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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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Keywords: phenazine biosynthesis, domain swapping, substrate channelling

FA1-MS12-P06

The secretome of parasitic nematodes: Analysis of host-parasite cross-talk. Markus Perbandt[a,c], Raphael Eberle[a], Kai Lüersen[b], Eva Liebau[b], Christian Betzel[a]. [a]*University of Hamburg, Department of Chemistry, Laboratory for Structural Biology of Infection and Inflammation, c/o DESY, Germany.* [b]*University of Münster, Institute of Animal Physiology, Department of Molecular Physiology, Germany.* [c]*University Medical Center Hamburg-Eppendorf, Department of Medical Microbiology, Virology and Hygiene, Germany.*
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We analyse functional macromolecular systems in filarial nematode worms causing major infectious diseases like river blindness and lymphatic filariasis. These helminths parasitize for long periods their immunocompetent hosts, provoking only restricted pathology in their tissue habitats although the pathogens can accumulate to large essemblies. Because of their size, helminths cannot sequester in niches like many bacteria, fungi and protozoa. By co-evolution, these "king-sized" pathogens have developed other strategies to secure survival. Recent research on the interaction between helminths and vertebrate hosts has provided information and insights about evasion, immunomodulation and protection mechanisms [1]. Here, molecules are most important that are secreted into the host tissue or are associated with the surface of the parasite. They represent the first molecules exposed to and affecting the host immune apparatus and are thus likely to be involved in the establishment and maintenance of the parasite within the host and in the avoidance, modulation and skewing of the host immune response. Immune modulation is suggested to be beneficial to both, the human host and the parasite, as it protects the worm from being eradicated, and at the same time protects the host from excessive inflammatory responses that may lead to tissue damage.
We analyse the composition of excretory-secretory products (ESPs) and selected surface proteins of parasitic nematodes, since these are likely to present the principle players in parasite-host cross-talk and have the capacity to actively shape the immunological environment [2,3].
We presently analyse the structures of all important and identified key proteins and here we present the structural insights of the first targets like the OvGST1 from Onchocerca *volvulus*, the causative agent of river blindness.

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Keywords: river blindness, immunmodulation, host-parasite cross-talk

FA1-MS12-P07

Crystal structure of *Enterococcus faecalis* Thymidylate synthase. C. Pozzi[a], M. Benvenuti[a], S. Ferrari[b], R. Luciani[b], A. Catalano[c], R.M. Stroud[d], M.P. Costi[b], S. Mangani[a]m [a]*Department of Chemistry,University of Siena, Italy.* [b]*Department of Pharmaceutical Sciences, University of Modena and Reggio Emilia, Italy.* [c]*Department of Pharmacy and Chemistry, University of Bari, Italy.* [d]*University of California at San Francisco, USA.*
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Thymidylate synthase (TS) is an enzyme that catalyzes the reductive methylation of 2'-deoxyuridine 5'-monophosphate (dUMP) to thymidyne 5'-monophosphate (dTMP), using the cofactor 5,10-methylene-5,6,7,8-tetrahydrofolate (mTHF) as a one–carbon donor and reductant [1]. This reaction is the only the novo source of thymidylate for the cells [2]. The inhibition of TS leads to pronounced changes in cellular protein and RNA, cessation of DNA replication and eventually cell death [3]. Because of its critical function, considerable effort has been focused on the design of TS inhibitors for the treatment of cancer [4]. Less attention has been directed towards the design of species-specific TS inhibitors aimed at treating diseases caused by bacterial, fungal or opportunistic pathogens. However, taking into account the rise in antibiotic resistant bacteria, the relative toxicity of treatments for fungal infections and the poor therapies available for several opportunistic infections in immunocompromised patients, the successful development of pathogen-specific TS inhibitors may offer an important alternative to current antibiotic, antiparasitic and antifungal drugs [5,6]. TS enzymes show an highly conserved structure, but some differences of the active site may be exploited for the design of inhibitors able to discriminate pathogen TSs vs. human TS [7].
In the effort to increase our knowledge about bacterial TS, we have determined the 2.18 Å crystal structure of *Enterococcus faecalis* TS (EfTS), which is the first structure determination of this enzyme, as prepared and in complex with a specific inhibitor.
EfTS is a homodimer showing subunit heterogeneity as only one of the subunits brings bound a derivative of the tetrahydrofolate intermediate of the catalyzed reaction. The binding of the inhibitor is accompanied by large rearrangement of the protein, determining the ordering of a mobile loop that appears disordered in the inhibitor free subunit. The designed inhibitor replaces the naturally occurring inhibitor while leaving the other subunit free.
The determination of the two crystal structures provides interesting clues about the catalytic mechanism of EfTS and its inhibition that might be relevant for the rational design of more powerful and selective inhibitors towards bacterial targets.

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Keywords: macromolecular crystallography drug design, thymidylate synthase, drug discovery and design

FA1-MS12-P08

Binary complex of 14-3-3σ/p53 pT387-peptide and implications for stabilization. Benjamin Schumacher, Justine Mondry, Philipp Thiel, Michael Weyand[a], Christian Ottmann[a,*]. *Chemical Genomics Centre of the*