The crystal structure of a bacterial intradiol dioxygenases involved in the biodegradation of polychlorinated aromatic compounds

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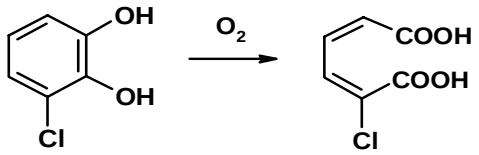
The aerobic metabolism of chloroaromatics, a class of compounds highly recalcitrant to biodegradation, generally occurs through two pathways depending on the number of chlorine substituents on the aromatic ring [1,2]. Those compounds having one or two chlorine are usually converted to chlorocatechols 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]. Both chlorocathecols and hydroxyquinols are aerobically degraded by iron(III) containing intradiol dioxygenases which have high substrate specificity.



Intradiol dioxygenases, involved in the biodegradation of polychlorinated aromatic compounds, have been purified from a variety of microorganisms in particular from Gram negative and also from Gram positive bacteria, but not much is known about the enzyme and the factors discriminating for substrate specificity of this novel group of intradiol dioxygenases.

The x-ray structures of chlorocathecol 1,2-dioxygenases from Rhodococcus opacus 1CP have been solved by multiwavelength anomalous dispersion using the weak anomalous signal of two catalytic irons (1 Fe/257 aminoacids).

The enzyme is an homodimer composed of two identical subunits in an α_2 type quaternary structure (molecular weight of about 60 kDa) and contain a catalitically essential Fe(III) ion per protomer. Hexagonal crystals of 4-ClC1,2DO grew in 1.6 M ammonium sulfate, 0.1 M sodium chloride, Tris.HCl pH= 9.0, 5-15% glycerol.

A complete data set at 100 K extending to a maximum resolution of 2.7 Å was collected at the EMBL beamline BW7A, Hamburg, Germany. Data were collected using a MAR CCD detector and a wavelength of 1.01 Å. Crystals belong to the primitive hexagonal space group $P6_322$ with unit cell dimensions a=90.42, c=307.53 [5]. 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 replacements attempts using coordinates of known intradiol dioxygenases structures as a model failed to provide a solution for 4-ClC1,2DO.

A MAD data set was then collected at the iron absorption edge, inflection and remote wavelenght at the EMBL beamline BW7A. Data sets were processed with DENZO and SCALEPACK. Data processing statistics are reported in table 1.

The program SOLVE was used to identify the two iron sites.The 3.5 Å MAD phases were improved and extended to 2.5 Å by solvent flattening and histogram mapping using the program DM from the CCP4 program suite. The resulting map was of good quality and allowed the manual tracing of 442 out of 514 amino acid residues using the program QUANTA. Refinement of the model using the program Refmac (version 5) from the CCP4 program suite is currently in progress.

Table1. Data collection statistics

Data Collection	Fe peak	Fe inflection	Fe remote
Wavelength (Å)	1.7436	1.7439	1.5806
Limiting resolution (Å)	3.0	3.0	3.0
Unique reflections	15072	14647	14376
R_{sym}	0.071 (0.31)	0.068 (0.32)	0.084 (0.38)
Multiplicity	13.2	12.6	12.7
Completeness overall (%)	95.5 (95.4)	92.4 (93.7)	94.5 (95.2)
I/•(I)	32.7 (5.7)	33.3 (6.3)	30.5 (5.3)

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] M. Ferraroni, M.Y. Ruiz Tarifa, F. Briganti., A. Scozzafava, S. Mangani, I.P. Solyanikova, M.P. Kolomytseva, L. Golovleva Acta Cryst. D58, 1074, 2002