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Water-splitting manganese complex controls light-induced redox changes of cytochrome b_{559} in Photosystem II

Rakesh Kumar Sinha · Arjun Tiwari · Pavel Pospíšil

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Abstract The effect of water-splitting Mn complex on light-induced redox changes of cytochrome b_{559} (cyt b_{559}) was studied in spinach photosystem II (PSII) membranes. Photoreduction of the heme iron in the intact PSII membranes was completely suppressed by DCMU, whereas photoreduction and photooxidation of the heme iron in the Mn-depleted PSII membranes were unaffected by DCMU. Interesingly, photoreduction and photooxidation of the heme iron in the Mn-depleted PSII membranes were completely diminished by exogenous superoxide dismutase (SOD), whereas no effect of SOD on photoreduction of the heme iron was observed in the intact PSII membranes. The current work shows that the light-induced redox changes of cyt b_{559} proceed via a different mechanism in the both types of PSII membranes. In the intact PSII membranes, photoreduction of the heme iron is mediated by plastoquinol. However, in the Mn-depleted PSII membranes, photoreduction and photooxidation of the heme iron are mediated by superoxide anion radical formed in PSII.

Keywords Cytochorome b_{559} · Photosystem II · Redox potential · Water-splitting manganese complex

Abbreviations

cyt b_{559}	cytochorome b_{559}
Em	midpoint redox potential
HP	high-potential form of cyt b_{559}
IP	intermediate potential form of cyt b_{559}
LP	low-potential form of cyt b_{559}
MES	2-[N-Morpholino]ethanesulfonic acid
PSII	photosystem II
SOD	superoxide dismutase
SOO	superoxide oxidase
SOR	superoxide reductase
Q_A	primary quinone electron acceptor of PSII
Q_B	secondry quinone electron acceptor of PSII
Pheo	pheophytin - primary electron acceptor of PSII
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
EMPO	5-ethoxycarbonyl-5-methyl-1-pyrroline *N*-oxide

R. K. Sinha · A. Tiwari · P. Pospíšil (✉)
Laboratory of Biophysics, Department of Experimental Physics, Faculty of Science, Palacký University,
tř. 17. listopadu 1192/12,
771 46 Olomouc, Czech Republic
e-mail: pospip@prfnw.upol.cz

Introduction

Photosystem II (PSII) is a multisubunit enzyme known to catalyze oxidation of water and reduction of plastoquinone in the thylakoid membranes of higher plants, algae, and cyanobacteria (Renger and Holzwarth 2005; Rappaport and Diner 2008). Light-driven oxidation of water takes place at the catalytic site of the water-splitting Mn complex via a consecutive series of four oxidation steps with concomitant release of protons (Messinger and Renger 2008; Dau and Haumann 2008). Reduction of plastoquinone occurs at the Q_B site in the PQ/PQH_2 cavity via two-electron reduction from Q_A^- with a concomitant proton uptake (Petrouleas and Crofts 2005).

Cytochrome b_{559} (cyt b_{559}) is an intrinsic component of PSII tightly bound to D1 and D2 homologous proteins. It is a heme-bridged heterodimer consisting of α and β subunits, encoded by *psbE* and *psbF* genes (Babcock et al. 1985; Tae et al. 1988). The heme iron of cyt b_{559} is coordinated by two histidine residues; His^{22} and His^{17} of the α and β subunits located near to the stromal side and

oriented perpendicular to the membrane plane. The crystal structures of PSII from thermophilic cyanobacteria *Thermosynechococcus elongatus* and *Thermosynechococcus vulcanus* reveal that the heme iron is distanced at about 30 Å from the Q_B site and 50–60 Å from the water-splitting Mn complex (edge-to-edge distance) (Kamiya and Shen 2003; Ferreira et al. 2004; Loll et al. 2005; Guskov et al. 2009).

