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Differential mechanism of light-induced and oxygen-dependent restoration of the high-potential form of cytochrome b_{559} in Tris-treated Photosystem II membranes

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ABSTRACT

The effect of illumination and molecular oxygen on the redox and the redox potential changes of cytochrome b_{559} (cyt b_{559}) has been studied in Tris-treated spinach photosystem II (PSII) membranes. It has been demonstrated that the illumination of Tris-treated PSII membranes induced the conversion of the intermediate-potential (IP) to the reduced high-potential (HP^{Fe2+}) form of cyt b_{559}, whereas the removal of molecular oxygen resulted in the conversion of the IP form to the oxidized high-potential (HP^{Fe3+}) form of cyt b_{559}. Light-induced conversion of cyt b_{559} from the IP to the HP form was completely inhibited above pH 8 or by the modification of histidine ligand that prevents its protonation. Interestingly, no effect of high pH or histidine modification was observed during the conversion of the IP to the HP form of cyt b_{559} after the removal of molecular oxygen. These results indicate that conversion from the IP to the HP form of cyt b_{559} proceeds via different mechanisms. Under illumination, conversion of the IP to the HP form of cyt b_{559} depends primarily on the protonation of the histidine residue, whereas under anaerobic conditions, the conversion of the IP to the HP form of cyt b_{559} is driven by higher hydrophobicity of the environment around the heme iron resulting from the absence of molecular oxygen.

1. Introduction

Light-driven water oxidation and plastoquinone reduction is catalyzed by photosystem II (PSII) embedded in the thylakoid membrane of higher plants, algae, and cyanobacteria [1–3]. An essential part of PSII is cytochrome b_{559} (cyt b_{559}), which is a heme-bridged protein consisting of α and β subunits [4–6]. It has been shown that the heme iron is coordinated by two histidine residues, His^{22} of the α and His^{17} of the β subunit of the heterodimer [7–9]. Crystal structures of PSII from thermophilic cyanobatecteria *Thermosynechococcus elongatus* and *Thermosynechococcus vulcanus* revealed that the heme is located near the stromal side of the membrane and is oriented perpendicular to the plane of the membrane [10–13]. The most recent crystal structure provides a more detailed map of cyt b_{559} in relation to the third quinone-binding Q_C site and binding of plastoquinone molecule to the Q_C site [14].

Cytochrome b_{559} can exist in several forms that differ in the redox potential properties of the heme iron: the high-potential (HP) form (midpoint redox potential *E*m ~ +400 mV), the intermediate-potential (IP) form (*E*m ~ +200 mV) and the low-potential (LP) form (*E*m ~ +50 mV) [15–18]. Several hypotheses have been proposed to explain the existence of different redox potential forms of cyt b_{559}: 1) orientation of the two histidine imidazole rings [7], 2) variation in the nature of the heme coordination [17], 3) different hydrophobicity of the heme environment [19], and 4) protonation state of the histidine imidazole ring [16,18,20]. It has been proposed that the protein environment around the heme iron in the HP form of cyt b_{559} is supposed to be more hydrophobic with a lower dielectric constant, whereas it is less hydrophobic and more open to the polar medium in the LP form of cyt b_{559} [18,19]. It has been suggested previously that binding of the PQ to the Q_C site is essential for the establishment of the HP form of cyt b_{559} [21,22]. The structural details of the Q_C site provided by recent crystal structure [14] show that the isoprenoid chain of a PQ molecule bound at the Q_C site is located close to the heme of cyt b_{559}. This finding strongly supports the hypothesis that the maintenance of the hydrophobic environment of the heme is necessary for the stability of the HP form of cyt b_{559}. The protonation of the imidazole group of histidine residues and the formation of hydrogen bonds with the polypeptide backbone are prerequisites for the conversion of cyt b_{559} into its HP form [18]. It has been proposed earlier that in the HP form of cyt b_{559}, hydrogen bonds are present between the imidazole NH of the both histidine residues and carbonyl groups of the peptide backbone. However, in the LP form of cyt b_{559}

Abbreviations: Capso, N-cyclohexyl-2-hydroxyl-3-aminopropanesulfonic acid; Chl, chlorophyll; cyt b_{559}, cytochorome b_{559}; DEPC, diethylpyrocarbonate; *E*m, midpoint redox potential; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HP^{Fe3+}, high-potential form of cyt b_{559} with oxidized heme iron; HP^{Fe2+}, high-potential form of cyt b_{559} with reduced heme iron; IP, intermediate-potential form of cyt b_{559}; LP, low-potential form of cyt b_{559}; MDGD, monogalactosyldiglycerol; MES, 2-[N-Morpholino]ethanesulfonic acid; PSII, photosystem II; Tricine, N-[tris (hydroxymethyl)methyl]glycine; SQDG, sulfoquinovosyldiacylglycerol

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one of these hydrogen bonds is missing [20]. Thus, the additional hydrogen bond was proposed to stabilize the HP form of cyt b_{559} by maintaining hydrophobic environment around the heme iron [18].

