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DIRECT EVIDENCE FOR A BACK-REACTION IN PHOTOSYSTEM II OF SPINACH CHLOROPLASTS FOLLOWING FLASH EXCITATION AT LOW TEMPERATURE

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Summary

We measured the absorption changes following excitation of spinach chloroplasts with a ruby laser, at —170° C. C-550 is fully reduced by the flash and 80% reoxidized in a back-reaction ($t_{1/2}$: 4.2 ms). A 518 nm effect behaves similarly, except for a different sensitivity to ferricyanide. A small amount of cytochrome b_{559} is oxidized within 5 ms after the flash.

It is known that, at room temperature, a short flash brings about the transfer of one electron from the oxygen-evolving system to the pool of plastoquinone in all the Photosystem II reaction centers [1,2]. A single flash also induces a nearly complete fluorescence induction [3]. At low temperature, however, one flash of saturating intensity can only slightly increase the fluorescence yield, whereas a series of such flashes (or continuous illumination) results in a complete fluorescence induction. This was found to be true both at liquid N_2 temperature [4—6] and in the S_2 and S_3 states at —50° C [7]. Similarly, we observed that C-550, a possible primary electron acceptor of Photosystem II, was in a partially reduced state after one saturating flash at —196° C [8], a result which has been confirmed by Butler and coworkers [9,10]. An identical result was obtained at —55° C using chloroplasts in the states S_2 or S_3 [11].

The small amount of reduced C-550 and the small increase of the fluorescence yield may be interpreted as due to either a back-reaction between the products of the primary reaction [4,6,11], or a low quantum efficiency for charge separation [5], or an equilibrium between a photoactive

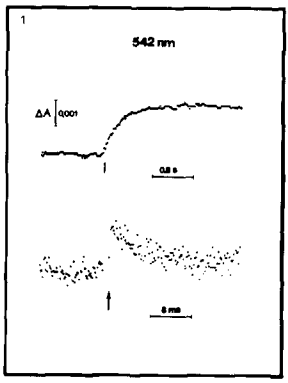
and a photoinactive form of the reaction center which could also occur at room temperature [12].

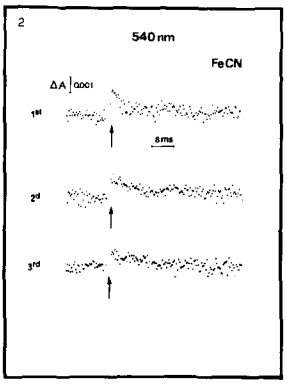
In this paper we report that C-550 is fully reduced by one flash at −170° C and that a partial back-reaction occurs within 5 ms. Part of the cytochrome b_{559} is oxidized in the same time. A similar back-reaction is observed for a 518-nm effect that we have previously found to be related with the primary photochemistry of Photosystem-II [13].

A suspension of spinach chloroplasts in 0.4 M sucrose, 0.02 M Tris buffer (pH 7.8), 0.01 M NaCl, was diluted with 2 vol. of glycerol. For comparisons between control and ferricyanide-treated chloroplasts, they were kept for 5 min at 4° C in a 5 times diluted buffer, with or without 10 mM potassium ferricyanide. The chloroplasts were then returned to the normal buffer concentration. The suspension was poured in a 1-mm cuvette and the kinetics of absorption changes were measured at −170° C as described in ref. 14. The only difference is that in this report the excitation is provided by a ruby laser. The laser pulse (10 ns, 400 mJ) was homogenized and attenuated by a ground glass and lenses. The overall time-resolution was 0.3 ms. We stored in different sub-groups of a digitizer the signals due to the first, the second, and the third flash given on the same cuvette, at a time interval of 30 s. We often added these three signals (Figs 3—6) in order to improve the signal to noise ratio.

