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A PATHWAY FOR THE REDUCTION OF CYTOCHROME *b*-559 BY PHOTOSYSTEM II IN CHLOROPLASTS

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Summary

Cytochrome *b*-559, which is normally reduced in the dark, was oxidized by preillumination in the presence of *N*-methyl-phenazonium methosulfate with low intensity far-red light. The average half-time for the photoreduction of oxidized cytochrome *b*-559 by a long actinic flash ranged from 90 to 110 ms. In the presence of 0.25 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea the half-time for the photoreduction increased to 230 ms although the extent of the absorbance increase was unchanged. Under similar conditions inhibition of electron transport by 3-(3,4-dichlorophenyl)-1,1-dimethylurea and the increase in the chlorophyll fluorescence show that a large fraction of the Photosystem II reaction centers are blocked. These results are consistent with the concept that electrons are shared between different photosynthetic units by a common pool of plastoquinone and imply that the principle pathway for the reduction of cytochrome *b*-559 by Photosystem II occurs through plastoquinone. In the presence of the uncoupler gramicidin which stimulates non-cyclic electron transport, the rate of photoreduction of cytochrome *b*-559 is slower ($t_{1/2}$ = 180 ms), from which it is inferred that cytochrome *b*-559 competes with cytochrome *f* for electrons out of this pool. Comparison of cytochrome *b*-559 photoreduction and electron transport rates using untreated and KCN-treated chloroplasts indicate that, under conditions of basal electron transport from water to ferricyanide, approximately one-fifth of the electrons from Photosystem II go through cytochrome *b*-559 to ferricyanide. Further support for this pathway is provided by a comparison of the effect of 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (dibromothymoquinone) on the rates of reduction of cytochrome *b*-559 and ferricyanide.

Introduction

Recent measurements showing that the rate of cytochrome *b*-559 reduction by Photosystem II is much slower than that of plastoquinone imply that high

potential cytochrome *b*-559 is not a component of the main electron transport chain (ref. 1, see also refs. 2—6). The pathway for cytochrome *b*-559 reduction by Photosystem II is determined only to the extent that the electron donor for cytochrome *b*-559 is between the primary acceptor for Photosystem II and cytochrome *f*. The experimental evidence for this conclusion is based on the observation that 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (dibromothymoquinone), which blocks electron transport between plastoquinone and cytochrome *f* [7,8], inhibits the photooxidation but not the photoreduction of cytochrome *b*-559 [1,8], although 3-(3,4-dichlorophenyl)-1,1-dimethylurea can block its photoreduction completely [9].

One method available to determine the pathway of cytochrome *b*-559 photoreduction depends upon the concept that electrons are shared between different electron transport chains by a common pool of plastoquinone [10—15]. Evidence supporting this model results from observations of the effect of 3-(3,4-dichlorophenyl)-1,1-dimethylurea upon the redox state of Photosystem I reaction centers following a long flash of light. At a 3-(3,4-dichlorophenyl)-1,1-dimethylurea concentration inhibiting most of the Photosystem II reaction centers, nearly all of the Photosystem I reaction centers remain active [10]. If the electron transport chains were independent units, inhibition of Photosystem II reaction centers should prevent electrons from reaching the corresponding Photosystem I reaction centers. The fact that most Photosystem I reaction centers remain active is most easily explained in terms of electron sharing. Other results consistent with this suggestion include the observation that the inhibitory effect of 3-(3,4-dichlorophenyl)-1,1-dimethylurea upon oxygen evolution depends upon the intensity of the exciting light [13,16]. The concentration of 3-(3,4-dichlorophenyl)-1,1-dimethylurea which inhibits the rate of oxygen evolution by 50% at sub-saturating light intensities has little or no effect upon the rate of oxygen evolution at saturating light intensities, since under the latter condition the rate-limiting step is between plastoquinone and cytochrome *f*. Although the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea may block a large fraction of the Photosystem II reaction centers, at saturating light intensities electron donation by the remaining active centers can be sufficient, and redistribution by the plastoquinone pool rapid enough, so that transfer from plastoquinone to cytochrome *f* remains the rate-limiting step. Under these conditions the oxygen evolution rate is not inhibited. However, under low light conditions the rate limitation is determined by the light intensity and, as a consequence, the rate of oxygen evolution is proportional to the number of active reaction centers and is therefore much more sensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

The work reported here indicates that high potential cytochrome *b*-559 is reduced by Photosystem II through the electron-sharing plastoquinone pool, and may transfer electrons to ferricyanide from Photosystem II.

