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pH dependence of oxygen evolution and reduction kinetics of photooxidized chlorophyll a_{II} (P-680) in Photosystem II particles from *Synechococcus* sp.

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The oxygen flash yield and the kinetics of Chl a_{II}^+ (P-680$^+$) reduction have been measured under repetitive excitation as a function of pH between pH 4.0 and pH 9.0 in oxygen-evolving PS II particles from *Synechococcus* sp. (i) The optimum of oxygen yield is observed between pH 6.5 and pH 7.5. The inhibition in the acidic pH region is reversible and can be described by a monoprotic binding site with a p*K* value of about 4.5. In the alkaline pH region the inhibition is half maximal at pH 8.3 and might be described by the titration of three binding sites or more. The loss of oxygen evolution at pH 9.0 is caused by reversible inhibition and irreversible inactivation. (ii) Between pH 4.0 and pH 7.5 the fraction of Chl a_{II}^+ decaying in the nanosecond time range and the oxygen yield follow the same pH dependence. (iii) Both in Photosystem II centers reversibly inhibited at low pH and in Photosystem II centers inactivated at high pH, Chl a_{II}^+ is reduced by a donor Z, different from the normal immediate donor D_1 or a modified state of D_1, and, in part, by back reaction. (iv) Below pH 5.0, the decay in the nanosecond range can be explained by the existence of two phases with $t_{1/2} \approx 42$ ns and $t_{1/2} \approx 300$ ns (ratio of amplitudes, 1.3:1). A reduction phase with $t_{1/2} = 20$ ns that is the major phase around the pH optimum is not observed below pH 5.0.

Introduction

Light-induced charge separation between a specialized chlorophyll *a* molecule, Chl a_{II} (P-680) [1,2] and the first stable acceptor, a special plastoquinone, Q_A (X-320) [3,4], starts the reaction sequence leading to water oxidation. In order to oxidize 2 H_2O into 1 O_2 and 4 H^+, the stepwise accumulation of four oxidizing equivalents in the O_2-evolving complex by four consecutive photooxidations of Chl a_{II} is required. Thereby the O_2-evolving complex passes through four different redox states (the so-called S-states, S_0–S_3) before oxygen is released during the transition $S_3 \rightarrow S_0$ [5–7]. Protons are released with a pattern (1, 0, 1, 2) during the transitions $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$, respectively [8–11].

Chl a_{II}^+ reduction kinetics depend on the oxidation state of the O_2-evolving complex [12]. The phase with $t_{1/2} \approx 20$ ns is associated with states S_0 and S_1, respectively. Slower biphasic reduction

Abbreviations: Q_A, primary quinone acceptor of Photosystem II; Chl, chlorophyll; Mes, 4-morpholineethanesulphonic acid; PS I, Photosystem I; PS II, Photosystem II; D_1, immediate electron donor to Chl a_{II}^+; D_2, electron carrier between D_1 and the oxygen-evolving complex; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; Z, electron donor to Chl a_{II}^+ after inhibition of O_2 evolution characterized by EPR Signal II_f; SB 12, *N*-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate; Mops, 4-morpholinepropanesulphonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

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with half-life times of 50 and 260 ns occurs in state S_2 as well as S_3 [12]. The retardation of electron transfer to Chl a_{II}^+ in states S_2 and S_3 was explained by Coulomb attraction due to a positive excess charge located in the O_2-evolving complex in states S_2 and S_3 [12]. The formation of excess charges in S_2 and S_3 was also shown by electrochromic absorption changes [13]. In the nanosecond time range the multi-phasic reduction kinetics of Chl a_{II}^+ under repetitive excitation could be explained by a superposition of the S-state-dependent electron-transfer times. They were explained by the existence of three nanosecond phases with half-life times of 20, 50 and 260 ns (together about 85% of the total amplitude) [14,15]. Minor phases (about 15%) in the microsecond range were observed under repetitive excitation [15]. The amplitude of a 35-μs phase varies as a function of the S-states, being maximal in states S_2 and S_3 [15].

The experiments summarized so far were performed around the pH optimum of oxygen evolutin (pH 6–8). A pH dependence of the amplitude of microsecond decay phases has been observed in former measurements of the absorption changes in thylakoids around 690 nm [16].

Because of the biphasic nanosecond reduction kinetics of Chl a_{II}^+ in the S_2 and S_3 state we proposed the existence of two intermediate carriers, D_1 and D_2, between Chl a_{II} and the O_2-evolving complex [12]. The direct donor to Chl a_{II}^+, D_1, has not yet been identified. On the basis of EPR [17,18] and optical spectroscopy [19–21] D_2 has been proposed to be a plastoquinol. D_2^+ is reduced by manganese bound in the O_2-evolving complex. The kinetics of D_2^+ reduction [22] and manganese oxidation [23] are both dependent on the S-states and coincide as far as known.

