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Positive Charges of Polyamines Protect PSII in Isolated Thylakoid Membranes During Photoinhibitory Conditions

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The effects of the positive charges of amines such as spermine (SPM), putrescine (PUT) and methylamine (MET) on the protection of PSII against excessive illumination were investigated in isolated thylakoid membranes. Under photoinhibition conditions, water oxidation, the kinetics of the Chl fluorescence rise and charge recombination in PSII were affected. A low concentration of SPM (1 mM) added before photoinhibition produced a significant improvement of F_v/F_0, the oxygen yield and the amplitude of the B-band of thermoluminescence compared with the other amines. Amongst the amines studied, only SPM could protect the photosynthetic apparatus under photoinhibition conditions. This protection was probably provided by the polycationic nature of SPM (four positive charges at physiological pH), which can stabilize surface-exposed proteins of PSII through electrostatic interaction.

Keywords: Photoinhibition • Polyamines • Thylakoid membranes.

Abbreviations: D1 and D2, heterodimeric core of PSII; DA, decylamine; DD, 1,10-diaminodecane; FI, chlorophyll fluorescence induction; F_m, maximal level of Chl fluorescence; F_v, variable Chl fluorescence; F_o, basal level of Chl fluorescence; LHC, light-harvesting complex; MET, methylamine; MSP, manganese-stabilizing protein; OEC, oxygen-evolving complex; Pheo, pheophytin; PI, photoinhibited sample; PQ, plastoquinone; P680, primary electron donor of PSII; PUT, putrescine; Q_A and Q_B, primary and secondary quinone acceptors of PSII; RC, reaction center; ROS, reactive oxygen species; SPD, spermidine; SPM, spermine; TL, thermoluminescence.

Introduction

PSII is a membrane-bound protein complex that contains Chl, plastoquinone (PQ), manganese (Mn) and several other bound cofactors, and catalyzes the light-driven oxidation of water and the reduction of PQ in cyanobacteria and plants (Nanba and Satoh 1987). Water oxidation occurs at an Mn cluster located on the luminal side of PSII (Sproviero et al. 2008). The photochemical events are initiated by the capture of incident photons by the antenna complexes. The absorbed energy is quickly transferred to the photochemical reaction centers (RCs), located on the D1 protein, where the excited singlet states of the special Chl *a*, P680, reduces a pheophytin (Pheo) molecule. Stabilization of the charge-separated state occurs with the electron transfer from $Pheo^-$ to Q_A, the primary quinone of PSII, forming $P680^+Q_A^-$. The $P680^+$ radical oxidizes tyrosine Y_Z (Tyr161 of D1). The latter is reduced by electrons originating from the Mn cluster. At this site, water oxidation is performed through the so-called S-state cycle, $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow (S_4) \rightarrow S_0$, requiring four successive quanta of excitation. Concurrent with the release of dioxygen from water, the S_4 state decays to the S_0 state after the fourth step. At the acceptor side, Q_A^- reduces the secondary quinone, Q_B, in a two-step process leading to the formation of plastoquinol (PQH_2).

PSII is considered as the photosystem most sensitive to excessive illumination and is primarily affected by photoinhibition (Powles 1984). This usually occurs when photosynthetic organisms are exposed to high light intensity, causing the imbalance between the rate of absorption of light energy by photosynthetic pigments and the rate of consumption of CO_2 in the chloroplast (Powles 1984, Adams and Adams 1992, Melis 1999). However, the molecular mechanism of photoinhibition of PSII has been the subject of controversy. Some investigations suggested the acceptor side as the predominant initial photoinactivation site. In fact, under high light intensity the PQ pool remains in the fully reduced state, blocking the electron flow between Q_A and Q_B. Consequently, the yield of recombination of the primary radical pair $P680^+Pheo^-$ increases, leading to the formation of Chl triplet states and generation of singlet oxygen molecules ($^1O_2$) which are responsible for photodamage of the D1 protein of PSII (Mathis et al. 1989, Styring et al. 1990, Vass et al. 1992, Tyystjarvi 2008). However, several studies have considered the donor side as the primary target of photoinhibition (Callahan et al. 1986, Jegerschold et al. 1990, Jegerschold et al. 1991, Murata et al. 2007). This condition is produced when the oxygen-evolving complex (OEC) is partially or fully inactivated after absorption of light by the Mn cluster (Allakhverdiev and Murata 2004,

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Hakala et al. 2005). Consequently, the electron flow from OEC to $P680^+$ is blocked. As a result, the levels of $P680^+$ and Yz^+ remain high. These radicals are thought to be responsible for photoinhibition damage (Hakala et al. 2005, Ohnishi et al. 2005, Zsiros et al. 2006).

Plants respond and adapt to photoinhibition with appropriate physiological, developmental and biochemical changes to prevent the damage occurring due to this stress condition. Polyamines (PAs), such as putrescine (PUT), spermidine (SPD) and spermine (SPM), are low molecular weight biogenic amines distributed in all organisms. Their mechanism of action is based on their chemical and physical interactions with macromolecules such as DNA, RNA, acid phospholipids and proteins (Tabour and Tabour 1984). In plants, PAs play important roles in defense against environmental stresses (Kotzabasis and Dornemann 1998, Legocka and Zajchert 1999, Sfichi et al. 2004). It has been observed that plants significantly increase the amount of various PAs, reaching a 900% increase in the SPD content in tobacco plants exposed to UV-B irradiation (Lütz et al. 2005). Also, it was shown that overexpression of spermidine synthase in Arabidopsis mutants improves tolerance to several stress conditions (Kasukabe et al. 2004). However, the precise role of PAs and the physiological significance of this increase remain unclear. Addition of exogenous PAs can prevent lipid peroxidation of thylakoid membranes, thus retaining their structural integrity and avoiding the release of the manganese-stabilizing protein (MSP) of PSII and Cyt *f* from the thylakoid membranes (Stoynova et al. 1999, Peters and Chin 2003). Moreover, exogenous application of SPD may stabilize the native structure of D1 and D2 proteins and Cyt in oat plants in response to osmotic stress (Kusnetsov et al. 2006).

