s8a.m1.p43 Structural studies of chitinase A from *S. marcescens* complexed with substrates and the inhibitor allosamidin.

Y. Papanikolau, G. Prag[+], G. Tavlas, M. Papadovasilaki, S. Dimitriou, C.E. Vorgias[#], A.B. Oppenheim[+] and K. Petratos, *IMBB-FORTH, PO Box 1527, 711 10 Heraklion, Greece.* [+]*Dept. of Mol. Gen. and Biotechnology, The Hebrew University, P.O. Box 12272, 91120 Jerusalem, Israel.* [#]*National and Kapodistrian Univ. of Athens, Dept. of Biochemistry Mol. Biology, 15701 Athens, Greece.*

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Chitinase A (chiA) from *Serratia marcescens* is a hydrolytic enzyme, which catalyses the cleavage of ß(1? 4) glycosidic linkages between the linearly arranged 2-deoxy-2-N-acetyl-D-glucosamine (NAG) residues of the natural biopolymer chitin. It belongs to the family no. 18 of glycosyl hydrolases, which is believed to act *via* a retaining mechanism[1] of the configuration of the anomeric carbon (C1) of the scissile bond. The structure of the native chitinase A had been determined earlier[2].

ChiA has been crystallized under salting out conditions (0.75 M Na-citrate) using the vapour diffusion method. The native structure has been refined at 1.55 Å resolution using synchrotron data. In an attempt to study the mechanism of the enzyme, the crystal structures of the complexes of native chiA with the natural inhibitor allosamidin as well as the inactive mutants (E315Q and D313A) with the oligosaccharide substrate $(NAG)_8$ have been determined and refined at 1.8 Å resolution. The crystals were produced under salting out conditions as mentioned above in the presence of excess of the inhibitor or the oligosaccharide (co-crystallisation). Diffraction data were collected at 100 ^{o}K using the in-house rotating anode generator for the chiA/allosamidin complex and synchrotron radiation for the complexes of the chiA mutants with substrate. In all cases the added substances could be positioned unambiguously using difference Fourier maps. The entire chain of N-acetyl-chito-octaose could be modelled in the active site groove of the enzyme.

Based on these structural data, chiA appears to be a chitobiosidase, cleaving $(NAG)_2$ disaccharide units from the reducing-end of the substrate. A critical ‘chair’ ($^{4}C_{1}$) to ‘boat’ ($^{1,4}B$) conformational change at the cleavage site (subsite –1) was clearly visible in the electron density maps. The orientation of the critical 2-acetamido moiety of the substrate does not lend support to a substrate-assisted mechanism. Moreover, the conserved residue Tyr390 among the enzymes of the family seems to play an important role in the catalysis worth to be further investigated.

[1] Davies, G. & Henrissat B. “Structures and mechanisms of glycosyl hydrolases”, Structure, (1995), 3: 853-859.
[2] Perrakis A., Tews I., Dauter Z., Oppenheim B.A., Chet I., Wilson K.S. & Vorgias C.E. “Crystal structure of a bacterial chitinase at 2.3 Å resolution”, Structure, (1994), 2: 1169-1180.

s8a.m1.p44 The type II Dehydroquinate dehydratase from *Bacillus subtilis* – an intriguing innactive enzyme.

D.A. Robinson*, A.W. Roszak[+], J.R. Coggins* and A.J. Lapthorn[+]. **Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences and* [+] *Department of Chemistry, University of Glasgow, Glasgow, G12 8QQ.*

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Dehydroquinate dehydratase [dehydroquinase or DHQase EC 4.2.1.10] catalyses the dehydration of 3-dehydroquinate to 3-dehydroshikimate, a reaction common to two metabolic pathways, (i) the biosynthetic shikimate pathway (essential in plants and micro-organisms but absent in animals) and (ii) the catabolic quinate pathway in fungi. Two distinct classes of DHQase are found in micro-organisms, Type I, found for example in *Escherichia coli*, and Type II, found in the majority of eubacteria such as *Mycobacterium tuberculosis*. *Bacillus subtilis* is the only bacteria so far that has been shown to contains genes for both type I and type II enzymes. We have cloned and overexpressed the type II DHQase from *B. subtilis* and shown that it does not possess any DHQase activity. We have solved the 3-D structure of the enzyme to 2.1Å resolution and will discuss the structural implications of this enzyme with respect to the active DHQases from *M. tuberculosis* and *Streptomyces coelicolor*.