



Title	Functions, structures, and applications of enzymes acting on trehalose and its derivatives [an abstract of dissertation and a summary of dissertation review]
Author(s)	田口, 陽大
Degree Grantor	北海道大学
Degree Name	博士(農学)
Dissertation Number	甲第14383号
Issue Date	2021-03-25
Doc URL	https://hdl.handle.net/2115/81099
Rights(URL)	https://creativecommons.org/licenses/by/4.0/
Type	doctoral thesis
File Information	Taguchi_Yodai_abstract.pdf, 論文内容の要旨



学位論文内容の要旨

博士の専攻分野名称：博士（農学）

氏 名：Yodai Taguchi

学位論文題名

Functions, structures, and applications of enzymes acting on trehalose
and its derivatives

（トレハロースおよびその誘導体に作用する酵素の構造，機能
ならびに応用に関する研究）

Trehalose (Glc α 1-1 α Glc) is a non-reducing disaccharide used as an energy source and a compatible solute in various organisms. In the universal pathway, trehalose is synthesized *via* trehalose 6-phosphate (Tre6P, Glc α 1-1 α Glc6P). Tre6P synthase (TPS) produces Tre6P from UDP-Glc and Glc6P, and Tre6P phosphatase (TPP) dephosphorylates Tre6P to produce trehalose. Trehalose is hydrolyzed to Glc by trehalase. In plants, the endogenous amount of Tre6P is very small, but Tre6P acts as an essential signal substance for controlling growth, flowering, and nutrition metabolism. Plants have multiple isozymes responsible for trehalose-synthesis. Since these enzymes are differently expressed in various organs, growth stages, and environments, plants strictly regulate the level of Tre6P using the multiple-enzyme system. In contrast to the trehalose synthesizing enzymes, plants possess a single trehalase responsible for the degradation of trehalose. Despite the physiological importance of trehalose related enzymes to regulate Tre6P signaling, enzyme functions and structures have been scarcely understood thus far. For precise functional and structural analysis of the enzymes, Tre6P and its derivatives are required abundantly. In this study, enzymatic synthesis of Tre6P and its derivative is established, and functions and structures of TPP isozymes and trehalase from *Arabidopsis thaliana* are investigated to provide a better understanding of the trehalose metabolism in plants.

1. Chemoenzymatic synthesis of Tre6P and its derivative using the reverse phosphorolysis catalyzed by Tre6P phosphorylase

Tre6P phosphorylase (TrePP) reversibly phosphorolyzes Tre6P into β -Glc1P and Glc6P. For Tre6P synthesis, the reverse phosphorolysis of Tre6P catalyzed by *Lactococcus lactis* TrePP was used. The TrePP produced 91.9 mM Tre6P from 100 mM β -Glc1P and 100 mM Glc6P. The one-pot reaction of TrePP and maltose phosphorylase enabled the production of 65 mM Tre6P from 100 mM maltose (Mal), 100 mM Glc6P, and 20 mM inorganic phosphate (Pi). By the addition of β -phosphoglucosyltransferase, converting β -Glc1P to Glc6P, Glc6P was eliminated from starting materials. Besides, the addition of yeast, consuming Glc produced in the phosphorolysis of Mal, increased the yield of Tre6P. Finally, from 1 M Mal and 0.6 M Pi, 0.375 M Tre6P was synthesized in a 100 mL scale reaction, and 9.4 g (19 mmol) of highly purified Tre6P dipotassium salt was obtained by the purification procedures. Acceptor screening in the reverse phosphorolysis of TrePP showed that

Man6P also served as an acceptor substrate. From β -Glc1P and Man6P, a novel sugar phosphate, Glc α 1-1 α Man6P, was synthesized at 51% yield.

2. Comprehensive evaluation of enzymatic properties of TPP isozymes from *A. thaliana*

A. thaliana has 10 TPP isozymes, AtTPPA–AtTPPJ, with 45–82% identity in the amino acid sequences. For the investigation of the enzymatic functions of these TPP isozymes, recombinant AtTPPs were produced in *E. coli* and purified. All AtTPPs except for AtTPPI showed the dephosphorylation activity toward Tre6P. The k_{cat}/K_m of AtTPPs for Tre6P was diverse ranging from 0.113 s⁻¹mM⁻¹ to 23.0 s⁻¹mM⁻¹. AtTPPA and AtTPPB, whose gene expression level is high at the upper ground tissues and root, respectively, showed higher k_{cat}/K_m than the other isozymes. All active AtTPPs dephosphorylated Glc α 1-1 α Man6P as well as Tre6P. Since AtTPPs did not have the detectable activity to α -Glc1P, β -Glc1P, Glc6P, Fru6P, Gal6P, Man6P, α -Mal1P, and Suc6P, AtTPPs are considered to recognize the α 1-1 α glucosyl group of substrates strictly.

3. Molecular mechanism of substrate recognition of plant trehalase which changes substrate specificity depending on pH conditions

Trehalase from *A. thaliana* (AtTRE1) was characterized using truncated enzyme of the N-terminal transmembrane domain. AtTRE1 showed hydrolytic activity not only toward trehalose but also its analogs containing Tre6P. This substrate specificity was common in *Oryza sativa* trehalase. MS analysis of ¹⁸O-labeled reaction products from Tre6P showed that Glc and Glc6P residues bind to subsite -1 and +1, respectively. AtTRE1 showed two peaks at pH 3.7 and pH 6.9 in the pH- k_{cat} curve of trehalose hydrolysis, indicating that AtTRE1 forms two types of ES complex, designated as ES_{acid} and ES_{neutral}, respectively, depending on pH conditions. This enzyme showed much higher activity to Tre6P at acidic pH than at neutral pH and showed a single peak at pH 3.9 in the pH- k_{cat} curve for the hydrolysis of Tre6P. Thus, AtTRE1 is considered to take a suitable structure for the hydrolysis of Tre6P at acidic pH. The structural analysis of inactive AtTRE1 mutant (D380A) in complex with trehalose revealed that α 1 $\rightarrow$ α 2 loop and α 11 $\rightarrow$ α 12 loop covered the substrate binding site, and the substrate binding site was closed (closed-form). The residues, Arg224 and Glu595, on these loops could form hydrogen bonds with 6-OH of Glc in subsite +1. By co-crystallization with Tre6P, the distorted structure of AtTRE1 was obtained. In this structure, the residues, located on α 1 $\rightarrow$ α 2 and α 11 $\rightarrow$ α 12 loops, flipped to open the substrate binding site (open-form). Since the structure of the closed-form AtTRE1 is not suitable for binding to Tre6P due to possible steric hindrance against the phosphoryl group, the open-form was considered to be suitable for the reaction with Tre6P. In the ES_{acid} and ES_{neutral}, AtTRE1 presumably takes the structures similar to the open- and closed-forms, respectively. Ser223 and Thr600 on α 1 $\rightarrow$ α 2 and α 11 $\rightarrow$ α 12 loops, respectively, could form hydrogen bonds differently in the closed- and open-forms. Since S223G mutation enhanced the preference for Tre6P, S223 was important for stabilizing the closed-form (open-form was more preferred in this mutant than in the wild type). On the other hand, T600D mutation to stabilize the closed-form reduced the preference for Tre6P. The mechanism for the interconversion of ES_{acid} and ES_{neutral} through the structural change of the α 1 $\rightarrow$ α 2 and α 11 $\rightarrow$ α 12 loops is proposed.