ELSEVIER

Biochimica et Biophysica Acta 1274 (1996) 1–8

BBA Biochimica et Biophysica Acta

Net charge oscillation and proton release during water oxidation in photosynthesis. An electrochromic band shift study at pH 5.5–7.0

H. Kretschmann, E. Schlodder, H.T. Witt *

Max-Volmer-Institut für Biophysikalische und Physikalische Chemie, Technische Universität Berlin, Str. des 17. Juni 135, 10623 Berlin, Germany

Received 21 April 1995; revised 18 October 1995; accepted 17 November 1995

Abstract

In the S-state cycle of water oxidation, a local electric field was measured in states S_2 and S_3. This was indicated by the strongly retarded reduction kinetics of the oxidized primary electron donor of PS II in these states (Brettel, K., Schlodder, E. and Witt, H.T. (1984) Biochim. Biophys. Acta 766, 403–415) as well as by electrochromic band shifts in state S_2 and S_3 (Saygin, Ö. and Witt, H.T., FEBS Lett. 176 (1984) 83–87; 189 (1985) 224–226). The electric field oscillation of 0:0:1:1 in S_0:S_1:S_2:S_3 is strictly coupled with the pattern of manganese redox changes measured at 365 nm and of O_2 evolution under very different conditions (Kretschmann, H. and Witt, H.T. (1993) Biochim. Biophys. Acta 1144, 331–345). In this work with PS II complexes from the cyanobacterium *Synechococcus elongatus* it is shown that the electric field oscillation as well as the pattern of redox changes of manganese are practically pH-independent between pH 5.5 and pH 7.0; i.e., in the range in which the pattern of O_2 evolution and water oxidation, respectively, is pH-independent. It was suggested that a net charge created as charge difference between electron extraction and proton release from the catalytic center may be the origin of the electric field. With this explanation it follows that, with the $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$ transitions, a pH-independent proton release of 1:0:1:2 from the catalytic center takes place. The proton release into the medium is, however, generally pH-dependent. For PS II complexes from cyanobacteria a mechanism is proposed which may be responsible for the modification of the supposed pH-independent proton release from the catalytic center into the pH-dependent proton release into the medium. It is proposed that in the pH 5–7 range an amino acid residue with a pK value of approx. 6 releases a proton induced by a pK shift through electrostatic interaction with the local electric field set up in state S_2. When, subsequently, the created base traps a proton released from the catalytic center in the $S_3 \rightarrow S_0$ transition, this results in a pH-dependent non-integer oscillation of the proton release into the medium. The predicted values have been compared with the directly measured ones.

Keywords: Photosynthesis; Water oxidation; Proton release; Net charge; Electrochromism; Photosystem II; Oxygen-evolving complex

1. Introduction

In the primary act of photosynthesis, a transmembrane charge separation takes place at the reaction centers (RC) I and II which is indicated by the creation of a transmembrane electrical field [1]. At RC II this is realized through an electron ejection from the excited chlorophyll a_{II} (P_{680}) [2,3] located at the inner side of the membrane to the first plastoquinone acceptor, Q_A, at the outer side [4]. The redox potential of P^+_{680}/P_{680} is the driving force for water oxidation. P^+_{680} oxidizes the electron carrier Y_Z, tyrosine-161 of the polypeptide D1 [5,6], which is the immediate electron donor to P^+_{680} [7]. Y^+_Z, in turn, extracts an electron from a manganese cluster located in the water-oxidizing complex. The water oxidation requires the extraction of 4 electrons from 2 H_2O with the release of 1 O_2 and 4 H^+. It was shown that for this process the complex cycles through five redox states, $S_0 \rightarrow S_4$, whereby O_2 evolution takes place in the transition from state S_4 to S_0 [8,9]. The subscripts indicate the number of electrons extracted step-by-step from the complex. The period-four oscillation of the complex is driven by four turnovers of $P_{680}Q_A \rightarrow P^+_{680}Q^-_A$. It is generally accepted that a cluster of four manganese (Mn) is located within the catalytic center. The

Abbreviations: BBY, membrane fragments of enriched PS II; Chl, chlorophyll *a*; DCBQ, 2,5 dichloro-*p*-benzoquinone; Hepes, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; Mes, 2N-morpholino-ethane sulfonic acid; OEC, O_2-evolving complex; PS II, Photosystem II; P680, Chl a_{II} primary electron donor of PS II; RC, reaction center; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl] glycine.

* Corresponding author. Fax: +49 30 31421122.

SSDI 0005-2728(95)00150-6

S-state transitions correspond most likely to the successive oxidation of the Mn cluster that acts as a storage device for the oxidizing equivalents.

With respect to the four protons released from water, it was accepted for many years that the stoichiometric pattern is 1:0:1:2 for the transitions $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$ [10,11]. More recent work based on measurements with pH-indicating dyes showed, however, that the stoichiometry of proton release into the medium depends strongly on the type of preparation (thylakoids, BBY particles or PS II core complexes), the experimental material (spinach, pea, cyanobacteria, etc.) and the pH. Furthermore, non-integer stoichiometries of proton release have been reported ([12–15], for a review see Ref. [16]).

