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Quality Control of Photosystem II during Photoinhibition

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The reaction center D1 protein of photosystem II is the target of photodamage by excess illumination. The D1 protein is damaged by reactive oxygen species generated by photochemical reactions and then degraded by specific proteolytic enzymes. We found that the D1 protein also cross-links with the surrounding polypeptides, such as D2 and CP43 in isolated thylakoids or photosystem II-enriched membranes from spinach under the illumination with strong visible light. The cross-linking was observed in spinach leaf discs as well when they were illuminated at higher temperature (40°C). It was also shown that the cross-linked products are digested efficiently by a protease(s) in the stroma. Thus the cross-linking/digestion processes of the D1 protein seem to comprise a new pathway in the turnover of the photodamaged D1 protein. It should be noted, however, that the cross-linked products of the D1 protein and CP43 induced by endogenous cationic radicals in the donor-side photoinhibition are resistant to proteolytic digestion. Accumulation of these cross-linked products in the thylakoids may lead to the decay of the function of chloroplasts and finally to the death of plant cells. Thus, we suggest that the quality control of photosystem II, especially removal of the cross-linked products of the D1 protein, is crucial for the survival of chloroplasts under the light stress.

Key words: photosynthesis, photoinhibition, D1 protein, reactive oxygen species, turnover of photosystem II

INTRODUCTION

Photosystem (PS) II in the thylakoid membranes of chloroplast is vulnerable to light, and the reaction center D1 protein is photodamaged and degraded rapidly. This process, referred to as photoinhibition of PS II, has attracted much attention, and the mechanism has been studied extensively.

Photoinhibition of photosystems is caused by reactive oxygen species and other radicals formed by photochemical reactions. By excess illumination of thylakoids, reactive oxygen species are produced both at PS I and II. At the reducing side of PS I, electrons are transferred to molecular oxygen to form superoxide and hydrogen peroxide, but these reactive oxygen

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species are efficiently removed by enzymes such as SOD and ascorbate peroxidase, and also by reductants. Strong illumination of PS II induces singlet oxygen. As the PS II complexes are mostly located in the stacked region of thylakoids, the enzymes and reductants which are effective in scavenging the reactive oxygen species are not accessible to the site of production of singlet oxygen, and therefore the D1 protein is photodamaged. The photodamaged D1 proteins are efficiently degraded by proteases and removed from the PS II complex. In this report, I show our resent results on the degradation pathways of the photodamaged D1 protein.

MATERIALS AND METHODS

Preparation of thylakoids and PS II-enriched membranes from spinach, illumination of the samples, SDS/urea-PADE, Western blot analysis, fluorography with ECL (enhanced chemiluminescence) were carried out as described previously [1]. Tobacco cells were cultured according to the method described in ref. [2]. Fluorescence of dichlorofluoresein diacetate (DCFH/DA)was monitored according to the protocol provided by Molecular Probes, Inc.

RESULTS AND DISCUSSION

For the analysis of the cleavage and degradation of the D1 protein under strong illumination, PS-II enriched membranes were often used. When we illuminated spinach PS II membranes by light with various intensities for various periods, we could detect not only the cleavage/degradation products but also the cross-linked products of the D1 protein (Table 1). Three kinds of the cross-linked products were detected; D1/D2, D1/the α-subunit of cytochrome b559 and D1/CP43 [3]. A question here is what kind of reactive oxygen species (ROS) is responsible for the induction of the protein cross-linking. We compared the effects of various ROS and found that hydroxyl radicals are the most effective species (data are shown by Uchida et al. in this volume). The hydroxyl radicals may be produced by the reaction of hydrogen peroxide produced at the reducing side of PS II and Fe(II) released from photodamaged cytochrome b559 under strong illumination.

The observation of the cross-linked products of the D1 protein with the nearby polypeptides is not confined to the in vitro conditions. We detected the D1/D2 cross-linked products in the spinach leaf discs illuminated at 40°C and also D1/CP43 in the illuminated tobacco cells (data not shown). Thus, it is probable that under physiological stress conditions, ROS are formed at PS II and, especially hydroxyl radicals induce cross-linked products of the D1 protein in plant cells.

