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Crystallization and preliminary crystallographic study of the yeast *Malassezia sympodialis* allergen Mala s 1

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The opportunistic yeast *Malassezia sympodialis* can act as an allergen and elicit specific IgE- and T-cell reactivity in patients with atopic eczema. The first identified major allergen from *M. sympodialis*, Mala s 1, is present on the cell surface of the yeast. Recombinant Mala s 1 was expressed in *Escherichia coli*, purified and refolded in a soluble form. Crystals of Mala s 1 were obtained in 25% PEG 8K, 0.2 *M* $(NH_4)_2SO_4$. Crystals belong to space group $P2_12_12$, with unit-cell parameters a = 44.4, b = 163.7, c = 50.6 Å, and diffract to 1.35 Å resolution.

1. Introduction

The chronic inflammatory skin disease atopic eczema (AE) affects about 10–20% of the population in industrialized countries (Leung *et al.*, 2004). This skin disorder has a great impact on quality of life at all ages; it interferes with work, play and nightly rest and affects family functions and relations. In childhood, it is often the first symptom of allergic disease, later developing into allergic rhino-conjunctivitis and asthma. Although the pathogenesis of AE is not fully understood, a combination of genetic predisposition and environmental factors seem to contribute to the symptoms (Leung *et al.*, 2004). The yeast *Malassezia*, which belongs to the normal human cutaneous flora, has been shown to act as an allergen and elicit specific IgE- and T-cell reactivity in patients with AE (Scheynius *et al.*, 2002). Several different *Malassezia* species have been identified, *M. sympodialis* being the most frequently isolated species from the skin of AE patients and healthy controls in Sweden, Russia and Canada (Scheynius *et al.*, 2002). To date, the genes for eight different *M. sympodialis* allergens, designated Mala s 1 and Mala s 5–11, have been cloned and published (Schmidt *et al.*, 1997; Lindborg *et al.*, 1999; Rasool *et al.*, 2000; Andersson *et al.*, 2004). The first identified allergen from *M. sympodialis*, Mala s 1 (previously denoted Mal f 1), is a major allergen present in the cell wall and is exposed on the cell surface of the yeast (Zargari *et al.*, 1997). This allergen exhibits a sequence identity of 60% to the hypothetical protein UM04915.1 from *Ustilago maydis* (NCBI accession No. XM_755969). However, the function of both these proteins is still unknown.

The aim of the present study was to establish a system for structural studies of the *M. sympodialis*-derived allergen Mala s 1. Elucidation of the structure will provide insights into the function of Mala s 1 and facilitate the search for potential structural resemblances to other protein families. Furthermore, the structure of Mala s 1 will be used as a template for the mapping of B- and T-cell epitopes.

2. Materials and methods

2.1. Cloning, expression and purification of Mala s 1

Recombinant Mala s 1 (rMala s 1) with a hexahistidine affinity-purification tag on the C-terminus was produced as previously described (Zargari *et al.*, 1999). The cDNA encoding Mala s 1 (ExPASy accession No. Q01940), without the signal sequence at the N-terminus of the protein, was amplified with *Pfu* polymerase (Stratagene, La Jolla, CA, USA) using PCR. The amplified PCR product was then ligated into the pET 17b expression vector

Table 1
Statistics of data collection.

Values in parentheses are for the highest resolution shell.

X-ray source	I711
Wavelength (Å)	0.911
Resolution range (Å)	50–1.35 (1.45–1.35)
Space group	$P2_12_12$
Unit-cell parameters (Å)	a = 44.4, b = 163.7, c = 50.6
Total No. of unique reflections	82360
No. of observed reflections	1180323
Completeness (%)	99.6 (99.9)
R_{sym}	0.056 (0.487)
$I/\sigma(I)$	21.4 (2.71)
Mosaicity (°)	0.33

(Novagen, Abingdon, England) and transformed into *Escherichia coli* strain BL21 DE3 pLysS (Novagen). When the cell concentration reached an OD_{600} of 0.6, protein production was induced through the addition of 400 µ*M* IPTG (Sigma, St Louis, MO, USA). Inclusion bodies were solubilized for 2 h at room temperature in solubilization buffer containing 6 *M* guanidine–HCl, 20 m*M* Tris–HCl pH 8.0 and 100 m*M* NaCl. Recombinant Mala s 1 was bound to Talon metal-affinity resin (Clontech Laboratories, Palo Alto, CA, USA) and washed with PBS in order to gradually refold the protein. Finally, rMala s 1 was eluted with PBS containing 100 m*M* imidazole (Sigma). The eluted sample was dialysed against 20 m*M* Tris buffer pH 7.4 containing 400 m*M* NaCl. The concentration of the recombinant protein was determined by measuring the absorbance at 280 nm. The purity of the product was assessed by SDS–PAGE under denaturing conditions.