PAs differ not only in size but also in the number of positive charges at physiological pH (e.g. two in PUT, four in SPM). In order to examine the effect of positive charges of PAs during photoinhibition conditions, we added SPM, PUT or methylamine (MET) (one positive charge at physiological pH) to thylakoid membranes illuminated with strong white light. Water oxidation and the advancement of the S-states, the kinetics of the Chl fluorescence rise, and electron transfer at both sides of PSII were affected. We concluded from the experimental data that SPM, which contains the greatest number of positive charges, was able to limit the damage occurred by photoinhibition, compared with the other amines.

Results

In order to clarify the influence of amines on the thylakoid membranes illuminated with strong light, we measured the yield of oxygen evolution in thylakoid membranes using a train of 12 saturating single turnover flashes. A periodicity of four in the yield of oxygen evolution is observed in relation of the advancement of the S-states of the OEC (Kok et al. 1970). Oxygen evolution was strongly decreased in the photoinhibited samples and the oscillation pattern was slightly modified (**Fig. 1**, trace 5). A milder decrease in the oxygen yield was observed when 1 mM SPM was present during the strong illumination (**Fig. 1**, trace 2). However, the other amines used could not ameliorate the oxygen yield significantly (**Fig. 1**, traces 3 and 4). A more general view is shown in the inset of **Fig. 1** where the total yield of the first four flashes is represented. SPM added to the sample before photoinhibition clearly increased the oxygen yield compared with the photoinhibited sample without amines by about 45%. However, MET and PUT exhibited much weaker effects. These results indicate that SPM could partly prevent the damage occurring when thylakoids were exposed to excessive light.

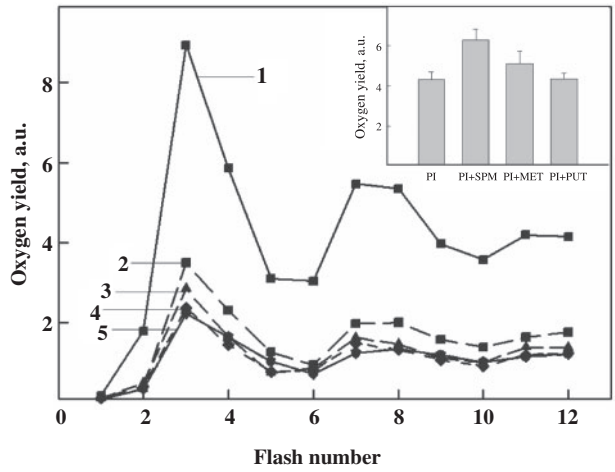

Fig. 1 Period four oscillation of the yield of oxygen evolution in thylakoid membranes using a train of 12 saturating (4 J) single turnover flashes (10 μs): (1) control; (2) photoinhibited sample (PI) + SPM; (3) PI + MET; (4) PI + PUT and (5) PI without amine. Assays were carried out in media containing 400 mM sucrose, 40 mM HEPES-NaOH (pH 7.6), 10 mM NaCl and 5 mM $MgCl_2$. Details are given in the Materials and Methods. Inset: effects of photoinhibition in the presence of various amines on the yield of oxygen evolution in thylakoid membranes. Each point represents the total yield of the first four flashes from the experiment of **Fig. 1**.

To analyze further the action of amines added before photoinhibition, we analyzed the O–J–I–P induction traces that display the progressive reduction of the plastoquinones located at the acceptor side of PSII with three main phases corresponding to O–J, J–I and I–P (Pospisil and Dau 2000, Zhu et al. 2005, Boisvert et al. 2006). **Fig. 2** (trace 4) shows the damping of the Chl fluorescence induction in the control photoinhibited sample without amine. The decline of the amplitudes of the O–J and I–P phases together with the simultaneous increase of Chl fluorescence at the start of the fluorescence induction (an approximation of F_0) is consistent with the photoinhibitory action of the strong illumination, thus decreasing the maximum quantum yield of PSII, F_v/F_m, where $F_v = F_m - F_0$. Addition of 1 mM SPM before photoinhibitory illumination could partly prevent this damping (**Fig. 2**, trace 2). However, the addition of PUT or MET had no

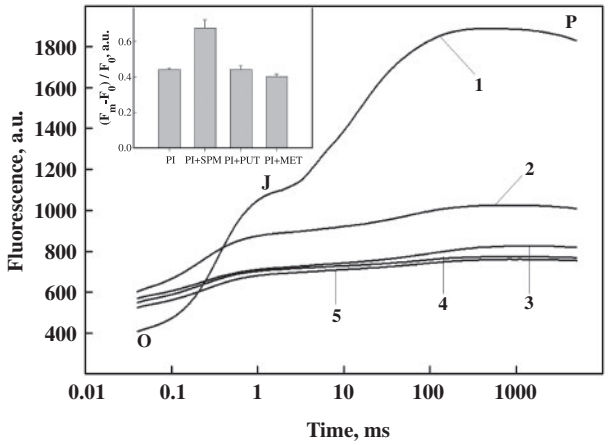

Fig. 2 Chl fluorescence induction traces of thylakoid membranes photoinhibited with 1 mM SPM, PUT or MET: (1) control; (2) photoinhibited sample (PI) + SPM; (3) PI + PUT; (4) PI without amine; (5) PI + MET. Inset: Chl fluorescence parameter F_v/F_0 measured in thylakoid membranes photoinhibited with or without 1 mM SPM, PUT or MET. See details in the Materials and Methods.

