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Cloning, purification, crystallization and preliminary crystallographic studies of *Bradyrhizobium fucosyltransferase* NodZ

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The α-1,6-fucosyltransferase NodZ from *Bradyrhizobium* sp. WM9 (*Lupinus*), composed of 325 amino acids with a molecular weight of 37 kDa, has been cloned, expressed and purified. Protein crystals suitable for X-ray diffraction were obtained under optimized crystallization conditions using ammonium dihydrogen phosphate as a precipitant. The crystals are hexagonal and belong to space group $P6_122$ or $P6_522$, with unit-cell parameters a = 125.5, c = 95.6 Å, and contain 56.8% solvent and a single protein molecule in the asymmetric unit. Native data were collected to 2.85 Å using synchrotron radiation and cryogenic conditions. The native crystals were soaked in a mother-liquor solution containing 2.5 m*M* $[Ta_6Br_{12}]^{2+}$ cluster for derivatization and SAD data were collected to 3.4 Å at the tantalum L_{III} absorption peak.

1. Introduction

Symbiosis between legumes and *Rhizobium* bacteria depends on the exchange of specific molecular signals (Denarie *et al.*, 1992; Fisher & Long, 1992). This process results in the formation of root nodules in which the bacteria fix atmospheric nitrogen (Long, 1989). In the initial phases of nodulation, the host plant secretes flavonoids and the activation of bacterial *nod* genes is induced. The *nod* genes are involved in the synthesis of lipochitin oligosaccharides (LCO), called Nod factors, which are necessary for the infection of root hair by the symbiotic bacteria. Additionally, bacterial signals are also necessary for the development of infection (Spaink, 2000). The Nod factors are composed of a chitin backbone formed by a combination of 3–6 β-1,4-linked *N*-acetyl-D-glucosamine (GlcNAc) residues, N-acetylated at the non-reducing end and O-acetylated at the other residues (Fisher & Long, 1992; Lerouge *et al.*, 1990; Price *et al.*, 1992). The differences between Nod factors are based on the number of GlcNAc residues, the nature of the acyl chain and the presence or absence of additional substituents, and determine the host specificity of the bacterium. The reducing GlcNAc residue may be substituted with a sulfate, acetate or with saccharide residues such as D-arabinose or L-fucose. The additional saccharide unit may be also substituted with a methyl group, acetyl or sulfate group (Carlson *et al.*, 1994; Denarie *et al.*, 1992). L-Fucose is frequently observed at C6 of the reducing GlcNAc residue as a Nod-factor substituent and may play a role in host specificity and/or protection of the Nod factors against degradation (Bras *et al.*, 2000; D'Haeze *et al.*, 2000; Ovtsyna *et al.*, 2000). Attachment of L-fucose to Nod factors is encoded by *nodZ* genes in many rhizobia (Mergaert *et al.*, 1996; Quesada-Vincens *et al.*, 1997; Quinto *et al.*, 1997; Stacey *et al.*, 1994). The NodZ protein catalyzes α-1,6-fucosylation of the chitin oligosaccharide core.

Generally, fucosyltransferases catalyze the transfer of fucose from GDP-fucose to various oligosaccharide-acceptor substrates. This class of enzymes is involved in the synthesis of biologically important oligosaccharides in eukaryotes and prokaryotes (Costache *et al.*, 1997; Quesada-Vincens *et al.*, 1997). All fucosyltransferases transfer fucose either in an α-1,2-linkage to a galactose residue or in an α-1,3-, α-1,4- or α-1,6-linkage to an *N*-acetylglucosamine residue.

Eukaryotic fucosyltransferases are membrane proteins that share topology with other Golgi-resident glycosyltransferases (Paulson & Colley, 1989). In contrast, bacterial transferases lack the transmembrane domain. There is no sequence identity to other glycosyltransferases, but a highly conserved motif is present in the catalytic domain of all prokaryotic and eukaryotic α-1,2- and α-1,6-fucosyltransferases (Breton *et al.*, 1998). This motif is absent in α-1,3-fucosyltransferases, which form a distinct family (Breton *et al.*, 1996, 1998; Oriol *et al.*, 1999). There is currently no structural information for fucosyltransferases and the detailed mechanism of action is not clear.

