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Specificity of Auxin-binding Sites on Maize Coleoptile Membranes as Possible Receptor Sites for Auxin Action[1]

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ABSTRACT

Dissociation coefficients of auxin-binding sites on maize (*Zea mays* L.) coleoptile membranes were measured, for 48 auxins and related ring compounds, by competitive displacement of ^{14}C-naphthaleneacetic acid from the binding sites. The sites bind with high affinity several ring compounds with acidic side chains 2 to 4 carbons long, and much more weakly bind neutral ring compounds and phenols related to these active acids, most phenoxyalkylcarboxylic acids, and arylcarboxylic acids except benzoic acid, which scarcely binds, and triiodobenzoic acids, which bind strongly. Specificity of the binding is narrowed in the presence of a low molecular weight "supernatant factor" that occurs in maize and other tissues. Activity of many of the analogs as auxin agonists or antagonists in the cell elongation response was determined with maize coleoptiles. These activities on the whole roughly parallel the affinities of the binding sites for the same compounds, especially affinities measured in the presence of supernatant factor, but there are some quantitative discrepancies, especially among phenoxyalkylcarboxylic acids. In view of several factors that can cause receptor affinity and biological activity values to diverge quantitatively among analogs, the findings appear to support the presumption that the auxin-binding sites may be receptors for auxin action.

Binding sites for 1-NAA[2] and certain other auxins in membrane particle preparations from maize coleoptiles were first reported by Hertel *et al.* (13), and were subsequently characterized chemically (22) and localized mainly to membranes of the ER (21). These binding sites might represent receptor sites for auxin action. The principal means available in general for judging whether a given kind of binding site may actually function as a receptor site is to compare the specificity of binding with the specificity of the hormonal response toward a series of analogs. In this report we characterize the specificity of the major NAA-binding site of maize membranes toward NAA, IAA, and a series of analogs by measuring the ability of the analogs to compete for the NAA-binding sites, and compare this with estimates of activity in the auxin response both from experiments on maize coleoptiles and general information in the literature.

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[2] Abbreviations: DA: dichlorophenoxyacetic acid; DB: dichlorobenzoic acid; DP: dichlorophenoxy-2'-propionic acid; IBA: 3-indole-4'-*n*-butyric acid; IPA: 3-indole-3'-propionic acid; NAA or 1-NAA: naphthalene-1-acetic acid; 2-NAA: naphthalene-2-acetic acid; 2-NOA: 2-naphthoxyacetic acid; PA: phenylacetic acid; PU: phenylurea; SF: supernatant factor (22); TIBA: triiodobenzoic acid.

MATERIALS AND METHODS

Plant Material. Seedlings of maize (*Zea mays* L., hybrid WF9 × Bear 38, Bear Hybrid Corn Co., Decatur, Ill.) were grown, coleoptiles were harvested and homogenized, and particles were isolated for NAA-binding assay, as previously described (22).

Binding Assay and Evaluation. The standard binding assay described previously (22) was used throughout. Assays contained approximately 50 nM ^{14}C-NAA and 1 or 2% ethanol or acetonitrile as vehicle for the addition of test compounds. Each assay series included controls containing ^{14}C-NAA only (plus the same vehicle as used for the addition of test compounds), samples containing 0.1 mM unlabeled NAA (to determine the level of nonspecific binding), and samples containing a series of concentrations (normally a 10-fold dilution series) of compounds to be tested, care being taken to avoid precipitation of test compounds in the make-up procedure. In tests requiring addition of acidic compounds at concentrations greater than 1 mM, the citrate buffer concentration was increased to 60 mM (normal concentration, 10 mM).

Radioactivity in the assay pellets was plotted against log concentration of each test compound. The concentration of the given compound (referred to as its K_D) that reduces specifically bound radioactivity to half, *i.e.* reduces radioactivity in the pellet halfway from the control value to the value for nonspecific binding (the radioactivity obtained with 0.1 mM unlabeled NAA), was determined by linear interpolation between points in the log plot lying on either side of the halfway point (Fig. 1). In some instances of very weak binding, values of K_D above 1 mM were estimated by linear extrapolation (on the log plot) from the radioactivity found at the highest concentration tested, provided that this point represented at least 15% reduction of specific binding. To make data presentation more compact, K_D values are given as $pK_D = -\log K_D$ and are rounded to the nearest 0.1 log unit. In Tables II through IV any pK_D values estimated by extrapolation are shown in brackets.

Supernatant was prepared and used, in assays to determine the effect of SF on K_D values, as described previously (22). Tests of SF were made using a medium containing 20% supernatant, *i.e.* 1 part of supernatant diluted with 4 parts by volume of standard assay medium.

