BL-17A/2016G636

Structure of a two-domain family GH19 chitinase allergen from Japanese cedar (*Cryptomeria japonica*) pollen

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1 Introduction

Japanese cedar (*Cryptomeria japonica*) pollen is a major source of aeroallergens in Japan that causes various types of pollinosis including rhinitis and conjunctivitis. Approximately 25 million people in Japan currently suffer from seasonal pollinosis. Among numerous allergens in *C. japonica* pollen, Cry j 1 and Cry j 2 were found to have pectate lyase and polymethylgalacturonase activities, respectively. CJP-6 was also identified as an important allergen from the pollen; this protein has homology to the isoflavone reductase family. On the other hand, Fujimura et al. identified CJP-4, an allergenic chitinase from *C. japonica* pollen that has high IgE-binding affinity.

Chitinases (EC 3.2.1.14) hydrolyze β-1,4-glycosidic linkages of chitin, a linear homo-polymer of N-acetylglucosamine (GlcNAc), and are classified into the GH18 and GH19 families based on the CAZy database (http://www.cazy.org/Glycoside-Hydrolases.html). According to an independent classification system for plant chitinases, at least five classes (classes I, II, III, IV, and V) have been recognized based on their domain organization and loop deletions.

The allergenicity of plant chitinase was first identified in class I chitinase from avocado (*Persea americana*). Subsequently, allergenic class I chitinases were identified in several fruits. In the early 2000s, class IV chitinases from maize, grapes, and Japanese cedar pollen were shown to bind IgE in the sera of allergic patients. In this study, to clearly understand the structure-allergenicity relationship of plant chitinases, we determined the crystal structure of CJP-4-Cat by X-ray crystallography and found potential epitopes. Functional and structural properties of CJP-4 were also compared to those of other GH19 chitinases.

2 Experiment

The recombinant CJP-4 was prepared using pETB-CJP-4 as an expression vector, as described previously. [1] Prior to crystallization, the protein was concentrated to 5 mg/ml. Crystallization was achieved by screening with commercially available crystallization kits from Hampton Research using the sitting-drop vapour-diffusion method. Sitting drops were prepared by mixing 1 µl of protein solution (5 mg/ml in water) with 1 µl of reservoir solution containing 0.2 M BIS-TRIS, pH 6.5, 15% polyethylene glycol 3350. Quadrangular prism crystals grew within 2 weeks under all conditions. For data collection, the crystals were cryoprotected in a solution consisting of 0.2 M BIS-TRIS, pH 6.5, 15% Polyethylene glycol 3350, and 20% glycerol, and then flash-cooled in a nitrogen stream at 95 K. X-ray diffraction data were collected at the beam-line BL-17A of the Photon Factory (Ibaraki, Japan) using an ADSC Q270 CCD detector at a cryogenic temperature (95 K). Diffraction data were integrated and scaled with HKL2000. The crystals belong to the monoclinic space group $P2_1$, with unit cell dimensions of, a = 33.0 Å, b = 74.3 Å, c = 35.8 Å, α = 90°, β = 99.9°, and γ = 90°. The processing statistics are summarized in Table 1.

Table 1: Data collection and refinement statistics.

Data collection	
Space Group	$P2_1$
Cell dimensions	
a, *b*, *c* (Å)	33.01, 74.30, 35.81
α, *β*, *γ* (°)	90.0, 99.9, 90.0
Wavelength (Å)	0.98
Resolution (Å)	50 - 1.19 (1.21-1.19)
R_{merge}	0.059 (0.156)
$\langle I/\sigma I \rangle$	54.1 (20.5)
Completeness (%)	93.8 (83.4)
Redundancy	7.7 (7.3)
Refinement	
Resolution (Å)	37.15 – 1.19
No. reflections	48394
R_{work} [a] / R_{free} [b]	0.146 / 0.164
No. of atoms	
Protein	1600
Water	183
Average B-factors (Å^2)	
Protein	6.92
Water	17.5
RMS deviations	
Bond lengths(Å)	0.007
Bond angles (°)	1.155

The values in parentheses are for the outermost shell.
[a] $R_{work} = \Sigma|F_o - F_c|/\Sigma F_o$ for reflections of working set.
[b] $R_{free} = \Sigma|F_o - Fc|/\Sigma F_o$ for reflections of test set (5.0% of total reflections).

