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pH-dependence of the four steps in the S-cycle in Photosystem II

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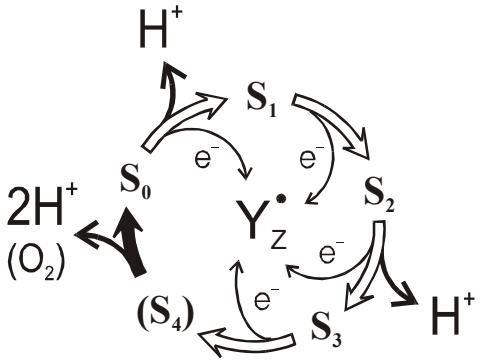
Introduction

Water oxidation by PSII is a key reaction in the biosphere. It occurs on the lumenal side of PSII and is carried out by a triad of highly oxidizing components which are the primary donor P680, Tyrosine$_Z$ (Y_Z) and the $(Mn)_4$ cluster. Y_Z and the $(Mn)_4$ cluster constitute the catalytic site for water oxidation in the oxygen evolving complex (OEC). The donor side triad carries out a sequential series of electron transfer reactions ultimately oxidizing water. During turnover, the OEC cycles through the S-cycle (Scheme 1). The oxidation of water to molecular oxygen involves the release of four protons. Scheme 1 shows one experimentally determined pattern for the H^+ release. The H^+ release pattern is very preparation dependent and it also varies very much with the measuring pH (Lavergne and Junge, 1993; Schlodder and Witt, 1999).



Scheme 1.

In PSII membranes, the oxygen evolution is maximal at pH 6.0-7.0. On the acidic and alkaline sides, the oxygen evolution decreases with pK's of 4.8-5.5 and 7.4-8.0 (Vass and Styring, 1991). It is not clear which step(s) in the S-cycle that is (are) responsible for this inhibition pattern. In this study we have measured the pH-dependence between pH 4-9 for each individual S-transition. We have prepared PSII in the S_n state by flashes at normal pH. Then, the samples were exposed to a rapid pH-jump. The ability to advance to the S_{n+1} state was then probed by the appearance or disappearance of specific EPR signals from the 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. OEC was synchronized in the S_1 state by a preflash procedure (Styring and Rutherford, 1988; Geijer et al., 2000). Then PSII was 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 EPR samples were either frozen at this level of sample preparation or exposed to one more laser flash, the probe flash.

To study the $S_1 \rightarrow S_2$ and $S_3 \rightarrow S_0$ transitions the formation of the S_2 and S_0 multiline signals, respectively, were followed. In these experiments the samples were frozen after the pH-jump and the probe flash. The $S_2 \rightarrow S_3$ and $S_0 \rightarrow S_1$ transitions we followed by the disappearance or decrease of the S_2 and S_0 multiline signals. Here two parallel samples were made at each pH. One sample was frozen before and one after the probe flash. After the EPR measurements were finished the samples were thawed and the final pH-, 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

Figure 1 shows the result for the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition at three different pH values. In this experiment two flashes were given to induce the S_3 state in about 70% of the centres. The pH was then changed and the probe flash was given. This induced the S_0 multiline signal when the transition worked but did of course not form the S_0 multiline signal when the transition was blocked. At pH 6.2 (spectrum b) the S_0 multiline signal has its maximal amplitude while the signal intensity was much smaller at pH 4.4 (spectrum a) and 8.0 (spectrum c). Fig. 1B shows the amplitude of the S_0 multiline signal as a function of pH.

