MANGANESE STIMULATION OF OXYGEN CONSUMPTION IN CHLOROPLASTS WITH DIBROMOTHYMOQUINONE

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1. Introduction

Dibromothymoquinone (DBMIB)* at low concentrations (0.5 μM) is a very effective inhibitor of electron transport from photosystem II to photosystem I blocking on the reducing side of plastoquinone [1]. In the presence of electron acceptors such as ferricyanide which oxidizes DBMIB, a strictly photosystem II oxygen evolution is observed [2,3]. Further, DBMIB alone at high concentrations (20 μM) and at a pH above 8.0 will catalyze the photosystem II reaction with a consumption of oxygen and formation of H_2O_2 in a Mehler-type reaction [4]. The photosystem II reaction includes a low efficiency region for ATP synthesis.

This paper reports a previously undescribed characteristic of the DBMIB photosystem II reaction. When DBMIB is present with Mn^{2+}, an interaction takes place allowing oxygen consumption to be observed at low concentrations of DBMIB. This oxygen uptake reaction is coupled to the electron transport which drives ATP synthesis. The magnitude of the photosystem II reactions is comparable to the normal rate of the Hill reaction. The characteristics of this photosystem II reaction with Mn^{2+} and DBMIB are described.

**Abbreviations*: DCMU = 3-(3,4-dichlorophenyl)-1,1-dimethylurea. EDAC = 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide. DBMIB = 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone. DPC = diphenylcarbazide. EDTA = ethylene-diaminetetraacetic acid.

2. Materials and methods

Chloroplasts were isolated from spinach (*Spinacia oleracea* L.) [5] or from maize (*Zea mays* L.) as described earlier [6], and resuspended in a medium containing sucrose, 0.4 M; NaCl, 10 mM; Tricine, pH 7.8, 20 mM and 0.1% bovine serum albumin. Tris-(0.8 M) treated chloroplasts were prepared by a standard procedure [7], KCN-treated chloroplasts were prepared with 30 mM KCN, pH 7.8, 30 min. [8]. All chloroplasts were resuspended in the isolation buffer and the amount of chlorophyll was determined using the absorption coefficients of MacKinney [9].

Electron transport was followed by changes in oxygen concentration with the Clark electrode using an apparatus as reported before [6]. The reaction mixture with 20 μg chlorophyll per ml in a 5 ml chamber contained Tricine, 40 mM; NaCl, 30 mM; $MgCl_2$, 5 mM; NH_4Cl, 4 mM at pH 7.8.

ATP synthesis was assayed by a firefly luminescence technique [10] in the presence of 80 μg of chlorophyll contained in a medium consisting of sucrose, 0.4 M; $MgCl_2$, 2 mM; Na_2HPO_4, 1.3 mM; K_2HPO_4, 1.3 mM; ADP, 0.3 mM; and indicated electron acceptor at pH 7.8. The photometer for the assay consisted of an EMI 9536B photomultiplier tube, a Keithley 414 picoammeter and a Moseley 680 Strip-chart recorder. Firefly extract was from Nutritional Biochemicals. DBMIB was synthesized and recrystallized twice by a modification of the procedure reported in [1].

3. Results and discussion

Table 1 compares the characteristics of electron transport in isolated spinach chloroplasts catalyzed by ferricyanide or DBMIB in the presence of $MnCl_2$. As previously documented [1–4], DBMIB functions as an electron acceptor in the presence of ferricyanide and Mn^{2+} has little effect on the rate of oxygen evolution. When DBMIB is used at a concentration too low and a pH too low to catalyze a Mehler reaction by itself, a rapid rate of oxygen uptake results from addition of $MnCl_2$. This enzymatic, light dependent oxygen consumption is blocked by DCMU, and other inhibitors limiting electron transport between photosystem II and DBMIB site, but is not inhibited by compounds functioning between the DBMIB site and photosystem I, i.e. EDAC [11], and KCN-treatment [8]. Tris washing (0.8 M) blocks electron transport on the oxidizing side of photosystem II [7] and abolishes oxygen uptake with DBMIB, Mn^{2+} also.

These results suggest that DBMIB is the electron acceptor for a Mehler reaction and tests with catalase (5000 units) confirm the presence of H_2O_2 (data not shown).