2. Methods

2.1. Cloning

The coding seqence of the *nodZ* gene was amplified by PCR from genomic DNA of

Bradyrhizobium sp. WM9 (*Lupinus*). A C-terminal His_6-tag sequence was introduced by a reverse primer and the tagged gene was cloned into *Nde*I/*Bam*HI-digested pET3a expression vector. The recombinant plasmid was sequenced to confirm the correct sequence of the insert and the construct was used to transform the BL21-CodonPlus(DE3)-RP strain of *Escherichia coli*.

2.2. Expression and purification

25 ml of TB medium containing 34 µg ml^{-1} chloramphenicol and 100 µg ml^{-1} ampicillin was inoculated with a single colony and grown overnight at 310 K. The overnight culture was used for inoculation of 2.5 l of TB medium with appropiate antibiotics and grown to an OD_{600} of 2.0. The temperature was decreased to 298 K and protein expression was induced by isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.2 m*M*. The cells were harvested 16 h after induction. The cell pellet was resuspended in lysis buffer (5 m*M* imidazole, 500 m*M* NaCl, 20 m*M* Tris–HCl pH 7.9) with addition of protease inhibitors (1 m*M* PMSF and 5 m*M* β-mercaptoethanol). Cells were disrupted by sonication on ice and centrifuged to remove cell debris. The supernatant was loaded onto an $NiSO_4$-charged HiTrap column and the protein was eluted with an imidazole gradient (0.1–1 *M* in 500 m*M* NaCl, 20 m*M* Tris–HCl pH 7.9). SDS–PAGE confirmed the size of the expressed protein (about 38 kDa including the His-tag sequence). The protein solution was dialized against buffer *A* (20 m*M* Tris–HCl pH 8.0, 10 m*M* β-mercaptoethanol, 5% glycerol) and concentrated to 4 ml. Size-exclusion chromatography was applied as the second step of purification. The protein solution was loaded onto Sephacryl S300 High Resolution (Pharmacia) gel-filtration column previously equilibrated with buffer *B* (50 m*M* NaCl, 20 m*M* Tris–HCl pH 8.0, 10 m*M* β-mercaptoethanol, 5% glycerol). The protein was eluted with buffer *B*. Fractions containing NodZ protein were dialyzed against buffer *A* and concentrated to 7 mg ml^{-1} using Amicon Ultra 30 filters. The protein concentration was estimated using the Bradford method (Bradford, 1976).

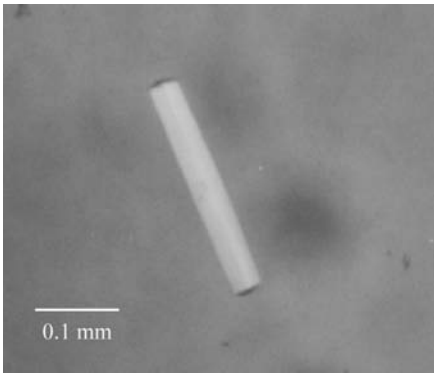

Figure 1
A single crystal of native α-1,6-fucosyltransferase NodZ (approximate dimensions 0.05 × 0.05 × 0.3 mm).

Table 1
X-ray data-collection details and processing statistics for native and anomalous data sets.

Values in parentheses correspond to the last resolution shell.

	Native	$[Ta_6Br_{12}]^{2+}$ derivative
Space group	$P6_122$ or $P6_522$	$P6_122$ or $P6_522$
Unit-cell parameters (Å)	a = 125.5, c = 95.6	a = 124.1, c = 95.2
Temperature (K)	100	100
Mosaicity (°)	0.46	0.17
Wavelength (Å)	1.2533	1.2533
Resolution limits (Å)	30.0–2.85 (2.95–2.85)	25.0–3.4 (3.52–3.40)
No. observations	259535	112864
Symmetry for merging	6/*mmm*	622
No. unique reflections	10849	11293
Redundancy	23.9	10.0
Completeness (%)	100 (100)	100 (100)
$\langle I/\sigma(I)\rangle$	34.9 (6.5)	11.2 (5.1)
R_{int}†	0.081 (0.502)	0.098 (0.436)

† $R_{int} = \sum_h \sum_j |I_{hj} - \langle I_h\rangle| / \sum_h \sum_j I_{hj}$, where I_{hj} is the intensity of observation j of reflection h.

