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# A general method for co-crystallization of concanavalin A with carbohydrates

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A small grid of conditions has been developed for co-crystallization of the plant lectin concanavalin A (conA) and polysaccharides. Crystals have been obtained of complexes of conA with $\alpha$1-2 mannobiose, 1-methyl $\alpha$1-2 mannobiose, fructose, a trisaccharide and a pentasaccharide. The crystals diffract to resolutions of 1.75–2.7 Å using a copper rotating-anode source. The crystals are grown in the presence of polyethylene glycol 6K [10–20%(*w*/*v*)] at around pH 6.0. Optimization for each particular carbohydrate requires small adjustments in the conditions; however, all complexes give some crystalline precipitate in this limited grid. The $\alpha$1-2 mannobiose complex crystals diffract to 1.75 Å with space group *I*222 and cell dimensions $a$ = 91.7, $b$ = 86.8, $c$ = 66.6 Å. One monomer is present in the asymmetric unit. The 1-methyl $\alpha$1-2 mannobioside complex crystallizes in space group $P2_12_12_1$, cell dimensions $a$ = 119.7, $b$ = 119.7, $c$ = 68.9 Å and diffract to 2.75 Å. One tetramer is present in the asymmetric unit. Two crystal forms of the conA–fructose complex have been obtained. The first has space group $P2_12_12_1$, cell dimensions $a$ = 121.7, $b$ = 119.9, $c$ = 67.3 Å with a tetramer in the asymmetric unit and diffracts to 2.6 Å. The second crystallizes in space group $C222_1$, cell dimensions $a$ = 103.3, $b$ = 117.9, $c$ = 254.3 Å with two dimers in the asymmetric unit and diffracts to 2.42 Å. Structures and crystallization of the trisaccharide–conA and pentasaccharide–conA complexes have already been reported. In all complexes, the protein is found as a tetramer, although varying combinations of non-crystallographic and crystallographic symmetry are involved in generating the tetramer. The precise packing of the tetramer varies from crystal to crystal and it is likely that this variability facilitates crystallization.

## 1. Introduction

Protein–carbohydrate interactions are ubiquitous in biology and are important in inflammation, immune response and infection (Dwek, 1996; Ni & Tizard, 1996; Lowe & Ward, 1997). As such, these interactions are clear targets for therapeutic intervention. Current modelling approaches are unsatisfactory for protein–carbohydrate complexes. Understanding the basis of protein specificity for oligosaccharides not only has application in designing appropriate force fields for modelling, but is also of considerable intellectual interest. This knowledge can only be obtained by probing the contributions of surface area, hydrogen bonding, van der Waals interactions, sugar conformation and solvent reorganization to binding energy. This requires thermodynamic and structural characterization of a wide range of protein–carbohydrate complexes. Chemical synthesis of novel sugars serves to extend the range of carbohydrates beyond naturally occurring sugars and to test specific hypotheses. If crystallography is to provide valuable information, it must be able to do so quickly and reliably. We have chosen to work with the plant lectin, concanavalin A (conA). The protein has several advantages for a study of protein–carbohydrate interactions: it is readily available and possesses an increasingly well characterized specificity for oligosaccharides.

Although conA was crystallized in a form suited to X-ray diffraction in the 1970s (Hardman & Ainsworth, 1972) well diffracting crystals of conA in complex with a sugar proved elusive and were not reported until the conA–glucose complex in 1987 (Yariv *et al.*, 1987). This was followed in 1989 by the structure of conA complexed with $\alpha$-D-mannopyranoside (Derewenda *et al.*, 1989). These two complexes were obtained under very different crystallization conditions and the delay between native and complex crystallization reflects the difficulty in obtaining co-crystals. Soaking of the protein crystals in carbohydrate-containing solution results in crystal disruption. The mannose–conA complex unambiguously identified the binding site and

the interactions involved in monosaccharide recognition by the protein. This work has been developed by higher resolution studies of the mannose and glucose complexes (Naismith *et al.*, 1994; Harrop *et al.*, 1996). The lectin itself has now been studied at atomic resolution (Deacon *et al.*, 1997).

