Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

The crystals of a mannose-specific jacalin-related lectin from *Morus nigra* are merohedrally twinned

A. Rabijns,[a] C. Verboven,[a] H. Novoa de Armas,[a] E. J. M. Van Damme,[b] W. J. Peumans[b] and C. J. De Ranter[a]*

[a]Laboratorium voor Analytische Chemie en Medicinale Fysicochemie, Faculteit Farmaceutische Wetenschappen, K. U. Leuven, Van Evenstraat 4, B-3000 Leuven, Belgium, and [b]Laboratorium voor Fytopathologie en Plantenbescherming, Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, K. U. Leuven, W. de Croylaan 42, B-3001 Leuven, Belgium

Correspondence e-mail: camiel.deranter@farm.kuleuven.ac.be

Received 21 August 2000
Accepted 31 January 2001

MornigaM, a lectin from *Morus nigra*, belongs to the mannose-binding subgroup of the family of jacalin-related plant lectins. It was crystallized in the $P6_5$ space group, with unit-cell parameters $a = b = 110.74$, $c = 159.28$ Å. The partially merohedrally twinned crystals could be detwinned and a subsequent molecular-replacement solution could be found using the coordinates of jacalin. Preliminary analysis clearly shows the tetrameric assembly of this protein. Furthermore, data from MornigaM crystals soaked in a mannose solution were collected.

1. Introduction

Seven families of structurally and evolutionary related carbohydrate-binding proteins (lectins) have been identified in plants (Van Damme *et al.*, 1998). Detailed structural analysis of members of the legume lectins, chitin-binding lectins, monocot mannose-binding lectins, amaranthins, type 2 ribosome-inactivating proteins and jacalin-related lectins demonstrated that higher plants have developed at least six structurally different carbohydrate-binding motifs (Peumans, Barre *et al.*, 2000).

Interestingly, some simple mono- or oligosaccharides are recognized by multiple binding motifs, *e.g.* mannose-binding lectins are found within the families of the legume lectins, the monocot mannose-binding lectins and the jacalin-related lectins. The identification of mannose-binding proteins within the family of jacalin-related lectins was surprising because jacalin and the closely related *Maclura pomifera* agglutinin clearly exhibit specificity towards galactose and according to structural studies possess a binding site that preferentially accommodates the T-antigen disaccharide (Galβ1-3GalNAc). Based on this apparent difference in specificity and based on differences in molecular structure, biosynthesis and sub-cellular location, the jacalin-related lectins are subdivided into two different subgroups. The so-called galactose-specific subgroup (containing, for example, jacalin and *Maclura pomifera* agglutinin) exhibit specificity towards galactose and are built up of four protomers consisting of a short β-chain and a long α-chain. Lectins of the mannose-specific subgroup (*e.g. Helianthus tuberosus* lectin, *Calystegia sepium* agglutinin, banana lectin, rice lectin, KM+ from jack-fruit seeds) exhibit an exclusive specificity towards mannose and are built up of two, four or eight protomers consisting of a single polypeptide chain (Bourne *et al.*, 1999). Worthwhile mentioning is that the mannose-specific jacalin-related lectins are cytoplasmic proteins, whereas their galactose-specific counterparts are located in the storage vacuoles (Peumans, Hause *et al.*, 2000).

Bark tissue of *M. nigra* (mulberry) contains reasonable quantities of a mixture of two different jacalin-related lectins exhibiting different specificity towards galactose and mannose (Van Damme *et al.*, 1998). According to their specificity, these lectins have been named *M. nigra* galactose-specific agglutinin (MornigaG) and *M. nigra* mannose-specific agglutinin (MornigaM). Both lectins are very potent agglutinins and exert different biological activities on animal and human cells, the most important being a stimulatory effect on immunocompetent cells. Though MornigaG and MornigaM have a very similar molecular structure and share a high sequence similarity, they definitely differ with respect to their specificity. To explain why a similar site accommodates either galactose or mannose, a structural analysis is required at the atomic level of both lectins and of their respective carbohydrate-binding sites. Since *M. nigra* is one of the few plants in which two closely related lectins from the jacalin family with a different specificity occur simultaneously, the above-mentioned lectins are an ideal study object for this structural comparison. Here, we describe the crystallization and the preliminary X-ray analysis of MornigaM, as well as the preliminary structural analysis of MornigaM crystals soaked with mannose.

