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Abstract of Thesis

Name (Linda Juniar)	
Title	Structural Analysis of the Electron Transfer Complex Between Ferredoxin–Thioredoxin Reductase and Thioredoxin （フェレドキシン–チオレドキシン還元酵素とチオレドキシンが形成する電子伝達複合体の構造研究）

Abstract of Thesis

Plants have developed regulatory systems to survive through fluctuating light environments. For optimization of metabolic flux in the stroma under such condition, chloroplasts have to tune their metabolic reactions in a light-dependent manner. Thiol-based redox regulation is one of the mechanisms developed to control the enzymatic reactions in chloroplast. In this system, two soluble redox-mediator proteins, known as ferredoxin–thioredoxin reductase (FTR) and thioredoxin (Trx), sequentially transfer the electron from the photosynthetic electron transport chain to the target enzymes such as fructose 1,6-bisphosphatase, NADP-malate dehydrogenase or 2-Cys peroxiredoxins.

Completion of *Arabidopsis thaliana* genome sequencing has indicated that 10 Trx isoforms are localized in chloroplasts and classified into five subtypes (two Trx-*f*, four Trx-*m*, one Trx-*x*, two Trx-*y*, and one Trx-*z*). On the other hand, the catalytic subunit of FTR is encoded by a unique gene associated with a variable subunit encoded by two genes. FTR acts as an important protein that transfers reducing equivalents from the photosynthetic electron transport chain to Trxs. Several structures of Trxs have been determined by X-ray crystallography. Despite the common Trx-fold, each Trx shows different molecular characteristics, such as the protein surface charge and midpoint redox potential (*E*m), leading to their differences in target and protein-protein interaction. Based on their FTR-dependent kinetic parameters, *A. thaliana* Trxs can be clustered into three classes: high, middle, and low efficiency group. The comparative kinetic analysis showed that the electron transfer efficiency from FTR to Trx is not fully controlled by *E*m but also by other factors. To date, the three-dimensional structure of *Synechocystis* FTR and spinach Trx complexes have been reported. The interaction of Trx-*m* with FTR is similar to that of Trx-*f*. The conformational difference exists in small rotation angles of Trx molecule to the flat FTR molecule. Since this published FTR:Trx complex consisted of proteins from different organisms, I could not refer those non-physiological complex structures to explain the differences in FTR affinity to three classes of Trx from *A. thaliana*. Therefore, I focused on the physiological complex between FTR and Trx isoforms from *A. thaliana*. After extensive screening of crystallization, I have chosen Trx-*y*1, Trx-*f*2 and Trx-*m*2 as a representative for high, middle and low efficiency group, respectively.

In this research, I have successfully determined three structures of FTR:Trx protein complex; FTR:Trx-*y*1; FTR:Trx-*f*2; and FTR:Trx-*m*2, at 1.59 Å, 1.79 Å and 2.4 Å, respectively. FTR:Trx-*f*2 and FTR:Trx-*m*2 complexes contained Trx, catalytic and variable subunit of FTR, whereas FTR:Trx-*y*1 contained Trx and FTR catalytic subunit only. The overall structure of three FTR:Trx complexes was similar with the rmsd values of 0.588 Å based on the Cα atoms of FTR, and the values ranging from 1.2 to 1.5 Å based on those of Trxs. Trx molecules interacted mostly with catalytic subunit of FTR with different rotational angles of 7.9-16.1 degree in polar angle of kappa.

I performed superposition of FTR and Trx in the complex and single form to study the structural changes upon complex formation. I used *Synechocystis* FTR structure (PDB ID 1DJ7) for comparison with *A. thaliana* FTR in FTR:Trx complexes, and *A. thaliana* Trx-*m*1 (determined at 1.1 Å resolution) and spinach Trx-*f* (PDB ID 1FAA) for comparison with Trx in the complexes. Since there are no available structures of Trx-*y* monomer, I could not do the same analysis for Trx-*y*1. The structural comparison results showed that there were microscopic changes of FTR or Trx upon complex formation. The biggest structural differences were found in the side chain conformations located at the molecular interface.

