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学位論文題目　Molecular-Biological and Physiological Characterization of Thioredoxin Peroxidase in Cyanobacteria

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論文内容の要旨

Active oxygen species (AOS), such as O_2^-, ・OH and H_2O_2, are generated as a result of the incomplete reduction of O_2 during respiration and photosynthesis. Organisms living in oxygenic environments have an absolute requirement for mechanisms that detoxify AOS. AOS is extremely reactive and can cause severe damage of cell components *in vitro*, for example, by inactivating proteins, cleaving DNA and causing peroxidation of unsaturated fatty acids in cell membranes [1]. In plant chloroplasts, O_2^-, the primary product in the oxygen reduction in chloroplasts, is immediately dismutated to H_2O_2 and O_2 by superoxide dismutase, and H_2O_2 is reduced to H_2O by ascorbate peroxidase, which uses ascorbate as the electron donor. The univalently and divalently oxidized products of ascorbate, monodehydroascorbate and dehydroascorbate, respectively, are then re-reduced to ascorbate via the Halliwell-Asada pathway [2].

In cyanobacteria, H_2O_2 is scavenged by peroxidases and/or catalases [3]. The peroxidases use electrons generated during the photosynthetic electron transport and such peroxidase activity is not observed in the presence of DCMU or in the dark. However, the physiological and enzymological properties of "light-dependent peroxidases" and the donors of electrons have not been fully clarified [4].

Several years ago, Kim *et al.* isolated a novel antioxidant enzyme, thioredoxin peroxidase (TPX), from yeast [5]. The enzyme catalyzes not only the reduction of H_2O_2 to H_2O but also the reduction of alkyl hydroperoxides to the corresponding alcohols and H_2O, with thioredoxin as the electron donor [6,7]. Baier and Dietz reported that some plants have a gene for a homolog of TPX, bas1, and that the product of this gene is localized in the chloroplast stroma [8,9]. Furthermore, the genome of the cyanobacterium *Synechocystis* sp. PCC 6803 includes an open reading frame (ORF) designated sll0755 that encodes a putative homolog of TPX [9,10]. However, the existence of alkyl hydroperoxide reductase and TPX in cyanobacteria and chloroplasts has not been verified. Characterization of TPX in cyanobacteria will contribute to precise understanding of the detoxification of alkyl hydroperoxide in cyanobacteria and chloroplasts, and to our knowledge of the machinery of dissipation of excess photons as well as the water-water cycle in chloroplasts.

The aims of the present study are: (1) biochemical identification of the product of ORF sll0755 from *Synechocystis* as TPX, and (2) characterization *in vivo* of the product of ORF sll0755 as a "light-dependent peroxidase" that reduces peroxides with electrons donated from the photosynthetic electron transport system.

Chapter 1. General introduction

In this chapter, he reviewed the previous studies on peroxiredoxin in various

organisms and the machineries and enzymes involved in the detoxification of active oxygen species in chloroplasts and cyanobacteria.

Chapter 2. Cloning of genes for thioredoxin peroxidase of cyanobacteria, *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942 and characterization of the recombinant protein expressed in *Escherichia coli*

In this chapter, he described the cloning of thioredoxin peroxidase genes (tpx) of cyanobacteria and biochemical characterization of recombinant TPX protein expressed in *E. coli*. The amino acid sequence deduced from the ORF designated sll0755 in *Synechocystis* is similar to the amino acid sequences of TPXs from other organisms. The product of ORF sll0755 was overexpressed as a fusion protein with a histidine tag in *E. coli* under the control of T7 promoter. The fusion protein purified by affinity chromatography showed the activity to reduce peroxides with thioredoxin and NADPH:thioredoxin oxidoreductase-coupling system from *E. coli* as an electron donor [11]. These results indicated that the ORF sll0755 encodes TPX. Furthermore, a 6.2-kb fragment of DNA that contained the *tpx* gene from *Synechococcus* was isolated by inverse PCR and normal PCR. The amino acid sequence deduced from the *tpx* gene from *Synechococcus* was also similar to those of TPXs from red algae and some land plants and conserved two catalytic cysteine residues.

