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 $H_2O \rightarrow pBQ$ 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 affected 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 $0.4 \, \text{mM} \, \text{d-sorbitol}, 10 \, \text{mM} \, \text{NaCl}, 1 \, \text{mM} \, \text{MnCl}_2, 5 \, \text{mM} \, \text{MgCl}_2, 2 \, \text{mM} \, \text{EDTA}$ and $50 \, \text{mM HEPES (pH 7.5)}$ [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 $-80^\circ \text{C}$ to $+80^\circ \text{C}$ using equipment similar to that in [6]. Samples were illuminated with white light at $10 \, \text{W/m}^2$ for 5 min during continuous cooling from $+20^\circ \text{C}$ to $-80^\circ \text{C}$, then heated at a constant rate of $10 \, \text{°C/min}$ 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$_{\text{red}} \rightarrow \text{MV}$ was not inhibited even by very high trifluralin concentrations, but the electron flow from $H_2O \rightarrow \text{MV/FeCy}$ was strongly inhibited confirming earlier observations [2]. In our experiments the reaction from $H_2O \rightarrow pBQ$ was not inhibited by trifluralin in the concentration range from $0.5 \, \mu\text{M}$ to $30 \, \mu\text{M}$. Higher concentration of trifluralin strongly inhibited electron flow from $H_2O \rightarrow pBQ$ and complete inhibition was obtained over $300 \, \mu\text{M}$ (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 $300 \, \mu\text{M}$ trifluralin and the site II phosphorylation ($H_2O \rightarrow \text{DMQ}$) was not inhibited at all [2].
The fact that lower concentrations of trifluralin inhibited the reaction from $\text{H}_2\text{O} \to \text{MV}$ but not the reactions from $\text{H}_2\text{O} \to p\text{BQ}$ and $\text{DCIP}_{\text{red}} \to \text{MV}$ suggests an inhibitory site of trifluralin behind the action site of $p\text{BQ}$, 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 $\mu$M the definite inhibition of the reaction from $\text{H}_2\text{O} \to p\text{BQ}$ indicates the existence of a second inhibitory site of the herbicide before the action site of $p\text{BQ}$.

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 $+20^\circ\text{C}$ (Fig. 2). After DCMU treatment of chloroplasts the main band at $+20^\circ\text{C}$ is replaced by a new band appearing at $+6^\circ\text{C}$. After addition of low concentrations of trifluralin, where the electron flow from $\text{H}_2\text{O} \to p\text{BQ}$ was not inhibited, the glow curves were similar to that of the control. Although the reaction from $\text{H}_2\text{O} \to \text{MV}$ 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 μM), where the reaction from H₂O → pBQ was strongly inhibited, the herbicide treatment of chloroplasts produced an effect on thermoluminescence similar to that obtained with DCMU (one main band at +6 °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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