Evidence for the electrochromic identification of the change of charges in the four oxidation steps of the photoinduced water cleavage in photosynthesis

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After dark adaptation of oxygen-evolving photosystem (PS) II complexes, oscillatory absorption changes (stable >0.5 s) with a period of 4 were previously detected at 514 nm, probably due to electrochromic band shifts of carotenoids [(1984) FEBS Lett. 176, 83–87]. To support this interpretation, we looked for corresponding phenomena in the 650–714 nm range. Here, oscillations were also observed with a difference spectrum giving evidence for an electrochromic band shift of the chlorophyll *a* absorption band. The oscillation pattern 0:0:+:+ corresponds to those at 514 nm, supporting the former conclusion that the states S_2 and S_3 have a surplus charge compared to S_0 and S_1. This is possible if the electron release pattern 1:1:1:1 is accompanied by an intrinsic proton release pattern 1:0:1:2 for the transition $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$.

Photosynthesis Electrochromic absorption change Photosystem II Water-splitting enzyme S Oscillation Proton release pattern S-state charge

1. INTRODUCTION

The chlorophyll (Chl) a_{II} in system II of photosynthesis extracts 4 electrons in 4 turnovers from the water-splitting complex S. The latter thereby goes through 4 distinct long-lived (>0.5 s) redox states called $S_0 \longrightarrow S_3$ states. At the end of such a cycle, 2 H_2O are decomposed into 4 electrons and one O_2. In our previous work [1] we observed by a special technique at 514 nm oscillatory absorption changes with a period of 4. Under the proposal that the observed absorption changes are due to the electrochromic band shift of carotenoids [2], we interpreted these oscillations as charge oscillations 0:0:+:+ of the water-splitting complex, S; i.e. that the complex generates a surplus charge in its states S_2 and S_3. This meant that the electron release pattern 1:1:1:1 had to be accompanied by an 'intrinsic' proton release pattern of 1:0:1:2 during the transitions $S_0 \longrightarrow S_1 \longrightarrow S_2 \longrightarrow S_3 \longrightarrow S_0$ (see discussion in [1]). These results, obtained in the time range of seconds due to electrochromism, correspond to those obtained in the nanosecond range due to the analysis of the Chl a_{II}^{+} reduction kinetics in single flashes (see [3]). If our electrochromic interpretation is correct, the charge changes of S should also induce electrochromic band shifts of other pigments in our PS II particles, provided that they are in the close neighborhood of S. To prove this assumption and the conclusions of our previous work, we studied the wavelength region 650–714 nm of Chl *a*. It is shown that also in this range oscillatory changes like those at 514 nm can be observed. The obtained difference spectrum gives evidence for an electrochromic shift of Chl *a*.

2. MATERIALS AND METHODS

Oxygen-evolving PS II particles from the thermophilic cyanobacterium *Synechococcus* sp. were prepared according to [4]. The suspension used for

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measurements contained 4–5 × 10^{-8} M Chl a_{II} centers, 0.01 M $MgCl_2$, 0.5 M mannitol, 2 × 10^{-2} M Mes/NaOH (pH 6.8) and 5 × 10^{-5} M silicomolybdate (or 2 × 10^{-3} M FeCy). Optical changes were measured with the same apparatus as described in [1], except that in most experiments the measuring beam, after passing through a cut-off filter (RG 665 or RG 645), was split behind the cuvette and directed onto two photodiodes. The beams passed through interference filters having different transmission wavelengths but adjusted to the same intensity. Only the difference of the signals was amplified and recorded by a storage oscilloscope. The PS II particles were dark-adapted for 1 h before the measurements. Every 0.5 s after each flash, absorption changes of the solution were measured for 2 ms. This was done by switching on the measuring beam for 2 ms after 0.5 s dark time. Since silicomolybdate accepts electrons directly from Q_A, stable oscillatory absorption changes under these conditions should be due only to stable (>0.5 s) changes of the S-states.

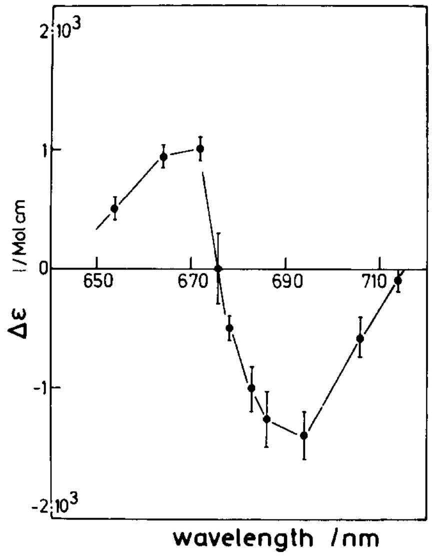

Fig.1. Difference spectrum of the oscillatory absorption changes. The spectrum is obtained from absolute measurements at single wavelengths (with FeCy as acceptor) as well as from difference measurements at 2 wavelengths (with SiMo as acceptor).

3. RESULTS AND DISCUSSION

Absorption measurements between 650 and 714 nm using SiMo as acceptor showed, besides oscillations with a period of 4, overlapping light-induced irreversible absorption changes. These irreversible changes are partly due to silicomolybdate reduction and partly due to particle bleaching below 690 nm (probably antennae chlorophyll). The amplitude of the oscillations at 714 nm were negligible, so that difference measurements between any wavelength (in the region 650–714 nm) minus 714 nm allowed elimination of the irreversible drifts to a great extent.