Following illumination of chloroplasts at −170° C, an absorption increase is observed at 540—542 nm (Fig.1). It has the same magnitude in either continuous light or laser flashes. However in the latter case, a biphasic decay occurs consisting of a fast component ($t_{1/2}$ = 4.2 ± 0.5 ms) and an irreversible phase. Within a series of 3 successive flashes the time course of the signal remains the same, but the magnitude of the absorption change decreases progressively. This behaviour is exemplified in Fig. 2, for chloroplasts treated with ferricyanide, a treatment which has very little effect at 540 nm. For the results presented in Figs 3—6 we added and averaged the effect of the first three flashes in order to improve the signal:noise ratio.

The previous properties are observed between 535 and 550 nm. In that range the difference spectrum is characteristic of the reduction of C-550 (Fig.3). From these data, we conclude that C-550 is fully reduced by one flash; about 80% of it is then reoxidized in a dark back-reaction leading to a state that can be activated by a second flash, while about 20% of the reduced C-550 stays in a stable reduced state. Small deviations from pure C-550 absorption changes are apparent in Figs 4—5. At 540 nm, the relative magnitude of the irreversible phase is slightly greater without than with ferricyanide and the reverse is true at 547 nm. We attribute the differences to a small Photosystem I absorption change which is positive in that spectral range [13] and which does not reverse in the ms time range. This is confirmed by the occurrence of a small absorption increase (irreversible in our time domain) upon laser excitation at −170° C of chloroplasts that received, before cooling, one flash in the presence of DCMU (20 μM) and hydroxylamine (100 μM),





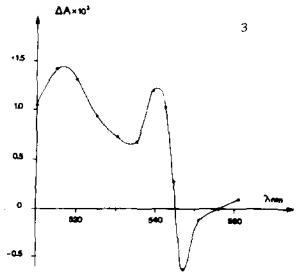
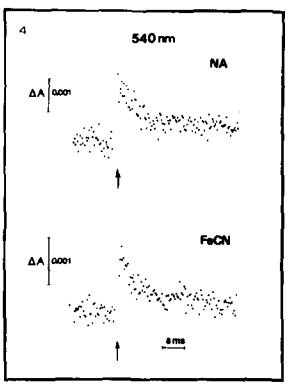
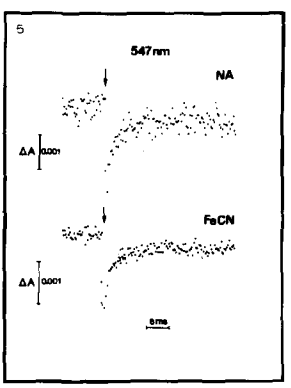


Fig.1. Kinetics of absorption changes induced at 542 nm on chloroplast suspensions from the same batch (chlorophyll concentration: 500 $\mu g \cdot ml^{-1}$) by: upper trace; continuous light (630 nm). Average of 3 experiments. Lower trace: one laser flash. Average of 15 experiments.

Fig.2. Absorption changes at 540 nm induced by three successive flashes on the same suspension. 10 mM Ferricyanide (FeCN) was added prior to freezing. Chlorophyll concentration: 435 $\mu g \cdot ml^{-1}$. Average of 5 experiments.

Fig.3. Difference spectrum of absorbance changes measured 3 ms after the laser flash. Chlorophyll concentration: 435 $\mu g \cdot ml^{-1}$. Average of 3 experiments (with different cuvettes) and of the first 3 successive flashes (on the same cuvette).





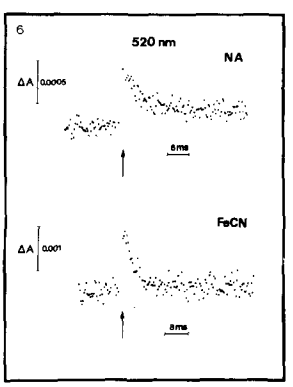


Fig.4. Kinetics of absorption changes at 540 nm, for a same batch of chloroplasts (435 $\mu g \cdot ml^{-1}$). Upper trace: no addition (NA). Average of the first 3 successive flashes in 7 experiments. Lower trace: addition of 10 mM ferricyanide (FeCN). Average of the first 3 successive flashes in 5 experiments.

