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Crystallization and preliminary X-ray analysis of birch-pollen allergen Bet v 1 in complex with a murine monoclonal IgG Fab′ fragment

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The human type I allergic response is characterized by the presence of allergen-specific serum immunoglobulin E (IgE). Allergen-mediated cross-linking of receptor-bound IgE on the surface of mast cells and circulating basophils triggers the release of mediators, resulting in the development of the clinical symptoms of allergy. In order to study the structural basis of allergen–antibody interaction, a complex between the major birch-pollen allergen Bet v 1 and a Fab′ fragment isolated from the murine monoclonal Bet v 1 antibody BV16 has been crystallized. Complex crystals belong to space group $P1$, with unit-cell parameters a = 91.65, b = 99.14, c = 108.90 Å, α = 105.7, β = 98.32, γ = 97.62°, and diffract to 2.9 Å resolution when analyzed at 100 K using synchrotron-generated X-rays.

1. Introduction

The type I allergic immune response is characterized by the presence of allergen-specific serum immunoglobulin E (IgE) produced by the interaction of B-lymphocytes with allergen-specific T-helper cells, releasing cytokines of the Th2 type characteristic of the allergic immune response (Wierenga *et al.*, 1990; Parronchi *et al.*, 1991; Kapsenberg *et al.*, 1991). Allergen-specific serum IgE binds to the IgE high-affinity receptor FcεRI, which is localized on the surface of tissue mast cells and circulating basophils. The clinical symptoms of allergy, *i.e.* rhinitis, rhinoconjuctivitis and asthma, are triggered as a result of the cross-linking of receptor-bound IgE molecules and aggregation of FcεRI receptors by a multivalent allergen followed by the release of mediators such as histamine (Segal *et al.*, 1977; Holowka & Baird, 1996). The number of serum IgE-binding epitopes per allergen with affinities high enough to be of clinical significance is unknown, but an antigen must have at least two epitopes recognized by specific serum IgE in order to stimulate FcεRI aggregation and function as an allergen.

The major source of tree-pollen allergic sensitization in the temperate climate zone consists of pollens from the *Fagales* order, of which birch is the clinically most important. Over 90% of all birch-pollen allergic patients show specific serum IgE reactivity towards the 17.5 kDa major pollen allergen Bet v 1 (Ipsen & Løwenstein, 1983), illustrating its unique importance in tree-pollen allergy.

The structure of Bet v 1 has been determined by X-ray crystallography and NMR spectroscopy, revealing the presence of molecular surface areas conserved among the naturally occurring Bet v 1 isoforms and among the Bet v 1 homologous *Fagales* major allergens of alder, hornbeam and hazel pollen (Gajhede *et al.*, 1996). The murine monoclonal antibody BV16 is raised against Bet v 1 purified from birch pollen and shows cross-reactivity towards the major allergens Aln g 1 and Car b 1 present in alder- and hornbeam-pollen extracts, respectively (Mirza *et al.*, in preparation). The cross-reactivity pattern of BV16 is in agreement with the 70–90% sequence identity between the *Fagales* major allergens (Larsen, 1995) and indicates that the B-cell epitope defined by BV16 is located in one of the conserved molecular-surface patches predicted to be involved in tree-pollen allergic cross-reactivity (Gajhede *et al.*, 1996). Here, we report the crystallization and X-ray analysis of Bet v 1 in complex with the Fab′ fragment from the murine monoclonal IgG_1 antibody BV16.

2. Experimental

Recombinant Bet v 1 was expressed in *Escherichia coli* and purified as described previously (Spangfort *et al.*, 1996). Monoclonal hybridoma IgG1 antibody BV16 was derived from BALB/c mice immunized with purified Bet v 1 isolated from birch-pollen extract. Antibody BV16 was isolated from ascites fluid by protein G affinity chromatography using a resin of protein G GammaBindPlus (Pharmacia, Sweden).

BV16 Fab′ fragments were generated by pepsin cleavage as described previously (Stura *et al.*, 1989) and were isolated by FPLC molecular-sieve chromatography using Sephadex G-75 HiLoad 16/60 (Pharmacia, Sweden) in

Table 1
Data-collection statistics.

Space group	*P*1
Unit-cell parameters (Å, °)	*a* = 91.7, *b* = 99.1, *c* = 108.9, α = 105.7, β = 98.3, γ = 97.6
Maximum resolution (Å)	2.9
Outer shell (Å)	3.00–2.9
No. of observations (outer shell)	181008 (11123)
No. of unique observations (outer shell)	80241 (6763)
Completeness (%) (outer shell)	95.5 (83.8)
R_{sym}† (outer shell)	0.047 (0.376)
$I/\sigma(I) > 2$ in outer shell (%)	58.2
R_{merge} (outer shell)	0.051 (0.268)
$I/\sigma(I) > 2$ in outer shell (%)	57.6

† $R_{sym} = \sum_{hkl}\sum_i[|I_{i(hkl)} - \langle I_{hkl}\rangle|]/\sum_{hkl}\sum_i I_{i(hkl)}$, where $\langle I_{hkl}\rangle$ is the average of *I* over all symmetry-related reflection for R_{sym} in one data set and for R_{merge} in the merged data sets.

