Principles of IAA action in plants

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The processes that predetermine the growth-regulating effect of the phytohormone indole-3-acetic acid (IAA), as well as the chemical IAA fund composition and a possible role of separate IAA fund parts are analyzed. The metabolitic systems participating in the regulation of IAA level in growing cells are defined. The resulting IAA metabolites are identified by the GC–MS method.

Characterizing the action of the growth-regulating mechanism of the phytohormone, data on the peculiarities of the formation of possible IAA-receptory complexes, their transduction and realization through changes in gene expression are presented. The possible relations between IAA basipetal transport and the functioning of IAA-receptory complexes are discussed.

Key words: IAA metabolism, mechanism of growth regulating action

INTRODUCTION

The essence of the plant growth-regulating effect of the phytohormone indole-3-acetic acid is a balance of free IAA and IAA bound to other chemical substances, which ensures the hormone effect homeostasis, and the interaction between IAA basipetal transport and its receptory system. Application of biochemical methods offered the possibilities to reveal their course in detail and to show the metabolic links that predetermine the course and peculiarities of the growth-regulating processes. These, first of all, are studies into peculiarities of IAA synthesis and metabolism in the cell, specific binding of the hormone by proteins, as well as compartmentation of IAA–protein complexes in the cell and changes caused by these complexes in gene expression processes that depend on the hormone. The experimental data presented in the paper deal with the analysis of the above-mentioned processes and their role in plant growth regulation.

MATERIALS AND METHODS

The study object was etiolated, intensively growing by elongation segments of 3–4-d-old wheat coleoptiles (*Triticum aestivum* L. cvs. "Arcus", "Selpek") and germinating (up to 10 days) field bean seeds (*Vicia faba* L., *cvs.* "Ausra"), cotyledons and shoots separately.

Purification of indole specimens was performed by the method of several-staged gel-filtration chromatography using PVPP (Serva), Sephadex G-10 and Sephadex LH-20 (Pharmacia) columns in succession [1–3]. Identification was done by traditional and modern methods of physico-chemical analysis [1, 4, 5]. The chemical structure of IAA metabolites was determined by a combined gas-chromatography–mass-spectrometry (GC-MS) method [2,4].

The membrane fraction enriched with plasmalemma vesicles was separated from wheat coleoptile segments by differential centrifugation and purification in sucrose density gradient [6].

Nuclei isolated from wheat coleoptiles were suspended in the media used to determine RNA-polymerase I and II activity [7]. Triphosphates GTP, UTP, CTP and [^{14}C] ATP (0.1 mM, sp. act. 3.1 MBq $\cdot$ g^{-1}), IAA and IAA-treated plasmalemma preparations were added into incubation medium according to test conditions.

RESULTS AND DISCUSSION

The IAA fund in reserve tissues (cotyledons) has been determined to consist of high-molecular IAA conjugates – IAA peptides, low-molecular mass IAA conjugates – labile-bound IAA-amides and IAA-esters. Amide-type conjugates comprise about 70%. In maize endosperm, nearly all reserve IAA fund consists of ester-type compounds – high-molecular IAA-glucane complexes and IAA-esters [8].

In germinating bean seeds, free IAA is releazed from the reserve compounds. Its part, in the form of IAA molecule, is transported to the seedling [4, 7]. The size of the IAA pool transported to the shoot depends not on the total IAA fund,

but on the content of free IAA in the cotyledons. The IAA transported into shoots participates in the processes of their growth and is metabolized. The IAA fund in bean shoots during the 1–7th day grows from 360 to 7671 ng/10 shoots, but the part of the fund in the form of IAA molecule comprises only 10–14%.

The results obtained using a model system of intact seedlings (part of IAA fund in the cotyledons was replaced by ^{14}C-IAA) showed that IAA concentration in bean seedling tissues (Fig.) is formed and maintained under the effect of the reversive IAA conjugation and its oxidative catabolism systems. By means of reversive conjugation (substituting the carboxyl group of IAA molecule /-OH/ by amino acid or a carboxydrate molecule) IAA-amides and IAA-esters are formed. This way is considered to support the ratio of reserve IAA forms and physiologically active IAA in growing cells [3]. By way of oxidative catabolism (oxidating the IAA molecule in the 2nd position of the ring) the IAA concentration is lowered; the process is irreversible [1, 3]. At considerably higher IAA concentrations in bean shoot tissues, also the IAA decarboxylation system can be involved to give 3-methyl-indole.

A comparison of our results [2, 9] with the literature data on IAA metabolism in maize (monocotyledonous) shoot cells [9–11] allowed to state that in regulating the IAA level (homeostasis) the same metabolic systems take part.

