The Effect of Bialaphos on Ammonium-Assimilation and Photosynthesis

II. Effect on Photosynthesis and Photorespiration

Christine Ziegler and Aloysius Wild

Institut für Allgemeine Botanik der Johannes Gutenberg-Universität, Saarstraße 21, D-6500 Mainz, Bundesrepublik Deutschland

Z. Naturforsch. 44c, 103–108 (1989); received September 23, 1988

Ammonium-Accumulation, Bialaphos, Phosphinothricin, Photorespiration, Photosynthesis

The application of bialaphos (phosphinothricyl-alanyl-alanine) effects a quick photosynthesis inhibition under atmospheric conditions (400 ppm CO₂, 21% O₂). However, under conditions (1000 ppm CO₂, 2% O₂) under which photorespiration cannot occur there is no photosynthesis inhibition. In the previous investigation it could be shown that bialaphos splits in plants into phosphinothricin and alanine. The inhibition of glutamine synthetase through freed phosphinothricin results in an NH₄⁺-accumulation and a decrease in glutamine. With the addition of glutamine, photosynthesis inhibition by bialaphos can be reduced. An NH₄⁺-accumulation takes place under atmospheric conditions as well as under non-photorespiratory conditions; though in the latter case, in less amounts. After adding glutamine and other amino acids the NH₄⁺-accumulation increases especially. This indicates that NH₄⁺-accumulation cannot be the primary cause for photosynthesis inhibition by bialaphos. The investigations indicate that for the effectiveness of either bialaphos or phosphinothricin, a process in connexion with photorespiration plays a considerable role. The glyoxylate transamination in photorespiration could be inhibited, which results probably on a glyoxylate accumulation. Corresponding investigations showed inhibition of photosynthesis as well as a direct inhibition of RuBp-carboxylase with glyoxylate.

Introduction

Two additional alanine residues distinguish bialaphos from PPT (glufosinate). Our previous investigations into the effect of bialaphos and PPT on NH₄⁺-assimilation enzymes show that, through cleavage of the alanine residues in bialaphos upon application to plants, PPT is also the only active herbicidal component in bialaphos [1].

PPT inhibits photosynthesis under photorespiratory conditions (400 ppm CO₂, 21% O₂). Under non-photorespiratory conditions (1000 ppm CO₂, 2% O₂) no inhibition of photosynthesis occurs, although even under these conditions a notable quantity of NH₂⁺ is accumulated through GS inhibition [2]. In addition to this NH₂⁺-accumulation, a process connected with photorespiration appears to play an important role in the herbicidal effectiveness of PPT.

The purpose of this investigation was to examine the effect of bialaphos on photosynthesis and photorespiration. In addition, differences in effectiveness of bialaphos and PPT were investigated.

Materials and Methods

Plant material

Sinapis alba plants were grown as described [3].

Chemicals

The Na⁺-salt of bialaphos (phosphinothricyl-alanyl-alanine) was supplied by Hoechst AG (Frankfurt/Main, West Germany) [1].

Measurement of photosynthesis

Photosynthesis was measured as the CO₂ fixation rate by means of an infrared gas analyser as described [4]. The experiments were performed on entire plants and on excised primary leaves. The excised primary leaves could be fed with bialaphos and other compounds via the petiole.

Determination of the content of free ammonium

The assay is based on a reaction between NH₄⁺ and phenol in the presence of sodium hypochlorite with formation of a blue phenylquinone monimine complex [5]. The concentration can be determined...
spectrophotometrically at a wavelength of 625 nm. Addition of sodium nitroprusside as catalyzer intensifies this reaction.

*Measurement of the electron transport rate (Hill reaction)*

The isolation of chloroplasts was achieved according to [6].

The electron transport rate of broken chloroplasts was measured as photoreduction of the electron acceptor DCPIP. The final concentrations of the reaction medium contained 50 mM tricine, pH 8.3, 50 mM KCl, 5 mM MgCl₂, 5 mM KH₂PO₄, pH 8.3, 30 mM DCPIP, 10.9 ml H₂O. The reaction medium was distributed into two test-tubes. One test-tube was illuminated for 60 s and the other one was stored in darkness for the same time. The DCPIP reduction was determined spectrophotometrically by 605 nm (ε = 21 cm² . µmol⁻¹).

