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Selective and specific degradation of the D1 protein induced by binding of a novel Photosystem II inhibitor to the Q_B site

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Abstract

When Photosystem (PS) II membranes were incubated with PNO8 (*N*-octyl-3-nitro-2,4,6-trihydroxybenzamide) classified as a phenol-type PS II inhibitor, the D1 protein of PS II reaction center was degraded into two fragments of 23 and 9 kDa in complete darkness, while the D2 protein was not affected at all by incubation with PNO8. Other typical PS II inhibitors, including DCMU, atrazine, ioxynil and dinoseb, showed no degradation activity. Occupation by another PS II inhibitor, DCMU, of the binding site of the secondary quinone acceptor, Q_B, prevented the D1 protein from PNO8-induced degradation. The degradation was inhibited at low temperature, but occurred even in the absence of oxygen. Moreover, an inhibitor of serine-type proteinase, PMSF, was effective in suppressing the D1 protein degradation. Photoinhibitory treatment of the membranes also induced a degradation product with an apparent molecular mass of 23 kDa that corresponds to the PNO8-induced 23 kDa fragment. The data were interpreted as indicating a selective and specific cleavage of the D1 protein triggered by binding of PNO8 to the Q_B site, and the results were discussed in relation to the features of proteolytic degradation of the D1 protein in photoinhibition.

Keywords: Photosystem II; D1 protein; Herbicide; Protein degradation; Photoinhibition

1. Introduction

The reaction center of Photosystem (PS) II is comprised of the heterodimer of D1 and D2 proteins that bind all redox components required for basic PS II functions [1]. The D1 protein carries the secondary quinone acceptor, Q_B, and the tyrosine donor, Y_z, while the D2 protein binds the primary quinone acceptor, Q_A [2–4]. By analogy with the reaction center of photosynthetic bacteria [5], the primary electron acceptor, pheophytin, probably is associated with the D1 protein, whereas the primary electron donor, P680, presumably is bound by both the D1 and D2 proteins. The D1 protein has been characterized by its high turnover rate in a light-dependent manner [6]. The turnover has been considered to involve a selective degradation of photodamaged D1 protein and its repair by de novo synthesis. Under strong-light conditions, PS II is photoinhibited and the amount of the D1 protein decreases, since D1 synthesis cannot compensate the high rate of D1 degradation [7–9]. Interestingly, it has been reported that photoinhibitory illumination given at low temperatures does not induce any degradation of the D1 protein, but the degradation starts in complete darkness when the photoinhibited sample is transferred to ambient temperatures [10]. This phenomenon has been interpreted to mean that D1 protein degradation itself does not require light, but an enzymatic proteolysis is triggered by a light-dependent modification of the D1 protein. The existence of a putative proteinase in PS II has been proposed from the observations that inhibitors of serine-type proteinase suppress the light-dependent D1 protein degradation [7–9,11].

Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone; PNO8, *N*-octyl-3-nitro-2,4,6-trihydroxybenzamide; PMSF, phenylmethanesulfonyl fluoride; PS II, Photosystem II; PY35, 3,5-dibromo-4-hydroxy-6-methyl-2-(3-(4-phenylphenoxy)-1-bromopropyl)pyridine; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; Q_B, secondary quinone acceptor of Photosystem II.

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Photoinhibitory illumination gives rise to several degradation products of the D1 protein. Among them, a fragment with apparent molecular mass of 23 kDa has been commonly detected after both in vitro [12–14] and in vivo [15] photoinhibition as a primary product of D1 protein degradation, and identified to originate from the N-terminal portion of the D1 protein cleaved at the loop domain exposed between helices D and E [12–15]. It is also reported that occupation of the Q_B site by plastoquinone [16] or DCMU, a PS II inhibitor, [6,17–21] alters the extent of D1 degradation during photoinhibition. These observations seem to be consistent with the above proposal that a conformational change around the Q_B site brought about by photoinhibitory illumination makes the D1 protein become susceptible to a putative proteinase [7–9,11]. Conformational changes of the Q_B site by the binding of some PS II inhibitors has been suggested by the finding that these inhibitors impaired the apparent accessibility of trypsin to the D1 protein at Arg-238 located in a vicinity of the Q_B site [22]. It is, therefore, reasonable to hypothesize that the degradation of the D1 protein could be initiated without any photoinhibitory treatments, if the conformation around the Q_B site required for the putative proteinase to function is achieved by some other means.

In the present study, we studied the effect of PS II inhibitors on the stability of the D1 protein. It has been demonstrated that the D1 protein is selectively degraded into 23 and 9 kDa fragments in complete darkness when the Q_B site is occupied by PNO8, which has recently been developed as a highly potent PS II inhibitor that interrupts specifically the electron transfer between Q_A and Q_B [23,24]. PNO8 is classified as phenol-type PS II inhibitor but is quite unique in having a phloroglucinol nucleus.

