character of the antigenic surface.

3.4. Cross reactive epitopes of the Bet v1 family predicted from 3D structure

Mapping of conserved residues on the surfaces of cross-reactive allergens led to the identification of three solvent-exposed patches (cross-reactive epitopes) of the Bet v 1 allergen family (Schirmer et al., 2005). Fig. 3 a and b show the corresponding residues mapped on the surface of protein T1. We found that the patches were disrupted by the replaced amino acid residues. Absolute and relative solvent accessibility values of the conserved residues of the patches are shown in Tables 1a and 1b. In Protein T1, cluster I, II and III contain seven, six and five conserved residues respectively. Although the number of conserved residues are least in cluster III, it contains largest

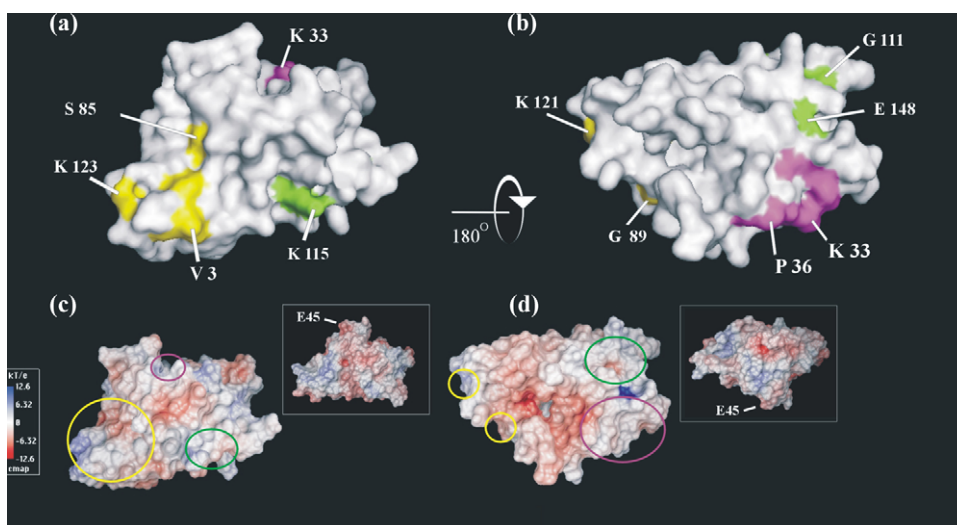


Fig. 3. (a) Mapping of conserved residues (which form three predicted 'cross-reactive' epitopes of the Bet v 1 allergen family) on the surface of protein T1. The clusters are coloured in yellow, green and magenta respectively (following Shirmer et al., 2005). (b) The molecule has been rotated 180° along the horizontal axis. (c) and (d) the electrostatic potential of the residues mapped on the protein surface. Inset diagrams show the comparative surface of Bet v 1, in the same orientation. The positions of the green, blue and magenta coloured patches have been shown by circles of respective colours. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Table 1a

SASA (in Å²) of the conserved residues of protein T1: only seven, six and five residues are conserved in cluster I, cluster II and cluster III respectively

I	G ₁	V ₃	S ₈₅	G ₈₉	I ₉₉	H ₁₂₁	K ₁₂₃
	19.7	80.9	17.7	26.7	0.9	42.3	86.8
	23.5	49.8	14.1	31.8	0.49	21.3	41.8
II	E ₉	S ₁₂	G ₁₁₁	S ₁₁₂	K ₉₀	E ₁₄₈	
	114.1	11.5	12.5	3.8	93.0	29.3	
	61.0	9.16	14.9	3.02	44.8	16.6	
III	D ₂₆	K ₃₃	P ₃₆	A ₁₄₆	Y ₁₅₀		
	55.0	139.7	81.6	21.9	74.8		
	35.4	67.3	56.4	18.8	31.4		

For comparison, the values have been listed in Bet v 1 monomer (B_{mo}), dimer (B_{di}) and in protein T1. Relative accessibility values are in italics.

conserved surface (more than 370 Å²), most of which (84%) comes from the side-chains and only 16% (approximately 60 Å²) has been contributed by the main chain atoms, as calculated by the GETAREA 1.1 program (http://www.scsb.utmb.edu/cgi-bin/get_a_form.tcl) using a 1.4 Å probe. The conserved surface area of the other clusters are even less. As most of the Fab fragments are known to bind to relatively contiguous 600–900 Å² surface patches (Braden and Poljak, 1995), none of these patches are likely to form cross-reactive epitopes in case of Protein T1.