2.2. Crystallization of Mala s 1

Crystals of rMala s 1 were obtained using hanging-drop vapour diffusion. Crystal Screens (Hampton Research, Aliso Viejo, CA, USA) were used to establish initial crystallization conditions for Mala s 1, which were then refined in a finer grid. Typically, 1 µl of 3 mg ml^{-1} rMala s 1 in 20 m*M* Tris, 400 m*M* NaCl pH 7.4 was mixed in a 1:2 ratio with the crystallization reservoir solution [25% PEG 8K, 0.2 *M* $(NH_4)_2SO_4$] and allowed to equilibrate at room temperature. Streak-seeding was used to improve crystal quality and size.

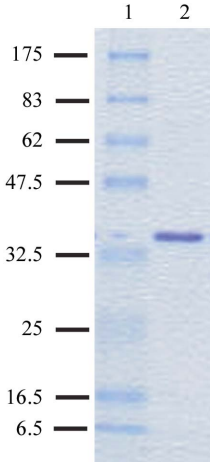

Figure 1
Protein separation by SDS–PAGE (12% gel) under denaturing and reducing conditions stained with Coomassie Brilliant Blue. Lane 1 shows molecular-weight markers (kDa) and lane 2 1 µg of purified rMala s 1.

2.3. Data collection and processing

Crystals were soaked in cryoprotectant (80% mother liquor and 20% glycerol) before flash-freezing in a cold nitrogen stream. A data set for Mala s 1 was collected at beamline I711 at MaxLab (Lund, Sweden) to a resolution of 1.35 Å. A total of 360 images were collected with 0.5° oscillation per frame. A further 360 images for a low-resolution data set were collected with 1° oscillation. Space group and unit-cell parameters were determined using the auto-indexing option in *DENZO* (Otwinowski & Minor, 1997). Owing to overlap problems, the initial predictions from *DENZO* were used to integrate the images using the *ProW* program (Bourgeois, 1999), which can deconvolute spatially overlapped spots. The data set was scaled in the program *SCALEPACK* from the *HKL* suite (Otwinowski & Minor, 1997).

3. Results and discussion

The expressed Mala s 1 construct encodes for the mature Mala s 1 sequence with a hexahistidine tail at the C-terminus. The expressed protein is composed of 334 amino-acid residues with a calculated molecular weight of 36 812.8 Da and a theoretical pI of 8.53 (EMBL accession No. X96486; Schmidt *et al.*, 1997). The His-tag linked recombinant Mala s 1 protein was produced in *E. coli* and isolated using a Talon metal-affinity resin. A minimum of 10 mg was obtained from 1 l of culture. The protein was refolded into a soluble form and concentrated. When analysed on SDS–PAGE, rMala s 1 showed a single band at approximately 37 kDa, which is in accordance with the calculated molecular weight (Fig. 1). We have previously demonstrated that the IgE-binding capacity of rMala s 1 is similar to that of native Mala s 1, indicating that rMala s 1 is correctly folded (Zargari *et al.*, 1999).

Crystals of rMala s 1 appeared after two months in 25% PEG 8K and 0.2 *M* $(NH_4)_2SO_4$ (Fig. 2). Interestingly, both the quality and the growth rate were improved by lowering the initial protein concentration by 50% or more. This is in accordance with our previous experiments when solving the crystal structure of Fel d 1, a major allergen from the domestic cat (Kaiser, Gronlund, Sandalova,

Figure 2
A representative rMala s 1 crystal. The longest dimension is ∼0.3 mm.

Ljunggren, Schneider *et al.*, 2003; Kaiser, Gronlund, Sandalova, Ljunggren, van Hage-Hamsten *et al.*, 2003).

The rMala s 1 crystals diffracted to 1.35 Å resolution using synchrotron radiation. The statistics from the collected data set are listed in Table 1. The crystals belong to space group $P2_12_12$, with unit-cell parameters $a = 44.4$, $b = 163.7$, $c = 50.6$ Å. The average B factor as derived from a Wilson plot was 12.7 Å^2. The crystals are very likely to contain one Mala s 1 molecule per asymmetric unit, which would give a Matthews coefficient V_M of 2.3 Å^3 Da^{-1}, corresponding to 47% solvent.

We have produced, isolated, refolded and determined the conditions for crystallization of the *M. sympodialis*-derived major allergen Mala s 1. The crystal structure of this allergen will provide us with important insights into both the structure and the function of Mala s 1 and hopefully lead to a better understanding of the role of Mala s 1 in the induction of allergic responses in AE patients.

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