2. Materials and methods

Thylakoid membranes were prepared from market spinach as described in [25], and BBY-type O_2-evolving PS II membranes were prepared as described in [26]. O_2-evolving PS II core particles were prepared from the PS II membranes by solubilization with *n*-heptyl thioglucoside essentially according to Ref. [27]. Sample materials were stored in liquid nitrogen until use. After thawing, the PS II membranes and the core particles were washed and suspended in 400 mM sucrose, 40 mM Mes-NaOH, 20 mM NaCl (pH 6.5), and then incubated with PNO8 in complete darkness at 25° C unless otherwise noted. PNO8 (1 mM stock solution in dimethylsulfoxide) was added to the incubation mixture, while the final concentration of dimethylsulfoxide in the suspension was less than 1%. After incubation, the treated membranes were quickly frozen and stored in liquid nitrogen until analysis of protein composition. Anaerobic condition was achieved by a 10 min bubbling of argon gas into the incubation medium prior to the addition of the membranes, followed by continuous gas flow above the mixture during dark incubation. PNO8 was added to the suspension after 5 min of the gas flow above the suspension. For photoinhibition, the PS II membranes (200 μg Chl/ml) were gently stirred in a temperature-controlled glass cylinder (2.5 cm diameter) and were illuminated at 15° C with strong white light (200 mW/cm^2) from a 500 W halogen lamp through heat-absorbing filters.

Protein compositions of the sample materials were analyzed by SDS-PAGE according to Laemmli [28] with a gel containing 5 M urea and 13% (w/v) acrylamide. Separated polypeptides were electroblotted onto a nitrocellulose membrane (Schleicher and Schuell) as in [29] but in the presence of 0.05% (w/v) SDS with a semi-dry type blot apparatus and probed with rabbit antisera raised against spinach D1 and D2 proteins (kind gifts from Dr. M. Ikeuchi). Immunoreacted protein bands were immunodetected with goat antibody against rabbit IgG conjugated with alkaline phosphatase (Jackson ImmunoResearch), and visualized by reaction with nitroblue tetrazolium and bromochloroindolyl phosphate. Densitometric determination of immunodetected bands was carried out at 540 nm with a Shimadzu CS-9000 chromatoscanner.

O_2 evolution was measured with a Clark-type oxygen electrode at 25° C in the presence of 0.5 mM phenyl-*p*-benzoquinone as an electron acceptor.

N-Octyl-3-nitro-2,4,6-trihydroxybenzamide (PNO8) and its related chemicals were synthesized as in [30,31] and purified by high performance liquid chromatography. Structures of the synthesized compounds were confirmed by ^{1}H-NMR, IR and mass spectroscopy. No derivatives were detected during storage in dimethylsulfoxide.

3. Results

Fig. 1 shows the dependence of inhibition of O_2 evolution activity on the concentrations of PNO8 and DCMU in

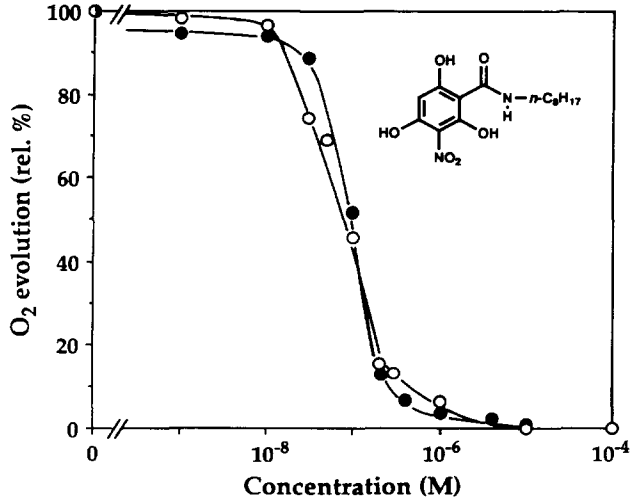

Fig. 1. Inhibition of O_2 evolution activity by PNO8 (closed circles) and DCMU (open circles). The chemical structure of PNO8 is shown.

