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Evidence that cytochrome *b*₅₅₉ is involved in superoxide production in Photosystem II: effect of synthetic short-chain plastoquinones in a cytochrome *b*₅₅₉ tobacco mutant

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Abbreviations used: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; desferal, deferoxamine mesylate; DPC, diphenylcarbazide; EMPO, 2-ethoxycarbonyl-2-methyl-3,4-dihydro-2*H*-pyrrole-1-oxide; EPR, electron paramagnetic resonance; PQ-*n*, plastoquinone with *n* isoprenoid units in the side chain; PSII, photosystem II; Q_A, primary quinone electron acceptor in PSII; Q_B, secondary quinone electron acceptor in PSII; ROS, reactive oxygen species.

ABSTRACT

Light-induced production of superoxide ($O_2^{\cdot-}$) in spinach PSII membrane particles was studied using EPR spin-trapping spectroscopy. The presence of exogenous plastoquinones with a different side chain length enhanced $O_2^{\cdot-}$ production in the following order: PQ-1>PQ-2>>PQ-9. In PSII membrane particles isolated from tobacco mutant of cytochrome b_{559} (cyt b_{559}) carrying single-point mutation in the β -subunit, which has also reduced amount of the α -subunit, the effect of PQ-1 was smaller than in the wild type. The increase in LP cyt b_{559} content, induced by incubation of spinach PSII membrane particles at low pH, resulted in a significant increase in $O_2^{\cdot-}$ formation in the presence of PQ-1, whereas it had little effect on $O_2^{\cdot-}$ production in the absence of PQ-1. The enhancement of $O_2^{\cdot-}$ formation induced by PQ-1 was not abolished by DCMU. Under anaerobic conditions, dark oxidation of LP cyt b_{559} increased as pH was decreased. The presence of molecular oxygen significantly enhanced dark oxidation of LP cyt b_{559} . Based on these findings it is suggested that short-chain plastoquinones stimulate $O_2^{\cdot-}$ production via mechanism that involves electron transfer from Pheo $^-$ to LP cyt b_{559} and subsequent autooxidation of LP cyt b_{559} .

INTRODUCTION

Photosystem II (PSII) is a pigment-protein complex embedded in thylakoid membranes of higher plants, algae and cyanobacteria. It is involved in conversion of light energy into chemical energy by transferring electrons from water to plastoquinone [1,2]. Cytochrome *b*₅₅₉ (cyt *b*₅₅₉), which is an integral part of PSII, mediates cyclic electron flow around PSII [3,4]. An important feature of cyt *b*₅₅₉ is its occurrence in different potential forms. Typically, these are a high-potential form (HP cyt *b*₅₅₉) with a midpoint redox potential of 330–400 mV and a low-potential form (LP cyt *b*₅₅₉), whose midpoint redox potential varies from 20 to 80 mV [5,6]. Under strong light illumination, LP cyt *b*₅₅₉ was found to accept electrons from Pheo preventing PSII against acceptor side photoinhibition [7]. Under conditions, when electron donation from water-oxidizing complex to P680 is inhibited, HP cyt *b*₅₅₉ reduces oxidizing species and prevents thus PSII against donor side photoinhibition [8].

Among several prenyllipids found in thylakoid membranes, the long-chain plastoquinones (PQ-9, PQ-B, PQ-C) are only believed to participate in the photosynthetic electron transport [9,10]. Free plastoquinones act as electron and proton carriers across the thylakoid membrane, whereas permanently (Q_A) and transiently (Q_B -binding site) bound plastoquinones serve as primary and secondary quinone electron acceptors, respectively. In our previous study, using synthetic short-chain plastoquinones (PQ-1, PQ-2), two specific binding sites in the vicinity of cyt *b*₅₅₉ have been proposed for natural long-chain plastoquinones [11]. Plastoquinone bound to the first binding site was suggested to mediate reduction of LP cyt *b*₅₅₉ via the semiquinone form, whereas PQ molecule bound to the other binding site was proposed to oxidize LP cyt *b*₅₅₉. Under aerobic conditions, molecular oxygen has been proposed to serve as electron acceptor from LP cyt *b*₅₅₉ [12].

Although the main site of superoxide ($O_2^{\cdot-}$) production in thylakoid membranes is photosystem I [13,14], the light-induced production of $O_2^{\cdot-}$ in PSII membrane particles was also demonstrated by an assay involving cytochrome *c* reduction in the presence of xanthine/xanthine oxidase [15] and by EPR spin-trapping spectroscopy [16]. The primary electron acceptor ($Pheo^-$) and the primary quinone electron acceptor (Q_A^-) were proposed to reduce O_2 [15,17].

