Absorption changes of Photosystem II donors and acceptors in algal cells

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Received 30 April 1984; revised version received 21 May 1984

Flash-induced absorption changes were measured in the 390–450 nm region, using whole cells of a double mutant strain of *Chlorella sorokiniana*, lacking PS I centers and part of the pigment antenna. Spectral changes associated with PS II acceptors (Q_a and Q_b) and donors (P_{680}, Z and L) are described. In this region, the spectrum obtained for Q_b^-/Q_b differs markedly from that of Q_a^-/Q_a. Different spectra were also obtained for the secondary donor (Z^+/Z) when measured after one or three flashes.

Photosystem II *Primary donor* *Secondary donor* *Primary acceptor* *Secondary acceptor* *Absorption change*

1. INTRODUCTION

The study of photosynthetic electron transfer by means of absorption spectroscopy encounters severe difficulties when using algae or isolated chloroplasts. This is generally so because of interference of unwanted photo-induced signals and because of a poor signal to noise ratio due to the high background absorption and flattening effect, especially in the blue and red peaks of chlorophyll. One way of overcoming these difficulties is the use of algal mutants which may have a lower pigment content and may lack some part of the photosynthetic chain. This paper reports on absorption changes in the blue region of the spectrum caused by redox changes of PS II donors and acceptors, using a double mutant strain (S56) of the green alga *Chlorella sorokiniana*. This strain lacks both PS I centers and part of the LHC, which results in the absence of PS I signals, a reduced amplitude of the electrochromic absorbance change, and a higher signal to noise ratio for measurements in the regions where chlorophyll absorbs strongly.

The generally accepted view of electron transfer around PS II may be summarized by the following reactions [1–3]:

$$P_{680}^* \, Q_a \longrightarrow P_{680}^+ \, Q_a^- \quad (1)$$

$$Q_a^- + PQ \longrightarrow Q_a \, Q_b^- \quad (2)$$

$$Q_a Q_b^- (+2H^+) \longrightarrow Q_a + PQH_2 \quad (3)$$

$$Z \, P_{680}^+ \longrightarrow Z^+ \, P_{680} \quad (4)$$

$$S_i \, Z^+ \longrightarrow S_{i+1} \, Z \quad (5)$$

where P_{680} is the primary donor, Z a secondary donor, S_0–S_4 the charge storage states of the oxygen evolving system, Q_a the primary quinonic acceptor, PQ a plastoquinone of the pool which is reduced by Q_a^-, and remains bound to the center in its semiquinone form (denoted Q_b^-), until a further reductive step promotes the formation and release of plastoquinol (PQH_2). Reaction (1) occurs in the ns time range, (2) and (3) have a $t_{1/2}$ around 500 μs (possibly faster, 100–200 μs, for (2) [4]), (4) has a $t_{1/2}$ of 30–50 ns after the first, and slower after subsequent flashes [5], and the $t_{1/2}$ of (5) depends on S_i, being fast ($t_{1/2} \simeq 70$ μs) in state S_1 [6], slower (1–2 ms) in state S_3 [6,7]. According to the generally accepted pattern for proton release

Abbreviations: PS, Photosystem; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LHC, light-harvesting chlorophyll *a/b*–protein

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by the S states [3], S_2 and S_3 bear one net positive charge compared to S_0 and S_1, and this charge storage has been associated with an absorption change in the UV ascribed to a carrier 'L' [6]. This carrier would be oxidized (L^+) in states S_2 and S_3, reduced (L) in states S_0–S_1. The possible existence of two distinct secondary donors (Z_1 and Z_2) has been suggested [2], Z_1^+ being the oxidant for states S_0 and S_1 (or L), Z_2^+ for states S_2 and S_3 (or L^+).