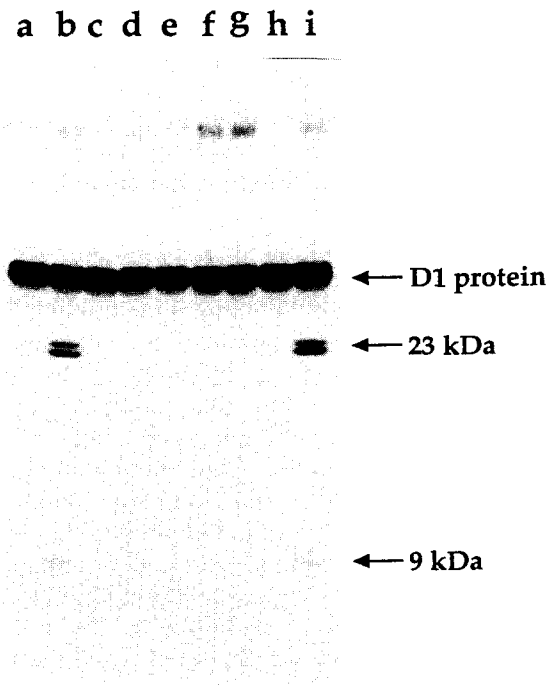

Fig. 2. Effects of PS II inhibitors on the stability of the D1 protein during dark incubation. PS II membranes were incubated for 60 min at 25° C in the dark. D1 protein and its degradation products were detected immunologically with D1 antiserum. Additions were: (a) no addition (control); (b and i) 10 μM PNO8; (c) 10 μM DCMU; (d) 50 μM atrazine; (e) 100 μM ioxynil; (f) 100 μM dinoseb; (g) 50 μM PY35; (h) 200 μM DBMIB. All chemicals were dissolved in dimethylsulfoxide as a stock solution and added to the incubation mixture, while the final concentration of dimethylsulfoxide was less than 1% (v/v).

the PS II membranes in which assays were carried out at Chl concentration of 5 μg Chl/ml. The apparent I_{50} value for PNO8 ($7.9 \cdot 10^{-8}$ M) was close to that found for DCMU ($9.5 \cdot 10^{-8}$ M), indicating that PNO8 inhibits PS II electron transport as strongly as DCMU. The site of inhibition by PNO8 was found to be the Q_A-to-Q_B electron transfer, based on the fact that PNO8-treated PS II membranes exhibit the thermoluminescence band arising from charge recombination between S_2 and Q_A^- (data not shown) as in DCMU-treated thylakoids [24]. It is of note that the dependence curve shifted upward by nearly one order of concentration when O_2 evolution assay was carried out at Chl concentration of 200 μg Chl/ml, which was applied to PNO8 treatment for the D1 protein degradation (data not shown). Unless otherwise noted, a PNO8 concentration of 10 μM was used throughout this study, which effected complete inhibition of O_2 evolution at 200 μg Chl/ml.

Fig. 2 shows the effects of various PS II inhibitors on the stability of the D1 protein in which the PS II membranes were incubated with the inhibitors at 25° C in the dark for 60 min. DCMU and atrazine belong to urea-type and triazine-type PS II inhibitors, and ioxynil, dinoseb and PY35 [32] belong to phenol-type PS II inhibitors, while DBMIB may also acts as an electron acceptor in the PS II membranes. The polypeptide compositions of the treated membranes were resolved with SDS-PAGE and the D1 protein was immunologically detected with the D1 antiserum. No polypeptide band was detected except for the native D1 protein in the membranes treated with DCMU, atrazine, ioxynil, dinoseb, DBMIB and PY35, of which concentrations were enough for complete inhibition of the electron transfer between Q_A and Q_B except for DBMIB. To our surprise, however, dark incubation of the membranes with PNO8 induced a degradation of the D1 protein to yield two fragments at 23 and 9 kDa (lanes b and i). The band of the 23 kDa fragment appeared as a doublet due to interference in binding of antibody to the fragment by the 22 kDa intrinsic protein (*psb* S gene product) superimposed on the 23 kDa fragment [33].

Fig. 3 shows the effects of dark incubation with PNO8 in the thylakoid membranes, PS II membranes and O_2-evolving core particles. Dark incubation with PNO8 led to generation of the identical two fragments of the D1 protein at 23 and 9 kDa in all three preparations and no other degradation products of the D1 protein were detected. The amounts of the PNO8-induced fragments were rather small in the core particles probably because of the reduced affinity of the Q_B site for PNO8 in the core particles by one order of magnitude below that in the PS II membranes (data not shown). The result indicates that the PNO8-in-

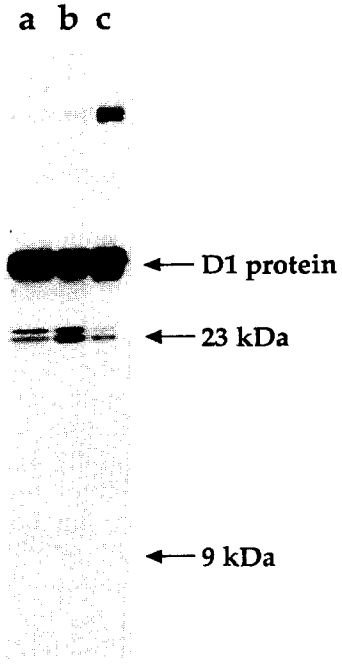

Fig. 3. Degradation of the D1 protein by PNO8. Thylakoid membranes (a), PS II membranes (b) and O_2-evolving core particles (c) were incubated with 10 μM PNO8 for 60 min at 25° C in the dark. D1 protein and its degradation products were detected immunologically with D1 antiserum. The sample concentrations during incubation with PNO8 and the sample amounts loaded in each well of SDS-PAGE were 400 μg Chl/ml and 200 μg Chl/ml, 80 μg Chl/ml, and 3 μg Chl, and 1 μg Chl and 0.4 μg Chl for the thylakoid membranes, PS II membranes and O_2-evolving core particles, respectively.

