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X-ray structure of fungus-derived FAD glucose dehydrogenase

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1 Introduction

The flavin adenine dinucleotide (FAD)-dependent glucose dehydrogenases (FADGDHs) comprise oxidoreductases that catalyze the oxidation of first hydroxyl group of glucose and other sugar molecules, using FAD as the primary electron acceptor. FADGDHs use a variety of external electron acceptors (but not oxygen). Recently, fungus-derived FADGDH has received attention as the enzyme for glucose monitoring, especially self-monitoring of blood glucose, exploiting its oxygen insensitivity and narrow substrate specificity. We determined X-ray structure of fungus-derived FADGDH, *Aspergillus flavus* glucose dehydrogenase (AfGDH) in complexes with D-glucono-1,5-lactone (LGC) [1].

2 Experiment

The expression and purification of recombinant AfGDH have been reported previously [2]. The purified protein solution was concentrated to 11.5 mg/ml for crystallization. After initial screening and optimization of the crystallization condition, well-diffracting crystals were obtained in a droplet containing 1.0 μ L of protein solution (11.5 mg/mL in 10 mM of potassium phosphate buffer, pH 6.5) and 1.0 μ L of reservoir solution (0.1 M of BisTris, pH 6.5, 22–25% PEG3350) against 80 μ L of the reservoir solution by the sitting-drop method at 293 K.

X-ray diffraction data were collected on the BL-5A in the PF. Diffraction data were processed using the programs HKL2000 and the CCP4 program suite. For obtaining a ligand-complexed structure, a single crystal was soaked in 15% (w/v) LGC in reservoir solution.

Initial phase determination and model building of AfGDH were performed by molecular replacement using the structure of AnGOx (PDB ID:1CF3) as a probe model.

3 Results and Discussion

The overall structures of AfGDH alone and in complex with LGC (AfGDH/LGC) are almost same and similar to that of the fungal glucose oxidases, *A. niger* glucose oxidase AnGOx (1CF3, 1GAL, 3QVP) reported till date, with r.m.s.d. 1.7 Å and 35% identity, and *Penicillium amagasakiense* glucose oxidase (PaGOx, 1GPE) with r.m.s.d. 1.7 Å and 34% identity by Dali search.

The structure of AfGDH consist of two major domains. The FAD-binding domain includes a three-layer ($\beta_3\beta_5\alpha_3$) [(B9, B10, B11,), (B8, B2, B1, B12, B20,), (H1, H5, H18)] sandwich structure with short eight α helices (H2, H3, H4, H6, H7, H8, H9, H17) and an irregular β sheet (B3, B4, B6, B7), and a long loop containing a short antiparallel β sheet (B17, B18). The C-terminal domain contains a large six-stranded antiparallel β sheet (B5, B14, B15, B16, B13, B19) surrounded by six α helices (H11, H12, H13, H14, H15, H16) and an additional short α helix (H10) (Fig. 1).

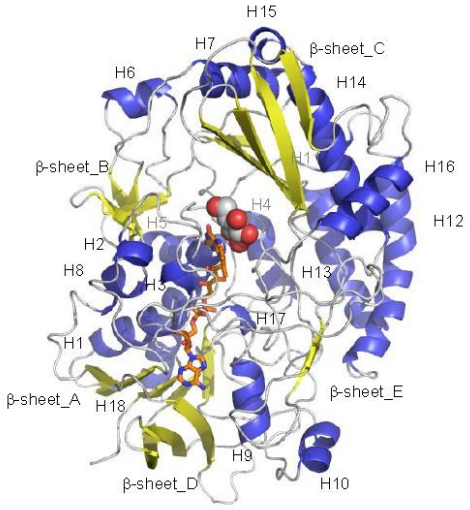

Fig. 1: Structure of FAD glucose dehydrogenase in complexed with glucono-δ-lactone.