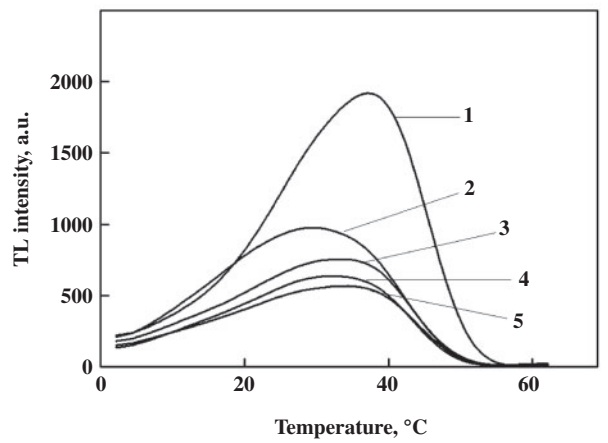

Fig. 3 Thermoluminescence glow curves (B-band) from thylakoid membranes after photoinhibition in the presence of different amines: (1) control without amine; (2) photoinhibited sample (PI) + SPM; (3) PI + MET; (4) PI + PUT; (5) PI without amine. The samples were heated from 2 to 62°C at a rate of 0.5°C s^{-1}.

significant effect (**Fig. 2**, traces 3 and 5). A more quantitative view is presented in the inset of **Fig. 2** that shows the loss of F_v/F_0, a parameter that accounts for the simultaneous variations in F_m and F_0 in determinations of the maximum quantum yield of PSII induced by strong illumination. Exogenously supplied SPM led to significantly stronger F_v/F_0 compared with the other amines.

Thermoluminescence (TL) was also used to obtain further insight into the effect of amines on charge recombination processes in PSII under photoinhibition conditions. **Fig. 3** shows the TL glow curves produced by a linear increase in temperature from 2 to 62°C at the rate of 0.5°C s^{-1} following a single turnover (1 μs) white flash. The amplitude of the TL signal reached a maximum at about 38°C in control thylakoid samples (**Fig. 3**, trace 1). This major TL emission, the B-band (30–40°C), originates from $S_2Q_B^-$ charge recombination (Sane 2004). This band was strongly decreased after photoinhibition treatment (**Fig. 3**, trace 5). Addition of 1 mM SPM before photoinhibition treatment increased the amplitude of the B-band of photoinhibited samples with a shift of the maximum toward lower temperatures (**Fig. 3**, trace 2). This shift may be explained by the heterogeneity in the population of PSII centers and their differential sensitivity toward photoinhibitory treatment. So, SPM may protect the population of PSII having a lower activation energy requirement for charge recombination. Moreover, 1 mM MET added to the sample before photoinhibition treatment slightly increased the TL intensity (**Fig. 3**, trace 3). However, no significant modification of the B-band was observed after addition of 1 mM PUT (**Fig. 3**, trace 4).

The above results show that only SPM, with the greatest number of positive charges, was able to protect PSII against photoinhibition conditions. To confirm that the observed effect of SPM was due to the strength of the positive charges and not to molecular size, we compared SPM with two inorganic amines: 1,10-diaminodecane (DD) and decylamine (DA) which contain two and one positive charges, respectively, with the same number of carbon atoms (C10) as SPM. Our results confirmed that only SPM with the greatest number of positive charges was able to increase F_v/F_m under photoinhibition conditions (data not shown). In addition, SPD which has a similar chemical structure to SPM with three positive charges was used to estimate its protective effect under photoinhibition conditions. The result showed a very similar effect compared with SPM (data not shown), indicating that the polycationic nature is important for protection.

Chl fluorescence emission at 77 K was used to study the effect of amines on the association of light-harvesting complex II (LHCII) antenna with the PSII core under photoinhibition conditions. At cryogenic temperature, two separate emission bands appear at 680 nm with shoulders at 690 and 735 nm that originate from PSII and PSI, respectively (Bredenkamp and Baker 1990, Siffel et al. 2000). **Fig. 4** shows the fluorescence emission in thylakoid membranes normalized at 736 nm. After photoinhibition treatment, the relative amplitude of the peak at 683 nm associated with PSII complexes strongly decreased (**Fig. 4**, trace 5). This result indicates the disconnection of LHCII antenna from the PSII complexes due to de-stacking. However, this change was completely abolished in the presence of the exogenous amines added before photoinhibition irrespective of the amine used (SPM, MET or PUT) (**Fig. 4**, traces 1–3). In that case, the traces were similar to the trace obtained from the control non-photoinhibited sample (**Fig. 4**, trace 4), showing that the amines prevented the disconnection of LHCII from the PSII complexes.

As the protection against strong illumination was specific for SPM, F_v/F_m was measured to determine the effect of SPM on the time course of photoinhibition. **Fig. 5A** shows that the

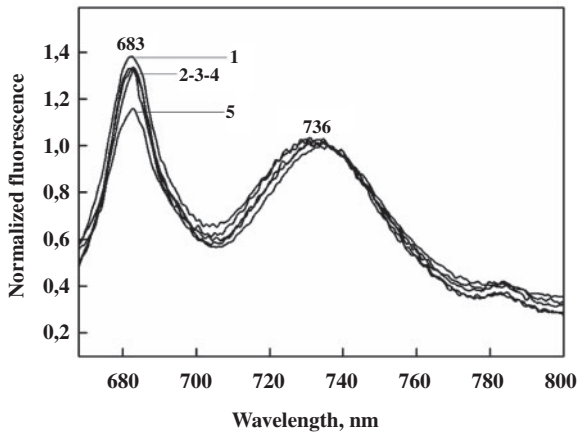

Fig. 4 Fluorescence emission spectra measured in isolated thylakoids at 77 K: (1) photoinhibited sample (PI) + SPM; (2) PI + PUT; (3) PI + MET; (4) control without amine; (5) PI without amine. All traces were normalized to 736 nm. These traces are the average of 6–9 assays.

