Mechanism of Action of the Herbicide 4,6-Dinitro-*o*-cresol in Photosynthesis

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The herbicide 4,6-dinitro-*o*-cresol inhibits electron transport to ferricyanide and non-cyclic photophosphorylation for 50% at about 15 μM. At higher concentrations the photosystem I dependent Mehler reaction ascorbate/dichlorophenolindophenol to methyl viologen is stimulated, while cyclic photophosphorylation is inhibited. The herbicide thus is an inhibitory uncoupler.

Although the chemical structure of 4,6-dinitro-*o*-cresol is different from that of the diuron-type herbicides, its site and mechanism of action is similar. Both 4,6-dinitro-*o*-cresol and diuron inhibit electron transport between the primary electron acceptor of Photosystem II and the plastoquinone pool. This causes a closing of the reaction centers of Photosystem II. The interaction with the inhibited molecule however is different for the two herbicides.

Introduction

4,6-Dinitro-*o*-cresol (DNOC) is widely used as a contact herbicide controlling broadleaf weeds in monocotyledonous crops. Because its chemical structure is very similar to that of 2,4-dinitrophenol, one of its modes of action is believed to be uncoupling of oxidative phosphorylation. However, after application its action starts very rapidly, leaving doubt that uncoupling of oxidative phosphorylation is an important mechanism of action.

Kerr and Wain [1] reported that DNOC inhibits the ferricyanide Hill reaction in isolated chloroplasts. Expecting an effect of DNOC on photosynthesis we have started investigations on the influence of this herbicide on various photoreactions in isolated chloroplasts. We have found that DNOC inhibits the Hill reaction and non-cyclic photophosphorylation. However, at higher concentrations of DNOC, the Photosystem I dependent electron transport is stimulated, due to uncoupling.

While we do not think that the effect of DNOC on photosynthesis is its primary phytotoxic mode of action, its mechanism of inhibition of photosynthesis is important because its chemical structure is very different from that of the DCMU-type herbicides. Therefore, its effects on photosynthesis are compared with those of DCMU.

Abbreviations: DBMIB, 2,5,dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DNOC, 4,6-dinitro-*o*-cresol; DTE, dithioerythritol; PMS, phenazine methosulfate.

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Materials and Methods

Spinach (*Spinacia oleracea* L. Nobel) and peas (*Pisum sativum* var. Rondo) were grown in a growth chamber at 20 °C. Broken chloroplasts were isolated as described before [2]. Electron transport was measured with a Gilson oxygraph as described earlier [3]. Photophosphorylation was determined from the rate of H^+ consumption as described by Dilley [4] according to the equation:

$$Mg - ADP^{1-} + 2\,Pi^{2-} + n\,H^+ \rightarrow Mg - ATP^{2-} + H_2O$$

where $n = 0.96$ at pH 8 in the presence of Mg^{2+} [5]. Light-induced pH changes were detected with a combination pH electrode connected to a Corning-Eel Model 12 Research pH meter and a recorder. The pH changes were calibrated by adding 10 μl 0.01 N HCl to the reaction medium. Reactions were run in a thermostated, 2 ml reaction vessels at 25 °C and at saturating light intensity.

Results and Discussion

The Hill reaction in isolated chloroplasts with ferricyanide as an electron acceptor is strongly inhibited by DNOC (Fig. 1). Non-cyclic photophosphorylation coupled to electron transport from water to methyl viologen is inhibited by DNOC to the same extent (Fig. 1). This suggests a common site of inhibition, *i. e.* in the electron transport chain between photosystem II and I.

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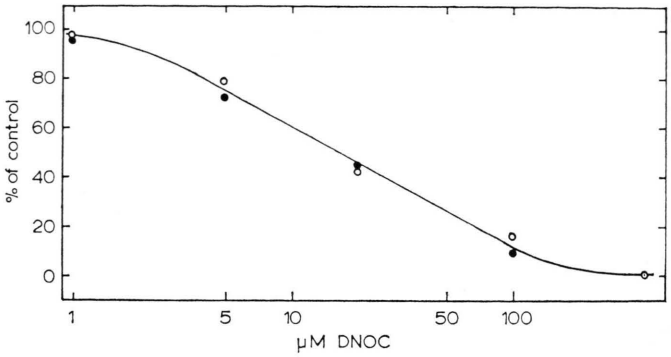

Fig. 1. Inhibition of the $H_2O \rightarrow$ ferricyanide Hill reaction (○) and non-cyclic photophosphorylation (●) by DNOC. For the Hill reaction the medium contained in 2 ml: 50 mM tricine-NaOH (pH 7.6), 0.3 M sorbitol, 5 mM $MgCl_2$, 5 mM NH_4Cl, 0.5 mM ferricyanide and broken chloroplasts, containing 50 μg chlorophyll; control activity was about 260 μmol O_2 per mg chlorophyll per hour. The medium for non-cyclic photophosphorylation contained in 2 ml: 100 mM KCl, 5 mM $MgCl_2$, 100 μM methyl viologen, 1 mM ADP, 1 mM K_2HPO_4 and broken chloroplasts, equivalent to 50 μg chlorophyll. The reactions were started at pH = 8; control activity was about 310 μmol ATP per mg chlorophyll per hour.

