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Cross-linking of the D1 protein with the surrounding polypeptides in PS II and digestion of the cross-linked products by a stromal protease(s) comprise a crucial pathway of D1 turnover during photoinhibition of PS II

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Introduction

It is generally accepted that under excessive illumination of PS II, the D1 protein is damaged by radicals (either oxygen radicals or endogenous cationic radicals) and promptly digested by proteases. Several candidates for the proteases have been reported, including Clp-type proteases, FtsH (Andersson and Aro 1997) and DegP2 protease (Haußühl et al. 2001). All these proteases probably recognize the photo-damaged D1 proteins or their primary fragments as the substrates. Recently, we found that the photodamaged D1 protein cross-links with the surrounding polypeptides during the photoinhibition. The cross-linking of the D1 protein takes place in the wide range of light conditions with various samples from intact chloroplasts to PS II-enriched membranes (Ishikawa et al. 1999). It was also shown that the cross-linked products are digested by a protease(s) located in the stroma. In the present study, we showed by quantification of the cross-linked products that the cross-linking of the D1 protein represents a pivotal process in the degradation of the protein.

Materials and Methods

Preparation of thylakoid membranes and PS II-enriched membranes, Tris-treatment of the PS II membranes, isolation of the stroma fraction from intact chloroplasts, sample illumination, SDS/urea-PAGE and Western blot analysis were carried out as described previously (Ishikawa et al. 1999). To determine the relative amount of the native D1 protein, the degradation products of D1 and the cross-linked products of D1, the PS II samples and Tris-treated PS II membranes were illuminated with strong light (2500 $\mu E\ m^{-2}\ s^{-1}$) for various time periods and subjected to SDS/urea-PAGE and Western blot analysis. The D1 and D1-related bands on the fluorograms were determined by a Personal Scanning Imager PD110 (Molecular Dynamics ,U.S.A.). The stroma fractions containing a protease(s) were subjected to anion-exchange (Q-Sepharose) chromatography for further purification.

Results

Quantification of the cross-linked products of the D1 protein

The cross-linked products of the D1 protein in the illuminated PS II have been shown with SDS/urea-PAGE and Western blot analysis (Yamamoto and Akasaka 1995, Ishikawa et al. 1999). Here, we quantitatively determined D1, the degradation products of D1, and the cross-linked products of D1 (Fig. 1).

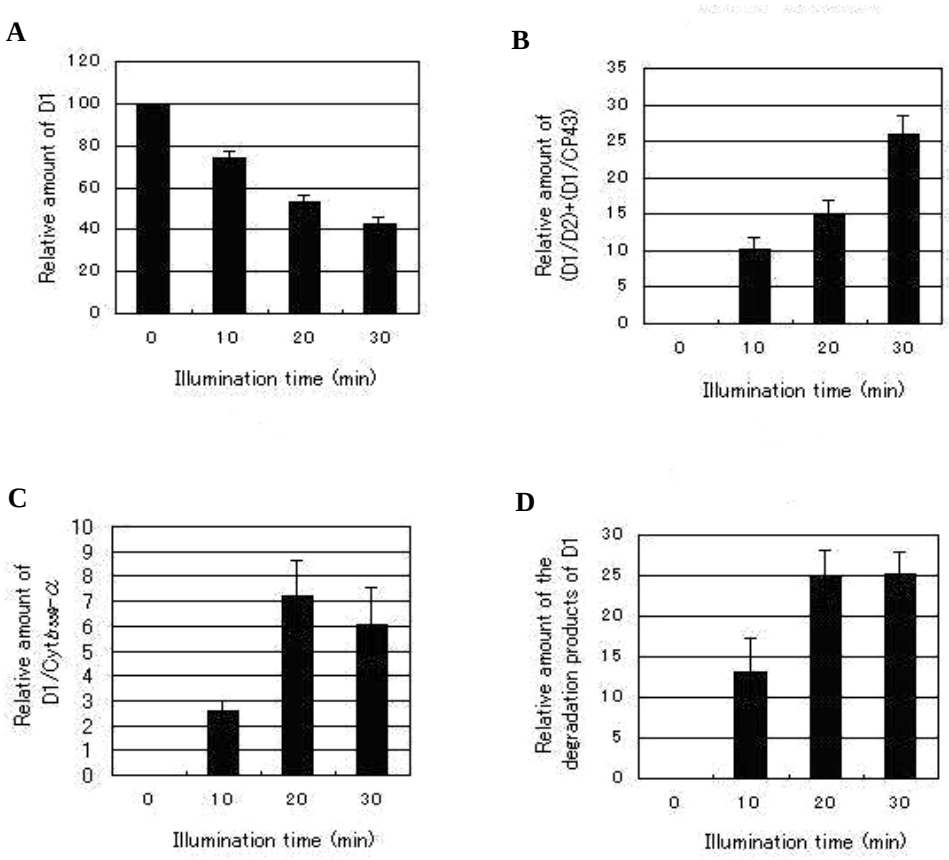
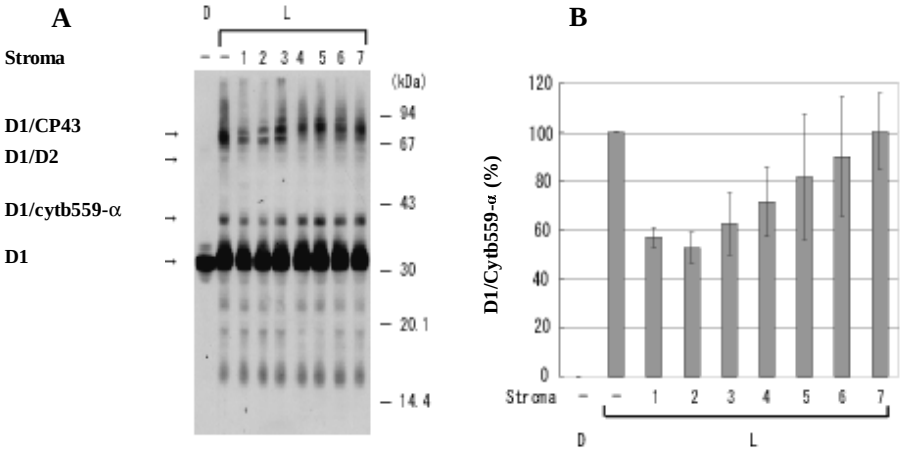