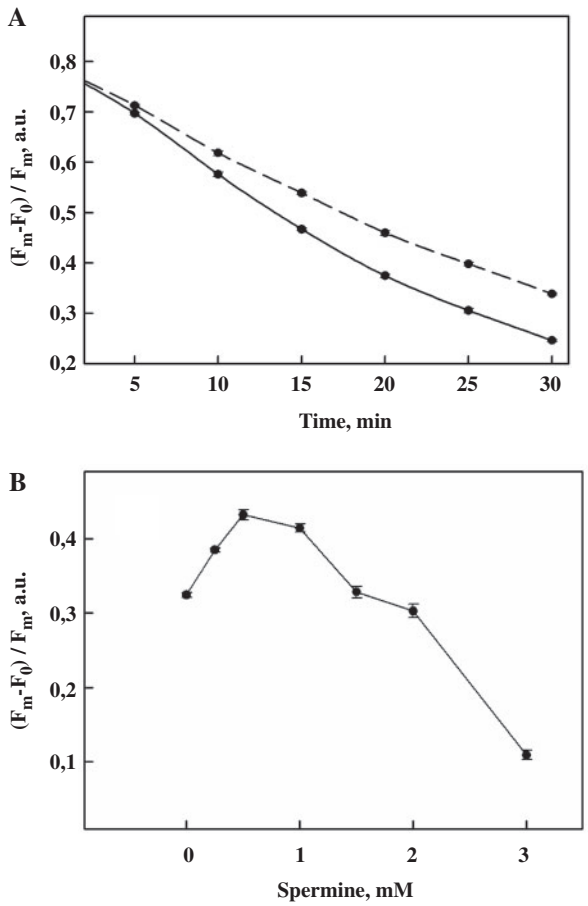

Fig. 5 (A) Chl fluorescence parameter F_v/F_m measured in thylakoid membranes with and without 1 mM SPM at different times of photoinhibition: solid line, photoinhibited sample (PI) without amine; dashed line, PI + SPM. (B) Effect of increasing the SPM concentration in thylakoid membranes photoinhibited for 30 min on the Chl fluorescence parameter F_v/F_m.

effect of SPM increases according to the time of photoinhibition. To gain more information about the optimal concentration of exogenous SPM involved in photoprotection, F_v/F_m was measured when different concentrations of SPM were added to the sample before photoinhibition. **Fig. 5B** shows that the maximal activity of PSII was reached at around 0.5–1 mM SPM.

To predict the site of binding of SPM on PSII, a docking experiment in which the polyamine was docked on PSII from *Thermosynechococcus elongates* was used. The docking results are shown in **Fig. 6**. Amongst the possible binding sites, the prediction suggested that the preferred binding site of SPM on PSII is located at the hydrophilic loop of the CP47 protein in the vicinity of the PsbO protein (**Fig. 6A**). At this position, SPM is surrounded by Glu1598, Asn1599, Val1601, Gly710, Val711 and Arg692 in the CP47 protein with a binding energy (ΔG) of -6.56 kcal mol^{-1} (**Fig. 6B**). It is known that CP47 and PsbO proteins of cyanobacteria are very similar to their homologs in higher plants. Thus, the above suggests that the interaction of SPM with the hydrophilic portion of proteins exposed to the lumen side of PSII would be involved in stabilizing the photosystem proteins against photoinhibition. The preferred binding site of PUT on PSII is located at the D1

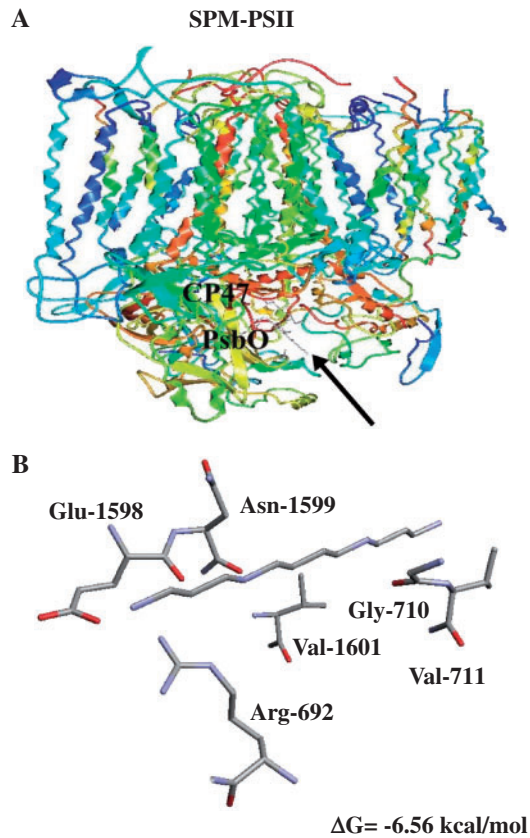

Fig. 6 (A) Best conformation for SPM docked into the PSII structure from *Thermosynechococcus elongatus* (PDB entry 3BZ1). The arrow indicates the location of SPM. (B) CP47 residues in the vicinity of SPM and the free binding energy (ΔG) of the docked complex.

protein with a ΔG of -7.32 kcal mol^{-1}. However, MET is located at the D2 protein with a ΔG of -5.55 kcal mol^{-1} (data not shown).

Discussion

In this contribution we investigated the role of positive charges of PAs in the protection of the photosynthetic apparatus upon photoinhibition conditions. Photoinhibition is known to affect both the acceptor and donor sides of PSII causing a decrease of F_v/F_0 and the loss of oxygen evolution as shown here in **Figs. 1** and **2**. It was shown that the donor-side photoinhibition of PSII could be due to long-lived and highly oxidative species, such as the oxidized tyrosine electron donor Tyr Z^+ and $P680^+$ (Yamamoto 2001). The latter species can oxidize the surrounding amino acid residues of proteins, especially the D1 protein, causing damage to the RC of PSII (Nishiyama et al. 2006). On the other hand, the impairment of the OEC might facilitate the access of free oxygen molecules to P680, producing hydroxyl radicals (OH·) and superoxide radicals ($O_2^{\cdot-}$) that can also damage the RC (Pospisil et al. 2004). Photoinhibition is accompanied by the proteolytic degradation of D1 protein that follows an initial step induced by the generation of reactive oxygen species (ROS).