For the protons released from the catalytic center it is, however, expected that these are strictly correlated with the S-state oxidations. Obviously, the proton release from the catalytic center is transiently superimposed by protons dissociated from other material-dependent groups, e.g., from amino acid residues located at the periphery of the PS II complex. The proton release from these unknown acids may be realized by pK shifts due to the electrostatic interaction between the charge of the protonatable residue of the amino acid and the positive charges created with the manganese oxidation in the catalytic center or even through the oxidized tyrosine, Y_Z^+, preceding the Mn oxidation [16]. Therefore, on the basis of extrinsic proton measurements in the medium with pH-indicating dyes or pH electrodes, no direct conclusion can be made regarding the specific stoichiometry of the proton release from the catalytic center and bound water, respectively.

In this context it would be worthwhile if an intrinsic indicator were available to monitor the proton release from the catalytic center. We have suggested that the local electric field which has been observed to oscillate with the S-state transitions may indicate this proton release [17–19]. In the nanosecond range, the P_{680}^+ reduction kinetics show a period-four oscillation: fast kinetics in S_0 and S_1 (20 ns) and kinetics strongly retarded in states S_2 and S_3 (biphasic with 50 and 250 ns) [17]. This characteristic pattern, fast kinetics in S_0 and S_1 on the one one hand and slow kinetics in S_2 and S_3 on the other hand, was found to be independent of the pH in the range between 4.5 and 7.0 [20]. The drastic retardation of $P680^+$ reduction was attributed to a local electric field which may be caused by a positive charge localized in the catalytic center in states S_2 and S_3. It was assumed that the electric field retards the electron transfer from Y_Z to P_{680}^+ by Coulomb attraction. It has been estimated that the electrostatic effect of one positive elementary charge in the catalytic center may well explain the retardation of P_{680}^+ reduction in states S_2 and S_3 [17]. The formation of a local electric field was supported by the observation of electrochromic band shifts which occur on the $S_1 \rightarrow S_2$ transition and are reversed in the $S_3 \rightarrow S_0$ transition [18,19]. These shifts have been ascribed to the presence of a local electric field in the catalytic center in states S_2 and S_3. These electrochromic changes were discovered at 515 nm [18] and in the red around 680 nm [19]. The difference spectrum in the red indicates that the absorption band of a chlorophyll a molecule, possibly P_{680} itself, is blue-shifted. (These local electrochromic changes should not be confused with the delocalized electrochromic changes indicating the formation of the transmembrane electric field [1].) In summary, the retardation of the electron transfer and the electrochromic band shifts give strong evidence for a period-four oscillation of a local electric field: 0:0:1:1 in S_0:S_1:S_2:S_3. A local electric field was furthermore detected by an electrochromic band shift in state S_{-1} [21]. S_{-1} is obtained by reduction of the S_1 state, e.g., by hydroxylamine, to the over-reduced state, S_{-1}, which can be further reduced to the superreduced and terminal state, S_{-2} [22,23]. The reduction is due to the over- and super-reduction of the manganese cluster [21,23,24]. In this extended system, the oscillation of the local electric field therefore is 1:0:0:1:1 in S_{-1}:S_0:S_1:S_2:S_3. With respect to the origin of the electric field it has been assumed that a positive net charge is generated as a difference between electron abstraction and proton release from the catalytic center. Then the pattern can be explained if the 1:1:1:1 electron extraction in the $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$ transitions is accompanied by a proton release of 1:0:1:2 from the catalytic center. In the extended S-system, a proton release of 2:1:0:1:2 should take place in the $S_{-1} \rightarrow S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$ transitions.

The oscillation of the local electric field appears to be strongly coupled with the turnover of the catalytic center. Therefore, it is expected that the pattern is independent of the PS II material and the type of preparation. Indeed, the same oscillation of the retarded reduction kinetics of P_{680}^+ was observed in PS II from spinach [17] and PS II core complexes from cyanobacteria [17,20], pea (Schlodder and Drewenstedt, unpublished data), and spinach [25]. A corresponding material-independent behavior of the local electrochromic band shift was observed by the same period-four oscillation in different preparations of PS II complexes from cyanobacteria [18,19,23], PS II particles from spinach [12] and core complexes from spinach [13]. Furthermore, a strict correlation between the oscillation pattern of O_2 evolution, the absorption changes at 365 nm attributed to the redox changes of manganese [26] and of the electric field has been observed under very different conditions using PS II complexes from cyanobacteria [23].

In this work, we measured absorption changes at 695 nm indicating the local electric field and at 365 nm assigned to the redox reactions of manganese as a function of the flash number between pH 5.5 and pH 7.0. The patterns were found to be independent of the pH, suggesting that the proton release from the catalytic center is pH-independent. On the other hand, the proton release into the medium depends on the pH. A model is proposed which explains in a simple manner, how the pH-independent proton release from the catalytic center is transformed

into a pH-dependent non-integer proton release into the medium.