In the present study, $O_2^{\cdot-}$ production was studied in PSII membrane particles using EPR spin-trapping spectroscopy in the presence of synthetic short-chain plastoquinones normally not present in the thylakoid membrane. In agreement with our previous work [11], we show

that short-chain plastoquinones facilitate electron transfer from Pheo⁻ to LP cyt *b*₅₅₉. Here, direct evidence is given that synthetic short-chain plastoquinones (PQ-1, PQ-2) enhance O₂^{-•} generation supporting the suggestion that LP cyt *b*₅₅₉ reduces molecular oxygen forming O₂^{-•}.

MATERIALS AND METHODS

Plant material – Spinach (*Spinacia oleracea*) plants were obtained from local market. Tobacco (*Nicotiana tabacum* cv. *Petit Havana*) plants were grown in a greenhouse in soil during summer. The tobacco mutant plant grew considerably more slowly than the wild-type.

Sample preparation – PSII membrane particles from spinach and tobacco were prepared using Triton X-100 purification according to the method of Berthold *et al.* [18] with modifications described in Ford and Evans [19]. PSII membrane particles were stored in resuspension medium containing 400 mM sucrose, 15 mM NaCl, 5 mM MgCl₂, and 40 mM Mes (pH 6.5) at -80 °C. Mn-depleted PSII membranes were prepared by incubation of PSII membrane particles in 0.8 M Tris (pH 8.0) for 20 min [20]. After this treatment, PSII membrane particles were washed several times in resuspension buffer and stored at -80°C. Plastoquinones were added to samples before illumination as ethanol solution (the final concentration of ethanol in the sample did not exceed 1%). Short-chain plastoquinones (PQ-1 and PQ-2) were a kind gift from prof. H Koike (Hyogo, Japan) and PQ-9 was obtained as described in Kruk [21].

Oxygen uptake – Oxygen uptake by PSII membrane particles (150 µg Chl ml⁻¹) was measured using Clark-type electrode (Hansatech, UK) under saturating white light in the presence of 400 mM sucrose, 15 mM NaCl, 5 mM MgCl₂ and 40 mM Mes (pH 6.5). To eliminate O₂ evolution, the measurements were performed in the absence of artificial electron acceptors.

Absorption spectroscopy – To monitor the redox state of cyt *b*₅₅₉, absorption difference spectra were measured in the range 520-590 nm under continuous stirring using SLM Aminco DW2000 spectrophotometer (Urbana, USA). The absorption changes at 559-570 nm were used to determine the level of individual cyt *b*₅₅₉ forms. Absorption spectra and kinetic measurements were acquired in a split beam and dual wavelength mode, respectively. During kinetic measurements, photoreduction of cyt *b*₅₅₉ was achieved using white light from a halogen lamp passed through a 630 nm cut-off filter. An interference filter ($\lambda_{\text{max}} = 564$ nm, $T_{\text{max}} = 30\%$, FWHM = 12 nm) was used to protect photomultiplier from the scattered light. To stabilize the baseline and ensure O₂ consumption by the oxygen trap, all samples were incubated for 10 min before measurements under continuous stirring.

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 $\mu\text{g Chl ml}^{-1}$) 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) or acetate buffer (pH 4.5). Illumination was performed with continuous white light (900 $\mu\text{E m}^{-2} \text{s}^{-1}$) using a halogen lamp with a light guide (KL 1500 electronic, Schott, Germany) and spectra were recorded using EPR spectrometer MiniScope MS200 (Magnettech GmbH, Germany). Signal intensity was evaluated as a relative height of the central doublet of the first derivate of the absorption spectrum. 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

Production of $\text{O}_2^{\bullet-}$ in PSII membrane particles

The light-induced formation of $\text{O}_2^{\bullet-}$ in PSII membrane particles was measured by EPR spin-trapping spectroscopy using EMPO spin-trap compound. Due to high stability of EMPO-OOH adduct (five-fold more stable than the $\text{O}_2^{\bullet-}$ adduct of the commonly used spin trap DMPO), EMPO is suitable for $\text{O}_2^{\bullet-}$ detection on the time scale of several minutes [22]. In the dark, addition of EMPO to PSII membrane particles induced no EPR signal, whereas illumination of the sample in the presence of EMPO led to appearance of EPR signal of the EMPO-OOH adduct (Fig. 1). The model EPR spectrum of the EMPO-OOH adduct obtained using the xanthine/xanthine oxidase system is presented in Fig. 1. The four-line spectra exhibit all the characteristics of EPR spectra of the EMPO-OOH adduct as published in the literature [22].

Effect of plastoquinones on $\text{O}_2^{\bullet-}$ production

To test the involvement of plastoquinones in $\text{O}_2^{\bullet-}$ production, time dependence of EPR signal intensity of the EMPO-OOH adduct was measured in PSII membrane particles in the presence of exogenous plastoquinones. As evident from Fig. 2, intensity of EPR signal of the EMPO-OOH adduct was enhanced in the presence of plastoquinones in the following order: PQ-1 > PQ-2 >> PQ-9. In the presence of PQ-9, production of $\text{O}_2^{\bullet-}$ increased only slightly, whereas short-chain plastoquinones stimulated $\text{O}_2^{\bullet-}$ production by 53% after 10 min of illumination.