Whereas the monosaccharides (glucose and mannose) are bound weakly ($K_a$ = 0.82 × $10^4$ $M^{-1}$) by conA, oligosaccharides are bound more strongly ($K_a$ up to 1.41 × $10^6$ $M^{-1}$ for the pentasaccharide; Mandal *et al.*, 1994; Toone, 1994; Chervenak & Toone, 1995) and are specific to a particular lectin. Structures of the cognate trisaccharide and pentasaccharide complexes of conA have been reported (Loris *et al.*, 1996; Naismith & Field, 1996; Moothoo & Naismith, 1998). These studies highlighted the extended nature of the binding site and the role of a single structurally conserved water. The pentasaccharide structure showed that conA is capable of significant distortion of carbohydrate conformation. Relating these observations to the thermodynamic data obtained by calorimetry is the crux of developing a rational understanding of protein–carbohydrate interactions.

We now report a general method for obtaining carbohydrate complexes of conA and describe the preliminary X-ray characterization of these complexes. This method will allow rapid X-ray analysis and thus allow us to relate structural results to thermodynamic data.

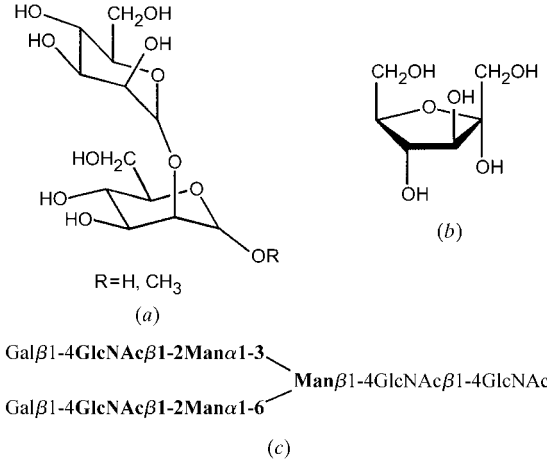


**Figure 1**
(*a*) α1-2 Mannobiose, R = H, Me. (*b*) Fructose. (*c*) The core of the N-linked glycan found on mammalian cell surfaces. The pentasaccharide is shown in bold.

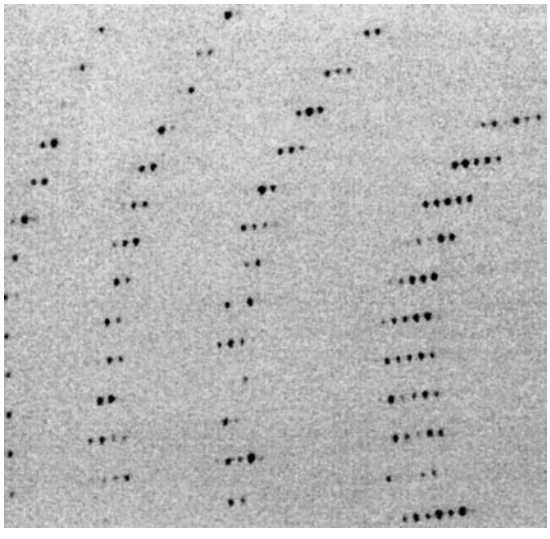

**Figure 2**
Section of a 0.8° oscillation diffraction pattern from a $C222_1$ crystal of the conA–fructose complex. Because of the long *c* cell edge (254.3 Å) the spots are close together.

## 2. Crystallization and data collection

Concanavalin A and fructose were obtained from Sigma (Poole, UK), α1-2 mannobiose was obtained from Dextra Laboratories (Reading, UK) and 1-methyl α1–2 mannobioside was synthesized at the University of St Andrews. The general protocol for crystallization and structure determination of the complexes follows.

