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CONTRASTING EFFECTS OF PLASTOCYANIN ON THE PHOTOREDUCTION AND PHOTOOXIDATION OF CYTOCHROME *f* IN CHLOROPLASTS

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SUMMARY

Removal of plastocyanin from Photosystem I subchloroplast particles had no effect on the Photosystem I photooxidation of cytochrome *f*. Chloroplasts depleted of plastocyanin by sonication lost the ability to reduce cytochrome *f* in Photosystem II light. Addition of plastocyanin restored the photoreduction of cytochrome *f*. These results are consistent with a plastocyanin site on the reducing side of cytochrome *f*.

INTRODUCTION

Reports from several laboratories in the last few years have presented contradictory conclusions regarding the relative positions of cytochrome *f* and the copper protein plastocyanin in the photosynthetic noncyclic electron transport chain. Levine and Gorman[1] reported that chloroplasts from a mutant of *Chlamydomonas reinhardti* lacking plastocyanin could photoreduce cytochrome *f* in Photosystem II light but could not photooxidize cytochrome *f* in Photosystem I light. The addition of plastocyanin restored the Photosystem I photooxidation of cytochrome *f* (ref. 2). Hind observed that the rate of cytochrome *f* photooxidation was accelerated by the addition of plastocyanin in Triton-treated chloroplasts[3]. These results seemed to indicate that plastocyanin functions as an electron carrier on the oxidizing side of cytochrome *f*. However, Knaff and Arnon reported that chloroplasts depleted of plastocyanin by sonication could still photooxidize cytochrome *f* and that addition of plastocyanin had no effect on this photooxidation[4]. Also, Murata and Fork have reported that Photosystem I particles that contain little plastocyanin can photooxidize cytochrome *f* at the same rate as can untreated chloroplasts[5].

Recently, Avron and Shneyour[6] reported that cytochrome *f* photooxidation in sonicated chloroplasts was markedly stimulated by plastocyanin. These authors suggested that the lack of plastocyanin dependence for cytochrome *f* photooxidation observed by Knaff and Arnon could have been due to the fact that the sonication treatment used "was insufficient to remove the required amount of plastocyanin."

In the previous work from this laboratory[4] only the plastocyanin that was

Abbreviation: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea.

released from the chloroplast lamellae by sonication could be assayed and there was no technique available to determine whether any residual plastocyanin remained bound to the chloroplasts. Recently, Malkin and Bearden[7] (see preceding paper) have used electron paramagnetic resonance (EPR) spectroscopy to determine the amount of bound plastocyanin in chloroplasts and chloroplast fragments. The effects of plastocyanin on the Photosystem I photooxidation of cytochrome *f* and on the Photosystem II photoreduction of cytochrome *f* in spinach chloroplasts have been reinvestigated in conjunction with this new plastocyanin assay.

METHODS

"Broken" spinach chloroplasts (P_{1S}) were prepared according to the method of Whatley and Arnon[8]. Chloroplasts were sonicated for 2 min as described previously[4]. Photosystem I particles (D-144) were prepared by the digitonin treatment procedure of Hauska *et al.*[9]. Sonicated D-144 particles were prepared by sonicating the D-144 particles at a chlorophyll concentration of 0.5 mg/ml for 2 min at power setting 3 with a Branson Model S125 sonifier. After sonication, the sonicated D-144 particles were sedimented by centrifuging for 60 min at $144000 \times g$ and the plastocyanin-containing supernatant was discarded. Chlorophyll was determined as described by Arnon[10].

Light-induced absorbance changes were measured with a dual wavelength spectrophotometer as previously described[11]. The half-band width of the measuring beam was 2.0 nm and the half-band widths of the actinic beams were 10 nm. The plastocyanin content of the chloroplasts and subchloroplast particles was determined by EPR spectroscopy at 25 °K in the presence of ferricyanide according to the procedure of Malkin and Bearden[7].

RESULTS

The effect of plastocyanin on cytochrome f photooxidation

The recent observation of Baszynski *et al.*[12] that Photosystem I subchloroplast particles contain significant amounts of plastocyanin suggested the possibility of studying the plastocyanin requirement for cytochrome *f* photooxidation in these particles. These particles are particularly useful because they contain neither C550 (ref. 13) nor cytochrome b_{559} (refs 14 and 15). These two chloroplast components have absorbance bands overlapping the α-absorbance band of cytochrome *f* and thus might interfere with measurements of cytochrome *f*.

