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Relation between microsecond reduction kinetics of photooxidized chlorophyll a_{II} (P-680) and photosynthetic water oxidation

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The reduction phases of chlorophyll a_{II}^+ (P-680$^+$) in the microsecond range have been studied in O_2-evolving Photosystem II particles from *Synechococcus* sp. and in spinach subchloroplasts. (1) In selected Photosystem II preparations only approx. 15% of chlorophyll a_{II}^+ is reduced under repetitive excitation in the microsecond time-range (approx. 85% are reduced in the nanosecond time-range). (2) The size of the microsecond fraction varies as a function of the flash number given to dark-adapted samples, suggesting a correlation to the oxidation states of the O_2-evolving complex (S-states). The oscillatory pattern closely follows the concentration of $S_2 + S_3$. (3) The microsecond decay can be deconvoluted into three exponential phases with half-life times of approx. 5, 35 and 200 μs. It is the amplitude of the 35 μs phase which depends on $S_2 + S_3$. Therefore, the 35 μs phase (approx. 10% under repetitive excitation) is connected with water oxidation. (4) Considerably higher values of the μs fraction (up to 50%) reported in former publications were probably due to Photosystem II centers which were inactive in O_2 evolution.

Introduction

The photooxidized primary donor of Photosystem II, Chl a_{II} (P-680) [1,2] is re-reduced by an electron which is ultimately donated by the oxygen-evolving complex. Photosynthetic O_2 evolution requires the accumulation of four oxidizing equivalents produced by four consecutive turnovers of the photoactive reaction center chlorophyll, Chl a_{II}. According to the Kok-model [3,4], the O_2-evolving complex passes through four different states (S_0, S_1, S_2, S_3) by four subsequent turnovers of Chl a_{II}. Oxygen is evolved only in the transition S_3 to S_0. This model is based on the observation that the yield of O_2 evolved by consecutive flashes given to a dark-adapted sample shows an oscillation with a periodicity of four [3–5].

The time-course of Chl a_{II}^+ reduction has been resolved by fast-flash spectroscopy monitoring absorption changes around 680 nm and 820 nm [6–9]. Under repetitive excitation, the kinetics of the re-reduction of Chl a_{II}^+ is multiphasic with half-life times of approx. 20 ns (≈ 35%), 50 ns (≈ 18%), and 280 ns (≈ 18%) [6,9]. The remaining portion of approx. 30% is reduced in the microsecond time range with half-life times between 5 and 400 μs [6,8,10]. Advances have been made recently (a) concerning time resolution and sensitivity of the flash absorption spectrometer [6,8,11] and (b) concerning the isolation of suitable PS II oxygen-

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Abbreviations: Chl, chlorophyll; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; Mes, 4-morpholineethanesulfonic acid; PS, Photosystem; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

evolving particles [12,13]. As a consequence, the Chl a_{II}^{+} reduction can be measured in single flashes with nanosecond time resolution [9]. This offers the possibility of using the reduction kinetics of Chl a_{II}^{+} as a probe monitoring the reaction pattern on the donor side leading to oxygen evolution and proton release. Recent single flash experiments [9] revealed that the reduction kinetics in the nanosecond time range depends on the oxidation state S_n of the O_2-evolving complex. The Chl a_{II}^{+} re-reduction correlated to the states S_0 and S_1 occurs with $t_{1/2} \approx 20$ ns, whereas in state S_2 as well as S_3 a biphasic reduction with $t_{1/2} \approx 50$ ns and ≈ 260 ns was observed. The retardation of the electron transfer in states S_2 and S_3 was explained by Coulomb attraction due to a positive charge located in the O_2-evolving complex in states S_2 and S_3. The multiphasic reduction kinetics of Chl a_{II}^{+} under repetitive excitation [6,9] has been explained quantitatively by a superposition of the different kinetics related to the four S-states.

In the present paper we extend the single flash experiments to the minor reduction phases of Chl a_{II}^{+} in the microsecond time-range, which have not been investigated in our previous work [9]. These experiments yield valuable information concerning the relationship between microsecond reduction phases of Chl a_{II}^{+} and photosynthetic water oxidation.

