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REVIEW

PHOTOINHIBITION OF PHOTOSYSTEM 1

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Summary. Till recently the term photoinhibition (PI) has been related to PS2 part of photosynthetic electron transport chain, in spite of some publications showing that PS1 can also be inhibited at definite conditions. At chilling temperature and low light intensity PS1 is selectively inhibited while PS2 remains practically unchanged. This review describes the nature of PS1 PI; the differences between PS1 and PS2 PI; PI of PS1 and chilling stress of photosynthesis; mechanisms of PS1 PI and their targets; reactive oxygen species as the primary cause of photoinhibitory injury of PS1; degradation of PsaB and PsaA gene products – the site of PS1 PI. In the conclusion some questions were put on to be answered in the near future.

Key word: chilling temperatures, low light, photoinhibition, photosystem 1 and photosystem 2, PsaB and PsaA gene products, reactive oxygen species.

Abbreviations: APX – ascorbate peroxidase; Chl – chlorophyll; DAD – diamino-diurene; ET – electron transport; FeS – iron-sulfur centres (clusters); PFD – photon flux density; PI – photoinhibition; PS1 and PS2 – photosystem 1 and photosystem 2; PSETC – photosynthetic electron transport chain; QY – quantum yield; TMs – thylakoid membranes; RC(s) – reaction centre(s); ROS – reactive oxygen species; SOD – superoxide dismutase.

Although light is an essential substrate for photosynthesis, strong illumination, often combined with other stresses, causes a decrease of photosynthetic activity. If the absorbed light energy which reaches reaction centre(s) (RCs) exceeds its consumption, the photosynthetic apparatus can be injured (Demmig-Adams and Adams, 1992; Horton et al.,1994). The term **photoinhibition** (PI) was defined originally by Kok

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(1956) as the decrease in photosynthetic activity that occured upon excess illumination. During the early stages of research into this phenomenon, inactivation of both PS2 and PS1 was reported (Satoh, 1970 a, b, c; Satoh and Fork, 1982). Inoue et al. (1986, 1989) investigated in spinach the site of PS1 complexes PI under both aerobic and anaerobic conditions. They observed that illumination of thylakoid membranes (TMs) with 30 000 lux under aerobic conditions decreased the photoreduction activity of $NADP^+$ to less than 10% of control, while 40% of iron-sulfur (FeS) centres remained intact. Nevertheless, until recently the term PI has been mainly used as a synonym for "PI of PS2" because D1 protein has long been considered the primary target for PI (Andersson and Styring, 1991; Barber and Andersson, 1992; Aro et al., 1993), and also because PS1 is more stable than PS2 under strong light (Powles, 1984). On the basis of sufficient experimental data accumulated so far, many authors have assumed PS2 as the site of photodamage (Powles, 1984). The suggestion was that this process proceeded only in this part of the photosynthetic electron transport chain (PSETC) and the injury included inhibition of PS2 and degradation of D1 protein – one of the two homologous subunits in hetherodimer, which comprised the PS2-RC complexes (Aro et al., 1993). The D1 protein, described by Ellis (1981) was first noticed as a rapid turnover protein in chloroplast. It was suggested that reactive oxygen species (ROS) (Miyato, 1994), or protease(s) (Andersson et al., 1994) took part in its degradation. Aro et al. (1993) proposed that the inhibition of PS2 acceptor side was first induced, then the destruction of RC occured, followed by the degradation of D1 subunits. This process was comlplished by a repair cycle. However, during last decade some publications appeared showing that at chilling temperature and low light intensity in some plants PS1 was more injured than PS2 (Terashima et al., 1994; Havaux and Davoud, 1994; Sonoike and Terashima, 1994; Tjus et al., 1998; Zak and Pakrasi, 2000). It was established that weak light (100–200 $\mu mol.m^{-2}.s^{-1}$) treatment of cucumber leaves at 4 °C for several hours caused a decrease in the relative quantum yield (QY) of electron transport (ET) in PS1 – from diamino-diurene (DAD) to $NADP^+$, with almost no inhibition of PS2 activity (Terashima et al., 1994).

Recognition of the PS1 PI in earlier studies

As was mentioned above, Satoh (1970 a, b, c) established that illumination of spinach TMs with saturated light inactivated both PS2 and PS1 activities. In contrast to the observations of Kok et al. (1965) the inactivation of PS1 showed a clear dependence on temperature: the higher was the temperature, the larger was the extent of inhibition.

Selective PS1 PI in isolated spinach TMs from plants grown at very low light intensity was observed by Sonoike (1995) in the presence of oxygen and electron flow from PS2. The recovery of selective PI of PS1 activity permitted to establish its physi-

ological significance in higher plants (Terashima et al., 1994; Havaux and Davaud, 1994; Sonoike and Terashima, 1994). The increased sensitivity of PS1 to light stress in isolated chloroplasts compared to the whole plant showed that the PS1 complex was well protected by mechanisms localized in the chloroplasts.

A question arises as to why this selective PS1 PI, if it is an intrinsic character of PS1, has not been noticed before? According to Sonoike (1998) the answer is: first, the PS2 activity could easily be estimated by analysing the fluorescence induction kinetics of intact tissues, while the measurements of PS1 activity is not so easy. PS1 PI is not accompanied by significant loss of total P700 itself and its chemical titration do not reveal any inhibition, when weak light is applied; the second and more important reason is that the PFD employed in past studies was non-physiologically strong, while PI of PS1 was induced by moderate light – 100–200 $\mu mol.m^{-2}.s^{-1}$ or less. Extremely strong illumination inhibits both PS1 and PS2 simultaneously (Sonoike and Terashima, 1994).

Huner et al. (1996) proposed that PI of PS2 was enhanced at chilling temperatures in chilling resistant plants through increased excitation pressure. Similar results were received also in chilling sensitive plants. Sonoike (1999) considered that PS1 was not the chilling sensitive site, even though it was the site of injury at light chilling treatment. PS1 PI in thylakoid membranes was suppressed by DCMU, an inhibitor of PS2, or methyl viologen (MV) an artificial electron acceptor of PS1. These results led to the suggestion that as in the case of PS2 the increased reduction power on the acceptor side of PSI was the cause for its PI.

