Two Sites of Inhibition of the Photosynthetic Electron Transport Chain by the Herbicide Trifluralin

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The effect of trifluralin on the photosynthetic electron transport has been investigated by oxygen evolution and thermoluminescence measurements. The results confirm the earlier observations that trifluralin at low concentrations blocks electron transport between the two photosystems probably at the same site as DBMIB does. At higher concentrations however, trifluralin inhibits the reaction from $H_2O \rightarrow p$BQ also and affects the thermoluminescence of chloroplasts in a manner similar to DCMU. These results suggest that trifluralin has a second inhibitory site therefore the use of trifluralin as a specific inhibitor of electron transport has to be questioned.

Introduction

The biochemical mode of action of trifluralin was first investigated by Moreland *et al.* [1]. They demonstrated that trifluralin inhibits the Hill reaction, but non-cyclic phosphorylation and PMS-cyclic phosphorylation was only slightly effected by this herbicide. Recently it was shown that the site of action of trifluralin is located between the two photosystems and it is distinctly different from that of DCMU [2]. Since the data obtained by trifluralin are reminiscent of the effects observed with DBMIB [2] it is accepted that trifluralin inhibits electron transport on the oxidizing side of plastoquinone [3, 4].

In this work we report that trifluralin has two inhibitory sites in the photosynthetic electron transport.

Materials and Methods

Intact chloroplasts were obtained from enzymatically isolated protoplasts of maize and resuspended in a medium containing 0.4 M d-sorbitol, 10 mM NaCl, 1 mM $MnCl_2$, 5 mM $MgCl_2$, 2 mM EDTA and

*Abbreviations: p*BQ, *p*-benzoquinone; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCIP, dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DMQ, 2,5-dimethyl-*p*-benzoquinone, FeCy, ferricyanide; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethane sulphonic acid; MV, methylviologen; PMS, phenazine methosulphate.

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50 mM HEPES (pH 7.5) [5]. Oxygen evolution and consumption were measured using a Clark-type electrode as described previously [6]. The measurement of thermoluminescence was carried out in the temperature region from $-80\,°C$ to $+80\,°C$ using equipment similar to that in [6]. Samples were illuminated with white light at 10 W/m² for 5 min during continuous cooling from $+20\,°C$ to $-80\,°C$, then heated at a constant rate of 10 °C/min to measure thermoluminescence. Samples contained 55% glycerol to prevent the distortion of the glow curves by the solid-liquid phase transition of water [6].

