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Review

Photoinhibition of photosystem II under environmental stress

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Abstract

Inhibition of the activity of photosystem II (PSII) under strong light is referred to as photoinhibition. This phenomenon is due to an imbalance between the rate of photodamage to PSII and the rate of the repair of damaged PSII. In the "classical" scheme for the mechanism of photoinhibition, strong light induces the production of reactive oxygen species (ROS), which directly inactivate the photochemical reaction center of PSII. By contrast, in a new scheme, we propose that photodamage is initiated by the direct effect of light on the oxygen-evolving complex and that ROS inhibit the repair of photodamaged PSII by suppressing primarily the synthesis of proteins *de novo*. The activity of PSII is restricted by a variety of environmental stresses. The effects of environmental stress on damage to and repair of PSII can be examined separately and it appears that environmental stresses, with the exception of strong light, act primarily by inhibiting the repair of PSII. Studies have demonstrated that repair-inhibitory stresses include CO_2 limitation, moderate heat, high concentrations of NaCl, and low temperature, each of which suppresses the synthesis of proteins *de novo*, which is required for the repair of PSII. We postulate that most types of environmental stress inhibit the fixation of CO_2 with the resultant generation of ROS, which, in turn, inhibit protein synthesis.
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1. Introduction

Exposure of photosynthetic organisms to strong light results in inhibition of the activity of photosystem II (PSII) [1–4]. This phenomenon is referred to as photoinhibition. Since light energy is the driving force for photosynthesis, photoinhibition is unavoidable in photosynthetic organisms [5–7]. However, photosynthetic organisms are able to overcome the toxic effects of light via the rapid and efficient repair of PSII [2–4]. Therefore, the extent of photoinhibition depends on the balance between photodamage to PSII and the repair of such damage. To analyze the mechanism of photoinhibition in detail, it is essential to determine the rates of photodamage and repair separately. In previous studies, we established methods for the separate monitoring of photodamage to and repair of PSII *in vivo* in both cyanobacteria and plants [5,8,9]. Monitoring the process of photodamage exclusively requires total inhibition of the repair process, which can be achieved by exposing cells or plants to an inhibitor of protein synthesis, such as chloramphenicol or lincomycin. The repair of PSII can be monitored after exposure of cells to very strong light, which reduces the activity of PSII to 10 to 20% of its initial level, and subsequent exposure of cells to weak light [5,8,9].

In the previously accepted "classical" scheme for the molecular mechanism of photoinhibition, photodamage was interpreted as follows: photosynthetically active light produces reactive oxygen species (ROS) either by excessive reduction of Q_A, the primary electron acceptor of PSII [10], or by charge recombination between the acceptor side and the donor side of PSII [11]. The resultant ROS then attack the photochemical reaction center of PSII directly. However, in recent studies in plants and in the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*), various results were obtained that cannot be interpreted by reference to the classical scheme for the molecular mechanism of photoinhibition, as follows. The initial

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rate of photodamage to PSII, which was determined in the presence of inhibitors of protein synthesis [6,12–14] and by kinetic analysis [7,15,16], was exactly proportional to the intensity of the incident light. This proportionality was unaffected by ROS [14]. Furthermore, the action spectrum of photodamage to PSII was completely different from the absorption spectra of chlorophyll and carotenoids [17–20]. Rather, it resembled the absorption spectra of manganese compounds [19–21]. In addition, increases in intracellular levels of H_2O_2, as a result of application of exogenous H_2O_2 or inactivation of genes for H_2O_2-scavenging enzymes, stimulated the apparent photoinhibition of PSII by inhibiting the repair of PSII but not by accelerating photodamage to PSII [13]. Increases in intracellular levels of $^1O_2$, as a result of illumination of cells in the presence of rose bengal or ethyl eosin, also stimulated the apparent photoinhibition of PSII by inhibiting the repair of PSII but not by accelerating photodamage to PSII [14]. These observations cannot be explained by the classical scheme.

