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

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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.).

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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

DbeA and DbeA1 was initiated to understand the structure-function relationships of the wild type and the insertion mutant.

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Keywords: haloalkane dehalogenase; catalytic peptide; halide-binding residues

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1,3-Propanediol Dehydrogenase from Klebsiella Pneumoniae: Decameric Quaternary Structure and Possible Subunit Cooperativity.

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Alcohol dehydrogenases are a rather old subject. They play a central role in the most ancient business of biotechnology: alcoholic fermentation. As a consequence, they also play an important role in our liver and stomach, providing a line of defense against a potentially dangerous molecule, ethanol. It is therefore not strange that they were subject of early attention, with the first alcohol dehydrogenase purified and crystallized in 1937 [1]. However, there are many different enzymes that interconvert alcohols, aldehydes and ketones. [2]. The enzyme 1,3-propanodiol dehydrogenase from *Klebsiella pneumoniae* is a type III iron-dependent dehydrogenase, a not so well studied group of enzymes, with very few known structures [3]. *Klebsiella pneumoniae* is a nosocomial pathogen frequently isolated from opportunistic infections, especially in clinical environments [4]. In spite of its potential pathogenicity, this microorganism has several metabolic potentials that could be used in biotechnology applications. *K. pneumoniae* is able to metabolize glycerol as a sole source of carbon and energy [5]. 1,3-Propanediol dehydrogenase is the core of the metabolic pathway for the use of glycerol [6]. We have determined the crystallographic structure of 1,3-propanediol dehydrogenase, a type III Fe-NAD-dependent alcohol dehydrogenase, at 2.7-Å resolution. The structure of the enzyme monomer is closely related to that of other alcohol dehydrogenases. The overall arrangement of the enzyme showed a decameric structure, formed by a pentamer of dimers, which is the catalytic form of the enzyme. Dimers are associated by strong ionic interactions that are responsible for the highly stable in vivo packing of the enzyme. Kinetic properties of the enzyme as determined in the article would suggest that this decameric arrangement is related to the cooperativity between monomers.

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Keywords: glycerol metabolism; type III alcohol dehydrogenase; 1,3-propanediol

FA1-MS03-P13

Crystal Structure of Shikimate Dehydrogenase from *Helicobacter Pylori.*

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Shikimate dehydrogenase (EC 1.1.1.25) catalyzes the NADPH-dependent reduction of 3-dehydroshikimate to shikimate, as well as its reverse, and has been developed as a promising target for the discovery of new antimicrobial agent, herbicides, and antiparasitic agents. It is the fourth enzyme in the shikimate pathway for aromatic amino acid biosynthesis in bacteria, fungi, and plants, but not mammals. The crystal structure of native shikimate dehydrogenase form *Helicobacter pylori* (HpSDH) was solved to 1.6Å resolution using single-wavelength anomalous dispersion methods, showing an N-terminal α/β domain and a C-terminal Rossmann domain. We have also determined the binary HpSDH·shikimate structure (1.4 Å) and the ternary HpSDH·shikimate·NADPH (2.0 Å) structure, respectively. These structures demonstrate that shikimate binds to the N-terminal domain, while NADPH binds to the Rossmann-fold domain. Furthermore, the apo-form adopts an open-state conformation, while the complex structures have a closed-form conformation. Crucial shikimate binding residues (Ser16, Ser18, Tyr21, Thr65, Lys69, Asn90,Asp105 and Gln237) are identified, which provide a basis for the structure-guided design of SDH inhibitors.

Keywords:crystal structure; shikimate dehydrogenase; shikimate pathway; *helicobacter pylori*

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Crystallization of Bifunctional Catalase-phenol Oxidase (CATPO) from *Scytalidium Thermophilum.*

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Catalase is a common enzyme in all aerobic and many