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LIGHT-INDUCED CHANGES OF C-550 AND FLUORESCENCE YIELD IN ULTRAVIOLET-IRRADIATED CHLOROPLASTS AT ROOM TEMPERATURE

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SUMMARY

Effects of ultraviolet irradiation of chloroplasts on several photochemical reactions mediated by Photosystem II were studied at room temperature. The Hill activity and fluorescence of variable yield were decreased by ultraviolet irradiation in parallel. The activity of 2,6-dichlorophenolindophenol (DCIP) photoreduction in irradiated chloroplasts was only slightly recovered by addition of diphenylcarbazide, an electron donor for Photosystem II. No restoration of the original fluorescence yield was observed on addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) or preincubating the irradiated chloroplasts with dithionite.

Photobleaching of C-550 was much more resistant than the Hill activity or fluorescence of variable yield toward ultraviolet irradiation. Chloroplasts, of which the Hill activity and variable fluorescence had mostly been eliminated by ultraviolet irradiation, still showed C-550 photobleaching, which was in magnitude more than 50 % of that in the original unirradiated chloroplasts. C-550 was shown not to respond to membrane potential. The photobleaching of C-550 is sensitized by Photosystem II and proceeds with unaltered quantum efficiency in ultraviolet-irradiated chloroplasts. These observations are interpreted to indicate that C-550 is not identical with Q, the hypothetical quencher of fluorescence, and that C-550 is a better indicator for the primary photoact in Photosystem II than Q.

INTRODUCTION

Knaff and Arnon [1, 2] described, in 1969, an occurrence of a new chloroplast component which undergoes a reversible spectral change on illumination of chloroplasts. Since its absorbance change showed a maximum at 550 nm, this component

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

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was designated C-550 [1]. They demonstrated that the photoresponse of C-550 was a Photosystem II-mediated reaction, by showing that the absorbance decrease at 550 nm was induced effectively only by red light, whereas far red light was ineffective [1]. A close association of C-550 with the reaction center of Photosystem II was indicated by their finding that the photoresponse of C-550 occurred in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and at liquid nitrogen temperature [1, 2].

Erixon and Butler [3] showed that there is a close correlation between C-550 and fluorescence yield. When C-550 was reduced the fluorescence yield of chloroplasts was high at liquid nitrogen temperature. They determined the light-induced changes of C-550, fluorescence yield and cytochrome b_{559} at low temperature as functions of the redox potential of the chloroplast suspension. The redox titration curves and the midpoint-potentials for these three photoresponses agreed with each other. They suggested, therefore, that C-550 is identical with, or serves as an index of, the primary electron acceptor of Photosystem II, Q, which acts as a quencher of fluorescence in its oxidized state.

On the other hand, Ben Hayyim and Malkin [4] found that at room temperature a good correlation between C-550 and fluorescence yield holds only at low intensities of short wavelength light but not with far red light. They concluded, therefore, that the identity of C-550 and Q is still questionable.

The aim of the present study was to reinvestigate the relationship between C-550 and fluorescence yield at room temperature. The effects of ultraviolet irradiation of chloroplasts on these light-induced changes were determined. Erixon and Butler [5] showed previously that ultraviolet irradiation of chloroplasts resulted in a destruction of C-550, roughly in parallel with the loss of fluorescence of variable yield at liquid nitrogen temperature. It will be shown, however, in this paper that, when the light induced changes were assayed at room temperature, the photobleaching of C-550 is much more resistant to ultraviolet irradiation than the fluorescence yield and the Hill activity, thereby indicating that C-550 is not identical to Q. It is suggested that Q is not the primary electron acceptor of Photosystem II, whereas C-550 is an excellent indicator of the primary photoact of Photosystem II.

MATERIALS AND METHODS

Spinach chloroplasts were prepared as described previously [6]. Chloroplasts suspended in the preparation medium (0.4 M sucrose, 0.05 M phosphate, pH 7.8, and 0.01 M NaCl) were placed in a petri-dish to form a thin layer of about 1 mm thickness and were irradiated with a Toshiba germicidal lamp GL 10, placed 5 cm above the surface of the suspension. The chloroplast suspension was chilled with ice-water and was continuously stirred by a magnetic stirrer during the irradiation.

All the assays of photochemical reactions were performed at room temperature. The Hill reaction with 2,6-dichlorophenolindophenol (DCIP) as electron acceptor was determined with a Hitachi EPU 2A spectrophotometer modified for cross-illumination as described previously [6]. Chloroplasts were illuminated with red light (650–800 nm) of $4 \cdot 10^5$ ergs/cm^2 per s. The reaction mixture contained, in a final volume of 2.0 ml; 50 mM phosphate, pH 7.8, 10 mM NaCl, 25 mM methylamine · HCl, 50 μM DCIP and chloroplasts equivalent to 5–20 μg chlorophyll. Where

indicated 1 mM of diphenylcarbazide was added.