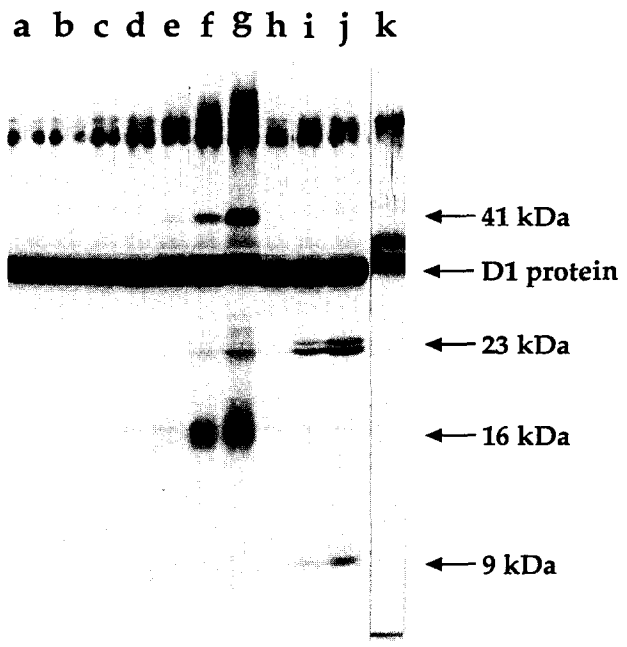

Fig. 4. Immunological detection of the degradation products of the D1 and D2 proteins. Control PS II membranes were incubated in the dark for 0 (a), 10 (b) and 60 min (c); PS II membranes were illuminated for 0 (d), 10 (e), 60 (f) and 120 min (g); PS II membranes were incubated with 10 μM PNO8 in the dark for 0 (h), 10 (i) and 60 min (j and k). Dark incubations and photoinhibitory treatments were carried out at 25° C and 15° C, respectively. Western blotting was performed using anti D1 (a–j) and anti D2 (k) polyclonal antisera. Degradation products seen faintly at 23 kDa at 0-min treatment with PNO8 (h) were due to adventitious degradation of the D1 protein during sample handling. The 67 kDa band seen in all lanes and the band at around 4 kDa in lane k were due to non-specific immunoreaction with unidentified substances.

duced cleavage of the D1 protein is not due to damage of the D1 protein inflicted during isolation of the PS II membranes and core particles with detergent. Therefore, the following experiments were performed using only the PS II membranes.

Fig. 4 shows the effects of photoinhibitory illumination and PNO8 treatment on D1 protein in the PS II membranes. Photoinhibitory treatment resulted in selective degradation of the D1 protein to produce 23 and 16 kDa fragments (lanes d-g). The two fragments became detectable after 10 min of illumination and continued to accumulate with illumination time. Very broad bands of the fragment of the D1 protein were detected at 8–10 kDa region in the sample photoinhibited for 120 min when the blotted membrane was overreacted with nitroblue tetrazolium and bromochloroindolyl phosphate (data not shown), although the fragment band was too faint to be revealed well in the figure. Photoinhibition also led to the appearance of a 41 kDa band, which has been reported to be an adduct between the D1 protein and the α-subunit of cytochrome *b*-559 [34]. No degradation product of the D1 protein was detected when the PS II membranes were incubated in the dark (lanes a–c). The PNO8-induced 23 kDa fragment migrated to almost the same position as that of the photo-degraded 23 kDa fragment (lanes h–j) and also detected as a doublet band due to the presence of *psb* S gene product although the fragment appeared to be much broader in the photoinhibited than in the PNO8-treated membranes. PNO8-induced protein degradation was highly specific for the D1 protein, and no degradation product of the D2 protein could be detected (lane k). No changes in the polypeptide profile on Coomassie stained gel were seen even after prolonged dark incubation with PNO8 (data not shown).

