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In Vitro Binding Affinities of 4-Chloro-, 2-Methyl-, 4-Methyl-, and 4-Ethylindoleacetic Acid to Auxin-binding Protein 1 (ABP1) Correlate with Their Growth-Stimulating Activities

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Abstract. 4-Chlorindole-3-acetic acid (4-CI-IAA), an endogenous auxin in certain plant species of Fabaceae, has a higher efficiency in stimulating cell elongation of grass coleoptiles compared with indole-3-acetic acid (IAA), particularly at low concentrations. However, some investigations reported a 1,000-fold discrepancy between growth stimulation and binding affinity of 4-CI-IAA to auxin-binding protein 1 (ABP1) from maize. Here we report binding data of 4-CI-IAA and three alkylated IAA derivatives using purified ABP1 in equilibrium dialysis. There is a clear correlation between the growth-promoting effects and the binding affinity to ABP1 of the different IAA analogues measured by competition of [^{3}H]naphthalene-1-acetic acid binding. Our data are consistent with the hypothesis that ABP1 mediates auxin-induced cell elongation.

Key Words. Auxin-binding protein 1—Binding affinities—Elongation growth

The main strategy to elucidate the molecular mechanisms of auxin action concentrated on the search for auxin receptor proteins. Despite 17 years of intense research on auxin binding site 1 (Dohrmann et al. 1978), identical with auxin-binding protein 1 (ABP1) (Löbler and Klämbt 1985 a, 1985b), there is no general agreement on its physiological role and functional localization. Recently some critical remarks concerning the acceptance of ABP1 as a mediator of auxin-induced cell elongation and plasma membrane hyperpolarization were raised (Hertel 1995).

The main objection is based on conflicting properties of 4-chloroindole-3-acetic acid (4-CI-IAA), comparing growth-stimulating activity and competition of naphthalene-1-acetic acid (NAA) binding to corn coleoptile microsomes. Hertel, who was the first to show auxin binding sites in corn coleoptiles (Hertel et al. 1972), claimed that 4-CI-IAA is 100 times less active than NAA in the competition assay but is 10 times more effective in promoting elongation growth of corn coleoptiles (Hertel 1993).

Although the major part of the auxin binding sites as well as of ABP1 resides in the ER (Jones et al. 1989, Ray 1977), the protein seems to be functional at the outer surface of the plasma membrane (Barbier-Brygoo et al. 1989, 1991, Diekmann et al. 1995, Löbler and Klämbt 1985b, Rück et al. 1993, Venis et al. 1990, 1992). For that reason, we believe using purified ABP1 is more suitable for auxin binding assays than using ABP1 still enclosed in vesicles.

This paper presents competition data of [^{3}H]NAA binding to ABP1 by the endogenous auxins indole-3-acetic acid (IAA) and 4-CI-IAA as well as three alkyl derivatives of IAA: 2-methylindole-3-acetic acid (2-Me-IAA), 4-methylindole-3-acetic acid (4-Me-IAA), and 4-ethylindole-3-acetic acid (4-Et-IAA), in correlation to their effectiveness in growth tests with maize coleoptile sections.

Materials and Methods

Chemicals

NAA and IAA were from Boehringer, Mannheim, FRG. The IAA derivatives 4-CI-IAA, 4-Me-IAA, and E-Et-IAA were synthesized by Dr. B. Kojic-Prodic (Rudger Boskovic Institute, Zagreb, Croatia); 2-Me-IAA was from Aldrich, Steinheim, FRG. [^{3}H]NAA (17 mCi/mmol) was obtained from Amersham Buchler, Braunschweig, FRG.

Abbreviations: ABP1, auxin-binding protein 1; 4-CI-IAA, 4-chloroindole-3-acetic acid, NAA, naphthalene-1-acetic acid; ER, endoplasmic reticulum; IAA, indole-3-acetic acid; 2-Me-IAA, 2-methylindole-3-acetic acid; 4-Me-IAA, 4-methylindole-3-acetic acid; 4-Et-IAA, 4-ethylindole-3-acetic acid; MES, 4-morpholineethanesulfonic acid, PAA, phenylacetic acid.

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Plant Material

Seeds of *Zea mays* L. cv. Mutin 240 (KWS, FRG) were soaked in tap water for 1 h, sown on wet cotton wool in plastic boxes, covered, and placed in a growth chamber. Seedlings were grown in the dark for 3 days at 26°C.