Figs.1 and 2 illustrate the concentration of DBMIB and Mn^{2+} required for oxygen uptake. DBMIB is at a very high concentration compared to

Table 1
Conditions for oxygen uptake in the presence of DBMIB or $K_3Fe(CN)_6$

Reaction conditions	μmol O_2 evolved or consumed/mg Chl·h
$K_3Fe(CN)_6$ μM	204
$K_3Fe(CN)_6$, DBMIB 10 μM	180
$K_3Fe(CN)_6$, DBMIB, $MnCl_2$ 100 μM	186
$K_3Fe(CN)_6$, $MnCl_2$	180
DBMIB	60
DBMIB, $MnCl_2$	−366
DBMIB, $MnCl_2$, DCMU 0.4 μM	6
DBMIB, $MnCl_2$, EDAC 200 μM	−330
DBMIB, $MnCl_2$, KCN-treated	−351
DBMIB, $MnCl_2$, Tris-treated	30

Reaction procedure and chloroplast treatment as described under Methods and materials. Final conc. refers to the last listed compound and it was used at that conc. throughout.

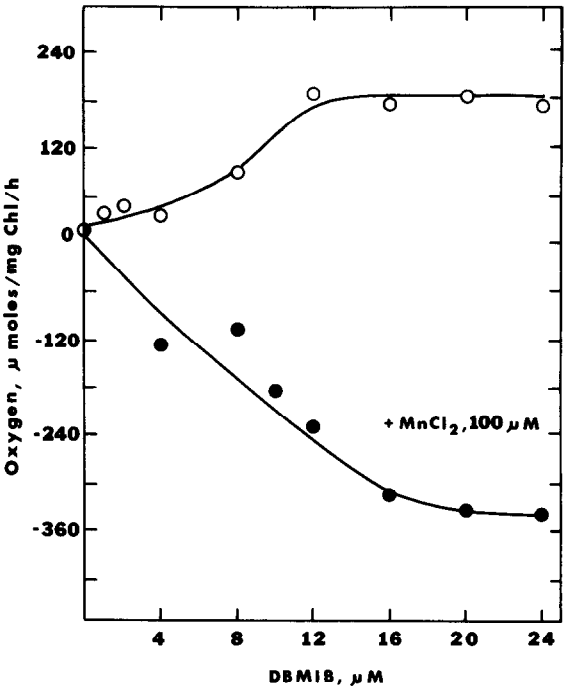

Fig.1. Effect of increasing conc. of DBMIB on oxygen uptake in spinach chloroplast with (•), or without (○), 100 μM $MnCl_2$.

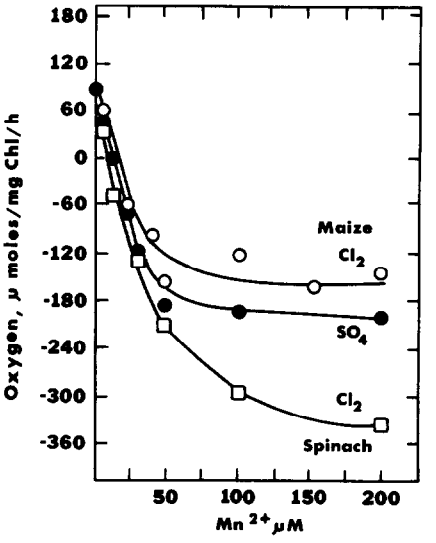

Fig.2. Effect of increasing conc. of Mn^{2+} on oxygen uptake in spinach or maize chloroplast with 10 μM DBMIB. Reaction conditions as in Methods and materials.

that which inhibits the methyl viologen Mehler reaction. The Mn^{2+} concentration for half-maximal rates of electron transport is approximately twice that required for DBMIB suggesting that perhaps two molecules of Mn^{2+} are required for each molecule of DBMIB. Other salts of Mn^{2+} were as effective as $MnCl_2$ (fig.2) but other divalent cations such as Ca^{2+} or Mg^{2+} could not replace Mn^{2+}.

Though the data presented in this paper is for spinach chloroplasts, we have duplicated the results with maize chloroplasts (fig.2) where this effect was first observed.

Mn^{2+} is not being oxidized in this reaction by photosystem II near the site for water splitting as it is in other reactions [12,13] since oxidation of Mn^{2+} to Mn^{3+} could not be observed with the procedure of Ben-Hayyim and Avron [12].

