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An agreement on the quaternary oscillation of ultraviolet absorption changes accompanying the water splitting in isolated Photosystem II complexes from the cyanobacterium *Synechococcus* sp.

H. Kretschmann, J.P. Dekker *, Ö. Saygin ** und H.T. Witt

Max-Volmer-Institut für Biophysikalische und Physikalische Chemie, Technische Universität Berlin, Berlin (Germany)

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Authors of controversial publications with regard to the S-transition spectra of the water-splitting enzyme system S together reinvestigated the transitions in isolated *Synechococcus* Photosystem II complexes. It is confirmed that the spectrum of the S_0–S_1 transition is different from the spectra of the S_1–S_2 and S_2–S_3 transitions: at 355 nm, the S_0–S_1 transition is more than three times smaller. The S_0–S_1 transition is possibly due to an Mn(II) → Mn(III) change, the latter ones due to Mn(III) → Mn(IV) changes.

During water splitting in photosynthesis four oxidation states, S_0–S_4, are engaged in one turnover of the water-splitting enzyme system 'S' [1]. The transitions between the different S states which are created successively through four turnovers of the photocenter Chl-a_{II} (P-680) are accompanied by absorbance changes in the ultraviolet [2]. Several attempts have been made to describe the absorption changes of the individual S-state transitions quantitatively [3–10], but different results were obtained. The discrepancies are mainly due to the different ways of correcting for binary oscillations from the acceptor side of PS II, the interpretation of absorbance changes caused by the 1st flash in dark-adapted preparations, and the deconvolution procedure for the S states overlapping one another. The absorbance difference spectra of the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions are characterized by a broad absorbance increase in the ultraviolet and are presumably caused by the oxidation of one Mn(III) to Mn(IV) [3,10]. Dekker et al. concluded from their experiments that in spinach membrane fragments also the $S_0 \rightarrow S_1$ transition gives rise to very similar absorbance changes and, therefore, attributed also this transition to an Mn(III)/Mn(IV) valence change (reviewed in Ref. 8). Saygin and Witt [9,10], using highly active PS II preparations from the thermophilic cyanobacterium *Synechococcus* sp., also observed the similarity of absorbance changes by the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions, but obtained rather strong evidence for a different contribution upon the $S_0 \rightarrow S_1$ transition. This evidence is based on experiments in the absence and presence of low concentrations of hydroxylamine; in the latter case the O_2 evolution is shifted backwards by two units.

* Present address: Department of Biology, University of Michigan, Ann Arbor, MI 48109, U.S.A.

** Present address: Bogazici University, Institute of Environmental Sciences, P.K. 2 Bebek, Istanbul, Turkey.

Abbreviations: Chl, chlorophyll; PS II, Photosystem II; Mes, 4-morpholineethanesulphonic acid; UV, ultraviolet.

Correspondence: H.T. Witt, Max-Volmer-Institut für Biophysikalische und Physikalische Chemie, Technische Universität Berlin, Strasse des 17. Juni 135, 1000 Berlin 12, Germany.

The aim of this contribution is the reinvestigation, by all the authors of the controversial publications with regard to the $S_0 \rightarrow S_1$ transition spectrum (see Refs. 4 and 10, respectively), of the oscillation patterns under normal, oxygen-evolving conditions (i.e., in the absence of hydroxylamine) on the same object (*Synechococcus* PS II complex). It is confirmed that the spectrum of the $S_0 \rightarrow S_1$ transition is different from the other two transitions, $S_1 \rightarrow S_2 \rightarrow S_3$, in this system.

Oxygen-evolving PS II complexes were extracted from membranes of the cyanobacterium *Synechococcus* sp., as reported by Schatz and Witt [11], and further purified to yield the SG-1 preparation according to Rögner et al. [1]. The suspension for measurement contained $4 \cdot 10^{-8}$ M Chl a_{II} centers, 0.01 M $MgCl_2$, 0.5 M mannitol, 0.02 M Mes/NaOH (pH 6.5) and $6 \cdot 10^{-4}$ M 2,5-dichloro-*p*-benzoquinone (DCBQ). Due to the unusually high acceptor concentration, the binary oscillations from the acceptor side could be eliminated [10]. Absorption changes were measured with a double-beam spectrophotometer as described in Ref. 13. The dark-adaptation time of the samples was at least 30 min, and the saturating laser flashes were spaced at 500 ms. For the deconvolution procedure of the S states which overlap one another, see Refs. 1, 4 and 10.

