Inhibitory Action of Glufosinate on Photosynthesis

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Glufosinate (phosphinothricin) irreversibly blocks the glutamine synthetase which subsequently gives rise to an accumulation of ammonium and to a strong decrease in some amino acids, especially glutamine and glutamate.

Under atmospheric conditions (400 ppm CO_2, 21% O_2) glufosinate causes a rapid inhibition of photosynthesis, too. However, under non-photorespiratory conditions (1000 ppm CO_2, 2% O_2) only a slight inhibition of photosynthesis occurs with glufosinate. Since under both conditions an accumulation of ammonium occurs, it is concluded that inhibition of photosynthesis is not induced by the higher concentrations of ammonium. The results rather suggest that the absence of amino donors in the glycolate pathway leads to a break-down of the transamination reaction of glyoxylate to glycine. This causes an inhibition of photorespiration and as a further consequence the inhibition of photosynthesis. There are two hypotheses for explaining this phenomenon. One of them supposes that the blockade in the glycolate pathway produces a lack of Calvin cycle intermediates which subsequently is the cause of the inhibition of photosynthesis. The other one suggests a direct inhibition of the ribulose-1,5-bisphosphate carboxylase by the accumulation of glyoxylate and P-glycolate.

After treatment with different intermediates of the Calvin cycle and photorespiration together with glufosinate no decrease in the inhibition of photosynthesis could be measured. This suggests that the inhibition of photosynthesis is not induced by a depletion of intermediates of the Calvin cycle.

Tests on the effect of glyoxylate and P-glycolate on ribulosebisphosphate carboxylase activity showed that in crude leaves extracts the enzyme activity can only be inhibited by high concentrations of these substances. However, in intact spinach chloroplasts the enzyme activity can be blocked by using much lower concentrations of glyoxylate. This may indicate that the ribulosebisphosphate carboxylase activase is affected by this metabolite and that this may be the reason for an inhibition of photosynthesis after treatment with glufosinate.

Introduction

Phosphinotricin (PPT, glufosinate), an active ingredient of a non-selective herbicide [1] irreversibly inhibits glutamine synthetase [2]. The inhibition of GS caused by PPT results in an accumulation of ammonium under atmospheric (400 ppm CO_2, 21% O_2) and non-photorespiratory (1000 ppm CO_2, 2% O_2) conditions [3–6].

In the presented studies the effects of PPT on the amino acid concentrations, on photosynthesis and on the CO_2 compensation point were examined. In addition, the influence of different intermediates of the Calvin cycle and photorespiration on the inhibition of photosynthesis by PPT and the effect of glyoxylate and P-glycolate on RuBPCase activity were studied.

Abbreviations: AOA, aminooxy acetate; GS, glutamine synthetase; GOGAT, glutamine-2-oxoglutarate aminotransferase; PPT, phosphinothricin (glufosinate), RuBPCase, ribulose-1,5-bisphosphate carboxylase.

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Materials and Methods

Plants of *Sinapis alba* (mustard) and *Brassica napus* (oilseed rape) were grown as described by Wild and Manderscheid [7]. Growth conditions for *Spinacia oleracea* (spinach) plants were described by Robinson [8]. Excised leaves were fed with PPT and other compounds in various concentrations *via* the petiole. Measurements of amino acids and photosynthetic rate were carried out as described [9]. Determination of RuBPCase activity and the isolation of intact spinach chloroplasts were carried out as described by Wendler *et al.* [10].

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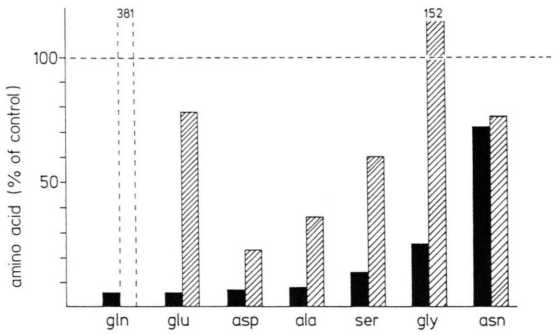

Fig. 1. Amino acid concentrations (in % of control) of excised rape leaves. The leaves were illuminated for 150 min after addition of PPT (1 mM) (■) or PPT (1 mM) + glutamine (20 mM) (▨).