Fig. 1 shows the EPR spectrum of plastocyanin in D-144 particles before and after sonication. Because only the oxidized form of plastocyanin can be detected by the EPR technique[7], the EPR spectra are measured in the presence of ferricyanide, which will oxidize any plastocyanin present. The D-144 particles contained 0.6 nmole of plastocyanin per mg of chlorophyll before sonication and no detectable plastocyanin after sonication, as indicated by the EPR signal at $g=2.05$ (ref. 7). As may be seen in Fig. 2, removal of plastocyanin affected neither the rate nor the extent of cytochrome *f* photooxidation by Photosystem I light (715 nm). It was not possible to test the effect of added plastocyanin on the photooxidation of cytochrome *f* in these Photosystem I particles because of competing spectral changes

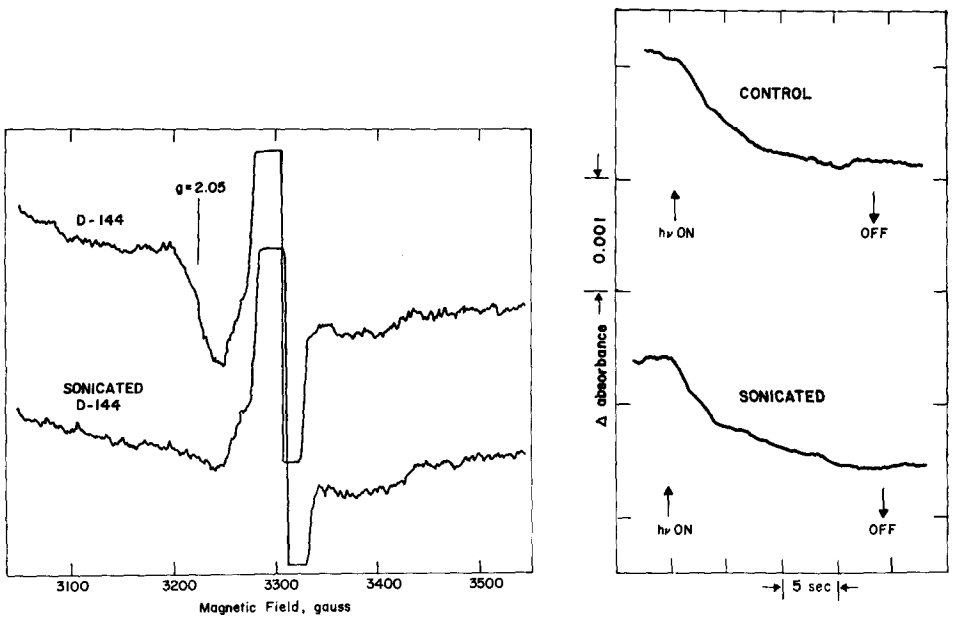

Fig. 1. Effect of sonication on plastocyanin content of Photosystem I subchloroplast fragments. Spectra of unsonicated D-144 fragments or sonicated fragments (1.0 mg chlorophyll per ml in each case) were recorded at 25 °K in the presence of 1 mM ferricyanide. Conditions of EPR spectroscopy: frequency, 9.26 GHz; power, 10 mW; modulation frequency, 100 kHz; and amplitude, 10 G; time constant, 0.1 s; scanning rate, 1000 G/min.

Fig. 2. Effect of plastocyanin depletion by sonication on cytochrome *f* photooxidation in D-144 particles (554 nm *minus* 540 nm). The reaction mixture contained (per 1.0 ml) D-144 or sonicated D-144 particles (equivalent to 75 μg chlorophyll) and the following in μmoles: Tricine [*N*-tris (hydroxymethyl)methylglycine] buffer (pH 7.9), 50; and sodium ascorbate, 1. The 715-nm actinic light had an intensity of 5.9 nEinsteins/cm^2 per s.

in the cytochrome α-band region caused by the photooxidation of the added plastocyanin. The large P700 content of the D-144 particles relative to cytochrome *f* (refs 14 and 15) made it difficult to study cytochrome *f* changes in the γ-band spectral region.

In addition to the measurements of cytochrome *f* photooxidation in D-144 particles, the effect of plastocyanin on cytochrome *f* photooxidation in sonicated chloroplasts has been reinvestigated. Sonicated chloroplasts, prepared as previously described[4], were shown to contain less than 5% of the plastocyanin of the unsonicated chloroplasts (see Fig. 2 in preceding paper[7]). In agreement with the earlier results from this laboratory[4], the sonicated chloroplasts were still capable of photooxidizing cytochrome *f*. No significant increase in the extent of cytochrome *f* photooxidation was observed when plastocyanin was added (data not shown).