Photoinhibition of PSII could also occur on the acceptor side where $O_2^{\cdot-}$ is produced. In fact, under excessive illumination, the electron acceptor Q_B interacts with molecular oxygen to form $O_2^{\cdot-}$, which stimulates the degradation of D1 protein (Yamamoto et al. 2008). Moreover, the over-reduction of Q_A accompanied by its dissociation from its binding site might increase the probability for charge recombination between $P680^+$ and $Pheo^-$, leading to an enhancement of the formation of P680 triplet states. The latter interact with molecular oxygen to produce $^1O_2$ and consequently damage the D1 protein (Hideg et al. 1994). It has been suggested that both donor and acceptor side photoinhibition occurred even during in vitro conditions (Yamamoto 2001).

It is known that PAs play important roles in plant defense against environmental stresses (Kotzabasis and Dornemann 1998, Legocka and Zajchert 1999, Sfichi et al. 2004). It has been shown that changes in the PUT/SPM ratio caused by an increase or decrease in the level of endogenous PUT or SPM are responsible for the regulation of responses of the photosynthetic apparatus under various environmental stresses (Demetriou et al. 2007, Ioannidis and Kotzabasis 2007, Navakoudis et al. 2007, Yamaguchi et al. 2007). However, the effect of exogenous PAs added in vitro is incompletely understood. During our experiments we have observed that the addition of 1 mM SPM could reduce the inhibition exerted by strong illumination. In fact, the yield of oxygen evolution, F_v/F_0 and the B-band intensity were significantly increased compared with the photoinhibited sample without SPM (**Figs. 1–3**). However, PUT and MET did not protect the photosynthetic apparatus under similar conditions. This result clearly shows that SPM, with a higher net positive charge compared with PUT and MET, would be appropriate to stabilize the proteins of PSII under photoinhibition conditions. Indeed, other amines with the same number of carbon atoms as SPM but with fewer positive charges (DD and DA) were ineffective, thus demonstrating that the polycationic nature of SPM is required. Thus, the increased number of amino groups in SPM might enhance the binding of PAs to proteins, providing the most stable conformation under photoinhibition conditions (Kusnetsov and Shevyakova 2007).

On the other hand, the detachment of LHCII antenna complexes from the PSII core under photoinhibition conditions is associated with the partial de-stacking of the thylakoid membranes known to occur during photoinhibition (Yamamoto et al. 2008). This detachment is known to be prevented in the presence of a low concentration of inorganic cations such as Mg^{2+} or K^+ due to the screening of surface charges on the LHCII proteins. Similar screening was shown here. The amines used all prevented the LHCII dissociation from PSII complexes (**Fig. 4**), showing that the number of positive charges was not important for this rather non-specific screening effect that differs from the photoprotective action of SPM.

Interestingly, **Fig. 5B** shows that the maximal activity of PSII in photoinhibited samples was reached at around 0.5–1 mM SPM. Above this optimal concentration SPM might enhance the inhibitory effect of strong illumination. This is in good agreement with our previous studies which reported that a high concentration of amines (SPM, PUT and MET) perturbs the protein secondary structure in PSII-enriched submembrane fractions, leading to a significant decrease in the percentage of α-helix with an increase in β-sheet. This modification is responsible for the drop in PSII activity (Beauchemin et al. 2007, Hamdani and Carpentier 2009, Hamdani et al. 2009).

Proposed mechanism of photoprotection by SPM

Our working hypothesis envisages that exogenously supplied SPM can stabilize the conformation of PSII proteins exposed to the hydrophilic media through electrostatic interaction owing to its polycationic nature. Polycations [poly(amino acids)] were shown to stabilize and even improve the photochemical activity of photosystems effectively while negatively charged or hydrophobic poly(amino acids) were ineffective (Matsumoto et al. 2010).

Exogenously added SPM, like other PAs, can penetrate to the luminal side of thylakoid membranes as mentioned previously (Ioannidis et al. 2006, Beauchemin et al. 2007). This might occur through ion channels, especially Ca^{2+}-permeable channels, which are efficiently modulated by this PA (Yamaguchi et al. 2007). At the luminal side, SPM may interact with hydrophilic fractions of proteins such as CP47 and the extrinsic polypeptides of the OEC, especially the PsbO protein, which is known as the MSP, as suggested by our simulation (**Fig. 6A**). Consequently, PSII activity may be preserved under high light stress (**Figs. 1–3**). The above proposal is strongly substantiated

by recent investigations that proposed the OEC as the primary target of photoinhibition due to the initial inactivation of the Mn cluster following light absorption (Callahan et al. 1986, Jegerschold et al. 1990, Jegerschold and Styring 1991, Hakala et al. 2005, Murata et al. 2007). Moreover, it was also reported that the PsbO protein is oxidatively damaged under excessive illumination (Yamamoto et al. 2008). An extended structure of PsbO probably interacts with a large surface of the D1 protein, conferring protection against ROS and cationic radicals (Yamamoto 2001, Pospisil et al. 2004, Nishiyama et al. 2006, Yamamoto et al. 2008). Photoprotection of the luminal side of PSII may therefore be of physiological relevance. However, other potential binding sites for SPM may also be present, such as the acceptor side of PSII.

Materials and Methods

Materials

SPM, SPD, PUT, MET, DD and DA were purchased from Sigma Chermical Co. and used as supplied.

Thylakoid membrane preparation

Thylakoid membranes were isolated from fresh market spinach (*Spinacia oleracea* L.) as described elsewhere (Joly et al. 2005) and kept in the dark. The Chl concentration was calculated following the procedure outlined in Porra et al. (1989).