Materials and Methods

Chloroplast preparation. Spinach leaves gathered from a controlled climate facility were used to isolate chloroplasts according to the technique of Ort and Izawa [17]. KCN-treated chloroplasts were prepared following the technique of

Izawa et al. [18]. Chlorophyll concentration was determined according to the method of Arnon [19].

Experimental. Oxygen evolution and uptake were measured polarographically using a Clark-type oxygen electrode as described previously [1]. The actinic light was filtered by a Corning 2-58 blocking filter and a $CuSO_4$ solution.

Light-induced cytochrome absorbance changes were measured using a modified Aminco-Chance dual wavelength spectrophotometer as described previously [1]. The reference wavelength used was 570 nm and the optical pathlength was 1 cm. The actinic light was provided by a 150 W tungsten-halogen lamp and passed through two 'heat' filters and a Corning 2-58 blocking filter. The intensity was $3.4 \cdot 10^5$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. The far-red background light was defined by a 732 nm Balzers interference filter. The intensity was approx. $4 \cdot 10^3$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}.

Chlorophyll fluorescence was measured using a Perkin-Elmer MPF-4 fluorescence spectrophotometer. The excitation wavelength was 440 nm and the fluorescence emission was measured at 680 nm.

All experiments were done at room temperature (22—23° C).

Results

In order to observe the photoreduction of cytochrome *b*-559, which is reduced in the dark, the chloroplast preparation was illuminated in the presence of *N*-methyl-phenazonium methosulfate by low intensity far-red light prior to each flash. During the far-red illumination cytochrome *b*-559 becomes oxidized [20], thereby enabling us to observe its photoreduction during a relatively long flash of actinic light [1]. Under these conditions the amount of cytochrome *b*-559 reduced in a single flash corresponds to 1.0—1.2 molecules per photosynthetic unit, which represents 50—70% of the total hydroquinone-reducible cytochrome *b*-559 observed in our chloroplast preparation. The cytochrome turning over is considered to be of relatively high potential ($E_{m\,7.8}$ = +395 mV [21]) since it is fully reducible by hydroquinone. (These calculations are based upon a photosynthetic unit of 500 chlorophyll molecules and a reduced-oxidized extinction coefficient of 15 mM^{-1} $\cdot$ cm^{-1} at 559—570 nm [6]).

The average absorbance increase at 559 nm induced by a single actinic flash is shown in Fig. 1A. The spectrum of the light-induced absorbance increase exhibits a peak at 559 nm corresponding to the reduced-oxidized α band of cytochrome *b*-559. This spectrum and the spectra for other absorbance changes presented here are not shown since they are essentially the same as published previously [1] and indicate that the signals are due primarily to cytochrome *b*-559. The average half-time for the photoreduction shown in Fig. 1A is 100 ± 15 ms. This value shows little variation from one chloroplast preparation to another, normally falling between 90 and 110 ms. Kinetics of the photoreduction do not depend upon the presence of *N*-methylphenazonum methosulfate since the half-time for photoreduction was unchanged when chloroplasts were pretreated with high intensity white light rather than *N*-methyl-phenazonum methosulfate to enable oxidation of cytochrome *b*-559 by far-red light. Increas-

