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Cloning, purification, crystallization and preliminary X-ray diffraction of the OleC protein from *Stenotrophomonas maltophilia* involved in head-to-head hydrocarbon biosynthesis

OleC, a biosynthetic enzyme involved in microbial hydrocarbon biosynthesis, has been crystallized. Synchrotron X-ray diffraction data have been collected to 3.4 Å resolution. The crystals belonged to space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 98.8$, $c = 141.0$ Å.

1. Introduction

The biological mechanisms of hydrocarbon biosynthesis have recently attracted attention as a means of producing important commercial chemicals from renewable resources (Rude & Schirmer, 2009; Steen *et al.*, 2010). Plants, animals and microbes have evolved several different biosynthetic pathways for generating hydrocarbons, but the biochemical details are only now beginning to be revealed. A head-to-head condensation of fatty acids that generates long-chain olefins has been known for more than 40 years (Albro & Dittmer, 1969), but only in the last year have the olefin (*ole*) biosynthetic genes been revealed (Beller *et al.*, 2010). The head-to-head condensation reaction requires a minimum of three gene products (OleACD). A genetic knockout of the *oleC* gene led to the loss of hydrocarbon biosynthesis in *Shewanella oneidensis* MR-1 (Sukovich, Seffernick, Richman, Gralnick *et al.*, 2010). In a recent study, 69 divergent bacteria were indicated to generate olefins *via* an OleC-dependent biosynthetic pathway (Sukovich, Seffernick, Richman, Hunt *et al.*, 2010). The genes have yet to be demonstrated in plants and animals, although marine eukaryotic algae make similar compounds (Rieley *et al.*, 1998).

OleC is a member of the LuxE acyl-protein synthetase superfamily based on a conserved-domain search at the National Center for Biotechnology Information (NCBI). This family includes LuxE, which is involved in bioluminescence, and fatty acyl-CoA synthase, which is involved in the ligation of fatty acids to a coenzyme A moiety with an AMP-activated acyl group as an intermediate. As of 8 April 2010, 63 crystal structures in the Protein Data Bank belong to this superfamily, with the most closely related being only 26% identical to OleC in amino-acid sequence. Only OleC is known to be involved in olefin biosynthesis. Previous studies suggested that the Ole proteins from *Stenotrophomonas maltophilia* would have a relatively broad specificity for different fatty-acid chain lengths and degrees of unsaturation (Yu *et al.*, 1988). Thus, the *S. maltophilia oleC* gene was selected for cloning and expression studies. The purification and crystallization of an OleC protein has not previously been described.

2. Experimental

2.1. Cloning of the *oleC* gene

DNA consisting of the *S. maltophilia* ATCC 17679 *oleC* gene sequence (Friedman & Rude, 2008) and flanking *NdeI* and *HindIII* restriction sites was synthesized by the GenScript Corporation (Piscataway, New Jersey, USA). The DNA was cloned into a pET30b vector (Novagen, Madison, Wisconsin, USA) containing a C-terminal His tag. The recombinant plasmid was transformed into *Escherichia coli* BL21 (DE3) pLysE One Shot cells (Invitrogen) for expression.

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2.2. Expression and purification of OleC

E. coli BL21(pOleC) cells were cultured in 500 ml LB medium containing kanamycin ($50 \mu\text{g ml}^{-1}$) and chloramphenicol ($34 \mu\text{g ml}^{-1}$) at 310 K. Cultures were induced with isopropyl β -D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM when the OD_{600} of the culture reached 0.65–0.70. After 4 h at 310 K, the induced cells were harvested by centrifugation at 3000g for 25 min and resuspended in 20 mM sodium phosphate, 500 mM NaCl pH 7.4 buffer with EDTA-free protease inhibitors (Roche). The cells were disrupted by three passes through a chilled French pressure cell at 8.3 MPa and centrifuged at 27 000g for 90 min to remove cell debris and insoluble protein. The soluble fraction was either filtered through a $0.45 \mu\text{m}$ filter or centrifuged for 30 min prior to loading onto a Pharmacia Biotech LCC 501 FPLC fitted with a 5 ml HisTrap HP (Amersham Biosciences) column complexed with Ni^{2+} and equilibrated with 20 mM sodium phosphate and 500 mM NaCl pH 7.4. The His-tagged OleC protein eluted at 200 mM imidazole. The purity of the protein was confirmed by SDS–PAGE, with a single band running at 60 kDa (Fig. 1). The protein was concentrated to 8–13 mg ml^{-1} and the buffer was exchanged for 20 mM HEPES, 500 mM NaCl pH 7.4 using a 50 ml Amicon pressure concentrator with a YM-10 membrane (Millipore). After centrifugation at 27 000g for 20 min to remove precipitated protein, 2.2 mM adenosine 5'-monophosphate (5'-AMP) was added. The protein was rocked gently on ice for 1.5 h prior to flash-freezing in liquid nitrogen for storage.