Differences between PS1 and PS2 photoinhibition

The conditions which induce PS1 PI are completely different from these leading to PS2 PI. The later requires very strong light that often exceeds the whole sun light intensity. By contrast, PS1 PI can be induced by weak light at 4–5 °C for a few hours. As Tjus et al. (1998) have mentioned, it may occur *in vivo*, when the light intensity is insufficient to cause a direct PS2 PI. In addition, PS1 PI can not proceed in the absence of oxygen suggesting that in this process ROS are involved. As for PS2, singlet oxygen has been proposed to play an important role in the photoinhibitory process (Barber and Andersson, 1992) while, according to Hideg and Wass (1995) singlet oxygen is not produced in PS1. Apparently ROS, converted from superoxide, produced at the reducing side of PS1 play an important role in the process. Besides PS1 and PS2 respond differentially to H_2O_2 treatment in darkness: It was shown that PsaB, a component of PS1 RC, was not degraded in darkness while D1 protein of PS2 was cleaved by this treatment (Miyao et al., 1995). Since the extent of PS1 PI is determined mainly on temperature whereas in PS2 PI both temperature and PFD are of major importance, it could be concluded that PS1 is the main site of injury at chilling tempe-

rature and weak light. In contrast to D1 protein of PS2, which experiences rapid turnover in light (Mattoo et al., 1994), the turnover rate of the PsaB protein of PS1 is not so high and could not contribute to the recovery from PI as much as the D1 protein. Besides, the degree of PS2 PI in cucumber *in vivo* correlates with the redox state of Q_A, while PS1 PI at chilling temperature is not related, thus suggesting different mechanisms of the PI occuring in PS1 and PS2. It has also been shown that chilling treatment at weak light enhances the cyclic ET in PS1, which has similar to PS2 ET temperature dependence with a threshold temperature at 10°C.

Photoinhibition of PS1 and chilling stress of photosynthesis

Inhibition of photosynthesis under light/chilling stress has various aspects (Sonoike, 1998). At chilling temperatures plants suffer damage to photosynthesis. The sites and mechanisms involved in this damage differ under different chilling conditions. In his review Lyons (1973) pointed out three important features of chilling injury: i) chilling injury is a phenomenon that differs from freezing injury; ii) it develops further if the damaged tissue is transferred to non chilling conditions and iii) there is a critical temperature (usually 10–12 °C) below which injury becomes significant.

According to many earlier investigations stomata closure is the primary cause of net photosynthesis chilling damage (Drake and Salisbury, 1972; Pasternak and Wilson, 1972; Ku et al., 1977). Besides, the chilling damage of photosynthesis in chilling sensitive plants can also be explained by excitation pressure (Huner et al., 1996) and some treatment(s) (as drought, salinity) that reduce intercellular CO_2 concentration (C_i) through stomatal closure. Under such conditions the site of damage was found to be Rubisco and the ET between PS1 and PS2 (Ishibashi et al., 1996, 1997a, b) but not PS2 itself. Thus, such inhibition must be caused independently of excitation pressure – the relative redox state of Q_A. In chilling resistant plants (rye, wheat, spinach) strong correlation between the extent of PS2 PI and the redox state of Q_A was established (Huner et al., 1996). A single curvilinear relationship was obtained regardless of PFD and temperature, when excitation pressure parameter 1-qP was plotted against F_v/F_m in conditions of different photon flux density (PFD) and temperatures (Öquist et al., 1993). This result indicates that the determining factor for PS2 PI was not PFD or temperature, but the redox state of Q_A.

Hodgson et al. (1987) showed that the inhibition of photosynthesis began at the temperatures at which polar lipids of TMs undergo phase separation, i.e. the phase separation of the TM lipids may also be the target of chilling damage to photosynthesis (Murata et al., 1982, 1992; Nishida and Murata, 1996). They found a positive correlation between chilling sensitivity of plants and the level of the saturated phosphatidylglycerol in TMs. However, Moon et al. (1995) did not establish a specific threshold temperature for inhibition of photosynthesis in transgenic tobacco plants.

Sonoike (1998) assumed that lipids which specifically bind to some proteins or protein complexes may play an important role in PS1 PI and hence in the sharp decrease of photosynthetic activity at chilling temperature. A conclusion was made that the marked decrease in photosynthetic activity was a concequence of other factors, rather than membrane lipid changes. Therefore, even this very short review of the data available in literature supports the assertion of Sonoike (1998) that inhibition of photosynthesis under light/chilling stress has various aspects.

The photosynthetic activity decrease caused by chilling temperature strongly correlates with PS1 PI increase. Terashima et al. (1994) showed that when cucumber leaves were illuminated with moderate light of 220 μmol.m^{-2}.s^{-1} for 5 h at 4°C, the maximum QY of photosynthetic ET through PS1 decreased by 70–80%, while that through PS2 decreased by less than 20% (Fig. 1). Similar inhibition of PS1 was observed also in other plants – potato (chilling sensitive) (Havaux and Davaud, 1994), spinach and barley (chilling tolerant) (Sonoike, 1995; Tjus et al., 1998). The inhibition of photosynthesis strongly correlates with PS1 PI (Fig.1). A sharp decrease in photosynthesis below the specific threshold temperature, around 10°C, was observed also by Hodgson et al. (1987) and Hodgson and Raison (1989). They did not observe such a sudden decrease in photosynthesis rate in chilling resistant and in intermediate species such as maize (Long et al., 1983).

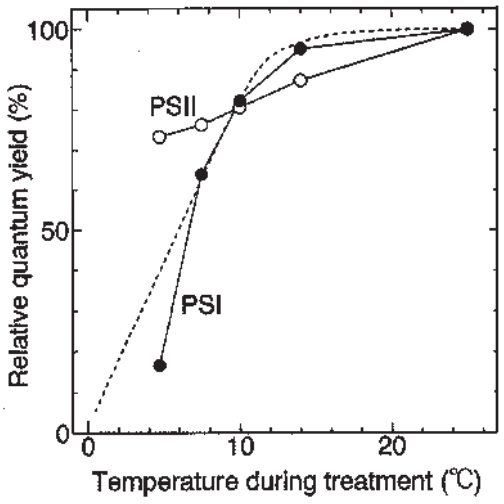

Fig. 1. The relative quantum yield of electron transfer through PS1 an PS2 (DAD to $NADP^+$ and H_2O to 2,6-dimethyl-*p*-quinone, respectively) in thylakoid membranes from photoinhibited cucumber leaves (closed circles and open circles, respectively). The broken line shows the dependence of photosynthetic activity on the temperature at which photoinhibitory treatments were applied (according to Terashima et al., 1994).

