GTP Enhances the Degradation of the Photosystem II D1 Protein Irrespective of Its Conformational Heterogeneity at the Q\textsubscript{B} Site\textsuperscript{*}

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The light exposure history and/or binding of different herbicides at the Q\textsubscript{B} site may induce heterogeneity of photosystem II acceptor side conformation that affects D1 protein degradation under photoinhibitory conditions. GTP was recently found to stimulate the D1 protein degradation of photoinactivated photosystem II (Spetea, C., Hundal, T., Lohmann, F., and Andersson, B. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 6547—6552). Here we report that GTP enhances the cleavage of the D1 protein D-E loop following exposure of thylakoid membranes to either high light, low light, or repetitive single turnover flashes but not to trypsin. GTP does not stimulate D1 protein degradation in the presence of herbicides known to affect the accessibility of the cleavage site to proteolysis. However, GTP stimulates degradation that can be induced even in darkness in some photosystem II conformers following binding of the PNO8 herbicide (Nakajima, Y., Yoshida, S., Inoue, Y., Yoneyama, K., and Ono, T. (1995) Biochim. Biophys. Acta 1230, 38—44). Both the PNO8- and the light-induced primary cleavage of the D1 protein occur in the grana membrane domains. The subsequent migration of photosystem II containing the D1 protein fragments to the stroma domains for secondary proteolysis is light-activated. We conclude that the GTP effect is not confined to a specific photoactivation pathway nor to the conformational state of the photosystem II acceptor side. Consequently, GTP does not interact with the site of D1 protein cleavage but rather enhances the activity of the endogenous proteolytic system.

Exposure of photosynthetic membranes to light intensities exceeding saturation of electron flow leads to inactivation of photosystem II (PSII)\textsuperscript{1} accompanied by degradation of the reaction center D1 protein (1, 2). Photoactivation occurs initially at the acceptor side of PSII, inhibiting electron flow between the primary and secondary plastoquinone electron acceptors Q\textsubscript{A} and Q\textsubscript{B}, bound to the reaction center D2 and D1 proteins, respectively. Impairment of the PSI acceptor side during photoinhibition has been assigned to the generation of stable Q\textsubscript{A}\textsuperscript{−} forms (3), a process that can be retarded by the presence of urea-type herbicides (4). On the other hand, Krieger-Liszkay and Rutherford (5) showed that binding of phenol-type herbicides at the Q\textsubscript{B} site can lower the redox potential of the Q\textsubscript{A}/Q\textsubscript{A}\textsuperscript{−}, increasing the sensitivity of PSII to light. The photoactivation of electron transport causes oxidative damage to the reaction center because of triplet chlorophyll (3Chl) and singlet oxygen (\textsuperscript{1}O\textsubscript{2}) formation (3). The primary cleavage site during the proteolytic removal of the damaged D1 protein under acceptor side photoinhibition conditions is located in the stroma-exposed loop connecting the D and E transmembrane helices (6). This is the same domain which is cleaved by externally added trypsin (7).

Not only very high light intensities but also low light intensities or single turnover flashes can efficiently damage PSII and induce subsequent degradation of the D1 protein (8—10). The mechanism of photodamage under these conditions involves back electron flow via charge recombination between Q\textsubscript{B}\textsuperscript{−} and the S\textsubscript{2,3} states of the oxygen-evolving complex. This process may lead to \textsuperscript{3}Chl and \textsuperscript{1}O\textsubscript{2} formation as in the case of high light-induced inactivation although via a different pathway (10).

A wide range of herbicides can interfere with the photoinhibition process. Such herbicides, derived from urea, phenol, or triazine, bind to the Q\textsubscript{B} site, competing with the binding of plastoquinone and thus inhibiting electron flow (11, 12). Various types of herbicides may interact with different amino acid residues within the Q\textsubscript{B} niche, hence affecting the conformation of this domain of the D1 protein in a specific manner. Such conformational changes can protect the cleavage site against the thylakoid endogenous protease as well as against externally added trypsin. Urea- and triazine-type herbicides but not phenol-type can induce such a protease resistance to the D1 protein (13—15).

