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Concanavalin A crystallized in complex with the trisaccharide 3,6-di-*O*-methyl-(α-D-mannopyranosyl)-α-D-mannopyranoside

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Abstract

Concanavalin A was co-crystallized in two crystal forms with 3,6-di-*O*-methyl-(α-D-mannopyranosyl)-α-D-mannopyranoside, which is primarily responsible for the high-affinity binding of *N*-linked carbohydrates to concanavalin A. Both crystal forms have space group $P2_1$ and contain a complete concanavalin A tetramer in the asymmetric unit. Form *A* was crystallized using polyethylene glycol methyl ether as the precipitant and has unit-cell dimensions $a = 59.83$, $b = 64.84$ and $c = 125.92$ Å, $\beta = 93.87°$. Form *B* was obtained using phosphate as the precipitant and has unit-cell dimensions $a = 81.94$, $b = 66.75$ and $c = 108.92$ Å, $\beta = 97.58°$. Form *B* was stable in the X-ray beam for several days and diffracted to 3.15 Å resolution. Form *A* crystals could not withstand X-ray radiation at room temperature, but produced high-quality data under cryogenic conditions. The latter are suitable for a 2.3 Å resolution structure determination by molecular replacement.

The biological activities of the mitogenic lectin concanavalin A (Con A) depend on the specific binding of the protein to polysaccharide and glycoprotein receptors. The minimum requirements that carbohydrates must fulfill to be bound specifically is the *arabino* configuration for the C atoms at the 3, 4 and 5 positions and unmodified 3-, 4- and 6- hydroxyl groups (Goldstein, Hollerman & Smith, 1965). The most specific monosaccharide has been found to be methyl α-D-mannopyranoside (MeαMan) (Debray, Decout, Strecker, Spik & Montreuil, 1981). However, the trimannoside 3,6-di-*O*-methyl-(α-D-mannopyranosyl)-α-D-mannopyranoside [Man(3,6)] binds with an affinity about 60-fold higher than MeαMan and is the major binding epitope for Con A on all *N*-linked oligomannose-type carbohydrates (Debray *et al.*, 1981). In contrast, other glucose/mannose specific *Leguminosae* lectins like the pea (*Pisum sativum*), lentil (*Lens culinaris*) and *Lathyrus ochrus* lectins, show no enhanced binding of Man(3,6) as compared to MeαMan (Debray *et al.*, 1981). The crystal structure of pea lectin complexed with Man(3,6) showed it to bind through a single terminal monosaccharide residue (Rini, Hardman, Einspahr, Suddath & Carver, 1993).

The thermodynamics of Con A–oligosaccharide complexation demonstrate that the higher affinity of Con A for the Man(3,6) epitope results from an extended recognition site on the lectin. A microcalorimetric investigation (Williams, Chervenak & Toone, 1992) suggested interaction of the two terminal mannose residues of the trisaccharide in distinct sites, as opposed to binding in a single high-affinity site. Recently Mandal *et al.* (1994) presented more detailed thermodynamic data on Man(3,6) interactions with Con A. They proposed that the α(1–6) linked mannose residue would bind in the high-affinity monosaccharide binding site known from the complex of Con A with MeαMan (Derewenda *et al.*, 1989). The α(1–3) linked mannose would bind elsewhere in a lower affinity site and interact through its 3-hydroxyl group. A third site appears to be involved in interactions with the 'core' mannosyl residue.

