Acta Biologica Szeged *13*, (3—4), pp. 107—112 (1967)

ON THE MECHANISM OF GIBBERELLIN-AUXIN INTERACTION

IV. EFFECT OF GIBBERELLIN TREATMENT ON THE OXIDATIVE DESTRUCTION OF INDOLEACETIC ACID IN BEAN HYPOCOTYL TISSUES

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(Received October 17, 1967)

Introduction

In our earlier works it was shown that due to the effect of gibberellin (GA) the quantity of the different indoleacetic acid (IAA) forms increases in the bean hypocotyl tissues. Namely, according to our experiments, not only the free IAA level is enhanced by GA treatment, but the formation of the IAA-conjugates and IAA-macromolecule complexes are promoted too (Varga and Bitó, 1968; Varga, 1968). Thereupon the question arose, by what kind of mechanism the GA-induced rise of IAA level is realized in the bean hypocotyls. According to our earlier results, the increase of the quantity of IAA is greatly the result of the fact that GA promotes the IAA synthesis; in detail, it enhances the utilization of tryptophan (TTP) precursor in the biosynthesis of auxin (Varga et al., 1968). However, since several authors have shown that GA can control the auxin concentration in plant tissues by affecting the IAA oxidase acivity, it may be supposed that also in bean hypocotyls — beside a stimulated TTP ⟶ IAA conversion — an „auxin-sparing" mechanism is in action. Consequently, the IAA-oxidase activity in GA-treated and untreated hypocotyls was examined and compared in detail.

Material and method

Phaseolus vulgaris (var. *Golden Rain)* seedlings were grown as described earlier (Varga and Bitó 1967). On the 5th day the seedlings of the same size were selected and having removed the cotyledons, hypocotyls of 3 to 3.5 cm were used for experiments. From one part of the hypocotyls the shoot apex was cut (decapitated hypocotyls) and kept on the others (intact hypocotyls). Hypocotyls were incubated for 24 hours — one series in light (6000 lx), another in dark —

in growth medium containing 0, 5 and 50 ppm GA_3. After 24-hours incubation the IAA content and the IAA oxidase activity in the tissues were measured.

Determination of IAA oxidase activity was carried out by a modified form of Gordon-Weber's colorimetric method (1951) and by paperchromatography, as described in our earlier works (Varga and Köves 1962, Varga and Zsoldos 1963).

All examinations were conducted with four replications.

Results

The IAA oxidase activity, observed in the GA-treated and untreated hypocotyls under different experimental conditions, is presented in Fig. 1. Ordinate indicates the amount of IAA broken down in 1 hour by an enzyme preparation corresponding to 1 g fresh weight. Without GA treatment (0 ppm), both in light and dark, a greater enzyme activity could be measured in the decapitated hypocotyls; which is in full agreement with our earlier statement that in these tissues the IAA content is lower than in the intact hypocotyls (Varga and Bitó, 1967). Another conclusion can be also drawn, i.e. the oxidative

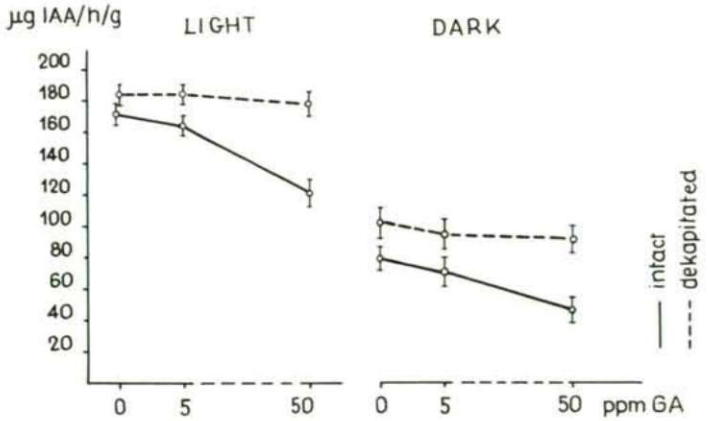

Fig. 1. Effect of GA treatment on the IAA oxidase activity.

destruction of IAA is greater in light than in dark; that is also reconcilable with the higher auxin concentration measured in dark. GA treatment apparently does not alter these conditions, however, in the intact hypocotyls — both in light and dark — it decreased the enzymatic degradation of IAA. On the other hand, GA treatment had no effect on the enzyme activity in the decapitated hypocotyls, i.e. the differences from the control are not significant.

Quite similar results were obtained with the paperchromatographic examinations too (Fig. 2), when the relative IAA quantity, remained after incubation with the enzyme, was estimated by comparing the spot size and colour intensity.

Having previously fed the hypocotyls with TTP, GA significantly enhanced the utilization of the precursor in IAA synthesis, i.e. the IAA

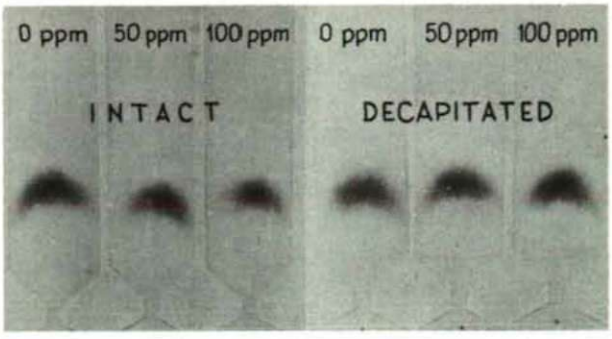

Fig. 2. Measurement of IAA oxidase activity with paper chromatography (comparison of the relative IAA quantities after incubation with the enzyme).

content of the tissues (V a r g a et al. 1967). At any rate, the IAA oxidase activity apparently was not influenced by the TTP pretreatment (Fig. 3), because the enzymatic destruction of IAA measured in this case — taking the standard error into consideration — was about the same as in the tissues not fed with TTP.