Fluorescence of chlorophyll *a* was determined as described previously [6]. Chloroplasts containing 5–10 μg chlorophyll were suspended in 3.0 ml of 50 mM phosphate, pH 7.8, containing 10 mM NaCl and illuminated with blue light (600 ergs/cm^2 per s. Maximum intensity at 480 nm; half bandwidth, 15 nm). Fluorescence emitted was measured at 685 nm with a photomultiplier (Hamamatsu TV, R 236).

Light-induced absorbance changes of C-550 and cytochrome *f* were determined with an Aminco–Chance Dual Wavelength spectrophotometer. Red light (650–800 nm; $2.8 \cdot 10^5$ ergs/cm^2 per s) was used as the actinic light. A combination of Toshiba glass filter VR 63 and an interference filter (maximum transmission, 650 nm; half bandwith, 15 nm) or of VR 69 and an interference filter (716 nm, 15 nm) was used to isolate red or far red light. A guard filter (Corning 4-96) was used to shield the photomultiplier from scattered actinic light. The basal reaction medium contained, in a final volume of 2.0 ml, 50 mM phosphate, pH 7.8, and 10 mM NaCl. Other additions were indicated in the legends for figures.

Chlorophyll was determined as described by Arnon [7].

RESULTS

The activities of DCIP-Hill reaction in chloroplasts which had been exposed for varied periods of time to ultraviolet light are shown in Fig. 1. The rate of DCIP photoreduction decreased with increasing doses of ultraviolet irradiation to become negligible after 30 min of irradiation.

Katoh and San Pietro [8] previously showed that in *Euglena* chloroplasts the effect of ultraviolet irradiation differed from that of heat treatment in that subsequent addition of ascorbate as electron donor for Photosystem II was effective in restoring a DCMU-sensitive photoreduction of NADP in heated chloroplasts but not in ultraviolet-irradiated chloroplasts. On the contrary, Yamashita and Butler [9] described

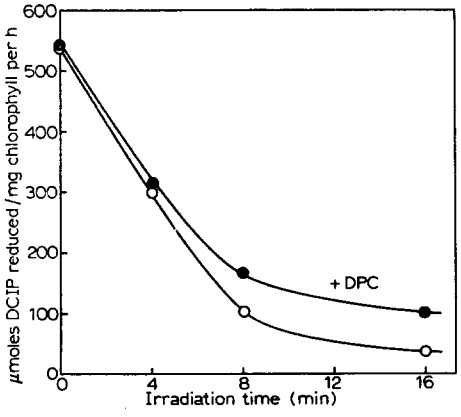

Fig. 1. Effect of ultraviolet irradiation of chloroplasts on DCIP-Hill reaction in the presence and absence of diphenylcarbazide. Reaction mixture contained, in a final volume of 2.0 ml: 50 mM phosphate, pH 7.8, 10 mM NaCl, 25 mM methylamine–HCl, 50 μM DCIP and chloroplasts equivalent to 17 μg chlorophyll. ○–○ and ●–● indicate activities determined in the absence and presence of 1 mM diphenylcarbazide, respectively.

that ultraviolet irradiation of spinach chloroplasts, like Tris washing, inhibited the electron transfer at a site between water and the reaction center of Photosystem II, since addition of electron donors for Photosystem II to the irradiated chloroplasts restored the Photosystem II-mediated NADP photoreduction. The extent of recovery thus attained was, however, far from being complete [5]. Fig. 1 shows that the rate of DCIP photoreduction in ultraviolet-irradiated spinach chloroplasts increased on addition of diphenylcarbazide, an electron donor for Photosystem II [10], only to a limited extent. This indicates that the major cause of ultraviolet inhibition is not a blocking of the electron transfer between water and the reaction center of Photosystem II, which can be bypassed by the addition of diphenylcarbazide.

