PLASTOQUINONE MEDIATES ELECTRON TRANSPORT BETWEEN CYTOCHROME *b*-559 AND CYTOCHROME *f* IN SPINACH CHLOROPLASTS

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1. Introduction

It has recently been reported [1, 2] that the quinone analog 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) at concentrations $\geqslant 10^{-6}$ M inhibits the photoreduction of low potential acceptors with water as a donor, along with the associated photophosphorylation. The Hill reaction with ferricyanide or dichlorophenolindophenol as acceptor was, however, only partially inhibited. Electron flow from system I donors to ferredoxin-NADP was not inhibited by DBMIB. The inhibition of non-cyclic electron flow and phosphorylation could be almost completely reversed by the presence of 10^{-4} M plastoquinone. These experiments show that DBMIB inhibits by interacting with structurally similar plastoquinone in the electron transport chain. The experiments reported here show that DBMIB blocks the reduction of cytochrome *f* by photosystem II light and the oxidation of cytochrome *b*-559 by photosystem I.

2. Methods

Chloroplasts were prepared from market spinach. 20 g of depetioled leaves were homogenized in a Sorvall Omnimixer for 3 seconds at 100 volts in 50 ml of medium (0.4 M sucrose, 0.05 M tricine-NaOH, 1 mM $MgCl_2$, and 1 mM $MnCl_2$). The homogenate was filtered through 8 layers of cheesecloth and 1 layer of 40 μm mesh nylon cloth (Tobler, Ernst, and Traber, Inc.). The chloroplast pellet was obtained through two centrifugations at 1500 *g* for five minutes. The preparatory procedure required about 15 min, and the resulting chloroplasts were well coupled for at least 3 hr, as judged by ADP stimulation of O_2 evolution with ferricyanide as acceptor by at least a factor of 1.7. Measurements of the cytochrome absorbance changes were made in an Aminco-Chance dual wavelength spectrophotometer using a PAR lock-in amplifier as previously described [3]. The measuring beam intensity was about 1 erg/(cm^2 sec) with a half band width of 2.1 nm. The actinic light intensities incident upon the cuvette were 6.3×10^4 erg/(cm^2 sec) and 7.0×10^4 erg/(cm^2 sec), respectively, at 645 nm and 713 nm. We are grateful for gifts of DBMIB, synthetic plastoquinone A, and *p*-CF_3O-CCP (FCCP), respectively, from Professor Achim Trebst, Professor David Krogmann, and Dr. P.G. Heytler.

3. Results and discussion

Fig. 1 shows the oxidation by 713 nm light and reduction by 645 nm light of cytochrome *f* in spinach chloroplasts, one of the classical experiments indicating the presence of two pigment systems in algae and chloroplasts [4–6]. 2 μM DBMIB completely inhibits the 645 nm photoreduction of cytochrome *f*, but not the photooxidation of cytochrome *f* by 713 nm light (fig. 1), placing the site of action of DBMIB on the system II side of cytochrome *f*. It should be noted that DBMIB also accelerates the rate of dark reduction of cytochrome *f* after red or far-red illumination. A similar experiment is shown in fig. 2, with the DBMIB inhibition of 645 nm

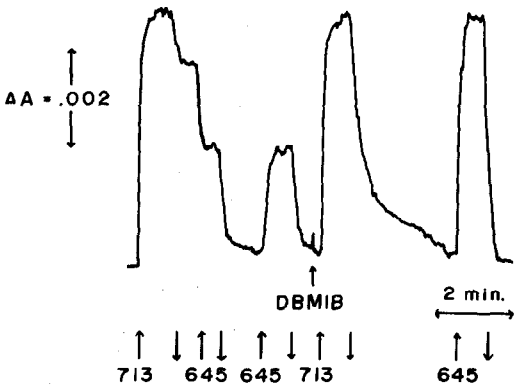

Fig. 1. Effect of DBMIB (2 μM) on the light-induced absorbance changes and the dark reduction of cytochrome *f*. DBMIB added in the dark before the second 713 nm illumination. Cytochrome *f* changes measured at 554 nm with 540 nm as reference. Upward arrows, actinic light on; downward arrows, light off. The reaction mixture contained, in mM: tricine-NaOH, pH 8.0, 25; $MgCl_2$, 5; Na_2HPO_4, 5; and chloroplasts. Chlorophyll concentration, 110 μg/ml.

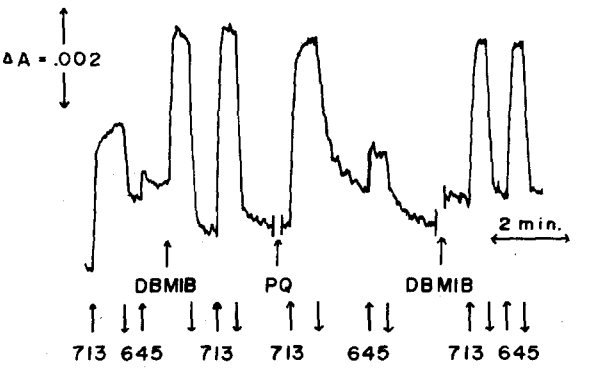

Fig. 2. Plastoquinone (0.15 mM) reversal of the DBMIB inhibition of the photoreduction of cytochrome *f* by 645 nm light. 2 μM DBMIB added first during 645 nm illumination; second addition of DBMIB (2 μM) in the dark as indicated. Chlorophyll concentration, 100 μg/ml; reaction medium as in fig. 1.

photoreduction of cytochrome *f* being relieved by plastoquinone (1.5×10^{-4} M added). A difference between the experiments shown in figs. 1 and 2 is that the chloroplasts in fig. 2 seem to have more endogenous reducing activity so that there is a rapid dark reduction of cytochrome *f* after 713 nm illumination.