2. Material and methods

Oxygen-evolving complexes (OEC) were prepared from the cyanobacterium *Synechococcus elongatus* according to Refs. [27], [28]. For the optical measurements the medium contained a concentration of 3 μM chlorophyll, 20 mM buffer (at pH 5.5–6.5, Mes; at pH 7.0, Hepes or Tricine), 0.3 M mannitol, 20 mM $CaCl_2$ and 10 mM $MgCl_2$; temperature 20°C. DCBQ (600 μM) was used as acceptor to eliminate the binary oscillation of the acceptor side [26]. The flash-induced electrochromic shift at 695 nm and absorption changes of manganese at 365 nm were measured as described in Refs. [19] and [24]. The samples were excited at 640 nm by saturating laser flashes with a pulse duration of about 300 ns from a dye laser (Phase R Model DL-1400). The absorption changes were monitored at 0.49 s after the flash for 10 ms. The time between the flashes was 0.5 s. The signals are the average of 20–30 measurements. The optical path for the measuring light was 1 cm in the UV and 5 cm in the red. For the reduction of the OEC to the overreduced state, S_{-2}, hydrazinium sulfate was used [23]. Proton release was monitored in the laboratory of W. Junge by absorption transients of the pH-indicating dye Bromcresol purple at 575 nm as described previously [29].

In order to obtain the correlation of the measured data with the unmixed individual S-state transitions, corrections were made with the Kok parameter (S_0/S_1 ratio of dark adapted samples, misses and double hits). The latter were determined by analysis of the UV-absorption changes at 367 nm (for details see Ref. [23]).

3. Results and discussion

Fig. 1A,B shows electrochromic absorption changes at 695 nm measured in a train of flashes at pH 5.5 and 7.0. After dark adaptation, S_1 is the most stable state, whereby the sequence practically starts with $S_1 \rightarrow S_2 \rightarrow S_3$,. etc. The signal in the first flash is overlapped by an unknown signal and is excluded from the considerations. The further pattern oscillates with a period of four and is practically the same at pH 5.5 and pH 7.0. The best fit is obtained with the Kok parameters given in the figure legend (see triangles), if the relative electrochromic band shifts are (0:0:1.1:0.9) in the states S_0:S_1:S_2:S_3 at pH 5.5 as well as at pH 7.0.

In [23] it was shown that under different conditions the period-four oscillation of the electrochromic band shifts is strictly coupled with the UV absorption changes at 365 nm assigned to the redox reaction of manganese, Mn. This should also apply to the pH dependency. Fig. 1C,D shows

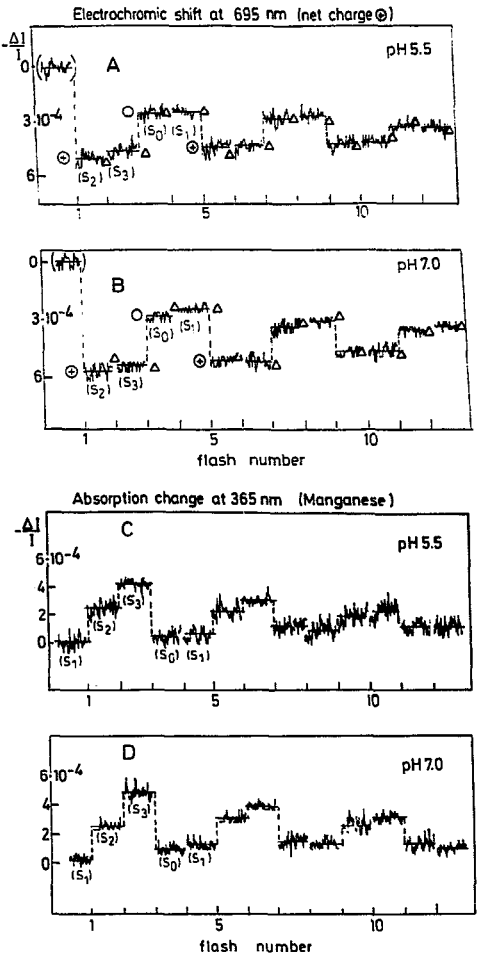


Fig. 1. Oscillation pattern of relative absorption changes measured 0.49 s after the flash as a function of the flash number (dark time between the flashes 0.5 s). (A) Electrochromic changes at 695 nm at pH 5.5 and (B) at pH 7.0. The symbol △ indicates the calculated values using the following Kok parameters: for pH 5.5 19% S_0, 81% S_1, 4% misses, 8% double hits and for pH 7.0 16% S_0, 84% S_1, 3% misses, 3% double hits. (C) UV absorption changes at 367 nm at pH 5.5 and (D) at pH 7.0.

the flash-induced absorption changes at 365 nm as a function of the flash number at pH 5.5 and pH 7.0. The pattern of the optical changes of Mn at pH 5.5 closely resembles that at pH 7.0, i.e., in both cases the pattern were found to be pH-independent.