The relative QY of PS2 also decreased at chilling temperatures, but its dependence on temperature was almost linear, without a specific threshold temperature. Similar results were obtained in other chilling sensitive plants, when PI was monitored by Chl fluorescence. It is well known that at room temperature Chl fluorescence is emitted mainly by PS2 whose activity is inhibited by less than 10–20%. That is why, Sonoike (1996b) concluded that PS1, and not PS2, limits the overall photosynthesis activity after chilling treatment of cucumber leaves at weak light.

Despite chilling temperatures (0–10°C, 4–6 h), at low light intensity PS1 PI in leaves of higher plants needed oxygen as well as normal electron flow from PS2. One of the

unique properties of PS1 PI is that it proceeds at rather low PFD (less than 10% of that required for PS2 PI) and low temperature which induce critical unfavorable changes in PS1.

Targets of PS1 photoinhibition

There are many candidates for this role. One of them are Calvin cycle enzymes. It was proposed that the activity of PS1 was inhibited when CO_2 fixation strongly decreaseds at chilling temperatures. However, the sharp temperature dependency ($Q_{10} > 5$ between 4–8 °C) observed during PS1 PI, or inhibition of photosynthesis was difficult to explain by thermodynamic temperature dependency of CO_2 fixation activity, i.e. by thermodynamics of carbon metabolism enzymes (Sonoike, 1998). Besides, this author established that it was the oxidative inhibition, but not the thermodynamic decrease of the activity of some enzymes, that was induced by chilling temperatures. It was shown that the two regulating enzymes, fructose-1,6-bisphosphatase (FBPase) and sedoheptulose-1,7-bisphosphatase (SBPase), activated via a thioredoxin-mediated reduction of specific sulfhydryl groups, were inactivated after light chilling treatment of tomato leaves (Sassenrath et al., 1990). It is possible that feredoxin:thioredoxin oxidoreductase is the site of damage. Alternatively, the phenomenon may also be attributed to the damage to PS1. It is known that the reduced enzymes are always subjected to oxidation by oxygen which is mediated by thioredoxin (Scheibe, 1990). Since full activity was obtained when these enzymes were reduced with dithiothreitol (DTT), a conclusion was made that the enzymes were inactivated through oxidation. Therefore, the inactivation of these stromal enzymes can be alternatively explained by PS1 PI. They were subjected to oxidation by O_2 and the reducing power must be provided by PS1. Later Brügemann et al. (1992), Brügemann (1995) and Byrd et al. (1995) showed that Rubisco was also inactivated through oxidation upon light-chilling treatment.

The inactivation of Calvin cycle enzymes through oxidation can also be explained by an increased production of H_2O_2 due to the loss of thylakoid bound ascorbate peroxidase (APX). But according to Sonoike (1998) more plausible is the assumption that under normal conditions the reducing equivalents are supplied to the stromal enzymes by ET through PS1 and PS2 via the thioredoxin system. Thus, oxidation of these enzymes at chilling temperature can be explained as a consequence of PS1 PI, which is responsible for the generation of reducing equivalents (Terashima et al., 1994).

On the other hand, there are reports showing that PS1 is not the target site of PI. This conclusion is based on the fact that the rate of ET from ascorbate/dichlorphenolindophenol (DCPIP) to MV in thylakoids remains unchanged upon treatment of attached cucumber leaves with strong light (Critchley, 1981). Similar results were obtained with *Nerium oleander* (Powles and Björkman, 1982) and pumpkin (Tyystjärvi

et al., 1989). These authors conclude that the site of PI is PS2, even though Tyystjärvi et al. (1989) have mentioned that the decrease in the rate of photosynthesis of chilled pumpkin leaves in the light could not be fully explained by the decrease in PS2 activity. It is also known that PS1 PI *in vivo* might not be observed at room temperature and that at a high concentration MV can accept electrons from the partially destroyed PS1 (Fuji et al., 1990; Sonoike and Terashima, 1994), so that PS1 activity might have been overestimated in such measurements. In the experiments of Terashima et al. (1994), 70% of ET activity to MV was maintained in TMs from photoinhibited cucumber leaves in which the rate of $NADP^+$ photoreduction was only 20–30% of the control.

Mechanisms of PS1 photoinhibition and the role of reactive oxygen species

There are three steps of PS1 inactivation by PI: i) inactivation of its acceptor side; ii) destruction of the RC Chl of PS1 and iii) degradation of the polypeptides of Chl-bound subunits of PS1 RC. Later, the EPR and flash induced changes investigations have shown that iron-sulfur centres, electron acceptors in PS1 complex, are destroyed after photoinhibitory treatment (Sonoike et al., 1995). Such selective PS1 PI was induced in isolated TMs, as well as in intact thermosensitive and thermotolerant (spinach) plants under chilling and room temperatures. These observations clearly showed that PS1 PI in chloroplasts itself was an universal phenomenon and was not limited to chilling sensitive plants only. Selective PS1 PI was established also in other chilling tolerant plants – potato (Havaux and Davaud, 1994), barley (Tjus et al., 1998) and also in cyanobacterium *Synechocystis* sp. PCC 6803 (Zak and Pakrasi, 2000) at low temperatures. Therefore, the sensitivity to PI is an inward feature of PS1 *in vivo*. Obviously, in PS1 some mechanisms, frequently lost during TM isolation procedures, have been developed for protection against PI. The protective component which might be associated with TMs is supposed to be chilling sensitive factor in chilling sensitive plants and might be responsible for the strong dependence on temperature.

