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New crystal forms of Diocleinae lectins in the presence of different dimannosides

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Studying the interactions between lectins and sugars is important in order to explain the differences observed in the biological activities presented by the highly similar proteins of the Diocleinae subtribe. Here, the crystallization and preliminary X-ray data of *Canavalia gladiata* lectin (CGL) and *C. maritima* lectin (CML) complexed with Man(α1-2)Man(α1)OMe, Man(α1-3)Man(α1)-OMe and Man(α1-4)Man(α1)OMe in two crystal forms [the complexes with Man(α1-3)Man(α1)OMe and Man(α1-4)Man(α1)OMe crystallized in space group $P3_2$ and those with Man(α1-2)Man(α1)OMe crystallized in space group $I222$], which differed from those of the native proteins ($P2_12_12$ for CML and $C222$ for CGL), are reported. The crystal complexes of ConA-like lectins with Man(α1-4)Man(α1)OMe are reported here for the first time.

1. Introduction

Lectins are carbohydrate-binding proteins which function as cognate receptors for various cell-surface glycoproteins, resulting in several important cellular-mediated events ranging from mitogenic processes to plant defence mechanisms (Weis & Drickamer, 1996).

Plant lectins, especially those purified from species of the Leguminosae family, represent the best studied group of carbohydrate-binding proteins (Van Damme *et al.*, 1998). Legume lectins have been used for decades as a model system for studying protein–sugar interactions. Fundamental insights obtained from investigating these protein model systems can often be readily applied to lectins outside this family, such as the pharmaceutically important C-type lectins and galectins (Hamelryck *et al.*, 1998).

The legume lectins from the Diocleinae subtribe are highly similar proteins that present significant differences in the potency/efficacy of their biological activities (Delatorre *et al.*, 2006). For instance, even though they only differ by three amino-acid residues, *Canavalia gladiata* lectin (CGL) and *C. maritima* lectin (CML) present different patterns in several activities, such as in aorta-relaxation experiments (to be published). In spite of these activities being very well documented, little is known about the receptors with which they interact and how this happens. The crystal structures of many plant lectins have revealed how they specifically recognize their carbohydrate ligands (Lis & Sharon, 1998) and this may give us a direction for elucidating their mechanism of action.

An outstanding feature of Diocleinae lectins is that although all the monomers have similar tertiary structures, they show different modes of quaternary association. Minor differences in the ratio between the dimeric and tetrameric forms, together with changes in the relative orientations of the carbohydrate-binding sites in the quaternary structures, have been hypothesized to contribute to the differing biological activities possessed by these lectins (Brinda *et al.*, 2004).

Native CGL and CML have been crystallized and their structures have been solved in space groups $C222$ and $P2_12_12$, respectively (Moreno *et al.*, 2004; Gadelha *et al.*, 2005). Both have tetramers composed of 237 amino-acid monomers in the asymmetric unit. Crystals of CML in complex with trehalose and maltose were subsequently obtained under the same conditions as described for the native protein and presented the same space group $P2_12_12$ (Delatorre *et al.*, 2006).

We report here the crystallization and preliminary data of six dimannoside-complexed crystals of CGL and CML in two different space groups, including the first crystal of a ConA-like lectin with the dimannoside Man(α1-4)Man(α1)OMe.

2. Material and methods

2.1. Crystallization

CML and CGL were purified according to the methods of Moreira & Cavada (1984) and Ceccatto *et al.* (2002), respectively. Each dimannoside [Man(α1-2)Man(α1)OMe, Man(α1-3)Man(α1)OMe and Man(α1-4)Man(α1)OMe; purchased from Sigma–Aldrich] was added in a 0.08 molar proportion to 10 mg ml^{-1} of the pure lectin dissolved in 20 m*M* Tris–HCl pH 7.6 containing 5 m*M* $CaCl_2$/$MnCl_2$ and incubated for 24 h at room temperature.