Elongation Tests. From maize coleoptiles 1.5 to 2.5 cm long a segment 8 mm long beginning 3 mm from the tip was cut, under dim green light, using two razor blades mounted 8 mm apart. The segments were floated on water during cutting and were then transferred to 10-cm Petri dishes, 10 to 15 segments/dish, with 20 ml of 1.5% (w/v) sucrose containing 10 mM

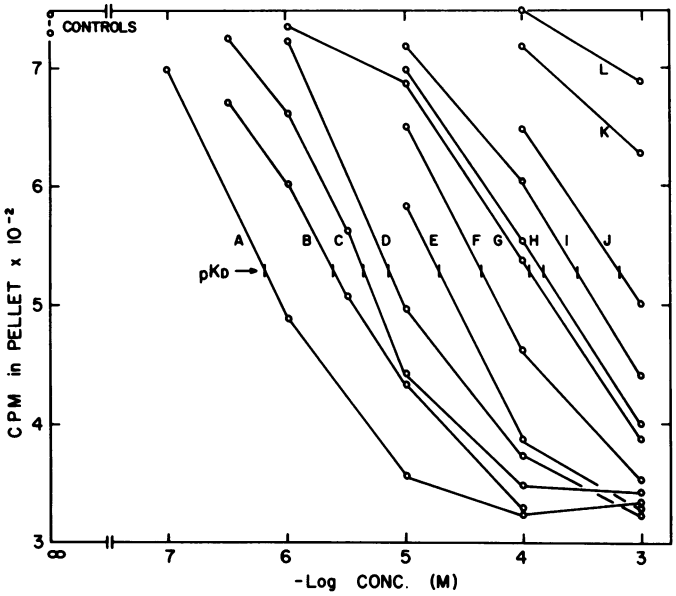

FIG. 1. Displacement of ^{14}C-NAA from centrifugally pelletable binding sites by unlabeled NAA and various analogs. Control radioactivity (upper left) is that in the pellet when no unlabeled NAA or analog was supplied; the plateau of radioactivity reached at high concentrations of NAA (lower right) constitutes nonspecifically bound NAA. Vertical bar on each curve shows estimated pK_D, or $-\log$ concentration for 50% reduction of specific ^{14}C-NAA binding. Tested compounds are: A: 1-NAA; B: 2-NAA; C: IAA; D: 2,3,5-TIBA; E: 3,5-DA; F: 2,5-DA; G: 2,4-DA; H: phenol; I: 2,4-DB; J: 4-chlorobenzoic acid; K: 2-chlorobenzoic acid; L: benzoic acid.

KH_2PO_4/10 mM Na_3 citrate (pH 6.3) plus 0.1 or 0.2% ethanol which served as vehicle during introduction of the test compound. Each compound was tested in a 10-fold dilution series, normally between at least 0.1 mM and 1 μM. All experiments included a control containing no test compound (but containing vehicle) and another control containing 0.1 mM IAA, which gives maximal stimulation of elongation. In all but the short term tests (see Fig. 5), the coleoptile segments were measured after 16 to 18 hr of incubation in the dark at 23 C. They were measured by butting all of those from one dish end to end in a V-shaped plastic guide and measuring their total length to the nearest 0.5 mm with a millimeter ruler.

Chemicals. 1'-^{14}C-NAA (196 Ci/mol) and 1'-^{14}C-IAA (54 Ci/mol) were from Buchler/Amersham, Braunschweig, Germany.

Compounds listed in Tables II through V were obtained from commercial sources, primarily Firma Merck-Schuchardt, Hohenbrunn bei München, Germany; Nutritional Biochemicals Corp., Cleveland, Ohio; and Sigma Chemical Co., St. Louis, Missouri. 2-NOA (from Merck-Schuchardt) was recrystallized twice from ethanol before use. Indole-3-*iso*butyric acid was a gift from B. B. Stowe of Yale University. The 2,5- and 3,5-dichlorophenoxy and 2,5-dimethylphenoxy compounds listed in Table IV were generously loaned by B. Åberg, Lantbrukshögskolan, Uppsala 7, Sweden, and had been prepared by A. Fredga (see refs. 2 and 3). 2,6-DA and the 2,4-DP isomers were from Amchem Products, Inc., Ambler, Pa.

RESULTS

Distribution of Auxin-binding Activity. A high level of specific NAA binding was found in membrane particle preparations from shoot tissues (Table I). The remaining tissues showed a lesser, but not negligible, capacity to bind auxin.

Antagonism of NAA Binding by Structural Analogs. Tables II through IV list compounds that were found to be effective in displacing ^{14}C-NAA, along with some related compounds that were virtually ineffective. We determined the concentration dependence for displacement of ^{14}C-NAA by each of the effective compounds, if possible up to or above a concentration that gives a 50% reduction of specific binding of ^{14}C-NAA. This concentration (called K_D for reasons given below) is expressed in the data as its negative logarithm or pK_D.

Examples of displacement curves are given in Figure 1. These curves have essentially the same shape, in a log plot, as the curve for displacement of ^{14}C-NAA by unlabeled NAA. The curves show that at sufficiently high concentrations of any of the more effective ^{14}C-NAA-binding becomes reduced to essentially the same plateau of nonspecific binding as is observed when unlabeled NAA is used to displace ^{14}C-NAA. This shows that the analogs affect specific but not nonspecific binding. None of the compounds tested yielded a displacement curve with a significant step or break, or which declined to a plateau value substantially higher than the nonspecific binding seen with 0.1 mM NAA, features which would have indicated the presence of two or more types of NAA-binding site with significantly different affinities for the displacing compound.