Materials and Methods

Subchloroplasts from spinach were prepared as described previously [9]. The reaction medium contained: $2 \cdot 10^{-2}$ M Tricine-NaOH (pH 7.5)/$3 \cdot 10^{-2}$ M sucrose/$2 \cdot 10^{-3}$ M $MgCl_2$/10^{-2} M NaCl/$5 \cdot 10^{-4}$ M Na_2HPO_4/0.5% (v/v) dimethyl sulfoxide/10^{-3} M $K_3Fe(CN)_6$/$1.6 \cdot 10^{-4}$ M Chl. Preparation of oxygen-evolving PS II particles from the thermophilic cyanobacterium *Synechococcus* sp. is described by Schatz and Witt [13]. They are characterized by a PS II : PS I ratio of more than 20 and by an O_2 flash yield of $3.6 \cdot 10^{-3}$ O_2/Chl per flash, corresponding to 70 Chl per Chl a_{II} which is active in O_2 evolution. The reaction medium for measurements at 680 nm contained: $2 \cdot 10^{-2}$ M Mes-NaOH (pH 6.5)/$1 \cdot 10^{-2}$ M $MgCl_2$/$2 \cdot 10^{-3}$ M KH_2PO_4/0.5 M mannitol/approx. 1.0% (v/v) glycerol/approx. 0.02% (w/w) sulfobetaine 12/$2 \cdot 10^{-4}$ M phenyl-*p*-benzoquinone/$2 \cdot 10^{-3}$ M $K_3Fe(CN)_6$/$3.6 \cdot 10^{-6}$ M Chl. The reaction medium for measurements at 824 nm contained: $2 \cdot 10^{-2}$ M Mes-NaOH (pH 6.5)/$1 \cdot 10^{-2}$ M $MgCl_2$/$2 \cdot 10^{-3}$ M KH_2PO_4/0.5 M mannitol/approx. 10% (v/v) glycerol/approx. 0.15% (w/w) sulfobetaine 12/$2.75 \cdot 10^{-5}$ M Chl/$1 \cdot 10^{-4}$ M phenyl-*p*-benzoquinone/10^{-3} M $K_3Fe(CN)_6$.

Absorption changes at 824 nm were measured using an apparatus described by Brettel et al. [9]. Absorption changes at around 680 nm were measured as described previously [6,11]. The samples were excited by 3 ns (FWHM) laser flashes at 532 nm from a frequency-doubled Nd/YAG laser (YG 441 from Quantel).

For measurements in the nanosecond range, the detection system (photodiode, C30952 E from RCA; amplifier, 461a from Hewlett Packard; transient recorder wtih 2 ns/point, Biomation 6500; signal averager, Nicolet 1170) had an electrical bandwidth of 1 kHz–50 MHz. In the microsecond range the measuring light was monitored by a photodiode (FND 100 from EG&G) loaded with 1 kΩ (5 kΩ). The signals were amplified (Tektronix AM 502) and digitized with 300 ns/point and 5 μs/point (Nic 1170 with plug-in Model 174 or 172/4B; Nicolet Corp.). The electrical bandwidth was 2 Hz to 1 MHz (0.3 MHz).

Measurement of absorption changes induced by each individual flash in a flash series given to dark-adapted samples was performed as follows: if the absorption change induced by the nth flash should be measured, $n-1$ oversaturating flashes were given to a new, dark-adapted sample followed by a flash that was attenuated by neutral density filters to an energy corresponding to approx. 50% saturation. Only for this attenuated flash, the absorption changes were recorded. The dark time between the flashes was 1 s. The signals were transmitted serially to an Apple II plus microcomputer and stored on floppy disks. Signals were fitted by means of least-squares curve fitting programs.

Results

Microsecond reduction of Chl a_{II}^{+} under repetitive excitation

Comparing a larger number of O_2-evolving PS

II preparations from *Synechococcus,* we observed preparations with very small fractions of Chl a_{II}^+ (15%) decaying in the microsecond time range ($t_{1/2} > 1$ μs). In our previous work this fraction amounted to approx. 30% [6]. The differences might be explained by the assumption that PS II centers with damaged oxygen-evolving complex contribute to the microsecond phases to a differing extent. It is namely well established that inactivation of water oxidation results in μs reduction phases of Chl a_{II}^+ [10,14–16]. In order to minimize contamination by damaged PS II centers, we analyzed those preparations with the smallest microsecond fraction.

Fig. 1 shows the time-course of the absorption change at 680 nm monitoring the photooxidation and the subsequent re-reduction of Chl a_{II} (P-680) in one of these samples under repetitive excitation. Most of the Chl a_{II}^+ is re-reduced in the nanosecond range, indicating a PS II preparation with μs phases of very small amplitudes. In agreement with previous work [6,9], the decay of Chl a_{II}^+ in the nanosecond time range can be well adapted by three exponential phases with half-times of approx. 20 ns (43%), 50 ns (21%) and 280 ns (21%) (see Fig. 1, broken line). The small rapid transient (half-time of decay is less than 5 ns, corresponding to the instrumental response time) is not related to Chl a_{II} [6]. The microsecond phases ($t_{1/2} > 1$ μs) have been extrapolated to $t = 0$ resulting in an amplitude of about 15%.