These findings led us to develop a new scheme for the molecular mechanism of photoinhibition. This new scheme allows us to interpret the above-mentioned and other previously reported results. Table 1 shows the differences between the classical and the new schemes for the molecular mechanism of photoinhibition of PSII. In the new scheme, photodamage occurs via a two-step process: the first step is the light-dependent destruction of the Mn cluster of the oxygen-evolving complex and the second step is the inactivation of the photochemical reaction center of PSII by light that has been absorbed by chlorophyll [19]. ROS increase the extent of photoinhibition by inhibiting the repair of PSII.

Among several steps in the repair of photodamaged PSII, the synthesis of the D1 protein, in particular, is markedly suppressed by elevated levels of intracellular H_2O_2 and $^1O_2$ in *Synechocystis* cells [12–14]. Moreover, among the several steps that lead to the synthesis of the D1 protein *de novo*, the translation of *psbA* mRNA and, in particular, the elongation of its product are specifically suppressed by H_2O_2 [13] and $^1O_2$ [14]. We can assume that, under oxidative conditions, ROS interrupt the normal redox signal(s) that would otherwise activate the translation of *psbA* mRNA. A recent biochemical study demonstrated that elongation factor 2 interacts with thioredoxin, suggesting that the reduction of disulfide bonds in elongation factors might be involved in the activation of translational elongation [22]. Excess ROS might interrupt such a reducing signal by oxidizing specific thiol-containing residues in elongation factors so that the translational elongation of the protein product of *psbA* mRNA is arrested.

A review of the new scheme for the mechanism of photoinhibition can be found in previously published reports by our group [23,24].

In natural environments, photosynthetic organisms are often exposed to unfavorable environmental conditions, such as high concentrations of NaCl and low and high temperatures. PSII is very sensitive to changes in the environment and, under unfavorable or stressful environmental conditions the activity of PSII declines more rapidly than many other physiological activities [3,4,25,26]. Initial studies directed toward an understanding of the mechanisms of the inhibition of PSII by environmental stress suggested that environmental stress might accelerate photodamage to PSII [17,27–30]; for reviews, see also [1,3,4,31–33]. However, recent studies demonstrated that the repair of PSII is much more sensitive to environmental stress than is the process of photodamage to PSII [5,12–14,34,35].

In this review, we summarize recent progress in studies of the mechanism of photoinhibition of PSII under environmental stress.

Table 1
Major differences between a newly proposed scheme and the previously accepted scheme for the mechanism of photoinhibition of photosystem II

	New scheme	Previous scheme
Action of ROS [a]	Inhibition of repair Inhibition of synthesis of proteins (in particular, the D1 protein)	Direct attack to inactivate RCII [a]
Photodamage	Two-step process: Step 1, Photodamage to OEC [a] (slow and rate-limiting) Step 2, Photodamage to RCII (fast)	One-step process: Photodamage to RCII
Effective light	Step 1, Light absorbed by the Mn cluster Step 2, Light absorbed by Chl	Light absorbed by Chl

[a] Abbreviations: ROS, reactive oxygen species; RCII, photochemical reaction center of photosystem II; OEC, oxygen-evolving complex.

2. Neither electron transport nor the synthesis of ATP affects photodamage to PSII

The effects of electron transport on photoinhibition have been studied with an inhibitor of electron transport in PSII, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU). The results have been controversial. Jegerschöld et al. [36] and Kirilovsky et al. [37] reported that DCMU did not affect the extent of photoinhibition in thylakoid membranes from spinach. In intact chloroplasts, in which both photodamage and repair occur, Krause and Behrend [38] and Barr et al. [39] found that DCMU appeared to accelerate photoinhibition of PSII (for review, see [40]). Komenda and Masojidek [41] reported that DCMU appeared to enhance the extent of photoinhibition in *Synechococcus* PCC 7942. By contrast, Kyle et al. [42] and Zer and Ohad [43] reported that DCMU retarded photoinhibition in *Chlamydomonas reinhardtii*. However, in the cited studies, the effects of DCMU were examined without separate measurements of the extents of photodamage and repair, perhaps explaining why the results differed among research groups and among the organisms examined.