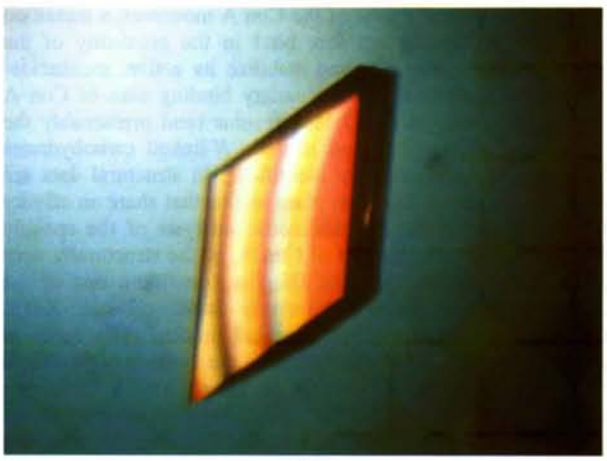
The stereochemistry of the monosaccharide–lectin interactions has been shown clearly in the crystal structure of the complex of Con A with MeαMan (Derewenda *et al.*, 1989), that has recently been refined at 2 Å resolution (Naismith *et al.*, 1994). The two metal ions of the Con A monomer, a transition metal ion and a calcium ion, bind in the proximity of the carbohydrate-binding site and stabilize its active, saccharide-binding conformation. The secondary binding sites of Con A that interact with the α(1-3)Man residue (and presumably the core Man) of Man(3,6) and related *N*-linked carbohydrates have not yet been similarly identified. No structural data are available for one of the other plant lectins that share an affinity for the trisaccharide. A calorimetric analysis of the specific Man(3,6) binding properties of Con A, of the structurally very homologous legume lectin of *Dioclea grandiflora* and of the distinctly related *Galanthus nivalis* lectin (Hester, Kaku, Goldstein & Wright, 1995), revealed marked differences in entropy and enthalpy contributions to the binding of Man(3,6) for all three lectins (Chervenak & Toone, 1995). Enthalpy–entropy compensations for Con A and the *Dioclea grandiflora* lectin clearly are manifested as a function of the lectin structure and dynamics. This emphasises the need for structural knowledge of lectin–saccharide complexes and their comparison with the saccharide-free lectin structures.

Con A was purified as described for pea lectin (Van Driessche, Smets, Dejaegere & Kanarek, 1982). Man(3,6) was purchased from Toronto Research Chemicals. The protein used for crystallization was dissolved in 250 m*M* NaCl; 1 m*M* $MnCl_2$; 1 m*M* $CaCl_2$; 0.02% NaN_3 and mixed with Man(3,6) to final concentrations of 8 mg ml^{-1} Con A and 12.5 m*M* trisaccharide (corresponding to a molar ratio trisaccharide:protein of 42:1). Hanging drops were prepared by mixing 5 µl of the above solution and 5 µl of the bottom precipitant solution. Two different crystal forms appeared. Form *A* (Fig. 1*a*) was crystallized by equilibration against a bottom solution of 13% polyethylene glycol methylether (PEGME) 5 kDa; 40 m*M* HEPES buffer at pH 6.5, and has unit-cell dimensions. $a = 59.83$, $b = 64.84$ and $c = 125.92$ Å, $\beta = 93.87°$. Crystals of form *B* (Fig. 1*b*) were grown by equilibration against 2 *M* NaH_2PO_4; titrated to pH 7.0 with NaOH and have unit-cell dimensions $a = 81.94$, $b = 66.75$ and $c = 108.92$ Å, $\beta = 97.58°$. Assuming a molecular mass of 26 500 Da for the Con A monomer (Min, Dunn & Jones, 1992), the asymmetric unit in both crystal forms must consist of a tetramer. This results in a V_m of 2.38 Å^3 Da^{-1} for form *A*, or a solvent content of 48.4%,

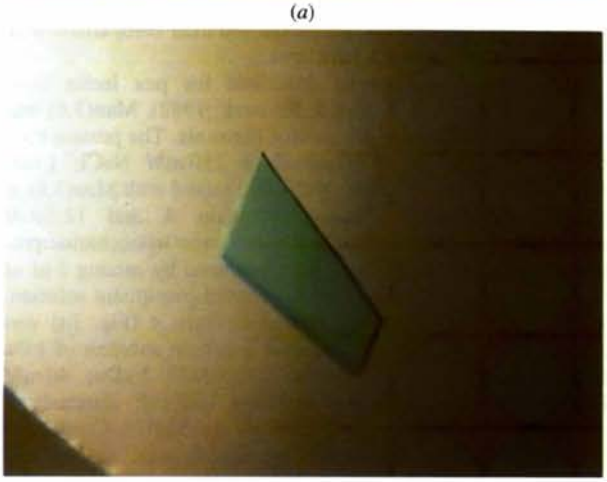
Table 1. *Statistics for the X-ray data as a function of the resolution*

No σ cut off on the intensity of the reflections was applied.