2.3. Crystallization

Initial crystallization trials of OleC were carried out by the Hauptman–Woodward Medical Research Institute High-Throughput Screening (HTS) laboratory. The HTS library tests 1536 different chemical conditions for crystallization *via* the microbatch-under-oil method. When very few hits resulted in crystals from the initial screen, crystallization trials of OleC were repeated in the presence of 2.2 mM 5'-AMP. The inclusion of 5'-AMP was based on the success of cocrystallization of other LuxE-superfamily proteins with an acyl-adenylate or acyl group and 5'-AMP substrate (Wu *et al.*, 2008). OleC crystals grew under multiple conditions (>100) in a week when 5'-AMP was included in the OleC sample, suggesting conformational

Figure 1

SDS–PAGE analysis of OleC. Proteins were analyzed on a 10% SDS–PAGE gel and stained with SimplyBlue Safe Coomassie stain. The left lane contains standard molecular-weight markers (kDa); the right lane contains purified OleC.

Table 1

Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Space group	$P3_121$ or $P3_221$
Unit-cell parameters	
$a = b$ (Å)	98.8
c (Å)	141.0
Wavelength (Å)	0.9784
Resolution range	50–3.40 (3.46–3.40)
No. of unique reflections	115600 (11407)
$R_{\text{merge}}^\dagger$ (%)	11.9 (40.9)
Completeness (%)	100 (100)
Redundancy	10.0 (9.8)

$^\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity and $\langle I(hkl) \rangle$ is the average intensity of multiple measurements.

heterogeneity in the absence of this substrate. The crystals used for X-ray data collection were grown in HTS condition 1303 (10% PEG 8000 and 10% PEG 1000), which contains no buffer. This condition was transferred to hanging-drop vapor diffusion in VDX plates (Hampton Research) using equal amounts (1.0 μl) of protein solution and reservoir solution. Optimization was achieved by a fine screen around the PEG 8000 and PEG 1000 concentrations.

2.4. X-ray diffraction data collection, processing and structure solution

The crystal was soaked in a cryoprotectant solution consisting of 50% Paratone-N and 50% paraffin oil (Hampton Research). When all of the aqueous solution had been washed away, the crystal was mounted in a nylon loop and flash-frozen in liquid nitrogen. Data were collected at a temperature of 100 K on beamline 4.2.2 at the Advanced Light Source, Berkeley, California. The resulting diffraction data were processed with *HKL-2000* (Otwinowski & Minor, 1997). Further diffraction data analysis was conducted with the *CCP4* suite (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

3.1. Crystallization

The final optimized conditions for crystal growth were 10% PEG 8000 and 11% PEG 1000 at 293 K. Crystals suitable for X-ray analysis appeared after 1 d by hanging-drop vapor diffusion. Crystals of OleC only grew in the presence of 5'-AMP. The crystals were clear, with a bipyrmaid-like morphology (Fig. 2).

Figure 2

OleC crystals grown in the presence of 5'-AMP. Average crystal dimensions are $150 \times 40 \times 40 \mu\text{m}$.

Figure 3
Section of an X-ray diffraction image from OleC crystals grown in the presence of 5'-AMP. The red-colored arcs indicate resolution (from left to right the arcs are at 3.5, 4.5 and 8.0 Å resolution).

3.2. Crystallization and X-ray diffraction data collection

The majority of OleC crystals grown in the presence of 5'-AMP did not diffract. The problem is likely to be one of crystal fragility towards handling, as the use of alternative cryoprotectants (glycerol, xylitol, 2-methyl-1,3-propanediol, PEG 400, PEG 600 and perfluoropolyether) had no impact in improving crystal diffraction. Overall, one out of every 30 crystals was suitable for data collection, with diffraction to 10 Å resolution in-house and diffraction to 3.4 Å resolution at the synchrotron. Data-collection statistics are provided in Table 1 and a diffraction image is shown in Fig. 3. Initial data processing demonstrated that the crystals belonged to the trigonal space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 98.8$, $c = 141.0$ Å.

Analysis of the Matthews coefficient (Matthews, 1968) suggested the presence of one OleC monomer (60 kDa) per asymmetric unit, with a solvent content of 62.73%. The data are of moderate quality, with an overall R_{merge} of 11.9% and an estimated mosaicity of 0.93°. The average B factor derived from the Wilson plot is 70.1 Å² (Wilson, 1949). Attempts to solve the structure by molecular replacement are currently under way.

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