THE EFFECT OF PHENYLSERINE AND RELATED COMPOUNDS ON THE INDOLEACETIC ACID OXIDASE ACTIVITY OF CUCUMBER HYPOCOTYL AND ROOT EXTRACTS

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SUMMARY

Feeding of DL-threo-β-phenylserine increases the IAA-oxidase activity of cucumber hypocotyl extracts and decreases that of root extracts. A change in the cofactor/inhibitor ratio seems to be responsible for this effect. Indirect evidence was obtained for a break-down of phenylserine into glycine and benzoic acid or derivatives thereof.

1. INTRODUCTION

While investigating the effect of amino acids on cucumber scab VAN ANDEL (1966) found L-threo-β-phenylserine to be an active chemotherapeutant. The compound, however, appeared ineffective against the fungus as such. When administered via the roots activity could only be obtained after a considerable time. Thus, not phenylserine itself but conversion products were considered responsible for the effects found. Van Andel attributed the activity to influences on indoleacetic acid oxidase activity in the treated plants.

About the fate of phenylserine in plant material nothing is known as yet. In bacteria evidence was obtained for the break-down of phenylserine into benzaldehyde and glycine (JANEČEK & HAVRÁNEK 1962). Van Andel suggested that an increased production of *p*-coumaric acid or other compounds acting as cofactors for IAA-oxidase in treated plants might explain the increased resistence against fungal attack.

My interest in IAA-oxidase led me to investigate the possible effects of phenylserine in more detail. In preliminary experiments similar effects were found for both L- and DL-threo-β-phenylserine. Most of the experiments were carried out with the DL-isomer.

2. MATERIAL AND METHODS

For the experiments ten-day-old seedlings, cv. "Lange gele tros" were used. The seedlings were dug from the sand, the roots were washed with tap water and 20 plants were put with their roots in 50 ml of a solution of the compound to be tested. After 1, 2, or more days the plants were used for the IAA-oxidase

test. The hypocotyls and/or roots were rinsed, ground in buffer and centrifuged. The IAA-oxidase activity was evaluated in a reaction mixture which contained 0.2 ml hypocotyl or root extract, 0.2 ml IAA solution 10^{-3} g/ml and 4.6 ml phosphate-citrate buffer pH 4.3. In some cases 0.5 ml *p*-coumaric- or caffeic acid in varying concentrations was added, whereas the amount of buffer was diminished to obtain the same 5.0 ml end volume. The residual amount was measured colorimetrically with Salkowski reagent (TANG & BONNER 1947). Acetone powders of the extracts were made according to the procedure described by JANSSEN (1969).

3. RESULTS AND DISCUSSION

In the first experiments the activity of phenylserine (PS) was evaluated. A one-day treatment with PS gave IAA-oxidase activity of both hypocotyl and root extracts similar to that of the control. Two-day treatment of the roots resulted in a considerable increase in IAA-oxidase activity in hypocotyl extracts and a decrease in that of root extracts (*fig. 1*). As far as the activity of the hypocotyl extracts is concerned this is in agreement with van Andel's results. A decrease in activity of the root extracts, however, does not seem to comply with the increase found in root length (VAN ANDEL 1962). Increased root growth points to a decrease in IAA concentration instead of to an increase as suggested by lower IAA-oxidase activity.

The change in IAA-oxidase activity may be due to changes in enzyme-synthesis as well as to a change in the cofactor/inhibitor range. To find out what actually occurs in this case, acetone powders of untreated cucumber hypocotyls were prepared. To this acetone powder boiled hypocotyl extracts of either PS-treated or untreated plants were added. As can be seen from *fig. 2* the boiled extracts of PS-treated plants induced higher activity than the control. This indicates that more of a cofactor (or less of an inhibitor) may be available in hypocotyls of PS-treated plants. Thus van Andel's suggestion on the production of compounds acting as cofactors for IAA-oxidase might be true for the hypocotyls. Whether *p*-coumaric acid may be involved, however, still remains to be seen. Biochemically, a break-down of PS into glycine and a benzoic aldehyde or acid seems more appropriate than a conversion into a cinnamic acid.