Fig. 5 shows the time course of the appearance of the 23 and 9 kDa fragments during incubation with PNO8, in which the relative absorbance of the fragment bands was plotted against incubation time. Accumulation of the fragments started with no lag-time after addition of PNO8, showing a high initial rate followed by progressive bending off with time, and then reached a constant level after 150 min. The time courses for the formation of the two fragments were almost the same, suggesting that the two fragments are the products of a single-site cleavage of the D1 protein. It is note that the amount of the cleaved D1 protein was estimated to be about 10% of the total D1 protein after the treatment for 150 min.

Fig. 6 shows the dependence of the D1 protein degradation on the concentration of PNO8 as detected by the formation of the 23 kDa fragment. The amount of the fragment was dependent on the concentration of PNO8 and reached a high level at 5 μM PNO8. Although the fragment formation increased gradually with PNO8 concentration above 5 μM, treatment with 10 times concentrated PNO8 resulted in only 34% augmentation in the amount of

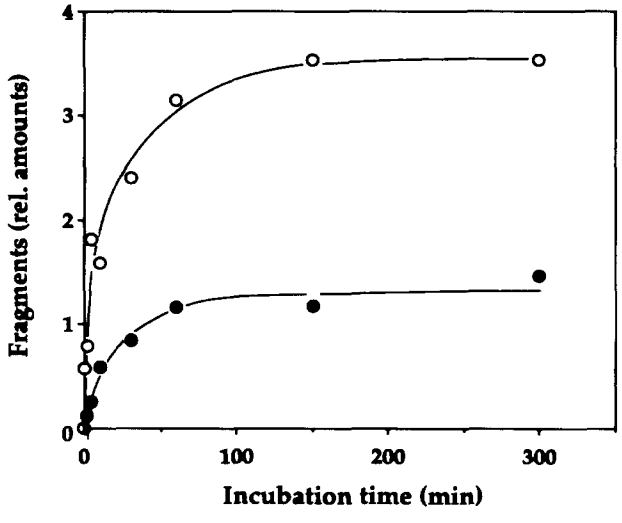

Fig. 5. Time course of accumulation of the degradation products of the D1 protein during dark incubation with PNO8: 23 kDa (open circle) and 9 kDa (closed circle) fragments. PS II membranes were incubated with 10 μM PNO8 for various time at 25° C in the dark. The fragments were detected immunologically with D1 antiserum, and quantified densitometrically. The sample amount loaded in each well of SDS-PAGE was 1 μg Chl which affords a linear relationship between the staining intensities of the two fragments bands and the amounts of the fragments.

the fragments. The result indicates that the binding of PNO8 to a specific binding site is responsible for the cleavage of the D1 protein to form the 23 and 9 kDa fragments.

Fig. 7 shows some of the factors that affected the PNO8-induced degradation of the D1 protein. The fragments were still formed even when the concentration of PNO8 was reduced as low as 1 μM (lane b). The fragment formation was not much affected by the absence of oxygen (lane d), indicating that no oxygen dependent process is involved in the degradation. Notably, however, the fragment formation was considerably suppressed to about 20% by the presence of excess DCMU (lanes g, h) which inhibits the electron transfer between Q_A and Q_B by binding to the Q_B site of the D1 protein in place of a plastoquinone molecule. Since the binding domain of PNO8 is known to overlap with that of DCMU [24], the result implies that the PNO8-dependent degradation of the D1 protein requires specific binding of PNO8 molecule to the Q_B site. This may explain why the D1 protein was the only protein cleaved by PNO8. It has been proposed that a specific proteinase is implicated in the D1 protein degradation during photoinhibition. The putative proteinase has been claimed to be associated closely with the PS II core and/or PS II reaction center [7–9], and to be inhibited by serine-type proteinase inhibitors such as PMSF and diisopropyl fluorophosphate [12,35,36]. As dark incubation with PNO8 resulted in the generation of the 23 and 9 kDa fragments in the core particles as shown in Fig. 3, the effects of a proteinase inhibitor, PMSF, were examined to check the possible participation of a proteinase in the PNO8-induced degradation of the D1 protein. Interestingly

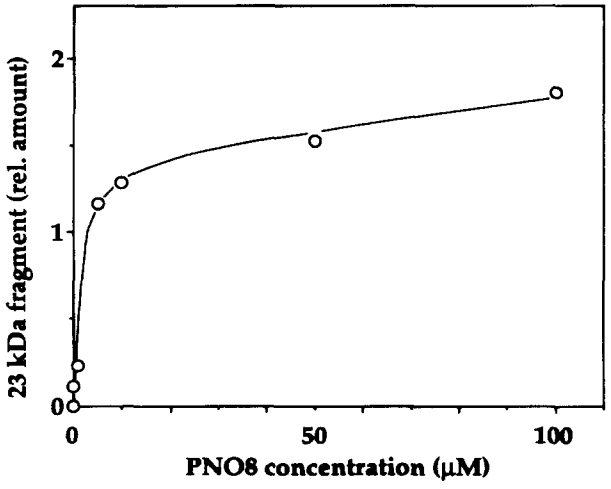

Fig. 6. Dependence on PNO8 concentration of the degradation of the D1 protein. PS II membranes were incubated with various concentrations of PNO8 for 60 min at 25° C in the dark. The 23 kDa fragment was detected immunologically with D1 antiserum, and quantified densitometrically. The sample amount loaded in each well of SDS-PAGE was 1 μg Chl, which affords a linear relationship between the staining intensities of the fragment band and the amount of the fragment.