After irreversible inhibition of oxygen evolution, Chl a_{II}^+ is reduced by a component giving rise to EPR Signal II_f in the oxidized form [24]. Since it is not clear if this component is a modified state of D_1 or D_2 or an alternative donor, we call it Z.

Several studies on the pH-dependence of oxygen evolution have been reported [16,25–28]. Some of these studies, however, may not be entirely convincing, since the measured O_2-evolving rate in continuous light may also be affected by pH effects on the acceptor side of PS II (see, e.g., Refs. 26 and 27). In other studies carried out with chloroplasts the internal thylakoid pH which controls the reactions on the donor side of PS II will be different from that of the external medium [16]. The analysis of the results requires therefore reliable determination of the internal pH. The pH-dependent inhibition of the oxygen evolution has also been investigated by fluorescence measurements [25,29–31], EPR studies [28,33–36], measurements of the release of both Mn^{2+} and peripheral proteins [28,33,34], and thermoluminescence studies [34]. It was found that the irreversible inactivation at alkaline pH (pH > 8) is accompanied by the loss of Mn^{2+} [32,33], the release of peripheral proteins (18, 24 and 33 kDa) [34–36], the appearance of EPR Signal II_f [28,33], and the loss of the light-induced multi-line and $g = 4.1$ EPR signal which were attributed to the S_2 state [28]. At acidic pH (pH < 5) release of Mn^{2+} and increase of the EPR Signal II_f amplitude have also been observed [29].

In this work we have measured the reduction kinetics of Chl a_{II}^+ under repetitive flash excitation as a function of pH in the nano- to millisecond time range. These measurements were carried out in connection with measurements of O_2 evolution under repetitive flash light. All experiments were performed with PS II particles from *Synechococcus* sp. [37].

Materials and Methods

Oxygen-evolving PS II particles were prepared from the thermophilic cyanobacterium *Synechococcus* sp. as described by Schatz and Witt [37]. The PS II particles were finally obtained in solution A (80% v/v), glycerol (20% v/v) and about 0.3% (w/w) of the detergent SB 12. Solution A contained $3 \cdot 10^{-3}$ M Mes-NaOH (pH 6)/$1 \cdot 10^{-2}$ M $MgCl_2$/$2 \cdot 10^{-3}$ M KH_2PO_4/0.5 M mannitol. The chlorophyll content was about 10^{-4} M in the stock suspension. It was stored at −80°C before further use. The PS II particles are characterized by a PS II/PS I ratio of about 50 and by an O_2-flash yield of $(2.5–3.6) \cdot 10^{-3}$ O_2 per Chl and flash corresponding to 70–100 Chl per Chl a_{II} active in O_2 evolution. Absorption changes at 824 nm were measured as described previously [12,15]. For measurements in the nanosecond range, the

detection system (photodiode FND-100 from EG &G; amplifier TVV 123 from Telemeter; Transient recorder with 2 ns/point, Biomation 6500; signal averager, Nicolet 1170) had an electrical bandwidth of 100 Hz–100 MHz. In the microsecond and millisecond range, the measuring light was monitored by a photodiode (FND 100 from EG&G) loaded with 1 kΩ. The signals were further amplified (Tektronix AM 502) and digitized by a Nicolet 1170 with plug-in Model 174. The electrical bandwidth was 2 Hz to 1 MHz. The samples were excited by 3 ns (FWHM) laser flashes at 532 nm from a frequency-doubled Nd/YAG laser (YG 441 from Quantel). The energy per flash corresponds to approx. 80% saturation. Most signals were transmitted to an Apple II microcomputer and fitted by means of least-squares curve-fitting programs as described previously [12].

Flash-induced oxygen evolution was measured with a zirconia oxygen sensor (Programmelectronic AG, Dornach, Switzerland). Purified N_2 gas (50 ppb O_2) was used as a carrier gas. Oxygen produced in the reaction cuvette was swept out by the carrier gas and transported to the zirconium dioxide high-temperature oxygen electrode. The oxygen content of the gas stream was recorded with a microcomputer (Commodore 64) and the peak due to oxygen evolution was integrated to give the absolute amount of oxygen produced. Calibration of the apparatus was achieved by injecting 50 µl of air-saturated water. The samples were illuminated with saturating Xe flashes of approx. 20 µs (FWHM). The repetition rate was 1 Hz. For comparison, flash-induced oxygen evolution was measured with a Clark-type electrode (Bachofer).

