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Formation of Cross-Linked Products of The Reaction Center D1 Protein in Photosystem II under Light Stress

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When illuminated with strong visible light, the reaction center D1 protein of photosystem II is photodamage and degraded. Reactive oxygen species and endogenous cationic radicals generated by photochemical reactions are the cause of the damage to the D1 protein. Recently we found that the photodamaged D1 protein cross-links with the surrounding polypeptides such as D2 and CP43 in photosystem II. As the cross-linking reaction is dependent on the presence of oxygen, reactive oxygen species are suggested to be involved. Among the reactive oxygen species examined, · OH was most effective in the formation of the cross-linked products. These results indicate that the cross-linking is mostly due to · OH generated at photosystem II. The cross-linking site of the D1 protein is not known. As several tyrosine residues exist at the D-E loop of the D1 protein, there is a possibility that di-Tyr is formed between the D-E loop of the D1 protein and surrounding polypeptides during the strong illumination. Therefore, we examined the formation of di-Tyr using the monoclonal antibody against di-Tyr under excess illumination of the photosystem II membranes. The results obtained here suggest that no di-Tyr is formed during the excess illumination of photosystem II.

Key words: photosystem II, reaction center, D1 protein, reactive oxygen species, di-tyrosine, photoinhibition

INTRODUCTION

In oxygenic photosynthetic organisms, light is necessary as the driving force of photosynthesis, but strong illumination results in loss of photosynthetic capacity. This phenomenon is called photoinhibition. The D1 protein, which is the reaction center of photosystem II (PS II) is the main target of photo-inhibition. The PS II is a supermolecular complex consisting of more than 30 different protein subunits encoded by both nuclear and chloroplast genomes. When illuminated with excess light, the D1 protein is damaged and electron transport of PS II is inactivated. The photodamaged D1 protein is degraded rapidly and replaced by a newly synthesized protein. Plants survive the light stress by the repair of the damaged D1 protein. Photoinhibition of PS II is considered to proceed by two different mechanisms, i.e., so-called accepter-

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side and donor-side photoinhibition. Under strong illumination, the D1 protein is damaged by reactive oxygen species (ROS) in the acceptor-side photoinhibiton and by cationic radicals in the donor-side photoinhibition. It was reported that cleavage of the D1 protein occurs by the action of the ROS [1]. Degradation of the protein by proteases may take place simultaneously. We have shown that the D1 protein also cross-links with the surrounding polypeptides in PS II, such as D2 and CP43, after the photodamage [2]. However, it is not known if the cross-linked products are formed by the action of ROS. In this study, we compared the effects of various ROS in the formation of the cross-linked products of the D1 protein. To see the nature of the cross-linking, we also examined formation of di-tyrosine in the cross-linked products.

MATERIALS AND METHODS

Preparation of thylakoids and PS II membranes. Thylakoids and PS II-enriched membranes were isolated from spinach leaves. Thylakoid membranes were suspended in TB buffer which contained 100mM sorbitol, 15mM NaCl, 5mM $MgCl_2$, and 50mM Tricine-KOH (pH7.6). The PS II membranes were suspended in SMN buffer which contained 400 mM sucrose, 10 mM, NaCl, and 40mM MES-NaOH (pH 6.5). The concentration of chlorophyll in thylakoids and PS II membranes was adjusted to 0.5 mg/ml. For Tris treatment, the samples were treated with 0.8M Tris-HCl (pH9.4).

Photoinhibitory treatment. The suspensions of the samples were illuminated at 25℃ with visible light (2000 μ $Em^{-2}s^{-1}$) for 120 min. Immediately after the illumination, these samples were subjected to SDS-PAGE. The concentration of polyacrylamide in the separation gel was 12.5%. Subsequently, Western blotting was carried out with a specific polyclonal antibody against the N-terminal part of the D1 protein and a monoclonal antibody against the di-tyrosine [3]. The immunoreaction was visualized by enhanced chemiluminescence (ECL).

Treatment with ROS. Thylakoids and PS II membranes were incubated with chemically generated ROS at 25℃ for 10 min in darkness. Four species of ROS were generated ; hydrogen peroxide(H_2O_2), hydroxyl radical(· OH), singlet oxygen($^1O_2$) and superoxide(O_2^-). Hydroxyl radicals were generated from H_2O_2 and Fe^{2+} (Fenton reaction). Singlet oxygen was generated either from H_2O_2 + NaClO or Rose-Bengal + green light. Superoxide was generated from xanthine + xanthine oxidase.

RESULTS AND DISCUSSION

The cross-linked products of the D1 protein were formed by the addition of chemically generated ROS to the thylakoids and PS II membranes (Fig.1).