Screening of a phage-displayed nona-peptide library by Bet v 1-specific human IgE identified a mimotope sequence. With this sequence, a computer-based 3D epitope search could locate two regions between residues 9–22 and 104–113. They are neighboring regions on the protein surface and are potential immuno-dominant IgE-epitopes (Gangenberger et al., 2000). Combining this information with the above-mentioned three patches, the IgE-binding residues of Bet v 1 could be correlated to the green colored residues (Fig. 1). However, a recent study (Mittag et al., 2006), which also used phage-display technology and computer-based mapping of the amino-acid sequences, supports the existence of the antigenic region that was identified by crystallographic studies (Mirza et al., 2000 and Spangfort et al., 2003).

Table 1b

Accessibility values (SASA in Å²) of 20 residues have been dropped in Bet v 1 dimer

Bet	K ₂₀	L ₂₄	D ₂₅	D ₂₇	N ₂₈	P ₃₁	K ₃₂	S ₄₀	V ₄₁	E ₄₂	N ₄₃	I ₄₄	E ₄₅	G ₄₆	N ₄₇	G ₄₈	G ₄₉	P ₅₀	K ₅₄	K ₅₅
B _{mo}	94	77	56	54	81	34	144	47	34	102	74	76	118	37	177	36	29	87	21	49
	45	39	36	35	48	23	69	37	21	54	44	40	63	44	69	43	35	66	10	24
B _{di}	20	27	12	47	13	14	81	33	6	11	3	45	116	13	43	18	11	80	12	36
	9.6	0.1	7.7	30	7.7	9.6	39	26	3.7	5.8	1.8	24	62	15	25	21	13	55	5.8	17
T1	K	L	D	G	T	N	K	S	V	E	I	L	Q	G	D	G	G	A	K	L
	129	70	64	34	89	39	140	29	33	93	73	101	125	46	155	34	34	77	22	42
	62	35	41	40	26	23	67	23	20	50	38	51	66	55	100	40	40	66	11	21

For comparison, corresponding the values have been listed in Bet v 1 monomer (B_{mo}), dimer (B_{di}) and in protein T1. Relative accessibility values are in italics.

3.5. The electrostatic surface

Electrostatic complementarity of the interacting surfaces is an important factor for protein–protein interaction in general, and antigen–antibody interactions in particular (Van Oss, 1995). The electrostatic potential of the residues have been mapped on the protein surface (Fig. 3c and d) which showed a clear difference to Bet v 1 (inset diagrams). On the electrostatic surface of Bet v 1, E45 has been found to be a red bump (as described by Spangfort et al., 2003), which is very distinctive, in contrast to protein T1. It seems that solvent-exposed residues (especially that of the conserved patches) with differences in the electrostatic potential might be responsible for the disruption of cross-reactivity among Bet v 1 and protein T1. Plotting of residue-wise electrostatic potential values, as calculated by the delphi (Rocchia et al., 2001; data not shown), with the respective SASA values can also support this view.

3.6. Analysis of the dimerized structure of Bet v 1

In addition to monomers, Bet v 1 molecule exists, in solution, as dimeric and even in tetrameric conditions. According to a recent investigation, the dimeric, but not the monomeric, molecules are capable of cross-linking the effector cell-bound IgE molecules, nor can the monomeric Bet v 1 molecules activate the B cells by cross-linking BCR for specific IgE production. As a result, monomeric forms are rather hypoallergenic (Schöll et al., 2005).