In active PSII membranes, cyt b_{559} is present in the reduced HP form (70%), whereas the remaining 30% of cyt b_{559} is in the oxidized LP form [16–18]. Several harsh treatments, which destabilize the integrity of PSII, are known to convert cyt b_{559} from the HP to the LP form [21–25]. One such treatment is the incubation of PSII membranes with Tris, which results in the dissociation of the water-splitting manganese complex on the PSII electron donor side. In Tris-treated PSII membranes, the reduced HP form of cyt b_{559} is absent and cyt b_{559} is present only in the oxidized LP form [26–29]. However, illumination or removal of molecular oxygen from Tris-treated PSII membranes was shown to convert the LP form of cyt b_{559} back into the HP form typical for untreated PSII [26–31]. Mizusawa et al. demonstrated that either continuous or pulsed light restored the HP form of cyt b_{559} during photoactivation [26,30,31]. Reversible transition between the LP and the HP form of cyt b_{559} was shown under transition from aerobic to anaerobic or oxidizing to reducing conditions in Tris-treated PSII membranes [28]. Recently, detailed study on the redox changes of cyt b_{559} from the IP to the HP form in PSII membranes with a different degree of disintegration of PSII electron donor side was presented [29]. As far as we know, the effect of pH on redox changes of cyt b_{559} induced by light or removal of molecular oxygen in Tris-treated PSII membranes has not been studied yet.

In this work, the effect of pH and histidine modification on the light- and oxygen-dependent redox changes of cyt b_{559} was studied in Tris-treated PSII membranes. We have demonstrated that the light-induced conversion of cyt b_{559} from the IP to the HP form is suppressed by high pH and histidine modification, whereas it was independent of pH and histidine modification, when induced by removal of molecular oxygen. Thus, based on the redox state of the heme iron and on the difference in pH dependence of these conversions, it has been suggested that the mechanism of conversion of cyt b_{559} from the IP to the HP form by illumination and by removal of molecular oxygen could be entirely different.

2. Materials and methods

2.1. PSII membrane preparation

PSII membranes were prepared from dark-adapted spinach leaves using the method of Berthold et al. [32] with the modifications described in Ford and Evans [33]. The isolated PSII membranes were stored at −80 °C in suspending medium containing 400 mM sucrose, 10 mM NaCl, 5 mM $CaCl_2$ and 40 mM Mes–NaOH (pH 6.5) until further use. Tris-treated PSII membranes were prepared by incubation of PSII membranes (1 mg Chl ml^{-1}) in a buffer containing 0.8 M Tris–HCl (pH 8) for 30 min at 4 °C, in the darkness under continuous gentle stirring. After the treatment, PSII membranes were washed twice in the suspending medium. All the measurements were performed using the same suspending medium (pH 6.5), unless stated otherwise.

2.2. Anaerobic conditions

Anaerobic conditions were established by mixing 50 U ml^{-1} glucose oxidase (EC 1.1.3.4, from *Aspergillus niger*) (Fluka), 500 U ml^{-1} catalase (EC 1.11.1.6, from Bovine liver) (Sigma) and 5 mM glucose with the suspending medium (pH 6.5). During addition of the oxygen trap, air was removed from the upper part of the cuvette by a gentle stream of argon and the cuvette was sealed with the help of a 1 cm thick rubber cap and paraffin wax. To maintain anaerobic conditions, reducing agents were added in the sealed cuvette with the help of a micro-syringe.