The light-induced reduction and oxidation of the heme iron is one of the important properties of cyt b_{559}, which is possibly involved in the regulation of photochemical efficiency in PSII (Whitmarsh and Pakrasi 1996; Stewart and Brudvig 1998; Faller et al. 2005). Within last two decades, a large effort has been made to characterize the actual reductant and oxidant of the heme iron in cyt b_{559} (Buser et al. 1992; Barber and De Las Rivas 1993; Faller et al. 2001; Tracewell et al. 2001). Supply of reducing and oxidizing equivalents on the electron acceptor and donor side of PSII has been shown as a major requirement for photoreduction and photooxidation of cyt $b_{559,}$ respectively. Based on the observation that DCMU diminished photoreduction of cyt b_{559}, it has been proposed that the bound plastosemiquinone Q_B^-, bound plastoquinol (Q_BH_2) or mobile plastoquinol (PQH_2) molecules provide an electron to cyt b_{559} (Buser et al. 1992). The finding that photoreduction of cyt b_{559} was observed in PSII reaction centers, which lacks PQ molecules, reveals that $Pheo^-$ is another potent candidate for the reduction of cyt b_{559} (Nedbal et al. 1992; Barber and De Las Rivas 1993; Ortega et al. 1995). Photooxidation of cyt b_{559} by $P680^+$ was demonstrated at cryogenic temperature, when electron donation form water-splitting Mn complex to $P680^+$ is inhibited (De Paula et al. 1985). Later, it has been specified that cyt b_{559} is oxidized by $P680^+$ with β-carotene (β-car) and monomeric chlorophyll z (Chlz) as an intermediate in a linear or a branched pathway (Hanley et al. 1999; Tracewell et al. 2001; Faller et al. 2001).

Recently, it has been demonstrated that in addition to intrinsic cofactors in PSII, superoxide anion radical ($O_2^{\bullet-}$) and its protonated form, perhydroxyl radical ($HO_2^{\bullet}$), serve as an exogenous reductant and oxidant of cyt b_{559}, respectively (Tiwari and Pospíšil 2009). The authors demonstrated that the reduction of ferric heme iron by $O_2^{\bullet-}$ occurs as an outer-sphere reaction, whereas the oxidation of ferrous heme iron by $HO_2^{\bullet}$ proceeds via the inner-sphere reaction. It was proposed that the IP form of cyt b_{559} serves as superoxide oxidase (SOO), whereas the HP form of cyt b_{559} acts as superoxide reductase (SOR).

The current work provides evidence that photoreduction and photooxidation of cyt b_{559} are brought about by the different mechanisms depending upon the integrity of PSII electron donor side. In PSII membranes with the intact water-splitting Mn complex, photoreduction of the heme iron was abolished by DCMU. On contrary, in the PSII membranes deprived of water-splitting Mn complex photoreduction and photooxidation of cyt b_{559} were prevented by exogenous SOD. Thus, it is evidenced here that in the PSII containing water-splitting Mn complex photoreduction of cyt b_{559} occurs via plastoquinol; however, it is mediated via $O_2^{\bullet-}$ in the PSII deprived of water-splitting Mn complex.

Materials and methods

PSII membranes preparation

PSII membranes were prepared from fresh spinach leaves purchased from a local market using the method of Berthold et al. (1981) with the modifications described in Ford and Evans (1983). PSII membrane were suspended in a buffer solution containing 400 mM sucrose, 10 mM NaCl, 5 mM $CaCl_2$ and 40 mM Mes-NaOH (pH 6.5) and stored at −80 °C at the final concentration of 3 mg Chl ml^{-1} until further use. PSII membranes deprived of water-splitting Mn complex and 17, 23 and 33 kDa extrinsic proteins were prepared by Tris treatment. PSII membranes (1 mg Chl ml^{-1}) were incubated in a buffer containing 0.8 M Tris-HCl (pH 8) for 30 min at 4 °C, in the darkness with continuous gentle stirring. After treatment, PSII membranes were washed twice in a medium containing 400 mM sucrose, 10 mM NaCl, 5 mM $CaCl_2$ and 40 mM Mes-NaOH (pH 6.5).