2. MATERIALS AND METHODS

Strain S56 is a double mutant of *C. sorokiniana* isolated by P. Bennoun. It is completely devoid of PS I centers (CPI complex), and lacks part of the pigment antenna (LHC). Details about culture conditions, isolation and characterization of various mutants of *C. sorokiniana* and their use in absorption spectroscopy experiments are given in [8,9]. A high level of reduction of the pool of plastoquinones was found in dark-adapted untreated cells of S56, causing inhibition of PS II centers after a few flashes. We therefore used the algae after treatment with 2×10^{-4} M benzoquinone (5 min incubation followed by centrifugation and resuspension in a medium containing 0.2 M sucrose, and 50 mM phosphate buffer, pH 7). After this treatment, a normal pattern of oxygen evolution in flash sequence experiments was obtained, and the plastoquinone pool and secondary acceptor Q_b were found mostly oxidized in the dark. The algae were used at room temperature at a concentration of about 10 μg chlorophyll/ml. All the results shown were normalized to the same amplitude of PS II signals.

Absorption changes were measured with the apparatus described in [10,11], using monochromatic flashes as a detecting beam. The actinic illumination consisted of saturating flashes from a xenon flash-lamp (duration at half height $\simeq 2$ μs) or a Q-switched ruby laser (50 ns). A typical r.m.s. noise on $\Delta I/I$ is about 2×10^{-5}. The results shown in section 3 were obtained by averaging 5–10 experiments at each wavelength

3. RESULTS

3.1. *Acceptor side*

Spectrum 1 in fig.1A is that of the absorbance change measured 100 ms after a flash in the presence of DCMU and hydroxylamine. The ionophore dicyclohexyl-18-crown-6 was also added to eliminate any contribution from the electrochromic change responding to the membrane potential. In the absence of ionophore, this contribution turned out to be small in the spectral region investigated here (due to the deficiency in pigments, the field-indicating change is intrinsically smaller in such strains [9]), and was neglected in other experiments. Since hydroxylamine rapidly reduces the PS II donor side, the only absorption change remaining under these conditions should be that of the reduced primary acceptor Q_a^-. In the absence of hydroxylamine (spectrum 2), a significant difference is observed around 440 nm, which must be associated with charge storage on the donor side.

When the algae are submitted to a sequence of saturating flashes (no DCMU, but hydroxylamine present), a binary pattern of the absorption change

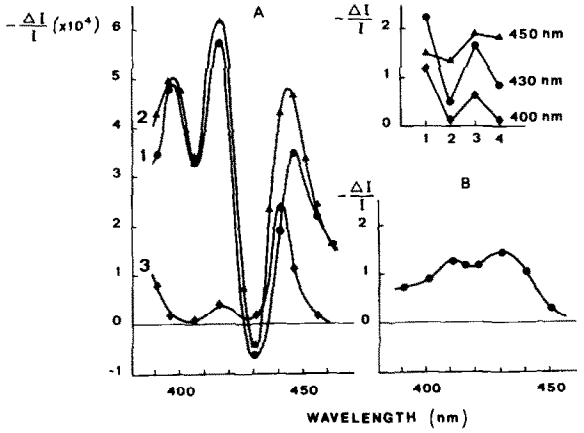

Fig.1. Spectra of Q_a, L and Q_b. (A) Curve 1, absorption change measured at 100 ms after 1 saturating flash in the presence of 10^{-5} M DCMU, 3 mM hydroxylamine and 10^{-4} M dicyclohexyl-18-crown-6. Curve 2, same, but in the absence of hydroxylamine. Curve 3, difference (2)–(1). (B) Spectrum of the period-2 component in the absorption change measured at 800 ms during a sequence of saturating flashes spaced 1 s apart. This component was estimated by subtracting the value measured 800 ms after the second flash from the average of the values after the first and third flashes. The inset shows the absorption changes measured during the flash sequence, referred to a common initial baseline before the first flash. Wavelength as indicated. The abscissa indicates the number of flashes.

at long times after the flashes may be observed, as shown in the inset of fig.1B. This period-2 oscillation is characteristic of the secondary acceptor Q_b. In quinone-treated algae, Q_b is found mostly oxidized in the dark-adapted state, so that after odd-numbered flashes, the majority of Q_b is reduced. The spectrum of the oscillating absorption change behaving as expected from Q_b is given in fig.1B. It was obtained by subtracting the change 800 ms after the second flash from the average of those following the first and third ones. This allows a better correction for non-oscillating drifts occurring in such measurements. A basically similar spectrum was obtained, however, for the difference (first)–(second) flash.