The effect of PQ-1 concentration on $O_2^{\cdot-}$ production is shown in Fig. 3. The increase in PQ-1 concentration resulted in the enhancement of EPR signal of the EMPO-OOH adduct. The production of $O_2^{\cdot-}$ was saturated at PQ-1 concentration about 30-40 μ M, which corresponds to prenyllipid/chlorophyll molar ratio of about 1:5.

Effect of PQ-1 on $O_2^{\cdot-}$ production in the cyt b_{559} tobacco mutant

Tobacco mutant, in which phenylalanine at position 26 in the β -subunit was changed to serine [23], was used to study the involvement of cyt b_{559} in $O_2^{\cdot-}$ production in the presence of PQ-1. It has been shown that tobacco mutant exhibits reduced amount of cyt b_{559} [24]. We have determined that in PSII membrane particles isolated from tobacco mutant, the total amount of cyt b_{559} is one seventh of the wild-type tobacco (Chl/cyt $b_{559} \sim 1850$). In PSII membrane particles isolated from tobacco mutant, PQ-1 caused only about 15 % increase in $O_2^{\cdot-}$ production after 10 min of illumination (Fig. 4), whereas in PSII membrane particles isolated from the wild-type of tobacco, the stimulation was similar to spinach PSII membrane particles (data not shown). However, the overall $O_2^{\cdot-}$ production in PSII membrane particles of tobacco mutant was higher compared to wild-type tobacco and spinach PSII membranes. The increased leakage of electrons to O_2 in PSII membrane particles of the mutant, might be explained by altered PSII assembly due to changes in the hydrophobic stretch in transmembrane helix of the β -subunit of cyt b_{559} .

Effect of pH on $O_2^{\cdot-}$ production

To figure out the actual electron donor to O_2 , the effect of pH on EPR signal intensity of the EMPO-OOH adduct was studied (Fig. 5). As demonstrated in Table 1, control PSII membrane particles at pH 6.5 contain 28 % of LP form cyt b_{559} , which is in agreement with the literature data [12,25]. When pH was lowered to 4.5, the amount of LP cyt b_{559} raised to about half of the total content of cyt b_{559} (Table 1). In the presence of PQ-1, the drop in pH to values close to 4.5 caused enhancement of $O_2^{\cdot-}$ formation, whereas in the absence of exogenous plastoquinones, $O_2^{\cdot-}$ production was unaffected in a pH range from 4.5 to 6.5 (Fig. 5). The decrease in EPR signal intensity of the EMPO-OOH adduct observed below pH 4.5 and above pH 6.5 was caused by a decrease in PSII electron transport activity (data not shown).

The effect of various plastoquinones on time dependence of EPR signal intensity of the EMPO-OOH adduct was measured at pH 4.5 (Fig. 6). During 2-3 minutes of illumination more than three-fold increase in $O_2^{\cdot-}$ production was observed in the presence of PQ-1,

whereas in the presence of PQ-9, EPR signal of the EMPO-OOH adduct was increased by about 50 %. The decrease in EPR signal intensity of the EMPO-OOH adduct observed for a longer period of illumination is probably caused by reduction of the adduct by an unspecified reductant generated in the sample during illumination.

Effect of oxygen on dark oxidation of LP cyt b_{559}

To test the effect of low pH on the oxidation of cyt b_{559} , time dependence of absorption changes at 559-570 nm were measured in the presence of PQ-1 (Fig. 7). Due to the fact that no photoreduction of LP cyt b_{559} was detectable under aerobic conditions [11], dark oxidation of LP cyt b_{559} was measured in the presence of glucose/glucose oxidase/catalase system, which is known to remove O_2 . When pH was decreased, cyt b_{559} oxidation was enhanced (Table 2). The enhancement of cyt b_{559} oxidation at low pH is probably caused by an increase in the amount of LP cyt b_{559} , as well as by the upshift of the redox potential of PQ/PQH₂ couple, which is the electron acceptor from LP cyt b_{559} under anaerobic conditions. It has been shown that, on the contrary to HP cyt b_{559} , the LP cyt b_{559} can be oxidized directly by molecular oxygen and plastoquinones [12].

To study oxidation of LP cyt b_{559} under aerobic conditions, H_2O_2 was injected into the sample at the offset of illumination (Fig. 7). Due to presence of catalase in the sample, molecular oxygen was produced immediately after H_2O_2 injection. The applied amount of H_2O_2 was adjusted to give 250 μM oxygen concentration in the sample as checked by Clark type electrode (data not shown). In the presence of O_2 , the cyt b_{559} oxidation evidently increased when compared to anaerobic conditions (Table 2).

