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Singlet oxygen scavenging activity of plastoquinol in photosystem II of higher plants: Electron paramagnetic resonance spin-trapping study

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ABSTRACT

Singlet oxygen ($^1O_2$) scavenging activity of plastoquinol in photosystem II (PSII) of higher plants was studied by electron paramagnetic resonance (EPR) spin-trapping technique. It is demonstrated here that illumination of spinach PSII membranes deprived of intrinsic plastoquinone results in $^1O_2$ formation, as monitored by TEMPONE EPR signal. Interestingly, the addition of exogenous plastoquinol (PQH_2-1) to PQ-depleted PSII membranes significantly suppressed TEMPONE EPR signal. The presence of exogenous plastoquinols with a different side-chain length (PQH_2-n, n isoprenoid units in the side chain) caused a similar extent of $^1O_2$ scavenging activity. These observations reveal that plastoquinol exogenously added to PQ-depleted PSII membranes serves as efficient scavenger of $^1O_2$.

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1. Introduction

Photosystem II (PSII) is a pigment–protein complex embedded in the thylakoid membrane of higher plants, algae and cyanobacteria. It is involved in the conversion of light energy into chemical energy by electron transfer from water to plastoquinone [1,2]. When higher plants, algae and cyanobacteria are exposed to high-light intensity illumination, PSII activity is inhibited in a process called photoinhibition [3–5]. Photoinhibition is considered to be caused by damage to the D1 protein, which forms a heterodimer with the D2 protein along with the subsequent rapid degradation of the D1 protein [6–9]. Damage to PSII is thought to be due to the action of reactive oxygen species (ROS) formed on the electron acceptor side of PSII, when the plastoquinones Q_A and Q_B are highly reduced [10–13]. Singlet oxygen ($^1O_2$) generated from triplet chlorophyll species is considered as the main ROS responsible for PSII damage [14–16]. Recently, formation of hydroxyl radical as another ROS responsible for PSII damage has been demonstrated on PSII electron acceptor site [17,18].

Singlet oxygen is generated by the interaction of molecular oxygen with the excited triplet state of chlorophyll formed via charge recombination pathway [10–12]. The charge recombination of radical pair $^3[P680^+Pheo^-]$ results in the formation of triplet excited state $^3P680^*$ [19,20]. When $^3P680^*$ is not efficiently scavenged by carotenoids or α-tocopherol, the interaction of $^3P680^*$ with triplet molecular oxygen $^3O_2$ results in the formation of $^1O_2$.

Singlet oxygen scavenging by carotenoids and α-tocopherols occurs either by excitation energy transfer (physical scavenging) or by electron transport reaction (chemical scavenging) [21–23]. In the physical type of $^1O_2$ scavenging, denoted as $^1O_2$ quenching, the excitation energy transfer from $^1O_2$ to quencher (carotenoid and α-tocopherol) results in the formation of the triplet excited state of the quencher, while $^3O_2$ is formed. Subsequently, the triplet excitation energy of the quencher is lost as heat. Typically, one molecule of quencher can deactivate several hundreds of $^1O_2$ molecules [24]. In the chemical type of $^1O_2$ scavenging, the interaction of $^1O_2$ with scavenger (α-tocopherol) was shown to form intermediate 8-hydroperoxy-tocopherone known to hydrolyze irreversibly to α-tocopherolquinone [25]. On the contrary, α-tocopherol is destroyed and thus a continuous re-synthesis of α-tocopherol is required to keep its level sufficient for the photoprotection [26,27].