Recently, it was reported that another compound, PNO8, that shares the characteristics of phenol-type herbicides, but in addition has a phloroglucinol nucleus, binds to the Q\textsubscript{B} site, thereby inhibiting PSII electron flow (16). However, unlike other phenol-type herbicides, the binding of PNO8 to PSII can induce the typical D1 protein degradation even in darkness. It is thought that this herbicide can induce conformational...
changes in the D-E loop of the D1 protein in a population of PSII centers, thereby exposing the cleavage site to the endogenous protease without any preceding photodamaging event (16). The primary products of the D1 protein cleavage were identified as a 23-kDa fragment containing the N-terminal domain and the corresponding 9–10-kDa C-terminal fragment (16) as is also the case during D1 protein degradation following acceptor side photoinactivation (17–19). It is also suggested that PNO8 triggers the degradation of the D1 protein in PSII centers having a certain conformation of the acceptor side. Short exposure of the thylakoid membranes to light (10 s), which by itself does not cause measurable photoinactivation, induces additional conformational changes of the site and results in an enhanced D1 protein degradation in darkness in the presence of PNO8 (20). However, this conformation(s) is not stable and decays with a half-life of 1 h.

We have recently discovered that GTP is required for the degradation of the D1 protein during acceptor side photoinactivation of PSII (21). The presence of GTP but not ATP accelerates the primary cleavage of the D1 protein and the formation of the 23-kDa N-terminal degradation fragment. The question arises whether GTP acts at the substrate level affecting the conformation of the susceptible Q_{b} domain or as a modulator of the endogenous proteolytic activity. The results obtained in this work demonstrate that, in dark-adapted thylakoids and in thylakoids photoinactivated under low excitation pressure or by high light, the acceptor side of PSII may assume different conformations which, upon binding of PNO8, expose to different degrees the D-E loop to the endogenous protease. In all cases, GTP promotes the primary cleavage irrespective of the mode whereby the D-E loop is influenced. Thus, we suggest that GTP may regulate the proteolytic activity at the enzymatic level, but it does not interact directly with the cleavage site in the D-E loop of the D1 protein substrate.

## EXPERIMENTAL PROCEDURES

### Plant Material and Thylakoid Preparation—
Thylakoid membranes were isolated from Pisum sativum (Pisum sativum) leaves as described previously (22) and finally resuspended in 50 mM HEPES/NaOH buffer (pH 7.4) containing 0.4 mM sucrose, 15 mM NaCl, and 5 mM MgCl_{2}.

### Photoinhibitory Treatments—
Pea thylakoids diluted in resuspension buffer to a final concentration of 0.4 mg of Chl/ml (3-m1 glass cuvettes, 1-cm optical path) were exposed to low light (30 μmol of photons/m²s) or to single-turnover light flashes (150 μJ/pulse, 9-ns pulse width, 532 nm) at a frequency of 1 flash/24 s for up to 4 h (600 total charge separation events) at 22 °C (20). In certain experiments, 50% inactivation of electron transport was reached by exposure of spinach thylakoids (0.2 mg of Chl/ml) to heat-filtered visible light (5,000 nm) at a frequency of 1 flash/24 s for up to 4 h (600 total charge separation events) at 22 °C (20). After dilution to 0.1 mg of Chl/ml in the resuspension buffer supplied with the indicated additions, the photoinactivated samples were transferred to darkness at 22 °C to allow proteolysis. Where indicated, spinach thylakoids (0.1 mg of Chl/ml) were illuminated with 1,500 μmol of photons/m²s visible light for up to 2 h at 22 °C.

Before illumination, 0.2 mM GTP or GTP·S were added to the thylakoids followed by incubation on ice in darkness for 30 min. Where indicated, various herbicides (10 μM DCMU, 50 μM ioxynil, and 100 μM diuron) were added at the beginning of the illumination. Control thylakoids or those subjected to 10 s precollimation were incubated with another herbicide, PNO8, in darkness at 22 °C. In some experiments, thylakoid subfractions (grana, grana margins, and stroma-exposed thylakoids) prepared by the digitonin method (23) were used.

### Trypsin Treatment—
Trypsin treatment of dark control and 50% photoinactivated thylakoids was performed as described (24) using 14% acrylamide and 6 M urea in the separation gel. Resolved thylakoid proteins were electrotransferred to poly(vinylidene difluoride) membranes according to Towbin et al. (25). The D1 protein and its 23-kDa N-terminal fragment were identified with antibodies raised against a synthetic peptide corresponding to amino acids 234–242 of the spinach protein. For quantification of the level of the D1 protein and the 23-kDa fragment, 0.2 and 0.8 μg of Chl were loaded per lane, respectively. The plotted data represent averages from at least three independent experiments with the indicated standard deviations.

