

Structure studies of γ -carboxymuconolactone decarboxylase from *Acinetobacter sp. ADP1*

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

Research into the biodegradation of aromatics has intensified over the years since many of these compounds, especially those synthesized by man, have deleterious effects on the environment. The biodegradation of xenobiotic compounds suggests that organisms such as bacteria can fairly rapidly evolve catabolic pathways that work on these new compounds. There are, however, many synthetic aromatics that are not readily degraded and tend to accumulate in the environment. It has been suggested that existing enzymes, which catalyze a similar but distinct chemical reaction, are recruited in the assembly of new catabolic pathways [1]. The elucidation of the three-dimensional structures of the enzymes in catabolic pathways, therefore, is crucial to determining their evolutionary pedigree. This knowledge may be used to shed light on why some aromatics are not amenable to biodegradation.

The β -keto adipate catabolic pathway transforms 3,4-dihydroxybenzoate, a crucial intermediate in aromatic catabolism, into the citric acid cycle molecules succinate and acetyl-CoA. The enzyme γ -carboxymuconolactone decarboxylase from *Acinetobacter sp. ADP1* (Ac CMLD) is an enzyme in this pathway and catalyzes the decarboxylation of γ -carboxymuconolactone to β -keto adipate enol lactone [2]. Each monomer of Ac CMLD consists of 134 amino acids, and most likely exists as a hexamer in solution [3, 4]. Ac CMLD has no sequence homology to any other proteins of known function except for other CMLDs from other soil bacteria. Since the sequence was of no assistance in determining Ac CMLD's evolutionary pedigree, we sought to determine its molecular structure. The utter lack of any homology to other proteins with available crystal structures suggests that Ac CMLD may have a novel structure. The present report describes the collection of a high-resolution cryogenic data set of Ac CMLD.

Methods and Materials

Ac CMLD was expressed and purified from an *Escherichia coli* clone [4]. Crystals with a cube morphology grew out of high concentrations of PEG and Mg acetate. This crystal form was used for crystallographic studies of Ac CMLD at Argonne National Laboratory at beamline 19-ID. The mother liquor had cryoprotectant qualities so no glycerol was needed during the cryofreezing of the crystals. Crystals were mounted in a cryo loop and snap-cooled to liquid nitrogen temperatures by plunging them directly into liquid nitrogen. For the heavy-atom data sets, crystals were soaked in various heavy-atom solutions, frozen, and then checked for adequate diffraction prior to travel to the synchrotron. A native data set was produced using monochromatic radiation

($\lambda = .00726 \text{ \AA}$). Multiple anomalous dispersion data sets were also collected on crystals derivatized with mercury or platinum. For each of these, complete data sets were collected at the peak wavelength, at the inflection point, and at the remote wavelength. All data sets were collected using a 3 x 3 CCD array imaging detector. Diffraction intensities were indexed with DENZO and scaled with SCALEPACK [5].

Results and Discussion

A native data set was collected to a maximal resolution of 1.5 \AA resolution, with an R_{merge} of 4.2%. This is a greater than 0.5 \AA improvement in the diffraction seen on the home rotating anode source prior to transportation to the synchrotron source. The crystals were found to belong to the cubic space group I23 ($a = b = c = 128.97 \text{ \AA}$). Assuming a molecular weight of 15,324 for a monomer of the protein and two monomers per asymmetric unit yields a V_m of 3.06 $\text{\AA}^3/\text{dalton}$. It is possible that there may be a trimer per asymmetric unit as this yields an acceptable V_m of 2.21 $\text{\AA}^3/\text{dalton}$. The structure determination of Ac CMLD using the isomorphous and multiple anomalous dispersion methods are in progress.

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 Argonne National Laboratory Structural Biology Center.

References

- [1] G.A. Petsko *et al.*, *Trends Biochem. Sci.* **18** (10), 372–376 (1993).
- [2] R.N. Patel *et al.*, *Biochemistry* **12** (18), 3531–3537 (1973).
- [3] W.K. Yeh *et al.*, *J. Biol. Chem.* **255** (13), 6347–6354 (1980).
- [4] R.C. Doten *et al.*, *J. Bacteriol.* **169** (7), 3168–3174 (1987).
- [5] Z. Otwinowski, *Proceedings of the CCP4 Study Weekend. Warrington: Daresbury Laboratory* (1993).