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## Crystallization and preliminary X-ray diffraction analysis of an anti-H(O) lectin from *Lotus tetragonolobus* seeds

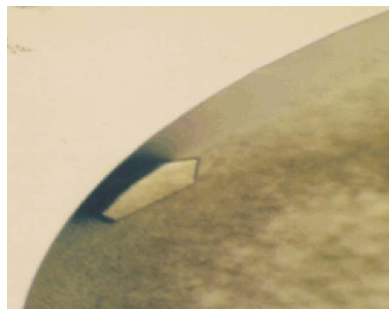
The seed lectin from *Lotus tetragonolobus* (LTA) has been crystallized. The best crystals grew over several days and were obtained using the vapour-diffusion method at a constant temperature of 293 K. A complete structural data set was collected at 2.00 Å resolution using a synchrotron-radiation source. LTA crystals were found to be monoclinic, belonging to space group  $P2_1$ , with unit-cell parameters  $a = 68.89$ ,  $b = 65.83$ ,  $c = 102.53$  Å,  $\alpha = \gamma = 90$ ,  $\beta = 92^\circ$ . Molecular replacement yielded a solution with a correlation coefficient and  $R$  factor of 34.4 and 51.6%, respectively. Preliminary analysis of the molecular-replacement solution indicates a new quaternary association in the LTA structure. Crystallographic refinement is under way.

### 1. Introduction

The legume lectins are a large group of homologous carbohydrate-binding proteins of non-immune origin that are found mainly in the seeds of most leguminous plants. These lectins act by deciphering specific glycodes encoded in the structure of glycans. The interaction between lectins and carbohydrates plays a biological role in cellular processes such as cell communication, host defence, fertilization, development, parasitic infection, tumour metastasis and plant defence against herbivores and pathogens (Vijayan & Chandra, 1999; Gallego del Sol *et al.*, 2005). Lectins are ubiquitous in animals, plants and microorganisms. Over 250 three-dimensional structures of lectins from diverse sources are available and legume lectins represent a significant proportion of these proteins. Lectins share a common structural fold but they differ in their carbohydrate specificities. An outstanding feature of the group of legume lectins is that although all the monomers have similar tertiary structures, they show different modes of quaternary association (Brinda *et al.*, 2004). This structural feature deserves more in-depth investigation in order to identify possible structural aspects that may dictate the determinants of the quaternary association of legume lectins.

Bianchet and coworkers characterized fucose-binding lectins as proteins that bind fucose and share a specific sequence motif whose function is associated with immune recognition in vertebrates and invertebrates (Bianchet *et al.*, 2002). Fucose-binding lectins are widespread among microorganisms, animals and plants, including *Pseudomonas aeruginosa* lectin (PA-IIL), *Anguilla anguilla* agglutinin (AAA), *Morone saxatilis* agglutinin (MsaFBP32), *Dicentrarchus labrax* agglutinin, *Chromobacterium violaceum* lectin (CV-IIL), *Ralstonia solanacearum* lectin (RS-IIL), *Ulex europaeus* agglutinin (UEA-I) and *Lotus tetragonolobus* agglutinin (LTA) (Bianchet *et al.*, 2002; Mitchell *et al.*, 2005; Vandonselaar & Delbaere, 1994; Konami *et al.*, 1990; Zinger-Yosovich *et al.*, 2006; Odom & Vasta, 2006; Cammarata *et al.*, 2001).

Fucose-binding lectins have been widely studied because the molecular-recognition properties of glycoproteins and glycolipids containing L-fucose are often characterized in terms of their interactions with fucolectins. Moreover, there have been several reports that L-fucosyl oligosaccharides are found in most common human cancers, particularly in adenocarcinomas and neuroblastomas. Several pieces of evidence suggest that fucosyl oligosaccharides are important cell-surface recognition determinants (Hakomori, 1984; Santer *et al.*, 1983; Cheng *et al.*, 1998).