Results and Discussion

The inhibition of the glutamine synthetase by PPT (phosphinothricin, glufosinate) gives rise to an accumulation of ammonium and, furthermore, to an impoverishment of some amino acids (Fig. 1 and 7). A rapid and strong decrease of glutamine, glutamate, aspartate, alanine, serine and glycine is measured. Glufosinate irreversibly blocks the glutamine synthetase which results in an impoverishment of glutamine. Glutamine cannot be synthesized in the absence of glutamine due to the lack of substrate for the GOGAT enzyme. Since in the glycolate pathway glutamate is the essential amino donor in the transamination reaction of glyoxylate to glycine, glycine cannot be produced under this condition. This also results in an impoverishment of serine, because the glycine that is formed by this sequence of reactions then diffuses into the mitochondria where serine, NH_3 and CO_2 are produced from two molecules of glycine. Furthermore, alanine and aspartate cannot by synthesized because the transamination reaction with pyruvate and oxaloacetate cannot proceed due to the absence of the amino donor glutamate.

However, when the excised leaves were fed *via* the petiole by PPT plus glutamine, glutamate can be produced *via* the GOGAT reaction and the other transamination reactions can subsequently proceed in order to synthesize various amino acids (Fig. 1).

Under atmospheric conditions (400 ppm CO_2, 21% O_2) a strong inhibition of photosynthesis occurs very rapidly after application of the herbicide solution. However, under non-photorespiratory conditions (1000 ppm CO_2, 2% O_2) only a small inhibition of photosynthesis by PPT is detected (Fig. 2A). Under both conditions an accumulation of ammonium can be measured [4–6]. Addition of the lacking amino acids – particularly glutamine and glutamate – results in a decrease in inhibition of photosynthesis by PPT (Fig. 2B).

The photosynthetic rates of excised leaves were also measured with aminooxy acetate, an inhibitor of the transamination reaction of glyoxylate to glycine. Under atmospheric conditions the photosynthetic activity was rapidly inhibited by very low concentrations of AOA, but there was only a slight inhibition of photosynthesis measured with AOA under non-photorespiratory conditions (Fig. 3). The effects of AOA can therefore be compared with those of PPT.

By these experimental results it can be suggested that the inhibition of the transamination reaction from glyoxylate to glycine in the glycolate pathway triggers the inhibition of photosynthesis observed in the presence of PPT. The consequences of the inhibition of transamination may be explained by two hypotheses. One of them proposes that the lack of Calvin cycle intermediates is the cause of the inhibition of photosynthesis, whereas the other one suggests a direct inhibition of RuBPCase with glyoxylate not having been transaminated.

After addition of different intermediates of the Calvin cycle and photorespiration to the PPT solution no decrease in the inhibition of photosynthesis inhibition is measured (Fig. 4). This indicates that the lack of intermediates of the Calvin cycle is not the cause of the inhibition of the photosynthesis by PPT.

Measurements of the CO_2-compensation point in a closed system show that leaves treated with PPT *via* the petiole continuously release CO_2 (Fig. 5). An explanation for this may be that the accumulating glyoxylate non-enzymatically oxidizes to formate and CO_2 [11, 12]. If glutamine and PPT are added at the same time the CO_2-compensation point is only a little bit higher compared with the reference (control with water). By adding glutamine as substrate for the GOGAT reaction it becomes possible to produce glutamate. Glutamate serves as amino donor for the transamina-