The reaction medium contained solution B/approx. 2% (v/v) glycerol/approx. 0.03% (w/w) SB $12/2 \cdot 10^{-4}$ M phenyl-*p*-benzoquinone/$2 \cdot 10^{-3}$ M $K_3Fe(CN)_6/1 \cdot 10^{-5}$ M chlorophyll. 3 ml of the reaction medium were divided into two parts for measurements of absorption changes and oxygen evolution. Solution B contained $5 \cdot 10^{-2}$ M buffer/10^{-2} M $MgCl_2/2 \cdot 10^{-3}$ M KH_2PO_4/0.5 M mannitol. The following buffers were used:
at pH 4.0 glycylglycine (pK 3.1)
at pH 4.0–5.0 succinic acid (pK_1 4.19, pK_2 5.57)
at pH 5.5–6.5 Mes (pK 6.15)
at pH 6.5–7.0 Mops (pK 7.2)
at pH 7.0–7.5 Hepes (pK 7.55)
at pH 7.5–9.0 Tricine (pK 8.15)
The buffers were adjusted to the appropriate pH by addition of NaOH or HCl.

Reversibility experiments were performed as follows: PS II particles were stored for 10 min in the dark at pH 4.0 or pH 9.0, respectively. Electron acceptors were added to the final concentrations of $2 \cdot 10^{-3}$ M $K_3Fe(CN)_6$ and $2 \cdot 10^{-4}$ M phenyl-*p*-benzoquinone. In some experiments the sample was additionally illuminated with 500 flashes. By addition of solution B (pH 8.5 or pH 6.0, respectively) the pH values of the samples were adjusted to pH 7.0. Final concnetrations of chlorophyll and electron acceptors were the same as in the reaction medium.

Results

Fig. 1 shows the time course of the 824 nm absorption changes in O_2-evolving PS II particles from *Synechococcus* sp. at different pH values. The absorption changes were attributed to photooxidation and re-reduction of Chl a_{II}. The small rapid transient ($t_{1/2} \leq 5$ ns corresponding to the instrumental response time) is probably caused by the phycobilin pigments which normally contaminate these preparations. The amplitude of Chl a_{II}^+ decaying in the nanosecond time range ($t_{1/2} <$ 1 µs) gets significantly smaller when the pH is lowered (Fig. 1a). We analyzed the decay in the 10–1500 ns time range by two-exponential phases which are sufficient for a good fit. Although the decay in the nanosecond range is a superposition of three nanosecond phases as derived from single flash experiments already for a two-exponential fit the deviations between measured signal and fitting function are not well above the noise. The slow phases decaying in the micro- and millsecond range were taken into account by a slightly declining straight line. The results of the fits of the signals in Fig. 1a are given in Table I.

On the average (taking into account differences in preparations, reaction media and sets of experiments) an adaptation of the decay kinetics between pH 8.0 and pH 6.0 by two-exponential phases yielded the following parameters. $t_{1/2} = 23 \pm 7$ ns, $t_{1/2} = 220 \pm 50$ ns, $a_1/a_2 = 2.5 \pm 0.8$; $a_3 = 0.15$ to 0.4. Between pH 4.5 and pH 4.0 the