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LTA and UEA-I are two homologous fucolectins from the Leguminosae family. Although they possess the same primary specificity for fucose, they display considerable diversity in their carbohydrate binding to fucosylated oligosaccharides. UEA-I recognizes the H-type 2 determinant [ $\alpha$ -L-Fuc(1 $\rightarrow$ 2) $\beta$ -D-Gal(1 $\rightarrow$ 4) $\beta$ -D-GlcNAc] but not the Le<sup>a</sup> and Le<sup>x</sup> determinants. However, LTA recognizes Le<sup>x</sup> and other different divalent L-fucosyl carbohydrates (Cheng *et al.*, 1998). Despite their similarity and apparently conserved sequences (41% amino-acid identity), the above lectins possess different biological activities. Furthermore, a previous study of LTA and other legume lectins with unknown structures have shown that the LTA structure should have a quaternary association type that differs from those of known legume lectins (Brinda *et al.*, 2004). Thus, structural studies of LTA will help us to understand its binding to carbohydrates, which is directly responsible for its biological activity, and to elucidate its quaternary association.

LTA is a member of the legume family (Leguminosae, Papilionoideae, Loteae) of lectins, which have been widely used to explore the properties of membranes from both normal and transformed cells (Shirahama *et al.*, 1993; Mansour *et al.*, 2005). The primary structure of LTA has been previously determined and is a glycoprotein containing 240 amino-acid residues with a molecular weight of 26 273.17 Da. Furthermore, the affinity of LTA for L-fucosyl oligosaccharides has been investigated by nuclear magnetic resonance and electron microscopy (Cheng *et al.*, 1998; Haselhorst *et al.*, 2001). In order to establish the crystal structure of this new member of the fucose-binding lectins, we carried out a crystallization study and preliminary X-ray diffraction analysis of LTA, a fucolectin from *Lotus tetragonolobus* seeds, with the aim of solving its native quaternary structure (Pereira & Kabat, 1974a,b; Konami *et al.*, 1990).

## 2. Material and methods

### 2.1. LTA crystallization, data collection and processing

LTA was purchased from Sigma–Aldrich (USA). The purified lectin was dissolved completely at a concentration of 5.0 mg ml<sup>-1</sup> in 20 mM Tris–HCl pH 7.6 containing 1 mM CaCl<sub>2</sub> and MnCl<sub>2</sub>. For hanging-drop crystallization trials, the sample was submitted to various crystallization conditions using Crystal Screens I and II and SaltRx, Index and PEG/Ion screens (Hampton Research, Riverside, CA, USA). The drops were composed of equal volumes (1  $\mu$ l) of protein solution and reservoir solution and were equilibrated against 500  $\mu$ l reservoir solution. Crystals were grown in Linbro plates at 293 K by the vapour-diffusion method (Jancarik & Kim, 1991).

X-ray diffraction data were collected at a wavelength of 1.47 Å using a synchrotron-radiation source (MX1 station, Laboratório Nacional de Luz Síncrotron, Campinas, Brazil) and a CCD detector (MAR Research). Data were collected from the LTA crystal at 100 K and to avoid freezing crystals were soaked in a cryoprotectant solution containing 75% mother liquor and 25% glycerol. Using an oscillation range of 1.5° and an exposure time of 90 s per frame, 150 images were collected to a maximum resolution of 2.0 Å. Data were processed, indexed and integrated using *MOSFLM* and scaled using *SCALA* (Collaborative Computational Project, Number 4, 1994; Leslie, 1992).

### 2.2. Molecular replacement

The molecular-replacement method was used to determine the crystal structure of LTA using *MOLREP* (Collaborative Computational Project, Number 4, 1994). Rotation and translation functions were obtained using the peanut lectin monomer coordinates (PDB code 1cr7; Ravishankar *et al.*, 2001) as a search model. Three space groups were tested (*P*<sub>2</sub>, *P*<sub>2</sub><sub>1</sub> and *C*<sub>2</sub>) and the best solution for each model was chosen based on the magnitude of the correlation coefficient and the *R* factor.