The most probable candidate for a chilling injury target of PS1 is the scavenging system of ROS. It was established that addition of n-propyl gallate, a scavenger of hydroxyl radicals ($OH^{\bullet}$), protected PS1 against PI (Sonoike, 1995). Moreover, the addition of H_2O_2 to TM suspension in the light enhanced PS1 PI although there was no inhibition in the dark (Sonoike et al., 1997). A hydroxyl radical which was produced by the reaction between H_2O_2 and light reduced FeS centres (Jakob and Heber, 1996) seemed to be the direct cause for the PS1 PI (Sonoike et al., 1997). These authors concluded that the most probable candidate for the chilling sensitive step was thylakoid-bound APX, which scavenged H_2O_2 and was located near the PS1 RC (Miyake and Asada, 1992). Obviously, this APX is the target that is injured in chilling-sensitive plants and maybe responsible for the strong dependence of PS1 activity on temperature. Sonoike (1996a, b) assumes that chilling treatment influences the interaction between APX and TMs, leding to enhanced H_2O_2 formation (see also Asada, 1994).

As was mentioned above, PS1 PI needs O_2 and ET from PS2 both *in vivo* (Terashima et al., 1994; Havaux and Davaud, 1994) and *in vitro* (Satoh, 1970b; Inoue et al., 1986; Sonoike, 1995). Asada (1994) has established that the major site of superoxide production in chloroplasts is the reducing side of PS1 and enzymes scavenging ROS – SOD and APX, are localized at/or near the PS1 RC (Miyake and Asada, 1992; Ogawa et al., 1995). A Cu-Zn SOD which converts superoxide to H_2O_2, is present at a high concentration in the chloroplast stroma, specifically around PS1 (Ogawa et al., 1995). H_2O_2 is reduced to H_2O by APX which is bound to the stromal thylakoids (Miyake and Asada, 1992). Therefore, most likely, the oxygen-scavenging enzymes in chloroplasts may serve to protect PS1 *in vivo*. It was shown that the generation of superoxide by xanthine/xanthine oxidase in darkness resulted in a decrease of FeS centres and PS1 activity in isolated TMs (Inoue et al., 1986). Later, Sonoike (1996a, b) demonstrated that the reagents scavenging $OH^{\bullet}$ – dimethyl-pyrolene-oxide and n-propyl gallate, suppressed PS1 PI. In the same publications it was reported that PI of PS1 was not induced when the FeS centres were oxidized by the addition of MV. It was speculated that produced $OH^{\cdot}$ reduced FeS centres in PS1 and immediately destroyed them once H_2O_2 escaped from the scavenging system of ROS. Therefore, the photoinhibition of PS1 can be considered as a series of phenomena.

According to Terashima et al. (1994) there are several views regarding the site of PI in PS1. Satoh and Fork (1982) suggested damage to the RC, but later Inoue et al. (1986) showed that the FeS centres sequentially arranged on the PS1acceptor side of PS1 were the sites of damage. At present, there are enough experimental data revealing that the acceptor side of PS1 is the primary site of damage and the extent of PS1 activity loss may potentially explain the extent of the irreversible inactivation of photosynthesis (Terashima et al., 1994; Sonoike and Terashima, 1994; Sonoike et al., 1997; Tjus et al., 1998).

The inactivation of the acceptor side of PS1 is belived to be the first step of its PI. Then the Chl of RC is distructed, i.e. at weak light in chilled leaves the P700 injury is preceded by the electron acceptors of PS1. According to Golbeck and Bryant (1991) the linear sequence of electron carriers is considered to be as follows: $P700 \rightarrow A_0 \rightarrow A_1 \rightarrow F_x \rightarrow F_A/F_B$ (see also Fig. 2) where A_0 is a Chl monomer, A_1 is phylloquinon (vit. K), F_x, F_A and F_B are FeS centres. The inactivation of electron transferring components leads to the degradation of Chl-bound subunits of PS1, when its acceptor side is fully reduced. It was established that charge recombination between a component on the acceptor side and P700 was inhibited by

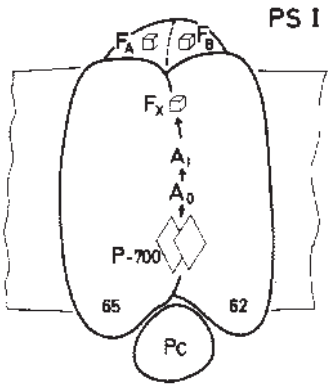

Fig. 2. Tentative presentation of the structure of PS1 reaction centre. The two large polipeptides are supposed to hold the primary donor P-700 and the first acceptors (according to Mathis and Rutherford, 1987).

the addition of MV (Sonoike and Terashima, 1994). Flash-induced absorption decay measurements revealed in barley progressive damage to (i) the FeS clusters F_A and F_B, (ii) the FeS clusters F_A, F_B and F_x and (iii) the phylloquinone A_1 and Chl A_0 or P700 of the PS1 electron acceptor chain (Tjus et al., 1998). Besides, the charge recombination between F_A/F_B^- and $P700^+$ as well as between F_x^- and $P700^+$ were inhibited by 1–3 μM and about 1 mM MV respectively. Sonoike and Terashima (1994) found that the light-induced absorption changes in the presence of 3 μM MV represented the amount of the PS1 fraction containing intact F_A/F_B clusters which donated electrons to the physiological electron acceptor ferredoxin. That is why the light-induced absorption changes in the presence of 3 μM MV are in a good correlation with relative QY for the ET (see Fig. 1). On the other hand, a very high concentration of MV was necessary to inhibit the triplet formation caused by the recombination between A_0^- or A_1^- and $P700^+$. The strong dependence of the absorption changes on increasing MV concentrations up to 10 μM observed in photoinhibited TMs, permitted Sonoike and Terashima (1994) to suggest that either A_1 or F_x was the site of this PI. They noted that the destruction of FeS centres rather than the double reduction of A_1 (observed under strong reducing conditions) could be the primary cause for damage to PS1 *in vivo*. The damage was suggested to be caused by superoxide and/or singlet oxygen produced in PS1. The authors speculated that the low temperature led to a decrease in the rate of CO_2 fixation resulting in the accumulation of reducing power on the acceptor side of PS1. It was also proposed that the chilling temperature introduced lesions into the lipid bilayer could inactivate the oxygen scavenging enzymes such as SOD (Terashima et al., 1994; Sonoike, 1995, 1998; Asada, 1994). Casano et al. (1997) showed in wheat that high levels of active oxygen, produced under severe stress conditions, directly inactivated and degraded chloroplastic SOD. When the acceptor side of PS1 is fully reduced, recombination between the radical pairs $P700^+/A_0^-$ or $P700^+/A_1^-$ can generate the triplet state of P700 (Shuvalov et al., 1986; Golbeck and Bryant, 1991). Chl triplets can react with molecular oxygen to create very toxic singlet oxygen that could cause photoinhibitory damage to PS1. It is interesting to note that MV which must increase the formation of superoxide and hence H_2O_2, protects PS1 rather than inactivates it. One plausible explanation is that MV oxidizes FeS centres which can be reduced during illumination, thus suppressing the formation of $OH^\bullet$ upon the interaction of H_2O_2 with reduced FeS centres (Sonoike et al. 1997). Jakob and Heber (1996) found that addition of MV decreased the rate of $OH^\cdot$ formation rather than enhanced it. On the other hans, it is suggest that the dependence of the extent of PS1 components inactivation on PFD reflects the succession of inactivation (Sonoike and Terashima 1994). Obviously, the inactivation of both FeS centres and P700 proceeds almost simultaneously at relatively strong light.