Optical measurements

Optical absorption spectroscopy was used to study redox properties of cyt b_{559} using Olis RSM 1000 spectrometer (Olis Inc., Bogart, Georgia, USA). The redox state and content of cyt b_{559} were determined form absorbance changes measured at 559 nm by additions of 20 μM potassium ferricyanide (reference cuvette), 8 mM hydroquinone, 5 mM sodium ascorbate, sodium dithionite (test cuvette) to PSII membranes (150 μg Chl ml^{-1}) in a 3 ml quartz cuvette at 20 °C. After addition of redox mediators, PSII membranes were slowly stirred for 5 min in the dark inside the spectrophotometer using a tiny bar magnet unless stated otherwise. After switching off the stirring, absorption spectra were recorded from 530 nm to 580 nm. The spectral slit width, the total band pass and the scan speed was 0.12 µm, 0.5 nm and 50 nm per min, respectively. The amount of different states of cyt b_{559} was calculated from the average spectra of five measurements. Different redox potential forms of cyt b_{559} were determined by treatment minus control spectrum. Total HP form of cyt b_{559} was determined by difference spectra of hydroquinone-reduced minus ferricyanide-oxidized cyt

b_{559}. The IP form of cyt b_{559} was determined by difference spectra of ascorbate-reduced minus hydroquinone-reduced cyt b_{559}, whereas the LP form of cyt b_{559} was obtained by difference spectra of dithionite-reduced minus ascorbate-reduced cyt b_{559}. The light-induced formation of the HP form of cyt b_{559} was obtained by difference of the absorbance spectrum measured after illumination for 100 s and the dark-adapted ferricyanide oxidized spectrum. Illumination was performed with continuous white light (1,000 μmol photons $m^{-2}s^{-1}$) by 90° rotating the cuvette at each 15 s interval using a halogen lamp with a light guide (Schott KL 1500, Schott AG, Mainz, Germany).

EPR spin-trapping spectroscopy

The spin-trapping was accomplished by EMPO, 5-(ethoxycarbonyl)-5-methyl-1-pyrroline N-oxide (Alexis Biochemicals, Lausen, Switzerland). PSII membrane particles (150 µg Chl ml^{-1}) were illuminated in a glass capillary tube (Blaubrand® intraMARK, Brand, Germany) in the presence of 25 mM EMPO, 100 µM desferal, 40 mM Mes (pH 6.5). Illumination was performed with continuous white light (1,000 μmol photons $m^{-2}s^{-1}$) using a halogen lamp with a light guide (Schott KL 1500, Schott AG, Mainz, Germany). Spectra were recorded at room temperature using EPR spectrometer MiniScope MS200 (Magnettech GmbH, Germany). EPR conditions were as follows: microwave power, 10 mW; modulation amplitude, 1 G; modulation frequency, 100 kHz; sweep width, 100 G; scan rate, 1.62 G s^{-1}.

Results

Characterization of redox form of cyt b_{559} in PSII membranes

In this study, spinach PSII membranes with a different integrity of PSII electron donor side were used to study the effect of illumination on redox properties of cyt b_{559}. Characterization of redox state of cyt b_{559} was performed by measuring the absorption changes at 559 nm after oxidation of cyt b_{559} with potassium ferricyanide and reduction by hydroquinone (HP form), sodium ascorbate (IP form) and sodium dithionite (LP form). In the PSII membranes with the intact water-splitting Mn complex, the composition of cyt b_{559} was determined as 38% of hydroquinone-reducible HP form, 26% of sodium ascorbate-reducible IP form and 36% sodium dithionite-reducible LP form (Fig. 1a, traces a, b, c, respectively). The PSII membranes deprived of water-splitting Mn complex by Tris treatment exhibited 47% IP and 53% LP form of cyt b_{559} (Fig. 1b).

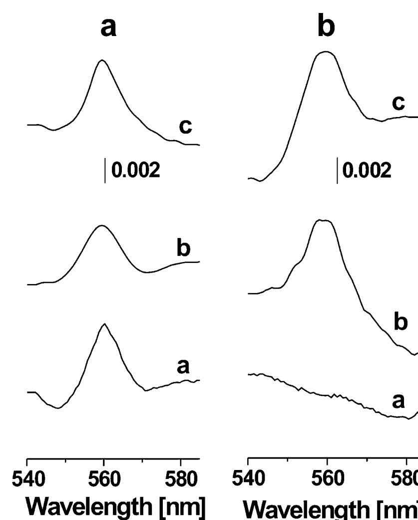

Fig. 1 Redox difference spectra of cyt b_{559} measured in spinach PSII membranes with the intact water-splitting Mn complex (**a**) and deprived of water-splitting Mn complex (**b**). The spectra represent the difference of hydroquinone-reduced minus ferricyanide-oxidized spectra (HP form of cyt b_{559}) (*a*), ascorbate-reduced minus hydroquinone-reduced spectra (IP form of cyt b_{559}) (*b*) and dithionite-reduced minus ascorbate-reduced spectra (LP form of cyt b_{559}) (*c*). The spectra were smoothed by using five points averaging of the experimental data by the Origin v4.1 software

These results confirm that removal of water-splitting Mn complex caused conversion of the HP form into the IP and the LP form of cyt b_{559}.