Crystallization trials were performed based on two different strategies: (i) varying the ammonium sulfate concentration from 0.25 to 3.0 *M* combined with different buffers varying from pH 3.5 to 9.0 (strategy supplied by McPherson, 2003) and (ii) varying the sodium formate concentration from 0.5 to 7.0 *M* with Tris–HCl pH 7.6. Both strategies were carried out at 293 K using the hanging-drop vapour-diffusion method in Linbro plates. The drops were composed of equal volumes (2 µl) of protein solution and reservoir solution and were equilibrated against 500 µl of the latter.

Table 1
Crystallization conditions, space groups and number of molecules per asymmetric unit for native CGL and CML and for their complexes with Man(α1-2)Man(α1)OMe, Man(α1-3)Man(α1)OMe and Man(α1-4)Man(α1)OMe.

	Crystallization conditions	Space group	Molecules per ASU
Native CGL	0.1 *M* Tris–HCl pH 8.5, 2 *M* ammonium sulfate	*C*222	4
CGL–Man(α1-2)Man(α1)OMe	0.1 *M* Tris–HCl pH 8.0–9.0, 1.8–2.6 *M* ammonium sulfate	*I*222	1
CGL–Man(α1-3)Man(α1)OMe	4.5–6.5 *M* sodium formate	$P3_2$	4
CGL–Man(α1-4)Man(α1)OMe	4.5–6.5 *M* sodium formate	$P3_2$	4
Native CML	0.1 *M* Na HEPES pH 8.48, 4% PEG 400, 2*M* ammonium sulfate	$P2_12_12$	4
CML–Man(α1-2)Man(α1)OMe	0.1 *M* Tris–HCl pH 8.0–9.0, 1.8–2.6 *M* ammonium sulfate	*I*222	1
CML–Man(α1-3)Man(α1)OMe	4.5–6.5 *M* sodium formate	$P3_2$	4
CML–Man(α1-4)Man(α1)OMe	4.5–6.5 *M* sodium formate	$P3_2$	4

2.2. Data collection and processing

X-ray diffraction data were collected at a wavelength of 1.43 Å using a synchrotron-radiation source (MX1 station, Laboratório