3.2. *Donor side*

Using the spectral shape of Q_a^- (fig.1A,1), its reoxidation kinetics may be studied. We measured for the first, second and third flash, half-times of about 260, 50 and 425 μs, respectively (thus slower than found in higher plant chloroplasts in [4], and lacking the period-2 behaviour reported by these authors). These half-times do not account for slower phases of Q_a^- reoxidation, and we found that, during the first 100 μs after the flash, only about 15% of Q_a^- is reoxidized. Fig.2A shows the absorption changes measured at 10 or 100 μs after the first or third flash of a series, using a baseline measured immediately before the last flash. As explained above, only little reoxidation of Q_a^- by the secondary acceptor occurs in this time range so that no significant contribution from Q_b^-/Q_b is expected in these spectra. The differential spectra (10 μs–100 μs) are plotted in fig.2B. After the first flash, the only reaction expected to occur to a large extent during this time interval is the reduction of the secondary donor Z^+ by the oxygen evolving

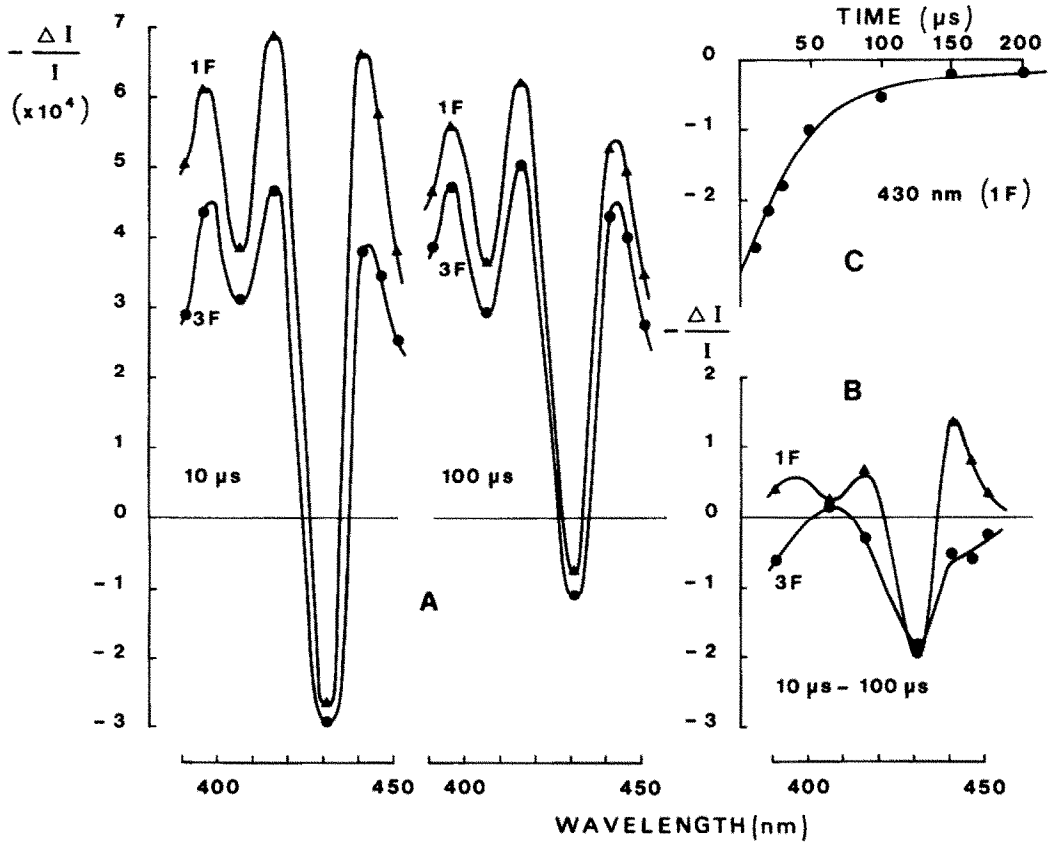

Fig.2. (A) Absorption changes measured at 10 and 100 μs after the first or third saturating flash of a series (time between flashes: 1 s), using baselines measured immediately before the relevant flash. (B) Difference spectra (10 μs–100 μs) computed from A. (C) Kinetics of the absorption change at 430 nm after the first flash.