2.3. Histidine modification treatment

Chemical modification of the imidazole rings of the histidine residues was performed using diethylpyrocarbonate (DEPC) (Aldrich) according to the method of Hegde et al. [34] with modification described in Roncel et al. [18]. Tris-treated PSII membranes (50 μg Chl ml^{-1}) were treated with 5 mM DEPC for 15 min at 25 °C under stirring. The reaction was terminated by the addition of a large volume of histidine solution to PSII membranes to the final concentration of 20 mM and incubated for 1 min in the dark. After incubation, PSII membranes were washed twice and re-suspended in 50 mM Mes (pH 5.5). Selective modification of the imidazole groups using histidine-modifying reagent DEPC caused carbethoxylation of imidazole rings. Diethylpyrocarbonate binds to the imidazole group as a covalent probe of protein surface structure and prevents its protonation by protons in the medium.

2.4. Optical measurements

Redox properties of cyt b_{559} were monitored by following the absorbance changes using Olis RSM 1000 spectrometer (Olis Inc., Bogart, Georgia, USA) as described [26,27]. Tris-treated PSII membranes (250 μg Chl ml^{-1}) were treated with 50 μM potassium ferricyanide (reference cuvette) and 8 mM hydroquinone, 5 mM sodium ascorbate or sodium dithionite (test cuvette) in a 3 ml quartz cuvette (path length 1 cm) at 20 °C. Addition of each reductant was followed by dark adaptation for 5 min under continuous slow stirring 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 bandpass and the scan speed were 0.12 mm, 0.5 nm and 50 nm per min, respectively. The amount of cyt b_{559} was calculated on the basis of its molar extinction coefficient (17.5 mM^{-1} cm^{-1}). The observed ratio of cyt b_{559}:Chl was 1:230. The amount of cyt b_{559} in different forms was determined from difference spectra observed in the presence of particular oxidant and reductant (average from 5 measurements). Total HP form was determined from a difference spectrum of hydroquinone-reduced minus ferricyanide-oxidized cyt b_{559}. The IP form was determined by difference spectra of ascorbate-reduced minus hydroquinone-reduced cyt b_{559}. The LP form was obtained by dithionite-reduced minus ascorbate-reduced spectrum. Previously, dithionite-reducible form has been considered as very low-potential form (VLP); however, the nomenclature used in this study to describe different forms of cyt b_{559} is according to the recently established facts [28,29], which is based on their redox potential range obtained from potentiometric titration. The light-induced formation of the HP form of cyt b_{559} was monitored as a difference between the dark-adapted ferricyanide-oxidized spectrum and the absorption spectrum measured after 100 s of illumination. Illumination was performed with continuous white light (1000 μmol photons m^{-2} s^{-1}) using halogen cold light source (Schott KL 1500, Schott AG, Mainz, Germany) by 90° rotating the cuvette at each 15 s intervals.

2.5. Potentiometric measurements

Potentiometric redox titrations of Tris-treated PSII membranes (250 μg Chl ml^{-1}) were performed at room temperature as described previously [18], using potentiometer Oxi 340i (WTW GmbH, Weilheim, Germany) equipped with the Pt–Ag/AgCl redox microelectrode (Pt 5900 A, Schott Instruments GmbH, Mainz, Germany). Potentiometric measurements were performed simultaneously with measurements of absorbance changes at 559 nm. Redox microelectrode (5 mm diameter) was placed inside the test cuvette of the spectrophotometer in such a way that it did not affect the light path. For anaerobic titration, molecular oxygen was removed from the cuvette using glucose/glucose oxidase/catalase system as described in the previous section. Prior to the reductive titration, PSII

membranes were oxidized with 2 mM potassium ferricyanide (Em = +430 mV, pH 7). Reductive titrations were carried out in the presence of the following redox mediators: 20 μM p-benzoquinone (Em = +280 mV, pH 7), 20 μM 2,5-dimethyl-p-benzoquinone (Em = +180 mV, pH 7), 20 μM N-methylphenazonium methosulfate (Em = +80 mV, pH 7), 20 μM sodium ascorbate (Em = +60 mV, pH 7), and 20 μM sodium dithionite (Em = −660 mV, pH 7). Stirring was 'switched on' before the addition of the mediators and 'switched off' before starting the measurements. The dilution resulting from the titration was compensated by the addition of buffer solution in the reference cuvette. Midpoint redox potential and fraction of reduced cyt b_{559} were determined by fitting of the redox titration curve to Nernst equation in accordance with one-electron process (n = 1) for one or three redox components.

