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Characterization of the stromal protease(s) degrading the cross-linked products of the D1 protein generated by photoinhibition of photosystem II

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Abstract

When photosystem (PS) II-enriched membranes are exposed to strong light, cross-linking of the intrinsic D1 protein with the surrounding polypeptides and degradation of the D1 protein take place. The cross-linking of the D1 protein with the α-subunit of cytochrome b_{559} is suggested to be an early event of photoinduced damage to the D1 protein (Barbato et al., FEBS Lett. 309 (1992) 165–169). The relationship between the cross-linking and the degradation of the D1 protein, however, is not yet clear. In the present study, we show that the addition of stromal extract from chloroplasts degrades the 41 kDa cross-linked product of D1/cytochrome b_{559} α-subunit and enhances the degradation of the D1 protein. Incubation of the preilluminated PS II-enriched membranes with the stromal extract at 25°C causes the degradation of the cross-linked product by more than 70%. The activity of the stromal extract showed a pH optimum at 8.0, and was enhanced by the addition of ATP or GTP. Consistent with the nucleotide effect, this stromal activity was eliminated by the preincubation of the stromal extract with apyrase, which hydrolyzes nucleotides. Also, the stromal activity was nearly fully inhibited by a serine-type protease inhibitor, 3,4-dichloroisocoumarin, which suggests participation of a serine-type protease(s).

Keywords: D1 protein; Cytochrome b_{559}; Cross-linked product; Stromal protease; Photoinhibition; Photosystem II

1. Introduction

The intrinsic D1 and D2 proteins of the photosystem (PS) II complex play a central role in the photochemistry of PS II as the reaction center-binding proteins. The D1 protein, however, is labile

Abbreviations: PS II, photosystem II; D1 and D2, the reaction center-binding proteins of photosystem II; P680, the primary electron donor of PS II; Pheo, the primary electron acceptor of PS II; Tyr_Z, the secondary electron donor of PS II; ClpP and ClpA, prokaryotic ATP-dependent proteases; FtsH, a membrane-bound protease in bacteria; PAGE, polyacrylamide gel electrophoresis; Tris, tris(hydroxymethyl)aminomethane; MES, 2-(*N*-morpholino)ethanesulfonic acid; Bis-tris, bis(2-hydroxyethyl)imino-tris(hydroxyl)methane; tricine, *N*-tris(hydroxymethyl)methylglycine; CHES, cyclohexylaminoethanesulfonic acid; CAPS, cyclohexylaminopropanesulfonic acid; 3,4-DCI, 3,4-dichloroisocoumarin; E-64, L-3-carboxy-*trans*-2,3-epoxypropyl-leucylamido(4-guanidino)butane; ECL, enhanced chemiluminescence; CP43, the antenna chlorophyll-binding protein of photosystem II with molecular mass of 43 kDa

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under illumination where it is the main target of photoinhibition. The D1 protein is continuously damaged, degraded, and replaced by newly synthesized protein [1,2]. Consequently, its efficient turnover is crucial for maintaining functional PS II activity.

In regard to photoinhibition of PS II, two mechanisms have been proposed, namely acceptor-side photoinhibition and donor-side photoinhibition [1]. It has been suggested that singlet oxygen produced by the photochemical reactions in PS II under excessive illumination is responsible for the damage in the D1 protein in the acceptor-side mechanism [3]. This toxic oxygen species is generated by the reaction of molecular oxygen with the primary electron donor P680 in its triplet state, which is formed by the charge recombination between $P680^+$ and the reduced primary electron acceptor $Pheo^-$. Actually, the singlet oxygen was detected by the illumination of isolated PS II reaction center complexes with steady-state and time-resolved luminescence measurements [4] and chemical methods [5]. In the donor-side photoinhibition mechanism, endogenous cationic radicals such as $P680^+$ or Tyr_Z^+ are responsible for the damage to the D1 protein [6]. These radicals are generated by illumination under the conditions where the water oxidation system is not functioning properly for some reasons. Irrespective of the inhibition mechanism, specific proteases may degrade the photodamaged D1 protein [2]. As chloroplasts have a prokaryotic origin, they may have bacterial type proteases. Homologues of ClpP and ClpA proteases, and FtsH protease in *Escherichia coli*, have been identified in chloroplasts [7,8] and a possible role of the FtsH protease in the degradation of the D1 protein was suggested [9].

In addition to the degradation of the D1 protein, cross-linking of the D1 protein with nearby polypeptides takes place during photoinhibition of PS II by excessive illumination [10–13]. Among the photoinduced cross-linked adducts of the D1 protein, a 41 kDa band detected by SDS-polyacrylamide gel electrophoresis (PAGE) and Western blot analysis with specific antibodies was shown to be the cross-linked product between the D1 protein and the α-subunit of cytochrome b_{559} [14]. In a later study, Barbato et al. showed that cross-linking takes place between the N-terminal serine of the α-subunit of cytochrome b_{559} and the 239Phe-244Glu region in the DE loop of the D1 protein [15]. In a more recent study, they used mutants of *Synechocystis* sp. PCC6803 having deletions in the DE loop of the D1 protein and observed that the D1 cross-linking site was different [16]. Although the cross-linking site of the D1 protein and the α-subunit of cytochrome b_{559} is not clearly established yet, the nature of the 41 kDa cross-linked product is characterized more than that of the other D1 adducts.

