THE RELATIONSHIP BETWEEN C-550 AND DELAYED FLUORESCENCE

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It is generally accepted that the mechanism for the production of delayed fluorescence from green plants involves the recombination of the reduced form of the primary electron acceptor of Photosystem II, Q^-, and the oxidized form of the primary electron donor of Photosystem II, Z^+ [1]. On the basis of this mechanism any treatment which destroys Q or Z or inactivates the primary photochemical separation should eliminate delayed fluorescence.

An isomorphic relationship was demonstrated between Q, as indicated by fluorescence yield, and C-550, as indicated by light-induced absorbance changes at liquid nitrogen temperature [2]. The strict relationship between these two parameters only holds when C-550 is assayed at low temperature. At room temperature the responses of C-550 are more complex [3]. C-550 (assayed at $-196°$) acts as if it were the primary electron acceptor of Photosystem II.

Spectroscopic analysis of mutants of Scenedesmus [4] and Chlamydomonas [5] showed that mutants with a high, invariant fluorescence yield lacked C-550. These mutants are also characterized by negligible levels of delayed fluorescence [6, 7]. In the present work the delayed fluorescence of spinach chloroplasts was examined after a treatment with pancreatic lipase which was shown to destroy C-550 [8, 9].

Spinach chloroplasts made by the method of Kraayenhof [10] (with the addition of 2 mM $MgCl_2$ to the grinding medium) were incubated with pancreatic lipase (25 mg lipase and 2 mg chlorophyll in 10 ml 50 mM tricine-KOH buffer, pH 1.5) for 1 hr at 20° as described previously [9].

A control sample of chloroplasts without lipase was also incubated for 1 hr at 20°. The chloroplasts were then collected by centrifugation at 15,000 *g* for 10 min and suspended in 1.0 ml of 50 mM tricine–KOH buffer pH 7.5. Delayed fluorescence (referred as 1 msec delayed fluorescence) was measured with a conventional phosphoroscope between 0.6 and 0.7 msec after the excitation flash. Prompt fluorescence was measured simultaneously in the same apparatus by a light pipe which transmitted the fluorescence to an external phototube. The chloroplasts were suspended at 20 μg chlorophyll/ml in a 50 mM TES, 100 mM KCl buffer, pH 7.5, with 10^{-7} M valinomycin and 5×10^{-7} M nigericin in a 1 mm cuvette. Delayed fluorescence and prompt fluorescence were excited by broadband blue light, 5 mwatts/cm^2. C-550 was measured by the light-induced absorbance changes between 550 and 540 nm at room temp. with an Aminco-Chance dual wavelength spectrophotometer. (A room temp. assay was adequate for this work since we are only seeking to establish the presence or absence of C-550.) Chloroplasts were suspended in a 1 cm cuvette at 100 μg chlorophyll/ml in 50 mM tricine, 10 mM KCl, 2 mM $MgCl_2$, 0.4 M sucrose buffer pH 7.5 with 3.3 mM ferricyanide to remove light-induced changes due to cytochromes [11].

A comparison of the absorbance changes, induced by red (660 nm) actinic light (3 mwatt/cm^2) with the control and lipase treated chloroplasts is shown in fig. 1. The control chloroplasts (curve A) showed the expected bleaching at 550 nm due to the photoreduction of C-550 while the lipase treated chloroplasts (curve B) showed no such change.

The measurement of prompt fluorescence and 1 msec delayed fluorescence are shown in fig. 2. The chloroplasts were suspended with valinomycin and nigericin to minimize contributions of membrane potentials and ion gradients to the stimulation of delayed light [12].

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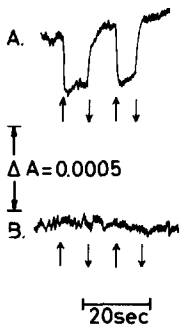

Fig. 1. Absorbance difference between 550 and 540 nm. Chloroplasts (100 μg chlorophyll/ml) suspended in 0.4 M sucrose, 10 mM KCl, 2 mM $MgCl_2$, 50 mM tricine buffer, pH 7.5 with 3.3 mM potassium ferricyanide. Red (660 nm) actinic light (3 mwatts/cm^2) on at upward arrows, off at downward arrows. (A) Control chloroplasts after 1 hr at 20°. (B) Lipase treated chloroplasts after 1 hr incubation with pancreatic lipase at 20°.

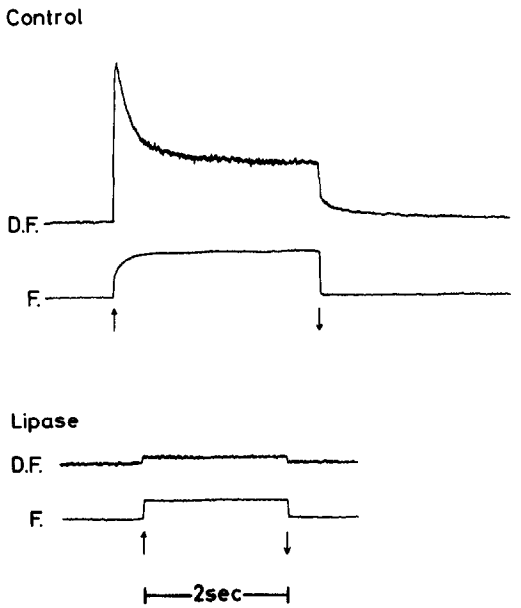

Fig. 2. Delayed fluorescence (D.F.) and prompt fluorescence (F.) from control and lipase treated chloroplasts. Blue excitation light (5 mwatts/cm^2) on at upward arrows, off at downward arrows.

The light-induced fluorescence yield increase is apparent in the curve of prompt fluorescence for the control chloroplasts. During the same initial period the curve for delayed light from the control chloroplasts reaches a maximum about 80 msec after the onset of irradiation and then decays to a lower steady-state level. The decay of delayed light during a period when the fluorescence yield continues to increase could be attributed to a decay in the concentration of Z^+. The lipase-treated chloroplasts show a low invariant fluorescence yield, comparable to the minimum yield of the intact chloroplasts, and a very low level of delayed light, 8 to 9% of the steady-state level of the control chloroplasts. After a sufficiently long dark period the lipase-treated chloroplasts also show an initial spike in the delayed light which reaches a peak value about twice that of the steady-state level. The low level of delayed fluorescence from the lipase-treated chloroplasts may be due to a small amount of Photosystem II activity which survived the treatment.

The reason why lipase digestion leaves spinach chloroplasts with the minimum fluorescence yield is unknown. Simple considerations would suggest that the destruction of Q should result in the maximum yield. Perhaps the treatment opens up Photosystem II to other fluorescence quenchers or quenching mechanisms. It was found previously that spinach and Chlamydomonas chloroplasts differed in that lipase digestion, which destroyed C-550 in both, left Chlamydomonas chloroplasts with a high, invariant fluorescence yield comparable to the maximum yield of untreated Chlamydomonas chloroplasts.

The loss of delayed light would be expected to result from any treatment which destroyed the reaction center complex or the photochemical activity of Photosystem II. In all of the known cases, however, e.g. the high fluorescence yield mutants and the lipase-treated chloroplasts, the absence of Photosystem II activity and delayed fluorescence are correlated with the absence or destruction of C-550.

Acknowledgements

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