The active site structure of AfGDH/LGC is compared with that of AnGOx in Fig. 2 (a) and (b). The residues interacting with LGC (Tyr53, Arg501, Asn503, His505, His548) were conserved in AnGOx. Only one residue, Glu413, was not conserved, and it corresponds to Asp424 in AnGOx. In AfGDH/LGC, the side chain of Glu413 causes reorientation and interacts with LGC [Fig. 2(a)]. In addition, Leu401 and Trp415 provide a hydrophobic environment around the bound LGC on the Si face, although the indole ring of Trp415 is unlikely to engage in a CH-π interaction with the pyranose ring of LGC. In the vicinity of the active site of AnGOx, Arg176 approaches the substrate-binding site and is located at the position forming hydrogen bonds with Asn217 and

Gly108 and has van der Waals contact with Gln347. Unlike AnGOx, the Arg176, Asn217, and Gln347 of AnGOx correspond to the Ser161, Ser201, and Ser333 of AfGDH. Thus, the Ser161 of AfGDH corresponding to the Arg176 of AnGOx belonging to the loop between B4 and H7 does not approach the active site, and a cavity is present in the vicinity of the substrate-binding site, whereas the conserved Arg and Gln of GOxs occupy a part of this cavity (Fig. 2 (c)).

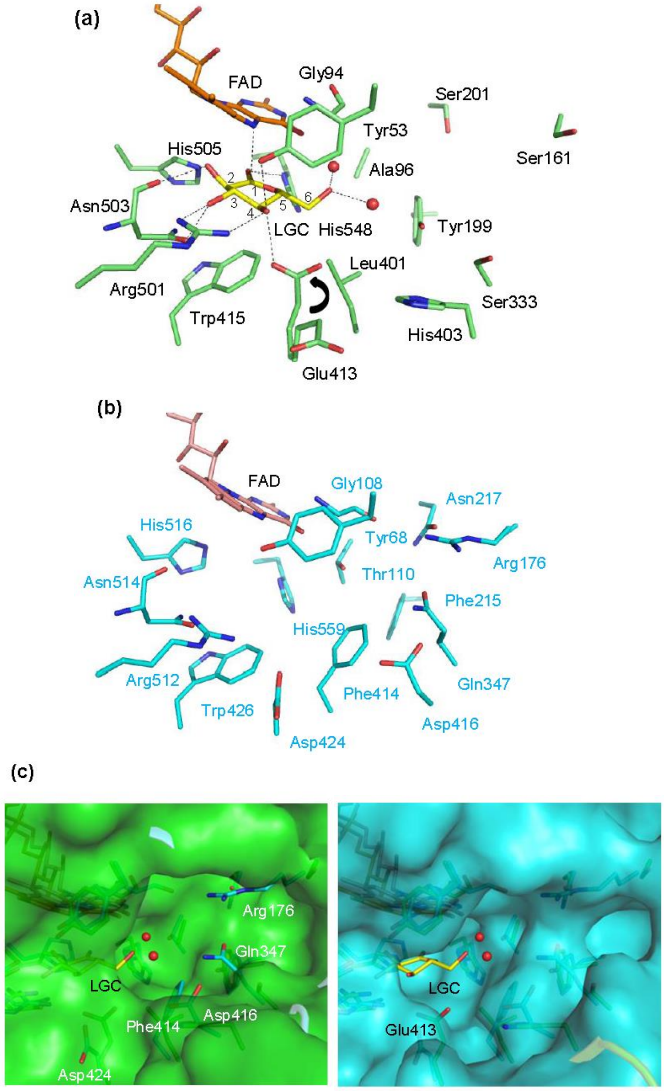

Fig. 2: Comparison of active site structures between AfGDH/LGC and AnGOx (1CF3).

Active site structures of (a) AfGDH/LGC (protein, green; FAD, orange; LGC, yellow) and (b) AnGOx (protein, cyan; FAD, pink). (c) Representation of the surface models of the active site in AfGDH/LGC (left, light green) and AnGOx (right, cyan). In the surface models, the active site structure of (b) was superimposed onto (a). Water molecules interacting with LGC are represented as red spheres.

Considering the difference of substrate specificity between AfGDH and AnGOx, the presence of the cavity in AfGDH may have a crucial role in the difference of the recognition of xylose compared with GOxs. The absence of residues that recognize the sixth hydroxyl group of the glucose of AfGDH and the presence of significant cavity in the active site may account for this enzyme activity toward xylose.

Acknowledgement

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References

[1] H. Yoshida et al., *Sci. Rep.* **5**, 13498 (2015).
[2] G. Sakai et al., *Biotechnol. Lett*. **37**, 1091-1099 (2015).

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