In our previous study, we showed that the cross-linked products of the D1 protein in PS II are removed by the incubation of the PS II-enriched membranes with a stromal extract at room temperature. These results indicate the presence of a protease(s) in the stroma that digests the cross-linked products [13]. The putative protease(s) in the stroma was resistant to SDS. Also, it recognized the cross-linked products of the D1 protein but not the native D1 protein. In the present study, we further characterized the stromal protease activity using the 41 kDa cross-linked products between the D1 protein and the α-subunit of cytochrome b_{559} as the substrate.

2. Materials and methods

2.1. Preparation of PS II-enriched membranes and illumination conditions

PS II-enriched membranes were prepared from spinach according to the method of Kuwabara and Murata [17] and stored at −80°C. Tris-treated PS II-enriched membranes were obtained by washing the membranes with a solution containing 0.8 M Tris-HCl and 3 mM EDTA (pH 9.3) as described previously [11]. Chlorophyll was determined in 80% acetone with the absorption coefficient reported by Mackinney [18]. For photoinhibition, the samples were suspended in a solution of 0.4 M sucrose, 10 mM NaCl and 40 mM MES-NaOH (pH 6.5) at a concentration of 0.5 mg chlorophyll ml^{-1} and put into microtubes. The microtubes were incubated in a circulating water bath at 25°C and illuminated with white light from a fluorescent lamp or from a slide projector (light intensity was 100–4000 μE m^{-2} s^{-1}). The optical path length of the sample suspensions was 17 mm. Where indicated, the samples were illu-

minated under anaerobic conditions, which was achieved by the addition to final concentrations of 0.1 mg ml^{-1} catalase, 0.1 mg ml^{-1} glucose oxidase, and 10 mM glucose to the samples.

2.2. Preparation of stromal extracts and protease assay conditions

Intact chloroplasts were isolated from spinach according to the method of Mullet and Chua [19]. Stromal extracts were prepared fresh before use from intact chloroplasts. Intact chloroplasts were suspended and lysed in a solution containing 5 mM $MgCl_2$ and 10 mM MES-NaOH (pH 6.5) at 2.0 mg chlorophyll ml^{-1}, incubated for 5 min on ice, and then centrifuged at $10\,000 \times g$ for 10 min. The supernatant was re-centrifuged at $40\,000 \times g$ for 1 h and the final supernatant was collected as the stromal extract. For pH dependence studies of the stromal protease activity, stromal extracts were prepared from intact chloroplasts which were suspended in solutions with different pH values (from pH 5.5 to 11.0) containing 5 mM $MgCl_2$ and 10 mM of Good's buffers (MES, pH 5.5–6.5; Bis-tris, pH 6.5–7.5; tricine, pH 7.5–8.5; CHES, pH 8.5–10.0; CAPS, pH 10.0–11.0). The effects of ATP and GTP were examined at final concentrations of between 2 and 10 mM of added nucleotide to the stromal extracts. Apyrase was added to 2 units/ml final concentration to stromal extracts and incubated for 30 min at 30°C for removal of endogenous nucleotides. In the experiments with protease inhibitors, 2–10 mM final concentration of either 3,4-dichloroisocoumarin (3,4-DCI), L-3-carboxy-*trans*-2,3-epoxypropyl-leucylamido(4-guanidino)butane (E-64), or EDTA, was added to stromal extracts, and incubated for 10 min at 30°C prior to use with PS II samples.

2.3. SDS/urea-polyacrylamide gel electrophoresis and Western blot analysis

SDS/urea-PAGE and Western blot analysis were carried out as described previously [11]. Polyclonal antibodies against the C terminus of the D1 protein, and the DE loop (25 amino acids from 225 Arg to 249 Val) of the D1 protein, were kindly provided by Dr. P. Nixon of the Imperial College of Science, Technology and Medicine, UK, and by Dr. M. Miyao-Tokutomi of the National Institute of Agrobiological Resources, Japan, respectively. Polyclonal antibodies against the α-subunit of cytochrome b_{559} were kindly provided by Dr. B. Andersson of the University of Stockholm, Sweden. Immuno-decorated proteins were detected by enhanced chemiluminescence (ECL) (Amersham, Japan) on X-ray film (Fuji, Japan). Densitometric measurements of the chemiluminescently produced bands on the X-ray films were quantified with a Personal Scanning Imager PD110 (Molecular Dynamics, USA).

