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Evidence for Receptor Function of Auxin Binding Sites in Maize[1]

RED LIGHT INHIBITION OF MESOCOTYL ELONGATION AND AUXIN BINDING

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ABSTRACT

When 3- to 4-day-old dark-grown maize (*Zea mays* L. WF9 × Bear 38) seedlings are given red light, auxin-binding activity localized on endoplasmic reticulum membranes of the mesocotyl begins to decrease after 4 hours; by 9 hours, it falls to 50 to 60% of that in dark controls, on either a fresh weight or total particulate protein basis. Endoplasmic reticulum-localized NADH:cytochrome *c* reductase activity decreases in parallel. Loss of binding is due to decrease in number of sites, with no change in their affinity for auxin (K_d 0.2 micromolar for naphthalene-1-acetic acid). Elongation of mesocotyl segments in response to auxin decreases with a similar time course. Elongation of segments from irradiated plants shows the same apparent affinity for auxin as that of the dark controls. Auxin-binding activity and elongation response also decrease in parallel down the length of the mesocotyl. These observations are consistent with a role of endoplasmic reticulum-localized auxin binding sites as receptors for auxin action in cell elongation.

Binding of natural and synthetic auxins to membrane-bound and soluble sites in a variety of plant tissues has been described (17). A site localized on the ER (often called site I) in maize coleoptiles has been most fully characterized (10, 16, 17). At least two other types of sites have been reported in maize (2, 6), although the reality of these has been doubted (10). However, there is no rigorous evidence that any of these auxin binding sites act as receptors for auxin action (10).

An approach rather widely used to show receptor function for binding sites for animal hormones is to correlate developmental or environmentally induced changes in hormone sensitivity with changes in the concentration or affinity of binding sites in the tissue (5, 8). We report here some evidence of this nature regarding site I auxin binding in maize seedlings.

MATERIALS AND METHODS

Plant Material. Maize seeds (*Zea mays* L. WF9 × Bear 38; CFS Research, Decatur, IL 62526) were soaked in tap water for 1 to 2 h in the dark and sown on wet cellulose packing material (Kimpak) in 33- × 23- × 10-cm plastic boxes. The boxes, with lids ajar, were kept in total darkness at 25°C and 50% RH. The lids were removed at 60 h, and additional water was supplied. After planting and prior to harvest or to the preharvest light treatments specified below, the seedlings received no light at any time.

Light treatments, when given, were started when the plants were about 85 h old. Light from four 80-w cool-white fluorescent lamps located 0.4 m above the plants was passed through three layers of red cellophane (intensity at plant level 0.74 $nE \cdot cm^{-2} \cdot s^{-1}$). Under a dim green safelight (546 nm), the apical 3 cm of each mesocotyl was harvested onto ice and processed as described below in normal room light. This light exposure during the limited time needed for harvesting and workup did not affect NAA[3] binding.

Auxin Binding Assay. Auxin binding was measured by centrifugation with 1-naphthalene-[1-^{14}C]acetic acid in a twice washed total crude microsomal preparation (16) (100,000*g* pellet from 5000*g* supernatant) using 10 mM citrate buffer at pH 5.5 (16). Binding values are corrected for nonspecific binding by subtracting from the total binding the amount of radioactivity bound in the presence of 0.1 mM [^{12}C]NAA. In comparative experiments, R-treated and control samples were always processed simultaneously.

Unlike auxin binding in coleoptiles, binding in mesocotyl membrane preparations was strongly inhibited by organic solvents (acetonitrile, methanol, or ethanol) used as solvent for NAA. Therefore, final concentration of any organic solvent was kept below 0.05% (v/v).

Protein was measured by the method of either Lowry *et al.* (9) or, for sucrose gradients, Bradford (3), with BSA as standard.

