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X-ray structure of L-lactate oxidase from *Enterococcus hirae* in complex with a product "D-lactate form ligand"

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1 Introduction

L-Lactate oxidase (LOx) belongs to a family of flavin mononucleotide (FMN)-dependent α-hydroxyacid oxidizing flavoproteins. To date, the structures of LOx and mutagenesis studies have been reported, and its substrate recognition mechanism has also been elucidated, however, its substrate/product inhibition mechanisms are yet to be elucidated. In this study, we determined the X-ray structure of LOx derived from *Enterococcus hirae* (EhLOx) in complex with "D-lactate form ligand", which was covalently bonded with Tyr211. This structure is the direct evidence to support a new hypothesis of the product-inhibition mechanism of LOx [1].

2 Experiment

The recombinant EhLOx was used for crystallization. Crystals were obtained in a droplet containing a mixture of 0.8 µl protein solution (10-12 mg/ml in 20 mM potassium phosphate buffer pH 7.0) and 0.8 µl reservoir solution (0.1 M Tris–HCl, pH 8.0, 30% (w/v) Polyethylene glycol monomethyl ether 2,000) in a well containing 50 µl reservoir solution using the sitting-drop method at 293 K. To obtain the structure in complex with substrate, protein solution containing 0.4 M sodium pyruvate was used in the above crystallization condition.

X-ray diffraction data were collected on the PF BL-5A in the KEK, and processed using the programs XDS and the CCP4 suite. The structure was determined by molecular replacement with the program MOLREP using the structure of LOx from *Aerococcus viridans* (PDB ID 4YL2).

3 Results and Discussion

The structure of EhLOx in complex with D-lactate form ligand was determined at 1.7 Å resolution. EhLOx forms a homotetramer (Fig. 1). Each monomer of EhLOx has $(\beta/\alpha)_8$ TIM-barrel fold with FMN, same as other α-hydroxyacid oxidizing flavoproteins. In the complex structure of EhLOx, the whole flexible loop region (187–216) was ordered. The simulated-annealing omit maps (sa-omit maps) contoured at 4σ revealed the presence of the strong and continuous electron density maps from Cα of the ligand to the hydroxyl group of Tyr211 (Fig. 2).

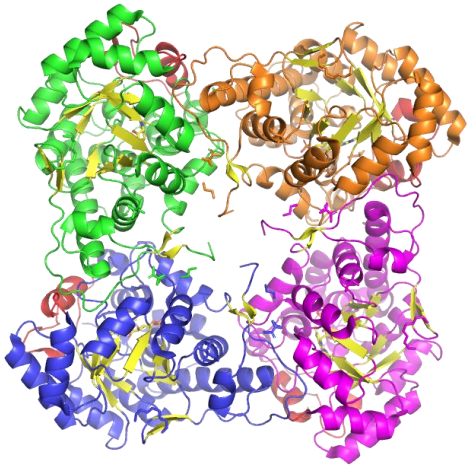

Fig. 1: Overall structure of homotetrameric EhLOx in complex with D-lactate form ligand. The flexible loop regions (187–218) are colored in red and all β-strands are colored in yellow.

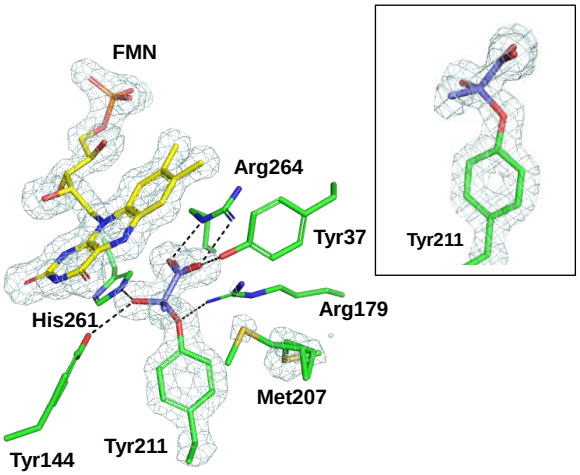

Fig. 2: Structure of EhLOx active site in complex with D-lactate form ligand (purple stick).

This observation revealed that Cα of the ligand was mainly bonded to Tyr211 in this complex structure, and the bound ligand does not fit an sp^2 configuration of pyruvate, but instead adapts sp^3 configuration of a Cα of D-lactate. Considering the additional covalent bond, the bound ligand was refined as "D-lactate" and hence called the ligand the "D-lactate form ligand." In this binding mode, the nucleophilic sp^3 N5 of reduced FMN cannot attack on α-proton of D-lactate form ligand because it orients to methyl group. Therefore, the observed D-lactate form ligand could bind in the active site as an inhibitor in EhLOx.

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Reference

[1] Hiraka, K. et al., Protein Science. 31, e4434 (2022).

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