Effect of ultraviolet irradiation of chloroplasts on the induction of chlorophyll *a* fluorescence is shown in Fig. 2. The effect of irradiation was most marked on the variable part of fluorescence. The rate and extent of the gradual fluorescence rise, subsequent to the initial rapid rise, were markedly reduced by 4 min of exposure of chloroplasts to ultraviolet light and variable part of fluorescence was completely

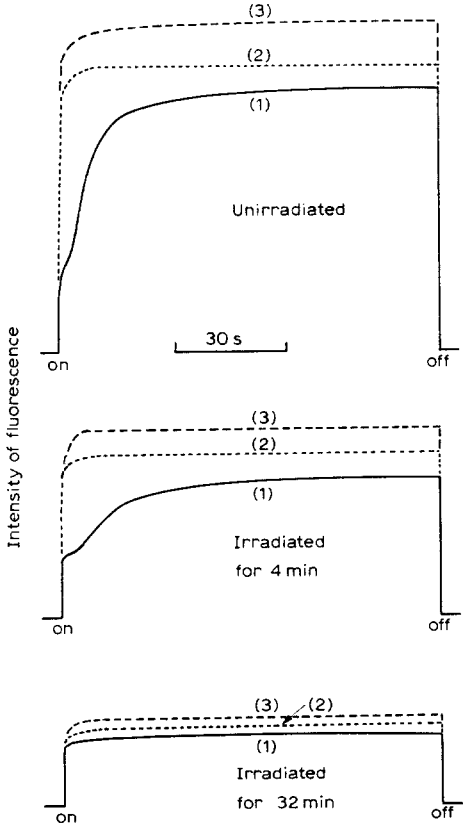

Fig. 2. Effect of ultraviolet irradiation of chloroplasts on time courses of fluorescence change. Chloroplasts were illuminated with blue light (maximum intensity, 480 nm; half bandwidth, 15 nm; 600 ergs/cm^2 per s). Reaction mixture contained, in a final volume of 3.0 ml: 50 mM phosphate, pH 7.8, 10 mM NaCl and chloroplasts equivalent to 9 μg chlorophyll. Curve 1, no addition. Curves 2 and 3, in the presence of 10 μM DCMU and a few grains of dithionite, respectively.

eliminated by 32 min of irradiation. On the other hand, the initial rapid rise of fluorescence was little affected by the irradiation. Effect of DCMU and dithionite in increasing the steady-state level of fluorescence decreased with increasing doses of ultraviolet irradiation. No significant increase in fluorescence yield was observed on addition of DCMU and dithionite to the 32-min irradiated chloroplasts; electron donor for Photosystem II, such as diphenylcarbazide and hydroxylamine was also without effect. These features of ultraviolet effect on fluorescence mostly agree with the previously observations [9, 11, 12].

Recently, Ikegami and Katoh [13] demonstrated that, although dithionite cannot reduce Q chemically, a slow reduction of Q occurs during incubation of chloroplasts with dithionite in the dark through a reverse electron transfer to Q from a pool of electron carrier between Photosystem I and II. If ultraviolet irradiation of chloroplasts caused an inactivation of photochemical reaction which drives the reduction of Q without destroying Q, incubation of chloroplasts with dithionite for a sufficiently long period in the dark should reduce Q and, consequently, increase extent of the initial rapid fluorescence rise. However, a prolonged preincubation of the irradiated chloroplasts with the reductant failed to increase the fluorescence yield. This suggests that the ultraviolet-induced quenching of variable fluorescence is due to a change in Q itself. In support to this is the following finding that the irradiated chloroplasts still exhibit a photochemical reaction which is closely associated with the primary reaction of Photosystem II.

Fig. 3 shows the effect of ultraviolet irradiation on the light-induced bleaching of C-550. Exposure of chloroplasts to ultraviolet light for 18 min caused about 80 % inhibition of the Hill reaction, while the magnitude of absorbance decrease at 550 nm was reduced only by 20 %. Ultraviolet irradiation did not cause any appreciable change in the light-on and light-off kinetics of the photoresponse (Fig. 3A). The light-minus-dark difference spectra determined with unirradiated and irradiated chloroplasts showed negative peaks having a maximum at 550–552 nm, indicating the absorbance changes observed at 550 nm are due to C-550 (Fig. 3B).

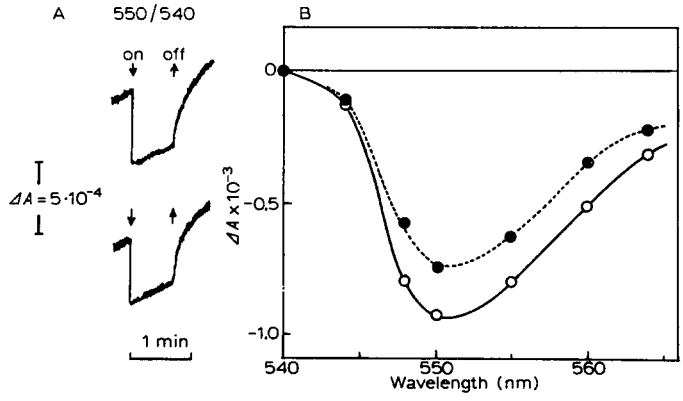

Fig. 3. Time courses of light-induced absorbance changes at 550 nm (A) and light-minus-dark difference spectra (B) in unirradiated (○–○) and 18 min ultraviolet-irradiated chloroplasts (●- - -●). Reference wavelength, 540 nm. Reaction mixture contained, in a final volume of 2.0 ml: 50 mM phosphate, pH 7.8, 10 mM NaCl, 5 mM $Fe(CN)_6^{3-}$, 20 μM gramicidin J and 10 μM DCMU. Chloroplasts added were equivalent to 75 μg chlorophyll per ml.