Degradation of PsaB and PsaA gene products – the site of PS photoinhibition

Convinsing evidence exists showing that FeS centres F_x, F_A and F_B are the site of inactivation in the reducing side of PS1 (Sonoike et al., 1995, 1996a; Tjus et al., 1998). Measurements by EPR showed the destruction of FeS centres in parallel with the loss of QY of ET from DAD to $NADP^+$ (Sonoike et al., 1995). Flash photolysis established the increase in the triplet states of P700 and antenna pigments along with a decrease of ET from P700 to F_A/F_B. This indicated an increase in charge recombination between $P700^+$ and A_0^-. The authors concluded that weak light treatment of cucumber leaves at chilling temperature can destroy F_x, F_A and F_B and possibly F_1. Specific degradation of PsaB gene product, one of the two large subunits of PS1 RC, was observed during PI of PS1 (Sonoike, 1996a). In *in vitro* photoinhibited spinach TMs, the degradation of PsaB gene product gave rise to 51 kDa and 45 kDa fragments. When n-propyl gallate, a scavenger of ROS, was added, to isolated TMs a suppression of the generation of these fragments was established. The addition of MV protecting PS1 from PI but increasing the production of H_2O_2 also suppressed the generation of 51 kDa polypeptid fragment, however it increased the formation of 45 kDa fragment (Sonoike, 1996a). A conclusion was made that the interaction of ROS with reduced electron acceptors in PS1 resulted in a PS1 inactivation and generation of 51 kDa fragments, whereas the 45 kDa fragment was generated solely by the presence of ROS independent of PS1 activity inhibition. This conclusion was illustrated by Sonoike (1996a) by the following experimental data. He checked the extent of PS1 PI in isolated spinach TM in the presence of several reagents and established that: addition of glucose oxidase and glucose completely suppressed the inhibition, suggesting an important role of oxygen in PS1 PI; addition of DCMU, an inhibitor of PS2, also completely protected PS1 from PI, resulting from a decrease of ET to PS1 rather than from a lowered oxygen concentration, due to the inhibition of PS2 oxygen evolution. It is known that dibromo-methyl-isopropyl-benzoquinon (DBMIB) and dodecyl maltoside (DM) also protect PS1 from PI. DBMIB inhibits ET at b_6/f complex, while DM inhibits ET at plastoquinone and plastocianin through the destruction of membrane structure; MV also suppresses the inhibition, suggesting that overreduction of the acceptor side of PS1 may be one of the causes for its PI.

As was mentioned above the RC complexes of PSI are also heterodimers as PS2 and consist of two homologous subunits – PsaA and PsaB gene products. Similar to PS2 PI, the degradation of the subunit(s) is caused by photoinhibitory treatment and this degradation is inhibited under anaerobic conditions in which PS1 activity is not inhibited. Interesting is also that both PS1 and PS2 PI proceed in several steps. The component(s) in PS1 acceptor side, between A_0 and F_A/F_B seems to be inactivated by weak illumination and chilling temperatures at first, and then the destruction of P700

and degradation of chlorophyll-binding large subunits (PsaA/B gene products) seem to follow (Sonoike and Terashima, 1994; Sonoike et al., 1995; Sonoike, 1996a, b). Light-induced PS1 damage in barley was also evidenced by partial degradation of PS1-A and PS1-B proteins and correlated with the appearance of smaller proteins. When the ET rate was saturated with respect to light, most of the Q_A was reduced. Given that the electron acceptors like $NADP^+$ were scarce at low temperature, the components of PSETC including FeS centres in PS1 had to be reduced. Then the presence of the H_2O_2 and the reduced FeS centres leads to production of $OH^\bullet$ which destroyed the FeS centres in PS1. It is suggested that $OH^\bullet$, produced through the Fenton reaction, are involved in PS1 PI *in vivo* in cucumber leaves. A protective role of the scavenging ROS system in PS1 has been previously proposed (see reviews of Asada, 1994, 1999 and Fig. 3). There are thylakoid and stromal scavenging systems which include CuZn-SOD, APX, MDAR, DHAR, GR and FNR.

It is well known that Cu-ZnSOD and APX are effective scavengers of ROS produced during PS1 PI. Both enzymes are inhibited by CN. In order to test the importance of these two oxygen-scavenging systems in the protection against light stress, Tjus et al. (1998) infiltrated barley leaves with 10 mM KCN prior to illumination. Aggravated photodamage was observed upon illumination of these leaves, which inhibited Cu-ZnSOD and APX. This indicates that the photodamage of PS1 in barley observed during low light illumination at chilling temperatures arises because the defence against ROS by active scavenging enzymes is insufficient under these specific conditions. The authors stress that PS1 PI at chilling temperatures is an important phenomenon in cold-tolerant plant species.

