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Comparison of different IAA–ABP complexes formed in kidney bean cell chloroplasts and mitochondria

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The molecular mechanism of phytohormone indole-3-acetic acid (IAA) action was investigated. The possibility of the formation of specifically bound IAA–ABP (auxin-binding protein) complexes in organelles (chloroplasts and mitochondria) and the importance of intactness and functionality of the organelles in the formation of these complexes have been revealed. Our results show that two different IAA–ABP complexes may be formed in the intact chloroplasts (optimal pH 5.5 and 7.5) and one (optimal pH 7.0) in mitochondria. IAA–ABP complexes differ in respect of their main characteristics (the content of specifically bound IAA, specificity, number of IAA binding sites (n), dissociation constant (K_D) and ligand effectiveness). They also differ from IAA–ABP complexes formed in the plasmalemma at pH 5.5 (having the type of structure of the auxin-binding site characteristic of the auxin receptor mediating plant growth by elongation in monocot and dicot plants) and from the second IAA–ABP complex (optimal pH 7.5) formed in this membrane as well as from both IAA–ABP complexes formed in the cytosol at pH 7.8 of kidney bean cells [11].

We suppose that the role of separate IAA–ABP complexes in IAA-dependent response(s) of the cell may be different.

Key words: IAA–ABP complexes, chloroplasts, mitochondria, plasmalemma, kidney bean

INTRODUCTION

IAA (indole-3-acetic acid or auxin) plays the principal role in the regulation of growth processes of the cell [1, 2]. IAA is considered to display its physiological activity by interacting with a specific auxin-binding protein (ABP)-receptor and modifies the manifestation of genetic information in the nucleus [1–4]. This assertion is once more supported by the fact that at least three families of the early auxin response genes – *Aux/IAAs, SAURs* and *GH_3*s – with the expression increasing at a time interval of about 5 min – have been identified [2, 3]. Small short-lived nuclear-localized Aux/IAA proteins (products of the large family of *Aux/IAA* genes), auxin response factors (ARFs) and components of the ubiquitin-proteosome pathways of Aux/IAA protein degradation are most significant for the realization of IAA function [2, 5]. The expression of other genes may be suppressed: at 20 min cellular priority increases the expression of *Aux/IAA, SAUR* and *GH_3* genes while decreasing the expression of genes encoding metabolic enzymes [3]. The products of gene expression, the amount of which increases several times after 30, 40, 60 or 120–480 min of treatment with IAA, are serine/treonine protein kinases, PIN7 auxin-transport protein, GH_3 family auxin conjugated proteins, etc. [6]. Thus, IAA binding with specific proteins is the first and essential step for its signaling to begin. Such a scheme is also characteristic of the function realization of other phytohormones [2, 7].

Recently, ABPs have been revealed in the plasmalemma [4, 7–9], tonoplast [10], cytosol [4, 11, 12] of monocot and dicot plants. The possible acceptance of auxin by ABPs in endoplasmic reticulum has been discussed [13, 14]. Other authors assert that IAA interacts with a specific protein, IAA-receptor TIR1 (transport inhibitor response 1), in the nucleus, the mission of this receptor being to mediate auxin responses throughout plant development [5]. Our attention was attracted by chloroplasts and mitochondria in which proteins, IAA–receptors, have not been investigated so far. The aim of our investigation was to determine whether the ABPs are localized in these organelles.

MATERIALS AND METHODS

For mitochondria subcellular fraction isolation, etiolated 4–5-d hypocotyls of kidney bean (*Phaseolus vulgaris* L. cv. "Baltija") and for chloroplasts 12–14-d leaves were used as the test objects.

The optimal conditions (plant material homogenization, differential centrifugation and purification) for intact (func-

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tioning) chloroplasts and mitochondria subcellular fraction preparations were chosen experimentally on the basis of methodical approaches used by other investigators for the subcellular fraction of these organelles, which ensured a high (>90%) purity and intactness of isolation [11, 15, 16]. The intactness and functionality of the subcellular fractions of both organelles were estimated by measuring the concentration of O_2 uptake or evolution using a chamber with a Clark-type electrode [17, 18]. For ascertaining chloroplast intactness, the ferricyanide test (λ = 420 nm) was used as well [16]. The purity (contamination with other compartments of the cell) of the chloroplast specimens obtained from the nucleus (stained with toluidine blue 1%) was determined microscopically. The contamination of both specimens was determined biochemically according to ATP(s) activity of the plasmalemma marker K^+Mg^{2+} ATP-ase, tonoplast marker H^+ ATPase and the effect of inhibitors such as vanadate, diethylstilbestrol (DES), potassium nitrate [19, 20]. The inhibition of mithochondria ATPase activity was revealed by azide [19]. The content of chlorophyll was determined according to Arnon [21]. Protein was monitored according to Bradford [22] in all cases.