Competitive Kinetics. Figure 2 shows the results of kinetic tests for competitive interaction between NAA and selected antagonists of ^{14}C-NAA binding, presented as Lineweaver-Burk plots of the reciprocal of the specific NAA binding against the reciprocal of total NAA concentration, with or without an antagonist. The tests include compounds that displace ^{14}C-NAA with high effectiveness (low K_D value) and also several compounds that displace ^{14}C-NAA only at rather high concentrations. In all cases the results conform with a competitive interaction at the NAA-binding sites, between NAA and these antagonists. The plots also conform with behavior to be expected if a single kinetic class of NAA-binding sites is present.

This evidence indicates that displacement of ^{14}C-NAA from its binding sites by the structural analogs under consideration represents competition for common binding sites. The concentration of a given analog needed for 50% displacement of specifically bound ^{14}C-NAA therefore represents (see ref. 8) the dissociation coefficient (K_D) of the NAA-binding site for that analog, divided by the factor $(1 + [NAA^*]/K_N)$ where $[NAA^*]$ is the concentration of labeled NAA used in the binding assay and K_N is the dissociation coefficient of these binding sites for NAA. Since the ^{14}C-NAA concentration used in the binding assay was much lower than the apparent K_N, we refer to the measured 50% displacement concentrations as K_D values, and regard these as indications of the affinity of the NAA-binding sites for different compounds.

Binding of Radioactive IAA and Phenylacetic Acid. ^{14}C derivatives of IAA and PA, which are two of the analogs that

Table I. *Distribution of NAA-Binding Activity in Maize Seedlings*

Particles were prepared from different tissues as described for coleoptiles in Methods; particles from 0.5 gfr.wt. per ml of resuspension medium were used in the binding assay.

Tissue	Specific ^{14}C-NAA binding	
	percent of total ^{14}C-NAA in assay	per mg particulate protein
	%	*p moles/mg*
coleoptile	4.9	4.0
primary leaf	9.3	5.5
node and shoot tip	0.62	0.3
mesocotyl	1.72	3.2
root	0.33	0.4
seed endosperm	0.17	0.2

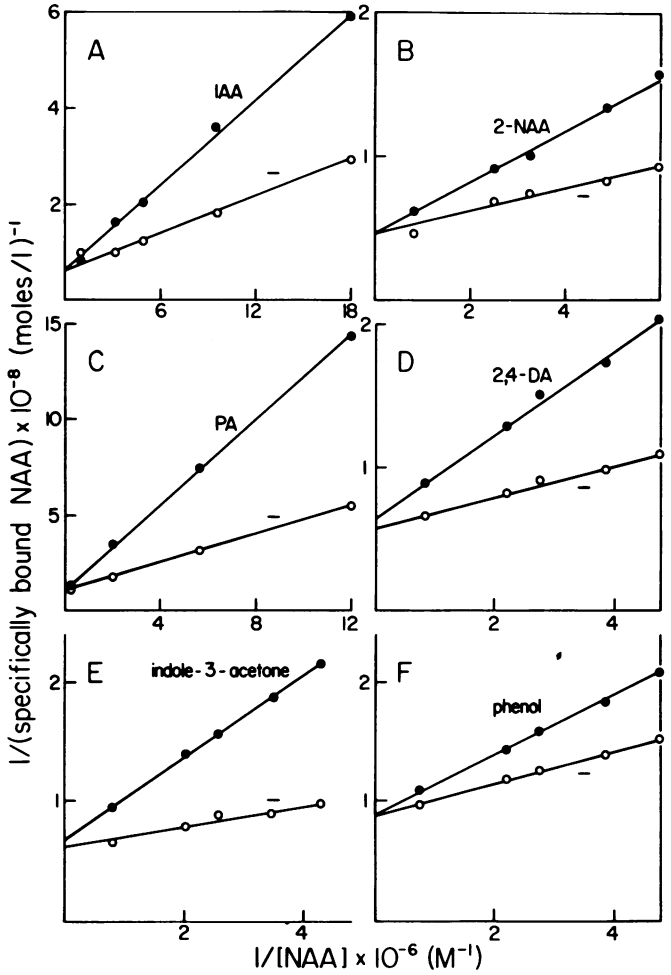

FIG. 2. Lineweaver-Burk double reciprocal plots of specifically bound NAA as a function of NAA concentration in the absence (○) and presence (●) of IAA or various analogs: A: 10 μM IAA; B: 0.2 μM 2-NAA; C: 5 μM PA; D: 0.1 mM 2,4-DA; E: 1 mM indole-3-acetone; F: 0.1 mM phenol. Specifically bound NAA was calculated by subtracting the nonspecifically bound radioactivity (that observed in the presence of 0.1 mM NAA) from the radioactivity observed in the pellets at each particular (lower) concentration of NAA, and dividing by the specific activity of the NAA at that concentration. The reciprocals of these values were plotted against the reciprocals of total NAA concentration (labeled plus unlabeled NAA) in the assay. Each point is the mean of three or more individual measurements.

compete with relatively high affinity (low K_D value) for the NAA-binding sites, can be directly shown to bind to specific binding sites on maize membranes using the same procedure as used for NAA. The concentration of IAA that is required for displacement of 50% of the ^{14}C-IAA from its binding sites is exactly the same as the K_D value for displacement by IAA of ^{14}C-NAA from NAA-binding sites (Fig. 3). Since the concentration of ^{14}C-IAA in the assay (0.4 μM) was low compared to the apparent K_D for IAA, as in the assay involving ^{14}C-NAA, it follows from the principles noted in the preceding section that the K_D values of the IAA-binding sites and of the NAA-binding sites for IAA are the same. This again indicates that NAA and IAA bind to the same sites.

