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Properties of a crystal of the complex of methyl α-D-arabinofuranoside with concanavalin A.

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Abstract

The complex of methyl α-D-arabinofuranoside with concanavalin A crystallizes in the orthorhombic space group $P2_122_1$ with cell dimensions $a = 97.5$, $b = 87.0$ and $c = 61.5$ Å. The asymmetric unit contains one dimer and the unit cell consists of two tetrahedral clusters of point-group symmetry 222. The crystals diffract to 2.0 Å resolution.

The structure of the complex of methyl α-D-mannopyranoside with concanavalin A has been described recently (Derewenda *et al.*, 1989; Naismith *et al.*, 1994). We report the preparation and the properties of a crystal of the complex of the five-membered-ring sugar, methyl α-D-arabinofuranoside, with this protein.

Concanavalin A is known to form precipitates with poly-D-arabinofuranosans and with poly-D-fructofuranosans (Goldstein, 1976). Evidence that the protein binds methyl α-D-arabinofuranoside comes from the observation that this saccharide causes dissolution of crystals of saccharide-free concanavalin A. Saccharide-free crystals of concanavalin A of space group *I*222 (Greer, Kaufman & Kalb, 1970) are perfectly stable in dilute salt as well as in solutions of saccharides that do not bind to the protein but they dissolve in contact with saccharides that do bind to the protein. Thus, the minimal concentration of a saccharide sufficient to dissolve these crystals can be used to estimate the affinity of that saccharide for concanavalin A. Results of such solubility experiments, summarized in Table 1, indicate that the affinity of methyl α-D-arabinofuranoside for the protein, expressed as the inverse of the dissociation constant, is roughly two orders of magnitude less than that of methyl α-D-mannopyranoside. This result was used to design crystallization experiments in which the concentration of methyl α-D-arabinofuranoside would be sufficient to achieve a high degree of saturation of the saccharide-binding sites.

Crystals of the methyl α-D-arabinofuranoside complex with concanavalin A were grown by equilibrating a 20 µl portion of an aqueous solution of the protein (7 m*M*), contained in a microdialysis cell, against a 1 ml portion of methyl α-D-arabinofuranoside (0.15 or 0.3 *M*) in 0.05 *M* PIPES buffer (pH 5.8) containing 0.1 *M* $NaNO_3$, 1 m*M* $MnCl_2$ and 1 m*M* $CaCl_2$. Orthorhombic crystals of maximum dimensions 3.0 × 1.0 × 0.4 mm grew in several days at either concentration of the saccharide.

Evidence that the new crystals are indeed a protein–saccharide complex comes from the observation that they do not survive removal of the saccharide. When a microdialysis cell containing crystals in equilibrium with the mother liquor from which they grew was allowed to equilibrate against the dilute salt solution described above, without added methyl α-D-arabinofuranoside, the crystals disintegrated within 24 h. Furthermore, a difference electron-density map based on phases from the correctly positioned saccharide-free model of the protein (PDB file 1CON, Bernstein *et al.*, 1977) and structure-factor amplitudes from the 2.7 Å resolution data set (see below for details of molecular-replacement analysis and data collection) has significant density in a region previously identified as the saccharide-binding site (Derewenda *et al.*, 1989) (see Fig. 1).

Table 1. *Relationship between affinity of a saccharide for concanavalin A and its effectiveness in dissolving saccharide-free crystals of the protein*

Saccharide	Minimal concentration to dissolve *I*222 crystal ($M \times 10^3$)	$K_{dissocn}$ ($M \times 10^3$)*
Methyl α-D-mannopyranoside	1.0	0.1
Methyl α-D-glucopyranoside	5.0	0.25
Methyl α-D-arabinofuranoside	200	10–20 (estimated)†
L-Fucose	Crystal is stable in 2 *M*	Does not bind

* Dissociation constants for the mannoside and glucoside complexes are from Yariv, Kalb & Levitzki, (1968) and Goldstein (1976).

† The method of estimating this dissociation constant is described in the text.

The new crystals diffract to approximately 2.0 Å resolution at room temperature in a conventional Cu $K\alpha$ X-ray beam. They were characterized by means of 3° unscreened and 9° screened precession photographs down the three major axes. The crystallographic parameters, based on direct measurement of the reciprocal-lattice spacings on the films, are presented in Table 2.