Density Gradient Fractionation. A mesocotyl homogenate was prepared by chopping with mechanically driven razor blades for 10 min in 1 ml homogenization medium (16)/g fresh weight, squeezing through nylon cloth, and centrifuging for 10 min at 1000*g*. The supernatant (18 ml) was layered onto a 20-ml linear 15 to 45% (w/w) sucrose gradient in gradient basal medium (14), and centrifuged for 3.5 h at 24,300 rpm (80,000*g* at r_{av}) in a Beckman SW 27 rotor at 0°C. Marker enzymes in 1.5-ml gradient fractions were assayed as described (14). Glucan synthetase I is a measure of incorporation of UDP-[^{14}C]glucose into 70% (v/v) ethanol-insoluble products in the presence of 0.5 μM UDP-glc and 10 mM Mg; glucan synthetase II is the same assay but in the presence of 10 mM UDP-glc and no added Mg (15).

Growth Measurements. Twenty 1-cm mesocotyl sections from directly below the node were cut under dim green light (which had no effect compared to total darkness) and incubated for the indicated time in 100 ml medium (fresh 10 mM KH_2PO_4, 77 μM chloramphenicol, and 30 mM sucrose [pH 6.0]) (19), in covered glass dishes 10 cm wide, on a slow rotary shaker in darkness at 23°C. Combined length of groups of 5 to 15 segments measured was to the nearest mm using a plastic guide and ruler.

RESULTS

Auxin binding correlated with the elongation response to auxin in successive 20-mm segments cut from the mesocotyl of maize seedlings (Fig. 1). As previously shown for *Avena* mesocotyl (11),

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[3] Abbreviations: NAA, naphthalene-1-acetic acid; R, red light.

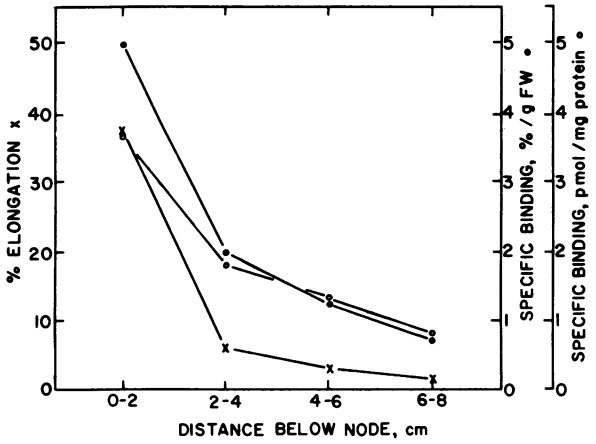

FIG. 1. Auxin-stimulated elongation and NAA binding as a function of position along the mesocotyl. Elongation of 20-mm segments was measured after 16 h treatment with 5.0 μM NAA. [^{14}C]NAA-specific binding in total membrane preparations from comparable segments is given on both a membrane protein and a fresh weight (FW) basis (per cent of offered radioactivity specifically bound, per g fresh weight).

Table I. *Effect of Red Light on NAA Binding and Elongation*

Dark-grown maize plants, 89-h old, were exposed to light for 30 min, then kept in darkness for an additional 10 h before harvest for binding and growth assays.

Tissue	Specific Binding[a]		NAA Bound		Elongation[b]	
	Dark	Light	Dark	Light	Dark	Light
	%		*pmol/mg protein*[c]		%	
Coleoptile	7.9	8.7	1.3	1.4	18	19
Primary leaf	11.0	8.7	0.8	0.8		
Mesocotyl	8.3	4.5	1.9	1.1	66	39

[a] Percentage of offered [^{14}C]NAA specifically bound by crude microsomal particles from 0.5 g fresh weight/ml assay medium. [^{14}C]NAA concentration 14.0 nM.

[b] Per cent elongation of segments measured after 16 h in 10 μM NAA.

[c] Total particulate protein of crude microsomal preparations.