In the tissues, free and immobile (bound, non-transportable forms of IAA including both active growth-regulating forms and reserve IAA conjugates) IAA fund is significant for plant growth: as long as free IAA moves through the tissues, the IAA-stimulated growth can be observed [12]. In ~2 cm long wheat coleoptiles, the exogenously supplied IAA stream is removed from their segments in about 8 hours, and the growth rate becomes equal to that of control segments. It seems reasonable to conclude that also growth regulation depends on the free IAA form, however, when the test was prolonged and a new portion of IAA was supplied to coleoptile segments the growth rate was greatest in the segments that had accumulated in their cells immobile IAA forms and were additionally supplied with free IAA. Thus, growth regulation can be accomplished through the interaction of the free and the immobile funds of IAA.

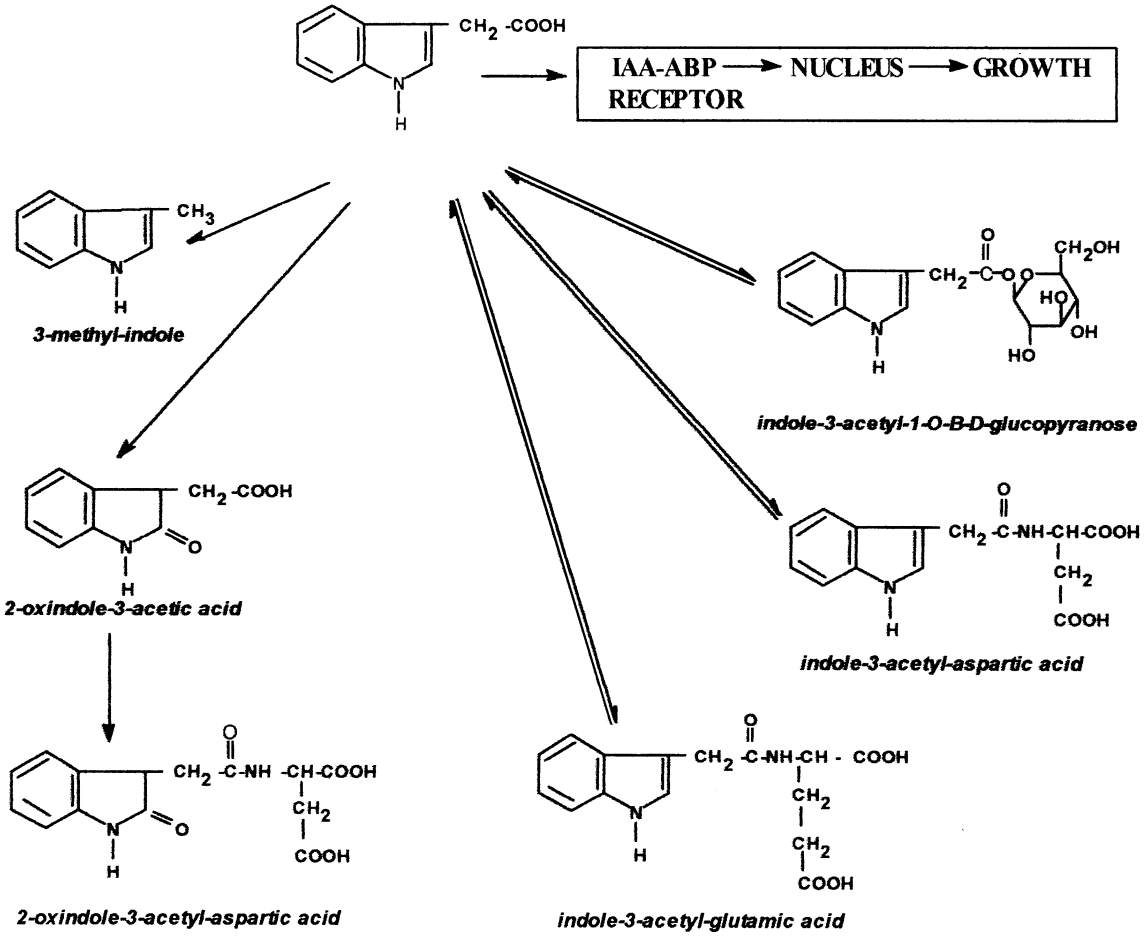

Figure. IAA fund transformation in growing tissues of field bean seedlings

At present, it is common knowledge that IAA realizes its functional activity through IAA–protein receptory complexes. These complexes, also in other laboratories, have not been studied so thoroughly as to allow a conclusion that these are the hormone receptory complexes, therefore most commonly IAA–protein complexes and their compartmentation are considered. Specific IAA–protein complexes are formed in the plant cell cytosol, tonoplast and, first of all, in the plasmalemma, which for the cell is a kind of the first selective barrier that receives IAA from the above-located cell and divides the hormone stream at least into three parts: one of them remains in the IAA transport system and goes basipetally further. This IAA again gets into the lower-located cell; the second part is bound, probably by the receptory protein whose external, ligand-binding domain is located on the external side of the cell plasmalemma and realizes its functional activity in the plasmalemma. IAA–protein complexes of this type have been described in maize, barley [13, 14], wheat coleoptiles [15] and in other plants. The third part of IAA fund in the cell is the IAA–protein complexes formed in the cell plasmalemma and realizing their functional activity in the nucleus via changes in specific IAA gene expression. We have no direct evidence that exactly these complexes stimulate IAA specific gene transcription processes in the cell, however, on compiling a system of isolated nuclei RNA-synthesis allowing to monitor RNA-polymerase I and II activity depending on the presence of IAA–protein-plasmalemmal complexes in that system, we observe an enhanced RNA-polymerase activity induced by these complexes, implying that the physiological function of the mentioned plasmalemmal IAA–protein complexes is related to RNA transcription processes in the nucleus.

On the other hand, changes in genome functioning caused by the phytohormones are one of the most extensively studied fields in plant physiology. The bulk of interest is attracted by the *Aux*/IAA gene family, which is rapidly and specifically induced by IAA [16]. Only in the *Arabidopsis* genome more than 20 representatives of this family have been found. The Aux/IAA proteins are localized in the nucleus and have a very short half-life period – 5–10 minutes, one of the shortest ever known. *Aux*/IAA genes have been found in dicotyledonous (peas, soya, lucerne, *Arabidopsis*, tomato, tobacco, cotton), monocotyledonous (maize