Singlet oxygen scavenging activity of plastoquinol molecules in thylakoid membrane, as well as in the liposomes, has been proposed in the previous studies [28–30]. Later, we have demonstrated that the addition of plastoquinone homologues to *Chlamydomonas reinhardtii* grown in the presence of a plastoquinone inhibitor prevented damage of D1 protein [27]. Recently, plastoquinol has been shown to decompose under illumination in *Chlamydomonas reinhardtii* cells as a consequence of reaction with $^1O_2$ generated in PSII [31]. The authors demonstrated that due to the irreversible oxidation of plastoquinol,

Abbreviations: PSII, photosystem II; MES, 4-morpholineethanesulfonic acid; TMPD, 2, 2, 6, 6-tetramethyl-4-piperidone; TEMPONE, 2, 2, 6, 6-tetramethyl-4-piperidone-1-oxyl; PQH_2, plastoquinol; DanePy, (3-[N-(β-diethylaminoethyl)-N-dansyl]amino-methyl-2,2,5,5-tetra-methyl-2,5-dihydro-1H-pyrrole)

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the continuous re-synthesis of plastoquinol is required to keep its level sufficient in PSII. Recently, we have demonstrated that plastoquinol is an active $^1O_2$ scavenger in organic solvents of different polarity [25]. As far as we know, no evidence has been provided on the $^1O_2$ scavenging activity of plastoquinol in higher plant PSII.

In the present work, we provide evidence on the $^1O_2$ scavenging activity of plastoquinol using electron paramagnetic resonance (EPR) spin-trapping spectroscopy. It is demonstrated here that the addition of exogenous plastoquinols to PQ-depleted PSII membranes scavenged $^1O_2$. On the basis of these observations, we conclude that plastoquinol serves as an efficient $^1O_2$ scavenger in PSII of higher plants.

2. Materials and methods

2.1. PSII membranes preparation

PSII membranes were isolated from fresh spinach leaves purchased from a local market using the method of Berthold et al. [32] with the modifications described in Ford and Evans [33]. PSII membranes suspended in a buffer solution containing 400 mM sucrose, 10 mM NaCl, 5 mM $CaCl_2$, 5 mM $MgCl_2$ and 50 mM MES-NaOH (pH 6.5) were stored at −80 °C at the final concentration of 3 mg Chl ml^{-1} until further use. PQ-depleted PSII membranes were prepared by plastoquinone extraction in a heptane-isobutanol solvent system using the methods of Wydrzynski and Inoue [34]. PSII membranes (0.3 mg Chl ml^{-1}) in plastic tube were shaken with heptane (the ratio of heptane/sample 3:1) and isobutanol (the ratio of isobutanol/sample 1:30) for 40 min. After formation of two phases, the lower phase (PSII membranes) was separated from the upper organic phase and bubbled with air for 10 minutes to remove the residual organic phase. PQ-depleted PSII membranes were stored at −80 °C at the final concentration of 3 mg Chl ml^{-1} until further use.

2.2. Singlet oxygen generation by rose bengal photosensitization

For the photosensitized generation of $^1O_2$, 5 μM rose bengal (Sigma) in 25 mM phosphate buffer (pH 7.0) was illuminated for 10 min with a continuous white light (1000 μmol photons m^{-2} s^{-1}) using a halogen lamp with a light guide (Schott KL 1500, Schott AG, Mainz, Germany). The absorption of light by rose bengal leads to a singlet excited state of the sensitizer, which is subsequently converted into its triplet excited state by intersystem crossing. In the presence of molecular oxygen, the triplet excited state of the rose bengal reacts with the ground triplet state of molecular oxygen. This process returns the sensitizer to its singlet ground state, while $^1O_2$ is formed [35,36].

2.3. EPR spin-trapping spectroscopy

The spin-trapping was accomplished by hydrophilic spin trap compound TMPD, (2, 2, 6, 6-Tetramethyl-4-piperidone) (Sigma). PQ-depleted PSII membranes (150 μg Chl ml^{-1}) were illuminated in the presence of 50 mM TMPD and 40 mM MES-NaOH (pH 6.5). Illumination was performed with continuous white light (1000 μmol photons m^{-2} s^{-1}) using a halogen lamp with a light guide (Schott KL 1500, Schott AG, Mainz, Germany). After illumination the sample was centrifuged at 5000*g* for 3 min to separate TEMPONE from the PQ-depleted PSII membranes. The separation of two phases was carried out to prevent the reduction of TEMPONE by non-specific reducing component in the PQ-depleted PSII membranes. After centrifugation, the upper phase was immediately transferred into the glass capillary tube (Blaubrand® intraMARK, Brand, Germany) and frozen in liquid nitrogen until the use. Prior to data collection, the capillary tube was taken away from the liquid nitrogen and EPR spin-trapping data were collected at room temperature. Spectra were recorded using EPR spectrometer MiniScope MS200 (Magnettech GmbH, Germany). Signal intensity was evaluated as a height of the central peak of EPR 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}.