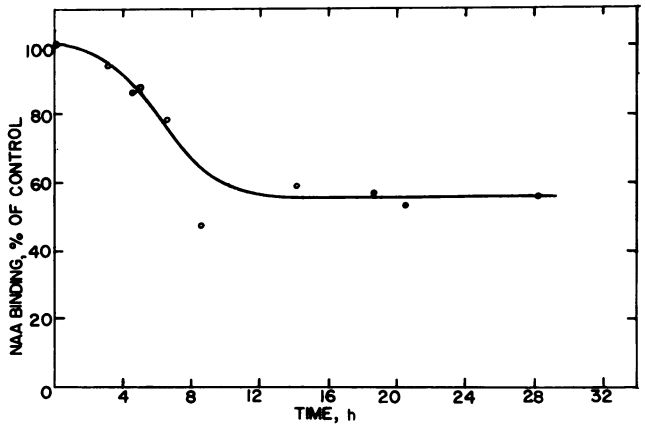

FIG. 2. Time course of light-induced change in specific NAA binding. Plants (80 h old) were placed under continuous R and harvested at the indicated times. Binding by membrane preparations from the apical 3 cm of the mesocotyls is expressed on a membrane protein basis relative to the binding in dark controls. The open and filled circles represent two different experiments, in which specific binding by controls was 5.4% and 11.9%, respectively.

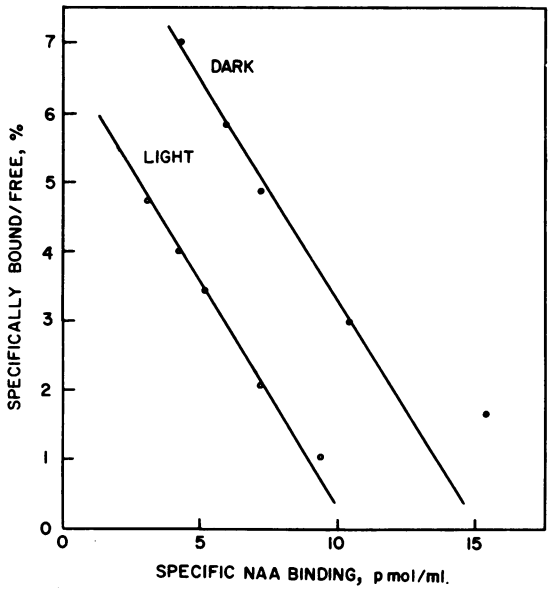

FIG. 3. Scatchard plots of specific NAA binding in membrane preparations from dark control and 15 h R-treated seedlings. Microsomes from 0.5 g fresh weight of mesocotyl tissue per ml binding assay medium (particulate protein 0.59 mg/ml for dark-grown and 0.53 mg/ml for light-treated tissue) were given 70 nM [^{14}C]NAA plus unlabeled NAA at different concentrations, four replications of each. The ratio of specifically bound (total minus nonspecific binding) to free NAA at each concentration is plotted *versus* the amount of NAA specifically bound at that concentration (16).

the capacity for elongation of segments in response to NAA decreased markedly with distance down the mesocotyl from the coleoptilar node. This can be regarded as a developmental sequence, from young tissue adjacent to the intercalary meristem at the coleoptilar node, to older nongrowing tissue at the base of the internode.

Effect of Red Light on Auxin Binding. When dark-grown maize seedlings were exposed to R at least 9 h before harvest, auxin binding in mesocotyl membrane preparations, expressed on either a fresh weight or a protein basis, was reduced by 30 to 50% compared with dark controls. Binding in either the primary leaf or the coleoptile was not reduced (Table I). Elongation in response to auxin of segments from the mesocotyl but not the coleoptile was decreased by R (Table I).

A time course for the decrease in binding in continuous R is shown in Figure 2. Binding began to decline at about 4 h and reached a minimum after about 10 h.

Scatchard analysis of NAA binding in membrane preparations from control and R-treated tissue (Fig. 3) indicated that the number of binding sites was decreased by light, but their affinity for NAA did not change. The K_d for NAA binding in mesocotyl preparations was 0.2 μM, about one-third that measured by Ray *et al.* (16) for binding in coleoptiles. The concentration of sites was about the same in the two tissues, as was the pH optimum (data not shown). The lowermost points in Scatchard plots were consistently displaced to the right, perhaps because of the presence of another class of receptors with low affinity for NAA, or to negative cooperativity within a single class of receptors.