### RESULTS AND DISCUSSION

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**Measurement of PSII Activity—** Oxygen evolution was measured with a Clark-type electrode under saturating visible light at 22 °C in resuspension buffer supplemented with 0.5 mM phenyl-p-benzoquinoine as an electron acceptor. Thermoluminescence (TL) signal in pea thylakoids photoinactivated or exposed to single turnover flashes was measured in 0.2 ml dark-adapted samples for 2 min at 22 °C. The samples were then rapidly cooled and excited with 2 flashes at 0 °C followed by freezing at −20 °C. The heating was of 0.5 °C/s, and the light emitted as a function of temperature was measured by photon counting (10).

**GTP Enhances D1 Protein Degradation Irrespective of the Photoinactivation Pathway at the PSII Acceptor Side—**
GTP has been previously demonstrated that infrequent excitation of PSII promotes formation of PQ^{+} but does not result in further reduction to plastoquinol. As in the case of the high light-induced photoinactivation, charge recombination between P_{680}^{+*} and Pheo^{+} generates 3Chl and O_{2} and thus induces degradation of the D1 protein. This phenomenon can be drastically enhanced by synchronizing the above charge separation/recombination process using series of single turnover light flashes (8–10). It was therefore of interest to investigate whether the effect of GTP on the degradation of the D1 protein may be related to photodamage to PSII in general and not specifically associated with the high light-induced acceptor side photoinactivation. We have consequently examined the degradation of the D1 protein in thylakoids exposed to single turnover flashes delivered at 24-s intervals in the absence and presence of 0.2 mM GTP or GTP·S. After 4 h of exposure (total of 600 charge separation events), 60% photoinactivation of electron transport occurred in all samples as indicated by thermoluminescence measurements (not shown). Notably, GTP accelerates the subsequent D1 protein degradation process, and about 40% of the initial D1 protein was degraded (not shown). This effect was paralleled by an enhanced appearance of the 23-kDa fragment of the D1 protein (not shown). As expected, addition of GTP·S slightly inhibited the degradation relative to control, as during high light-induced photoinhibition (21). Under illumination conditions leading to −20% inactivation, GTP·S completely prevented the loss of the D1 protein (not shown). It should be noted that the typical 23-kDa D1 fragment is generated under limiting light conditions irrespective of whether the thylakoids are exposed to repetitive single-turnover flashes or to continuous low light intensity (30 μmol of photons/m²s, not shown). This implies the involvement of the same GTP-dependent proteolysis for the D1 protein irrespective of the redox conditions of the quinone acceptors which lead to PSII inactivation and oxidative damage to the reaction center.

**GTP Does Not Stimulate D1 Protein Degradation by Trypsin—** The enhancement of the light-induced degradation by GTP could be because of the activation of the endogenous protease that induces the primary cleavage of the D1 protein or by alteration of the protein substrate conformation. In an attempt to distinguish between these two possibilities, we have used the exogenous protease trypsin. The trypsin sensitivity of the D1 protein is well known (26–27) and has been used as a tool in connection with studies on light-induced D1 protein degradation. As shown in Fig. 1A, 0.2 mM GTP did not enhance the degradation by trypsin in dark-control thylakoids but...
rather slowed down the process in the first 10 min of incubation. In thylakoids that had been 50% inactivated in PSII electron transport by pre-exposure to high light at low temperatures (21) and subsequently incubated in darkness, the GTP-stimulated reduction of the D1 protein cleavage by trypsin is more evident than in the dark-control samples (Fig. 1B). The D1 protein is also degraded in photoinactivated samples incubated without trypsin, although with slower kinetics, indicating that the endogenous GTP-dependent proteolysis has been initiated. These data suggest that the D1 protein degradation by trypsin does not require GTP at variance with the light-mediated process catalyzed by the thylakoid endogenous protease(s).

Effect of GTP on the D1 Protein Degradation in the Presence of Various Herbicides—The occupancy of the QB site by plastoquinone or plastoquinone analogues modulates the conformation of the D-E loop domain of the D1 protein and thus modulates its degradation (13–15, 28). Studies involving the use of various types of herbicides could be useful to elucidate the possible interaction of GTP with the QB site. Concentrations of various types of herbicides could be useful to elucidate the effect is not related to its direct interaction with the cleavage site of the protein substrate.