Similar results indicating the same conclusion were obtained with the binding of PA (data not presented).

Structure-Activity Relations in Binding. From the K_D data (Tables II–IV) it will be evident that all compounds that bind with high affinity ($pK_D > 5.0$) to the NAA-binding sites are ring compounds with short acidic side chains. An acetic acid side chain is best, while propionic or butyric side chains are somewhat less effective. Much less effective is a simple carboxyl group, as with benzoic acid which in numerous tests have proved to be almost without effect on NAA binding even at 1 mM, contrary to the report of Batt *et al.* (5). However, halogen substitution of the benzoic acid ring can confer significant affinity for the binding sites.

Neutral derivatives such as the methyl esters or amides of IAA and NAA, or the analogous nitriles, compete significantly for the binding sites but with low affinity. This might be questioned because of the possibility of contamination by small amounts of the corresponding acetic acids, which compete with high affinity. However, certain other neutral or effectively neutral compounds, such as phenols, PU, and indoxyl acetate, also compete for the NAA-binding sites with low affinity; the possibility that these compounds are significantly contaminated with active acids seems much more remote.

The presence of a basic group on the side chain virtually prevents interaction with the NAA-binding sites, even though the ring and side chain would otherwise appear conducive to binding, as in the case of tryptamine (Table II), tryptophan and phenylalanine (Table VIII of ref. 22).

Effect of Supernatant Factor on Binding Affinity. Low mol wt compound(s) in maize homogenates (SF) reduce(s) binding of NAA to its binding sites by lowering their affinity for NAA (22) Tables II through IV show that the influence of SF on K_D varies greatly among different compounds that compete in the NAA-binding assay. SF actually increases the affinity to the sites for certain compounds, notably 2,4-DA, 3,4,5-TIBA, and (most dramatically) 2-NOA. These findings add additional weight to the conclusion that SF is a noncompetitive modulator of binding site affinity (22).

Hormonal Effectiveness of Comparison Compounds. Examples of the elongation responses of *Zea* coleoptiles to several test compounds over a range of concentrations are shown in Figure 4. For each compound that was tested on elongation the concentration for half-maximum action (c_{50}) was determined, by linear interpolation on a log plot similar to Figure 4, and is listed as its negative logarithm or pc_{50} in Tables II through IV. For strongly stimulatory compounds, c_{50} is the concentration that would give half of the increase in elongation caused by 0.1

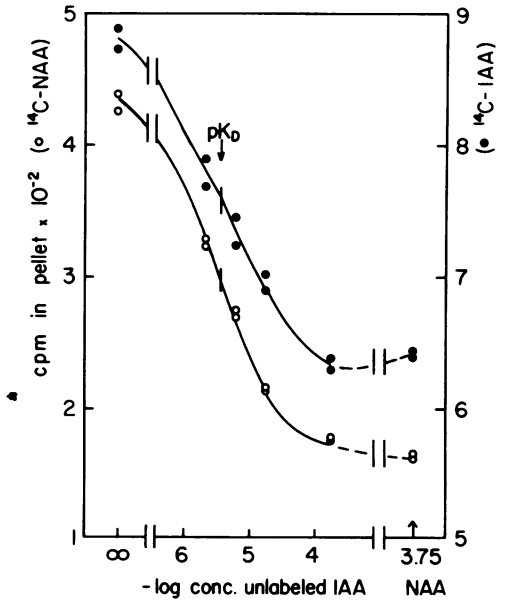

FIG. 3. Displacement of ^{14}C-NAA or ^{14}C-IAA from pelletable binding sites by different concentrations of unlabeled IAA and, for comparison, by 0.18 mM NAA (lower right). Each assay sample contained either 13,000 cpm ^{14}C-NAA (○) or 28,000 cpm ^{14}C-IAA (●). Note different scales on the respective ordinates.

Table II. *Activities of Indole and Naphthalene derivatives*
Molar concentration (K_D) giving 50% reduction of specific ^{14}C-NAA binding in the absence (-SF) and presence (+SF) of 20% (V/V) supernatant factor, and concentration (c_{50}) giving half maximum effect on elongation, are expressed as their negative logarithms, pK_D and pc_{50} respectively. Values for acetic derivatives are means of 3-5 determinations, for other compounds single determinations or the mean of 2. Spaces (..) indicate tests that were not made. Figures in ( ) were estimated by extrapolation (see Methods).