Effect of plastoquinones on O_2 uptake

To quantify the effect of plastoquinones on $O_2^{\cdot -}$ production, O_2 uptake was measured in PSII membrane particles. In the absence of plastoquinones, the rate of oxygen consumption in control PSII membrane particles was about 2 $\mu mol O_2 (mg Chl)^{-1} h^{-1}$ (Table 1). Low pH incubation resulted in the enhancement of O_2 uptake. In accordance with EPR spin-trapping data, more than three-fold increase in the rate of oxygen consumption was observed in the presence of PQ-1 at pH 4.5. When PQ-2 was added, O_2 consumption was enhanced by 50%, whereas PQ-9 caused only 21% enhancement of O_2 consumption at low pH.

In further experiments, O_2 uptake was measured in Tris-washed PSII membrane particles that contain higher amount of LP cyt b_{559} as compared to control PSII membrane particles

(Table 1). Similarly to low pH data, PQ-1 caused more than three-fold increase in the rate of O_2 consumption. Almost three-fold increase in O_2 uptake was observed also for PQ-2, whereas PQ-9 stimulated O_2 uptake by 33 % (Table 1).

Effect of DCMU on $O_2^{\cdot-}$ production

EPR spectra of the EMPO-OOH adduct were measured also in the presence of DCMU (Fig. 8). In the absence of PQ-1, addition of DCMU lowered EPR signal intensity of the EMPO-OOH adduct by 50% (Fig. 8). Similarly, when PQ-1 was added, DCMU lowered the EPR signal intensity by 50% (Fig. 8). It has been suggested that the decrease in $O_2^{\cdot-}$ production in the presence of DCMU is caused by suppression of charge separation due to the electrostatic effect of negatively charged Q_A^- (Pospíšil and Rutherford, unpublished results). These results suggest that PQ-1-induced enhancement of $O_2^{\cdot-}$ formation is not connected with the Q_B -binding site.

DISCUSSION

Production of $O_2^{\cdot-}$ by PSII

In the present study, production of $O_2^{\cdot-}$ in PSII membrane particles was studied using EPR spin-trapping spectroscopy. As demonstrated in Fig. 1, illumination of PSII membrane particles resulted in generation of $O_2^{\cdot-}$. It has been previously suggested that Pheo $^-$ and Q_A^- may serve as electron donors to molecular oxygen [17–19]. Pheo $^-$ has the highest reduction power ($E_m = -610$ mV, pH 7); however, the reduction of O_2 by Pheo $^-$ is limited due to fast electron transfer from Pheo $^-$ to Q_A^- (300–500 ps) [2]. On the other hand, reduction of O_2 by Q_A^- is less favourable from a thermodynamic point of view (the midpoint redox potential of Q_A^-/Q_A redox couple $E_m = -80$ mV, pH 7); however, it better fits kinetic criteria (time constant of electron transfer from Q_A^- to $Q_B^- \sim 200$ –400 μ s) [26]. Recently, we have proposed that the dominant reductant of O_2 changes over the time course of photoinhibition [16]. It has been suggested that Q_A^- serves as a reductant to O_2 in the early phase, whereas later it is Pheo $^-$ that donates electrons to O_2 .

Role of plastoquinones in $O_2^{\cdot-}$ production

The main objective of this work was to investigate the effect of plastoquinones on $O_2^{\cdot-}$ production in PSII membrane particles. In our previous work, we have demonstrated that in

the presence of short-chain plastoquinones, LP cyt b_{559} is reduced upon illumination and reoxidized to the original level in the dark [11]. Based on these results, it has been suggested that short-chain plastoquinones bound in the vicinity of cyt b_{559} facilitate electron flow from Pheo⁻ to LP cyt b_{559} . Recently determined crystal structure of PSII [27,28] shows that the edge-to-edge distance between Pheo⁻ and LP cyt b_{559} is too long to maintain direct electron transport. The structure data support our suggestion that plastoquinones might sufficiently fulfil this gap and serve as electron carriers between Pheo⁻ and LP cyt b_{559} .

The finding that short-chain plastoquinones are considerably more effective in the stimulation of O₂⁻ production than the long-chain PQ-9 (Fig. 6) is in agreement with our earlier observation that the rate of LP cyt b_{559} photoreduction increases with decreasing side chain length of plastoquinones [11].

Role of LP cyt b_{559} in O₂⁻ production

It has been shown that site directed mutagenesis of cyt b_{559} , which affects a highly hydrophobic stretch in the transmembrane helix of the β -subunit, results in lowering of the cyt b_{559} content [23,24]. The observation that in PSII membrane particles isolated from tobacco mutant, the stimulatory effect of PQ-1 on O₂⁻ production was suppressed, indicates evidently that cyt b_{559} is involved in O₂⁻ formation in the presence of PQ-1.

