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POST-ILLUMINATION KINETICS OF CYTOCHROME f REDUCTION IN CHLOROPLAST THYLAKOID IN THE PRESENCE OF DIBROMOTHYMOQUINONE

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SUMMARY Addition of dibromothymoquinone (DBMIB) to isolated chloroplast thylakoids reduces cytochrome f in the dark. Reduced cytochrome f is oxidised when the thylakoids are illuminated, and is re-reduced in the subsequent darkness. The rate of re-reduction in the dark is faster after red (650 nm) illumination than after far red (713 nm) illumination. In the presence of DCMU or upon heat treatment or at high (>10uM) concentration of DBMIB the rate of dark reduction after red illumination becomes slower and equal to that after far red illumination, suggesting that photosystem II electron transfer at least upto plastoquinone facilitates DBMIB-mediated reduction of cytochrome f in the thylakoids. © 1985 Academic Press, Inc.

INTRODUCTION Dibromothymoquinone (DBMIB) has been widely used in photosynthesis research, particularly to study the kinetics of cytochrome redox reactions in chloroplasts (1 for review). In chloroplasts the oxidation of reduced plastoquinone is blocked by low concentration (1 uM) of DBMIB (2). Although the exact site of action of DBMIB is not known, it has been suggested that DBMIB acts at or close to the Rieske iron-sulfar center (3). At high concentration (>10 uM) DBMIB blocks electron transfer to plastoquinone (4) and also acts as an electron acceptor (5). Chain and Malkin (3) have reported that addition of DBMIB to chloroplast reduces cytochrome f, plastocyanin and P700 in the dark. Bose (6) has reported recently that DBMIB interacts with isolated cytochrome f and cytochrome c in solution in the dark leading to the reduction of cytochromes and formation of a complex between DBMIB and the cytochrome. In this paper the

Abbreviations: DBMIB, 2,5-Dibromo-3-methyl-6isopropyl-p-benzoquinone (dibromothymoquinone); DCMU, 3-(3′, 4′-dichlorophenyl)-1, 1-dimethylurea; HEPES, N′-2-Hydroxyethylpiperazine-N′-2-propanesulfonic acid; EDTA, Ethylenediaminetetra acetic acid; PQ, Plastoquinone; PQH_2, reduced plastoquinone; PS II, Photosystem II; PS I, Photosystem I.

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post-illumination kinetics of cytochrome f reduction in the chloroplast thylakoids in the presence of DBMIB have been examined.

MATERIALS AND METHODS Chloroplasts were isolated following Mills and Hind (7) from spinach grown in growth chamber. Washed leaves with mid-ribs removed were ground in a pre-chilled Waring blender for 7 seconds in 30 mM HEPES buffer (pH 7.6) containing 0.2 M sucrose and 50 mM NaCl. The macerate was passed through 8 layers of cheese cloth and the filtrate centrifuged at 3000xg for 5 minutes. The pellet was washed in EDTA buffer containing 0.5 mM EDTA and 4 mM Tricine-NaOH at pH 7.6 and centrifuged at 3000xg for 5 minutes. The pellet was resuspended again in EDTA buffer, incubated for 15 minutes and centrifuged at 9000xg for 10 minutes. The pellet was resuspended in a small volume of 0.1 M sucrose, 35 mM NaCl and 5 mM HEPES (pH 7.6).

Light-induced absorbance changes in thylakoids were measured at regulated temperature in a single sample split-beam spectrophotometer. Actinic illumination was provided from two slide projectors using interference filters of 650 nm (11 nm half band width) and 713 nm (5 nm half band width) for red and far-red illumination respectively. The intensities were 5.4 x 10^{-3} J $cm^{-2}sec^{-1}$ for red and 3.4 x 10^{-4} J $cm^{-2}sec^{-1}$ for far red illumination. DBMIB was initially obtained from Prof. A. Trebst and later prepared by brominatiion of thymoquinone following the method by Izawa et al (5). Solution of DBMIB was freshly prepared in 95% ethanol. In final assay mixture ethanol concentration never exceeded 1%. DBMIB solutions were prepared and the experiments were conducted in semi-darkness to avoid the possibility of light-induced reduction of DBMIB in solution.

RESULTS Figure 1A shows the typical red-far red effects on the redox reactions of cytochrome f in washed thylakoids. In red illumination (650nm) an initial oxidation by PS I was observed which was followed by a reduction by PS II and attaining a steady state in 5-10 seconds. On turning off the light a further reduction by electrons from the PQ pool was observed (8). In subsequent far red illumination (713 nm) a complete oxidation of cytochrome f was observed. As expected, upon far red illumination alone (Fig.1A) or red illumination in the presence of DCMU (Fig.1B) of the dark adapted thylakoids, only oxidation of cytochrome f was observed.