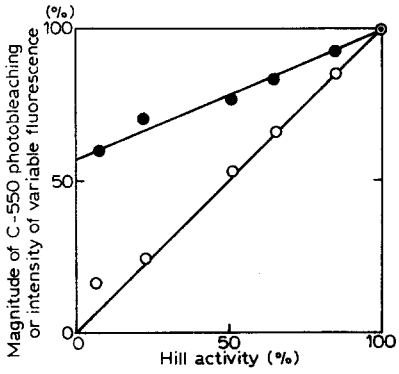

Fig. 4. Relationship between Hill activity, variable fluorescence and photobleaching of C-550 in chloroplasts irradiated with ultraviolet light for various periods. DCIP-Hill reaction, fluorescence and C-550 photobleaching were determined as described in Figs 1, 2 and 3, respectively; chloroplast concentrations, 5.5, 1.8 and 68 μg chlorophyll per ml of each reaction mixture. The Hill reaction activity in unirradiated chloroplasts was 217 μmoles DCIP reduced/mg chlorophyll per h. Variable fluorescence (○–○) was determined as the difference between the initial yield of fluorescence (f_i) in the dark adapted chloroplasts and the steady state of fluorescence determined in the presence of DCMU (f_{DCMU}). ●–●, C-550 photoresponse.

Fig. 4 compares the magnitudes of the C-550 photoresponse and variable fluorescence (determined in the presence of DCMU) as function of the surviving Hill activity in chloroplasts which had been exposed for varied periods to ultraviolet light. The magnitude of variable fluorescence decreased in parallel with the loss of Hill activity on increasing the length of ultraviolet irradiation. Similar observations were previously described by Malkin and Jones [11] and Mantai et al. [12]. In contrast, photobleaching of C-550 was markedly resistant to ultraviolet light. More than 50 % of C-550 photoresponse survived the ultraviolet irradiation which caused almost complete losses of Hill activity and variable fluorescence, indicating that there is no close correlation between C-550 and the change underlying the variable fluorescence so far as tested at room temperature. Since this finding was quite unexpected and in contrast to the results obtained by Erixon and Butler [5] from their low temperature experiments, we examined several possibilities for explaining the different sensitivities of the C-550 and fluorescence changes to ultraviolet irradiation.

Butler [14] has recently shown that the magnitude of C-550 photoresponse was markedly reduced by addition of nigericin and valinomycin. He suggested, consequently, that a part of C-550 reflects the change of membrane potential. It might be possible that the surviving C-550 reaction in ultraviolet-irradiated chloroplasts is induced by the light-induced membrane potential, but not by Photosystem II.

Fig. 5 shows the effects of gramicidin J and DCMU on the time course of light-induced absorbance decrease at 550 nm (reference wavelength, 540 nm). Curve a was determined in the presence of 5 mM $Fe(CN)_6^{3-}$ alone. The light-on response is biphasic; there is a rapid decrease at the onset of illumination, followed by a slow change. In accord to the observation of Butler [14], addition of an ionophore, gramicidin J, markedly reduced the magnitude of the first rapid absorbance decrease (Curve b). The effect of gramicidin J on the second slow phase varied significantly with chloroplast preparations. On further addition of DCMU, the second absorbance

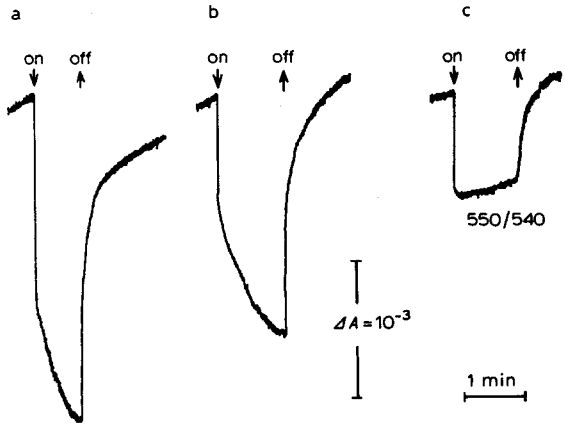

Fig. 5. Effect of gramicidin J and DCMU on light-induced absorbance change at 550 nm in chloroplasts in the presence of $Fe(CN)_6^{3-}$. Reference wavelength, 540 nm. (a) 5 mM $Fe(CN)_6^{3-}$; (b), (a) plus 20 μM gramicidin J; (c), (b) plus 10 μM DCMU. Chloroplast concentration was 78 μg chlorophyll per ml.

change was completely eliminated whereas the first rapid change remained essentially unaltered (Curve c). As has been described by Knaff and Arnon [1], DCMU slowed down the rate of the dark reversal.