Since the resolution of the complex structures were quite good, I comparatively studied the interaction between

FTR and Trxs. The Trx molecules interact with FTR mainly through hydrophobic interactions which can be clustered into two types, common interaction for all Trx isoforms and isoform-specific peripheral interaction. The FTR:Trx-*y*1 complex had more isoform-specific interactions, followed by FTR:Trx-*f*2 and FTR:Trx-*m*2, which was supported by the total buried surface area of FTR:Trx-*y*1 around 1622 Å^2 whereas Trx-*f*2 and Trx-*m*2 were 1470 Å^2, and 1509 Å^2, respectively. $S_{0.5}$ of Trx-*y*1 was 10-fold smaller than that of Trx-*f*2, while $S_{0.5}$ of Trx-*m*2 was not so different from that of Trx-*f*2. The electrostatic potential of each Trx may drive the differences in isoform-specific interaction leading to different affinity of FTR toward Trx.

Based on my determined three X-ray structures of FTR:Trxs, I have concluded that there were small but significant structural changes of FTR and Trx upon complex formation, and the number of isoform-specific interaction located peripherally is the key for the three classes of enzymatic efficiency, which may be facilitated by the differential charged surface of Trx isoforms.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

学位申請者は，光合成ではたらくレドックス蛋白質チオレドキシンに着目し「STRUCTURAL ANALYSIS OF THE ELECTRON TRANSFER COMPLEX BETWEEN FERREDOXIN–THIOREDOXIN REDUCTASE AND THIOREDOXIN(フェレドキシン–チオレドキシン還元酵素とチオレドキシンが形成する電子伝達複合体の構造研究)」と題する研究を行った．

光合成生物は，地球上の多様で変化に富んだ光環境に対応するため，生育条件に合わせて光合成機能を最適化させる光環境適応機構を発達させてきた．光環境適応機構の一つに，フェレドキシン-チオレドキシン還元酵素（FTR）とチオレドキシン（Trx）を介した代謝酵素の活性調整が重要であることが知られている．申請者は，シロイヌナズナのゲノム解析で見出された 10 種類の Trx アイソフォームが，FTR による還元活性で，High，Middle，Low の３つの活性グループに分類できることに着目した．すなわち単一の FTR 分子が 10 種類もの Trx アイソフォームを区別して認識し，活性調整の際に多様な光環境に応じて Trx を使い分けている可能性に着目したのである．しかし，単一の FTR がアミノ酸配列の似通った 10 種類の Trx をどのように区別して異なった活性を引き出しているのか，その構造基盤は明らかになっていなかった．本論文で，申請者は反応効率の高い FTR:Trx-*y*1 複合体の結晶構造を 1.59Å 分解能で，中程度の反応効率を示す FTR:Trx-*f*2 複合体の結晶構造を 1.79Å で，そして反応効率の低い FTR:Trx-*m*2 の結晶構造を 2.459Å 分解能で決定し，各複合体構造のＸ線結晶構造をアミノ酸レベルで議論できる分解能で明らかにしている．また，複合体形成前の FTR と Trx との立体構造比較を基に複合体形成に伴う構造変化の解析も行なって，FTR や Trx アイソフォームが複合体形成の際に主鎖骨格を大きく変化させることはなく，アミノ酸側鎖のコンフォメーションを変化させることで複合体を安定化させることを突き止めた．次に申請者は，３種類の活性効率の異なる複合体の分子間相互作用に着目して，FTR と Trx の相互作用を各 Trx アイソフォームに共通なものとアイソフォーム特異的なものの２種類に分類することで，各 Trx アイソフォームに特有な相互作用が分子界面の縁に相対的に配置されることを解明している．さらに申請者は，これら３種類の複合体構造が成す分子間相互作用面のうち，高活性の FTR:Trx-*y*1 が 1,622 Å^2 と最も広い相互作用面を示して，速度論パラメータとも整合することを考察している．

申請者は，環境適応機能を高度に発達させた高等植物が特徴的に保持している複数種類の Trx アイソフォームと FTR との相互作用に着目し，複数の結晶構造を高分解能で解析する事で，最終的に FTR と Trx アイソフォームの相互作用と活性調節反応との二つを結びつける構造基盤を提唱するに至っている．本論文の研究内容は，光合成電子伝達の制御機構を理解する上で，大変意義のある成果である。

よって，本論文は博士（理学）の学位論文として十分価値あるものと認める．