Photosystem I dependent electron transport from ascorbate/DCIP to methyl viologen is not inhibited by DNOC. Instead, at higher concentration the electron transport rate is stimulated (Fig. 2). Since

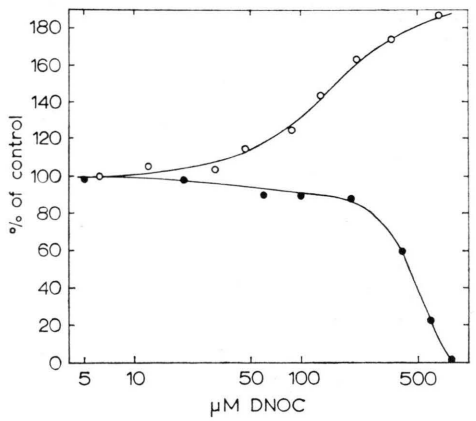

Fig. 2. Effects of DNOC on the ascorbate/DCIP → methyl viologen Mehler reaction (○) and cyclic photophosphorylation (●). For the Mehler reaction the medium contained in 2 ml: 50 mM tricine-NaOH (pH 7.6), 0.3 M sorbitol, 5 mM $MgCl_2$, 10 μM methyl viologen, 2 mM DTE, 50 μM DCMU, 0.2 mM DCIP, 6 mM sodium ascorbate and broken chloroplasts, containing 50 μg chlorophyll; control activity was about 170 μmol O_2 per mg chlorophyll per hour. The medium for cyclic photophosphorylation contained in 2 ml: 100 mM KCl, 5 mM $MgCl_2$, 20 μM PMS, 1 mM ADP, 1 mM K_2HPO_4 and broken chloroplasts equivalent to 50 μg chlorophyll. The reactions were started at pH = 8; control activity was about 510 μmol ATP per mg chlorophyll per hour.

cyclic photophosphorylation is inhibited at the same concentration range (Fig. 2) this is an uncoupling. These results confirm earlier results [3], which showed that the electron transport from ascorbate/DCIP to methyl viologen is stimulated by DNOC in the absence, but not in the presence of an uncoupler. Electron transport with ascorbate/TMPD as electron donor is not coupled to photophosphorylation [6]. This type of electron transport is not affected by DNOC [3].

Because DNOC inhibits electron transport at low concentrations and uncouples at higher ones, it is a typical example of an inhibitory uncoupler, according to the classification of Moreland [7].

A more detailed study [3] of the inhibition of electron transport by DNOC revealed that the silicomolybdate Hill reaction in the presence of DCMU is not influenced by DNOC. This means that DNOC inhibits electron transport beyond the site of electron accepting of silicomolybdate, which is the primary electron acceptor Q [8, 9]. Since DNOC does inhibit the phenylenediamine-mediated photoreduction of ferricyanide in the presence of DBMIB, it must act between Q and plastoquinone. This is also the site of inhibition of DCMU.

Most herbicidal inhibitors of photosystem II dependent electron transport share the common chemical structural element $-CX-NH-$, in which X is O or N [10, 11]. This was revised by Trebst and Harth [12] into $-CX-\overline{N}=$, in which the free electron pair at the nitrogen is of large importance. Because DNOC does not have this basic chemical structure we have further studied effects of this herbicide on various photoreactions in isolated chloroplasts and compared them with those of DCMU [2]. We have found that the percent inhibition of the ferricyanide Hill reaction at a given concentration of either DNOC or DCMU is the same at various light intensities. At very low light intensity the percent inhibition becomes larger. Also in whole algae the percent inhibition of oxygen evolution by DCMU is higher at light limiting intensities compared to saturating ones [13]. This means that these herbicides affect both photochemical and enzymatic dark reactions. The chlorophyll a fluorescence induction is influenced by DNOC in the same characteristic way as it is by DCMU: both herbicides cause a faster rise in fluorescence yield than in control chloroplasts, although a higher concentration of DNOC is required for the same effect. The faster

rise is explained by preventing the reoxidation of the reduced primary acceptor Q [14]. This confirms our earlier conclusion that both herbicides inhibit electron transport between Q and plastoquinone. Since Q remains in its reduced state the reaction centers are closed. In this way the herbicides affect photochemical reactions. Although the chemical structure of DNOC is completely different from that of DCMU, its site and mechanism of inhibition is similar to that of DCMU.

We have also demonstrated that the concentration of DCMU causing 50% inhibition of oxygen evolution decreases with decreasing chloroplast (and thus of chlorophyll) concentration [2]. With DNOC, the relative decrease is much less than with DCMU. From Hill plots we calculated that there appears to be a cooperative binding of DCMU with two binding sites at the inhibited molecule and a noncooperative binding of DNOC with only one binding site.

After treatment of isolated chloroplasts with trypsin inhibition of the Hill reaction by DCMU is lost [15]. Trypsin treatment also induces loss of inhibition by DNOC [16]. From these results we concluded that trypsin treatment makes Q accessible for ferricyanide, and that the site of inhibition by DNOC is beyond Q.

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