It is well known that in native PSII, cyt b_{559} is present mainly in the reduced HP form [3]. Based on difference absorption spectra of cyt b_{559} in the α -band region, we have determined that our PSII membrane particles contain about 28 % of cyt b_{559} in the LP form (Table 1) that is oxidizable by O₂. In order to increase the level of LP cyt b_{559} , PSII membrane particles were incubated at low pH. It has been demonstrated that the HP form of cyt b_{559} is unstable at low pH and is partially converted to the LP form [29]. After low pH incubation of PSII membrane particles, the amount of LP cyt b_{559} increased to about a half of the total content of cyt b_{559} (Table 1).

The observation that at pH close to 4.5, production of O₂⁻ was significantly enhanced in the presence of short-chain plastoquinones, suggests participation of LP cyt b_{559} in this stimulatory effect (Fig. 5–6). In accordance with this finding, it has been demonstrated that in the absence of O₂, autooxidation of LP cyt b_{559} increased at low pH (Fig. 7). It is suggested that under anaerobic conditions it is PQ that oxidizes LP cyt b_{559} . When O₂ was present, the rate of LP cyt b_{559} autooxidation was enhanced even more due to electron transfer to O₂ (Fig. 7).

In Tris-treated PSII membrane particles, the effects of PQ-1 and PQ-2 on $O_2^{\bullet-}$ production were greatly increased compared to untreated PSII. In Tris-treated PSII, almost all of cyt b_{559} is in the LP form [30, 31]. Our observation that the increase in the level of LP cyt b_{559} resulted in enhancement of $O_2^{\bullet-}$ production supports our idea that LP cyt b_{559} is involved in $O_2^{\bullet-}$ formation.

Our previous finding that under aerobic conditions, no photoreduction of LP cyt b_{559} was detected, indicates that LP cyt b_{559} is fast reoxidized by O_2 [12]. Our results presented in this study showed that exogenously added short-chain plastoquinones significantly enhanced $O_2^{\bullet-}$ production by PSII. This finding indicates that one-electron reduced plastoquinones are able to reduce LP cyt b_{559} , which in turn undergoes spontaneous autooxidation resulting in $O_2^{\bullet-}$ formation (Scheme 1).

Physiological relevance

In the presented study, production of $O_2^{\bullet-}$ by LP cyt b_{559} was stimulated by synthetic short-chain plastoquinones. Due to their increased polarity and smaller molecular size, they are able to bind in the vicinity of the heme of LP cyt b_{559} at the relatively polar membrane region [32, 33]. Even though short-chain plastoquinones are not natural components of thylakoid membranes, the involvement of native, long-chain plastoquinones in $O_2^{\bullet-}$ production may have relevance under physiological conditions.

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FIGURE LEGENDS

Figure 1 Light-induced EPR spectra of EMPO-OOH adduct measured in spinach PSII membrane particles after illumination for the time indicated.

EPR spectra were obtained after illumination of PSII membrane particles with white light ($900 \mu\text{E m}^{-2} \text{s}^{-1}$) in the presence of 25 mM EMPO, 100 μM desferal, 150 $\mu\text{g Chl ml}^{-1}$ and 40 mM Mes (pH 6.5). The upmost trace labelled x/xo shows EPR signal of the EMPO-OOH adduct generated by xanthine/xanthine oxidase system.

Figure 2 Time dependence of EPR signal intensity of EMPO-OOH adduct measured in spinach PSII membrane particles in the presence of plastoquinones with different side chain length.

PSII membrane particles were illuminated with white light ($900 \mu\text{E m}^{-2} \text{s}^{-1}$) in the presence of 25 mM EMPO, 100 μM desferal, 150 $\mu\text{g Chl ml}^{-1}$ and 40 mM Mes (pH 6.5). Plastoquinones were added before illumination at the concentration of 33 μM .

Figure 3 Effect of various PQ-1 concentrations on EPR signal intensity of EMPO-OOH adduct measured in spinach PSII membrane particles.

PSII membrane particles were illuminated with white light ($900 \mu\text{E m}^{-2} \text{s}^{-1}$) for 5 min in the presence of 25 mM EMPO, 100 μM desferal, 150 $\mu\text{g Chl ml}^{-1}$, 40 mM Mes (pH 6.5) and PQ-1 as indicated.

Figure 4 Time dependence of EPR signal intensity of EMPO-OOH adduct measured in tobacco mutant PSII membrane particles in the absence or presence of 33 μM PQ-1.

Experimental conditions as in Fig. 2.

Figure 5 Effect of pH on EPR signal intensity of EMPO-OOH adduct measured in spinach PSII membrane particles in the absence and presence of 33 μM PQ-1.