It was proposed that both PS1 PI and PsaB protein degradation were caused by ROS since n-propyl gallate, a scavenger of $OH^\bullet$, exerted a protective effect (Sonoike, 1996b; Sonoike et al., 1997). However, in this case the quantity of the degradation product was very low, even when more than 50% of the activity of RC was suppressed. On the basis of these results it was assumed that similar to PS2 PI, the subunits PsaB and PsaA degradation in PS1 was a consequence of electron transfer inactivation. The same conclusion was made by Tjus at al. (1998) mentioning that protein degradation of PS1 subunits was not the primary effect of photodamage in PS1 but it followed rapidly the initial damage to the ET cofactors.

The PsaB gene product was specifically degraded by illumination of spinach TMs leading to formation of N-terminal fragments of Mr 51 and 45 kDa (Sonoike et al., 1997). The authors established that the formation of the 51 kDa fragment was: i) partially suppressed by the addition of phenylmethylsulfonyl fluoride (PMSF) or 3.4-dichloroisocoumarin, which are inhibitors of serine proteases and ii) enhanced in the presence of H_2O_2 during photoinhibitory treatment, but iii) not detected following H_2O_2 treatment in the dark. They proposed the following mechanism for the formation of the 51 kDa fragment. First, superoxide is formed at the reducing side of PS1

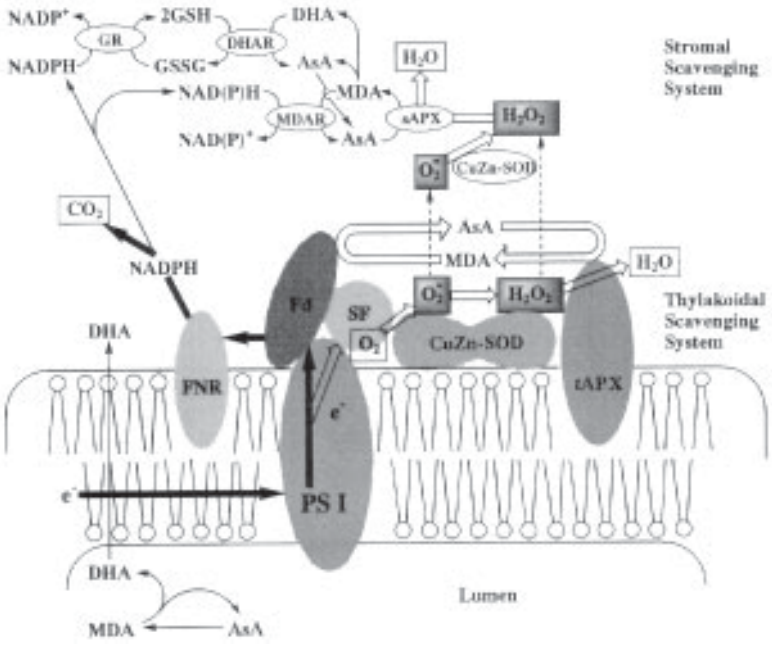

Fig. 3. Molecular mechanism of the photoreducing system of dioxygen to water in the water-water cycle and microcompartmentalization of the participating enzymes. O_2^- is photoproduced directly in the PS1 complex or indirectly mediated by a stromal factor (SF). A possible candidate of SP is monodehydroascorbate reductase (MDAR). The thylakoidal scavenging system is composed of CuZn-superoxide dismutase (SOD) attached on the thylakoids (in several plants, Fe-SOD), thylakoid-bound APX (t-APX), and ferredoxin (Fd). Fd reduces MDA directly to ascorbate (AsA). The stromal scavenging system is composed of CuZn-SOD localized in the stroma, stromal APX (sAPX), MDAR, dehydroascorbate reductase (DHAR), and glutathione reductase (GR). NAD(P)H for the reduction of either monodehydroascorbate (MDA) or dehydroascorbate (DHA) is photo-generated via ferredoxin-$NADP^+$ oxidoreductase (FNR). MDA is generated also in the lumen in the reaction of violaxanthin deepoxidase, and when AsA donates electrons to PS2 or PS1. MDA in the lumen is rapidly disproportionated, and DHA in the lumen penetrates through the thylakoid membranes and is reduced to AsA by the stromal scavenging system (according to Asada, 1999).

and converted to H_2O_2 by SOD. Then a hydroxyl radical is formed by the reaction of H_2O_2 with the reduced FeS centre of PS1. The destruction of FeS centres disturbs the structural conformation around the ligands for F_x accessible to a protease. The OH^- produced at the reduced FeS centres in the PS1 triggers the conformational changes of the PS1 complex, which allows an access of serine-type protease to PsaB. On the other hand, the formation of the 45 kDa fragment, which was changed in the presence of MV but did not accompany the PI of PS1, was not affected by the addition of H_2O_2 or protease inhibitors.

Aro et al. (1993) established that when photoinhibitory illumination was combined with chilling temperature, a degradation of D1 protein of PS2 was not observed, regardless of a significant inhibition of the electron transfer. Conversely, degradation of PS1 protein was observed in the dark, when the photoinhibited TMs were incubated at room temperature. It was also shown that the degradation of PsaB and PsaA subunits of PS1 could not be directly connected with the reparatory cycle of RC because their turnover was no so fast as that of the D1 protein, characterized by extremely high turnover (Ellis, 1981).

It was also suggested that the inactivation of PS2 can suppress the PS1 inactivation since PS1 PI was completely suppressed by the addition of DCMU, which blocked ET from PS2 to PS1 (Sonoike, 1995; Tjus et al., 1998). Under these conditions P700 will be oxidized into $P700^+$, which is able to dissipate excess excitation energy as heat (Nuijs et al., 1986) and thereby to effectively quench deleterious effects, otherwise caused by excess light. Accordingly, it would be predicted that PS1 PI *in vivo* may occur when the light intensity is sufficiently low not to cause a direct PI of PS2. Finally, inactivation of PS1, however, will inevitably cause overreduction of the acceptor side of PS2 and, consequently, will induce the damage of PS2. As was mentioned above, weak illumination is essential for the selective PI of PS1. Stronger illumination induces PI of PS2 and thus might protect PS1. However, very strong light induces inactivation of PS1 independently of PS2, so that both photosystems may be reduced once PS1 is photoinhibited. Under such conditions, PS2 is subjected to danger of "acceptor side PI" and the light intensity necessary for PI of PS2 may become weaker than is usually needed. Hence, PI of PS2 protects PS1 from PI while inhibition of PS1 might enhance the PS2 PI.

