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# The structure of major birch pollen allergens – epitopes, reactivity and cross-reactivity

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## Introduction

Determination of molecular structures of allergens and allergen-antibody complexes is important in our efforts to understand the molecular interactions between allergens and the human immunesystem. By revealing the amino acid composition of the molecular surface of allergens, three-dimensional structures forms the basis for a rational approach to map IgE-binding B cell epitopes and enables questions regarding antibody specificity and cross-reactivity to be addressed.

At present, allergen specific IgE-epitope mapping is hampered by the technical difficulties of obtaining relevant amounts of monoclonal allergen specific serum IgE antibodies from allergic patients. This problem can be addressed by structural determination of allergen-antibody complexes using specific murine monoclonals combined with site-directed mutagenesis and immunochemical assays.

## Results and Discussion

We have used tree pollen allergy as a model system and succeeded in solving the three-dimensional structure of the major birch pollen allergen Bet v 1 independently both by nuclear magnetic resonance spectroscopy and X-ray crystallography (1). A main difference between NMR spectroscopy and X-ray crystallography is that the structure is determined in aqueous solution and in the crystalline state, respectively. In the case of Bet v 1, the X-ray and NMR structures are close to identical, strongly suggesting that the experimentally determined three-dimensional model reflect the naturally occurring structure.

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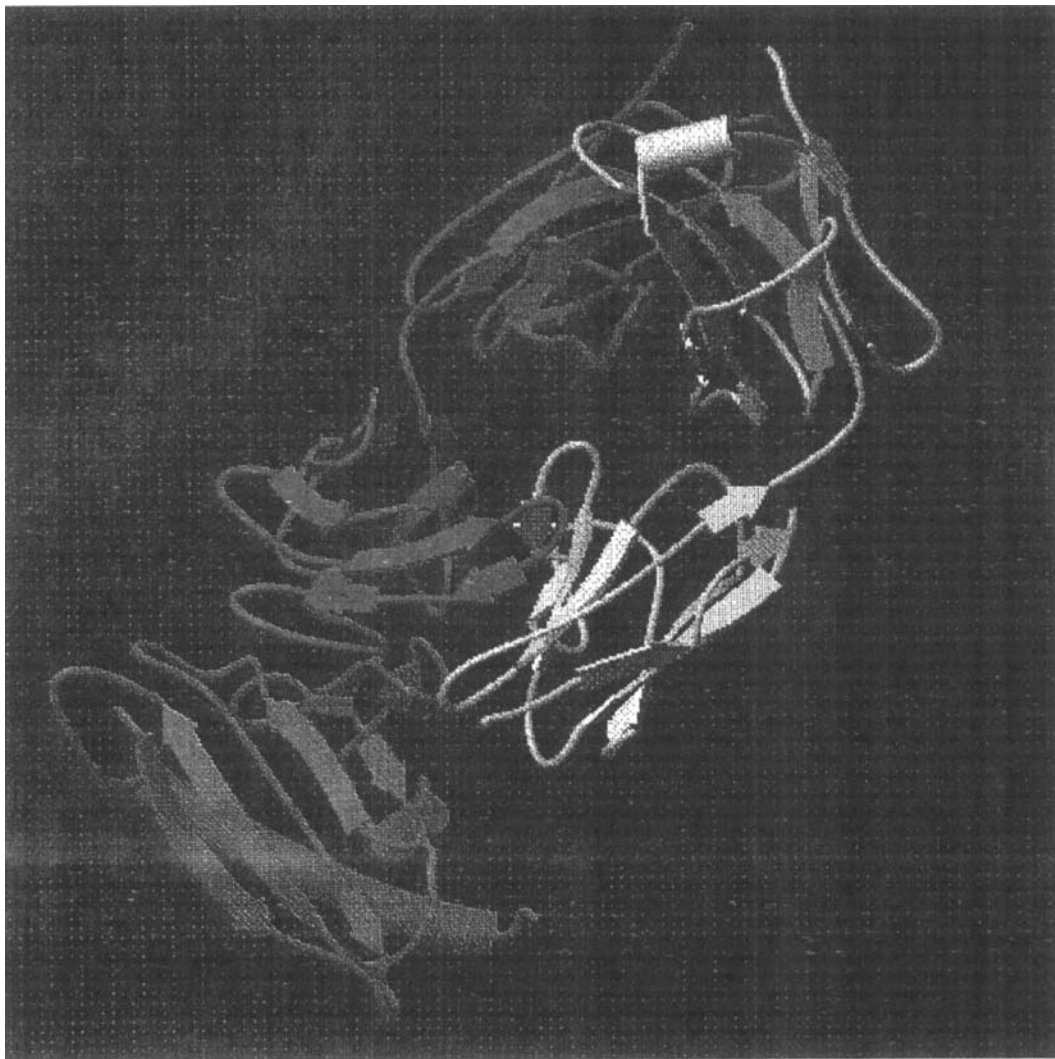