If *p*-coumaric acid is involved effects similar to that of PS would be expected from root-treatment with phenylalanine as a precursor of *p*-coumaric acid and from that with *p*-coumaric acid itself. When these compounds were fed to the roots, however, opposite results were obtained. A two-day treatment with 10^{-4} up to 10^{-1} M L-phenylalanine and with 5×10^{-4} to 10^{-3} M *p*-coumaric acid resulted in increased IAA-oxidase activity in the roots and a decreased one in the hypocotyls. These results might be explained, supposing a rapid turnover into IAA-oxidase inhibitors like for instance ferulic- or caffeic acid in the hypocotyl. They do not, however, aid to a better understanding of the mechanism of PS activity.

The question whether indeed more of a cofactor in the hypocotyls might

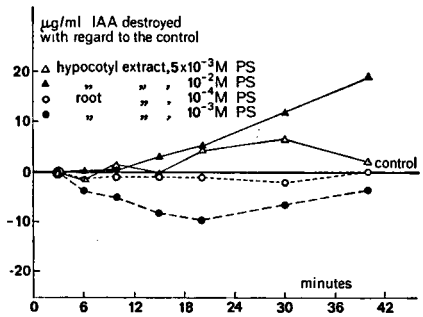

Fig. 1. Difference in IAA oxidase activity between extracts of plants treated for two days with DL-threo-β-phenylserine and those of control plants.

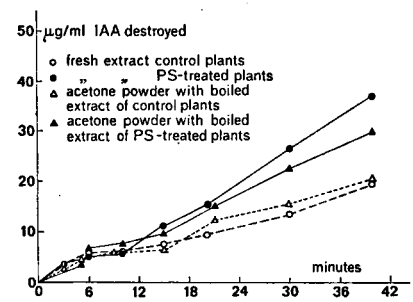

Fig. 2. IAA oxidase activity of cucumber hypocotyl acetone powder with boiled extracts of phenylserine treated and control plants. Ibid. of fresh extracts without acetone powder.

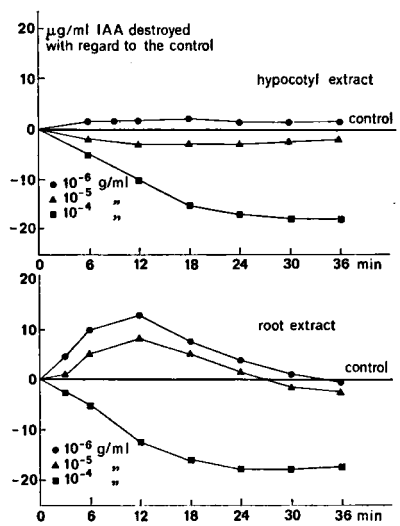

Fig. 3. Influence of the addition of *p*-coumaric acid on the IAA oxidase activity of cucumber hypocotyl and root extracts.

explain the PS effect, was further tested with hypocotyl extracts of untreated plants. Varying concentrations of *p*-coumaric were added to these extracts (*fig. 3*). No influence at all on the IAA-oxidase activity could be obtained with lower concentrations and inhibition occurred at a concentration of 10^{-4} g/ml. The root extracts were stimulated in their activity at 10^{-6} or 10^{-5} g/ml *p*-coumaric acid. Similar results were obtained after the addition of 2.4-dichlorophenol. Thus, in the hypocotyls of the cucumber seedlings an optimal or supra-optimal concentration of cofactors seems available. When indeed the different cofactors all stimulate IAA-oxidase in the same way the idea that cofactor production as a result of PS treatment is responsible for the increased IAA-oxidase activity has to be abandoned.

Apart from the cinnamic acids some other possible conversion products of PS like glycine and benzoic acid were investigated as well. Glycine appeared totally inactive. With benzoic acid varying results were obtained. In the roots we found mainly decreased IAA-oxidase activity. In the hypocotyls, however, in most cases no change in activity was apparent; in some cases an increase was obtained. Salicylic acid gave effects similar to those of PS. A two-day treatment with salicylic acid resulted in enhanced IAA-oxidase activity in the hypocotyl and a decreased effect of root extracts. Like PS it was inactive when added as such to a crude extract of untreated plants. The latter results might point to a break-down of phenylserine into glycine and benzoic acid or derivatives thereof.

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