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On the site of action of plastocyanin in isolated chloroplasts

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SUMMARY

Adding plastocyanin to plastocyanin-depleted chloroplast particles, restored both their ability to catalyse the photoinduced electron transfer from ascorbate–DCIP to NADP, and to induce the photooxidation of cytochrome *f*. It is concluded, therefore, that plastocyanin mediates the photoinduced oxidation of cytochrome *f*, as previously suggested.

Several reports have appeared in the last few years which arrived at contradictory conclusions regarding the site of action of plastocyanin in the electron transport path of photosynthesis[1]. Wessels[2] reported that in chloroplast fragments depleted of cytochrome *f* and plastocyanin, only the latter could restore the ability to photoreduce NADP, with ascorbate–2,6-dichlorophenolindophenol (DCIP) as the electron donor. Hind[3] observed that in Triton-treated chloroplasts, the rate of cytochrome *f* photooxidation was markedly accelerated by the addition of plastocyanin. Both of these reports when coupled with the earlier observations[1] seemed to establish plastocyanin as an electron carrier functioning between cytochrome *f* and photosystem I. However, recently Knaff and Arnon[4] reported that sonicated chloroplasts which were presumably plastocyanin-less could still photooxidize cytochrome *f*. Moreover, this oxidation was not affected by the addition of plastocyanin. They concluded therefore that plastocyanin is not required to mediate electron transport during the photooxidation of cytochrome *f*.

We have therefore attempted to recheck the effect of added plastocyanin on the photooxidation of cytochrome *f* in sonicated chloroplasts. In view of the conflicting reports described above, we tested in parallel on the same samples the loss and restoration of activity in the most well-established site of action of plastocyanin, the photoreaction from ascorbate–DCIP to NADP, and on the rate of cytochrome *f* photooxidation.

Fig.1 illustrates the cytochrome *f* photooxidation data. It is clear that the rate of

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

cytochrome *f* photooxidation was very much accelerated by the addition of plastocyanin to the sonicated chloroplasts. It may be noted that a small stimulation was observed even in the control chloroplasts. Also, the addition of plastocyanin caused a moderate (control) to a marked (sonicated) stimulation in the rate of the dark rereduction of cytochrome *f* by the low concentration of ascorbate present in the medium (see also ref. 3).

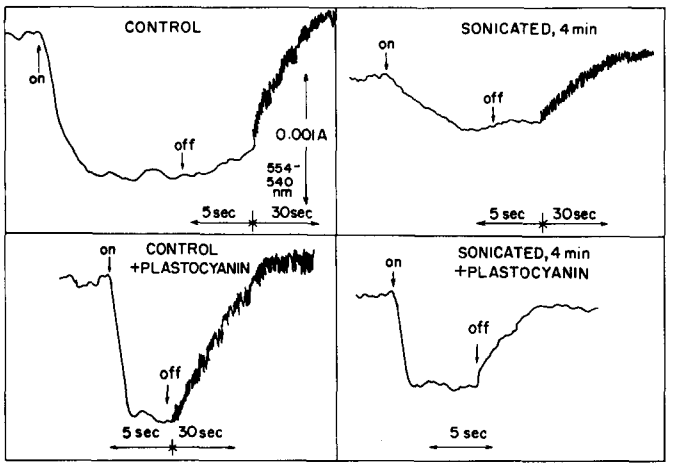

Fig. 1. The reaction mixture contained in a total volume of 3.0 ml: Tricine, pH 8.0, 15 mM; KCl, 20 mM; Diquat, 10 μM; ascorbate, 0.2 mM; DCMU, 5 μM; and chloroplasts prepared as previously described[5]. Where indicated sonication was performed in a Branson sonifier equipped with a microtip at 4 A at 0°. The chloroplasts were suspended at a concentration of 0.3 mg/ml and sonicated for 1-min periods, allowing for cooling between periods. After sonication (including the control), the chloroplasts were recentrifuged at 12 000 x *g* for 10 min and resuspended in the same medium (0.2 M sucrose, 0.1 M NaCl, 0.05 M Tris, pH 8.0) before use. Plastocyanin was isolated from swiss-chard leaves by a method similar to that described by Katoh *et al.*[6]. Where indicated 4.8 nmoles were added per cuvette. Measurements were performed in a modified Aminco–Chance dual-wavelength spectrophotometer as previously described[7]. Actinic light was provided by a projector filtered through a Baird Atomic 730 nm interference filter (half band width, 30 nm) at an intensity of 10^4 ergs · cm^{-2} · sec^{-1}

Table I presents a comparison between the ability of the chloroplasts to catalyse the photoinduced transfer of electrons from ascorbate–DCIP to NADP and the photooxidation of cytochrome *f*. Although the experiments were performed under quite different experimental conditions in order to optimize the conditions for each reaction, it is clear that the activity decreased and increased in parallel in both reactions. It seems clear therefore that these experiments fully support the previous conclusion that plastocyanin does mediate the photoinduced oxidation of cytochrome *f*.

It is difficult to reconcile these results with those of Knaff and Arnon[4]. It may be that their treatment (1 min sonication) was insufficient to remove the required amount of plastocyanin, since their data on the removal of plastocyanin indicate that such a treatment was just barely sufficient to cause the removal of plastocyanin in a similar, but presumably separate experiment.

TABLE I

COMPARISON OF THE EFFECT OF PLASTOCYANIN ON THE RATE OF PHOTOINDUCED OXIDATION OF CYTOCHROME *f* AND TRANSFER OF ELECTRONS FROM ASCORBATE–DCIP TO NADP IN SONICATED CHLOROPLASTS

The reaction conditions and mixture for photooxidation of cytochrome *f* were as described under Fig. 1. The reaction mixture for ascorbate–DCIP to NADP contained in a total volume of 3.0 ml: phosphate, pH 8.0, 50 mM; KCl, 12 mM; ascorbate, 8 mM; DCIP, 20 μM; 3(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU), 2.5 μM; NADP, 20 μM; a saturating amount of swiss-chard ferredoxin and lettuce chloroplasts isolated as described under Fig. 1 containing 30 μg chlorophyll. NADP reduction was continuously monitored in a Cary Model 14 spectrophotometer as previously described[8]. Illumination was provided by a 500-W projector filtered through a heat filter and corning C.S. 2-58 filter. Control activities were: cytochrome *f* photooxidation, 35 μmoles·mg $chl^{-1}\cdot h^{-1}$; cytochrome *f* dark reduction of 1.8 μmoles·mg $chl^{-1}\cdot h^{-1}$; ascorbate–DCIP to NADP, 36 μmoles electrons mg $chl^{-1}\cdot h^{-1}$.

Contents	*Rate of cytochrome f*		*Ascorbate–DCIP → NADP (% of control)*
	Photooxidation (% of control)	*Dark reduction (% of control)*	
Chloroplasts	(100)	(100)	(100)
Chloroplasts + plastocyanin	160	124	150
Chloroplasts, sonicated 2 min	11	–	28
sonicated 2 min+plastocyanin	160	–	85
sonicated 4 min	9	48	5
sonicated 4 min+plastocyanin	110	520	89

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