Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 9295–9299, December 1990
Biochemistry

Evidence for a biological role in photosynthesis for cytochrome *b*-559—a component of photosystem II reaction center

(photooxidation/*Nicotiana tabacum*)

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Communicated by Martin D. Kamen, July 11, 1990 (received for review December 21, 1989)

ABSTRACT **Absorbance changes in untreated intact leaf discs, produced upon excitation with high-intensity red light, were shown to be due to the photooxidation of cytochrome *b*-559. At low intensities (<100 W/m^2), photooxidation was almost undetectable. Photooxidation occurred with a half-time of 4.3 sec and an extent of 0.64 mol of cytochrome per 320 mol of chlorophyll. Upon transition to darkness, an additional oxidation occurred that exhibited faster kinetics ($t_{1/2}$ < 100 msec) and 0.32 mol of cytochrome was oxidized per 320 mol of chlorophyll. Photooxidation was inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea and was specifically induced by red light since far-red light did not cause any absorbance decrease. These results suggest that the redox changes of cytochrome *b*-559 are driven by photosystem II. Photooxidation was increased by 67% and its initial rate was doubled upon incubation of the leaf in carbonylcyanide *p*-trifluoromethoxyphenylhydrazone. Exposure of the leaf to mild water stress or mild heat stress resulted in an increase in the extent of photooxidation and in a 6-fold decrease in the rate constant. Mild heat stress also induced a large increase of the rate constant for the dark reduction of the cytochrome. The dependence of photooxidation on high-intensity red light, its inhibition criteria, the fast transient dark oxidation, and enhancement of both photooxidation and dark transient oxidation by treatments that affect Z, the primary donor to P680, indicate that cytochrome *b*-559 *in vivo* is involved in cyclic electron flow around photosystem II. Its primary role in photosynthesis is to divert excess photons from a linear to a cyclic electron flow at high light intensities for protection of the D_1 and D_2 proteins against photodamage. Dark oxidation of the cytochrome is suggested to reflect a second role, that of deactivation of the powerful oxidant Z^+ in the dark.**

The discovery and characterization of the isolated reaction center of photosystem II (PSII), which consists of the D-1 and D-2 polypeptides and cytochrome *b*-559, advanced our understanding of the mechanism of primary charge separation in photosynthesis (1). However, the role played by cytochrome *b*-559, a central component of the PSII reaction center (2), is still not known. The molecular structure of this cytochrome was recently established to be an intermolecular heme cross-linked dimer of 14 kDa (3), which could be either a heterodimer or a homodimer (4). Uncertainty over its role arose from the absence of light-induced redox changes at room temperature in isolated untreated chloroplasts (5–7). At room temperature, nonphysiological manipulations such as treatment with Tris (which inactivates the oxygen evolving complex) or with carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), which acts as an ADRY (acceleration of deactivation reactions of the water-splitting system) reagent resulted in light-induced absorbance changes of cytochrome *b*-559 (6, 7). The ambiguity of experimental findings concerning the role of cytochrome *b*-559 led researchers to formulate three major hypotheses for its function: (*i*) an electron transfer intermediate on the oxidizing side of PSII (5), (*ii*) in a bypass or cyclic electron transfer route around PSII (7, 8), (*iii*) in the noncyclic electron transfer linking the two photosystems (9) (for a review, see refs. 3 and 10).

In the present work, we demonstrate light-induced redox changes of cytochrome *b*-559 by direct observation of absorbance changes in untreated leaves of several species. These changes were larger but of slower kinetics in leaves undergoing mild water or heat stress. The effects of the photosynthetic inhibitors 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and FCCP are analyzed. In view of the *in vivo* light-induced changes in the oxidation state of the cytochrome, a role in photosynthesis is presented.