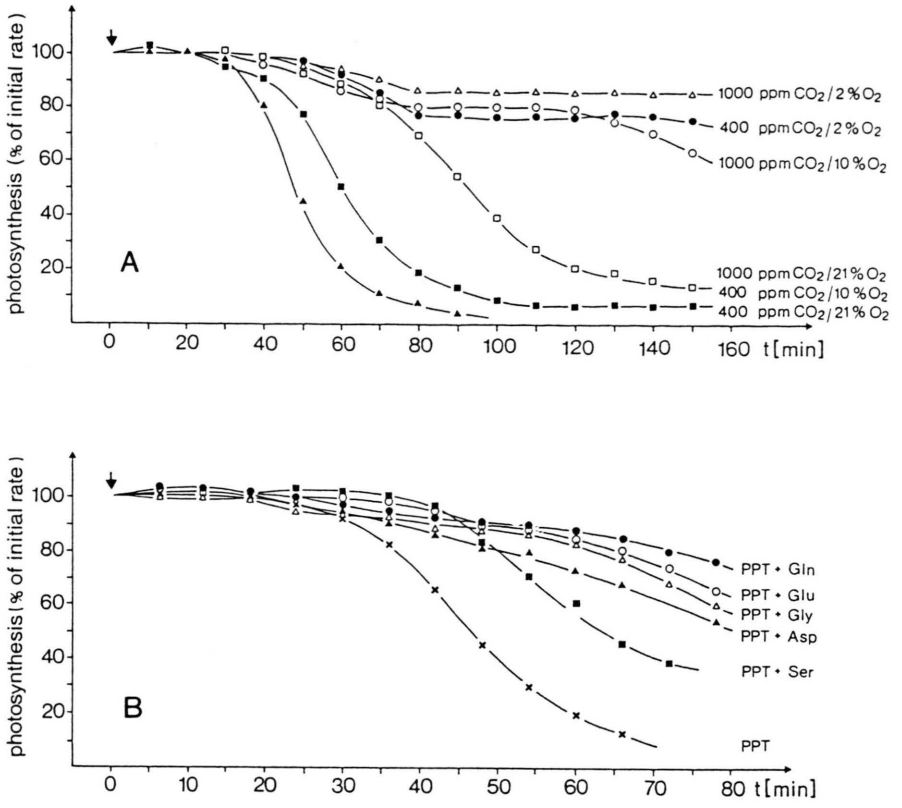

Fig. 2. Photosynthetic activity of excised rape leaves. After reaching a constant level of photosynthesis (100%) the substances were added *via* the petiole at zero time (↓). A: Photosynthetic rate after PPT treatment (1 mM) under different CO_2 and O_2 concentrations. B: Photosynthetic rate after treatment with PPT (1 mM) or with PPT (1 mM) and different amino acids (20 mM) under atmospheric conditions (400 ppm CO_2, 21% O_2).

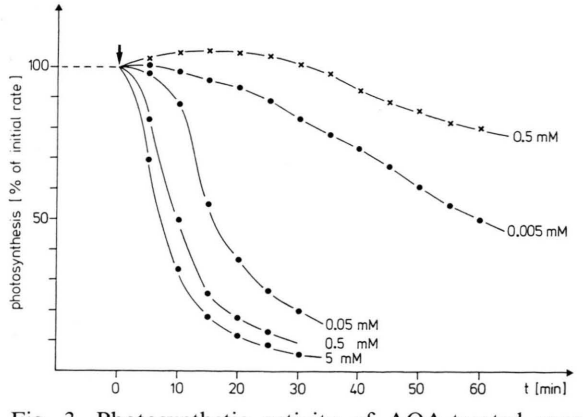

Fig. 3. Photosynthetic activity of AOA-treated rape leaves under atmospheric conditions (400 ppm CO_2, 21% O_2; ●−●) and non-photorespiratory conditions (1000 ppm CO_2, 2% O_2; ×−×). After reaching a constant level of photosynthesis (100%) AOA was added *via* the petiole at zero time (↓).

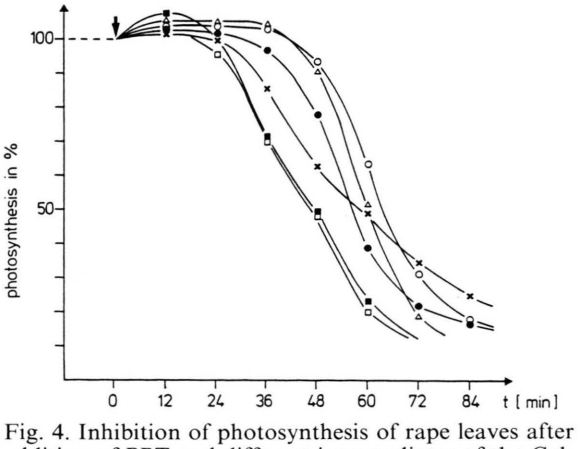

Fig. 4. Inhibition of photosynthesis of rape leaves after addition of PPT and different intermediates of the Calvin cycle and photorespiration under atmospheric conditions (400 ppm CO_2, 21% O_2). After reaching a constant level of photosynthesis (100%) the substances were added *via* the petiole at zero time (↓). ●−● = PPT (1 mM); △−△ = PPT (1 mM) + 3P-glycerate (10 mM); ○−○ = PPT (1 mM) + glycerate (10 mM); ×−× = PPT (1 mM) + hydroxypyruvate (1 mM); ■−■ = PPT (1 mM) + glycerinaldehyde-3-P (5 mM); □−□ = PPT (1 mM) + glycerinaldehyde (5 mM).

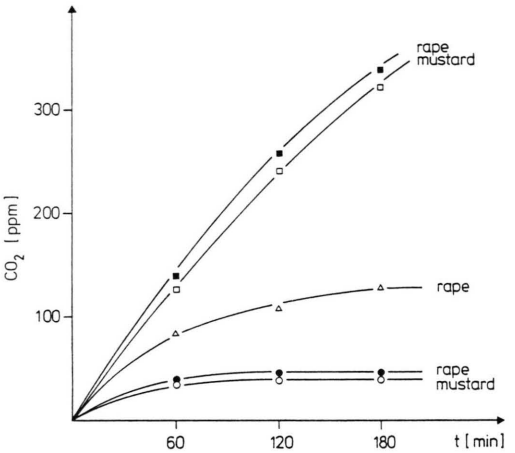

Fig. 5. CO_2-Release of rape or mustard leaves after addition of PPT or PPT + glutamine. ●−●, ○−○ = controls; △−△ = treated with PPT (1 mM) + glutamine (20 mM); ■−■, □−□ = treated with PPT (1 mM).

