Characterization of IAA–ABPs complexes in different compartments of dicotyledonous plant cells

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We have analyzed peculiarities of the formation of indole-3-acetic acid (IAA) and auxin-binding protein (ABP) complexes in the plasmalemma, cytosol and intact mitochondria sub-cellular fractions (compartments) of etiolated kidney *(Phaseolus vulgaris* L., sort 'Baltija') 4.5-day-old hypocotyl cells as well as an intact chloroplast fraction derived from 12-day-old kidney bean leaves.

We established the dependence of the formation of IAA–ABP complexes in various compartments on the binding medium pH, their specificity and the amount of ^{14}C-IAA associated with a unit of protein. By means of chelato-affinity chromatography, we demonstrated that ABP functioning in the plasmalemma of kidney bean (dicotyledonous plant) hypocotyl cells has the site of IAA carboxyl group binding /-His-Arg-His-/, which is analogous to ABP1, the auxin receptor in the plasmalemma of maize (monocotyledonous plant). The possibility of formation of two different IAA–ABP complexes in the cytosol is demonstrated.

We suggest that ABP may also be localized in mitochondria and chloroplast.

Key words: indole-3-acetic acid (IAA), auxin-binding proteins (ABPs), IAA–ABPs complex

INTRODUCTION

The understanding of the biochemical-molecular mechanisms of IAA involvement in the plant growth processes has been changing during the period of investigations. For a long time IAA was though to influence the growth by exerting through an effect on the cell wall, *i.e*. by stimulating its elongation [1]. Only in the 1970s, when a substantial experimental evidence was accumulated, a notion that IAA acts via the genetic information has developed [2, 3]. There is enough evidence to confirm that IAA induces expression of early-inducible genes in monocotyledonous and dicotyledonous plants [4–6]. The activity of RNA-polymerases I and II is enhanced in isolated nuclei systems of the same cells [7–9]. The auxin response factors that are unique to plants [7] have been characterized. Under the effect of IAA two types of proteins are synthesized or their pool may undergo modifications [6, 10, 11]. Peculiarities of the formation of auxin–protein complexes were most exhaustively studied in the plasmalemma and cytosol of elongating cells of coleoptiles of monocotyledonous plants, maize [12, 13] and wheat [8, 9, 11]. Several ABP were found. Most thoroughly the ABP1, an auxin-receptor acting in the plasmalemma of maize coleoptile cells, was characterized [12, 13]. ABP1 is regarded as the best candidate for the role of auxin receptor. It may take part in auxin-mediated plant cell elongation and expansion [13, 14]. Although, using various genetic and biochemical methods, ABP1 was found in many dicotyledonous plants [12, 15], its role as an IAA receptor in the plasmalemma of these plants is doubtful and still under discussion [16, 17].

So far, it is not clear in which cell compartments, besides cytosol and plasmalema, ABPs may be localized and what is their role in the realization of IAA function.

Our work was aimed at analysing of ABPs distribution in the cells of dicotyledonous plants (the plasmalemma, cytosol, mitochondria and chloroplast compartments) and characterization of the forming IAA–ABP complexes.

MATERIALS AND METHODS

As the test objects, kidney bean (*Phaseolus vulgaris* L., cv. 'Baltija') 4.5-d etiolated hypocotyls and their 12-d leaves were used. Sub-cellular fractions from the test objects were derived by traditional differential centrifugation procedures (cytosol and plasma-

lema – as descripted earlier [18]). Conditions of isolation (centrifugation regimes, buffers, pH and stabilizing additives) of the sub-cellular fractions were chosen experimentally, applying the methods of other authors used for isolation of intact chloroplast [19, 20] and mitochondria [19, 21] sub-cellular fraction. The fractions of intact organelles were purified on sucrose or Percoll gradient [19–21]. For sub-fractionation of the organelles – to obtain membranous, stroma or matrix specimens – we used the ordinary procedures of osmotic shock and differential centrifugation.

Peculiarities of the formation of IAA–ABP complexes in mitochondria and chloroplast sub-cellular fractions as well as their sub-compartments (membranes and liquid medium) were analyzed and estimated by the same methods as in the case of the plasmalemma and cytosol, *i.e.* customary laboratory methods [8, 9, 19]. In order to determine possibilities of the formation of IAA–ABP complexes in organelles we used ^{14}C-IAA in concentrations ranging from 10^{-7} M to 10^{-4} M. Cold IAA and other indole compounds containing carboxyl group in the 3rd position were used as competing ligands.