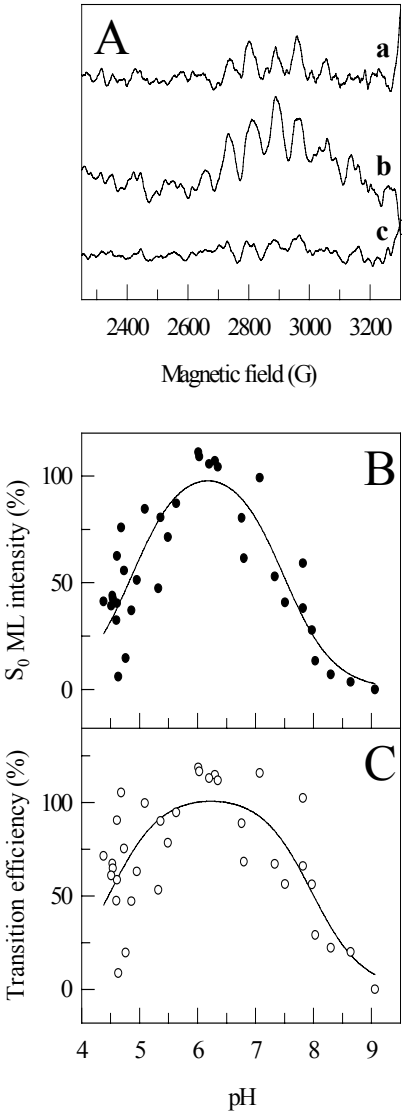


Figure 1: Efficiency of the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition. (A) Low field region of the S_0 state multilane EPR signal, induced by two saturating laser flashes at pH 6.2 followed by the pH-jump and subsequent flash. The final pH was (a) pH 4.8, (b) pH 6.2 and (c) pH 8.2. EPR settings: power, 50 mW; frequency, 9.41 GHz; T 7 K; mod. ampl., 15 G. (B) pH-dependence of the S_0 multiline signal intensities, induced by two saturating laser flashes, followed by the pH jump (to the indexed pH values) and a subsequent flash. The data points (solid circle) represent the amplitude of the S_0 multiline signal. (C) Yield of the $S_3 \rightarrow S_0$ as a function of pH. The points (open circle) are obtained from (B) by division of the experimental points shown and the titration curve of the S0 multiline signal amplitude (Geijer *et al.*, 2000)

Unfortunately this curve does not show the pH-dependence directly. Instead, the data must be corrected for the pH-dependence of the S_0 multiline

signal amplitude (Geijer et al., 2000). The corrected results are shown in Fig. 1C which shows that the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition is inhibited at both low and high pH. The data can be fitted well with two pK´s; $pK_1 = 4.5 \pm 0.2$ and $pK_2 = 8.0 \pm 0.2$, respectively.

Similar experiments were performed to probe all individual S-transitions and the results are shown in Table 1 and Fig 2A. The $S_0 \rightarrow S_1$ transition is blocked with a pK = 4.7 ± 0.1 in the acidic region while it is fully active at least up to pH 10. The $S_1 \rightarrow S_2$ transition is fully operational between pH 4.4 and 8.4 similar to what was found earlier (Damoder and Dismukes, 1984). The $S_2 \rightarrow S_3$ transition is inhibited with $pK_1 = 4.0 \pm 0.1$ in the acidic and $pK_2 = 9.4 \pm 0.2$ in the alkaline region, respectively. The sharpest inhibition pattern is found for the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition which shows sharp blocks both at acidic ($pK_1 \approx 4.5$) and alkaline pH ($pK_2 \approx 8.0$).

Table 1. pH-dependent inhibition of the individual steps in the S-cycle

Transition	pK_1[a]	pK_2[a]
$S_0 \rightarrow S_1$	4.7 ± 0.1	pH-independent
$S_1 \rightarrow S_2$	pH independent	pH-independent
$S_2 \rightarrow S_3$	4.0 ± 0.1	9.4 ± 0.2
$S_3 \rightarrow S_0$	4.5 ± 0.2	8.0 ± 0.2

[a] pK_1 and pK_2 is the pK's for the inhibition in the acidic and alkaline region, respectively.

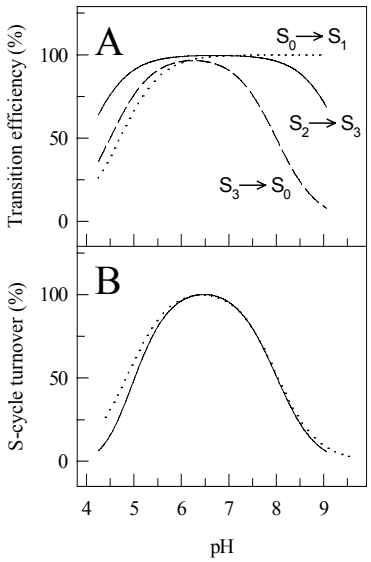


Figure 2. pH-dependence of the whole S-cycle. (A) The pH-blocks in the different S-cycle transitions. The $S_1 \rightarrow S_2$ transition is not pH-dependent. B. Steady-state oxygen evolution in our PSII preparation at different pH's (dotted line). Multiplication of the titration curves for the individual S-transitions (according to Table 1). The product of the multiplication gives the pH-dependence of the whole S-cycle as defined by the individual S-transitions (thick line).

