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Crystallization and preliminary X-ray crystallographic studies of a lectin from the mushroom *Marasmius oreades*

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The *Marasmius oreades* agglutinin (MOA) recognizes blood group B oligosaccharides. This mushroom lectin belongs to the ricin superfamily and is currently the only lectin known with exclusive specificity for Galα1,3Gal-structures, as occur in the subterminally fucosylated blood group B epitope Galα1,3(Fucα1,2)Galβ1,4GlcNAc (MOA's preferred ligand) or without fucosylation in the xenotransplantation epitope. MOA has been co-crystallized with the linear blood group B trisaccharide Galα1,3Galβ1,4GlcNAc using the hanging-drop vapour-diffusion technique at room temperature. MOA crystals were grown in the presence of ammonium formate and HEPES buffer. A 3.0 Å data set has been collected. Preliminary analysis of the X-ray data is consistent with space group $P3_1$ or $P3_2$ and unit-cell parameters $a = b = 105$, $c = 113$ Å, with two dimers per asymmetric unit.

1. Introduction

Lectins are carbohydrate-binding proteins that are found in all life forms. Structurally, they can be grouped into several dissimilar families having large differences in structure; functionally, they can be placed into groups showing differences in sugar specificities, which do not necessarily correlate with the structural differences. Several lectins display blood-group specificity (Judd, 1980) and have thus become important tools for the identification of different blood-group antigens.

The fairy ring mushroom *Marasmius oreades* first attracted interest because of its human blood group B erythrocyte agglutination activity (Elo *et al.*, 1951). Purification and detailed studies of the carbohydrate-binding specificity of the purified agglutinin (MOA) have revealed a high affinity for the linear disaccharide Galα1,3Gal and several oligosaccharides related to the human blood group B antigen (Winter *et al.*, 2002; Teneberg *et al.*, 2003). MOA also recognizes the extended linear oligosaccharide Galα1,3Galβ1,4GlcNAc, which is not present in humans but is a common antigenic component of tissues and organs in lower mammals and accordingly complicates xenotransplantation to humans.

The MOA monomer contains 293 amino acids and has a molecular weight of 32 kDa. The protein is synthesized without a signal peptide and is therefore believed to reside in the cytosol, where its native function is unknown (Kruger *et al.*, 2002). A sequence alignment revealed MOA to be a member of the ricin superfamily. The ricin fold containing the Q*X*W sequence repeat is found in the B-chain of type-II ribosome-inactivating proteins (RIPs), which exhibit a β-trefoil fold (Niwa *et al.*, 2003).

Recently, other structures of fungal lectins have been solved, including the orange-peel fungus *Aleuria aurantia* lectin with a six-bladed β-propeller fold (Wimmerova *et al.*, 2003; Fujihashi *et al.*, 2003) and the golden needle mushroom *Flammulina velutipes* lectin with a fibronectin III-type fold (Paaventhan *et al.*, 2003). Here, we report the crystallization of the mushroom lectin MOA from *M. oreades* in complex with the trisaccharide Galα1,3-β1,4GlcNAc.

2. Materials and methods

2.1. Crystallization

Crystals of recombinantly expressed MOA were obtained by the hanging-drop vapour-diffusion technique. The protein was prepared as described by Kruger *et al.* (2002), yielding homodimers of functional protein, and was concentrated to 20 mg ml^{-1}. The trisaccharide Galα1,3Galβ1,4GlcNAc was added to the protein solution in a molar ratio varying from 1:1 to 10:1 (sugar:protein) at least 1 h prior to crystallization setup. Immediately before setting up the drops, the mixture was centrifuged at 10 000 rev min^{-1} for 10 min at 277 K.

Several leads were obtained from Structure Screens I and II from Molecular Dimensions (Molecular Dimensions Ltd, England) and an intense effort was invested into lead optimization in order to improve the diffraction quality of the crystals and to reduce the crystallization time. However, thus far crystallization has not been improved significantly and the final conditions are very similar to

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell (3.16–3.00 Å).

No. crystals	1
Resolution range (Å)	22–3.0 (3.16–3.00)
No. observations	74922 (7468)
No. unique observations	26248 (2843)
$R_{\rm merge}$†	0.068 (0.068)
Completeness (%)	98.4 (91.1)
$\langle I/\sigma(I)\rangle$	9.7 (3.5)

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

condition No. 15 from the original Molecular Dimensions Structure Screen II: 2.0–2.5 *M* ammonium formate in 0.1 *M* HEPES buffer pH 6.5–7.5 (protein/sugar to reservoir ratio 1:1). In our laboratory, crystals appear reproducibly 2–3 months after equilibration at 293 K and grow to maximum dimensions of 0.4 × 0.4 × 0.1 mm (Fig. 1).