system (transition from S_1 to S_2 leading to the formation of the oxidized species L^+). The differential spectrum of fig.2B (first flash) should thus be mostly indicative of LZ^+/L^+Z absorption changes. The kinetics of this reaction measured at the negative 430 nm peak was plotted in fig.2C. A $t_{1/2}$ of 30–40 μs was found. After the third flash, kinetics with a similar rate and amplitude were also observed.

The spectrum of the S_2 state (i.e., that of L^+/L) may be obtained by subtracting the change measured 100 ms after a flash in the presence of hydroxylamine and DCMU (Q_a^-/Q_a, spectrum 1 in fig.1A) from that measured in the presence of DCMU alone ($L^+Q_a^-/LQ_a^-$, spectrum 2 in fig.1A). The resulting spectrum (3 in fig.1A) displays a sharp positive peak around 440 nm, is close to the baseline in the 400–430 nm range, and rises again below 400 nm.

The spectrum of Z^+/Z may be obtained by subtracting the spectrum of Q_a^-/Q_a (fig.1A,1) from that obtained 10 μs after one flash. This is shown in fig.3A (triangles). Alternatively, one may add the spectrum of L^+/L to that of LZ^+/L^+Z (fig.2B,1F). The result (fig.3A, squares) is very similar to that obtained by the first procedure, confirming the consistency of our working assumptions.

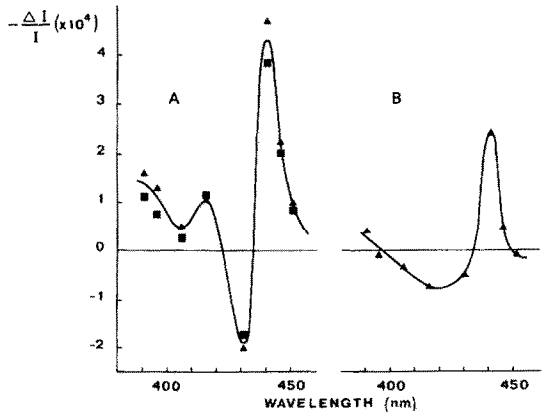

Fig.3. Spectra of the secondary donor after 1 (A) or 3 (B) flashes. (A) Triangles, obtained by subtracting spectrum (1) of fig.1A (Q_a^-/Q_a) from the first flash spectrum at 10 μs of fig.2A. Squares, obtained by adding spectrum (3) of fig.1A (L^+/L) to the IF difference spectrum of fig.2B. (B) Obtained as above (triangles) by subtracting curve 1 of fig.1A from the spectrum at 100 μs (third flash) of fig.2A.