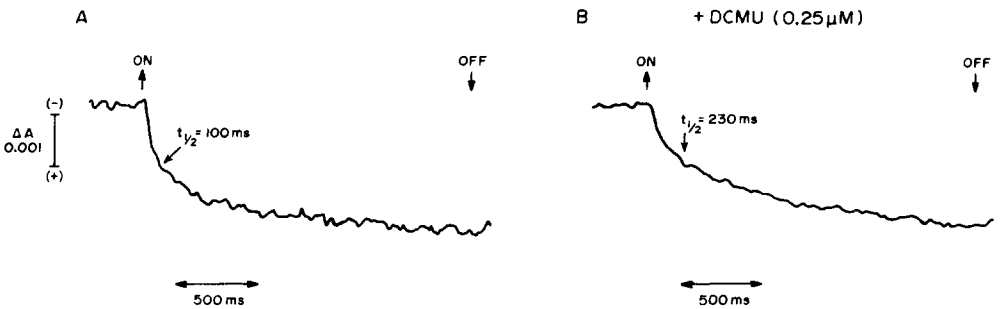


Fig. 1. Kinetics of the absorbance change at 558—570 nm induced by illumination of spinach chloroplasts. The sample was illuminated for 60—90 s prior to each flash by low intensity 732 nm light. The intensity of the actinic light was $3.4 \cdot 10^5$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. The half-bandwidth of the measuring beam was 3 nm. (A) The trace shown is the average of 15 runs during which the sample was changed three times. The reaction mixture contained 0.2 M sucrose, 30 mM Tricine/NaOH (pH 7.8), 2 mM $MgCl_2$, 10 mM KCl, 100 μM methyl viologen, 1 μM *N*-methyl-phenazonium methosulfate and 75 μg chlorophyll/ml. (B) The trace shown is the average of 30 runs during which the sample was changed six times. The reaction mixture was the same as described in A except that it contained 0.25 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). Further details are given in Materials and Methods.

ing the actinic flash intensity did not increase the reduction rate indicating that the photoreduction is light saturated.

In Fig. 2 the effect of pH upon the rate and extent of cytochrome *b*-559 photoreduction is shown. The rate of reduction appears to be maximal from pH 8.5 to 7.0 and then decreases to one-half of the maximum rate at pH 5.8. The extent of the turnover is maximal between pH 6.5 and 8 and is likely dependent upon the pH dependence of both the dark redox level and the amplitude of far-red-oxidation [9,22].

Effect of 3-(3,4-dichlorophenyl)-1,1-dimethylurea upon cytochrome b-559 photoreduction

In Fig. 1B the effect of 0.25 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea

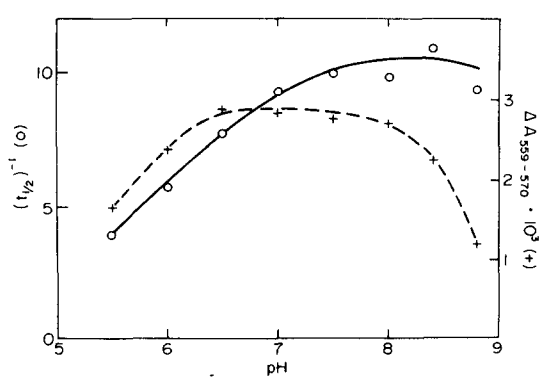


Fig. 2. The rate and extent of the light-induced absorbance change at 559—570 nm as a function of pH in chloroplasts; $t_{1/2}$ is given in seconds. The experimental conditions were essentially the same as described in Fig. 1A. The reaction mixture contained 0.1 M sucrose, 2 mM $MgCl_2$, 0.1 mM methyl viologen, 1 μM *N*-methyl-phenazonium methosulfate and was buffered at pH 5.5—6.5 by 30 mM 2-(*N*-morpholino)-ethane sulfonic acid (MES)/NaOH, at pH 7.0—7.5 by 30 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES)/NaOH, and at pH 8.0—8.8 by 30 mM Tricine/NaOH.

upon the photoreduction of cytochrome *b*-559 is shown. The extent of the absorbance change is not altered by 3-(3,4-dichlorophenyl)-1,1-dimethylurea although the average half-time for the photoreduction is extended to 230 ± 20 ms. The half-time in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea is dependent upon actinic light intensity under the conditions of Fig. 1B.

In order to determine the extent to which Photosystem II reaction centers are blocked by 0.25 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea, electron transport rates were determined using low and high light intensities in the absence and presence of 0.25 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (Table I). Using low light, under conditions similar to those described in Fig. 1 the rate of basal electron transport (i.e. in the absence of ADP and P_i) is 70% of the light-saturated rate and is inhibited approx. 50% by 0.25 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea, demonstrating that a sizeable fraction (50% or more) of the Photosystem II reaction centers are blocked. Using high light the rate of electron transport is unaffected by 0.25 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea, a result consistent with the concept of electron exchange between electron transport chains (see Introduction).

If cytochrome *b*-559 were reduced by Photosystem II in a linear pathway, independent of other identical pathways, then the addition of 0.25 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea, which blocks a large fraction of the Photosystem II reaction centers, should block the reduction of the corresponding cytochrome *b*-559 molecules. As a result we would expect the extent of the photoreduction to be inhibited by approx. 50%, while the rate would be unaffected. The results in Fig. 1, however, show that the extent of the absorbance increase is not inhibited by 0.25 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea, indicating that the photoreduction of cytochrome *b*-559 is complete even when half of the Photosystem II reaction centers are blocked. This implies that the site of reduction of cytochrome *b*-559 by the electron transport chain occurs subsequent to electron sharing by the plastoquinone pool. The decrease in the rate of reduction is a consequence of the fact that in the presence of 0.25 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea the rate is not light saturated.

