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DESTRUCTION OF C-550 BY ULTRAVIOLET RADIATION

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SUMMARY

Low-temperature absorption spectra of spinach chloroplasts measured after different periods of ultraviolet radiation show that C-550 is destroyed by the ultraviolet treatment. Cytochrome b_{559} is oxidized and denatured so that it is no longer reducible by ascorbate. The light-induced fluorescence yield changes induced at low temperature diminish as C-550 is destroyed.

INTRODUCTION

The mechanism by which ultraviolet radiation inhibits photosynthetic electron transport reactions has been studied in a number of laboratories[1–7]. JONES AND KOK[3] showed that ultraviolet radiation primarily inhibited Photosystem II acitivity and concluded that the Photosystem II reaction centers were inactivated by ultraviolet radiation. YAMASHITA AND BUTLER[4] demonstrated a site of ultraviolet inhibition between water and Photosystem II by showing that a dichlorophenylmethylurea (DCMU)-sensitive photoreduction of $NADP^+$ could be restored to ultraviolet-inhibited chloroplasts by adding artificial electron donors for Photosystem II. MANTAI *et al.*[5] found that ultraviolet inhibition of chloroplasts was similar to inhibition by lipase and protease and concluded that a primary action of ultraviolet radiation was the disruption of the structural integrity of the lamellar membranes with the resultant loss of Photosystem II activity.

KNAFF AND ARNON[8,9] demonstrated that two photoreactions, the oxidation of cytochrome b_{559} and the reduction of a new component, C-550, were mediated by Photosystem II at liquid nitrogen temperature. ERIXON AND BUTLER[10] showed that C-550 acted as if it were the primary electron acceptor of Photosystem II and that cytochrome b_{559} was oxidized by the primary electron donor. The low temperature photoreactions provide a convenient assay to examine a very limited and well-defined region of the electron transport system in the vicinity of the Photosystem II reaction centers. BUTLER AND OKAYAMA[11] and OKAYAMA *et al.*[12] used this assay to show that lipase destroys C-550. In the present paper we show that ultraviolet radiation has a similar effect.

Abbreviation: DCMU, dichlorophenylmethylurea.

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METHODS

Spinach chloroplasts were prepared by methods described previously[13]. A thin layer (approx. 1 mm thick) of chloroplasts (200 μg chlorophyll per ml) was irradiated at 0° in an enamel tray (9 cm × 18 cm) with a General Electric 15-W germicidal lamp (G15 T8) about 6 cm from the surface. After various times of irradiation with ultraviolet light a 0.3-ml sample of chloroplasts was removed from the irradiation tray and frozen to liquid nitrogen in cuvette and dewar[14].

Absorption spectra of chloroplasts at −196° were measured with a single-beam spectrophotometer on line with a small computer[15]. Absorption spectra of the frozen samples were measured before and after irradiation with red light (10^4 ergs/cm^2 per sec) for 30 sec. The spectra were stored as digital data on paper tape and differences between any two spectra could be plotted out on an X–Y recorder.

The intensity of fluorescence excited by the 650-nm measuring beam of the spectrophotometer was measured by replacing the 600-nm short pass interference filter (Optics Technology), normally used to block fluorescence from the phototube, with a 695-nm interference filter which passed fluorescence but blocked the exciting beam. The relative intensity of fluorescence of the frozen chloroplast sample was measured before (F_D) and after (F_L) the actinic irradiation with red light at −196°.

RESULTS

Light-*minus*-dark difference spectra due to the irradiation of the chloroplast samples at −196° with red light are shown in Fig. 1A for a series of samples irradiated with ultraviolet light for periods ranging from 0 to 40 min. The bleaching at 556 nm is due to the photooxidation of cytochrome b_{559} and the bleaching at 547 nm and absorbance increase at 542 nm are due to the photoreduction of C-550. The extent of the absorbance changes associated with the two photoreactions decreases with increasing times of ultraviolet radiation.

Differences between the spectra taken after different times of ultraviolet radiation are shown in Fig. 1B. (These difference spectra were calculated from the spectra measured before irradiation with red light.) During the first 5 min of ultraviolet radiation some of the C-550 was destroyed and a small amount of the cytochrome b_{559} was destroyed or oxidized. The 0 min-*minus*-5 min difference spectrum shows that less of the 546-nm absorption band of oxidized C-550 and less of the 556-nm band of reduced cytochrome b_{559} were present after 5 min of ultraviolet radiation. Loss of the 556-nm band could be due to oxidation of cytochrome b_{559} but loss of the 546-nm band can only be due to a destruction of C-550 (chemical reduction of C-550 results in a band shift from 546 to 544 nm with little change of extinction[11]). During the next 5 min of ultraviolet radiation (see 5 min *minus* 10 min difference spectrum) most of the cytochrome *f* and more of the cytochrome b_{559} were destroyed or oxidized. Some C-550 was also destroyed but the 546-nm absorption band is masked by the 548-nm band of the cytochrome *f*. Between 10 and 20 min and 20 and 40 min progressively more of the C-550 was destroyed and the rest of the cytochrome b_{559} destroyed or oxidized. Difference spectra due to addition of ascorbate and dithionite to the ultraviolet-irradiated samples (data not shown) showed that the cytochrome *f* and cytochrome b_{559} had been oxidized by the ultraviolet radiation treatment. The cytochrome *f* was

reducible by ascorbate after oxidation by the ultraviolet treatment but the cytochrome b_{559} was modified so that most of it was no longer ascorbate reducible but was reduced by dithionite.