After 3 flashes, the re-reduction of Z^+ ($S_3Z^+ \longrightarrow S_4Z \longrightarrow S_0 + O_2$) is expected to be slowed down to a half time of 1–2 ms [6,7]. Thus, the spectrum of Z^+ (or of the alternative secondary donor Z_2^+ which is possibly involved when L^+ is present (see section 1) should not be present in the 10–100 μs spectrum of fig.2B (3rd flash), but should rather be a component of the 3rd flash spectra of fig.2A. A tentative deconvolution of the 'Z_2^+' spectrum is shown in fig.3B, by subtracting the Q_a^-/Q_a spectrum of fig.1A,1 from the $Z_2^+Q_a^-/Z_2Q_a$ spectrum of fig.2A (100 μs, 3F). The result differs markedly from the Z_1^+ spectrum of fig.3A, with a smaller 440 nm peak and no large negative peak at 430 nm. Fig.4 is a plot of the absorption changes measured at 10 μs (triangles) and 100 ms (squares) during a sequence of saturating flashes (using a 'fresh' baseline before each flash). It confirms several features of the preceding experiments. At long times, the pattern is consistent with a predominant contribution of L^+/L at 440 nm and of Q_b^-/Q_b at 430 nm. At 10 μs, a period-4 oscillation is obtained at 440 nm, in agreement with the higher amplitude of Z_1^+ compared with Z_2^+ (fig.3), and also with the small amplitude at this wavelength of the fast decaying

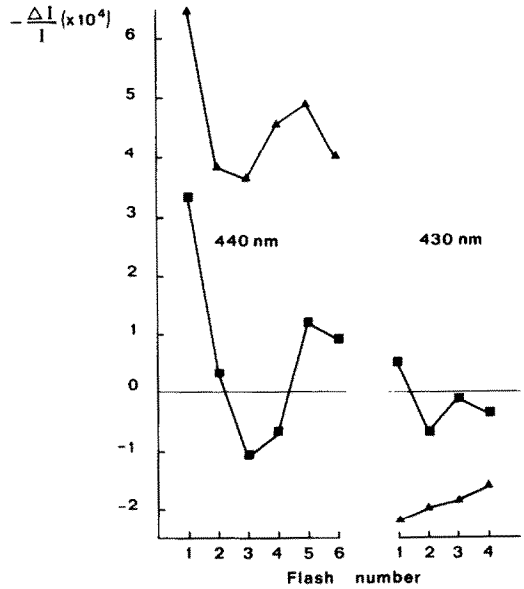

Fig.4. Absorption changes (referred to a fresh baseline measured before each flash) at 10 μs (triangles) and 300 ms (squares) during a sequence of saturating flashes spaced 1 s apart. Wavelength as indicated.

component after 3 flashes (fig.2B, 3F). At 430 nm, no oscillation appears at 10 μs, as expected from the similar amplitudes of the fast decaying component at 430 nm after 1 or 3 flashes (fig.2B).

4. DISCUSSION

4.1. *Acceptor side*

The spectrum we report for Q_a^- is in good agreement with that obtained in [12] using PS II particles (see [13]). Contrary to the changes measured in the UV [14], the spectrum of Q_a^- is considerably distorted in the blue region as compared to the plastosemiquinone anion spectrum obtained in vitro in [15]. This may be due, particularly the 430 nm trough, to a local electrochromic effect of the semiquinone on P_{680}.

The blue spectrum of Q_b^- (fig.1B) has not been reported before. Contrary to the results obtained in the UV region [14], it differs markedly from that of Q_a^-, with a peak instead of a trough at 430 nm. However, it still differs from the in vitro spectrum of plastosemiquinone, presumably due to interaction with the binding site of the 32-kDa protein.

4.2. *Donor side*

The spectrum obtained for L^+ (fig.1A,3) differs noticeably from that reported in [12]. These authors found a similar shape to that of Z^+ (i.e., fig.3A), with a negative peak at 430 nm and a positive peak at 440 nm of higher amplitude than that of Z^+. Our results confirm only the positive peak at 440 nm, but with a markedly smaller amplitude than the Z^+ peak. Indeed, we consistently observed an absorption decrease at 440 nm during the first 100 μs after a flash (as shown by the positive peak in the 10–100 μs difference spectrum of fig.2B) corresponding to the transition $LZ^+ \longrightarrow L^+Z$. Similarly, both the 10–100 μs spectrum and the DCMU ± NH_2OH difference spectrum strongly argue against a large negative peak at 430 nm associated with L^+. The characteristics of the period-4 oscillating pattern obtained at long times in flash sequence experiments (as shown in fig.4 at two wavelengths) are consistent with our implicit assumption that the only absorption change controlled by charge storage on the oxygen evolving system responds to the state of L rather than to the individual S_i states. However, the accuracy of this statement is necessarily limited due to interference of Q_b^-/Q_b changes in such experiments.

