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Ligand Specificity of Bean Leaf Soluble Auxin-binding Protein[1]

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ABSTRACT

The soluble bean leaf auxin-binding protein (ABP) has a high affinity for a range of auxins including indole-3-acetic acid (IAA), α-napthaleneacetic acid, phenylacetic acid, 2,4,5-trichlorophenoxyacetic acid, and structurally related auxins. A large number of nonauxin compounds that are nevertheless structurally related to auxins do not displace IAA from bean ABP. Bean ABP has a high affinity for auxin transport inhibitors and antiauxins. The specificity of pea ABP for representative auxins is similar to that found for bean ABP. The bean ABP auxin binding site is similar to the corn endoplasmic reticulum auxin-binding sites in specificity for auxins and sensitivity to thiol reagents and azide. Qualitative similarities between the ligand specificity of bean ABP and the specificity of auxin-induced bean leaf hyponasty provide further evidence, albeit circumstantial, that ABP (ribulose 1,5-bisphosphate carboxylase) can bind auxins *in vivo*. The high incidence of ABP in bean leaves and the high affinity of this protein for auxins and auxin transport inhibitors suggest possible functions for ABP in auxin transport and/or auxin sequestration.

We have presented evidence for the identity of the high incidence, soluble ABPs[2] of bean and pea leaves with RuBPCase (32). Because the $(NH_4)_2SO_4$ precipitation assay for IAA-ABP binding is highly reproducible and the basis for lack of IAA-binding by some ABP preparations in equilibrium dialysis is not known (32), the former procedure has been employed to examine the ligand specificity of ABP. This approach can provide the ligand specificity of a model high affinity ABP- and in addition will define the nature of a major plant auxin-binding site if the *in vitro* results directly reflect the behavior of an active conformer of ABP (RuBPCase) *in vivo*. We have shown previously (31) that the partially purified bean leaf ABP binds a variety of active synthetic auxins. The present paper describes the interaction of extensively purified bean leaf ABP with a wide range of naturally occurring and synthetic auxins, antiauxins, auxin transport inhibitors, and structurally related compounds. The binding of representative auxins to purified pea ABP is also reported.

MATERIALS AND METHODS

Purification of ABP. Bean leaf ABP was purified from about 10-day-old primary leaves of dwarf beans (*Phaseolus vulgaris* var. Royal Windsor) essentially as described previously (32). Because of the instability of bean ABP after chromatography on DEAE-Sephacel this chromatographic step was replaced with a further gel filtration through a column (2.5 cm^2 × 70 cm) of Ultrogel AcA-34 as described previously (32). Pea ABP was purified from about 10-day-old leaves of peas (*Pisum sativum* var. Greenfeast) as described previously (32).

Measurement of IAA-Binding. [2-^{14}C]IAA binding to bean and pea ABP was measured in the standard assay at pH 8.0 containing 2×10^{-7} M IAA (specific radioactivity of [2-^{14}C]IAA: 49 mCi/mmol) as previously described (32). Specific [2-^{14}C]IAA-binding is presented as [2-^{14}C]IAA binding to ABP in the standard assay that is abolished by inclusion of 2×10^{-4} M unlabeled IAA. The data in Table I alone are calculated on the basis of [2-^{14}C]IAA displaced by inclusion of 1×10^{-4} M unlabeled IAA (98% of that displaced by 2×10^{-4} M unlabeled IAA). Specific [2-^{14}C]IAA binding was routinely determined in triplicate and SDs in the binding assays were about 2.5% of the means. Titrations of [2-^{14}C]IAA binding to ABP by inclusion of increasing concentrations of test ligands in the assay were routinely performed with triplication or duplication of assays for each data point. Results from such titrations are expressed as C_{50} values (M): the concentration of test ligand for 50% inhibition of specific [2-^{14}C]IAA binding to ABP determined in the standard assay conducted in the presence or absence of 2×10^{-4} M unlabeled IAA. All IAA-binding assays conducted in this study contained 0.05% (w/v) dimethylsulfoxide in the 1 ml final assay volume.

Materials. Chromatographic materials and [2-^{14}C]IAA were obtained as described previously (32). IAA and most indole, purine, pyrimidine, and naphthalene derivatives in addition to tryptophan metabolic derivatives, organic thiol reagents, plant growth inhibitors, and amino acids were obtained from the Sigma. Most phenylacetic acid, phenoxyacetic acid, imidazole, and benzoic acid derivatives were obtained from the Aldrich Chemical Co., Gillingham, Dorset, U.K. 2,4-D, 2,4,5-T, and TIBA were obtained from the Sigma. 2-Naphthylmethylselenideacetic acid, α(+)-2,4,5-trichlorophenoxypropionic acid, and α(−)-2,4,5-trichlorophenoxypropionic acid were generously supplied by Professor B. Åberg, Växtfysiologiska Institutionen, Lantbrukshögskolan, Uppsala, Sweden. Benzisothiazoles, synthesized by the procedures by Davis *et al.* (13), were kindly supplied by Dr. M. Davis, Organic Chemistry Department, La Trobe University, Melbourne, Australia. Most auxin transport inhibitors listed in Tables VII and VIII were generously provided by Dr. G. Katekar, CSIRO Plant Industry, Canberra, ACT, Australia; erythrosin A, eosin, and rose bengal were obtained from British Drug Houses, Poole, U.K.

