ジャスモン酸グルコシドによる活性酸素種産生と運動細胞収縮誘導およびその構造活性相関に関する研究

博士論文

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Jasmonic acid glucoside-mediated motor cell shrinkage via ROS production and its structure-activity relationship studies

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博士論文要約

Introduction

Nyctinasty, a circadian rhythmic folding/opening movement of plant leaves, has been studied since Darwin`s era. Nyctinastic leaf movement is triggered by the swelling/shrinking of motor cells through regulation of K^+ channels. Recently, 12-*O*-β-D-glucopynranosyljasmonic acid (JAG, **1**), identified as endogenous leaf-closing factor of *Samanea saman*, was revealed to cause the extensor motor cell shrinkage leading to the leaf-folding movement. However, the molecular mechanism of JAG-induced cell shrinkage remains unknown. In this study, I report the unique features of JAG: induction of Reactive oxygen species (ROS) production to induce cell shrinkage and involvement of D-glycopyranoside structure for the bioactivity and target affinity.

Result and discussion

1. JAG-triggered ROS production induces cell shrinkage

In plant, it is well-known that plant hormone abscisic acid (ABA) functions as a ligand in the shrinkage of guard cells and induces the stomatal closure in which ROS act as secondary messengers. Extensor motor cells and guard cells have been considered to be close in response to the behavior against light stimuli.

In this thesis, it was revealed that JAG triggered ROS production in extensor motor cell protoplasts in the daytime but not in flexor motor cell protoplasts. Interestingly, ABA could not trigger ROS production in extensor motor cells. It was also found that JAG-induced ROS production increased ROS in the cytosol of extensor motor cell protoplasts. Time-course of JAG-triggered ROS production was completely consistent with that of JAG-induced shrinkage of extensor motor cell protoplasts. Exogenous application of ROS inhibitors could cancel both of the JAG-triggered ROS production and JAG-induced cell shrinkage. Additionally, the external application of H_2O_2 could induce shrinkage of extensor motor cell protoplasts as did JAG. This JAG-induced cell shrinkage was confirmed to be independent on apoptosis-like programmed cell death which is well known to be involved in the ROS-induced cell shrinkage. This JAG-induced cell shrinkage requires the expression of outward-rectifying K^+ channel (*SPORK2*) which is high in daytime and extremely low at evening. Extensor motor cell cannot shrink at all in the evening when the expression of *SPORK2* was very low. Considering that SPORK2 could be activated by H_2O_2 in vitro (unpublished data), these results strongly suggested that JAG triggered ROS production which activated the SPORK2 to induce cell shrinkage.

2. The involvement of D-glycopyranoside structure in the structure recognition of JAG

JAG is the glycoside of plant hormone jasmonic acid. Structure-activity relationship (SAR) studies on JAG revealed the unique feature of JAG in which glycon moiety plays an important role in the structure recognition.

SAR studies using JAG (**1**), *ent*-JAG (*ent*-**1**), 12-*O*-β-L-glucopynranosyljasmonic acid (**2**), 12-*O*-β-D-glucopynranosyl-*ent*-jasmonic acid (*ent*-**2**), and open-chain type 12-*O*-D-sorbitoyljasmonic acid (**3**) revealed that only **1** could induce the cell-shrinking. It should be notified that no cell-shrinking

activity in **2** and **3** strongly suggested the importance of D-glucopyranosyl structure. Furthermore, I developed four hybrid-type JAG-CMP stereoisomers (**4**, *ent*-**4**, **5** and *ent*-**5**) which are composed of (-)-12-OH-jasmonic acid (**6**)/D-galactopyranoside, (+)-(*ent*-**6**)/L-galactopyranoside, (-)-(**6**)/L-galactopyranoside, (+)-(*ent*-**6**)/D-galactopyranoside moieties, respectively. Because D-galactopyranosyl moiety was stable against hydrolysis by glucosidases in the living cell and substitution of glucopyranosyl moiety into galactopyranosyl moiety (4′-epimer) did not affect the cell-shrinking activity as well as previously reported leaf-folding activity. In photoaffinity-labeling experiment, probe **4** was shown the highest affinity with the putative target protein of JAG (MTJG). The results of photoaffinity-labeling experiment provided direct proof that stereostructure of glycon moiety was important for binding with MTJG.

Interestingly, *ent*-**5** was also observed to be weakly bind to MTJG while **5** cannot bind to MTJG. Weak affinity of *ent*-**5** with MTJG can be attributed to the thermodynamic equilibrium of *ent*-**5** and 7-iso-*ent*-**5**. The minor component 7-iso-*ent*-**5** corresponding to the 3-epimer of **4** is expected to be contributed to the weak affinity of *ent*-**5** with MTJG.

Conclusion

This study revealed that JAG triggered ROS production in the extensor motor cell and activated the SPORK2 to induce cell shrinkage. I also confirmed that both glycon and aglycon moieties of JAG contributed to cell-shrinking activity and target affinity. This is a unique feature of JAG in which aglycon moiety plays an important role in the structure recognition of ligand by its target protein.