**PNO8-induced D1 Protein Degradation and Its Stimulation by GTP**—At variance with urea- or triazine-type herbicides which prevent exposure of the cleavage site to the thylakoid protease, herbicides such as PNO8 can promote the proteolysis even in darkness without preceding photoinhibitory treatment (16). As shown in Fig. 3A, the D1 protein degradation after 60 min of incubation in darkness, is saturated at 20–30 μM PNO8 when about 10–15% of the initial D1 protein has been lost. This degradation is enhanced by more than 2.5-fold following preillumination of the thylakoids. The corresponding formation of the 23-kDa primary degradation fragment follows the same pattern (Fig. 3B). The calculated Kd values of PNO8 on the basis of the level of induced 23-kDa fragment were 7.8 and 3.4 μM for the dark-adapted and preilluminated thylakoids, respectively. The actual D1 protein degradation induced by the 10-s preillumination was calculated from the difference between the two curves (Fig. 3B). The obtained Kd value of 2.0 μM indicates a 4-fold higher affinity of PNO8 to the PSI11 complexes following photoinduced changes of the D1 protein, thereby increasing the efficiency of degradation.

Nakajima et al. (16) reported suppression of the PNO8-induced D1 protein degradation and formation of the 23-kDa fragment by addition of excess DCMU in dark-incubated PSII complexes suggesting that the two compounds compete for the same binding niche at the QB site. The inhibition of PSII electron flow is saturated at 1 μM by both PNO8 and DCMU (16). However, our present results demonstrate that the concentration of PNO8 required to saturate the onset of D1 protein degradation in darkness is significantly higher than that required to inhibit electron flow. Thus, one should consider that the mode of binding of these two herbicides at the QB niche may show subtle differences. Only the binding of PNO8 can induce the conformation recognized by the protease as a substrate. It is plausible that within the QB binding niche different herbicides interact with different affinities to various amino acids, resulting in different conformations. We have investigated the DCMU competition with PNO8 by analyzing the prevention of the D1 proteolysis in dark-incubated as well as in preilluminated thylakoids in the presence of PNO8 (Fig. 4). Increasing the ratio of DCMU/PNO8 while using a PNO8 concentration of 5 or 10 μM progressively inhibited the formation of the 23-kDa D1 protein fragment. However, this inhibition was reduced in the preilluminated samples.

These results indicate that following preillumination a change in the conformation of the QB site occurs, leading to a higher affinity for the PNO8 relative to DCMU and thereby making the D1 protein more susceptible to the primary proteolytic cleavage. Based on these results as well as on previous data (20) demonstrating that the preillumination effect decays in darkness with slow kinetics, we suggest that the QB binding site may assume at least two different types of conformations that bind PNO8, with different affinity conferring different susceptibility of the D1 protein to the primary endogenous
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FIG. 3. Concentration dependence of PNO8-induced D1 protein degradation. Spinach thylakoids (0.1 mg of Chl/ml) were incubated with various concentrations of PNO8 in darkness for 60 min at 22° C (○). In parallel, thylakoids were illuminated for 10 s with 1,500 μmol of photons/m²/s light and subsequently incubated in darkness with the same PNO8 concentrations (△). The levels of the D1 protein (A) and the resulting 23-kDa degradation fragment (B) were analyzed by immunoblotting and plotted as a function of PNO8 concentration. The actual degradation induced by the 10-s preillumination was calculated from the difference between the two curves (△, dashed line).

FIG. 4. Effect of DCMU on the PNO8-induced D1 protein degradation. Spinach thylakoids (0.1 mg of Chl/ml), control (○, ●) and preilluminated for 10 s (△, ▲), were subsequently incubated in darkness for 60 min at 22° C with 5 μM (○, △) or 10 μM PNO8 (●, ▲) and increasing concentrations of DCMU corresponding to DCMU/PNO8 ratios of 1:1, 5:1, and 10:1. The level of the 23-kDa fragment of the D1 protein was plotted against the DCMU/PNO8 ratio.

protease. There is an unstable form (t½ about 1 h) (20) that is induced by short illumination and a more stable form that persists in darkness for a longer period of time (hours). The latter form has the cleavage site in the D-E loop less accessible to the protease. It is possible that the stable form is the product of the decay of the unstable one.