3. Results

3.1. Light-induced conversion of cyt b_{559} from the IP to the HP form

In this study, Tris-treated PSII membranes deprived of water-splitting manganese complex and 17, 23, 33 kDa extrinsic polypeptides were used to study the effect of illumination on cyt b_{559}. Redox properties of cyt b_{559} were monitored by measuring the absorbance changes at 559 nm after oxidation of cyt b_{559} with potassium ferricyanide and reduction by hydroquinone (the HP form), sodium ascorbate (the IP form) and sodium dithionite (the LP form). In the dark, Tris-treated PSII membranes contained the dithionite-reducible LP form and the ascorbate-reducible IP form of cyt b_{559}, whereas the hydroquinone-reducible HP form of cyt b_{559} was absent (Fig. 1A, left column). Upon illumination of Tris-treated PSII membranes for 100 s, the reduced HP form (HP^{Fe2+}) of cyt b_{559} was formed, whereas the ascorbate-reducible IP form of cyt b_{559} was decreased (Fig. 1A, middle column). Potentiometric redox titration of illuminated Tris-treated PSII membranes performed under aerobic conditions confirmed the appearance of the HP form of cyt b_{559} with Em ~ +310 mV (Fig 1B, inset, closed square). Due to the interference of molecular oxygen below the ambient potential of +100 mV, the potentiometric redox titration under aerobic conditions was performed up to the ambient potential of +100 mV. Thus, the IP and the LP forms could not be detected under these conditions. Potentiometric redox titration of Tris-treated PSII membranes measured under aerobic conditions in the dark did not show any redox potential form at ambient potential above +100 mV (Fig. 1B, inset, open square).

3.2. Conversion of cyt b_{559} from the IP to the HP form under anaerobic conditions

Furthermore, the effect of molecular oxygen on redox properties of cyt b_{559} was studied in Tris-treated PSII membranes. The removal of molecular oxygen using glucose/glucose oxidase/catalase system lowered the content of the ascorbate-reducible IP form of cyt b_{559}, whereas the hydroquinone-reducible HP form of cyt b_{559} appeared (Fig. 1, right column). Therefore, it can be concluded that the removal of molecular oxygen resulted in partial conversion of cyt b_{559} from the IP to the oxidized HP form (HP^{Fe3+}). Potentiometric redox titration of Tris-treated PSII membranes under anaerobic conditions showed the existence of three redox forms with midpoint redox potential of +306 mV, +140 mV and −42 mV (Fig. 1B).

To quantify the effect of molecular oxygen on the conversion of cyt b_{559} from the IP to the HP form, the formation of the HP form of cyt b_{559} was studied under different concentrations of molecular oxygen. The concentration of molecular oxygen was regulated in the solution by changing the concentration of glucose substrate in glucose/glucose oxidase/catalase system. The conversion of cyt b_{559} from the IP to the HP form was triggered by lowering the concentration of molecular oxygen by approx. 20% (down to ~220 μM) and the complete conversion was achieved by about 40% drop in oxygen concentration (down to ~168 μM) (Fig. 2).

