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RELATIONSHIP BETWEEN PLANT GROWTH REGULATION AND PHOSPHORYLATION PROCESSES II. INFLUENCE OF PLANT GROWTH SUBSTANCES ON METABOLISM OF THE PHOSPHOROUS COMPOUNDS IN PLANT TISSUES

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It is known that the fundamental reactions of hormonal regulation are of genetic level and, apart from that, indole-auxins and several other compounds of hormonal effect have also a direct influence on some metabolic processes. Among these processes, that with the most general importance is the oxidative phosphorylation, i.e., ATP synthesis, the connection of which with hormonal regulation is not even to-day, a question cleared enough.

The influence of auxins, antiauxins, as well that of some phenolic and flavonoid compounds, e.g. the so-called β-inhibitor, upon the change of P/O ratio — in an isolated mitochondria suspension — was studied by a lot of authors (Switzer, 1957; Reid, 1957; Stenlid and Saddik, 1962; Flaig and Schmid, 1962; McDaniel and Sarkissian, 1966; Zhivukhina and Jakushkina 1966). In spite of these investigations, the role of plant growth substances in the oxidative phosphorylation cannot be evaluated in vivo relation, as yet.

The in vivo experiments discussed in this paper have had the aim to follow — by determining the ratio of inorganic phosphorus, nucleotide-polyphosphates and phosphorylated products — in the track of changes in phosphorylation with an indirect method, in plants treated with regulators.

Materials and Methods

For the investigation the materials and methods described in details in Part I of the publication (Sirokmán and Köves, 1968) have been used.

The course of experiments has been as follows.

Isolated plant parts — shoots and roots of pea seedlings or excised leaves — were floated on a nutrient solution containing the required regulator in appropriate concentration. The intact plants were kept in a nutrient culture. After an incubation of 18—24 hours, some samples were taken from the plant material and extracted with ethanol of 70 %. The extract was chromatographed after being

evaporated. The chromatograms were evaluated radiometrically and the total number of impulses of the single compounds, respectively fractions recorded. The compounds were identified with the usual chromatographic methods. For separating ADP and ATP we have cut out the nucleotide spots of the chromatograms prepared in the way described above, and rechromatographed them — on a paper washed with 2 n acetic acid and impregnated with ethylenedliaminetetraacetic acid — in a solvent methanol-ammonia-water 6:1:1. The quantitative determination of ADP and ATP took place on the basis of their UV-absorption observed at a wave length of 260 nm.

Results of experiments

1. Influence of indole-3acetic-acid (IAA) on the incorporation of ^{32}P in alcohol-soluble phosphate compounds of pea seedlings.

According to data of Table 1, the total impulse number of ^{32}P incorporated in pea shoots fed with IAA is lower than that of controls. Expressed in percentage of the total impulse number, the impulse number of the inorganic phosphate fraction is higher, and that of the nucleotide fraction is considerably lower, than those in control plants. As these changes correspond to those expressed by the decrease of P/O, we have investigated under identical conditions, the effect of an uncopuler, 2,4-dinitrophenole (DNP) too, on the alteration of alcohol-soluble phosphate fractions. It appears from Table 1 that the changes induced by DNP in the ratio of fractions compared with one another, are essentially the same as in case of an IAA-treatment; that is to say, the inorganic phosphate ratio increases and that of the nucleotids decreases. The effect of 2,4-DNP is, anyway, considerably greater that that of IAA.

Table 1. Incorporation of ^{32}P in the alcohol-soluble phosphate fractions of pea epicotyl treated with uncoupling and growth regulating compounds. (The data refer to 1 g fresh weight.)

Fraction	Control		IAA 10^{-4} M		2,4-DNP 10^{-4}M	
	C.p.m./ fraction	%	C.p.m./ fraction	%	C.p.m./ fraction	%
Inorg.-^{32}P	12642	43,4	6841	48,2	2105	72,6
Ester-^{32}P	5336	18,3	2653	18,6	427	14,7
"Indole"-^{32}P	444	1,6	594	4,2	-	-
Nucleotide-^{32}P	10754	36,7	4140	29,0	370	12,7
Total	29176		14228		2902	

2. Influence of naturally occurring plant growth substances of phenolic carboxylic-acid character on the incorporation of ^{32}P in the alcohol-soluble phosphate compounds.