## 3. Results and discussion

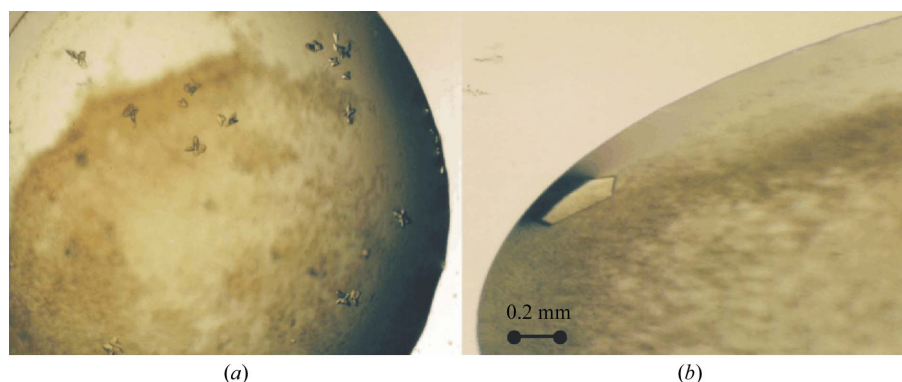
### 3.1. Optimization of LTA crystals

LTA has previously been purified and its primary sequence determined. The lectin was shown to be a glycolectin and its haemagglutinating activity was found to be inhibited by the presence of L-fucose (Konami *et al.*, 1990).

Small irregular crystals of LTA appeared in 0.1 M trisodium citrate pH 5.6 containing 20% 2-propanol and 20% PEG 4000 after a week (Hampton Research Crystal Screen II, condition No. 40). These crystals were not suitable for X-ray diffraction experiments. However, several optimization steps were performed, changing the pH and precipitant concentration. The LTA crystals grew over several days in 0.1 M trisodium citrate pH 5.6 containing 8% 2-propanol and 16% PEG 4000 to maximum dimensions of 0.1  $\times$  0.2  $\times$  0.4 mm (Fig. 1).

### 3.2. Data collection and processing

LTA crystals diffracted to a maximum resolution of 2.0 Å using a synchrotron-radiation source (LNLS, Campinas, Brazil). The complete data set (150 frames) was indexed, integrated and scaled in the resolution range 40.42–2.0 Å. LTA crystals were monoclinic,



**Figure 1** (a) Crystals from 0.1 M trisodium citrate pH 5.6 containing 20% 2-propanol and 20% PEG 4000. (b) Optimized LTA crystal suitable for X-ray diffraction experiments.

**Table 1**

Data-collection statistics.

Values in parentheses are for the highest resolution shell (2.11–2.00 Å).

