before [2]. Here, another c-diGMP binding mode is observed that cross-links the two DGC domains of the dimer. This new mode of domain immobilization may be of general importance for a large sub-class of DGC proteins.

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MS03 P18

Structural characterization of threonine aldolases

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Threonine aldolases (TAs) are PLP dependent enzymes which catalyze the reversible conversion of β-hydroxy-α-aminoacids into glycine and the corresponding aldehyde. In general it's possible to distinguish between two main types, L-selective and D-selective threonine aldolases, depending on their enantioselectivity with respect of the α-carbon of the amino acid. Threonine aldolases possess a high potential as biocatalysts for the synthesis of β-hydroxy-α-amino acids which are important chiral pharmaceutical intermediates.
We are specifically working with two bacterial enzymes, an LTA and a DTA, which share only very low sequence similarity.
For LTA, a microbatch screen was performed, using solutions of the Index Screen (Hampton Research) which yielded first conditions. After optimization it was possible to collect a dataset to a maximum resolution of 3.5 Å at our home x-ray source. The thus obtained LTA crystals are monoclinic (spacegroup $P2_1$) with unit cell dimensions a=106.5 Å; b=116.1 Å; c=106.8 Å and β=93.0°. The asymmetric unit consists of one homotetramer of the enzyme and contains about 70% solvent. First steps towards a structure solution by molecular replacement have been performed using this dataset and a previously generated homology model of LTA.
For DTA we had to employ reductive methylation of lysine residues (using formaldehyde and $NaBH_4$) in order to obtain diffraction quality crystals. With the thus modified DTA a standard crystallization screen was carried out. At our home x-ray source, first crystals yielded a diffraction pattern up to a maximum resolution in the range of 3.5 Å. They belong to spacegroup $P2_1$ with unit cell dimensions of a=62.4 Å; b=83.8 Å; c=73.3 Å and β=112.1°.
Further optimization of the crystallization conditions in order to improve the resolution of the diffraction data, as well as soaking experiments with substrates and inhibitors are underway.

MS03 P19

Crystallographic studies of an active-site mutant of plant-type L-asparaginase

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Keywords: plant-type asparaginase, isoaspartyl peptidase, Ntn-hydrolase, autoactivation

Plant asparaginases hydrolyze the side-chain amide bond of asparagine or of isoaspartyl dipeptides. Close homologs have been found not only in plants, but also in bacteria, with the *E. coli* protein, EcAIII, being particularly well studied. The enzymes from both sources belong to the family of Ntn-hydrolases with a catalytic Thr nucleophile located at the free N-terminus of subunit β, liberated during an autoproteolytic maturation event which generates two separate subunits (α and β) from a single-chain precursor. It is believed that the maturation mechanism involves the very same Thr nucleophile that is liberated in its consequence. However, the details of the maturation reaction are obscure, as the free amino group, supposed to activate the nucleophilic hydroxyl, is not present in the immature protein. To investigate the structural basis of EcAIII maturation, we have generated and crystallized an active-site mutant with the catalytic Thr residue substituted by alanine (T179A). The structure has been solved in two crystal forms, one of which corresponds to that of the wild-type enzyme. The protein exists as a dimer resembling the $(\alpha\beta)_2$ oligomeric form of the mature enzyme. The linker connecting the α and β portions of the precursor can be traced nearly in its entirety, allowing a discussion of a nucleophilic attack launched by residue 179 on the preceding peptide bond. In variance with the wild-type structure, a fragment of the linker chain is seen in the active-site cavity. A tightly coordinated sodium cation, which in the mature protein shapes a loop supporting the active site elements, is found in an analogous structure of the T179A mutant. This suggests that the metal cation is coordinated at an early stage during the protein folding process and that its presence is essential for both the maturation reaction and the final L-asparaginase activity.