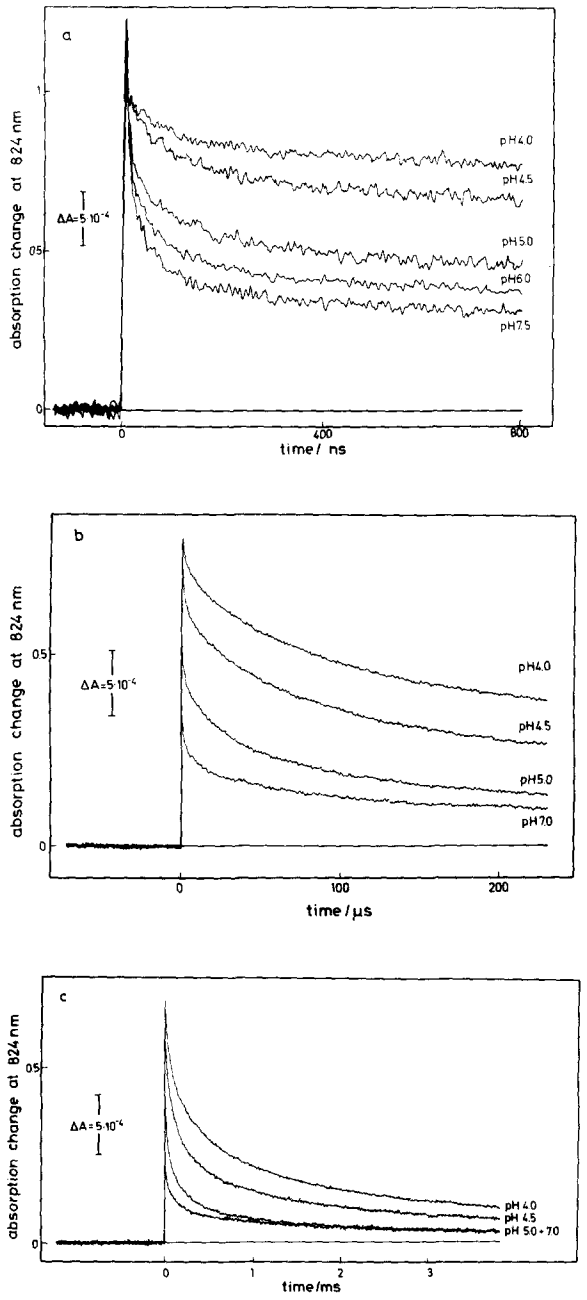

Fig. 1. Time-course of the absorption changes at 824 nm in O_2-evolving PS II paticles from *Synechococcus* sp. at different pH values. Excitation by approx. 80% saturating laser flashes (3 ns FWHM; 532 nm; repetition rate, 1 Hz). 10^{-5} M Chl/2·10^{-3} M $K_3Fe(CN)_6$ and $2 \cdot 10^{-4}$ M phenyl-*p*-benzoquinone as electron acceptors. (a) Time-course digitized with 2 ns/point, electrical bandwidth 100 Hz–100 MHz, 1024 averages; (b) time-course digitized with 300 ns/point, electrical bandwidth 2 Hz–1 MHz, 256 averages; (c) time-course digitized with 5 μs/point, electrical bandwidth 2 Hz–1 MHz, 256 averages.

TABLE I

RESULTS OF THE TWO-EXPONENTIAL FITS FOR THE SIGNALS IN FIG. 1a

pH	$t_{1/2}$ (ns)	a_1	$t_{1/2}$ (ns)	a_2	a_3
7.5	17	0.53	180	0.16	0.31
6.0	20	0.45	200	0.16	0.39
5.0	19	0.35	210	0.20	0.45
4.5	42	0.20	250	0.14	0.66
4.0	44	0.13	340	0.10	0.77

$t_{1/2}$, half-life time; $a_i (i = 1–3)$, relative amplitudes, a_3 is the initial amplitude of slow phases decaying in the micro- and millisecond range, which were taken into account by a slightly declining straight line.

result of the fit is significantly different: $t_{1/2} = 42 \pm 6$ ns, $t_{1/2} = 300 \pm 50$ ns and $a_1/a_2 = 1.3 \pm 0.5$.

The most striking feature is the decrease of the fraction of Chl a_{II}^+ decaying in the nanosecond time range $(a_1 + a_2)$ by lowering of the pH from 6.0 to 4.0 (see Table I). The acidification slows down the reduction of an increasing fraction of Chl a_{II}^+ (a_3) into the micro- and millisecond range. Fig. 1b and c shows the time-course of the 824 nm absorption changes at different pH values on a microsecond (Fig. 1b) and millisecond (Fig. 1c) time scale. It is observed that the lower the pH, the slower Chl a_{II}^+ is reduced. For a rough analysis of the pH-dependent decay in the micro- to millisecond range, the fractions of Chl a_{II}^+ decaying between 1.5 μs and 200 μs and between 200 μs and 3 ms are depicted in Fig. 2 as a function of pH. The increase of the fraction decaying between 1.5 μs and 200 μs below pH 6 is mainly caused by a reduction phase with a half-life time of about 5 μs at pH 6 (not shown) of about 15 μs at pH 5 and of about 30–40 μs at pH 4.5 and 4.0 (see Fig. 1b). Below pH 5.0 the amplitudes of slower reduction phases with half-life times of about 200 μs, 500–700 μs and about 5 ms increase (see Fig. 1c).

