Acta Biologica Szeged. *19* (1—4), pp. 93—98 (1973)

ON THE MECHANISM OF AUXIN – GIBBERELLIN INTERACTION VI. GIBBERELLIN-INDUCED CHANGE IN THE ACTIVITY OF IAA-FORMING ENZYME PREPARATAIONS

MAGDOLNA VARGA

Department of Plant Physiology, Attila József University, Szeged

(Received June 30, 1972)

Introduction

We have demonstrated in previous papers (VARGA, 1968; VARGA *et al.*, 1968) that after feeding the bean hypocotyl tissues with triptophan (TTP) and ^{14}C–2–TTP, the conversion of TTP to indoleacetic acid (IAA) or ^{14}C–IAA *(in vivo)* takes place in a higher degree in the GA-treated stems than in the untreated controls. It seems, therefore, that the utilization of the precursor in the IAA-biosynthesis is increased by GA.

It is anyway to take into consideration that at investigating the TTP ↑ IAA conversion *in vivo,* we could not follow simultaneously with attention and filter out several metabolic processes that can exert influence on the auxin yields. In case of *in vivo* experiments, for example, there was no possibility of gathering information about the degree of the oxidative degradation going on parallel with the IAA-synthesis. The concentration of IAA, however, measured at the given date, depends in a high degree upon the auxinoxidase activity. We regarded, therefore, desirable to carry out also the *in vitro* investigations of similar aim with cell-free enzyme preparations, for confirming the results of the *in vivo* experiments.

It has been proved in more works that cell-free enzyme preparations can perform the TTP → IAA conversion. GORDON wrote first in 1958 about an enzyme system extracted from shoot tips of *Phaseolus aureus* seedlings which catalyzed *in vitro* the process of the conversion of TTP to auxin. LANTICAN and MUIR (1967) described an IAA-producing enzyme system isolated from the apex of *Avena* coleoptiles, MOORE and SHANER (1967) from that of pea, VALDOVINOS *et al.*, (1967) similarly from that of pea and *Coleus,* and PHELPS and SEQUEIRA (1967) from that of tobacco shoots.

The degradation of TTP to auxin by cell-free plant tissue extracts can, therefore, be carried out *in vitro* too; and in the present experiments the effect of GA on the TTP → IAA conversion was investigated in this way, too. On the basis of the works of GORDON and PALEG (1961), GORDON and BUESS (1963) – who had referred to the TTP-degradation taking place spontaneously, as well, in the reaction mixtures – in the course of the experiments we took inti consideration the possible formation of IAA both in enzymatic and in non-enzymatic way.

Materials and Methods

The apical segments of seven days old shoots of bean seedlings (*Phaseolus vulgaris* L. var. Golden Rain), with the terminal bud and primordial leaves, were used for the experiments.

The cell-free enzyme preparation was made according to MOOR and SHANER (1967): the chilled tissues were homogenized with Mac Ilvain's buffer of double amount (pH 6.4 and 7.6), and after filtration were centrifuged with 105 000 x g for 90 minutes. In some cases the enzyme extract was dialyzed against 0.1 M buffer, for twenty hours, at 2 to 4°C.

With the enzyme preparation — in cases of both pH — four kinds of reaction mixtures were made, for the following aims:

1) Investigation of the enzymatic degradation of TTP in the presence of GA: 5 ml enzyme preparation + 5 ml TTP ($5 \cdot 10^{-4}$ M) + 10 ml GA (50 ppm).

2) Control of No. 1. without GA: 10 ml enzyme preparation +5 ml TTP ($5 \cdot 10^{-4}$ M) +10 ml buffer.

3) Investigation of the spontaneous conversion of TTP in the presence of GA: 5 ml preparation + 5 ml TTP ($5 \cdot 10^{-4}$ M) + 10 ml GA (50 ppm).

4) Control of No. 3 without GA: 10 ml TTP ($5 \cdot 10^{-4}$ M) +10 ml buffer.