Fig.5. Kinetics of absorption changes at 547 nm. Same conditions as for Fig.4. Upper trace: 11 experiments. Lower trace: 9 experiments.

Fig.6. Kinetics of absorption changes at 520 nm. Upper trace: no addition. Chlorophyll concentration: 210 $\mu g \cdot ml^{-1}$. Average of the first 3 successive flashes in 15 experiments. Lower trace: addition of 10 mM ferricyanide. Chlorophyll concentration: 435 $\mu g \cdot ml^{-1}$. Average of the first 3 successive flashes in 9 experiments.

a procedure that blocks Photosystem II. The distortion is also apparent in Fig.3; it is accentuated because we measured the absorption changes 3 ms after the flash in order to get rid of a stray-light artefact.

At the wavelength 556 nm, characteristic of cytochrome b_{559} absorption, we find no absorption change simultaneous with the flash. However, following the flash, a progressive absorption decrease, with $t_{1/2}$ = 4—6 ms, is observed, in agreement with a previous finding by Floyd et al. [15]. On one flash we observed the oxidation of only a small proportion (around 20%) of the cytochrome b_{559} that is photooxidized by continuous light. The signal disappears with chloroplasts treated with ferricyanide.

Around 510—520 nm a positive absorption increase is observed (Fig.6) which behaves almost exactly the same as the absorption changes due to the reduction of C-550, including a biphasic character with $t_{1/2}$ = 3.7 ± 0.4 ms for the fast phase, a maximum absorbance change due to the first flash identical with that obtained by continuous light as well as a progressive decrease within a series of successive flashes. This observation provides further support to our previous suggestion that the 518-nm effect (which might represent a local field effect) is directly related with the primary photochemistry of Photosystem II [13]. In contrast with its absence of effect on C-550 (540, 547 nm), ferricyanide nearly completely abolishes the irreversible component at 520 nm (Fig.6). This is also in agreement with what we found under continuous light [13] and indicates that this "field effect" is representative of both the reduction of the primary acceptor and the nature of the electron donor.

The back-reaction that we observe after the complete reduction of C-550 by one flash at −170° C is probably due to an hindered electron transfer reaction, either on the reducing side or on the oxidizing side of Photosystem II. This back-reaction accounts for the small amount of C-550 that was found in a reduced state after one flash, using conventional spectroscopic techniques [8—10]. It also seems to account for the small proportion of the fluorescence induction that can be effected by one flash at liquid N_2 temperature, a fact that supported at first the back-reaction hypothesis [4,6]:

$$\text{C-550—Chl—D} \underset{\text{dark (5 ms)}}{\overset{\text{light}}{\rightleftharpoons}} \text{C-550}^- \text{—Chl}^+ \text{—D} \xrightarrow[\text{dark (20 ms)}]{} \text{C-550}^- \text{—Chl—D}^+$$

However Den Haan et al. [5] did not find any change in fluorescence yield in the ms time domain, after a flash at liquid N_2 temperature. This last result is difficult to reconcile with the above simple scheme, unless there is a fortuitous balance between the fluorescence quenching by the C-550—Chl state and by the $C\text{-}550^-\text{—Chl}^+$ state.

A flash-induced absorption change peaking at 330 nm has been observed by Witt [16] and attributed to the primary acceptor of Photosystem II. At −160° C it is irreversible and fully saturated by one flash, two properties that differentiate it from C-550. If the assignments were correct, we would have

to deal with two different electron acceptors in Photosystem II at low temperature.

Around 680 nm, an absorption decrease with a half-time return of 4.5 ms has been found by Floyd et al. [15]. The properties of P_{680}, as reported by these authors (biphasic time course of the absorption change at times greater than 1 ms and progressive saturation within a series of successive flashes) are very similar to what we find for C-550. In conjunction with the results of Lozier and Butler [10] this seems to indicate that Floyd et al. did not detect P_{680} (considered as the primary electron donor) but another absorption change connected with the reduction of the primary electron acceptor.

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