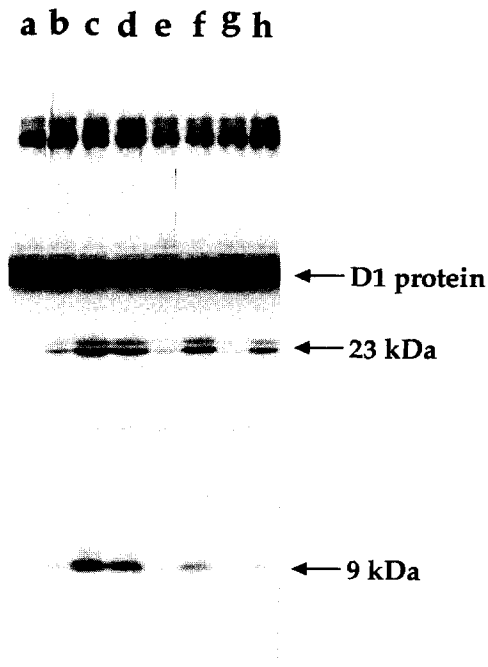

Fig. 7. Degradation of the D1 protein by PNO8 under various conditions. PS II membranes were incubated for 60 min at 25° C in the dark under aerobic condition unless otherwise noted. Degradation products were detected immunologically with D1 antiserum. Additions were: (a), no addition (control); (b), 1 μM PNO8; (c), 10 μM PNO8; (d) 10 μM PNO8, anaerobic; (e) 10 μM PNO8, 0° C; (f), 10 μM PNO8 *plus* 2 mM PMSF; (g), 1 μM PNO8 *plus* 100 μM DCMU; (h) 10 μM PNO8 *plus* 100 μM DCMU.

enough, the degradation was suppressed to about 60% by 2 mM PMSF (lane f). It is noted that PMSF at this concentration did not affect at all the inhibition of PS II electron transport by PNO8. The PNO8-induced degradation of the D1 protein was also suppressed to 40 ~ 60% by the addition of other inhibitors for serine-type proteinase including 4-amidinophenylmethanesulfonyl fluoride (APMSF), diisopropyl fluorophosphate (DFP), aminophenyl benzoate and benzamidine (data not shown). This suggests an involvement of a proteolytic process in PNO8-induced degradation, and is consistent with the result that the degradation was largely suppressed when the incubation temperature was lowered to 0° C (Fig. 6, lane e).

4. Discussion

The present study demonstrated that simple treatment of PS II membranes with PNO8, a potent electron transport inhibitor between Q_A and Q_B, induces a degradation of the D1 protein to give the 23 and 9 kDa fragments in complete darkness. The degradation was highly specific for the D1 protein, and other PS II proteins were not affected at all by PNO8 treatment. The protective effect of DCMU against PNO8-induced degradation of the D1 protein indicates that binding of a PNO8 molecule to the Q_B-pocket leads to

cleavage of the D1 protein. This view is further supported by the finding that the degradation of the D1 protein was saturated at the PNO8 concentration that was required for complete inhibition of the electron transfer between Q_A and Q_B as shown in Fig. 6. Two possible mechanisms may be assumed to explain this novel reaction: (i) the polypeptide chain of the D1 protein is cleaved by a direct chemical reaction with the PNO8 molecule bound to the Q_B-pocket or (ii) binding of PNO8 to the Q_B-pocket induces some structural modification in the D1 protein and triggers the degradation by a mechanism other than the direct reaction with PNO8. Judging from the protective effect of PMSF that suggests the involvement of a serine-type proteinase in this degradation process, the latter possibility seems to be more likely, although we can not completely exclude the former possibility at present.