RESULTS AND DISCUSSION

Inhibition of IAA Binding to Bean ABP by Indole Derivatives. Of indole derivatives tested at 10^{-4} M in the standard assay, the most effective inhibitors of IAA binding to bean ABP are acidic 3-indole derivatives (Table I, section A). Indole-5-carboxylic acid and indole-2-carboxylic acid are relatively ineffective as are carboxylic acid ester derivatives (Table I, section A). With the

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[2] Abbreviations: ABP: soluble bean or pea leaf auxin-binding protein; RuBPCase: ribulose-1,5-bisphosphate carboxylase; 2,4,5-T: 2,4,5-trichlorophenoxyacetic acid; TIBA: 2,3,5-triiodobenzoic acid; α-NAA: α-naphthaleneacetic acid; β-NAA: β-naphthaleneacetic acid; α-NPA: α-naphthylphthalamic acid.

Table I. *Effects of Indole Derivatives on IAA Binding to Bean ABP*

Specific IAA-binding was determined in triplicate in the standard assay conditions at pH 8.0 in the presence or absence of 0.1 mM test ligand in the final 1-ml assay volume. Inhibition of specific [2-^{14}C]IAA-binding is expressed as a percentage of the effect of inclusion of 0.1 mM unlabeled IAA (100%).

	Addition	Inhibition
		% control
(A)	IAA	100
	Indole-3-pyruvic acid	88
	Indoxyl sulfate	85
	Indole-3-butyric acid	50
	Indole-3-propionic acid	47
	Indole-3-acetonitrile	42
	Indoxyl-1,3-diacetate	30
	DL-Indole-3-lactic acid	19
	Indole-2-carboxylic acid	15
	Indole-5-carboxylic acid	0
	Indoxylacetate	1
	Indole-3-acetic acid ethyl ester	10
	5-Methoxyindole-2-carboxylic acid ethyl ester (0.2 mM)	2
(B)	5-Methoxyindole-3-carboxaldehyde	40
	3-Indolylacetone	22
	Indole-3-acetaldehyde	18
	Indole-3-carboxaldehyde	11
	1-Acetylindole-3-carboxaldehyde	1
	7-Methylindole-3-carboxaldehyde	−3
	3-Acetylindole	−9
(C)	Indole	−5
	7-Methylindole	12
	5-Methylindole	8
	2-Methylindole	−2
	6-Methylindole	0
	5-Hydroxyindole (0.2 mM)	2
	5-Methoxyindole (0.2 mM)	6
	3-Mercaptoindole (0.2 mM)	−2

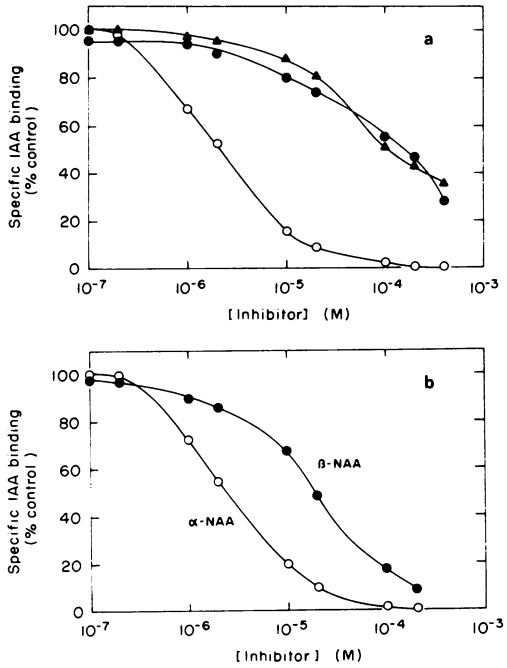

FIG. 1. Titration of specific [2-^{14}C]IAA-binding to bean ABP by naturally occurring and synthetic auxins. a: (○——○), IAA; (●——●), indole-3-propionic acid; (▲——▲), indole-3-butyric acid. b: (○——○), α-naphthaleneacetic acid; (●——●), β-naphthaleneacetic acid.

Table II. *Titration of the IAA-binding Site of Bean ABP with IAA, Phenylacetic Acid, and NAA Analogues*

Specific IAA-binding was determined in triplicate in the presence or absence of various concentrations of test ligands. The concentration of test ligand for 50% inhibition of specific [2-^{14}C]IAA-binding (C_{50}) was estimated from plots of specific IAA-binding *versus* test ligand concentration (*cf.* Fig. 1).