The component which decays in 100 μs after the third flash (fig.2B) should not involve the secondary donor, which, according to the EPR experiments on Signal II [7], is re-reduced only in the ms time range. Its spectrum resembles that of P_{680}^+ as reported in [13], although its amplitude would correspond only to a small part of the total P_{680}. It seems therefore that after 3 flashes a slow component ($t_{1/2}$ = 30–40 μs) of P_{680}^+ reduction takes place. The similar amplitude – and kinetics – observed at 430 nm after 1 or 3 flashes would be coincidental.

The spectrum found for Z^+/Z after one flash (fig.3A) is similar to that reported in [16] and still closer to that in [12], using PS II particles with an inactivated oxygen evolving system. A slightly different spectrum was also reported recently [17]. The UV part of the spectra reported by these authors resembles that of a semiquinone cation, such as that of 2,3-dimethyl-1,4-naphthoquinone [16], or of duroquinone [12]. Analysis of the EPR spectrum of Signal II also led authors in [18] to propose the involvement of a semiquinone cation, which they believe to be that of plastoquinone. In the blue region, the Z^+ spectrum bears no resemblance to the in vitro spectra, which suggests that local interactions predominate there, possibly a shift of the P_{680} spectrum caused by the neighbouring positive charge in Z^+. The kinetics of the $Z^+L \longrightarrow ZL^+$ reaction after one flash may be measured at 430 nm (fig.2C) or at 440 nm (where similar results were obtained; not shown). A $t_{1/2}$ of 30–40 μs was obtained, shorter than the 70 μs value obtained in [6] for L^+ formation in spinach chloroplasts.

The spectrum obtained for the secondary donor after 3 flashes is shown in fig.3B. Although we found some variability as to its precise shape for $\lambda \leqslant 430$ nm, we consistently obtained a spectrum markedly different from that of Z^+/Z after one flash, with a positive peak about two times smaller at 440 nm. This is confirmed by the shape of the oscillatory pattern obtained at 10 μs (fig.4), with a high value in the presence of L (1st, 4th and 5th flash), and a lower value in the presence of L^+ (2nd and 3rd flash). A similar pattern was also obtained at shorter times (2 μs) after the flash. These results give some support to the hypothesis of two dif-

ferent secondary donors [2], Z_1, operative in the $S_0 \longrightarrow S_1$ and $S_1 \longrightarrow S_2$ transitions, and Z_2 in the $S_2 \longrightarrow S_3$ and $S_3 \longrightarrow S_4$ transitions (i.e., when L^+ is present before the flash). Various models with Z_1 and Z_2 acting in parallel or in sequence have been discussed by authors in [2], who favoured the parallel scheme. However, taking into account the EPR data on Signal II, one has to face the following paradox. In the Z_1–Z_2 model, Signal II 'very fast', which is observed with sufficient accuracy after the third flash [7] should be identified with Z_2. On the other hand, it was claimed that the EPR spectrum of this signal is identical to that of Signal II 'fast' observed in Tris-washed material. Now, the optical spectrum of the donor responsible for Signal II fast was measured by authors in [16] and [12], who obtained, as mentioned earlier, a spectrum similar to the one we report here for Z_1 (fig.3A), not for Z_2. A possible explanation is that there is actually a single carrier Z, but that its spectrum in the blue region is markedly influenced by the state of L. This would be plausible if, as discussed above, the absorption change measured in this region is mainly a bandshift (of P_{680}?) responding to the oxidation of Z rather than a true oxidized/reduced spectral difference.

ACKNOWLEDGEMENTS

The author wishes to thank P. Joliot and B. Diner for many helpful discussions.

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