Inhibition of Photosynthesis by Bentazon in Intact Plants and Isolated Cells in Relation to the pH

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Z. Naturforsch. **34 c**, 944 – 947 (1979) ; received June 2, 1979

Bentazon, Dissociation of Bentazon, Penetration, Inhibition of Photosynthesis and CO_2 Assimilation, Chlorophyll Fluorescence Induction

Bentazon [3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide] causes an increase in the chlorophyll fluorescence in the leaves of mustard plants [*Sinapis alba* (L.)] as a result of its inhibitory effect on the photosynthetic electron transport. The rate of this fluorescence induction depends on the pH of the suspension medium. As the concentration of hydrogen ions diminishes, the maximum fluorescence that indicates a total inhibition of photosynthesis is reached at a later time.

In the case of single cells isolated from the leaves of soybean plants [*Glycine max* (L.) Mer "Harosoy"], bentazon inhibits photosynthetic CO_2 assimilation. The amount of inhibition is dependent on the pH of the suspension medium. The uptake of CO_2 is inhibited more at pH 6 than at pH 7 or 8. Bentazon is more readily absorbed by cells at pH 6 than at pH 7 or 8. The inhibition of CO_2 assimilation is proportional to the amount of bentazon taken up.

Introduction

The results of experiments with organic acid-type herbicides show that for certain herbicides the rate of penetration through the cuticula of the leaf may depend on the concentration of hydrogen ions in the suspension medium [1]. This is attributed to the difference between penetration of the dissociated and undissociated herbicide molecules. The more lipophilic properties of the undissociated molecule probably favor uptake [2 – 5].

Bentazon dissociates in aqueous solution as well. The rate of penetration of the bentazone through the cuticula of the leaf therefore is probably influenced by the pH of the suspension medium in much the same way as in the case of organic acid-type herbicides. If this is true, differences in the rate of the inhibitory effect on the photosynthetic electron transport and the CO_2 assimilation, which are responsible for bentazon's herbicidal activity [6, 7], can be expected as a function of the pH.

Experiments are carried out at various hydrogen ion concentrations to prove this hypothesis. The inhibitory effect on the photosynthetic electron transport is investigated in intact plants. The rate at which the bentazon is taken up and bentazon's effect on CO_2 assimilation is determined in cells isolated from leaf tissue. The experiments with isolated single cells should also provide information on whether the role of the hydrogen ion observed in the cuticular region also applies to the penetration of organic acid-type substances through the membranes of cells that are located in the interior of the leaf.

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0341-0382 / 79 / 1100-0944 $ 01.00/0

Method

1. *Determination of the inhibitory effect on the photosynthetic electron transport*

The inhibition of the photosynthetic electron transport in intact plants was illustrated by determining the rate at which fluorescence increases in the leaves of mustard plants *(Sinapis alba)* following an application of bentazon. The determination was made according to the method described by Pfister [8] to measure slow fluorescence changes.

2. *Isolation of the leaf tissue cells*

The leaf tissue cells were isolated from young but fully developed soybean leaves [*Glycine max* (L.) Mer "Harosoy"] by means of enzymatic maceration

of the leaf tissue based on the methods of Porter [9], St. John *et al.* [10], and Rehfeld and Jensen [11]. After several washings, the cells were suspended in an aqueous medium of 0.6 M/l of sorbose, 2.5 mM/l of $Ca(NO_3)_2$, 2.5 mM/l of KNO_3, 1.0 mM/l of $MgSO_4$, 0.5 mM/l of KCl, and 0.1 mM/l of KH_2PO_4. The cell concentration was such that 1 ml of suspension contained 200 μg of chlorophyll. Samples of the cell suspension were then mixed with bentazon in concentrations of 3×10^{-6} M/l, 6×10^{-6} M/l, 10^{-5} M/l, and 3×10^{-5} M/l. By adjusting the pH using MES (2-morpholinoethanesulphonic acid), HEPES (N-2-hydroethylpiperazin-2-ethanesulphonic acid), and TRIS [tris(hydroxy-methyl)-aminomethane] buffers in a final concentration of 100 mM/l to pH 6, pH 7 and pH 8, respectively, the proportion of the bentazon present in dissociated state was varied, while the total amount of bentazon remained constant.