**Figure 1.** Complex crystal structure of Bet v 1-BV16Fab. Bet v 1 colored blue and the remains of murine monoclonal antibody BV16 heavy and light chains colored red and yellow, respectively.

The main feature of the Bet v 1 structure is a seven-stranded anti-parallel  $\beta$ -sheet that wraps around a 25 residue long C-terminal  $\alpha$ -helix. The  $\beta$ -sheet and the C-terminal part of the long helix are separated by two consecutive helices. The seven stranded anti-parallel  $\beta$ -sheet consists of five longer strands of residues 2 $\rightarrow$ 11(I), 121 $\leftarrow$ 111(VII), 97 $\rightarrow$ 107(VI), 89 $\leftarrow$ 79(V), 66 $\rightarrow$ 75(IV), and two shorter strands 57 $\leftarrow$ 52(III), and 40 $\rightarrow$ 44(II). The C-terminal  $\alpha$ -helix, ( $\alpha$ 3), consists of residues 130-154 and the consecutive helices consist of residues 15-23, ( $\alpha$ 1), and 25-34, ( $\alpha$ 2), respectively.

Birch pollen allergic patients' serum IgE cross-react extensively with pollen allergens from other *Fagales* species for instance alder, hornbeam and hazel. Both species-specific (birch) and cross-reactive (common for *Fagales* allergens) antibody-binding epitopes have been demonstrated (2).

Antibodies raised by natural exposure interact primarily with their target antigens through conformational epitopes involving surface-exposed residues and crystallographic studies of Fab-antigen complexes suggest that a surface area covering up to about 600-900 Å<sup>2</sup> may be directly involved in antibody-antigen binding (3). An analysis of the known major pollen allergen sequences in combination with an analysis of the determined Bet v 1 surface showed that there are three patches larger than 600 Å<sup>2</sup> of coherently connected conserved surface residues on the determined Bet v 1 structure. These regions are potential candidates for harboring cross-reactive antibody-binding epitopes of *Fagales* allergens.

In order to address the question of cross-reactivity, the Fab-fragment from the murine monoclonal anti-Bet v 1 antibody BV16 was isolated by enzymatic digestion.

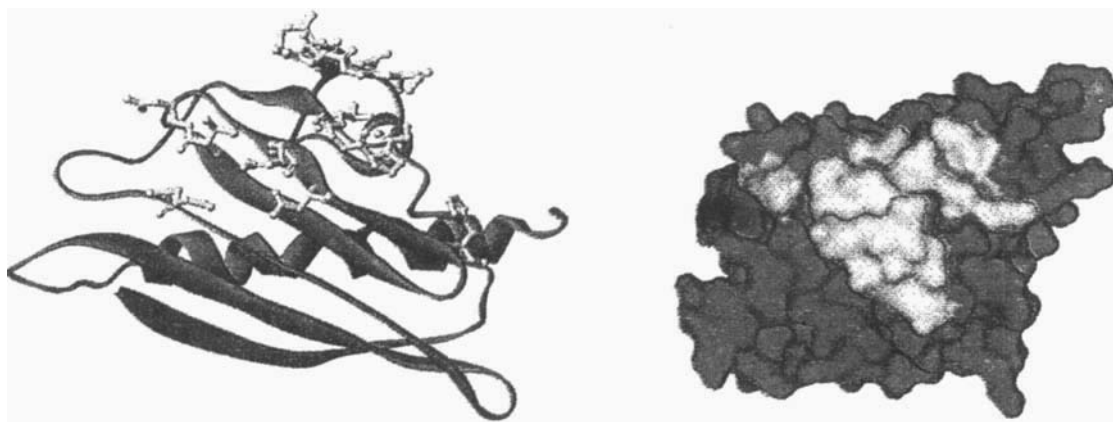


Figure 2. Bet v 1 with the B cell epitope defined by murine monoclonal antibody BV16. Left panel: Ribbon representation of Bet v 1 (green) with residues in contact with antibody BV16 in yellow. Right panel: molecular surface representation of Bet v 1 (green) with residues in contact with antibody BV16 in yellow.