When DBMIB was added to these chloroplasts at 2 uM, most of cytochrome f became reduced in the dark. This was concluded from the observation that on illumination with either red or far-red light, only a rapid oxidation was observed (Fig.1C) the extent of which was about 100% of the total

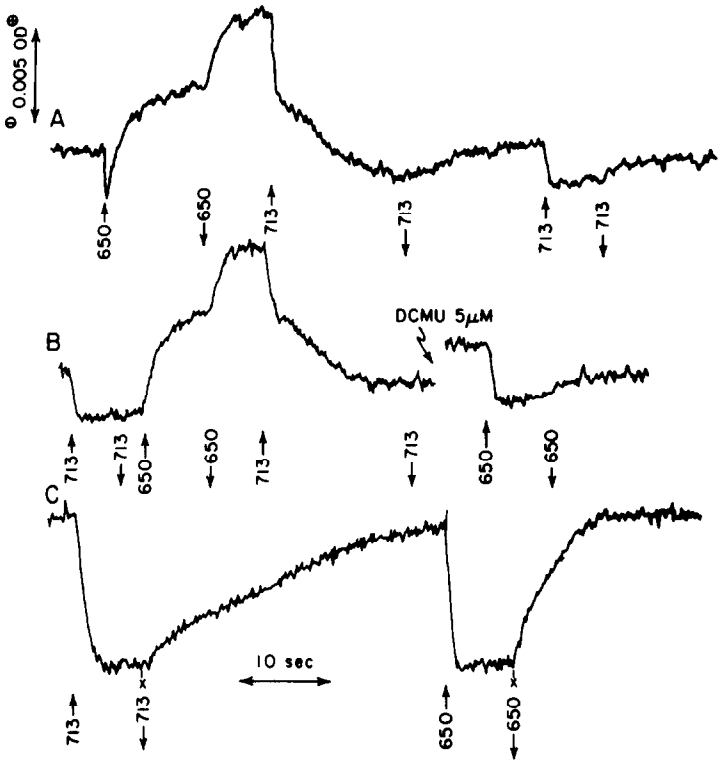

Fig.1. Light-induced absorbance changes monitored at 420 nm in chloroplasts thylakoids. The reaction mixture contained 5 mM HEPES (pH 7.6), 100 mM sucrose, 35 mM NaCl and 50 ug Chl/ml at 18°C. The light path was 0.5 cm. Upward arrows indicate turning on and downward arrows turning off of the actinic illumination. 650 and 713 stand for red and far red illumination. Traces A and B were for control chloroplasts and C with 3.3 uM DBMIB. Fresh samples of the stock preparation were used for each trace. All other conditions were described in Materials and Methods.

change observed in the control chloroplasts in Fig.1A and 1B. On turning the actinic light off, cytochrome f was reduced in the dark.

The rate of dark re-reduction (in the presence of DBMIB) following an illumination depended on the quality of illumination. The reduction was more rapid after red illumination than after far-red illumination (Fig.1C). However, in the presence of DCMU, which had no effect on the extent of oxidation during illumination or in the presence of high concentration (15 uM) DBMIB, the rate of re-reduction after red illumination slowed down to a magnitude similar to that after far-red illumination (data not shown). These observations are interpreted as follows. In the presence of low concentration of DBMIB electron transport proceeds at least upto plasto-

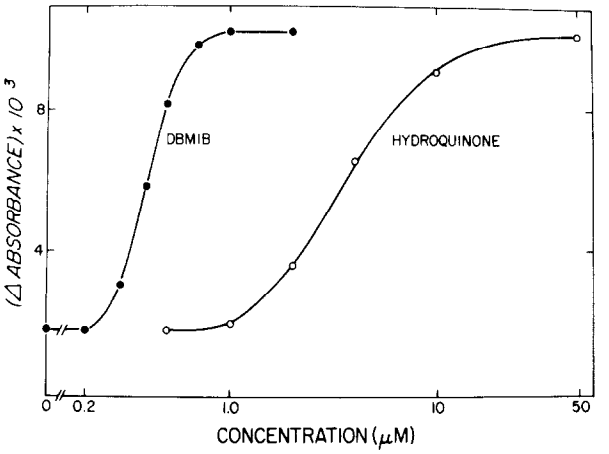

Fig.2. Light-induced absorbance changes at 420 nm in chloroplast thylakoids containing 5 uM DCMU and various concentrations of DBMIB or hydroquinone. Red (650 nm) light was used for actinic illumination. All other conditions were same as in Fig.1.

quinone (1). This electron transport or reduced plastoquinone itself facilitates DBMIB mediated re-reduction of cytochrome f. In the presence of DCMU or at high concentration of DBMIB the reduction of plastoquinone was blocked (9) and consequently the rate of dark re-reduction became independent of the preceding illumination.

