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Crystal structure of hydrogen sulfide-producing enzyme from a periodontal pathogen

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1 Introduction

Hydrogen sulfide (H_2S) is one of the predominant volatile sulfur compounds that are primarily responsible for oral malodor and contribute to the progress of periodontal disease. H_2S in the human oral cavity is generally produced by enzymatic reactions of oral bacteria. *Fusobacterium nucleatum*, a Gram negative periodontal pathogen, is known to be a heavy H_2S producer. For now, four genes (*fn0625*, *fn1055*, *fn1220*, and *fn1419*) encoding pyridoxal 5'-phosphate (PLP)-dependent H_2S-producing enzymes have been identified and characterized in *F. nucleatum* ATCC 25586. Of the four enzymes, Fn1220 protein is a unique H_2S-producing enzyme, which produces H_2S and an uncommon amino acid, L-lanthionine from L-cysteine [1]. Fn1220 displayed not only the highest turnover rate but also the highest transcription level of the gene. The contribution of Fn1220 to H_2S production from L-cysteine was tentatively estimated to be 87.6% [2]. The *cdl* gene idenfified in *F. nucleatum* ATCC 10953 [3] is a *fn1220* ortholog, and their gene products, Cdl and Fn1220, share 92% amino acid sequence identity.

Elucidation of the reaction mechanism of Fn1220 and Cdl will provide important insights into H_2S production in *F. nucleatum*. Here, we report the crystal structures of Cdl.

2 Experiment

Cdl was prepared as reported earlier [3, 4]. Crystals of Cdl were obtained by the hanging-drop vapor diffusion method at 20°C. After initial screening trials using commercially available kits, rod-like crystals were obtained [4]. A native dataset was collected at BL-5A using a Quantum CCD detector (Area Detector Systems Corporation). The diffraction data were indexed, integrated and scaled using MOSFLM and SCALA as implemented in XIA2. Phase determination was performed by molecular replacement technique. A homology model of Cdl was constructed on the basis of structure of *O*-acetyl- L-serine sulfhydrylase (PDB entry: 2q3b), and used for the search model. Model building and adjustment were performed using COOT and the structure was refined using REFMAC5.

3 Results and Discussion

The structure of Cdl was refined at 1.91 Å resolution. The *R*-factor and Free *R*-factor were 17.6% and 22.6%, respectively. The crystal contained four subunits in the asymmetric unit. Structure analysis by PIZA web server shows that subunits A-B and C-D form homodimers. Cdl is an α/β protein and the PLP cofactor is positioned at the bottom of active site cleft (Fig. 1a). The electron density from the ε-amino group of Lys42 to the C4′ atom of PLP indicates the formation of a covalent internal aldimine linkage (Fig. 1b). No structures in complex with substrate or intermediate have been determined yet.

In related enzymes such as *O*-acetyl-L-serine sulfhydrylases, active site closure associated with substrate binding has been observed. We expected that the subunits were in an open form. Although no substrates/its analogs were bound to the subunits, active sites in all four subunits were closed. It may be caused by crystal packing, and analysis of Cdl crystals with other forms will provide insights into the closure.

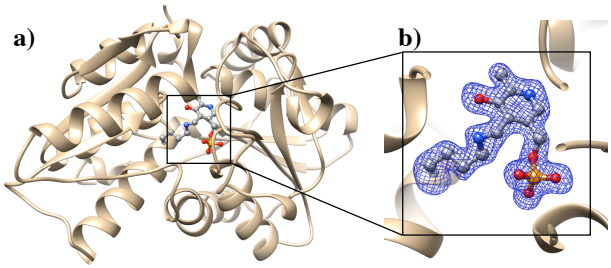

Fig. 1: Structure of Cdl a) Ribbon representation of Cdl subunit. The PLP cofactor covalently bound to Lys42 is shown by ball-and-stick drawings. b) Close-up view of the PLP. Final F_o-F_c omit electron density for the PLP and Lys side chain are superimposed.

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