Our group analyzed systematically the effects of the inhibition of electron transport and the synthesis of ATP on the rate of photodamage to PSII in *Synechocystis* using lincomycin [44]. We observed that the rate of photodamage was proportional to the intensity of incident light, as observed previously in leaves of higher plants [6,16]. We demonstrated that this proportionality was unaffected by the inhibition, by DCMU, of electron transport in PSII and by the acceleration, by *N*-methyl phenazonium methosulfate (PMS), of electron transport in PSI.

These findings suggested to us that the process of photodamage might not depend on the rate of electron transport.

We also examined the effects of the synthesis of ATP on photodamage in *Synechocystis* by monitoring such damage in the presence of lincomycin [44]. We found that the rate of photodamage, which was proportional to light intensity, was unaffected by the inhibition of ATP synthesis by *N,N*-dicyclohexylcarbodiimide (DCCD) or by a combination of nigericin and valinomycin (Nig/Val). All these findings suggested that the process of photodamage depended neither on the rate of electron transport nor on the intracellular level of ATP [44].

3. The synthesis of ATP is essential for repair

Several approaches have been used in attempts to characterize the roles of electron transport and the synthesis of ATP in the synthesis of the D1 protein. However, the results have been controversial. Mattoo et al. [45] suggested that both electron transport and the synthesis of ATP are important for the synthesis of D1 in *Spirodela oligorrhiza*. In *Chlamydomonas reinhardtii*, Trebitsh and Danon [46] showed that the redox signal associated with electron transport in PSI, as well as reduction of the plastoquinone pool, activated the initiation of translation of *psbA* transcripts. Studies in intact chloroplasts from spinach showed that the level of stromal ATP was correlated with the light-dependent synthesis of the D1 protein in intact chloroplasts [47]. Furthermore, Kuroda et al. [48] further demonstrated that electron transport via PSI was essential for the light-dependent translational elongation of the D1 protein. By contrast, Mühlbauer and Eichacker [49] suggested that a proton gradient, formed as a result of electron transport, might be important for the light-dependent translational elongation of the D1 protein in intact chloroplasts from barley. Thus, it seemed likely that electron transport and/or the synthesis of ATP might be required for the light-induced synthesis of the D1 protein. However, the direct effects of electron transport and the synthesis of ATP on the repair of PSII remained to be investigated.

We examined the effects of electron transport and the synthesis of ATP on the initial rate of repair of PSII in *Synechocystis* [44]. The rate of repair was diminished upon inhibition of the synthesis of ATP regardless of the type of electron transport involved, namely, that in PSI, which was accelerated by PMS, and that in PSII, which was inhibited by DCMU. It is likely that an adequate intracellular level of ATP is essential for repair [44]. Mühlbauer and Eichacker [49] reported that the stimulation by light of translational elongation depended on the formation of a proton gradient across the thylakoid membrane. However, our observation that DCCD abolished the repair of PSII suggested that it is the level of ATP, rather than a proton gradient, that is essential for the repair of PSII.

The repair of PSII involves several steps. We examined the effects of electron transport and the synthesis of ATP on the transcription of *psbA* genes in *Synechocystis* by Northern blotting analysis [44]. Our results demonstrated that either the presence of DCCD or that of Nig/Val depressed the level of *psbA* transcripts to some extent but did not completely eliminate them. By contrast, the presence of either DCCD or Nig/Val completely inhibited the synthesis of D1 and other proteins. These observations suggested that the translation of mRNAs might be the primary target of inhibition that results from a reduction in the intracellular level of ATP. Transcription is less sensitive to the level of ATP and is likely to be a secondary target.