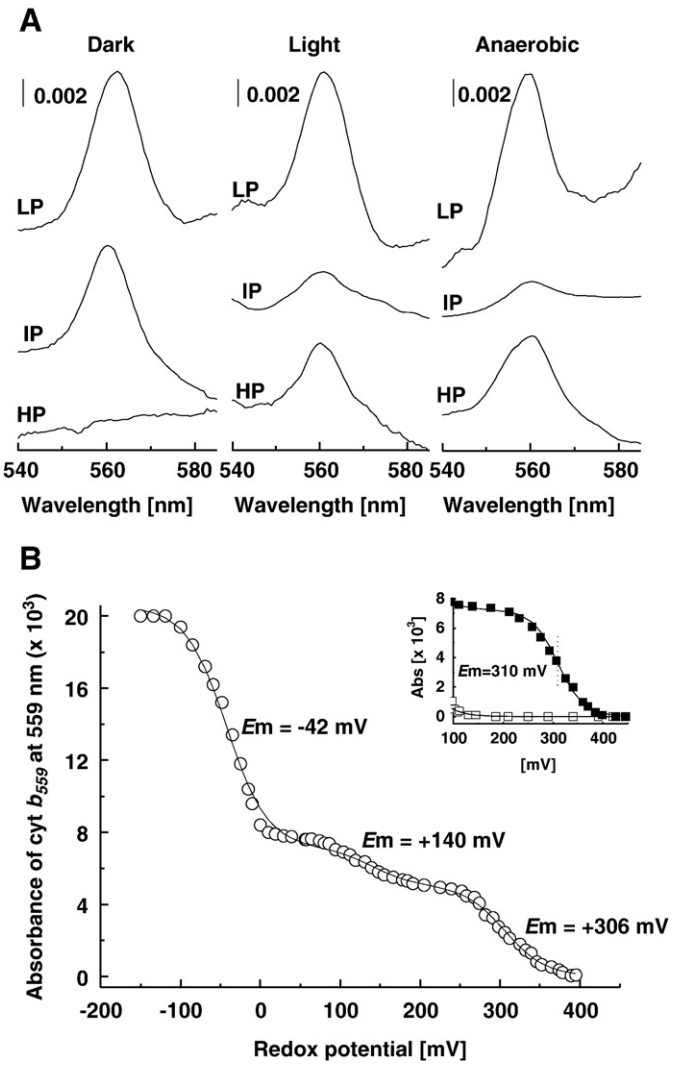

Fig. 1. (A), Redox changes of cyt b_{559} measured in Tris-treated PSII membranes in the dark (left column), after illumination for 100 s (middle column) and after removal of molecular oxygen (right column). In the middle column, sample was illuminated for 100 s with continuous white light (1000 μmol photons m^{-2} s^{-1}). In the right column, molecular oxygen was removed by using oxygen trap (5 mM glucose, 50 U ml^{-1}, glucose oxidase, 500 U ml^{-1} catalase). The LP form represents dithionite-reduced minus ascorbate-reduced difference spectra and the IP form represents ascorbate-reduced minus hydroquinone-reduced difference spectra. The HP form represents hydroquinone-reduced minus ferricyanide-oxidized difference spectra (the left and the right column) and light minus ferricyanide-oxidized difference spectra (middle column). The spectra were smoothed using five points averaging of experimental data by the Origin v4.1 software. (B), Reductive potentiometric titrations of cyt b_{559} in Tris-treated PSII membranes measured under anaerobic conditions. The inset shows reductive potentiometric titrations of cyt b_{559} in Tris-treated PSII membranes measured under aerobic conditions in the dark (□) and after illumination with white light (1000 μmol photons m^{-2} s^{-1}) for 100 s (■). The symbols show data points of the titration curve, whereas solid lines represent the best fits of the experimental data to Nernst equation in accordance with the one-electron process (n = 1) for one or three redox components. Reductive potentiometric titrations were repeated three times with the similar profile of the redox titration curve. In all the measurements, Tris-treated PSII membranes (250 μg Chl ml^{-1}) suspended in medium containing 400 mM sucrose, 10 mM NaCl, 5 mM $CaCl_2$ and 40 mM Mes–NaOH (pH 6.5) were used.

Fig. 1A shows that neither illumination nor removal of molecular oxygen affected the content of the dithionite-reducible LP form of cyt b_{559} in Tris-treated PSII membranes. Therefore, further experiments have been designed to study only two forms of cyt b_{559} *i.e.* the light- or hydroquinone-reducible HP form and the ascorbate-reducible IP form.

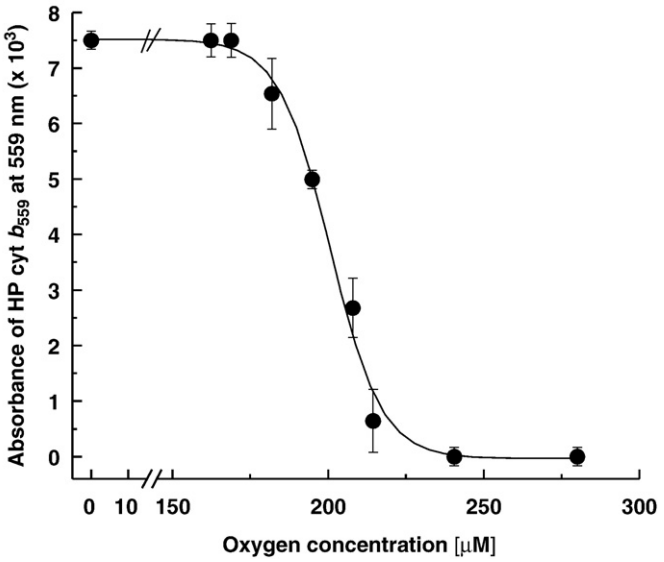

Fig. 2. Effect of oxygen concentration on the formation of the HP form of cyt b_{559} in Tris-treated PSII membranes. Concentration of molecular oxygen was measured with a Clark-type electrode (Hansatech, UK) assuming a saturation concentration of dissolved oxygen of 280 nmol ml^{-1} in salt containing buffer at 23 °C. The intensity of the absorption signal was calculated as the height of the peak at 559 nm from a reference line connecting the lowest points near 545 and 575 nm. Each data point represents the mean value of at least three measurements. Medium composition and pH were as described in Fig. 1.