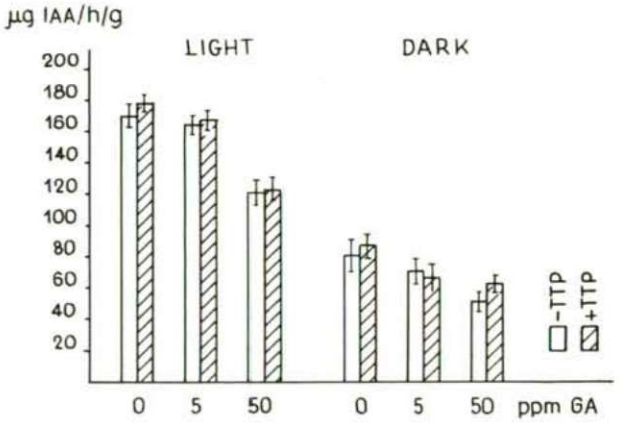

Fig. 3. Effect of TTP feeding on the IAA oxidase activity (intact hypocotyls).

Discussion

The effect of GA on the IAA-oxidizing enzyme system was studied several times in the last years and many of the authors rendered account of a decrease in IAA activity, due to the effect of GA treatment (P i l e t 1957; P i l e t and W u r g l e r 1958; G a l s t o n and M c C u n e 1961; K o n i n g s 1961; H a l e v y 1963). In some papers the observed endogen „auxin sparing" is explained indirectly, by stating that GA increases the formation of an IAA oxidase inhibitor in the tissues

(Stutz and Watanable 1957; Galston 1957, 1959; Housley and Deverall 1961; Gaspar and Bouillenne-Walrand 1966; Bouillenne-Walrand et al., 1967). According to other views, changes are induced by GA not in the inhibitor level but in the spectra of the IAA-oxidizing peroxidase system. For example, in the dwarf corn six peroxidase isozymes were found and GA treatment caused a decrease in the quantity of some of them and an increase in others (McCune and Galston 1959; Galston and McCune 1961).

On the other hand, some works lead to the conclusion that GA has no effect on IAA oxidase. E.g. the results of Kato and Katsumi (1958), Sági and Garay (1961), Kefford (1962), Varga and Bálint (1966) as well as Procko et al. (1966) do not support the idea that GA would influence the endogen IAA content by an auxin sparing mechanism.

In our experiments, in bean hypocotyl tissues, the enzymatic destruction of IAA was decreased by GA only in the present of the shoot apex and not influenced in the decapitated hypocotyls. Furthermore, the relatively slight decrease of IAA oxidase activity, observed in the intact hypocotyls, is not proportionate to the rate of the simultaneous rise of IAA level, nor to that of the stem elongation. Consequently, we can conclude that the GA-induced enhancement of IAA can not be ascribed merely to sparing the auxin from decomposition, as GA increased the IAA content even in cases when it did not decrease the enzyme activity, i.e. in decapitated hypocotyls. Thus, the higher IAA level induced by GA can be attributed only partly to an auxin sparing effect; this phenomenon — as results prove — can be caused rather by the direct promotion of IAA synthesis.

Pretreatment of the hypocotyls with TTP resulted in a significant rise of IAA concentration in the stem, but the IAA oxidase activity remained unchanged. Hence, in our experiments no adaptive formation of the enzyme could be observed, i.e. rising the substrate concentration the IAA oxidase activity (or synthesis) increases (Galston and Dalberg 1954; Gaspar 1965; Garay 1967).

In our experiments, both in the GA treated and untreated hypocotyls, the enzymatic destruction of IAA was greater in light than in dark. Similar statement was made by Galston (1957) working with dwarf pea, further by Stutz (1957 and Garay (1967) with *Lupinus albus*. According to the latter author, light increases the breakdown of IAA in the stem very likely by inducing a *de novo* synthesis of IAA oxidase.

Moreover, the results proved that the presence of the shoot apex plays also an important role in the connection of IAA oxidase with GA, since GA treatment influenced (decreased) the auxin destruction only in the intact hypocotyls. In all probability this is a symptom of the correlation; and that IAA oxidase activity can be influenced also by correlative effects, has been observed by other authors too (Garay 1967).

Summary

In bean hypocotyl tissues, in the presence of the shoot apex, GA treatment decreased the IAA oxidase activity, but it had no effect in the decapitated hypocotyls. Since GA enhanced the IAA content in hypocotyls even in cases when it did not alter the enzyme action, the GA-induced increase of IAA level can be ascribed only partly to saving the auxin from the enzymatic destruction. The higher IAA concentration in the GA treated tissues can be attributed rather to the direct promotion of IAA biosynthesis.

The action of GA exerted on IAA oxidase is influenced also by light and the presence of the shoot apex.

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