and rice) and even in pine needles. However, these genes have not been encountered in bacteria, animal and fungal genomes. They are considered to be the solely plant genes [17]. Aux/IAA proteins interact with ARF proteins. The *ARF* gene class encodes auxin response factors. In the *Arabidopsis* genome, also over 20 of such genes have been found. Mutations in *ARF* genes reduce the IAA-sensivity, cause dearrangements in gravitropic response and IAA-regulated gene expression. *Aux*/IAA gene mutations evoke the appearance of the fenotype, characterized by a short hypocotyl, wavy leaves, agravitropic roots and shoots, etc.

Additional auxin-responsive genes have been identified and many have AREs (auxin response elements) in their promoters, implicating ARFs and *Aux*/IAAs in their regulation. The functional importance of some of these genes in mediating auxin responses is now beginning to be established. For example, the *DFL1* gene of *Arabidopsis* influences auxin sensivity and stem elongation [18].

Alongside the facts that IAA response is regulated on the gene expression level, also the interactions on the physiological level should be mentioned. In this sense, of particular significance is the plasmalemma, a cell protoplasma-covering membrane. In the plasmalemma, IAA transport carriers and proteins – eventual IAA receptors are located. An interaction is possible between these two systems: in the case when the already isolated plasmalemmal fraction is exposed to IAA transport inhibitors (NPA, TIBA) in the presence of an excess of the hormone, specific IAA binding with proteins is strongly inhibited. These experimental results allow a suggestion that basipetal IAA transport is not only a source of supplying IAA to the cell, but also can be one of the factors responsible for the functioning of the IAA growth-regulating system.

CONCLUDING REMARKS

IAA level and state in the plant cell are closely related to the functioning of the IAA–protein receptory complexes, therefore, to support IAA homeostasis in the cell a complex metabolic system is employed (Fig.). The possible multifunctional plant cell responses to the phytohormone are determined on several levels: during the formation of the hormone-receptory complexes, transduction of this signal to the nucleus, and inducing the hormone-dependent gene expression processes. In the realization of the growth-regulating process, an interaction between basipetal IAA transport and IAA receptory systems becomes manifested. The pivotal role in this interaction belongs to the functioning of IAA receptory systems. The sum total of all enumerated processes endows the phytohormones with their characteristic polyfunctionality, which manifests itself as the growth-regulating effect of IAA.

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IAR VEIKIMO PRINCIPAI AUGALUOSE

S a n t r a u k a

Darbe analizuojami procesai, nulemiantys fitohormono – indolil-3-acto rūgšties (IAR) – augimą reguliuojantį veikimą, taip pat cheminė IAR fondo sudėtis ir galima atskirų IAR fondo dalių reikšmė augimui. Nustatytos metabolitinės sistemos, reguliuojančios IAR lygį augančiose ląstelėse ir audiniuose. Susiformavę metabolitai identifikuoti GC-MS metodu.

Apibūdinant hormono augimą reguliuojantį mechanizmą, pateikiami duomenys apie galimų IAR receptorinių kompleksų susidarymą, transdukciją ir realizavimą per genų ekspresijos pokyčius. Aptariami galimi ryšiai tarp bazipetalaus IAR transporto ir IAR receptorinių kompleksų funkcionavimo.