Our findings in *Synechocystis* demonstrate that the generation of ATP is essential not only for the synthesis of the D1 protein *de novo* but also for the total repair of photodamaged PSII. It seems likely that the requirement for ATP reflects the energy required for operation of the translational machinery. The addition of each amino acid to a polypeptide chain during translation requires at least one molecule of ATP for aminoacylation of the cognate tRNA and two molecules of GTP for binding of aminoacyl-tRNA to the ribosome and the subsequent translocation of the peptidyl tRNA [50]. The tight correlation between the synthesis of ATP and the repair of PSII suggests that the supply of ATP might be the rate-limiting factor in the complete repair of photodamaged PSII.

4. CO_2-limitation stress inhibits the repair of PSII

Early observations suggested that the suppression of the fixation of CO_2 might enhance the extent of photoinhibition of PSII [51,52]. It was generally accepted, for many years, that suppression of the fixation of CO_2 accelerates photodamage to PSII via the excessive reduction of Q_A [31]. However, a recent study by our group demonstrated that, in *Chlamydomonas reinhardtii*, suppression of CO_2 fixation by application of exogenous glycolaldehyde, an inhibitor of phosphoribulokinase, or by a missense mutation in the gene for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) failed to accelerate photodamage to PSII but did inhibit the repair of photodamaged PSII [53]. The synthesis of D1 protein in intact chloroplasts from spinach leaves was also inhibited by a limited supply of CO_2 [54].

Rubisco catalyzes the production of glycerate-3-phosphate (3-PGA) from ribulose-1,5-bisphosphate and CO_2. 3-PGA is then converted to triosephosphates, such as glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, at the expense of NADPH and ATP, which are produced via the photosynthetic transport of electrons. Our recent study of intact chloroplasts from spinach revealed that an exogenous supply of 3-PGA abolished the inhibition of the synthesis of D1 protein by glycolaldehyde [54], suggesting that depletion of 3-PGA during the suppression of CO_2 fixation might inhibit protein synthesis. Depletion of 3-PGA decreases the utilization of NADPH in the Calvin cycle, with a resultant drop in the level of $NADP^+$. Since $NADP^+$ is a major acceptor of electrons in PSI, depletion of $NADP^+$ accelerates the reduction of O_2 to generate ROS [55]. In fact, interruption of CO_2 fixation enhances the production of H_2O_2 [56–59]. H_2O_2 inhibits the synthesis of proteins, in particular, the D1 protein, in chloroplasts [54] and cyanobacteria [13]. Inhibition of protein synthesis by suppression of CO_2 fixation might, therefore, be attributable to the generation of H_2O_2 [54]. This hypothesis is supported by the observation that the effects of glycolaldehyde on the photoinhibition of PSII

depend on the presence of molecular oxygen, which is the precursor to O_2^- that is converted to H_2O_2 [60].

5. Moderate heat stress inhibits the repair of PSII

Strong heat stress inactivates the oxygen-evolving complex in PSII directly [61]. However, moderate heat stress, which does not directly inactivate the oxygen-evolving complex, stimulates photoinhibition of PSII [25]. Recently, this phenomenon was demonstrated in a symbiotic alga in cnidarian host cells [35,62,63]. In *Symbiodinium* within corals, moderate heat stress-dependent photoinhibition was attributed to inhibition of the repair of photodamaged PSII and not to be acceleration of photodamage to PSII [35]. However, in *Symbiodinium* cells isolated from corals, moderate heat stress accelerated photodamage to PSII [62]. Therefore, it is likely that moderate heat stress induces both inhibition of the repair of PSII and photodamage to PSII in some strains of *Symbiodinium*. Moderate heat stress also increases the extent of photoinhibition in higher plants [64]. Separate measurements of photodamage and repair demonstrated that moderate heat stress inhibits the repair of photodamaged PSII but does not affect the extent of photodamage to PSII in tobacco leaves [64].