3.3. Effect of pH on light- and oxygen-dependent redox changes of cyt b_{559}

Fig. 3 shows the effect of high pH on light- and oxygen-dependent redox changes of cyt b_{559} in Tris-treated PSII membranes. Above pH 8, light-induced conversion of cyt b_{559} from the IP to the HP form was completely suppressed (Fig. 3A). A sigmoid curve with pK_a value of ~7.4 was obtained by fitting of the experimental data. This observation indicates that light-induced conversion of cyt b_{559} from the IP to the HP form is dependent on the concentration of protons in the polar phase.

Interestingly, upon removal of molecular oxygen, the IP form of cyt b_{559} was readily converted into the HP form of cyt b_{559} even at high pH (up to pH 10) (Fig 3B, open circles). This indicates that the conversion of the IP to the HP form of cyt b_{559} under anaerobic conditions is largely independent on pH. To study the stability of the HP form at high pH, first molecular oxygen was removed using oxygen trap to form the HP form of cyt b_{559} and then pH was changed. Fig. 3B (closed circles) shows that the stability of the HP form of cyt b_{559} is unaffected at high pH. These results indicate that under anaerobic conditions the conversion of the IP to the HP form of cyt b_{559} as well as the stability of the HP form of cyt b_{559} is independent on pH, i.e. on proton concentration in the polar phase.