For data collection at 100 K, cryosolution was pipetted onto the crystal-containing drops and the crystals were transferred directly to liquid nitrogen. Several different cryoprotectants (glycerol, PEG 400 or ethylene glycol) and freezing protocols (*e.g.* cryoannealing) were tested and all worked equally well.

Crystallization without trisaccharide has not been attempted.

2.2. Data collection

Final diffraction data were collected at beamline I711 at the MAX II synchrotron in Lund, Sweden using a MAR CCD detector. Data were collected on a crystal cocrystallized in the presence of a twofold molar excess of trisaccharide in 2.4 *M* ammonium formate and 0.1 *M* HEPES pH 7.5. The crystal was pre-frozen in liquid nitrogen with 30%(*v*/*v*) glycerol as a cryoprotectant and showed diffraction to a maximum resolution of 2.5 Å. The temperature was set to 100 K and the wavelength to 1.089 Å. Fig. 2 shows an example of the diffraction pattern. The oscillation angle was 0.5° and a total of 180 images were collected. X-ray data were processed using the program *MOSFLM* v.6.2.3 and scaled using *SCALA* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). Data-collection statistics are summarized in Table 1.

Figure 1
Picture of typical MOA crystals.

3. Preliminary X-ray diffraction analysis

The MOA crystals belong to space group $P3_1$ or $P3_2$, with unit-cell parameters $a = b = 105$, $c = 113$ Å. Assuming two MOA dimers per asymmetric unit, the Matthews coefficient $V_{\rm M}$ (Matthews, 1968) was calculated to be 2.8 $Å^3$ Da^{-1}, corresponding to a solvent content of 56%. The diffraction data suffer from anisotropy along the c^*-axis direction and therefore a complete data set could only be obtained to 3.0 Å resolution (Table 1; for comparison, completeness at 2.6 Å is 73%). X-ray data collected at room temperature show a similar degree of anisotropy in the diffraction pattern (data not shown), indicating that it is neither the cryoprotectant solution nor the low temperature that introduces the disorder in the MOA crystals.

Phasing by molecular replacement has been attempted using the coordinates of the ricin B-chain (Montfort *et al.*, 1987; PDB code 2aai), which gave the best hit in a BLAST search against the Protein Data Bank (Berman *et al.*, 2000). So far, we have not succeeded in solving the structure by this approach; however, this is not surprising owing to the low sequence identity of the two proteins (16% overall and 22% in the stretch of 130 aligned residues). We are currently performing soaking trials with various heavy-atom compounds in order to obtain model-independent phases. In parallel, crystallization experiments have been set up for an SeMet variant of the protein produced for MAD experiments.

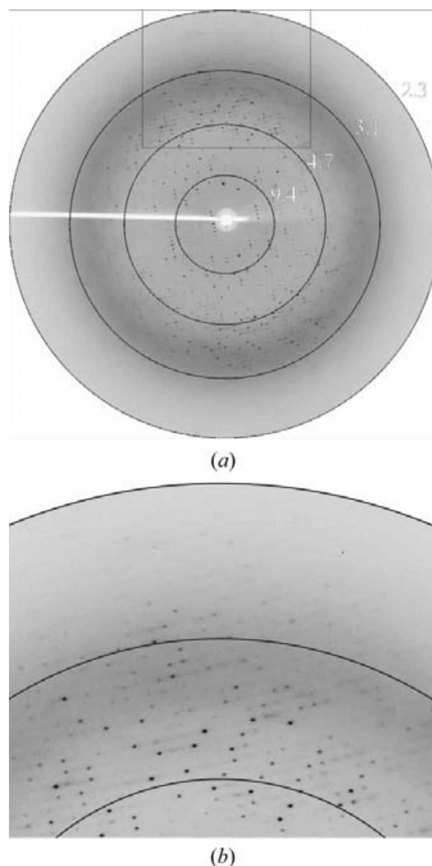

Figure 2
Diffraction image from a MOA crystal recorded at beamline I711, MAX II, Lund, Sweden. Resolution rings are indicated (*a*) and a zoom into the outer rings (*b*) shows that the crystal diffracts to a maximum resolution of 2.5 Å.

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