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Crystal structures of βC-S lyase from *Streptococcus anginosus* in complex with its reaction intermediates

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1 Introduction

Hydrogen sulfide (H_2S), a pungent gas, is one of the predominant volatile sulfur compounds responsible for oral malodor. Previous studies have suggested a correlation between periodontal diseases and oral malodor. Investigation of H_2S production by oral bacteria showed that *Streptococcus anginosus* has a high H_2S-producing capacity. One reason for this is the unique enzymatic character of βC-S lyase (Lcd), encoded by *lcd* gene, found in *S. anginosus*. βC-S lyase is a pyridoxal 5′-phosphate (PLP)-dependent enzyme that catalyzes the α,β-elimination of the sulfur-containing amino acids, such as L-cysteine and L-cystathionine. When Lcd acts on L-cysteine, H_2S is produced along with pyruvate and ammonia. The H_2S-producing capacity of βC-S lyases from the anginosus group represented by *S. anginosus* was much higher than that from other oral bacteria characterized to date.

As initial steps toward elucidating the relationship between the structure and properties of Lcd, we determined the crystal structures of substrate-free Lcd (internal aldimine form) and two reaction intermediate complexes (external aldimine and α-aminoacrylate forms).

2 Experiment

Crystals were obtained by the hanging-drop vapor-diffusion method at 20°C [1, 2]. Lcd crystals with reaction intermediates were prepared by soaking using the reservoir solution containing 25 mM L-serine, a substrate analog of Lcd. Structures were solved by molecular replacement technique with a homology model based on Cystalysin from *Treponema denticola* (PDB ID: 1C7N) as a search model. Detailed experimental procedures were previously described [2].

3 Results and Discussion

Lcd is an α/β protein composed of two domains. The crystals used for the analysis contained four subunits in the asymmetric unit. Structure analysis by PISA web server inditcated that Lcd forms a homodimer whose subunits are related by a two-fold axis. In the absence of any substrates and substrate analogs, the continuous electron density from the ε-amino group of Lys234 to the C4′ atom of PLP indicates the formation of a covalent internal aldimine linkage (Fig. 1a). The PLP is located at the bottom of the active site pocket formed by both domains of one subunit and by parts of the large domain from the other subunit. The pyridine ring of PLP is faced to the phenol ring of Tyr119. The phosphate group of PLP has a direct hydrogen bond with Tyr60 from the other subunit. These interactions by Tyr119 and Tyr60 are kept for binding to two reaction intermediates, identified as an external aldimine and α-aminoacrylate (Fig. 1b and 1c). These intermediates were trapped after 27 hours and >7 days of soaking, respectively. Furthermore, the guanidino group of Arg365 forms a salt bridge with the carboxyl moiety of the bound L-serine in the two forms (Fig. 1b and 1c). The structural information on the intermediate complexes enables us to suggest the catalytic mechanism in more detail [2], because the structures are thought to be snapshots along the catalytic cycle.

References

[1] Kezuka, Yoshida and Nonaka, *Acta Crystallogr. Sect. F* **65**, 874-877 (2009).

[2] Kezuka, Yoshida and Nonaka, *Proteins* **80**, 2447-2458 (2012).

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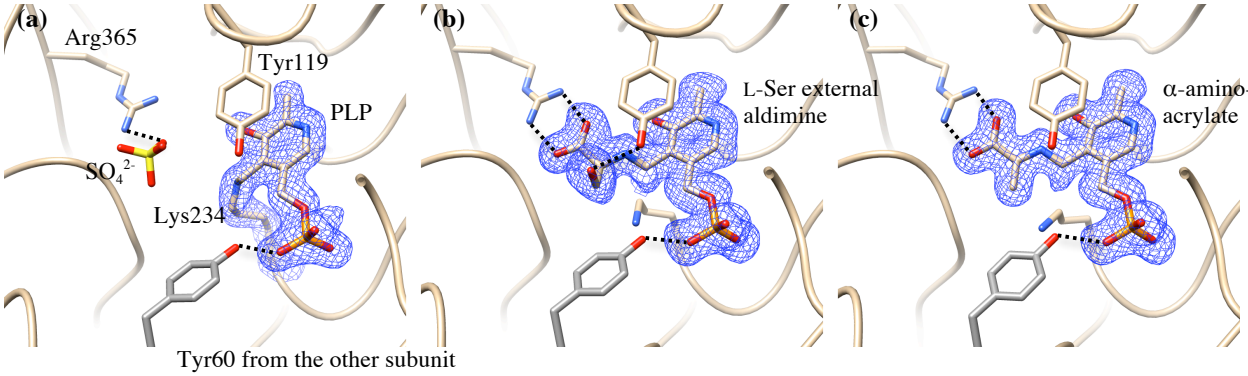

Figure 1 Close-up views of the active site with (b and c) and without (a) the substrate analog. Final F_o-F_c omit electron densities for the PLP and reaction intermediates are superimposed.