The incubation took place two hours long in the dark, with a continuous current of air. After stopping the reaction and acidifying to pH 2.8, the IAA content of the mixtures was shaken into peroxide-free ethyl-ether and separated with paper chromatography (Sch & Sch 2043 b paper, isopropanol — 7% ammonia-water 8 : 1 : 1 solvent). The quantity of IAA was compared partly on the basis of spot size and colour intensity obtained by Ehrlich's reagent and defined in relation to the standard series, partly by photometric measurement of the eluate of the chromatogram-spots at 280 nm. For expressing enzyme activity, the following formula was used: mμg IAA/min. x ml enzyme (i.e. IAA mμg produced in the reaction mixture in one minute and multiplied with the present ml enzyme). It is to be noticed that the incubation time of two hours means about the saturation level, the curve of the IAA yield being linear for 120—130 minutes. Referring the efficiency of the enzyme preparation to protein-N, also the specific activity was expressed: mμg IAA/min. x mg nitrogen.

Every investigation was carried out with two parallels, in four replications.

Results and discussion

The IAA-synthesizing ability of the cell-free enzyme preparation was studied with the four sorts of reaction-mixtures described in the Methods, at pH 6.4 and 7.6. The parallel work at both pH was considered necessary because

Table 1. GA-induced change in the IAA-synthesizing ability of cell-free enzyme extracts from seven days old bean shoots (pH 7.6).

Incubation mixtures		mμg IAA/min.	Enzyme* activity	Protein—N mg/ml	Specific** activity	Activity percentage
1	enzyme TTP GA	764.0	3820	1.12	4278	215
2	enzyme TTP	354.1	1770	1.12	1982	100
3	buffer TTP GA	86.5	432	1.12	484	24
4	buffer TTP	92.8	464	1.12	520	26

* mμg IAA/min. x ml enzyme

** mμg IAA/min. x mg protein—N

the *in vitro* functioning of the TTP → IAA converting enzyme system has – on the basis of our previous investigations and KÖVES's paper (1965) – its optimum between pH 7 and 8 and then there is practically no IAA-oxidase activity. On the other hand, pH 6.4, which is less favourable to IAA-formation, is the pH optimum of the IAA-oxidase in bean.

In the course of the experiments TTP → IAA conversion was observed, in a lower degree, in the enzyme-free reaction mixtures Nos. 3 and 4 as well; the demonstrable IAA amount was, however, in both cases ± identical. The spontaneous degradation of TTP to auxin was, therefore, by the presence of GA perceptibly not influenced (Fig. 1). All the more was noticed the stimulating effect of GA on the enzymatic IAA-formation; its presence namely strongly increased in the first reaction mixture the TTP-converting activity of the enzyme extract, that is to say, the yield of IAA. The results referring hereto are given in Table 1. The conclusions that can be drawn from the data can be accorded essestially well with MUIR's (1964) results of similar aim obtained, however, with another method and on other objects. It was namely observed by the author, too, that after GA-treatment the enzyme preparations from apical tissue of dwarf pea and from tomato ovary performed the conversion of TTP to auxin in a higher degree.

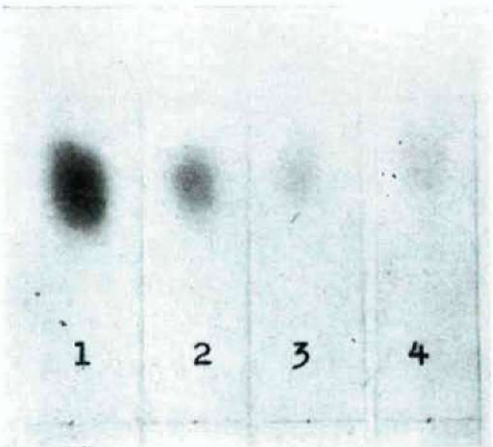

Fig. 1. Relative quantity of IAA formed in the reaction mixtures, on the basis of spot size and colour intensity (pH 7.6).
1, 2, 3, 4 = reaction mixtures

In the experiments, the GA given to the cell-free enzyme preparation at pH 7.6 increased the yield of IAA to be more than double (Table 2). Carrying out the investigations under completely sterile conditions and in the presence of 200 μg/ml penicillin and streptomycin, the yield of IAA did not decrease (Table 2); it is not probable, therefore, that the TTP → IAA conversion observed would be of bacterial origin, as published in the papers of some authors (LIBBERT and WICHNER, 1963; LIBBERT *et al.*, 1966; WINTER, 1966; WICHNER and LIBBERT 1968, etc.). Some participation of epiphytic bacteria in

Table 2. Effect of GA, dialysis, and antibiotica on the IAA-synthesizing ability of the cell-free enzyme preparations made from bean shoot.