Although, there are substantial evidences to believe that the monomer of Bet v 1 is not allergenic, all the presently available experimentally deduced structures of Bet v 1 describe only the monomer form of the protein. So it is not known which parts of the molecule mediate the dimerization or even if is a symmetrical or asymmetrical interface. But, as there is also evidence for oligomerization of Bet v 1, an asymmetrical interface, is a distinct possibility, at least in case of the oligomeric form. However, the recently available crystal structure of Api g 1 (2BK0.pdb), a Bet v 1-homologue, shows that Api g 1 is a dimer with its chains held together by isologous contacts.

The only available probable structure of the Bet v 1 dimer can be obtained from the Macromolecular Structure Database (MSD) of European Bioinformatics Institute (EBI).

This database, determines the possible oligomeric states of the proteins by using the program PQS (<http://pqs.ebi.ac.uk>), which tries to predict the quaternary structure by putting in symmetry-related copies of a chain within a defined distance of the monomer and assessing the extent of buried surface area. If a symmetry-related second chain results in the burial of 400 Å² or more of surface area, relative to the monomers, the database scores it as biologically meaningful.

Our study of this bioinformatically predicted Bet v 1 dimer structure shows two important facts: (1) in the homodimeric structure of this allergen, the p-loop region is almost buried. With respect to the monomeric form, the accessibility values of the residues S₄₀ to P₅₀ of the dimer, drops drastically, except E₄₅, which remains almost the same. Unexpectedly, most of these residues are important constituents of BV 16 binding surface, but they seem to be less important in case of the dimeric form. Notably, the significance of E₄₅ has been documented by crystallographic and mutagenesis studies (Mirza et al., 2000; Spangfort et al., 2003). So if we consider the structure of this dimer as a valid one, it can be said that these buried residues might be significant for the binding of IgG (BV16) and IgE to the monomers, but for *in vivo* cross-linking, which is mediated by the dimer molecules, other epitopes are involved. (2) Accessibility values of a total of 20 residues drop due to dimerization. They are listed in Table 1b. Most notable are the two Asparagine residues N₄₃ (74 Å² in monomer and 3 Å² in dimer) and N₄₇ (177 Å² in monomer, while 43 Å² in dimer). We also found that the main chain nitrogen atom of Asparagine 43 of each monomer is in favourable orientation to form H-bond with the side chain oxygen atom of aspartic acid 25 of the other monomer, which may lead to the stability of the dimer structure. In protein T1, this N₄₄ has been replaced by a isoleucine residue. Changes in interacting residues are most likely to hinder dimerization, resulting in a loss of allergenicity.

3.7. Structural docking studies to evaluate the function as a brassinosteroid carrier

From the sequence data it was found that most of the residues involved in the binding of brassinolides are conserved throughout Bet v 1 allergen family, including T1. So it was proposed that T1 may also be a BR carrier and that it can be a substitute for Bet v 1—related allergenic proteins to construct viable transgenic plants, which will neither release allergenic proteins via their pollen nor accumulate allergens in the tissues used for the production of food (Laffer et al., 2003).

In the energy minimized structure of T1-brassinolide complex, it has been observed that the alkyl side chain of the inner brassinolide is interacting with the hydrophobic residues V₆₇, I₈₅, I₉₈, and F₁₂₀ lining the binding cavity. The hydroxyl groups at positions 22 and 23 of brassinolide form hydrogen bonds with the amino acid Y₈₃ and Y₁₀₀, respectively. The hydroxyl groups at positions 2 and 3 are nearly within hydrogen binding distance with the strongly conserved residues K₅₄ and Y₈₁. The alkyl side chain of outer brassinolide molecule favorably interacts with hydrophobic residues L₉₅ and I₁₂₈. The hydroxyl substituents at positions 22 and 23 are in a favorable orienta-

tion for hydrogen bonding interactions with E₁₃₂. The observed mode of brassinolide binding to T1 (see Fig. 2d) is very similar to that observed in Bet v 1 crystal.

