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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

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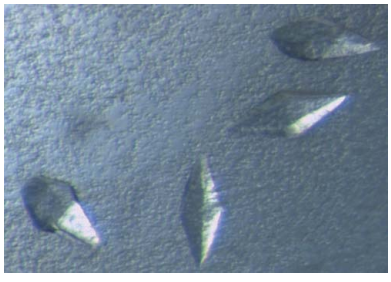
OleC, a biosynthetic enzyme involved in microbial hydrocarbon biosynthesis, has been crystallized. Synchrotron X-ray diffraction data have been collected to 3.4 Å resolution. The crystals belonged to space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 98.8$, $c = 141.0$ Å.

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 *Nde*I and *Hin*dIII 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.

2.2. Expression and purification of OleC

E. coli BL21(pOleC) cells were cultured in 500 ml LB medium containing kanamycin (50 µg ml^{-1}) and chloramphenicol (34 µg ml^{-1}) at 310 K. Cultures were induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.1 m*M* 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 3000*g* for 25 min and resuspended in 20 m*M* sodium phosphate, 500 m*M* 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 27 000*g* for 90 min to remove cell debris and insoluble protein. The soluble fraction was either filtered through a 0.45 µm 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 m*M* sodium phosphate and 500 m*M* NaCl pH 7.4. The His-tagged OleC protein eluted at 200 m*M* 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 m*M* HEPES, 500 m*M* NaCl pH 7.4 using a 50 ml Amicon pressure concentrator with a YM-10 membrane (Millipore). After centrifugation at 27 000*g* for 20 min to remove precipitated protein, 2.2 m*M* 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 m*M* 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 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.

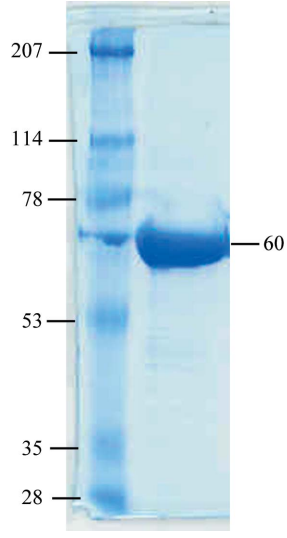


Figure 1
SDS–PAGE analysis of OleC. Proteins were analyzed on a 10% SDS–PAGE gel and stained with SimplyBlue Safe Coomassie stain. The left lane contains standard molecular-weight markers (kDa); the right lane contains purified OleC.

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_{merge}† (%)	11.9 (40.9)
Completeness (%)	100 (100)
Redundancy	10.0 (9.8)

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

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 *CCP*4 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 bipyramid-like morphology (Fig. 2).

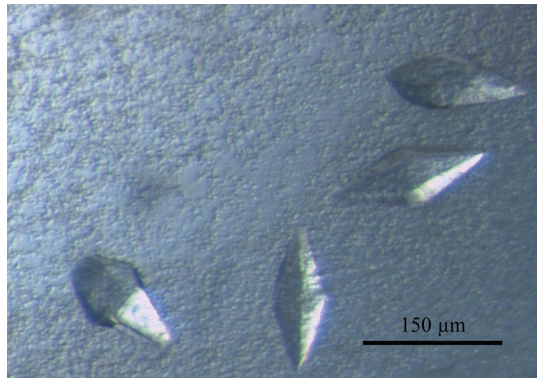


Figure 2
OleC crystals grown in the presence of 5′-AMP. Average crystal dimensions are 150 × 40 × 40 µm.

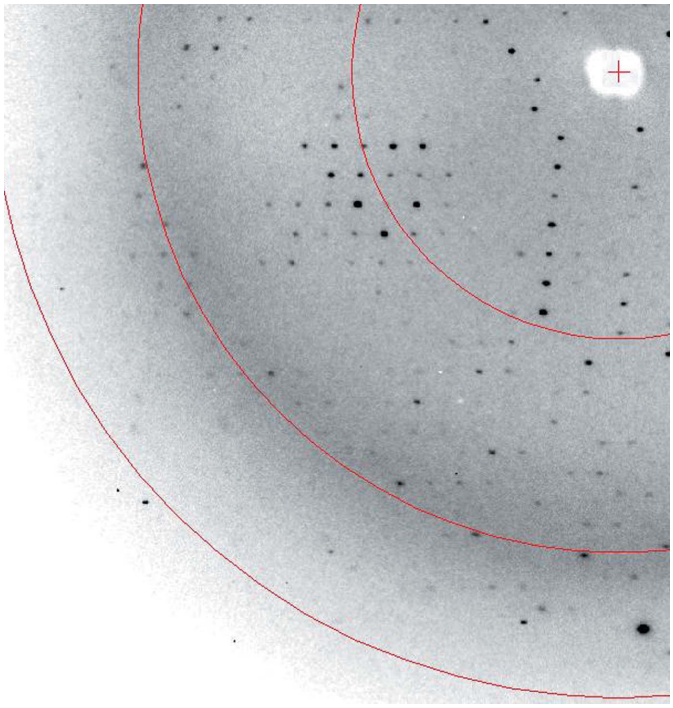


Figure 3
Section of an X-ray diffraction image from OleC crystals grown in the presence of 5′-AMP. The red-colored arcs indicate resolution (from left to right the arcs are at 3.5, 4.5 and 8.0 Å resolution).

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 $R_{\rm merge}$ of 11.9% and an estimated mosaicity of 0.93°. The average B factor derived from the Wilson plot is 70.1 Å^2 (Wilson, 1949). Attempts to solve the structure by molecular replacement are currently under way.

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