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X-ray Crystallographic Studies of Thermostable Extradiol Dioxygenases, Catechol 2,3-dioxygenase and Homoprotocatechuate 2,3-dioxygenase

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Introduction

Oxidative cleavage of catechol ring is one important catabolic process of the bacterial degradation of aromatic compounds. Dioxygenases catalyze the cleavage of catechol ring in either intradiol (*ortho*) of extradiol (*meta*) manner with incorporation of two atoms of oxygen. These enzymes have been used in bioremediation of polluted groundwater or soil contaminated with various aromatic compounds, ranging from monocyclic to polycyclics.

Catechol 2,3-dioxygenase (metapyrocatechase, MPC; EC 1.13.11.2) and homoprotocatechuate 2,3-dioxygenase (3,4-dihydroxyphenylacetate, HPCD; EC 1.13.11.15) from an extreme thermophile, *Thermus thermophilus* HB8 consists of 324, and 319 amino acid residues, respectively, with molecular weight of 37 kDa. Both MPC and HPCD are iron-dependent enzymes with non-heme Fe(II) as a sole cofactor, and form homotetramer in solution.

Results

T. thermophilus MPC and HPCD are expressed, purified and crystallized. The crystals of MPC were obtained from PEG 3,000 and isopropanol solutions by the hanging-drop vapour-diffusion method. The crystals of MPC and HPCD belong to space group *C*2 and $P6_{1/5}22$, respectively (Fig. 1).

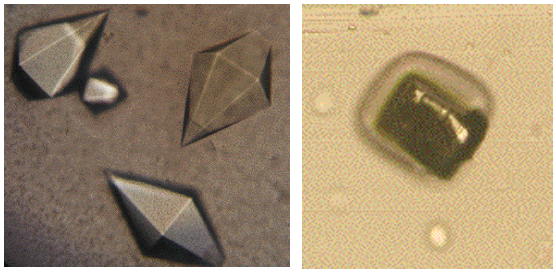

Fig. 1 The crystals of *T. thermophilus* HPCD (left) and MPC (right)

HPCD crystals diffracted up to 1.8 Å resolution, whereas MPC crystals diffracted up to only 3.35 Å resolution. The diffraction data from MPC crystals were collected at BL-18B beamline. Diffraction data statistics are given in Table 1.

Table 1: Diffraction data statistics

Dataset	MPC native
Beamline	BL-18B
Wavelength (Å)	1.0
Space group	*C*2
Unit-cell parameters	a = 121.3 Å, b = 111.3 Å , c = 82.2 Å, β = 97.1 ˚
Resolution limits (Å)	3.35
Observed reflections	37,621
Unique reflections	13.345
Completeness (%)	96.5
Redundancy	5.7
R_{meas} (%)	13.0

Molecular replacement of *T. thermophilus* MPC is now in progress.

References

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