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The role of transmembrane potential of plant cell plasmalemma *in vitro* in the functional activity of IAA–receptor complexes

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In model experiments on plasmalemma vesicles isolated from etiolated wheat (*Triticum aestivum* L.) coleoptiles, it has been shown that formation of the K^+-diffusive potential enhances the functional activity of IAA–protein complexes, evaluated with respect to RNA-polymerase II activity. This change does not follow from the increased IAA-binding capacity of the plasmalemma and seems to reflect an intensified functional activity of the hormone-receptor system.

Key words: plasmalemma, TMP, IAA

INTRODUCTION

The phytohormones, indole-3-acetic acid (IAA) in particular, are known to realize their regulatory function through receptory complexes that form on the plasmalemma [1, 2]. A number of works perfomed on animal cells, yeast and other microorganisms have been carried out to elucidate the mechanism of the perception and transduction of environmental signals to cell compartments where it is realized [3]. Data obtained on plant cells are more scanty, although over the recent years such works have gained more intensity [4, 5].

It is clear a priori that the information received by the cell during the formation of hormone-receptory complexes can be realized either on the plasmalemma itself [1, 6, 7] or be transmitted to the other cell compartments, to the nucleus in particular, where it performs the expression of definite genes [8, 9]. On the other hand, it has been also undeniably proved that on the cytoplasmic membrane of practically all functioning cells (*in situ*) a transmembrane potential of the order 100–150 mV is generated and maintained at a certain level [10]. It turns out that many processes localized on the plasmalemma are potential-dependent. This is a consequence of the functioning of ionic channels (*e.g.*, Ca^{2+}, K^+), action of the protone pump [11], integral processes of cell elongation regulation in plants, etc. [12]. Thus, it seems reasonable to ask a question: is there any cause-and-effect relationship between the formation and functioning of the hormone–receptor complexes and the value (or change) of the transmembrane potential (TMP)? In other words, is the hormone-receptor and specifically the IAA–receptor system potential-dependent? The present paper is an attempt to answer this question.

MATERIALS AND METHODS

The object of the work was decapitated, prepared from the leaf coleoptiles of etiolated 4-d wheat (*Triticum aestivum* L. cv. *"Nandu"*) shoots grown in a thermostat at a temperature of +25 ± 2 °C. The fraction enriched with plasmalemma vesicles was obtained by the method of [13] modified with respect to the object [7, 14]. The transmembrane potential on closed plasmalemma vesicles was obtained by the method described in [6, 15].

The physiological activity of IAA–protein complexes formed in the plasmalemma (pH 7.2) was evaluated from RNA-polymerase II activity in a system of nuclei isolated from wheat coleoptile cells [16]. CTP, GTP, UTP and 8-^{14}C-ATP (ammonium salt, 2.11 GBk/mmole at a final concentration 0.1 mM) were added into the system. The plasmalemma preparations containing IAA–protein complexes were dialysed against the medium to provoke RNA-polymerase II activity, added to the system of isolated nuclei and after a 35-min incubation at +37 °C sedimented with 5% trichloracetic acid. RNA-polymerase II activity was sensitive to α-amanitine.

The label content in the vesicles was determined with the aid of an LS 1801 scintillation counter (Beckman, USA).

Protein concentration was determined according to Bradford [17].

The exact procedures and solution concentrations are given in the text or in figure legend and table head.

The following chemical substances were used: IAA, Tris (Sigma, USA), Mes, DTT (Reanal, Hungary), EDTA, sucrose (Lachema, Czechia), valinomycine (Serva, Germany), dis-C_3-(5) (A.S. Waggoner, USA), CTP, GTP, UTP (Boehringer, Germany), ATP (Merck, Germany), ^{14}C-ATP, ^{3}H-IAA (Amersham, U. K.).

The differences significant at $P \geq 0.95$ were considered. The figures represent mean arithmetical values of 3–5 tests (with no less than 2–3 replications each) and their standard deviations.

RESULTS AND DISCUSSION

On the present-day methodical level, it is practically impossible to elucidate the role of the transmembrane potential (TMP) generated on the plasmalemma in the formation and activity IAA–protein complexes in intact plants or their tissues *in situ*. Therefore we employed tests *in vitro* on closed fragments of the cytoplasmic membrane (plasmalemma) of a plant cell. This choice has been predetermined by the fact that, first of all, model experiments allow to set and maintain an optimum concentration level of separate components of the intervesicular and external media. Second, employment of purified preparations of separate cell compartments (plasmalemma, isolated nuclei) as a model allows segregating the processes of the formation of IAA–protein complexes and realization of their functional activity both in time and in space. These undoubtful pluses outweigh the difficulties of the methodical aspects of TMP generation, in spite of their being rather numerous. It would be very attractive to set the TMP by physical methods with the aid of microelectrode technology and related high-ohmic amplifiers. However, such an approach is too intricate, because the vesicle diameter ranges within 0.1–0.2 μm and thus does not allow microelectrodes to be introduced (the diameter of the tip of a glass microelectrode measures about 0.1 μm). Another possibility is to create on plasmalemma vesicles $\Delta\mu H^+$ at the expense of a protonic pump of, *e.g.*, the ATPase nature. Such a method seems promising because of its nativity, but it is still unacceptable, as it requires introduction into the incubation medium of ATP, reaction co-factors, which can influence the functioning of the receptory system.