Light-minus-dark difference spectra for the rapid phase of the photoresponse are illustrated in Fig. 6 (reference wavelength, 550 nm). In the presence of $Fe(CN)_6^{3-}$ alone, there is a large absorbance increase with a maximum at 515 nm, which is indicative of an occurrence of membrane potential [15]. That a through at 550 nm is

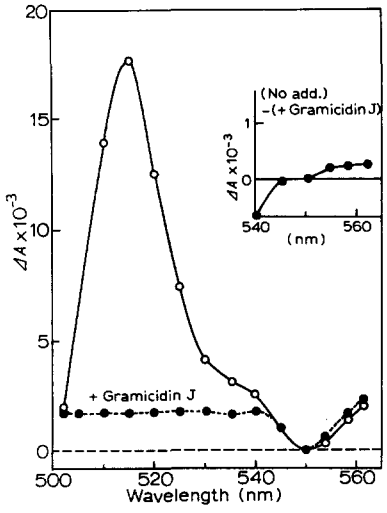

Fig. 6. Light-minus-dark difference spectra for light-induced rapid absorbance change in chloroplasts. Reference wavelength, 550 nm. Reaction mixtures were the same as that for Trace a (○–○) and for Trace b (●- - -●) in Fig. 5, respectively. Chloroplast concentrations were 80, 32 and 16 μg chlorophyll per ml at 540–562 nm, 535–525 nm and 515–502 nm, respectively. The insert shows the difference between the two difference spectra.

due to C-550 is clearly seen in the presence of gramicidin J which completely eliminates the 515-nm peak. On the other hand, the difference spectrum for the second slow phase shows that C-550 is not involved in the slower phase (data not shown). The insert of Fig. 6 shows difference between the light-minus-dark difference spectra determined in the presence and absence of gramicidin J. There is no absorption peak corresponding to C-550, indicating that C-550 photoresponse is not sensitive to gramicidin J. On the other hand, it is noted that gramicidin J decreased magnitude of light-induced absorbance increase at 540 nm, which most likely represents tailing of the 515-nm peak. This effect of gramicidin J must be responsible for an apparent inhibitory effect of the ionophore on light-induced absorbance decrease at 550 nm in Fig. 5, where 540 nm was used as reference wavelength. It is concluded from those observations that C-550 does not respond to membrane potential. In the present work, C-550 photoresponse was always determined in the presence of gramicidin J in order to avoid interference of the 515-nm peak.

The second possibility examined was that ultraviolet irradiation might reduce quantum efficiency of the primary photochemical reaction of Photosystem II. If this is the case, the accumulation of reduced Q is prevented at the relatively low light intensity (600 ergs/cm^2 per s) employed for the fluorescence experiment; while, an appreciable photobleaching of C-550 would occur in ultraviolet-irradiated chloroplasts since it is determined at a high light intensity ($2.8 \cdot 10^5$ ergs/cm^2 per s). Since C-550 photobleaching at high light intensities is too fast to be followed with the spectrophotometer employed in the present study, the rate of the light-on response was determined using a camera shutter according to the method described by Ben Hayyim and Malkin [4]. Fig. 7 shows typical curves obtained at $2.8 \cdot 10^5$ ergs/cm^2 per s. Although the magnitude of the photobleaching induced by longer flashes decreased

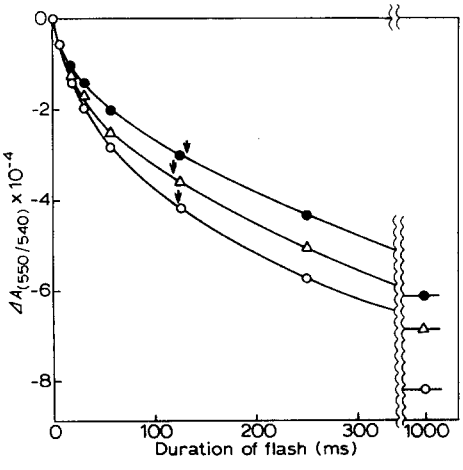

Fig. 7. Time courses of light-induced change of C-550. Experimental conditions were the same as described in Fig. 3. Flashes of varied durations were given with a camera shutter and magnitudes of the absorbance decreases attained were plotted as a function of durations of flashes. Arrows indicate half-maximum changes. ○–○, unirradiated chloroplasts. △–△ and ●–●, chloroplasts irradiated for 8 and 20 min, respectively. Activities of DCIP-Hill reaction were 230, 110 and 42 μmoles DCIP reduced/mg chlorophyll per h for 0, 8 and 20 min irradiated chloroplasts, respectively.