4. Conclusion

Our present understanding regarding the characteristics that confer upon proteins the ability to induce allergic responses in susceptible individuals is still rudimentary (Huby et al., 2000). Interestingly, it has been observed that the plant allergens chiefly clustered into four families and a membership of one of these families is likely to be associated with a greater potential for a protein to be an allergen (Radauer and Breiteneder, 2006). Protein T1 belongs to such a family of cross-reactive allergens, the ‘Bet v 1-like proteins’ (pfam accession number PF00407), which are present in pollen and in plant-derived foods. But, it is exclusively noted for its non-allergenic character and for the absence of cross-reactivity with Bet v 1 group.

Recent data shows that dimerization of the Bet v 1 molecule is necessary for its *in vivo* cross-linking potential. But, as most of the residues in crystallographically determined IgG (BV16)-binding epitope (and the p-loop) are almost buried in the predicted dimerized form, they seem to be of less importance for cross-linking. On the contrary, the surface-exposed conserved patches that are believed to be the cross-reactive epitopes (Schirmer et al., 2005), seems to be significant.

Although a previous study reports some difference in secondary structural contents of recombinantly expressed Bet v 1 and T1 (shown by CD curve-fitting experiments), homology modeling suggests a Bet v 1-like secondary structure. But it shows that the antigenic surface (both experimentally deduced epitope region and the epitope regions suggested by structure-based prediction methods) of protein T1 is markedly distinct from Bet v 1.

The molecular weight (16.17 kDa) and solvent-accessible surface area (8966.7 Å²) of protein T1 are comparable to that of Bet v 1. So, dimerization might be needed to retain its allergenicity, as in the case of Bet v 1. Interestingly, we found that the residues of Bet v 1 that are involved in dimerization, are not conserved in T1, supporting the notion that T1 is essentially monomeric and so it can neither cross-link the B-cell receptors nor the mast cell bound IgE molecules.

Lastly, our docking result indicates that T1, like other Bet v 1 family of proteins, could fulfill a BR-carrier function. Thus T1 could elicit similar biological function as Bet v 1 as well as it is non-allergenic in nature. Therefore, T1 can be considered as an ideal candidate for constructing transgenic hypo-allergenic plants as previously suggested (Laffer et al., 2003).

In conclusion, our comparative study of the modeled structure of protein T1, along with Bet v 1 monomer and dimer structure can explain the properties of protein T1 in relation to allergenicity and cross-reactivity. The 3D model of protein T1 might provide valuable clues to design experiments for better understanding of structural requirements for allergenicity and to develop safer vaccination strategy.