Measurements of the absorption changes due to Chl-a_{II}, at 824 nm, corresponding to those of Fig. 1, yielded a μs portion of approx. 23% under repetitive excitation (not shown). The larger portion, compared to the measurement at 680 nm, might be caused by the superposition of a signal at 824 nm that does not reflect the Chl a_{II} reaction. This explanation is supported by the observation of a DCMU-insensitive signal at 824 nm (see Fig. 2). Additional evidence results from our previous observations [6] that the amplitude of decay phases with $t_{1/2} > 1$ μs at 824 nm can still be increased with increasing flash energy, although the amplitudes of the nanosecond phases are already saturated. Contamination may be caused by some chlorophyll triplet states. Triplet states decay at room temperature with a half-life time of approx. 5 μs, if the chlorophylls cannot transfer the energy to carotenoid [17]. If we correct for DCMU-insensitive absorption changes at 824 nm (see Fig. 2), the fraction of Chl a_{II}^+ decaying with microsecond half-life times under repetitive excitation corresponds to about 15% in accordance with the 680 nm measurements shown in Fig. 1.

Microsecond reduction of Chl a_{II}^+ in single flashes

The following measurements, using dark-

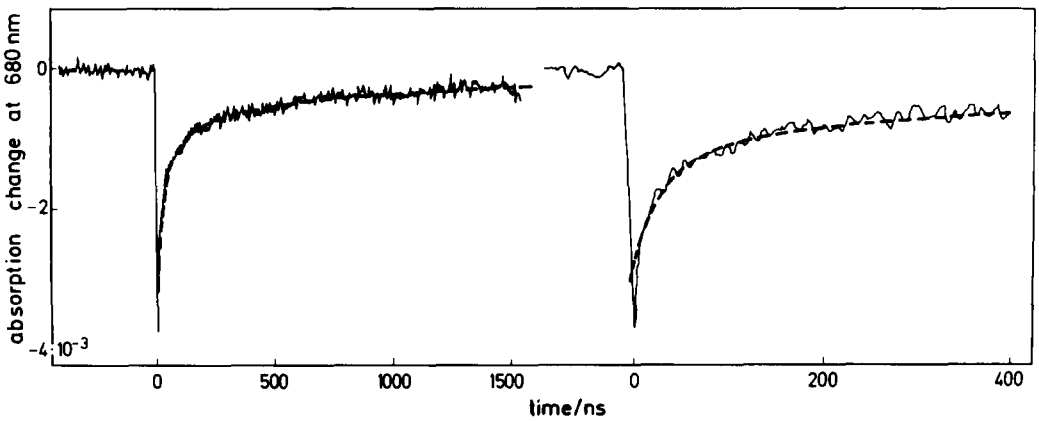

Fig. 1. Time-course of flash-induced absorption changes at 680 nm in O_2-evolving PS II particles from *Synechococcus* sp. Excitation by approx. 50% saturating laser flashes (full width at half-maximum, approx. 3 ns; 532 nm; repetition rate, 5 Hz); $3.6 \cdot 10^{-6}$ M Chl/$2 \cdot 10^{-3}$ M $K_3(Fe(CN)_6)$/$2 \cdot 10^{-4}$ phenyl-*p*-benzoquinone (pH 6.5); optical pathlength, 2 cm, electrical bandwidth, 1 kHz–50 MHz; average of 512 flashes; incident measuring light intensity, approx. 300 μW/cm². The broken line was calculated using three exponential phases with 20 ns (43%), 50 ns (21%) and 280 ns (21%), plus extrapolated microsecond decay phases (15%).