Compound	Binding pK_D		Elongation	
	-SF	+SF	Effect[1]	pc_{50}
Naphthalene-				
-1-acetic acid	6.1	5.6	++	5.3
-2-acetic acid	5.9	4.65	=	5.6
-2-oxyacetic acid	3.7	4.5	-[2]	5.5
			+[2]	4.2
-1-acetate, methyl[3]	4.6	4.5	..	..
-1-acetamide	4.0	3.3	-	(<3.5)[4]
-1-acetonitrile	3.6	-	..	..
-1-ol	4.4	4.0	..	..
-2-ol acetate[5]	4.1	..	-	3.5
-1-isocyanate	4.7	..	..	..
Indole-				
-2-carboxylic acid	3.5	3.1	..	..
-3-acetic acid	5.4	5.15	++	5.4
-3-propionic acid	5.2	4.0	-[2]	5.7
			+[2]	4.5
-3-*n*-butyric acid	5.0	4.0	++	4.7
-3-acrylic acid	4.0	..	..	..
-3-*iso*butyric acid	3.8	4.0	=	4.8
-3-acetonitrile	4.0	4.15	..	..
-3-acetone	2.9	..	..	..
-3-aldehyde	2.7	..	..	..
-3-ethylamine[6]	(2.1)	(2.0)	..	..
-3-ol acetate[7]	3.5	..	..	..

[1]++ strong stimulation, + weak stimulation, - inhibition, = weak inhibition, with partial inhibition as maximum effect.
[2]Inhibitory and stimulatory concentration ranges for this compound.
[3]Methyl ester of naphthalene-1-acetic acid.
[4]Value estimated from modest inhibition by this compound at 0.3 mM (Table V).
[5]Acetate ester of 2-naphthol.
[6]Tryptamine.
[7]Indoxyl acetate.

mM IAA; for strongly inhibitory compounds, c_{50} is the concentration that would give 50% inhibition of elongation compared with the minus-auxin control; for weakly stimulatory compounds giving a maximum stimulation much less than that of IAA, and for inhibitory compounds giving partial inhibition as their maximum inhibitory effect, c_{50} is the concentration that would give half of this maximum effect.

The results conform generally with the structure-activity relations seen using other test systems as described in the literature (14, 25).

The short term elongation response to 2,4-DA, NAA, and IAA was measured, by determining the elongation of multiple samples of coleoptile segments after a 2-hr exposure to test solutions. The results (Fig. 5) indicate that the sensitivity to 2,4-DA is considerably lower, compared to NAA or IAA, than in long term experiments (Fig. 4).

To check whether those analogs that inhibit elongation may act as auxin antagonists, a number of these were tested also in the presence of 1 μM and 0.1 mM IAA (Table V). In most cases, high auxin largely or completely reversed the inhibition, as would be expected if the inhibitory compound acts as a competitive antagonist at the receptor site. This agrees with more extensive kinetic data on several of these compounds in other test systems (14, 17, 25).

Certain analogs yield partial inhibitions of elongation as their maximum effect (Fig. 4B). This could be because the analog acts as a very weak auxin, with a stimulatory effect smaller than that of endogenous auxin of the tissue. Other analogs inhibit elongation at low concentration and stimulate it at higher concentration (Fig. 4A). These kinds of effects have been observed in other test systems (2-4), attributable to action

Table III. *Activities of Phenyl derivatives*
Data presentation and symbols as in Table II; 0 indicates no effect up to 1 mM concentration.

Compound	Binding pK		Elongation	
	-SF	+SF	Effect	pc_{50}
Benzoic acids				
Unsubstituted	(2.1)	(0)	-	(<< 3.0)[1]
2-chloro-	(2.5)	(0)	..	..
3-chloro-	3.4	(2.5)	..	..
4-chloro-	3.1	2.5	-	3.3
2,4-dichloro-	3.4	(2.7)	-	3.7
3,4-dichloro-	3.7	..	-	3.8
2,6-dichloro-	0	(<2.0)	-	(<3.0)
2,3,5-triiodo-	5.1	4.5	+	5.0
			-	4.1
3,4,5-triiodo-	4.5	5.0	-	5.0
Phenyl-				
-acetic acid	5.5	4.4	-	3.1
-urea	4.1	(2.5)	0	..
-thiourea	3.6	..	..	..

[1]Only slight inhibition at 1 mM.

Table IV. *Activities of Phenoxy compounds*
Data presentation and Symbols as in Tables II-III, except as noted.

Compound	Binding pK_D		Elongation	
	-SF	+SF	Effect	pc_{50}
Phenoxyacetic acids				
2,4-dichloro-	4.0	4.4	++	5.2
2,5-dichloro-	4.4	4.1	++	4.5
2,6-dichloro-	3.5	2.9	-	3.4
3,5-dichloro-	4.7	4.5	-	3.6
4-Cl, 2-methyl-	4.8	4.7	++	5.3
2,5-dimethyl-	5.1	4.6	(-)[3]	(<4.0)
Unsubstituted	3.8	..	..	..
Phenoxy-2'-propionic acids[1]				
D(+)-2,4-dichloro-	3.7	3.3	++	5.5
L(-)-2,4-dichloro-	3.7	3.0	++[4]	4.7
(+)-2,5-dichloro-	4.1	3.5	++	5.2
(-)-2,5-dichloro-	4.1	3.25	(0)[5]	..
(+)-2,5-dimethyl-	4.7	4.0	+	4.1
(-)-2,5-dimethyl-	4.5	3.0	(-)[3]	(<4.0)
Phenoxybutyric acids[2]				
2,4-dichloro-4'-*n*-	4.6	4.0	+	4.0
4-chloro-2'-*iso*-	4.0	3.0	-	3.3
Phenol	3.8	(2.1)	..	..