0.1 *M* $NaHCO_3$ pH 8.3. After dialysis against 10 m*M* Tris pH 8.0, BV16 Fab′ was further purified by ion-exchange chromatography using a Mono Q column (Pharmacia, Sweden) equilibrated with 10 m*M* Tris pH 8.0.

For complex formation, purified recombinant Bet v 1 and BV16 Fab′ fragments were mixed in a 1:1 molar ratio followed by isolation of the complex by FPLC molecular-sieve chromatography using Sephadex G-75 HiLoad 16/60 (Pharmacia) in 20 m*M* Tris–HCl pH 8.0.

The isolated complex was concentrated to about 15 mg ml^{-1} and was stable for several months when stored at 277 K.

3. Results and discussion

Initially, crystals of the Bet v 1 Fab′ complex were grown by vapour diffusion at 277 K at a protein concentration of about 7 mg ml^{-1} using PEG 6000 as a precipitating agent. For crystallization, 5 µl of protein solution was mixed with 5 µl of 12% PEG 6000, 0.1 *M* sodium citrate, 0.01% sodium azide pH 4.0 and equilibrated against 1 ml of 12% PEG 6000, 0.1 *M* sodium citrate, 0.01% sodium azide pH 4.0. For cryo-crystallography (see below), 10% glycerol was added to the reservoir solution followed by crystallization as described above. The addition of glycerol did not affect the outer morphology of the crystals.

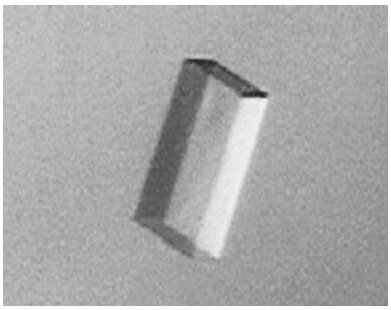

Figure 1
Crystal of Bet v 1–BV16Fab′ complex.

Crystals grown in the absence of glycerol showed diffraction to 3.5 Å resolution when analyzed by X-rays from a Rigaku RU200 rotating-anode generator and a Rigaku R-AXIS IIC image-plate system. Using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997), it was determined that these crystals belonged to space group *P*1 with unit-cell parameters *a* = 63.37, *b* = 84.11, *c* = 93.22 Å, α = 88.49, β = 76.27, γ = 83.96°. Using the Matthews formula (Matthews, 1968) and an expected complex molecular weight of 67 kDa, the most plausible unit-cell content is two complex molecules in the unit cell, giving a solvent content of about 70%.

In order to increase the resolution limit, crystals were tested on beamline 711 at the MAX-Laboratory synchrotron facility, University of Lund, Sweden. At room temperature these crystals suffered badly from radiation damage, although diffraction to 2.9 Å was observed. Consequently, a number of cryo-protectants were tested (glycerol, MPD, glucose, 2-propanol, PEG 600) at several concentrations in the precipitation solution (hereafter referred to as the cryo-buffer). When transferred to cryo-buffers containing most of the cryo-protectants, the crystals showed visible signs of damage. However, crystals grown in the presence of 10% glycerol showed an increased stability when transferred to a cryo-buffer containing 30% glycerol as the protectant. When collecting data at the MAX-Laboratory at 100 K, these crystals (Fig. 1) showed diffraction to 2.9 Å resolution (Table 1) without significant intensity decay as a function of time. The space group was still *P*1, but the unit-cell parameters were increased to *a* = 91.65, *b* = 99.14, *c* = 108.90 Å, α = 105.7, β = 98.32, γ = 97.62°. Maintaining a solvent content of about 70%, this implies a total of four complexes in the unit cell.

Both in-house and synchrotron data sets have been collected and structure solution and refinement is in progress. The complex structure is expected to serve as a starting point for identifying allergic B-cell epitopes and to give insight in the structural basis of allergic cross-reactivity.

Synchrotron data were collected during the commissioning period of beamline 711 (BL711) at the MAX-Laboratory, Lund, Sweden. The beamline is funded by the Swedish Council for Planning and Coordination of Research (FRN) and the Swedish Natural Science Research Council (NFR), to whom we are most grateful. We are also grateful to Fil. Lic. Yngve Cerenius (MAX-Laboratory) and Annette Gisselsson for excellent technical assistance.

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