3. Results

3.1. Formation of the 41 kDa cross-linked product of the D1 protein and the α-subunit of cytochrome b_{559}

Illumination of spinach PS II membranes with strong white light (intensity, 1500 μE m^{-2} s^{-1}) generates a 41 kDa cross-linked product of the D1 protein and the α-subunit of cytochrome b_{559} [14], cross-linked products with larger molecular masses (70–100 kDa) corresponding to the D1/D2 heterodimers and the D1/CP43 adducts [11,13]. Also, several degradation fragments of the D1 protein were generated. These products were detected by SDS/urea-PAGE and Western blot analysis with polyclonal antibodies against the DE loop of the D1 protein (Fig. 1A, left). The 41 kDa cross-linked product was also detected by the antibody against the α-subunit of cytochrome b_{559} (Fig. 1A, right), which confirmed the cross-linked products of the D1 protein and the α-subunit of cytochrome b_{559}. The immunodetected quantity of the 41 kDa adduct increased with increasing illumination times from 20 to 120 min. Formation of the 41 kDa adduct was also dependent on light intensity (Fig. 1B). The 41 kDa adduct was generated even under weak light, 100 μE m^{-2} s^{-1} for 30 min, and its level increased in proportion to the light intensity up to 4000 μE m^{-2} s^{-1}. Similar results were previously reported with PS II reaction center preparations [15], but cross-linking took place within a shorter illumination time.

The 41 kDa cross-linked product was detected only under aerobic conditions (Fig. 2). These results indicate that the adduct is formed by the action of