[1]Chloro and methyl derivatives of 2'-phenoxypropionic acid.
[2]Chloro derivatives of 4'-phenoxy-*n*-butyric acid and 2'-phenoxy-*iso*-butyric acid.
[3]These compounds gave weak inhibition beginning at 0.1 mM, but in case of the acetic derivative no inhibition at 0.6 mM, the highest concentration that was tested.
[4]This compound causes a weak inhibition at 1 μM (see Fig. 4).
[5]No effect up to 0.1 mM.

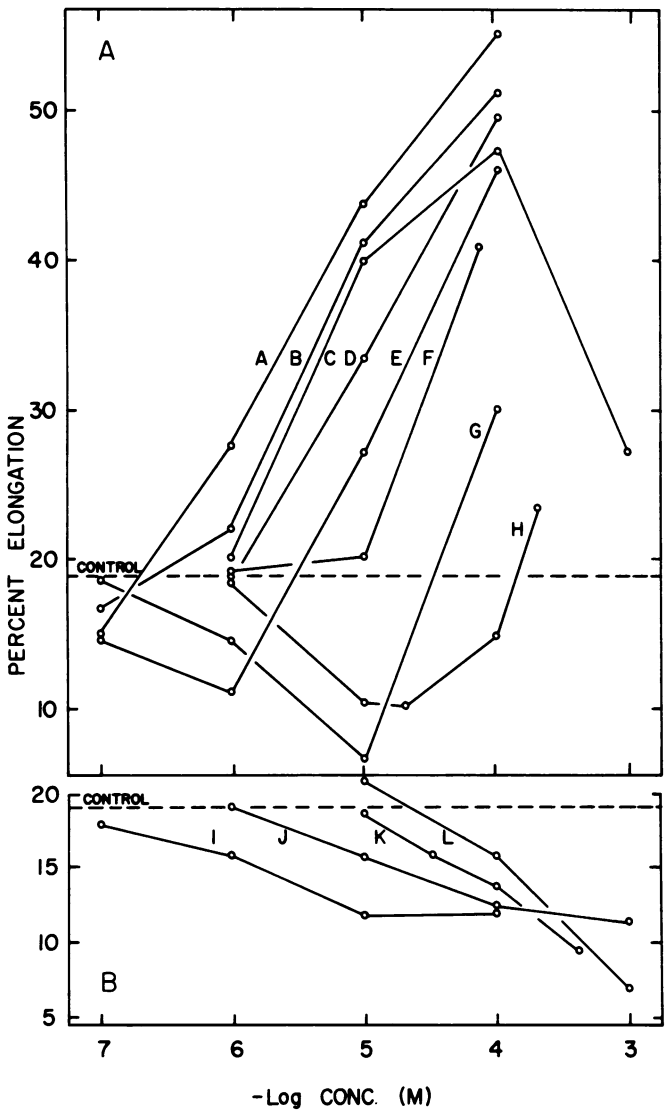

FIG. 4. Elongation responses of maize coleoptiles to stimulatory (upper panel) and inhibitory (lower panel) auxins and analogs, measured as segment elongation in 16 hr as per cent of initial length. Compounds illustrated are: A: IAA; B: 2,4-DA; C: 1-NAA; D: IBA; E: (−)-2,4-DP; F: (+)-2,5-dimethylphenoxy-2′-propionic acid; G: IPA; H: 2-NOA; I: 2-NAA; J: 3-indole-2′-*iso*butyric acid; K: 4-chlorophenoxy-2′-*iso*butyric acid; L: 3,5-DA.

intermediate between active auxins and strong auxin antagonists (1).

DISCUSSION

The data presented here and in reference 22 on competition between ^{14}C-NAA and unlabeled NAA or analogs, conform with binding by one principal type of saturable site. Batt *et al.* (5) inferred the presence of two classes of sites, from nonlinear Scatchard and Lineweaver-Burk plots that were made without correcting for unsaturatable ("nonspecific") binding, which forces such plots to be nonlinear even when only a single class of saturatable binding sites is present. Although features of the displacement of ^{14}C-NAA by analogs suggest (9, 20) the possible presence of binding sites that differ, in affinity for certain analogs, from the sites that are responsible for the bulk of the specific NAA binding, the following discussion concerns only these latter sites, for which relatively accurate K_D values for analogs can be obtained from competition data.

The NAA-binding sites might constitute active sites for conjugation processes that convert auxins *in vivo* to aspartyl, glucosyl, or other derivatives (24). However, coleoptile tissue forms the same types of major conjugation products from benzoic acid as from IAA, while it conjugates NAA much less vigorously and 2,4-DA hardly at all (15). These features conflict with the specificity of the NAA-binding sites, suggesting that the binding sites probably do not represent sites for conjugation.

