Crystallization and preliminary X-ray diffraction analysis of naphthalene dioxygenase from *Rhodococcus* sp. strain NCIMB 12038

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The three-component naphthalene dioxygenase (NDO) enzyme system carries out the first step in the aerobic degradation of naphthalene to (+)-*cis*-(1*R*,2*S*)-dihydroxy-1,2-dihydronaphthalene by *Rhodococcus* sp. strain NCIMB 12038. The terminal oxygenase component (naphthalene 1,2-dioxygenase) that catalyzes this reaction belongs to the aromatic ring hydroxylating dioxygenase family and has been crystallized. These enzymes utilize a mononuclear non-heme iron centre to catalyze the addition of dioxygen to their respective substrates. In this reaction, two electrons, two protons and a dioxygen molecule are consumed. The *Rhodococcus* enzyme has only 33 and 29% sequence identity to the corresponding α- and β-subunits of the NDO system of *Pseudomonas putida* NCIMB 9816-4, for which the tertiary structure has been reported. In order to determine the three-dimensional structure of the *Rhodococcus* NDO, diffraction-quality crystals have been prepared by the hanging-drop method. The crystals belongs to space group $P2_12_12_1$, with unit-cell parameters $a = 87.5$, $b = 144$, $c = 185.6$ Å, $\alpha = \beta = \gamma = 90°$, and diffract to 2.3 Å resolution.

1. Introduction

Xenobiotic compounds can be metabolized by the genus *Rhodococcus*, which is present in many environmental niches (Finnerty, 1992; Warhurst & Fewson, 1994). Naphthalene is released into the environment as coal tar and coal-tar products such as creosote (Mueller *et al.*, 1989). Naphthalene-degrading bacteria such as *Pseudomonas* sp. (Cerniglia, 1992) carry out the oxidation of naphthalene (Fig. 1) with the help of the naphthalene dioxygenase (NDO) enzyme system (EC 1.14.12.12). This system adds two atoms of oxygen to the aromatic nucleus of naphthalene (Jeffrey *et al.*, 1975) to form 1,2-dihydroxynaphthalene, which serves as a substrate for ring cleavage.

The NDO system from *P. putida* has three components: an iron–sulfur flavoprotein reductase, an iron–sulfur ferredoxin and a terminal oxygenase component. The first two components transfer electrons from NAD(P)H to the catalytic oxygenase component (Ensley *et al.*, 1982; Ensley & Gibson, 1983; Haigler & Gibson, 1990*a*,*b*), which is composed of large and small subunits, α and β, in an $\alpha_3\beta_3$ configuration (Kauppi *et al.*, 1998). NDO is a member of a large family of oxygenases whose α-subunits contain a Rieske [2Fe–2S] center and a mononuclear non-heme iron (Butler & Mason, 1997). In this system, electrons are transferred from the Rieske center of the ferredoxin to the Rieske center of the oxygenase α-subunit. The reduced Rieske center in one α-subunit transfers an electron to the mononuclear iron at the active site in an adjacent α-subunit (Kauppi *et al.*, 1998; Parales *et al.*, 1999). The α-subunits of oxygenase determine the substrate specificities of NDO, whereas the β-subunit is not directly involved in catalysis or substrate specificity (Parales, Emig *et al.*, 1998; Parales, Parales *et al.*, 1998).

This class of enzymes is important in the bioremediation of several toxic and carcino-
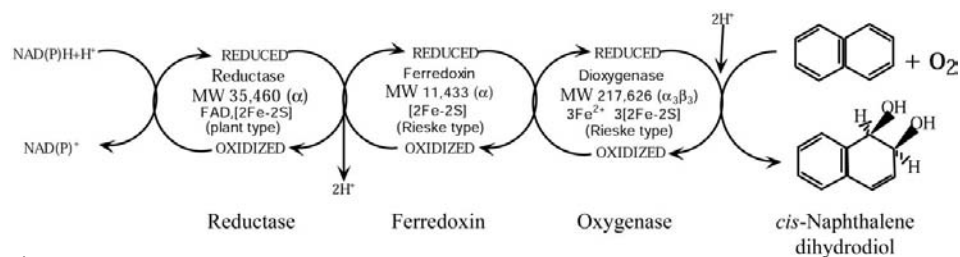
Figure 1
Schematic presentation of naphthalene oxidation by the three-component NDO system.

crystallization papers

genic compounds. The only NDO structure to have been reported is that from *P. putida*. Elucidation of the structure of NDO from *Rhodococcus* will help to identify the amino acids near the active site and provide insight into the substrate specificity and structure–function relationships of these enzymes.