Solutions were made up containing 0.6 m*M* conA, 18 m*M* oligosaccharide, 1 m*M* $MnCl_2$, 1 m*M* $CaCl_2$, 20 m*M* Tris pH 7.0 and 50 m*M* NaCl. An equal volume of this solution was mixed with the reservoir solution and the resulting mixture was equilibrated against the reservoir solution at 293.5 K.

The reservoir grid consists of 10, 15, 20 and 25% polyethylene glycol (PEG) 6K, pH 4.0, 5.0, 6.0, 7.0 and 8.0. This grid is repeated in the presence of 1.0 *M* LiCl. Optimization of PEG 6K concentration, pH and sugar concentration then follows. Data were collected on a Nonius DIP2000 from crystals sealed in glass capillaries with a small amount of the mother liquor. Data were processed and merged using *DENZO* and *SCALEPACK* (Otwinowski, 1993). Structures were determined using the molecular-replacement procedure *AMoRe* (Navaza, 1994) as implemented in the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994).

Details are given below for crystallization and structure determination for each complex currently being studied. In the absence of carbohydrate, native conA crystallizes under these conditions. The trimannoside and pentasaccharide complexes, which have already been published (Naismith & Field, 1996; Moothoo & Naismith, 1998), are not discussed here but were obtained in an analogous manner.

### 2.1. α1-2 Mannobiose

Crystals were obtained with a reservoir containing 10% PEG 6K and 0.1 *M* citric acid pH 5.0. Crystals which grew in one week were prone to cracking, whilst slower growing crystals, which took up to two weeks to grow, were less prone to cracking. Data were collected from a crystal of dimensions 1.2 × 1.0 × 0.8 mm which diffracted to a resolution of 1.75 Å. Data were recorded as 141 non-overlapping 25 min 0.75° oscillations. A second low-resolution data-collection pass enabled low-resolution data which was obscured by the backstop to be collected. The data were processed and merged in an *I*-centred orthorhombic lattice with unit-cell dimensions *a* = 91.7, *b* = 86.8, *c* = 66.6 Å and gave an $R_{\rm merge}$ of 6.1%, an average $I/\sigma$ of 11.3, a completeness of 95% and a multiplicity of 3.9. The highest resolution shell (1.81–1.75 Å) has an $R_{\rm merge}$ of 23.9%, a completeness of 80% and a multiplicity of 2.8. The asymmetric unit contains one monomer with a Matthews' number of 2.59 $Å^3$ $Da^{-1}$ (Matthews, 1968). Molecular-replacement calculations confirmed the space group as *I*222. In the first difference Fourier maps, electron density was visible for the carbohydrate. The structure is being refined to 1.75 Å; this, and a comparison to the atomic resolution native structure (Deacon *et al.*, 1997), will be reported elsewhere.

### 2.2. 1-Methyl α1-2 mannobioside

Crystals were obtained with a reservoir containing 13.5% PEG 6K, 1.0 *M* LiCl and 0.1 *M* Tris pH 7.0. The crystals were elongated rods and varied considerably in size. Some crystals grew as hollow rods. The crystals took 2–4 weeks to grow and a crystal with dimensions 0.5 × 0.2 × 0.2 mm was used for X-ray analysis. Data to 2.75 Å were recorded as 190 non-overlapping 32 min 0.8° oscillations. Autoindexing with *DENZO* suggested two different lattice types: primitive tetragonal and primitive orthorhombic. Data were integrated in both lattices.

