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Crystallization and X-ray diffraction analysis of chondroitin lyase from baculovirus: envelope protein ODV-E66

Baculovirus envelope protein ODV-E66 (67–704), in which the N-terminal 66 amino acids are truncated, is a chondroitin lyase. It digests chondroitin and chondroitin 6-sulfate efficiently, but does not digest chondroitin 4-sulfate. This unique characteristic is useful for the preparation of specific chondroitin oligosaccharides and for investigation of the mechanism of baculovirus infection. ODV-E66 (67–704) was crystallized; the crystal diffracted to 1.8 Å resolution and belonged to space group $P6_2$ or $P6_4$, with unit-cell parameters $a = b = 113.5$, $c = 101.5$ Å. One molecule is assumed to be present per asymmetric unit, which gives a Matthews coefficient of $2.54 \text{ Å}^3 \text{ Da}^{-1}$.

1. Introduction

Chondroitin is a linear polysaccharide which consists of repeating [–4 D-glucuronic acid (GlcUA) β 1–3 N-acetyl-D-galactosamine (GalNAc) β 1–]_n disaccharide units (Esko *et al.*, 2009; Habuchi, 2000). In many cases chondroitin exists as chondroitin sulfate, which is modified with a sulfate group at different positions. Chondroitin sulfate interacts with many proteins including cytokines and growth factors, regulating their signal transduction. Chondroitin sulfate is thought to be a regulatory factor that influences development, organ morphogenesis, inflammation and infection. Chondroitin lyase degrades chondroitin sulfate at the GalNAc β 1–4 GlcUA bond and belongs to polysaccharide lyase family 8 (PL8). Recently, three types of chondroitin lyase have been identified (Michaud *et al.*, 2003): chondroitin AC lyase, which can degrade chondroitin 4-sulfate (chondroitin sulfate A) and chondroitin 6-sulfate (chondroitin sulfate C), chondroitin B lyase, which can degrade dermatan sulfate (chondroitin sulfate B), and chondroitin ABC lyase, which can degrade all of the above-mentioned chondroitin sulfates.

We have recently reported a novel chondroitin lyase from the baculovirus envelope protein ODV-E66 (truncated version consisting of residues 67–704) of *Autographa californica* nucleopolyhedrovirus (Sugiura *et al.*, 2011). ODV-E66 (67–704) can degrade chondroitin and chondroitin 6-sulfate but cannot degrade chondroitin 4-sulfate. The enzyme is active over a wide pH (pH 4–9) and temperature (303–333 K) range and is unaffected by divalent metal ions (Sugiura *et al.*, 2011). ODV-E66 was identified from medium conditioned by baculovirus-infected insect cells as a truncated form starting at amino acid 67 (Sugiura *et al.*, 2011). The truncated mature protein has higher activity than full-length ODV-E66. Although the identity between ODV-E66 and other chondroitin lyases is very low (less than 12%), ODV-E66 has been predicted to have a PL8 domain in the N-terminal region (83–291). Because of these unique characteristics, ODV-E66 (67–704) is expected to be useful for producing site-specific sulfated chondroitin over a very wide pH and temperature range. In order to understand why ODV-E66 (67–704) shows these unique characteristics, we intend to perform structural analysis.

2. Expression and purification

The truncated form of ODV-E66 (Swiss-Prot Q00704) consisting of amino acids 67–704 was constructed using pET15b [pET15b-ODV-E66 (67–704)] as an N-terminally His-tagged protein with the attached amino-acid sequence MGSSHHHHHHSSGLVPRGSHM. *Escherichia coli* BL21 (DE3) cells (Novagen) were transformed with the abovementioned expression plasmid and cultured in LB medium containing 50 µg ml⁻¹ ampicillin at 310 K to an OD₆₀₀ of 0.5. The cultures were cooled to 298 K and then supplemented with 1 mM isopropyl β-D-1-thiogalactopyranoside. After a 15 h culture, the cells were harvested by centrifugation at 5000g for 10 min and resuspended in a sonication buffer consisting of 50 mM Tris–HCl pH 8.0, 500 mM NaCl. After ultrasonic cell disintegration, the cell suspension was centrifuged at 20 000g for 30 min. The supernatant was collected and applied onto a Ni-agarose gel column (Qiagen) equilibrated with 50 mM Tris–HCl pH 8.0, 500 mM NaCl, 20 mM imidazole. His-tagged proteins were eluted with 50 mM Tris–HCl pH 8.0, 500 mM NaCl, 500 mM imidazole. The eluted protein solution was applied onto a Superdex 200 HiLoad 16/60 gel-filtration column (GE Healthcare). The fractions containing ODV-E66 (67–704) were then collected and the buffer was exchanged to 50 mM Tris–HCl pH 8.0, 100 mM NaCl buffer using a Sephadex G-25 buffer-exchange column (GE Healthcare). The purified protein was analyzed by SDS–PAGE.