The space group of the new crystalline complex is $P2_122_1$ however, at low resolution, the reciprocal *b* axis shows a systematic absence of odd reflections. The first odd reflection along this axis is 0 13 0, just visible in an overexposed precession photograph (Fig. 2). This suggests that, in the *y* direction, the molecules are located at intervals of approximately one half the axial length. The similarity of the cell dimensions of the new crystal to those of saccharide-free (*I*222) and demetallized ($P2_122_1$) concanavalin A crystals (Table 2) further suggests that, here too, there are eight protein subunits in the unit cell. These conditions can be satisfied simultaneously if the protein is arranged as two tetrameric molecules of point symmetry 222 located near 0,0,0.25 and 0.5,0.5,0.75. This tetrahedral 222 cluster is the quaternary motif found in all known crystal forms of concanavalin A.

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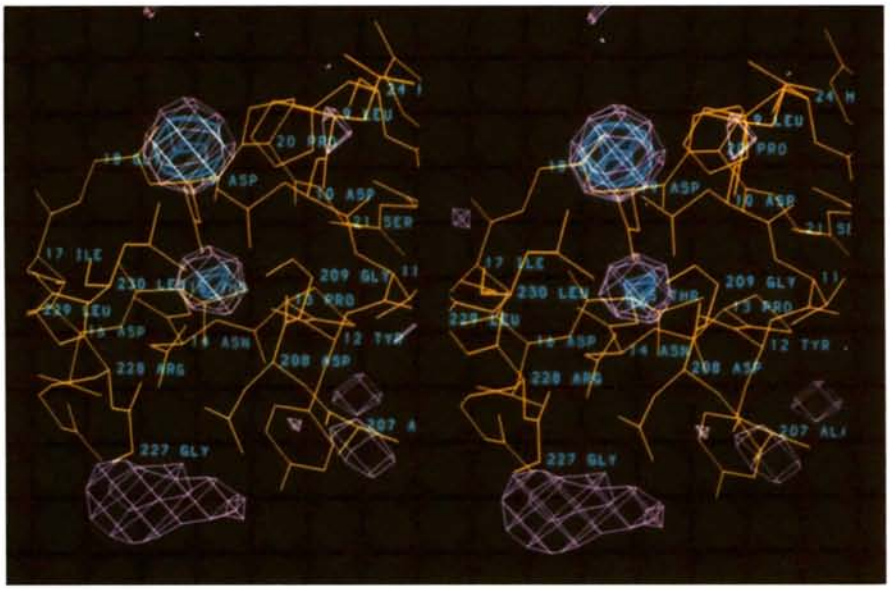

Fig. 1. Stereoscopic view of the difference electron-density map in the region of the metal-binding and saccharide-binding sites of concanavalin A. The map displays values greater than 3σ (pink) and values greater than 5σ (blue). Positive contours correspond to features of the structure not included in the model. The transition metal is the large, spherical feature at the top of the figure, the calcium ion is almost directly below and the saccharide site is the elongated feature at the bottom of the figure.

Table 2. *Crystal parameters of the complex of methyl α-D-arabinofuranoside with concanavalin A and comparison with saccharide-free and demetallized crystals*

		This complex	Saccharide-free*	Demetallized*
Cell dimensions (Å)	*a*	97.5	87.2	85.4
	b	87.0	89.2	91.5
	c	61.5	62.9	61.3
Space group		$P2_122_1$†	*I*222	$P2_122_1$†
Cell volume ($Å^3 \times 10^{-5}$)		5.22	4.89	4.79

* Crystal parameters for saccharide-free and demetallized concanavalin A are from Greer *et al.* (1970) and Jack *et al.* (1971).

† $P2_122_1$ is an unconventional setting of $P2_12_12$ (No. 18 in *International Tables for X-ray Crystallography*). The rationale for preferring this setting is given in the text. Equivalent positions for $P2_122_1$ are: x, y, z; $\frac{1}{2}-x, -y, \frac{1}{2}+z$; $-x, y, -z$; $\frac{1}{2}+x, -y, \frac{1}{2}-z$.

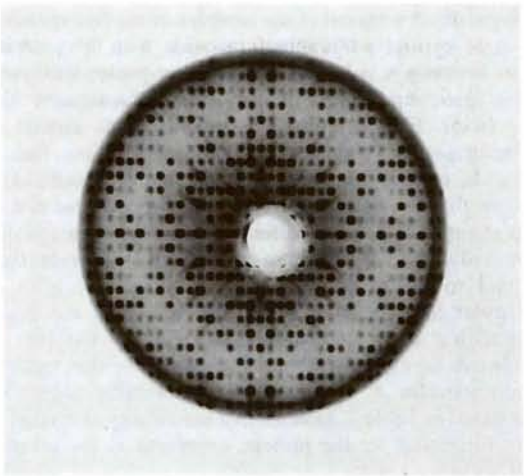

Fig. 2. 9° screened precession photograph of the 0*kl* zone of the complex of methyl α-D-arabinofuranoside with concanavalin A. Reciprocal *b* axis is horizontal. This photograph was purposely overexposed because it was suspected that an apparent screw axis might be a proper dyad. The weak 0 13 0 reflection shows that the *b* axis is actually a proper twofold. This assignment has been confirmed by the appearance in the 2.7 Å resolution data set of several additional reflections on the reciprocal *b* axis for which $k = 2n + 1$.