In the following we ask for a correlation between the reduction kinetics of Chl a_{II}^+ and the oxygen-evolving activity. The dependence of the O_2-flash yield on the pH is shown in Fig. 3a. The optimum of the oxygen yield is observed between pH 6.5 and pH 7.5. The yield decreases sharply in the alkaline region and is at a half maximum at

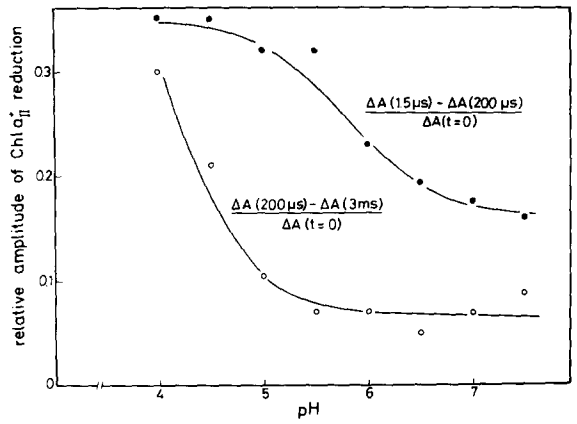

Fig. 2. Difference between the relative absorption change at 1.5 μs and 200 μs (●) and between 200 μs and 3 ms (○) as a function of pH. For experimental details see Fig. 1.

pH 8.3. In the acid region, the inhibition is half maximal at pH 4.5. The fraction of Chl a_{II}^{+} decaying in the nanosecond time range ($a_1 + a_2$) which was normalized to the result at pH 7.0 is depicted as a function of pH in Fig. 3b. Within the experimental error characterized by the scattering of data in different sets of experiments the O_2 yield and the amplitude of nanosecond decay components of Chl a_{II}^{+} follow the same dependence on the pH (for a better comparison the curves drawn in Fig. 3a and b are the same).

At pH 9.0, where O_2 evolution is zero, Chl a_{II}^{+} was mainly reduced with a half-life time of about 180 ns (Fig. 4a). It was found that the amplitude of this phase depends on the laser-flash frequency (not shown). With increasing repetition rate the amplitude of the 180-ns pahse decreases and a larger proportion of slower phases ($t_{1/2} \geqslant 200$ μs) was observed. This flash-frequency effect is characteristic of PS II particles in which oxygen evolution is inhibited [38]. Therefore, we measured the reduction of Chl a_{II}^{+} in Tris-treated PS II particles from *Synechococcus* sp. At pH 9.0 the same half-life time of 180 ns was observed (Fig. 4b). This indicates that the half-life time of 180 ns observed in untreated PS II particles at pH 9.0 can be attributed to electron donation from donor Z to Chl a_{II}^{+}. The pH dependence of Chl a_{II}^{+} reduction by the donor Z in Tris-treated PS II particles from *Synechococcus* sp. is given in Table II. Above pH 7.5 the nanosecond decay of Chl a_{II}^{+} in untreated

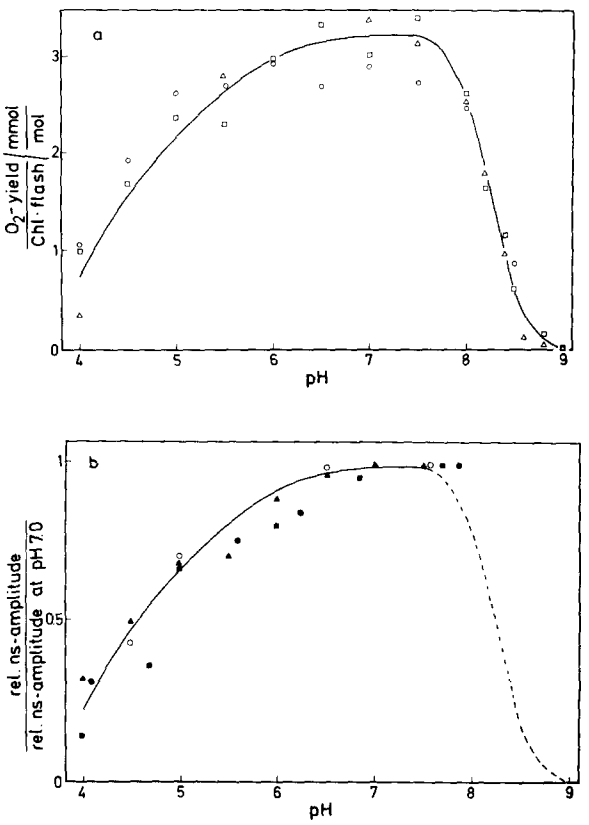

Fig. 3. (a) O_2 yield per flash and chlorophyll in dependence on the pH value measured with a zirconia oxygen sensor (□, △, two different sets of experiments) and with a Clark-type electrode (○). Samples were excited by saturating xenon flashes (20 μs FWHM). (b) Fraction of Chl a_{II}^{+} decaying with nanosecond half-life times as a function of pH. The data are normalized to the fraction maximum at pH 7.0. The different symbols represent different sets of experiments (conditions as in Fig. 1a). For further details, see text.

