

The toxic extracellular endopeptidase AsaP1 is the causative agent of *Aeromonas salmonicida* achromogenes and leads to atypical furunculosis, a systematic disease in Atlantic salmon and other farmed fish [1].

AsaP1 is 343 amino acids long zinc-metallopeptidase containing a signal sequence of 22 amino acids and an N-terminal propeptide of 171 amino acids, which is released by autocleavage leading to an active enzyme of 22 kDa.

For biochemical and structural characterisation an *E. coli* expression system was established and the structure elucidation for the two inactive mutants AsaP1_E294Q and AsaP1_E294A was performed by X-Ray crystallography.

The structure was solved by molecular replacement using the search model of the peptidyl-Lys metalloendopeptidase from *Grifola frondosa* composed of a 172 amino acid protease domain without propeptide (pdb entry 1g12).

Due to inactivity of AsaP1 mutants the propeptide remains still as a part of the protein, but its function, whether it plays a role in protease folding and acts as an intramolecular chaperone or whether it inhibits proteinase activity in the intracellular space, still remains unclear.

The complex build of propeptide domain and protease domain reveals insights for substrate interaction and substrate specificity of AsaP1.

[1] Gudmundsdottir, B. K. 1996. *J. App. Bacteriol.* 80 (1):105-13

Keywords: metalloendopeptidase, aspincin, propeptide

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Structure and Biochemistry of the APP E2-domain.

Sven O. Dahms, Dirk Roeser, Manuel E. Than.

Leibniz Institute for Age Research – Fritz Lipman Institute (FLI), Protein Crystallography Group, Jena, Germany.

E-mail: sdahms@fli-leibniz.de

The Amyloid Precursor Protein (APP) and its sequential cleavage by the proteases beta- and gamma-secretase are generally believed to be of central importance for the development of Alzheimer's disease (AD) [1]. The resulting neurotoxic peptide Aβ is found in the disease typical senile plaques. Especially the biological function of APP remains mostly unclear until now, not least because of insufficient structural knowledge about the Protein. We will present structural and biochemical data of the E2-domain of APP, which provides new functional insights.

[1] K. Blennow, M. J. de Leon and H. Zetterberg, *Lancet* 368 (2006), p. 387

Keywords: Amyloid Precursor Protein (APP), Alzheimer's Disease (AD), Crystal structure

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Crystal structure of N-formimino-L-Glutamate Iminohydrolase.

Alexander Fedorov^a, Elena Fedorov^a,

Ricardo Marty-Arbona^b, Frank Raushel^b, Steve Almo^a

^a*Department of Biochemistry, Albert Einstein College of Medicine, New York, USA.* ^b*Department of Chemistry, Texas A&M University, College Station, Texas, USA.*

E-mail: fedorov@aecom.yu.edu

The members of the aminohydrolase superfamily of protein molecules are found in every organism sequenced to date and are structurally characterized by metal center embedded at the C-terminal end of (β/α)₈-barrel protein fold.

Here we present the crystal structure of N-formimino-L-Glutamate Iminohydrolase from *Pseudomonas aeruginosa* – the member of aminohydrolase superfamily. The crystal structure of this enzyme in the presence of the inhibitor, N-formimino-L-aspartate also will be presented.

These crystal structures have provided insight into the mechanism for the deimination reaction and identified conserved residues within the active site that are required for substrate recognition.

Keywords: protein crystal structure, Iminohydrolase, enzyme mechanism

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Structure of the tetracycline-degrading

monooxygenase TetX2.

Gesa Volkert^a, Gottfried Palm^a, Manfred S. Weiss^b, Gerard D. Wright^c, Winfried Hinrichs^a. ^a*Institute for Biochemistry, Molecular Structural Biology, Ernst-Moritz-Arndt-Universität Greifswald, Felix-Hausdorff-Str. 4, D-17489, Greifswald, Germany.* ^b*EMBL, c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany.* ^c*Michael G. DeGroot Institute for Infectious Disease Research, Department of Biochemistry and Biomedical Sciences, McMaster University, 1200 Main St W, Hamilton, Canada.*

E-mail: gesa.volkert@uni-greifswald.de

The flavin-dependent monooxygenase TetX2 from anaerobic *Bacteroides thetaiotaomicron* confers resistance against tetracyclines on aerobically grown *Escherichia coli* [1]. The enzyme modifies several tetracycline antibiotics including the recently approved 3rd generation antibiotic tigecycline under regioselective hydroxylation of the substrate which leads to non-enzymatical degradation associated with weaker antibiotic properties [2]. In contrast to efflux or ribosomal protection mechanisms, this resistance mechanism is only partly understood. TetX2 has also been found in aerobic *Sphingobacterium* sp. which may be the ancestral source of the *tetX*-genes. The crystal structure was solved in a 3-WL MAD experiment with a SeMet-containing crystal in space group *P*₂₁. The native protein crystallized in *P*₁ and data were collected to a resolution of 2.5 Å. The self-rotation function of the *P*₂₁ data revealed two independent twofold non-crystallographic axes which occur also in the *P*₁ data but with a slightly different orientation of the four monomers in the asymmetric unit. TetX2 shares highly conserved homologous domains with other structurally known FAD-binding monooxygenases like PhzS hydroxylase, despite low sequence identity and different substrates. The ADP moiety of the flavin cofactor is bound by a glutathione reductase fold which is comparable to other FAD-binding enzymes. The supposed substrate binding domain contains a seven stranded β-sheet. Two strands connect the FAD-binding domain with the substrate binding domain additional to a helix composed of eight turns. In the absence of a substrate complex molecular modeling studies are now under way to position a tetracycline molecule in the supposed active site of TetX2.