MATERIALS AND METHODS

Light-induced absorbance changes of cytochrome *b*-559 and cytochrome *f* were measured in an AMINCO-CHANCE (Philadelphia) DW-2 dual-wavelength spectrophotometer at 20°C on intact leaf discs. Spectra in the α-band region of cytochromes were obtained by measuring the absorbance change every 2 nm with the reference wavelength at 540 nm within the range 540–570 nm. The spectra for photooxidation of cytochrome *b*-559 were corrected at each wavelength by subtracting the contribution of cytochrome *f*. This contribution appeared as an initial fast phase ($t_{1/2}$ < 100 msec) and had the spectrum of cytochrome *f*. The spectrum of dark oxidation was not corrected. Leaf discs were exposed to water stress as described (11). Heat stress was imposed by incubating leaf discs in water at 47°C as reported (12). DCMU (10 μM) and FCCP (1 μM) were administered by incubating a leaf disc in darkness for 2 hr 45 min and for 40 min, respectively. DCMU-treated leaf discs were inserted in darkness in the DW-2 apparatus and actinic plus measuring lights were switched on simultaneously. The effective cytochrome *b*-559 concentration was estimated by using the assumptions in refs. 13 and 14. To express the extents on a chlorophyll basis, we measured the total chlorophyll density in the leaves as described (15), which gave approximately 40, 44, and 49 μg of chlorophyll per cm^2 of bean, spinach, and tobacco leaf area, respectively. The quantum yield of oxygen evolution was measured by the photoacoustic technique as described (11, 16). Fluorescence rise curves were monitored as described (17). Changes in the status of thylakoid energization were monitored by the relative changes of the maximum level of chlorophyll fluorescence (18).

Abbreviations: PSII, photosystem II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; CCCP, carbonylcyanide *m*-chlorophenylhydrazone; ADRY, accelerating of deactivation reactions of the water-splitting system.

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RESULTS

Irradiation of dark-adapted untreated tobacco leaf disc with high-intensity red light caused a decrease in the absorbance of the leaf at 559 nm in reference to 540 nm (Fig. 1*A*). The kinetics of the decrease fitted a monoexponential over 75% of its extent with a half-time ($t_{1/2}$) of 4.3 ± 0.17 sec. A second consecutive slower phase, with a $t_{1/2}$ of 34.6 ± 3.2 sec, accounted for the final 25% of the absorbance change. An example of such a kinetic analysis is shown later in Fig. 2*C*. When the red light was turned off, a fast decrease was observed with a time constant of <100 msec (instrument limited). It was followed by a monoexponential increase in absorbance over 75% of its extent ($t_{1/2}$ = 4.6 ± 0.02 sec) and a slow increase accounting for 25% of the total extent, with a $t_{1/2}$ of 30.1 ± 2.2 sec. The spectrum for the absorbance decrease induced by red light peaked at 559 nm and is shown in Fig. 1*B*. Similarly, the transition from red light to dark conditions induced a very rapid initial phase (<100 msec), with a spectrum indicating a 559-nm maximum (Fig. 1*C*), which is clearly due to the dark oxidation of cytochrome *b*-559. These results indicate that cytochrome *b*-559, which in the dark is in a reduced state, becomes oxidized by light and to a smaller extent in the transition from light to dark and is then reduced in the dark. The extent of cytochrome *b*-559 oxidation is 0.10 nmol·cm^{-2} (Table 1), which amounts to the photooxidation of 2 mol of the cytochrome per 1000 mol of chlorophyll.

The absorbance decrease monitored at 554 nm was biphasic. The first fast phase of decrease ($t_{1/2}$ < 100 msec) amounted to 50% of the total absorbance decrease (Fig. 1*D*). The difference spectrum peaked at 554 nm, indicating that the fast phase is due to cytochrome *f* photooxidation (Fig. 1*E*). The fast phase was followed by a slow phase ($t_{1/2}$ = 7.6 sec). The slow phase clearly displayed the spectrum of cytochrome *b*-559 (Fig. 1*B*). The photooxidation of cytochrome *b*-559 measured by the extent of the slow phase (Fig. 1*D*) was monitored as a function of the intensity of the red light (Fig. 1*F*). At low light intensities (<100 W/m^2), the absorbance change due to cytochrome *b*-559 could hardly be detected. The extent increased and was saturated at 600 W/m^2, since no further increase of photooxidation occurred at 1200 W/m^2. On the other hand, the saturation curve of photooxidation of cytochrome *f* measured by the extent of the fast phase (Fig. 1 *D* and *E*) as a function of red light intensity shows that cytochrome *f* was completely oxidized at a relatively low intensity of 10 W/m^2 (Fig. 1*G*).