On the grounds of the above reasons we limited our choice by the electrochemical, passive (not requiring metabolic energy) methods. Specifically, we employed the generation of the K^+-diffusion potential. For this purpose the plasmalemma vesicles purified in sucrose density gradient were loaded with potassium ions by means of hypoosmotic shock. The shock was induced in 150 mM K_2SO_4 solution prepared on 1 mM Tris-Mes, pH 7.2. Then the vesicles loaded with K^+ ions were transferred into a Na^+-medium (150 mM Na_2SO_4 on 1 mM Tris-Mes, pH 7.2), and the K^+-permeability of the membrane was induced with the help of a highly selective ionophore, valinomycine. The generation of K^+-diffusion TMP was registered with a SFR-1 (Russia) according to fluorescence quenching of a potential-sensitive probe dis-C_3-(5). The subsequent potential titration with potassium ions corroborated the generation of exactly a K^+-diffusion potential on the vesicles. The TMP value calculated by titration according to the Nernst eguation was about 100 mV [6]. We consider such a method of TMP generation as most promising for tests *in vitro*.

To answer the question posed by the title of the present paper, it is necessary to prove that separate components of the generation medium of the K^+-diffusion potential themselves have no effect on gene expression evaluated by the activity of IAA-protein complexes in a system of nuclei isolated from the same object. As is shown by the results presented in figure (A), neither valinomycine nor K^+ and Na^+ ions at the above-mentioned concentrations changed RNA-polymerase II activity. It is evident that in these two variants only the action of valinomycine, potassium and sodium is controlled, since no TMP generation occurs here: in the first case valinomycine induces K^+-permeability, but no ion gradient is present, and in the second case the potassium and sodium ion gradient is present, but ionic streams are not provoked. Quite another matter is that in the latter variant (potassium and sodium ion gradient + valinomycine) the K^+-diffusion potential is generated on the vesicles, however, in this case no changes occur in RNA-polymerase activity, either.

If the plasmalemma fraction was pre-incubated with IAA ($5 \cdot 10^{-7}$ M), even in these conditions neither valinomycine nor potassium and sodium ions enhanced the RNA-polymerase activity of the isolated nuclei (Figure, B).

The picture was absolutely different when the plasmalemma fraction was incubated with IAA during introduction of valinomycine as well as potassium and sodium ion gradient into the IAA-binding medium, i.e. when on the membrane the K^+-diffusion potential was generated and the RNA-polymerase II activity abruptly (nearly by 80%) increased.

Thus, it seems reasonable to speak about a certain dependence of the IAA-receptory system on the potential. However, open remains the question whether the activity of IAA–protein complexes formed on the plasmalemma undergoes changes at the expence of the very process of complex formation or we deal here with an increase in the activity of

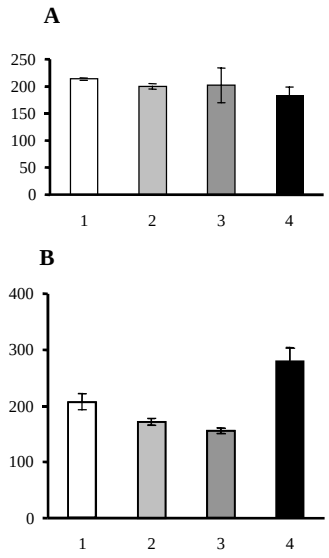

Figure. The effect of valinomycine and K^+ and Na^+ ions on RNA-polymerase II activity in a system of nuclei isolated from wheat coleoptile cells. A – incubation without IAA, B – incubation in the presence of IAA, $5 \cdot 10^{-7}$ M. 1 – control; 2 – valinomycine; $8.3 \cdot 10^{-9}$ M; 3 – inside the vesicles K_2SO_4, 150 mM, outside – Na_2SO_4, 150 mM; 4 – in the conditions of K^+–diffusion potential generation as in 3, plus valinomycine, $8.3 \cdot 10^{-9}$ M

IAA–protein complexes without any increase of their number.

We made an attempt to verify these two mechanisms. It turned out that specific IAA binding with plasmalemma proteins did not increase while generating a K^+-diffusion potential on it (Table).

Table. **Effect on IAA specific binding of generation with valinomycine of K^+-diffusion transmembrane potential on plasmalemma vesicles**

Variant	Specific binding	
	Imp.	%
Control	200	100
Valinomycine ($8.33 \cdot 10^{-9}$M)	190	90

Thus, the obtained experimental data suggest that generation on the plasmalemma of an electrochemical potential, which results in a considerable increase of RNA-polymerase activity, takes place not at the expence of the formation of an additional pool of IAA–protein complexes.

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TRANSMEMBRANINIO POTENCIALO AUGALO LÀSTELËS PLAZMOLEMOJE *in vitro* REIKÐMË IAR RECEPTORINIØ KOMPLEKSØ FUNKCINIAM AKTYVUMUI

Santrauka

Modeliniais eksperimentais su plazmolemos vezikulëmis, izoliuotomis ið etioliuotø kvieèiø (*Triticum aestivum L. cvs "Nandu"*) koleoptiliø, nustatyta, kad K^+ difuzinio potencialo sukûrimas padidina IAR baltymø kompleksø funkciná aktyvumà, kuris buvo vertinamas pagal RNR polimerazës II aktyvumà. Ðie pokyèiai nëra IAR sujungimo su baltymais gebëjimo iðdava ir tikriausiai atspindi hormonà receptuojanèios sistemos funkcinio aktyvumo padidëjimà.