Effect of plastocyanin on cytochrome f photoreduction

Fig. 3 illustrates that chloroplasts sonicated for 2 min to remove the plastocyanin could not photoreduce cytochrome *f* in Photosystem II light (664 nm). These sonicated chloroplasts contained less than 5% of the plastocyanin of control

chloroplasts, as indicatid by the EPR assay (see Fig. 2 in preceding paper[7]). The extent of the photooxidation of cytochrome *f* in Photosystem I light (715 nm) is small because most of the cytochrome *f* is oxidized during the sonication procedure. Reduction of cytochrome *f* by addition of ascorbate prior to illumination resulted in a much larger Photosystem I photooxidation of cytochrome *f* (data not shown). Addition of plastocyanin (final concentration 0.5 μM) restored the ability of the sonicated chloroplasts to photoreduce cytochrome *f* in Photosystem II light, as

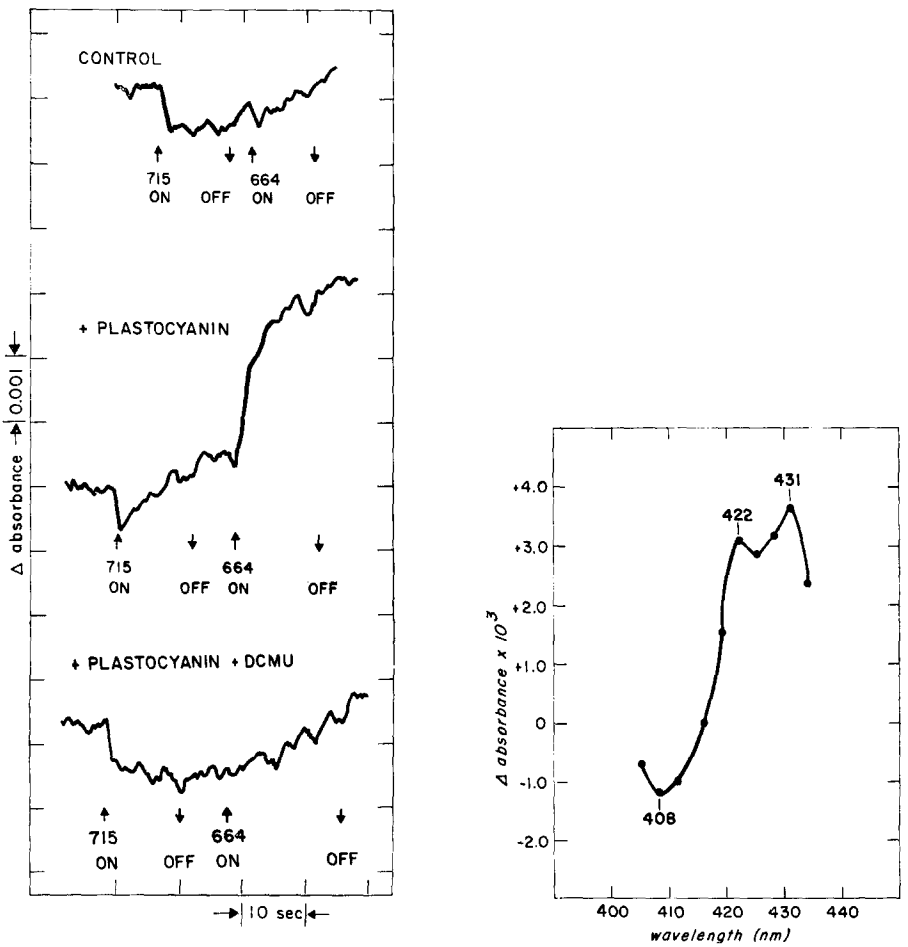

Fig. 3. Effect of plastocyanin and DCMU on cytochrome *f* photoreduction in sonicated chloroplasts (422 nm *minus* 416 nm). The reaction mixture contained (per 1.0 ml) sonicated spinach chloroplasts (equivalent to 25 μg chlorophyll) and the following in μmoles: 2-(*N*-morpholino)-ethanesulfonic acid buffer (pH 6.2), 50; $MgCl_2$, 2; K_2HPO_4, 5; and, where indicated, plastocyanin, $5 \cdot 10^{-4}$, and DCMU, $1 \cdot 10^{-3}$. The 664-nm actinic light had an intensity of 8.3 nEinsteins/cm^2 per s and the 715-nm actinic light was as in Fig. 1.