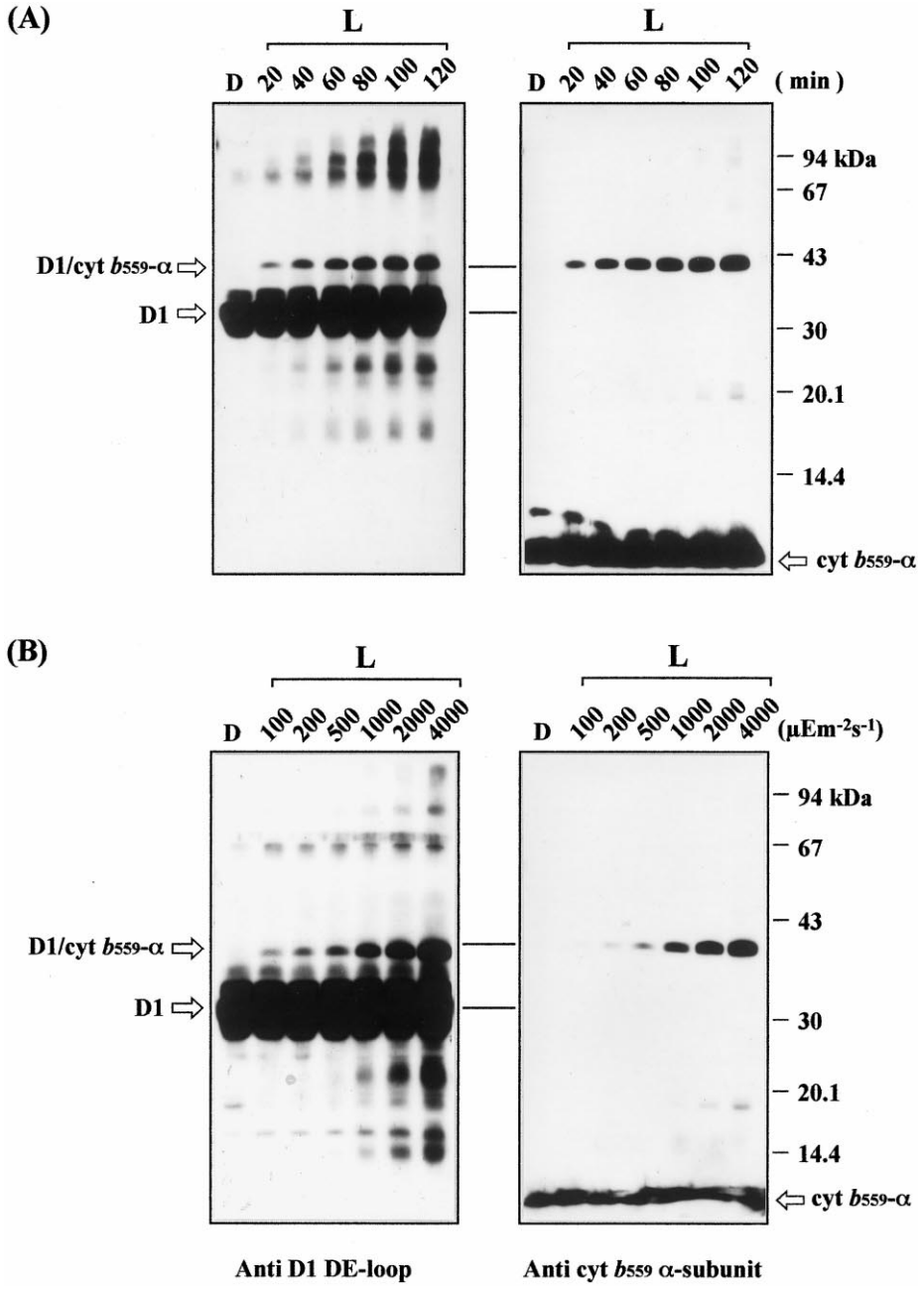


Fig. 1. Light-induced formation of the 41 kDa cross-linked adduct of the D1 protein and the α-subunit of cytochrome b_{559}. The effects of illumination time (A) and light intensity (B) are shown. The PS II-enriched membranes (0.5 mg chlorophyll ml^{-1}) under aerobic conditions were illuminated with white light (1500 μE m^{-2} s^{-1} in A and 100–4000 μE m^{-2} s^{-1} in B, respectively) for the periods indicated at the top of the gels (A) or for 30 min at 25°C at the light intensity shown at the top of the gels (B). The PS II membranes were then subjected to SDS/urea-PAGE. The samples equivalent to 2.5 mg chlorophyll were loaded to each lane of the gels. After that, Western blot analysis was carried out with antibodies against the DE loop of the D1 protein (left) and against the α-subunit of cytochrome b_{559} (right). The immuno-decorated bands were detected by fluorography with ECL. D and L denote the dark control and the illuminated samples, respectively. The bands of the D1 protein, the α-subunit of cytochrome b_{559}, and the 41 kDa cross-linked adduct are shown on both sides of the gels. Molecular markers are shown on the right side of the gels.

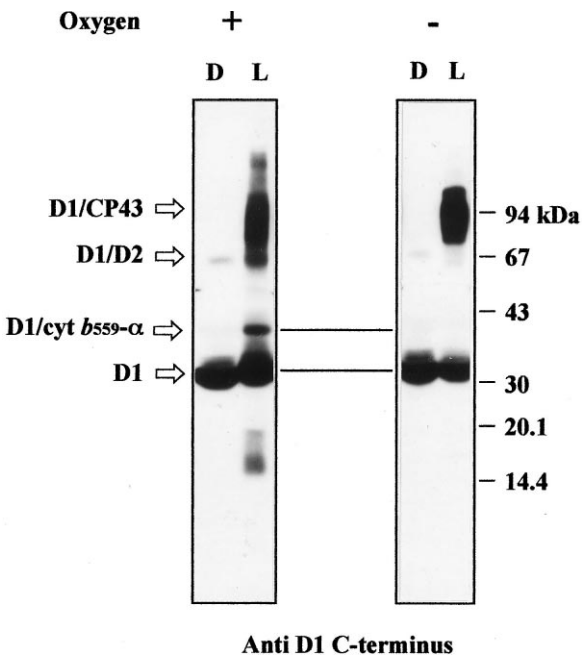


Fig. 2. The effects of oxygen on the light-induced formation of the cross-linked products of the D1 protein and the α-subunit of cytochrome b_{559}. Tris-treated PS II-enriched membranes were illuminated with white light (2500 μE m^{-2} s^{-1}) for 30 min at 25°C under aerobic (denoted +) and anaerobic (denoted −) conditions. Antibodies against the C terminus of the D1 protein were used for Western blot analysis. Other experimental conditions were the same as those described in the legend to Fig. 1.

reactive oxygen molecules produced by the illumination of PS II. In connection with this, it was observed that the light-induced cross-linking of the D1 protein with the α-subunit of cytochrome b_{559} was more prominent in Tris-treated PS II samples compared to that for the non-Tris-treated PS II samples. In Tris-treated samples, the extrinsic proteins of PS II (OEC subunits) and Mn clusters are removed, and the illumination of those samples should induce the donor-side photoinhibition of PS II. Although the reason why the cross-linking reaction was enhanced by the illumination of Tris-treated samples is not yet clear, it is likely that the generation of reactive oxygen is increased in the Tris-treated PS II membranes when illuminated compared to the non-Tris-treated membranes. We therefore used Tris-treated PS II membranes to analyze the light-induced cross-linking of the D1 protein and the stromal protease activity in the following studies.

3.2. Proteolysis of the 41 kDa cross-linked product by a stromal protease(s)

When the illuminated Tris-treated PS II membranes were incubated with stromal extracts for 30 min at 25°C, a significant decrease in the amounts of the 41 kDa cross-linked product, as well as those of D1/D2 and D1/CP43, was observed by Western blot analysis (Fig. 3). Densitometric analysis showed that the amount of the 41 kDa adduct in the samples (equivalent to 75 μg chlorophyll) decreased by approx. 40% with the addition of stromal extract (equivalent to 0.3 mg chlorophyll). The loss of the 41 kDa adduct was dependent on temperature [13],