Another potential role of auxin-binding sites is as carrier sites involved in polar transport of auxins. The sensitivity of IAA transport to inhibition by various auxins and analogs (20) parallels fairly well the affinities of the NAA-binding sites for these compounds. However, polar transport most likely depends upon carrier sites located in the plasma membrane (7, 12, 23), whereas most of the specific NAA binding is due to sites localized on the ER membranes (21). Therefore, most of the NAA binding cannot logically be assigned a function in polar transport, even though it remains possible that some of the NAA-binding sites are located on the plasma membrane and

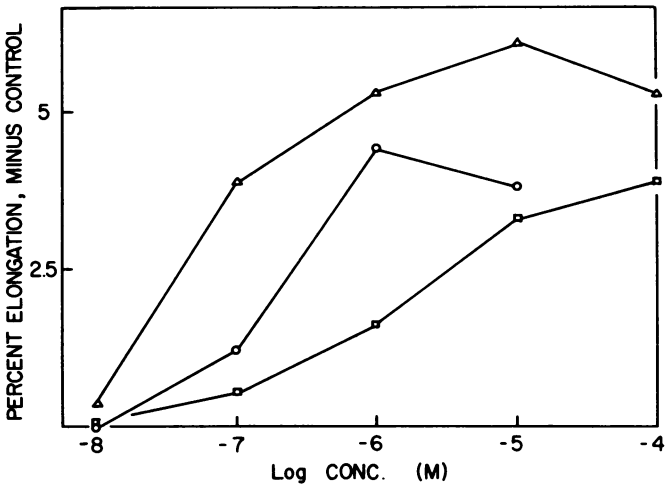

FIG. 5. Short term elongation responses of maize coleoptile segments to IAA (△), NAA (○), and 2,4-DA (□). Triplicate lots of 10 segments each were kept in water for 1 hr, then incubated 2 hr on a shaker (60 excursions/min) at 23 C in 20 mM Na/K phosphate (pH 5) containing the test compound, and immediately measured. Data show the mean elongation above the minus auxin control, in per cent of initial length, averaged from two similar experiments.

Table V. *Reversal by IAA of inhibition of elongation by auxin analogs*
Data show percent inhibition of elongation by the analog, relative to a control lacking the analog but containing the same concentration of IAA. Percent elongation of controls in 0, 1 μM, and 0.1 mM IAA were 22, 31 and 53% respectively.

analog	concentration	Percent inhibition of elongation in presence of: no IAA	10^{-6} M IAA	10^{-4} M IAA
	mM		%	
4-chlorophenoxy-*iso*butyric acid	0.3	48	41	-2
3,4-dichlorobenzoic acid	0.3	61	55	30
3,5-dichlorophenoxyacetic acid	0.3	70	33	28
2,5-dimethylphenoxyacetic acid	0.2	0	19	9
indole-3-*iso*butyric acid	0.1	44	..	1
indole-3-*n*-propionic acid	0.01	46	-1	0
naphthalene-2-acetic acid	0.03	39	31	3
naphthalene-1-acetamide	0.3	23	0	13
2-naphthoxyacetic acid	0.02	56	29	4
	0.2	-19[1]	31	4
2-naphthyl acetate	0.3	58	57	63
phenylacetic acid	0.3	31	2	9
	1.0	28	27	4
3,4,5-triiodobenzoic acid	0.02	75	83	56

[1] Stimulation above the minus-auxin control.

play a role in polar transport (9).

Comparison of Binding with Hormonal Action. The data on tissue distribution of binding activity (Table I), although necessary general background, are not decisive regarding the possible role of the binding sites as receptors for hormone action, because of the relative lack of target tissue specificity for plant hormones.

The competition tests showed the analog specificity of the NAA-binding sites to be rather broad, as is the auxin hormonal response (14, 25). All active auxins that we have tested bind to the sites, as do a variety of analogs which, according to the literature and to our data, act as competitive antagonists of auxin action. Within a given series of derivatives, for example among the indole derivatives or among the naphthalene derivatives, the structure-activity relations cited above for binding strength are at least qualitatively similar to rules that are well known for auxin action. The low affinity of the sites for benzoic acid and its chloro derivatives compared with the high affinity for TIBA also agrees with the relative effectiveness of the respective groups of compounds in influencing the auxin growth response (18).

The fact that neutral ring compounds as well as phenols compete, albeit with low affinity, for the binding sites seems at first sight to constitute a serious discrepancy since neutral compounds are usually considered inactive as auxins. Because of questions about stability of many of these compounds *in vivo* and the high concentrations which the binding assay suggests should be required to interact with the auxin response, it seems dubious whether their possible biological activity could be checked unambiguously. There are reports, however, of auxin activity (11, 25) or possible auxin antagonist activity (see ref. 17) of certain phenols and neutral ring compounds.

In Figure 6A, values for effectiveness of different compounds on elongation (pc_{50}) have been plotted against effectiveness of these same compounds in the NAA-binding assay (pK_D). The points scatter rather widely from the line of equality. The more conspicuous part of this scatter is due to phenoxy acids that stimulate elongation at concentrations lower than might be expected on the basis of their binding affinity for the NAA-binding sites, and to phenyl derivatives (PA, PU) which bind more strongly than their effects on elongation seem to warrant.

Several of the most important discrepancies between binding affinity and biological action are substantially reduced if the K_D values for binding measured in the presence of SF are considered, as plotted in Figure 6B. SF lowers the affinity of the binding sites for NAA and IAA while raising it for 2,4-DA, thus bringing the values for these highly active auxins closer together. SF drastically reduces the affinity of the sites for PA and PU, bringing these values more into line with biological activity in the case of PA, and with the lack thereof in the case of PU.