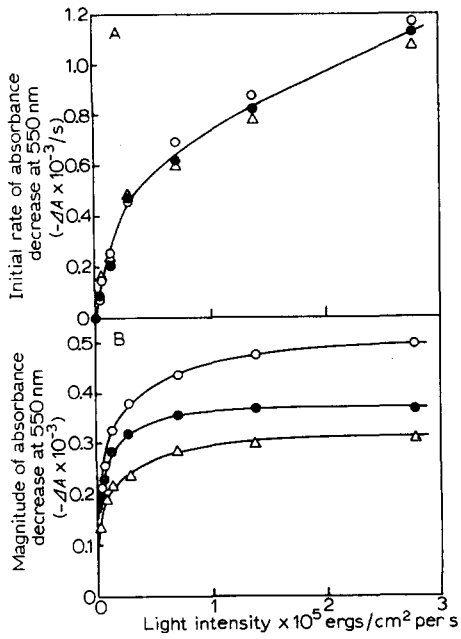

Fig. 8. Effect of light intensities on the rate (A) and magnitude (B) of light-induced C-550 responses in unirradiated and ultraviolet-irradiated chloroplasts. Experimental conditions were the same as described in Fig. 3. Initial rates were determined, at low light intensities, directly from the initial slope of the recorder tracing and, at high light intensities, with a camera shutter as described in Fig. 7. Light intensity was varied with neutral density filters. Chlorophyll concentration was 130 μg chlorophyll per ml. ○–○, unirradiated chloroplasts. ●–● and △–△, chloroplasts irradiated with ultraviolet light for 10 and 35 min, respectively. Activities of DCIP-Hill reaction were 290, 130 and 25 μmoles DCIP reduced/mg chlorophyll per h for chloroplasts irradiated for 0, 10 and 35 min, respectively.

to some extent, time required for 50 % bleaching was not markedly affected by ultraviolet irradiation of chloroplasts.

Fig. 8A shows initial rates of light-induced absorbance decrease at 550 nm at varied light intensities. In this figure, the rates are normalized for the maximum magnitudes of photobleaching in each chloroplast preparation (see Fig. 8B). The curves obtained with unirradiated and irradiated chloroplasts fairly coincide with each other, thus indicating that the quantum efficiency of C-550 photobleaching is not affected by the ultraviolet irradiation. Fig. 8B shows the light intensity dependency of the magnitude of C-550 photobleaching as affected by ultraviolet irradiation. In unirradiated chloroplasts the magnitude of the photoresponse was saturated at about 10^5 ergs/cm^2 per s, and the half maximal size of the photoresponse was attained at a light intensity lower than 10^4 ergs/cm^2 per s. Ultraviolet irradiation decreased the magnitude of the photoresponse, indicating that a part of C-550 was destroyed by the irradiation. However, there was no marked change in light intensity for half maximal signal size of the surviving C-550 response.

For a comparison, fluorescence yield was determined in the presence of DCMU at varied light intensities using a high pressure mercury lamp as a light source. In

ultraviolet-irradiated chloroplasts, fluorescence yield was found to be constant over a wide range of light intensities (10^3–$1.2 \cdot 10^5$ ergs/cm^2 per s) and never reached the level of fluorescence yield in the unirradiated chloroplasts (data not shown). Since light intensities used were sufficiently high to induce an appreciable magnitude of C-550 photobleaching, it is concluded that there is no good correlation between magnitude of C-550 photobleaching and yield of variable fluorescence in the irradiated chloroplasts.

Ben Hayyim and Malkin [4] showed that at room temperature far red light mainly exciting Photosystem I induced a C-550 response as large as that induced by red light exciting Photosystems I and II. They could not observe a red drop in the photobleaching of C-550 in the absence of $Fe(CN)_6^{3-}$, whereas the quantum yield of fluorescence rise showed a distinct red drop. This suggests a possibility that a part of C-550 response is insensitive to ultraviolet irradiation, since it is sensitized by Photosystem I.