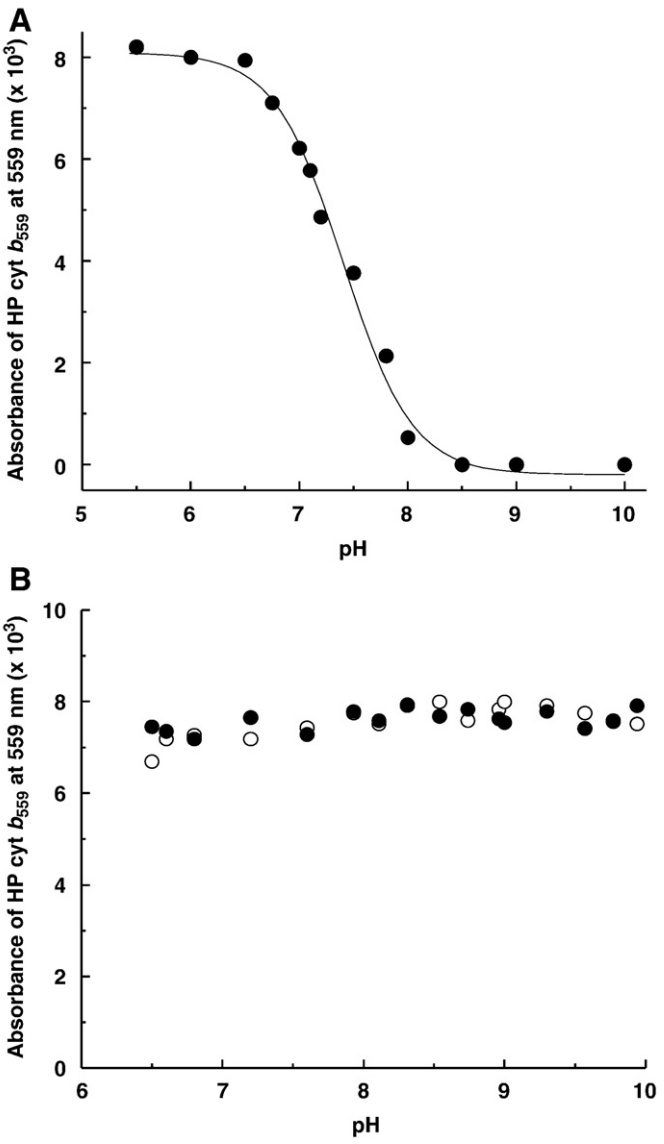

Fig. 3. Effect of pH on the formation of the HP form of cyt b_{559} measured in Tris-treated PSII membranes after (A) illumination for 100 s and (B) removal of molecular oxygen. Data points represent the height of the absorption maximum at 559 nm obtained from the light minus ferricyanide-oxidized difference spectra (A) and the hydroquinone-reduced minus ferricyanide-oxidized difference spectra (B). In B, to study the formation of the HP form of cyt b_{559} molecular oxygen was removed after adjusting the pH (open circles), whereas to explore the stability of the HP form of cyt b_{559} pH was adjusted after the removal of molecular oxygen (closed circles). The measurements were performed using suspending medium containing 400 mM sucrose, 10 mM NaCl, 5 mM $CaCl_2$ and 40 mM Mes–NaOH (pH 5.5–6.5) or 40 mM Hepes–NaOH buffer (pH 7–8), or 40 mM Tricine (pH 8.5), or 40 mM Capso (pH 9–10). Other experimental conditions were the same as described in Fig. 1.

3.4. Effect of histidine modification on light- and oxygen-dependent redox changes of cyt b_{559}

To find out whether the protonation of histidine plays any role in light- and oxygen-dependent redox changes of cyt b_{559}, histidine-modified PSII membranes were used. Histidine-modifying reagent DEPC is known to covalently bind to nitrogen (N1) of the histidine residue, replacing thus the hydrogen normally present on the imidazole ring [34,35]. In histidine-modified Tris-treated PSII membranes, cyt b_{559} was present in the ascorbate-reducible IP form, whereas the hydroquinone-reducible HP form was completely absent (Fig. 4, left column). Upon illumination of histidine-modified PSII membranes for 100 s, no HP form of cyt b_{559} was detected and the amount of the IP form of cyt b_{559} was not changed (Fig. 4, middle column). The finding that the light-induced conversion of the IP to the HP form of cyt b_{559} is completely suppressed in histidine-modified PSII membranes indicates that the protonation of the histidine residue is essential for this conversion. Surprisingly, the modification of histidine did not affect the conversion of the IP to the HP form of cyt b_{559} induced by anaerobic conditions. The removal of molecular oxygen in histidine-modified Tris-treated PSII membranes led to the formation of the hydroquinone-reducible HP form at the expense of the ascorbate-reducible IP form (Fig. 4, right column).

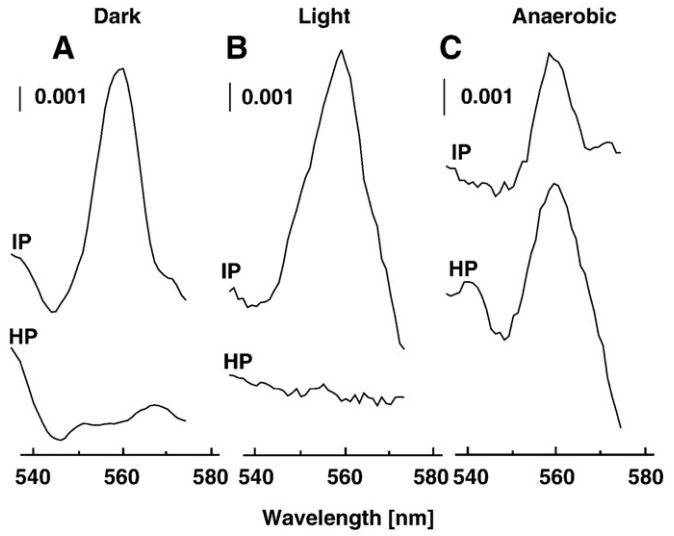

Fig. 4. Effect of histidine modification on redox changes of cyt b_{559} measured in Tris-treated PSII membranes in the dark (left column), after illumination for 100 s (middle column) and after removal of molecular oxygen (right column). Chemical modification of the imidazole ring of the histidine residue was performed using diethylpyrocarbonate as described in Materials and methods. The suspending medium and other experimental conditions were as described in Fig. 1.

4. Discussion

In agreement with previous studies [26–31], we have demonstrated that illumination and removal of molecular oxygen in Tris-treated PSII membranes resulted in the conversion of cyt b_{559} from the IP to the HP form. However, for the first time we have studied how this conversion is affected by pH and by the protonation of histidine residues, which coordinate the heme iron of cyt b_{559}. The light-induced conversion of cyt b_{559} from the IP to the HP form was found to be pH-dependent, whereas the conversion induced by the removal of molecular oxygen was unaffected by pH. Based on these observations, it is proposed that pH of the reaction medium is a limiting factor for the conversion of cyt b_{559} from the IP to the HP form during illumination but not under anaerobic environment.

