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# KINETICS OF MANGANESE REDOX TRANSITIONS IN THE OXYGEN-EVOLVING APPARATUS OF PHOTOSYNTHESIS

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**The kinetics of the S-state transitions of the oxygen-evolving complex were analyzed in dark-adapted, oxygen-evolving Photosystem-II preparations supplied with the electron acceptor 2,5-dichloro-*p*-benzoquinone. The kinetics of flash-induced absorbance changes at 350 nm, largely due to the successive S-state transitions $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$, confirm the +1, +1, +1, −3 sequence of manganese oxidation reported earlier (Dekker, J.P., Van Gorkom, H.J., Wensink, J. and Ouwehand, L. (1984) Biochim. Biophys. Acta 767, 1–9), and reveal half-times of 30, 110, 350 and 1300 μs, respectively, for these transitions.**

The oxygen-evolving complex of photosynthesis carries out the oxidation of two water molecules to one dioxygen molecule by four successive photoreactions of PS II (for a review, see Ref. 1). Therefore, the system can be in five different oxidation states, the so-called S-states $S_0$–$S_4$, the suffix indicating the number of oxidizing equivalents in the system. $S_0$ and $S_1$ are stable in the dark, $S_2$ and $S_3$ revert to $S_1$ in minutes, and $S_4$ is reduced to $S_0$, releasing oxygen. Thus, illumination of dark-adapted PS II by single-turnover flashes results in a period-4 oscillation, which is damped due to misses and double hits.

Such a period-4 oscillation has also been detected in ultraviolet absorbance [2]. Recently, we analyzed these changes in detail in oxygen-evolving PS-II preparations, and concluded that they are caused by the successive oxidation of three Mn(III) ions to Mn(IV) in the $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions, respectively, and by the reduction of the three Mn(IV) ions to Mn(III) in the $S_3 \rightarrow (S_4) \rightarrow S_0$ transition [3,4]. The half-time of this last transition was found to be about 1–1.5 ms in these preparations [3]. Presumably, the other S-state transitions proceed more rapidly [cf. 1].

The kinetics of the manganese redox reactions have now been measured. PS-II preparations were isolated according to Berthold et al. [5] with modifications as in Ref. 3. The preparations were suspended in 20 mM Mes-NaOH/5 mM $MgCl_2$/15 mM NaCl (pH 6.0) at a Chl concentration of 200 μg/ml and dark-adapted for at least 15 min, after which the electron acceptor DCBQ (100 μM) was added, and a series of saturating, 10 μs xenon flashes was fired. The absorbance changes were analyzed in a single-beam apparatus described in more detail in Ref. 4 (relevant settings are indicated in the legend of Fig. 1).

We analyzed the absorbance changes at 350 nm. At this wavelength the changes due to the manganese redox transitions are relatively large, while no changes are expected of the secondary donor Z, and only relatively small changes are

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Abbreviations: Chl, chlorophyll; DCBQ, 2,5-dichloro-*p*-benzoquinone; Mes, 4-morpholinethanesulfonic acid; PS II, Photosystem II.

observed of the acceptor system [3,4]. Fig. 1A shows the changes due to the first five flashes. The third flash clearly induced the $S_3 \rightarrow (S_4) \rightarrow S_0$ transition. After proper subtraction of the 20 ms phase of $Q^-$ reoxidation [3] a $1.3 \pm 0.1$ ms half-time for the rereduction of Mn(IV) was calculated. The amplitude of this phase on the flashes shown, and on the subsequent flashes, could be fitted with the same parameters of the period-4 oscillation as in Ref. 3: a dark S-state distribution of 75% $S_1$ and 25% $S_0$, and 9% misses and 9% double hits on all transitions.

Fig. 1B shows similar measurements on a 10-fold expanded time-scale (the smooth lines will be discussed below). They reveal, in addition to instantly rising signals which must be ascribed to reduction of the primary acceptor Q [3,4], the relatively slow kinetics of manganese redox changes. The changes were corrected for the expected 1.3 ms decay phase on each flash (see Fig. 1A), and analyzed on a semi-logarithmic scale. The first trace was found to contain a major phase of $110 \pm 20$ $\mu$s half-time, presumably reflecting the $S_1 \rightarrow S_2$ transition. The second flash induced a much slower rise; here a main component of $350 \pm 50$ $\mu$s half-time was observed, which may reflect the $S_2 \rightarrow S_3$ transition. Probably, $S_0 \rightarrow S_1$ proceeds more rapidly than $S_1 \rightarrow S_2$. A minor rapid phase on the first flash, and the fact that the rise on the fourth flash is faster than that on the fifth, may be ascribed to this rapid $S_0 \rightarrow S_1$ transition.

