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Crystallization and preliminary crystallographic analysis of a novel haemolytic lectin from the mushroom *Laetiporus sulphureus*

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The novel haemolytic lectin from the parasitic mushroom *Laetiporus sulphureus* (LSL) is a homotetramer (~140 kDa) composed of subunits associated by non-covalent bonds. It exhibits haemagglutination and haemolytic activities, both of which are inhibited by *N*-acetyllactosamine. The structural similarity found between LSL and the bacterial pore-forming toxins mosquitocidal toxin (MTX2) from *Bacillus sphaericus* and α-toxin from *Clostridium septicum* points to a mechanism of biological action involving the formation of pores in the target membranes. LSL has been crystallized using the hanging-drop vapour-diffusion method at 291 K. Diffraction-quality hexagonal crystals have unit-cell parameters $a = b = 101.8$, $c = 193.9$ Å and belong to space group $P6_322$. A 2.7 Å native data set was collected with an $R_{\rm merge}$ of 9.2%.

1. Introduction

Lectins are sugar-binding proteins of non-immune origin which agglutinate cells and precipitate glycoconjugates (Goldstein *et al.*, 1980). Some lectins lyse as well as agglutinate cells. These are termed haemolytic or toxic lectins. Several of these haemolytic lectins have been isolated and characterized. One of these, the novel haemolytic lectin (LSL) from the mushroom *Laetiporus sulphureus*, was first isolated by Konska *et al.* (1994). They reported it to be a heterotetrameric protein of 190 kDa with subunits of 36 and 60 kDa. Our recent investigations have shown that isolation of the lectin in the presence of protease inhibitors prove it to be a homotetramer with subunits of molecular weight 35 kDa (Tateno & Goldstein, 2003). LSL agglutinated and lysed rabbit, human and sheep erythrocytes by a colloid-osmotic mechanism compatible with the formation of pores of about 4 nm. Both of the activities were inhibited by *N*-acetyllactosamine, indicating that LSL binds to *N*-acetyllactosamine-containing glycoconjugates on the cell membrane and creates ion-permeable pores. LSL has a unique structural feature. It contains a ricin B chain-like (QXW)3 domain at its N-terminus and shows sequence homology to pore-forming bacterial toxins from *Bacillus sphaericus* and *Clostridium septicum*. According to the results obtained by circular dichroism, β-structure would be the predominant regular secondary structure in LSL. Interestingly, a C-terminal deletion mutant retained its cell-agglutination activity but lost its haemolytic activity, indicating that the N-terminal domain is a carbohydrate-recognition domain, whereas the C-terminal domain probably functions as an oligomerization and structural stabilization domain.

It would be of great interest and value to gain additional information on the structural basis of pore formation of this novel type of haemolytic lectin. In this communication, we present preliminary X-ray crystallographic data on the native *L. sulphureus* lectin.

2. Experimental

2.1. LSL purification

The lectin was prepared according to the procedure of Tateno & Goldstein (2003). *L. sulphureus* mushrooms were harvested in Ann Arbor, Michigan in late August. The mushroom fruit (100 g) were macerated and homogenized in a Waring Blendor at 277 K in 500 ml PBSE (10 m*M* sodium phosphate pH 7.2, 0.15 *M* NaCl, 0.04% sodium azide, 1 m*M* EDTA) and 1 ml l^{-1} protein–inhibitor mixture. The extract was filtered through cheesecloth and centrifuged at 12 000*g* for 15 min. The supernatant was loaded onto a Sepharose 4B affinity column (2.5 × 15 cm) equilibrated with PBSE and washed until the absorbance at 280 nm reached approximately 0.05. The absorbed protein was eluted with 0.1 *M* lactose in PBSE, dialyzed against water and lyophilized. The yield was approximately 7 mg from 100 g of fresh mushrooms.

