THE EFFECT OF A PLASTOQUINONE ANTAGONIST ON THE OXIDATION–REDUCTION REACTIONS OF CHLOROPLAST CYTOCHROME b_{559}

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1. Introduction

Trebst and co-workers [1,2] have recently introduced 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) as an inhibitor of photosynthetic electron transport. The inhibition of both noncyclic and cyclic electron transport caused by this substituted benzoquinone can be reversed by the addition of plastoquinone A [2], making it likely that DBMIB inhibits electron transport by acting as a plastoquinone antagonist.

It became of interest to investigate the effect of this inhibitor in relation to the sequence of electron carriers for noncyclic electron transport proposed by this laboratory [3–6]. Of special interest was the effect of DBMIB on cytochrome b_{559}, which has been placed in an electron transport chain linking two short-wavelength (Photosystem II) photoacts. The result obtained with this new inhibitor are not consistent with the previous interpretation [3–6] of the role of cytochrome b_{559} in noncyclic electron transport. Possible reasons for this discrepancy are discussed.

2. Methods

Tris-treated spinach chloroplasts were prepared by a modification [4] of the method of Yamashita and Butler [7]. Sonicated spinach chloroplasts were prepared as described previously [3–5]. Chlorophyll was determined by the method of Arnon [8]. DBMIB was a gift of Professor A. Trebst and Dr. H. Böhme.

Light-induced absorbance changes were measured with a dual wavelength spectrophotometer as described previously [9]. The half-band width of the measuring beam was 2.0 nm. The 664-nm actinic light was isolated with an interference filter (10-nm half-band width) and had an intensity of 2.5×10^4 ergs/cm^2 per sec.

3. Results

Fig. 1. shows that 1 μM DBMIB had no effect on the rate or extent of the Photosystem II oxidation of cytochrome b_{559} in Tris-treated chloroplasts. This

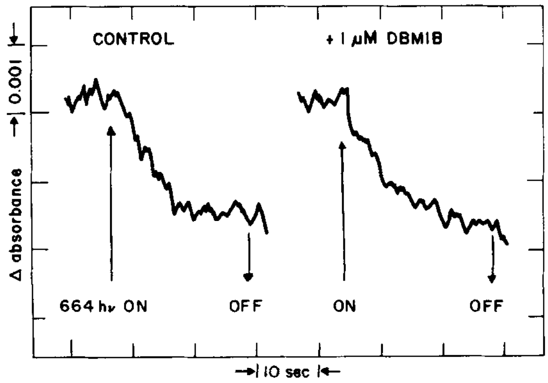

Fig. 1. Effect of DBMIB on the Photosystem II photooxidation of cytochrome b_{559} in Tris-treated chloroplasts (561 minus 570 nm). The reaction mixture contained (per 1.0 ml) Tris-treated spinach chloroplasts (equivalent to 75 μg chlorophyll) and the following, in μmoles: Tricine [*N*-Tris (hydroxymethyl) methylglycine] buffer (pH 8.2), 50; $MgCl_2$, 2; K_2HPO_4, 5; sodium ascorbate, 1; NADP, 1; spinach ferredoxin, 0.01; and, where indicated, DBMIB, 0.001.

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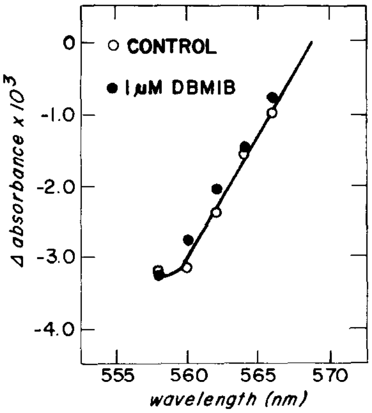

Fig. 2. Spectrum of the light-induced absorbance changes in Tris-treated chloroplasts in the presence and absence of DBMIB. Experimental conditions were as in fig. 1. Reference wavelength, 570 nm.

concentration of DBMIB gave 83% inhibition of electron transport from the substitute Photosystem II donor, hydroquinone [10], to NADP with the Tris-treated chloroplast preparation used for the cytochrome b_{559} photooxidation measurement. Fig. 2 shows the spectrum of cytochrome b_{559} photooxidation in Tris-treated chloroplasts in the presence and absence of 1 μM DBMIB. Absorbance changes below 560 nm caused by the photooxidation of cytochrome *f* [7] make it difficult to measure the complete spectrum of cytochrome b_{559} photooxidation. The form of cytochrome b_{559} that is photooxidized in these experiments is the high-potential form (E_0 = + 330 mV) [5, 11] as indicated by the fact that it is hydroquinone-reducible.