We report here the crystallization and preliminary crystallographic studies of NDO from *Rhodococcus* sp. strain NCIMB 12038, the fourth member of this large class of dioxygenases to be crystallized (Lee *et al.*, 1997; Imbeault *et al.*, 2000; Nam *et al.*, 2002).

2. Materials and methods

NDO from *Rhodococcus* sp. NCIMB 12038 was purified using previously published protocols (Larkin *et al.*, 1999).

2.1. Crystallization

After purification (Larkin *et al.*, 1999), the protein (in 50 mM Tris pH 7.2, 10% glycerol, 10% ethanol, 0.5 mM DTT) was concentrated using a Vivascience Vivaspin Centrifuge Ultrafiltration Cartridge (Vivascience Ltd, Unit 6, Stonedale Road, Stonehouse, Gloucestershire GL10 3RQ, England) at 5000 rev min^{-1} and 277 K. Initial crystallization conditions were established using the hanging-drop vapor-diffusion technique using Hampton Crystal Screen I and II kits (Jancarik & Kim, 1991). A hanging drop was prepared by mixing equal volumes (2 µl each) of the protein and reservoir solution on a cover slip, which was then sealed with silicon grease over a well containing 1 ml of the respective crystallization solution and incubated in a cold room at 279 K. The first crystals were obtained using solution 35 [70%(v/v) MPD (2-methyl-2,4-pentanediol), 0.1 M HEPES pH 7.5] from the Hampton Crystal Screen II kit (Jancarik & Kim, 1991).
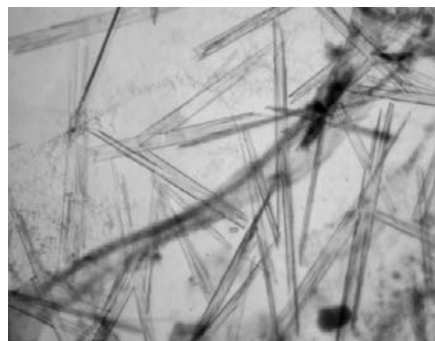
Figure 2
Crystals of NDO-R (naphthalene dioxygenase from *Rhodococcus*) appear red owing to the presence of a Rieske Fe–S cluster. The largest crystal dimension is 1.7 × 0.6 × 0.3 mm.

Table 1
Data-collection and processing statistics of the NDO crystals.

Values in parentheses are for the outermost shell (2.37–2.3 Å).

Images	1170
Exposure time (s)	5
Detector distance (mm)	210
Resolution (Å)	23–2.3
Multiplicity	3.6 (3.7)
Unique reflections	120880
Completeness (%)	97.1 (95)
$I/\sigma(I)$	7.5 (2.2)
R_{sym}† (%)	7.8 (28.2)

† $R_{sym} = \sum_h \sum_i |I_{hi} - \bar{I}_h| / \sum_h \sum_i \bar{I}_h$, where I_{hi} is the observed intensity of the ith measurement of reflection h and $\bar{I}_h$ is the mean intensity of reflection h.

The initial crystallization conditions were further refined by changing the pH, the precipitant concentrations and the ratio of protein to reservoir solution in the hanging drop. This produced diffraction-quality crystals from 0.1 M HEPES pH 7.8, 67.5–69.5%(v/v) 2-methyl-2,4-pentanediol (MPD) and 40 mg ml^{-1} protein.

2.2. Data collection and analysis

The data were collected (from flash-cooled crystals at 100 K) at the SBC-CAT beamline 17-ID at the Advanced Photon Source, Argonne National Laboratories. Complete data sets were collected from single crystals using 0.1° oscillation steps.

Data were indexed, integrated and scaled using the program *d*TREK* (Pflugrath, 1999). The scaled data were truncated to obtain structure-factor amplitudes using the *CCP*4 (Collaborative Computational Project, Number 4, 1994) program *TRUNCATE* (French & Wilson, 1978).

3. Results and discussion

Crystals were needle-like in shape and grew within 2–7 d to approximate dimensions of 1.7 × 0.6 × 0.3 mm (Fig. 2). No extra cryoprotectant was added when flash-cooling the crystals. Crystals were removed from drops using a fiber loop and were immediately flash-cooled in liquid nitrogen for low-temperature data collection.

Crystals grew in the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 87.5$, $b = 144$, $c = 185.6$ Å, and diffracted to 2.3 Å. The data statistics are shown in Table 1. Preliminary analysis (66.7% solvent content) suggested the possibility of a full hexamer in the asymmetric unit. Molecular replacement using *AMoRe* (Navaza, 1994) and a polyalanine model of the NDO from *Pseudomonas* sp. was used to determine the structure. This was followed by density modification including threefold averaging using *DM* (Cowtan, 1994). This produced maps that have been used to trace the entire chain. Model building and refinement are now in progress.

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