*Determination of RubP-carboxylase*

The RubP-carboxylase assay in a crude enzyme extract was described by Braun et al. [7].

*Measurement of catalase activity*

The method of catalase determination by means of the oxygen electrode was described by Del Rio et al. [8].

**Results**

**Effect of bialaphos on photosynthesis**

*Measurement with entire plants.* Entire Sinapis alba plants were sprayed with either bialaphos solution or PPT solution and placed in darkness overnight. Then the photosynthetic rate was measured. After 15 min a significant decrease of photosynthesis occurred in bialaphos treated as well as PPT treated plants (Fig. 1). The measurements were taken under atmospheric conditions (400 ppm CO₂, 21% O₂).

However, under non-photorespiratory conditions (1000 ppm CO₂, 2% O₂) there were no photosynthetic inhibitions in plants treated with either bialaphos or PPT (Fig. 2).

*Measurement on excised primary leaves.* The photosynthetic rates of bialaphos treated excised primary leaves were measured under various concentrations of CO₂ and O₂ (Fig. 3). A 1 mM bialaphos solution was applied through the petiole.

Under atmospheric conditions (400 ppm CO₂, 21% O₂) a strong inhibition of photosynthesis occurred very rapidly after application of herbicide solution. About 80 min after application, photosynthesis was almost completely inhibited.

However, under non-photorespiratory conditions (1000 ppm CO₂, 2% O₂) no inhibition by bialaphos was detected.

Further measurements of photosynthesis were performed under the following conditions after adding bialaphos: 1000 ppm CO₂/21% O₂; 400 ppm CO₂/
10% O₂; 400 ppm CO₂/2% O₂. The degree of intactness of photosynthesis can be taken as an indicator for the suppression of photorespiration. The greatest decrease in photorespiration could be reached under 400 ppm CO₂ and 2% O₂. Total suppression of photorespiration could be achieved through simultaneous increase of CO₂ content to 1000 ppm and decrease of O₂ content to 2%.

**Effect of glutamine on bialaphos toxicity**

Measurements were carried out, in which different glutamine concentrations were applied in addition to bialaphos (1 mM).

The measurements of primary leaves showed that, with increasing glutamine concentrations, the photosynthesis inhibition through bialaphos was reduced (Fig. 4). The measurements were taken under atmospheric conditions (400 ppm CO₂, 21% O₂).

**Effect of other amino acids on bialaphos toxicity**

Measurements of excised primary leaves were carried out in which asparagine, glutamate and glycine were added to the bialaphos solution. The amino acids showed a slight decrease in inhibition of the photosynthesis with bialaphos.

Glutamine however, causes the strongest decrease in inhibition of photosynthesis with bialaphos (Table I).

**Ammonium-accumulation after treatment with bialaphos**

Immediately after the gas exchange measurements, the NH₄⁺-content of bialaphos treated primary leaves was measured. The treated leaves showed a clear increase of NH₄⁺-concentration (Fig. 5). There was an NH₄⁺-accumulation under normal atmospheric conditions (400 ppm CO₂, 21% O₂) as well as under non-photorespiratory conditions (1000 ppm CO₂, 2% O₂). After addition of various amino acids, there was a particularly high NH₄⁺-accumulation.

**Effect of glyoxylate on photosynthesis**

Instead of bialaphos, glyoxylate was given to the excised primary leaves. The glyoxylate concentrations consisted to 10 mM and 30 mM and the measurements took place under atmospheric conditions (400 ppm CO₂, 21% O₂).

With 10 mM glyoxylate, the photosynthesis inhibition was only slight. With 30 mM glyoxylate a strong inhibition occurred (Table II).
Effect of bialaphos on the electron transport rate

The electron transport rate was measured after exposing bialaphos treated mustard plants to light for different periods of time.

Table I. The effect of different amino acids on bialaphos toxicity to photosynthesis under atmospheric conditions (400 ppm CO₂, 21% O₂). The excised primary leaves were fed with the compounds via the petiole. Photosynthetic rate in %.