2.2. Crystallization

The LSL solution for crystallization experiments was prepared as follows: the lyophilized protein was dissolved in 10 m*M* Tris pH 8.0

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containing 0.1 *M* NaCl and subsequently centrifuged at 10 000 rev min^{-1} for 2 min. The protein concentration was determined from absorbance measurements employing a *E*(0.1%, 280 nm, 1 cm) of 0.55 on a Cintra 5 UV–Vis spectrophotometer (GBC Scientific Equipment) with 1 cm quartz cells. The protein solution was further concentrated to a final concentration of ~40 mg ml^{-1}. The initial crystallization conditions were established using the sparse-matrix sampling technique (Jancarik & Kim, 1991) with the hanging-drop vapour-diffusion method at 291 K using Crystal Screen I (Hampton Research). Drops containing equal volumes (2 µl) of protein and reservoir solution were equilibrated against 500 µl reservoir solution. Small crystals appeared in all solutions of the kit that contained $(NH_4)_2SO_4$ after ~2 h, in condition 24 [20%(*v*/*v*) 2-propanol, 0.1 *M* sodium acetate trihydrate pH 4.6, 0.2 *M* calcium chloride dihydrate] after 24 h and in condition 14 [28%(*v*/*v*) polyethylene glycol 400, 0.1 *M* Na HEPES pH 7.5, 0.2 *M* calcium chloride dihydrate] after ~3 d. Crystals suitable for diffraction were obtained by optimization of condition 24 to 14%(*v*/*v*) 2-propanol, 0.1 *M* sodium acetate trihydrate pH 4.5, 0.1 *M* calcium chloride dihydrate (Fig. 1). Crystals grown in solutions containing polyethylene glycol 400 diffracted poorly (up to ~4.5 Å resolution).

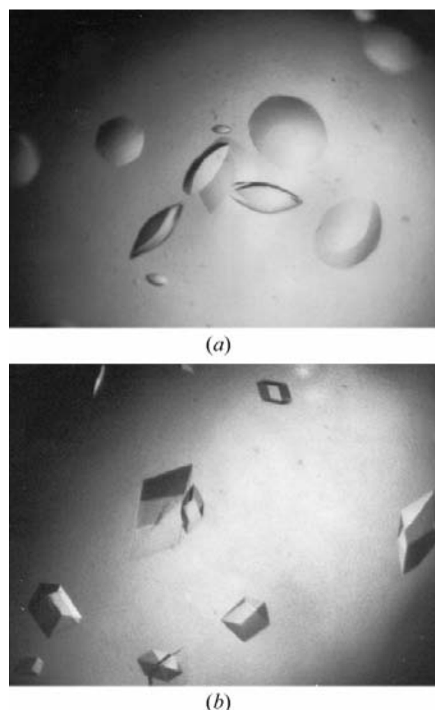

Figure 1
Crystals of LSL grown at 291 K (*a*) in 0.1 *M* calcium chloride dihydrate, 0.1 *M* sodium acetate trihydrate pH 4.5, 14%(*v*/*v*) 2-propanol and (*b*) in 0.1 *M* calcium chloride dihydrate, 0.1 *M* HEPES pH 7.5, 26%(*v*/*v*) polyethylene glycol 400.

2.3. X-ray diffraction experiments

Diffraction data were collected in-house on a MAR 345 image-plate detector with Cu *Kα* X-rays generated by an Enraf–Nonius rotating-anode generator equipped with a double-mirror focusing system operated at 40 kV and 90 mA and also at ESRF (Grenoble, France) on beamline BM16 using a CCD detector. Crystals were cryoprotected by a quick soak (10 s) in reservoir solution containing 30%(*v*/*v*) glycerol. Diffraction data consisted of 204 images with 1° oscillation and extended to 2.70 Å resolution. The crystal-to-detector distance was set to 200 mm. All data were processed and scaled using the programs *MOSFLM* (Leslie, 1992) and *SCALA* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). The self-rotation function was calculated with *MOLREP* (Vagin & Teplyakov, 1997) from the *CCP*4 package.