PS II particles is presumably a superposition of electron donation by the donor D_1 in centers still active in oxygen evolution and by the donor Z in centers which are inhibited. Therefore, in Fig. 3b data points are not marked in the alkaline pH region above pH 8.0 and the curve is shown as a broken line. Compared to data published for Tris-trated chloroplasts [38] the pH dependence found in PS II particles from *Synechococcus* (see Table II) is more pronounced. The change of the half-life time per pH unit is a factor of 3–3.5 rather than about 2 as observed in spinach chloroplasts [38]. It is remarkable that the half-life time

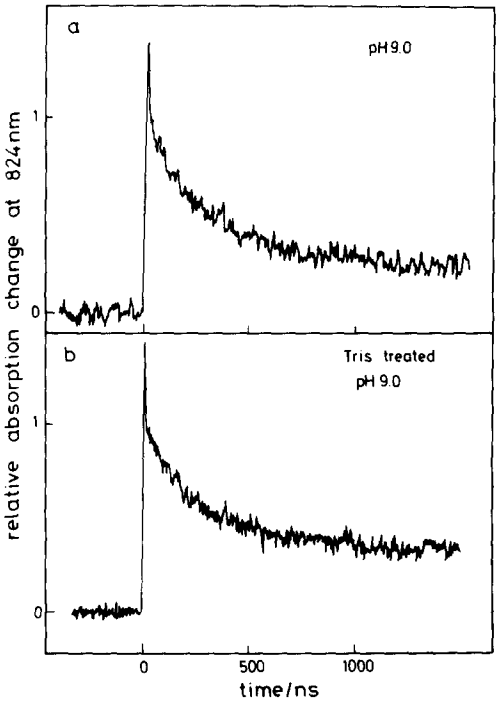

Fig. 4. Time-course of the relative absorption change at 824 nm (a) in untreated PS II particles at pH 9.0 and (b) in Tris-treated PS II particles at pH 9.0. Repetition rate: 0.5 Hz, other conditions as in Fig. 1a.

at pH 9.0 is lower by a factor of about 6 in PS II particles from *Synechococcus*.

In the following we asked for the reversibility of the inhibition of oxygen evolution at extreme pH values (see Table III). After the indicated pre-treatment the activity was measured at the pH 7.0. Table III shows that the inhibition of oxygen evolution observed at low pH (Fig. 3a) is reversible. Reversibility of the retardation of Chl a_{II}^{+} reduction by low pH is demonstrated in Fig. 5. The reduction is strongly retarded at pH 4. Returning the sample to pH 7 after incubation at pH 4, the Chl a_{II}^{+} reduction kinetics (see curve b) is nearly the same as in the control experiment (see curve a), where Chl a_{II}^{+} reduction was measured at pH 7.0 without pretreatment.

After incubation at high pH (pH 9.0) in the dark, oxygen yield is restored to only about 65% returning the sample to pH 7.0. Irreversible inactivation is significantly increased if the sample is illuminated at the alkaline pH (see Table III).

TABLE II

pH DEPENDENCE OF THE HALF-LIFE TIME OF CHL a_{II}^{+} REDUCTION BY THE DONOR Z IN TRIS-TREATED PS II PARTICLES FROM *SYNECHOCOCCUS* sp

pH	half-life time
4.5	30 μs
5.5	10 μs
6.5	4 μs
7.5	650 ns
9.0	180 ns
10.0	180 ns

TABLE III

OXYGEN-FLASH YIELD AT pH 7.0 AFTER PRETREATMENT AT pH AS INDICATED

pH	Incubated for 10 min in the dark at pH indicated, measured at pH 7.0	Incubated for 10 min at pH indicated plus illumination with 500 flashes, measured at pH 7.0
7.0	100%	88%
4.0	92%	81%
9.0	65%	28%

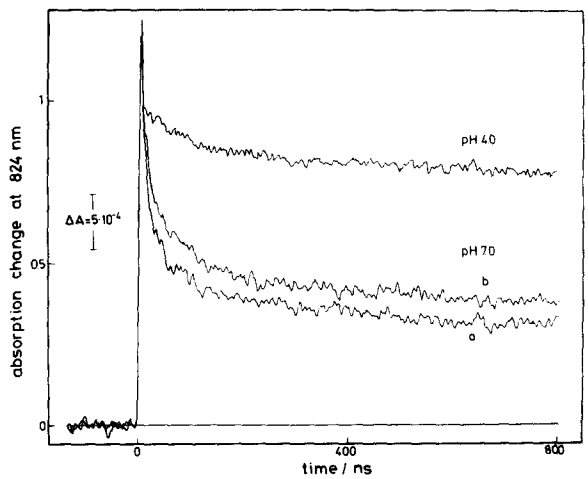

Fig. 5. Time-course of the 824 nm absorption change at pH 4.0, at pH 7.0 after incubation at pH 4.0 (trace b) and at pH 7.0 without pretreatment (trace a). Experimental conditions as in Fig. 1a.

