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ON THE MECHANISM OF GIBBERELLIN – AUXIN INTERACTION VII. EFFECTS OF GIBBERELLIC ACID ON THE UTILIZATION OF AUXIN PRECURSORS IN INDOLEACETIC ACID SYNTHESIS

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Introduction

We have demonstrated in earlier works that in bean hypocotyl tissues the gibberellic acid (GA_3) treatment raises the level of the endogenous indoleacetic acid (IAA). In the GA-treated stem tissues, in vivo, the concentration both of the free and bound IAA increased considerably (VARGA and BITÓ, 1968; VARGA, KÖVES and SIROKMÁN, 1968). According to our data, the GA-induced increase of IAA level ensues much more by promoting the biosynthesis of auxin from tryptophan (TTP) than by decreasing the auxin destruction (VARGA et al., 1968; VARGA and BITÓ, 1967). De demonstrated the stimulation of the TTP → IAA conversion by GA also in an in vitro growth system (VARGA, 1972).

SASTRY and MUIR, (1965) observed in their experiments carried out with Avena coleoptiles that the TTP-induced elongation was of comparatively low degree but it increased in the presence of GA, showing that the conversion of TTP into IAA in the coleoptiles was stimulated by GA directly. Taking into consideration these results, we have carried out further investigations with bean stem segments concerning the influence of GA on the utilization of TTP and other auxin precursors in the IAA synthesis. These experiments were also suitable for studying the problem in what path-ways the TTP → IAA conversion in bean stem tissues is realized.

Materials and Methods

10 mm segments were cut out, immediately under the cotyledons, from the hypocotyl of five-day-old bean seedlings (*Phaseolus vulgaris* L. var. *Golden Rain*) grow in the dark. Ten pieces of segments were floated in a Petri dish on 5 ml test solution, at 24°C, in dark, for 24 hours. The solution contained 0,05 M phosphate buffer (pH 6.0), 0.05 M sucrose, as well as GA, IAA, various auxin precursors or their mixtures in different concentrations. As auxin precursors TTP, indoleacetonitrile (IAN), indoleacetaldehyde (IAAld) and tryptamine (TNH_2) were used. The length of the segments was measured after incubation and the elongation was expressed as Δ mm exceeding the control value ($n = 4 \times 10$).

Origin of the chemicals used: GA_3: Phylaxia Budapest; IAA: Merck AG Darmstadt; TTP, IAN and THN_2: Fluka AG Buchs SG; IAAld: Sigma Chem. Co. St. Louis.

Results and discussion

The effect of various concentrations of TTP, IAN, IAAld and TNH_2, inducing stem elongation, can be studied — as compared to that of IAA — in Fig. 1. The highest degree of lengthening was produced — although on a lower level as compared to IAA — by TTP, the reaction optimum is at 10^{-5} M. The effect of IAN was somewhat smaller than that of TTP but still pronounced,

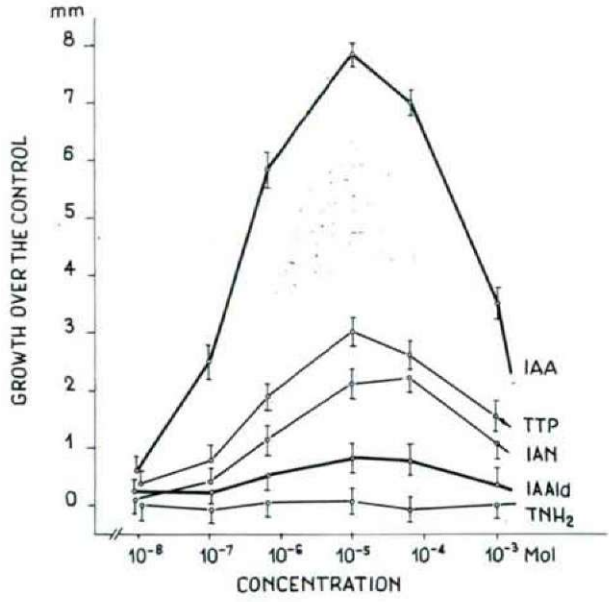

Fig. 1. Effect of some IAA-precursors on the elongation of the subapical hypocotyl segments of bean.

with a concentration optimum of 10^{-4} M. After applying IAAld in an interval of 10^{-6} to 10^{-3} M, only a little growth-stimulating effect could be observed; and TNH_2 proved to be ineffective in any concentration. The hypocotyl segments of bean contain, therefore, enzymes converting TTP, IAN and probably IAAld into auxin, as well. The most important intermediary of the TTP → IAA pathway is apparently IAN, in a smaller degree IAAld; but the reaction-way leading through TNH_2 seems to be missing in the stem tissues of bean.

