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研究課題名（和文）Dissecting role of sucrose and GA recognition in SWEET transporters

研究課題名（英文）Dissecting role of sucrose and GA recognition in SWEET transporters

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研究成果の概要（和文）：近年、糖輸送体SWEET が、糖だけでなく植物ホルモンであるGAも輸送することが報告された。糖と植物ホルモンは、植物の発生・生育に不可欠である。しかし、SWEETがショ糖とGAという異なる基質をどのように輸送できるのか、また、SWEETがどのような基質を輸送できるのかについては、これまで不明であった。本研究では、GAとショ糖を輸送するSWEETの基質選択性について、計算化学的および生化学的アプローチを用いて解析を行った。その結果、SWEETにおいてGAとスクロースを認識する残基を決定し、シロイヌナズナの稔性にスクロースとGAの輸送活性が生理的に関与しているかどうかを明らかにした。

研究成果の学術的意義や社会的意義
酵素や輸送体は無数の基質と相互作用するが、その中で生理的に意義があるものはごくわずかである。SWEETの基質選択性の分子メカニズムや輸送活性の生理的意義を解析することで、輸送体や酵素の持つ複数の基質活性の理解が深まる。本研究で得られた知見は、農薬や抗菌剤の開発などの創薬分野にも役に立つと考えられる。

研究成果の概要（英文）：Substrate selectivity of transporters is critical to accomplish the complex systems in biological processes. However, the molecular mechanisms of substrate selectivity of transporters have not been fully elucidated. SWEETs play essential roles through the transport activity for sucrose and/or glucose. In addition to sugars, SWEETs can transport GA, a hormone that regulates many important aspects of plant growth. Sugars and phytohormones are essential to the fundamental processes of plant development and growth. It has remained unclear, however, which spectrum of substrates SWEETs can transport, and how SWEETs can transport substrates as different as sucrose and GA. In this project, we investigated the substrate selectivity of GA and sucrose transporting SWEETs using computational and biochemical approaches. We have determined the residues responsible for GA and sucrose recognition in SWEETs and whether sucrose and GA are physiologically relevant for male fertility in Arabidopsis.

研究分野：Plant physiology

キーワード：Sucrose　Gibberellin　SWEET　substrate selectivity

１．研究開始当初の背景

Plants carry out myriads of reactions, orders of magnitude more than the number of genes in a given genome. We also know that xenobiotics are substrates of enzymes and transporters, indicating that a single protein must be able to carry out a large number of reactions; in the case of transporters move a large number of different molecules across the membrane. Well-known examples are oligopeptide transporters that can transport diverse drugs in the human body. Seo's group at RIKEN and others have found that members of the related plant nitrate-oligopeptide transporter family (NPF) can transport, besides their known substrates also structurally diverse plant hormones such as ABA, GA, JA-Ile and auxin. Recently, Seo's group also found that several members of a new class of sugar transporters, SWEETs, transport GA as well. Apparently, both nitrate and sucrose transporters are critical for their role in nutrition, and thus plant growth and development but possibly also in the context of hormone action. These finding lead to a number of important questions: how do the transporters recognize multiple substrates; how do the structurally very different substrates interact with the binding pockets; are they capable of transporting multiple substrates in vivo and are these activities physiologically relevant? And can we separate the activities?

We have recently discovered the family of SWEET proteins as sugar transporters in plant plasma and vacuolar membranes and the Golgi of humans. SWEETs were shown to not only be critical for carbon allocation, i.e. phloem loading and seed filling but also play key role in pathogen susceptibility. A more detailed understanding of the relative role of hormone and sucrose transport is thus also relevance for practical applications in agriculture.

２．研究の目的

SWEETs were discovered as sugar transporters, and we have demonstrated the ability of clade III Arabidopsis SWEETs family are essential for pathogen interactions as well as phloem loading, nectar production, seed filling and pollen nutrition. Recently, GA transport activity of Arabidopsis thaliana SWEET13 and 14 were detected. GA is known as the important key player in many cellular processes where it promotes stem elongation, overcomes dormancy in seeds and induces flowering. SWEETs could thus be critical in GA translocation. However, open questions are: (i) How do SWEETs recognize and transport different compounds like GA and sucrose? (ii) Can we separate these functions? (iii) Can we identify other substrates? (iv) Can we identify inhibitors that can be used to manipulate plant functions such as carbon allocation, GA transport and pathogen susceptibility? (v) How does GA transport GA transport of SWEETs contribute to plant developments and plant response to biotic and abiotic factors? To lay the basis for answering these questions, we propose computational approaches in addition to genetic and biochemical approaches to elucidate the molecular mechanisms of substrate selectivity in SWEETs.

３．研究の方法

(1) Molecular docking and molecular dynamics were used to predict the amino acid residues in the binding pocket of AtSWEET13 responsible for their transport activities.

(2) Mutations were introduced at sites predicted by molecular docking studies, and their transport activities for sucrose and GA was measured by in-cell transport activity assays.

(3) AtSWEET13 with shifted substrate preference has been introduced into the mutants to test the physiological relevance of the substrates.

