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Crystallization and preliminary crystallographic analysis of the hydroxyquinol 1,2-dioxygenase from *Nocardioides simplex* 3E: a novel dioxygenase involved in the biodegradation of polychlorinated aromatic compounds

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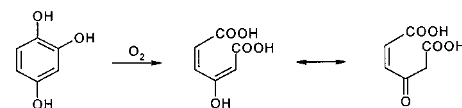
Hydroxyquinol 1,2-dioxygenase (HQ1,2O) from *Nocardioides simplex* 3E, an enzyme involved in the aerobic biodegradation of a large class of chloroaromatic compounds such as 2,4-dichlorophenoxyacetate (2,4-D) and 2,4,5-trichlorophenoxyacetate (2,4,5-T), has been crystallized. HQ1,2O, which specifically catalyzes the intradiol cleavage of hydroxyquinol (1,2,4-trihydroxybenzene), an intermediate in the degradation of a variety of aromatic pollutants, to maleylacetate, has been recently purified to homogeneity. The enzyme is an homodimer composed of two identical subunits in a α_2-type quaternary structure, has a molecular weight of about 65 kDa and contains a catalytically essential Fe(III) ion. Crystals of HQ1,2O obtained using 2% PEG 400 and 2 *M* ammonium sulfate at pH 7.5 as precipitants belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 81.15 (6), b = 86.79 (7), c = 114.93 (8). Assuming one dimer per asymmetric unit, the V_m value is 2.51 Å^3 Da^{-1}. A complete native data set to 1.8 Å resolution has been collected on a laboratory source. This is the first intradiol dioxygenase which specifically catalyzes the cleavage of hydroxyquinol to give diffraction-quality crystals.

1. Introduction

The aerobic metabolism of chloroaromatics, a class of compounds highly recalcitrant to biodegradation, generally occurs through two pathways depending on the number of chlorine substituents on the aromatic ring (Chaudhry & Chapalamadugu, 1991; Reineke & Knackmuss, 1988). Those compounds having one or two chlorines are usually converted to chlorocatechols and then catabolized through the well known modified *ortho*-cleavage pathway, whereas those containing two or more chlorine substituents (the most persistent pollutants) are converted to hydroxyquinol or chlorohydroxyquinol (Apajalahti & Salkinoja-Salonen, 1987; Joshi & Gold, 1993; Kozyreva *et al.*, 1993; Li *et al.*, 1991; Sangodkar *et al.*, 1988; Sze & Dagley, 1984). Both chlorocatechols and hydroxyquinols are aerobically degraded by intradiol dioxygenases. Generally these dioxygenases have high substrate specificity, *i.e.* a catechol dioxygenase cannot catalyze the hydroxyquinol cleavage and a hydroxyquinol dioxygenase cannot cleave catechol. The hydroxyquinol 1,2-dioxygenase (HQ1,2O) catalyzes the intradiol cleavage of hydroxyquinols to 3-hydroxy-*cis*,*cis*-muconates (keto form, maleylacetate).



Recently, several HQ1,2Os have been purified from a variety of microorganisms such as Gram-negative (*Burkholderia cepacia* AC1100, *Azotobacter* sp.) and Gram-positive (*Nocardioides simplex* 3E, *Streptomyces rochei* 303) bacteria and also yeast (*Trichosporon cutaneum*, *Phanerochaete chrysosporium*), but very little is known about the enzyme or the factors discriminating for substrate specificity of this novel group of intradiol dioxygenases (Daubaras *et al.*, 1996; Latus *et al.*, 1995; Zaborina *et al.*, 1995; Travkin *et al.*, 1997; Sze & Dagley, 1984; Rieble *et al.*, 1994). HQ1,2O is an homodimer of molecular weight of about 65 kDa, containing one Fe(III) ion essential for its activity and having quaternary structure α_2Fe(III). A recent X-ray absorption spectroscopy study shows that in the native enzyme the iron is five-coordinated, with an average Fe–L distance of 1.98 Å, and that histidines are present in the metal coordination sphere

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Table 1
Data-collection statistics for HQ1,2O.

Space group	$P2_12_12_1$
Unit-cell dimensions (Å, e.s.d. in parentheses)	
a	81.15 (6)
b	86.79 (7)
c	114.93 (8)
Total reflections measured	142485
Unique reflections	66716

Data-collection parameters *versus* resolution

Resolution bins (Å)	Number of reflections	Completeness (%)	Redundancy	$I/\sigma(I)$	R_{sym}†
To 3.88	7931	100.00	3.90	29.03	0.067
3.00	15565	100.00	3.84	17.44	0.069
2.69	22974	99.51	3.69	9.85	0.072
2.44	30085	98.30	3.57	7.20	0.075
2.27	36933	96.98	3.46	5.74	0.076
2.13	43564	95.66	3.37	4.88	0.082
2.03	49889	94.13	3.27	4.16	0.087
1.94	55837	92.37	3.18	3.86	0.122
1.86	61461	90.52	3.08	3.70	0.161
1.80	66716	88.61	2.99	4.20	0.185

† $R_{sym} = \sum |I_i - \langle I \rangle| / \sum \langle I \rangle$, where I_i is an individual intensity measurement and $\langle I \rangle$ is the average intensity for this reflection with summation over all the data.

(Briganti *et al.*, 1998). The study also shows that five-coordination is maintained in the enzyme–substrate complex and that the first metal coordination sphere is not affected by substrate binding.