	Ligand	C_{50}
		M
(A)	IAA	2×10^{-6}
	Indole-3-butyric acid	1×10^{-4}
	Indole-3-propionic acid	2×10^{-4}
(B)	4-Phenylbutyric acid	8×10^{-5}
	p-Hydroxyphenylacetic acid	3×10^{-5}
	Phenylacetic acid	2×10^{-5}
(C)	α-Naphthalene acetic acid	3×10^{-6}
	β-Naphthalene acetic acid	2×10^{-5}
	β-Naphthoxyacetic acid	2×10^{-6}
	2-Naphthylmethylselenide acetic acid	1×10^{-5}

exception of 5-methoxyindole-3-carboxaldehyde, neutral 3-indole derivatives with a carbonyl residue are ineffective (Table I, section B). Indole at 0.1 mM does not inhibit IAA binding to ABP nor do a variety of methylindole isomers (Table I, section C). While *N*-acetyl D-tryptophan and *N*-acetyl-L-tryptophan at 0.1 mM inhibit specific IAA-binding by 28 and 10%, respectively, a range of other tryptophan derivatives do not inhibit at 0.1 mM concentration, namely: L- and D-tryptophan, 5-hydroxy-L-tryptophan, 5-hydroxy-D-tryptophan, L-tryptophan ethyl ester, L-tryptophan methyl ester, D-tryptophan methyl ester, tryptophol, tryptamine, 5-methoxytryptamine, *N*-acetyl-5-methoxytryptamine, 5-hydroxytryptamine, and L-tryptophanamide. IAA is the most efffective indole ligand found, having an inhibitory effectiveness two orders of magnitude greater than that for indole-3-butyric acid or indole-3-propionic acid (Fig. 1; Table II), these latter indole derivatives being biologically much less active than IAA (28). With the exception of indole-3-acetonitrile, indoxyl-1,3-diacetate, and 5-methoxyindole-3-carboxaldehyde (all relatively weak ABP ligands with K_d values $>10^{-4}$ M) the indole derivatives that substantially inhibit IAA-binding at 0.1 mM have an acidic indole-3-substituent (Table I).

Inhibition of IAA Binding to ABP by Phenylacetic Acid Analogues. Since a variety of naturally occurring indole auxins were shown to bind to ABP, the effects of the naturally occurring auxin phenylacetic acid and analogs of this compound on IAA-ABP binding were examined. Phenylacetic acid and a variety of phenylacetic acid analogues inhibit IAA binding to ABP (Table III). The C_{50} values for phenylacetic acid and its more effective analogs are an order of magnitude higher and lower than those for IAA and indole-3-propionic acid, respectively (Table II). The *o*-, *m*-, and *p*-chlorophenylacetic acids are all comparable to phenylacetic acid as inhibitors of IAA binding (Table III). The *m*- and *o*-isomers of hydroxyphenylacetic acid are less effective than the *p*-isomer, as are the 3-methoxy- and 3-hydroxy-substituted derivatives of *p*-hydroxyphenylacetic acid (Table III). The auxin activities of the *o*-, *m*-, and *p*-isomers of hydroxyphenylacetic acid and chlorophenylacetic acid have been described by Åberg (3). While *m*- and *o*-nitrophenylacetic acids are comparable to phenylacetic acid as inhibitors, *p*-nitrophenylacetic acid does not inhibit IAA binding to ABP (Table III). The *m*- and *o*-isomers of nitrophenylacetic acid are auxins but *p*-nitrophenylacetic acid is inactive as an auxin (3, 9) and thus the ligand specificity of ABP qualitatively correlates with the biological activity of these phenylacetic acid derivatives.

Inhibition of IAA Binding by Auxin and Antiauxin Phenoxya-

Table III. *Effects of Phenylacetic Acid Analogues on IAA Binding to Bean ABP*

Inhibition of specific IAA-binding was determined in the standard assay conditions at pH 8.0 in the presence or absence of test ligands at the indicated concentrations in the final 1-ml assay volume. Inhibition of specific [2-^{14}C]IAA-binding is expressed as percentage of the effect of inclusion of 0.2 mM unlabeled IAA (100%).

Addition	Concentration	Inhibition
	M	% control
IAA	2×10^{-4}	100
Phenylacetic acid	2×10^{-5}	22
	2×10^{-4}	78
4-Phenylbutyric acid	2×10^{-4}	86
p-Hydroxyphenylacetic acid	10^{-5}	24
	10^{-4}	70
m-Hydroxyphenylacetic acid	10^{-5}	6
	10^{-4}	39
o-Hydroxyphenylacetic acid	10^{-5}	2
	10^{-4}	37
3-Methoxy-4-hydroxyphenylacetic acid	10^{-5}	10
	10^{-4}	40
3,4-Dihydroxyphenylacetic acid	10^{-5}	1
	10^{-4}	38
m-Chlorophenylacetic acid	2×10^{-5}	61
	2×10^{-4}	85
p-Chlorophenylacetic acid	2×10^{-5}	57
	2×10^{-4}	85
o-Chlorophenylacetic acid	2×10^{-5}	52
	2×10^{-4}	83
o-Nitrophenylacetic acid	2×10^{-5}	20
	2×10^{-4}	67
m-Nitrophenylacetic acid	2×10^{-5}	17
	2×10^{-4}	74
p-Nitrophenylacetic acid	2×10^{-4}	4

IV. *Titration of the IAA-binding Site of Bean ABP with Phenoxyacids*

The concentration of test ligand for 50% inhibition of specific [2-^{14}C]IAA-binding in the standard assay (C_{50}) was estimated from plots of specific IAA-binding *versus* test ligand concentration (*cf.* Fig. 1).