The fixation of CO_2 in the Calvin cycle is sensitive to moderate heat stress [65–69]. Although Rubisco from higher plants is stable under heat stress, Rubisco activase is unstable under moderate heat stress [68,70,71]. Moreover, moderate heat stress accelerates the production of H_2O_2 [63,64], inhibiting protein synthesis and the repair of PSII, as in the case of the CO_2 limitation-induced inhibition of the repair of PSII [53,54].

6. Salt stress inhibits the repair of PSII

Salt stress is an important environmental factor that limits the growth and productivity of plants [72]. In natural environments, salt stress often occurs in combination with light stress and there have been several studies of the effects of salt stress on PSII under strong light. Such studies suggest that salt stress might enhance photodamage to PSII in *Chlamydomonas reinhardtii* [73]; in leaves of barley (*Hordeum vulgare*) [74], sorghum (*Sorghum bicolor*) [74], and rye (*Secale cereale*) [75]; and in a cyanobacterium *Spirulina platensis* [76]. However, it is unclear in the cited reports whether salt stress accelerated photodamage to PSII or inhibited the repair of PSII.

We examined the effects of salt stress on photodamage and repair separately in *Synechocystis* [12,34,77]. Our result clearly demonstrated that salt stress, due to 0.5 M NaCl, inhibited the repair of photodamaged PSII but did not directly accelerate photodamage to PSII. Labeling of proteins in *Synechocystis* cells revealed that the synthesis *de novo* of the D1 protein was inhibited by the salt stress [12,34]. Moreover, salt stress suppressed not only the synthesis of the D1 protein *de novo* but also the synthesis of almost all other proteins. In an investigation of the reactions that lead to the synthesis of the D1 protein *de novo*, Northern and immunoblotting analyses revealed that salt stress, due to 0.5 M, suppressed the synthesis of the D1 protein at the translational level [34].

Although it seems clear that salt stress inhibits protein synthesis, the molecular mechanisms of such inhibition remain to be determined. Several possible mechanisms seem plausible, as follows. (1) The first possible mechanism is based on the finding that high concentrations of NaCl inactivate the translational machinery (or ribosomes) *in vitro* [78,79]. Therefore, an increase in the intracellular concentration of NaCl, due to the influx of NaCl into the cell [80], might inhibit protein synthesis directly. (2) Another possible mechanism is based on the finding that Rubisco from *Tamarix jordanis* is inactivated by the presence of a high concentration of NaCl [81]. Thus, it seems reasonable to postulate that the primary target of salt stress is Rubisco and that the inhibition of CO_2 fixation by salt stress induces the generation of ROS, which, in turn, inhibit protein synthesis. (3) However, it is also possible that an increase in the intracellular concentration of NaCl inactivates ATP synthase and decreases the intracellular level of ATP, which is essential for protein synthesis, as noted above [44].

7. Low-temperature stress inhibits the repair of PSII

Low-temperature stress also has a significant negative effect on the growth and productivity of plants [82]. Such stress apparently enhances photoinhibition of PSII under strong light [83,84]. We examined the effects of low-temperature stress on photodamage and repair separately in cyanobacteria [5,8,12] and in plants [9]. Our results demonstrated that low-temperature stress inhibits the repair of PSII but does not affect photodamage to PSII.

Labeling of proteins in *Synechocystis* cells demonstrated that the synthesis *de novo* of the D1 protein is suppressed at low temperatures [12]. The extent of suppression depends on temperature and it seems likely that low-temperature stress suppresses the synthesis of a variety of proteins *de novo* [12]. The step in the synthesis of the D1 protein that is suppressed by low temperature remains to be identified. Low-temperature stress also inhibits the processing of the precursor to the D1 protein that generates the mature D1 protein [85], which is necessary for the assembly of the active PSII complex. It remains to be determined whether the effects of low temperature on the synthesis and processing of proteins are direct or indirect via, for example, the production of ROS and/or a decrease in the intracellular level of ATP.