Enzyme preparations	Presence of GA	Yields of IAA	
		mμg/g fresh weight	μmoles/g fresh weight
Non-dialyzed	—	71.1	406
	+	152.7	872
Dialyzed	—	102.3	584
	+	211.6	1208
Non-dialyzed + penicillin and streptomycin	—	74.0	422
	+	148.9	850
Dialyzed + penicillin and streptomycin	—	100.7	575
	+	215.8	1233

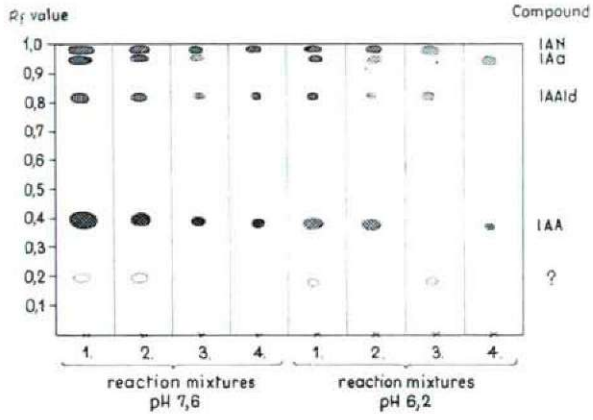

Fig. 2. Chromatogram-spots of IAA and IAA-precursors formed from tryptophan in the reaction mixtures.
(Solvent: isopropanol — 7% ammonia — water 8 : 1 : 1)

the IAA-production is, of course, not excluded completely by our experiments. But we do not believe that this could be considerable in this case – or in other similar experiments – as compared with the IAA-production of the enzyme system of apical stem tissues.

On the other hand, the yield of IAA was observably increased in the reaction mixtures, containing GA or not, by the dialysis of the enzyme preparation (against 0.1 M buffer, for 20 hours, at 2 to 4 °C). That can be explained by the removal of the compounds disturbing the reaction, resp. of the phenolic co-factors stimulating the IAA-oxidase activity.

For determining also the simultaneous IAA-destruction in the reaction mixtures, IAA of known quantity was added to the enzyme preparations (pH 7.6) and after two hours incubation the remaining auxin concentration was measured (VARGA and BÁLINT, 1966). The IAA-destruction proved to be of very low degree: 1.35 to 1.80 μg/h per ml of enzyme. That means that the

IAA could be regained in 92 to 94 per cent. In the tissue extract of the bean shoot applied, at pH 7.6, is therefore no considerable IAA-oxidase activity. The values given in Table 1 can therefore be considered as the actual auxin yields during the time unit.

The IAA quantity produced at pH 6.2 – although its presence was doubtless observed in the Nos. 1 and 2 reaction mixtures – was much less than that obtained at pH 7.6. That is obviously because in this case the *in vitro* TTP → IAA conversion of lower degree was followed by IAA-destruction of higher degree. The auxin-oxidase activity of the enzyme preparation was here 10.8 to 11.7 μg/h per ml of enzyme. This corresponds to regaining not more than 45 ± 3 per cent of the IAA added.

On the chromatograms of the ether extracts of the reaction mixtures, apart from the IAA spot, there could be observed, of course, other spots of indole compounds formed from TTP as well; and at pH 6.2, too, about in the same amount as at pH 7.6. From among these IAA precursors at R_f 0.83 indoleacetaldehyde (IAAld), at 0.92 indoleacetamide (IAa), and at 0.98 indoleacetonitrile (IAN) could be identified. Identification took place on the basis of R values, colour reactions, UV fluorescence and UV absorption spectrum, as compared to those of authentic compounds.

The result of the *in vitro* experiments carried out with cell-free tissue extracts have therefore confirmed again our previous statement that in bean shoots the biosynthesis of IAA from TTP is mainly realized through IAN – IAa, but another pathway through indolepyruvic acid (IPyA) – IAAld can also be proved (VARGA *et al.*, 1968; VARGA, 1971). It seems that the presence of GA exerts a stimulatory effect on these biochemical processes.