In Fig. 2A,B, the patterns of the electrochromic absorption changes starts with state S_{-2}. The super-reduced S_{-2} state has been attained by reversible reduction of the S_1 states and manganese, respectively, with hydrazine (details

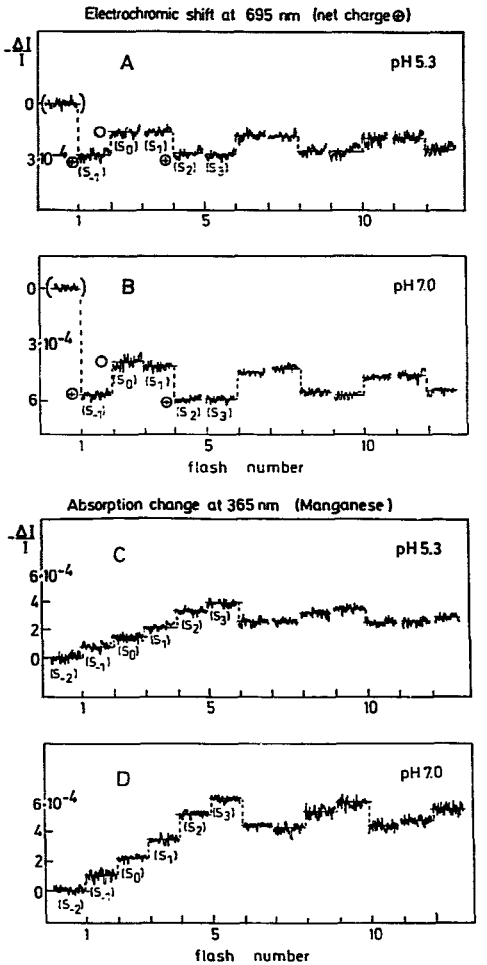


Fig. 2. Oscillation pattern as in Fig. 1 but after incubation with 100 μM hydrazine for 2.5 h. (A) electrochromic changes at 695 nm at pH 5.3 and (B) at pH 7.0. (C) UV absorption changes at 367 nm at pH 5.3 and (D) at pH 7.0.

see Ref. [23]). S_{-2} is the most reduced state which is reversible. The signal in the 1. flash is overlapped by an artefact which is indicated by brackets. The subsequent patterns at pH 5.3 and pH 7.0 are very similar. As shown in Ref. [23], the pattern at pH 7.0 can be fitted best with the Kok parameters if the relative electrochromic band shifts are (1:0:0:1.1:0.9) in the states S_{-1}:S_0:S_1:S_2:S_3. This stoichiometry is regarded as relevant also at pH 5.3. The small deviations of the stoichiometries of the electrochromic changes from the integer values in Figs. 1 and 2 are close to the experimental accuracy. Therefore, these values are regarded as integer between pH 5 and 7.

In Fig. 2C,D the corresponding pattern of the absorption changes at 365 nm are shown at pH 5.3 and pH 7.0. In comparison with Fig. 1C,D, beyond the S_1 state three additional reduction steps become visible up to the S_{-2} state, which are assigned to the Mn reduction by hydrazine [23]. The flash-induced pattern of the redox changes starting at state S_{-2} is similar at pH 5.3 and pH 7.0.

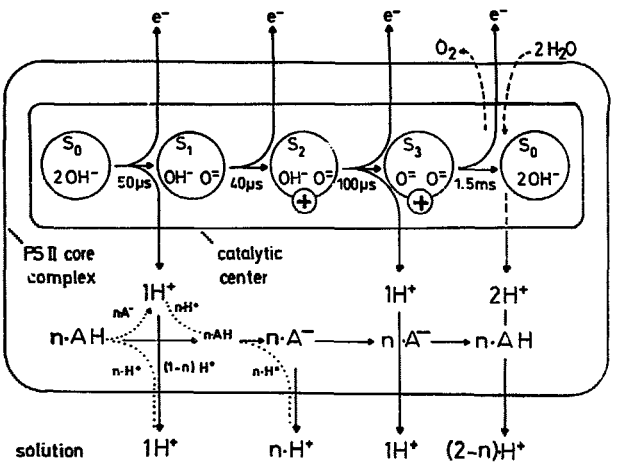
The smaller amplitudes of the pattern of the electrochromic absorption changes as well as of the UV absorption changes in Figs. 1 and 2 at the acid pH values in comparison with those at pH 7 are due to the deactivation of a fraction of the PS II complexes. The deactivation is reversible, because within the sequence of the measurement pH 7 → pH 5 → pH 7, we observed at pH 7 practically the same amplitude at the end compared to that at the beginning (not shown). The results presented in Figs. 1 and 2 show that the oscillation of the local electric field as well as of the redox changes of the manganeses are practically pH-independent in a range in which also the oscillation of the O_2 evolution is pH-independent [20].

Different events may be considered as cause for the local electric field and its pH independence. The possibility that local ion displacements on the protein matrix may be responsible for the electric field [25] is unlikely because this should depend on the pH. On the other hand, ion displacements of $\lesssim 3$ Å within the catalytic center may be too small to induce a significant electrochromic shift considering the distance from the shifted chlorophyll *a* (possibly P_{680}) (≥ 20 Å). It is also unlikely that such displacements in the order of ≈ 3 Å can induce a retardation of the kinetics of the electron transfer from Y_Z to P_{680}^+ by a factor of 10 (see above). Therefore, it is most likely, as proposed earlier [17–19], that a net charge created as difference between the electron abstraction and the proton release from the catalytic center may be the origin of the electric field. A high dielectric strength in the vicinity of the Mn cluster would facilitate the formation of this net charge energetically. (This intrinsic net charge should not be confused with the extrinsic net charge defined, for example in Ref. [29], as charge difference between electron abstraction and proton release into the medium.) On this basis, between pH 5.5 and 7.0, the stoichiometry of proton release from the center would then be 1:0:1:2 for the transition $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$ and 2:1:0:1:2 for the transition $S_{-1} \rightarrow S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$ (see Introduction). With the specific assumption that the proton released from the catalytic center is originating from bound water, the release of 4 protons between S_{-1} and S_3 is only consistent with 2 H_2O in S_{-1} and 2 OH^- in S_0. Consequently, the possible water derivatives in the other S-states should be those indicated in Scheme 1.