PSII membrane particles were illuminated with white light ($900 \mu\text{E m}^{-2} \text{s}^{-1}$) for 2.5 min in the presence of 25 mM EMPO, 100 μM desferal, 150 $\mu\text{g Chl ml}^{-1}$, 40 mM acetate buffer (pH 3.5 – 5.0) or 40 mM Mes (pH 5.5 – 6.5) or 40 mM Hepes (pH 7.0 – 8.0).

Figure 6 Time dependence of EPR signal intensity of EMPO-OOH adduct measured at pH 4.5 in spinach PSII membrane particles in the presence of plastoquinones with different side chain length.

PSII membrane particles were illuminated with white light ($900 \mu\text{E m}^{-2} \text{s}^{-1}$) in the presence of 25 mM EMPO, 100 μM desferal, 150 $\mu\text{g Chl ml}^{-1}$ and 40 mM acetate buffer (pH 4.5). Plastoquinones were added before illumination at the concentration of 33 μM .

Figure 7 Effect of molecular oxygen on photoreduction and dark oxidation of cyt b_{559} measured in spinach PSII membrane particles in the presence of PQ-1 at different pH.

The kinetics of reduction and oxidation of cyt b_{559} was measured by absorption changes at 559-570 nm after onset (\uparrow) and offset (\downarrow) of the illumination, respectively. Under anaerobic conditions, the measurements were performed using the oxygen trap (1 mM glucose, 50 U ml^{-1} glucose oxidase, 500 U ml^{-1} catalase) in the presence of 33 μM PQ-1, 1 mM MnCl_2 , 0.2% Triton, 40 mM acetate buffer (pH 4.5) or 40 mM Mes (pH 5.5 and 6.5) or 40 mM Hepes (pH 7.5). To achieve aerobic conditions, H_2O_2 was injected into the measuring assay at the end of illumination period to give 0.8 mM H_2O_2 final concentration. Other experimental conditions were the same as under anaerobic conditions.

Figure 8 Effect of DCMU on EPR spectra of EMPO-OOH adduct in spinach PSII membrane particles measured at pH 4.5 in the absence and presence of PQ-1.

PSII membrane particles were illuminated with white light ($900 \mu\text{E m}^{-2} \text{s}^{-1}$) for 2.5 min in the presence of 25 mM EMPO, 100 μM desferal, 150 $\mu\text{g Chl ml}^{-1}$ and 40 mM acetate buffer (pH 4.5). Prior to illumination, 33 μM PQ-1 and 10 μM DCMU were added into the reaction mixture.

Table 1 Effects of various plastoquinones on oxygen uptake measured in control, low pH-incubated and Tris-treated PSII membranes.

Oxygen uptake was measured under saturating white light in the presence of either 400 mM sucrose, 15 mM NaCl, 5 mM MgCl₂ and 40 mM Mes (pH 6.5) (control and Tris-treated PSII membrane particles) or 400 mM sucrose, 15 mM NaCl, 5 mM MgCl₂ and 40 mM acetate buffer (pH 4.5) (low pH incubation). In Tris-treated PSII membranes, electron transport was supported by the addition of 1 mM DPC. The values in brackets represent percent values of O₂ uptake measured in the absence of plastoquinones. The amount of HP cyt *b*₅₅₉ and LP cyt *b*₅₅₉ was determined from difference absorption spectra at 559-570 nm. The amount of HP cyt *b*₅₅₉ was calculated from absorbance change after addition of potassium ferricyanide (control-FeCy), whereas the content of oxidized HP and LP cyt *b*₅₅₉ was estimated from absorbance change after sequential reduction by hydroquinone (HQ-control) and sodium ascorbate (Asc-HQ), respectively. No oxidized HP cyt *b*₅₅₉ was found.

	control PSII	low pH-incubated PSII	TRIS-treated PSII
LP cyt <i>b</i> ₅₅₉	28 %	49%	75%
O ₂ uptake (μmol O ₂ (mg chl) ⁻¹ h ⁻¹)			
without PQ	2.18 (100%)	5.69 (100%)	6.01 (100%)
+ 33 μM PQ-9	2.45 (112%)	6.86 (121%)	7.99 (133%)
+ 33 μM PQ-2	2.66 (122%)	8.56 (150%)	16.43 (274%)
+ 33 μM PQ-1	2.76 (126%)	18.48 (324%)	20.00 (333%)

Table 2: Effect of oxygen on dark oxidation of LP cyt b_{559}

The rate of dark oxidation LP cyt b_{559} expressed as half-time of absorption changes at 559-570 nm after offset of illumination.