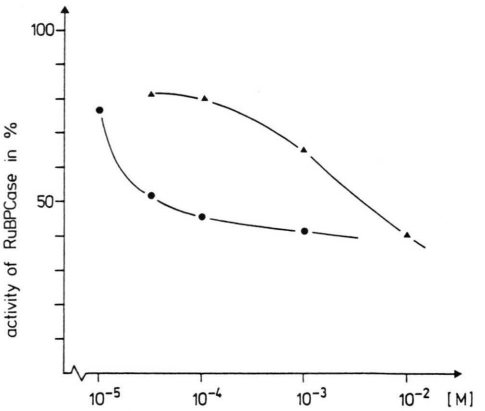

Fig. 6. RuBPCase activity (5) of intact spinach chloroplasts following addition of different glyoxylate (●−●) and P-glycolate (▲−▲) concentrations. Chloroplasts were preincubated in the dark for 1 min together with the substances prior to an illumination of 5 min.

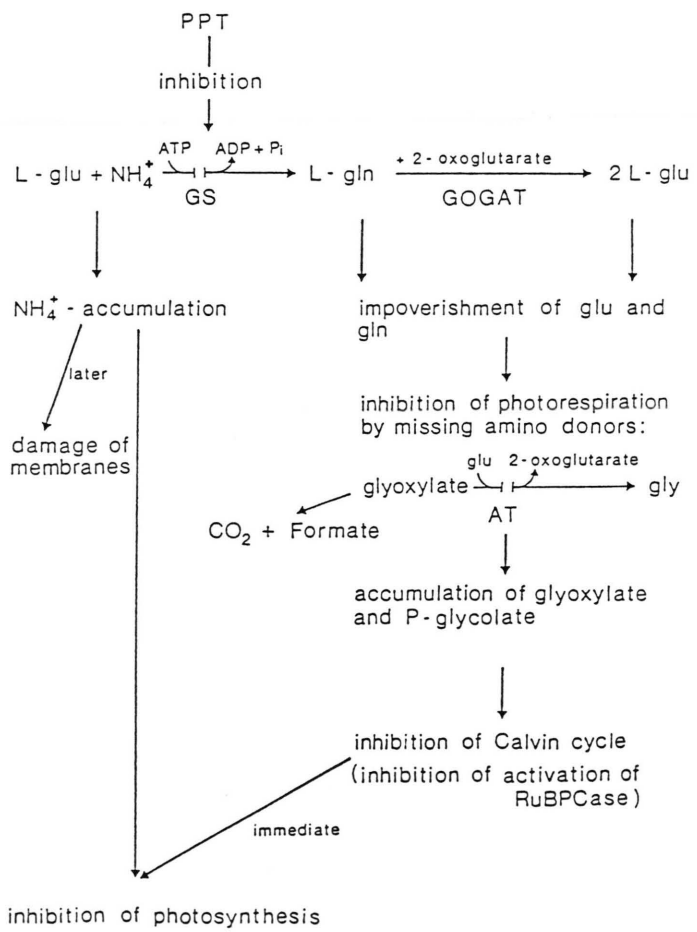

Fig. 7. Consequences of the inhibition of glutamine synthetase by glufosinate.

tion of glyoxylate to glycine; thus, glyoxylate can again be transformed in a large quantity.

Glyoxylate, that cannot be further converted, does not accumulate in large quantities because most of it oxidizes [10]. This could be the reason that no direct inhibition of RuBPCase takes place since high concentrations of glyoxylate or its preproduct P-glycolate would be necessary [10]. If, however, intact spinach chloroplasts are activated by light, small concentrations of glyoxylate already suffice to inhibit RuBPCase (Fig. 6).

It is to be accepted, therefore, that it is not the RuBPCase reaction that is directly influenced by glyoxylate and P-glycolate, but rather the activation conditions of the RuBPCase enzyme seem to be affected by these metabolites.

Fig. 7 summarizes the effects of the inhibition of glutamine synthetase by glufosinate. This inhibition gives rise to an accumulation of ammonium and to a strong decrease in glutamine and glutamate. The absence of sufficient amino donors in the glycolate pathway leads to a break-down of the transamination reaction of glyoxylate to glycine. The accumulation of glyoxylate and of its preproduct P-glycolate causes as a further consequence the inhibition of photosynthesis.

Acknowledgements

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