Ligand	C_{50}
	M
p-Chlorophenoxyacetic acid	8×10^{-7}
α-(*p*-Chlorophenoxy)isobutyric acid	4×10^{-6}
3,4-Dichlorophenoxyacetic acid	2×10^{-6}
2,3-Dichlorophenoxyacetic acid	1×10^{-5}
2,4-Dichlorophenoxyacetic acid	3×10^{-5}
α(+)2,4,5-Trichlorophenoxypropionic acid	5×10^{-6}
α(−)2,4,5-Trichlorophenoxypropionic acid	8×10^{-7}
2,4,5-Trichlorophenoxyacetic acid	8×10^{-6}

cetic Acid Derivatives. ABP binds all the auxin or antiauxin phenoxyacids examined (Table IV). The auxin *p*-chlorophenoxyacetic acid (2) binds more tightly than the antiauxin α-(*p*-chlorophenoxy)isobutyric acid (8), which nevertheless binds tightly to ABP (Table IV). Both α(+)-2,4,5-trichlorophenoxypropionic acid and α(−)-2,4,5-trichlorophenoxypropionic acid have been described as antiauxins by Åberg (5) with the (−)-isomer being a more active antiauxin than the (+)-isomer. The (−)-isomer binds more tightly to ABP than the (+)-isomer (Table IV) and is one of the best ABP ligands found to date. One would expect antiauxins (antagonists) to bind to the receptor site for auxins (agonists) and the binding of these antiauxins to ABP provides further evidence for the receptor-like ligand specificity of this protein. While the 3,4- and 2,4-isomers can be stronger auxins than 2,3-dichlorophenoxyacetic acid (4), the affinity of 2,4-D for ABP is lower than the affinity of the 2,3-isomer (and of the 3,4-isomer) and of the auxin 2,4,5-T (28) for ABP (Table IV). The affinity of 2,4-D for the corn ER auxin-binding site is an order of magnitude lower than the affinity of this site for IAA, indole-3-propionic acid, and indole-3-*n*-butyric acid (27) and 2,4-D also has a relatively low affinity for the solubilized corn membrane auxin-binding protein (11). The relatively low affinity of ABP for 2,4-D is discussed later in this paper in relation to a possible physiological auxin sequestering function for ABP.

Table V. *Effects of Naphthalene Acetic Acid Analogues on IAA Binding to Bean ABP*

Inhibition of specific IAA-binding was determined in the standard assay conditions as described in Table III.

Addition	Concentration	Inhibition
	M	% control
IAA	2×10^{-4}	100
α-Naphthalene acetic acid	10^{-4}	97
β-Naphthalene acetic acid	10^{-4}	81
β-Naphthoxyacetic acid	10^{-4}	92
α-Naphthylphosphate	2×10^{-5}	−10
	2×10^{-4}	4
α-Naphthylpropionate	2×10^{-5}	−5
	2×10^{-4}	5
β-Naphthylpropionate	2×10^{-5}	−5
	2×10^{-4}	3
β-Naphthylsulfate	2×10^{-5}	78
	2×10^{-4}	99
α-Naphthalene acetamide	10^{-5}	−2
	10^{-4}	10
α-Naphthylphthalamic acid	2×10^{-4}	99
β-Naphthylphthalamic acid	2×10^{-4}	110
N-Maleyl-β-naphthylamine	2×10^{-4}	94
β-Naphthylcarboxylate	10^{-5}	1
	10^{-4}	6

Inhibition of IAA Binding by NAA Analogues. The highly active synthetic auxins α-NAA and β-NAA (28) bind more tightly to ABP than the less active auxin β-NAA (15) (Table II). The nonacidic derivatives α-naphthylpropionate, β-naphthylpropionate, and α-naphthalene acetamide do not displace IAA from ABP at 2×10^{-4} M and an acidic α- or β-substituent is the common feature of all the effective naphthalene-derived ABP ligands found (Table V). However β-naphthylcarboxylate and α-naphthylphosphate do not inhibit IAA-binding, whereas α-naphthylsulphate ($C_{50} < 2 \times 10^{-5}$ M) is a potent inhibitor (Table V). While ABP has a high affinity and remarkable specificity for auxins related structurally to IAA, NAA, phenylacetic acid, and phenoxyacetic acid, this protein also binds the auxin transport inhibitor α-NPA (28) and β-naphthylphthalamic acid (Table V). The powerful antiauxin 2-naphthylmethylselenideacetic acid (1) also binds tightly to ABP (Table II).

Inhibition of IAA Binding by Halogen-substituted Benzoic Acid Derivatives. While benzoic acid is ineffective, a range of mono- and dihalogenated benzoic acid derivatives at 10^{-4} M cause substantial displacement of IAA from ABP in the standard assay (Table VI). Benzoic acid is not an auxin and appropriate mono- or dihalogenation of benzoic acid confers auxin activity (28). However the relative affinities of these compounds for ABP do not correlate with their relative biological activities. Thus while the 3-chloro- and 3-iodo-substituted benzoic acids are more effective ligans than the *ortho*-isomers, only the *ortho*-isomers are effective as auxins (28). A similar result obtains with cyclohexylacetic acid which is not an auxin (28) but which inhibits specific IAA-binding to ABP by 59 and 95% at 2×10^{-5} M and 2×10^{-4}

Table VI. *Effects of Halogenated Benzoic Acid Derivatives on IAA-Binding to Bean ABP*

Inhibition of specific IAA-binding by halogenated benzoic acid derivatives was determined in the standard assay as described in Table III.

