

Crystallization and Preliminary X-ray Analysis of *dmpFG*-Encoded-4-Hydroxy-2-Ketovalerate Aldolase-Aldehyde Dehydrogenase (Acylating) from *Pseudomonas* Sp. Strain CF600

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Introduction: Environmental pollution by recalcitrant organic chemicals is a widespread problem that requires new technologies to solve. One promising approach is the use of microbial degradation activities to transform or destroy these compounds. Microbial degradation of one class of recalcitrant compounds, aromatics, involves oxygenative ring cleavage followed by one of a few central pathways for conversion to primary metabolites that can sustain microbial growth. One such pathway is the *meta*-cleavage pathway for catechol, which is involved in the degradation of phenols, toluates, naphthalene, biphenyls, and other compounds. Enzymes of the phenol degradation pathway of *Pseudomonas* sp. Strain CF600 are encoded by the *dmp* operon of the megaplasmid, pV1150. In the *meta*-cleavage pathway, the final steps for catechol degradation involve conversion of 4-hydroxy-2-ketovalerate to pyruvate and acetyl coenzyme A by the enzymes 4-hydroxy-2-ketovalerate aldolase (HOA) and aldehyde dehydrogenase (acylating) (ADA)(Scheme 1). Biochemical studies indicate that these two enzymes comprise a bifunctional heterodimer (DmpFG, a molecular mass 71kDa). In order to examine the organization of the active sites, the possibility of substrate channeling, and the catalytic mechanisms of the two reactions, we have undertaken structural studies of this heterodimeric bifunctional enzyme.

Methods and Materials: The DmpFG complex was purified from phenol-grown *Pseudomonas* sp. Strain CF600 following a modification of the previously described procedure (Powlowski *et al.*, 1993). Crystals of DmpFG complex grow in multiple fan-like clusters of thin plates by the hanging drop method and are improved by streak seeding. Heavy atom derivative screening identified three isomorphous derivatives. Diffraction data of native and the three heavy atom derivatives were measured at beamline X8C, Brookhaven National Laboratory, Upton, NY. All data were processed and scaled with DENZO and SCALEPACK from the HKL suite of software. Initial MIR phases were derived by using the automated program SOLVE. Density modification by solvent flattening and non-crystallographic averaging calculations were undertaken using program DM of the CCP4 suite of software.

Results: The crystals belong to orthorhombic space group $P2_12_12_1$ with unit cell dimensions of $a = 102.0\text{\AA}$, $b = 140.7\text{\AA}$ and $c = 191.3\text{\AA}$ and diffract to 2.1\AA resolution. Assuming four DmpFG complex molecules in the asymmetric unit, the V_m value is $2.4\text{\AA}^3/\text{Da}$, which is within the expected range. The V_m value corresponds to a solvent content of approximately 48%. A self-rotation function, calculated using the program POLARRFN revealed three significant peaks in the $\kappa = 180^\circ$ section suggesting the presence of three non-crystallographic two fold axes relating the four DmpFG molecules in the asymmetric unit. A search for suitable heavy atom derivatives for MIR phasing yielded three derivatives. Because of the low number of heavy atom sites and the relatively poor occupancies of these sites the automated program SOLVE was unable to phase the full structure. Initial phases, developed by MIR, were further improved by using solvent flattening and non-crystallographic averaging procedures but the resulting electron density map is discontinuous, making it difficult to trace the entire structure.

Conclusions: Most recently we have succeeded in expressing and crystallizing a selenomethionine variant of DmpFG and anticipate that the structure will be readily solved by incorporating carefully measured MAD data as well as the MIR phases that we currently have.

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References: Powlowski *et al.*, Journal of Bacteriology **175**, 377(1993)