In the present study, a relatively dense suspension of chloroplasts was illuminated with the actinic light beam at right angle to the measuring beam. Under this experimental condition, relative size of C-550 photobleaching induced by red light

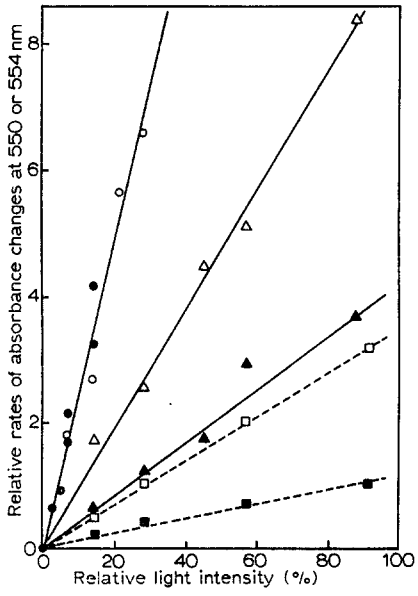

Fig. 9. Effect of red and far red light on initial rates of C-550 photobleaching and cytochrome *f* photooxidation. Photobleaching of C-550 was determined as described in Figs 3 and 7. Photooxidation of cytochromes *f* was determined at 554 nm (reference wavelength, 540 nm) in the presence of 2 mM sodium ascorbate and 10 μM DCMU. At high light intensities, initial rates of photooxidation were determined with a camera shutter. The same concentration of chloroplasts (120 μg chlorophyll per ml) was used for the assay of C-550 and cytochrome *f* changes. 100 % intensities of red (650 nm) and far red (716 nm) light were $4 \cdot 10^4$ and $1.8 \cdot 10^4$ ergs/cm^2 per s, respectively. Triangles, C-550 photobleaching in unirradiated chloroplasts. Squares, C-550 photobleaching in chloroplasts irradiated with ultraviolet light for 30 min. Circles, cytochrome *f* photooxidation in unirradiated chloroplasts. Open and solid symbols represent experiments under red and far red light, respectively. Activities of DCIP-Hill reaction were 211 and 18 μmoles DCIP reduced/mg chlorophyll per h in unirradiated and irradiated chloroplasts, respectively.

(650 nm; $4 \cdot 10^4$ ergs/cm^2 per s) to that induced by far red light (716 nm; $1.8 \cdot 10^4$ ergs/cm^2 per s) was found to change significantly with concentrations of chloroplasts. Red light induces a larger signal than far red light at chloroplast concentration lower than 80 μg chlorophyll/ml, whereas the reverse is true at chloroplast concentration higher than 100 μg chlorophyll/ml. This is due to much stronger absorption of chloroplasts at 650 nm than at 716 nm; effective intensity of red light is reduced much more strongly than that of far red light before the light beams reach the part of chloroplast suspension where the measuring light beam traverses. Thus, which photosystem mediates C-550 change cannot be determined simply by comparing relative size of the photoresponses induced by red and far red light.

Effects of red and far red light on C-550 photoresponses were, therefore, compared with those on photooxidation of cytochrome *f* at the same chloroplast concentrations (Fig. 9). Photooxidation of cytochrome *f* is strictly a Photosystem I reaction in the presence of DCMU and can be used as a measure of effective intensities of Photosystem I light of the two actinic lights used. For the convenience of comparison, intensities of red and far red light are adjusted so as to give a similar rate of cytochrome *f* photooxidation at the same relative intensities on the abscissa of the figure. It is seen that in unirradiated chloroplasts, the initial rate of C-550 response is much faster with red light than with far red light at the same relative light intensities, indicating that C-550 change is a Photosystem II reaction. Effectiveness of red light relative to that of far red light was more marked in irradiated chloroplasts. It is concluded, therefore, that C-550 photoresponses in ultraviolet-irradiated chloroplasts are mediated by Photosystem II.

DISCUSSION

Since an earlier work of Holt et al. [16] on the inhibitory effect of ultraviolet irradiation of chloroplasts on the Hill activity, divergent views have been reported on the mechanisms and sites of ultraviolet inhibition of the photosynthetic electron transport system [5, 9, 11, 12, 16–19]. Evidence has accumulated to indicate that ultraviolet irradiation of chloroplasts affects at multiple sites, e.g., a destruction or modification of plastoquinone [17–19], C-550 [5], cytochrome b_{559} [5], a blocking of the electron transfer between water and the reaction center of Photosystem II [9], or modification of membrane structure of thylakoid [12]. Of special interest in this respect is a fact that ultraviolet irradiation specifically affects the variable part of fluorescence emission, leaving the rapid initial phase of fluorescence unaltered (Fig. 2). It is generally assumed that fluorescence of variable yield is quenched when the primary electron acceptor of Photosystem II, Q, is oxidized (21]. Based on this hypothesis, the decrease in fluorescence yield is explained by assuming that ultraviolet irradiation of chloroplasts inhibits accumulation of reduced Q. Since the loss of variable fluorescence closely parallels the inactivation of the Hill activity (Fig. 4 and refs 11 and 12), the inhibition of photoreduction of Q is most likely the major cause of ultraviolet inactivation of the overall electron transport in chloroplasts.

