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Crystal structure of glycine oxidase from *Geobacillus kaustophilus*

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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 Gox from *Geobacillus kaustophilus* (GoxGk). GoxGk has high thermal stability, but it is subject to substrate inhibition. To elucidate the characteristic properties of GoxGk, the three-dimensional structural information is needed. Here we report the crystal structure of GoxGk.

2 Experiments

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

GoxGk was crystallized at 20ºC using the hanging drop vapor diffusion method. 1 µl of GoxGk was mixed with the same volume of reservoir solution (7 % (v/v) 2-propanol, 0.1 M phosphate-citrate pH 4.7, 0.2 M $LiSO_4$). X-ray diffraction data collections were performed at KEK Photon Factory macromolecular crystallography beamlines at 95 K with reservoir solution added to 20 mM glycine as a substrate and 28 % (v/v) 2-propanol as a cryoprotectant. The structure was solved by molecular replacement method using Phaser with model structures of Gox from *Bacillus subtilis* (GoxBs; PDB code, 1RYI) [1]. The crystal structure was refined using COOT and REFMAC5.

3 Results and Discussion

The GoxGk crystal belongs to the hexagonal space group $P6_522$, with unit cell constants of a = 87.99 Å, b = 152.29 Å, c = 413.44 Å, and contains two protein molecules per asymmetric unit. The structure was refined to 2.2 Å resolution (PDB code 4YSH; R_{work} = 23.3%, R_{free} = 26.6%). The crystal structure and gel filtration chromatography analysis show that GoxGk forms tetramer in the same way as GoxBs. Each GoxGk monomer comprises eight α-helices, four 3_{10} helix, and sixteen 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. In the two monomer molecules of GoxGk per asymmetric unit, the substrate, glycine, bound to one molecule and did not bind to another molecule. The monomeric structures of GoxGk and GoxBs overlap well (r.m.s.d. = 1.2–1.3 Å). Analysis by Protein Interactions Calculator (PIC) [2] shows that their differences in types of intersubunit interactions: there are more hydrophobic and ionic interactions in intersubunit of GoxGk than those of GoxBs.

In the active site pockets of GoxGk and GoxBs, the conformations of several residues are highly conserved. The substrate-binding residues, Tyr246 and Arg302 of GoxBs, correspond to Tyr253 and Arg309 of GoxGk, suggesting their common mechanism of enzymatic reaction.

The present study provides new structural insights of GoxGk. To understand the characteristic properties of GoxGk in detail, further analysis is currently in progress.

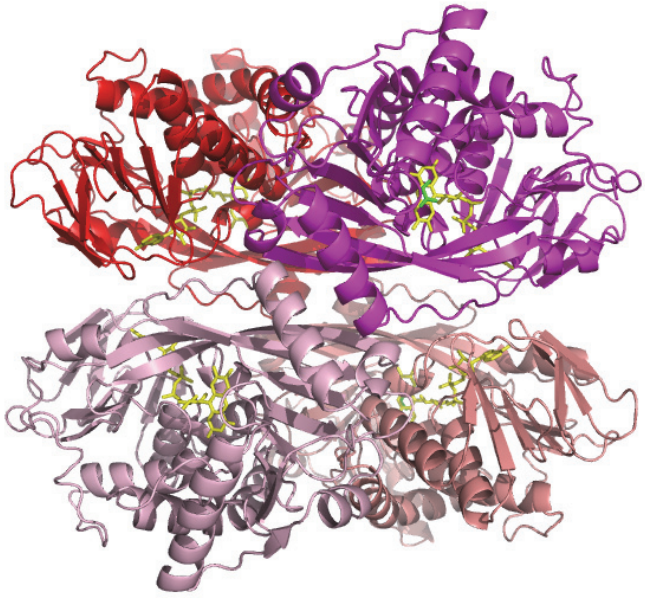

Fig. 1: Crystal structure of the GoxGk tetramer.

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References

[1] M. Mörtl *et al.*, *J. Biol. Chem.* **279**, 29718 (2004).
[2] K. G. Tina *et al.*, *Nucleic Acids Res.* **35**, W473 (2007).

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