The pH-independent proton release from the catalytic center derived from the local electric field is different from the proton release into the medium which is pH-dependent

(see Introduction). Therefore, the latter cannot be the cause of the electrochromic shift. This is in accordance with results of Van Leeuwen [13] obtained with core particles from spinach. Although the pattern of proton release into the medium was 1:1:1:1, he nevertheless observed a period-four oscillation of the local electric field effects. According to Van Leeuwen [13], electrochromic absorption changes around 435 nm associated with the S-state transitions $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ are practically pH-independent between pH 5.5 and 7.2. In contrast to our results, these workers observed a pH-dependent band shift for the $S_0 \rightarrow S_1$ transition. In spite of this difference, our conclusion and those in Refs. [13] and [29] are, however, in contradiction to measurements of Rappaport and Lavergne [12]. They reported that in BBY particles from spinach the pattern of the electrochromic changes are strongly correlated with the proton release into the medium. The reason for these discrepancies is not clear.

In the following, a mechanism is discussed for PS II complexes from cyanobacteria which explains how in the pH 5–7 range the proposed pH-independent and integer proton release of the catalytic center may be modified into the pH-dependent non-integer proton release into the solution (see scheme). It is proposed that this is due to the electrostatic interaction of the positive net charge in the catalytic center with amino acid residues of the protein matrix. In order to elucidate the application of the mechanism, it seems useful to focus the consideration on a pH range in which an interaction with only one amino acid residue takes place. PS II of cyanobacteria do not contain the subunits of 17 and 24 kDa found in the PS II of higher plants. In these samples, we consider in the pH 5–7 range the presence of one effective amino acid residue AH per PS II with a pK value of about 6. In PS II complexes from cyanobacteria a protonation of a group with a pK of about 5.3 was observed [20], retarding the electron transfer from Y_Z to P_{680}^+. In BBY particles from spinach a protolytic group with a pH 5.8–6 has been found which retards the electron transfer from Y_D to the catalytic center [30]. Both effects may be due to one and the same group (histidine?) which may be identical to that considered in our model. It is assumed that in the dark the residue of the amino acid is predominantly protonated in the acid medium ($n \approx 1$) and is successively deprotonated ($n < 1$) towards the alkaline medium ($n \approx 0$). It is $1 - n = \alpha =$ degree of dissociation. For a weak acid it is $\mathrm{pH} - \mathrm{p}K \cong \log(\alpha/1-\alpha)$, i.e., $n = 0.5$ if $\mathrm{pH} = \mathrm{p}K = 6$. nAH indicates the fraction of the amino acid residue in the protonated state. It is further assumed that in the $S_1 \rightarrow S_2$ transition the release of all n protons from nAH into the medium takes place with the formation of nA^- bases. This should be induced by a pK shift towards $\mathrm{p}K \approx 4.0$ due to electrostatic interaction between the charge of the protonated residue of the amino acid and the positive net charge of the catalytic center in state S_2 (see above). The base nA^- remains up to S_3 because the net charge remains up to this state; nA^- becomes reprotonated when in the $S_3 \rightarrow S_4 \rightarrow S_0$ transition the net charge disappears through the release of two protons and uptake of 2 OH^-, respectively, at the catalytic center (see scheme). Thereby, $n\mathrm{H}^+$ of the two protons



Scheme 1. Proposed mechanism for PS II complexes of cyanobacteria functioning in the range of pH 5.5–7.0. Top: Electron and proton release from the catalytic center and derivatives of water in the different S states. The times indicate the electron transfer times [26]. Bottom: AH, deprotonatable amino acid residues with $\mathrm{p}K \approx 6$. n, pH-dependent value between one (acid medium) and zero (alkaline medium). Deprotonation of the amino acid residue AH and formation of A^- is assumed to take place through a pK shift induced by the electric field and net charge ⊕, respectively, in state S_2 and its reprotonation when ⊕ disappears with the S_3~S_0 transition. Dotted lines: possible preceding proton release by pK shift through oxidation of Y_Z. For details see text.