Fig.1 shows the difference spectrum of the oscillatory absorption change measured in this way. The shape of the fairly symmetric spectrum indicates a shift of an absorption band to lower wavelengths. The zero intercept at 676 nm suggests that Chl *a* is the pigment responsible for the band shift. Fig.2 shows the oscillations at 2 different wavelength differences. The particular wavelengths 672 – 676 nm and 694 – 706 nm were chosen because under these conditions the drifts are practically zero.

Concerning the pattern of oscillatory changes in fig.2, we find a similarity to the pattern observed previously at 514 nm, interpreted to be due to electrochromic shift of carotenoids: after the first and second flashes (mainly states S_2 and S_3, respectively) the absorption of the particles is higher than after the 3rd and 4th flashes (mainly states S_0 and S_1, respectively). We further notice in fig.2 that the absorption change by the first flash is larger than expected from the rest of the pattern, indicating an additional contribution from an unknown process occurring only once. We fitted the pattern of fig.1, bottom, to the equation

$$(\Delta I/I)_i = k_1(S_2 + S_3)_i + k_0$$

with $(\Delta I/I)_i$ being the absorption change after the *i*-th flash relative to the absorption level of the dark-adapted sample; k_0 considers the additional contribution in the first flash.

Fig.3 shows the fitting of the $\Delta\epsilon$ values with the population of the $S_2 + S_3$ states for the parameter $\alpha = 0.07$ (for misses), $\beta = 0.04$ (for double hits) and assuming $S_1 \simeq 100\%$ in the dark-adapted state.

In the states S_2 and S_3 a higher absorption is observed than in the states S_0 and S_1. One can interpret this result by assuming that in the states S_2 and S_3 one elementary excess charge is present in

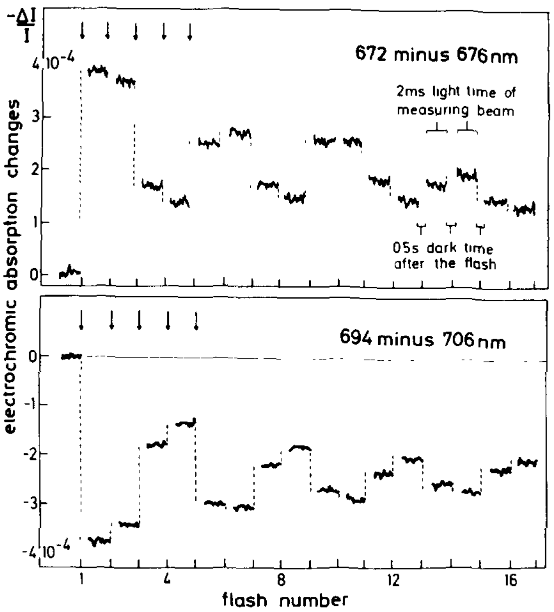

Fig.2. Absorption changes induced by single laser flashes at isolated O_2-evolving PS II complexes as a function of flash number. Arrows and dotted lines indicate the position of the flash excitation. Seven measurements (top) and 6 measurements (bottom) were averaged. The PS II particles were dark-adapted for 1 h before measurements. The suspensions used are described in section 2. The acceptor was silicomolybdate.

comparison to states S_0 and S_1; i.e., a charge pattern of 0:0: + : +. This can be explained if the pattern of the electrons released from the water-splitting complex 1:1:1:1 is accompanied by an intrinsic proton release pattern of 1:0:1:2 during the transitions $S_0 \longrightarrow S_1 \longrightarrow S_2 \longrightarrow S_3 \longrightarrow S_0$ (for details see discussion in [1]). If the electrons extracted by Chl a_{II}^{+} from the water-splitting complex are taken in the transformation S_0–S_3 primarily from the Mn ions and not from water [5] and if water is bound in the form of 2 H_2O in state S_0, water should be converted according to the H^+ pattern to one OH^- in S_1 and two OH^- in S_3.

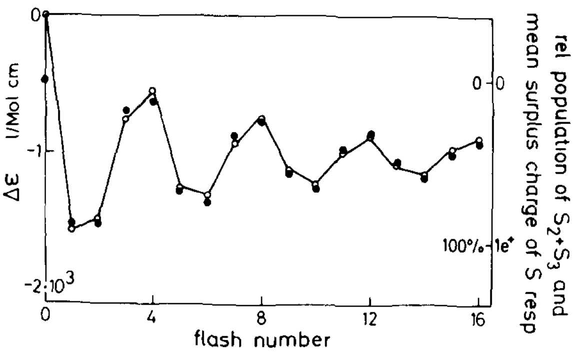

Fig.3. Comparison of the experimental values of absorption changes of fig.2 (bottom) (open circles) with relative population of the $S_2 + S_3$ states and excess charge, respectively (filled circles). α, β and initial S_i distribution values obtained from the best fit are given in the text. The difference between theory and experiment at the beginning reflects the amplitude of an additional contribution to the first flash as discussed in the text.

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