Effect of Phosphinothricin (Glufosinate) on Photosynthesis and Photorespiration

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Phosphinothricin (PPT) causes a rapid inhibition of photosynthesis under atmospheric conditions (400 ppm CO_2, 21% O_2). However, under conditions (1000 ppm CO_2, 2% O_2) under which photorespiration cannot occur, there is no or only a very low rate of photosynthesis inhibition by phosphinothricin. Under both conditions, a strong NH_4^+-accumulation is apparent caused through the inhibition of glutamine synthetase by phosphinothricin. This indicates, that NH_4^+-accumulation cannot be the primary cause for photosynthesis inhibition by phosphinothricin, but a process in connexion with photorespiration plays a central role. Through the lack of amino donors, the transamination of glyoxylate to glycine in photorespiration cannot take place. PPT causes a great decrease in glutamine, glutamate, aspartate, serine, and glycine. Following addition of these amino acids to PPT, there is a decrease in photosynthesis inhibition by PPT. With the addition of glutamine or glutamate to PPT no decrease in serine and glycine is detected, because the transamination of glyoxylate to glycine in photorespiration can occur.

Introduction

Phosphinothricin (glufosinate, PPT) is an active ingredient of a non-selective herbicide [1, 2]. PPT is an analogue of glutamate and irreversibly inhibits the glutamine synthetase (GS) in plants [3]. The inhibition of GS by PPT results in an accumulation of ammonium [4–6].

Furthermore, PPT causes a rapid inhibition of photosynthesis under atmospheric conditions (400 ppm CO_2, 21% O_2), but no or only a slight photosynthesis inhibition occurs under non-photorespiratory conditions, although an NH_4^+-accumulation can be measured [6–8].

This indicates, that the photosynthesis inhibition by PPT is actually based on an inhibition of a part of the photorespiratory process [9]. Through the lack of amino donors, the transamination of glyoxylate to glycine in photorespiration cannot take place [10].

The purpose of the present investigations was to examine the effect of PPT on photosynthesis under different CO_2 and O_2 conditions. Furthermore, photosynthesis was measured after addition of different amino acids to the PPT-solution. In addition, the influence of PPT, PPT and glutamine, PPT and glutamate on amino acid levels was investigated.

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Materials and Methods

Plant material

Plants of *Sinapis alba* (mustard) and *Brassica napus* (oilseed rape) were grown as described [5]. For the experiments 17 to 20 day old mustard plants and 26 to 30 day old rape plants were used.

Chemicals

The active ingredient, DL-homoalanin-4-yl-(methyl)phosphinic acid (glufosinate, code no. Hoe 035 956) was supplied by Hoechst AG (Frankfurt/Main, F.R.G.).

Measurement of photosynthetic rate

The CO_2 fixation rate was measured by means of an infrared gas analyzer (URAS 2T, Hartmann and Braun, Frankfurt/Main) at 150 $W \cdot m^{-2}$ and 20 °C. Different CO_2 and O_2 concentrations were adjusted with gas mixer pumps. The experiments were conducted on excised primary mustard leaves which could be fed with PPT and other compounds *via* the petiole. The transpiration rate was measured parallel to CO_2 gas exchange [6].

Measurement of different amino acids

Excised rape leaves were fed with PPT and other compounds *via* the petiole and illuminated for 150 min. A crude extract was prepared as described by [11] and [12]. The derivatization of the

amino acids was carried out with FMOC-Cl (9-fluorenylmethyl-chloroformate) [12, 13]. The amino acids were determined with high performance liquid chromatography as described [14].

Results

The effect of PPT on photosynthesis under different CO_2 and O_2 concentrations

The photosynthetic rates of PPT-treated excised mustard leaves were measured under different CO_2 and O_2 conditions (Fig. 1). Under atmospheric conditions (400 ppm CO_2, 2% O_2) a great photosynthesis inhibition was measured after PPT-application. Under 1000 ppm CO_2 and 2% O_2 there was only a low level of photosynthesis inhibition by PPT detected. Under these conditions photorespiration cannot occur. The suppression of photorespiration was caused by an increase in CO_2 level, as well as by a decrease in O_2 level. The decrease in photosynthesis inhibition by PPT resulted from a decrease in photorespiration.

The effect of PPT on photosynthesis after addition of different amino acids

The photosynthetic rates of excised mustard primary leaves were measured after treatment with PPT-solution (1 mM) containing the amino acids glutamine, glutamate, aspartate, serine, and glycine (Table I). The addition of these amino acids – particularly glutamine and glutamate – resulted in a decrease in photosynthesis inhibition by PPT. Whereas there was no effect on photosynthesis after the addition of these amino acids without PPT (controls).

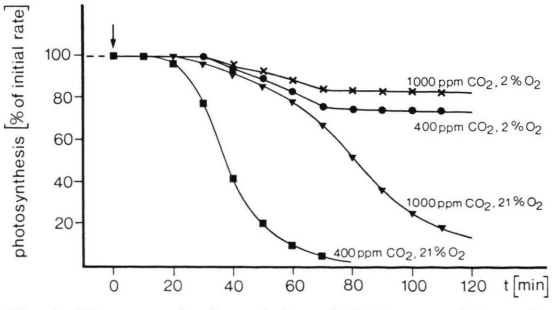

Fig. 1. Photosynthetic activity of PPT-treated (1 mM) excised mustard primary leaves under various CO_2 and O_2 concentrations (v/v). Photosynthetic rate in %. After reaching a constant level of photosynthesis (100%) PPT was added *via* the petiole at zero time (↓).

