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Contrasting requirement for plastocyanin in the photooxidation of chloroplast cytochromes

We have recently reported[1,2] that the chloroplast copper protein plastocyanin is required for the photooxidation of cytochrome b_{559}, in a reaction that is induced effectively only by short wavelength light ($\lambda < 700$ nm) characteristic of System II of photosynthesis. This and related findings[2,3] led us to subdivide System II into two photoreactions (IIa and IIb) and to link them by a "dark" electron transport chain that includes (but is not limited to) cytochrome b_{559}, plastocyanin, and C550, a newly discovered chloroplast component, distinct from cytochromes, that undergoes photoreduction by System II light[3]. According to this concept, the photoreduction of ferredoxin and NADP is accomplished solely by System II reactions (Scheme 1).

Scheme 1:

$$H_2O \rightarrow h\nu_{IIb} \rightarrow C550 \rightarrow \text{cyt. } b_{559} \rightarrow PC \rightarrow h\nu_{IIa} \rightarrow Fd \rightarrow NADP$$

where PC is plastocyanin and Fd ferredoxin.

Other investigators[4–6] have attributed the photooxidation of cytochrome b_{559} to System I functioning best at long wavelength light ($\lambda > 700$ nm) and have placed plastocyanin[7–17] along with cytochrome b_{559} and cytochrome f (refs. 6, 18, 19) in an electron transport chain that joins System II with System I (Scheme 2, abbreviated).

Scheme 2:

$$H_2O \rightarrow h\nu_{II} \rightarrow \ldots \text{cyt. } b_{559} \rightarrow \text{cyt. } f \rightarrow PC \rightarrow h\nu_{I} \rightarrow Fd \rightarrow NADP$$

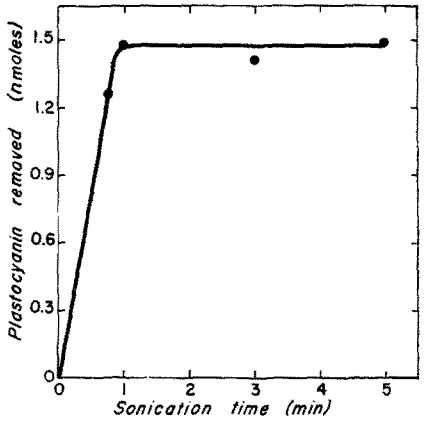

Fig. 1. Effect of sonication time on removal of plastocyanin from spinach chloroplasts. Isolated chloroplasts[22] were placed in 0.8 M Tris buffer, pH 8.0, at a concentration of approx. 0.13 mg chlorophyll per ml. After 10 min, the suspension was centrifuged for 1 min at 200 × g and the pellet discarded. The green supernatant was centrifuged for 7 min at 1000 × g. The pellet was suspended in 0.035 M NaCl. The Tris-treated chloroplasts were sonicated at 0° (0.5 mg chlorophyll per ml) and then centrifuged for 30 min at 144000 × g. The plastocyanin released by sonication was assayed by determining the oxidized *minus* reduced (ferricyanide *minus* ascorbate) difference spectrum of the 144000 × g supernatant on a Cary 14 spectrophotometer after the supernatant had been concentrated 5-fold on DEAE. $\varepsilon_{ox\text{-}red}$, 597–500 nm was taken to be 7.2 $mM^{-1} \cdot cm^{-1}$ and the amount of plastocyanin released was computed per 1 mg chlorophyll in the starting material.

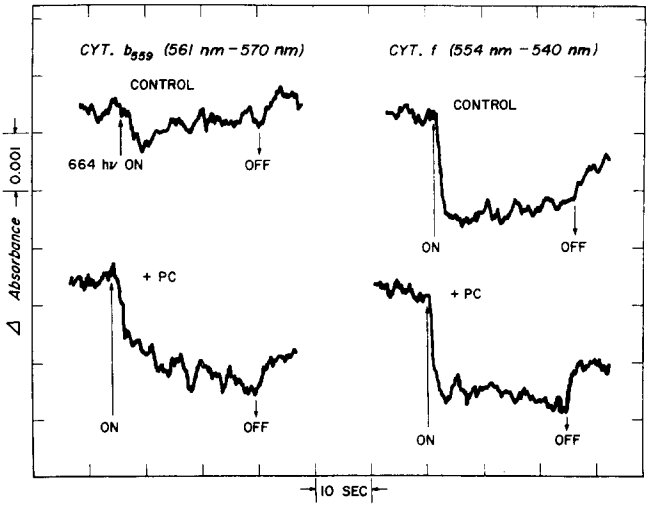

Fig. 2. Effect of added plastocyanin on photooxidation of cytochromes b_{559} and f by sonicated chloroplasts in System II light. The reaction mixture contained, per 1.0 ml, sonicated (1 min), Tris-treated chloroplasts (see legend to Fig. 1) (equivalent to 75 μg chlorophyll) and the following in μmoles: $MgCl_2$, 2; K_2HPO_4, 5; ascorbate, 1; MES [2-(*N*-morpholino)ethanesulfonic acid] buffer (pH 6.2), 33.3; and where indicated, plastocyanin, 0.015. In addition, the samples used for measuring photooxidation of cytochrome b_{559} contained the following in μmoles: NADP, 1; and ferredoxin, 0.01. Gas phase, N_2. Illumination, monochromatic light beam (664 nm) of approx. $1.5 \cdot 10^4$ ergs/cm^2/sec. (The addition of ferredoxin–NADP reductase had little effect on the photooxidation of the cytochromes.)