Further evidence supporting this pathway is provided by comparison of the extent of the photoreduction of cytochrome *b*-559 and the increase in steady-state relative chlorophyll fluorescence yield as a function of 3-(3,4-dichloro-

TABLE I

THE EFFECT OF 3-(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA UPON ELECTRON TRANSPORT UNDER CONDITIONS OF SATURATING AND NON-SATURATING LIGHT

The intensity under "high light" was saturating and was greater than $2 \cdot 10^6$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. The intensity under "low light" was $3.4 \pm 0.5 \cdot 10^5$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. The data presented is the average of the number of experiments shown in parentheses. The reaction mixture was as described in Fig. 1 except *N*-methyl-phenazonium methosulfate was not present and 1 mM NaN_3 was added. The electron transport rate is given in μequiv./mg chlorophyll per h.

	Conditions	
	High light (3)	Low light (6)
Control	195	134
+ 0.25 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea	195	62

phenyl)-1,1-dimethylurea concentration (Fig. 3). The steady-state fluorescence yield is an indicator of the redox state of the primary acceptor of Photosystem II [23]. Upon the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea the observed increase in the fluorescence yield is directly related to the number of Photosystem II reaction centers blocked except at very low light intensities. The concentration of 3-(3,4-dichlorophenyl)-1,1-dimethylurea needed to inhibit the extent of cytochrome *b*-559 reduction by 50% (1.5 μM) is nearly seven times that necessary to cause 50% of the maximum fluorescence yield increase (0.22 μM). Thus, in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea concentrations which inhibit the majority of the Photosystem II reaction centers, electrons are still available to reduce the oxidized cytochrome *b*-559, implying that it is reduced after an electron sharing pool.

Comparison of the kinetics of cytochrome b-559 photoreduction and rates of electron transport

In Fig. 4 the kinetics of the photoreduction of cytochrome *b*-559 are shown using untreated (Figs. 4A and 4B) and KCN-treated chloroplasts inhibited at the plastocyanin site near Photosystem I [18], in the absence and in the presence of the uncoupler gramicidin (Figs. 4C and 4D). In untreated chloroplasts the half-time for the photoreduction of cytochrome *b*-559 increases from the control value of 95 ± 10 (Fig. 4A) to 180 ± 15 ms in the presence of gramicidin (Fig. 4B). At this light intensity gramicidin stimulates the rate of electron transport by a factor of 1.3—1.5 [1]. In KCN-treated chloroplasts electron transport to methyl viologen is inhibited by at least 95%. Under these conditions the

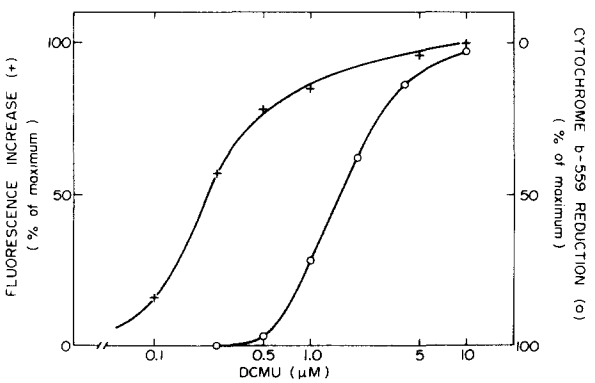


Fig. 3. Comparison of the dependence of cytochrome *b*-559 reduction and the steady-state fluorescence increase in the presence of an electron acceptor upon the concentration of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). (A) The percent fluorescence increase is defined as the increase in the steady-state relative fluorescence yield induced by a given concentration of 3-(3,4-dichlorophenyl)-1,1-dimethylurea, compared to the maximum increase induced by 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea. The addition of 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea caused an increase in fluoresence of 3.4 times the control level. The intensity of the exciting light was $5 \cdot 10^4$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. The results were the same at $3 \cdot 10^4$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. The reaction medium was as described in Fig. 1A except no *N*-methylphenozium methosulfate was present. (B) The percent reduction of cytochrome *b*-559 is defined as the change in absorbance at 559—570 nm induced by a flash of light at a given concentration of 3-(3,4-dichlorophenyl)-1,1-dimethylurea compared to the change in absorbance in the absence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea. The intensity of the actinic light was $3.4 \cdot 10^5$ $\cdot$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. Reaction mixture as described in Fig. 1A.