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References

- Baker, N.A., Sept, D., Joseph, S., Holst, M.J., McCammon, J.A., 2001. Electrostatics of nanosystems: application to microtubules and the ribosome. *Proc. Natl. Acad. Sci. USA* 98, 10037–10041.
- Braden, B.C., Poljak, R.J., 1995. Structural features of the reactions between antibodies and protein antigens. *FASEB J.* 9, 9–16.
- Breiteneder, H., Mills, C., 2006. Structural bioinformatics approaches to understand cross-reactivity. *Mol. Nutr. Food Res.* 50, 1–5.
- Carpin, S., Laffer, S., Schoentgen, F., Valenta, R., Chénieux, J.C., Rideau, M., Hamdi, S., 1998. Molecular characterization of a cytokinin-inducible periwinkle protein showing sequence homology with pathogenesis-related proteins and the Bet v1 allergen family. *Plant Mol. Biol.* 36 (5), 791–798.
- Davies, D.R., Padlan, E.A., Scheriff, S., 1990. Antibody-antigen complexes. *Annu. Rev. Biochem.* 59, 439–473.
- Ferreira, F., Ebner, C., Kramer, B., Casari, G., Briza, P., Kungl, A.J., Grimm, R., Jahn-Schmid, B., Breiteneder, H., Kraft, D., Breitenbach, M., Rheinberger, H.J., Scheiner, O., 1998. Modulation of IgE reactivity of allergens by site directed mutagenesis: potential use of hypoallergenic variants for immunotherapy. *FASEB J.* 12 (2), 231–242.
- Fiers, M.W., Kleter, G.A., Nilhand, H., Peijnenburg, A.A., Nap, J.P., van Ham, R.C., 2004. Allermatch, a webtool for the prediction of potential allergenicity according to current FAO/WHO Codex alimentarius guidelines. *BMC Bioinformatics* 5, 133.
- Gajhede, M., Osmark, P., Paulsen, F.M., Ipsen, H., Larsen, J.N., Joost van Beeren, R.J., Schou, C., Løwenstein, H., Spangfort, M.D., 1996. X-ray and NMR structure of Bet v1, the origin of birch pollen allergy. *Nat. Struct. Biol.* 3 (12), 1040–1045.
- Gangenberger, E., Grünberger, K., Sponer, B., Radauer, C., Breiteneder, H., Boltz-Nitulescu, G., Scheiner, O., Jemsen-Joralim, E., 2000. Allergen mimotopes for 3-dimensional epitope search and induction of antibodies inhibiting human IgE. *FASEB J.* 14, 2177–2184.
- Guex, N., Peitsch, M.C., 1997. Protein modeling for all. *Electrophoresis* 18 (15), 2714–2723.
- Hagler, A.T., Lifson, S., Dauber, P., 1979. Consistent force field studies of intermolecular forces in hydrogen-bonded crystals. A benchmark for the objective comparison of alternative force fields. *J. Amer. Chem. Soc.* 101, 5122–5130.
- Huby, R.D.J., Dearman, R.J., Kimber, I., 2000. Why are some proteins allergens? *Toxicol. Sci.* 55, 235–246.
- Jenkins, J.A., 2005. Structural relatedness of plant food allergens with specific reference to cross-reactive allergens: an *in silico* analysis. *J. Allerg. Clin. Immunol.* 115, 163–170.
- Joralim, E., Rumpold, H., Endler, A.T., Ebner, H., Breitenbach, M., Scheiner, O., 1989. IgE and IgG antibodies of patients with allergy to birch pollen as tools to define the allergen profile of *Betula verucosa*. *Allergy* 44, 385–395.
- Kabsch, W., Sander, C., 1983. Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers* 22 (12), 2577–2637.
- Laffer, S., Hamdi, S., Lupinek, C., Sperr, W.R., Valent, P., Verdino, P., Keller, W., Grote, M., Hoffmann-Sommergruber, K., Scheiner, O., Kraft, D., Rideau, M., Valenta, R., 2003. Molecular characterization of recombinant T1, a non-allergenic periwinkle (*Catharanthus roseus*) protein, with sequence similarity to the Bet v 1 plant allergen family. *Biochem. J.* 373 (Pt 1), 169–261.
- Laskowski, R.A., McArthur, M.W., Moss, D.S., Thomson, J.D., 1993. PROCHECK: a program to check the stereochemistry of protein structure. *J. Appl. Cryst.* 26, 183–291.
