s8a.m1.p17 **Structural studies of HPr-kinases /phosphatases.** S. Fieulaine*, A. Galinier°, S. Poncet³, J. Deutscher³, S. Nessler*. * *Laboratoire d'Enzymologie et de Biochimie Structurales (LEBS), CNRS bât. 34, F-91198 Gif/Yvette, ° IBCP, 7 passage du Vercors, F-69367 Lyon cedex 07, ³ LGM, INRA and CNRS, ERS-567, F-78850 Thiverval-Grignon.*
Keywords: bacteria; new protein-kinases family; enzymatic mechanism.

PTS is one of sensitive systems wich allows bacteria to adapt to their environment. Carbohydrates translocation through the membrane coupled to their phosphorylation is mediated by HPr phosphorylation on His-15, by PEP-dependent enzyme I. In Gram-positive bacteria, HPr can also be phosphorylated on Ser-46, by the ATP-dependent HPr-kinase /phosphatase (HPr-K/P). HPr-P-Ser is able to interact with the transcriptional modulator CcpA, forming a complex which binds to DNA, resulting on transcriptional regulation. This is the carbon catabolite repression (CCR). As HPr plays a role of molecular switch in this paradigm of signal transduction, it is very important to understand how HPr-K/P phosphorylates HPr. Sequence alignments have shown that HPr-K/Ps don't have any homology sequence with other proteins. That's why they are classified into a new protein-kinases family. Besides, some HPr-K/Ps were found in pathogene Gram-negative bacteria. In *Neisseria meningitidis*, the enzyme could be implicated in cell adhesion, important for virulence. So we are very interested to compare HPr-K/Ps of Gram-positive and Gram–negative bacteria.

HPr-K/Ps of *Bacillus subtilis*, *Lactobacillus casei* and *Neisseria gonorrhoeae* were expressed, and purified with a His-tag. The kinase activity is tested, and biochemical assays for proteins characterization were done. Interactions between HPr-K/P and HPr, ATP, Mg^{2+} and/or FBP are analysed. As preliminary results for crystallography, we have obtained crystals with a truncated HPr-K/P of *L.casei*, which diffract at 2.7-Å resolution. Currently, two approaches are developed for data phasing: multiple isomorphous replacement (MIR) or expression of the protein by incorporating selenomethionine.

s8a.m1.p18 **Crystallographic study of the maize cytokinin gluco-side-specific β-glucosidase Zm-p60.1.** J. Marek[1], J. Vé-vodová[1], Xiao-Dong Su[2], J. Zouhar[1] & B. Brzoboha-tý[1,3]. [1]*Faculty of Science, Masaryk University, Kotlárská 2, CZ 611 37 Brno, Czech Republic.* [2]*Department of Molecular Biophysics, Lund University, S-221 00 Lund, Sweden.* [3]*Institute of Biophysics, Academy of Sciences of the CR, Královopolská 135, CZ 61265 Brno, Czech Republic.*
Keywords: cytokinin, beta-glucosidase, hydrolase.

Maize β-glucosidase, Zm-p60.1, has been suggested to be one of the key enzymes involved in regulation of plant development by releasing biologically active cyto-kinins from their storage and transport forms, cytokinin-O- and N3-glucosides [1, 2]. The mature catalytically active form of Zm-p60.1 is a homodimer located in plastids/chloroplasts. When expressed in transgenic to-bacco plants, Zm-p60.1 can alter ratio between free and conjugated cytokinins (Brzobohatý *et al*., in preparati-on). For preparing of pure enzyme in mg quantities we developed a single-step purification protocol based on immobilized metal affinity chromatography [3].

Recently we modified this method slightly, we prepared Zm-p60.1 single crystals diffracting with synchrotron X-ray and under cryoconditions to at least 2.05 Å and we successfully applied MR with white clover glucosidase [4] as a model. Model building and refining is in the progress. The resulting 3-D model of the enzyme will be presented at the poster.

This work is supported by grants VS96095 and VS96096 from the Czech Ministry of Education. JV thanks Socrates/Erasmus program for support during her stay in Sweden.

[1] Campos, N., Bakó, L., Feldwisch, J., Schell, J. and Palme, K. (1992) A protein from maize labelled with azido-IAA has novel β-glucosidase activity. *Plant J* **2**, 675-684 (1992)
[2] Brzobohatý, B., Moore, I., Kristoffersen, P., Bakó, L., Campos, N., Schell, J., and Palme, K. Release of Active Cytokinin by a β-Glucosi-dase Localized to the Maize Root Meristem. *Science* **262**, 1051 (1993).
[3] Zouhar, J., Nanak. E. & Brzobohatý, B.: Expression, Single Step Purification, and Matrix-Assisted Refolding of a Maize Cytokinin Glucoside-Specific β-Glucosidase. *Protein Expression Purif.* **17**, 153-162 (1999).
[4] Barret, T., Suresh, C. G., Tolley, S. P., Dodson, E. J. & Davies, G.: The crystal structure of a cyanogenic β-glucosidase from white clover, a family 1 glycosyl hydrolase. *Structure* **3**, 951-960 (1995).