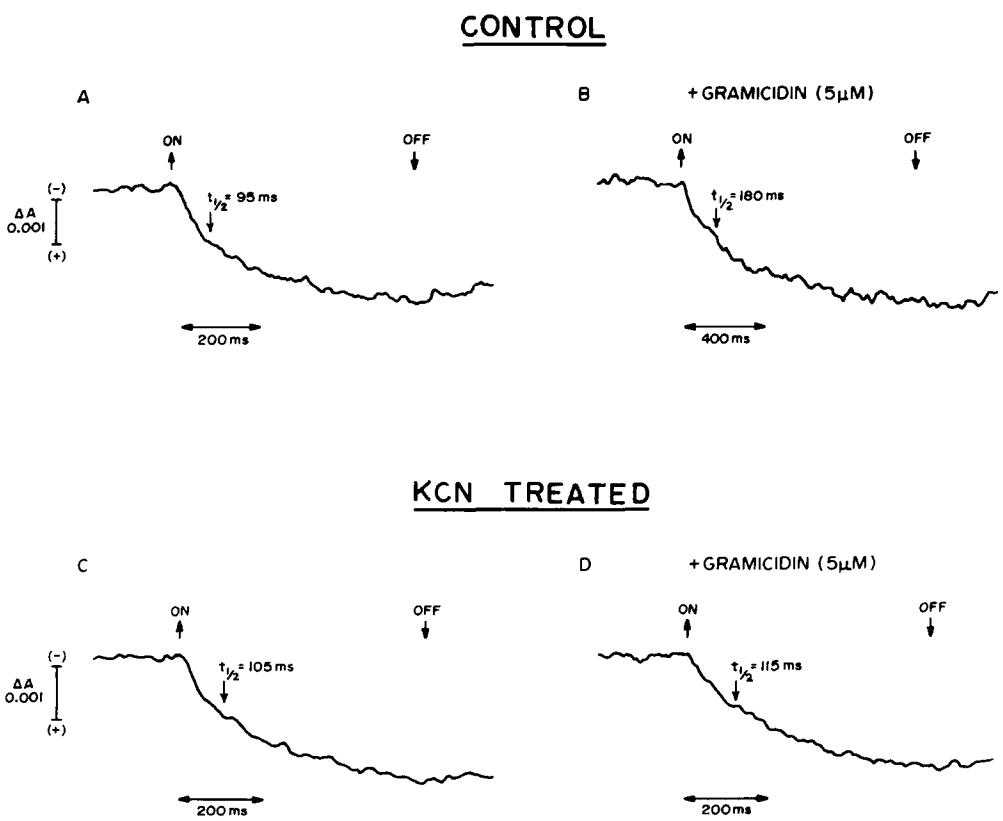


Fig. 4. Kinetics of the absorbance change at 560—570 nm induced by a flash of light in untreated and KCN-treated chloroplasts. (A) The trace shown is the average of 12 runs during which the sample was changed three times. The conditions were as in Fig. 1A. (B) Conditions as in A except 5 μM gramicidin was added. Note that the time scale is different from A. (C) Conditions as in A except KCN-treated chloroplasts were used. For details see text. (D) Conditions as in C except 5 μM gramicidin was added.

average half-time for the reduction of cytochrome *b*-559 (105 ± 10 ms, Fig. 4C) is the same as in untreated chloroplasts. In the presence of gramicidin the average half-time (115 ± 10 ms, Fig. 4D) does not show the large increase observed in untreated chloroplasts and, within the experimental error, is the same as in the absence of gramicidin. The fact that gramicidin causes little or no change in the half-time of photoreduction in KCN-treated chloroplasts implies that the increase in the reduction half-time in untreated chloroplasts is related to the flow of electrons from plastoquinone to Photosystem I, and is most likely due to the effect of gramicidin upon the rate-limiting step between plastoquinone and cytochrome *f*. Except for trace 4B, the signals shown in Fig. 4 were light saturated.

