**Maureen B. Quin,* Grayson
Wawrzyn and Claudia Schmidt-
Dannert***BMBB/BTI, University of Minnesota,
1479 Gortner Avenue, Saint Paul, MN 55108,
USACorrespondence e-mail: mbquin@umn.edu,
schmi232@umn.eduReceived 21 February 2013
Accepted 19 April 2013

Purification, crystallization and preliminary X-ray diffraction analysis of Omp6, a protoilludene synthase from *Omphalotus olearius*

Basidiomycetes produce a wide range of industrially relevant natural products. One of the main classes of natural products isolated from fungi are terpenoids, a highly diverse group of secondary metabolites, many of which are bioactive and have been adapted for pharmaceutical purposes. The discovery of a suite of novel sesquiterpene synthases from *Omphalotus olearius* via genome sequencing and bioinformatic analyses has recently been described. Here, the expression, purification and crystallization of one of these enzymes (Omp6), a protoilludene synthase, is reported. A native crystal diffracted to a resolution of 2.9 Å and belonged to space group $P2_1$, with unit-cell parameters $a = 43.67$, $b = 76.76$, $c = 107.22$ Å, $\alpha = \gamma = 90^\circ$, $\beta = 95^\circ$. A diffraction data set was collected on a home-source Rigaku/MS MicroMax-007 X-ray generator.

1. Introduction

Basidiomycetes are a rich resource for many industrially relevant natural products owing to the fact that they produce an extremely diverse range of secondary metabolites (Wasser, 2011). The unique properties of the chemical structures and bioactivities of these secondary metabolites have enabled the robust survival and evolvability of fungal species. Notably, many of these bioactive compounds have been isolated and adapted for pharmaceutical purposes, including as antimicrobial, anti-inflammatory and anticancer drugs (Zhong & Xiao, 2009).

The main type of bioactive secondary metabolite that has been isolated from Basidiomycetes is a class of compounds known as terpenoids, in particular the sesquiterpenes (Abraham, 2001). Sesquiterpenes are produced by a family of enzymes called sesquiterpene synthases. These enzymes cyclize the 15-carbon molecule farnesyl pyrophosphate (FPP), yielding several hundred different types of volatile cyclic hydrocarbon backbones (Miller & Allemann, 2012; Christianson, 2006). Despite this wide product diversity, all sesquiterpene synthases share a common three-dimensional fold and a catalytic mechanism involving the metal-ion-mediated dephosphorylation of FPP to produce a reactive carbocation. This carbocation undergoes a series of rearrangement reactions until a final quenching by water, releasing the final hydrocarbon product from the catalytic active site (Yamada *et al.*, 2012).

We recently described the genome sequencing, genome mining and subsequent discovery of a suite of novel sesquiterpene synthases from *Omphalotus olearius*, a Basidiomycete which had previously been shown to produce the anticancer sesquiterpene scaffold illudin (McMorris *et al.*, 1990, 2002; Wawrzyn *et al.*, 2012). Two of these novel sesquiterpene synthases, Omp6 and Omp7, have been shown to be highly active and product-specific protoilludene synthases, producing Δ -6 protoilludene via a likely 1,11 ring closure of FPP to create a *trans*-humulyl cation intermediate followed by two cyclization steps to yield the final volatile sesquiterpene (Fig. 1; Wawrzyn *et al.*, 2012). Recent studies have shown that Δ -6 protoilludene is also produced by active-site mutants of the closely related pentalenene synthase via premature deprotonation of the carbocation. These data strongly

Table 1

Data-collection and processing statistics for Omp6.

Values in parentheses are for the outermost resolution shell.

Data collection	
Space group	$P2_1$
Unit-cell parameters ($\text{\AA}$, $^\circ$)	$a = 43.67$, $b = 76.76$, $c = 107.22$, $\alpha = \gamma = 90$, $\beta = 95$
Wavelength ($\text{\AA}$)	1.54
Resolution ($\text{\AA}$)	23.14–2.90 (3.06–2.90)
Matthews coefficient V_M ($\text{\AA}^3 \text{Da}^{-1}$)	2.23
Solvent content (%)	44.77
Monomers in asymmetric unit	2
Data processing	
No. of observed reflections	36143
No. of unique reflections	14699
Completeness (%)	94.0 (85.7)
Multiplicity	2.5 (1.8)
$R_{\text{merge}}^{\dagger}$ (%)	11.2 (22.7)
Mean $I/\sigma(I)$	6.4 (2.7)

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the i th observation of reflection hkl and $\langle I(hkl) \rangle$ is the weighted average intensity for all observations i of reflection hkl .

sucrose and were mounted in a 0.1 mm CryoLoop (Hampton Research) before flash-cooling by immediate immersion into liquid nitrogen at 100 K.