3. *Determination of the rate of CO_2 assimilation*

The amount of CO_2 assimilated by the cells under these conditions was determined by the use of ^{14}C. The cell suspension was mixed with $NaH^{14}CO_3$ (3 μM/ml of solution, spec. activity 1.6 mCi/mM) and then exposed to light (5000 lux).

Samples were removed from the exposed cell suspension at hourly intervals and the CO_2 which had not been assimilated by the cells was released from solution by adding 2 ml of concentrated formic acid. The amount of ^{14}C incorporated by the cells per unit of time was then determined with a scintillation counter and recorded as a measure of the rate of assimilation.

4. *Determination of the rate of bentazon penetration*

The tests for determining the amount of bentazon penetrating the cells at varying pH values were carried out in the same manner as the experiments for determining CO_2 fixation, except that radioactive [^{14}C]bentazon (spec. activity 7.5 mCi/mM) and unlabeled sodium hydrogen carbonate were used. The cells from the samples removed 2, 4 and 6 h after addition of [^{14}C]bentazon were separated from the solution by centrifuging (3 min, at $80 \times g$) and then washed three times. The washing was carried out with solution which contained unlabeled bentazon at a concentration equivalent to the amount of [^{14}C]bentazon used in the test. The labeled material remaining in the cells after the washing was determined with a scintillation counter.

Results and Discussion

Bentazon molecules form anions in aqueous solution upon the loss of a proton (Fig. 1). The pk_A value for this dissociation step is 3.45. The relatively low pk_A shows clearly that during experiments with bentazon within the pH range normally used for biological tests in the aqueous milieu, there are considerable quantities of the active ingredient present in the form of hydrophilic anions, in addition to undissociated bentazon molecules. The values given in the Table (Fig. 2) on the partition of bentazon in the system octanol/water at various concentrations of hydrogen ions indicate the extent to which the ratio between lipophilic undissociated bentazon and hydrophilic bentazon anions changes as the pH changes.

Within the tested range of pH 5 to pH 8, bentazon causes a rise in the chlorophyll fluorescence in leaves of mustard plants due to its inhibitory effect

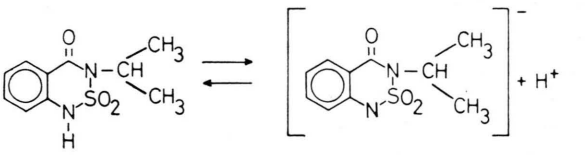

Fig. 1. Dissociation of bentazon.

pH	BENTAZON CONCENTRATION OCTANOLPHASE Co = µg/ml	WATERPHASE Cw = µg/ml	PARTITION COEFFICIENT q = Co / Cw
2	15,602	0,650	24,003
4	16,055	1,020	15,740
6	7,466	9,511	0,785
8	2,575	14,425	0,179

Fig. 2. pH-dependent partition of bentazon in the system octanol/water (supplemented according to Redecker [12]).

on the photosynthetic electron transport. The rate of this fluorescence induction is dependent on the pH of the applied bentazon solution.

The length of time that is required to reach maximum fluorescence (indicating a complete blockage of photosynthesis) from the moment of the active compound application increases when the concentration of hydrogen ions diminishes and the ionization of the bentazon rises in the suspension medium (Fig. 3). If we assume that the inhibitory effect on the photosynthetic electron transport in the plant most probably is proportional to the amount of herbicide taken up, then the result suggests a diminished rate of penetration of the dissociated bentazon molecules.

Analogous to the observations made on intact plants, there is also inhibition in isolated leaf tissue cells due to bentazon in connection with the inactivation of photosynthesis. The inhibition of photosynthetic CO_2 assimilation in the isolated leaf tissue cells following the addition of equal amounts of bentazon to the suspension medium depends on the pH of the suspension medium (Fig. 4). At the pH levels of 6, 7 and 8, the inhibition of CO_2 assimilation decreased as alkalinity increased.