Light-induced redox changes of cyt b_{559} in the intact PSII membranes

Illumination of the PSII membranes with the intact water-splitting Mn complex caused reduction of cyt b_{559} (Fig. 2a, trace a). Figure 3a shows that photoreduction of cyt b_{559} has been reached within 100 s of continuous illumination and reduced form of cyt b_{559} was found stable within the whole period of illumination upto 300 s. The addition of hydroquinone in the sample after illumination for 100 s did not cause any further reduction of the heme iron (Fig. 2a, trace b), whereas sodium ascorbate and sodium dithionite resulted in reduction of cyt b_{559} (Fig. 2a, traces c and d). These results confirm that illumination of the PSII membranes with the intact water-splitting Mn complex caused photoreduction of the HP form of cyt b_{559}, whereas the IP and the LP forms of cyt b_{559} were unaffected.

Light-induced redox changes of cyt b_{559} in the Mn-depleted PSII membranes

Illumination of PSII membranes deprived of water-splitting Mn complex caused reduction of cyt b_{559} (Fig. 2b, trace a, solid line). Figure 3b shows that photoreduction of cyt b_{559} observed within 100 s of continuous illumination was followed by photooxidation of the heme iron. It has been recently

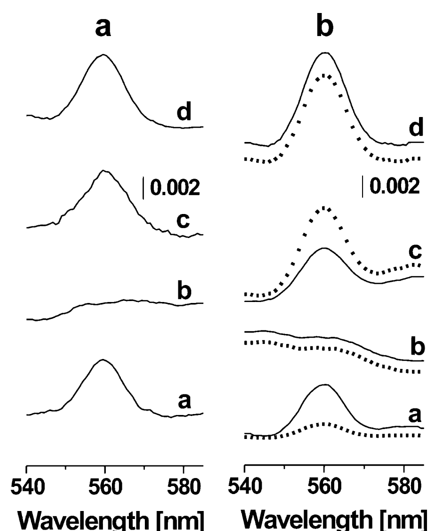

Fig. 2 Light-induced redox changes of cyt b_{559} measured in spinach PSII membranes with the intact water-splitting Mn complex (**a**) and deprived of water-splitting Mn complex (**b**). PSII membranes (100 µg Chl ml^{-1}) were illuminated with continuous white light (1,000 μmol photons m^{-2}s^{-1}) for 100 s (*solid line*) and 300 s (*dotted line*). The spectra represent the difference of light minus ferricyanide-oxidized spectra (*a*), hydroquinone-reduced minus light spectra (*b*), ascorbate-reduced minus hydroquinone-reduced spectra (*c*) and dithionite-reduced minus ascorbate-reduced spectra (*d*)

demonstrated that photoreduction and photooxidation of heme iron were followed by an upshift of *E*m to +310 mV and downshift of *E*m to +125 mV, respectively (Tiwari and Pospíšil 2009; Pospíšil and Tiwari 2010). The addition of hydroquinone in the sample after illumination for 100 s did not cause any further reduction of the heme iron (Fig. 2b, trace b, solid line), whereas it was reduced by sodium ascorbate and sodium dithionite (Fig. 2b, traces c and d, solid lines). After illumination for 100 s, the ascorbate-reducible form (Fig. 2b, traces c, solid lines) showed lowered value than the ascorbate-reducible form observed before illumination (Fig. 1b, traces c). The addition of hydroquinone in the sample after illumination for 300 s did not cause further reduction (Fig. 2b, trace b, dotted line), whereas sodium ascorbate and sodium dithionite resulted in reduction of cyt b_{559} (Fig. 2b, traces c and d, dotted lines). These results confirm that illumination of the PSII membranes deprived of water-splitting Mn complex caused reduction and oxidation of the IP and HP form of cyt b_{559}, respectively.

