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

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 (NH₄)₂SO₄. Crystals belong to space group *P*2₁2₁2, 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 μM 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 *mM* Tris-HCl pH 8.0 and 100 *mM* 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 *mM* imidazole (Sigma). The eluted sample was dialysed against 20 *mM* Tris buffer pH 7.4 containing 400 *mM* 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 *mM* Tris, 400 *mM* NaCl pH 7.4 was mixed in a 1:2 ratio with the crystallization reservoir solution [25% PEG 8K, 0.2 *M* (NH₄)₂SO₄] 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* (NH₄)₂SO₄ (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 Å². 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 Å³ 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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