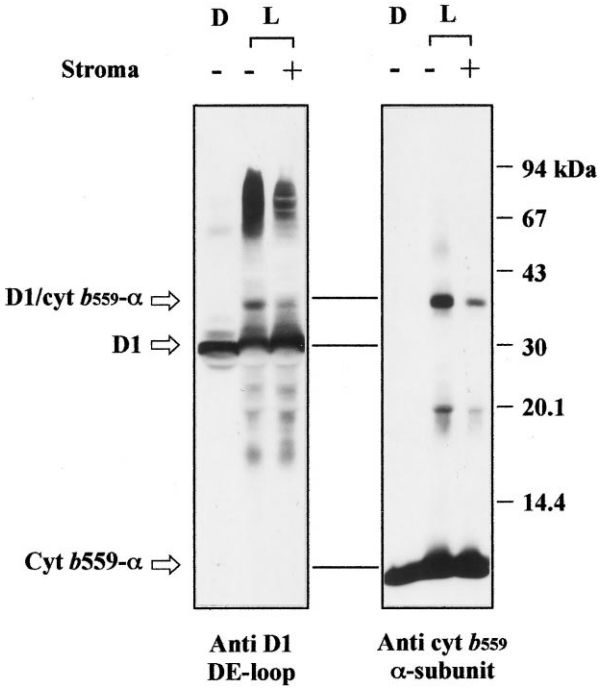


Fig. 3. The effects of stromal extracts on the light-induced 41 kDa cross-linked adduct of the D1 protein and the α-subunit of cytochrome b_{559}. Tris-treated PS II membranes were illuminated with white light (2500 μE m^{-2} s^{-1}) for 30 min at 25°C under aerobic conditions. Illuminated samples containing 75 μg chlorophyll were then incubated in the presence (+) or absence (−) of stromal extracts equivalent to 0.3 mg chlorophyll for 30 min at 25°C in darkness. In the absence of stromal extract addition, a solution containing 10 mM MES-NaOH and 5 mM $MgCl_2$ (pH 6.5) was used. Other experimental conditions were the same as those described in the legend to Fig. 1.

