

Poster Presentations

[MS5-P06] Structure-function Studies of Glycosyl Hydrolases.

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Glycosyl hydrolases have been in the centre of our structure-function studies for many years because of their high potential for biotechnological and biomedical applications. Our original structural study of a GH2 β -galactosidase from psychrophilic bacterium *Arthrobacter* sp. C2-2 revealed its unique hexameric arrangement [1] and more data have now been gathered on ligand binding in its active site and accompanying structural changes of the enzyme. This cold active β -galactosidase capable of maintaining significant activity at 10° C can bind its saccharide ligands in a shallow binding mode even if the key residue for this binding mode, Trp999 of mesophilic *E. coli* β -galactosidase is in the cold-active enzyme replaced by Cys999. X-ray structures of three complexes of the enzyme with a product, transition state analogue, and inhibitor identify structural changes near and apart from the active site connected with ligand binding [2]. Chitinases of GH18 from a human commensal bacterium *Clostridium paraputrificum* J4 can be potentially exploited in treatment of diseases caused by pathogenic fungi. This bacterium discovered, isolated and

studied by our team attracted our attention by a large number of chitinolytic enzymes secreted into medium [3].

Recently, we have sequenced the genome of the bacterium and discovered at least 12 protein coding sequences annotated as chitinolytic enzymes. Chitinase Chit62J4 of GH18 is a four-domain enzyme, with mostly exochitinase activity and its catalytic domain highly similar to Nctu2 from *Bacillus cereus*. ChitBJ4 is homologous to ChiB of *Clostridium paraputrificum* M-21. Several of the enzymes of the chitinolytic complex undergo crystallization experiments and tests of their effects on pathogens [4,5]. As the catalytic mechanisms have been explained our work is focused on the role of the additional domains on activity and on the catalytic efficiency of the whole enzymatic complex in chitin degradation. Support by the Czech Science Foundation (no. 310/09/1407) and by the Czech Ministry of Education, Youth and Sports (no. EE2.3.30.0029) is acknowledged.

[1] Skálová, T., Dohnálek, J., Spiwok, V., Lipovová, P., Vondráčková, E., Petroková, H., Dušková, J., Strnad, H., Králová, B., Hašek, J. (2005) *J. Mol. Biol.* 353, 282-294.

[2] Štěpánková, A. (2012) X-ray structural analysis of enzymes from extremophiles and their complexes with bound ligands. PhD thesis, Czech Technical University in Prague.

[3] Šimůnek J., Kopečný J., Hodrová B., Bartoňová H. (2002) *Folia Microbiol.* 47, 559-564.

[4] Dušková J., Tishchenko G., Ponomareva E., Šimůnek J., Koppová I., Skálová T., Štěpánková A., Hašek J., Dohnálek J. (2011) *Acta Biochim. Pol.* 58, 261-263.

[5] Šimůnek J., Koppová I., Tishchenko G., Dohnálek J., Dušková J. (2012) *Folia Microbiol.* 57, 335-339.

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