Molecular-replacement analysis by means of the general rotation function (Tong & Rossmann, 1990) as implemented in the program package *REPLACE* (Tong, 1993) confirms these predictions. A complete data set to 2.7 Å, collected with a Xentronics area detector at a Rigaku RU300 rotating anode (Cu $K\alpha$) and processed by the *XDS* suite (Kabsch, 1988) was used. A self-rotation search, using 1026 large terms (20% of independent reflections between 10 and 3.5 Å) and a radius of integration of 50 Å, found the non-crystallographic twofold of the concanavalin A dimer that constitutes the asymmetric unit to be parallel to the *xz* plane and rotated 7° from the direction of the *z* axis. The rotation function for this solution was 30% of the origin peak (3.5σ) and was three times as high as the next largest peak. Cross rotation using a dimer of saccharide-free concanavalin A (PDB file 1CON, Bernstein *et al.*, 1977) in a *P*2 cell ($a = b = c = 130$ Å, $\alpha = \beta = \gamma = 90°$) as model and 688 strong reflections (16% of all independent reflections between 10 and 4 Å resolution) gave an unequivocal solution (10σ) for Eulerian angles 0, 174, 88 (*zyz* convention) in agreement with the self-rotation function. A translation search, using the appropriately oriented dimer as model and 2380 strong reflections (55% of all independent reflections measured between 10 and 4 Å resolution) provided a clear cut solution corresponding to translation of $\frac{1}{4}$ along the *y* axis. The *R* factor for this model was 0.420 for all reflections ($F > 2\sigma$) between 8 and 2.7 Å resolution. Rigid-body refinement by means of *X-PLOR* (Brünger, Kuriyan & Karplus, 1992) against 13 817 reflections with $F > 2\sigma$ in the resolution range between 8 and 2.7 Å reduced the *R* factor to 0.345.

Accordingly, each of two tetrahedral clusters is located with one of its dyads coincident with a crystallographic twofold axis and with its other two dyads in the *xz* plane, rotated approximately 6° from the crystallographic axes. This arrangement is quite similar to that of the demetallized crystal of concanavalin A. Consequently, we have preserved the non-standard space-group assignment, $P2_122_1$, adopted previously for the demetallized form (Jack, Weinzierl & Kalb, 1971), in order to emphasize the relationship between the new crystal and the saccharide-free crystal, namely, that it is the *y* axis of the *I*222 crystal which remains twofold and that there is a small rotation of the tetrameric cluster about this axis.

Solution and refinement of the structure of this complex, now in progress, has required conformational analysis of methyl α-D-arabinofuranoside in order to select energetically favourable conformers as models for the saccharide, whose crystal structure is as yet unknown (*cf.* Furberg & Hammer, 1961). (See Fig. 3.) Solution of the structure of this complex should be relevant to the study of the large body of biological systems in which furanosides play a key role. Examples of such systems from the current literature are the β-fructofuranosidases of *Lolium rigidum* plants (Bonnet & Simpson, 1993), the fructosyl transferase of *Streptococcus salivarius* (Jacques, 1993) and the galactofuranosyl residue that has been identified as the antigenic determinant in the extracellular polysaccharides of *Penicillium* and *Aspergillus* moulds (van Bruggen-van der Lugt *et al.*, 1992).

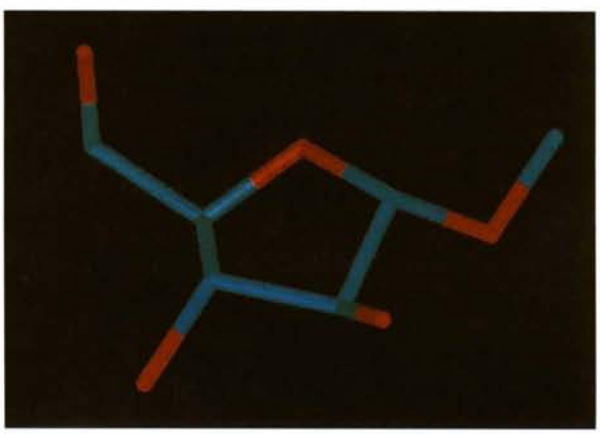

Fig. 3. A low-energy conformer of methyl α-D-arabinofuranoside.

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