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Crystal structure of glycine oxidase from *Bacillus thuringiensis*

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1 Introduction

Glycine oxidase (Gox, EC 1.4.3.19) catalyzes the oxidative deamination of small amines and D-amino acids. This enzyme has potential for industrial applications. We investigated Goxs derived from several gram-positive *Bacillus* species. However, those specific activities and stabilities were lower than industrial requirements. In the present study, we targeted a novel Gox from *Bacillus thuringiensis* (GoxBt). In preliminary experiments, GoxBt had resistance against to SDS, a commonly used anionic detergent. To elucidate the unique property of GoxBt, the three-dimensional structural information is needed. Here we report the crystal structure of GoxBt.

2 Experiment

GoxBt was expressed in *E. coli*, and purified by the following steps: ammonium sulfate fractionation, hydrophobic chromatography, cation exchange chromatography, and gel filtration chromatography. The purified enzyme showed a single band in SDS-PAGE.

GoxBt was crystallized at 20ºC by the hanging drop vapor diffusion method. 1μl of GoxBt was mixed with the same volume of reservoir solution (0.1M sodium acetate, pH 5.5, 0.2 M calcium acetate, 26% PEG 400). X-ray diffraction data collections were performed at KEK Photon Factory Structural Biology Beamlines (BL-5A etc.) at 95 K with reservoir solution added to 30% PEG 400 as a cryoprotectant. The structure was solved by molecular replacement method using Phaser with model structures of Gox from *Geobacillus kaustophilus* and *Bacillus subtilis* (PDB: 1RYI) [1]. The crystal structure was refined using COOT and REFMAC5.

3 Results and Discussion

The GoxBt crystal belongs to the *I*-centered orthorhombic space group *I*222, with unit cell constants of a = 74.25 Å, b = 237.05 Å, c = 307.61 Å, and contains six protein molecules per asymmetric unit. The structure was refined to 2.8 Å resolution. The crystal structure and gel filtration chromatography analysis show that GoxBt forms tetramer as Gox from *B. subtilis* (GoxBs). Each GoxBt monomer comprises nine α-helices, one 3_{10} helix, and seventeen β-strands, and contains one noncovalently-bound FAD molecule. The FAD-binding domain has the conserved Rossmann fold βαβ motif, which serves as a dinucleotide-binding motif. The monomeric structures of GoxBt and GoxBs overlap well (r.m.s.d. = ~1.5 Å). However, the tetrameric structures of GoxBt and GoxBs overlap roughly (r.m.s.d. = ~2.7 Å), suggesting that there is slight difference in subunit assembly between them. Analysis by Protein Interactions Calculator (PIC) [2] shows that their differences in types of intersubunit interactions: there are more hydrogen bonds and less hydrophobic interactions in intersubunit of GoxBt than in intersubunit of GoxBs.

In the active site pockets of GoxBt and GoxBs, the conformations of several residues are highly conserved. The substrate-binding residues, Tyr246 and Arg302 of GoxBs, correspond to Tyr252 and Arg308 of GoxBt, suggesting their common mechanism of enzymatic reaction. However, some differences near the active site, such as Phe247, Arg250, and flexible loop region (residues 53-59) of GoxBt may change its substrate specificity.

The present study provides new structural insights of GoxBt. To understand the unique property of GoxBt in detail, further study is currently in progress.

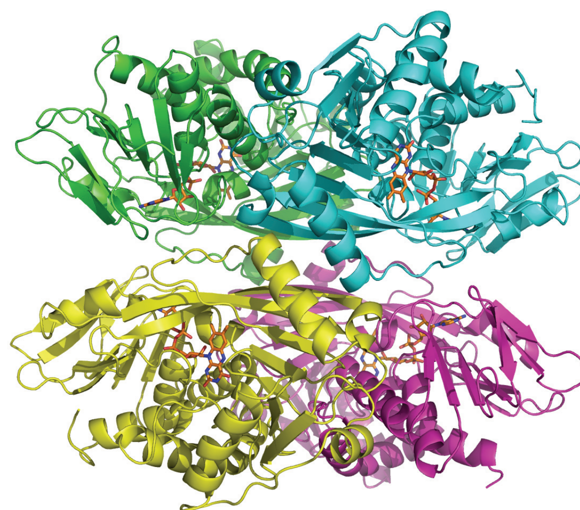

Fig. 1: Tetrameric structure of the GoxBt crystal structure.

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