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Crystallization and preliminary X-ray studies on the lectin from the seeds of *Cratylia mollis*

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Abstract

The lectin from the seeds of *Cratylia mollis* shows strong binding to human malignant cancerous tissues, particularly those from many glands, uterus, rectum and brain. The *C. mollis* lectin has been crystallized using the hanging-drop method with polyethylene glycol 6000 as a precipitant. Two different crystal forms were grown from the same drops and they belong to space groups $I222$ and $P2_12_12_1$, respectively. The cell parameters obtained were $a = 63.26\,(4)$, $b = 77.45\,(8)$ and $c = 105.22\,(8)$ Å, for the $I222$ form, and $a = 88.83\,(5)$, $b = 183.24\,(9)$ and $c = 61.70\,(2)$ Å for the $P2_12_12_1$ crystals. The solution of both structures is currently being attempted by means of molecular replacement techniques.

1. Introduction

Lectins constitute a class of proteins that specifically bind carbohydrates in a non-covalent fashion (Sharon & Lis, 1989). Carbohydrate–lectin association is a primary event in many biological processes such as infection, cell differentiation, host–bacteria symbiosis, organ formation and metastasis (Sharon & Lis, 1986). Besides its important roles in cell recognition, the lectin–carbohydrate interaction has been explored in various branches of research where saccharide specificity is essential, resulting in useful biological tools such as blood typing, cancer-cell staining and lymphocyte agglutination (Sharon, 1993). Although lectins are widely found in plants, their biological role in these systems is still unknown. Nevertheless, their specific glycan-binding properties make them an ideal object for the detailed study, at atomic level, of protein–carbohydrate interactions. In particular, lectins of the family Fabaceae are used as model systems to study the nature of such interactions because of their abundance in mature seeds and their broad range of carbohydrate specificities.

C. mollis is a legume belonging to the family Fabaceae, tribe Phaseoleae, subtribe Dioclinae, which contains the genus *Canavalia*, botanically related to *Cratylia*. The plant is native to the Northeast of Brazil. Some of us have recently reported the purification and characterization of isoform I of the lectin from the seeds of *C. mollis* (Correia & Coelho, 1995). This lectin is glucose–mannose specific, similar to concanavalin A, a well characterized lectin from *Canavalia ensiformis* (Naismith *et al.*, 1993, 1994). However, *C. mollis* lectin and concanavalin A showed different structural characteristics, such as the elution pattern in Superose 12 and VYDAC C4 by fast protein liquid chromatography and high-pressure liquid chromatography, respectively, and isoelectric focusing under denaturing conditions. Also, partial identity was observed between the two lectins, with double immunodiffusion using an antiserum against the *C. mollis* lectin. Native *C. mollis* lectin has been used to raise antibodies which were purified by affinity chromatography and conjugated to peroxidase. They have cytotoxic (KB cells) and antitumoral activity (sarcoma 180), and revealed differentiated specific binding to benign and malignant human tumors; the lectins, in different proportions, modified hormone–receptor affinities on hypophyseal receptors of gonadoliberine (GnRH) in rats. Futhermore, in contrast to concanavalin A, the *C. mollis* lectin induces plantar edema reduction (Correia & Coelho, personal communication). As legume lectins with the same monosaccharide specifity but distinct biological activities can discriminate between closely related but different oligosaccharides, further knowlege of their three-dimensional structures could contribute to the understanding of the molecular mechanisms involved in cellular recognition (Loris *et al.*, 1993).

2. Methods and results

The protein used for the crystallization experiments was purified by affinity chromatography on Sephadex G-75, followed by ion-exchange chromatography on CM-Cellulose. The lectin has a molecular weight of 31 kDa with a p*I* of 8.5 to 8.6 as described by Correia & Coelho (1995). Crystals were grown at 291 K using the hanging-drop vapor-diffusion method (McPherson, 1982) with a drop that contained 5 µl of protein solution at 12 mg ml^{-1} and 5 µl of the reservoir solution containing 0.01 *M* sodium acetate at pH 4.5, 0.15 *M* NaCl buffer, 9%(*w*/*v*) polyethylene glycol 6000, 0.01 *M* NaN_3 and 0.15 *M* methyl-α-D-mannopyranoside. Crystal growth was visible after a week and was usually complete within a month. The crystallization droplets consistently showed two different crystal morphologies: crystal form I with an elongated prismatic shape, with typical dimensions of $0.40 \times 0.15 \times 0.15$ mm and diffracting a resolution limit of to 2.0 Å resolution and crystal form II, which grew as large plates with sizes up to $0.9 \times 0.5 \times 0.1$ mm and which diffracted up to 2.2 Å. Autoindexing procedures, combined with the analysis of the diffraction patterns and averaging of equivalent intensities was used in the characterization of the Laue symmetry. Crystal form I has the orthorhombic space group $I222$ and has average unit-cell dimensions of $a = 63.26\,(4)$, $b = 77.45\,(8)$ and $c = 105.22\,(8)$ Å, obtained from the measurements of four crystals. The volume of the unit cell is $515\,(1) \times 10^3$ Å^3, compatible with one monomer in the asymmetric unit with a V_m value (Matthews, 1968) of 2.079 Å^3 Da^{-1}. Assuming a value of 0.74 cm^3 g^{-1} for the protein partial specific volume, the calculated solvent content in the crystal is 41% and the calculated crystal density 1.21 g cm^{-3}. Crystal form II is also orthorhombic, with space group $P2_12_12_1$ and with unit-cell dimensions $a = 88.83\,(5)$, $b = 183.24\,(9)$ and $c = 61.70\,(2)$ Å with standard deviations estimated from the setting reflections of two still photographs. This crystal form results in similar packing indicators when a tetramer is assumed per asymmetric