In order to relate the photoreduction of cytochrome *b*-559 to electron transport we measured the rate of basal electron transport from water to various acceptors in untreated and KCN-treated chloroplasts (Table I). In untreated chloroplasts, using either ferricyanide or methyl viologen as acceptor, the rates of electron transport were nearly the same, 217 and 227 μequiv./mg chlorophyll per h, respectively. In the presence of dibromothymoquinone the flow of

TABLE II

THE EFFECT OF DIBROMOTHYMOQUINONE ON FERRICYANIDE REDUCTION

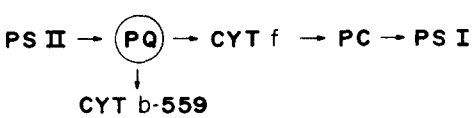
The 2 ml reaction mixture contained 0.2 M sucrose, 30 mM Tricine/NaOH (pH 7.8), 2 mM $MgCl_2$, 10 mM KCl, 40—64 μg chlorophyll (except in the experiments indicated with an asterisk, 140 μg chlorophyll was used), other additions as indicated, and when methyl viologen was present, 1 mM NaN_3. The electron transport rate is given in μequiv./mg chlorophyll per h.

$H_2O \rightarrow$	Methyl viologen (0.1 mM)	Ferricyanide (0.5 mM)	Dibromothymoquinone (1 μM), methyl viologen (0.1 mM)	Ferricyanide (0.5 mM), dibromothymoquinone (1 μM), methyl viologen (0.1 mM)
No. of Expts.	5	3	3	5
Control	227	217	0	209
$H_2O \rightarrow$	Methyl viologen (0.1 mM)	Ferricyanide (1 mM)	Dibromothymoquinone (1 μM), methyl viologen (0.1 mM)	Dibromothymoquinone (1 μM), ferricyanide (1 mM)
No. of Expts.	3	6	3	6
KCN-treated	9 *	45	0 *	195

electrons from water to methyl viologen was completely inhibited, whereas the rate of ferricyanide reduction was nearly the same as in the absence of dibromothymoquinone. In KCN-treated chloroplasts where electron transport from water to methyl viologen was inhibited by over 95% the rate of ferricyanide reduction was 45 μequiv./mg chlorophyll per h in the absence of dibromothymoquinone, and increased to 195 μequiv./mg chlorophyll per h after addition of dibromothymoquinone, a rate approaching the control basal value. These results are similar to earlier findings [15] and indicate that a site for ferricyanide reduction exists before the plastoquinone-cytochrome *f* step [15,24]. The rate of ferricyanide reduction through this site, in the absence of dibromothymoquinone, is approx. 20% of the basal rate (Table II) and 7—10% of the coupled rate obtained in the presence of ADP and P_i.

Discussion

Comparison of the effects of 3-(3,4-dichlorophenyl)-1,1-dimethylurea upon the photoreduction of cytochrome *b*-559, the rate of electron transport, and the chlorophyll fluorescence yield, lead to the conclusion that the pathway for the photoreduction of high potential cytochrome *b*-559 by Photosystem II includes the plastoquinone pool. In this model the cytochrome is considered to be accessible to the outer aqueous phase near the outside of the membrane [25]. A schematic representation of this pathway is shown below:



The circle around PQ indicates the pool function of plastoquinone. PSI, PSII, Photosystems I and II; PQ, plastoquinone; Cyt, cytochrome; PC, plastocyanin.

It appears that oxidized cytochrome *b*-559 competes with cytochrome *f* for electrons coming from the plastoquinone pool. The competition is constrained by the fact that the rate-limiting step for electrons from plastoquinone to cytochrome *b*-559 is 100 ms, as inferred from the lack of effect of KCN treatment on the photoreduction and saturation of this rate at a relatively low light intensity [1]. The existence of the competition is implied by the observation that the addition of gramicidin causes the rate of cytochrome *b*-559 photoreduction to decrease as the rate of electron transfer from plastoquinone to cytochrome *f* increases. In the case of KCN-treated chloroplasts, in which electron transport to cytochrome *f* is blocked, gramicidin causes little or no change in the rate of cytochrome *b*-559 reduction, indicating that the increase in the reduction halftime is not due to the action of gramicidin upon cytochrome *b*-559, but rather to its effect upon the plastoquinone-cytochrome *f* electron transfer rate.