In order to resolve the kinetics of the $S_0 \rightarrow S_1$ transition, we analyzed the changes due to the first flash in more detail. The xenon flash was replaced by a 250 ns laser flash, and a 500 $\mu$s sweep was measured (Fig. 1C). After subtraction of the 110 $\mu$s phase of the $S_1 \rightarrow S_2$ transition a half-time of $30 \pm 10 \mu$s for the $S_0 \rightarrow S_1$ transition was obtained.

Thus, the advance of the S-states was found to proceed with half-times of about 30, 110, 350 $\mu$s and 1.3 ms, respectively, in the PS-II preparations. In order to verify this conclusion quantitatively, we computed the signals on each flash using these half-times, using the +1, +1, +1, −3 sequence of manganese oxidation, and using the parameters of the period-4 oscillation as indicated above. The calculated curves are shown in Fig. 1B and C by smooth lines. They show an excellent fit with the measured signals when another, instantly present and stable (during the sweep-time) signal is assumed, the amplitude of which is indicated by the horizontal arrows. The latter contribution is probably caused by reduction of the primary acceptor Q, which has about the same differential extinction coefficient at 350 nm as the formation of one Mn(IV) [4]. The fact that the amplitude of this signal is larger on the first flash than on the subsequent flashes was noted already in Ref. 3. The striking similarity between the measured and calculated kinetics of the manganese absorbance changes on all five flashes again indicates the absence of complications at the donor-side: the advance of the S-states can be explained quantitatively by the simplest model and seems to proceed with exponential kinetics, which become 3–4-times slower on each successive step.

Our results contradict the conclusions of Boska and Sauer [6], who proposed only two different S-state transition times. Their mesurements, however, are not inconsistent with our results. Our 110 $\mu$s $S_1 \rightarrow S_2$ transition is somewhat slower than the 70 $\mu$s reported by Velthuys [7] for intact chloroplasts on the basis of similar experiments as described here. Velthuys, however, did not correct for the faster $S_0 \rightarrow S_1$ transition on the first flash. Nevertheless, the S-state transitions may actually be somewhat slower in the PS-II preparations than in chloroplasts; also the 1.3 ms half-time for the $S_3 \rightarrow (S_4) \rightarrow S_0$ transition is somewhat slower than normally observed in chloroplasts [cf. 1]. The kinetics of the $S_2 \rightarrow S_3$ transition are in good agreement with earlier suggestions, made on the basis of the EPR signal of the secondary donor Z [8], and on proton release [9]. The kinetics of the $S_0 \rightarrow S_1$ transition were not reported before. $S_0 \rightarrow S_1$ transition times estimated from the turnover times of PS II as measured in oxygen flash yield [10] are limited by the much slower reoxidation of $Q^-$. It should be noted that slow decay phases of the oxidized primary donor P-680$^+$ could in principle disturb the patterns at 350 nm. At this wavelength, however, the sign of the $P^+$ relaxation signals is opposite to that of Mn(IV) formation [11].

In conclusion, the results described here confirm the proposed +1, +1, +1, −3 sequence of absorbance changes [3], reflecting the oxidation of Mn(III) to Mn(IV) [4], and show that the succes-