Now it is questionable whether the elongation of the segments incubated in TTP and IAN can be increased by adding GA at the same time. The joint effect of TTP and GA is demonstrated in Fig. 2. As perceptible, the interaction of TTP and GA depends on the concentration. The synergistic effect between 10^{-5}, 10^{-4} and 10^{-3} M GA and 10^{-5} M TTP is obvious. But synergism can be observed, however to a lower degree, also with the two lower TTP concentrations. That means that the TTP → IAA conversion is increased by GA, at least in cases of TTP concentrations like these.

After simultaneously applying GA and IAN (Fig. 3), definite synergism manifested between 10^{-5} to 10^{-3} M GA and any concentration of the precursor and particularly 10^{-5} M. In this growth system, the biological effect of IAN is

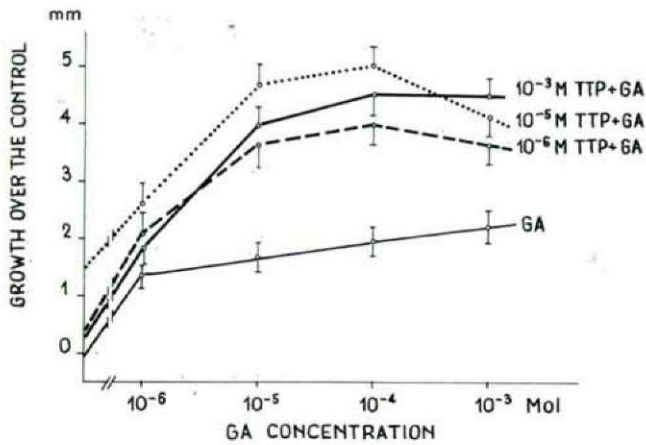

Fig. 2. Joint effect of GA and TTP on the elongation of the stem segments of bean.

therefore increased by GA, what is possible obviously only by increasing in some way the utilization of the precursor in IAA-formation.

In the presence of 10^{-5} M IAAld with 10^{-5} to 10^{-3} M GA, a synergism exceeding the standard error but in a small degree was observed; GA exerted, therefore, only a very little effect on the elongation induced by this precursor.

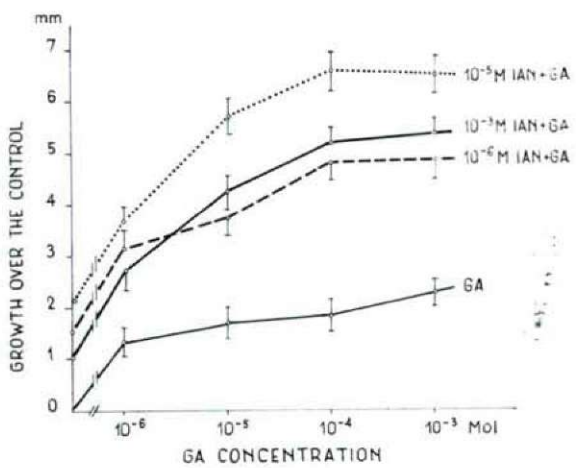

Fig. 3. Joint effect of GA and IAN on the elongation of the hypocotyl segments of bean.

GA had in the experiments of SASTRY and MUIR (1965) also a little effect on the IAAld-induced growth, from what it was concluded that GA took part in the TTP → IAA conversion before the aldehyde state. We interpret the small physiological effect of IAAld rather by saying that the path-way of IAA-biogenesis through indolepyruvic acid — indoleacetaldehyde in the bean stem tissues is of lower importance.

the pigment components chlorophyll-a is decreasing the most expressedly and,

In connection with the above-mentioned experiments it seemed to be necessary to investigate the kinetics of the stem elongation stimulated by IAA and TTP, as well as by TTP + GA. The time curves of the elongation of stem segments are demonstrated in Figs. 4 and 5.