<table>
<thead>
<tr>
<th>min</th>
<th>Bialaphos</th>
<th>Bialaphos+ Asn (30 mM)</th>
<th>Bialaphos+ Glu (30 mM)</th>
<th>Bialaphos+ Gly (30 mM)</th>
<th>Bialaphos+ Gln (30 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>101 ± 1</td>
<td>102 ± 1</td>
<td>103 ± 3</td>
<td>102 ± 3</td>
<td>101 ± 3</td>
</tr>
<tr>
<td>20</td>
<td>98 ± 2</td>
<td>98 ± 1</td>
<td>102 ± 4</td>
<td>102 ± 3</td>
<td>99 ± 4</td>
</tr>
<tr>
<td>30</td>
<td>81 ± 6</td>
<td>81 ± 6</td>
<td>91 ± 1</td>
<td>98 ± 2</td>
<td>84 ± 8</td>
</tr>
<tr>
<td>40</td>
<td>40 ± 6</td>
<td>59 ± 8</td>
<td>68 ± 3</td>
<td>75 ± 4</td>
<td>74 ± 8</td>
</tr>
<tr>
<td>50</td>
<td>23 ± 3</td>
<td>43 ± 5</td>
<td>55 ± 2</td>
<td>50 ± 4</td>
<td>69 ± 6</td>
</tr>
<tr>
<td>60</td>
<td>18 ± 2</td>
<td>32 ± 7</td>
<td>37 ± 2</td>
<td>40 ± 4</td>
<td>66 ± 6</td>
</tr>
<tr>
<td>70</td>
<td>15 ± 2</td>
<td>23 ± 7</td>
<td>29 ± 2</td>
<td>30 ± 3</td>
<td>62 ± 5</td>
</tr>
</tbody>
</table>

Table II. The effect of glyoxylate on photosynthesis under atmospheric conditions (400 ppm CO₂, 21% O₂). The excised primary leaves were fed via the petiole. Photosynthetic rate in %.

<table>
<thead>
<tr>
<th>min</th>
<th>Glyoxylate (10 mM)</th>
<th>Glyoxylate (30 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>100 ± 2</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>20</td>
<td>98 ± 3</td>
<td>87 ± 17</td>
</tr>
<tr>
<td>30</td>
<td>93 ± 4</td>
<td>68 ± 22</td>
</tr>
<tr>
<td>40</td>
<td>88 ± 4</td>
<td>52 ± 20</td>
</tr>
<tr>
<td>50</td>
<td>84 ± 6</td>
<td>42 ± 18</td>
</tr>
<tr>
<td>60</td>
<td>78 ± 8</td>
<td>34 ± 15</td>
</tr>
<tr>
<td>70</td>
<td>74 ± 8</td>
<td>28 ± 15</td>
</tr>
<tr>
<td>80</td>
<td>70 ± 9</td>
<td>23 ± 13</td>
</tr>
</tbody>
</table>

Table III. Electron transport rate per chlorophyll of *Sinapis alba* leaves after treatment with bialaphos (0.05%) • 1 = H₂O, control; 2 = bialaphos treated.

<table>
<thead>
<tr>
<th></th>
<th>90 min</th>
<th>180 min</th>
<th>300 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron transport rate [μmol e⁻ ⋅ mg⁻¹ Chl ⋅ h⁻¹]</td>
<td>±61 ±53</td>
<td>±56 ±53</td>
<td>±75 ±51</td>
</tr>
</tbody>
</table>

The measurements show that the photosynthetic electron transport via DCPIP is not inhibited by bialaphos (Table III).
Discussion

As was shown in the previous investigation into the effect of bialaphos, bialaphos splits in plants into PPT and alanine [1]. Accordingly, PPT appears to be the only herbicidal active substance in bialaphos application. It was therefore concluded that the herbicidal effects of bialaphos and PPT are the same.

Investigations on the effect of bialaphos and PPT on photosynthesis in whole plants have shown that, under atmospheric conditions (400 ppm CO₂, 21% O₂), the inhibition of photosynthesis with PPT and bialaphos proceeds quickly (Fig. 1). Under atmospheric conditions bialaphos as well as PPT [9] show a rapid inhibition of photosynthesis in excised primary leaves (Fig. 3). However, photosynthesis is not inhibited with either bialaphos (Fig. 2, Fig. 3) or PPT (Fig. 2) under non-photorespiratory conditions (1000 ppm CO₂, 2% O₂). Thus, photorespiration seems to play an important role for the effect of both bialaphos and PPT. This becomes particularly obvious after measurements of photosynthesis using bialaphos in different CO₂/O₂ concentrations. The inhibition of photosynthesis by bialaphos decreases strongly with increasing photorespiratory suppression. Similar results were previously obtained with PPT (Wild et al., unpublished results). In different CO₂/O₂ concentrations, the extent of inhibition of photosynthesis can be taken as an indicator for the intensity of photorespiration.

