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Jun a 1, the major allergen of pollen from the mountain cedar *Juniperus ashei*, has been crystallized using the hanging-drop vapor-diffusion method at 277 K. The crystals are monoclinic, space group $P2_1$, with unit-cell parameters a = 53.38, b = 113.48, c = 72.44 Å, β = 96.36° and four molecules in the unit cell. A complete 2.5 Å data set has been collected at 100 K with X-rays from a Cu $K\alpha$ rotating-anode generator.

1. Introduction

Pollen from the mountain cedar *Juniperus ashei* is a major cause of seasonal hypersensitivity in the central United States (Schwietz *et al.*, 2000). The problem is so severe in some communities that the planting of certain pollen-producing trees such as cedars, cypresses and junipers has been banned. Current treatment for cedar hypersensitivity is mainly directed towards relieving the symptoms. There has been limited success with desensitization therapy, with only about 30% of patients responding after 2 y of weekly injections. An understanding of the three-dimensional structure of the allergen would be a significant milestone in the development of more effective immunotherapeutic agents (Valenta & Kraft, 2002).

Pollen from *J. ashei* is cross-reactive with those from other cedars and cypresses in the northern hemisphere (Schwietz *et al.*, 2000). In 1999, we isolated and characterized the major allergen, Jun a 1, from mountain-cedar pollen (Midoro-Horiuti, Goldblum, Kurosky, Goetz *et al.*, 1999). The Jun a 1 allergen was found to be a glycoprotein similar to the group 1 allergens isolated from other cedars and cypresses: Jun v 1 from the North American eastern red cedar *J. virginiana* (Midoro-Horiuti *et al.*, 2003), Cup a 1 from the Arizona cypress *Cupressus arizonica* (Aceituno *et al.*, 2000), Cup s 1 from the Mediterranean Italian cypress *C. sempervirens* (Ford *et al.*, 1991), Cha o 1 from the Japanese cypress *Chamaecyparis obtusa* (Suzuki *et al.*, 1996) and Cry j 1 from the Japanese cedar *Cryptomeria japonica* (Yasueda *et al.*, 1983; Sone *et al.*, 1994). The amino-acid sequences of these group 1 allergens are highly conserved, implying that the cedar allergens have similar tertiary structures. If the cedar allergens indeed have similar tertiary structures, then it is likely that the epitopic sites will have similar if not identical spatial arrangements, which would explain the extensive allergenic cross-reactivities.

It has been shown that the Cry j 1 allergen has pectate-lyase activity (Taniguchi *et al.*, 1995). Recent experiments have shown that Jun a 1 also has low levels of pectate-lyase activity (data not shown). Pectate lyase is an enzyme secreted by microorganisms and is important in plant pathogenesis (Pilnik & Rombouts, 1981). Pectate lyase cleaves the α-1,4 glycosidic bond of pectate, the major component of plant cell walls. Pectate lyase also promotes germination by pollen grains (Carpita & McCann, 2000). Cry j 1 and Jun a 1 have amino-acid sequences that are over 80% identical and are likely to have very similar if not identical structures. Although Jun a 1 and Cry j 1 have a sequence identity to pectate lyase of only 25–50%, the putative active-site residues of pectate lyase are conserved in the Jun a 1 and Cry j 1 sequences (Midoro-Horiuti, Goldblum, Kurosky, Wood *et al.*, 1999). It is not known if the three-dimensional structures of the cedar allergens are similar to that of pectate lyase, but it is a very likely possibility. However, until the tertiary structure of at least one group 1 cedar allergen has been determined, it remains a possibility that the cedar allergens possess an entirely different peptide backbone fold.

The crystal structures of several pectate lyases from *Aspergillis*, *Erwinia chrysanthemi* and *Bacillus subtilis* have been reported (Akita *et al.*, 2001; Mayans *et al.*, 1997; Pickersgill *et al.*, 1994; Thomas *et al.*, 2002; Yoder *et al.*, 1993). However, no three-dimensional structure of a cedar or cypress allergen has been reported, nor has there been a report of the crystallization of a cedar or cypress allergen. We report here the first crystallization of a cedar allergen that is related to pectate lyase.

2. Materials and methods

2.1. Purification

Jun a 1 allergen was isolated from mountain-cedar pollen collected in northwestern Bexar

County, Texas, USA. The allergen was purified using Con A-Sepharose (Pharmacia) chromatography as described previously (Midoro-Horiuti, Goldblum, Kuroksy, Goetz *et al.*, 1999).

2.2. Crystallization

The initial crystallization screening was performed using Hampton Crystal Screen 1 and the hanging-drop vapor-diffusion method at 277 K. 2 µl of 4 mg ml^{-1} protein solution was mixed with 2 µl reservoir solution (Jancarik & Kim, 1991). Several of the drops, including the solution yielding crystals, formed a slight precipitate upon addition of the reservoir solution. After 4–5 weeks, crystals appeared in Hampton Crystal Screen 1 formulation 9 [0.2 *M* ammonium acetate, 0.1 *M* trisodium citrate dihydrate pH 5.6, 30%(*w*/*v*) polyethylene glycol 4000]. Crystallization conditions were optimized to 100 m*M* sodium acetate pH 5.5 buffer containing 200 m*M* ammonium acetate and 23% PEG 4000. These conditions typically yield crystals with dimensions of approximately 0.1 × 0.2 × 0.4 mm after 6–7 weeks at 277 K (Fig. 1).

