Sulfurous ping-pong in the urinary tract

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Transfer of information is a basic property of biological systems. Common examples include transfer of genetic information or nerve impulses. Transmission of signals occurs at an even more fundamental level between and within cells, including signaling molecules, which bear a phosphate or a sulfate group. The latter contain a sulfur atom.

Since these processes are of supreme importance, they have been extensively studied and a number of mechanisms and related protein structures have been revealed. Thus, it is even more surprising that ETH Zurich researchers studying transfer processes among sulfurylated molecules discovered a protein, sulfotransferase, whose function is known but which exhibits a previously unknown structure. The group of Rudi Glockshuber recently published a paper about the protein, called ASST, in the scientific journal *PNAS*.

The discovery of the signal transfer mechanism happened accidentally, as is often the case in scientific research. The Glockshuber group studies protein folding mechanisms, where bonds between two sulfur atoms in a protein chain, disulfide bridges, play an important role. While examining gene data banks, the researchers stumbled upon an unusual gene combination present in strains of *E. coli* which cause urinary tract infections: two genes for the disulfide bond formation machinery were clustered with the gene for ASST.

Hence, they decided to elucidate the structure of ASST. This turned out to be a tantalizing task because this protein is large and present in only minute amounts in a bacterial compartment called periplasm. By

growing large-scale bacterial cultures the scientists could obtain sufficient material for crystallographic studies. The crystals of ASST were analyzed at the Swiss Light Source at Paul Scherrer Institut in Villigen, Switzerland.

This analysis, down to 2 Ångström resolution, revealed that ASST indeed contains an extremely short disulfide bond which can presumably only be formed by the action of the disulfide bond formation machinery genetically associated with ASST. This disulfide bridge is a prerequisite for proper folding of this protein and could also play a role in regulating its catalytic activity.

However, these features were almost outweighed by other unusual discoveries: the researchers found a previously never-observed protein structure to catalyze this process. This structure consists of two equal propeller-like parts which contain active sites in the center of the two propellers, built of beta-pleated sheets. Such a structure has never been observed for a sulfotransferase.

How does this two-propeller machine function? To answer this question, the scientists replaced individual amino acids, i.e. building blocks of the protein. In addition, they used molecules acting as sulfuryl-donors and repeated crystallographic analyses. Now they saw that five amino acids containing nitrogen are essential for the function of ASST. They built a reaction cage that accommodates both the donor and the acceptor of the sulfuryl group. Furthermore, during the transfer, the sulfuryl group is directly, covalently bound to a histidine side chain of ASST. Thus, the signal is first transferred from the donor to ASST and subsequently from ASST to the acceptor. Such a ping-pong mechanism is unique in the processes of sulfuryl transfer.

A new structure, a new mechanism – this opens up medically relevant perspectives. Goran Malojčić; the first author of this study, explains

several interesting points. Since ASST is not present in mammals, the protein could be a feasible target for antibacterial drugs. Furthermore, since ASST is present exclusively in *E. coli* strains causing urinary tract infections, a selective action against these bacteria would leave the other, useful bacteria intact.

In addition, Malojčić intends to collaborate with in-silico chemists, who use computers to design molecules, and develop inhibitors of ASST. He also plans to use ASST for the synthesis of novel molecules bearing sulfuryl groups.

Paper: Malojèiã G, Owen RL, Grimshaw JP, Brozzo MS, Dreher-Teo H, Glockshuber R: A structural and biochemical basis for PAPS-independent sulfuryl transfer by aryl sulfotransferase from uropathogenic Escherichia coli. Proc Natl Acad Sci USA. 2008 Dec 9, 105, 19217-19222. doi:10.1073/pnas.0806997105

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