Effect of Light on Elongation Response. Mesocotyl segments cut from R-pretreated seedlings gave markedly less elongation in response to auxin than segments from untreated plants (Fig. 4). This is true whether the elongation was measured over 4 h or 16.5 h. This decrease in responsiveness occurred over a time scale similar to that for decrease in auxin binding (Fig. 2). Light pretreatment reduced the elongation rate, not merely the duration

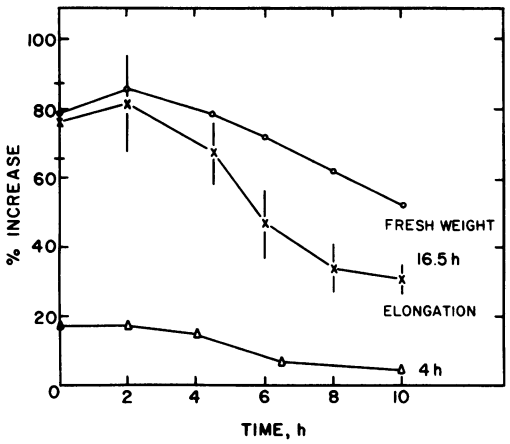

FIG. 4. Effect of duration of R pretreatment on short term and long term elongation, and long term increase in fresh weight, of mesocotyl segments in response to auxin. Plants were placed in R for different lengths of time (abscissa) prior to harvest, apical mesocotyl segments 1.0 cm long (initial fresh weight 29 mg per segment) were harvested from all lots at the same time under dim green light, and measured after 4 h or 16.5 h incubation in buffered 10 μM NAA.

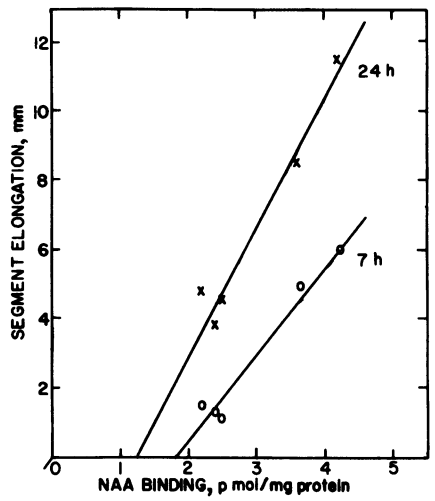

FIG. 6. Correlation between elongation and NAA binding of mesocotyl segments from the same lot of seedlings. Plants 96 h old had been exposed to continuous R for different times from 0 to 28 h prior to harvest. Elongation of 1-cm segments after 7 h (O) and 24 h (x) in 5 μM NAA is shown.

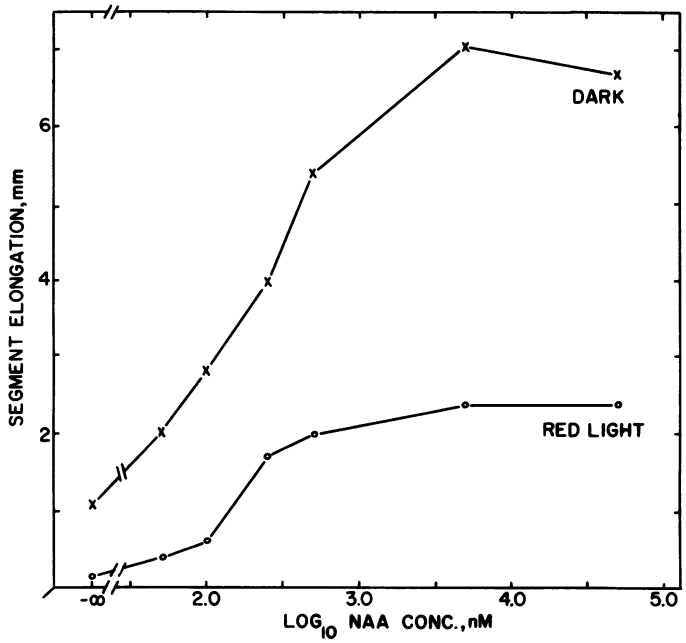

FIG. 5. Elongation of mesocotyl segments from dark-grown and light-pretreated plants, as a function of NAA concentration. Light-treated plants received 2 h of R (total dose 5.3 $\mu E \cdot cm^{-2}$) 15 h before apical 1-cm segments were cut from their mesocotyls. Elongation was measured after 24 h incubation.