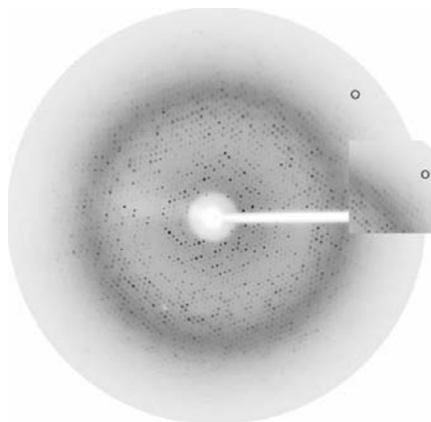

Figure 2
X-ray diffraction pattern of a hexagonal LSL crystal collected in-house on a MAR 345 image-plate detector. The exposure time was 15 min, the crystal-to-detector distance 200 mm and the oscillation angle 1.0°. The edge of the plate corresponds to 2.44 Å resolution. The circles indicate the same 2.68 Å reflection.

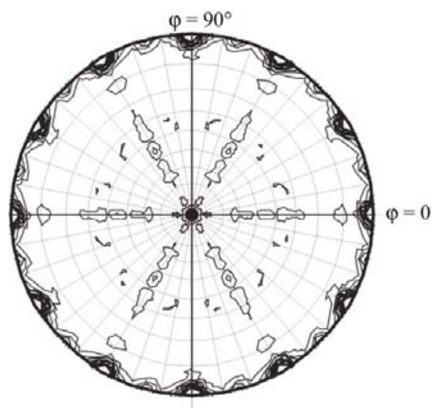

Figure 3
Stereographic projection of the self-rotation function calculated for χ = 180° with crystal data in the 15–3 Å resolution range with an integration radius of 30 Å using *MOLREP* (Vagin & Teplyakov, 1997). The results indicate that the point-group symmetry is 622 and also indicate the existence of a non-crystallographic symmetry axis at (θ, φ, χ) = (90, 15, 180°).

Table 1
Data-collection and processing statistics.

Values for the outermost shell (2.85–2.70 Å) are given in parentheses.

Space group	$P6_322$
Unit-cell parameters (Å)	*a* = *b* = 101.8, *c* = 193.9
Resolution range (Å)	32.3–2.7
No. measured reflections	286793
No. unique reflections	17071
R_{merge} (%)	9.2 (42.7)
Completeness (%)	100 (100)
Multiplicity	16.8 (13.8)
Average $I/\sigma(I)$	6.1 (1.8)

3. Results and discussion

LSL crystals diffract to 2.70 Å resolution (Fig. 2) and belong to the hexagonal space group $P6_322$ (unit-cell parameters *a* = *b* = 101.8, *c* = 193.9 Å). Data-collection and processing statistics are summarized in Table 1. A total of 286 793 measured reflections were merged into 17 071 unique reflections with an R_{merge} of 9.2%. The merged data set was 100% complete to 2.70 Å resolution. The number of molecules in the crystallographic asymmetric unit was estimated using the Matthews probability calculator, with the resolution as an additional input parameter (Kantardjieff & Rupp, 2003). The highest probability (0.96) was obtained with two molecules in the asymmetric unit, giving a Matthews coefficient (V_M value; Matthews, 1968) and solvent content of 2.07 Å^3 Da^{-1} and 40%, respectively. Further evidence pointing to this result comes from analysis of the local symmetry using *MOLREP* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). The self-rotation function calculated from the crystal data shows strong features at χ = 180° showing the 622 point-group symmetry and also the existence of a local twofold axis at (θ, φ, χ) = (90, 15, 180°) (Fig. 3). The peak intensity with respect to the crystallographic axis was 21.2% (Rf/σ = 2.3). The observation of non-crystallographic twofold symmetry is in agreement with the previous result indicating the presence of a dimer in the asymmetric unit. Taking into account the fact that LSL is a homotetramer (Tateno & Goldstein, 2003), these results indicate that LSL can be described as a dimer of dimers related by a twofold axis, the monomers within each dimer being related by another

twofold axis, *i.e.* LSL is a tetramer that exhibits a 222 symmetry. Determination of the structure of LSL by MIR phasing is under way.

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