For IAA binding activity determination, the procedures common in our [4, 9] and other [7, 12, 23] laboratories for the formation and characterization of IAA–ABP complexes were used. To ensure the intactness and functionality of the organelles, an osmotic (sorbitol or mannitol) was added to the binding assay medium. The ^{14}C-IAA concentrations 5×10^{-7} M and ^{12}C-IAA 1×10^{-4} M were used. Two indole compounds, IAA and indole-3-carbonic acid (ICA), possessing an analogous chemical structure and a carboxyl group, were used for the analysis of competition for IAA binding sites (Table 2). The formed IAA complexes were characterized by the content of total IAA and IAA specifically bound with a protein unit, complex specificity (%) and K_D (unlabelled IAA concentration needed for a 50% displacement of specifically bound ^{14}C-IAA). Data represent the mean arithmetical values of four tests (with no less than 2–3 replications). The differences significant at P – 0.95 were considered.

RESULTS AND DISCUSSION

The obtained results [2, 5] reveal that, from the biochemical viewpoint, determination of IAA receptors, clarification of the peculiarities of IAA and protein–receptor interactions, and characterization of IAA–ABP complexes determining auxin-mediated response(s) are the most important issues for elucidation of the realization mechanism of IAA physiological function. Taking into consideration that chloroplasts and mitochondria have their own genetic information which is not identical to that of the nucleus, as well as an individual protein synthesis machinery [24], and formation of IAA–ABP complexes has not been investigated so far, we searched for the localization of IAA binding sites on these organelles and the possibility of the formation of specifically bound IAA–ABP complexes.

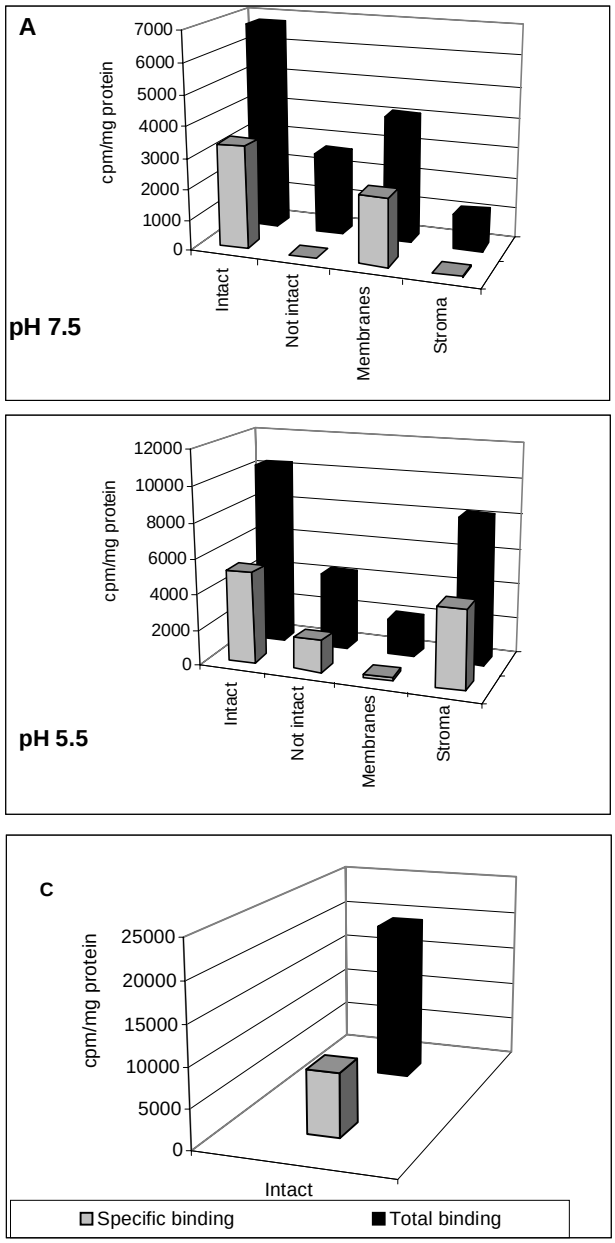


Figure. Comparison of total and specifically bound IAA amount in IAA–ABP complexes formed in the chloroplast (A, B) and mitochondria (C)