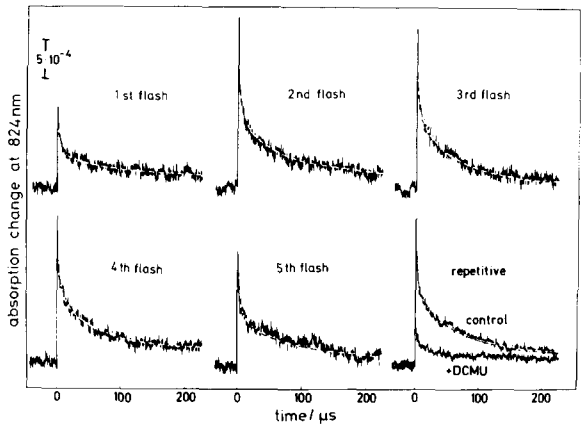

Fig. 2. Time-course of the absorption changes at 824 nm in the microsecond time range in dependence on the flash number, n. The signal of the nth flash results from an approx. 50% saturating flash preceded by $n-1$ oversaturating flashes. The series of flashes is given to dark-adapted samples of O_2-evolving PS II particles of *Synechococcus*. The dark time between flashes was 1 s. Each trce is the average of four measurements. Under repetitive excitation 16 measurements are averaged. The signal (+DCMU) was obtained without addition of an e^--acceptor and in the preence of $2 \cdot 10^{-5}$ M DCMU, after about 20 preflashes (repetition rate, approx. 1 Hz); $2.73 \cdot 10^{-5}$ M Chl (pH 6.5); electrical bandwidth, 2 Hz–1 MHz; optical pathlength, 5 cm. The broken lines were calculated as described in the text (see the Results section).

adapted samples, were performed at 824 nm, where the measuring light is not absorbed before excitation and, therefore, does not disturb dark adaptation.

Fig. 2 shows absorption changes at 824 nm in the microsecond time range after the 1st–5th flash, given after dark adaptation and under repetitive excitation (±DCMU) in O_2-evolving PS II particles from *Synechococcus*. All signals contain a fast peak ($t_{1/2} \lesssim 1$ µs) which reflects the instrumental response (electrical bandwidth, 2 Hz–1 MHz) to the nanosecond decay phases of Chl a_{II}^+. The higher amplitude of the fast peak after the 2nd–4th flash is in acordance with the recent finding that the nanosecond reduction kinetics are slower after the 2nd–4th flash than after the 1st and 5th one [9]. For the analysis of the microsecond reduction kinetics of Chl a_{II}^+ the fast peak was not taken into account. It is apparent that the amplitude of the microsecond decay phases varies in dependence on the flash number. The amplitude of Chl a_{II}^+ decaying in the microsecond time range ($t_{1/2} > 1$ µs) is significantly smaller after the 1st and 5th flash than after the ones in between.

Experiments similar to those with O_2-evolving PS II particles have been performed with spinach subchloroplasts.. Fig. 3 shows the time-course of the 824 nm absorption changes in the microsecond time range after the 1st–5th flash given to dark-adapted subchloroplasts from spinach. The amplitude of the microsecond decay, which can be attributed to the re-reduction of Chl a_{II}^+ under the chosen experimental conditions [8], depends on the flash number similar to what was observed for O_2-evolving particles. Under repetitive excitation, the fraction of Chl a_{II}^+ decaying with microsecond

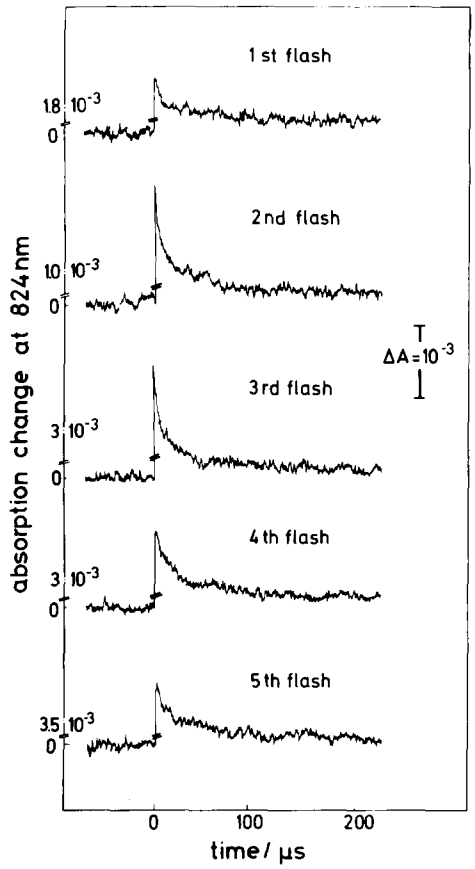

Fig. 3. Time-course of the absorption changes at 824 nm in the microsecond time range in subchloroplasts from spinach as a function of the flash number. Each trace is the average of four measurements. Note that slow decaying phases ($t_{1/2} > 1$ ms) due to the re-reduction of Chl a_I^+ (the primary donor of PS I) have been suppressed as indicated by the break. Electrical bandwidth, 2 Hz–0.3 MHz; optical pathlength, 5 cm.