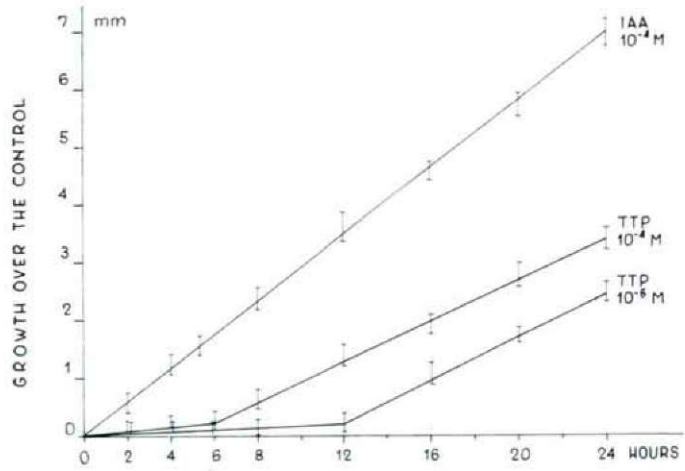

Fig. 4. Time curves of the growth-stimulatory effect of IAA and TTP.

According to the data in Fig. 4, the TTP-induced stimulation begins only after a definite lag-period; the lag proved to be 5 to 6 hours in case of TTP of maximum effect 10^{-5} M, and 10 to 12 hours after applying 10^{-6} M TTP inducing a smaller elongation. In all probability, this time is necessary for the critical amount of auxin to be produced from TTP in the stem tissues. This suppositon is strongly supported by the experience that the stimulatory effect of IAA has no perceptible lag-period.

The duration of the lag observed at the incubation in TTP was shortened by the simultaneous adding of GA (Fig. 5). The explanation of that is obviously that the TTP → IAA conversion is promoted by the increase of the endogenous GA-concentration.

Summary

In bean stem segment test, the growth-stimulating activity of tryptophan (TTP), indoleacetonitrile (IAN), indoleacetaldehyde (IAAld) and tryptamine (TNH_2) was investigated, in the presence and absence of gibberellic acid (GA_3).

Applying TTP + GA, IAN + GA, as well as IAAld + GA jointly, a definite synergism manifested itself in the elongation of segments. The biological effect of these auxin-precursors is stimulated by GA apparently by the increase of their utilization in the IAA-formation.

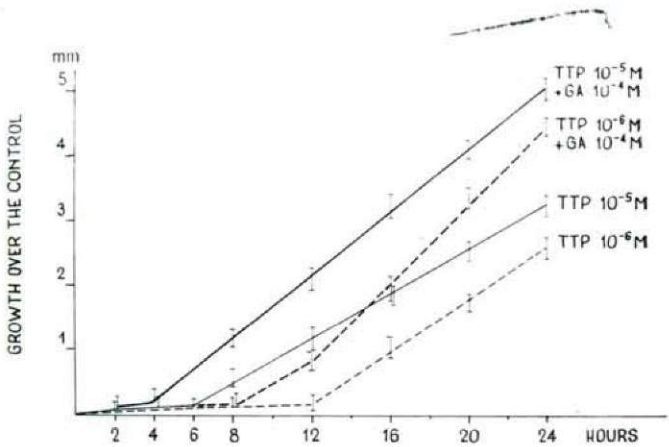

Fig. 5. Effect of GA on the kinetics of the TTP-induced growth

The stimulation of the TTP-induced growth takes place, dependent upon concentration, only after a lag-period of 5 to 12 hours. The duration of lag is shortened by the presence of GA that similarly indicates the promotion of TTP → IAA conversion by GA.

In bean shoots, the biosynthesis of IAA from TTP is mainly realized through IAN, but a less important path-way through IAAld can also be demonstrated. On the other hand, the conversion of TNH_2 into auxin could not be observed in the test object.

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