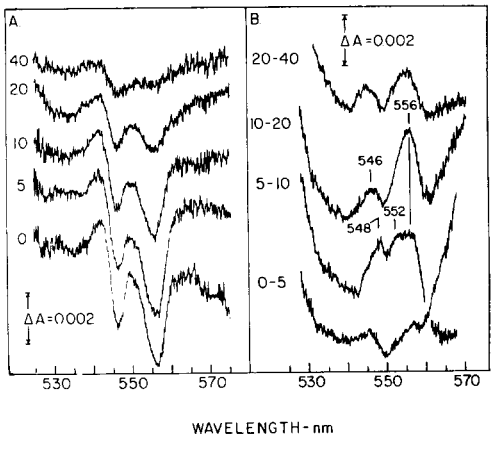

Fig. 1. A. Light-*minus*-dark difference spectra of spinach chloroplasts at −196° after different periods (the number beside each spectrum indicates the minutes) of ultraviolet radiation. B. Difference spectra at −196° between samples irradiated with ultraviolet light for different periods.

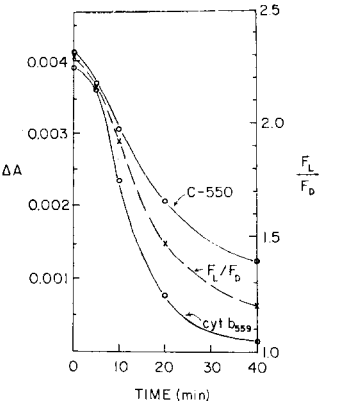

Fig. 2. Light-induced absorbance changes of C-550 (calculated from the data of Fig. 1A as the absorbance difference between the maximum at 542 nm and the minimum at 547 nm), and cytochrome b_{559} (calculated as the bleaching at 556 nm relative to an estimated baseline) and the light-induced fluorescence-yield changes at low temperature (calculated as the ratio of the intensity of fluorescence at 695 nm after irradiation with red light at −196°, F_L, to the intensity before irradiation, F_D) as a function of the time of irradiation with ultraviolet light.

The light-induced absorbance changes are compared with the low temperature light-induced fluorescence-yield changes in Fig. 2 as a function of the time of ultraviolet radiation. The absorbance changes due to photoreduction of C-550 were calculated as the absorbance difference between the maximum at 542 nm and the minimum at 547 nm while those due to photooxidation of cytochrome b_{559} were calculated as the bleaching at 556 nm relative to an estimated baseline. Previous studies[4] showed that ultraviolet radiation destroyed the fluorescence of variable yield leaving the fluorescence yield near the low minimum level of unirradiated chloroplasts. The data of Fig. 2 shows that the decrease in the fluorescence of variable yield (manifest at low temperature) roughly parallels the loss of C-550. From our previous work[10] showing that fluorescence yield changes are correlated with redox changes of C-550 we would predict that the loss of C-550 would result in the loss of fluorescence-yield changes.

We also showed previously that the photooxidation of cytochrome b_{559} at low temperature required the concomitant photoreduction of C-550. Thus, the photooxidation of cytochrome b_{559} should also decrease as C-550 is lost. With the ultraviolet-irradiated chloroplasts, however, the photooxidation of cytochrome b_{559} should decrease more rapidly than the destruction of C-550 because the ultraviolet radiation progressively oxidizes cytochrome b_{559} so that less is available for photooxidation.

DISCUSSION

The previous work from this laboratory showing that ultraviolet radiation inhibited photosynthetic electron transport between water and Photosystem II employed relatively low doses of ultraviolet radiation (a 10-min radiation with a low pressure mercury lamp). In that case, approx. 30% of the $NADP^+$ photoreduction activity could be restored by adding hydroquinone and ascorbate to donate electrons to Photosystem II. With larger doses of ultraviolet radiation restoration of activity was not achieved. Thus, as should be expected, ultraviolet radiation has multiple sites of inhibition. The site between water and Photosystem II, which is analogous to the site inhibited by Tris[13] and chaotropic agents[16], is one of the more sensitive sites to ultraviolet radiation. The degree to which Photosystem II electron donors can restore electron transport to ultraviolet-inhibited chloroplasts depends upon how much C-550 remains. After more prolonged irradiation treatments the Photosystem II reaction centers are inactivated and inhibition is irreversible.

The irreversible inhibition by ultraviolet radiation is similar to the inhibition by lipase[11,12] in that both treatments destroy C-550. Our work with lipase suggested that disruption of the structural integrity of the lamellar membranes leads to the loss of C-550. The fact that the photochemical change of C-550 involves only the shift of the absorption band from 546 to 544 nm with little change in the intensity of the band[11] suggests that the absorption band of C-550 indicates the state of the membrane or a pigment–protein association. Operationally, however, C-550 acts as if it is the primary electron acceptor of Photosystem II.

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