SF confers a difference in affinity of the sites toward (+) and (−) isomers of optically active phenoxy-2′-propionic acids (Table IV) that conforms at least qualitatively with the relative auxin activity of the members of these pairs. However, as a group, the K_D values for the phenoxypropionic acids in the presence of SF exhibit the most substantial departure from the general trend between hormonal activity and binding affinity (Fig. 6B). Another serious discrepancy appears in the case of 2,4-dimethylphenoxyacetic acid, which competes for NAA binding more effectively than 2,4-DA but which has only a weak effect on elongation (Table IV) except at higher concentrations that we tested (2).

Although the relation between hormonal activity and binding affinity in Figure 6B is not as good as can be demonstrated for some animal hormone receptors, deviations of as much as 50-fold from a proportional relationship (equivalent to 1.7 units on the log scales of Fig. 6) are encountered in activity-affinity relationships that are considered excellent correlations (6, 16, 19, 26). Reasons why a strictly proportional correlation cannot be expected include differences between compounds in respect to uptake, sequestration, metabolic alteration, and in stimulatory activity once combined with a receptor site. In maize coleoptiles, for example, 2,4-DA is more extensively taken up (10) and much less subject to destruction and conjugation (15) than IAA, which would tend to offset a relatively low affinity of receptor sites for 2,4-D. The much lower sensitivity of elongation to 2,4-DA than to IAA in short term measurements (Fig. 5 and ref. 10) suggests that the auxin receptor system actually has a relatively low affinity for 2,4-DA, as do the NAA-binding

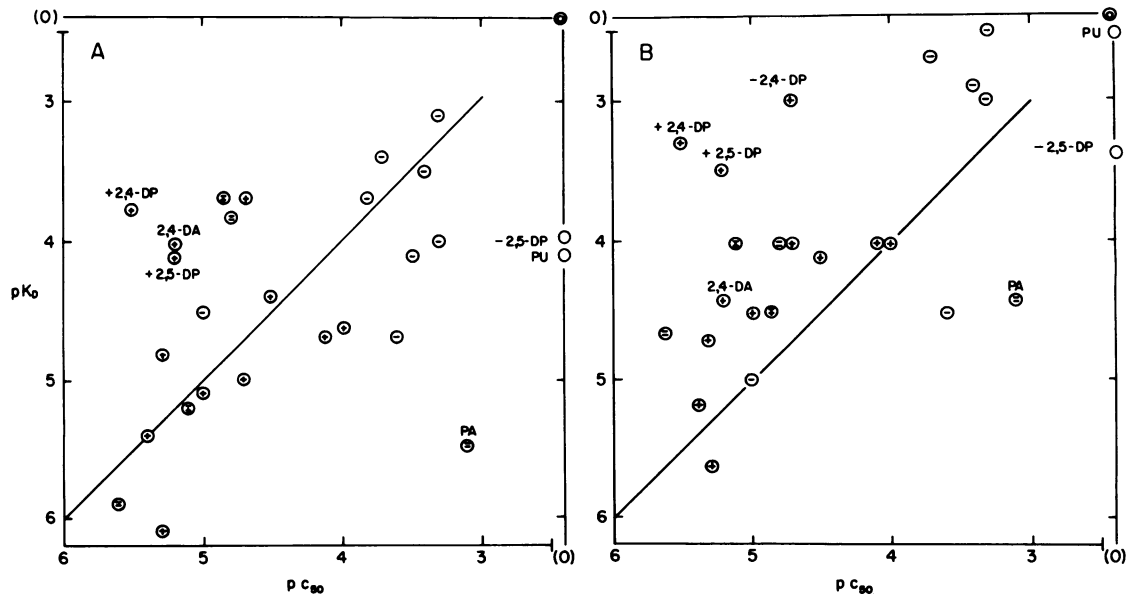

FIG. 6. pK_D values for competition by different compounds in the NAA-binding assay without (A) and with (B) supernatant factor, plotted against pc_{50} values for effects of the same compounds on elongation (+: stimulation; −: inhibition; =: weak inhibition [see footnote 1 of Table II]; ±: compound with inhibitory and stimulatory concentration ranges, the pc_{50} value plotted being the mean of those for the two actions). Data are from Tables II through IV. For purposes of discussion the points for certain compounds are identified by the abbreviations listed in text footnote 2. The line of equality between numbers on abscissa and ordinate is drawn in each graph.

sites. Differences between conditions *in vivo* and in the *in vitro* binding assay may also influence the relative sensitivity to different analogs, as the results with SF illustrate; cytoplasmic pH is likely to be higher than the optimum pH of 5.5 for binding which was used in the assays to facilitate accurate determination of K_D values. Considering all of these sources for quantitative divergence between receptor affinity and biological sensitivity toward hormone analogs, the specificity characteristics described here for NAA-binding sites seem to warrant at least a presumption that these sites may be physiological hormone receptor sites. Certain discrepancies remain to be adequately accounted for. The known properties of the binding sites do not explain the behavior of "intermediate" auxins, which at low concentrations antagonize the action of active auxins but at higher concentrations stimulate the response. As has been pointed out (1), this suggests the involvement of more than one type of receptor site.

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