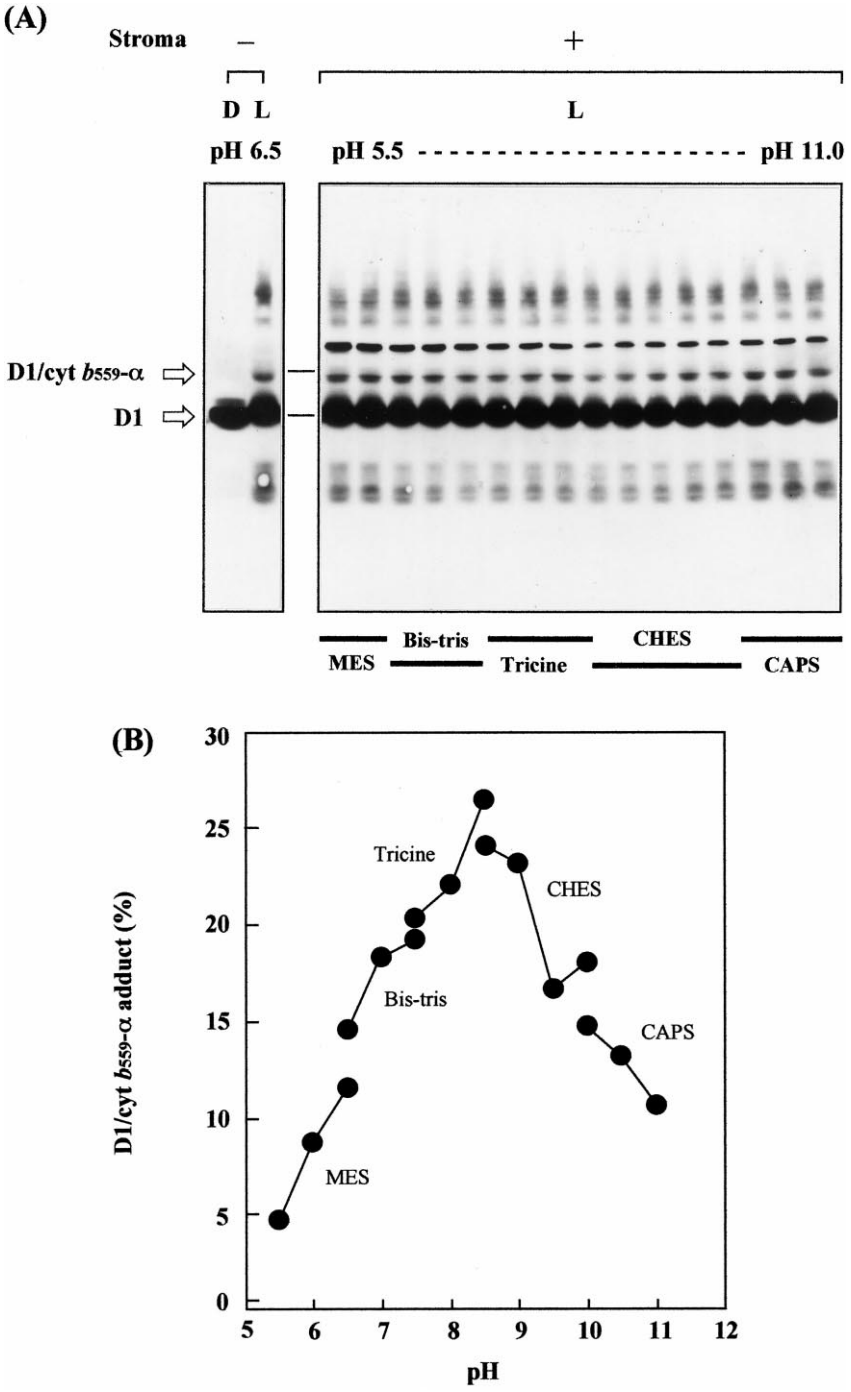


Fig. 4. The effects of pH on the stromal protease activity. (A) A fluorogram showing the results of the stromal protease activity at different pH values. Tris-treated PS II membranes were illuminated with white light (1500 μE m^{-2} s^{-1}) for 30 min under aerobic conditions, followed by incubation in darkness in the presence (+) or absence (−) of stromal extract equivalent to 0.3 mg chlorophyll for 30 min at 25°C. Stromal extracts were prepared from intact chloroplasts equivalent to 2.0 mg chlorophyll that were suspended in five different solutions containing 5 mM $MgCl_2$ and one of the Good's buffers (MES, pH 5.5–6.5; Bis-tris, pH 6.5–7.5; tricine, pH 7.5–8.5; CHES, pH 8.5–10.0; CAPS, pH 10.0–11.0). (B) The pH profile of the stromal protease activity obtained from the data in panel A. Other experimental conditions were the same as those described in the legend to Fig. 1.

and also on the amount of the stromal extract added and incubation time (data not shown).

It was noted that when the illuminated PS II membranes were subjected to Western blot analysis with the antibody against the α-subunit of cytochrome b_{559}, an additional band with a molecular mass of approx. 20 kDa was detected between the bands corresponding to the 41 kDa cross-linked adduct and to the native α-subunit of cytochrome b_{559} (Fig. 3, right). The 20 kDa band may be either a degradation product of the 41 kDa adduct, or a cross-linked product of a D1 fragment and the α-subunit of cytochrome b_{559}. The 20 kDa band becomes less obvious by the addition of stromal extract to illuminated samples, indicating the digestion of this molecular species as well.

3.3. Characterization of the stromal protease(s)

We next looked at the nature of the stromal protease activity responsible for the loss of the 41 kDa cross-linked product more closely. First, the pH dependence of the stromal protease activity was examined (Fig. 4). The optimum pH was 8, and the peak was relatively narrow. It should be noted that not only the 41 kDa adduct was degraded, but the cross-linked products with higher molecular masses, which correspond to D1/D2 and D1/CP43 adducts, and the partially degraded products of the D1 protein were also degraded by the addition of the stromal extracts with a similar pH dependence.

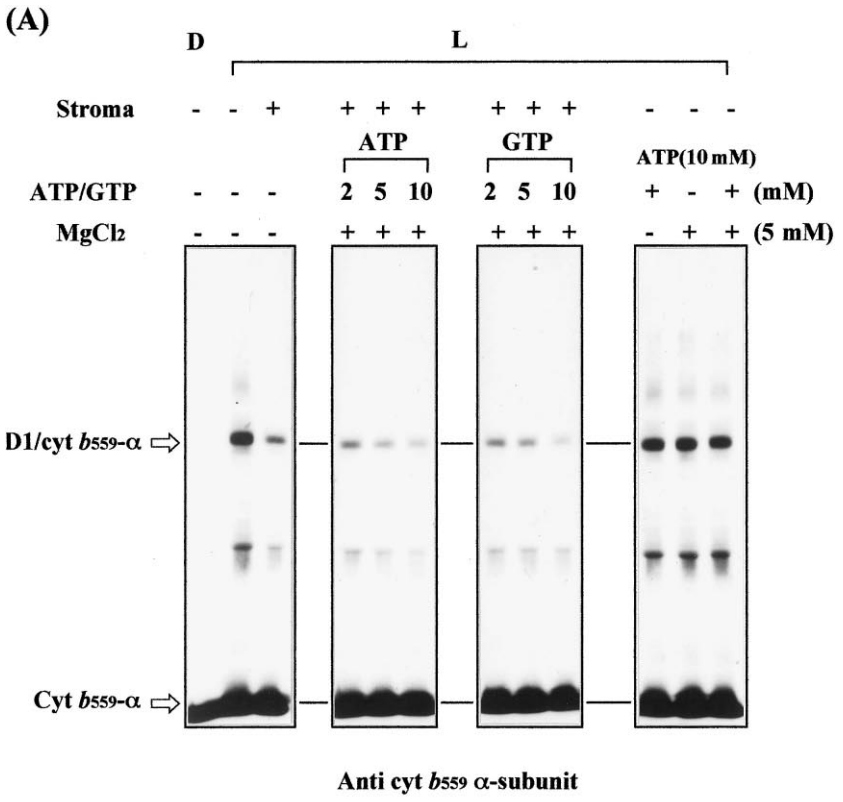
Addition of ATP or GTP stimulated the activity of the stromal protease(s) (Fig. 5A). In the presence of these nucleotides, the amount of the 41 kDa adduct was decreased by 70% or more. In accordance with this result, when preilluminated samples were incubated with stromal extracts, in which endogenous nucleotides were previously hydrolyzed by the addition of apyrase, the D1/cytochrome b_{559} adduct remained intact (Fig. 5B). Thus, the stromal protease activity was eliminated by the destruction of endogenous nucleotides. The stimulatory effects of the nucleotides were not observed with PS II membranes in the absence of stromal extracts (Fig. 5A).