3 Results and Discussion

Obtaining the three-dimensional structures of allergens is important in order to understand the molecular basis of allergenicity. Furthermore, knowledge of structure in relation to the information on B-cell and T-cell epitopes is also necessary to develop allergen-specific immunotherapy. During the current study, we attempted to determine the three-dimensional structure of CJP-4. However, we could not obtain crystals of CJP-4, perhaps due to the flexibility of the linker region connecting the N-terminal CBM18 and C-terminal GH19 domains. Hence, we crystallized CJP-4-Cat, and the structure was successfully solved by molecular replacement. The crystal structure of CJP-4-Cat was bilobal with eleven distinct α-helices (Figure 1A), which is a typical structure of GH19 family members. To date, only two crystal structures of the catalytic domain of class IV chitinase have been solved: PaChi(s) from Norway spruce and ChitA from maize. The overall structure of CJP-4-Cat was similar to that of PaChi(s) (PDB code 3HBD) over the corresponding 192 Cα atoms, with an RMSD of 1.166 Å. Similarities to ChitA (PDB code 4MCK) were also found over 186 Cα atoms with an RMSD of 1.823 Å (Figure 1B).[2] Crystallization of full-length class IV chitinases has been unsuccessful. However, this is a critical gap in our knowledge, and further investigation is required to elucidate whether the structural conservation of these class IV chitinases brings about immunological cross-reactivity among these proteins. This is especially important, given that the class IV chitinases endochitinase 4A, ChitA, and CJP-4 are all reported to be allergenic. ADFS (Allergen Database for Food Safety) is a web server database system which is comprised of allergenic proteins for food safety and allergenicity prediction tools. The database contains allergens classified into 8 categories (pollen, mite, animal, fungus, insect, food, latex,and others) and identified at least four potential allergenic epitopes in CJP-4. These epitopes are well conserved among class IV chitinases. Of these, predicted epitope 1 is present in the CBM18 domain and other three epitopes are in the GH19 domain. Since a number of conserved residues are found in the GH19 domains of allergenic class I, II and IV chitinases, IgE-binding epitopes responsible for cross-reactivity among GH19 enzymes may be present in this domain. This should be investigated in future studies.

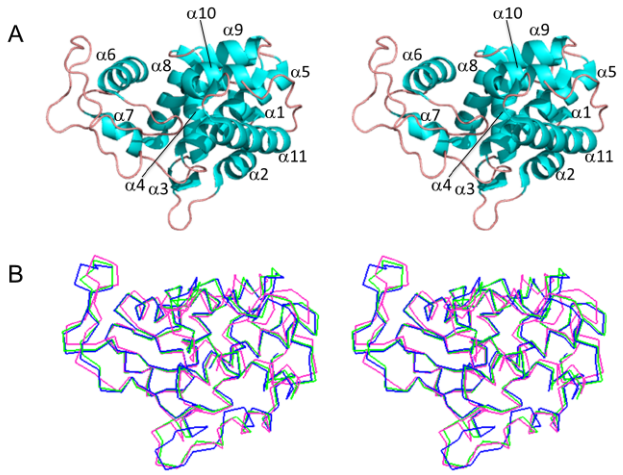

Fig. 1: Stereo view of a ribbon representation of the main chain structure of CJP-4-Cat (A). Superimposition of Cα traces of CJP-4-Cat (blue; PDB code 5H7T), PaChi(s) (green; PDB code 3HBD) and ChitA (magenta; PDB code 4MCK) (B).

Acknowledgement

We are thankful to BL-17A staff at Photon Factory, KEK in Japan.

References

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