Addition	Concentration	Inhibition
	M	% control
IAA	2×10^{-4}	100
Benzoic acid	2×10^{-4}	10
2-Chlorobenzoic acid	2×10^{-5}	18
	2×10^{-4}	49
3-Chlorobenzoic acid	2×10^{-5}	36
	2×10^{-4}	79
4-Chlorobenzoic acid	2×10^{-5}	14
	2×10^{-4}	29
2-Iodobenzoic acid	2×10^{-5}	32
	2×10^{-4}	66
3-Iodobenzoic acid	2×10^{-5}	54
	2×10^{-4}	88
2,4-Dichlorobenzoic acid	2×10^{-5}	18
	2×10^{-4}	54
2,5-Dichlorobenzoic acid	2×10^{-5}	36
	2×10^{-4}	79
2,6-Dichlorobenzoic acid	2×10^{-5}	18
	2×10^{-4}	51
3,4-Dichlorobenzoic acid	2×10^{-5}	28
	2×10^{-4}	75
3,5-Dichlorobenzoic acid	2×10^{-5}	23
	2×10^{-4}	70

Table VII. *Titration of the IAA-binding Site of Bean ABP with Auxin Transport Inhibitors*

The concentration of test ligand for 50% inhibition of specific [2-^{14}C]IAA-binding (C_{50}) was estimated from plots of specific IAA-binding *versus* test ligand concentration (*cf.* Fig. 1).

Ligand	C_{50}
	M
2-(1-Pyrenoyl) benzoic acid	1×10^{-6}
5-(2-Carboxyphenyl)-3-phenylpyrazole	1×10^{-6}
1-(2-Carboxyphenyl)-3-phenylpropane-1,3-dione	3×10^{-6}
2,3,5-Triiodobenzoic acid	5×10^{-6}
2,3,6-Trichlorobenzoic acid	2×10^{-5}
α-Naphthylphthalamic acid	5×10^{-5}
3-Amino-2,5-dichlorobenzoic acid	1×10^{-4}

M, respectively. These results do not necessarily argue against a physiological function for ABP in view of possible antiauxin (antagonist) functions for these compounds (*cf.* Table IV) and the possibility that poor auxin activity may derive both from high affinity for a high incidence auxin-sequestering protein and low affinity for the auxin receptor (cf. 24). Benzoic acid binds tightly to auxin-binding "site I", but not "site II", distinguished in corn coleoptile membranes by Batt *et al.* (6; see also 14). Benzoic acid is also a poor ligand for ABP (Table VI) and for the solubilized (11) and ER-located high affinity auxin-binding site of corn coleoptile membranes (26, 27). TIBA binds more strongly to ABP (Table VII) than mono- or dichlorobenzoic acids (Table VI), as has been found for the ER-located site by Ray *et al.* (27).

Inhibition of IAA Binding by Auxin Transport Inhibitors. A variety of structurally disparate compounds shown to inhibit auxin transport bind relatively tightly to the auxin-binding site of ABP (Tables VII and VIII). In addition to the classic inhibitors of auxin transport TIBA and α-NPA (28), other ABP ligands in this category include a variety of polycyclic aromatic carboxylic acids described by Katekar and Geissler (19–21) as potent inhibitors of auxin transport and the morphactin methyl-2-chloro-9-hydroxy-fluorene-9-carboxylate (chlorflurenol) (29). Chlorflurenol has a relatively low affinity for ABP ($C_{50} > 2 \times 10^{-4}$ M) (Table VIII) compared with those of TIBA, α-NPA, and the polycyclic aromatic carboxylic acids such as 2-(1-pyrenoyl) benzoic acid (Table VII). While TIBA and α-NPA have comparable activities as inhibitors of IAA transport in bean petioles (20), the affinity of ABP for TIBA is an order of magnitude greater than that for α-NPA (Table VII). It has been proposed that α-NPA and the polycyclic aromatic carboxylic acids may have a common binding site in target cells that is distinct from morphactin- and TIBA-binding sites (21). The binding of these auxin transport inhibitors to ABP suggests a possible involvement of this protein in auxin transport *in vivo*.

Table VIII. *Effects of Auxin Transport Inhibitors on IAA Binding to Bean ABP*

Inhibition of specific IAA-binding by 0.2 mM test ligand is expressed as per cent of the effect of inclusion of 0.2 mM unlabeled IAA (100%).

Ligand	Inhibition
	% control
IAA	100
Methyl-2-chloro-9-hydroxy-fluorene-9-carboxylate (chlorflurenol)	43
2-Phenylbenzoic acid	96
2-(4-Chlorophenyl)benzoic acid	102
Eosin	80
Erythrosin A	89
Rose bengal	91

Table IX. *Effects of Non-indole Tryptophan Metabolic Derivatives, Imidazole Derivatives, and Benzisothiazoles on IAA Binding to ABP*

All ligands tested were present at 0.2 mM in the final 1-ml standard IAA-binding assay volume. Inhibition of specific [2-^{14}C]IAA-binding is expressed as per cent of the effect of inclusion of 0.2 mM unlabeled IAA (100%).