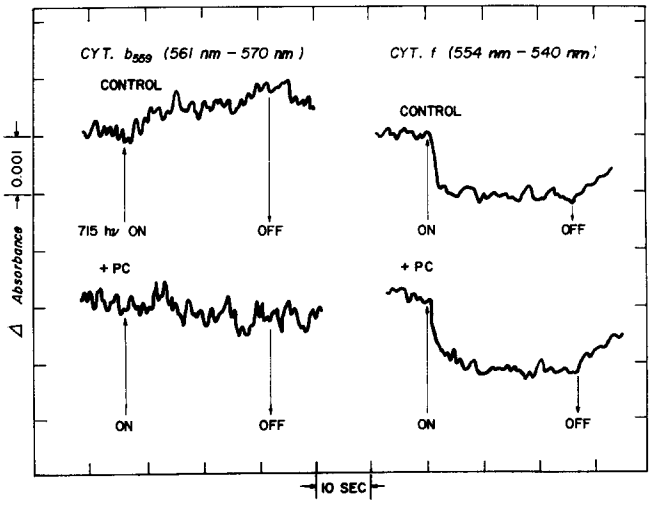

Fig. 3. Effect of added plastocyanin on photooxidation of cytochromes b_{559} and f by sonicated chloroplasts in System I light. Experimental conditions were as for Fig. 2 except that illumination consisted of a monochromatic light beam (715 nm) of approx. $0.9 \cdot 10^4$ ergs/cm^2/sec.

The relative merits of these conflicting interpretations of the role of plastocyanin in electron transport were tested by comparing directly the role of added plastocyanin on the photooxidation of cytochromes b_{559} and f in chloroplasts from which plastocyanin was removed by sonication. The methods used were described elsewhere[2].

After 1 min the sonication treatment gave maximum removal of plastocyanin (Fig. 1). The effect of plastocyanin removal and its subsequent restoration on the photooxidation of cytochromes b_{559} and f was investigated by sonicating chloroplasts treated with Tris, a treatment[20] that inactivates the electron flow from water but does not otherwise interfere with the photosynthetic activity of chloroplasts. As reported earlier[1,2], the Tris treatment of chloroplasts facilitates the measurement of photooxidation of cytochrome b_{559} at room temperature.

Fig. 2 shows that removal of plastocyanin by sonication from Tris-treated chloroplasts had little effect on the photooxidation of cytochrome f but seriously impaired the photooxidation of cytochrome b_{559}. The addition of plastocyanin had no significant effect on the photooxidation of cytochrome f, but it markedly stimulated the photooxidation of cytochrome b_{559} by System II light (664 nm). However, in agreement with previous results that this is a System II reaction[1,2], no photooxidation of cytochrome b_{559} occurred in System I light, even when plastocyanin was added (Fig. 3). By contrast, the photooxidation of cytochrome f occurred in either System I or System II light, whether plastocyanin was added or not. YAMASHITA AND BUTLER[20] have shown that, although cytochrome f is a component of System I, in Tris-treated chloroplasts it is photooxidized equally well in System II as in System I light. These findings in spinach chloroplasts differ from those of LEVINE AND GORMAN[6,9], who found that chloroplast fragments from plastocyanin-deficient *Chlamydomonas reinhardi* mutants were unable to photooxidize cytochrome f and from the observation of HIND[21] that plastocyanin stimulates the rate of cytochrome f photooxidation in Triton-treated chloroplasts.

These experiments show that, in spinach chloroplasts, plastocyanin (removable by sonication) is required for the photooxidation of cytochrome b_{559} but is not required for the photooxidation of cytochrome f. Moreover, the photooxidation of cytochrome b_{559}, in contrast to that of cytochrome f, is a System II reaction.

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On the relationship of the energy-linked transhydrogenase to energy-linked NAD^+ reduction in *Rhodospirillum rubrum*

Chromatophores of *Rhodospirillum rubrum* catalyze a light-dependent reduction of NAD^+ using succinate[1] or other electron donors[2,3]. KEISTER AND YIKE[4] demonstrated that this reaction is sensitive to inhibitors and uncouplers of photophosphorylation. The latter authors concluded that NAD^+ reduction reflects reversed electron flow in the oxidative electron-transport chain supported by some high energy intermediate of photophosphorylation (Eqn. 1).

$$\text{Succinate} + NAD^+ + X \sim Y \leftrightharpoons \text{fumarate} + NADH + H^+ + X + Y \quad (1)$$

Another energy-linked reduction observed in *R. rubrum* chromatophores is the photoreduction of $NADP^+$ during energy-linked transhydrogenation. This reaction which required NADH as a specific electron donor, was found to be sensitive to uncouplers and inhibitors of phosphorylation[4,5] and has in a similar way been assumed to be driven by the energized intermediate of the conservation mechanism (Eqn. 2).

$$NADH + NADP^+ + X \sim Y \longrightarrow NAD^+ + NADPH + X + Y \quad (2)$$

Work from this laboratory[6,7] has shown that the system responsible for the catalysis of the energy-linked transhydrogenase of *R. rubrum* can be resolved into a soluble protein factor and an insoluble membrane component. The partially purified soluble protein is not itself a transhydrogenase, since it does not carry out the energy- or nonenergy-linked reaction in the absence of the membrane component[8]. It also does not influence the rate of light-induced ATP synthesis in transhydrogenase factor-resolved particles[6], indicating that it is not functional in the formation or stabilization