2. Experiments and results

2.1. Purification

MornigaM was purified from *M. nigra* bark using affinity chromatography on immobilized

Table 1
Data-collection and reduction statistics.

Values in parentheses indicate data in the highest resolution shell.

	Unbound MornigaM	MornigaM–mannose
Space group	$P6_5$	$P6_5$
Unit-cell parameters (Å)	$a = b = 110.74$, $c = 159.28$	
Resolution limit (Å)	1.8 (1.83–1.80)	2.0 (2.03–2.00)
Total observations	250868	211071
Unique reflections	100939 (5072)	74409 (3763)
Completeness (%)		
All data	98.4 (99.7)	99.1 (100.0)
Data with $I > 2\sigma(I)$	90.6 (70.4)	89.8 (71.1)
Mean $I/\sigma(I)$	20.84 (4.96)	21.31 (4.92)
R_{sym} value (%)	4.2 (16.0)	4.5 (18.9)

mannose following the procedure described for the isolation of the lectin from *Calystegia sepium* rhizomes (Peumans *et al.*, 1997). The lectin was dissolved in a solution of 0.1 *M* sodium acetate buffer at pH 5.0 to a final concentration of 22 mg ml^{-1}.

2.2. Crystallization, data collection and processing

To determine initial crystallization conditions, Hampton Crystal Screen I (Jancarik & Kim, 1991) and a screening of ammonium sulfate at different concentrations and different pH values was applied to a sample of MornigaM. The protein sample used in these experiments was obtained by diluting the original sample with 50 m*M* Tris buffer pH 8.0 to a final concentration of 5 mg ml^{-1}.

Crystallization was carried out by the hanging-drop vapour-diffusion technique at 277 K using Linbro multi-well tissue plates. Each well was filled with 700 µl of reservoir solution; drops consisting of 1.5 µl protein solution and 1.5 µl of reservoir solution were placed on coverslips and set to equilibrate against these reservoir solutions.

Good quality crystals (Fig. 1) could be grown from a solution containing 55% saturated ammonium sulfate and 0.1 *M* imidazole buffer pH 7.0. Crystals of the complex between MornigaM and mannose were subsequently prepared by soaking an uncomplexed MornigaM crystal with a 50 m*M* mannose solution for approximately 48 h.

Diffraction data on the free lectin crystals as well as on the mannose-bound form were collected at 100 K using synchrotron radiation (X11 beamline, DESY, Germany) after soaking the crystal for 30 s in a solution of the mother liquor containing 25% glycerol. The crystals grow in space group $P6_5$, with unit-cell parameters $a = b = 110.74$, $c = 159.28$ Å. All data processing was performed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The final data set for the unbound crystals is 98.4% complete to 1.80 Å and is characterized by an R_{sym} of 4.2%, whereas the mannose-bound crystals have an R_{sym} of 4.5% with a completeness of 99.1% to 2.0 Å. Further statistics are summarized in Table 1.

2.3. Twinning

The first indications for the presence of merohedrally twinned crystals of MornigaM were obtained when the diffraction data for certain crystals could be reduced in the $P6_522$ space group with only moderately higher R_{sym} values (ranging from 14 to 26%) and when statistical analysis of the intensity data in *XPREP* (Bruker Analytical X-ray Systems) consistently showed low $\langle|E^2 - 1|\rangle$ values. Furthermore, all efforts to find a clear molecular-replacement solution in space group $P6_5$ using the structure of jacalin (PDB code 1jac) failed, indicating a problem in the data. Checking of the diffraction data at the twin server (Yeates, 1997) showed that the crystals were indeed partially merohedrally twinned with variable twin fractions (ranging from 14 to 35%). The twin fractions for the above-described data sets were 14% for the unbound crystal and 18% for the mannose-bound crystal. Subsequently, both data sets could be detwinned using the algorithm described by Yeates (1997), yielding a data set in which all reflections had their twin component removed.