Fig. 1 shows the absorption changes at 355 nm of dark-adapted PS II particles from *Synechococcus* sp. as a function of flash number. From such measurements we determined three extinction coefficient changes indicated in Fig. 1 as $\Delta\epsilon_{rev}$, $\Delta\epsilon_{irrev}$ and $\Delta\epsilon_{max}$, $\Delta\epsilon_{rev}$ mainly represents the absorbance decrease due to the $S_3 \rightarrow S_0$ transition and, to a minor extent, a non-oscillating decay from the acceptor side [4,10]. The oscillating amplitude of $\Delta\epsilon_{rev}$, which is proportional to the O_2 evolution, was determined by extrapolation, assuming exponential and identical kinetics at all flash numbers (in Fig. 1 the half-time of the decay kinetics was 1.8 ms). $\Delta\epsilon_{irrev}$ and $\Delta\epsilon_{max}$ are correlated with S_0–S_3 transitions (for details, see Ref. 10).

Fig. 2 shows the three $\Delta\epsilon$ values in dependence on the flash number. The best fit of $\Delta\epsilon_{rev}$ (Fig. 2, top, closed circles) is obtained with a dark S-state distribution of 90% S_1 and 10% S_0, with 9.3% misses (α) and 7.5%) double hits (β) upon each flash (Fig. 2a, open circles). With these S-state parameters the uncorrected $\Delta\epsilon_{irrev}$ values can be deconvoluted (Fig. 2, center, closed circles). The best fit is obtained when for the corrected individual values $\Delta\epsilon_{S_0 \rightarrow S_1} = 750\ M^{-1} \cdot cm^{-1}$, $\Delta\epsilon_{S_1 \rightarrow S_2} = 2350\ M^{-1} \cdot cm^{-1}$ and $\Delta\epsilon_{S_2 \rightarrow S_3} = 2450\ M^{-1} \cdot cm^{-1}$ are used (Fig. 2, center, open circles).

As discussed in Refs. 9 and 10, the value of $\Delta\epsilon_{max}$ (Fig. 2, bottom, closed circles) yields further information on the individual extinction coefficient changes of the S-state transitions. The ab-

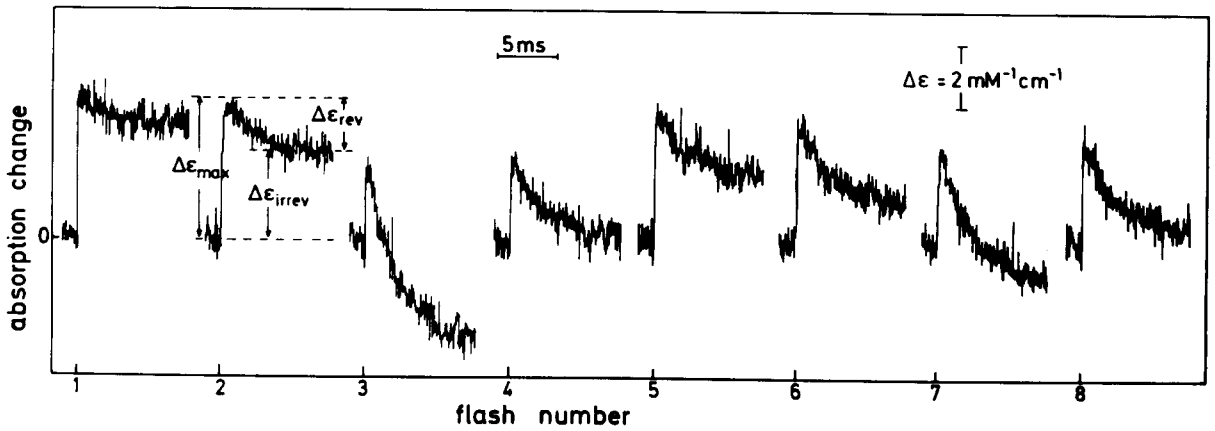

Fig. 1. Time-course of flash-induced absorption changes of dark-adapted PS II complexes from *Synechococcus* at 355 nm as a function of flash number. The dark time between the flashes was 0.5 s. The concentration of the particles was $4 \cdot 10^{-8}$ M. The external acceptor was DCBQ ($6 \cdot 10^{-4}$ M) at pH 6.5. The pattern is the average of 14 measurements.