Photoinhibitory treatment and amine addition

Thylakoid membranes (500 μg Chl ml^{-1}) were illuminated by an intense white light (2,000 μmol of photons m^{-2} s^{-1}) from a 150 W quartz–halogen projector lamp for 30 min with continuous stirring at 24°C controlled by a water bath. The assay medium contained 250 mM sorbitol, 20 mM Tricine KOH (pH 7.8), 10 mM KCl, 10 mM NaCl and 5 mM $MgCl_2$. Before photoinhibition, 1 mM SPM, PUT or MET were added. The Chl content remained constant during the photoinhibitory treatment.

Oxygen evolution

Flash-induced oxygen evolution was measured in the thylakoid membranes (after treatment) at 22°C using a laboratory-built instrument. A complete description of the oxygen electrode system can be found elsewhere (Zeinalov 2002). The electrode consists of two compartments separated by a cellophane membrane. The silver anode is filled by an electrolyte buffer containing 400 mM sucrose, 40 mM HEPES-NaOH (pH 7.6), 100 mM KCl, 10 mM NaCl and 5 mM $MgCl_2$. Thylakoid membranes deposited in the cathode chamber were diluted to 200 μg Chl ml^{-1} in a medium containing 400 mM sucrose, 40 mM HEPES-NaOH (pH 7.6), 10 mM NaCl and 5 mM $MgCl_2$ in a total volume of 90 μl. After a 5 min incubation, the sample was illuminated by a train of 12 saturating (4 J) single turnover flashes (10 μs). The quantitative estimation of photosynthetic oxygen production and the S-state transitions were carried out using an analytical solution for the fitting of experimental data as described previously (Messinger et al. 1997).

Chl fluorescence induction

Fluorescence induction measurements were performed at room temperature using the Plant Efficiency Analyser (Hansatech). The assay medium contained 250 mM sorbitol, 20 mM Tricine KOH (pH 7.8), 10 mM KCl, 10 mM NaCl, 5 mM $MgCl_2$ and 25 μg Chl ml^{-1}. Thylakoid membranes (after treatment) were excited with saturating red actinic light (655 nm and an intensity of 3,000 μmol m^{-2} s^{-1}) provided by light-emitting diodes. As the fluorescence signal during the first 40 μs is ascribed to artifacts due to the response time of the instrument, these data were not included in the analyses of fluorescence induction traces. The signal at 40 μs was taken as F_0, the initial fluorescence. Variable fluorescence, F_v (the difference between F_0 and the maximal fluorescence, F_m, in dark-adapted samples), was used to calculate the F_v/F_m and F_v/F_0 ratios.

Fluorescence spectroscopy

Low temperature (77 K) spectra of fluorescence emission as well as their excitation spectra were measured with a Perkin-Elmer LS55 spectrofluorimeter. Chl fluorescence was excited at 436 nm during the measurements of emission spectra. The excitation and emission spectral widths were fixed at 5 and 2.5 nm, respectively. The Chl content of the sample was adjusted to 5 μg ml^{-1}.

Thermoluminescence

TL measurements were carried out with a laboratory-built instrument. The description of the design and the functional aspects are presented elsewhere (Ducruet 2003, Gauthier et al. 2006). Thylakoid membranes (after treatment) were diluted to 200 μg Chl ml^{-1} in a medium containing 20 mM Tricine NaOH (pH 7.8), 400 mM sucrose, 10 mM KCl, 10 mM NaCl and 5 mM $MgCl_2$. About 200 μl of the suspension was added to the sample compartment (15 mm diameter) positioned just above the Peltier plate and covered with a Hellma 202-OS disc window. The sample chamber was closed with a holder bearing the light guide connected to the photomultiplier. The sequence of pre-incubation periods and flash illumination of thylakoid is presented below. First, samples were incubated for 120 s at 20°C in the dark. Following this step, the temperature was brought down to 2°C within 5–8 s and maintained for 60 s. This incubation temperature was selected in order to avoid freezing-induced damage to the OEC that may give rise to artifacts in thylakoid membranes without cryoprotectant (Gauthier et al. 2006). An actinic single turnover saturating white flash of about 1 μs width (setting 10, XE-STC, Walz) was applied to initiate charge separation in PSII. During the last step, linear warming of samples in total darkness activated the recombination of PSII charge pairs that can be detected by

the appearance of emission bands with characteristic TL peak temperatures (Ducruet 2003, Gauthier et al. 2006).

Molecular modeling and docking

The docking studies were performed with ArgusLab 4.0.1 software (Planaria Software LLC; http://www.arguslab.com). The PSII structure from *T. elongatus* is drawn from coordinates obtained by Guskov et al. (2009) (PDB entry: 3BZ1) and the SPM three-dimensional structures were generated from PM3 semi-empirical calculations using Chem3D Ultra 6.0. The docking runs were performed on the ArgusDock docking engine using high precision with a maximum of 150 candidate poses. The conformations were ranked using the Ascore scoring function, which estimates the free binding energy. Upon docking of SPM to PSII, the current configurations were optimized using a steepest descent algorithm until convergence, within 40 iterations, and amino acids residues within a distance of 3.5 Å relative to SPM were involved in the complexation.

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References

Adams, B.D. and Adams, W.W. (1992) Photoprotection and others responses of plants to high light stress. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43: 599–626.

Allakhverdiev, S.I. and Murata, N. (2004) Environmental stress inhibits the synthesis de novo of proteins involved in the photodamage–repair cycle of photosystem II in *Synechocystis* sp. PCC 6803. *Biochim. Biophys. Acta* 1657: 23–32.

Beauchemin, R., Gautier, A., Harnois, J., Boisvert, S., Govidachary, S. and Carpentier, R. (2007) Spermine and spermidine inhibition of photosystem II: disassembly of the oxygen evolving complex and consequent perturbation in electron donation from Tyr_Z to $P680^+$ and the quinone acceptors Q_A^- to Q_B. *Biochim. Biophys. Acta* 1767: 905–912.