Effect of DCMU on photoreduction and photooxidation of cyt b_{559}

Further, the effect of DCMU on light-induced reduction and oxidation of cyt b_{559} was studied in the PSII membranes with the different integrity of PSII electron donor side. In the presence of DCMU, illumination of the PSII membranes with the intact water-splitting Mn complex for 100 s did not cause any photoreduction of the HP form of cyt b_{559} (Fig. 4a, trace a, Fig. 6a), whereas it was reduced by hydroquinone, sodium ascorbate and sodium dithionite (Fig. 4a, traces b-d). These results reveal that in the PSII membranes with the intact water-splitting Mn complex

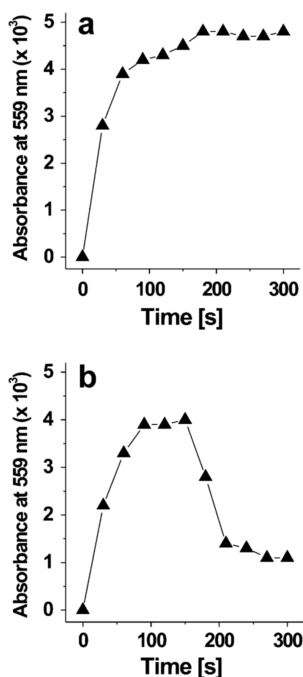

Fig. 3 Time course of light-induced redox changes of cyt b_{559} measured in spinach PSII membranes with the intact water-splitting Mn complex (**a**) and deprived of water-splitting Mn complex (**b**). The intensity of the absorption signal was calculated as the height of peak at 559 nm from the reference line connecting the lowest points near 545 and 575 nm. Each data point represents the mean value of at least three experiments

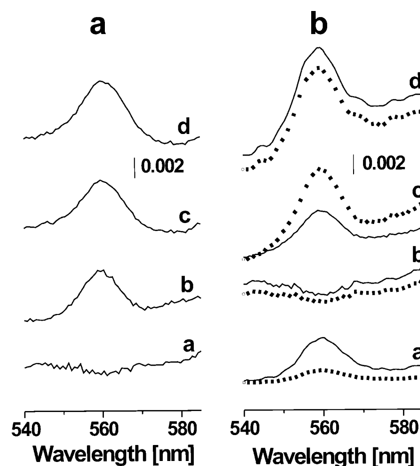

Fig. 4 Effect of DCMU on light-induced redox changes of cyt b_{559} measured in spinach PSII membranes with the intact water-splitting Mn complex (**a**) and deprived of water-splitting Mn complex (**b**). PSII membranes (100 µg Chl ml^{-1}) were illuminated with continuous white light (1,000 μmol photons m^{-2}s^{-1}) for 100 s (solid line) and 300 s (dotted line). Prior to illumination, 10 µM DCMU was added to PSII membranes. The spectra represent the difference of light minus ferricyanide-oxidized spectra (*a*), hydroquinone-reduced minus light spectra (*b*), ascorbate-reduced minus hydroquinone-reduced spectra (*c*) and dithionite-reduced minus ascorbate-reduced spectra (*d*)

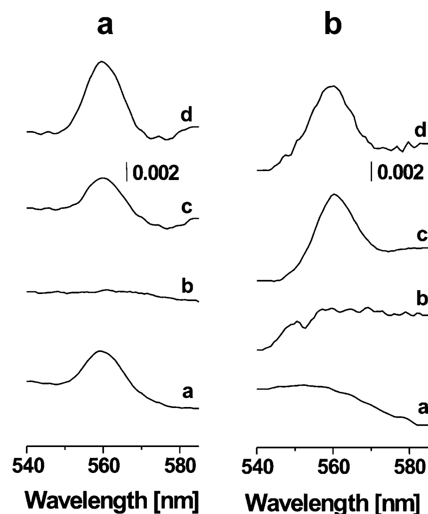

Fig. 5 Effect of SOD on light-induced redox changes of cyt b_{559} measured in spinach PSII membranes with the intact water-splitting Mn complex (**a**) and deprived of water-splitting Mn complex (**b**). PSII membranes (100 μg Chl ml^{-1}) were illuminated with continuous white light (1,000 μmol photons $m^{-2}s^{-1}$) for 100 s. Prior to illumination 400 U ml^{-1} SOD was added to PSII membranes. The spectra represent the difference of light minus ferricyanide-oxidized spectra (*a*), hydroquinone-reduced minus light spectra (*b*), ascorbate-reduced minus hydroquinone-reduced spectra (*c*) and dithionite-reduced minus ascorbate-reduced spectra (*d*)