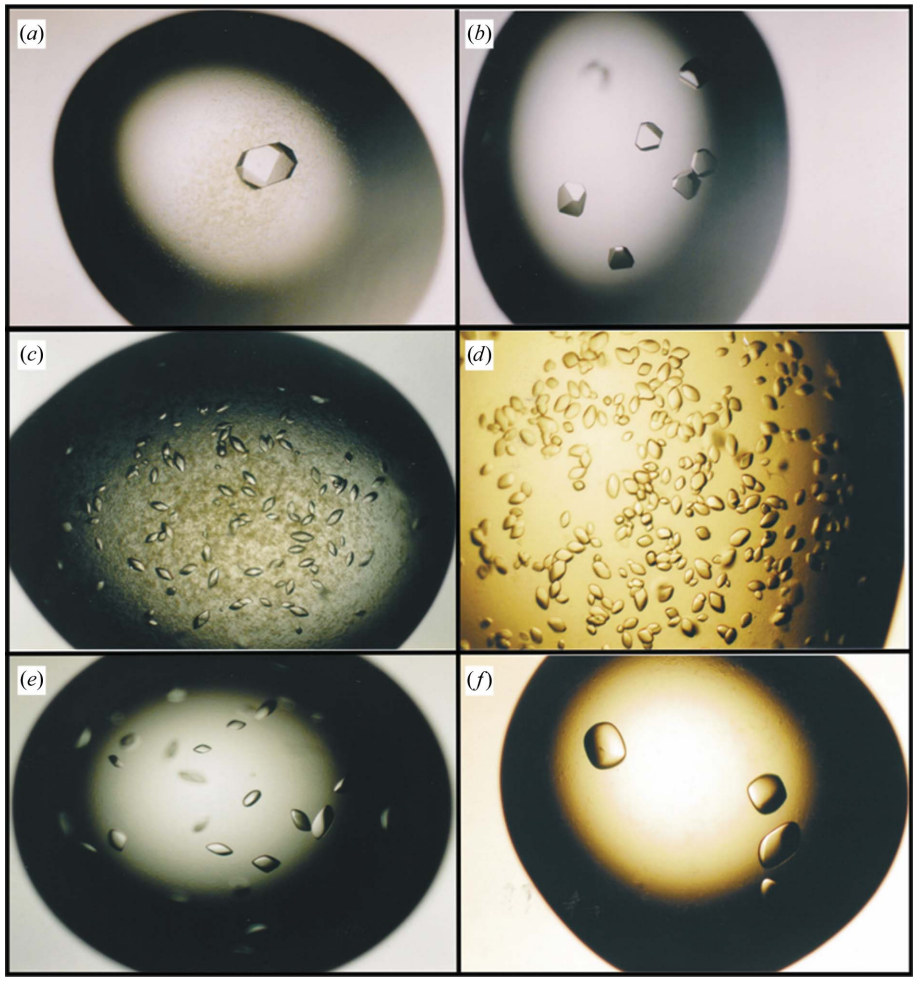

Figure 1
Crystals of (*a*) CGL–Man(α1-2)Man(α1)OMe, (*b*) CML–Man(α1-2)Man(α1)OMe, (*c*) CGL–Man(α1-3)Man(α1)OMe, (*d*) CML–Man(α1-3)Man(α1)OMe, (*e*) CGL–Man(α1-4)Man(α1)OMe and (*f*) CML–Man(α1-4)Man(α1)OMe.

Table 2
Statistics of data collection for CGL complexes.

Values in parentheses are for the highest resolution shell.

Data collection	CGL–Man(α1-2)Man(α1)OMe	CGL–Man(α1-3)Man(α1)OMe	CGL–Man(α1-4)Man(α1)OMe
R_{merge}	6.1 (32.8)	8.8 (40)	6.8 (23.3)
Resolution limit (Å)	21.03–1.50	40.29–2.07	40.096–1.980
$I/\sigma(I)$	7.6 (2.0)	10.7 (2.0)	8.8 (3.2)
Completeness (%)	99.9 (100.0)	91.5 (86)	99.4 (99.4)
Redundancy	19.20	2.5	10.20
Unit-cell parameters (Å)	a = 63.89, b = 86.19, c = 88.73	a = 69.37, b = 69.37, c = 161.21	a = 69.01, b = 69.01, c = 160.44
Matthews coefficient (Å^3 Da^{-1})	2.4	2.2	2.2
Solvent content (%)	48.2	43.6	42.5
Space group	$I222$	$P3_2$	$P3_2$
Wavelength (Å)	1.431	1.431	1.431
Total No. of reflections	760001	119394	607712
Total No. of unique observations	39568	48437	59558

Table 3
Statistics of data collection for CML complexes.

Values in parentheses are for the highest resolution shell.

Data collection	CML–Man(α1-2)Man(α1)OMe	CML–Man(α1-3)Man(α1)OMe	CML–Man(α1-4)Man(α1)OMe
R_{merge}	5.5 (45.2)	9.1 (29.6)	9.9 (34.8)
Resolution limit (Å)	20.96–1.4	40.49–2.10	34.73–2.10
$I/\sigma(I)$	15.5 (2.4)	15.8 (3.0)	15.6 (3.9)
Completeness (%)	97.9 (95.43)	98.9 (98.1)	96.5 (94.8)
Redundancy	4.0 (3.9)	4.2 (4.0)	5.2 (5.1)
Unit-cell parameters (Å)	a = 63.63, b = 85.44, c = 85.71	a = 69.39, b = 69.39, c = 161.29	a = 69.47, b = 69.47, c = 161.52
Mathews coefficient (Å^3 Da^{-1})	2.4	2.2	2.2
Solvent content (%)	48.5	43.6	43.8
Space group	$I222$	$P3_2$	$P3_2$
Wavelength (Å)	1.431	1.431	1.431
Total No. of reflections	183774	212393	252952
Total No. of unique observations	46457	50169	49112

Nacional de Luz Síncrotron, Campinas, Brazil) and a CCD detector (MAR Research) at 100 K. To avoid freezing, crystals were soaked in a cryoprotectant solution containing between 30 and 50% glycerol in mother liquor. Data were processed, indexed and integrated using *MOSFLM* and scaled using *SCALA* (Collaborative Computational Project, Number 4, 1994; Leslie, 1992).