RESULTS AND DISCUSSION

Although the physiological processes that regulate and/or involve IAA are known, the entirety of biochemical processes leading to this or that response remain obscure. The results accumulated while investigating the molecular mechanism of IAA action from the biochemical perspective allow to suggest that the interaction of IAA with the specific proteins – ABP is the first and most important stage, *i.e.*, that the essential components of the mechanism of molecular action of IAA are the IAA-"recognizing" and specifically interacting proteins.

Analyzing peculiarities of the formation of specifically bound ^{14}C-IAA–ABP complexes in the plasmalemma, chloroplast and mitochondria subcellular fractions in a routine way, varying pH between 4.0 and 9.0 in steps of 0.5, we revealed the differences of their formation with respect to pH, the number of IAA molecules associated with a unit of protein and the specificity of the formed complexes (Fig. 1). The binding activity of IAA (the amount of IAA associated with protein unit as well as the specificity of these complexes) in intact chloroplast and mitochondria fractions increases, when the concentration of ^{14}C-IAA in the binding medium increases up to 5×10^{-5} M. In this case, another IAA binding site was detected in the chloroplast. Its optimal pH is 7.5 and the affinity is lower if compared to the site forming at pH 5.5. According to preliminary results, the ABPs may be localized in membranous structures of both organelles.

Two different IAA binding proteins are revealed in kidney bean cytosol fraction. The amount of IAA associated with 100 μg protein is 9048 and 22095 cpm./min; the specificity 21% and 46%; the retention time in the column Sephadex G-100 differs by no less than 30 min, respectively. Judging from the elution of protein markers from the column, their native molecular masses are ≈ 290 and 50 kDa.

Based on the results obtained to date [9, 13, 16] it is possible to assume that some different IAA-binding proteins may be functioning in the cells of both monocotyledonous and dicotyledonous plants. According to the characteristics of the forming complexes, effects on the genetic apparatus, the resul-

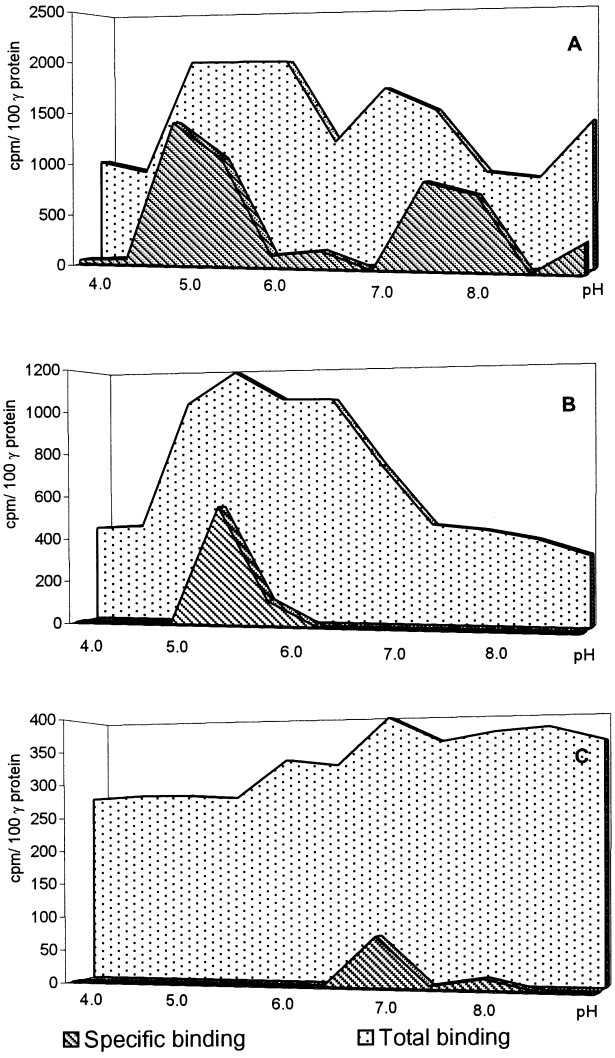

Fig. 1. Comparison of IAA–ABP complexes formed in different cell compartments
A – plasmalemma vesicles, B – chloroplast fraction, C – mitochondria fraction. ^{14}C-IAA conc. 5×10^{-7} M.

ting products can be evaluated as IAA-receptory complexes [9, 12, 14], IAA-enzymatic complexes [22] or IAA-transporter complexes, IAA-influx carriers [23] and efflux carriers [24].

