Technical perspective of investigating proteins involved in the plant hormone system

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This study is mainly focused on two proteins involved in plant hormone cytokinin metabolism and signalling, β-D-glucosidase and CRE1/AHK4 receptor.

β-D-glucosidase releases physiologically active cytokinins from their *O*-glucosides. We used mutagenesis to study structural relationships determining β-D-glucosidase specificity. The saturation mutagenesis was performed by combination of random saturation mutagenesis and site-direct mutagenesis. Mutants were expressed in *E. coli* and purified using two-step purification process (affinity chromatography and gel filtration). The initial velocities using one concentration of the natural as well as artificial substrate were determined.

β-D-glucosidase was also used as a model enzyme to develop an automated method for enzyme kinetics using artificial as well as natural substrates. For the automation using natural substrate was used the previously developed method that was based on glucose detection. These methods were optimized using liquid handling system BioNex Nanodrop II.

The cytokinin receptor CRE1/AHK4 transduces the hormone signal via interaction with its agonist including compounds released by β-D-glucosidase. In this study bacteria *E. coli* KMI001 transformed with plasmid carrying gene for CRE1/AHK4 was used. The modified bacterial signaling pathway leads into expression of reporter gene coding β-D-galactosidase. High throughput screening method based on this assay was optimized for seeking receptor agonists and antagonist across huge library of compounds. The method also includes process to identify compounds that interfere with the determination of the fluorescent product after β-D-galactosidase enzymatic reaction.

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