A further simple indication of the importance of Mn^{2+} in this reaction is that the chelator EDTA which binds Mn^{2+}, completely eliminates the DBMIB, Mn^{2+}

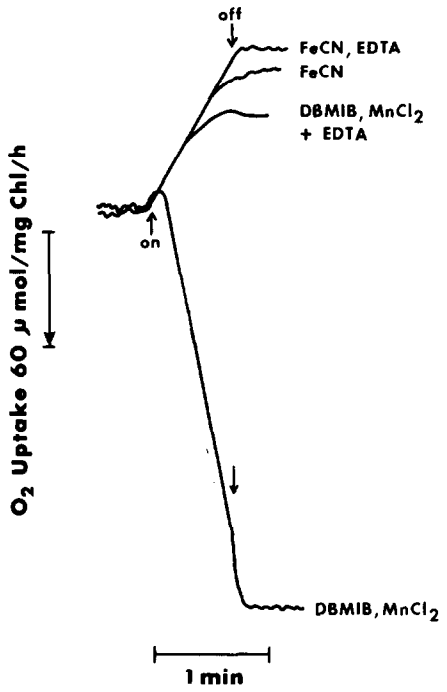

Fig.3. Rate of oxygen change with ferricyanide (100 μM) or DBMIB (10 μM), and Mn^{2+} (100 μM) in the presence or absence of 0.4 mM EDTA. Reaction conditions as in Methods and materials.

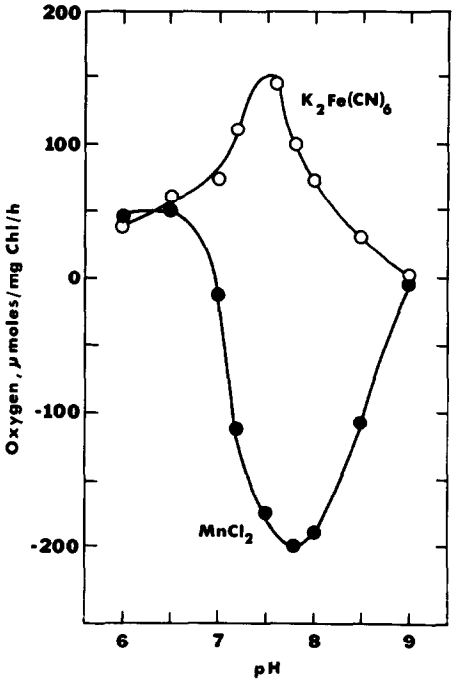

Fig.4. pH Optima for oxygen change with DBMIB and ferricyanide, (○); or DBMIB and $MnCl_2$, (●). Other conditions as in Methods and materials.

oxygen uptake while not altering oxygen evolution with ferricyanide (fig.3).

Fig.4 shows that the pH optimum (about 7.5) for DBMIB, Mn^{2+} oxygen uptake is similar to that for DBMIB, ferricyanide oxygen evolution. One difference which may be of importance is that oxygen uptake begins abruptly at or above pH 7.0. (Note that oxygen evolution occurs with both acceptors below pH 7.0). Mn^{2+} in alkaline solution (but not in acidic solutions) can exist as a hydroxide which is readily oxidized. We could speculate that DBMIB promotes the formation or oxidation of that hydroxide. No matter what the interaction may be, it is clear that other types of electron acceptor or cations do not interact in the same special way that DBMIB and Mn^{2+} do.

An important question now is whether electron flow with Mn^{2+} and DBMIB is coupled to phosphorylation. Table 2 shows ATP synthesis with DBMIB and $MnCl_2$ which requires light, requires ADP, and is eliminated by the uncoupler gramicidin. Synthesis requires Mn^{2+} but proceeds to some extent when DBMIB is omitted. These rates of ATP synthesis are

Table 2
Photophosphorylation with DBMIB and $MnCl_2$

Reaction conditions	μmol/mg Chl·h
DBMIB, Mn^{2+}	22
DBMIB, Mn^{2+}, dark	2
DBMIB only	3
Mn^{2+} only	10
DBMIB, Mn^{2+}, −ADP	0
DBMIB, Mn^{2+}, gramicidin, 0.1 mM	1

Assay procedure as under Methods and materials. Concentration of DBMIB was 10 μM, $MnCl_2$ (100 μM).

similar to those reported for photosystem II with DBMIB [4].

It has long been observed that Mn^{2+} stimulated the native Mehler reaction [1] and it is suggested here that Mn^{2+} stimulates oxygen uptake as well with this electron acceptor which only poorly catalyzes a Mehler reaction by itself.

This reaction could provide a good measure of photosystem II activity since it operates at a high rate and avoids the possible multiple effects of having a second acceptor present (ferricyanide).

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