Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

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

Yoshirou Kawaguchi,[a] Nobuo Sugiura,[b] Momo Onishi,[c] Koji Kimata,[d] Makoto Kimura[a,c] and Yoshimitu Kakuta[a,c]*

[a]Laboratory of Structural Biology, Graduate School of System Life Sciences, Kyushu University, Hakozaki 6-10-1, Fukuoka City, Fukuoka 812-8581, Japan, [b]Institute for Molecular Science of Medicine, Aichi Medical University, Yazako, Nagakute, Aichi 480-1195, Japan, [c]Faculty of Agriculture, Kyushu University, Hakozaki 6-10-1, Fukuoka City, Fukuoka 812-8581, Japan, and [d]Research Complex for the Medicine Frontiers, Aichi Medical University, Yazako, Nagakute, Aichi 480-1195, Japan

Correspondence e-mail: kakuta@agr.kyushu-u.ac.jp

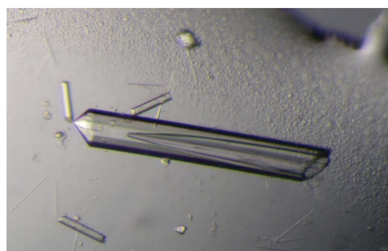
Received 4 November 2011
Accepted 9 December 2011

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 $Å^3\ Da^{-1}$.

1. Introduction

Chondroitin is a linear polysaccharide which consists of repeating [–4 D-glucuronic acid (GlcUA) β1–3 *N*-acetyl-D-galactosamine (GalNAc) β1–]$_n$ 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 β1–4 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.



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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 OD_{600} of 0.5. The cultures were cooled to 298 K and then supplemented with 1 m*M* isopropyl β-D-1-thiogalactopyranoside. After a 15 h culture, the cells were harvested by centrifugation at 5000*g* for 10 min and resuspended in a sonication buffer consisting of 50 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl. After ultrasonic cell disintegration, the cell suspension was centrifuged at 20 000*g* for 30 min. The supernatant was collected and applied onto an Ni–agarose gel column (Qiagen) equilibrated with 50 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 20 m*M* imidazole. His-tagged proteins were eluted with 50 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 500 m*M* 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 m*M* Tris–HCl pH 8.0, 100 m*M* 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 solution and 2 µl reservoir solution and was equilibrated against 200 µl reservoir solution in the well.

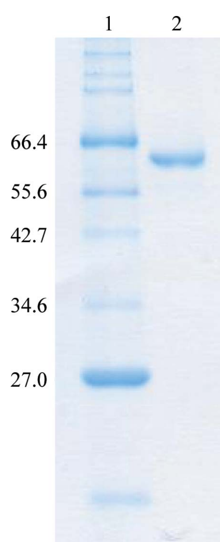

Figure 1
SDS–PAGE of purified ODV-E66 (67–704). Lane 1, molecular-weight markers (labelled in kDa); lane 2, purified ODV-E66 (67–704) (0.5 µg).

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	$P6_2$ or $P6_4$
Unit-cell parameters (Å, °)	$a = b = 113.5$, $c = 101.5$, $\alpha = \beta = 90.0$, $\gamma = 120.0$
Resolution range (Å)	50.0–1.80
R_{merge} (%)	9.4 (90.1)
$\langle I/\sigma(I)\rangle$	36.92 (2.96)
Completeness (%)	100.0 (100.0)
Multiplicity	8.1 (7.9)
V_M (Å^3 Da^{-1})	2.54
Solvent content (%)	51.6

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 m*M* Tris–HCl pH 8.0, 100 m*M* 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 $P6_2$ or $P6_4$, with unit-cell parameters $a = b = 113.5$, $c = 101.5$ Å. Matthews calculations suggested the presence of one molecule per asymmetric

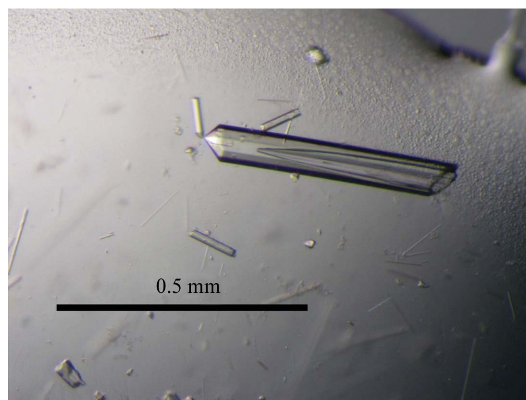

Figure 2
Crystal of ODV-E66 (67–708)

unit (Matthews coefficient V_M of 2.54 $Å^3 Da^{-1}$, 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 & Jedrzejas, 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.

We thank the staff members of beamline BL38B1 at SPring-8 for their help with data collection. The synchrotron-radiation experiments on BL38B1 at SPring-8 were performed with the approval of the Japan Synchrotron Radiation Research Institute (JASRI; Proposal No. 2011A2014).

References

Esko, J. D., Kimata, K. & Lindahl, U. (2009). *Essentials of Glycobiology*, 2nd ed., edited by A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart & M. E. Etzler, ch. 16. New York: Cold Spring Harbor Laboratory Press.
Habuchi, O. (2000). *Biochim. Biophys. Acta*, **1474**, 115–127.
Li, S., Kelly, S. J., Lamani, E., Ferraroni, M. & Jedrzejas, M. J. (2000). *EMBO J.* **19**, 1228–1240.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 499–501.
McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
Michaud, P., Da Costa, A., Courtois, B. & Courtois, J. (2003). *Crit. Rev. Biotechnol.* **23**, 233–266.
Nukui, M., Taylor, K. B., McPherson, D. T., Shigenaga, M. & Jedrzejas, M. J. (2003). *J. Biol. Chem.* **278**, 3079–3088.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Rigden, D. J. & Jedrzejas, M. J. (2003). *J. Biol. Chem.* **278**, 50596–50606,
Sugiura, N., Setoyama, Y., Chiba, M., Kimata, K. & Watanabe, H. (2011). *J. Biol. Chem.* **286**, 29026–29034.
Vagin, A. & Teplyakov, A. (2010). *Acta Cryst.* D**66**, 22–25.