Table 1
Expected stoichiometry of proton release into the solution between the acid and alkaline medium according to the mechanism depicted in the scheme (details see text)

	$S_0 \rightarrow S_1$	$S_1 \rightarrow S_2$	$S_2 \rightarrow S_3$	$S_3 \rightarrow S_0$
Acid medium				
$n \approx 1$ ($\leq$ pH 5)	1	1	1	1
0.5	1	0.5	1	1.5
0.3	1	0.3	1	1.7
$n \approx 0$ ($\geq$ pH 1)	1	0	1	2.0
Alkaline medium				

must be trapped by nA^- because of the electrostatic balance and the necessary restoration of the initial nAH state. Therefore, in the $S_3 \rightarrow S_0$ transition only $(2-n)$ protons of the two protons released from the catalytic center are released into the medium. For amino acid residues with effective $pK > 7$, it is assumed that a possible pK shift induced by the net charge in S_2 does not pass over values which would noticeably contribute to proton releases at $\leq$ pH 7.

The presence of nA^- in states S_2 and S_3 could, however, contribute to the extent of the electrochromic response and retardation of the P_{680} reduction kinetics. Such a contribution must depend on the locus of A^- within the protein subunit and its distance from the shifted chlorophyll(s). This effect would, however, not affect the pattern of the oscillation and scarcely the sensitivity of its indication, if A^- is located towards the protein periphery.

The values of the proton release into the medium, which are expected according to the outlined model are depicted for some n values between 0 and 1 in Table 1. A characteristic feature of the data is the indication of a change of a non-oscillating proton release in acid medium towards a period-four oscillation with increasing pH values. A pH-independent release of one proton is expected in the $S_0 \rightarrow S_1$ and $S_2 \rightarrow S_3$ transitions. Changing from an acidic to an alkaline medium, a decrease of the proton release from one to zero is predicted in the $S_1 \rightarrow S_2$ transition and an increase from one to two in the $S_3 \rightarrow S_0$ transition.

To test the quality of the model and its predictions, direct measurements of the proton release into the medium with the same material which has been used in this work were carried out with a pH-indicating dye in the laboratory of W. Junge (Haumann, M., unpublished results). The stoichiometries measured with the $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$ transitions are

*pH*5.5	1/1	:	0.9/0.9	:	1/1	:	1.1/1.1
*pH*6.5	0.5/1	:	0.4/0.3	:	1/0.7	:	2.1/2.0
*pH*7.5	0.5/1	:	0.3/0.3	:	0.9/0.6	:	2.3/2.1

The slash separates data obtained with two different Kok parameters. In PS II samples from another cyanobacteria (*Synechococcus vulcanus* Copeland) at pH 6.5 a stoichiometry of (1:0:1:2) was measured [31]. The predicted data in the table are in moderate agreement with the measured values. The predicted non-oscillating proton release in an acidic medium towards a period-four oscillation with increasing pH is in accordance with the measured values. The predicted pH-independent release of one proton in $S_0 \rightarrow S_1$ as well as in $S_2 \rightarrow S_3$ is also similar to the measured proton release. The pH dependence of the proton release in the $S_1 \rightarrow S_2$ and in $S_3 \rightarrow S_0$ transitions is also in agreement with the experimental results.

The mechanism outlined has been applied to a pH range (pH 5–7) where obviously only one amino acid residue is effective ($pK \sim 6$). According to the same mechanism, other amino acids with $pK > 7$ should be responsible for the pH-dependent proton release into the solution in the range of pH > 7. However, in this range, the proof for the calculations is more complicated. Obviously, at pH > 7 three proton-binding sites or even more are present at least in *Synechococcus elongatus* [32]. Furthermore, in the alkaline medium at pH > 8, the deactivation of water oxidation is in part irreversible, in contrast to the reversible deactivation in the acid medium [20,32].

If a large number of different protolytic groups (AH, BH, CH etc.) are present with a large spectrum of appropriate pK values, a deprotonatable acid is present at practically each pH value. Under these conditions, at all pH values one proton should be released in the $S_1 \rightarrow S_2$ state transition ($n = 1$) from an amino acid. Consequently, at all pH values the pattern of the proton release into the medium should be 1:1:1:1. Such a pH-independent stoichiometry has been observed in PS II core complexes from spinach [14]. The extrinsic 17 kDa and 24 kDa polypeptides which shield the water oxidizing complex from the aqueous medium are removed during the preparation of these core complexes and the lifetime of the higher S-states is drastically reduced compared to thylakoids [33], indicating a modification of the donor side. Possibly thereby a less hydophobic environment at the protein surface is created which may decrease the initial pK value of a large number of otherwise 'silent' amino acid residues. In this way, the electrostatic response of these groups to a local electric field may become effective [16]. However, the proton release induced under such conditions does not yield information on the mechanism of the proton release in the intact native system and the catalytic center, respectively. In PS II core complexes from cyanobacteria used in this work a pH-independent 1:1:1:1 pattern has not been observed (see above). This might be expected. Cyanobacteria have already by nature no 17 and 24 kDa subunits and the donor side is shielded from the hydrophilic surface by different means. This is also supported by the fact that lifetimes of the higher S states are not reduced.

With respect to the kinetics of the proton release from the catalytic center, it is plausible to assume that the release is coupled with the electron transfer from the manganese-cluster to Y_Z^+ (50 μs–1.5 ms, see scheme).

This might be expected because of the strong electrostatic interaction of the created positive charge on the manganese with the bound water in its vicinity. Also, the speed of deprotonation of acid, AH, should depend on the Mn oxidation because its pK shift is induced by the net charge formation in the transition $S_1 \rightarrow S_2$.