	$\tau_{1/2}$ (s)	
	anaerobic	aerobic
pH = 7.5	107.5	21.7
pH = 6.5	36.1	11.4
pH = 5.5	12.6	3.5
pH = 4.5	6.7	1.8

Scheme 1 The involvement of PQ-1 in $O_2^{\cdot -}$ production in PSII

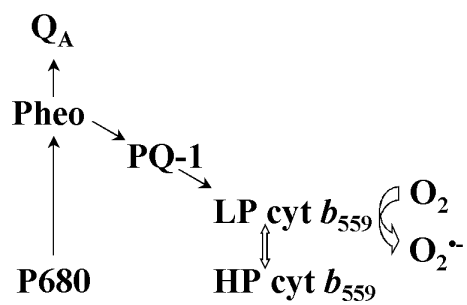


Figure 1

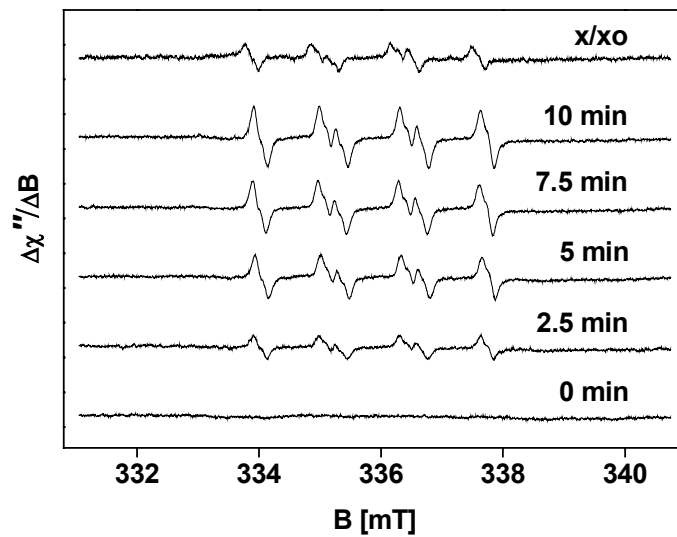


Figure 2