On the basis of the information available, we can offer the following schematic presentation of the processes occurring during PS1 PI (Fig. 4). A combination of chilling temperature and low light decreases the rate of reducing power utilization by Calvin enzymes and an overreduction of acceptor side of PS1 takes place (2). Low temperature inhibits also Rubisco activity (1). Under chilling temperature and low light the capacity of defence (scavenging) ROS systems is diminished (3). Such situation favours recombination of separated charges in PS1 RC between $P700^+$ and A_0^- or A_1^- and formation of extremely toxic single chlorophyll (^{1}Chl) from triplet chlorophyll (^{T}Chl) (4, 5). The interaction between ferredoxin and molecular oxygen (Mehler reaction) leads to superoxide anion radical (O_2^-) and H_2O_2 formation (6). The interaction of H_2O_2 with reduced FeS clusters (Fenton reaction) results in hydroxyl radical (OH^-) formation which destroys FeS clusters (7, 8). Inactive FeS clusters (FeS_{in}) induce conformational changes of PS1 core complex proteins, facilitating its access to proteases (9, 10); then a degradation of PsaB and PsaA gene products and release of 51 and 45 kDa proteins proceeds. The processes 8 (destruction of FeS clusters) and 10 (degradation of PsaB and PsaA subunits) are an indication of PS1 PI.

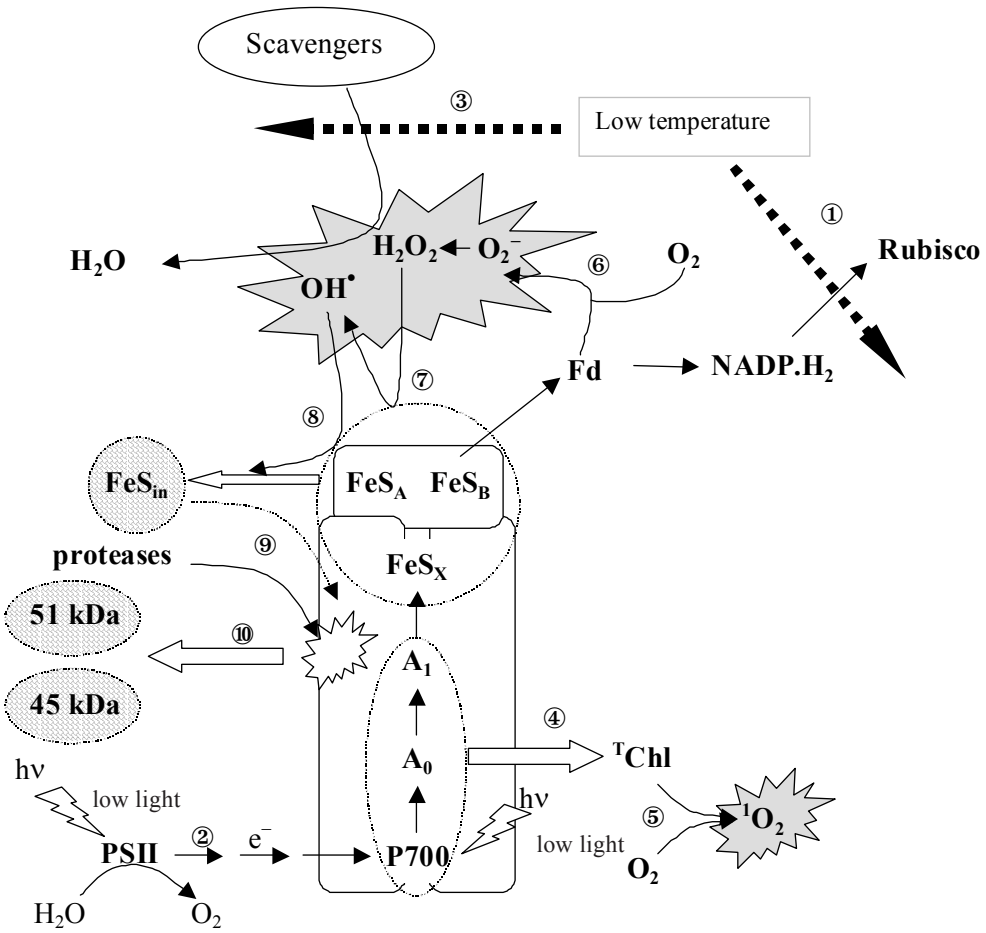

Fig. 4. Schematic presentation of processes during of PSI photoinhibition.
Processes:

1. Decreased rate of reducing power utilization by Calvin cycle enzymes (Rubisco) at low temperature;
2. Photoinduced electron transfer from PS2 and reduction of PS1 electron acceptors (FeS centres, Fd, NADP);
3. Cold-induced diminish of oxidative defense system (tAPX, sAPX etc.) capacity;
4. Recombination of separated charges in PS1 reaction centres between $P700^+$ and A_0^- or A_1^- and Chl triplet formation;
5. Energy migration from ^TChl to O_2 and production of singlet oxygen $^1O_2$;
6. Superoxide anion radical and H_2O_2 production in Mehler reaction;
7. Fenton reaction ($OH^•$ formation as result of interaction of H_2O_2 with reduced FeS-clusters);
8. Destruction of FeS-clusters by $OH^•$;
9. Inactive FeS-clusters (FeS_{in}) induce the conformational changes of PS1 core complex proteins facilitating its access for proteases;
10. Degradation of PsaB and PsaA gene products and release of 45 kDa and 51 kDa proteins;
11. Processes (8) and (10) result PS1 photoinhibition.