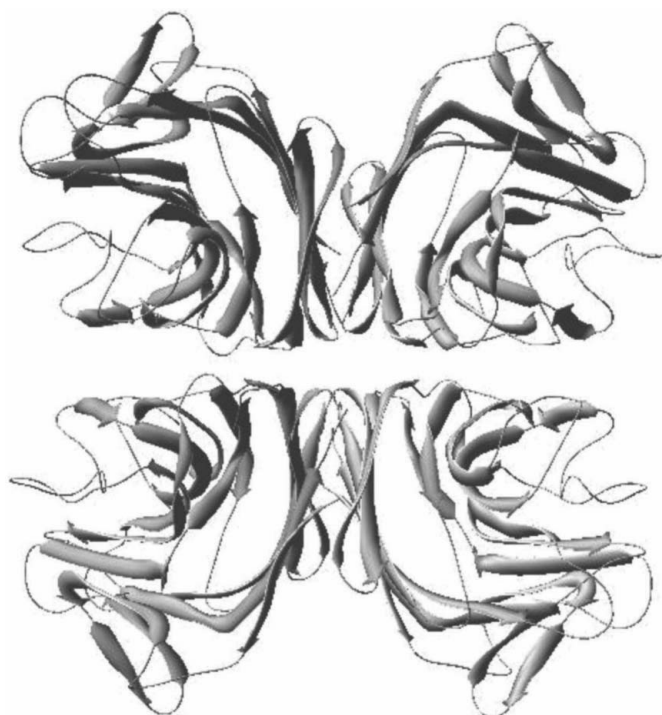
Wavelength (Å)	1.47
Space group	$P2_1$
Unit-cell parameters (Å, °)	$a = 68.89, b = 65.83, c = 102.53,$ $\alpha = \gamma = 90, \beta = 92$
Resolution range (Å)	40.42–2.00 (2.11–2.00)
Unique reflections	61847 (8835)
Completeness (%)	99.4 (97.9)
$\langle I/\sigma(I) \rangle$	10.8 (2.6)
$R_{\text{sym}}$ (%)	9.5 (45.9)
Asymmetric unit content	One tetramer (960 residues)

belonging to the  $P2_1$  space group, with unit-cell parameters  $a = 68.89$ ,  $b = 65.83$ ,  $c = 102.53$  Å,  $\alpha = \gamma = 90$ ,  $\beta = 92^\circ$ . The volume of the unit cell was 464 772.31 Å<sup>3</sup>, with a  $V_M$  value of 2.2 Å<sup>3</sup> Da<sup>-1</sup> (Matthews, 1968). The LTA crystal contained one tetramer per asymmetric unit. A summary of data-collection statistics is given in Table 1.

### 3.3. Molecular replacement

Sequence-alignment analysis was performed using the *BLASTP* software at the National Center of Biotechnology Information to compare the LTA primary sequence with those deposited in the PDB (Altschul *et al.*, 1990).

Molecular replacement was performed using the *MOLREP* software (Vagin & Teplyakov, 1997). The best result was obtained using the peanut lectin monomer. We found four monomers per asymmetric unit in the LTA crystal structure. The rotation and translation functions yielded a planar homotetramer with a correlation coefficient and  $R$  factor of 34.4 and 51.6%, respectively (Fig. 2). The model produced was then submitted to initial crystallographic refinement using *REFMAC5* (Collaborative Computational Project, Number 4, 1994). The first initial step of refinement was performed using rigid-



**Figure 2**

Molecular-replacement homotetramer solution for the LTA crystal using the peanut lectin monomer as a model.

body followed by restrained refinement (maximum-likelihood method), resulting in a model with an  $R$  factor of 29.4% and an  $R_{\text{free}}$  of 35.4%. Complete refinement of the LTA structure is in progress.

The crystallographic packing of the LTA structure is shown in Fig. 2. Preliminary comparison of the LTA tetramer structure with the quaternary association of other legume lectins indicates that it has a different quaternary association. As described previously, the peanut lectin consists of a homotetramer composed of two different dimers. Its tetramer is made up of a canonical dimer and an unusual dimer interface, leading to an open quaternary structure. The canonical dimer is found in several legume lectins, as described for ConA, which is a homotetramer composed of two canonical dimers linked via a specific dimer–dimer interface. Normally, legume lectin tetramers are essentially dimers of dimers and the different modes of tetramerization are a consequence of the diverse combination of dimeric interfaces seen in these quaternary structures. Our present work shows that the preliminary initial model of LTA is a tetramer composed of two back-to-back interconnected dimers (non-canonical), yielding a planar homotetramer. Although GS4, a lectin from *Griffonia simplicifolia*, possesses the same type of dimer–dimer interface as LTA, its tetramers are significantly different. The molecular-replacement solution leads to acceptable crystal packing, but further refinement results will clarify the particular structural properties of LTA.

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