The effect of ADRY reagent FCCP (1 μM) on the photooxidation of cytochrome *b*-559 is shown in Fig. 2. In an untreated leaf disc (Fig. 2*A*), first-order dependence of the photooxidation (up to 84%; Fig. 2*C*) allowed the calculation

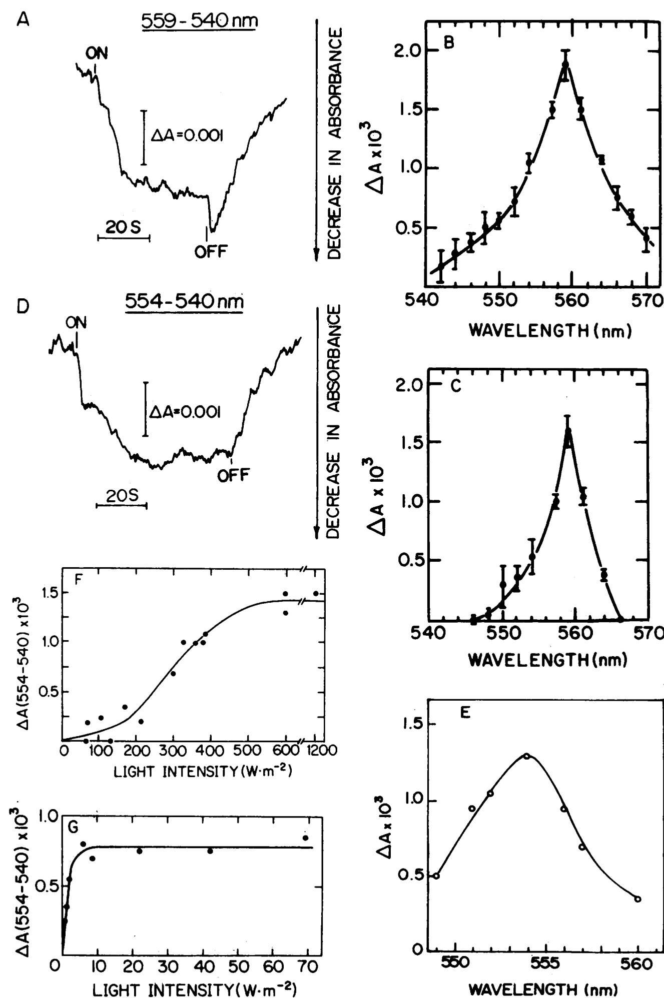


FIG. 1. (*A*) Photooxidation of cytochrome *b*-559 induced by broad-band red light (620–680 nm) with an intensity of 560 W/m^2, measured at 559 nm relative to 540 nm in untreated tobacco leaf. (*B*) Spectrum of photooxidation induced by the same red light. (*C*) Spectrum of fast dark oxidation phase ($t_{1/2}$ < 100 msec). (*D*) Absorbance decrease measured at 554 nm relative to 540 nm, induced by red light as described in *A*. (*E*) Spectrum of the fast phase of photoinduced absorbance decrease shown in *D*, indicating photooxidation of cytochrome *f*. (*F*) Light-intensity dependence of the slow phase ($t_{1/2}$ = 7.6 sec) reflecting the photooxidation of cytochrome *b*-559. (*G*) Light-intensity dependence of the fast phase ($t_{1/2}$ < 100 msec) reflecting the photooxidation of cytochrome *f* shown in *D* and *E*.

Table 1. Effect of leaf treatment on extents of light-induced absorbance changes of cytochrome *b*-559 oxidation

Treatment	Red light intensity, W/m^2	Amount of oxidized cytochrome nmol/cm^2	mol per 1000 mol of chlorophyll
Untreated tobacco leaf	560	0.10	2.0 ± 0.2
Untreated young* tobacco leaf	320	0.06	1.20 ± 0.1
FCCP in young tobacco leaf	320	0.10	2.0 ± 0.2
Untreated tobacco leaf	400	0.04	0.8 ± 0.08
Heat stress	400	0.10	2.0 ± 0.2
Untreated spinach leaf	400	0.04	0.8 ± 0.08
Untreated bean leaf	400	0.04	0.8 ± 0.08

Leaf discs were mounted between two glass slides and placed at 45° to the measuring beam, thus increasing the optical pathlength by $\sqrt{2}$. Actinic illumination of PSI or PSII was obtained from a slide projector. Light was passed through a 3-cm layer of water and either a far-red filter (RG 715 Schott filter; λ > 705 nm) or a red filter (RG 630 Schott filter; λ > 620 nm) in combination with a long-pass filter 680 nm (Ditric Opticals, Hudson, MA; λ < 680 nm). In some experiments, a low-intensity red light (λ = 640 nm) was provided by Ditric interference filter (10-nm band width). The cross-section of the beam on the leaf disc was 1.4 × 0.3 cm. The amount of oxidized cytochrome *b*-559 was calculated from the absorbance difference at 559 nm relative to 540 nm (Fig. 1) using the equation derived by Heber *et al.* (13). The values obtained represent lower limits, since the reduction of cytochrome *b*-563 by red light was neglected. Each determination is the average of three different experiments and the standard deviation was calculated.