8. Protective effects of glycinebetaine against the stress-dependent inhibition of repair

Glycinebetaine (hereafter, betaine) is a compatible solute that accumulates in certain plants and microorganisms in response to various types of stress [86]. We transformed plants, namely, *Arabidopsis*, rice and tomato, with the *codA* gene for choline oxidase, which catalyses the synthesis of betaine from choline. The resultant transgenic plants synthesized and accumulated betaine and, in addition, they exhibited enhanced tolerance to various environmental stresses, such as high salt, high and low temperatures, and freezing [86–95].

Table 2
Categorization of environmental stresses according to their effects on the photoinhibition of photosystem II

Damage-inducing stress	Repair-inhibitory stress
Strong visible light	Salt stress
UV light	Low temperature
	CO_2 limitation
	Moderate heat (oxidative stress)

We also transformed the cyanobacterium *Synechococcus* sp. PCC7942 (hereafter, *Synechococcus*) with the *codA* gene. A line of transformed *Synechococcus* cells, which we designated PAMCOD, synthesized betaine *in vivo* from exogenously supplied choline and accumulated betaine at levels of 60–80 mM [96]. PAMCOD cells that are grown in the presence of 1 mM choline can continue to grow in the presence of 400 mM NaCl, whereas wild-type cells, which do not synthesize betaine from choline, cannot grow at all at this concentration of NaCl. PAMCOD cells exhibit similarly enhanced tolerance to low temperatures when supplied with exogenous choline. This enhanced tolerance to low temperature was ascribed to acceleration of the repair of photodamaged PSII and not to protection of PSII against photodamage [97].

We examined the effects of salt stress and the synthesis of betaine on the photoinhibition of PSII under salt stress [98]. Salt stress due to 220 mM NaCl enhanced the photoinhibition of PSII, while betaine, which had been synthesized *in vivo*, protected PSII against photoinhibition under these conditions. However, neither salt stress nor the synthesis of betaine affected the photodamage to PSII. By contrast, salt stress inhibited the repair of photodamaged PSII and betaine reversed this inhibitory effect of salt stress. Pulse-chase labeling experiments revealed that salt stress inhibited the synthesis of the D1 protein *de novo* and the degradation of D1 in photodamaged PSII. By contrast, betaine protected PSII against inhibition of the degradation and synthesis of the D1 protein under salt stress. Neither salt stress nor betaine affected levels of *psbA* transcripts. These observations suggested that betaine might counteract the inhibitory effects of salt stress, with resultant accelerated repair of photodamaged PSII.

Sodium chloride impedes the intermolecular association of protein subunits and it is possible that betaine might compete with NaCl to enhance such association. The integrity and activity of the translational machinery might be reduced by salt stress and enhanced by betaine. There is ample evidence that betaine stabilizes the conformation of proteins and, in particular, the quaternary structure of complex proteins. For example, betaine protects the oxygen-evolving complex of PSII from the NaCl- and heat-induced dissociation of the extrinsic proteins [99].

Yang et al. [64,100] examined the effects of moderate heat stress and the synthesis of glycinebetaine on photoinhibition in tobacco plants, which had been transformed to synthesize glycinebetaine *in vivo*. They found that moderate heat stress inhibited Rubisco activase and, as a result, limited the fixation of CO_2. These conditions accelerated the generation of ROS, which, in turn, inhibited the repair of PSII. It is likely that betaine protects Rubisco activase against moderate heat-induced inhibition. The mechanisms that protect the PSII-repair system against salt stress and mild heat stress seem to have some features in common.

9. Conclusion

Separate analysis of the details of photodamage to and repair of PSII has allowed us to develop a new scheme for the mechanism of photoinhibition [24]. As presented in Table 1, the main differences between our new scheme and the previously accepted “classical” scheme are related to the actions of ROS and the primary target of photodamage. In the new scheme, ROS inhibit the repair of PSII. The primary target of photodamage is the oxygen-evolving complex and, in particular, the Mn cluster; the secondary target is the photochemical reaction center. Our new scheme led us to analyze the effects of the photosynthetic transport of electrons and the synthesis of ATP on photoinhibition, as well as the relationships between the effects of various forms of environmental stress on photoinhibition.