To investigate further whether the effect of the stromal extract on the degradation of the 41 kDa adduct is due to a protease(s), we next examined the effect of protease inhibitors in the assays. The inhibitors 3,4-DCI, a serine-type protease inhibitor, E-64, a cysteine protease inhibitor, or EDTA, a metalloprotease inhibitor, were each added to stromal extracts. Among these inhibitors, 3,4-DCI suppressed the stromal protease activity (Fig. 6).

4. Discussion

In a recent work, we showed that the cross-linked products of the D1 protein are formed not only in the PS II membranes, but also in thylakoids and intact chloroplasts [13]. These results, as well as those reported previously [20], suggest that the adduct formation is a phenomenon that occurs in vivo. It was also obvious from previous results that the extent of cross-linking observed is less in chloroplasts than in thylakoids and PS II membranes. This indicates that in chloroplasts the photoinduced cross-linking of the D1 protein is much reduced by the presence of stromal factor(s), or that the cross-linked products are formed and turned over by stromal factor(s). It is known that the presence of reductants, such as ascorbate or glutathione, in the stroma protects chloroplasts from oxidative damage. However, generation of the reactive oxygen species such as singlet oxygen may be localized within the PS II complexes, and the PS II complexes are enriched in the stacked thylakoid regions. Hence, it is likely that the PS II complex is inaccessible to the soluble reductants of the stroma. Once oxidative stress becomes prominent, especially under strong light intensity, damage to the D1 protein and cross-linking of the photodamaged D1 protein with surrounding polypeptides are observed [13]. It is expected then that a mechanism for turning over the photodamaged cross-linked products of the PS II complex be in effect.

Previously, we showed by Western blot analysis that adducts of the D1 protein and antenna chlorophyll-binding CP43 disappeared by the addition of the stromal extracts [13]. In that study, however, we could not determine whether the loss of the D1/CP43 adduct was due either to digestion of the adducts by a protease(s) or to dissociation of the adducts to reproduce native proteins by the action of a molecular chaperon(s) and/or the related components, because the nature of the binding of the two proteins is not known exactly. In contrast with the D1/CP43


(A)
D
L
Stroma
ATP
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(B)
D
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D1/cyt b559-α
D1
Anti D1 DE-loop

Fig. 5. The effects of nucleotides and apyrase on the stromal protease activity. (A) Stimulation of the stromal protease activity by ATP or GTP. ATP or GTP was added to the stromal extracts at concentrations of 2, 5 and 10 mM, with 5 mM $MgCl_2$. Tris-treated PS II membranes were preilluminated with white light (2500 μE m^{-2} s^{-1}) for 30 min at 25°C. Preilluminated samples were suspended in a solution containing 10 mM tricine-NaOH and 5 mM $MgCl_2$ (pH 8.0) and incubated in the presence or absence of stromal extract containing ATP or GTP. The symbols + and − at the top of the gels denote the presence and absence of stroma fraction, each nucleotide, and $MgCl_2$. Western blot analysis used the antibody against the α-subunit of cytochrome b_{559}. (B) Inhibition of the stromal protease activity by apyrase. Tris-treated PS II-enriched membranes were illuminated as described above, and then incubated in darkness in the presence (+) or absence (−) of apyrase (2 units/ml) for 30 min at 30°C. Western blot analysis used the antibody against the DE loop of the D1 protein. Other experimental conditions are the same as those shown in the legend to Fig. 1.
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adduct, the 41 kDa adduct is relatively well characterized. The 41 kDa adduct was detected not only in vitro [14] but also in vivo [20]. The site of cross-linking of the D1 protein and the α-subunit of cytochrome b_{559} was previously shown to be the stroma-exposed DE loop of the D1 protein and the N terminus of the α-subunit of cytochrome b_{559} [15]. More recently, however, the cross-linking site on the DE loop is questioned with the study of *Synechocystis* mutants which have a deletion in the DE loop [16].

The relationship between the formation of the 41 kDa adduct and turnover of the D1 protein has received considerable attention. In the present study, we examined the effects of stromal extracts on the covalently cross-linked 41 kDa product in illuminated PS II membranes. Despite the uncertainty of the cross-linking site, the 41 kDa adduct has merit for use as a substrate to characterize the observed stromal protease activity. The 41 kDa adduct is easily generated even under low light intensities [13], and is easily detected and quantified by SDS/urea-PAGE and Western blot analysis. Our present results indicate that a serine-type protease(s) in the stroma proteolyzes the 41 kDa adduct. The optimum pH of the protease(s) was 8, and is similar to the values reported with other serine-type proteases (pH 7.5–8.5) (Fig. 4) [21]. It is noted that by the addition of stromal extracts to preilluminated PS II membranes, the other D1 cross-linked products, such