We also compared the pH-dependence of the individual S-transitions and the pH-dependence of the steady-state oxygen evolution. In our PSII preparation we found that the oxygen evolution is inhibited with half-inhibition values around pH 5.0 and 8.0 (Fig. 2B). Multiplication of the functions describing the pH-dependence of the individual steps reveals the efficiency of the whole oxygen evolving cycle as a function of pH (if the pH-dependence is dominated by the S-cycle). This calculation (Fig. 2B) resulted in a titration curve for the oxygen evolution with half-inhibition values at pH 5.1 and 8.0. This is in remarkably good agreement with the inhibition of the steady-state oxygen evolution. Thus, the decrease of the steady-state oxygen evolution at low pH is a consequence of the simultaneous inhibition of three of the S-transitions. In contrast, at high pH the inhibition of the oxygen evolution is almost completely determined by the inhibition of the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition.

Discussion

The inhibition at low pH occurs with pK 4.0-4.7 and seems similar in each S-transition except in the $S_1 \rightarrow S_2$ transition. It is not likely that one or several carboxylic residues in the point of contact between the solvent and the hydrogen bond network involving Y_Z and the Mn cluster. In this case, it (they) are likely to become protonated with p*K*'s around 4.5 which is normal for carboxylic amino acids. If this was the case, protonation of the proton expulsion site would clog the proton release pathway from Y_Z thereby preventing turnover of the system.

It has also been proposed that the low pH actually releases Ca^{2+} from its site in the OEC (Krieger et al, 1993). This would explain a block in the S-cycle between S_2 and S_3 but it is less obvious how it would affect the other transitions that are blocked at low pH. We do not favor this hypothesis and it is unlikely that our conditions would give rise to release of Ca^{2+} from the OEC. In our experience, Ca^{2+} depletion is achieved at pH 3.0 in the presence of a chelator that binds Ca^{2+} and not at pH 4-5 for 30 sec in the presence of 5 mM $CaCl_2$ (our conditions) (Ono and Inoue, 1989).

The inhibition at alkaline pH is probably easier to explain. We recently found that a pH-jump in the S_3 state induced the formation of the $S_2Y_Z^{\bullet}$ state (Geijer et al., 2001). We proposed that this reflected a pH-induced shift of the redox potential of the $Y_Z^{\bullet}/Y_Z$ couple making this less oxidizing that the S_3 state of the Mn cluster. We propose that this mechanism applies to the $Y_Z^{\bullet}/Y_Z$ couple in all S-states. It explains well the inhibition of the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition since it would imply that $Y_Z^{\bullet}$ can not oxidize the S_3 state above pH 8-8.5 fast enough to avoid side reactions that reduce $Y_Z^{\bullet}$. In contrast, both the S_0 and the S_1 states are low enough in potential to be oxidized by $Y_Z^{\bullet}$ even at high pH (the oxidizing potential of the $Y_Z^{\bullet}/Y_Z$ couple approaches + 800mV at pH 8.5). Thus, there is no alkaline block in the $S_0 \rightarrow S_1$ transition or the $S_1 \rightarrow S_2$ transitions. The situation is less clear for the $S_2 \rightarrow S_3$ transition which was found to be blocked with a p$K \approx 9.4$. The S_2 state is considered to be about 50 mV less oxidizing than $Y_Z^{\bullet}$ at pH 6-7. This would mean that $Y_Z^{\bullet}$ would become unable to oxidize the S_2 state above pH ≈ 8 if the oxidizing potential of the S_2 state was unaffected by pH. We find, however, that the $S_2 \rightarrow S_3$ transition is inhibited with p$K \approx 9.4$. An explanation to this is that the redox potential of the S_2/S_1 state is pH-dependent in parallel to the $Y_Z^{\bullet}/Y_Z$ couple similar to what was proposed earlier from different arguments (Geijer et al., 2001).

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