Resolution (Å)	R_{merge} (%)	No. of unique reflections	Completeness (%)	Multiplicity	$I/\sigma(I)$	$I > \sigma$ (I) (%)	$I > 3\sigma$ (I) (%)
15.00–6.61	5.3	1409	98.7	2.6	9.2	98.7	97.4
6.61–4.92	5.7	2218	100.0	2.5	10.5	98.8	96.9
4.92–4.09	5.7	2536	97.3	2.7	8.5	98.0	96.3
4.09–3.57	7.2	877	76.8	1.5	9.2	92.2	88.6
3.57–3.22	8.2	619	76.5	1.2	8.5	94.1	89.3
3.22–2.95	9.3	700	77.7	1.2	7.5	92.1	83.7
2.95–2.74	11.9	672	76.1	1.2	6.0	89.4	78.2
2.74–2.56	15.0	691	73.8	1.2	5.1	87.2	71.6
2.56–2.42	18.6	665	70.3	1.1	4.2	84.3	66.2
2.42–2.30	19.9	87	22.6	1.1	3.8	82.0	60.6
Overall	6.3	29495	71.3	1.5	8.3	91.4	82.4



(*a*)



(*b*)

Fig. 1. Crystals of Con A in complex with 3,6-di-*O*-methyl-(α-D-mannopyranose)-α-D-mannose. (*a*) Form *A*, $P2_1$, grown in PEGME, $a = 59.83$, $b = 64.84$ and $c = 125.92$ Å, $\beta = 93.87°$. (*b*) Form *B*, grown in phosphate, $a = 81.94$, $b = 66.75$, $c = 108.92$ Å, $\beta = 97.58°$.

and a V_m of 2.79 Å^3 Da^{-1} for form *B*, or 55.84% solvent (Matthews, 1968).

Form *A* crystals diffract to 2.4 Å on a FAST area detector in combination with a rotating-anode source, form *B* only to 3.15 Å. Crystal form *A* suffered from extreme radiation sensitivity. After less than 10° of data collection the crystals were shattered. Form *B* has first been used for data collection of 23 666 unique reflections between 13 and 3.15 Å resolution from two crystals. The poor quality of the data, with a merging *R* factor over 15%, prompted us to look for conditions under which the form *A* crystals could be maintained in the X-ray beam. A search for cryogenic solvents suitable for use with these crystals in a 100 K liquid nitrogen cold stream delivered good results for a solution of 35%(*v*/*v*) polyethylene glycol 400 in water. The crystal was mounted by the method of Teng (1990), using a 60 μm diameter wire loop. Because it was difficult to capture the crystal straight from the drop with cryosolvent into the mounting loop, we first introduced the crystal into a glass capillary together with the cryosolvent. The wire loop was then held under the opening of the capillary, allowing the crystal to slide into place. Data were collected at 100 K up to 2.3 Å resolution (Table 1).

The change in space group compared to saccharide-free Con A strongly suggests that Man(3,6) is bound to the lectin in the crystal, because the *I*222 space group of saccharide-free Con A crystals involves the carbohydrate-binding site in crystal lattice contacts that are incompatible with the binding of a saccharide. Moreover, the space groups obtained upon co-crystallization of Man(3,6) with Con A have not been previously encountered for crystals of Con A free or in complex (Table 2). The MeαMan–Con A co-crystal structure adopts the $P2_12_12_1$ space group that allows for monosaccharide binding and that establishes new intermolecular lattice contacts involving the saccharide-binding residues. It is obvious from Table 2 that space-group changes accompany the crystallization of Con A with different saccharides.

Molecular replacement is under way with the tetramer of the MeαMan–Con A complex as search model. The solution conformations of the trimannose unit determined by proton nuclear resonance (Brisson & Carver, 1983; Taguchi *et al.*, 1995) will be tried as models for the saccharide. The binding

Table 2. *Space groups and unit cells known for crystals of Con A free or in complex*

Con A form	Space group	Unit-cell dimensions (Å)			Reference
		a	*b*	*c*	
Saccharide-free	$I222$	88.7	86.5	62.5	Greer *et al.* (1970)
MeαMan	$C222_1$	118.6	102.6	253.1	Hardman & Ainsworth (1976)
MeαGlc	$C222_1$	118.1	103.5	251.8	Becker *et al.* (1976)
MeαMan	$P2_12_12_1$	123.7	128.6	67.2	Derewenda *et al.* (1989)
MeαGlc	$I2_13$	167.8	167.8	167.8	Harrop *et al.* (1996)
MeαAra	$P2_122_1$	97.5	87.0	61.5	Kalb (Gilboa) *et al.* (1995)
Metal-free	$P2_12_12$	61.4	85.6	91.5	Jack *et al.* (1971)

mode of Man(3,6) and the accommodation of the saccharide by the lectin structure will contribute to the understanding of the mechanism of specific carbohydrate recognition by lectins.

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Note added in proof: a structure of the Con A–trimannoside complex at 2.3 Å resolution in a crystal form closely related to our form *B* has been reported independently by Naismith & Field (1996).

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