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. Interestingly, 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 Cytochrome $b_{559}$ · Photosystem II · Redox potential · Water-splitting manganese complex

Abbreviations
cyt $b_{559}$ cytochrome $b_{559}$
$E_m$ 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

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 QA$^+$ 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 $\alpha$ and $\beta$ 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 $\alpha$ and $\beta$ 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 Q₈ 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; Gusakov et al. 2009).

The light-induced reduction and oxidation of the heme iron is one of the important properties of cyt *b₅₅₉*, 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 *b₅₅₉* (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 *b₅₅₉*, respectively. Based on the observation that DCMU diminished photoreduction of cyt *b₅₅₉*, it has been proposed that the bound plastosemiquinone Q₈, bound plastoquinol (Q₈H₂) or mobile plastoquinol (PQH₂) molecules provide an electron to cyt *b₅₅₉* (Buser et al. 1992). The finding that photoreduction of cyt *b₅₅₉* was observed in PSII reaction centers, which lacks PQ molecules, reveals that Pheo⁺ is another potent candidate for the reduction of cyt *b₅₅₉* (Nedbal et al. 1992; Barber and De Las Rivas 1993; Ortega et al. 1995). Photooxidation of cyt *b₅₅₉* 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 *b₅₅₉* 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 (O₂⁻*) and its protonated form, perhydroxyl radical (HO₂⁺), serve as an exogenous reductant and oxidant of cyt *b₅₅₉*, respectively (Tiwari and Pospíšil 2009). The authors demonstrated that the reduction of ferric heme iron by O₂⁻* occurs as an outer-sphere reaction, whereas the oxidation of ferrous heme iron by HO₂⁺ proceeds via the inner-sphere reaction. It was proposed that the IP form of cyt *b₅₅₉* serves as superoxide oxidase (SOO), whereas the HP form of cyt *b₅₅₉* acts as superoxide reductase (SOR).

The current work provides evidence that photoreduction and photooxidation of cyt *b₅₅₉* 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 *b₅₅₉* were prevented by exogenous SOD. Thus, it is evidenced here that in the PSII containing water-splitting Mn complex photoreduction of cyt *b₅₅₉* occurs via plastoquinol; however, it is mediated via O₂⁻* 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 CaCl₂ and 40 mM Mes-NaOH (pH 6.5) and stored at −80 °C 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 CaCl₂ and 40 mM Mes-NaOH (pH 6.5).

**Optical measurements**

Optical absorption spectroscopy was used to study redox properties of cyt *b₅₅₉* using Olis RSM 1000 spectrometer (Olis Inc., Bogart, Georgia, USA). The redox state and content of cyt *b₅₅₉* 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 *b₅₅₉* was calculated from the average spectra of five measurements. Different redox potential forms of cyt *b₅₅₉* were determined by treatment minus control spectrum. Total HP form of cyt *b₅₅₉* was determined by difference spectra of hydroquinone-reduced minus ferricyanide-oxidized cyt