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pH-dependent oxidation of Cytochrome b_{559} is different in the different S-states

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Introduction

Photosystem II (PSII) is a large multisubunit enzyme localized in the thylakoid membrane, catalyzing plastoquinone reduction by using the energy of light to extract electrons from water. The oxygen evolving complex (OEC) consists of a $(Mn)_4$ cluster, Ca and Cl ions as cofactors and a redox active $Tyrosine_Z$ (Y_Z) residue. The OEC cycles through five S-states, denoted from S_0 to S_4. S_1 is the dark stable state. S_4 is the most oxidized, transient state which rapidly converts to the S_0 state as one oxygen molecule is released.

Cytochrome b_{559} (cyt b_{559}) is an essential and integral part of PSII. It consists of two small subunits (α, β), each with one transmembrane helix and one heme between the helices. The recent crystal structure of PSII (Zouni et al., 2001) has shown that its heme is localized close to the stromal surface of the membrane. An important feature of cyt b_{559} is that it exhibits different redox potential forms ranging from the high potential (HP) form (>360 mV) through the intermediate (IP, ~200mV) and low (LP, 0-80 mV) to the very low potential form (VLP, ~45 mV). The redox potential of cyt b_{559} varies with the pH. The E_m of the HP form is reported to be pH-independent, while the E_m of the LP form is pH-independent above and pH-dependent below 7.6 (Ortega et al., 1988). Low or high pH can also induce conversion between the cyt b_{559} forms.

Despite numerous research efforts the function of cyt b_{559} is still under debate. Many reports show that different forms of cyt b_{559} may participate in secondary electron transfer reactions in PSII thus protecting PSII against photoinhibition (Thompson and Brudvig, 1988; Nedbal et al., 1992; Barber and De Las Rivas, 1993; Magnuson et al., 1999). In these reactions cyt b_{559} is in redox equilibrium with different donor and acceptor side components. The pH-dependence of these equilibria might be a critical factor to determine the electron transfer pathway under extreme physiological conditions. In contrast, interactions between the OEC and cyt b_{559} during the S-cycle are not well known. In the present study we investigate the pH-dependence of the redox state of cyt b_{559} when the OEC is in different S-states.

Materials and methods

PSII membrane fragments were prepared (Pace et al., 1991) from spinach plants cultivated on liquid culture medium. The oxygen evolution was 350-400 μmol (mg Chl)$^{-1}$h^{-1}. The PSII membranes were transferred to EPR tubes in a low-buffering medium (0.5 mM Mes, 400 mM sucrose, 10 mM $MgCl_2$, 10 mM NaCl, 5 mM $CaCl_2$ at pH 6.0) in the presence of 5% (v/v) methanol. The OEC was synchronized in the S_1 state by a preflash procedure (Styring and Rutherford, 1988; Geijer et al., 2000), and PSII was then advanced to the appropriate S-state

at pH 6.0 by providing 0 (S_1), 1 (S_2), 2 (S_3) or 3 (S_0) saturating laser flashes. After the exciting flashes, the pH of the medium was adjusted by addition of a stronger buffer in the range of pH 4.0-9.5: DL-glutamic acid/KOH (pH 4.0-5.0), Mes/KOH (pH 5.0-7.0), Hepes/KOH (pH 7.0-8.0), glycyl-glycine/KOH (pH 8.0-9.5) (Vass and Styring, 1991; Geijer et al., 2000). The final buffer concentration was 14 mM. The samples were incubated after the pH-jump at room temperature for 30 s and frozen in liquid nitrogen. The S_2, S_0 multiline signal intensities and the intensity of the g_z component of the cyt b_{559} signal were measured by EPR spectroscopy, and all data were normalized to the Y_D signal. The total integral of the g_z component of oxidized cyt b_{559} (HP + LP) was used for evaluation. We also estimated the HP and LP intensities by measuring the cyt amplitude at $g \approx 3.05$ and $g \approx 2.97$, respectively. When the measurements were finished the samples were thawed and the final pH, the O_2 evolution at pH 6.0 and the Chl concentration were determined.

