

Structure of a cysteine (hydroxyl) lyase from hydrogen sulfide-producing oral pathogen, *Fusobacterium nucleatum*

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1 Introduction

Hydrogen sulfide (H₂S) is one of the predominant volatile sulfur compounds that are primarily responsible for oral malodor and is an important contributor to periodontitis. In the human oral cavity, H₂S is generally produced by enzymatic reactions of oral bacteria. β C-S lyase catalyzing the α,β -elimination reaction of L-cysteine to produce H₂S, pyruvate, and NH₄⁺ is known to be a common H₂S-producing enzyme. In *Fusobacterium nucleatum*, a heavy H₂S producer in the human oral cavity, two unique pyridoxal 5'-phosphate (PLP)-dependent H₂S-producing enzymes have been identified and characterized [1], in addition to β C-S lyase. One of the two enzymes is Fn1055 protein (cysteine (hydroxyl) lyase), which catalyzes the production of L-serine and H₂S from L-cysteine and H₂O (β -replacement reaction). This enzyme strictly prefers L-cysteine as a substrate. Interestingly, *F. nucleatum* lacks genes encoding the three enzymes essential to bio-synthesize L-serine from 3-phosphoglycerate, which are generally found in bacteria such as *Escherichia coli*. Therefore, Fn1055 seems to be important to control L-serine levels in this bacterium. As a first step to reveal the reaction mechanism of the enzyme, we performed the structural analysis.

2 Experiment

Fn1055 from *F. nucleatum* subsp. *nucleatum* ATCC 25586 was overproduced in *Escherichia coli* and purified as described earlier [2]. Crystals of Fn1055 were obtained by the hanging-drop vapor diffusion method. Rod-like crystals were obtained under condition #42 of PEGRx1 [4% (w/v) PEG8000 and 0.1 M Hepes pH 7.5] after initial screening trials of approximately 1000 conditions. A dataset from the crystal was collected at the beamline NW12A. The data was indexed, integrated, and scaled using the programs MOSFLM and SCALA, as implemented in XIA2. The phase determination was performed by the molecular replacement technique using a homology model on the basis of the structure of *O*-acetylserine sulfhydrylase from *Thermotoga maritima* (PDB ID: 1O58). The model was fixed and refined using COOT and REFMAC5.

3 Results and Discussion

The crystal structure of Fn1055 was refined at 2.9 Å resolution. A monomer of Fn1055 is situated in the asymmetric unit. The gel-filtration experiment suggests

that the enzyme is present as a dimer (data not shown) and the two subunits are related by a crystallographic two-fold axis.

The structure revealed an Fn1055-specific residue, Asp²³², which is likely to be important for the nucleophilic attack of water molecules to the α -aminoacrylate intermediate. The amino acid sequences of the loop containing Asp²³² differ between Fn1055 and related β -replacement enzymes. Therefore, the loop structure could contribute the substrate specificity of the enzymes.

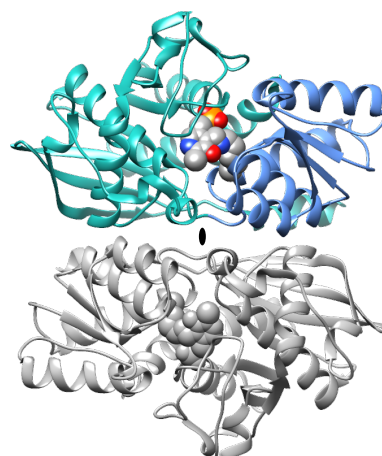


Fig. 1 Dimeric overall structure of Fn1055

The figure is shown in a ribbon representation. Whereas one subunit is colored in blue (N-terminal domain) and green (C-terminal domain), the other in gray. The PLP-cofactor is positioned between the domains and covalently bound to the ϵ -amino group of Lys⁴⁶.

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References

- [1] Y. Yoshida *et al.*, *Microbiology*, **157**, 2164-2171 (2011).
- [2] Y. Kezuka *et al.*, *Biochem. J.* **475**, 733-748 (2018).

The atomic coordinates and structure factors of Fn1055 have been deposited to Protein Data Bank as # 5B53.

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