It has been proposed that in photoinhibition the selective and specific degradation of the D1 protein is effected by a proteolytic process rather than a direct photochemical reaction [7,9,11], in which a light-dependent modification in the D1 protein is assumed to convert D1 to a substrate for the putative proteinase. Our observations in the present study as described in the above paragraph are in accord with the scenario proposed for the degradation of the D1 protein in photoinhibition, i.e., both require a structural modification of the D1 protein to trigger the proteolytic process. In this context, it may be of interest to note that the 23 and 9 kDa degradation products observed in this study coincide with those reported in acceptor side photoinhibition [12–14], in which the primary cleavage takes place at the loop connecting transmembrane helices D and E of the D1 protein to yield a 23 kDa N-terminal fragment [12–15] and a 10 kDa C-terminal fragment [12,13,37]. Since this loop domain provides the binding site for Q_B plastoquinone as well as for PS II inhibitors including PNO8, it is reasonable to assume that the D1 protein is cleaved at this loop upon binding of PNO8 to yield 23 kDa N-terminal and 9 kDa C-terminal fragments. Our preliminary analysis using a site-specific antibody raised against the oligopeptide that corresponds to the residue Leu326-Glu333 of the spinach D1 protein (unpublished data) supports this view. Based on these considerations, we may assume that a common mechanism is involved in the process of the degradation of the D1 protein by both photoinhibition and PNO8 treatment. Although the detailed molecular basis for the degradation of the D1 protein is not clear at present, we may speculate that the conformation of the Q_B site occupied by PNO8 molecule would be similar to that induced by photoinhibitory treatment, and both allow proteolytic cleavage by the putative proteinase at the same site of the D1 protein.

As shown in Figs. 2 and 5, only a limited population of the D1 protein was degraded by PNO8. Since we may assume that PNO8 binds to all the PS II centers on the basis of the complete inhibition of the O_2 evolution activity, this implies that PNO8-binding to the Q_B-pocket does not necessarily trigger the degradation of the D1 protein. One simple explanation for this would be the contamination of a chemical species that is effective in inducing the D1 protein degradation in place of PNO8. However, this does not appear to be the case for the following reasons: (i) careful inspection by high-performance liquid chromatography of the purity of our PNO8 preparation detected no contamination; (ii) chemical species possibly derived from PNO8 showed no or markedly less affinity for the Q_B site as far as tested; (iii) some phloroglucinol derivatives also induced D1 degradation and their capabilities showed a good correlation with their activities as PS II inhibitors (data not shown). In the D1 protein degradation under photoinhibitory conditions, it has been proposed that a damaged D1 protein is tagged by structural and/or conformational modifications to be discriminated from the native D1 protein [38,39]. The non-stoichiometric degradation of the D1 protein by PNO8 binding, therefore, seems to be reasonably explained, if we assume that PNO8 triggers the degradation of the D1 protein only in some special state and/or conformation.

It has been reported that several kinds of PS II inhibitor protect the D1 protein from degradation induced by illumination with UV and/or visible light and suppress the loss of O_2 evolution by photoinhibition [6,17–21], while any PS II inhibitor has not been reported to lead the degradation of the D1 protein as far as we know. It is, therefore, reasonable to assume that the structural requirement for the D1 protein degradation is rather different from that for the inhibition of the electron transfer and the protection of the D1 protein against photodegradation, although those may partly overlap with each other. PNO8 seems to be classifiable as a phenol-type PS II inhibitor as it contains the characteristic phenol nucleus, and also structural features essential for inhibitory activity are fit to the structural requirements for phenol-type inhibitors [40]. The nitro and octylamide groups in PNO8 may be regarded as the strongly electron-withdrawing group and either the slightly electron-withdrawing group with strict steric requirements or a lipophilic group without steric requirements in the proposed structural requirement, respectively. PNO8 is, however, quite unique in containing a phloroglucinol nucleus because this compound has been designed based on the structure of grandinol and homograndinol found in *Eucalyptus grandis* [23,24]. Thus, we have synthesized some derivatives of PNO8 to test the structural relevance of phloroglucinol nucleus to the D1 protein degradation. Preliminary experiments showed that no derivative possessed D1 protein degradation activity when the structure of phloroglucinol nucleus was modified, even though derivatives still retained the activity as PS II inhibitor. We tentatively assume as a working hypothesis that some interaction between the phloroglucinol nucleus and amino acid residues in the Q_B-niche induces the structural change which is required for the cleavage of the D1 protein.

The PNO8-induced degradation of the D1 protein would

provide a useful tool for understanding the molecular mechanisms of the selective and specific degradation of the D1 protein under photoinhibitory conditions and the rapid turnover of the D1 protein under weak-light conditions, too.