The question arises whether GTP enhances the D1 protein degradation induced by PNO8 in darkness without photoinactivation of PSII. To address this question, thylakoids were incubated in darkness for 90 min in the presence of 5 μM PNO8 and 0.2 mM GTP or GTPγS with or without 10 s of preillumination. Notably, the extent of D1 protein degradation was stimulated by addition of GTP in both types of samples (Fig. 5A). Addition of GTPγS inhibited the PNO8-induced degradation to a level below the dark control.

The stimulation by GTP of the PNO8-induced D1 protein loss was paralleled by enhanced accumulation of the 23-kDa fragment in the dark-adapted thylakoids but not in the preilluminated samples (Fig. 5B). The latter observation can be explained if one considers that preillumination may enhance the further proteolysis of this D1 fragment. The degradation of the 23-kDa fragment in photo inhibited thylakoids has been suggested to be mediated by an ATP-dependent FtsH-type metallopeptase (21). Addition of ATP and zinc ions did, however, not reduce the level of the PNO8-induced fragment in the dark-incubated thylakoids. In contrast, in the preilluminated samples, these effectors stimulated the degradation of the 23-kDa fragment by approximately 25% (Fig. 5B). The possibility that the secondary proteolytic cleavage is light-activated should, therefore, be considered. More likely, the lateral migration of the damaged PSII centers from the grana to the stroma regions, where the final step(s) of D1 protein degradation takes place (21) and where the FtsH protease is located (29), might be the specific light-dependent event in the degradation process of the 23-kDa fragment (see below).

FIG. 5. Effect of GTP, ATP, and zinc ions on the PNO8-induced D1 protein degradation. Spinach thylakoids (0.1 mg of Chl/ml) were incubated in darkness on ice for 30 min with various nucleotides. Aliquots were withdrawn and illuminated for 10 s (L). Subsequently, all samples were transferred to darkness at 22° C in the presence of 5 μM PNO8. A, the extent of D1 protein degradation after 90 min of incubation in the absence (open bars) and presence of 0.2 mM GTP or GTPγS, (black and gray bars, respectively). B, the level of the PNO8-induced 23-kDa D1 protein fragment in samples incubated for 90 min in darkness in the absence (−) or presence (+) of 0.2 mM GTP, 1 mM ATP and 0.5 mM zinc acetate.

PN08 Enhances D1 Protein Degradation in Photoinactivated PSII—The data presented so far indicate that PNO8 interacts with a modified form of PSII, thereby exposing the D-E loop of the D1 protein to the endogenous primary protease. The question arises whether this modified form can accumulate as a result of photoinactivation. Thylakoid membranes were exposed to high light at low temperature, thereby inducing acceptor side photoinactivation of PSII but preventing the subsequent degradation of the D1 protein (21, 30). The thylakoids were then further incubated in darkness at ambient temperature in the presence of GTP, and the degradation was allowed to occur in the absence or presence of PNO8 (Fig. 6).

In control thylakoids incubated in darkness for 90 min in the presence of PNO8, 15% of the D1 protein was lost (Fig. 6A). Preillumination for 10 s followed by transfer to darkness further increases the extent of degradation to about 25%. The effect of PNO8 on the degradation process in darkness following photoinactivation at low temperature for 45 and 90 min, respectively, are shown in Fig. 6, B and C. In the thylakoids illuminated for 45 min (75% active) (Fig. 6B), addition of PNO8 at the beginning of the dark period induced further loss of the D1 protein as compared with the control sample without PNO8. A 10-s illumination increased the effect only slightly. The same stimulation of D1 protein degradation by PNO8 was observed in the case of thylakoids photoinactivated for 90 min (50% active) (Fig. 6C). It should be noted that the enhancement of the D1 protein degradation by PNO8 in the photoinactivated thylakoids approaches the extent of loss in electron transport activity.

As mentioned above, photoinactivation of PSII also occurs in thylakoids exposed to low light or series of saturating single turnover flashes because of charge recombination between Qb...
and the $S^2$–$S^3$ states while the $Q_A$ acceptor is oxidized (10). Hence, the effect of PNO8 on D1 protein degradation was investigated in pea thylakoids exposed to a series of single turnover flashes (Fig. 7). The TL measurements revealed peak temperatures of $33 \pm 2$°C in the absence (●) and presence (●, ▲) of 5 μM PNO8. 10 min after the addition of PNO8, some samples were further illuminated for 10 s (▲). A, time course for D1 protein degradation (●, ▲, △) in dark-controls: B and C, time course for loss of oxygen evolution (●) and D1 protein degradation (●, ▲, △) in samples photoactivated (PI) for 45 and 90 min, respectively. The arrow indicates the onset of incubation of the photoactivated samples in darkness at 22° C.

