The role of basipetal IAA transport in the hormonal regulation of plant cell growth

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With the aim to determine the role of the basipetal transport of indole-3-acetic acid (IAA) in hormone-dependent regulatory processes, the inhibitors of basipetal IAA transport, 2,3,5-triiodobenzoic (TIBA) and 1-N-naphtylphtalamic (NPA) acids, have been used. TIBA and NPA have been shown to inhibit, both *in vivo* and *in vitro,* the processes of specific IAA binding and physiological activity (according to RNA-polymerase II activity in the system of RNA synthesis in isolated nuclei) of the resulting IAA-protein complexes of spring wheat (*Triticum aestivum* L., 'Selpek') coleoptile cell plasmalemma. Basipetal IAA transport is suggested to be one of the factors responsible for the functioning of the growth-regulating system of IAA.

Key words: IAA binding in plasmalemma, basipetal IAA transport, 2,3,5-triiodobenzoic and 1-N-naphthylphtalamic acids

INTRODUCTION

Strictly oriented basipetal transport is a characteristic feature of IAA, distinguishing it from other phytohormones. Probably the basipetal transport through the cells is the process that provides in the cell conditions favouring the realization of the growth-regulating function of IAA. There is no doubt that the main component of this realization is providing the cell with the phytohormone. Nevertheless, the presence of IAA in the cell is not enough to activate its growth. In our earlier works [1] it has been shown that when wheat coleoptile cells are saturated with immobile forms of IAA, *i.e.* when the tissues are rich in bound IAA, including conjugates with low-molecular compounds which, when hydrolized, can restore the level of unbound IAA optimal for the cell [2], this is not enough to activate the growth which is restored with restoring the basipetal flow of IAA [1, 3]. These data allow to suppose that the process of IAA transport, apart from supplying the cell with the phytohormone, can somehow participate in the realization of the growth-regulating function of IAA. Considering the above facts, the aim of the present work was to determine the influence of inhibitors (TIBA and NPA) of basipetal IAA transport on the physiological activity of IAA–protein complexes of the plasmalemma of wheat coleoptile cells.

MATERIALS AND METHODS

The object of the work was etiolated, intensively elongating 3–4-d-old spring wheat (*Triticum aestivum* L., 'Selpek') coleoptiles. The membrane fraction enriched with plasmalemma was obtained by differential centrifugation [4, 5]. Specific IAA binding in plasmalemma preparations was determined as a difference between the total binding of radioactive IAA ($5 \cdot 10^{-9}$ M) and residual label quantity at aliguot fraction incubated with the same label and high concentration ($5 \cdot 10^{-5}$ M) of unlabelled (cold) IAA [6]. Separation from the free hormone was carried out by equilibrium dialysis [7]. As a marker for determination of physiologicl activity of plasmalemmal IAA-protein complexes, the activity of RNA-polymerase II in the RNA synthesis system of isolated nuclei was used [8].

High-labelled ^{3}H-IAA (sp. act. 4.6 TBk·g^{-1}, Amersham, UK), ^{14}C-ATP (sp. act. 3.1 MBk·g^{-1}) were used. Radioactivity of samples was measured with a scintillation counter (Beckman LS 1801). The protein was determined according to Bradford [9]. The experimental data were evaluated statistically. The data presented are arithmetical means of 3–5 experiments and their statistical deviations. Only the differences significant at $P \geq 0.95$ were accounted for [10].

