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Structure analysis of characteristic enzymes regarding phosphate addition or release

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1 Introduction

A free serine kinase (SerK) was recently identified in *Thermococcus kodakarensis* [1]. SerK is involved in the cysteine biosynthesis and phosphorylates L-serine (Ser) using ADP to produces *O*-phospho-L-serine (Sep), which is a precursor of L-cysteine.

SerK is expected to possess a novel reaction mechanism because of its low sequence similarity to known kinases. This enzyme had been annotated as a chromosome-partitioning protein ParB based on the primary structure. However, no kinase activity of ParB has been reported. Thus, we determined the crystal structures of SerK complexed with the substrate or product and proposed its reaction mechanism.

2 Experiment

We crystallized the wild-type (WT) or its mutants (E30A and E30Q) using poly(ethylene glycol) 3350 as a precipitant. Diffraction data sets were collected using the beamlines of BL-1A and BL-5A as well as some other X-ray sources. Phases were determined by molecular replacement with the atomic coordinates of PF0380 (Protein Data Bank ID: 1VK1). This protein is 85% identical to SerK, but it was annotated as a ParB-like nuclease. Finally, the six complex structures were determined at 1.43–2.0 Å resolution: WT-AMP, E30A-products, E30A-AMP, E30A-ADP, E30Q-products, and E30Q-AMP.

3 Results and Discussion

The WT enzyme was crystallized with Ser and AMP, but the determined structure possesses only AMP. No electron density map corresponding to Ser was observed. The overall structure is divided into two domains: domain I and II (Fig. 1A). AMP is bound to domain I.

We chose four acidic residues (E4, E30, E36, and D69) near to the α-phosphate of AMP as candidates for the catalytic residue (Fig. 2A), because many kinases deprotonate a hydroxyl group of the phosphate acceptors using the acidic residue. The four residues were each replaced with alanine, and the activities of the resultant mutants were measured. No activity of E30A and D69A mutants were detected, while E4A and E36A mutants exhibited significant activity. This suggests that E30 and D69 may be important for the catalysis.

The E30A and D69A mutants were crystallized with Ser and ADP in order to determine the structure just before the reaction. The E30A structure was determined, whereas that of D69A was not due to its poor diffraction. Unexpectedly, the products (Sep and AMP) are found in the E30A structure (Fig. 1B), suggesting that E30A can catalyze the reaction in a long crystallization period. The E30A-product complex possesses Sep in the cavity between the two domains and is in the closed conformation (Fig. 1B). This implies that the two-ligand binding leads to the conformational closure, which may occur with two substrates, Ser and ADP. Consistent with this implication, the E30-AMP complex is in an open form.

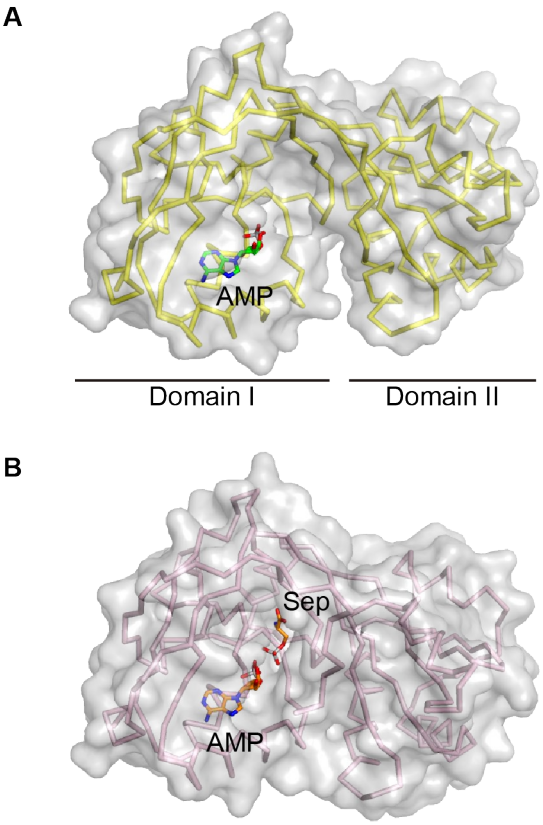

Fig. 1: Overall structures of the WT-AMP complex (A) and the E30A-product complex (B).

