

## Structure of a phosphotransacetylase from *Porphyromonas gingivalis*, a keystone pathogen of periodontitis

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### 1 Introduction

*Porphyromonas gingivalis* is known to be a keystone pathogen of periodontitis and is an asaccharolytic bacterium that gains its metabolic energy by fermenting amino acids such as glutamate and aspartate. In the metabolic pathways, acetyl-CoA is produced as an intermediate and then is converted to acetate. Phosphotransacetylase (Pta, EC 2.3.1.8) and acetate kinase (Ack, EC 2.7.2.1) play a role for production of ATP from excess acetyl-CoA. Pta produces acetyl phosphate from acetyl-CoA and resulting acetyl phosphate is subsequently converted to acetate by Ack with the concomitant production of ATP through substrate-level phosphorylation. Despite the importance of Pta and Ack in energy metabolism in *P. gingivalis*, these enzymes have not been characterized. We report herein the crystal structure of Pta from *P. gingivalis*.

### 2 Experiment

Pta from *P. gingivalis* ATCC 33277 (*PgPta*) was overproduced in *Escherichia coli* BL21(DE3) strain as a glutathione *S*-transferase-fusion protein and purified as previously reported [1]. Crystals of substrate-free *PgPta* were obtained under the conditions, 20% (w/v) polyethylene glycol 3350 and 0.2 M ammonium tartrate dibasic using the hanging-drop vapor diffusion method. Crystals of *PgPta* in complex with acetyl-CoA were prepared by cocrystallization in the presence of 5 mM acetyl-CoA. Crystals were cryoprotected by soaking in mother liquor containing 10% (v/v) 2-methyl-2,4-pentanediol, and then data from the crystals were collected at the beamlines NW12A and NE3A. The data were indexed, integrated, and scaled using the programs DIALS 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 *Methanosarcina thermophila* Pta (*MtPta*) (PDB ID: 2AF4). The models were fixed and refined using COOT and REFMAC5.

### 3 Results and Discussion

The crystal structures of *PgPta* and its complex with acetyl-CoA were determined at 1.7 Å and 2.0 Å resolution, respectively. The subunit is an  $\alpha\beta$  protein composed of two  $\alpha\beta$  domains (Fig. 1).

Two acetyl-CoA molecules were bound to the dimer, and the binding site in each subunit is identical. Arg89 and Arg135 interact with the 3'-phosphate and  $\beta$ -phosphate of acetyl-CoA, respectively. Replacement of these residues to

alanine resulted in a 58% and 21% decrease in the specific activity, respectively. In *MtPta*, two CoA molecules were bound to each subunit [2], and the two binding sites were shown to have different affinities. One site (site 1) is suggested to be the active site on the basis of the mutational study. The other site (site 2) is suggested to be a loading site to preorient the substrate or a regulatory site to control the activity. Superposition of the structures of *PgPta* and *MtPta* revealed that acetyl-CoA molecules were bound only to site 2 in both subunits of *PgPta*, even though crystal packing of *PgPta* does not appear to interfere with binding of acetyl-CoA to site 1. Since interference of the binding of acetyl-CoA to site 1 of *PgPta* has not been observed in solution, such contradictory findings are possibly due to crystallization artifacts. Dynamic aspects, such as domain motion, can affect substrate binding and are difficult to estimate from the static picture revealed by a crystal structure. Further analyses to investigate the binding of acetyl-CoA to *PgPta* are required.

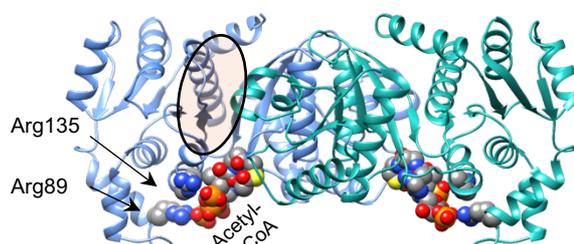


Fig. 1: Dimeric overall structure of *PgPta* in complex with acetyl-CoA. Each subunit is shown with independent colors. Bound acetyl-CoA, Arg89, and Arg135 are shown as spheres. The position with a circle is likely to correspond to the putative active site.

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### References

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The atomic coordinates and structure factors of *PgPta* and its complex with acetyl-CoA have been deposited to Protein Data Bank as # 6IOW and 6IOX, respectively.

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