Growth Measurements

Coleoptiles 15–20 mm in length were cut. From the coleoptiles 10-mm sections were excised, starting 3 mm from the tip, and the primary leaves were removed.

Growth was measured by a high resolution isotonic extensiometer measuring the extension of five coleoptile sections, fixed upright on a steel needle, in 2 ml of 10 mM MES-KOH buffer, pH 5.5, with constant aeration. Columns of sections were under a 0.10 g weight. Data obtained from 1-min interval measurements were analyzed with commercially available software (DASY*Lab*, DATALOG GmbH, Mönchengladbach, FRG).

All experiments were performed at 26°C.

Purification of ABP1

ABP1 from maize coleoptiles was prepared according to Viola (1991) with minor modifications. Maize coleoptiles were ground with an equal volume of homogenization buffer (HB: 50 mM Tris acetate, pH 8.0, 1 mM EDTA), and the homogenate was filtered through gauze. The filtrate was centrifuged for 20 min at 6,000 ×*g*. $CaCl_2$ was added to a final concentration of 10 mM to the supernatant. After 15 min of stirring, membranes were sedimented by centrifugation (30 min, 10,000 ×*g*) (Shimomura et al. 1986). To get the water-soluble luminal proteins, the resulting pellet was resuspended in HB, mixed with the same volume of ice-cold 1-butanol, and stirred vigorously for 20 min (Maddy et al. 1972). After centrifugation for 15 min at 3,400 ×*g*, the aqueous phase was dialyzed overnight against HB. The dialysate was applied to a DEAE-cellulose column (Servacel, Serva, Heidelberg, FRG) equilibrated with HB, washed with binding buffer (BB: 10 mM Na citrate, pH 6.0, 5 mM $MgCl_2$), and eluted with 0.3 M NaCl in BB. The eluate was loaded on a PAA affinity column prepared by coupling 4-OH-phenylacetic acid to epoxy-activated Sepharose 6B (Pharmacia, Uppsala, Sweden). After washing with 1M NaCl in BB, the bound ABP1 was eluted by shifting the pH with borate buffer (0.1 M Na borate, pH 9.6) and lyophilized. For use in the binding assays, the protein was redissolved and the buffer exchanged by a gel filtration column (Excellulose GF-5, Pierce, Rockford, IL, USA).

Binding Assays

Competition assays were performed by equilibrium dialysis for 3 hr at 4°C in Teflon dialysis chambers (Dianorm, München, FRG) with cellulose dialysis membranes (cutoff 5,000 Da). Binding of 10^{-7} M [^{3}H]NAA to ABP1 was determined in the presence or absence of the various derivatives of IAA in the range of 10^{-7} to 10^{-4} M at pH 5.5 in BB.

Results

The ability of IAA, 4-Cl-IAA, and various alkylated IAA derivatives to displace NAA binding to ABP1 was tested by equilibrium dialysis (Fig. 1a). Considering the concentrations needed to displace 50% of [^{3}H]NAA binding, 4-Cl-IAA and 4-Me-IAA together with IAA all are about ten times less active in competing NAA binding to ABP1 compared with NAA itself, indicating that the K_d of these derivatives is similar to that of IAA. On the contrary, 4-Et-IAA and 2-Me-IAA are not able to displace NAA in the same range. 4-Et-IAA is 50-fold less effective and 2-Me-IAA about 2,000-fold less effective than NAA.

The physiological sensitivity of maize coleoptile sections to these compounds was determined by comparing early elongation growth rates (60 min after auxin application) with dosage of the compounds (Fig. 1b). Each compound was added when the endogenous growth rate was stable, usually after 1 h of incubation. All compounds except 2-Me-IAA caused a clear rise in elongation rate. At a concentration of 10^{-6} M, roughly the optimum concentration of all compounds, the growth-promoting activities of the tested compounds correlate with the binding data.

Discussion

Elongation growth is a complex process mediated and influenced by many factors yet unknown. Since we consider ABP1 to be the hormone binding site, all growth-promoting compounds chemically similar to naturally occurring auxins should bind to ABP1. The growth-promoting activity of the different auxins should be reflected qualitatively by their binding affinities.