The light minus dark difference spectrum in the presence of 2 uM DBMIB showed (data not shown) a peak around 420 nm indicating that the changes observed in Fig.1 were indeed due to cytochrome f. Figure 2 shows the effect of DBMIB concentration on the degree of reduction of cytochrome f. About 1 uM DBMIB was sufficient to reduce cytochrome f almost completely, while at least ten-fold higher concentration of hydroquinone was required for complete reduction. Moreover, the rate of dark re-reduction in the presence of hydroquinone was much slower (data not shown) than in the presence of DBMIB, suggesting that DBMIB, unlike hydroquinone, was not a typical reductant. In case of hydroquinone, as one would expect, the rate of re-reduction increased with the increase of hydroquinone concentration in a wide range; and was independent of the quality of the previous illumination and was insensitive to DCMU (data not shown).

DISCUSSION The reduction of cytochrome f on addition of DBMIB to thylakoids in the dark is apparently surprising because oxidized DBMIB was used in these experiments. Although DBMIB has more negative potential (+170 mV, ref 10) than that of cytochrome f, it is difficult to perceive how oxidised DBMIB can act as a direct reductant by donating an electron from itself. A comparison with hydroquinone as a reductant of cytochrome f also suggests that DBMIB-mediated reduction has a different mechanism. We have shown recently (6) that DBMIB forms a complex with cytochrome c in solution leading to the reduction of the heme. The presence of a catalytic amount of a reductant, like ascorbate, enhances the rate of reduction. In case of thylakoids also, addition of DBMIB reduces cytochrome f, and it appears that the presence of a reductant, presumably PQH_2 produced by PS II electron transfer, enhances the rate of reduction. It will be interesting to examine whether this similarity between in vitro and in vivo reduction is fortuitous or reflects a similar mechanism of DBMIB-mediated cytochrome reduction.

Whatever the mechanism of reduction is, this communication points out that the rate of reduction of cytochrome f in the dark followed by illumination is altered by the presence of DBMIB, which may be a consequence of DBMIB-mediated reduction of cytochrome f in the dark. The kinetics of cytochrome redox reactions in thylakoids in the presence of DBMIB are generally interpreted in terms of inhibition of electron transfer by DBMIB (11, 12). Our observations suggest that the reducing property of DBMIB, which may alter the kinetics of cytochrome f reduction, should be taken into account to interpret such results. The complex nature of interaction between DBMIB and thylakoid components has also been emphasized by Bendall (13).

REFERENCES

1. Izawa, S. (1977) in Encyclopedia of Plant Physiology, New Series (Trebst, A. and Avron, M., eds.) Vol.5, pp.266-282.
2. Trebst, A., Harth, E. and Draber, W. (1970) Z. Naturforsch. 25b, 1157-1159.

3. Chain, R.K. and Malkin, R. (1979) Arch. Biochem. Biophys. 197, 52-56.
4. Guikema, J.A. and Yocum, C.F. (1978) Arch. Biochem. Biophys. 189, 508-515.
5. Izawa, S., Gould, J.M, Ort, D.R., Felker, P. and Good, N.E. (1973) Biochim. Biophys. Acta. 305, 119-128.
6. Bose, S. (1985) Biochem. Biophys. Res. Comm. 127, 578-583.
7. Mills, J.D. and Hind, G. (1979) Photochem. Photobiol. 28, 67-73.
8. Avron, M. and Chance, B. (1966) in Currents in Photosynthesis (eds. J.B. Thomas and J.G. Goedheer) pp.455-463.
9. Trebst, A., Reimer, S., Draber, W. and Knops, H.J. (1979) Z. Naturforsch. 34C, 831-840.
10. Hauska, G., Reimer, S. and Trebst, A. (1974) Biochim. Biophys. Acta 357, 1-13.
11. Anderson, J.M., Than-Nyant and Boardman, N.K. (1973) Arch. Biochem. Biophys. 155, 436-444.
12. Koike, H., Satoh, K. and Katoh, S. (1978) Plant cell Physiol. 19, 1371-1380.
13. Bendall, D.S. (1982) Biochim. Biophys. Acta 683, 119-151.