Proposed mechanisms for protection of PS1 from photoinchibition

Some mechanisms for PS1 protection from PI as a mode of excessive light energy dissipation have been proposed: so-called Asada pathway, the functional dissociation of LHC from PS2 core complex by means of protein phosphorylation, the xanthophyll cycle, the cyclic electron flow around PS1, a down regulation of PS2 activity by means of proton gradient created in TMs, and even by D1 protein degradation. Most likely, the Asada pathway is the most important. When ROS are scavenged in TMs or in the stroma and electrons flow from H_2O to H_2O (Asada pathway, 1994, 1999), oxygen accepts electrons from PS1 and produces superoxide, which is scavenged by SOD and APX being converted to H_2O in the reaction involved reduced Fd, with no net change in oxygen and electrons. This pathway functions not only to weaken PS1 overrerduction, but also to generate a pH gradient regulating the electron flow from PS2 to PS1 (Schreiber et al., 1995). Except for Asada pathway, the xanthophyll cycle can play also a definite role as a protective mechanism. Its components were found in PS1 fraction (Lee and Thornber, 1995). The cyclic electron flow around PS1 can dissipate the light energy absorbed by PS1, but electron flow from PS2 is not enough diminished. Hence, the reduction of PS2 activity by means of a down regulation is needed in order to protect PS1 from PI. The degradation of D1 protein and PS2 inactivation could also be considered as a mechanism for PS1 protection from PI which is more dangerous for plants.

At the end of 1998 and in the beginning of 1999 some publications (Barth and Krause, 1998, 1999) appeared, demostrating that the preferential PS1 PI in cucumber was not a general phenomenon in chilling-sensitive plants. These authors investigated the responses of PS1 and PS2 to light stress at 4°C and 20°C in leaf discs from three chilling-sensitive plant species, *Cucumber sativus, Cucurbita maxima* and *Nicotiana tabacum* and in the chilling-tolerant *Spinacea oleracea* (Barth and Krause, 1998, 1999). The P700 absorption change around 820 nm served as a relative measure of PS1 activity. It was found that in cucurbita, tobacco and spinach PS1 was not at all or only slightly inhibited by 2 h illumination with 200 $\mu mol.m^{-2}.s^{-1}$ at 4°C, or with 2000 $\mu mol.m^{-2}.s^{-1}$ at 20°C. In leaves of cucurbita and tobacco, the exposure at 2000 $\mu mol.m^{-2}.s^{-1}$ and 4°C resulted in a decline in both PS1 activity and potential PS2 efficiency almost to the same extent – 50% within 2 h. By contrast, in cucumber both moderate and high light combined with low temperature caused PS1 PI that proceeded considerably faster than the decline in PS2 efficiency. Such preferential PI of PS1 was not observed in the other three species tested. According to the authors the lower susceptibility of spinach PS1 and PS2 PI at 4°C was associated with a faster deepoxidation kinetics of violaxanthine (Vx) as compared to the three chilling-sensitive species. A conclusion was made that in the species tested despite PS2, PS1 might also be inhibited *in vivo* under light stress, but the preferential PI of PS1 was not a general phenomenon in chilling-sensitive plants. At low temperatures, a reduced functional activity of the xan-

thophyll cycle as well as of the antioxidative scavenging system may account for the enhanced PS1 and PS2 inhibition *in vivo*. Until now it is not known whether xanthophyll cycle which operates in PS1may contribute to its protection. According to Barth and Krause (1999) the factors leading to PS1 PI are not limited to low temperatures at moderate light. Most probably, at 4°C in all plants tested, PS1 is affected by high light to a great extent than by low light intensity. It is also possible at strong light a fraction of reduced FeS centres to be responsible for a production of $OH^{\bullet}$ from H_2O_2. In general, the high activity of the systems scavenging ROS is important for the protection of photosynthetic apparatus from light stress at suboptimal and superoptimal temperatures. Now it is very difficult to say who is right – the authors considering the preferential PS1 PI in cucumber as a general phenomenon in chilling-sensitive plants or those contending that not only in chilling-sensitive but also in chilling-tolerant plants PS1 PI can proceed under chilling temperature and low light. In support of the second conclusion experimental data obtained with spinach and barley isolated TMs (Sonoike et al., 1997; Tjus et al., 1998), potato leaves (Havaux and Davaud, 1994) have been reported. Recently, a new paper of Zak and Pakrasi (2000) has appeared showing a specific inhibition of PS1 under low temperature conditions in the cyanobacterium *Synechocystis* sp. strain PCC 6803. It was shown that the growth of this bacterium at 20°C (the optimal temperature was 30°C) caused an inhibition of PS1 activity and an increased degradation of the PS1 RC proteins PsaA and PsaB, while no significant changes were found in the level and activity of PS2. BtpA (a PS1-complex specific regulatory extrinsic TM protein in *Synechocystis* PCC 6803) was found to be a necessary regulatory factor for the stabilization of PsaA and PsaB proteins under such low temperature conditions (Bartesevich and Pakrasi, 1997). At normal growth temperature (30°C) the BtpA protein was present in the cell and its deletion caused an increase in the degradation rate of the PS1 RC proteins. However, the growth of *Synechocystis* cells at 20°C or shifting of cultures from 30°C to 20°C led to the rapid accumulation of the BtpA protein, presumably to stabilize the PS1 complex by lowering the rates of degradation of the PsaA and PsaB proteins. It was also shown that BtpA deletion mutant strain could not grow photoautotrophically at low temperature and exhibited a rapid degradation of the PS1 complex after transfer of cells from normal to low temperature. Therefore, it is clear that in order to answer indisputably the question put on above, more experimental data based on different species and families of plants are needed.

Conclusion

In conclusion we would like to note that there are many experimental data supporting the presence of PS1 PI in chilling-sensitive and some chilling-tolerant plants un-

der low temperature and weak light. The molecular basis of this phenomenon has also been established to a greater extent. As Sonoike (1998) has noted the most important question in the future is how the chilling sensitivity of plants can be determined. Another important task is to answer the question which type(s) from the many ones of damages is of importance and which is not.

A very important problem is also to accumulate enough experimental data, using different informative methods on this subject from different plant species and families in order to answer the question whether the PS1 PI is an universal phenomenon which proceeds in chilling-sensitive and chilling-tolerant plants, or it is specific only for some chilling-sensitive species under low temperature and weak light. Furthermore, the question about the physiological significance of PS1 PI must be better answered.

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