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# Structure of an acetate kinase from *Porphyromonas gingivalis*, a keystone pathogen of periodontitis

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## 1 Introduction

*Porphyromonas gingivalis* is an asaccharolytic periodontal pathogen which mainly gains its metabolic energy from amino acids such as glutamate and aspartate. In the metabolic pathways, this bacterium has phosphotransacetylase ($CH_3COSCoA + HPO_4^{2-} \leftrightarrow CH_3CO_2PO_3^{2-} + HS\text{-}CoA$) and acetate kinase ($CH_3CO_2PO_3^{2-} + ADP \leftrightarrow CH_3COO^- + ATP$) for the conversion of acetyl-CoA to acetate with formation of ATP .

We reported that inactivation of phosphotransacetylase gene (*ack*) or acetate kinase gene (*ack*) in *P. gingivalis* by homologous recombination was successful only when the inactivated gene was expressed *in trans* [1]. These results strongly suggest that both genes are essential for this pathogen. Despite the importance of these two enzymes in energy metabolism in *P. gingivalis*, these enzymes have not been characterized. We report herein the crystal structure of acetate kinase from *P. gingivalis*.

## 2 Experiment

Acetate kinase from *P. gingivalis* ATCC 33277 (*Pg*Ack) was overproduced in *Escherichia coli* BL21(DE3) strain as a hexahistidine tag-fusion protein and purified as described earlier [1]. Crystals of *Pg*Ack were obtained under the conditions, 0.2 M lithium sulfate monohydrate, 0.1 M Tris/HCl pH 8.5, and 24% (*w*/*v*) polyethylene glycol (PEG) 3350 using the hanging-drop vapor diffusion method. Crystals were soaked in mother liquor containing 30% (*v*/*v*) PEG 3350 to cryoprotect, and then a dataset from the crystals was collected at the beamline NE3A. The data was 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 *Thermotoga maritima* Ack (PDB ID: 2IIR). The model was fixed and refined using COOT and REFMAC5.

## 3 Results and Discussion

The crystal structure of *Pg*Ack was refined at 1.9 Å resolution (Fig. 1). Two dimers are contained in the asymmetric unit of the $P2_1$ cell. The subunit is composed of the N-terminal domain (1-149 and 384-398) and the C-terminal domain (150-383). Comparison of the four subunits in the asymmetric unit indicated that there are four distinct conformations, with root mean square deviations ranging from 0.8 to 5.2 Å using all 398 Cα atoms. As shown in Fig. 2, the structure of C-terminal domain is almost identical, and the position of the N-terminal domains relative to the C-terminal domain are different, which is leading to opening and closing of the putative catalytic cleft. While the cleft of subunit A is the most open, that of subunit D is the most closed (Fig. 2). Subunits B and C take the intermediate conformations (data not shown). In the cleft of subunit D, there are two bound sulfate ions probably derived from mother liquor. This may mimic binding of the phosphate groups of ADP and/or ATP, resulting the most closed conformation. These results indicate a remarkable conformational change in *Pg*Ack during catalysis.

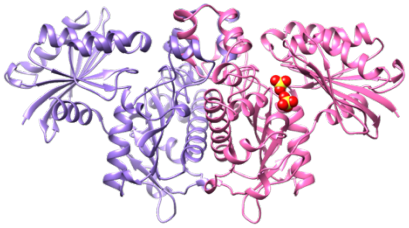

Fig. 1: Dimeric overall structure of *Pg*Ack (subunits C and D colored in purple and pink, respectively). Bound two sulfate ions are shown as spheres.

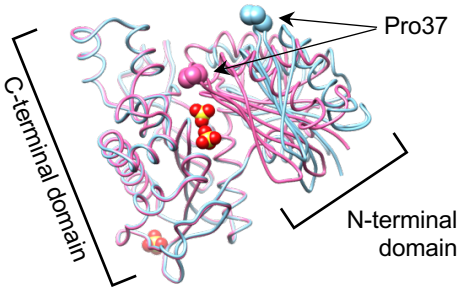


Fig. 2: Superposition of *Pg*Ack subunits A (light blue) and D (pink) using Cα atoms of the C-terminal domain. The bound sulfate ions and Pro37 positioned at the edge of the cleft are shown as spheres. The difference between the two Pro37 is ~18 Å.

## Acknowledgement

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## References

[1] Yoshida *et al.*, *J. Oral Microbiol.* **11**, 1588086 (2019).

The atomic coordinates and structure factors of *Pg*Ack have been deposited to Protein Data Bank as # 6IOY.

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