3. Results and discussion

Crystallization conditions, space groups and the number of molecules per asymmetric unit of the dimannoside complexes and native crystals are listed in Table 1. The preliminary crystallographic data for CGL complexes is presented in Table 2 and for CML complexes in Table 3. The crystal complexes are depicted in Fig. 1.

Although the crystallization conditions are different for the crystal complexes and the native proteins, the main cause of the change in the symmetry seems to be the presence of the dimannosides. CGL and CML complexed with Man(α1-2)Man(α1)OMe crystallized in space group $I222$ with one molecule in the asymmetric unit after 72 h, while the crystals of CGL and CML complexed with Man(α1-3)Man(α1)OMe and Man(α1-4)Man(α1)OMe belonged to space group $P3_2$ with a tetramer in the asymmetric unit, crystallizing after 48 h.

The importance of the dimannosides in the crystallization process is noteworthy. Native CGL and CML crystallize under different conditions and in different space groups: 0.1 *M* Tris–HCl pH 8.5, 2.0 *M* ammonium sulfate ($C222$) and 0.1 *M* Na HEPES pH 8.48, 4% PEG 400, 2.0 *M* ammonium sulfate ($P2_12_12$), respectively. In the presence of the carbohydrates, the crystallization conditions become the same for each sugar: for CML and CGL with Man(α1-2)Man(α1)OMe the condition was 0.1 *M* Tris–HCl pH 8.0–9.0, 1.8–2.6 *M* ammonium sulfate, while crystals of CML and CGL complexed with Man(α1-3)Man(α1)OMe and Man(α1-4)Man(α1)OMe only grew in the presence of 4.5–6.5 *M* sodium formate.

It is well established that legume lectins possess three types of hydrophobic sites based on different ligand affinities (Sharon & Lis, 1990). One of these sites is adjacent to the monosaccharide-binding site and participates in interactions involving several hydrophobic sugars. Bouckaert and coworkers described that the O3-linked mannose of Man(α1-3)Man(α1)OMe and the O6-linked mannose of Man(α1-6)Man(α1)OMe bind to the hydrophobic subsite formed by Tyr12, Tyr100 and Leu99 (Bouckaert *et al.*, 1999).

Investigations of the binding of Man(α1-2)Man(α1)OMe, Man(α1-3)Man(α1)OMe and Man(α1-6)Man(α1)OMe to ConA in this same hydrophobic subsite revealed significant differences in their affinity (Moothoo *et al.*, 1999). Based on this, we have crystallized CML and CGL complexed with the dimannosides Man(α1-2)Man(α1)OMe and Man(α1-3)Man(α1)OMe in order to compare them with the previously reported complexes from ConA and correlate their structure and affinity. The crystal complexes of CGL and CML in the presence of Man(α1-4)Man(α1)OMe represent the first ConA-like structure with this carbohydrate. The differences between the affinities of these mannosides may reflect how the protein binds to receptors related to lectin-mediated responses in plants or in other organisms. Therefore, solving the structures of CGL and CML complexed with dimannosides may be important to understanding many of their biological activities. Our data may clarify the understanding of how the interactions between the dimannosides and the hydrophobic subsite formed by Tyr12, Tyr100 and Leu99 occur.

The complex crystals were obtained in space groups that differed from those of the native lectins. Since crystal packing has an influence on the protein conformation (Kanellopoulos *et al.*, 1996), our work may be important in revealing interactions distinct from those in the native structures.

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