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Table 1. *Data-collection statistics for the I222 crystal form of the Cratylia mollis lectin*

Resolution in range (Å)	No. of reflections measured	No. of independent reflections	R_{merge} in range	Completeness in range (%)
7.78	975	341	0.082	97.7
5.50	1709	562	0.080	96.1
4.49	2282	719	0.086	97.7
3.89	2700	831	0.096	96.7
3.48	3081	934	0.111	96.3
3.18	3420	1003	0.121	94.8
2.94	3881	1071	0.136	93.5
2.75	4082	1072	0.146	87.1
2.59	4456	1082	0.162	82.8
2.46	3326	1040	0.161	75.9
Total	29912	8655	0.108	89.9

Table 2. *Data-collection statistics for the $P2_12_12_1$ crystal form of the Cratylia mollis lectin*

Resolution in range (Å)	No. of reflections measured	No. of independent reflections	R_{merge} in range	Completeness in range (%)
5.47	6136	2468	0.040	67.3
4.34	5585	2547	0.040	73.3
3.79	4586	2434	0.050	70.4
3.44	3793	2189	0.059	63.6
3.20	3178	1899	0.068	57.6
3.01	2848	1808	0.082	52.9
2.86	2510	1624	0.102	48.5
2.74	2018	1458	0.112	43.2
Total	30654	16427	0.049	59.8

unit [$V_{cell} = 1004\ (1) \times 10^3$ Å^3, $V_m = 2.025$ Å^3 Da^{-1}, solvent content 39.3% and calculated crystal density 1.21 g cm^{-3}].

Diffraction data sets were collected for both crystal forms using an image-plate detector R-AXIS IIC from RIGAKU with a rotating-anode generator RU200B operating at 40 kV and 120 mA. During all diffraction experiments the crystals were kept at 277 K with a nitrogen flow from a Enraf–Nonius cooling system. Systematic radiation damage prevented integration to the highest resolution observed in the initial exposures. For the *I*222 crystal form data was collected to a resolution limit of 2.46 Å from four crystals. A total of 29 912 reflections were measured and subsequently merged to 8655 independent reflections with $I > \sigma(I)$ with an overall $R_{merge} = 0.108$, corresponding to a completeness of 89.9%. Detailed data collection statistics are given in Table 1. For the $P2_12_12_1$ crystal form, the large *b* axis required a larger crystal-to-detector distance which further limited the data collection to 2.74 Å resolution. For this crystal form the initial data collection from one crystal yielded 30 654 measured reflections of which 16 427 were independent with $I > \sigma(I)$, $R_{merge} = 0.049$, corresponding to a completeness of 59.8%, as detailed in Table 2. The images were processed with the R-AXIS II processing program* and merged with the *CCP*4 package of programs (Collaborative Computational, Project Number 4, 1994). The structure solution is currently being attempted by molecular replacement techniques using the coordinates of concanavalin A as starting model (Naismith *et al.*, 1994). Similar to concanavalin A, whose binding to carbohydrate is mediated by two different ions, frequently Ca^{2+} and Mn^{2+} (Naismith *et al.*, 1994), the *C. mollis* lectin is also expected to bind metal ions. As no ions were added to the crystallization droplets, we expect that only a fraction of protein molecules will be found to bind metals ions from their native environment and that the two simultaneously grown crystal forms observed may correspond to the lectin in different states of ion and/or sugar binding.

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* Instruction Manual for R-AXIS IIC, Manual No. ME200KA1, Rigaku Corporation.

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