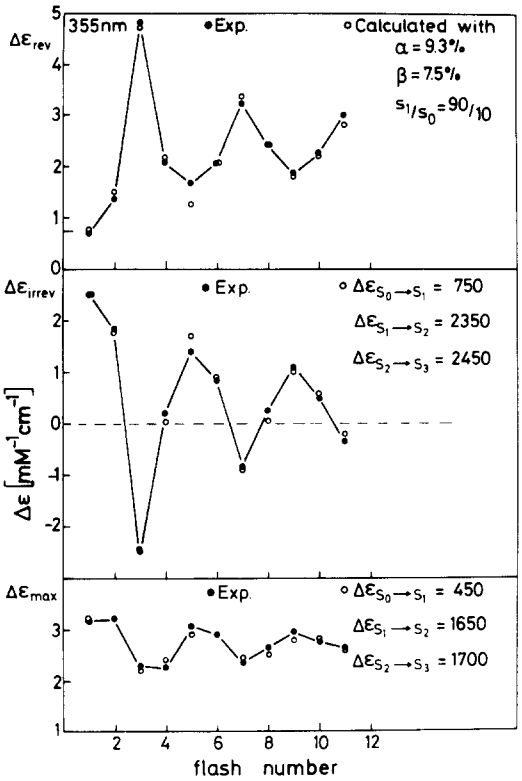

Fig. 2. Molar extinction coefficients $\Delta\epsilon_{rev}$, $\Delta\epsilon_{irrev}$ and $\Delta\epsilon_{max}$ corresponding to Fig. 1 as a function of the flash number. Full circles: measured values; open circles: calculated values. Top: the oscillation of $\Delta\epsilon_{rev}$ is proportional to the O_2 evolution. From the fitting procedure one gets the outlined parameters α, β and S_1/S_0, responsible for the mixing of the S states. Center and Bottom: with the parameters of the mixing, the uncorrected measured $\Delta\epsilon_{irrev}$ values and $\Delta\epsilon_{max}$, respectively (full circles) can be deconvoluted (open circles), if one uses for the individual unmixed $\Delta\epsilon_{S_n \to S_{n+1}}$ values those that are outlined in the figures.

solute values of ϵ depend strongly on the way of reading the numerical data from Fig. 1. Unaffected, however, is the result of the fitting that at 355 nm the changes of the $S_0 \to S_1$ transition are again more than 3 times smaller than those of the $S_1 \to S_2$ and $S_2 \to S_3$ transitions (Fig. 2, bottom, open circles). Similar experiments at 300 nm indicate that here also $S_0 \to S_1$ gives rise to a smaller absorption change (not shown). The absorption change of the $S_0 \to S_1$ transition should therefore be due to a different process, possibly an Mn(II) → Mn(III) transition as outlined in Ref. 10. Why the difference between the $S_0 \to S_1$ and the $S_1 \to S_2 \to S_3$ transitions could not be observed (see also Ref. 8) in spinach PS II preparations [4], and also in preparations of spinach in which interference by a period 2 oscillation from the acceptor side is excluded [14], is not known and will be reinvestigated. The overall structure of the manganese cluster appears to be very similar in high plants and in cyanobacteria [15,16], so that differences in the Mn-oxidation states in the individual S states might not be the reason for the discrepancy.

Note added in proof

After this report was accepted for publication, LaVergne had published results on the spectroscopic properties of the S states in Ref. 17. He also found that the difference spectrum of $S_0 \to S_1$ is different from that of $S_1 \to S_2$ and $S_2 \to S_3$. His absorption changes at 355 nm are, however, practically zero; whereas, in this report and in Ref. 10 $\Delta\epsilon(S_0 \to S_1) \approx 750\ M^{-1} \cdot cm^{-1}$ (355 nm). With respect to the $\Delta\epsilon(S_0 \to S_1)$ values in the whole UV region Saygin and Witt [10] found for $S_0 \to S_1$ a very characteristic UV spectrum but smaller than for $S_1 \to S_2$ and $S_2 \to S_3$; whereas LaVergne observed practically no spectral changes in the UV. In Ref. 10 the conclusions were also stronger because, besides the theoretical procedures for deconvoluting overlapping S states used in Ref. 10 as well as in Ref. 17, Saygin and Witt additionally established conditions under which the characteristics of the $S_0 \to S_1$ spectrum could be observed without a deconvolution procedure (the $S_0 \to S_1$ transition was shifted to the front of the flash train by addition of NH_2OH in low concentration).

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