half-life times has been determined (not shown) to be about 12% (after correction for the DCMU-insensitive signal). The slow decay phase with a half-life time of several milliseconds, which reflects the re-reduction of Chl a_I^+ (P-700$^+$) [18,19], has been suppressed in Fig. 3 as indicated by the breaks. The amplitude (see figures at the ordinate) of this millisecond phase is very different in dependence on the flash number .The reason is the following. In the presence of $K_3Fe(CN)_6$, the secondary donors of PS I and, to some extent, Chl a_I itself are oxidized in the dark. The remaining Chl a_I (depending on the time of preincubation) is photooxidized by the 1st flash. With the secondary donors to Chl a_I oxidized, Chl a_I^+ is re-reduced only very slowly. Therefore, the 2nd flash finds a low amount of photooxidizable Chl a_I. After a few more flashes, however, electrons are supplied by PS II via the plastoquinone pool, so that Chl a_I^+ can now be re-reduced with $t_{1/2} \approx 20$ ms during the dark time (1 s) between the flashes.

Fig. 4 shows the difference between the absorption changes at 5 and 225 μs which constitutes an approximate measure for the fraction of Chl a_{II}^+ decaying with microsecond half-life times as a function of the flash number. In O_2-evolving PS II particles from *Synechococcus* (two sets of experiments presented by open and full circles) and in spinach subchloroplasts (squares) the same dependence is observed. In each set of experiments the data are normalized to the maximal amplitude after the 3rd flash.

Analysis of the microsecond reduction kinetics

The kinetics of the microsecond decay have been analyzed on the basis of the signals shown in Fig. 2. In addition, measurements up to the millisecond range (not shown) were taken into account. The microsecond decay under repetitive excitation can be deconvoluted into three exponential phases with half-life times of 4 μs ($\Delta A = 3.8 \cdot 10^{-4}$), 35 μs ($\Delta A = 6.4 \cdot 10^{-4}$) and approx. 200 μs ($\Delta A = 2.4 \cdot 10^{-4}$) (see Fig. 2, repetitive, broken line). A small contribution ($\Delta A = 0.9 \cdot 10^{-4}$) is from a component with a half-life time of more than 1 ms. The extrapolated initial amplitude is $\Delta A = 1.35 \cdot 10^{-3}$ (the fast peak due to the unresolved ns phases has not been taken into account).

A comparison of the microsecond reduction kinetics after the 1st–5th flash (see Fig. 2) suggests that the signals differ from one another mainly by the amplitude of an intermediate (approx. 35 μs) component. Therefore, we performed the following quantitative analysis. For the sake of simplicity, the decays in the 3–225 μs range were fitted by three exponential phases under the assumption that only the amplitude of the intermediate phase varies in dependence on the flash number. The other parameters were fixed. Their values were taken from the deconvolution of the signal under repetitive excitation ($t_{1/2} = 4$ μs ($\Delta A = 3.8 \cdot 10^{-4}$), $t_{1/2} = 35$ μs (ΔA fitted) and $t_{1/2} = 200$ μs ($\Delta A = 2.4 \cdot 10^{-4}$). The 4-μs and 200-μs phases may be due to the following reactions which indeed should not vary with flash number: (a) a DCMU-insensitive component which gives rise mainly to a fast phase ($t_{1/2} \approx 5$ μs); (b) phases with half-times of approx. 4 and 200 μs are known to occur in PS II centers with damaged water oxidation. The 4 μs phase has been attributed to the reduction of Chl a_{II}^+ by an unknown intrinsic donor, when the O_2-evolving system is inactive [10,14]. The 200 μs phase has been attributed to a back reaction of Q_A^- with Chl a_{II}^+, also under conditions where the O_2-evolving system is inactive [15,16]. The results of the fits are depicted in Fig. 2 by the broken lines. Small contributions with $t_{1/2} > 1$ ms were taken into account by addition of a constant as a second fit parameter (for all fits, less than 10^{-4}). Fig. 2 shows that the signals are well described by the results of the fit. The amplitudes of the 35 μs phase resulting from the shift are listed in Table I. It is obvious that the amplitude of the 35 μs phase varies strongly in dependence on the flash number.

TABLE I

RESULTS OF THE FIT TO THE SIGNALS IN FIG. 2

For details, see text.