Cytochrome *b*-559 is oxidized by 713 nm light and reduced by 645 nm actinic light in the presence of *p*-CF_3O-CCP (3 μM) and 25 μM FMN (fig. 3a). FCCP is added at uncoupling concentrations to enhance the amplitude of the cytochrome *b*-559 photooxidation [6], which is more dependent than the cytochrome *f* response on the coupling conditions [6–9]. After a dark interval, 645 nm light causes a partial reduction of the *b*-559 which becomes complete upon addition of the DBMIB (9×10^{-7} M). The DBMIB markedly inhibits the photooxidation induced by subsequent illumination with 713 nm light. The lower concentration of DBMIB needed for inhibition in this experiment is probably just a consequence of the chlorophyll concentration being 50 μg/ml instead of 100 μg/ml as in figs. 1, 2. The inhibitory effect of the DBMIB again appears to be due to an interaction with plastoquinone, as addition of plastoquinone (6×10^{-5} M) to the cuvette prior to addition of DBMIB again reverses the effect of DBMIB on *b*-559 photooxidation and photoreduction (fig. 3b). DBMIB also inhibits cyt. *b*-559 photooxidation in the absence of FCCP (data not shown). A difference spectrum for the increase in cytochrome *b*-559 photoreduction produced by DBMIB addition as in fig. 3a is shown in fig. 4. The peak is at 559 nm. The sign of the absorbance change does not reverse for cytochrome *f* in this experiment because of scattering changes which increase in amplitude as the wavelength separation from the 570 nm reference is increased.

The data presented above indicate that the site of action of the DBMIB inhibitor in non-cyclic electron flow is between cyt. *b*-559 and cytochrome *f* in the electron transport chain linking photosystem II to photosystem I. Reversal by plastoquinone A of the inhibitor effects on the cytochromes shown above, and on photophosphorylation shown previously [1, 2], indicates that plastoquinone A mediates electron flow from cytochrome *b*-559 to cytochrome *f*. Since it is estimated that the midpoint potential of plastoquinone A is about +110 mV [10], the above data support the conclusion that it is a low potential ($E_m < +100$ mV) form of cytochrome *b*-559 which is photooxidized by photo-

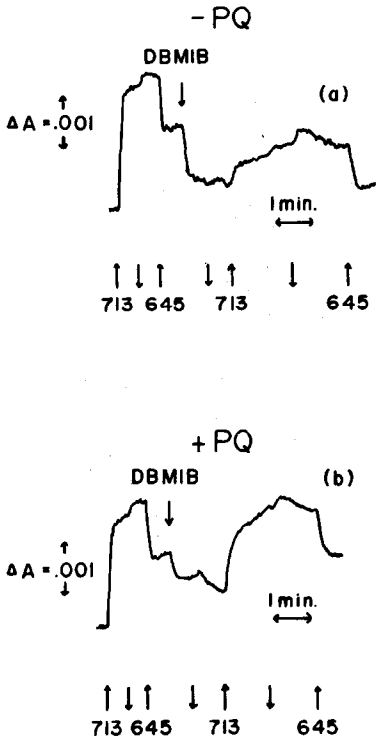

Fig. 3. Effect of DBMIB (0.9 μM) on the light-induced absorbance changes of cytochrome *b*-559 without (a) and with (b) prior 2 min incubation with 60 μM plastoquinone. DBMIB added during 645 nm illumination. Cytochrome *b* measured at 562 nm to avoid interference with cytochrome *f*; 570 nm reference. Chlorophyll concentration, 50 μg/ml; 25 μM FMN. 3 μM *p*-CF_3O-CCP was added to the reaction mixture of fig. 1, but phosphate was omitted.

system I [9]. Because the amplitude of the cyt. *b*-559 photooxidation by system I is enhanced by some uncouplers, the question exists as to whether the pathway from low potential *b*-559 through plastoquinone to system I operates in an electron transport pathway coupled to phosphorylation.

Acknowledgements

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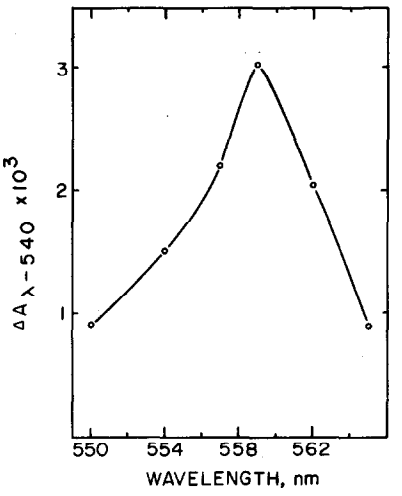

Fig. 4. Difference spectrum for the absorbance change caused by adding DBMIB to the suspension in the presence of 645 nm light as shown in figs. 3a, b. Reference wavelength, 570 nm; chlorophyll concentration, 100 μg/ml; 25 μM FMN; 3 μM *p*-CF_3O-CCP; reaction mixture as in fig. 1.

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