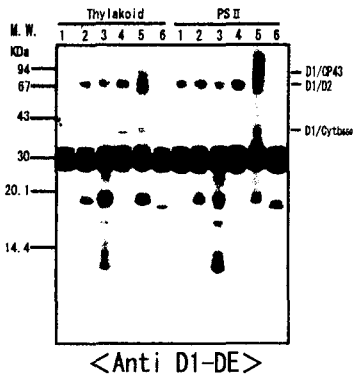

Fig.1 The cross-linked products of the D1 protein induced by several ROS. Thylakoids were suspended in TB buffer, and PS II membranes were suspended in SMN buffer. Each sample was exposed to several ROS described below. Lane 1 : control. Lane 2 : +10mM H_2O_2. Lane 3 : + $^1O_2$ generated by 10mM H_2O_2 and 5mM NaClO. Lane 4 : + $^1O_2$ generated by green light illumination (200 μ $Em^{-2}s^{-1}$) in the presence of 100 μ M Rose-Bengal. Lane 5 : + · OH generated with 10mM H_2O_2 and 5mM Fe^{2+}. Lane 6 : + O_2^- generated by 500 μ l xanthine and 0.05U/ml xanthine oxidase.

The cross-linked products increased by the treatment with · OH, H_2O_2 and $^1O_2$, but not with O_2^-. Hydroxyl radicals were most effective. Judging from these results, we suggest the following mechanism for formation of the D1 cross-linked products. At the reducing side of PS II, strong illumination may induce reduction of oxygen to form O_2^- and then H_2O_2. H_2O_2 then reacts with divalent metal ions and generates · OH. Under strong illumination $Cytb_{559}$ is damaged and degraded [4]. H_2O_2 may react with Fe^{2+} released from $Cytb_{559}$ and · OH is generated. This reactive species should induce the cross-linking of D1 protein.

We then examined the formation of di-Tyr in the cross-linked products induced by either strong illumination (2000 μ $Em^{-2}s^{-1}$) or · OH-treatment with Western blot analysis using a specific antibody against di-Tyr (Fig.2).

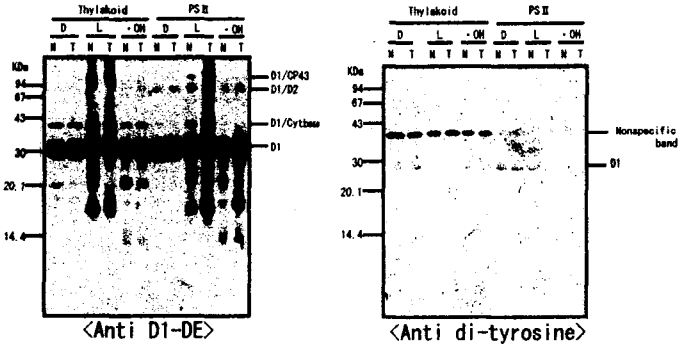

Fig.2 Western blot analysis to detect the di-Tyr in the cross-linked products of the D1 protein formed by the strong illumination or by the addition of hydroxyl radicals. Control thylakoids and PS II membranes (N) and Tris-treated samples (T) were illuminated with high light (2000 μ $Em^{-2}s^{-1}$) at 25℃ for 120 min (denoted as L). D indicates dark control. The treatment of the samples with · OH produced by 10mM H_2O_2 + 5mM Fe^{2+} was carried out as described in the legend to Fig.1.

In spite of the assays under various conditions, we could not detect di-Tyr in the D1 cross-linked products both in the illuminated and · OH-treated samples. These results show the possibility that the interaction of the polypeptides to form the cross-linked products of the D1 protein is rather non-specific.

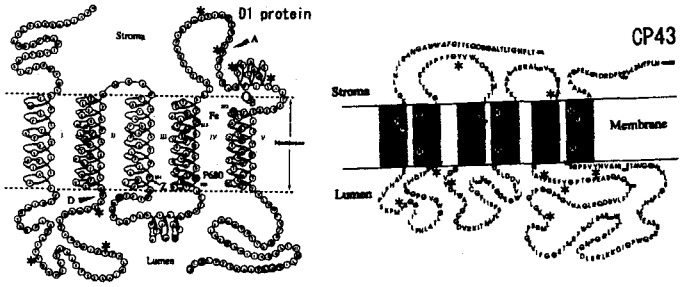

Fig.3 Amino acid sequences and structural models of the D1 protein and CP43. Tyrosine residues are marked in asterisk.

REFERENCES

1. Okada K., M. Ikeuchi, N. Yamamoto, T. Ono and M. Miyao (1996) Selective and specific cleavage of the D1 and D2 proteins of photosystem II by exposure to singlet oxygen: factors responsible for the susceptibility to cleavage of the proteins. *Biochim. Biophys. Acta* 1274, 73-79.

2. Yamamoto Y. (2001) Quality control of photosystem II. *Plant Cell Physiol.* 42, 121-128.

3. Kato Y., N. Kitamoto, Y. Kawai and T. Osawa (2001) The hydrogen peroxide/copper ion system, but not other metal-catalyzed oxidation systems, produces protein-bound dityrosine. *Free Rad. Biol. Med.* 31, 624-632.

4. Ortega J. M., M. Roncel and M. Losada. (1999) Light-induced degradation of cytochrome b559 during photoinhibition of the photosystem II reaction center. *FEBS Lett.* 458, 87-92.