Analysis of the IAA binding capability in specimens of the subcellular fraction of intact organelles at pH from 4.0 to 9.5 revealed that two different IAA–ABP complexes (optimal pH 5.5 and 7.5) could be formed in the chloroplasts (Fig. A, B) and one in the mitochondria (optimal pH 7.0) (Fig. C). The dependence of IAA–ABP complex formation on the intactness and functionality of both organelles was found. The IAA binding site, having a pH optimum at 7.5, is functioning only when the intactness and functionality of the chloroplasts are ensured (Fig. A). The intactness of the mitochondria had a significant positive effect on the amount of IAA specifically bound to a protein unit and the specificity of the formed IAA–ABP complexes.

A preliminary analysis in which the sub-compartment of chloroplasts (derived by osmotic shock and differential centrifugation [25]), the IAA–ABP complexes could form, allowed a presumption that IAA–ABP complexes are formed at optimal pH 5.5 in stroma (Fig. B). The formation of IAA–ABP complexes at pH 7.5 may be related to the proteins functioning in membranous structures (Fig. A) of this organelle.

In order to determine in which sub-compartment of the mitochondria ABPs are localized and functioning, the sub-cellular fractions of their membrane and matrix have been used separately. According to the obtained results, membrane proteins retained the attribution to recognize and interact with IAA. The specificity of the formed IAA–ABP complexes reaches 10% and the amount of both total and specifically protein bound IAA is lower in comparison with undamaged (intact and functioning) mitochondria (Table 1). Besides, the amount of specifically bound IAA in IAA–ABP complexes formed in the mitochondria was also 1.4 times lower when isolation as an osmotic sucrose had been used [16] for mitochondria sub-cellular fraction, and the functionality of mitochondria had not been ensured. From these results, the methodical conclusion is that destruction of mitochondria leads to a reduction of their ability to interact with IAA.

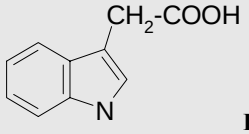
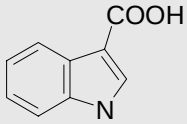
A comparison of all these three formed IAA–ABP complexes (Table 1), according to the main characteristics applied for the identification of such complexes (capability of binding auxin specifically, saturation of ABP binding site and n, K_D), revealed some differences among these complexes. All these complexes significantly differed in ABP binding site saturation, and separate IAA–ABP complexes formed in the chloroplast differed in the amount of binding sites (Table 1). The IAA–ABP complexes formed in the organelles differed from IAA–ABP complex formed in the kidney bean plasmalemma, which has an optimal pH 5.5 and the structure of IAA binding site (carboxyl group binding domain -/-His-Arg-His-/) specific to the IAA receptor mediating a rapid response of the cell by elongation in both monocot and dicot plants [7, 9]. The saturation of the protein binding site in the plasmalemma is 7–8 $\times$ 10^{-7} M, n – 2.07 $\times$ 10^{-10} mol/mg protein [26]. The complexes formed in the chloroplasts and mitochondria are not identical or analogous to the second IAA–ABP complex formed in the plasmalemma at pH 7.5: the saturation of ABP binding site is 1.25–1.5 $\times$ 10^{-6} M, K_D – 5.86 $\times$ 10^{-7} M [26] as well as low and high affinity IAA–ABP complexes formed in the cytosol at pH 7.8 [11].

To estimate the ability to displace the labeled hormone from IAA receptor sites in the chloroplasts and mitochondria, two indole compounds analogous in their chemical structure but not in physiological significance according to growth activity (Table 2), have been used: physiologically active phytohormone IAA and physiologically inactive ICA [1, 26, 27]. The results show that an auxin interacting with its receptor cannot be displaced by ICA a compound that does not induce cell growth by elongation. They are in good agreement with the supposition [13, 14, 23] that the effectiveness of indole compounds for IAA binding site competition is related to their biological activity. We suppose that the value

Table 1. **Comparison of main characteristics of IAA–ABP complexes formed in chloroplasts and mitochondria of kidney bean cells**