Discussion

In this study the reduction kinetics of photooxidized Chl a_{II} were investigated parallel to the oxygen-flash yield as a function of pH under repetitive excitation. The experiments were performed with isolated O_2-evolving PS II particles from *Synechococcus* sp. in order to insure that the medium pH controls O_2 evoltuion and electron-transfer reactions in PS II directly.

Between pH 6.5 and pH 7.5 the oxygen-flash yield and the fraction of Chl a_{II}^+ decaying in the nanosecond time range is maximal and independent of pH. The inhibition of oxygen evolution observed at pH < 6.5 and pH > 7.5 is accompanied by a drastic change of the Chl a_{II}^+ reduction kientics (see Figs. 1 and 5).

In the acidic pH region the decrease of oxygen evolution is half maximal at pH 4.5. The pH dependence of the inhibition can be roughly described by a monoprotic binding site with a pK value of 4.5. Inhibition of oxygen evolution is accompanied by a retardation of Chl a_{II}^+ reduction into the microsecond and millisecond time range. Lowering the pH we observed at first an increase of the Chl a_{II}^+ fraction decaying between 1.5 μs and 200 μs (see Fig. 2). This increase is mainly caused by a reduction phase with a half-life time of about 5 μs at pH 6, of about 15 μs at pH 5, and of about 30–40 μs at pH 4.5 and 4.0. Since the electron donation from Z to Chl a_{II}^+ in Tris-treated PS II particles shows a similar pH dependence of the half-life time (see Table II), we assume that the increase of the fast microsecond phase reflects an increasing fraction of PS II centers, where Chl a_{II}^+ is reduced by Z after inhibition of oxygen evolution. This conclusion is supported by the observation that inhibition of oxygen evolution at low pH is correlated with an increase of the amplitude of EPR Signal II_f [29].

Below pH 5.0 the amplitudes of slower reduction phases with half-life times of about 200 μs, 500–700 μs and about 5 ms increase (see Figs. 2 and 1c). The Chl a_{II}^+ reduction phase with $t_{1/2} \approx$ 200 μs has been attributed to the Chl a_{II}^+ Q_A^- charge recombination [39,40]. In accordance with our results it has been reported that the amplitude of this phase increases significantly in thylakoids below pH_{in} 4.5 [16,39]. The 500–700 μs phase of Chl a_{II}^+ reduction which has also been observed in PS II particles from *Phormidium* [41,42] is presumably also caused by charge recombination. As proposed for PS II particles from chloroplasts, the different half-life times may represent back reactions from different states of inhibited PS II centers (e.g., a faster back reaction from the state Z^+Chl a_{II}^+ $Q_A^-Q_B^-$, a slower back reaction from the state Z Chl a_{II}^+ $Q_A^-Q_B^-$) [43]. The origin of the small reduction phase with a half-life time of about 5 ms (up to 10% at pH 4) is not yet clear.

An influence of acidic pH on the amplitude of decay phases of Chl a_{II}^+ in the microsecond time range has been reported for broken spinach chloroplasts [16]. The authors measured absorption changes at 690 nm with an instrumental response time of about 12 μs. The results were analyzed as a function of pH of the internal thylakoid phase which was estimated in consideration of the light-induced transmembrane pH gradient. The amplitude of a 35 μs phase increased from about 15% to about 50% of the total Chl a_{II}^+ reduction when the pH_{in} was lowered from 5.6 to 4.5 [16]. It has been shown [15] that at pH 7.0 the amplitude of a 35 μs reduction phase depends on the S-states of the oxygen-evolving complex. Therefore, it was suggested that this 35 μs phase (about 10% of the total Chl a_{II}^+ reduction) is connected to water oxidation [15]. Our finding that at low pH the normal electron transfer at the donor side is interrupted and electron donation from Z occurs, makes it probable that the large amplitude of Chl a_{II}^+ reduction with $t_{1/2} = 35$ μs (see Fig. 1b and Ref. 16) at pH 4.5 is mainly due to PS II centers which are inactive in oxygen evolution.