Fig. 4. Plastocyanin-dependent, light-induced absorbance changes in sonicated chloroplasts. The reaction mixture and illumination were as in Fig. 2. The absorbance change induced by 664-nm actinic light after preillumination with 715-nm actinic light is plotted as a function of the measuring wavelength. Reference wavelength, 416 nm.

illustrated in Fig. 3. The Photosystem II photoreduction of cytochrome *f* is completely eliminated by the Photosystem II inhibitor 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU) as would be expected if the electrons that reduce cytochrome *f* come from the photooxidation of water.

To eliminate any spectral changes caused by the photoreduction of plastocyanin, these measurements were made in the cytochrome γ-band region, rather than the α-band region. The loss of the ability to photoreduce cytochrome *f* in Photosystem II light caused by sonication has also been measured in the α-band spectral region. However, absorbance changes caused by the reduction of added plastocyanin make it difficult to study the restoration of cytochrome *f* photoreduction by plastocyanin in this spectral region.

The spectrum of the absorbance change caused by Photosystem II light (664 nm) in sonicated chloroplasts supplemented with plastocyanin is shown in Fig. 4. No absorbance changes were observed in this spectral region in the absence of added plastocyanin or in the presence of DCMU. The decrease in absorbance with a peak at 408 nm and the increase in absorbance with a peak at 422 nm are consistent with the absorbance changes expected for the reduction of cytochrome *f* (refs 16–22). The positive peak at 431 nm is probably caused by the photoreduction of P700 (refs 23, 18, 20 and 24). The photoreduction of cytochrome b_{559} (refs 20 and 25) or cytochrome b_6 (refs 19 and 20) would also result in an increase in absorbance in the 430-nm region. However, the photoreduction of cytochrome b_{559} does not require plastocyanin[1,26], while the absorbance increase at 431 nm is dependent on plastocyanin. The photoreduction of cytochrome b_6 has been shown to be a Photosystem I reaction[19,27,28] but the absorbance increase at 431 nm occurs only in the short-wavelength light characteristic of Photosystem II. [The component responsible for the slow increase in absorbance observed after the initial oxidation caused by 715-nm light (see Fig. 3) has not yet been identified.] It is thus unlikely that the increase in absorbance at 431 nm is caused by the photoreduction of either of these *b*-type cytochromes.

DISCUSSION

The earlier report from this laboratory[4] that sonicated chloroplasts devoid of plastocyanin (< 5% of control) are able to carry out the Photosystem I photooxidation of cytochrome *f* has been confirmed. In addition, it has been shown that the removal of plastocyanin from Photosystem I subchloroplast fragments (prepared by digitonin treatment) has no effect on the cytochrome *f* photooxidation in these fragments (see Figs 1 and 2 above). These results are in agreement with the recent report of Murata and Fork[5] that Photosystem I subchloroplast fragments (prepared by French pressure cell treatment) with only 15% of the plastocyanin of control chloroplasts can still photooxidize cytochrome *f* as rapidly as can control chloroplasts ($t_{½} < 1$ ms). It thus seems unlikely that plastocyanin is required for the Photosystem I photooxidation of cytochrome *f*, although other laboratories[1–3,6] have reported stimulation of cytochrome *f* photooxidation by plastocyanin.

Unlike the photooxidation of cytochrome *f*, its reduction in sonicated chloroplasts was dependent on the addition of plastocyanin. These findings in sonicated spinach chloroplast differ from those of Levine and Gorman[1] who reported cyto-

chrome *f* photoreduction in a mutant of *C. reinhardti* lacking plastocyanin. Observations made with sonicated chloroplasts must be used with great caution since the sonication treatment may introduce reaction pathways that differ from those found in untreated chloroplasts[29]. Bearing this possibility in mind, it can be noted that the effect of plastocyanin on the photoreduction of cytochrome *f* and P700 appears to be inconsistent with an earlier proposal from this laboratory[4,26,30,31] that plastocyanin functions only in Photosystem II, parallel to and unconnected with the Photosystem I components, cytochrome *f* and P700. However, these results can be explained by an hypothesis in which Photosystem II and Photosystem I function in series and plastocyanin, cytochrome *f*, and P700 are part of the same electron transport chain (see refs 32 and 33 for a more detailed discussion of this hypothesis). Furthermore, the results reported above suggest that plastocyanin functions on the reducing side of cytochrome *f*.

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