The kinetics of the proton release might, however, be faster if in a preceding reaction a proton is released from an amino acid residue by electrostactic interaction with the oxidized Y_Z^+, preceding the Mn oxidation as reported by Haumann and Junge [15] for PS II samples from pea (see below). If we also consider this protolytic reaction in our model, it is nAH that could be deprotonated first by the Y_Z^+ formation preceding the S_0–S_1 transition. When Y_Z^+ is reduced by oxidation of the manganese, the pK shift induced by the electric field of Y_Z^+ is reversed and the base nA^- will immediately trap that proton which is subsequently released from the catalytic center with the oxidation of the manganese (see dotted pathway in the scheme). The nAH created can again be deprotonated by the Y_Z^+ formation preceding the S_1–S_2 transition. In this way, the time course of proton release from the catalytic center could become apparently fast in the $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ transitions. The extent of the fast portion should be pH-dependent because n has been introduced as pH-dependent (see above). Magnetic resonance studies [34] indicate the formation of a neutral tyrosyl radical, $\dot{Y}_Z$, upon Y_Z oxidation, which would exclude proton releases induced by Y_Z^+ as discussed above. Indeed, due to the very low pK value of oxidized tyrosine in water (p$K \leq 0$) it is most likely that a deprotonation of the tyrosine occurs upon its oxidation. The similarity between the UV-difference spectrum of $Y/\dot{Y}$ in water and the oxidized-minus-reduced tyrosine spectrum measured in O_2-evolving PS II complexes [7] supports this assumption. However, this suggestion is in contrast to the observation that the oxidized Y_Z induces an electrochromic band shift of a nearby chlorophyll [35]. Both results could be consistent with each other if the phenol proton of the oxidized Y_Z, is reversibly bonded to a base, B, in the immediate vicinity, i.e., $Y_Z \rightarrow \dot{Y}_Z \ldots H\text{–}B^+$, as discussed especially for Y_D (D2-Tyr160) in Ref. [34]. Thereby, the proton is hydrogen bonded to the oxygen of the neutral radical $\dot{Y}_Z$. In this case, fast proton releases from amino acids in general – and from AH in our model – would not be induced by electrostatic interaction with Y_Z^+ but with $\cdots H\text{–}B^+$.

Haumann and Junge observed in their PS II samples that under certain conditions the time course of the major portion of proton release takes place with phases much shorter than the rise time of the electron transfer from Mn to Y_Z^+ in any of the four transitions [15]. From this result they concluded that in each transition an amino acid residue is deprotonated by Y_Z^+, whereby the four corresponding bases created should be stable up to the S_4 state. The release of the four protons from water is assumed to take place only in the $S_4 \rightarrow S_0$ transition. These should be trapped by the four bases produced before in $S_0 \rightarrow S_4$. This should occur rapidly and is therefore not detectable. This mechanism would correspond to a concerted abstraction of four electrons and protons from 2 H_2O in the $S_4 \rightarrow S_0$ transition. Rappaport et al. reported recently [36] that a fast proton release does not occur in all S-state transitions (230 μs in the $S_2 \rightarrow S_3$ transition); it was also concluded that in the $S_3 \rightarrow S_0$ transition 1.5 protons are released from the catalytic center which are not trapped by compensating bases. But in spite of these differences in [15] and [36], the principal conclusion in Ref. [15] that practically no proton release occurs up to state S_4 differs from our interpretation of the electrochromic absorption changes presented in this work. The net charge indicates proton releases from the catalytic center in the $S_0 \rightarrow S_1$ as well as in the $S_2 \rightarrow S_3$ transitions (see Scheme 1). Furthermore, according to the concept in Ref. [15], an accumulation of four negative charges (or lack of positive charges) of the four bases would take place up to the S_4 state. Additionally, the accumulation of four positive charges within the catalytic center (mainly oxidized manganeses in approx. 3 Å distance) would occur up to the S_4 state without a compensating effect preventing the expected very strong Coulomb repulsion. (The compensating influence of the negative charges of the bases supposed to be located at the protein periphery would be too weak.) These features are not consistent with the measurements shown in Figs. 1 and 2. The accumulation of the four positive charges in the catalytic center should induce a strong four-step electrochromic band shift. This is, however, not observed in Figs. 1 and 2.

4. Conclusion

The S-state-dependent oscillation of a local electric field of 0:0:1:1 starting with S_0 is coupled with the pattern of oxygen evolution and of absorption changes at 367 nm, attributed to the redox changes of manganese under very different conditions [23]. These patterns are material- and pH-independent between pH 5.5 and 7.0. It is suggested that a net charge created as difference of the electron abstraction and proton release from the catalytic center is the origin of the electric field. Then, the pattern indicates between pH 5.5–7.0 a pH-independent proton release from the catalytic center with a stoichiometry of about 1:0:1:2. The proton release into the solution is, however, generally pH-dependent. For the PS II complexes from cyanobacteria, it is assumed that in the pH 5–7 range from an amino acid residue with a p$K \approx 6$ releases a proton induced by a pK shift through electrostatic interaction with the local electric field and net charge, respectively, in state S_2. When subsequently the created base traps a proton released from the catalytic center in the S_3–S_0 transition, it results a pH-dependent non-integer oscillation of proton

release into the medium. The predicted values are in moderate agreement with the protons measured in the medium. Regardless of our interpretation, the observed oscillation of the local electric field must provide essential information on the water-oxidizing mechanism.

