Elucidation of the plant immune system by using the elicitor peptide PIP-1 as a chemical probe

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Introduction

PIP-1 (Plant immunity-activating peptide, YGIHTH-NH_2) is a peptide elicitor discovered by screening of a synthetic combinatorial random hexapeptide library. PIP-1 induces typical plant immune responses, such as oxidative burst, phytoalexin biosynthesis, and defense-related gene expressions, via a jasmonic acid signaling pathway, when applied to suspension-cultured tobacco cells. Although its receptor protein has not been identified in tobacco so far, the structure-activity relationship study revealed that the N-terminus 4 residues of PIP-1 are essential elements to induce the immune responses. Interestingly, PIP-1 induces a different set of immune responses, depending on its concentration, in which maximum level of H_2O_2 generated is induced by treatment with 10 μM of PIP-1, whereas 10-fold higher concentration of PIP-1 is needed for full induction of capsidiol, the major phytoalexin in tobacco. Even though the exact fate of PIP-1 after the treatment in the culture medium of tobacco cells has not been examined yet, it is possible that PIP-1 may be gradually degraded in the incubation medium by tobacco cells due to its structural property. This implies that a decrease in concentrations of PIP-1 by degradation may not allow continuous stimulation of the immune system at the low concentration, leading to failure of capsidiol production. To explore this possibility, the author studied the relationship between the degradation of PIP-1 and induction of plant immune systems, and found important factors regulating the plant immune responses using degradation-resistant PIP-1 analogs.

Chapter 1. Design of Degradation-Resistant PIP-1 Analogs

The degradation profile of PIP-1 in the presence of tobacco cells was first investigated, and found that PIP-1 was rapidly hydrolyzed from its C-terminal end in the incubation medium. To examine the potential impact of such degradation on the induction of late-phase immune responses, degradation-resistant PIP-1 analogs were designed using several peptide modification strategies: substitution of the C-terminal residue, N-methylation of the amide bond, and introduction of an additional residue at the C-terminus. The newly synthesized PIP-1 analogs exhibited outstanding degradation resistance in the presence of tobacco cells without loss of biological activity. This result indicated that the C-terminal His residue is recognized by proteases secreted from tobacco cells, and that enzymatic stability of PIP-1 is

effectively improved by modification at the C-terminal residue.

Chapter 2. Effects of Degradation-Resistance of the PIP-1 Analog on Plant Immune Response Induction

To evaluate the effect of PIP-1 degradation on plant immune response induction, the responses induced by PIP-1 were compared with those by the degradation-resistant analog MePIP-1. H_2O_2 generation and defense-related gene expressions in tobacco cells induced by MePIP-1 were similar to those induced by PIP-1. However, significant differences were observed between PIP-1 and MePIP-1 in the late-phase immune responses, such as capsidiol production and HR-like cell death, in which the concentration of MePIP-1 10-times lower than that of PIP-1 was enough to induce those responses. Further investigation of the mechanism of action of MePIP-1 showed that continuous elicitor stimulation for 3-6 h was required to initiate capsidiol production, which coincided with long-lasting MAP kinase activation. Thus, the capsidiol production is likely to be regulated either by post-translational regulation or changes in metabolic flux, which is associated with long-lasting MAP kinase activity induced by continuous stimulation with MePIP-1. On the other hand, an expression pattern of phytoalexin biosynthesis-related genes were similar between MePIP-1 and PIP-1. Up-regulation of these gene expressions was not observed when tobacco cells were pretreated with kinase inhibitor, suggesting that the transient MAP kinase activation induced by early short stimulation with PIP-1 may be sufficient for transcription of these genes.

Chapter 3. Investigation of Temporal Factors Important for Phytoalexin Biosynthesis Induced by PIP-1 Using a Photocleavable PIP-1 Analog

To investigate temporal factors governing the induction of capsidiol production, the activity of the PIP-1 must be “switched off” at specific time points. In the experiments performed in Chapter 2, the cells were thoroughly washed with fresh media to halt the stimulation by PIP-1, which may provide undesired effects on the cells due to mechanical stress. In this chapter, a photocleavable PIP-1 analog (PcPIP-1) was synthesized using

substitution of the Thr residue of another degradation-resistant analog [Asp^6]PIP-1 with a photocleavable amino acid to “switch off” the activity by UV-irradiation at any time point. PcPIP-1 was highly resistant to enzymatic degradation by tobacco cells and induced a significant level of phytoalexin capsidiol production unless UV-irradiated. This response was effectively diminished after cleavage of the analog by UV-irradiation, and no negative effects of UV-irradiation were observed. Using this photocleavable analog, it was found that the prolonged activation of a MAP kinase, induced by the continuous action of PIP-1, caused an elevation of HMGR activity, which is likely to be associated with the initiation of the capsidiol production.

Chapter 4. Elucidation of Signaling Factors Important for Acetosyringone Accumulation in Tobacco Cells Induced by PIP-1

Acetosyringone, one of the representative extracellular phenolics in suspension-cultured tobacco cells, was accumulated in the incubation medium after PIP-1 treatment at the concentration where no capsidiol production was induced. When PIP-1 was removed by washing cells 1 h after elicitation, a significant level of acetosyringone was still induced, indicating that short-term stimulation is sufficient for the initiation of acetosyringone production. Based on the inhibitor studies, it was found that early signaling events induced by PIP-1, such as activation of kinase cascades and induction of oxidative burst, are closely associated with initiation of acetosyringone production. On the other hand, it was revealed that acetosyringone accumulation was involved in the phenylpropanoid synthetic pathway where some of the biosynthesis-related enzymes seem to be activated by the elicitation with PIP-1. In particular, the activity of PAL, an initial enzyme in the phenylpropanoid synthetic pathway, was shown to be regulated by NADPH oxidase-mediated ROS signals. These results suggested that NADPH oxidase-mediated oxidative burst plays a crucial role in acetosyringone accumulation through activation of PAL in the phenylpropanoid pathway.