Under conditions of electron transport from water to methyl viologen or NADP(H), cytochrome *b*-559 remains reduced in the steady state [6], and in the absence of a suitable oxidant does not offer a pathway for electrons from Photosystem II. Ferricyanide (E_{m7} = +430 mV), however, would work energetically as an acceptor for high potential cytochrome *b*-559, and in practice is a more efficient dark oxidant of cytochrome *b*-559 than of cytochrome *f* [25]. The time for oxidation of cytochrome *b*-559 by ferricyanide in a stirred sample appears in fact to be faster than 100 ms, since in the presence of 1.0 mM ferricyanide in continuously illuminated chloroplasts cytochrome *b*-559 remains primarily in the oxidized state (data not shown), implying that the rate of cytochrome *b*-559 oxidation by ferricyanide is faster than its rate of photoreduction. The accessibility and apparent reactivity of cytochrome *b*-559 to ferricyanide suggest the possibility that ferricyanide could be an electron acceptor for Photosystem II through cytochrome *b*-559. The rate constant for reduction of oxidized cytochrome *b*-559 implies that under conditions of basal electron transport approx. 20% of the electrons from water may go through cytochrome *b*-559 to ferricyanide.

Support for this suggestion is provided by electron transport data using KCN-treated chloroplasts. Although the rate of electron transport to methyl viologen was 9 μequiv./mg chlorophyll per h, when ferricyanide was added, it increased to 45 μequiv./mg chlorophyll per h, implying a site of ferricyanide interaction before the KCN block. The increase in rate corresponds to one-half an electron per photosynthetic unit every 100 ms, which is consistent with the rate-limiting step of cytochrome *b*-559 photoreduction. Another argument supporting this pathway for ferricyanide reduction is again based upon a comparison of cytochrome *b*-559 reduction kinetics and electron transport rates. In the presence of dibromothymoquinone the rate of photoreduction of cytochrome *b*-559 increases 3—4-fold ($t_{½}$ = 25—35 ms [1]). In KCN-treated chloroplasts the addition of dibromothymoquinone in the presence of ferricyanide caused the rate of electron transport to increase to 195 μequiv./mg chlorophyll per h, which corresponds to one-half an electron per photosynthetic unit every 19 ms. Given the indicated uncertainty in the reduction rate of cytochrome *b*-559 in the presence of dibromothymoquinone we feel that these results are consistent with the involvement of cytochrome *b*-559 in the reduction of ferricyanide.

A question of some interest concerns the mechanism by which dibromothy-

moquinone accelerates the photoreduction of cytochrome *b*-559. In terms of the pathway for reduction presented here, the possibility exists that the effect is a consequence of the blocking of the plastoquinone-cytochrome *f* step, thereby making more electrons available for cytochrome *b*-559 reduction. This explanation, however, is in conflict with the fact that the rate of cytochrome *b*-559 reduction saturates at relatively low light intensities [1], and that in KCN-treated chloroplasts, in which electron flow from plastoquinone to Photosystem I is blocked, the rate of reduction does not change. A more likely explanation would seem to be that dibromothymoquinone acts as a bridge between plastoquinone and cytochrome *b*-559, thereby removing the rate-limiting 100 ms step.

We wish to draw attention to the fact that the pathway for the photoreduction of high potential cytochrome *b*-559 presented here may not be easily reconcilable with the observation that it is photooxidized by Photosystem II at 77°K [26,27]. Low temperature photooxidation is thought to imply a close structural proximity with the Photosystem II reaction center, which appears at odds with a site of interaction with the main electron transport chain after electron sharing by the plastoquinone pool. One possible explanation is that the cytochrome *b*-559 population undergoing photooxidation at 77°K and that undergoing photoreduction at room temperature constitute different groups. Stoichiometries for both cases of approximately one molecule of cytochrome *b*-559 per photosynthetic unit do not rule out this possibility, although photooxidation of two molecules of cytochrome *b*-559 per Photosystem II at −50°C has been reported [27]. Another possibility is that the same molecules are involved but structural changes affect the accessibility of the cytochrome *b*-559 to the Photosystem II reaction center. Finally, the physical structure of Photosystem II may be so closely integrated and organized that the site of oxidation on the cytochrome *b*-559 protein is fairly close to the Photosystem II reaction center, even though the protein is reduced through the plastoquinone pool and apparently accessible to the aqueous phase.

Although the results presented here indicate that high potential cytochrome *b*-559 can be reduced by Photosystem II with significant efficiency, it has not yet been possible to identify a naturally occurring high potential oxidant which would allow such turnover under physiological conditions.

Acknowledgments

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