The salicylic acid, and some arylhidroxy-cinnamic acid derivates, in a concentration of $10^{-3}M$—$10^{-4}M$, inhibit the elongation as well the auxininduced elongation in several isolated plant organs. The basis of their growth inhibitory effect is — at least partly — the inhibition of the oxidative phosphorylation, as demostrated in vitro by Reid in 1957, by Marinos and Hemberg in 1960, by Stenlid and Saddik in 1962.

The result of our investigations in vivo is shown in Table 2.

Table 2. The radioactivity incorporated in the alcohol-soluble ^{32}P-fractions of pea roots treated with salicylic acid and o-coumaric acid expressed in the percentage of the total activity.

Fraction	$10^{-4}M$ salicylic acid	$10^{-4}M$ o-coumaric acid	control
Inorganic-^{32}P	92,2	95,0	65,2
Ester-^{32}P	3,9	2,4	14,6
"Indole"-^{32}P	1,5	0,8	2,2
Nucleotide-^{32}P	2,4	1,8	18,0

In some ^{32}P-fractions of pea roots, as a result of treatment, the same changes occur like in presence of 2,4-DNP, i.e., activity increased in fractions of the inorganic-^{32}P, but considerably decreased in fraction of the nucleotdie-^{32}P, as compared to the control.

3. Change of the ADP/ATP ratio in the plants treated with $10^{-4}M$ IAA.

Table 3 is demonstrating the amount of ATP, resp. ADP measured by spectrofluorometry in shoots of pea treated with IAA and untreated, further that of the ADP/ATP ratio. According to the data, the total

Table 3. ATP and ADP content of pea shoots treated with IAA.

Sample	µg ATP/g fresh weight	µg ADP/g fresh weight	ADP/ATP
Control	3,0	4,5	1,5
$10^{-4}M$ IAA	1,0	5,5	5,5

amount of the two nucleotides is higher in the control plant, and inside it, the ADP/ATP ratio is increased by IAA-treatment.

4. Incorporation of ^{32}P in the indole compounds of pea shoots in time.

We have established on the basis of earlier radiochromatographic investigations that ^{32}P incorporates in the indole compounds, the F_f-value of which in the chromatograms approaches very much the R_f-values of IAA and tryptophan. Some of them contain a sugar component, as well (Köves and Sirokmán, 1963; 1965; 1966).

Their quantitative change in shoots and roots of seedlings of the intact pea are shown by Figure 1 in the function of time. According to experimental data, the ^{32}P-incorporation is increased by light, both in root and shoot. The curve demonstrating the radioactivity of shoots has a maximum in light, showing that these compounds are incorporated in insoluble compounds and that their incorporation is in light more intensive. (The maximum is considerably smaller in case of measurement results referred to dry matter.)

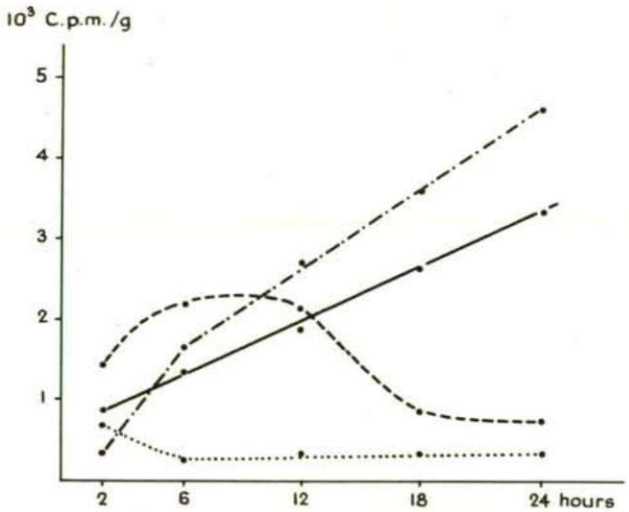

Figure 1. Incorporation of ^{32}P in indole compounds in tissue of pea shoots and roots.

— — — — — — shoot in light
. . . . . shoot in darkness
—.—.—.—.—.—. root in light
——————— root in darkness

The data of Table 4 are showing the influence of regulators applied in the experiments on the degree of the indole compounds in question being phosphorylated. The indices are showing the percentage of the ^{32}P activity incorporated in the indole compounds as compared with the total incorporated activity, in case of the single treatments. It can be ascertained that the salicylic acid and DNP, added into the nutrient

solution, are inhibiting, and IAA is increasing the incorporation of ^{32}P in the indole compounds.

Table 4. Relationship of growth intensity with incorporation of ^{32}P indole compounds in pea epicotyl treated with inhibitors and IAA.