MS03 P20

Structural Properties and Electrostatics of Cold-active beta-Galactosidase

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Keywords: beta-galactosidase, cold-active, hexamer

Arthrobacter sp. C2-2, a soil bacteria found on an island near Antarctica, belongs to psychrotrophic, i.e. cold tolerant, microorganisms. Structure of its beta-galactosidase (hydrolase cleaving lactose into galactose and glucose), iso-enzyme C-2-2-1, was solved up to 1.9 Å resolution.
The beta-galactosidase belongs to glycosyl hydrolase structural family 2 and has 30% sequence identity with *Escherichia coli* beta-galactosidase. In spite of the chain similarity, both enzymes differ in their oligomerization states.

Escherichia coli beta-galactosidase is known to be active only in the form of tetramers, while the cold-active *Arthrobacter* sp. C2-2 beta-galactosidase forms compact hexamers with active sites oriented into an internal cavity, connected by three types of channels with exterior solvent. Additionally, sequence differences between both enzymes exist in the active site.

Acknowledgement: The research was supported by the Grant Agency of the Czech Republic (project 204/02/0843/A) and by the Grant Agency of the Academy of Sciences of the Czech Republic (project B500500512).

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MS03 P21

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The biosynthesis of pyrroloquinoline quinone (PQQ), a novel vitamin and redox cofactor of quinoprotein dehydrogenases, is facilitated by an unknown pathway that requires the expression of six genes (pqqA-F) to derive PQQ from glutamate and tyrosine residues encoded in the precursor peptide PqqA. In previous work we successfully characterized the last step of PQQ biosynthesis and discovered that PqqC (EC 1.3.3.11) is a novel cofactor-less oxygenase (PNAS 2004; JACS 2004; Proteins 2004). PqqC catalyzes the final step in the pathway in a reaction that involves ring cyclization and eight electron oxidation of 3a-(2-amino-2-carboxy-ethyl)-4,5-dioxo-4,5,6,7,8,9-hexahydro-quinoline-7,9-dicarboxylic-acid to PQQ. Here, we describe the crystal structures of PqqC and its complex with PQQ and determine the stoichiometry of H_2O_2 formation and O_2 uptake during the reaction. The PqqC structure reveals a compact seven-helix bundle that provides the scaffold for a positively charged active site cavity. Product binding induces a large conformational change, which results in the active site recruitment of amino acid side chains proposed to play key roles in the catalytic mechanism. PqqC is unusual in that it transfers redox equivalents to molecular oxygen without the assistance of a redox active metal or cofactor. The structure of the enzyme-product complex shows additional electron density next to R179 and C5 of PQQ, which can be modeled as O_2 or H_2O_2, indicating a site for oxygen binding. We propose a novel reaction sequence that involves base-catalyzed cyclization and a series of quinone-quinol tautomerizations that are followed by cycles of O_2/H_2O_2-mediated oxidations.

MS03 P22

Structural studies of the dual-substrate TIM-barrel phosphoribosyl isomerase A Helena Wright[a], Lianet Noda-García[b], Adrián Ochoa-Leyva[b], David A Hodgson[a], Francisco Barona-Gómez[a,b] and Vilmos Fülöp[a],[a]*Department of Biological Sciences, University of Warwick,UK.*[b]*Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM).*
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Keywords: $(\beta\alpha)_8$-barrel, histidine and tryptophan biosynthsesis, evolution.

Phosphoribosyl isomerase A (PriA) is a $(betaalpha)_8$ TIM barrel enzyme involved in both histidine (HisA activity) and tryptophan (TrpF activity) biosynthesis [1]. Despite the availability of a 3D structure of PriA, its structure / function relationship remains poorly defined. Since PriA has ancient-like features, i.e. a dual-substrate specificity within a physiological context, this $(betaalpha)_8$-barrel represents a suitable model system to explore enzyme function evolutionary hypotheses. Thus, elucidating the structure / function relationship of this enzyme may have a bearing on the evolution of the $(betaalpha)_8$-fold as a whole. Here, we will discuss a novel crystallographic structure of PriA resolved at 1.8 Å, which shows the regions that appear disordered in a previously reported structure (PDB: 1vzw). Based on these structural differences, the function of this novel $(betaalpha)_8$-barrel enzyme has been predicted. These predictions, have been validated through mutagenesis and functional analysis.

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