2.3. Data collection and processing

The crystal shown in Fig. 1 was soaked in reservoir solutions containing increasing amounts of glycerol to a maximum of 30%(*v*/*v*) over a period of 12 h. The crystal was then flash-cooled in liquid nitrogen and mounted on the goniometer in a nitrogen stream at 100 K. Cu $K\alpha$ X-ray diffraction data were collected on a MacScience DIP 2030H image-plate system mounted on a MacScience M06HF rotating-anode generator equipped with Bruker Goëbel optics and running at 50 kV and 90 mA. 400 data frames were collected with an oscillation angle of 0.5°, 21 oscillations and an exposure time of 21 min per frame. Using a crystal-to-detector distance of 20 cm, a native data set was collected to 2.5 Å. The data were indexed, integrated and scaled using *DENZO* and *SCALEPACK* from the *HKL* package (Otwinowski & Minor, 1997). There were 108 027 measurements, which reduced to 29 648 unique reflections with an average $R_{\rm merge}$ of 6.6% on intensities. Data-collection statistics are summarized in Table 1. The data set was 99.6% complete, which is less than the completeness of the highest resolution shell. The lowest resolution shell (<6.79 Å) was only 98.3% complete, which lowered the overall completeness. Each of the remaining resolution shells are complete to more than 99.3%. These data, coupled with the high percentage of completeness at the edge of the data-collection sphere, indicate that the crystal diffracts beyond the 2.5 Å limit imposed by the data-collection setup.

Figure 1
A typical crystal of Jun a 1. Dimensions are 0.46 × 0.18 mm.

3. Results and discussion

The unit-cell parameters (Table 1) and the systematic absences for the 0*k*0, $k = 2n + 1$ reflections indicate that the Jun a 1 crystal belongs to the monoclinic space group $P2_1$. The crystal diffracted to better than 2.5 Å resolution. The protein moiety of Jun a 1 has a calculated molecular weight of 37.6 kDa. The carbohydrate content has not been determined. However, the molecular weight of the intact glycoprotein was determined to be 43 kDa by gel electrophoresis and to be 41 kDa by mass spectrometry (Midoro-Horiuti, Goldblum, Kurosky, Goetz *et al.*, 1999). Assuming two molecules in the asymmetric unit, the Matthews coefficient $V_{\rm M}$ is calculated to be 2.5 Å^3 Da^{-1}, which is well within the 1.7–3.5 Å^3 Da^{-1} range usually found for proteins (Matthews, 1968). Based on a specific volume of 0.74 cm^3 g^{-1}, the calculated solvent content is approximately 52%. Self-rotation function calculations using the program *GLRF* (Tong & Rossmann, 1997) support the assumption of two molecules in the asymmetric unit (Fig. 2). Only the $\kappa = 180°$ section shows significant peaks other than those belonging to the crystallographic space group.

The amino-acid sequence of Jun a 1 is 78% identical to the sequence of Cry j 1 (Sone *et al.*, 1994; Midoro-Horiuti, Goldblum, Kurosky, Wood *et al.*, 1999). If one includes the conservative differences as being identities, the similarity between the two sequences is 90%. Including less conservative substitutions increases the amino-acid sequence similarity of Jun a 1 and Cry j 1 to 95%. The carbohydrate moities on Jun a 1 are likely to be similar to those of Cry j 1 (Hino *et al.*, 1995). Therefore, it is likely that the conditions used to obtain crystals of the Jun a 1 allergen would also yield crystals of Cry j 1; these crystallization experiments are in progress.

Table 1
Data-collection and reduction statistics.

Values in parentheses are for the highest resolution shell (2.54–2.50 Å).

No. observations	108027
No. unique reflections	29648
Data completeness (%)	99.6 (99.9)
Average $I/\sigma(I)$	14.7 (7.5)
$I/\sigma(I) > 3$ (%)	85.0 (66.6)
Redundancy	3.7 (3.6)
$R_{\rm merge}$† (%)	6.6 (19.2)
Space group	$P2_1$
Unit-cell parameters	
a (Å)	53.38
b (Å)	113.48
c (Å)	72.44
β (°)	94.36

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i - \langle I\rangle|/\sum\langle I\rangle$.

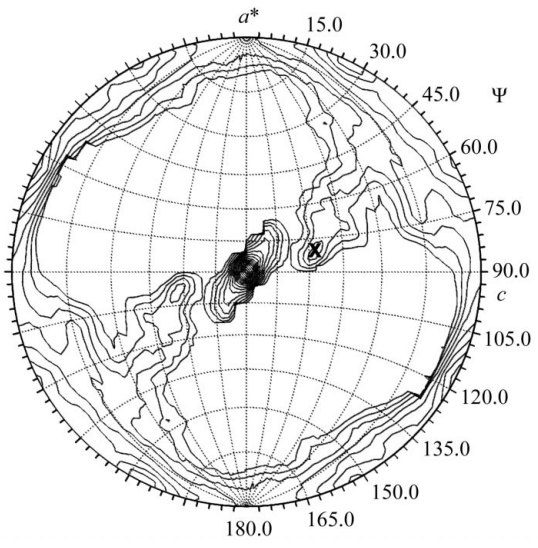

Figure 2
Self-rotation function plot at $\kappa = 180°$. The view is down the *b* axis; *a**, *c* and Ψ are as indicated. The plot was generated with the program *GRLF* using data in the resolution range 12–6 Å; the integration radius is 25 Å. The strong peaks are consistent with the monoclinic crystal symmetries. The X at $\Phi = 57$, $\Psi = 81°$ (peak/signal ≃ 4) indicates the location of the non-crystallographic twofold rotation axis relating the two molecules in the asymmetric unit.

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