Flash number	Amplitude of the 35 μs phase × 10^4
1	1.8
2	6.2
3	7.5
4	6.2
5	4.2
Repetitive excitation	6.4

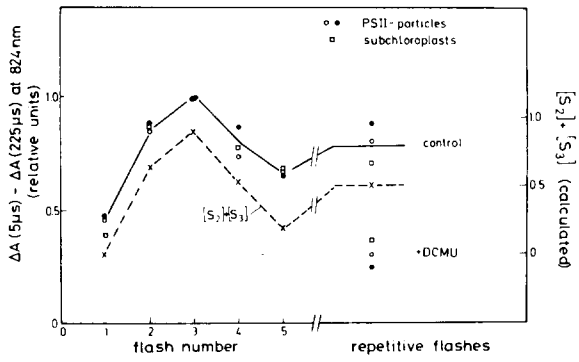

Fig. 4. Difference between the absorption change at 5 μs and 225 μs at 824 nm in dependence on the flash number and under repetitive excitation (with and without DCMU). ×------×, sum of the population of S_2 and S_3. For details see text.

The evaluation of the microsecond decay kinetics in spinach chloroplasts (see Fig. 3) leads qualitatively to the same result.

The dependence of the Chl a_{II}^+ reduction in the microsecond range (see Figs. 2–4 and Table I) suggests a correlation to the S-states of the oxygen-evolving complex (see Discussion). Further evidence for a correlation to the S-states can be drawn from the following experiment.

Fig. 5 shows the time-course of the 824 nm

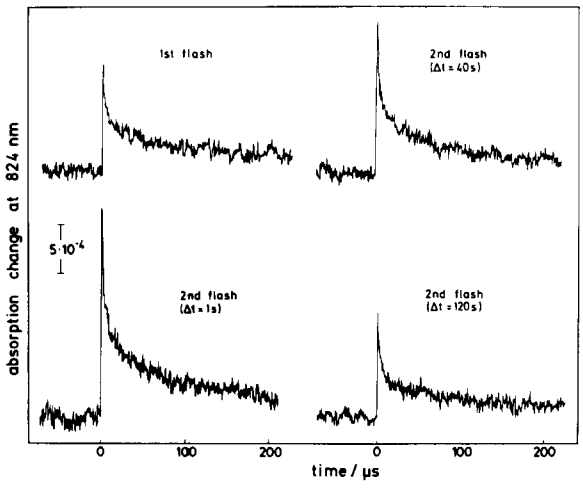

Fig. 5. Time-course of the absorption change at 824 nm in O_2-evolving PS II particles from *Synechococcus* in the microsecond time-range after the first and second flash, varying the time interval, Δt, between the flashes. Other conditions as in Fig. 2.

absorption change in the microsecond time-range after the 1st and 2nd flash, varying the time interval, Δt, between both flashes. If the dark time after the 1st flash is longer than about 2 min, the absorption change observed after the 2nd flash equals that measured after the 1st flash. The half-life time of this dark relaxation is probably attributable to the charge recombination of the negative charge on the acceptor side and the positive charge on the donor side of PS II, thereby reducing S_2 into S_1. The half-life time has been determined according to Fig. 5 to be about 25 s. This is in accordance with the decay time of the S_2 state evaluated from O_2 measurements [4]. The same result was found for the rate of Chl a_{II}^+ re-reduction in the nanosecond time range. In the presence of DCMU the half-life time is significantly shorter (approx. 2 s) (not shown).

Discussion

The aim of this work was a detailed investigation of the minor phases of Chl a_{II}^+ reduction in the microsecond range. Comparing a larger number of preparations of O_2-evolving PS II particles from *Synechococcus* and of spinach subchloroplasts, we observed that the amplitude of microsecond phases under repetitive excitation was at best as small as about 15% of the total amplitude (see Fig. 1). Possibly this portion still contains some PS II centers which are inactive in O_2 evolution. Non-oscillating absorption changes due to PS II particles with inactive water oxidation can be of an amplitude maximum as high as the lowest microsecond amplitude in a series of flashes corrected for DCMU-insensitive contributions. This value is induced by the first flash and can be estimated to be approx. 7%. Since the amplitude of the microsecond phases under repetitive excitation corresponds to approx. 15%, we can conclude that 8% (lower limit) of the total Chl a_{II}^+ is reduced with $t_{1/2} \approx 35$ μs under repetitive excitation in PS II centers with intact water oxidation. The magnitude of the fraction of Chl a_{II}^+ decaying in the microsecond time range has been estimated earlier by Conjeaud et al. [10] in untreated spinach chloroplasts to less than 25%.