2.3. Crystallization

Initial screening for crystallization conditions was performed according to the sparse-matrix method (Jancarik & Kim, 1991) using Structure Screen 1 from Molecular Dimensions. Initial crystals were obtained from 1 *M* ammonium dihydrogen phosphate and 0.1 *M* sodium citrate pH 5.6. The conditions were refined by adjusting the concentration of ammonium dihydrogen phosphate and the pH. The crystals were grown using the hanging-drop vapour-diffusion technique at 293 K. Drops containing 2 µl of the protein solution (7 mg ml^{-1}) in buffer *A* and 2 µl of the precipitant solution were equilibrated against 1.0 ml of the same precipitant solution.

2.4. Data collection and processing

A single crystal measuring 0.05 × 0.05 × 0.3 mm was cryoprotected by a quick soak (5 s) in reservoir solution supplemented with 30%(*v*/*v*) glycerol, mounted in a nylon loop and vitrified at 100 K in a stream of cold N_2 gas. Diffraction data consisting of 120 images with 1° oscillation and extending to 2.85 Å resolution were collected using synchrotron radiation (EMBL c/o DESY, Hamburg, beamline BW7A) and a MAR CCD 165 mm detector. Indexing and integration of all images was performed in *DENZO* and scaling of the intensity data in *SCALEPACK*, both from the *HKL* program package (Otwinowski & Minor, 1997). The final native data set is 100% complete and is characterized by R_{int} = 0.081 and $\langle I/\sigma(I)\rangle$ = 34.9 (Table 1). The crystals were derivatized by soaking for 24 h in mother liquor containing 2.5 m*M* $[Ta_6Br_{12}]Br_2$ complex (Banumathi *et al.*, 2003). Preliminary diffraction data for this derivative were collected analogously to the native data set using radiation with λ = 1.2533 Å at the white line of the L_{III} absorption peak for tantalum (as determined from a fluorescence scan). The derivative data set extends to 3.4 Å resolution and is characterized by R_{int} = 0.098 and $\langle I/\sigma(I)\rangle$ = 11.2. The presence of anomalous signal was confirmed using *SCALEPACK* (Otwinowski & Minor, 1997). Table 1 summarizes the data-collection and processing statistics.

3. Results and discussion

The expression and purification protocol allowed us to obtain a pure protein preparation. Typical yields were 10 mg of homogeneous NodZ protein from 2.5 l of culture. The effects of precipitant concentration and pH have been investigated for crystallization optimization. Crystals grew in the range 0.3–1 *M* ammonium dihydrogen phosphate. The best crystals were obtained from 350 m*M* ammonium dihydrogen phosphate and 100 m*M* Tris–HCl pH 7.5 at a protein concentration of 7 mg ml^{-1}. Under the optimized conditions, the crystals grew within 10 d (Fig. 1). The use of sodium citrate pH 5.6 as a buffer also produced crystals, but they only diffracted to 3.5 Å resolution.

The crystals belong to the hexagonal space group $P6_122$ (or $P6_522$), with unit-cell parameters $a = 125.5$, $c = 95.6$ Å. Analysis of the Matthews volume (Matthews, 1968) for the native protein crystals indicates the presence of one molecule in the asymmetric unit (V_M = 2.9 Å^3 Da^{-1}, solvent content 56.8%). For derivatization experiments, the $[Ta_6Br_{12}]Br_2$ complex was used. The soaking improved the mosaicity of the crystals (from about 0.5 to 0.2°), but reduced the diffraction limit from 2.8 to 3.4 Å. Analysis of the SAD data set collected at the high-energy slope of the tantalum L_{III} absorption peak in *SCALEPACK* (Otwinowski & Minor, 1997) indicates the presence of the anomalous signal to 3.4 Å resolution (overall and last resolution shell R_{int} values were 0.154 and 0.461 for 6/*mmm* scaling, and 0.098 and 0.436 for 622 scaling, respectively). An anomalous difference Patterson map analyzed in *SOLVE* (Terwilliger & Berendzen, 1999) is consistent with five anomalous sites.