The competition assay data of IAA and all substituted IAA analogues tested are in good agreement with their abilities to stimulate elongation growth of maize coleoptile sections. Hertel (1993) claimed 4-Cl-IAA to be 100 times less active in binding to the auxin binding site 1 compared with NAA. The competitive effect of NAA on [^{14}C]NAA binding to ABP1 is ten times higher than that of IAA (Löbler and Klämbt 1985a, Napier and Venis 1990, Ray et al. 1977). For this reason, according to Hertel, there should be a tenfold difference between the affinities of 4-Cl-IAA and IAA. We do not find such a difference.

The lower affinity of 4-Cl-IAA measured by Stotz and Hertel (1994), compared with our data obtained with purified ABP1, may be due to the experimental setup differences. By using a crude microsome fraction with the binding protein inside the vesicles, there may be different factors besides the K_d which also influence the binding data. For example, the effective concentrations of different auxins at the binding site may be altered by their different translocation across the vesicle membrane.

Our results support the present hypothesis that ABP1 is the functional auxin-binding protein mediating the auxin signal that leads to cell elongation.

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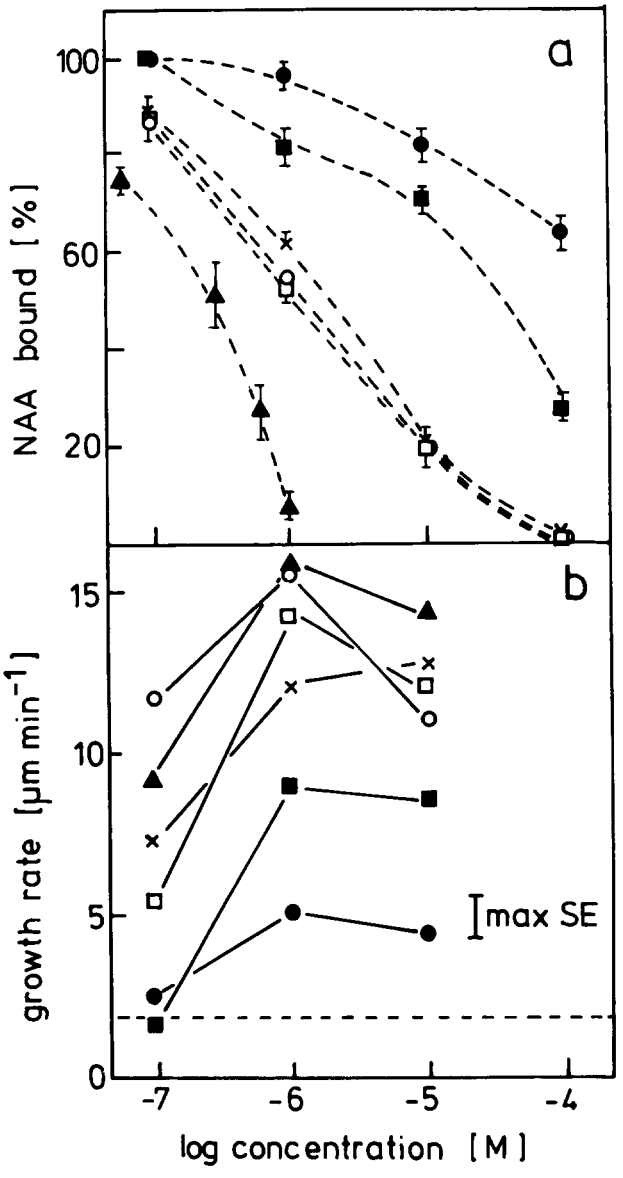

Fig. 1. Displacement of [^{3}H]NAA binding to maize ABP1 by NAA, IAA, and various IAA analogues compared with their growth-promoting activities. a, binding of 10^{-7} M labeled NAA in the presence of NAA (▲), IAA (×), and the IAA derivatives 4-Cl-IAA (○), 4-Me-IAA (□), 4-Et-IAA (■), and 2-Me-IAA (●) at pH 5.5 was determined as a percentage of [^{3}H]NAA binding in the absence of competitors. Each data point is the mean of five departments ± standard error. b, growth rates of sections (µm elongation of coleoptile section per min) incubated at 26°C in 10 µM MES-KOH buffer, pH 5.5, were measured in 1-min intervals. Compounds were added after about 1 h when the endogenous growth rate was stable. Growth rates recorded 1 h after the addition of compounds are presented as a function of concentrations applied. Control (no substance added) is indicated as broken line. The mean of three experiments ± maximum standard error (max SE) is shown.

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