2.4. Molecular replacement

The phase problem for the MornigaM structure was solved with the molecular-replacement technique in *X-PLOR* (Brünger *et al.*, 1987) using the detwinned data in space group $P6_5$. When the original twinned data were used in a similar molecular-replacement search, the high noise level hampered an unambiguous identification of the correct peaks. Using the coordinates of half a jacalin molecule (chains *A* and *C*) as a search model (Sankaranarayanan *et al.*, 1996; PDB code 1jac) with the detwinned data, four peaks were found which stood out above the mean. Two of these peaks corresponded to the two dimers, together composing a tetramer. Owing to the internal rotational symmetry of the dimers, the other two peaks are symmetry-related (in a purely rotational way) to the first two peaks. Combining the two dimers into a tetramer gave an *R* factor of 46% (R_{free} = 47%) after initial rigid-body refinement to 3 Å in *X-PLOR* (Brünger *et al.*, 1987). Subsequent conjugate-gradient minimization to 2 Å lowered the *R* factor to 39.7% and the R_{free} value to 45.6%; an initial run of simulated annealing further decreased these values to 36.5 and 41.4%, respectively. A correct molecular-replacement solution could also be found using a jacalin monomer or tetramer as a search model. However, we opted to use half a tetramer since the original coordinates of 1jac consisted of two half tetramers.

Initial electron-density interpretation showed some extra density in the sugar-binding site for the mannose-soaked crystals, indicating that the mannose molecule was indeed bound. Further cycles of refinement will be performed in the program *SHELXL*-97 (Sheldrick, 1990), as this program allows the refinement of the twin fraction.

Hitherto, the structures of only three members of the jacalin family have been determined: jacalin (galactose-specific; Sankaranarayanan *et al.*, 1996), *Maclura pomifera* agglutinin (galactose-specific; Lee *et al.*, 1998) and *Helianthus tuberosus* lectin (mannose-specific; Bourne *et al.*, 1999). The two galactose-specific lectins form a tetramer, whereas the only mannose-specific lectin analysed (from *H. tuberosus*) forms a doughnut-shaped octameric assembly. Hence, the structure of MornigaM is the first known mannose-specific jacalin-related lectin in a tetrameric assembly.

In conclusion, diffraction-quality crystals of MornigaM were obtained. Quality data sets were collected for both the uncomplexed lectin and the mannose-bound form, which will allow a successful structure determination. Preliminary analysis already shows that this lectin forms a tetrameric assembly, which until now has been unique to the mannose-specific jacalin-related lectins.

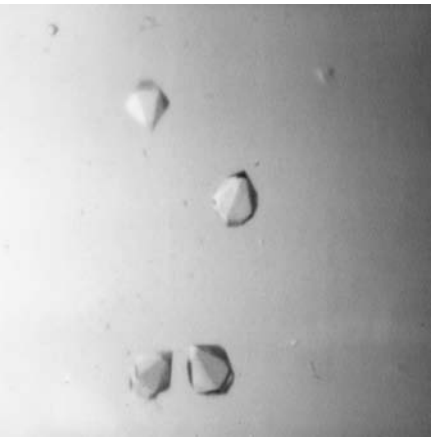

Figure 1
Crystals from MornigaM. Typical dimensions of the crystals are 0.3 × 0.2 × 0.2 mm.

AR and EJMVD are Postdoctoral Research Fellows of the Fund for Scientific Research-Flanders (Belgium) and WJP is a Research Director of this fund. CV is a Postdoctoral Research Fellow of the K. U. Leuven Research Fund (OT/98/38) and HNA is supported by an K. U. Leuven IRO fellowship. Walter Jottier is acknowledged for technical assistance during in-house data collection. We thank the beamline scientists at DESY for technical support and the European Union for support of the work at EMBL Hamburg through the HCMP to Large Installations Project, contract No. CHGE-CT93-0040. This work was supported in part by grants from the Katholieke Universiteit Leuven (OT/98/17), and the Fund for Scientific Research-Flanders (grant G.0223.97).

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