BIOLOGIA PLANTARUM (PRAHA)

27 (2—3) : 110—113, 1985

Genotype-Specific Soluble Auxin-Binding in Etiolated Pea Epicotyls

H.-J. JACOBSEN and KARIN HAJEK

Institut für Genetik der Universität Bonn, Kirschallee 1, D-5300 Bonn-1, FRG

Abstract. Using different independent procedures for assaying soluble auxin-binding in etiolated pea epicotyls, we could prove the reliability of the $(NH_4)_2SO_4$-pelleting assay both for crude cytosols as well as for specific protein fractions obtained after chromatofocusing. Three distinct genotypes (two parent lines, one tall recombinant) investigated so far exhibit characteristic differences with respect to soluble auxin-binding kinetics in their cytosols.

Although widely accepted in cAMP research, the ammonium sulphate precipitation assay meets some severe criticism when used in soluble auxin-binding research (STODDART and VENIS 1980), since this assay is suspected to create artefacts rather than indicating high-affinity binding. So, in order to reconsider previously reported results (JACOBSEN 1982) and to find a more convenient assay, we compared the characteristics of four alternative procedures to ascertain soluble high-affinity auxin-binding in etiolated pea epicotyls. The assays we used were the dextran-coated charcoal method (DCC; OOSTROM *et al.* 1975), nitrocellulose filters (NC), polyethylenimine-treated glass-fiber filters (PEI; BRUNS *et al.* 1983) and equilibrium dialysis in comparison with the ammonium-sulphate precipitation assay (ASP; WARDROP and POLYA 1977). Crude cytosols and specific cytosol fractions obtained after improving conventional chromatofocusing (JACOBSEN 1984) were used to elucidate the characteristics and limitations of the respective binding assays.

MATERIAL AND METHODS

Seeds of pea (*Pisum sativum* L. cv. Dippes Gelbe Victoria (DGV)) were germinated and grown in moist vermiculite in the dark generally for 9—10 days. For studies on the time course of auxin-binding kinetics, the seeds were germinated and grown for 8, 9, 10 or 12 days in the dark. Three independent binding experiments were carried out with the pea genotypes "Dippes Gelbe Victoria", its fasciated mutant 489C and a recombinant of these two lines (R4111).

Crude cytosols were prepared as previously described (JACOBSEN 1982), and directly used for the binding assays or applied to a pressure resistant glass column (12.7 × 1090 mm), filled with PBE 94 (Pharmacia Fine Chemicals,

Sweden). This column was connected to a peristaltic pump, equilibrated with 25 mM imidazole-HCl, pH 7.3, and eluted after binding the cytosol proteins to the gel matrix with 100 mM Na-cacodylate-HCl, pH 5.0. Strongly bound acidic proteins with a pI < 5.0 were removed from the column with 1 M NaCl in elution buffer.

Binding Assays

The DCC-method was performed according to OOSTROM *et al.* (1975) with modifications according to VAN DER LINDE (personal communication). Equilibrium dialysis was carried out with a Diachema equilibrium dialysis device, using dialysis cells with 250 µl free volume in each of the two chambers for up to 18 h. For the NC-method, nitrocellulose membranes (Millipore VMWP 02500, pore size 0.05 µm) were washed with 2 ml Tris-HCl (50 mH, pH 7.8), then cytosol, incubated with various amounts of hormone, is applied to the membranes, followed by twice washing with the Tris-buffer and counting the membranes in a LSC-counter. The PEI-method was essentially the same as described by BRUNS *et al.* (1983): glass-fiber filters (Schleicher und Schüll, West Germany, 370398) were soaked in fresh 0.03 % polyethylenimine, pH 10, for 30 min to several hours, then the cytosols incubated with various amounts of labelled hormone are applied, rinsed twice with buffer and the membranes counted.

TABLE 1

Results of 5 different binding assay procedures obtained with a) crude cytosols and b) specific cytosol fractions obtained by chromatofocusing

a) Crude cytosol		DCC	ED	NC	PEI	ASP
Nr. of experiments	+	2	0	4	1	18
	−	15	6	0	1*	6
b) CF-fractions						
I	+	/	/	/	/	0
	−					2
II	+	/	0	/	/	0
	−		1			2
III**	+	/	/	1	/	2
	−			0		3
IV	+	/	2	/	1	3
	−		0		0	0
V (NaCl)	+	/	/	/	/	0
	−					2

+: High-affinity binding detectable; −: No high affinity binding detectable; *: Glass-fiber filters weakened after overtime exposure to PEI; **: occurrence of high-affinity binding in fraction III obviously growth-stage dependent.