The cause of this diminishing inhibition of CO_2 assimilation seems to be an alteration in the bentazon concentration at the site of activity in the cell interior which is controlled via the pH of the suspension medium. As can be seen from the results of tests with ^{14}C-labeled bentazon (Fig. 4), less active ingredient penetrates the cells per unit of time when the pH value is raised from pH 6 to pH 7 or pH 8.

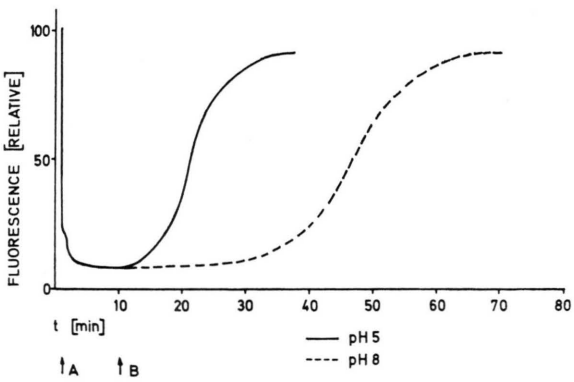

Fig. 3. Rate of the chlorophyll fluorescence induction in the case of *Sinapis alba* leaves following the application of a bentazon solution buffered to pH 5 and pH 8. A: Light on, B: Application of bentazon.

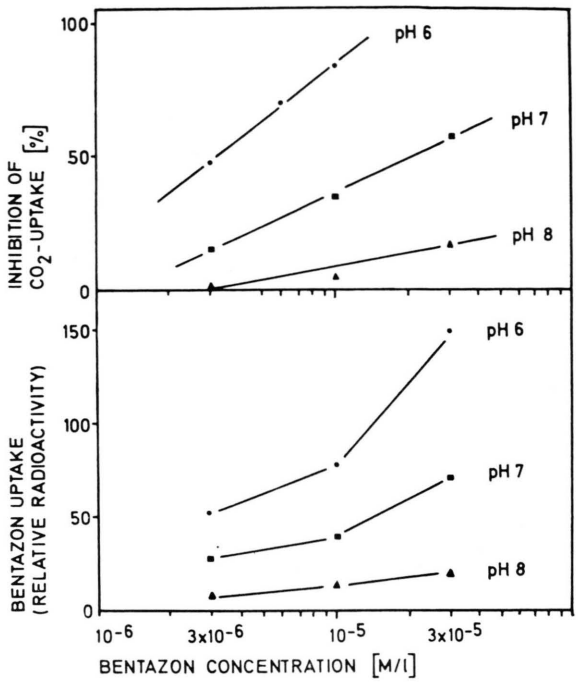

Fig. 4. Bentazon uptake and inhibition of CO_2 assimilation as a function of pH in isolated leaf tissue cells of soybeans *(Glycine max)*.

The inhibition of CO_2 assimilation caused by bentazon is proportional to the active compound quantity taken up by the isolated cells from the suspension medium under the influence of the different pH values (Fig. 5). Based on our data, a multiple correlation of 0.98 can be derived from the relationship.

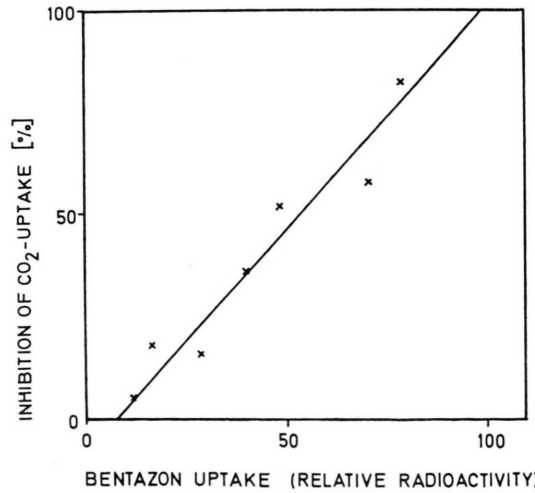

Fig. 5. Correlation between bentazon uptake and inhibition of CO_2 assimilation in isolated leaf tissue cells of soybeans *(Glycine max)*.

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