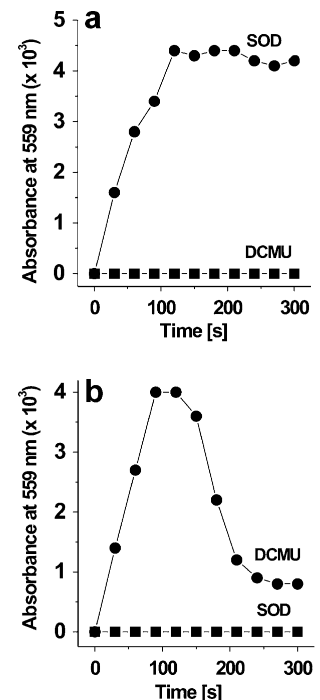

Fig. 6 Effect of DCMU and SOD on time course of light-induced redox changes of cyt b_{559} measured in spinach PSII membranes with the intact water-splitting Mn complex (**a**) and deprived of water-splitting Mn complex (**b**). Other experimental conditions were as described in Fig. 3

photoreduction of the heme iron was completely suppressed by DCMU. Contradictory, in the presence of DCMU illumination of PSII membranes deprived of water-splitting Mn complex caused photoreduction of cyt b_{559} (Fig. 4b, trace a, solid line, Fig. 6b), whereas further illumination upto 300 s resulted in photooxidation of cyt b_{559} (Fig. 4b, trace a, dotted line, Fig. 6b). Further addition of hydroquinone did not cause any reduction of cyt b_{559}, whereas the addition of sodium ascorbate and sodium dithionite caused reduction of cyt b_{559} (Fig. 4b, traces b-d). These results suggest that photoreduction and photooxidation of the heme iron in the Mn-depleted PSII membranes were unaffected by DCMU. Based on these observations, it is concluded that in the PSII membranes with the intact water-splitting Mn complex photoreduction of the heme iron is mediated by reducing compound formed at the Q_B site, whereas in the PSII membranes deprived of water-splitting Mn complex the heme iron is reduced by the compound formed prior to the Q_B site.

Effect of SOD on photoreduction and photooxidation of cyt b_{559}

To study the involvement of $O_2^{\bullet-}$ in light-induced redox changes of cyt b_{559}, the effect of exogenous SOD on photoreduction and photooxidation of the heme iron was measured in the PSII membranes with the different integrity of PSII electron donor side. It was observed that in the PSII membranes with the intact water-splitting Mn complex, photoreduction of the heme iron was unaffected by scavenging of $O_2^{\bullet-}$ using exogenous SOD (Fig. 5a, trace a, Fig. 6a). When hydroquinone was added, no further reduction of cyt b_{559} was observed (Fig. 5a, trace b), whereas the addition of sodium ascorbate and sodium dithionite caused reduction of cyt b_{559} (Fig. 5a, traces c and d). Contradictory, photoreduction and photooxidation of the heme iron of cyt b_{559} in the PSII membranes deprived of water-splitting Mn complex were completely diminished by exogenous SOD (Fig. 5b, trace a, Fig. 6b). Further addition of hydroquinone did not cause any reduction of cyt b_{559}, whereas the addition of sodium ascorbate and sodium dithionite causes reduction of cyt b_{559} (Fig. 5b, traces b-d). The effect of an exogenous SOD on photoreduction and photooxidation of the heme iron suggests that in the PSII membranes with the intact water-splitting Mn complex photoreduction of the heme iron is unaffected by $O_2^{\bullet-}$, whereas in the PSII membranes deprived of water-splitting Mn complex $O_2^{\bullet-}$ is involved in photoreduction and photooxidation of the heme iron. Based on these considerations it seems likely that in the PSII membranes deprived of water-splitting Mn complex, the heme iron of cyt b_{559} is reduced by $O_2^{\bullet-}$ formed prior to the Q_B site.