PNO8 can induce the conformational change required for proteolytic cleavage. However, following flash exposure the degradation of the D1 protein in this PSII population does not lead to a comparable increase in the amount of 23-kDa fragment. This could be because of the duration (4 h) of the flash treatment that may allow a slow migration of the centers containing the cleaved D1 protein to the stroma domains where the fragments are further degraded.

**Subfractionation of Thylakoid Membrane for Localization of the PSII Centers Susceptible to PNO8-induced Cleavage of the D1 Protein**—Following high light-induced photoinactivation, the PSII core dissociates from the rest of the complex and migrates to the stroma thylakoid region (31–32), where the D1 protein is degraded and the remaining components can reassemble with newly synthesized precursor D1 protein (33). The question arises at what stage the primary cleavage of the D1 protein occurs and whether the sequence of events is the same under high light-induced photoinactivation of PSII and incubation of active PSII complexes with PNO8 in darkness. To answer this question we have subfractionated the thylakoids into grana, grana margins (intermediate), and stroma membrane domains. The thylakoid membranes were photoinactivated or incubated in darkness with or without preillumination in the presence of 10 μM PNO8 for 90 min at 22° C prior to subfractionation. The relative amount of the 23-kDa fragment was determined in intact thylakoids (Fig. 8A) and in the different membrane fractions (Fig. 8, B and C). The 23-kDa fragment generated following high light treatment was found preferentially in the stroma membrane domain irrespective of the presence or not of PNO8 during the illumination (Fig. 8C). In contrast, the 23-kDa fragment formed during incubation of the thylakoid membranes in darkness with PNO8 with or without a 10-s preillumination was localized mostly in the grana membrane domains. These results strongly indicate that the primary cleavage induced by photoinactivation and/or PNO8 in darkness occurs in PSII complexes located in the grana regions. The D1 fragments produced by the primary cleavage are still assembled with the PSII core complexes, in line with previous
observations (34). The subsequent lateral migration of the PSII cores to the stroma membrane regions where the secondary proteolysis takes place (21) requires prolonged illumination.

In conclusion, our results demonstrate several novel aspects concerning the process of D1 protein turnover: the heterogeneity of the PSII population with respect to the conformation of the D-E loop, as well as the modulation of the proteolytic activity by GTP, as summarized in Scheme 1. The binding of PNO8 to the Q$_{b}$ site induces degradation of the D1 protein and loss of PSII electron flow in darkness in a limited population of PSII centers, indicating that this population is not photoinhibited but does possess, however, a changed conformation of the cleavage site as a result of previous light exposure (Scheme 1, (PSII active)). This conformer of PSII is stable, and the D1 protein is not degraded unless PNO8 binds to the Q$_{b}$ site and further alters its conformation to expose the cleavage site to the protease. The light-induced alteration of these PSII centers is not “reversed” and thus, they may persist in the thylakoid membrane for a long time (>12 h). Further short illumination (10 s) may induce additional but reversible changes in the conformation of the acceptor side in this population of PSII centers (Scheme 1, (PSII active)). This conformer is not stable and reverses rather rapidly to the initial state (t$_{1/2}$ about 1 h) (16). The susceptibility of this conformer to PNO8-induced degradation is higher relative to that of DCMU (Fig. 4). Prolonged exposure to high light leads to PSII inactivation and may cause further changes in the cleavage domain. Some of these photoinactivated PSII population is still stable toward the endogenous protease activity. Binding of PNO8 to the Q$_{b}$ site of these conformers induces the D1 protein degradation (Scheme 1, (PSII inactive)). Additional illumination further alters the conformation of the PSII complex, exposing the D-E loop in the absence of PNO8 (Scheme 1, (PSII inactive)). However, irrespective of the mechanism whereby the cleavage site is exposed to the protease, GTP enhances the D1 protein degradation in all the above PSII conformer populations.

The stimulation of the D1 protein degradation by GTP may be because of: (i) direct interaction of GTP with the susceptible D-E loop of the D1 protein; (ii) activation by GTP of the proteolytic system responsible for the degradation of the D1 protein. We interpret the results presented here as evidence for the second possibility. In this case, one may consider that GTP may interact directly with the protease or indirectly by binding to a component remote from the cleavage site, thereby affecting the conformation of the PSII core so as to expose the D1 protein to the primary protease.

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