The experiments presented in Figs. 2–4 demonstrate that the fraction of Chl a_{II}^+ decaying in the

microsecond time-range varies as a function of the flash number. The variation is similar in spinach subchloroplasts and O_2-evolving PS II particles from *Synechococcus* (see Fig. 4). The variation in the amplitude is most likely related to the S-states of the O_2-evolving complex. Strong evidence for that is given in Fig. 4. The oscillation pattern suggests a correlation of the amplitude of the microsecond decay phases of Chl a_{II}^+ with the sum of population of S_2 and S_3. In states S_2 and S_3 the amplitude of microsecond decay phases (see Fig. 4) corresponds to $(15 \pm 5)\%$ of total Chl a_{II}^+. In states S_0 and S_1 the portion is $(3 \pm 3)\%$. The population of the S-states prior to the flashes (see broken line in Fig. 4) has been calculated assuming the following parameters (dark distribution: $(S_0) = 25\%$, $(S_1) = 75\%$, $(S_2) = (S_3) = 0$; probability of misses: 0.15, and for double hits: 0, because of the short excitation pulse (3 ns)). These parameters are based on data fitting O_2-evolution patterns in flash series. Further evidence for the dependence of the microsecond amplitude on the S-states is yielded by the experiment shown in Fig. 5. Increasing the dark-time after the first flash from 1 s to 2 min, the signal observed after the second flash equals that after the first flash. The half-life time of this relaxation process (approx. 25 s) is in qualitative agreement with the decay constant of the S_2-state [4]. The analysis of the microsecond decay kinetics shows that our experimental results (Figs. 2 and 3) can be satisfactorily described, if we assume that only the amplitude of the 35 μs phase depends on the S-states.

An effect of the S-states on the amplitude of the microsecond decay phases has been first reported by Gläser et al. [20]. These authors measured the absorption changes at 690 nm, but could detect only slower microsecond phases ($t_{1/2} = 35$ μs and $t_{1/2} \approx 200$ μs) because of their limited instrumental response time. They concluded from their measurements that under repetitive excitation at least 40% of the total photooxidized Chl a_{II}^+, which is connected with the water oxidation, is reduced in the microsecond range. The half-life times are 35 and 200 μs. They reported that the microsecond amplitude oscillates in the same characteristic pattern as oxygen evolution, i.e., the maximal microsecond amplitude is related to state S_3. These results differ remarkably from the data presented in this work which have been obtained under improved experimental conditions. We have shown that under repetitive excitation less than 15% of Chl a_{II}^+, which is connected with the water oxidation, is reduced in the microsecond time range ($t_{1/2} \approx 35$ μs). The oscillation pattern closely follows the concentration of S_2 plus S_3.

The reason for the remarkable differences is not quite clear. They might be explained in part by the rather different experimental conditions in Ref. 20: the presence of 2-(3-chloro-4-trifluoromethyl)anilino-3,5-dinitrotiophene (ANT 2p) and a pH decrease of the internal space of the thylakoids caused by the measuring light (690 nm) and by the excitation with flash groups (5 ms dark time between flashes within a group of five flashes, 1 s between the flash groups). The investigation of the pH dependence of the Chl a_{II}^+ re-reduction over the whole time-range shows that the amplitude of the nanosecond decay phases decreases (and correspondingly the amplitude of the μs decay phases increases) with decreasing pH, from pH 7.5 to pH 4.0 (Schlodder, E., Brettel, K. and Witt, H.T., unpublished data). An influence of the pH on the amplitude of the microsecond decay phases has been reported earlier by Renger et al. [21].

An influence of the S-states on the fraction of Chl a_{II}^+ decaying with microsecond half-life times has been derived indirectly from measurements of the fluorescence yield and the amplitude of delayed fluorescence in the microsecond range [22–24]. It is generally agreed that the S-state transitions showing a maximal amplitude of delayed fluorescence and a minimal rapid fluorescence yield change are those in which the initial state is S_2 or S_3; i.e., the oscillatory pattern closely follows the concentration of S_2 plus S_3 before the flash [22]. This has been interpreted kinetically by the assumption that the concentration of longer-lived Chl a_{II}^+ ($t_{1/2} \gtrsim 1$ μs) depends on the S-states [23]. Our direct measurements of the time course of Chl a_{II}^+ re-reduction as a function of the flash number support this assumption.