The cross-linked products of the D1 protein were efficiently removed by the addition of the stromal fraction to the illuminated samples. We demonstrated a protease activity recognizing the cross-linked products of the D1 protein in the stroma [3]. The protease activity may be due to several proteases including a serine-type protease. One of them had an apparent M. Wt of 15 kDa. The candidates of the protease recognizing the photodamaged D1 protein are listed in a table

Table 1. Cross-linked products of the D1 protein induced by illumination of thylakoids and PS II membranes

Sample	Light intensity ($\mu E\ m^{-2}\ s^{-1}$)	Illumination time (min)	Cross-linked product
Thylakoids	500	>20	D1/D2
	500	>60	D1/D2, D1/Cytb559
PSII membranes	500	>20	D1/D2
	500	>50	D1/D2, D1/Cytb559
	1000	>30	D1/D2
	1000	>60	D1/D2, D1/Cytb559
	1000	>90	D1/D2, D1/Cytb559, D1/CP43

Table 2. The proteases possibly participating in the degradation of the photodamaged D1 protein

Protease [Ref]	M.Wt.	Type	Characteristics
FtsH [4]	78 kDa	Metallo	Bound to the thylakoids, ATP-dependent, expressed by light, stimulated by Zn^{2+}, involved in the secondary degradation of the D1 protein
DegP2 [5]	60 kDa	Serine	Associated with the stromal side of the thylakoids, apparently GTP-dependent, involved in the primary cleavage within the D-E loop of the D1 protein
Clp [6]	100 kDa (ClpC), 23 kDa (ClpP)	Serine	Localized in the stroma, ATP-dependent, constitutively expressed, recognizes protein aggregates ?
Spa [1,3]	15 kDa + others	Serine	Localized in the stroma, ATP/GTP-dependent, includes SDS-stable proteases

(Table 2). Although the cross-linked products of the D1 protein are digested by a stromal protease(s) [we call the protease(s) Spa: a stromal protease recognizing protein aggregates], a part of the cross-linked products of D1/CP43 is resistant to the protease(s). The apparently protease-stable cross-linked products are formed by the donor-side photoinhibition of PS II. These cross-linked products were formed even in the absence of oxygen (data not shown), and therefore, the photodamage to the D1 protein is caused by light-generated endogenous cationic radicals, i.e. $P680^+$, $TyrZ^+$ and chlorophylls$^+$. The donor-side photoinhibition of PS II can be

induced by illumination of the samples depleted of Mn and the oxygen-evolving complex (OEC) subunits. More physiologically, however, this kind of situation may occur when the assembly of the D1, Mn and the OEC subunits is not nicely coordinated in the regeneration process of PS II.

If cross-linked products of the D1 protein which are resistant to proteolytic digestion are accumulated in PS II, the efficient turnover of the D1 protein may be deteriorated. There is a possibility that the light-induced cross-linking of proteins in PS II results in the decay of the activity of chloroplasts and finally the death of plant cells in some cases. We tested this possibility using a suspension culture of tobacco cells. Tobacco cells were illuminated at 25°C for 30 min and the D1/D2 and D1/CP43 cross-linked products were detected in the chloroplasts by SDS/urea-PAGE, Western blot analysis with specific antibody against the D1 protein and fluorography with ECL. Under the same illumination conditions, we observed fluorescence of DCFH/DA in the cells, which indicates the generation of hydrogen peroxide. Staining with Trypan Blue showed the partial death of the tobacco cells (data not shown). Thus, all these data point out that photodamage to the D1 protein could be lethal for plant cells. Recently it is strongly suggested that protein aggregates can be toxic to cells. The protein aggregates generated in the process of protein folding and unfolding are associated with some of the very serious human diseases. We now expect that the protein aggregates may also be very critical for various fundamental processes in plant cells, including photosynthesis.

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