	Addition	Inhibition
		% control
(A)	*N*-Phenylanthranilic acid	100
	3-Hydroxyanthranilic acid	45
	3-Hydroxykynurenine	29
	Anthranilic acid	22
	Xanthurenic acid	19
	Kynureninic acid	17
	Fusaric acid	17
	N-Formyl-L-kynurenine	12
	Picolinic acid	3
	Quinolinic acid	−3
	Isonicotinic acid	2
	L-Kynurenine sulfate	2
(B)	Imidazole-4-acetic acid	19
	L-β-Imidazolelactic acid	1
	Urocanic acid	5
	2-Imidazolidone	1
	2-Imidazolidonethione	1
(C)	(2,1-Benzisothiazol-3-yl)-carboxylic acid	100
	(1,2-Benzisothiazol-3-yl)-carboxylic acid	90
	(2,1-Benzisothiazol-3-yl)-acetic acid	75
	7-Nitro-2,1-benzisothiazole	36
	(2,1-Benzisothiazol-3-yl)-carboxylic acid methyl ester	2
	5-Methoxy-2,1-benzisothiazole	−6

Effects of Nonindole Heterocyclics and Plant Growth Inhibitors on IAA Binding to Bean ABP. Despite the specificity of ABP for

compounds known to interact with the auxin receptive/auxin translocation systems the possibility exists that the IAA-binding site may be involved in indole compound metabolism *in vivo*. A range of nonindole tryptophan metabolic derivatives was, therefore, examined for effects on IAA-binding to ABP (Table IX, series A). 3-Hydroxyanthranilic acid has an inhibitory effectiveness comparable to that of indole-3-propionic acid (*cf.* Table I). *N*-Phenylanthranilic acid at 0.2 mM completely abolishes IAA binding to ABP, whereas anthranilic acid is weakly inhibitory at this concentration (Table IX, series A).

The specificity of ABP for IAA, NAA, and phenylactic acid analogs was further examined using imidazole derivatives. Imidazole-4-acetic acid and urocanic acid [β-4(5)-imidazolylacrylic acid] at 0.2 mM are ineffective (Table IX, series B). Imidazole at 0.1 mM is ineffective.

(1,2-Benzisothiazol-3-yl)-acetic acid is a strong auxin (7) and a variety of benzisoxazole and benzisothiazole derivatives are also active auxins (13, 17). (2,1-Benzisothiazo-3-yl)-carboxylic acid (C_{50}: 8×10^{-7} M) is a strong inhibitor of IAA-binding, whereas the corresponding methyl ester is ineffective. (1,2-Benzisothiazole-3-yl)-carboxylic acid (C_{50}: 4×10^{-6} M) and (2,1-benzisothiazol-3-yl)-acetic acid (C_{50}: 2×10^{-5} M) are also inhibitory whereas the nonacidic derivatives 5-methoxy-2,1-benzisothiazole and 7-nitro-2,1-benzisothiazole are noninhibitory and weakly inhibitory, respectively (Table IX, series C).

We have previously shown that a high affinity soluble cytokinin-binding protein from wheat embryo (CBP) (25) binds a variety of indole compounds (24). ABP shows a marked specificity for certain acidic indole derivatives and has a low affinity for neutral indole compounds (Table I). In contrast, the best indole-derived ligands for CBP are nonacidic derivatives such as L-tryptophan ethyl ester and the L- and D-tryptophan methyl esters (24; *cf.* Table I). The interaction of ABP with purine derivatives was therefore examined. While purine, 6-carboxypurine, and 6-(carboxymethyl) mercaptopurine are ineffective in displacing IAA from ABP, 6-(4-carboxybutyl) mercaptopurine at 0.2 mM causes significant displacement of IAA (Table X, series A). The 6-substituted part of this purine derivative is structurally similar to S-(carboxymethyl)-dimethyldithiocarbamate and related auxins (16, 34). Various N^6-substituted adenine derivatives that are active as cytokinins, notably zeatin and 6-(γ,γ-dimethylamino)purine and the corresponding ribosides, displace IAA from ABP, albeit only when present at the relatively high concentration of 0.1 mM (Table X, series B). Kinetin, BA, and N^6-dimethyladenine were ineffective at 0.1 mM in the assay. A variety of adenine nucleotides tested at 0.1 mM, including adenine, 3'-AMP, 2'-AMP, 5'-AMP, 3',5'-cyclic AMP, 2',3'-cyclic AMP, N^6-monobutyryl 3',5'-cyclic AMP, N^6, 2-O'-dibutyryl 3',5'-cyclic AMP, 5'-ADP, and 5'-ATP are ineffective in displacing IAA from ABP. A variety of other purine and pyrimidine derivatives at 0.1 mM are also ineffective in displacing IAA from ABP, including xanthosine, guanosine, 6-mercaptoguanosine, 8-mercaptoguanosine, 5'-GMP, 5'-GDP, 5'-GTP, 2',3'-cyclic GMP, thymidine, cytidine, uridine, inosine, 2'-deoxyguanosine, 2'-deoxyuridine, and 2'-deoxyadenosine. None of the 20 common L-amino acids cause significant inhibition when included at 0.1 mM concentration in the standard assay.