3. Crystallization

The purified ODV-E66 (67–704) protein was concentrated to 6.6 mg ml⁻¹. Crystallization experiments were performed using the sitting-drop and hanging-drop vapour-diffusion methods at 293 K. Initial screening was carried out by the sitting-drop vapour-diffusion method using the PEG/Ion 1 and 2 crystallization screening kits (Hampton Research) with a Mosquito nanolitre dispenser system (TTP LabTech) by mixing 100 nl protein solution with 100 nl reservoir solution. A single crystal (of approximate dimensions 0.05 × 0.05 × 0.05 mm) was obtained in 1 d from condition No. 41 of PEG/Ion 2 [0.02 M citric acid, 0.08 M bis-tris propane pH 8.8, 16% (w/v) polyethylene glycol 3350]. To optimize the condition, the hanging-drop vapour-diffusion method was used, varying the PEG 3350 concentration from 10 to 20% (w/v). Each drop consisted of 2 µl protein

Figure 1
SDS–PAGE of purified ODV-E66 (67–704). Lane 1, molecular-weight markers (labelled in kDa); lane 2, purified ODV-E66 (67–704) (0.5 µg).

Table 1

Data-collection and processing statistics for ODV-E66 (67–704).

Wavelength (Å)	1.3
Crystal-to-detector distance (mm)	170
Exposure time (s)	5
Space group	<i>P</i> 6 ₂ or <i>P</i> 6 ₄
Unit-cell parameters (Å, °)	<i>a</i> = <i>b</i> = 113.5, <i>c</i> = 101.5, α = β = 90.0, γ = 120.0
Resolution range (Å)	50.0–1.80
<i>R</i> _{merge} (%)	9.4 (90.1)
<i>I</i> / <i>σ</i> (<i>I</i>)	36.92 (2.96)
Completeness (%)	100.0 (100.0)
Multiplicity	8.1 (7.9)
<i>V</i> _M (Å ³ Da ⁻¹)	2.54
Solvent content (%)	51.6

solution and 2 µl reservoir solution and was equilibrated against 200 µl reservoir solution in the well.

4. Data collection and processing

A data set was collected on beamline BL38B1 at SPring-8, Hyogo, Japan. The ODV-E66 (67–704) chondroitin lyase crystal was soaked for 30 s in cryoprotectant buffer [15% (v/v) glycerol, 50 mM Tris–HCl pH 8.0, 100 mM NaCl, 0.02 M citric acid, 0.08 M bis-tris propane pH 8.8, 18% (w/v) PEG 3350] and then flash-cooled in a nitrogen-gas cryostream (Rigaku). A total of 360 frames of data were collected using a 0.5° oscillation range with 5 s exposure. Data were indexed, integrated and scaled with the *HKL*-2000 package (Otwinowski & Minor, 1997).

5. Results and discussion

The chondroitin lyase ODV-E66 (67–704) from the baculovirus envelope was expressed in *E. coli* and purified using Ni-agarose and gel-filtration column chromatography (Fig. 1). It was successfully crystallized by the sitting-drop vapour-diffusion method using 0.02 M citric acid, 0.08 M bis-tris propane pH 8.8, 16% (w/v) PEG 3350 as the reservoir solution. After optimization of the crystallization conditions, crystals suitable for X-ray diffraction were obtained using 18% (w/v) PEG in the same buffer with the hanging-drop vapour-diffusion method. These crystals had typical dimensions of 0.5 × 0.1 × 0.1 mm (Fig. 2). X-ray data collection was performed at SPring-8, Hyogo, Japan. The crystal diffracted to 1.8 Å resolution. The results of scaling suggested that the crystal belonged to space group *P*6₂ or *P*6₄, with unit-cell parameters *a* = *b* = 113.5, *c* = 101.5 Å. Matthews calculations suggested the presence of one molecule per asymmetric

Figure 2
Crystal of ODV-E66 (67–708)

unit (Matthews coefficient V_M of $2.54 \text{ \AA}^3 \text{ Da}^{-1}$, 51.6% solvent content; Matthews, 1968). Complete data statistics are given in Table 1. Molecular-replacement calculations with *Phaser* (McCoy *et al.*, 2007) and *MOLREP* (Vagin & Teplyakov, 2010) using the three-dimensional structures of homologues (PDB entries 1n7o, 1egu and 1ojm; Nukui *et al.*, 2003; Li *et al.*, 2000; Rigden & Jedrzejewski, 2003) as search models were attempted. The MR trials did not provide a solution, probably because the identities of the search models to ODV-E66 (67–704) were less than 12%. The preparation of SeMet-labelled ODV-E66 (67–704) and of heavy-atom derivatives using methylmercury chloride for SAD or MAD experiments is currently under way.

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