X-RAY CRYSTALLOGRAPHIC STUDIES OF CATECHOL 2,3-DIOXYGENASE, METAPYROCATECHASE

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Catechol dioxygenases catalyze the ring cleavage of catechol and its derivatives in either an intradiol or extradiol manner. These enzymes have a key role in the degradation of aromatic molecules in environment by soil bacteria. Catechol 2,3-dioxygenase catalyzes the incorporation of dioxygen into catechol and the extradiol ring cleavage to form 2-hydroxymuconate semialdehyde.

Catechol 2,3-dioxygenase (metapyrocatechase, MPC) from *Pseudomonas putida* mt-2 was the first extradiol dioxygenase to be obtained in a pure form and has been studied extensively. The initial crystals of MPC were obtained from sodium citrate solutions by the vapour-diffusion method. After the stepwise macroseeding, best crystals those were suitable for diffraction works were obtained [1]. The three-dimensional structure of MPC has been determined at 2.8 Å resolution by the multiple isomorphous replacement method. The diffraction data were collected with a screenless Weissenberg camera at BL-6A beamline. The enzyme is a homotetramer with each subunit folded into two similar domains. The structure of the MPC subunit resembles that of 2,3-dihydroxybiphenyl dioxygenase, although there is low amino acid sequence identity between these enzymes. The active-site structure reveals a distorted tetrahedral Fe(II) site with three endogenous ligands (His 153, His 214 and Glu 265), and an additional molecule that is most probably acetone [2].

To elucidate the ring-cleavage reaction mechanism, we planned the structure analysis of MPC complexed with *o*-nitrophenol, that is the inhibitor of MPC. The crystals were prepared by soaking to 0.5 or 0.1 mM *o*-nitrophenol solutions. X-ray diffraction data were collected up to 3.2 Å resolution. The large electron density was appeared near the Fe(II) atom and it is assumed to be the molecule of *o*-nitrophenol (Fig. 1).

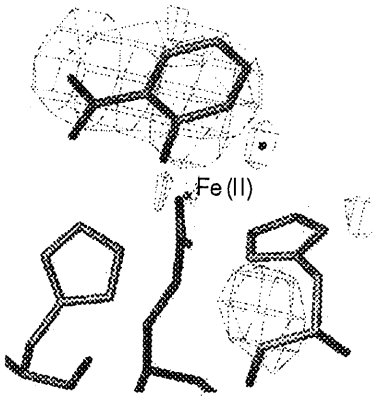

Fig. 1. Electron density around Fe(II)

References

[1] A. Kita, *et al*., *J. Biochem*. 122, 201-204 (1997).
[2] A. Kita, *et al*., *Structure* 7, 25-34 (1999).