The structure indicates that three of the four catalytic residue candidates (E4, E36, and D69) are involved in the magnesium binding. The other (E30) is the probable catalytic residue because structural comparison between the WT-AMP and E30A-product complex suggests that E30 is located near to the Oγ atom of Sep (Fig. 2B). Supporting this suggestion, Q30 in the E30Q-product complex is nearer to the Oγ atom than that in the E30Q-AMP complex (Fig. 2C).

We also determined the structure of the E30A-ADP complex. The electron density corresponding to the β-phosphate of ADP (phosphate donor of the reaction) was ambiguous. Thus, we collected the data sets using X-ray

of the wavelength of 2.70 Å. The anomalous Fourier map corresponding to the β-phosphate was clearly observed. Comparison between the E30A-ADP and E30A-product complex led us to propose that, in the closed form, the β-phosphate of ADP may be recognized in the same manner as that of the phosphate group of Sep (Fig. 2D).

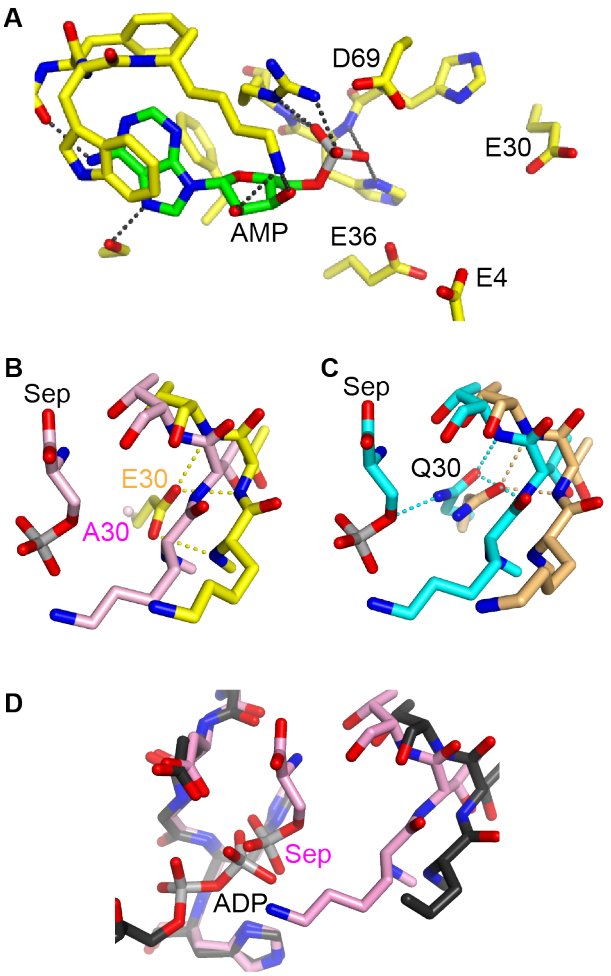

Fig. 2: Structures of the ligand-binding site. (A) AMP binding in the WT-AMP complex. (B, C) Comparison of E30 and the corresponding residues. The structures of WT-AMP, E30A-product, E30Q-product, and E30Q-AMP complexes are shown in yellow, pink, cyan, and light orange, respectively. (D) Structural comparison of the ligand-binding site between the E30A-product complex (pink) and the E30A-ADP complex (black).

Our structural analyses on SerK lead us to propose the reaction mechanism with the conformational shift. SerK is in the open form without its substrates, Ser and ADP, in solution. The substrate binding to the ligand-binding site results in the conformational closure. Then, the side chain of E30 approaches the hydroxyl group of Ser and deprotonates it. The deprotonated hydroxyl group attacks the phosphorus atom of the β-phosphate of ADP to produce Sep and AMP. Finally, Sep and AMP are released with opening of the cavity [2].

References
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[2] R. Nagata *et al., ACS Chem. Biol.* **12**, 1514–23 (2017)

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