Fig. 3 shows that 1 μM DBMIB had no effect on the Photosystem II reduction of cytochrome b_{559} in sonicated chloroplasts. This concentration of DBMIB gave 92% inhibition of electron transport from water to NADP (in the presence of plastocyanin) with the sonicated chloroplasts used for the cytochrome b_{559} photoreduction measurement. Fig. 4 shows the spectrum of cytochrome b_{559} photoreduction in sonicated chloroplasts in the presence and absence of 5 μM DBMIB. The cytochrome b_{559} photoreduced in these experiments is again the high-potential form, as indicated by the fact that it is hydroquinone-reducible.

In addition to the DBMIB inhibition of NADP re-

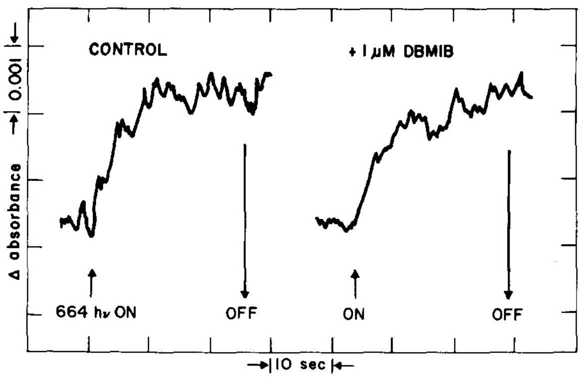

Fig. 3. Effect of DBMIB on the Photosystem II photoreduction of cytochrome b_{559} in sonicated chloroplasts (560 nm minus 570 nm). The reaction mixture contained (per 1.0 ml) sonicated spinach chloroplasts (equivalent to 75 μg chlorophyll) and the following, in μmoles: MES [2(*n*-morpholino) ethane sulfonic acid] buffer (pH 6.2), 50; $MgCl_2$, 2; K_2HPO_4, 5; potassium ferricyanide, 0.025; and, where indicated, DBMIB, 0.001.

duction with treated chloroplasts reported above, two further tests of the efficacy of the inhibitor were made. It gave marked inhibition of NADP reduction by H_2O in untreated chloroplasts (80 to 90% inhibition at 1 μM DBMIB). Also, DBMIB (1 μM) completely inhibited the Photosystem II reduction of cytochrome *f*, in agreement with the findings of Böhme and Cramer [12].

4. Discussion

The proposal by this laboratory of two Photosystem II light reactions in electron transport from water to NADP was based on measurements of two partial reactions of cytochrome b_{559} obtained with treated chloroplasts. The photooxidation of cytochrome b_{559} [3, 6, 9] was measured in chloroplasts which were Tris-treated to block electron flow from water [7]. The photoreduction of cytochrome b_{559} [5, 6] was studied in chloroplasts which had been sonicated to remove plastocyanin [4]. If the overall noncyclic electron transport pathway in untreated chloroplasts is simply the sum of these two partial reactions, at least one of the partial reactions should be inhibited by an inhibitor such as DBMIB that inhibits the overall process of electron transport from

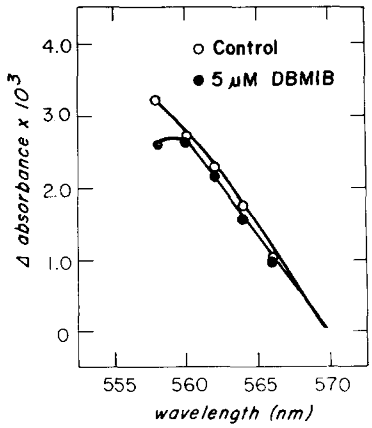

Fig. 4. Spectrum of the light-induced absorbance changes in sonicated chloroplasts in the presence and absence of DBMIB. Experimental conditions were as in fig. 2 except that DBMIB was present at a concentration of 0.005 μmoles/ml. Reference wavelength, 570 nm.

H_2O to NADP. However, neither the photooxidation of cytochrome b_{559} in Tris-treated chloroplasts nor the photoreduction of cytochrome b_{559} in sonicated chloroplasts is inhibited by DBMIB.

The possibility that some now unknown peculiarity affects the action of DBMIB on Tris-treated or sonicated chloroplasts cannot be excluded. Aside from that, these results raise the question whether the physiological role of cytochrome b_{559} in noncyclic electron transport can indeed be deduced from the sum of the two partial reactions. It is possible that sonication and Tris-treatment introduce reaction pathways for cytochrome b_{559} that are not typical of these in untreated chloroplasts.

Acknowledgement

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