A range of acidic plant growth inhibitors was examined in the standard assay (Table X, series C). Chlorogenic acid, *p*-coumaric acid and quinaldic acid—the more effective inhibitors—inhibit specific IAA binding by only about 40% at the relatively high concentration of 0.2 mM (Table X, series C). These compounds are two orders of magnitude less effective than the most effective inhibitors of IAA binding to ABP found in this study.

Inhibition of IAA Binding to Bean ABP by Thiol Reagents and Azide. Since auxin binding sites associated with *Zea mays* membranes are sensitive to thiol-reactive compounds such as Hg^{2+} (6) and *p*-chloromercuribenzoate (11) and since RuBPCase is also inhibited by such compounds (30), the effects of thiol reagents on IAA binding to ABP were examined. A range of thiol reagents abolishes IAA-binding (Table XI). The concentrations for 50% inhibition of IAA binding by *p*-chloromercuribenzoate and 2,2'-dipyridyldisulphide are 4×10^{-5} M and 3×10^{-5} M, respectively. The structural disparity of the thiol reagents effective in inhibiting IAA-binding to ABP (Table IX) (only *p*-chloromercuribenzoate is structurally similar to the major classes of effective ABP ligands) indicates that these thiol reagents are not acting as auxin analogues and that unmodified thiols are required for IAA binding to ABP. Evidence for a similar requirement has been obtained by Cross and Briggs (11) with respect to the solubilized corn membrane auxin-binding protein. Because of the evidence for the identity of ABP and RuBPCase, various RuBPCase ligands were tested for their effects on IAA-binding. However, RuBP (0.4 mM) and 3-P-glycerate (0.4 mM) are ineffective. Although KCN (a RuBP-dependent inhibitor of RuBPCase [30]) is ineffective, NaN_3 inhibits specific IAA binding by about 40% at 20 mM in the standard assay. NaN_3 (but not CN^-) inhibits NAA binding to solubilized auxin-binding protein from corn coleoptiles (10). This further strengthens the possibility that the "site 1" membrane-bound auxin-binding sites described previously (11) may be structurally similar (but not necessarily identical) to the ABP auxin-binding site. In this connection we note that RuBPCase (ABP) is a major

Table X. *Effects of Purine Derivatives, Cytokinins, and Plant Growth Inhibitors on IAA Binding to Bean ABP*

Specific IAA-binding was determined in duplicate in the standard assay at pH 8.0 in the presence or absence of test ligands at final concentrations of 0.2 mM (series A and C) or 0.1 mM (series B). Inhibition of specific [2-^{14}C]IAA-binding is expressed as percent of the effect of inclusion of 0.2 mM unlabeled IAA (100%).

Addition	Inhibition
	% control
(A) Purine	−2
6-Carboxypurine	7
6-(Carboxymethyl)mercaptopurine	5
6-(4-carboxybutyl)mercaptopurine	58
(B) 6(γ,γ-Dimethylallylamino)purine	40
6(γ,γ-Dimethylallylamino)purine-9-riboside	45
Zeatin (*trans* isomer)	42
Zeatin (mixed *cis*, *trans* isomers)	34
Zeatin-9-riboside	25
(C) *p*-Coumaric acid	44
Quinaldic acid	39
Chlorogenic acid	37
trans-Cinnamic acid	27
cis, *trans*-Abscisic acid	17
Caffeic acid	11

Table XI. *Inhibition of IAA Binding to Bean ABP by Thiol Reagents*

Inhibition of specific [2-^{14}C]IAA-binding by thiol reagents was determined in the standard assay as decribed in Table III.

Addition	Concentration	Inhibition
	mM	*% control*
N-Ethylmaleimide	0.2	44
	1.0	83
Cadmium acetate	0.2	64
	1.0	86
$HgCl_2$	0.2	95
	1.0	96
$Pb(NO_3)_2$	0.2	67
	1.0	86
p-Chloromercuribenzoate	0.2	99
2,2'-Dipyridyldisulfide	0.2	75

peripheral protein of thylakoid membranes (18) and therefore a possibility exists that ABP or an auxin-binding ABP subunit may bind as a peripheral protein to other plant cell membranes. However we have been unable to demonstrate IAA-binding to thylakoids nor to RuBPCase from maize leaves (32). Further, the large difference in pH optima for auxin binding to ABP (32) and to the maize ER-located sites (26) suggests nonidentity of these auxin-binding species.

Interaction of Auxins with Pea ABP. We have examined the binding to pea ABP of representative compounds shown above to bind tightly to bean ABP. Pea ABP has a specificity for the ligands tested (Table XII) similar to that found for bean ABP. Thus, in terms of relative affinities for pea ABP IAA ≫ indole-3-propionic acid and indole-3-butyric acid (*cf.* Table II); α-(−)-2,4,5-trichlorophenoxypropionic acid > α-(+)-2,4,5-trichlorophenoxypropionic acid (*cf.* Table IV); 2,4,5-T > 2,4-D (*cf.* Table IV); α-NAA, β-naphthoxyacetic acid > β-NAA (*cf.* Table II); phenylacetic acid > phenylbutyric acid (*cf.* Table II). TIBA binds to both pea ABP (Table XII) and to bean ABP (Table VII).