EPR measurements were performed with an ESP500e spectrometer using a SuperX EPR049 bridge and a Bruker ER4122SHQ cavity.

Results

Fig. 1 shows the effect of pH on cyt b_{559} spectra of PSII membranes poised in the S_1 (upper panel) and S_3 (lower panel) states, respectively. In these experiments 0 (S_1) or 2 (S_3) flashes were given to induce the correct S-state. The flashes were followed by a pH-jump and 30 s dark-incubation before freezing. (Note! no light was provided *after* the pH was changed). The spectral intensities are highly dependent on the pH and S-state. In the S_1 state the intensities of the oxidized cyt b_{559} are small and exhibit a gradual decrease when the pH was increased from pH 4.3 to pH 8.8. By contrast, in the S_3 state the signal intensities from oxidized cyt b_{559} are significantly larger. They also show a complicated pH-dependence: the EPR signal is big at low and high pH and smaller in between.

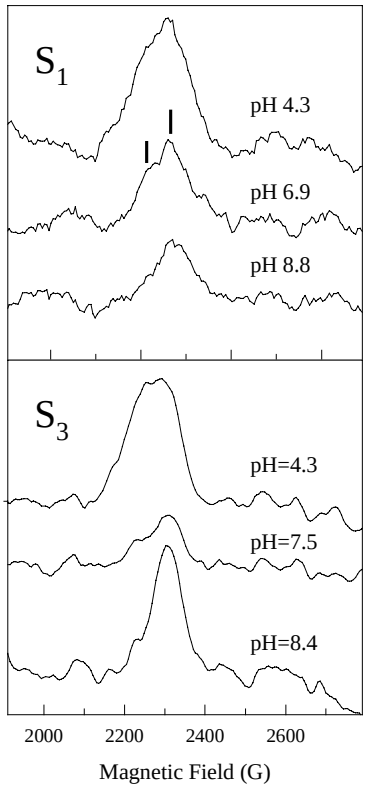

Figure 1. The effect of pH on the g_z EPR peak of the oxidized cyt b_{559} in the S_1-state (upper panel) and S_3-state (lower panel). The S_1 and S_3 states were induced by 0 and 2 flashes, respectively, at pH 6.0 followed by the pH-jump and 30 s dark-incubation at room temperature. For clarity, the spectra in the upper panel are magnified by a factor of 2 as compared to the spectra in the lower panel. EPR settings: power, 5 mW; frequency 9.41 GHz; T, 15 K, mod. ampl. 15 G. The bars indicate the field positions of HP ($g \approx 3.05$; 2235 G) and LP ($g \approx 2.97$; 2320 G) forms.

The lineshape of the signal also varies with pH in both S-states. At low pH, both the oxidized HP ($g \approx 3.05$) and LP forms ($g \approx 2.97$) of cyt $b_{559,}$ indicated by bars in Fig. 1, appear in the spectra with about equal intensity. At elevated pH (8.4-8.8) this changes and only the LP form was detected. Similar experiments were done to measure the oxidation level of cyt b_{559} also in the S_0 and S_2 states. The effect of pH on the spectra from oxidized cyt b_{559} in those cases was similar to the S_3 state.