*First leaf on the tobacco plant stem showed larger absorbance decrease than the fourth (middle) leaf at the same red light intensity.

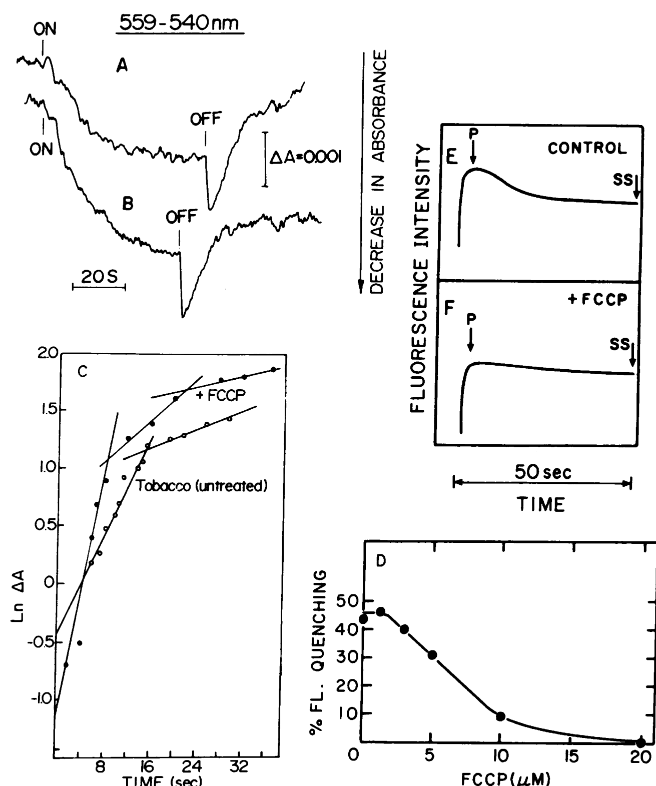


FIG. 2. Effect of FCCP on the photooxidation of cytochrome *b*-559 by broad-band red light (320 W/m^2) measured at 559 nm relative to 540 nm. (*A*) Untreated young tobacco leaf. (*B*) Same leaf treated with FCCP (1 μM). (*C*) Kinetic analysis of the decrease in absorbance induced by red light in untreated and in FCCP-treated leaves. (*D*) Effect of FCCP on the high-energy fluorescence quenching calculated as (Fp−Fss)/Fp where Fp and Fss are the maximum and steady-state fluorescence levels shown in *E* and *F*. (*E*) Fluorescence induction curve of a tobacco leaf disc incubated in water or in 1 μM FCCP for 60 min. (*F*) Same as in *E* but incubation was in 20 μM FCCP for 190 min. Measuring light was 10 W/m^2.