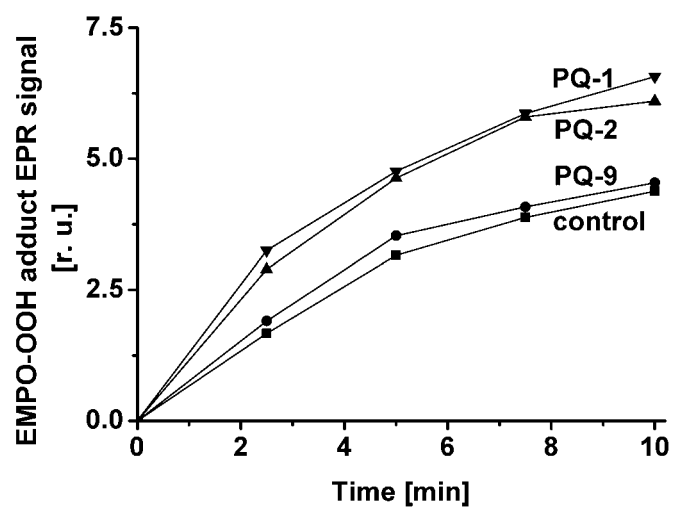


Figure 3

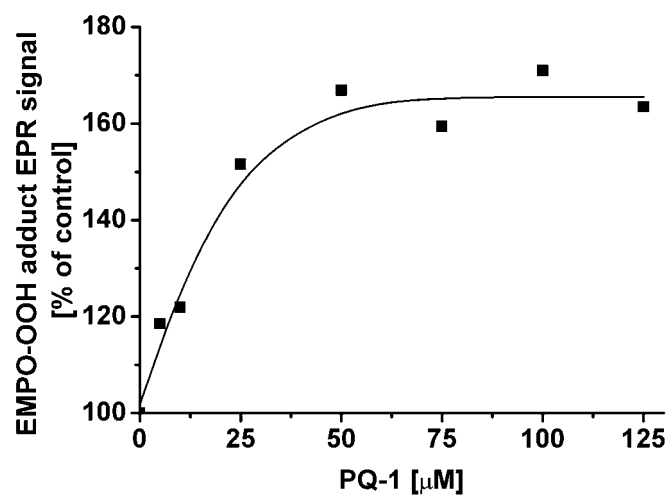


Figure 4

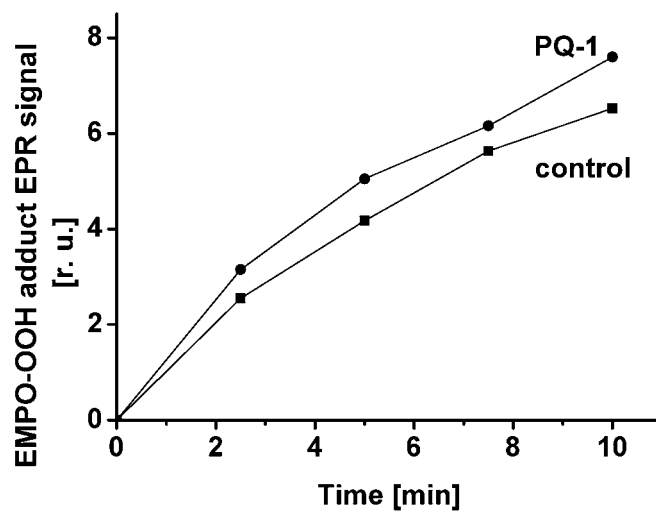


Figure 5

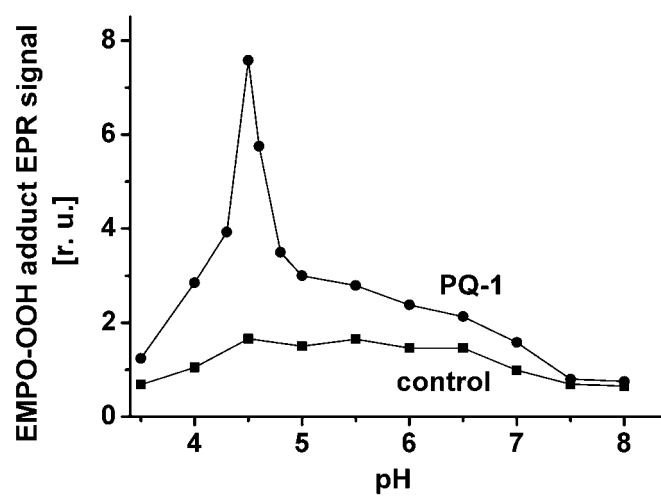


Figure 6

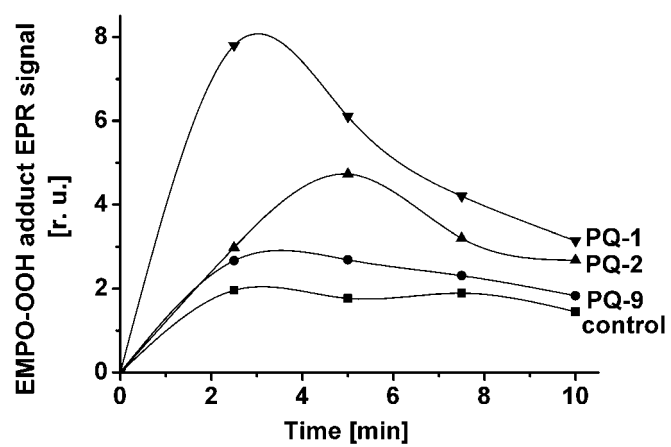


Figure 7

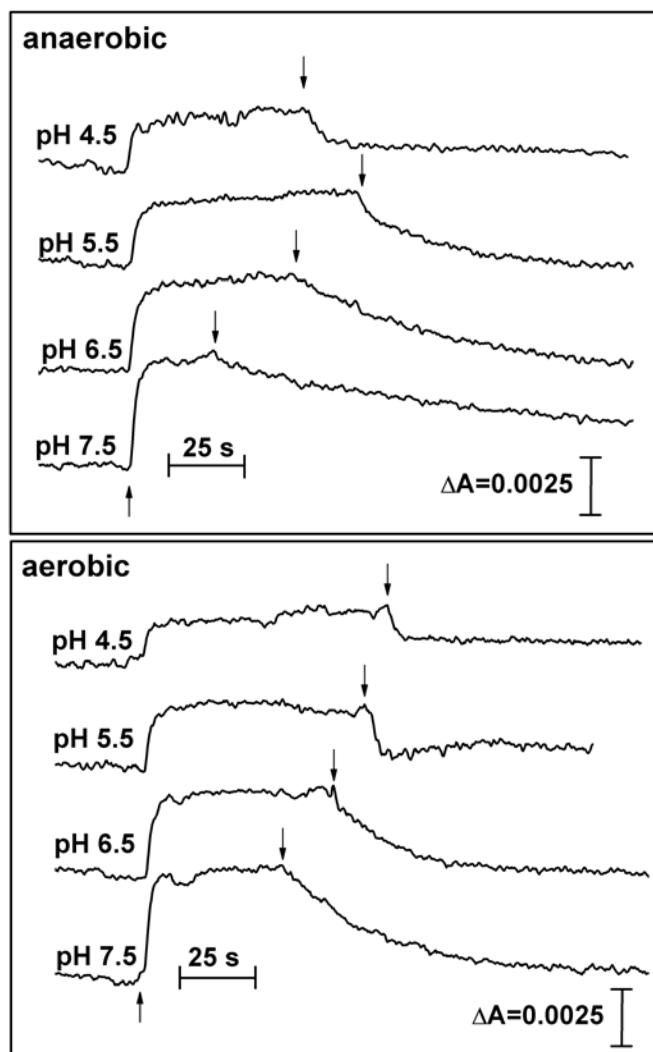


Figure 8