Although antibody BV16 was raised against naturally occurring Bet v 1 it cross-reacts with other major tree pollen allergens of Fagales (4) suggesting that the B cell epitope defined by antibody BV16 is located in one of the common patches described previously (1). In addition, the BV16 antibody partially inhibits the binding of specific serum IgE to Bet v 1 suggesting an overlap between the murine IgG and human specific serum IgE epitopes (4).

The complex between Bet v 1 and BV16Fab was formed by mixing the two purified components in a 1:1 (mol:mol) ratio, followed by crystallization of the Bet v 1 BV16Fab complex (4). The complex structure was then determined by x-ray diffraction analysis at 2.9 Å resolution (5), see Fig. 1.

The area of the Bet v 1 molecular surface covered by BV16 Fab upon binding, i.e the B cell epitope, is 931 Å<sup>2</sup> covering about 10% of the solvent accessible molecular surface of Bet v 1. The B cell epitope defined by antibody BV16 overlap one of the conserved molecular surface areas clearly demonstrating that such common patches form the structural basis of tree pollen allergen cross-reactivity.

The epitope defined by BV16 is conformational, i.e composed of residues originating from different parts of the Bet v 1 polypeptide chain brought in proximity in the three-dimensional structure, see Fig. 2. There are 16 Bet v 1 residues in contact with BV16Fab and sequence alignment shows that these contact residues are close to 100% conserved among the Bet v 1 isoallergens. Although it is clearly a conformational epitope, 10 of the 16 contact residues occurs in a linear sequence consisting of Bet v 1 residues 42-52 suggesting that a

peptide could be used to block the interaction between antibody BV16 and Bet v 1.

However, a synthetic Bet v 1 peptide, 39-53 was not able to inhibit the binding of BV16 to Bet v 1 when analyzed by ELISA inhibition demonstrating a limitation in using peptides for B cell epitope mapping.

The ability of antibody BV16 to partially inhibit the binding of specific serum IgE to Bet v 1 (4) suggests an overlap between the epitope defined by the murine IgG BV16 antibody and specific serum IgE epitopes raised by natural exposure to birch pollen allergen. This was experimentally tested by site-directed mutagenesis where Bet v 1 point mutations were introduced within the BV16 epitope defined in the crystallographic study. Glutamic acid in position 45 shows a high degree of solvent-exposure (40%) and is a contact residue with a central location in the B cell epitope defined by BV16. A serine residue was found to occupy position 45 in some of the Bet v 1 homologous plant pathogenesis-related PR-10 proteins arguing for that glutamic acid can be replaced by serine without distortion of the three-dimensional structure. In addition, as none of the known Fagales allergen sequences have serine in position 45, the substitution of glutamic acid with serine gives rise to a non-naturally occurring Bet v 1 molecule.

The Bet v 1 Glu45Ser mutant was expressed, purified and tested in a specific serum IgE-inhibition assay using a pool of serum IgE derived from birch allergic patients. Compared to recombinant Bet v 1, the mutant Bet v 1 showed a significant decrease in affinity for serum IgE suggesting that the epitope defined by the murine antibody BV16 also represents a major IgE-binding epitope.

The point mutation Glu45Ser also completely abolished the binding of antibody BV16 to Bet v 1 as judged by ELISA inhibition assay.

The crystal structure of the Bet v 1 mutant was solved and showed that the Glu45Ser mutation did not affect the structure of Bet v 1. Thus, any change in immunochemical activity of the Bet v 1 mutant is directly related to the single amino acid substitution.

Single point mutations introduced in the two remaining conserved molecular surface areas also gave rise to a similar decrease in IgE-reactivity supporting the concept that conserved allergen molecular surface areas harbors high-affinity IgE-binding epitopes as opposed to a more random distribution of IgE-binding epitopes over the entire allergen molecular surface.

## Conclusions:

- B cell epitopes are conformational and depends on the allergen three-dimensional structure.
- Peptides have a limited use for B cell epitope mapping.
- Murine monoclonal antibodies and human specific serum IgE can recognize the same allergen B cell epitope(s).
- A single amino acid substitution can abolish antibody binding.
- Conserved allergen molecular surface region forms the structural basis for cross-reactivity and harbors major IgE-binding epitopes.

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