4.1. Light-induced conversion of cyt b_{559} from the IP to the HP form

Illumination of Tris-treated PSII membranes caused the conversion of the IP to the HP form of cyt b_{559} and reduction of the heme iron (Eq. (1)).

$$IP\,cyt\,b_{559}(Fe^{3+}) \xrightarrow{light} HP\,cyt\,b_{559}(Fe^{2+})\,(pH-dependent). \quad (1)$$

Recently, we have proposed a detailed mechanism of light-induced conversion of cyt b_{559} from the IP to the HP form in Tris-treated PSII membranes [27]. It has been demonstrated that the light-induced conversion of the IP to the HP form of cyt b_{559} is mediated by superoxide anion radical ($O_2^{\bullet-}$) generated by PSII. It has been proposed that the interaction of $O_2^{\bullet-}$ with ferric heme iron of the IP form induced the conversion of cyt b_{559} into ferrous heme iron of the HP form and the release of molecular oxygen. Our finding that the light-induced conversion of cyt b_{559} from the IP to the HP form is completely suppressed at high pH indicates that the protonation of the heme-binding histidine residue is essential (Fig. 3A). Thus, at high pH there is a loss of the ability to form a hydrogen bond between the imidazole ring and the α helix, which is required to restore the HP form of cyt b_{559} under illumination. The loss of the ability to form the light-induced HP form of cyt b_{559} in histidine-modified PSII membranes supported this conclusion (Fig. 4, middle column).

4.2. Oxygen-dependent conversion of cyt b_{559} from the IP to the HP form

Removal of molecular oxygen using oxygen trap resulted in the conversion of the IP to the HP form of cyt b_{559}, while the heme iron remained in the oxidized state (Eq. (2)).

$$IP\,cyt\,b_{559}(Fe^{3+}) \xrightarrow{anaerobic} HP\,cyt\,b_{559}(Fe^{3+})\,(pH-independent). \quad (2)$$

The observation that conversion from the IP to the HP form of cyt b_{559} proceeds independently of pH in the medium reveals that the proton concentration in the polar phase is unlikely to be the limiting factor for oxygen-dependent conversion from the IP to the HP form of cyt b_{559} (Fig. 3B). This proposal is supported by the fact that oxygen-dependent conversion from the IP to the HP form of cyt b_{559} was observed in histidine-modified PSII membranes (Fig. 4, right column). Based on these observations one can deduce that oxygen-dependent conversion from the IP to the HP form of cyt b_{559} is unlikely to be dependent on protonation of the histidine residue.

These findings support the hypothesis that the mechanism behind the conversion of the IP to the HP form of cyt b_{559} under illumination and anaerobic conditions is different. It has been previously proposed that molecular oxygen attacks hydrogen bonds between the imidazole group of histidine and the polypeptide backbone and thus maintains cyt b_{559} in the LP form under aerobic conditions [28]. The establishment of highly hydrophobic environment around the heme group is widely considered as crucial for the formation and maintenance of the unstable HP form of cyt b_{559} [18,20]. A recent study performed on PSII membranes with a sequential depletion of extrinsic polypeptides and water-splitting manganese complex proposed that damage of oxygen channels alters the environment around the heme iron and thus changes redox properties of cyt b_{559} [29]. Recent crystal structure provided the evidence that the heme of cyt b_{559} is covered by the fatty acids of SQDG4, MDGD7 and the isoprenoid chain of the plastoquinone molecule in the PQ-PQH_2 exchange channel II maintaining thus hydrophobic environment around the heme [14]. In agreement with these proposals it seems likely that the removal of molecular oxygen changes the microenvironment around the heme. It is proposed that the absence of molecular oxygen caused an enhancement in the hydrophobicity around the heme and restoration of the HP form of cyt b_{559}.

5. Conclusion

The light-induced conversion of cyt b_{559} from the IP to the HP form is a photochemical process that involves the interaction of $O_2^{\bullet-}$ with the heme iron. However, conversion of cyt b_{559} from the IP to the HP form caused by the removal of molecular oxygen seems to be a biochemical process that is induced by changes in the microenvironment around the heme of cyt b_{559}. This seems to be the possible reason behind why the heme iron remains in the oxidized state during oxygen-dependent conversion of the IP to the HP form of cyt b_{559}.

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