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Proteomics of *Physcomitrella patens* to elucidate the functions of

12-oxo-phytodienoic acid

(プロテオミクスを用いたヒメツリガネゴケにおける

12-オキソファイトジエン酸の機能解析)

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Introduction

Plant hormones regulate a wide variety of physiological events in plants. Among them, jasmonic acid (JA) plays an important role for adaptation of stresses such as wounding and pathogenic infection. 12-Oxo-phytodienoic acid, an intermediate of JA biosynthesis in flowering plants, is also considered to have a signaling system, which is independent from JA. However, the detailed signaling mechanism of OPDA is still unknown.

Many studies have demonstrated that plant hormones such as auxin, cytokinins, and abscisic acid control physiological responses in a model moss, *Physcomitrella patens*. In *P. patens*, the first half of the octadecanoid pathway exists, however, JA is not synthesized. OPDA inhibits the growth of *P. patens*. Moreover, wounding induces OPDA accumulation in *P. patens*. Since OPDA, not JA, was accumulated in response to wounding, OPDA is suggested to be an important oxylipin as a signaling molecule in *P. patens*. Understanding of OPDA roles in *P. patens* would help to elucidate OPDA functions in plants.

There are two main developmental stage in *P. patens*; protonema and gametophore. Previous proteomic analysis of *P. patens* gametophore treated with OPDA showed that abundance of proteins was affected by OPDA in gametophore. In this research, proteomic analysis of *P. patens* protonema treated with OPDA was performed to compare the OPDA functions in protonema with those in gametophore. Moreover, the comparison of proteomic data between wild-type and OPDA-deficient *P. patens* mutant, which was disrupted *PpAOS1* and *PpAOS2* genes (*aos* mutant), was conducted to elucidate the OPDA functions in response to wounding.

1. Proteomic analysis of wild-type *P. patens* protonema treated with OPDA

In *P. patens*, a spore develops into a filamentous structure, protonema, and then differentiates onto gametophore that has leaf-like structure. OPDA retarded the growth of *P. patens* protonema in a dose-dependent manner. When OPDA concentration was more than 10 μM , the protonema growth was clearly inhibited by OPDA. Proteomics of *P. patens* gametophore treated with OPDA has been analyzed previously: OPDA increased the abundance of proteins related to photosynthesis and mainly decreased the abundance of proteins related to proteins synthesis. To compare OPDA effects in gametophore with those in protonema, proteomic analysis of wild-type *P. patens* protonema treated with OPDA was conducted. The data showed that the abundance of 41 proteins was significantly altered by OPDA with fold changes of more than 1.5 ($p < 0.05$). The abundance of 40 proteins decreased; only one protein increased in abundance due to OPDA treatment in protonema. Based on their biological properties, these differentially changed proteins were grouped into the following six categories such as defense, energy and carbohydrate metabolism, photosynthesis, protein metabolism (proteins synthesis, folding and degradation), others and unknown. The data showed that the proteins for which abundance decreased in response to OPDA at the protonema developmental stage were mainly involved in the metabolism of proteins and carbohydrates. The inhibition for protein accumulation is suggested to be a major physiological function of OPDA in *P. patens*. This study also showed that OPDA suppressed to accumulate histones and mRNA of histone genes at the protonema stage. To the best of our knowledge, this is first report that OPDA is involved in gene expression of histones. It is possible that suppression of histone expression at both steps, transcription and translation, is an OPDA-specific function in *P. patens* protonema. In *P. patens*, a subset of the physiological responses caused by OPDA is shown to differ between protonema and gametophore developmental stages. Our results suggest that the main function of OPDA is the repression of protein synthesis and energy consumption processes at both developmental stages. Additionally, OPDA is suggested to activate photosynthesis in both gametophore and protonema.

2. Comparative proteomic analysis of wild-type *P. patens* and OPDA-deficient *P. patens* mutant after wounding

To investigate the mechanism of the adaptation to wounding stress and the role of a biosynthetic enzyme for OPDA, allene oxide synthase (AOS), in response to wounding, three mutants with disrupted *PpAOS1* and *PpAOS2* genes were constructed. In contrast to wild-type, wounding did not cause to accumulate OPDA in these mutants. The morphological difference

between wild-type and these mutants was not found. The phenotypes between the three mutants were same, therefore a mutant (*aos* mutant) was selected for further experiments. Proteomic analysis showed that wounding increased the abundance of 114 proteins in the wild-type and 88 proteins in the *aos* mutant, while the abundance of 22 proteins in the wild-type was decreased due to wounding. Remarkably, the proteins in the *aos* mutant was not decreased significantly in response to wounding.

In wild-type, wounding mainly promoted the accumulation of proteins involved in protein synthesis, amino acid synthesis, photosynthesis, protein folding, and glycolysis in wild-type. Since these proteins response to wounding in *P. patens* are similar to those in flowering plants, the accumulation of these proteins in response to wounding may be a conserved physiological event in land plants.

Comparative proteomic analysis of wild-type *P. patens* and *aos* mutant after wounding showed that abundance of proteins involved in proteins synthesis was increased in both wild-type and *aos* mutant. It was possible that wound-induced accumulation of proteins involved in protein synthesis is possibly unrelated to AOS-related physiological event such as increase of OPDA in *P. patens*. The proteome data revealed that the increased abundance of proteins involved in amino acid metabolism, protein folding, photosystems, glycolysis, and energy synthesis were found in only wounded wild-type, suggesting that accumulation of these proteins by wounding was possibly influenced by AOS gene expression, which caused increase of OPDA concentration. Moreover, it is possible that the expression of *PpAOS1* and *PpAOS2* is involved in photosynthesis and effective energy utilization in response to wounding in *P. patens*. Additionally, these data also suggested that OPDA plays an important role in these processes. Wounding stimulates a wide variety of signaling systems. OPDA-mediated signaling pathway is considered to be one of wound-induced physiological responses in *P. patens*. On the other hand, the proteins that are presumably regulated by OPDA in wounded *P. patens* do not completely correspond to the proteins whose abundance are altered in *P. patens* treated with OPDA. Various types of signaling triggered by wounding are probably involved in crosstalk with OPDA signaling in *P. patens*.

The STRING (Search Tool or the Retrieval of Interacting Genes) database was used to calculate all direct interactions between the 136 and 88 proteins identified in the wild-type and the *aos* mutant. A complicated network of protein-protein interactions was found in wild-type. The proteins categorized in each group were closely related each other. In contrast to wild-type, the interactions between each protein group, which were clustered based on functions, were remotely related in the *aos* mutant. These data suggested that *PpAOS* genes disruption, which lowered the concentration of OPDA, reduces multiple protein-protein interactions in response to

wounding stress.

Wounding was particularly shown to induce gene expression and protein abundance of chaperonins, which are related to correct protein folding in wild-type. The transcription levels of three chaperonin genes were also increased in wild type after wounding. However, wounding did not induce the expressions levels of three chaperonin genes in *aos* mutant. When the *aos* mutant subjected to wounding and treated with 10 μ M OPDA at the same time, the transcriptional levels of three chaperonin genes were significantly increased. These results indicate that OPDA, which synthesized through AOS reaction, played an import role to regulate the expression of chaperonin in response to wounding in *P. patens*.

The elucidation of the detailed OPDA signaling mechanism would shed light on *P. patens* physiology and plant evolution. This study will support to advance the knowledge of how OPDA regulates physiology in *P. patens* and increases our understanding of the function of OPDA as a signaling compound in plants.