Earlier investigations have shown that bialaphos [1], similar to PPT [10], inhibits GS in vivo. A glutamine shortage and an NH₄⁺-accumulation results through inhibition of GS. The glutamine deficiency could be compensated by supplying exogenous glutamine. Accordingly, the photosynthesis inhibition through bialaphos decreases significantly with increasing exogenously supplied glutamine concentrations (Fig. 4). Again, similar results were obtained with PPT and glutamine, the photosynthesis inhibition is likewise greatly reduced [2]. Glutamine reacts with 2-oxoglutarate catalyzed by the GOGAT to form two molecules of glutamate, the substrate of transamination reactions. Thus glutamine deficiency results e.g. in general amino acid deficiency. However, in contrast to glutamine, amino acids as for instance asparagine, glycine, serine, glutamate show only a slight compensating effect on the inhibition of photosynthesis through bialaphos (Table I).

The NH₄⁺-concentration of bialaphos treated plants was measured. There was a high NH₄⁺-accumulation for bialaphos (Fig. 5) and PPT [9] under atmospheric as well as non-photorespiratory conditions. An even further rise in NH₄⁺-concentration was measured after adding various amino acids to the bialaphos solution (Fig. 5). Under non-photorespiratory conditions photosynthesis is not inhibited. Nevertheless, the NH₄⁺-concentration rises. Then, after adding glutamine to bialaphos or PPT solution there is a strong decrease in photosynthesis inhibition, though the NH₄⁺-accumulation increases a great deal (Fig. 4, Fig. 5).

Our results indicate that a toxic NH₄⁺-accumulation is not the sole cause of the effectiveness of either bialaphos or PPT. A process occurring during photorespiration must play an important role in the inhibition of photosynthesis by either bialaphos or PPT. In order to gain more precise information, the following experiments were carried out:

It was investigated whether the catalase activity is inhibited by bialaphos. Catalase destroys toxic H₂O₂ produced in photorespiration. However, catalase activity is not influenced by bialaphos (data not shown).

As stressed above GS inhibition results in glutamine and consequently in amino acid deficiency. Thus the glyoxylate transamination in photorespiration could be inhibited, which results in a glyoxylate accumulation.

Glyoxylate should inhibit RubP-carboxylase [11, 12] and the regeneration of RuBP [13], which leads to a photosynthesis inhibition.

In a corresponding investigation it was shown that glyoxylate inhibits photosynthesis, provided it was applied in high concentrations.

Preliminary experiments indicate that the inhibition of RubP-carboxylase by glyoxylate could be the cause of photosynthesis inhibition. The plants were treated with 20 mM glyoxylate. After an acting period of 60 min there was a 56% RubP-carboxylase inhibition.

Figure 6 gives a preliminary overview of the course of the herbicide effect of bialaphos based on the investigation results until now.

Acknowledgements

We gratefully acknowledge to the Hoechst Inc. (Frankfurt/Main) for kindly supporting this work by the donation of bialaphos and PPT. We thank Prof. Dr. W. Wernicke for critical reading the manuscript.
bialaphos — (PPT-ala-ala)

\[
\text{PPT + 2 ala} \quad \text{inhibition}
\]

\[
\begin{align*}
\text{ATP} & \quad \text{ADP + Pi} \\
\text{L-glu + NH}_3 & \quad \text{GOGAT}
\end{align*}
\]

\[
\begin{align*}
\text{GS} & \quad \text{L-gln} \quad \text{2L-glu} \\
\text{accumulation} & \quad \text{impoverishment}
\end{align*}
\]

\[
\begin{align*}
\text{damage of membranes} & \quad \text{inhibition of photorespiration by missing amino donors:}
\end{align*}
\]

\[
\begin{align*}
\text{glyoxylate} & \quad \text{2-oxoglutarate} \\
\text{gly} & \quad \text{AT}
\end{align*}
\]

\[
\begin{align*}
\text{inhibition of RubP-carboxylase} & \quad \text{immediate}
\end{align*}
\]

\[
\begin{align*}
\text{inhibition of photosynthesis} & \quad \text{later}
\end{align*}
\]

Fig. 6. The action of bialaphos in plants.