Light-induced production of $O_2^{\bullet-}$ in PSII membranes

Light-induced production of $O_2^{\bullet-}$ in PSII membranes was monitored using EPR spin-trapping spectroscopy. The spin-trapping was accomplished by spin trap compound EMPO known to react with $O_2^{\bullet-}$, forming the EMPO-OOH adduct. Exposure of the PS II membranes with the intact water-splitting Mn complex to continuous white light resulted in

the generation of EMPO-OOH adduct EPR spectra (Fig. 7a). Similarly, illumination of PSII membranes deprived of water-splitting Mn complex showed formation of EMPO-OOH adduct EPR spectra (Fig. 7b). The addition of exogenous SOD completely prevented the formation of EMPO-OOH adduct EPR spectra. Model EMPO-OOH adduct EPR spectrum was generated using xanthine/xanthine oxidase system. These results show that exposure of the both PSII membranes with the intact water-splitting Mn complex and PSII membranes deprived of water-splitting Mn complex caused $O_2^{\bullet-}$ production.

Discussion

Light-induced redox changes of cyt b_{559} were studied in the PSII membranes with the different integrity of PSII electron donor side. In agreement with previous reports, we have demonstrated that the exposure of PSII membranes to continuous white light caused reduction and oxidation of the heme iron (Buser et al. 1990; Buser et al. 1992; Barber and De Las Rivas 1993; Tiwari and Pospíšil 2009; Pospíšil and Tiwari 2010). We present here the convincing evidence that the mechanism of light-induced redox changes of cyt b_{559} depends upon the integrity of PSII electron donor side. We demonstrated that photoreduction of cyt b_{559} in the PSII membranes containing water-splitting Mn complex was supressed by DCMU (Fig. 4a), whereas in the PSII membranes lacking water-splitting Mn complex photoreduction and photooxidation of cyt b_{559} were abolished by exogenous SOD (Fig. 5b). Based on these observations, it is proposed that in PSII membranes with the intact water-splitting Mn complex photoreduction of the heme iron is mediated by plastoquinol, whereas in the PSII membranes deprived of water-splitting Mn complex, photoreduction and photooxidation are mediated by $O_2^{\bullet-}$ formed in PSII.

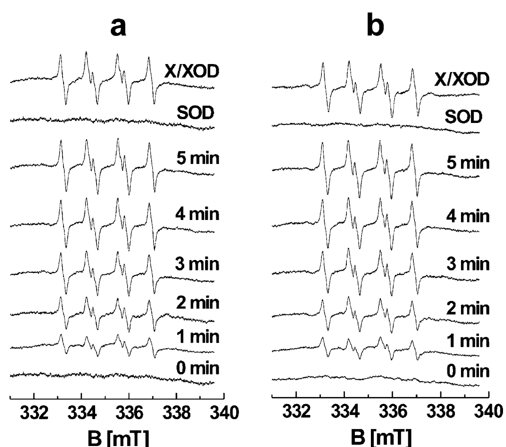

Fig. 7 Light-induced EPR spectra of the EMPO-OOH adduct measured in spinach PSII membranes with the intact water-splitting Mn complex (**a**) and deprived of water-splitting Mn complex (**b**). EPR spin-trapping spectra were obtained after the illumination of the PSII membranes (150 μg Chl ml^{-1}) with white light (1,000 μmol photons m^{-2}s^{-1}) in the presence of 25 mM EMPO, 100 μM desferal and 40 mM MES (pH 6.5). Superoxide anion radical production was completely prevented, when 500 U ml^{-1} SOD was added to the sample prior to the illumination. The upmost trace labeled X/XOD shows the EPR signal of the EMPO–OOH adduct generated by 1 mM xanthine and 50 U ml^{-1} xanthine oxidase

