Chlorocatechol 1,2-dioxygenase from the Chlorophenol-utilizing Gram-positive Rhodococcus opacus 1CP: Crystallization and preliminary crystallographic analysis

Maria Yolanda Ruiz Tarifa¹, Marta Ferraroni¹, Fabrizio Briganti¹, Andrea Scozzafava¹, Stefano Mangani², Ludmilla Golovleva

¹ Dipartimento di Chimica, Università di Firenze, Via Gino Capponi, 7 I-50121 Firenze, Italy
² Dipartimento di Chimica, Università di Siena, Pian dei Mantellini, 44 I-53100 Siena, Italy
³ Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, 142292 Pushchino Moscow region, Russia.

The capability of Gram-positive bacteria to use chloroaromatics compounds as sole carbon and energy sources has been only recently observed. The majority of the representatives of the nocardiaform genus Rhodococcus do not show the presence of a modified ortho-cleavage pathway, first identified in Gram-negative strains for the assimilation of chloroaromatics.

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 [1-2]. Those compounds having one or two chlorine are usually converted to chlorocatechols and then catabolized through the modified ortho-cleavage pathway, well studied in Gram-negative bacteria, whereas those containing two or more chlorine substituents are converted to hydroxyquinol or chlorohydroxyquinol [3-4]. Chlorocatechol 1,2-dioxygenases are key enzymes of the modified ortho-cleavage pathway and they generally show high substrate specificity.

The chlorocatechol 1,2-dioxygenase (ClC1,2O) catalyzes the intradiol cleavage of chlorocatechols to chloro-cis,cis-muconates:

\[
\begin{align*}
\text{OH} & \quad \text{OH} \\
\text{Cl} & \quad \text{Cl} \\
\text{O2} & \\
\text{Cl} & \quad \text{COOH} \\
\text{COOH} & \\
\end{align*}
\]

Several ClC1,2Os have been purified from a variety of microorganisms but not much is known about the enzyme and the factors discriminating for substrate specificity of this novel group of intradiol dioxygenases.

ClC1,2O from Rhodococcus opacus (erythropolis) 1CP is an homodimer of molecular weight of about 64 kDa, containing one Fe(III) and one Mn(II) ions [5]. A recent X-ray absorption spectroscopy study shows that, in the native enzyme, the iron is five-coordinated with average Fe-L distance of 1.93 Å and that histidines are present in the metal coordination sphere [6].

To date, only the X-ray structures of a few intradiol dioxygenases, the 3,4-protocatechuate dioxygenase (3,4PCD) from Pseudomonas aeruginosa and Acinetobacter Sp. ADP1, the Catechol 1,2-Dioxygenases from Acinetobacter Sp. ADP1 have been determined [7-8].
Chlorocatechol 1,2-dioxygenase from *Rhodococcus opacus* (erythropolis) 1CP was crystallized using sitting drop vapour diffusion method. 1 µl of a 20 mg/ml protein solution in Tris·SO₄ 20 mM pH=7.2 with 1 µl reservoir solution and equilibrated against 50 µl of precipitant solution. Optimized crystallization condition contains 1.6 M ammonium sulfate, 0.1 M sodium chloride, 100 mM Tris·HCl pH= 9.0, 5-15% glycerol.

Crystals were successfully frozen under liquid nitrogen adding 30% glycerol to the mother liquor solution as cryoprotectant. A complete data set at 100 K was collected at the EMBL beamline BW7A, at the DORIS storage ring, Hamburg, Germany. Data were collected at 2.8 Å using a MAR CCD detector and a wavelength of 1.01 Å. The crystals belong to the primitive hexagonal space group P6,22 with cell dimensions a= 90.42 Å, c= 307.53 Å. Assuming one molecule per asymmetric unit, the solvent content is about 61% of the unit cell (Vm=3.13 Å³/Da). Data processing with Denzo and Scalepack gave 17339 unique reflections, an Rsymmetric of 8.3 and an overall completeness of 89.9%. All molecular replacement attempts using coordinates of the structures of intradiol dioxygenases known as a model have so far failed to provide a solution for chlorocatechol dioxygenase. Our efforts are currently being directed towards a search for heavy atoms derivatives and the solution of the structure using multiple isomorphous replacement.

Figure 1. Crystals of Chlorocatechol 1,2-dioxygenase from *Rhodococcus opacus* 1CP.

References