Acknowledgements

We are very grateful to M. Haumann and Prof. Dr. W. Junge of the Biophysik Department of the Universität Osnabrück for making available to us the unpublished data of proton releases into the medium measured on our PS II preparation from *Synechococcus elongatus*. The financial support from the Deutsche Forschungsgemeinschaft (Sfb 312, TP A3 and A5) is gratefully acknowledged.

References

[1] Junge, W. and Witt, H.T. (1968) Z. Naturforsch. 23b, 244–254.
[2] Döring, G., Stiehl, H.H. and Witt, H.T. (1967) Z. Naturforsch. 22b, 639–644.
[3] Döring, R., Renger, G., Vater, J. and Witt, H.T. (1969) Z. Naturforsch. 24b, 132–139.
[4] Stiehl, H.H. and Witt, H.T. (1968) Z. Naturforsch. 23b, 1588–1598.
[5] Debus, R.J., Barry, B.A., Babcock, G.T. and McIntosh, C. (1988) Proc. Natl. Acad. Sci. USA 85, 427–430.
[6] Vermaas, W.F.J., Rutherford, A.W. and Hannson, Ö. (1988) Proc. Natl. Acad. Sci. USA 85, 8477–8481.
[7] Gerken, S., Brettel, K., Schlodder, E. and Witt, H.T. (1988) FEBS Lett. 237, 69–75.
[8] Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475.
[9] Joliot, P., Barbieri, G. and Chabaud, R. (1969) Photochem. Photobiol. 10, 309–329.
[10] Fowler, C.F. (1977) Biochim. Biophys. Acta 462, 414–421.
[11] Saphon, S. and Crofts, A. (1977) Z. Naturforsch. 32c, 617.
[12] Rappaport, F. and Lavergne, J. (1991) Biochemistry 30, 10004–10012.
[13] Van Leeuwen, P.J. (1993) Thesis, University of Leiden.
[14] Lübbers, K., Haumann, M. and Junge, W. (1993) Biochim. Biophys. Acta 1183, 210–214.
[15] Haumann, M. and Junge, W. (1994) Biochemistry 33, 864–872.
[16] Lavergne, J. and Junge, W. (1993) Photosynth. Res. 38, 279–296.
[17] Brettel, K., Schlodder, E. and Witt, H.T. (1984) Biochim. Biophys. Acta 766, 403–415.
[18] Saygin, Ö. and Witt, H.T. (1984) FEBS Lett. 176, 83–87.
[19] Saygin, Ö. and Witt, H.T. (1985) FEBS Lett. 187, 224–226.
[20] Meyer, B., Schlodder, E., Dekker, J.P. and Witt, H.T. (1989) Biochim. Biophys. Acta 974, 36–43.
[21] Saygin, Ö. and Witt, H.T. (1985) Photobiochem. Photobiophys. 10, 71–82.
[22] Messinger, J., Pauly, S. and Witt, H.T. (1991) Z. Naturforsch. 4c, 1033–1037.
[23] Kretschmann, H. and Witt, H.T. (1993) Biochim. Biophys. Acta 1144, 331–345.
[24] Kretschmann, H., Pauly, S. and Witt, H.T. (1991) Biochim. Biophys. Acta 1059, 208–214.
[25] Van Leeuwen, P.J., Heimann, C., Kleinherenbrink, F.A.M. and Van Gorkom, H.J. (1991) in Research in Photosynthesis (Murata, N., ed.), pp. 341–344.
[26] Saygin, Ö. and Witt, H.T. (1987) Biochim. Biophys. Acta 893, 452–469.
[27] Schatz, G.H. and Witt, H.T. (1984) Photobiochem. Photobiophys. 7, 1–14.
[28] Rögner, M., Dekker, J.P., Bokema, E.J. and Witt, H.T. (1987) FEBS Lett. 219, 207–211.
[29] Haumann, M., Bögershausen, O. and Junge, W. (1994) FEBS Lett. 355, 101–105.
[30] Vaas, I and Styring, S (1991) Biochemistry 30, 830–839.
[31] Wacker, U. (1994) Thesis, Technische Universität Berlin.
[32] Schlodder, E. and Meyer, B. (1987) Biochim. Biophys. Acta 89, 23–31.
[33] Van Leeuwen, P.J., Heimann, C., Gast, P. Dekker, J.P. and Van Gorkom, H.J. (1993) Photosynth. Res. 38, 169–176.
[34] Babcock, G.T., Barry, B.A., Debus, R.J., Hoganson, C.W., Atamian, M., McIntosh, L., Sithole, I. and Yocum, C.F. (1989) Biochemistry 28.
[35] Dekker, J.P. (1985) Thesis, Amsterdam.
[36] Rappaport, F., Blanchard, Desce, M. and Lavergne, J. (1994) Biochim. Biophys. Acta 1184, 178–192.