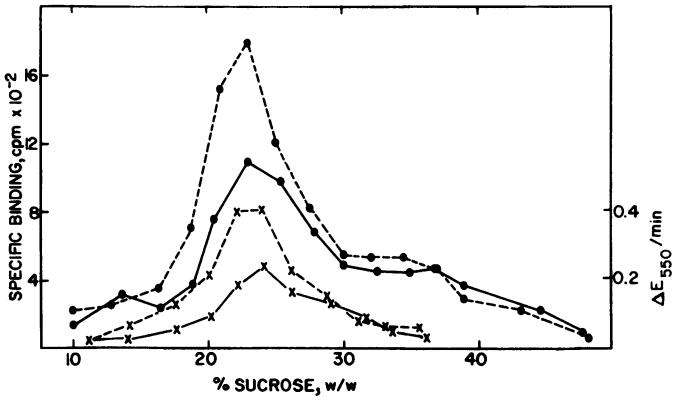

FIG. 7. Isopycnic distribution of NAA binding (O, ordinate at left) and NADH:Cyt *c* reductase activity (x, ordinate at right) in linear sucrose density gradient separation of mesocotyl membranes from dark control (---) and light-treated (——) plants. Treated plants had been given R for 12 h before harvest.

of segment elongation and reduced only slightly the elongation of segments in the absence of auxin. Segments cut from R-treated seedlings tended to swell in response to auxin, since their per cent increase in fresh weight exceeded their per cent elongation (Fig. 4). However, their volume increase was nevertheless considerably reduced by R, compared with dark controls.

Figure 5 shows NAA dose-response curves for elongation of mesocotyl segments from control and R-treated seedlings. They attained half-maximum growth at about the same NAA concentration (about 0.2 μM), but gave different maximal responses.

Figure 6 shows the correlation between elongation in response to NAA and auxin-binding activity, measured on parallel lots of

Table II. *NAA Binding and Enzyme Activities in Density Gradient-Separated Membranes*

Mesocotyls from dark-grown seedlings and from seedlings exposed to R for 12 h prior to harvest were fractionated, and the respective gradients were assayed for indicated activities and protein. Data show the sucrose concentration at which maximum activity of each kind was found in each gradient and give the relative activity, at this peak, for the gradient from R-treated tissue as a percentage of that from dark control tissue.

Activity	Isopycnic Peak (Sucrose concn.)	Light Relative to Dark Activity	
		Total Activity	Protein Basis[a]
	%(w/w)	%	
NAA binding	23.5	52	79
NADH:Cyt *c* reductase	23.5	60	91
Glucan synthetase I	29.0	12	14
Glucan synthetase II	32.5–33.0	107	113
Cyt *c* oxidase	37.5–38.5	96	104

mesocotyl segments cut from seedlings pretreated for different times with R.

Intracellular Localization of Light-Induced Changes. As in maize coleoptiles (14), in density gradient centrifugation most of the auxin-binding activity from both control and irradiated mesocotyl tissue banded isopycnically at a density of about 1.1 $g \cdot cm^{-3}$ (23.5% w/w sucrose) along with NADH:Cyt *c* reductase (Fig. 7), and therefore appears to be carried by ER membranes. The peak of binding activity was clearly separated from other cellular organelles and membranes such as Golgi cisternae (glucan synthetase I peak), plasma membrane (glucan synthetase II peak [15]), and mitochondria (Cyt *c* oxidase peak). Treatment of seedlings with light did not alter substantially the density characteristics of these membranes (Table II).

Density gradients from light-treated mesocotyl tissue consistently showed a similar reduction of both NAA binding and NADH:Cyt *c* reductase activity, compared with the dark control, at the coincident peaks of these respective activities (Fig. 7; Table II). Because dark controls showed somewhat more membrane protein at this point in the gradient than did gradients from light-treated tissue, the specific activity of neither binding nor reductase, on a protein basis, showed as great a difference between light treatment and control as the difference in total activity. This suggests that light may cause a decrease in total ER membrane material in this tissue. Auxin binding expressed on the basis of total tissue membrane protein (Table I) decreased because total membrane protein (due mainly to membranes other than ER) per g fresh weight was not decreased by R.