Photoreduction of cyt b_{559} by plastoquinol

Illumination of PSII membranes with the intact water-splitting Mn complex caused photoreduction of the HP form of cyt b_{559} (Fig. 2a). The observation that photoreduction of the heme iron of cyt b_{559} was completely suppressed by DCMU reveals that an electron carrier, which provides electron to the heme iron, is reduced at Q_B site. Based on the fact that the midpoint redox potential of the HP form of cyt b_{559} is 310–400 mV (pH 7) (Cramer and Whitmarsh 1977; Mizusawa et al. 1999; Roncel et al. 2003; Kaminskaya et al. 2007), the reduction of the HP form of cyt b_{559} by both plastosemiqunone (Q_B^-) and plastoquinol (Q_BH_2) is thermodynamically feasible. Due to the fact that midpoint redox potential of Q_B/Q_B^- redox couple is −45 mV (pH 7) (Hauska et al. 1983; Crofts and Wraight 1983), plastosemiquinone has redox power high enough for providing an electron to the HP form of cyt b_{559}. However, recent X-ray crystal structural analysis of PSII complexes in thermophilic cyanobacteria *Thermosynechococcus elongatus* showed that the heme iron is distanced at about 30 Å from the head group of plastoquinone bound at the Q_B site (Loll et al. 2005). On the opposite side, the fact that the midpoint redox potential of Q_B^-/Q_BH_2 redox couple is 290 mV (pH 7) (Hauska et al. 1983; Crofts and Wraight 1983) reveals that plastoquinol has less redox power for reduction of the HP form of cyt b_{559}. However, its ability to diffuse to the heme iron makes the plastoquinol more likely candidate for electron donation to the heme iron. Based on these considerations it seems likely that prior to the reduction of the heme iron, plastoquinol liberates from the Q_B site and diffuses to the vicinity of the heme iron. It has been recently proposed that exchange of plastoquinol molecule by plastoquinone at the Q_B site might occur via three possible mechanisms (Guskov et al. 2009). In the so-called altering and wriggling mechanisms, plastoquinol passes through the Q_C site, where the additional plastoquinone was proposed to be bound. Based on the X-ray PSII structural data (Guskov et al. 2009) the heme iron is distanced at 17 Å from the head group of plastoquinol bound at the Q_C site. It is proposed here that possible rebinding of plastoquinol molecule at Q_C site might provide an appropriate condition for electron donation to the heme iron.

Photoreduction and photooxidation of cyt b_{559} superoxide anion radical

Illumination of PSII membranes deprived of water-splitting Mn complex caused photoreduction and photooxidation of the IP and the HP form of cyt b_{559}, respectively (Fig. 2b). Based on the fact that the midpoint redox potential of the IP form of cyt b_{559} is 125–240 mV (pH 7) (Mizusawa et al. 1999; Roncel et al. 2003; Kaminskaya et al. 2007; Tiwari and Pospíšil 2009; Pospíšil and Tiwari 2010), the reduction of the IP form of cyt b_{559} by plastoquinol is not thermodynamically feasible and thus it is likely that another reductant might provide an electron to the heme iron. The observation that photoreduction and photooxidation of the heme iron of cyt b_{559} were completely diminished by exogenous SOD indicates that $O_2^{\bullet-}$ serves as reductant and oxidant of the heme iron. In the agreement with this proposal, we have recently demonstrated that $O_2^{\bullet-}$ interacts with the heme iron (Tiwari and Pospíšil 2009). It has been proposed that the IP form of cyt b_{559} serves as SOO known to catalyze the oxidation of $O_2^{\bullet-}$ to O_2. Based on the fact that the midpoint redox potential of $O_2/O_2^{\bullet-}$ redox couple is –160 mV (pH 7) (Wood 1987), the reduction of the IP form of cyt b_{559} by $O_2^{\bullet-}$ is thermodynamically feasible. On the other hand, the HP form of cyt b_{559} has been demonstrated to act as SOR known to catalyze the reduction of $O_2^{\bullet-}$ to H_2O_2. As the midpoint redox potential of $O_2^{\bullet-}/H_2O_2$ redox couple is 890 mV (pH 7) (Wood 1987), the oxidation of the HP form of cyt b_{559} by $O_2^{\bullet-}$ is favored from thermodynamic point of view. X-ray crystal structural analysis of PSII complexes showed that the heme iron is located in the vicinity of two channels, *i.e.* channel I and II (Guskov et al. 2009). The authors demonstrated that the channel I passes through the Q_C site, whereas both channels circumvent the Q_B site. It is proposed here that these channels facilitate diffusion of $O_2^{\bullet-}$ toward the heme iron and thus maintain reduction of the heme iron by $O_2^{\bullet-}$.

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