Boisvert, S., Joly, D. and Carpentier, R. (2006) Quantitative analysis of the experimental O-J-I-P chlorophyll fluorescence induction kinetics apparent activation energy and origin of each kinetic step. *FEBS J.* 273: 4770–4777.

Bredenkamp, G.J. and Baker, N.R. (1990) Modification of excitation energy distribution to photosystem I by protein phosphorylation and cation depletion during thylakoid biogenesis in wheat. *Photosynth. Res.* 23: 111–117.

Callahan, F.E., Becker, D.W. and Cheniae, G.M. (1986) Studies on the photoactivation of the water-oxidizing enzyme. *Plant Physiol.* 82: 261–269.

Demetriou, G., Neonaki, C., Navakoudis, E. and Kotzabasis, K. (2007) Salt stress impact on the molecular structure and function of the photosynthetic apparatus—the protective role of polyamines. *Biochim. Biophys. Acta* 1767: 272–280.

Ducruet, J.M. (2003) Chlorophyll thermoluminescence of leaf discs: simple instruments and progress in signal interpretation open the way to new ecophysiological indicators. *J. Exp. Bot.* 54: 2419–2430.

Gauthier, A., Govindachary, S., Harnois, J. and Carpentier, R. (2006) Interaction of N,N,N′,N′-tetramethyl-p-phenylenediamine with photosystem II as revealed by thermoluminescence: reduction of the higher oxidation states of the Mn cluster and displacement of plastoquinone from the QB niche. *Biochim. Biophys. Acta* 1757: 1547–1556.

Guskov, A., Kern, J., Gabdulkhakov, A., Broser, M., Zouni, A. and Saenger, W. (2009) Cyanobacterial photosystem II at 2.9-A resolution and the role of quinones, lipids, channels and chloride. *Nat. Struct. Mol. Biol.* 16: 334–342.

Hakala, M., Tuominen, I., Keränen, M., Tyystjärvi, T. and Tyystjärvi, E. (2005) Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of photosystem II. *Biochim. Biophys. Acta* 1706: 68–80.

Hamdani, S. and Carpentier, R. (2009) Interaction of methylamine with extrinsic and intrinsic subunits of photosystem II. *Biochim. Biophys. Acta* 1787: 1223–1229.

Hamdani, S., Tajmir-Riahi, H.A. and Carpentier, R. (2009) Methylamine interaction with proteins of photosystem II: a comparison with biogenic polyamines. *J. Photochem. Photobiol. B Biol.* 96: 201–206.

Hideg, E., Spetea, C. and Vass, I. (1994) Singlet oxygen production in thylakoid membranes during photoinhibition as detected by EPR spectroscopy. *Photosynth. Res.* 39: 191–199.

Ioannidis, N.E. and Kotzabasis, K. (2007) Effects of polyamines on the functionality of photosynthetic membrane in vivo and in vitro. *Biochim. Biophys. Acta* 1767: 1372–1382.

Ioannidis, N.E., Sfichi, L. and Kotzabasis, K. (2006) Putrescine stimulates chemiosmotic ATP synthesis. *Biochim. Biophys. Acta* 1757: 821–828.

Jegerschold, C. and Styring, S. (1991) Fast oxygen-independent degradation of the D1 reaction centre protein in photosystem II. *FEBS Lett.* 280: 87–90.

Jegerschold, C., Virgin, I. and Styring, S. (1990) Light-dependent degradation of the D1 protein in photosystem II is accelerated after inhibition of the water splitting reaction. *Biochemistry* 29: 6179–6186.

Joly, D., Bigras, C., Harnois, J., Govindachary, S. and Carpentier, R. (2005) Kinetic analyses of the OJIP chlorophyll fluorescence rise in thylakoid membranes. *Photosynth. Res.* 84: 107–112.

Kasukabe, Y., He, L.X., Nada, K., Misawa, S., Ihara, I. and Tachibana, S. (2004) Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress regulated genes in transgenic *Arabidopsis thaliana*. *Plant Cell Physiol.* 45: 712–722.

Kok, B., Forbush, B. and McGloin, M. (1970) Cooperation of charges in photosynthetic O_2 evolution-I. A linear four step mechanism. *Photochem. Photobiol.* 11: 457–475.

Kotzabasis, K. and Dornemann, D. (1998) Differential changes in the photosynthetic pigments and polyamine content during photoadaptation and photoinhibition in the unicellular green alga Scenedesmus obliquus. *Z. Naturforsch. C* 53: 833–840.

Kuznetsov, V.V., Radyukhina, N.L. and Shevyakova, N.I. (2006) Polyamines at stress: biological role, metabolism and regulation. *Russ. J. Plant Physiol.* 53: 658–684.

Kuznetsov, V.V. and Shevyakova, N.I. (2007) Polyamines and stress tolerance of plants. *Plant Stress* 1: 50–71.

Legocka, J. and Zajchert, I. (1999) Role of spermidine in the stabilization of the apoprotein of the light-harvesting chlorophyll

a/b-protein complex of photosystem II during leaf senescence process. *Acta Physiol. Plant.* 21: 127–132.

Lütz, C., Navakoudis, E., Seidlitz, H.K. and Kotzabasis, K. (2005) Simulated solar irradiation with enhanced UV-B adjust plastid- and thylakoid-associated polyamine changes for UV-B protection. *Biochim. Biophys. Acta* 1710: 24–33.

Mathis, P., Satoh, K. and Hansson, O. (1989) Kinetic evidence for the function of Z in isolated photosystem II reaction centers. *FEBS Lett.* 251: 241–244.

Matsumoto, K., Zhang, S. and Koutsopoulos, S. (2010) Enhanced electron transfer activity of photosystem I by polycations in aqueous solution. *Biomacromolecules* 11: 3152–3157.

Melis, A. (1999) Photosystem II damage and repair cycle in chloroplast: what modulates the rate of photodamage in vivo. *Trends Plant Sci.* 4: 130–135.