of a $t_{1/2}$ of 6.9 sec. However, second-order kinetics fits equally well with the same apparent $t_{1/2}$ and a rate constant of $1.1 \times 10^6 M^{-1} \cdot sec^{-1}$, indicating a complex kinetics. The subsequent phase of photooxidation (14%) was slow ($t_{1/2}$ = 35 sec). Upon cessation of illumination, a fast (<100 msec) additional oxidation occurred in the dark. When the leaf disc was treated with FCCP, the extent of photooxidation increased by 67% (Table 1). The kinetic analysis in the presence of FCCP involved at least two phases (Fig. 2*C*). The initial phase displayed a faster rate of $t_{1/2}$ = 2.6 sec compared to the control untreated leaf (Fig. 2*C*). The initial phase and the subsequent slow phase ($t_{1/2}$ = 15 sec) amounted to 73% and 20%, respectively, of the total photooxidation. The extent of the very fast ($t_{1/2}$ < 100 msec) dark oxidation also increased by 20% due to the presence of FCCP. It was followed by only partial dark reduction with a $t_{1/2}$ of 2.5 sec. Our results are in agreement with the extent of photooxidation in carbonylcyanide *m*-chlorophenylhydrazone (CCCP)-treated mutant pea leaves (14). However, Hiller *et al.* (14) reported that no absorption changes of cytochrome *b*-559 could be detected in these leaves when untreated because they used a low light intensity of 15 W/m^2. The effect of a low concentration of FCCP (1 μM) on intact leaf discs is primarily due to its ability to act as an ADRY reagent, since much higher concentrations of FCCP are required for its function as an uncoupler, as is demonstrated in Fig. 2*D*. It is well established that the large decrease in maximal fluorescence yield (Fp) to the steady-state fluorescence yield (Fss) occurring within 50 sec of illumination can be correlated to changes of the thylakoid pH gradient (18). Fig. 2*D* shows that a relatively high FCCP concentration (20 μM) was required to abolish the high-energy fluorescence quenching (Fp−Fss/Fp) in an intact leaf (Fig. 2 *E* and *F*). However, at 1 μM FCCP did not act as an uncoupler, since no change was detected in the high-energy fluorescence quenching.

Table 2. Effects of water stress and heat shock on the relative quantum yield of oxygen evolution and variable chlorophyll fluorescence

Stress time	Quantum yield of O_2 evolution, % of control	Variable chlorophyll fluorescence [(Fm−Fo)/Fo], % of control
Control	100	100
2 hr of water stress	80	90
5 hr of water stress	44	59
6 hr of water stress	21	40
Control	100	—
1 min at 47°C	+52	—
3 min at 47°C	−23	—
15 min at 47°C	0	—

The quantum yield of oxygen evolution was determined by dividing the photoacoustic oxygen signal by the photothermal signal. Negative value indicates oxygen uptake. Fm and Fo are maximum and minimum fluorescence yields, respectively (17). Fo did not change during the stress periods shown.

Rapid dehydration of leaf discs (11), resulted in inhibition of both O_2 evolution and variable fluorescence (Table 2). The latter could be restored with water analog donors, indicating a drought damage site in the oxidizing side of PSII (17). Irradiation of untreated tobacco leaf disc with red light caused photooxidation of both cytochrome *f* (fast component; $t_{1/2}$ < 100 msec) and cytochrome *b*-559 (slow component; $t_{1/2}$ = 7.6 sec). Upon switching from red to far-red light, an apparent increase in absorbance was observed (Fig. 3*A*). However, an even greater increase was observed on switching to darkness (Figs. 3*A* and 1*D*). Thus, the far-red light did not cause an increased reduction but, on the contrary, kept the cytochrome somewhat more oxidized in the steady state. Furthermore, a leaf disc irradiated with far-red light exhibited only the fast phase of photooxidation with a spectrum of cytochrome *f* (Fig. 3*B*). In a mildly water stressed leaf (2 hr of dehydration), the extent of photooxidation of cytochrome *b*-559 increased by a factor of 1.7 with no apparent change in

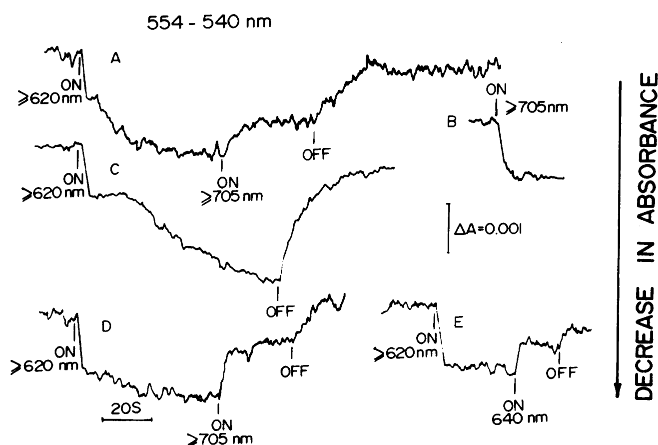


FIG. 3. The effect of water stress on the absorbance decrease induced by either broad-band red light (560 W/m^2) or far-red light (56 W/m^2) measured at 554 nm relative to 540 nm in tobacco leaves. (*A*) Red light effect on untreated leaf. (*B*) Far-red light effect on untreated leaf. (*C*) Leaf subjected to 2 hr of water stress. (*D*) Leaf subjected to 5 hr of water stress. (*E*) Leaf subjected to 6 hr of water stress.

the extent of photooxidation of cytochrome *f* (Fig. 3*C*). In severe water stress (5–6 hr of dehydration), the extent of photooxidation of cytochrome *b*-559 decreased substantially (Fig. 3 *D* and *E*). Far-red light (Fig. 3*D*) or red light (Fig. 3*E*) of low intensity both caused an increase in absorbance. These results indicate that water stress affects electron transfer through cytochrome *b*-559.