RESULTS AND DISCUSSION

a) Comparison of Binding Assay Procedures

The results of the different binding assays are shown in Table 1: For the crude cytosols, only nitrocellulose membranes, PEI-treated filters and ammonium-sulphate precipitation gave clear indications of high-affinity binding,

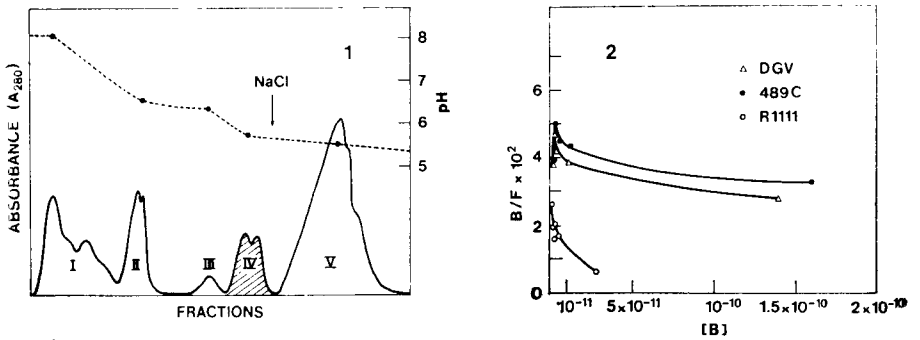

Fig. 1. Separation pattern of crude cytosol by chromatofocusing with PBE 94.
Fig. 2. Scatchard plots showing apparent cooperative auxin-binding in the cytosols of etiolated seedlings from the parent lines (DGV, 489C) and non-cooperativity in the tall recombinant (R4111).

whereas with DCC or equilibrium dialysis most of the assays were negative. At least for equilibrium dialysis the reason for negative results is evident: with highly concentrated crude cytosols osmotic problems occurred, which inhibited the equilibrium; with low protein concentrations obviously the number of high-affinity binding sites was below the level of detection, or these sites were degraded in the crude cytosol during the long incubation periods. However, when we investigated the soluble auxin-binding behaviour in distinct protein fractions obtained after an improved chromatofocusing of the crude cytosol (Fig. 1a), no soluble high-affinity auxin-binding could be detected in fractions I (pI $>$ 7.3), II (pI 6.8 — 7.3) or V (NaCl-elutable proteins with pI $<$ 5.0). With fraction III (pI 6.0 — 6.8), high-affinity auxin-binding can only be detected with epicotyls from older seedlings (10—12 d), but not from young seedlings (7—9 d) (JACOBSEN 1984), so both positive and negative results are obtained with nitrocellulose membranes and the ASP-assay. With the entire fraction IV (pI 5.0—6.0), equilibrium dialysis, PEI-treated filters and ASP-assay indicated the presence of high-affinity auxin-binding. Both from the fact that ASP fails to indicate high-affinity binding in CF-fractions I, II and V (as does ED in fraction II) and from the positive results obtained with ED, PEI and ASP for fraction IV, we consider the observed soluble auxin-binding a reality, despite the possible functions.

b) Genotype-Specific Soluble Auxin-Binding in Pea

When soluble auxin-binding was assayed in the cytosols of etiolated epicotyls of the three different genotypes DGV (parent I), its fasciated mutant 489C (parent II) and their recombinant R4111, differing from DGV by its almost doubled internode length (JACOBSEN and LÖNNIG, in preparation), the tall recombinant, which exceeds the length of DGV after 9 days in the dark by 5.7 cm (DGV: 6.0 cm, R4111: 11.7 cm, 489C: 10.1 cm), shows a con-

siderably different binding kinetics than both parents (Fig. 2). Obviously only one class of soluble auxin-binding sites is present, whereas in the parents the situation appears to be somewhat more complex. The recombinant, which is characterized during this particular growth stage by accelerated elongation growth, resembles an auxin-binding kinetics, which can be found in DGV at earlier growth stages (JACOBSEN 1982, 1984). Although the present data do not permit an unequivocal proof for this hypothesis, it seems likely that genetically caused differences in growth and morphology reflect typically different patterns of auxin-binding kinetics.

Since the number of seeds from this recombinant was limited, further studies on its unexpected auxin-binding characteristics will be carried out after propagation of seeds in the present vegetation period.

REFERENCES

BRUNS, R., LAWSON-WENDLING, K., PUGSCET, T.: A rapid filtration assay for soluble receptors using polyethylenimine treated filters. — Anal. Biochem. **132** : 74—81, 1983.

JACOBSEN, H.-J.: Soluble auxin-binding proteins in pea epicotyls. — Physiol. Plant. **56** : 161 to 162, 1982.

JACOBSEN, H.-J.: Two different soluble auxin-binding sites in pea. — Plant Cell Physiol. **25** : 867—873, 1984.

OOSTROM, H., VAN LOOPIK-DETMERS, M. A., LIBBENGA, K.: A high affinity receptor for indoleacetic acid in cultured tobacco pith callus. — FEBS Lett. **59** : 194—197, 1975.

STODDART, J. L., VENIS, M. A.: Molecular an subcellular aspects of hormone action. — In: MAC MILLAN, J. (ed.): Hormonal Regulation of Development. Encyclopedia of Plant Physiology. New Series Vol. 9. Springer Verlag, Berlin 1980.

WARDROP, A. J., POLYA, G. M.: Properties of a soluble auxin-binding protein from dwarf bean seedlings. — Plant Sci. Lett. **8** : 155—163, 1977.