Sample	Elongation in mm	"Indole"-^{32}P acitivity in percentage of the incorporated ^{32}P
10^{-4}M IAA	48	4,2
10^{-4}M salicylic acid	30	1,4
10^{-4}M 2,4-DNP	28	0,0
Control	32	1,6

Discussion of results

The basis of investigating connection between growth regulation and oxidative phosphorylation is the perception of the fact that sytheses accompanying the growth processes do realize only in case of a good energy supply. This has immediately implicated the supposition, that the degree of oxidative phosphorylation i.e., the amount of ATP at disposition, increases by growth-promoting auxins and decreases by compounds of growth-inhibiting effect. But the experimental works carried out on the basis of this supposition have not verified a direct connection like that. For explaining the effect of some growth-inhibiting substances, we can perhaps accept that they uncouple the oxidative phosphorylation. An interpretation of the auxin-effect and of the auxin-synergism is much more complicated.

Marré and Forti (1958) as well as Sen-Gupta and Sen (1961) found an unambiguous connection between oxidative phosphorylation and the growth-promoting effect of IAA. Stenlid and Saddik (1962) demonstrated about several types of regulators, among them compounds of auxin and antiauxin types, as well, that in vitro they decrease the P/O ratio. According to the authors, however, a direct connection is only possible between the effect of antiauxins and the inhibition of oxidative phosphorylation. Flaig and Schmid (1962) found IAA to have an uncoupling effect and explained the growth regulatory and inhibitory effects of auxin with the degree of uncoupling: the oxidative phosphorylation being inhibited in a low degree induces a promotion of growth owing to an increase of the inorganic phosphate level and a better utilization of the intermediary products of metabolism in the syntheses and being inhibited, it induces growth-inhibition owing to the low ATP level.

McDaniel and Sarkissian (1966), as well Zhivukhina and Jakushkina (1966) observed at physiological IAA concentration the increase of the oxidative phosphorylation, and at high IAA concentration its inhibition. In the plants used by them, however, the effect of IAA depended, apart from the concentration, on genotype and endogenous IAA-level, too. Spring and Rowan (1966) demonstrated a decrease of ATP concentration and an increase of the ADP/ATP ratio in plants fed with ^{32}P and treated with IAA; and they found the specific activity of these compounds identical with that of control. Trewavas, Johnston and Crook (1967) demontsrated the increase of the ADP/ATP ratio in case of a 5.10^{-5}M IAA concentration, owing to a decrease of ATP-level. According to their supposition, the decrease is caused by an increase of RNA synthesis induced by auxin.

In agreement with these author's results, it is shown also by our own experiments that in some way, a decrease of ATP level is brought about in the tissue by IAA. Coming up to the expectations, the accumulation of ATP, respectively nucleotide-^{32}P in tissues is decreased in vivo, too, by the salicylic acid known for its uncoupling effect, as well by o-coumaric acid.

We had succeeded, already in the course of our earlier experiments, in demonstrating the presence of some substances in the tissues treated with IAA that contain indole, sugar and phosphate components being similar, therefore, to nucleotides in view of the principle of their structural pattern (Köves and Sirokmán, 1963; 1966). From the fact that their chromatographic behaviour and their UV absorption spectra are almost identical with IAA, respectively tryptophan, the conclusion can be drawn that they are the derivates of these. Their disappearance from the plant tissues as a consequence of 2,4-DNP treatment is showing them being similar to the phosphorous compounds, described but not determined nearer — by Sen-Gupta and Sen (1961), the formation of which is inhibited by DNP. These properties of theirs are showing that they have some role in the phosphorylation processes.

We think so that by establishing the nature and physiological importance of these compounds, the connection between growth regulation and oxidative phosphorylation can be more illuminated. A further question to be cleared is, by what kind of mechanisms the decrease of nucleotides respectively ATP concentration in the plants treated with IAA occurs.

Summary

According to the experiments performed with ^{32}P, in the pea epicotyl treated with IAA the incorporation of ^{32}P in the nucleotides has decreased as compared with control; at the same time, however, the radioactivity of inorganic phosphate has increased. This effect of IAA is similar to that of 2,4-DNP but a little less. The ADP/ATP ratio in pea leaves is increased by IAA.

Growth-inhibiting phenolic compounds inhibit, in a higher degree than IAA, the incorporation of ^{32}P in nucleotides and increase the radio-

activity of inorganic phosphate in tissues.

Among phosphorous products, in different organs of pea seedling indole compounds can be demonstrated, incorporating ^{32}P in their molecules. This incorporation is increased by IAA but decreased by inhibitors.

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