Exposing an intact leaf disc to mild heat stress resulted in a decrease in O_2 evolution yield and was followed by an uptake of oxygen (ref. 12; Table 2). After mild heat stress, the extent of cytochrome *b*-559 oxidation by red light increased by 150% compared to the control, untreated leaf and the kinetics of photooxidation dramatically decreased from $t_{1/2}$ = 5.4 sec to $t_{1/2}$ = 32 sec (Fig. 4 *A* and *B*). The extent of the fast oxidation phase ($t_{1/2}$ < 100 msec) observed upon transition to dark also increased by 100% (Fig. 4 *A* and *B*). A prolonged heat stress resulted in abolishment (<12%) of photooxidation, dark oxidation, and reduction (Fig. 4*C*). Surprisingly, the dark reduction in heat-stressed leaf ($t_{1/2}$ = 1.5 sec) was faster than in untreated leaf ($t_{1/2}$ = 4.8 sec) (Fig. 4*D*). These experiments indicate that although the photooxidation of cytochrome *b*-559 due to mild heat stress is retarded, the reduction in the dark is fast and the extent of the oxidation–reduction changes is increased (Table 1).

The herbicide DCMU inhibits electron transfer from Q_A, the primary stable acceptor of PSII (19). DCMU did not affect the fast phase of oxidation of cytochrome *f* by far-red light (Fig. 5*A*). Irradiation with low intensity red light induced a very small absorbance increase, which was followed by a larger extent of dark reduction (Fig. 5*A*). Irradiation of a DCMU-treated leaf disc with high intensity red light induced a very small absorbance decrease. Switching from red light to far-red light caused an additional small fast oxidation ($t_{1/2}$ < 100 msec). Upon turning off the far-red light, a slow dark reduction took place (Fig. 5*B*). This experiment shows that the photooxidation of cytochrome *b*-559 by red light was abolished in the presence of DCMU and only changes in the oxidation of cytochrome *f* were observed (Fig. 3*B*).

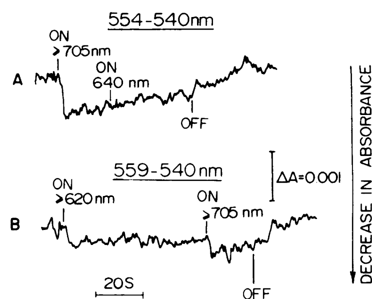


FIG. 5. The effect of DCMU (10 μM) on the photooxidation of cytochrome *f* and cytochrome *b*-559 in intact tobacco leaves. (*A*) Absorbance changes in the presence of DCMU measured at 554 nm relative to 540 nm and induced by either far-red light (λ > 705 nm; intensity, 56 W/m²) or red light (640 nm; intensity, 12 W/m²). (*B*) Absorbance changes in the presence of DCMU measured at 559 nm relative to 540 nm and induced by either high-intensity (560 W/m²) broad-band red light or by far red light (λ > 705 nm; intensity, 56 W/m²).

The photooxidation and dark reduction changes of cytochrome *b*-559 were also examined in other leaf species. Spinach and bean leaf discs exhibited photooxidation half-times of 12 sec and 7.5 sec, respectively (Fig. 6). However, the extents of photooxidation were equal in bean and spinach (Table 1). In spinach leaf disc, the extent of the fast (<100 msec) transient oxidation in the dark corresponded to 50% of the total absorbance change, in contrast to tobacco and bean (35%). The slow dark reduction was also faster in the bean ($t_{1/2}$ = 7 sec) than in spinach leaf disc ($t_{1/2}$ = 13 sec). This experiment suggests that in different leaf species it is possible to observe, under physiological conditions, a slow photooxidation of cytochrome *b*-559 *in vivo* followed by a fast dark oxidation phase and a slow dark reduction phase.