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References

Banumathi, S., Dauter, M. & Dauter, Z. (2003). *Acta Cryst.* D**59**, 492–498.

Bradford, M. M. (1976). *Anal. Biochem.* **72**, 248–254.

Bras, C. P., Jorda, M. A., Wijfjes, A. H., Harteveld, M., Stuurman, N., Thomas-Oates, J. E. & Spaink, H. P. (2000). *Mol. Plant-Microbe Interact.* **13**, 475–479.

Breton, C., Oriol, R. & Imberty, A. (1996). *Glycobiology*, **6**, vii–xii.

Breton, C., Oriol, R. & Imberty, A. (1998). *Glycobiology*, **8**, 87–94.

Carlson, R. W., Price, N. P. J. & Stacey, G. (1994). *Mol. Plant Microbe Interact.* **7**, 684–695.

Costache, M., Cailleau, A., Fernandez-Mateos, P., Oriol, R. & Mollicone, R. (1997). *Transfus. Clin. Biol.* **4**, 367–382.

Denarie, J., Debelle, F. & Rosenberg, C. (1992). *Annu. Rev. Microbiol.* **46**, 497–531.

D'Haeze, W., Mergaert, P., Promé, J.-C. & Holsters, M. (2000). *J. Biol. Chem.* **275**, 15676–15684.

Fisher, R. W. & Long, S. R. (1992). *Nature (London)*, **357**, 655–660.

Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.

Lerouge, P., Roche, P., Faucher, C., Maillet, F., Truchet, G., Prome, J.-C. & Denarie, J. (1990). *Nature (London)*, **334**, 781–784.

Long, S. R. (1989). *Cell*, **56**, 203–214.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Mergaert, P., D'Haeze, W., Fernandez-Lopez, M., Geelen, D., Goethals, K., Promé, J.-C., van Montagu, M. & Holsters, M. (1996). *Mol. Microbiol.* **21**, 409–419.

Oriol, R., Mollicone, R., Cailleau, A., Balanzino, L. & Breton, C. (1999). *Glycobiology*, **9**, 233–334.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Ovtsyna, A. O., Schultze, M., Tikhonovich, I. A., Spaink, H. P., Kondorosi, E., Kondorosi, A. & Staehelin, C. (2000). *Mol. Plant Microbe Interact.* **13**, 799–807.

Paulson, J. C. & Colley, K. J. (1989). *J. Biol. Chem.* **264**, 17614–17618.

Price, N. P. J., Relic, B., Talmont, F., Lewin, A., Prome, D., Pueppke, S. G., Maillet, F., Denarie, J., Prome, J.-C. & Broughton, W. J. (1992). *Mol. Microbiol.* **6**, 3575–3584.

Quesada-Vincens, D., Fellay, R., Nasim, T., Viprey, V., Burger, U., Promé, J.-C., Broughton, W. J. & Jabbouri, S. (1997). *J. Bacteriol.* **179**, 5087–5093.

Quinto, C., Wijfjes, A. H. M., Bloemberg, G. V., Blok-Tip, L., López-Lara, I. M., Lugtenberg, B. J. J., Thomas-Oates, J. E. & Spaink, H. P. (1997). *Proc. Natl Acad. Sci. USA*, **94**, 4336–4341.

Spaink, H. P. (2000). *Annu. Rev. Microbiol.* **54**, 257–288.

Stacey, G., Luka, S., Sanjuan, J., Banfalvi, Z., Nieuwkoop, A. J., Chun, J. Y., Forsberg, L. S. & Carlson, R. (1994). *J. Bacteriol.* **176**, 620–633.

Terwilliger, T. C. & Berendzen, J. (1999). *Acta Cryst.* D**55**, 849–861.