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

Maria Yolanda Ruiz Tarifa[1], Marta Ferraroni[1], Fabrizio Briganti[1], Andrea Scozzafava[1], Stefano Mangani[2], Ludmilla Golovleva

[1] *Dipartimento di Chimica, Università di Firenze, Via Gino Capponi, 7 I-50121 Firenze, Italy*

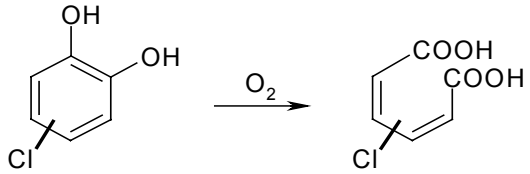
[2] *Dipartimento di Chimica, Università di Siena, Pian dei Mantellini, 44 I-53100 Siena, Italy*

[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:



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 $P6_322$ 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 $Å^3$/Da). Data processing with Denzo and Scalepack gave 17339 unique reflections, an R_{symm} 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

[1] G. O. Chaudhry, S. Chapalamadugu, Microbiol. Rev. 55, 59 (1991)
[2] W. Reineke, H-J. Knackmuss, Annu. Rev. Microbiol. 42, 263 (1988)
[3] J. H. A. Apajalahti, M. S. Salkinoja-Salonen, J.Bacteriol. 169, 5125 (1987)
[4] D. K. Joshi, M. H. Gold, Appl. Environ. Microbiol 59, 1779 (1993)
[5] O. V.Maltseva, I. P. Solyanikova, L. A. Golovleva, Eur. J. Biochem. 226, 1053 (1994)
[6] F. Briganti, S. Mangani, L. Pedocchi, A. Scozzafava , L. A. Golovleva, A. P. Jadan, I. P. Solyanikova, FEBS Lett. 433, 58 (1998)
[7] T. E. Elgren, A. M. Orville, K. A. Kelly, J. D. Lipscomb, D. H. Ohlendorf, L. Jr. Que, Biochemistry 36, 11504 (1997)
[8] D. H .Ohlendorf, J. D. Lipscomb, P.C. Weber, Nature(London) 336, 403 (1988)
[9] M. W, Vetting, D. H., Ohlendorf, *Structure (London)* 8, 429 (2000)