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# Crystal structure analysis of peptide ligase PGM1 from *Streptomyces cirratus*.

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## 1 Introduction

Peptide antibiotics form a large subclass of natural products, including clinically important pharmaceuticals. *Streptomyces cirratus* produces structurally unique pheganomycins (PGMs), which show anti-mycobacteria activity. PGM contains a nonproteinogenic amino acid and a proteinogenic core peptide. The biosynthetic gene cluster from *S. cirratus* responsible for PGM production was identified [1]. PGM1 was found in the cluster as potentially capable of linking between the precursor peptides and the nonproteinogenic amino acid with guanidinoacetic acid scaffold. PGM1 also accepted a variety of peptides as the nucleophile. To clarify the ligation mechanism between amino acid and peptide and understand the substrate's promiscuity, PGM1 structure was determined.

## 2 Experiment

*Crystallization* – 20 mg/ml selenomethionine-labeled PGM1 with or without adenylyl imidodiphosphate (AMPPNP) were crystallized with sitting-drop vapor diffusion method. 2 µl of PGM1 and 2 µl of reservoir solution were mixed, and equilibrated against 500 µl of reservoir solution at 20ºC. Diffraction-quality apo crystals were finally obtained in 0.1 M Tris-HCl, pH 8.3, 1.2 M lithium sulfate, and 10 mM L-methionine after a few days incubation. PGM1 with AMPPNP was crystallized 0.1 M Tris-HCl, pH 8.1, 1.2 M lithium sulfate and 10 mM AMPPNP after a few days incubation.

*Data collection and structure determination* – Crystals were transferred into the reservoir solution containing 10%(v/v) glycerol as a cryoprotectant, and were flash-frozen in a nitrogen stream. Single-wavelength anomalous diffraction (SAD) data of PGM1 apo and AMPPNP complex crystals were collected at beamline BL-17A and BL-5A under cryogenic condition at -173ºC, respectively. Wavelength of 0.97939 Å at BL-17A and 0.97901 Å at BL-5A were used for data collection on the basis of the fluorescence spectrum of the Se *K* absorption edge. The diffraction data was processed and scaled using *XDS*. Se sites were determined and refined and the initial phase of PGM1 was calculated with *AutoSol* in *PHENIX*. The initial phase of PGM1-AMPPNP complex was calculated by the molecular replacement (MR)-SAD method with *AutoSol* in *PHENIX* using PGM1 apo as the search model. The structure was rebuilt using *AutoBuild* in *PHENIX*, modified manually with *Coot* and refined with *phenix.refine* in *PHENIX*. The coordinates and structure factors have been deposited under accession number 3WVQ for the PGM1 apo, and 3WVR for the PGM1-AMPPNP complex.

## 3 Results and Discussion

X-ray structures of PGM1 apo and AMPPNP complex were refined at 1.96 and 2.17 Å resolutions, respectively. The final *R*-values of apo and complex structures were 19.4% and 20.2% ($R_{free}$ = 23.1% and 24.6%), respectively. Asymmetric unit contains four monomers, which form the two set of biologically active symmetric dimers. PGM1 consisted of four domains to form a central core (N, A, and C domains) and a upper (B domain) structures as seen in those of the ATP-grasp superfamily enzymes (Fig 1a). The central core and upper structures formed a large cavity at the center of the PGM1 structure. The ATP-binding site was located deeply inside the cavity. Most of the conserved resides lining PGM1's ATP-binding site were in a location and orientation similar to that in other ATP-grasp enzymes. PGM1 also formed a long cleft from the large cavity. The large cavity and the cleft created an unusual active site (Fig 1b). The site-directed mutagenesis stidies of E255, S316, R335, and S339 formed the cavity and those of A14 and S190 formed the cleft were suggested that the large cavity and the long cleft are enable to accept the guanidinoacetic acid scaffold substrates and the several peptides.

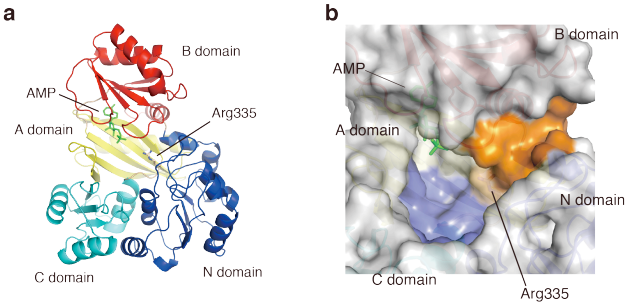


Fig. 1: Crystal structure of *S. cirratus* PGM1. (a) Overall structure of PGM1-AMPPNP complex. The electron densities of β- and γ-phosphates of AMPPNP were weak. Thus, the structure was built as PGM1-AMP complex. (b) Close-up view of the active site. The bottom of the large cavity and the cleft are highlighted in blue and orange, respectively.

## References

[1] M. Noike *et al*., *Nat. Chem Biol*. **11**, 71-76 (2015).

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