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Keywords: MRSA, drug design, Vitamin B1 metabolism

FA1-MS12-P04

Structural Characterization of the Aromatic Monooxygenases PhzO and TcpA. Emerich-Mihai Gazdag[a], Dmitri V. Mavrodi[b], Luying Xun[c], Linda S. Thomashow[b], Wulf Blankenfeldt[a]. [a]*Max Planck Institute of Molecular Physiology Dortmund, Germany.* [b]*Department of Plant Pathology, Washington State University, Pullman, Washington 99164.* [c]*School of Molecular Biosciences, Washington State University, Pullman, Washington 99164-4234.*
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A large number of oxygenases has been isolated and studied over the past decades. They are able to catalyze a wide variety of oxidative reactions such as regio- and stereoselective hydroxylation. Oxidation reactions are difficult to be achieved using chemical approaches, which makes monooxygenases interesting catalysts for biotechnological applications.
Our current work focuses on the structural and biochemical characterization of the two two-component flavine-diffusible monooxygenases PhzO and TcpA. These enzymes belong to an understudied class of monooxygenases that utilize FAD as a cosubstrate rather than as a cofactor.
PhzO from *Pseudomonas aureofaciens* is a monooxygenase that hydroxylates phenazines [1], a class of redox-active bacterial secondary metabolites that act as virulence factors in infections of humans. At the same time, the phenazines also have beneficial effects in the biological control of plant disease because root-colonizing phenazine producers such as *P. aureofaciens* can protect the plant against other microbial pathogens. PhzO is responsible for the generation of a large spectrum of hydroxylated phenazine derivatives that *P. aureofaciens* produces, and insight into its structure/activity relationships may provide opportunities to enhance the plant-protecting properties of this strain.
TcpA from *Ralstonia eutropha*, on the other hand, initiates the sequential dechlorination of polychlorophenols through an oxidative process [2]. A better understanding of the dechlorination procedure could lead to better applications of these polychlorophenol-degrading microorganisms in the bioremediation of polychlorophenols, a pesticide derivative that is one of the most persistent environmental pollutants.
We present here the crystal structures of PhzO and TcpA in the ligand-free form. Comparison the related 4-hydroxyphenylacetate 3-monooxygenase HpaB [3] allows for the modeling of enzyme-substrate complexes and the identification of specificity determinants. Biochemical assays for the investigation of substrate turnover have been developed and demonstrate that at least for the phenazine-modifying PhzO reduction of the substrate precedes hydroxylation.

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Keywords: phenazine derivatives, polychlorophenols, two-component flavine-diffusible monooxygenases

FA1-MS12-P05

Structural and functional studies of phenazine biosynthesis protein PhzE, a 2-amino-2-desoxyisochorismate synthase. QiAng Li[a], Dmitri V. Mavrodi[b], Linda S. Thomashow[b,c], Manfred Roessle[d], Wulf Blankenfeldt[a]. [a]*Max Planck Institute of Molecular Physiology, Department of Physical Biochemistry, Otto-Hahn-Straße 11, 44227 Dortmund, Germany.* [b]*Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430 (U.S.A).* [c]*USDA, Agricultural Research Service, Root Disease and Biological Control Unit, Pullman, WA 99164-6430 (U.S.A).* [d]*European Molecular Biology Laboratory-Hamburg Outstation, c/o Deutsches Elektronen Synchrotron, 22603 Hamburg, Germany.*
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Phenazines are nitrogen-containing heterocyclic pigments produced by a number of bacterial genera, including fluorescent *Pseudomonas*, *Burkholderia*, *Brevibacterium* and *Streptomyces*. Historically, it was believed that phenazines are solely used as redox-active antibiotics in microbial competitiveness. Recently, however, it has been recognized that these compounds have diverse physiological functions because they also act as signalling molecules and also as respiratory pigments under anoxic conditions as met e.g. in the deeper layers of biofilm. This indicates that phenazine biosynthesis may be an attractive target for pharmaceutical intervention [1].Phenazine biosynthesis requires five genes encoding the proteins PhzB, PhzD, PhzE, PhzF and PhzG, which catalyze the reactions responsible for the synthesis of phenazine-1-carboxylic acid from chorismic acid. PhzE catalyzes the first reaction in this pathway, producing 2-amino-2-desoxyisochorimate (ADIC). The enzyme is highly similar to bacterial anthranilate synthases, which are known to be feedback-inhibited by tryptophan. We have therefore studied PhzE by structural and biochemical methods to assess if it acts as a point of allosteric control of the phenazine biosynthesis pathway.We present here the crystal structure of PhzE of *Burkholderia lata* 383 in a ligand-free open and ligand-bound closed conformation at 2.9 and 2.1 Å resolution, respectively. PhzE arranges as an unusual intertwined dimer, which was also confirmed by small angle x-ray scattering. The dimer possesses relatively weak interactions along the dyad axis but makes more intimate contact between the glutamine amidotransferase and ADIC synthase domains of the two opposite chains, leading to the formation of an ammonia transport channel with approx. 25 Å in length. Large structural rearrangements accompany the binding of chorismic acid, which was found converted to benzoate and pyruvic acid in the ADIC synthase active center of the closed form. Unlike anthranilate synthase, PhzE is not allosterically inhibited, which can be attributed to a tryptophan residue of the protein blocking the respective potential regulatory site. Additional electron density in the active center of the GATase1 domain was identified as zinc and it could be demonstrated that Zn^{2+} and Ni^{2+} indeed reduce the activity of PhzE.

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