The remaining ns decay of Chl a_{II}^+ at low pH shows significantly different kinetics, compared to those around pH 7.0 (see Fig. 1 and Table I). Under repetitive excitation, the reduction kinetics in the nanosecond time range have been adapted by two phases with half-life times of 42 ns ($\approx$ 20%) and 250 ns ($\approx$ 14%) at pH 4.5 and 44 ns ($\approx$ 13%) and 340 ns ($\approx$ 10%) at pH 4.0. A 20 ns-phase that was associated with states S_0 and S_1 based on single flash experiments at pH 6.5 (12) is not observed. Instead, the kinetics under repetitive excitation at low pH (pH < 4.5) are similar to the slower biphasic kinetics ($t_{1/2} \approx$ 50 ns and $\approx$ 300 ns) which have been linked to states S_2 and S_3

[12]. The retardation of the electron transfer in states S_2 and S_3 has been explained by Coulomb attraction due to a positive excess charge located in the oxygen-evolving complex in states S_2 and S_3 [12]. The absence of a 20 ns phase that is the major phase around the pH optimum indicates that below pH 5.0 a retardation of the electron donation to Chl a_{II}^+ occurs. This retardation might be explained by the protonation of a binding site close to D_1 at low pH.

A retardation of the electron transfer from the oxygen-evolving complex to Chl a_{II}^+ might also be caused by the increase of the standard free enthalpy, ΔG^O, of water oxidation lowering the pH. For example, lowering of the pH from 8 to 4 leads to a decrease of the redox potential difference between Chl a_{II}/Chl a_{II}^+ and 2 H_2O_2/O_2, 4 H^+ by 240 mV.

Down to pH 4.0 the inhibition of oxygen evolution and the change of Chl a_{II}^+ reduction kinetics were reversible (see Table III and Fig. 5). Performing single-flash experiments at pH 4.5 we found that the electron transfer to Chl a_{II}^+ is already slowed down into the microsecond time range after the first flash given to a dark-adapted sample (not shown). This indicates that the inhibition occurs in the dark and may be caused by a reversible protonation of a functional group which regulates the electron transfer from D_1 to Chl a_{II}^+. Irreversible inactivation, which has been observed in spinach chloroplasts in connection with release of Mn^{2+} [29] below pH 5.0, is probably shifted to lower pH values in PS II particles from *Synechococcus*.

At alkaline pH the oxygen-flash yield decreases strongly between pH 8.0 and 9.0 with a half-maximum at pH 8.3 (see Fig. 3a). A Hill plot of

$$\log \frac{Y_{O_2}^{max} - Y_{O_2}}{Y_{O_2}} \text{ against pH } (Y_{O_2} = O_2 \text{ flash yield})$$

between pH 8.0 and 9.0 yields a straight line of slope $n = 2.6$ (not shown). The Hill coefficient n yields a minimum number of three binding sites for H^+ or OH^- involved in the inhibition of oxygen evolution caused by deprotonation or binding of OH^-. This inhibition is accompanied by a change of the Chl a_{II}^+ reduction kinetics. Increasing the pH above pH 8.0, an increasing fraction of Chl a_{II}^+ is reduced by the donor Z. Consistent with our results, the amplitude of EPR Signal II_f due to the oxidized form of Z increases under continuous illumination with increasing alkalinization [28,33]. At pH 9.0 the half-life time of electron donation by Z is 180 ns (see Fig. 4). Significantly slower Chl a_{II}^+ reduction kinetics are probably due to charge recombination (see above).

Table III shows that at pH 9.0 reversible inhibition and irreversible inactivation of oxygen evolution are superimposed. As a possible mechanism of the reversible inhibition it has been proposed that bound Cl^- from the catalytic site of the oxygen-evolving complex is displaced by OH^- [44,45]. It has been shown that the irreversible inactivation is time-dependent and increases significantly by illumination (Refs. 32 and 33; see also Table III). This has been explained by specific sensitivity of the S_2 state to alkalinization [32]. The inactivation is accompanied by loss of Mn^{2+} and the release of the peripheral 33 kDa protein [33,34].

The results of this work show that the electron donation from the immediate donor, D_1, to Chl a_{II}^+ is sensitive to both reversible inhibition at low pH and irreversible inactivation of oxygen evolution at high pH. We assume that under these conditions Chl a_{II}^+ is reduced by the donor Z, which might be a modified state of D_1 and, in part, by charge recombination. Z^+ is ultimately reduced by an electron from the acceptor side. The nanosecond decay connected with water oxidation shows altered kinetics below pH 5.5. Single-flash experiments are in preparation in order to clarify (a) the pH dependence of the pattern of the positive excess charge located in the oxygen-evolving complex and (b) if the reversible inhibition of O_2 evolution at low pH can be explained by an increase of misses in all centers or by an increase of a fraction of centers which are completely blocked.

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