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Crystallographic analysis of complex structures of xenobiotics-degrading enzyme CumA1A2

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Introduction

The cumene degradation pathway of *Pseudomonas fluorescens* IP01 [1] begins with the hydroxylation of the aromatic ring by a cumene dioxygenase electron transport system (CumA) composed of three components. A flavin-containing ferredoxin reductase (CumA4) and a Rieske [2Fe-2S] ferredoxin (CumA3) transfer electrons from NADH to the terminal oxygenase component (CumA1A2). Resultant diol compound is converted to a catechol derivative (3-isopropylchatecol) by a dihydrodiol dehydrogenase (CumB). 3-Isopropylcatechol is attacked by an extradiol dioxygenase (CumC), resulting in a formation of a *meta*-cleavage product (MCP), namely 2-hydroxy-6-oxo-7-methylocta-2,4-dienoic acid (HOMODA). HOMODA is hydrolyzed by MCP hydrolase (CumD), yielding isobutyric acid and 2-hydroxypenta-2,4-dienoic acid (HODA). HODA is eventually converted into the intermediates of the central metabolism through the tricarboxylic acid cycle. We have already determined the crystal structure of CumD S103A mutant complexed with a cleavage product, isobutyric acid [2]. We conducted a comprehensive structure-based study on the cumene degradation system.

Results and Discussion

CumA1A2

We solved the crystal structure of cumene dioxygenase (CumA1A2) by the molecular replacement method using the structure of naphthalene dioxygenase (NDO; 1EG9) as a search model. The structure is refined to R = 19.1% and R_{free} = 21.4% at 2.3 Å resolution [3]. The overall structure was similar to NDO and composed of $\alpha_3\beta_3$ hexamer. The α subunit contains a Rieske [2Fe-2S] cluster and the active site with a non-heme mononuclear ferrous ion. Main chain traces of the loops forming the active site of CumA1A2 was largely deviated form those of NDO, and the shape of inner surface was also different.

CumA4

We succeeded in producing yellow crystals of CumA4. Diffraction data was collected at BL-6A up to 2.3 Å resolution. The space group was $C222_1$ and the cell constants were, a = 79.1, b = 178.3, c = 236.8 Å. The structure was solved by the molecular replacement method using the structure of ferredoxin reductase component of biphenyl dioxygenase (1F3P) as a search model. An asymmetric unit contained three subunits. The structure is currently under refinement.

CumC

Diffraction data of a crystal of CumC was collected at BL-18B up to 2.4 Å resolution. The structure was solved by the molecular replacement method using the structure of 2,3-dihydroxybiphenyl 1,2-dioxygenase (1LGT) as a search model. The structure is currently refined to R = 25.6% and R_{free} = 30.4%. However, one of the two CumC molecules in the asymmetric unit appeared severely disordered, and the electron density peak of the ferrous ion at the active site appeared to be too weak. We are trying to obtain more reliable structure of CumC.

CumD

In addition to the previously reported two complex structures of CumD (acetate and isobutyrate), we collected datasets of 6 complex structures (propionate, *n*-butyrate, *n*-valerate, isovalerate, (*S*)-2-methylbutyrate, and benzoate) at BL-6A and BL-18B [4]. Superimposition of the complex structures revealed that benzoic acid was bound in a significantly different direction compared with others, providing the structural basis of the strict substrate specificity of CumD enzyme. Moreover, the crystal structure of a mutant enzyme showing high activity was determined using the diffraction data collected at BL-6A [5].

References

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