Auderset *et al.* (1) recently reported an apparent decrease in auxin binding, expressed on a membrane protein basis, in certain density gradient fractions from soybean hypocotyl membranes, only 10 min after the plants had been exposed to bright light. The reported findings (1) differ, in almost all respects studied, from those described above for light-induced changes in maize mesocotyl membranes. We have no explanation for the differences, but there is perhaps no reason to expect maize mesocotyl and soybean hypocotyl tissues to show closely comparable light responses.

As indicated in Table II, light treatment caused a large decrease in Golgi-localized glucan synthetase I activity of maize mesocotyl tissue. We shall report on this in more detail elsewhere.

DISCUSSION

We observed a strong correlation between light-induced changes in ER-localized auxin-binding activity and in auxin-inducible cell enlargement in maize mesocotyl tissue, both in timing (Figs. 2 and 4), and extent (Fig. 6), suggesting that the binding sites may be auxin receptors that mediate the auxin cell enlargement response. As judged from the NAA concentration required for half-maximal cell enlargement, the depressed response of light-treated mesocotyls showed a sensitivity to NAA comparable to that of control tissue (Fig. 5). This supports the view that the auxin binding sites are receptor sites because light reduced the number of NAA binding sites but not their affinity for NAA (Fig. 3). Also consistent with a receptor function for the auxin binding sites are the observations that (a) capacity for elongation in response to auxin, and binding, varied in parallel along the length of the mesocotyl; and (b) in coleoptiles, in contrast to mesocotyls, neither auxin-binding activity nor the auxin cell enlargement response was reduced by comparable R treatments (Table I).

Both auxin binding and auxin-inducible elongation of mesocotyl tissue fell to a non-zero plateau after about 10 h of light exposure (Figs. 2 and 4). That the correlation between them (Fig. 6) had a non-zero intercept on the abscissa may indicate that some of the binding sites are inactive as receptors for cell enlargement, or that a certain minimum receptor occupancy is needed for any enlargement response, or that the relation between receptor occupancy and response is in fact nonlinear at least at low occupancies.

Vanderhoef *et al.* (20) have separated the inhibition of intact maize seedling mesocotyl growth by continuous R into two phases. The first phase begins 20 min after the start of illumination and reaches a plateau at about 50% of the dark growth rate after 2 h. Since during this time R causes a decrease in diffusible auxin (4, 13), and the growth inhibition is reversible by auxin (19), the first phase may be attributed simply to a decreased auxin supply in the mesocotyl. The second phase begins after about 4 h, reaching nearly zero elongation rate after 7 h. The changes in auxin binding described in this paper match rather closely this second phase, except that auxin binding does not fall to near zero. However, the combination of a decrease in auxin supply and in receptor number might be responsible for the severe inhibition of mesocotyl growth in intact plants by R.

Normand *et al.* (12) have reported that 20 min of R with or without an additional 10 min of far-red light causes no change in microsomal auxin binding activity of maize coleoptiles or mesocotyls. Since their tissue samples were homogenized immediately after the light treatment, their results are consistent with ours. We have not studied the effects of far-red light on the long term loss of auxin binding in mesocotyls.

Despite the correlation between binding and growth, the light-induced loss of auxin response in mesocotyl sections could be due to an effect of R on some process other than auxin binding. For example, Goodwin (7) observed that transition from annular to pitted xylem elements accompanied R inhibition of mesocotyl elongation in *Avena* seedlings. Formation of inextensible secondary walls might thus cause the reduced auxin response of segments from R-treated plants, except that the presence of pitted xylem elements should completely inhibit elongation, whereas some elongation does occur in segments (Figs. 4 and 5). Alternatively, since our data indicate that R may cause a decrease in total ER membrane material and in a Golgi-localized glucan synthetase activity, change in some of these rather than in auxin binding might cause the mesocotyl growth inhibition. While alternative explanations such as the foregoing cannot be ruled out, the observed correlations still suggest that "Site I" auxin binding represents hormone receptor activity.

Trewavas (18) has proposed that hormonally mediated developmental processes in plants may often be regulated by changes in sensitivity to hormone (for example, by receptor changes) as against changes in hormone concentration. The response of maize mesocotyl elongation to R appears to include both types of control.

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