There has been some controversy about the effects of electron transport, the proton gradient across the thylakoid membrane, and the synthesis of ATP on photoinhibition. Our systematic analysis, with separate measurements of photodamage and repair in combination with the use of suitable inhibitors [44], has clearly demonstrated that photodamage is affected neither by the rate of electron transport nor by the intracellular level of ATP. By contrast, repair of PSII does depend on the intracellular level of ATP.

Separate measurements of photodamage and repair have allowed us to analyze the mechanisms of action of various types

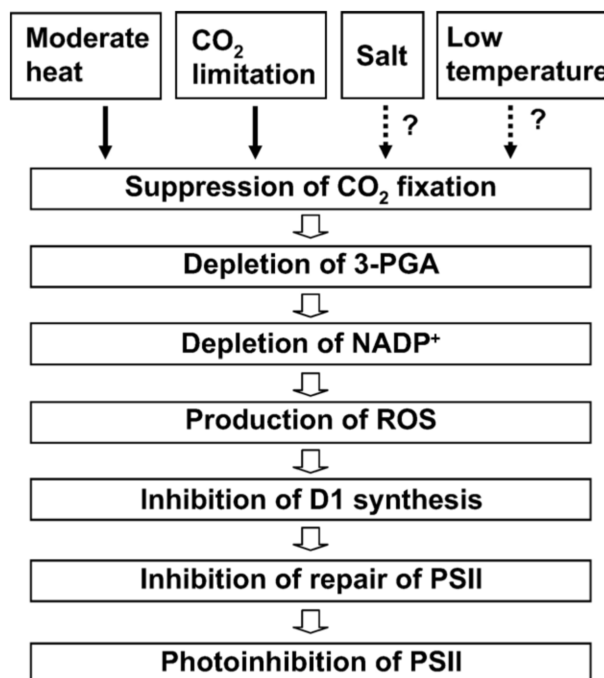

Fig. 1. A hypothetical scheme for the stress-enhanced photoinhibition of PSII. Solid and dashed lines indicate, respectively, steps that have been demonstrated and steps that require further examination.

of environmental stress and of the protective effect of betaine on photoinhibition. It is now clear that salt stress, low temperature, moderate heat, and CO_2 limitation all inhibit repair but have no effect on photodamage (with the exception that moderate heat might affect photodamage in some symbiotic algae in corals). It is possible to divide environmental stresses into two groups according to their effects on photoinhibition. Table 2 shows that damage-inducing stresses include strong visible light and UV light, whereas the repair-inhibiting stresses include salt stress, low temperature, CO_2 limitation, and moderate heat. Oxidative stress, which is generated by strong light and the presence of some herbicides, such as methyl viologen, inhibits the repair of PSII. Thus, strong visible light has two effects: a direct effect on photodamage and an inhibitory effect on repair via the production of ROS. There are many other types of environmental stress, such as osmotic stress, heavy-metal stress and nutrient limitation. Future studies will reveal how these various stresses affect photodamage and repair.

Are the effects of various types of stress on photoinhibition mediated by a common mechanism? If so, how might ROS be involved in such a mechanism? Fig. 1 shows a hypothetical scheme for the actions of various types of stress. As mentioned above, it is very likely that CO_2 limitation and moderate heat stress generate ROS by suppressing the fixation of CO_2 and decreasing levels of 3-PGA. These ROS, in turn, inhibit protein synthesis and, thus, the repair of PSII. It remains to be determined whether salt stress and low-temperature stress also inhibit the fixation of CO_2 as their primary target. In this context, it is of interest to note that these environmental stresses close stomata and suppress the fixation of CO_2 [101,102]. Thus, they might be expected to accelerate the production of ROS. Further studies are necessary for a full understanding of the mechanisms responsible for the effects of various kinds of environmental stresses on photoinhibition and photosynthesis.

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