The question is how to explain the different amplitudes of the intermediate (≈ 35 μs) decay phase of Chl a_{II}^+ as a function of the S-states of the O_2-evolving complex. We assume that the rate constants for the electron transfer from the oxygen-evolving complex to Chl a_{II}^+ are influenced

by the S-states. From the proton release pattern (1,0,1,2) [25–27] the oscillation of the nanosecond Chl a_{II} kinetics [9] and the electrochromic absorption change pattern [28] for the transitions ($S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, $S_3 \rightarrow S_0$) it follows that in states S_2 and S_3 the O_2-evolving complex carries one positive charge. Therefore, it seems likely that the oscillation of the microsecond amplitude with S_2 plus S_3 (see Fig. 4) is caused by the positive charge located in the oxygen-evolving complex in states S_2 and S_3. The proposed explanation will be put into more concrete terms using the kinetic model outlined in Scheme I for the electron transfer on the donor side of PS II:

$$\text{Chl } a_{II}^{+} D_1 D_2 S_n \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Chl } a_{II} D_1^{+} D_2 S_n \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{Chl } a_{II} D_1 D_2^{+} S_n \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \text{Chl } a_{II} D_1 D_2 S_{n+1}$$

Scheme I

The O_2-evolving complex in the different S-states, S_n, donates the electrons via two electron carriers, D_1 and D_2, to Chl a_{II}^{+}. This model has been used to give a quantitative explanation of the different nanosecond reduction times of Chl a_{II}^{+} in dependence of the S-states [9].

For the following discussion, the two questions are essential.

(1) In which way can the kinetic model (Scheme I) also account for the Chl a_{II}^{+} fraction decaying in the microsecond time-range?

(2) Is the electron ultimately donated by H_2O or by a side donor (or a back reaction) which is not considered in Scheme I?

(A) Since k_1 and k_2 correspond to half-life times in the nanosecond range but k_3 to a half-life time of an order larger than 1 μs (electron transfer time of S_n to D_2^{+}), a preceding rapid equilibrium is created within less than 1 μs, characterized by:

$$\frac{[P^{+} D_1 D_2 S_n]}{[P^{+} D_1 D_2 S_n] + [P D_1^{+} D_2 S_n] + [P D_1 D_2^{+} S_n]}$$

In states S_2 and S_3, respectively, this ratio would have to amount to approx. 15% in order to explain a longer-lived fraction of Chl a_{II}^{+} (15%) which is re-reduced within $t_{1/2} \approx 35$ μs. According to Scheme I, the decay of this fraction should qualitatively reflect the re-reduction of D_2^{+} in the transition $D_2^{+} S_n \rightarrow D_2 S_{n+1}$. This would be expected to occur in states S_2 and S_3 with 0.4–1 ms [29], if D_2^{+} gives rise to ESR Signal II_{vf} [30].

Since the longer-lived fraction of Chl a_{II}^{+}, which is maximal in states S_2 and S_3, is re-reduced with a half-life time of approx. 35 μs and not with $t_{1/2} \approx$ 0.4–1 ms, we can exclude that the microsecond decay reflects the re-reduction of D_2^{+} by the S-states.

(B) In a second approach, one might assume that due to an appropriate equilibrium constant $K_3 = k_3/k_{-3}$ for the reaction:

$$\text{Chl } a_{II} D_1 D_2^{+} S_{n-1} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \text{Chl } a_{II} D_1 D_2 S_n$$

about 15% of PS II centers would be present as Chl $a_{II} D_1 D_1^{+} S_{n-1}$ in states S_n ($n = 2, 3$). For this fraction, the following flash gives rise to the state Chl $a_{II}^{+} D_1 D_2^{+} S_{n-1}$. It seems possible that the electron donation from D_1 to Chl a_{II}^{+} is retarded (up to 35 μs) in this fraction of PS centers because of the presence of a positive charge on D_2.

In the presence of Chl $a_{II}^{+} D_1 D_2^{+} S_{n-1}$, also the following possibility can be discussed. It may be that Chl a_{II}^{+} is reduced by a side donor (or a back reaction) with $t_{1/2} \approx 35$ μs in this case. The amplitude of the 35 μs decay phase would then be a direct indication for the extent of misses. The existence of a side donor is, however, unlikely, because this donor could deactivate the S_2 and S_3 states in the dark time between the flashes. This would be in contrast to the high stability of S_2 and S_3 (approx. 30 s). The possibility of a back reaction from Q_A^{-} to Chl a_{II}^{+} is, however, not ruled out by this objection, because Q_A^{-} is present only for a few 100 μs after the flash.

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