The crystal structure of *Streptomyces* sp. sulfotransferase *St*Cpz8 involved in the biosynthesis of the antibiotic caprazamycin

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The actinomycete *Streptomyces* sp. MK730-62F2 produces liponucleoside antibiotics caprazamycins (CPZs), which have potent activity against *Mycobacterium tuberculosis* [1]. A previous study identified several kinds of CPZs containing a sulfate group at 2”-hydroxy of the aminoribosyl moiety [2], and a unique two-step sulfation mechanism during the biosynthesis of the CPZs [3]. In the first step, *St*Cpz8 uses universal sulfate donor PAPS to catalyze the sulfation of presulficidins, yielding PAP and sulficidins. In the second step, *St*Cpz4, a PAPS-independent sulfotransferase, transfers sulfate groups from sulficidins to CPZ. However, the structural insight into the two-step sulfation during CPZ synthesis remains unclear. In this study, we determined the crystal structure of *St*Cpz8 in complex with presulficidin analog KA4. The structure reveals the structural basis of the substrate recognition of *St*Cpz8.

We determined the two crystal structures: The *St*Cpz8-PAP binary complex and the *St*Cpz8-PAP-KA4 ternary complex. A clear electron density of KA4 in the substrate binding site of *St*Cpz8 was observed. The pyrone moiety of bound KA4 makes interactions with Asp67, Trp108, and Tyr120. Asp67 could serve as a catalytic base to facilitate sulfation reaction. The aliphatic chain portion is surrounded by many hydrophobic residues. The C-terminal α-helix of *St*Cpz8 covers the substrate-binding site. Compared to the *St*Cpz8-PAP structure, the C-terminal α-helix of the *St*Cpz8-PAP-KA4 structure is distinct position, suggesting that *St*Cpz8 undergoes a conformational change to form its substrate-binding site. The structure also revealed that the terminus of the aliphatic chain portion is exposed to the outside of the protein. This is consistent with the fact that *St*Cpz8 exhibits sulfation activity to presulficidins with various lengths of aliphatic chain portion. This study provides structural insight into the substrate specificity of *St*Cpz8 and is the first report on the mechanism of donor-substrate biosynthesis of PAPS-independent sulfotransferase.

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