3. Results

3.1. Effect of PQH_2-1 on rose bengal-induced $^1O_2$ formation

Effect of short-chain plastoquinol (PQH_2-1) on $^1O_2$ generation by photosensitization of rose bengal was studied using EPR spin-trapping technique. Spin trapping was accomplished by utilizing the oxidation of diamagnetic 2, 2, 6, 6-tetramethyl-4-piperidone (TMPD) by $^1O_2$ to yield paramagnetic 2, 2, 6, 6-tetramethyl-4-piperidone-1-oxyl (TEMPONE) [37]. Addition of TMPD spin trap compound to rose bengal in the dark did not result in the appearance of TEMPONE EPR spectra (Fig. 1). Small TEMPONE EPR signal observed in the dark was due to impurity of the spin trap. Illumination of rose bengal in the presence of molecular oxygen resulted in the formation of TEMPONE EPR signal (Fig 1). When rose bengal was illuminated in the presence of PQH_2-1, TEMPONE EPR signal was significantly suppressed. The observation that the addition of PQH_2-1 to paramagnetic TEMPONE does not affect TEMPONE EPR signal reveals that PQH_2-1 was not able to reduce TEMPONE to diamagnetic hydroxylamine (data is not shown). These observations indicate that plastoquinol serves as an efficient scavenger of $^1O_2$ generated from the photoactivation of rose bengal.

3.2. Singlet oxygen formation in PQ-depleted PSII membranes

In order to study selectively $^1O_2$ scavenging activity of the exogenous plastoquinol in spinach PSII membranes, $^1O_2$ formation was measured in PSII membranes deprived of the intrinsic plastoquinone and other prenyllipid (e.g. α-tocopherol) by the heptane/isobutanol extraction procedure. Fig. 2A shows TEMPONE EPR spectra measured in PQ-depleted PSII membranes. The addition of TMPD to PQ-depleted PSII membranes in the dark did not result in the appearance of TEMPONE EPR signal. Small TEMPONE EPR signal observed in non-illuminated PQ-depleted PSII membranes was due to impurity of the spin trap compound. Exposure of PQ-depleted PSII membranes to continuous white light resulted in the generation of TEMPONE EPR spectra. Due to the fact that TMPD is a hydrophilic nitroxide spin trap, the formation of TEMPONE EPR signal reflects $^1O_2$ production predominantly in the polar phase. When the height of the TEMPONE EPR signal was plotted against the illumination time, the remarkable enhancement in the TEMPONE EPR signal was observed in the initial period of illumination, whereas moderate increase in the TEMPONE EPR signal was detected during the later period of

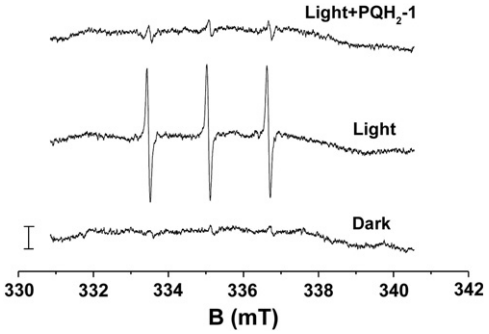

Fig. 1. Effect of exogenous plastoquinol (PQH_2-1) on the rose bengal-induced TEMPONE EPR spectra. Rose bengal (5 μM) was illuminated with white light (1000 μmol m^{-2} s^{-1}) for 10 min in the presence of 50 mM TMPD and 25 mM phosphate buffer (pH 7.0). Prior to illumination, 100 μM PQH_2-1 was added to the reaction mixture. Vertical bar represents 1000 relative units.

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