Structure of a manganese-containing homoprotocatechuate 2,3-dioxygenase from *Arthrobacter globiformus* CM-2 at 1.5 Å resolution

Matthew W. Vetting and Douglas H. Ohlendorf
Center of Metals in Biocatalysis and Department of Biochemistry, Molecular Biology and Biophysics, 6-155 Jackson Hall, 321 Church Street, S.E., University of Minnesota Medical School, Minneapolis, MN 55455-0347 USA

Introduction

The biodegradation of natural and man-made aromatics is critical to both the global carbon cycle and the health of the environment. A class of enzymes termed "aromatic ring cleaving dioxygenases" are believed to be the most crucial step in catabolic pathways of bacteria that degrade aromatic compounds. Catechol dioxygenases catalyze the cleavage of dihydroxybenzene rings concomitant with the incorporation of molecular oxygen. Homoprotocatechuate 2,3-dioxygenase from *Arthrobacter globiformus* CM-2 (Ag 2,3-HPCD) is a member of a subclass termed extradiol dioxygenases that cleave the ring between the carbons adjacent to the two hydroxyls. These enzymes typically contain iron in the ferrous state, which is used to bind the substrates (dihydroxy aromatic compound and molecular oxygen), altering their electronic state to facilitate their interaction leading to catalysis.

Despite having over 85% sequence homology with homoprotocatechuate 2,3-dioxygenase from *Brevibacterium fuscum* (Bf 2,3-HPCD), a ferrous-containing extradiol dioxygenase, it was found Ag 2,3-HPCD used manganese as its catalytic metal [1, 2]. We have recently determined the structure of Bf 2,3-HPCD to 1.6 Å resolution using multiple isomorphous replacement, density modification techniques, and a high-resolution data set collected on beamline 19-ID at Argonne National Laboratory (ANL). The present report describes the collection of a high-resolution cryogenic data set of Ag 2,3-HPCD to yield insight into the metal specificity between the homologous enzymes.

Methods and Materials

Ag 2,3-HPCD was expressed and purified from an *Escherichia coli* clone to a specific activity of 14 U/mg [1]. A monoclinic form grew out of 10% Peg8000, 0.2 M Mg acetate, and 50 mM NaCacodylate (pH = 6.8). This crystal form had a plate-like morphology and belonged to space group C2 (a = 137.80 Å, b = 59.04 Å, c = 102.25 Å, and $\beta = 119.0^{o}$). A trypsin digest (Δ21 residues carboxy terminus) similar to that used for Bf 2,3-HPCD led to an Ag 2,3-HPCD exhibiting a more controlled nucleation and growth phase yielding thicker plates.

A high-resolution cryogenic data set was collected at ANL at beamline 19-ID. Cryofreezing was accomplished by exchanging the crystal mother liquor solution with a cryoprotectant solution consisting of 10% PEG8000, 0.2 M Mg acetate, 100mM Mops (pH = 7.5), and 15% glycerol. Crystals were mounted in a cryo loop and immersed in liquid nitrogen prior to placement on the x-ray source. Numerous crystals were frozen and checked for adequate diffraction prior to travel to the synchrotron. Only 5% of the crystals survived the freezing process, as they tended to freeze in an asymmetrical fashion leading to twinning and smearing of spots. Data were produced using monochromatic radiation (λ = 1.03321 Å) and collected using a 3 x 3 CCD array imaging detector. Diffraction intensities to a resolution of 1.5 Å were indexed with DENZO and scaled with SCALEPACK [3].

Results and Discussion

The 1.5 Å resolution of the synchrotron data set is an improvement over the resolution obtained for Bf 2,3-HPCD (1.6 Å) and that obtained for Ag 2,3-HPCD on a home source (1.8 Å). The structure of Bf 2,3-HPCD was used as a model to arrive at a molecular replacement solution for Ag 2,3-HPCD.

The structure of Ag 2,3-HPCD places it clearly as a type II extradiol dioxygenase. Type II extradiol dioxygenases are composed of two domains (N-terminal and C-terminal). Each of these domains is made of two structurally homologous $\beta\alpha\beta\beta\beta$ motifs that combine to form a β-barrel [4, 5]. As in the case of the ferrous iron in Bf 2,3-HPCD, the active site manganese of Ag 2,3-HPCD is coordinated by two axial ligands (His214^{NE2} and a water molecule) and four equatorial ligands (His155^{NE2}, Glu267^{O1}, and two water molecules) in an octahedral geometry. The quality of the model obtained (high resolution) is crucial to the comparison of two structures that appear to have high sequence and structural homology in the area of the active site. We are in the process of analyzing the limited number of differences between the two structures, especially in the active site cavity, that may explain the metal selectivity. These include differences in residues in the second and third sphere from the iron.

Acknowledgments

This work was supported by the National Institute of Health grant (GM-46436 to DHO) and the National Institute of Health Biophysics Training Grant (GM-07323 to M.W.V.). Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38.

I would like to thank Stephan L. Ginell, Ph.D.; Norma E.C. Duke, Ph.D.; Rongguang Zhang, Ph.D.; and Andrzej Joachimiak, Ph.D. who assisted in the collection of data sets obtained on beamline 19-ID at the ANL Structural Biology Center.

References

[1] Y.R. Boldt *et al*., *J. Bacteriol*. **177** (5), 1225–1232 (1995).

[2] A.K. Whiting *et al*., *Biochemistry* **35** (1), 160–170 (1996).

[3] Z. Otwinowski, *Proceedings of the CCP4 Study Weekend. Warrington: Daresbury Laboratory* (1993).

[4] S. Han *et al., Science* **270** (5238), 976 -980 (1995).

[5] L.D. Eltis *et al., J. Bacteriol*. **178** (20), 5930–5937 (1996).