Results and Discussion

The effect of trifluralin on the photosynthetic electron transport chain is shown in Fig. 1. Electron flow from $CDIP_{red} \rightarrow MV$ was not inhibited even by very high trifluralin concentrations, but the electron flow from $H_2O \rightarrow MV/FeCy$ was strongly inhibited confirming earlier observations [2]. In our experiments the reaction from $H_2O \rightarrow p$BQ was not inhibited by trifluralin in the concentration range from 0.5 µM to 30 µM. Higher concentration of trifluralin strongly inhibited electron flow from $H_2O \rightarrow p$BQ and complete inhibition was obtained over 300 µM (Fig. 1). This result is at variance with the observation of Robinson *et al.* who obtained only about 50% inhibition of electron transport even after the addition of 300 µM trifluralin and the site II phosphorylation ($H_2O \rightarrow DMQ$) was not inhibited at all [2].
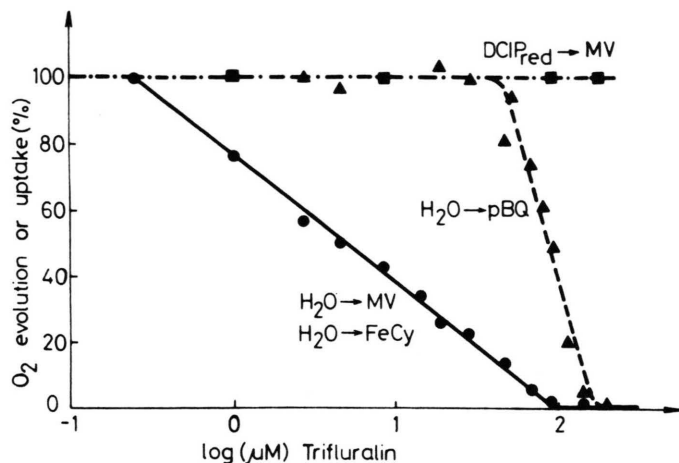
Fig. 1. Effects of trifluralin on photosynthetic electron transport. The rate of O_2 evolution and uptake versus the concentration of trifluralin is given as a percentage of the values obtained with untreated controls. Samples contained 50 µg chlorophyll in 3 ml.
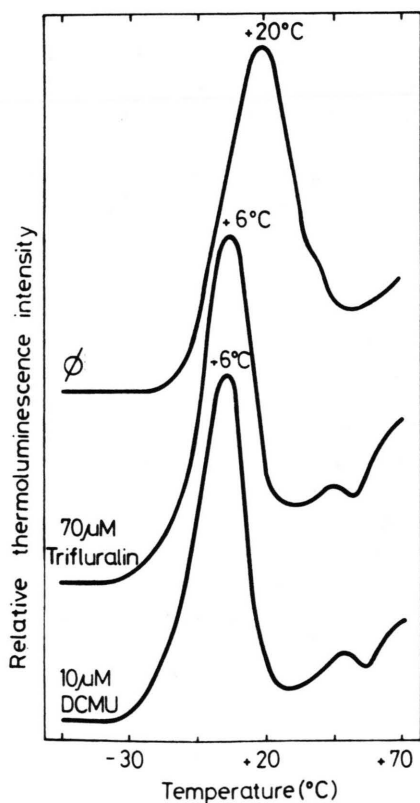

Fig. 2. Effects of trifluralin and DCMU on the thermoluminescence of isolated chloroplasts. Samples contained 80 µg Chl/ml. Details of measurements are given in Materials and Methods.

The fact that lower concentrations of trifluralin inhibited the reaction from $H_2O \rightarrow MV$ but not the reactions from $H_2O \rightarrow p$BQ and $DCIP_{red} \rightarrow MV$ suggests an inhibitory site of trifluralin behind the action site of pBQ, that is between the two photosystems, probably on the oxidizing side of plastoquinone, as it was suggested previously [2–4]. At higher trifluralin concentrations than 30 µM the definite inhibition of the reaction from $H_2O \rightarrow p$BQ indicates the existence of a second inhibitory site of the herbicide before the action site of pBQ.

Since trifluralin inhibits the electron flow in photosystem II also, we tried to localize its sites of action by thermoluminescence measurements. Thermoluminescence originates in photosystem II and the bands of the glow curve can be related to the different components of the electron transport chain [7]. This method offers a good opportunity to localize the action sites of this inhibitor. The untreated chloroplasts exhibit a main band at about +20 °C (Fig. 2). After DCMU treatment of chloroplasts the main band at +20 °C is replaced by a new band appearing at +6 °C. After addition of low concentrations of trifluralin, where the electron flow from $H_2O \rightarrow p$BQ was not inhibited, the glow curves were similar to that of the control. Although the reaction from $H_2O \rightarrow MV$ was strongly inhibited in this concentration range, this effect was not reflected in the thermoluminescence since electron

transport components located behind the plastoquinone pool do not participate in the generation of thermoluminescence [7]. However, at higher concentrations ($> 30\,\mu\text{M}$), where the reaction from $H_2O \rightarrow p\text{BQ}$ was strongly inhibited, the herbicide treatment of chloroplasts produced an effect on thermoluminescence similar to that obtained with DCMU (one main band at $+6\,^\circ\text{C}$) (Fig. 2). This result also confirms that one more action site of trifluralin exists and it is probably similar to the inhibitory site of DCMU.

Summarizing our results we can state that trifluralin has two inhibitory sites in the electron transport chain and therefore the use of trifluralin as a specific inhibitor of electron transport has to be questioned.

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