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FORMATION OF AUXIN-MACROMOLECULE COMPLEXES IN PLANT ORGANS OF VARIOUS AGES

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Introduction

In the course of previous studies of the bound forms of indole-3-acetic acid (IAA) it proved indisputable that the IAA is capable of binding to the macromolecules to be found in the cells (SIEGEL and GALSTON, 1953; WINTER and THIMANN, 1964; GALSTON et al., 1964; ZENK, 1964; MUKHERJEE et al., 1966; MORRIS et al., 1969; MERKYS et al., 1966; 1969; DAVIES and GALSTON 1970; FELLENBERG, 1970). Because of the low intracellular endogeneous concentration of these molecular complexes, there are barely any quantitative data available (SIEGEL and GALSTON, 1953; ZENK, 1964; WHEELER, 1968), and the situation is similar with regard to what correlation is shown by these bund IAA forms with the extent of the extension (MERKYS, 1969). Nor is it known what role is played by the auxin-macromolecule complexes in the growth-regulation; in this connection only theoretical considerations have so far been put forward (ARMSTRONG, 1966; GALVSTON et al., 1964; FELLENBERG, 1968; 1969).

In order to establish a relation between the extent of the formation of the endogeneous IAA-macromolecule complexes, the age of the organs and the elongation, the IAA content incorporated in the macromolecules precipitable by trichloroacetic acid (TCA) was determined on the first 8 days of the individual development in various organs of bean shoots. In the course of the experiment the extent of the hypocotyl extension was measured, together with the protein content of the samples.

The results so obtained were compared with the results of experiments in which the total activities and specific activities were measured for the macromolecule fractions of plant organs immersed in sulutions containing ^{14}C-labeled IAA. These experiment provide information on the binding of the exogeneous IAA to the macromolecules.

Experimental methods

The IAA bound to the macromolecules was determined by the modified method of GALSTON et al. (1964). The plant material was homogenized with cold methanol and extracted until it no longer contained free IAA. The residue was suspended in a buffer of pH 7.5, centrifuged, and the supernatant solution purified on a Sephadex G—50 column. After gel-filtration 0.5 M TCA was added and the resulting precipitate was washed several times. The TCA-insoluble fraction, completely free of salts and low molecular weight compounds, was hydrolyzed for 4 hours at 60 °C with 0.5 M NaOH. The IAA set free was shaken into ether

and paper-chromatographed. The IAA was washed out of the chromatogram with methanol and its amount determined spectrophotometrically at 280 nm.

In the incubation experiments IAA was used which had been labelled with ^{14}C on the carboxyl group. The specific activity of the preparation was 0.35 mCi/mM, the concentration of IAA in the solution was 10^{-4} M, the incubation time was 16 hours and the incubation temperature was 24 °C. After the completion of the 16 hours the plant material was subjected to the procedure described above in order to determine the radioactivity incorporated into the macromolecule fraction. An aliquot part of the fraction insoluble in TCA (the macromolecule fraction) was dissolved in NH_4OH, and the radioactivity resulting from the ^{14}C—IAA was measured with a Packard—Tricarb liquid scintillation spectrometer. The data given in both Tables are the averages of four experimental series.

Results and discussion

The data of Table 1 exhibit the following relations:

The bound IAA content of the cotyledon — calculated on the basis both of the fresh weight and the protein content — decreases gradually during the first eight days of the germination. It is worthy of attention that in parallel with this the protein content of the cotyledon also decreases. The amount of bound IAA found in the cotyledon is fairly low compared with the free IAA, and so it can not be considered that this form of the bound auxin plays a part in the stored from.

Table 1. Endogeneous bound IAA and protein contents of bean-plant organs. (The data referring to the determination of the bound IAA can be found in the relevant section; the protein was determined by the method of Lowry et al (1951).

Sample	μg IAA per g fresh weight	μg IAA per mg protein	Protein mg/g	Growth mm/24 hours
Cotyledon				
0 day old	0.30	0.003	89.4	—
4 „ „	0.22	0.003	72.0	—
6 „ „	0.17	0.028	62.0	—
Hypocotyl				
6 day old	0.64	0.037	17.0	8.9
8 „ „	0.11	0.009	12.6	2.3
Shoot apex				
6 day old	0.72	0.028	25.0	—
8 „ „	0.21	0.105	20.6	—

Simultaneously with the determination of the bound IAA content of the decapitated hypocotyl, a measurement was also made of the elongation during 24 hours. During the studied period the intensity of the growth and the protein content of the organ decrease in parallel with the bound IAA content.

Bound IAA is not present in detectable amounts in the elongated hypocotyl.

The change with time of the bound IAA content in the apical part cantaining the bud and primary leaves is similar to that in the hypocotyl: it decreases in inverse proportion to the age of the organ, and together with it the protein content also decreases. The high bound IAA content is striking compared with

the other two organs. It must be noted, however, that the highest bound IAA content calculated for the protein is connected not with the shoot apex, but with the intensively extended hypocotyl.

The presence of bound IAA in the roots could be confirmed only qualitatively. It is probable that the roots too contain very small amounts of bound auxin.

Based on the data of Table 2, the incubation experiments confirm that the labelled auxin present in the solution is taken up by the tissues and incorporated into the macromolecule fraction. The experiments carried out with the labeled IAA support the results obtained in the quantitative determinations with regard to the relation between the bound IAA content, the protein content and the extension. The greatest incorporation calculated for the fresh weight was observed in the young shoot apex, followed in turn by the hypocotyl and the cotyledon, while the highest specific activity of the protein was found in the intensively extending stage.

Table 2. Radioactivity and specific activity incorporated into macromolecule fraction of bean hypocotyls incubated with 10^{-4} M IAA.

Sample	Imp. per min per g fresh weight	Spec. activity: imp. per min per mg protein	mg protein per g fresh weight
Hypocotyl			
6 day old	13 640	718	19.0
8 „ „	1 440	107	13.4
Shoot apex			
6 day old	16 620	585	28.4
8 „ „	3 360	145	23.1

It is a general finding that the concentration of bound IAA in the bean sprouts and the change of this with time are parallel with the protein content of the organs and the quantitative change of these, and that this is observed in the same way for the same organs of different ages as for different organs of the same age. In this way, the extension is correlated with not only the free, but also the bound IAA content. These findings are in agreement with the data published by MERKYS (1969). According to these, the IAA is bound to the RNA and DNA with a greater intensity on the more strongly growing side of the geotropically curving stems, than on the more slowly growing side. Our results also support those theories which see the explanation of the mode of action of the hormones in the regulation of the syntheses of certain proteins.

Summary

Experiments were carried out in order to characterize quantitatively the binding of endogenous IAA to the mocromolecule fraction of the cells in the different organs of various ages in bean sprouts. For this purpose precipitates were obtained with TCA from the shoot apex, hypocotyl and cotyledon of the sprouts, and these precipitates were hydrolyzed with NaOH and the IAA set free was measured spectrophotometrically.

In another experimental series a study was made of the incorporation of ^{14}C-labelled IAA into the macromolecule fraction. The experimental results show that the bound IAA endogen and its level during incubation are proportional to the protein content of the organ. There is a similar relation between the extension of the hypocotyl and the bound IAA content. There is an inverse relation between the bound IAA level and the age of the organs.

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