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IDENTIFICATION OF THE SUBSTRATE BINDING SITES IN *ACTINOBACTERIA* SULFOTRANSFERASE CPZ8

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The understanding of natural product biosynthetic pathways has received increased interest of the modern scientific community. Recently, our collaborators from the University of Tübingen dissected the biosynthetic pathway of the antibiotic caprazamicin (CPZ) in *Streptomyces* sp., identifying genuine sulfate donors and acceptors of an unprecedented two-step sulfation pathway [1]. In this work, they identified a PAPS dependent sulfotransferase, Cpz8, which provides phenolic sulfate esters as sulfate donors for a PAPS-independent arylsulfate sulfotransferase (Cpz4) in the pathway, the latter generating sulfated CPZs. Interestingly, the Cpz8 primary sequence displays low homology with known sulfotransferases (19 and 29% identity for human (hSULT) and *S. mansoni* (smSULT) [2], respectively) and clusters with hypothetical proteins, predicted to be involved in sulfate metabolism of microorganisms. Cpz8 and its closest homologues do not contain the conserved 5′-phosphosulfate-binding loop, which has been described as essential for PAPS-dependent sulfotransferases. Furthermore, the molecular bases for sulfate acceptor recognition by Cpz8 were not clear, since Cpz8 could accept *p*-nitrophenol, 4-methylumbelliferone, 4-hydroxy-6-methyl-2-pyrone and 2-naphthol as substrates, but not 3-hydroxy-2-methyl-4-pyrone, phloroglucinol and resorcinol [1]. Therefore, we attempted to determine the crystal structure of Cpz8 aiming the identification and characterization of its substrates binding sites. For this, the enzyme was heterologous expressed, purified and subjected to crystallization experiments. Diffraction data sets were collected at the MX-2 beam line (LNLS, Campinas, SP) with resolution better than 2 Å. SAD experiments were carried out to phase the Cpz8 X-ray data, since molecular replacement was inefficient. Cpz8 crystals were subjected to X-ray data collection after quick cryo-soaking with halide solutions [3]. After data processing, the phases were recovered using the SAD data sets with iodine. The calculated electron density map allowed for the determination of the crystal structure of Cpz8 at 1.8 Å resolution. The atomic model was refined and analyzed. It was found that the Cpz8 3D-structure presents a large cavity compatible with the PAPS binding site, which is similar in architecture to the PAPS binding sites in hSULT and smSULT. Furthermore, the important chemical groups to interact with PAPS and sulfate transfer are preserved in this putative Cpz8 PAPS binding site, despite the difficulty in aligning the Cpz8 primary sequence with known sulfotransferases, as well as in identifying the PAPS binding motif of Cpz8. A second cavity was also found and predicted to bind the sulfate acceptors. However, the putative acceptor binding site in Cpz8 is restricted by the C-terminal helix, being smaller than the cavities of smSULT and hSULT. Structural and biochemical analyses are ongoing to verify if protein readjustments are needed to accommodate the sulfate acceptor or if this binding site is indeed smaller in Cpz8.

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