Table I. Photosynthetic activity of excised mustard primary leaves after addition of PPT solution (1 mM) containing different amino acids (20 mM) under atmospheric conditions (400 ppm CO_2, 21% O_2). After reaching a constant level of photosynthesis the amino acids together with the PPT-solution were added *via* the petiole. Photosynthetic rate in % of control.

Time after addition [min]	PPT	PPT + gln	PPT + glu	PPT + gly	PPT + asp	PPT + ser
10	100,0	101,6	101,2	98,0	104,0	102,9
20	100,0	101,1	100,9	97,7	102,3	103,4
40	78,4	92,1	95,9	92,7	88,8	95,8
60	19,0	87,5	85,6	82,5	72,7	58,2
80	8,0	77,5	65,9	59,7	53,5	34,2

Concentration of amino acids after PPT treatment

The concentrations of various amino acids were determined following PPT-treatment (1 mM) of rape leaves (Fig. 2). PPT caused a great decrease in glutamine, glutamate, aspartate, serine, glycine, and alanine and a low decrease in asparagine. However, after PPT-treatment an increase in isoleucine, leucine, phenylalanine, valine, and lysine was measured. Preliminary investigations with PPT and chlorsulfuron (an inhibitor of branched-chain amino acid synthesis) show, that the PPT induced accumulation of the branched-chain amino acids is probably the result of *de novo* synthesis.

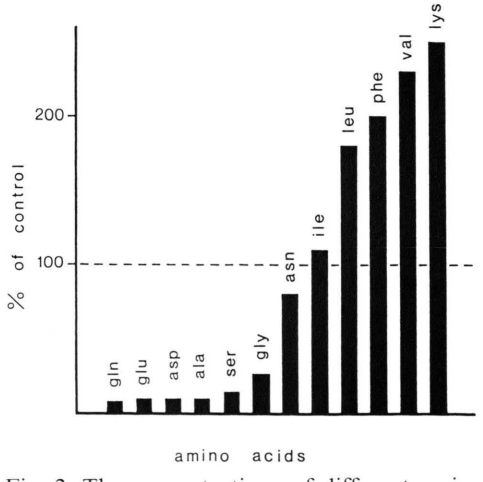

Fig. 2. The concentrations of different amino acids of PPT-treated (1 mM) excised rape leaves. Amino acid concentrations in %. PPT was fed *via* the petiole and the leaves were illuminated for 150 min.

Concentration of glycine and serine after addition of glutamine and glutamate to the PPT-solution

The concentrations of glutamine, glutamate, serine, and glycine of excised rape leaves were measured after treatment with PPT and glutamine or PPT and glutamate (Fig. 3). In relation to PPT-treated leaves there was an increase in glutamate, serine, and glycine after addition of glutamine to PPT. After addition of glutamate to PPT there was an increase in serine and glycine.

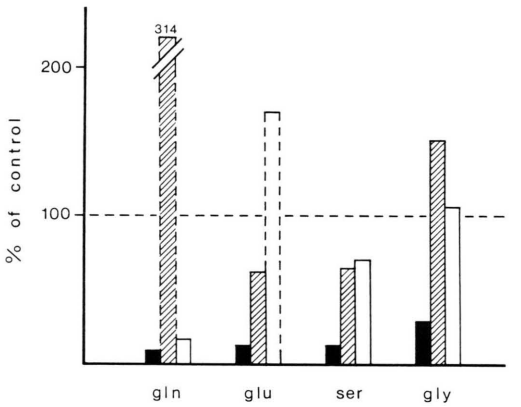

Fig. 3. The concentrations of glutamine, glutamate, serine, and glycine of excised rape leaves after addition of PPT (1 mM), PPT and glutamine (20 mM), PPT and glutamate (20 mM). Amino acid concentrations in %. The compounds were fed *via* the petiole and the leaves were illuminated for 150 min. ■ = PPT-treated; ▨ = treated with PPT and glutamine; □ = treated with PPT and glutamate.

Discussion

The investigations indicate that a process in photorespiration plays an essential role on photosynthesis inhibition by PPT. The lack of amino donors prevents the transamination of glyoxylate to glycine in photorespiration. A rapid and strong impoverishment of glutamine, glutamate, serine, glycine, and aspartate in *Brassica napus* leaves after PPT-treatment was measured.

With the addition of these amino acids photosynthesis inhibition by PPT can be reduced. Following the addition of glutamine together with PPT, glutamate is produced *via* the GOGAT enzyme to serve as an amino donor in transamination of glyoxylate to glycine. Aspartate is probably also converted to glutamate in the presence of 2-oxoglutarate *via* aspartate-aminotransferase. Serine is also an amino donor for the transamination reaction in photorespiration and glycine can be converted in serine.

After application of PPT and glutamine or PPT and glutamate to rape leaves, no decrease in glycine and serine was measured (Fig. 3). This indicates that the transamination of glyoxylate to glycine in photorespiration can occur and thus produces glycine and serine.

The measurements indicate clearly, that the lack of transamination of glyoxylate to glycine in photorespiration plays the central role for the photosynthesis inhibition by PPT. It has to be examined now, if the inhibition of the transamination reaction results in an accumulation of glyoxylate and other compounds involved in photorespiration. Glyoxylate is known to inhibit the RuBP-carboxylase [15, 16] and the CO_2-fixation accordingly.

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