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References

[1] Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
[2] Hansson, Ö. and Wydrzynski, T. (1990) Photosynth. Res. 23, 131–162.
[3] Debus, R.J. (1992) Biochim. Biophys. Acta 1102, 269–352.
[4] Barber, J. and Andersson, B. (1994) Nature 370, 31–34.
[5] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Nature 318, 618–624.
[6] Mattoo, A.K., Hoffman-Falk, H., Marder, J.B. and Edelman, M. (1984) Proc. Natl. Acad. Sci. USA 81, 1380–1384.
[7] Barber, J. and Andersson, B. (1992) Trends. Biochem. Sci. 17, 61–66.
[8] Prášil, O., Adir, N. and Ohad, I. (1992) in The Photosystems: Structure, Function and Molecular Biology (Barber, J., ed.), pp. 295–348, Elsevier, Amsterdam.
[9] Aro, E.-M., Virgin, I. and Andersson, B. (1993) Biochim. Biophys. Acta 1143, 113–134.
[10] Aro, E.-M., Hundal, T., Carlberg, I. and Andersson, B. (1990) Biochim. Biophys. Acta 1019, 269–275.
[11] Andersson, B., Ponticos, M., Barber, J., Koivuniemi, A., Aro, E.-M., Hagman, Å., Salter, A.H., Dan-Hui, Y. and Lindahl, M. (1994) in Photoinhibition of photosynthesis (Baker, N.R. and Bowyer, J.R., eds.), pp. 143–159, βIOS Scientific Publishers, UK.
[12] Salter, A.H., Virgin, I., Hagman, Å. and Andersson, B. (1992) Biochemistry 31, 3990–3998.
[13] De Las Rivas, J., Andersson, B. and Barber, J. (1992) FEBS Lett. 301, 246–252.
[14] De Las Rivas, J., Shipton, C.A., Ponticos, M. and Barber, J. (1993) Biochemistry 32, 6944–6950.
[15] Greenberg, B.M., Gava, V., Mattoo, A.K. and Edelman, M. (1987) EMBO J. 6, 2865–2869.
[16] Gong, H. and Ohad, I. (1991) J. Biol. Chem. 266, 21293–21299.
[17] Trebst, A. and Depka, B. (1990) Z. Naturforsch. 45c, 765–771.
[18] Jansen, M.A.K., Malkin, S. and Edelman, M. (1990) Z. Naturforsch. 45c, 408–411.
[19] Kuhn, M. and Böger, P. (1990) Photosynth. Res. 23, 291–296.
[20] Kirilovsky, D., Rutherford, A.W. and Etienne, A.-L. (1994) Biochemistry 33, 3087–3095.
[21] Jansen, M.A.K., Depka, B., Trebst, A. and Edelman, M. (1993) J. Biol. Chem. 268, 21246–21252.
[22] Trebst, A., Depka, B., Kraft, B. and Johanningmeier, U. (1988) Photosynth. Res. 18, 163–177.
[23] Honda, I., Yoneyama, K., Iwamura, H., Konnai, M., Takahashi, N. and Yoshida, S. (1990) Agric. Biol. Chem. 54, 1227–1233.
[24] Yoneyama, K., Konnai, M., Honda, I., Yoshida, S., Takahashi, N., Koike, H. and Inoue, Y. (1990) Z. Naturforsch. 45c, 317–321.
[25] Arntzen, C.J., Vernotte, C., Briantais, J.-M. and Armond, P. (1974) Biochim. Biophys. Acta 368, 39–53.
[26] Ono, T. and Inoue, Y. (1989) Biochim. Biophys. Acta 973, 443–449.
[27] Enami, I., Kamino, K., Shen, J.-R., Satoh, K. and Katoh, S. (1989) Biochim. Biophys. Acta 977, 33–39.
[28] Laemmli, U.K. (1970) Nature 227, 680–685.
[29] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[30] Honda, I., Yoneyama, K., Konnai, M., Takahashi, N. and Yoshida, S. (1990) Agric. Biol. Chem. 54, 1071–1072.
[31] Honda, I., Shibagaki, M., Yoneyama, K., Nakajima, Y., Konnai, M., Takahashi, N. and Yoshida, S. (1993) Z. Naturforsch. 48c, 159–162.
[32] Asami, T., Baba, M., Koike, H., Inoue, Y. and Yoshida, S. (1993) Z. Naturforsch. 48c, 152–158.
[33] Funk, C., Schröder, W.P., Green, B.R., Renger, G. and Andersson, B. (1994) FEBS Lett. 342, 261–266.
[34] Barbato, R., Friso, G., Rigoni, F., Frizzo, A. and Giacometti, G.M. (1992) FEBS Lett. 309, 165–169.
[35] Virgin, I., Salter, A.H., Ghanotakis, D.F. and Andersson, B. (1991) FEBS Lett. 287, 125–128.
[36] Shipton, C.A. and Barber, J. (1992) Biochim. Biophys. Acta 1099, 85–90.
[37] Cánovas, P.M. and Barber, J. (1993) FEBS Lett. 324, 341–344.
[38] Ohad, I., Kyle, D.J. and Hirschberg, J. (1985) EMBO J. 4, 1655–1659.
[39] Aro, E.-M., Kettunen, R. and Tyystjärvi, E. (1992) FEBS Lett. 297, 29–33.
[40] Trebst, A., Donner, W. and Draber, W. (1984) Z. Naturforsch. 39c, 405–411.