Diffraction data were collected to a resolution of 2.9 $\text{\AA}$ using a wavelength of 1.54 $\text{\AA}$ on a Rigaku/MSC MicroMax-007 X-ray generator at the Kahlert Structural Biology Laboratory at the University of Minnesota. A total of 264 images were collected with an oscillation angle of 0.5 $^\circ$ and an exposure time of 90 s per image. The data were indexed using *iMOSFLM* (Battye *et al.*, 2011) and were scaled using *SCALA* in *CCP4* (Winn *et al.*, 2011; Evans, 2006). Data-collection and processing statistics are summarized in Table 1.

3. Results and discussion

Recombinant Omp6 from *O. olearius* was expressed as a soluble fusion with Trx in *E. coli* C2566 cells. The protein was purified to near-homogeneity by Ni-affinity chromatography and size-exclusion chromatography, resulting in a yield of 1.5 mg purified protein from 3 l culture. SDS-PAGE analysis revealed a single band corresponding to a molecular weight of about 40 kDa, which is close to the calculated molecular weight of 41.07 kDa. Omp6 eluted from a Superdex

Figure 2

Purification of Omp6 by size-exclusion chromatography. The chromatogram shows two main peaks; SDS-PAGE (15%) analysis of the proteins contained within these fractions shows that the peak at the void volume (8.0 ml) contains unwanted proteins, while the 41 kDa Omp6 (highlighted with an arrow) elutes as a distinct peak (15.2 ml) from the Superdex 200 column.

200 size-exclusion column as a single peak at 15.2 ml, suggesting that it behaves as a monomer in solution, while contaminating proteins eluted in the void volume (8.0 ml) (Fig. 2). Enzyme assays carried out using the purified protein confirmed Omp6 to be a highly active and specific protoilludene synthase (Fig. 3).

Crystals of Omp6 were obtained using 0.01 M ZnCl_2 , 20% (w/v) PEG 6000, 0.1 M MES pH 6.0. Initial high-throughput crystallization screens produced crystals that were deemed to be too small to mount in a X-ray beam. The crystallization conditions were successfully reproduced manually using the hanging-drop technique, allowing the growth of Omp6 crystals to dimensions of $100 \times 50 \times 20 \mu\text{m}$ (Fig. 4). While these crystals were still reasonably small, it was possible to harvest them and to test them for diffraction quality. The best diffraction was obtained from crystals which were cryoprotected in mother liquor supplemented with 1% (w/v) sucrose. It was found that the choice of cryoprotectant was important in obtaining suitable diffraction; screening of other cryoprotectants at a range of concentrations, including PEG 400, glucose and glycerol, yielded diffraction to 6 $\text{\AA}$ resolution at best. Upon soaking several Omp6 crystals in gradually decreasing concentrations of sucrose, the resolution limits of diffraction of each crystal gradually increased. A crystal soaked in cryoprotectant supplemented with 1% (w/v) sucrose diffracted to

Figure 3

Activity of purified Omp6. GC-MS analysis of an *in vitro* assay of Omp6 incubated with (E,E)-FPP. The enzyme produces Δ -6 protoilludene as its sesquiterpene product.

Figure 4

Crystals of Omp6. The scale bar represents 100 μm .

Figure 5

Diffraction pattern from a single Omp6 crystal. The crystal diffracted to a resolution of 2.9 Å.

2.9 Å resolution (Fig. 5), allowing a complete native data set to be collected.