RESULTS AND DISCUSSION

Since the main growth-regulating unit in the cell, at least in the elongating cell of the coleoptile, is IAA-receptory complex, while determining the role of basipetal IAA transport in growth-regulating processes first of all it was reasonable to elucidate the effect of TIBA and NPA, basipetal IAA transport inhibitors, on the processes of specific IAA binding and the physiological activity of the resulting IAA–protein complexes. We checked the plasmalemmal IAA–protein complexes able to induce changes in RNA-polymerases I and II activity in a system of isolated nuclei [11]. The data obtained have shown that treating with NPA of isolated plasmalemma vesicles evokes a dramatic inhibition of specific IAA binding (Table 1). In the experiments under discussion, the isolated plasmalemma vesicles were treated with basipetal IAA transport inhibitors, and then ^{3}H-IAA was applied to create IAA–protein complexes. As ATP was absent in the system, IAA could get into the vesicles in a passive way only and its shortage inside the normally oriented vesicles could prevent the formation of IAA–protein complexes (pH 7.2). With the aid of alameticine which in membranes forms the pores dependent on membrane potential and transducing rather large molecules (*e.g.*, ATP), IAA had been provided a possibility to get freely into the vesicles. Data presented in Table 1 show that treating with alameticine just reduced the negative effect of NPA on the formation of IAA–protein complexes of the plasmalemma, however, no restoration up to the contol values occurred. TIBA used in the same conditions completely inhibited specific ^{3}H-IAA (pH 7.2) binding with cell plasmalemma proteins, and no IAA–protein complexes were formed upon enriching the vesicle contents with IAA. Analogous results were also obtained when on IAA-treated plasmalemma vesicles conditions were formed to favour the formation of IAA–proteins complexes in the plasmalemma, and they were added into a system of isolated nuclei to provoke RNApolymerase II activity (data not shown). Inhibition of specific ^{3}H-IAA binding in wheat coleoptile plasmalemma (pH 7.2) was observed also when wheat coleoptile segments were treated with NPA and TIBA *in vivo* (Table 2). Additional treatment with IAA through agar-IAA blocks on the apical sections of NPA-pretreated coleoptile segments resulted in a doubling of specific ^{3}H-IAA binding in the vesicles of isolated plasmalemma. This means that the action of NPA reaches far beyond the blocking of basipetal IAA transport.

Table 1. **The *in vitro* effect of treating the fraction of isolated wheat coleoptile* cell plasmalemma with NPA and TIBA ($1\cdot10^{-5}$ M, pH 7.2)**

Variant	^{3}H-IAA specific binding	
	cpm/100 µg protein	%
Control	2215 ± 385	100
NPA	539 ± 6	24
NPA + alameticine	1556 ± 29	70
TIBA	0	0
TIBA + alameticine	0	0

*Separated from primary leaflets, non-decapitated wheat coleoptiles.

Table 2. **The *in vivo* effect of treating wheat coleoptile segments with NPA and TIBA ($1\cdot10^{-4}$ M) on specific IAA binding in cell plasmalemma (pH 7.2)**

Variant	^{3}H-IAA specific binding	
	cpm/100 µg protein	%
Control	1177 ± 100	100
NPA	0	0
TIBA	111 ± 21	9
TIBA+IAA	300 ± 40	26
NPA+IAA*	2283 ± 371	194

*Unlabelled IAA ($1\cdot10^{-5}$ M) was used to treat wheat coleoptile segments through the agar block on the apically cut surfaces

CONCLUSIONS

The sum total of data obtained earlier and those presented in this paper implies that for IAA to reveal its growth-regulating action indispensable are both IAA receptory complexes that realize their function by changing the synthesis of proteins necessary for the growth processes to occur and a flow of unbound IAA in the basipetal direction from cell to cell. The role of basipetal IAA transport seems not to be limited by merely providing IAA to the sites where its receptory complexes are formed. Recently, while elucidating the mechanism of NPA action on IAA transport it has been shown that NPA is bound with the integral plasmalemma proteins, which differ from IAA-binding proteins [12, 13]. The resulting NPA-protein complexes influence the activity of tyrozinkinases, which phosphorylate minute soluble proteins. As the processes of phosphorylation are important links in transducing a hormonal signal, it is possible that the effect of IAA on the IAA-receptory system can be mediated by these processes.

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BAZIPETALAUS IAR TRANSPORTO VAIDMUO HORMONAMS REGULIUOJANT AUGALO LĄSTELĖS AUGIMĄ

S a n t r a u k a

Bazipetalaus indolil-3-acto rūgšties (IAR) transporto reikšmės hormonų reguliuojamiems procesams įvertinti panaudoti IAR transporto inhibitoriai 2,3,5-trijodbenzoinė ir 1-N-naftilftalamo rūgštys. Parodyta, kad, paveikus šiais inhibitoriais *in vivo ir in vitro,* slopinami IAR specifinis surišimas vasarinių kviečių (*Triticum aestivum* L., 'Selpek') koleoptilių ląstelių plazmolemos baltymais ir susidariusių IAR-baltymų kompleksų fiziologinis aktyvumas (sprendžiant iš RNR-polimerazės II aktyvumą izoliuotų branduolių RNR sintezės sistemoje). Daroma prielaida, kad bazipetalus IAR transportas gali būti vienas iš veiksnių, atsakingų už IAR augimą reguliuojančios sistemos funkcionavimą.