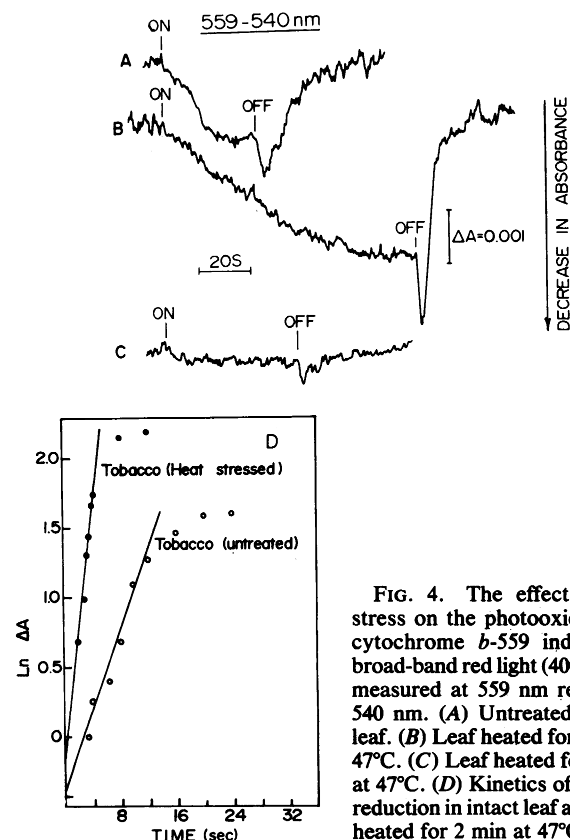


FIG. 4. The effect of heat stress on the photooxidation of cytochrome *b*-559 induced by broad-band red light (400 W/m²), measured at 559 nm relative to 540 nm. (*A*) Untreated tobacco leaf. (*B*) Leaf heated for 2 min at 47°C. (*C*) Leaf heated for 15 min at 47°C. (*D*) Kinetics of the dark reduction in intact leaf and in leaf heated for 2 min at 47°C.

DISCUSSION

In the present work, light-induced absorbance changes of cytochrome *b*-559 are demonstrated in untreated intact leaf discs. Our results show that high intensity (>100 W/m²) red light-induced oxidation of this cytochrome (Fig. 1), which could not be detected in far-red light, was abolished by DCMU, indicating that it is driven by PSII. At saturating light intensity (560 W/m²), we observed the photooxidation of 0.64 mol of the cytochrome per 320 mol of chlorophyll (Table 1). Another fraction of 0.32 mol of cytochrome per 320 mol of chlorophyll was oxidized by fast kinetics upon transition from red light to darkness. The oxidation of 1 mol of

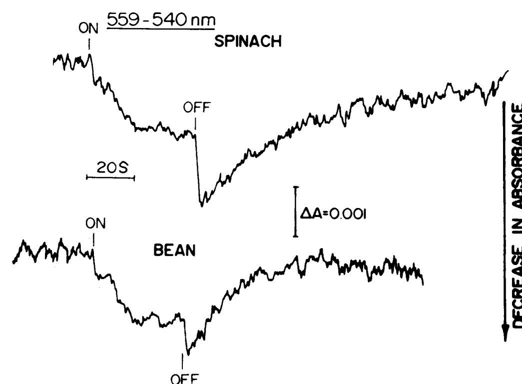


FIG. 6. Photooxidation of cytochrome *b*-559 induced by broad-band red light (400 W/m²) measured at 559 nm relative to 540 nm in spinach leaf and bean leaf.

cytochrome *b*-559 per 320 mol of chlorophyll points to the possibility of a dimer of cytochrome *b*-559 in the PSII reaction center. In isolated chloroplasts, chemical titration resulted in a stoichiometry of 1 cytochrome *b*-559 per 320 chlorophylls (20).

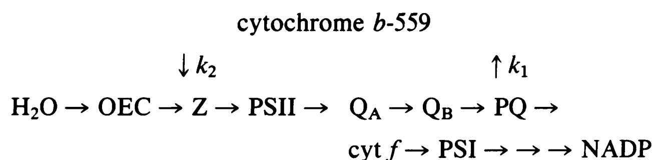
Biphasic chemical-induced oxidation kinetics was reported in chloroplasts and it was suggested that the high potential cytochrome exists in the thylakoid membrane in two different environments (20). Our results indicate that in intact leaves, photooxidation of only one major population of cytochrome *b*-559 occurs, with a half-time of 6.9 sec (84% in extent). The second slow (35 sec) kinetic phase was <14% in a tobacco leaf and was undetected in bean leaves. However, treatments that affect PSII, such as water or heat stress, resulted in a 100% conversion of the fast phase (6.9 sec) into a slow phase (35 sec). Therefore, we suggest that the slow phase most likely resulted from some change in the cytochrome's environment due to the various treatments.

