FA1-MS03-P09

A Hexameric Acylaminoacyl Peptidase: Substrate Selectivity and Stabilization. Veronika Harmat[a,b], Éva Tichy-Rács[b], Balázs Hornung[b], András L. Kiss[c], Krisztina Rádi[b], Gábor Náray-Szabó[a,b], László Polgár[c]. [a]*Protein Modelling Group, Hungarian Academy of Sciences-Eötvös Loránd University, Budapest, Hungary.* [b]*Laboratory of Structural Chemistry and Biology, Institute of Chemistry, Eötvös Loránd University, Budapest, Hungary.* [c]*Institute of Enzymology, Hungarian Academy of Sciences, Budapest, Hungary.*
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Acylaminoacyl peptidase (AAP) as a member of prolyl oligopeptidase family can cleave only oligopeptides, which is ensured by its beta-propeller domain restricting the access to the active site. AAP removes N-terminally blocked amino acids from peptide chains. It is involved in clearing cytotoxic denatured proteins from cells. It was reported to be a more sensitive target for cognitive-enhancing organophosphorus compounds than acetylcholinesterase. It was found in oligomerisation states in different species.
We report the structure of an unusually hexameric variant of AAP from the thermophilic archaeon *Pyrococcus horikoshii*. The phase problem was solved by MIRAS methods using Pt, I and U derivatives. The structure was refined to 1.9 Angstrom resolution.
Our previous enzyme kinetic studies revealed endopeptidase activity of the enzyme. The structural basis of that is explored. The most interesting feature of the structure is an unusual insertion loop of the propeller domain stabilizing the hexamer. The substrate size selectivity and stabilization of the environment of the active site are realized by the novel type of quaternary structure.
Support from EMBL, ESRF and Hungarian Scientific Research Fund (OTKA) grants NK67800 and F67937 is acknowledged.

Keywords: oligomeric proteases; protease selectivity; beta-propeller domain

FA1-MS03-P10

Structural Studies of Mycocypins, A New Family of Cysteine Protease Inhibitors. Miha Renko[a], Jerica Sabotič[b], Jože Brzin[b], Dušan Turk[a]. [a]*Department of Biochemistry and Molecular and Structural Biology, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia.* [b]*Department of Biotechnology, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia.*
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Protease inhibition with endogenous inhibitors is the basic example of regulation of its activity in biological processes.
The two known member of mycocypin family, macrocypin and clitocypin are found in basidiomycete species. They are the only two known members of a new family I48 according to MEROPS classification. Clitocypin inhibits endopeptidases in pikomolar range and is also a weak inhibitor of some exopeptidases. Macrocypin has a similar inhibition spectrum. Both inhibitors also inhibit legumain.
The determined crystal structure of macrocypin (1.64 A), clitocypin (1.92 A) and of the complex of clitocypin with cathepsin V (2.23 A) are revealing the structural properties of these inhibitors. The basic fold is a unique beta barrel, formed by 8 long and 4 shorter antiparalel beta sheets. Macrocypin has a short alpha helix (2 turns), but this one is absent in clitocypin.
The inhibition with two loops, which bind into the active site of proteases, is slightly similar to the inhibition with cystatins. Loop Asp19-Glu25 takes places of a N-terminal region in stefin A. Another loop Glu39-Ile50 is much wider than one in cystatins and takes out the role of both remaining loops in cystatins.
The selectivity of macrocypin and clitocypin towards endopeptidases can be explained by structural differences in loop-binding into the active site cleft. Two broader loops are much more rigid than three loops in cystatins, which can easily adopt to additional features in exopeptidases (like occluding loop, mini chain etc.).

FA1-MS03-P11

Structural Analysis of a Novel Type of Haloalkane Dehalogenase DbeA and Mutant DbeA1. Tatyana Prudnikova[a], Pavlina Rezacova[d], Tomas Mozga[c], Jiri Damborsky[c], Michal Kuty[a,b], Ivana Kuta Smatanova[a,b*]. [a]*Institute of Physical Biology, Nove Hrady, Czech Republic.* [b]*Institute of Systems Biology and Ecology, Nove Hrady, Czech Republic.* [c]*Department of Experimental Biology and National Centre for Biomolecular Research, Brno, Czech Republic.* [d]*Institute of Molecular Genetics, Prague, Czech Republic.*
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Haloalkane dehalogenases (EC 3.8.1.5) make up one very important class of microbial enzyme because of their ability to catalyze detoxification reactions and act on a broad range of halogenated aliphatic compounds arising because of environmental problems with production and usage of halogenated hydrocarbons [1]. A novel enzyme DbeA was isolated from *Bradyrhizobium elkani* USDA94. To understand it altered specificity and activity mutant (designated DbeA1) containing unique insertion was prepared [2]. DbeA1 was constructed to study the importance of the insertion in the N-terminus of the cap domain for activity and specificity of these enzymes. A novel haloalkane dehalogenase DbeA and its mutant variant DbeA1were crystallised using the sitting-drop vapour-diffusion procedure and the crystal structures have been solved and refined to 2.2 Å resolution. The DbeA crystals belong to the primitive orthorhombic space group $P2_12_12_1$, while the crystals of the mutant DbeA1 belong to the monoclinic space group $C2$. Comparison characteristic of both structures showing the importance of the 9 unique aminoacid residues extension. Crystallographic analysis of