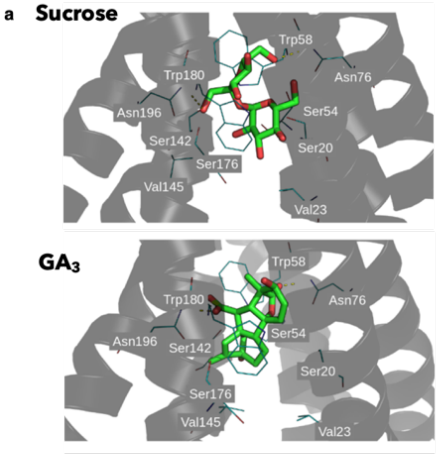
４．研究成果

(1) Investigate the substrate selectivity of the GA and sucrose transporting SWEETs from *Arabidopsis* using computational approaches

Molecular docking is known as an important tool for drug discovery. This structure-based method can be used to model the interaction between transporter and substrate at the atomic level, providing us with a prediction of the ligand-receptor complex structure. We used this powerful approach to characterize the substrate selectivity of the transporter. To understand the interaction between transport and two substrates, we have performed molecular docking studies based on the published crystal structure of *Arabidopsis* SWEET13 (PDB ID: 5XPD, Han et al., 2017) using AutoDock 4.2. The docking studies have identified the residues of binding pocket of AtSWEET13 for sucrose and GA_3 (Ser20, Val23, Ser54, Trp58, Asp76, Ser142, Val145, Trp180 and Asn196) (Fig. 1a).

(2) Determine the residues responsible for GA and sucrose recognition in Arabidopsis SWEETs using biochemical approaches

Using the predicted side chains that interact with GA and sucrose we performed site directed mutagenesis of AtSWEET13 to test the prediction and to shift the relative substrate selectivity either towards sucrose or GA. Sucrose and GA transport activities were quantified using transport activity assay. To quantify sucrose transport activity AtSWEET13 and sucrose fluorescent biosensor FLIPsuc-90μ∂1 were coexpressed in mammalian HEK293T cells (Chen et al., 2012). To quantify GA transport activity of AtSWEET, we have developed the GA transport activity assay using HEK293T expressing AtSWEET13 and GA biosensor GPS1. The results of in-cell transport activity assays indicate that $AtSWEET13^{N76Q}$ and $AtSWEET13^{N196Q}$ preferentially transport GA_3 over sucrose, whereas $AtSWEET13^{S142N}$ and $AtSWEET13^{V145L}$ preferentially transport sucrose over GA (Fig. 1b).



b	GA_3 (Y2H)	GA_3 (β-gal)	GA_3 (HEK293T)	Sucrose (HEK293T)
AtSWEET14	++++	++++	n.a	n.a
AtSWEET13	++++	+++	++++	++++
N76Q	++++	+++	+++	−
N196Q	+++	n.a	+++	−
S20N	+++	n.a	+	++++
S142L	++	+~++	+/++	++
S142N	++	++	+/++	+++
V145L	+	+	−	+~++

Fig. 1 (a) Sucrose and GA_3 binding position in AtSWEET13 predicted by docking studies. **(b)** Transporter activity assay using yeast two-hybrid system and mammalian cells with fluorescent biosensors.

(3) Test physiological relevance of transport activities by introducing transporters with shifted relative substrate selectivity into loss-of-function mutants

Fertility is reduced in the *sweet13; sweet14* mutants. To test whether GA transport activity are physiologically relevant in the context of fertility, $AtSWEET13^{N76Q}$ and $AtSWEET13^{S142N}$ were introduced into *sweet13; sweet14* double mutants. The results showed that sucrose transport activity, but not GA transport activity of AtSWEET13, is required for fertility.

<引用文献>

L. Han, *et al.*, Molecular mechanism of substrate recognition and transport by the AtSWEET13 sugar transporter. *Proc. Natl. Acad. Sci. USA* **114**, 10089–10094 (2017)

L.-Q. Chen, *et al.*, Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science* **335**, 207–211 (2012).

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　2件）

１．著者名 Isoda Reika, Yoshinari Akira, Ishikawa Yuuma, Sadoine Mayuri, Simon Rüdiger, Frommer Wolf B., Nakamura Masayoshi	４．巻 105
２．論文標題 Sensors for the quantification, localization and analysis of the dynamics of plant hormones	５．発行年 2020年
３．雑誌名 The Plant Journal	６．最初と最後の頁 542～557
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/tpj.15096	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Wu Lin‐Bo, Eom Joon‐Seob, Isoda Reika, Li Chenhao, Char Si Nian, Luo Dangping, Schepler‐Luu Van, Nakamura Masayoshi, Yang Bing, Frommer Wolf B	４．巻 234
２．論文標題 OsSWEET11b, a potential sixth leaf blight susceptibility gene involved in sugar transport‐dependent male fertility	５．発行年 2022年
３．雑誌名 New Phytologist	６．最初と最後の頁 975～989
掲載論文のDOI（デジタルオブジェクト識別子） 10.1111/nph.18054	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計1件（うち招待講演　1件／うち国際学会　1件）

１．発表者名 Wolf Frommer
２．発表標題 Genetically encoded biosensors - design and use in plants
３．学会等名 EMBO Course Functional Live Cell Imaging（招待講演）（国際学会）
４．発表年 2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関