A very interesting aspect of cytochrome *b*-559 electron transfer properties is the extremely fast dark oxidation phase, which peaks at 559 nm and occurs within <100 msec after photooxidation. It was increased by treatments inhibitory to the oxidizing side of PSII (FCCP, heat treatment; Figs. 2 and 4). It was absent in the absorbance change at 554 nm induced by far-red light and elicited by cytochrome *f* oxidation (Fig. 3*B*) and in a DCMU-treated leaf disc (Fig. 5). The mechanism of ADRY destabilization of the oxidizing side of PSII is by catalyzing cyclic electron flows around PSII (21). The photooxidation of an ADRY reagent (CCCP) and its subsequent re-reduction by cytochrome *b*-559 has been reported in PSII preparations, suggesting that the cytochrome is oxidized by Z^+ through an ADRY reagent acting as an intermediate (22). In a FCCP-treated leaf disc, the initial rate constant of photooxidation increased by a factor of 2 compared to an untreated leaf (Fig. 2). It is possible that FCCP in the intact leaf facilitates the oxidation of cytochrome *b*-559 by Z^+ so that the cytochrome can better compete with electron donation from water. Since FCCP and heat stress increased both photooxidation and dark oxidation, it is suggested that Z^+ oxidizes a large fraction (65%) of cytochrome *b*-559 in the light in steady state (in an apparent kinetics of several seconds), which occurs via a cyclic electron transfer around PSII and a smaller fraction (35%) in a transient reaction upon removal of the light, in a fast (<100 msec) reaction.

In the presence of DCMU, photooxidation is abolished (Fig. 5), since the plastoquinone pool is oxidized and cannot reduce the cytochrome. The reduction of cytochrome *b*-559 by plastohydroquinone has been suggested (23). In heat-treated leaf discs, the half-time of the dark reduction decreased (Fig. 4), and the large oxygen uptake indicated an inactivation of the Calvin cycle (12). Therefore, the plastoquinone pool is probably highly reduced, causing a faster reduction of the cytochrome *b*-559 in the dark.

The apparent slow (6.9 sec) absorbance decrease of cytochrome *b*-559 elicited by red light is kinetically complex, probably reflecting a combination of k_1 and k_2, the rate constants of photoreduction and photooxidation of the cytochrome occurring simultaneously in a cyclic electron pathway around PSII. Since the half-time of photoreduction of oxidized cytochrome *b*-559 by a flash was reported to be 100 msec (23), the photooxidation must necessarily be faster than 100 msec if a net photooxidation at a steady state is to be observed. Indeed, in the transition from light to dark, the true oxidation rate in the millisecond range (or possibly even faster) is detected because the cytochrome is rapidly oxidized by Z^+, as compared to its slow reduction in the dark.

We suggest the following scheme for the role of cytochrome *b*-559 *in vivo*:



where OEC is oxygen-evolving complex and PQ is plastoquinone.

At low light intensities, linear electron transport is fast (24) and has a high quantum yield. Under high-intensity light, the plastoquinone is highly reduced and will transfer electrons with some efficiency to the cytochrome. The cytochrome is then oxidized by Z^+. A cyclic PSII-driven electron flow at saturating light intensities involving 15% of total electron flow has been claimed (25). At low temperature, it was reported that cytochrome *b*-559 is oxidized through an intermediate of oxidized chlorophyll (26). Proposals for the role of cytochrome in the PSII cycle have been presented (7, 8, 10, 26, 27). However, it was difficult to corroborate them because conflicting results were obtained with regard to the effects of light in chloroplasts treated in various ways.

We propose that the cytochrome plays two roles in photosynthesis. The first role is to protect PSII at high light intensities by maintaining a cyclic pathway for diverting away excess photons. The second role is to deactivate the powerful oxidant Z^+ in the dark, which is reflected by the very fast dark oxidation of cytochrome *b*-559 in transition from light to dark.

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