Form 2

Dissertation Abstract

Report no.	(Course-based) No. 1003	Name	YUTTHANASIRIKUL RAYAKORN
Dissertation title	Molecular Mechanism of Oxidative Damage to Translation Factor EF-Tu in the Cyanobacterium *Synechocystis* sp. PCC 6803 (シアノバクテリア Synechocystis sp. PCC 6803における翻訳因子 EF-Tu の酸化傷害の分子機構)		

In the doctoral thesis, molecular mechanism of the oxidation of translational factor EF-Tu was studied in the cyanobacterium *Synechocystis* sp. PCC 6803.

Chapter 1 summarizes the response of cyanobacteria to oxidative stress and the role of EF-Tu in translation system. Previous studies on the response of photosynthesis to oxidative stress and the defense mechanisms to protect photosynthesis against oxidative damage are reviewed. In addition, the sensitivity of elongation factors to oxidation and, in particular, recent findings on the mechanism of oxidation of translation factor EF-G are reviewed. The functions of elongation factors in translation are also reviewed. The aim and experimental design of the present study are described. The aim is to clarify the molecular mechanism by which translation factor EF-Tu is oxidized and inactivated by reactive oxygen species (ROS).

Chapter 2 describes the molecular mechanism of the oxidation of EF-Tu that was revealed in the present study. The structure of EF-Tu changes dramatically depending on the bound nucleotide. Therefore, I investigated the sensitivity to oxidation *in vitro* of GTP- and GDP-bound EF-Tu, as well as that of nucleotide-free EF-Tu. Assays of translational activity with a reconstituted translation system from *Escherichia coli* revealed that GTP-bound and nucleotide-free EF-Tu were sensitive to oxidation by H_2O_2, whereas GDP-bound EF-Tu was resistant to H_2O_2. The

inactivation of EF-Tu was the result of oxidation of Cys82, a single cysteine residue, and subsequent formation of both an intermolecular disulfide bond and sulfenic acid. Replacement of Cys82 with serine rendered EF-Tu resistant to inactivation by H_2O_2, confirming that Cys82 was a target of oxidation. Furthermore, oxidized EF-Tu was reduced and reactivated by thioredoxin. Gel-filtration chromatography revealed that some of the oxidized nucleotide-free EF-Tu formed large complexes of more than 30 molecules. Atomic force microscopy revealed that such large complexes dissociated into several smaller aggregates upon addition of dithiothreitol. Immunological analysis of the redox state of EF-Tu *in vivo* showed that levels of oxidized EF-Tu increased under strong light. Thus, resembling EF-G, EF-Tu appears to be sensitive to ROS via oxidation of a cysteine residue and its inactivation might be reversed in a redox-dependent manner.

Chapter 3 describes the conclusions deduced from the results of the present study and the perspectives for future study. Future study should be directed toward fuller understanding of the mechanism by which the function of EF-Tu in translational elongation is suppressed by its oxidation and also toward detection of the large complexes of oxidized EF-Tu *in vivo* and understanding of their physiological significance.