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Article / Book Information

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Title(English)	
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Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
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論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学 ﾗｲﾌｴﾝｼﾞﾆｱﾘﾝｸﾞ	系 コース	
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申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of （ 理学 ）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

In chloroplasts, redox regulation system plays a central role in regulation of photosynthesis activities. Various photosynthesis-related enzymes such as four Calvin-Benson cycle enzymes fructose-1,6-bisphosphatase (FBPase), glyceraldehyde-3-phosphate dehydrogenase, sedoheptulose-1,7-bisphosphatase, and phosphoribulokinase, NADP-malate dehydrogenase (MDH) working in malate valve, and γ subunit of ATP synthase possess Cys pairs for redox-regulation. These Cys pairs can reversibly form disulfide bond and serve as redox switches of the enzyme activities. In 1977, thioredoxin (Trx) was identified as a protein involved in the reduction of the disulfide bond. After the discovery of the whole genome sequence of *Arabidopsis thaliana* in 2000, it was revealed that chloroplasts possess 5 types of Trx, Trx-*f*, Trx-*m*, Trx-*x*, Trx-*y*, and Trx-*z*, whose target selectivity is different from each other.

Since Trx-*f* and Trx-*m* was reported in 1970s, many studies have attempted to clarify the determinants of the target selectivity of these Trx isoforms. However, these studies mainly used Trx-*f* and Trx-*m* from spinach, and did not consider other types of Trx isoforms and Trxs from other plants. To address these issues, I first studied the residues specifically conserved in each type of Trx by comparison of the amino acid sequences of chloroplast Trxs from various plants. I especially focused on Trx-*f*, most well-studied chloroplast Trx, and used FBPase and artificial Trx-targeted protein "change in redox state of Trx 1 (CROST1)" as Trx-*f*–specific target proteins. Consequently, I could identify the key residues that determine the target selectivity of Trx-*f* by the mutational analysis using Trx-*f*1 (one of the isoforms of Trx-*f* in *A. thaliana*) mutants. The impact of the identified residues on Trx-*f*–specific target recognition was then confirmed by the biochemical analysis with Trx-*m*2 (one of the isoforms of Trx-*m* in *A. thaliana*) mutants and molecular dynamics simulation with a complex structure model of Trx-*f*1 and FBPase.

I also focused on Trx-like proteins which have structures and activities similar to Trx. Recently, Trx-like 2 (TrxL2), one of the Trx-like proteins, was identified as an oxidation factor for Trx-targeted proteins, which mediates a reverse reaction of Trx-mediated reduction process. I found that other Trx-like proteins named atypical Cys His-rich Trx 1 (ACHT1) and ACHT2a can also serve as oxidation factors. ACHT1 and ACHT2a possess several key residues identified on Trx-*f*, and they could efficiently oxidize Trx-*f*–specific targets FBPase and CROST1. This result indicates that target selectivity also exist in the target oxidation process mediated by these proteins.

Hence, TrxL2.1 and TrxL2.2 (the isoforms of TrxL2 in *A. thaliana*), ACHT1, and ACHT2a have been identified as oxidation factors in vitro, whereas there is no evidence that these proteins can oxidize their target proteins in plant cells. I therefore generated mutant plants deficient in these Trx-like proteins and investigated the impact on target oxidation. Consistent with the results of biochemical analyses, the results of in vivo analyses indicate that these Trx-like proteins serve as oxidation factors and they also have target selectivity like Trxs.

Finally, I investigated the physiological significance of the redox regulation system. Because non-plastidial–type homologs of redox-regulated enzymes in chloroplasts are switch-free and constitutively active, deactivation of the chloroplast enzymes in the dark should be important for chloroplast function. MDH, which is one of the redox-regulated enzyme, works in malate valve. Because malate valve exports the reducing power of NADPH in chloroplasts to the outside, MDH is thought to be tightly inactivated in the dark to prevent excessive export of reducing power from chloroplasts. To examine the validity of this hypothesis and investigate the physiological significance of the redox regulation system, we prepared a mutant plant in which a redox switch of MDH is deleted. Using this mutant plant, we revealed that the redox regulation of MDH is vital under certain physiological conditions, but not much critical under normal growth conditions in laboratories. Metabolome analysis and chlorophyll fluorescence measurement revealed that the redox switch of MDH is required for maintaining NADPH pool in chloroplasts.

Taken together, this doctor thesis study provide important insight to understand the molecular mechanism and physiological significance of the redox regulation in chloroplasts.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。
Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1copy of 800 Words (English).

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