The Omp6 crystal belonged to the monoclinic space group $P2_1$, with unit-cell parameters $a = 43.67$, $b = 76.76$, $c = 107.22$ Å, $\alpha = \gamma = 90^\circ$, $\beta = 95^\circ$. Assuming the presence of two molecules in the asymmetric unit, the calculated Matthews coefficient and solvent content are $2.23 \text{ Å}^3 \text{ Da}^{-1}$ and 44.77%, respectively (Matthews, 1968). Attempts to solve the structure of Omp6 by molecular replacement with *Phaser* (McCoy *et al.*, 2007) and *MOLREP* (Vagin & Teplyakov, 2010) using PDB entries 3kb9 (24% sequence identity; Aaron *et al.*, 2010) and 1ps1 (26% sequence identity; Lesburg *et al.*, 1997) as search templates were unsuccessful. Further crystallization trials are currently under way with the aim of producing crystals of suitable diffraction quality to allow SAD phasing. Solution of the structure of Omp6 would represent the first crystal structure of a protoilludene synthase and would provide key information about the catalytic mechanism of this industrially relevant enzyme.

The authors would like to thank Professor Carrie Wilmot for critical reading of this manuscript, Dr Ke Shi of the Nanoliter Crystallization Facility at University of Minnesota for assistance with crystallization and Ed Hoeffner of the Kahlert Structural Biology Laboratory at University of Minnesota for advice and assistance. MBQ conceived, designed and performed the experiments, analyzed the data and wrote the manuscript. GW supplied the Omp6-pUCBB plasmid. CS-D conceived and designed the experiments, supplied the reagents and wrote the manuscript. This research was supported by the National Institute of Health Grant GM080299 (to CS-D).

References

- Aaron, J. A., Lin, X., Cane, D. E. & Christianson, D. W. (2010). *Biochemistry*, **49**, 1787–1797.
- Abraham, W. R. (2001). *Curr. Med. Chem.* **8**, 583–606.
- Battye, T. G. G., Kontogiannis, L., Johnson, O., Powell, H. R. & Leslie, A. G. W. (2011). *Acta Cryst. D* **67**, 271–281.
- Christianson, D. W. (2006). *Chem. Rev.* **106**, 3412–3442.
- Evans, P. (2006). *Acta Cryst. D* **62**, 72–82.
- LaVallie, E. R., DiBlasio-Smith, E. A., Collins-Racie, L. A., Lu, Z. & McCoy, J. M. (2003). *Methods Mol. Biol.* **205**, 119–140.
- Lesburg, C. A., Zhai, G., Cane, D. E. & Christianson, D. W. (1997). *Science*, **277**, 1820–1824.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- 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.
- McMorris, T. C., Kashinatham, A., Lira, R., Rundgren, H., Gantzel, P. K., Kelner, M. J. & Dawe, R. (2002). *Phytochemistry*, **61**, 395–398.
- McMorris, T. C., Kelner, M. J., Wang, W., Moon, S. & Taetle, R. (1990). *Chem. Res. Toxicol.* **3**, 574–579.
- Miller, D. J. & Allemann, R. K. (2012). *Nat. Prod. Rep.* **29**, 60–71.
- Seemann, M., Zhai, G., de Kraker, J. W., Paschall, C. M., Christianson, D. W. & Cane, D. E. (2002). *J. Am. Chem. Soc.* **124**, 7681–7689.
- Vagin, A. & Teplyakov, A. (2010). *Acta Cryst. D* **66**, 22–25.
- Vick, J. E., Johnson, E. T., Choudhary, S., Bloch, S. E., Lopez-Gallego, F., Srivastava, P., Tikh, I. B., Wawrzyn, G. T. & Schmidt-Dannert, C. (2011). *Appl. Microbiol. Biotechnol.* **92**, 1275–1286.
- Wasser, S. P. (2011). *Appl. Microbiol. Biotechnol.* **89**, 1323–1332.
- Wawrzyn, G. T., Quin, M. B., Choudhary, S., López-Gallego, F. & Schmidt-Dannert, C. (2012). *Chem. Biol.* **19**, 772–783.
- Winn, M. D. *et al.* (2011). *Acta Cryst. D* **67**, 235–242.
- Yamada, Y., Cane, D. E. & Ikeda, H. (2012). *Methods Enzymol.* **515**, 123–162.
- Zhong, J.-J. & Xiao, J.-H. (2009). *Adv. Biochem. Eng. Biotechnol.* **113**, 79–150.
- Zu, L., Xu, M., Lodewyk, M. W., Cane, D. E., Peters, R. J. & Tantillo, D. J. (2012). *J. Am. Chem. Soc.* **134**, 11369–11371.