Fig. 1. Determination of the ratio of the native D1 protein, the cross-linked products of D1 and the degradation products of D1 after illumination of Tris-washed PS II membranes with strong light (2,500 μE m^{-2} s^{-1}). SDS/urea-PAGE and Western blot analysis were carried out. (A) D1; (B) (D1/D2)+(D1/CP43); (C) D1/Cytb_{559}-α; (D) the degradation products of D1.

By the illumination of the samples for 30 min, the native D1 protein decreased by 60%, which is due to generation of either the degradation products (25%) or the cross-linked products (35%). Although significant cross-linking of the D1 protein was detected by SDS/urea-PAGE, it is likely that some D1 aggregates still escaped detection. To examine this possibility, we omitted mercaptoethanol in the electrophoresis. Apparently, the amount of the D1/CP43 cross-linked products increased significantly in the absence of mercaptoethanol (data not shown). These results indicate that disulfide bonds are involved in the cross-linking of D1/CP43. When LDS-PAGE was carried out at low temperature, the D1/CP43 cross-linked products increased considerably (data not shown).



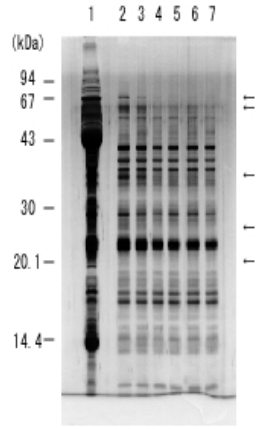


Fig. 2. Partial purification of the stromal protease(s). (A) Tris-treated PS II membranes were illuminated with white light (2,500 μE m^{-2} s^{-1}) for 14 min at 25 ºC under aerobic conditions, then incubated in the absence (-) or presence (1-7) of stromal fraction. The samples were subjected to SDS/urea-PAGE, and Western blot analysis was carried out with antibodies against the D1 DE-loop. 1, total stromal fraction; 2-7, the stromal fraction not adsorped to the Q-Sepharose at pH6.0, 6.5, 7.0, 7.5, 8.0, and 8.5, respectively. (B) D1/Cytb_{559}-α was quantified from fluorograms by densitometry. Data are the averages of three independent measurements with S.D. (C) The profile of polypeptides each stromal fraction (1-7) obtained by the batch adsorption method. The bars on right side of the gel show the candidates of the protease(s), which were detected only in acidic fractions after adsoption to the anion exchange resin.

Digestion of the cross-linked products of the D1 protein by a protease(s) in the stroma

The cross-linked products formed during the excessive illumination of PS II were digested by the addition of a stromal fraction to the illuminated samples (Ishikawa et al. 1999). The stromal protease(s) were characterized thereafter (Ferjani et al. 2001). The protease(s) responsible for the degradation of the D1 cross-linked products is serine-type and requires ATP or GTP for the optimum activity.

Isolation of the protease

The stromal fraction was subjected to batch adsorption to Q-Sepharose at different pHs from 6 to 8.5, and the protease activity of the fractions not adsorped to the anion exchange resin at each pH was assayed (Fig.2). In the fractions that have relatively strong protease activity, several protein bands were identified by SDS/urea-PAGE and silver-staining of the gel (shown by the bars).

Discussion

Cross-linking of the D1 protein with the surrounding polypeptides in PS II is an inherent process in photoinhibition. In the present work, we demonstrated with denaturing gel electrophoreis (SDS/urea-PAGE) that the extent of the cross-linking is comparable to or

larger than that of the degradation of the D1 protein (Fig. 1). These results suggest again the importance of the D1 cross-linking reaction.
The cross-linked products can be digested efficiently by a stromal protease(s). Although the protease(s) is not identified or purified yet (Fig. 2), the presence of the proteolytic process of the D1 cross-linked products strongly suggests that the cross-linking of the D1 protein and digestion of the cross-linked products play a significant role in the turnover of the D1 protein in photoinhibition (Yamamoto 2001).

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