Chapter 3. The function of thioredoxin peroxidase as a light-dependent peroxidase in *Synechocystis* sp. PCC 6803

In this chapter, he described the targeted disruption of the *tpx* gene in *Synechocystis* cells and biochemical and physiological properties of the *tpx*⁻ mutant. In cyanobacteria, H_2O_2 is scavenged by peroxidase and/or catalase peroxidase [3]. *Synechocystis* has the activity of light-dependent peroxidase that reduces H_2O_2 to water with electrons donated from the photosynthetic electron transport system. However, the functions of light-dependent peroxidase and its electron donor have not been clarified. The function of TPX as a "light-dependent peroxidase" *in vivo* has been examined by targeted disruption of the *tpx* gene in *Synechocystis* cells by insertional mutagenesis with a spectinomycin/streptomycin resistance gene cassette. *tpx*⁻ cells were able to grow under low-intensity light (30 μmol photons m^{-2} s^{-1}), indicating that TPX is not essential for the growth of *Synechocystis* cells under non-oxidative conditions. In contrast to wild-type cells, the H_2O_2-dependent and tertiary-butyl hydroperoxide-dependent photosynthetic evolution of oxygen and the electron flow in photosystem II by adding H_2O_2 or tertiary-butyl hydroperoxide to the cell suspension were absent in the cells of *tpx*⁻ [11]. These results indicated that TPX functions as a "light-dependent peroxidase" whose activities are coupled to the photosynthetic electron transport system in *Synechocystis* cells. These findings

provided for the first fine evidence that the TPX-dependent flow of electrons is operating in living photosynthetic cells.

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論文の審査結果の要旨

酸素が励起または還元されて生じる活性酸素は、生物に酸化的ストレスを引き起こす物質である。酸素発生型光合成生物において光化学反応系を通じて多量の活性酸素が生成するため、活性酸素消去系を発達させてこれに対応している。本研究では、現在まで未知であったシアノバクテリアの活性酸素消去系の存在を生理・生化学的、ならびに分子生物学的に解明することを目的としている。本論文は次の３章から構成されている。

第１章：General Introduction　近年報告された新規な過酸化水素消去酵素であるチオレドキシンペルオキシターゼおよびペルオキシレドキシンファミリーの酵素学的特性および生理学的機能についてまとめている。さらに現在までに報告されている高等植物葉緑体とシアノバクテリアにおける活性酸素消去系および構成する酸素の種類と特徴についてまとめ、チオレドキシンペルオキシターゼが光合成生物の新規な活性酸素消去系を構成する可能性を論じている。最後に、本研究を遂行する目的と意義を記述している。

第２章：Cloning of genes for thioredoxin peroxidase of cyanobacteria, Synechocystis sp. PCC 6803 and Synechococcus sp. PCC 7942 and characterization of the recombinant protein expressed in Escherichia coli シアノバクテリア Synechocystis sp. PCC 6803 の ORFsll0755 にコードされるタンパク質がチオレドキシンペルオキシターゼであることをリコンビナント酵素を用いた酵素学的解析により明らかにした。また Synechococcus sp. PCC 7942 からのチオレドキシンペルオキシターゼ遺伝子のクローニングをおこない、その結果からシアノバクテリアにおけるチオレドキシンペルオキシターゼの存在の普遍性を示唆した。

第３章：The function of thioredoxin peroxidase as a "light-dependent peroxidase" in Synechocystis sp. PCC 6803 in vivo におけるチオレドキシンペルオキシターゼの機能形態を明らかにするために、Synechocystis sp. PCC 6803 株のチオレドキシンペルオキシターゼ遺伝子を挿入破壊した。作成した変異株と野生株の過酸化水素およびヒドロペルオキシドに対する応答を比較検討することにより、細胞内においてチオレドキシンペルオキシターゼは光化学で生じた光還元力をフェレドキシン-チオレドキシン系を介して受け取り、過酸化水素を還元する新規な活性酸素消去系を構成することを明らかにした。

以上のように、申請者の研究の内容は、thioredoxin peroxidase が光合成生物において新規な活性酸素消去系を構成することを明確に示したものであり、博士論文にふさわしいものと判断し、合格と判定した。

さらに、専門分野及びその基礎となる分野に関して審査し、博士論文の内容についても討論した。その結果、申請者は十分な学力を有するものとして判断された。

また、英語能力に関して審査した。既に英文原著論文を発表していることから、十分な英語能力を備えているものと判断した。