- Mirza, O., Henricksen, A., Ipsen, H., Larsen, J.N., Wissenbach, M., Spangfort, M.D., Gajhede, M., 2000. Dominant epitopes and allergic cross-reactivity: complex formation between a fab' fragment of a monoclonal murine IgG antibody and the major allergen from birch pollen Bet v1. *J. Immunol.* 165 (1), 331–338.
- Mittag, D., Batori, V., Neudecker, P., Wiche, R., Friis, E.P., Ballmer-Weber, B.K., Vieths, S., Roggen, E., 2006. A novel approach for investigation of specific and cross-reactive IgE epitopes on Bet v 1 and homologous food allergens in individual patients. *Mol. Immunol.* 43, 268–278.
- Mogensen, J.E., Wimmer, R., Larsen, J.N., Spangfort, M.D., Otzen, E.E., 2002. The major birch pollen allergen, Bet v1 shows affinity from a broad spectrum of physiological ligands. *J. Biol. Chem.* 277 (26), 23684–23692.
- Morković-Housely, Z., Degano, M., Lamba, D., Von Roepenack-Lahaye, E., Clemens, S., Susani, M., Ferreira, F., Scheiner, O., Breiteneder, H., 2003. Crystal structure of a hypoallergenic isoform of the major birch pollen allergen Bet v1 and its likely biological function as a plant steroid carrier. *J. Mol. Biol.* 325 (1), 123–133.
- Neudecker, P., Schweimer, K., Nerkamp, J., Scheurer, S., Vieths, S., Sticht, H., Rosch, P., 2001. Allergenic cross reactivity made visible; Solution structure of the major cherry allergen Pru av1. *J. Biol. Chem.* 276 (25), 22756–22763.
- Neudecker, P., Lehmann, K., Nerkamp, J., Haase, T., Wangorsch, A., Fotisch, K., et al., 2003. Mutational epitope analysis of Pru av 1 and Api g1, the major allergen of Cherry (*Prunus avium*) and Celery (*Apium graveolens*): correlating IgE reactivity with three dimensional structure. *Biochem. J.* 376, 97–107.
- Radauer, C., Breiteneder, H., 2006. Pollen allergens are restricted to few protein families and show distinct patterns of species distribution. *J. Allergy Clin. Immunol.* 117, 141–147.
- Ramachandran, G.N., Sashisekharan, V., 1968. Conformation of polypeptides and proteins. *Adv. Prot. Chem.* 23, 238–283.
- Rocchia, W., Alexov, E., Honig, B., 2001. Extending the applicability of the non-linear Poisson-Boltzmann equation: multiple dielectric constants and multivalent ions. *J. Phys. Chem. B.* 105, 507–514.
- Roy, D., Ghosh, D., Gupta-Bhattacharya, S., 2003. Homology modeling of allergenic cyclophilin: IgE-binding site and structural basis of cross-reactivity. *BBRC* 307, 422–429.
- Sali, A., Bundell, T.L., 1993. Comparative protein modelling by satisfaction of spatial restraints. *J. Mol. Biol.* 234 (3), 779–815.
- Sanner, M.F., 1999. Python: A Programming Language for Software Integration and Development. *J. Mol. Graphics Mod.* 17, 57–61.
- Schirmer, T., Hoffmann-Sommergrube, K., Susani, M., Breiteneder, H., Morovic-Housley, Z., 2005. Crystal structure of the major Celery allergen Api g1. Molecular analysis of cross reactivity. *J. Mol. Biol.* 351 (5), 1101–1109.
- Schöll, I., Kulkarna, N., Schedziankova, Y., Bergmann, A., Verdino, P., Knittelfelder, R., Kopp, T., Hantusch, B., Betzel, C., Dierks, K., Scheiner, O., Boltz-Nitulescu, G., Keller, W., Jensen-Joralim, E., 2005. Dimerization of major birch pollen allergen Bet v 1 is important for its *in vivo* IgE cross-linking potential in Mice. *J. Immunol.* 175, 6645–6650.
- Spangfort, M.D., Mirza, O., Ipsen, H., Joost van Neerven, R.J., Gajhede, M., Larsen, J.N., 2003. Dominating IgE binding epitopes of Bet v1, the major allergen of Birch pollen, characterized by X-ray crystallography and site directed mutagenesis. *J. Immunol.* 171 (6), 3084–3090.
- Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighing, position-specific gap penalties and weight matrix choice. *Nucleic Acid Res.* 22 (22), 4673–4680.
- Van Oss, C.J., 1995. Hydrophobic, hydrophilic and other interactions in epitope-paratope binding. *Mol. Immunol.* 32 (3), 199–211.