Inhibition of accumulation of reduced Q in illuminated chloroplasts might be explained by one of three possible mechanisms; inhibition of electron transfer from water to the reaction center of Photosystem II, inactivation of the primary photochemical reaction which drives the reduction of Q, or destruction of Q itself.

Addition of DCMU or electron donor for Photosystem II such as diphenylcarbazide or hydroxylamine was shown to be mostly ineffective in restoring the original level of DCIP photoreduction or variable fluorescence in ultraviolet-irradiated chloroplasts. This is in contrast to the effectiveness, in the same respect, of these reagents observed in the case of chloroplasts of which electron transfer on the oxidizing side of Photosystem II has been blocked by Tris or heat treatment of chloroplasts or by addition of inhibitor such as carbonylcyanide *m*-chlorophenylhydrazone, salicylaldoxime or antimycin A [9, 22–25]. We conclude, therefore, that a blocking of electron transfer between water and the reaction center of Photosystem II is not the primary cause of inhibition by ultraviolet irradiation.

If the loss of variable fluorescence in ultraviolet-irradiated chloroplasts was solely due to an inhibition of the primary photochemical reaction reducing Q, and not to an inactivation of Q itself, preincubation of the irradiated chloroplasts with dithionite should have increased fluorescence yield since the reductant has been shown to be able to reduce Q in the dark [13]. No such recovery was found in the irradiated chloroplasts. This strongly indicates that ultraviolet irradiation specifically affects Q so that the variable fluorescence is permanently quenched and electron transfer through Q is blocked.

If Q is the primary electron acceptor of Photosystem II, one would expect that destruction of Q causes a parallel inactivation of the primary photochemistry of Photosystem II. It was, however, shown in the present work that the chloroplasts, of which the Hill activity and fluorescence of variable yield are mostly eliminated by ultraviolet irradiation, still have a capacity for exhibiting a reversible photobleaching of C-550.

It was demonstrated in the present study that C-550 does not respond to membrane potential. The secondary effect of pH gradient through thylakoid membrane on fluorescence yield was also negligible under our experimental conditions; there was indeed no appreciable change in fluorescence yield on addition of gramicidin J. Therefore, difference in sensitivity towards ultraviolet light between C-550 and fluorescence of variable yield at room temperature cannot be ascribed to these secondary effects of electrochemical changes in membrane on C-550 and fluorescence yield.

Recently, Okayama and Butler [26] reported an interesting finding that fluorescence yield is controlled by a factor other than the redox state of Q. They suggested that a strong oxidant associated with the water-side of Photosystem II, possibly the primary electron donor, can quench chlorophyll *a* fluorescence of Photosystem II. However, the influence of the oxidant on fluorescence yield can be observed only under a limited conditions and must be negligible under the present experimental condition at room temperature [26, 27]. In addition, the apparent quantum efficiency of C-550 photoresponse was not significantly altered by ultraviolet irradiation (Fig. 8). This excludes a possibility that ultraviolet irradiation induces a formation of an additional quencher in the chloroplasts and consequently eliminates variable fluorescence.

In ultraviolet-irradiated chloroplasts, C-550 photobleaching was shown to be mediated by Photosystem II (Fig. 9). This, together with the facts that C-550 photoresponse takes place in the presence of DCMU or at liquid nitrogen temperature [1–5], strongly indicates that the primary photochemical reaction of Photosystem II is functioning in ultraviolet-irradiated chloroplasts. Evidence supporting this view

will be published elsewhere. It follows that yield of variable fluorescence is not directly controlled by the primary photochemistry of Photosystem II. If fluorescence yield is controlled by the redox state of Q, Q cannot be the primary electron acceptor of Photosystem II. Of interest in this respect is a recent report of Mauzerall [28] which showed that increase in fluorescence yield does not reflect the primary photochemical reaction, since the rise of fluorescence is not so fast as expected the primary photoact should be.

It seems, however, premature to conclude that C-550 is the primary electron acceptor of Photosystem II until we learn much more about chemistry and photochemistry of C-550. Mechanism of regulation of fluorescence yield by Q is still largely unknown. In spite of these uncertainties, we would conclude from the results obtained in the present study that C-550 is not identical with, or not always an excellent indicator of Q. This agrees with the conclusion of Ben-Hayyim and Malkin [4]. Boardman [29] also concluded from experiments on fluorescence and C-550 changes in subchloroplast particles that C-550 does not appear to be identical with Q.

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