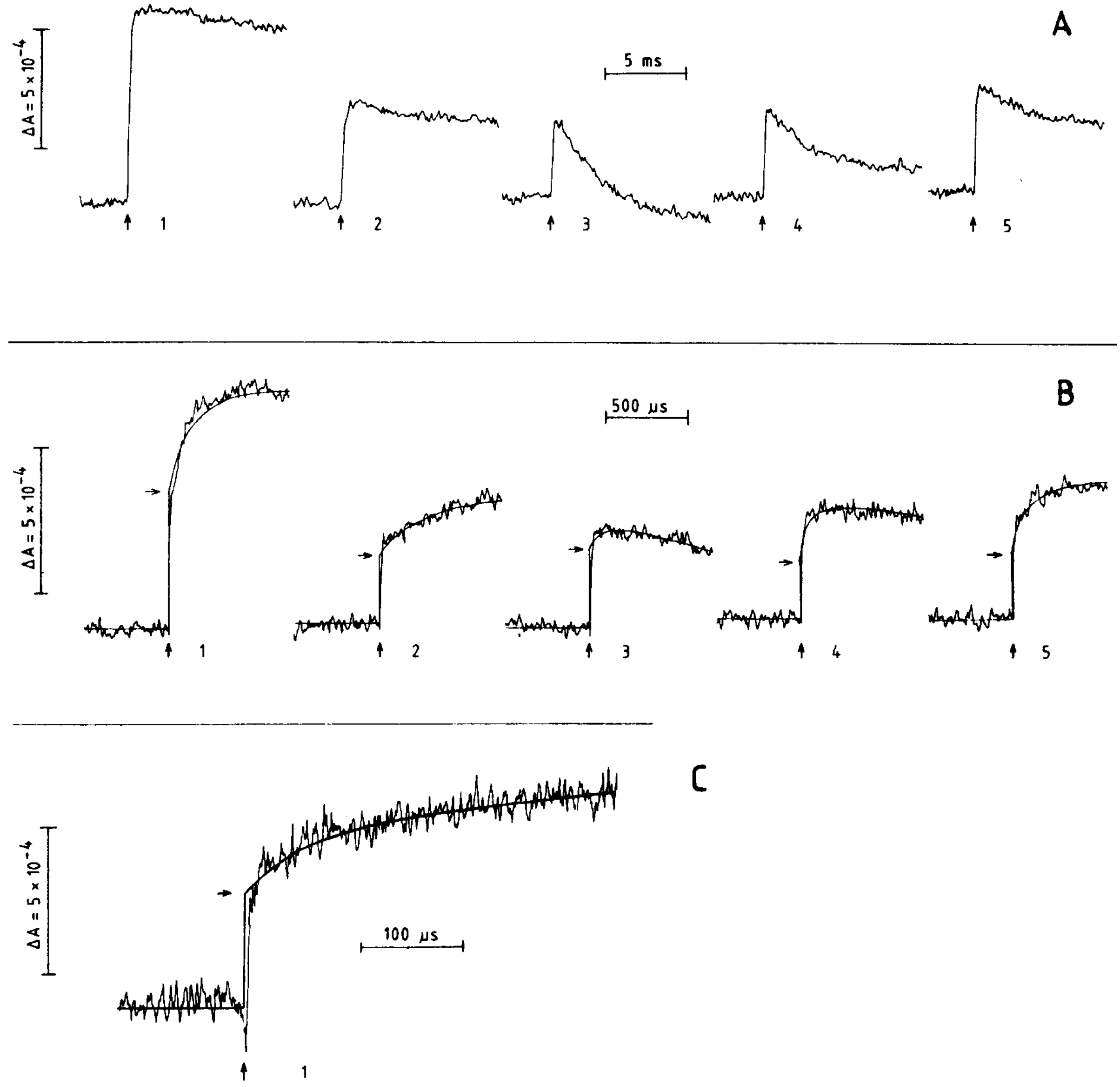

Fig. 1. (A) Absorbance changes at 350 nm induced by five xenon flashes, spaced at 300 ms, in dark-adapted PS-II preparations, suspended at a Chl concentration of 200 µg/ml in 20 mM Mes-NaOH (pH 6.0)/5 mM $MgCl_2$/15 mM NaCl/100 µM DCBQ. The optical pathway was 1.2 mm. The traces show 12.5 ms sweeps, measured with a response time of the apparatus of 100 µs, averaged 100×. (B) Same as (A), but now measured as 1.25 ms sweeps, with a response time of 15 µs, averaged 300×. The smooth lines above the horizontal arrows indicate computed signals of S-states advance, calculated as described in the text. (C) Same as (A), first flash, except that a 250 ns dye laser flash was used; measured as a 500 µs sweep with 7.5 µs response time and averaged 700×. The smooth line indicates the computed signal, as in (B).

sive transitions occur with half-times of 30, 110, 350 and 1300 µs, respectively. Apparently, the oxidation of the oxygen-evolving complex becomes about $3\frac{1}{2}$-times slower on each step. The possibility to detect each S-state transition separately by absorbance measurements may prove useful in future studies on the mechanism of oxygen evolution.

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