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Activation of Plant Signal Peptide Peptidase by Saline Solutions

Tomiko Asakura, Masako Hoshi

Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences,
The University of Tokyo

Summary

Signal peptide peptidase (SPP) is a homologue of presenilin, which is involved in Alzheimer disease. Presenilin cleaves and releases the amyloid peptide from the plane of the plasma membrane. On the other hand, SPP cleaves the signal peptide from the ER membrane after shedding by a signal peptidase. SPP and SPP analogues have been found in various organisms, in some plants as well. The absence of these enzymes causes serious phenotype. *Arabidopsis thaliana* SPP (AtSPP) deletion mutant becomes lethal.

To reveal the functions of AtSPP, we produced a plant with knocked down AtSPP by using RNAi method. Besides, overexpressed AtSPP was produdced using 35S cauliflower mosaic virus promoter by the floral dip method with *Agrobacterium tumefaciens*. Comprehensive gene expression of AtSPP KD flower and that in the wild type were analyzed by DNA microarray analysis. The DNA microarray data were normalized with the DFW algorithm using the statistical language R and Bioconductor. Differentially expressed genes (DEGs) between AtSPP KD and the wild type were extracted, and the Gene Ontology Enrichment Analysis was performed. About the gene expression in the overexpressed AtSPP flower, comprehensive gene expression was also analyzed in the same method. As a result, we found that the expression of stress-related genes was significantly increased in the AtSPP KD plant. In addition, 42% of these genes were transcription factors. This result indicates that AtSPP negatively regulates the expression of stress-related genes regulating transcription factors. On the other hand, we found that the expression of oxidation-reduction process related genes was significantly increased in the overexpressed AtSPP plant. These result indicate that AtSPP might be related oxidation stress.

Next, we examined whether AtSPP exhibit the proteolytic activity or not. An *n*-dodecyl-*ß*-maltoside (DDM)-solubilized membrane fraction from *Arabidopsis* cells digested the myc-Prolactin-PP-Flag peptide, a human SPP substrate, and this activity was inhibited by $(Z\text{-}LL)_2$-ketone, an SPP-specific inhibitor. The proteolytic activities from the membrane fractions solubilized by other detergents were not inhibited by $(Z\text{-}LL)_2$-ketone. To confirm the proteolytic activity of AtSPP, the protein was expressed as either a GFP fusion protein or solely AtSPP in yeast. SDS-PAGE analysis showed that migration of the fragments that were cleaved by AtSPP were identical in size to the fragment produced by human SPP using the same substrate. These membrane-expressed proteins digested the substrate in a manner similar to that in *Arabidopsis* cells. We concluded that plant SPP possesses proteolytic activity and may be involved in RIP. Subsequently, application of AtSPP to food processing was examined. Salts are often used during food processing. There were no effect

to activity of protease until 200mM NaCl in reaction buffer. It is known that concentration of salt are 2.7M in soy sause. So we tested about more than 1.0 M of NaCl and KCl. The protease activity was detected at 1M NaCl, but not 2.5M NaCl. From the result of testing about KCl, enzyme activity were inhibited with100mM KCl. After considering these results, we specluated that AtSPP can be used for food processing with high content phosphatides (e.g. egg, soy bean) and under high concentration of salt.