Messinger, J., Seaton, G., Wydrzynski, T., Wacker, U. and Renger, G. (1997) S-3 state of the water oxidase in photosystem II. *Biochemistry* 36: 6862–6873.

Murata, N., Takahashi, S., Nishiyama, Y. and Allakhverdiev, S.I. (2007) Photoinhibition of photosystem II under environmental stress. *Biochim. Biophys. Acta* 1767: 414–421.

Nanba, O. and Satoh, K. (1987) Isolation of a photosystem II reaction center consisting of D-1 and D-2 polypeptides and cytochrome b-559. *Proc. Natl Acad. Sci. USA* 84: 109–112.

Navakoudis, E., Vrentzou, K. and Kotzabasis, K. (2007) A polyamine- and LHCII protease activity-based mechanism regulates the plasticity and adaptation status of the photosynthetic apparatus. *Biochim. Biophys. Acta* 1767: 261–271.

Nishiyama, Y., Allakhverdiev, S.I. and Murata, N. (2006) A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II. *Biochim. Biophys. Acta* 1757: 742–749.

Ohnishi, N., Allakhverdiev, S.I., Takahashi, S., Higashi, S., Watanabe, M., Nishiyama, Y. et al. (2005) Two-step mechanism of photodamage to photosystem II: step 1 occurs at the oxygen-evolving complex and step 2 occurs at the photochemical reaction center. *Biochemistry* 44: 8494–8499.

Peters, J.S. and Chin, C.K. (2003) Inhibition of photosynthetic electron transport by palmitoleic acid is partially correlated to loss of thylakoid membrane proteins. *Plant Physiol. Biochem.* 41: 117–124.

Porra, R.J., Thompson, W.A. and Kriedemann, P.E. (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta* 975: 384–394.

Pospısil, P., Arato, A., Liszkay, A.K. and Rutherford, A.W. (2004) Hydroxyl radical generation by photosystem II. *Biochemistry* 43: 6783–6792.

Pospisil, P. and Dau, H. (2000) Chlorophyll fluorescence transients of photosystem II membrane particles as a tool for studying photosynthetic oxygen evolution. *Photosynth. Res.* 65: 41–52.

Powles, S.B. (1984) Photoinhibition of photosynthesis induced by visible light. *Annu. Rev. Plant Physiol.* 35: 15–44.

Sane, P.V. (2004) Thermoluminescence: a technique for probing photosystem II. *In* Photosynthesis Research Protocols. Edited by Carpentier, R. pp. 229–248. Humana Press, Totowa, NJ.

Sfichi, L., Ioannidis, N. and Kotzabasis, K. (2004) Thylakoid-associated polyamines adjust the UV-B sensitivity of the photosynthetic apparatus by means of light-harvesting complex II changes. *Photochem. Photobiol. B* 80: 499–506.

Siffel, P., Hunalov, I. and Rohacek, K. (2000) Light-induced quenching of chlorophyll fluorescence at 77 K in leaves, chloroplasts and photosystem II particles. *Photosynth. Res.* 65: 219–229.

Sproviero, E.M., Gascon, J.A., McEvoy, J.P., Brudvig, G.W. and Batista, V.S. (2008) Quantum mechanics/molecular mechanics study of the catalytic cycle of water splitting in photosystem II. *J. Amer. Chem. Soc.* 130: 3428–3442.

Stoynova, E.Z., Karanov, E. and Alexieva, V. (1999) Subcellular aspects of the protective effect of spermine against atrazine in pea plants. *Plant Growth Regul.* 29: 175–180.

Styring, S., Virgin, I., Ehrenberg, A. and Andersson, B. (1990) Strong light photoinhibition of electron transport in photosystem II. Impairment of the functions of the first quinone acceptor, Q_A. *Biochim. Biophys. Acta* 1015: 269–278.

Tabour, C.W. and Tabour, H. (1984) Polyamines. *Annu. Rev. Biochem.* 53: 749–790.

Tyystjarvi, E. (2008) Photoinhibition of photosystem II and photodamage of the oxygen evolving manganese cluster. *Coord. Chem. Rev.* 252: 361–376.

Vass, I., Styring, S., Hundal, T., Koivuniemi, A., Aro, E.M. and Andersson, B. (1992) Reversible and irreversible intermediates during photoinhibition of photosystem II. Stable reduced Q_A species promote chlorophyll triplet formation. *Proc. Natl Acad. Sci. USA* 1102: 195–201.

Yamaguchi, K., Takahashi, Y., Berberich, T., Imai, A., Takahashi, T., Michael, A.J. et al. (2007) A protective role for the polyamine spermine against drought stress in Arabidopsis. *Biochem. Biophys. Res. Commun.* 352: 486–490.

Yamamoto, Y. (2001) Quality control of photosystem II. *Plant Cell. Physiol.* 42: 121–128.

Yamamoto, Y., Aminaka, R., Yoshioka, M., Khatoon, M., Komayama, K., Takenaka, D. et al. (2008) Quality control of photosystem II: impact of light and heat stresses. *Photosynth. Res.* 98: 589–608.

Zeinalov, Y. (2002) An equipment for investigations of photosynthetic oxygen production reactions. *Bulg. J. Plant Physiol.* 28: 57–67.

Zhu, X.G., Govindjee, N.R., Bakereric, D., Ort, D.R. and Stephen, P.L. (2005) Chlorophyll a fluorescence induction kinetics in leaves predicted from a model describing each discrete step of excitation energy and electron transfer associated with photosystem II. *Planta* 223: 114–133.

Zsiros, O., Allakhverdiev, S.I., Higashi, S., Watanabe, M., Nishiyama, Y. and Murata, N. (2006) Very strong UV-A light temporally separates the photoinhibition of photosystem II into light-induced inactivation and repair. *Biochim. Biophys. Acta* 1757: 123–129.