Frames 1–30 showed a high $R$ factor and low $\langle I/\sigma \rangle$ compared with the remaining data. Inspection of the images showed that the cell contracted ~1 Å along the $c$ dimension during the first 30° of data collection, after which the cell ceased to contract. The diffraction intensity increased as the cell decreased. Frames 1–30 were excluded from all further analysis. Merging the data in Laue groups 4/$m$, 4/$mmm$ and 2/$mmm$ gave $R_{\rm merge}$ values of 47.4, 50.8 and 10.1%, respectively. The space group was assigned as $P2_12_12_1$ (on the basis of systematic absences) with unit-cell parameters $a$ = 119.7, $b$ = 119.7, $c$ = 68.9 Å to give an $R_{\rm merge}$ of 10.1%, an average $I/\sigma$ of 8.9, a multiplicity of 3.29 and a completeness of 99.7%. The highest resolution shell (2.85–2.75 Å) has an $R_{\rm merge}$ of 29.0%, 99.5% completeness and a multiplicity of 4.9. The asymmetric unit contains one tetramer and approximately 49% solvent. Molecular-replacement calculations confirm the space group and density for the sugar is visible in preliminary maps. Refinement of the complex is proceeding and will be reported elsewhere.

### 2.3. Fructose

Crystals were obtained from a protein solution containing 30 m$M$ fructose equilibrated against a reservoir containing 20% PEG 6K and 0.1 $M$ HEPES pH 6.5. Data were collected from one crystal of dimensions 0.5 × 0.3 × 0.2 mm as 90 non-overlapping 25 min 1.0° oscillations to a resolution of 2.6 Å. Autoindexing suggested a primitive orthorhombic lattice. On the basis of systematic absences the space group was assigned as $P2_12_12_1$, with cell dimensions $a$ = 121.7, $b$ = 119.9 and $c$ = 67.3 Å. Merging the data gave an $R_{\rm merge}$ of 11%, a completeness of 83.7%, an average $I/\sigma$ of 7.1 and a multiplicity of 3.7. The highest resolution shell (2.69–2.60 Å) has an $R_{\rm merge}$ of 29.1%, a completeness of 62.8% and a multiplicity of 2.3. The asymmetric unit contains one tetramer with a Matthews number of 2.41 Å$^3$ Da$^{-1}$ (Matthews, 1968). Molecular-replacement calculations confirm the space group. Electron density is visible for the sugar in one subunit. Refinement is proceeding and details will be reported elsewhere.

Crystals of a second conA–fructose complex were obtained from a protein solution containing 40 m$M$ fructose equilibrated against 10% PEG 6K and 0.1 $M$ HEPES pH 7.0 in a hanging drop. Large crystals (0.8 mm$^3$) were obtained after 2 weeks and diffracted to 2.42 Å. Data were collected as 135 non-overlapping 20 min 0.8° oscillations. Fig. 2 shows one 0.8° oscillation image of this data. Autoindexing with this data suggested a $C$ orthorhombic lattice with unit-cell parameters $a$ = 103.3, $b$ = 117.9, $c$ = 254.3 Å. The data merged with an $R_{\rm merge}$ of 8.1%, a completeness of 97.2%, an average $I/\sigma$ of 8.3 and a multiplicity of 4.7. The highest resolution shell (2.51–2.42 Å) has an $R_{\rm merge}$ of 27.5%, a completeness of 87% and a multiplicity of 2.9. The asymmetric unit contains two dimers, approximately 67% solvent. Molecular-replacement calculations using a dimer as the search model gave two solutions in space group $C222_1$. Electron density is visible for the saccharide in the binding sites. Refinement of this structure is ongoing and will be reported elsewhere.

## 3. Discussion

A general protocol involving optimization of a narrow range of parameters for the crystallization of conA–saccharide complexes has been used to obtain several structures of these complexes. The protein is consistently found as a tetramer. The sugar always binds at a region of crystal contacts and leads to a variation in the crystal packing. We hypothesize that it is this flexibility which leads to the ease of crystallization of conA with oligosaccharides of varying sizes. The combination of current thermodynamic studies with the information gained from the rapid structure determination of these complexes provide a powerful insight into the factors which govern protein–carbohydrate interactions.

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