Compartment	Optimum pH	Characteristics of IAA–ABPs			
		Specificity, %	Saturation, M	K_D, M	n, mol/mg protein
Chloroplasts	5.5	51.02 ± 2.90	8–9 $\times$ 10^{-6}	1.14 $\times$ 10^{-5}	2.0 $\times$ 10^{-9}
	7.5	43.23 ± 3.89	7–8 $\times$ 10^{-5}	1.72 $\times$ 10^{-5}	4.4 $\times$ 10^{-10}
Mitochondria	7.0	41.32 ± 3.62	4–5 $\times$ 10^{-4}	1.90 $\times$ 10^{-5}	3.85 $\times$ 10^{-9}

Table 2. **Comparison of two indole compounds with analogous molecular structure in competition for IAA binding sites**

Compound		Ligand specificity, %			Physiological characteristics of compounds
		Chloroplasts		Mitochondria	
		pH 5.5	pH 7.5	pH 7.0	
	**IAA**	48.12 ± 4.57	40.53 ± 3.98	38.26 ± 4.21	Phytohormone auxin
	**ICA**	0.00	7.46 ± 2.12	0.00	Physiologically inactive product of IAA destruction

7.46 ± 2.12 obtained for the chloroplast (at pH 7.5) may be a bias. There are data to show that the growth activating effect of IAA cannot be induced by other compounds that have no indole ring in their molecule or lack acetic acid in β position, or contain a larger number of /-CH_2-/ in the side-chain and thus cannot be converted into IAA during the oxidation [1].

Thus, the results presented here for the first time show that two different IAA–ABP complexes may be formed in the chloroplasts and one in the mitochondria. They also confirm the possibility of several different ABPs to function in the organelles. The formed IAA–ABP complexes differ according to optimal pH, saturation of binding sites, amount of total and specifically bound IAA, n, etc. The possibility of IAA binding site localization and IAA–ABP complex formation in the chloroplasts and mitochondria has not been investigated so far. For a definite clarification of the possibility of formation of specifically bound IAA–ABP complexes in these organelles and elucidation of their possible role in the mechanism of IAA action, additional experiments must be carried out and other methodical approaches must be used.

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SKIRTINGŲ IAR-ASB KOMPLEKSŲ, SUSIFORMUOJANČIŲ PUPELIŲ LĄSTELIŲ CHLOROPLASTUOSE IR MITOCHONDRIJOJOSE, PALYGINIMAS

S a n t r a u k a

Tirtas indolil-3-acto rūgšties (IAR) veikimo molekulinis mechanizmas. Parodyta specifiškai sujungtų IAR-ASB (auksiną prisijungiančio baltymo) kompleksų formavimosi galimybė organoiduose (chloroplastuose ir mitochondrijose). Atskleista šių organoidų intaktiškumo ir funkcionalumo svarba IAR-ASB kompleksų formavimuisi. Gauti rezultatai rodo, kad du skirtingi IAR-ASB kompleksai gali formuotis intaktiniame chloroplaste (pH optimumas 5,5 ir 7,5) ir vienas (pH optimumas 7,0) – mitochondrijoje. Pagal susiformuojančių IAR-ASB kompleksų charakteristikas (specifiškai prijungtos IAR kiekį, kompleksų specifiškumą, prijungimo vietų skaičių (n), disociacijos konstantą (K_D) ir konkuruojančių ligandų efektyvumą) jie skiriasi vienas nuo kito. Taip pat jie skiriasi nuo IAR-ASB komplekso, susiformuojančio pupelių hipokotilių plazmolemoje, kai pH 5,5, ir turinčio IAR prijungimo saito struktūrą, būdingą auksino receptoriui, kuris nulemia vienaskilčių ir dviskilčių augalų atsaką tįstamuoju augimu; nuo antrojo IAR-ASB komplekso, susiformuojančio šioje membranoje, kai pH 7,5, ir nuo abiejų IAR-ASB kompleksų, susiformuojančių šių ląstelių citozolyje, kai terpės pH 7,8 [11].

Rezultatai rodo, kad keletas skirtingų IAR-ASB kompleksų gali formuotis ląstelėje, ir leidžia daryti prielaidą, kad atskirų IAR-ASB kompleksų vaidmuo nuo IAR priklausomoje(se) ląstelės atsakomojoje(siose) reakcijoje(se) gali būti skirtingas.

Raktažodžiai: IAR-ASB kompleksai, chloroplastai, mitochondrijos, plazmolema, pupelė