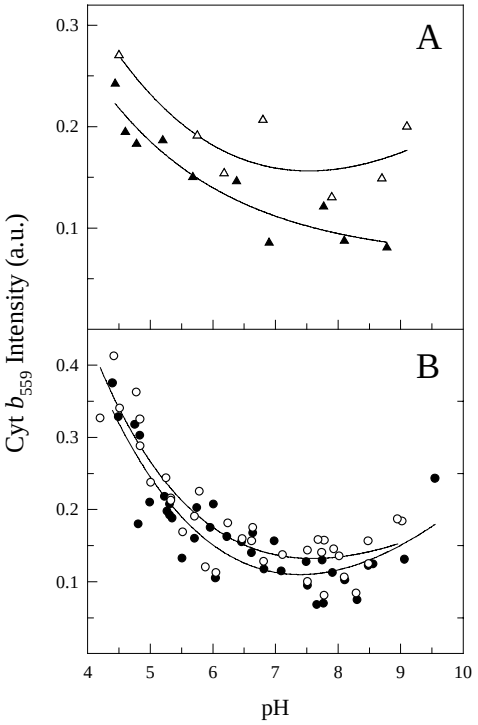

Figure 2. pH-titration of cyt b_{559} oxidation in the S_1, S_2 (panel A), S_3 and S_0-states (panel B). The S_1 (closed triangles), S_2 (open triangles), S_3 (closed circles), and S_0 (open circles) states were induced by 0-3 saturating laser flashes, respectively, at pH 6.0 followed by the pH-jump and 30 s dark-incubation at room temperature. Data points represent the integral of cyt b_{559} g_z component at $g \approx 3$ as presented in Fig. 1. Note the different scales in A and B.

The titration curves of the oxidized cyt b_{559} fraction in different S-states is shown in Fig. 2. In the S_1 state (panel A, filled triangles) only a small fraction was oxidized. The size of this fraction decreases monotonically by increasing the pH from pH 4.2 to pH 9.4. The shape of the titration curves is different in the S_2 (panel A, open triangles), S_3 and S_0 states (panel B, open and filled circles, respectively). The intensities are minimal between pH 7 and 8 and the oxidation of cyt b_{559} increased greatly when the pH was decreased while it increased moderately when the pH was increased.

The pH-dependence of the oxidation of the HP and LP cyt b_{559} forms is also different. The oxidation level of the HP form exhibits a decreasing tendency with pH and has an apparent p*K* around pH 6.0-6.5. Contrary to this, the intensity of the oxidized LP fraction (which is about the same as the HP form at pH 4.5) decreases more steeply with pH up to pH 7. Above pH 7 the intensity sharply increases.

Discussion

We have studied the oxidation state of cyt b_{559} at different pH. Similar studies were carried out either by using dark-adapted PSII (Buser et al., 1992) or different inhibited PSII preparations (Ortega et al., 1995). Here we present the first report on the pH-dependence of the cyt b_{559} oxidation level during the entire S-cycle. The redox state was followed by EPR spectroscopy, which can distinguish the different cyt b_{559} forms without the addition of any reductants.

Our results show that the extent of the pH-dependent cyt b_{559} oxidation is (very) S-state dependent. The cyt b_{559} is mostly reduced in the S_1 state at all pH's but a significant oxidation can be observed in the higher S-states and in the S_0 state. However, in the case of S_0 it should be noted that there is a significant S_3 state population (≈ 30%) due to misses (≈ 15%) over the sequential three flashes. Consequently the oxidation of cyt b_{559} we observe in the 3 flash sample partly reflects PSII centers in the higher S-states (S_2 and S_3).

We recently reported (Feyziyev et al., 2001), that the S_2 state is in direct redox interaction with cyt b_{559} and S_2 can oxidize cyt b_{559} in a slow reaction at pH 6.2. Seemingly, this reaction is pH-dependent and more efficient at low pH. It also appears to occur in the S_3 state. In

contrast, at pH 6.2 we did not observe any oxidation of cyt b_{559} by the S_3 state (Feyziyev et al., 2001). This is similar to what we find here at pH 6-7. However, at low pH (in particular) and at high pH it seems that S_3 interacts even more efficiently with cyt b_{559} and we observe very high oxidation (after 30 s dark-incubation at 20°C). Presumably this reflects direct electron transfer from cyt b_{559} to the OEC in the S_3 state. The pH-range where this occurs (pH 4.5-5) is reasonable and might occur on the lumenal side of PSII (the OEC side) in high light. Thus, our observations indicate that cyt b_{559} can directly reduce the OEC in the S_3 state. At present we are not clear about the situation in S_0 (our samples are contaminated with S_3) and our results need more analysis. It also seems clear that the reactions are of little importance in the S_1 state.

Finally it is worth mentioning that the two major forms (HP, LP) of cyt b_{559} show different pH-dependence. This could indicate that a second redox partner is also involved in these reactions.

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