Summary

The ability of the cell-free enzyme preparations from apical tissues of bean shoots to realize TTP → IAA conversion was studied, in presence and absence of GA. Addition of GA to the enzyme preparation did not exert any influence on the spontaneous degradation of TTP to auxin; it did however considerably stimulate the enzymatic IAA production and multiplied many times the yields of auxin. The IAA-synthesizing ability of the tissue extracts was therefore definitely increased by the presence of GA. These *in vitro* experiments have confirmed the results of our earlier *in vivo* investigations.

Carrying out the experiments under sterile conditions and in presence of antibacterial compounds, the yield of IAA did not decrease. The TTP → IAA conversion observed cannot be, therefore, of bacterial origin.

References

GORDON, S. A. (1958): Intracellular localization of the tryptophan-indoleacetate enzyme system. — Plant Physiol. *33*, 23—27.

GORDON, S. A.—BUESS, E. (1963): Inactivity of hexitols and hexose in biosynthesis of auxin from tryptophan. — Plant Physiol. *38*, 11.

GORDON, S. A.—PALEG, L. G. (1961): Formation of auxin from tryptophan through action of polyphenols. — Plant Physiol. *36*, 838—845.

KÖVES, E. (1965): Adatok a természetesen előforduló fenolkarbonsavak növekedésszabályozó hatásának mechanizmusához. — Kandidátusi értekezés. (Thesis).

LANTICAN, B. P.—MUIR, P. M. (1967): Isolation and properties of the enzyme system forming indoleacetic acid. — Plant Physiol. *42*, 1158—1160.

LIBBERT, E.—WICHNER, S. (1963): Trytophan-Abbau zu Indolessigsäure durch pflanzenbesiedelnde Mikroorganismen. — Naturwiss. *50*, 451.

LIBBERT, E.—WICHNER, S.—SCHIEWER, U.—RISCH, H.—KAISER, W. (1966): The influence of epiphytic bacteriae on auxin metabolism. — Planta *68*, 327—334.

MOORE, T. C.—SHANER, C. A. (1967): Biosynthesis of indoleacetic acid from tryptophan—C^{14} in cell-freee extracts of pea shoot tips. — Plant Physiol. *42*, 1787—1796.

MUIR, R. M. (1964): Conversion of trytophan to auxin by plant tissue preparations. — Plant Physiol. *39*, Suppl. XVII.

PHELPS, R. H.—SEQUEIRA, L. (1967): Synthesis of indoleacetic acid via tryptamine by a cell-free system from tobacco terminal buds. — Plant Physiol. *42*, 1161—1163.

VALDOVINOS, J. G.—ERNEST, L. C.—HENRY, E. W. (1967): Effect of ethylene and gibberellic acid on auxin synthesis in plant tissues. — Plant Physiol. *42*, 1803—1806.

VARGA, M. (1971): On the mechanism of gibberellin — auxin interaction. VII. Effect of gibberellic acid on the utilization of auxin precursors in indoleacetic acid synthesis. — Acta Biol. Szeged *17*, 113—118.

VARGA, M.—BÁLINT, I. (1966): The effect of gibberellin on the growth, indoleacetic acid content and on the activity of indoleacetic acid oxidase in rice seedlings. — Acta Biol. Hung. *16*, 243—253.

VARGA, M.—KÖVES, E.—SIROKMÁN, F.—BITÓ, M. (1968): On the mechanism of gibberellin — auxin interaction. III. Effect of gibberellin on the biosynthesis of IAA from tryptophan. — Acta Bot. Hung. *14*, 435—442.

WICHNER, S.—LIBBERT, E. (1968): Interactions between plants and epiphytic bacteria regarding their auxin metabolism. I. Detection of IAA-producing epiphytic bacteria and their role in long duration experiments on tryptophan metabolism in plant homogenates. — Physiol. Plant. *21*, 227—241.

WINTER, A. (1966): A hypothetical route for the biogenesis of IAA. — Planta *71*, 229—239.

Address of the author
Dr. MAGDOLNA VARGA
Department of Plant Physiology
A. J. University
H — 6701 Szeged P. O. Box 428,
Hungary