To date, only the X-ray structures of one intradiol dioxygenase, the 3,4-protocatechuate dioxygenase (3,4PCD) from *Pseudomonas aeruginosa*, and its adducts with substrates and inhibitors have been determined (Elgren *et al.*, 1997; Ohlendorf *et al.*, 1988, 1994; Orville *et al.*, 1997). Diffraction-quality crystals have also been obtained and X-ray diffraction studies are in progress for a few intradiol dioxygenases with different substrate specificities, such as the catechol 1,2-dioxygenase isoenzymes from *Pseudomonas putida (arvilla)* C-1 and the 3,4PCD from *P. cepacia*, *Brevibacterium fuscum* and *Acinetobacter calcoaceticus* (Earhart, Hall *et al.*, 1994; Earhart, Radhakrishnan *et al.*, 1994; Ludwig *et al.*, 1984; Vetting *et al.*, 1993).

In this paper, we report the crystallization of hydroxyquinol 1,2-dioxygenase. X-ray data have been also collected on native enzyme crystals providing a complete data set at 1.8 Å. This is the first dioxygenase which catalyzes the degradation of 1,2,4-trihydroxybenzene to be crystallized and shown to produce diffraction-quality crystals. The elucidation of the three-dimensional structure of this enzyme appears to be the only means for providing information about the specificity of the site and to allow progress in understanding the mechanistic aspects of HQ1,2O.

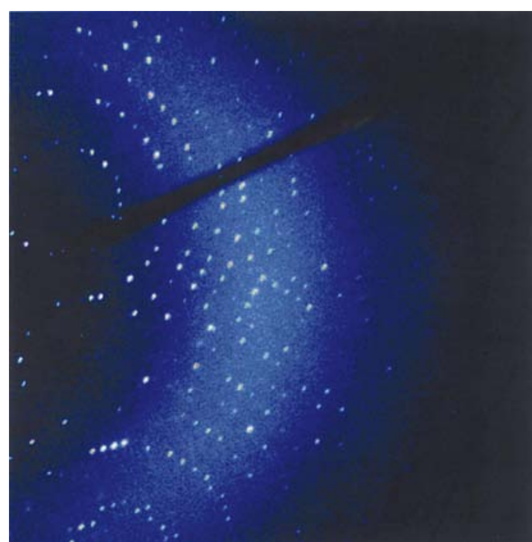

Figure 1
Area-detector frame.

2. Materials and methods

HQ1,2O from *Nocardioides simplex* 3E was purified as previously reported (Travkin *et al.*, 1997) and then dialyzed and concentrated against 50 m*M* potassium phosphate pH 7.2 buffer to a final concentration of 24 mg ml^{-1}. Initial crystallization trials were carried out using the sparse-matrix approach (Crystal Screen kit I; Hampton Research) and sitting-/hanging-drop methods. 5 µl of a 5–15 mg ml^{-1} enzyme solution in 50 m*M* potassium phosphate buffer were mixed with 5 µl of precipitant solution and then equilibrated by vapour diffusion against 100 µl of precipitant solution. Crystals were obtained from different precipitants, including 15–32% PEG 4000 at pH 8.0–8.5 and 20% PEG 6000 at pH 8.5 in the presence of salts such as 0.1–0.2 *M* sodium citrate and sodium acetate. The most promising crystal form was obtained at 283 K from PEG 400 and ammonium sulfate. Crystallization conditions were then refined by varying the pH and the protein and precipitant concentrations. The best results were obtained with a 12 mg ml^{-1} enzyme solution, 2–10% PEG 400 and 2.0–2.5 *M* ammonium sulfate in 100 m*M* HEPES buffer at pH 7.5. Attempts to grow large and regularly shaped crystals were made using different additives to reduce nucleation and to slow the crystal growth. Very large and regularly shaped prismatic crystals were finally obtained by the hanging-drop method from a solution containing ammonium sulfate 2.0 *M*, 2% PEG 400 and 10% glycerol at pH 7.5 in 100 m*M* HEPES buffer at 283 K.

3. Results and discussion

Diffraction data were collected with a Siemens SMART 1K CCD detector using Cu $K\alpha$ radiation from a Siemens M12X-HF rotating-anode X-ray generator operating at 45 kV and 90 mA. Göbel mirror optics were used to obtain a monochromated and highly parallel X-ray beam. Data were collected at 277 K to a maximum resolution of 1.65 Å from one crystal sealed in a 1.0 mm Lindemann glass capillary tube with a small portion of mother liquor. The HQ1,2O crystal was stable in the X-ray beam for 24 h at 277 K. The dimensions of the crystal used for data collection were approximately 0.4 × 0.4 × 1.0 mm. Data collection consisted of 0.25° ω scans at different φ values using a crystal-to-detector distance of 50 mm and a detector swing angle (2θ) of 25°. A total of 774 frames were collected to cover the full reciprocal-space unique portion with a redundancy of 3.0. The crystal was only slightly affected by radiation damage in the last steps of data collection after about 24 h of irradiation, by which time a complete data set had already been collected. The diffraction data were processed and reduced using the *SAINT* software and resulted in 66716 unique observations at 1.8 Å resolution. Details of the data collection are reported in Table 1.

The crystal unit cell was initially determined by indexing 512 reflections from 75 frames collected by 0.25° ω scans on the CCD detector, and subsequently refined by using 8129 reflections from a complete native data set. The crystal space group has been univocally assigned by systematic absences to be orthorhombic $P2_12_12_1$, with unit-cell parameters a = 81.15 (6), b = 86.79 (7), c = 114.93 (8). On the assumption of one α_2 dimer per asymmetric

unit, the V_m value was calculated to be 2.5 Å^3 Da^{-1} (Matthews, 1968).

The search for suitable heavy-atom derivatives is in progress.

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