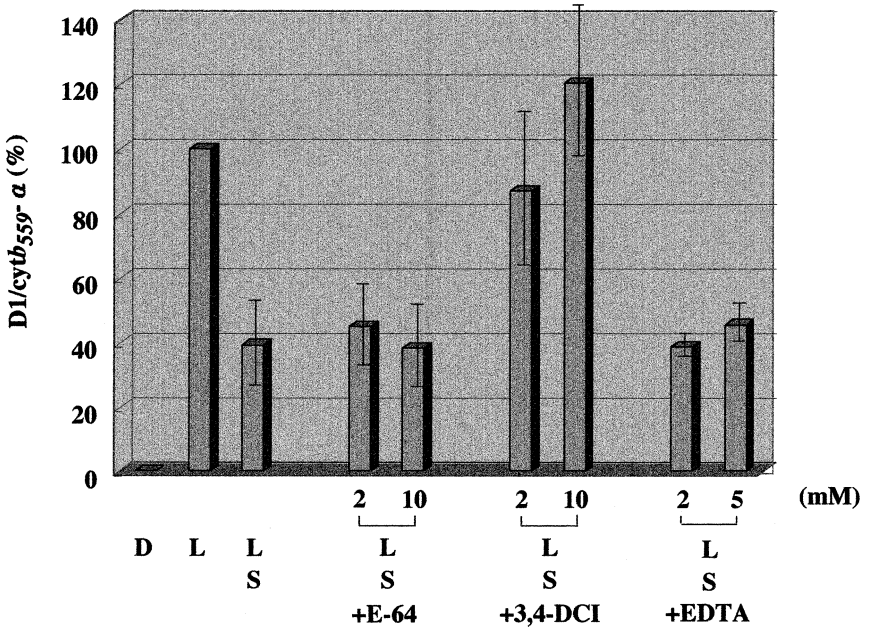

Fig. 6. Inhibition of the stromal protease activity by protease inhibitors. The protease inhibitors used are: E-64 (a cysteine protease inhibitor), 3,4-DCI (a serine-type protease inhibitor), and EDTA (a metalloprotease inhibitor). The 41 kDa adduct was quantified from fluorograms by densitometry as described in Section 2. Averages of data from three independent measurements with standard deviations are shown. Other experimental conditions are the same as those shown in the legend to Fig. 1.

as D1/D2 and D1/CP43 adducts, were removed as well. It is likely that the putative serine protease(s) has a broad substrate specificity functioning as a housekeeping enzyme to digest damaged and unneeded proteins, such as the photoinduced protein adducts.

The results of this study suggest that the stromal protease(s) are energy-dependent, because its (their) activity was enhanced by the addition of ATP or GTP (Fig. 5A). This was demonstrated by depleting the stromal extracts of endogenous nucleotides with apyrase treatment prior to its addition to PS II membranes, which inhibited the proteolytic activity of the extract (Fig. 5B). It was shown recently that GTP bound to thylakoid membranes is required for the light-induced degradation of the D1 protein [22,23]. The relationship between the requirement of GTP in the protease activity in our study and that in thylakoid membranes is currently unknown and should be further investigated.

When incubating preilluminated PS II membranes with stromal extracts, we observed a small increase in the quantity of D1 degradation products, by Western blot analysis (Fig. 3, left). This increase in D1-derived degradation products may be due, in part, to the degradation of the larger cross-linked products. The 20 kDa band detected by both the antibodies against the D1 protein and the α-subunit of cytochrome b_{559}, which may be the degradation products of the 41 kDa adduct or a cross-linked product of a D1 fragment and the α-subunit of cytochrome b_{559}, was also digested by the putative stromal protease(s) (Fig. 3, right). As we could not detect fragments that are newly produced by the addition of the stroma and cross-react with the antibody against the α-subunit of cytochrome b_{559}, it is suggested that the amino acid sequence at or very close to the covalent binding site of the two proteins is recognized by the protease(s), and thereby, the α-subunit of cytochrome b_{559} remains almost intact without being digested. The putative cross-linking site in the DE loop of the D1 protein contains a Phe-Gly-Gln-Glu-Glu-Glu motif [15]. As many serine-type endopeptidases are known to also recognize a Glu residue for substrate cleavage [24], the short poly-Glu sequence may be the site of cross-linking with the N-terminal serine of the α-subunit of cytochrome b_{559}. This stretch of amino acids also may be the cleavage site of the putative stromal protease(s). We must, however, await the determination of the exact cross-linking site between the D1 protein and the α-subunit of cytochrome b_{559} to estimate the cleavage site by the protease(s). These results indicate that the stromal protease activity plays a primary functional role in the dissolution of the 41 kDa cross-linked adduct freeing the α-subunit of cytochrome b_{559} and in the subsequent turnover of the D1 protein.

The digestion of the cross-linked products of the D1 protein and the α-subunit of cytochrome b_{559} appears as one of the quality control mechanisms to maintain the functional integrity of the PS II complex under light stress. Protease systems functioning in chloroplasts are most probably similar to those in prokaryotes. In *E. coli*, several protease families, such as the serine-type Clp endopeptidase [25] and metalloprotease FtsH [26], are well characterized. Proteases homologous to those in *E. coli* were found in chloroplasts and characterized [7–9]. In a previous study, we showed that the cross-linked products of the D1 protein generated by illumination are removed by SDS-resistant protein components in the stroma [13]. A 15 kDa protein was most prominent in the activity. Apparently the 15 kDa protein was able to digest all the cross-linked products of the D1 protein including the 41 kDa D1/cytochrome b_{559} adduct. Purification of the 15 kDa SDS-resistant protease is now in progress.

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