To characterise the ABPs of kidney bean cells (natural molecular mass, polypeptide composition and structure of their binding sites – (amino acid sequences) the method of chelato-affinity chromatography was chosen [18, 25]. A Sepharose type matrix with an immobilised specific ligand (iminodiacetic acid, 26 μM/ml) was loaded on the column. The proteins were immobilized in the column via amino acid histidine (chelators Cu^{2+}) or two histidines (chelator Zn^{2+}). Imidazole was used as a competing ligand. In this case proteins were fractionated into two major fractions: IAA-'recognising' and IAA-non-'recognising' proteins. In the total preparation of solubilized kidney bean plasmalemma protein specimens by SDS-PAGE method, 36 polypeptides were revealed, five of them belonging to the IAA-'recognising' fraction. Formation of specifically bound IAA–ABP complexes was stated at the medium pH 5.5 and 7.5.

The obtained data confirm presence of two histidine molecules at the site of the binding of the carboxyl group of IAA molecule with ABP at pH 5.5 (Fig. 2). The molecular mass of polypeptide is about 26 kDa [25]. Before answering the question whether one or two ABPs are functioning in the kidney bean plasmalemma, complementary experiments must be done. Thus, we have succeeded in demonstrating that kidney bean hypocotyl plasmalemma ABP possesses the IAA binding site /-His-Arg-His-/, the same as or analogous to that in

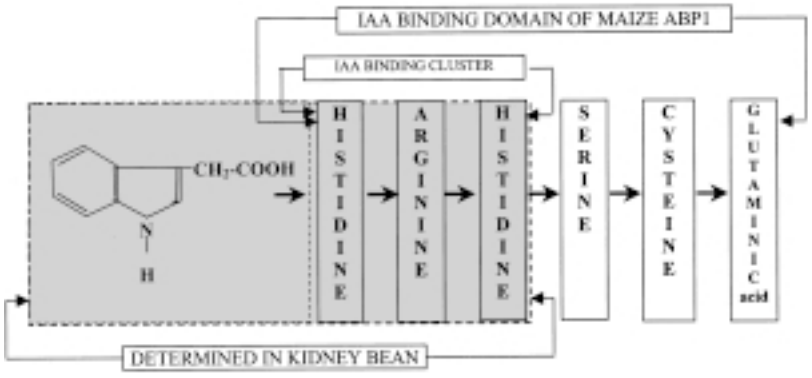

Fig. 2. IAA binding site

ABP1 – the IAA-receptor in the plasmalemma of maize [12, 13].

On the ground of the results obtained to date, it is possible to assume that ABPs can function not only in the plasmalemma and cytosol of the cell. Here we present some evidence that ABPs may be localized also in the chloroplast and mitochondria.

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IAR-ASB KOMPLEKSŲ, SUSIFORMUOJANČIŲ DVISKILČIŲ AUGALŲ LĄSTELIŲ SKIRTINGUOSE KOMPARTMENTUOSE, CHARAKTERIZAVIMAS

S a n t r a u k a

Analizuotos IAR-ASB kompleksų formavimosi ypatybės 4,5 paros amžiaus etioliuotų pupelių (*Phaseolus vulgaris* L., sort 'Baltija') hipokotilių ląstelių plazmolemos, citozolio ir intaktinių mitochondrijų bei 12 parų amžiaus lapų chloroplastų kompartmentuose. Nustatyta IAR–ASB kompleksų formavimosi priklausomybė nuo terpės pH, jų specifiškumas, su baltymo vienetu asocijavusios ^{14}C-IAR kiekis.

Chelato-afininės chromatografijos metodu parodyta, kad pupelių hipokotilių (dviskiltis augalas) plazmolemoje funkcionuoja ASB, turintis IAR karboksilo grupės prijungimo saitą /-His-Arg-His-/, analogišką ABP1 – auksino receptoriaus kukurūzų (vienaskiltis augalas) plazmolemoje. Dviejų skirtingų IAR-ASB kompleksų formavimosi galimybė pademonstruota citozolyje.

Daroma prielaida, kad ASB gali būti mitochondrijose ir chloroplastuose.