Physiological Significance of Auxin-ABP Interactions. Bean ABP has a high affinity for all naturally occurring and synthetic auxins tested and also binds a variety of antiauxins and auxin-transport inhibitors. Pea ABP has a specificity similar to that of bean ABP for the ligands examined (Table XII). As discussed above, within the different classes of compounds examined the relative affinities for ABP are consistent with the hypothesis that ABP (or a protein with a similar ligand specificity) can be a physiologically significant target in experiments in which these compounds are applied. However, a hormone receptor function for ABP would appear to be unlikely because the concentration of ABP (RuBPCase) in bean leaves is about 100 times greater than the K_d for IAA and the concentration of "nonbound" IAA (33) in the tissue (32). We have established a similar disparity between the tissue concentration of a soluble high affinity cytokinin-binding protein (CBP) from wheat germ and its K_d values for cytokinins (24, 25). Auxin sequestration and/or auxin translocation functions for ABP are clearly more plausible than a receptor function and are supported by the auxin-binding specificity of ABP and the interaction of a variety of auxin transport inhibitors with ABP. Total IAA (including a minor component of readily extracted IAA and a major component of "bound," but not necessarily covalently bound, IAA) in 12-day-old dwarf bean leaves (33) is similar on a molar basis to total ABP (RuBPCase) (32), consistent with an auxin-sequestering function for this protein. We have proposed that the low affinity of CBP for zeatin is consistent with a cytokinin buffering or sequestering function for this protein (24). A similar argument can be applied to the relatively low affinity of 2,4-D for ABP (Table IV), namely that the high biological effectiveness of this synthetic auxin could be due to its low affinity for a high incidence auxin-sequestering protein (ABP) in addition to its requisite high affinity for the actual auxin receptor.

Table XII. *Titration of the IAA-binding Site of Pea ABP with Auxins*

C_{50} values in the standard assay were determined as described in Table II.

Ligand	C_{50}
	M
IAA	3×10^{-6}
Indole-3-butyric acid	2×10^{-4}
Indole-3-propionic acid	4×10^{-4}
α(−)-2,4,5-Trichlorophenoxypropionic acid	3×10^{-6}
α(+)-2,4,5-Trichlorophenoxypropionic acid	3×10^{-5}
α-Naphthaleneacetic acid	5×10^{-6}
β-Naphthaleneacetic acid	3×10^{-5}
β-Naphthoxyacetic acid	4×10^{-6}
Phenylacetic acid	3×10^{-5}
4-Phenylbutyric acid	2×10^{-4}
2,3,5-Triiodobenzoic acid	2×10^{-5}

While a receptor function for ABP appears implausible we have nevertheless sought to compare the ligand specificity of bean leaf ABP with the specificity of a bean leaf auxin bioassay. Auxin-induced bean leaf hyponasty is maximal at relatively high applied IAA levels (about 0.1 μmol/leaf) (23) that exceed leaf ABP (RuBPCase) levels (32) *i.e.* the high incidence of ABP does not disqualify this protein from possible mediation of this particular auxin response. The ligand specificity of bean ABP and the specificity of the auxin-induced bean leaf blade hyponastic response determined by Lippincott and Lippincott (23) are in qualitative agreement with respect to some 40 pertinent compounds tested. The only compounds found that induce the hyponastic response but which bind poorly to ABP are β-(indol-3-yl)ethanol(tryptophol) and indole-3-acetaldehyde, compounds for which one can envisage ready conversion to IAA *in vivo*. Conversely, 3,4-dihydroxyphenylacetic acid, 2,3-dichlorophenoxyacetic acid, and indoxylsulphate are the only compounds we have found which significantly displace IAA from ABP (at 0.1 mM) but which fail to induce hyponasty in bean leaves at 1 mM (23). However, if ABP (RuBPCase) mediates the hyponastic response, the inducing compound must cross as many as three membranes for passage to the chloroplast stroma and initiation of the response. 3,4-Dichlorophenoxyacetic acid, while inactive in inducing hyponasty (23), is an active auxin (4), in addition to binding to ABP (Table IV). Bean leaf auxin-induced hyponasty is inhibited by the antiauxin α(*p*-chlorophenoxy)isobutyric acid (8) which, like other antiauxins tested, has a high affinity for ABP (Table IV). Hyponasty is induced by TIBA (23) and bean leaf ABP has a high affinity for this and other auxin transport inhibitors (Tables VII and VIII).

Our *in vitro* ligand specificity studies provide evidence largely consistent with an involvement of bean leaf ABP in auxin-induced bean leaf hyponasty and hence with auxin binding to ABP (RuBPCase) *in vivo*. Since auxin-induced bean leaf hyponasty is light-modulated (23), as is the activity of RuBPCase (12, 22), we are currently seeking evidence for *in vivo* light modulation of auxin binding to ABP (RuBPCase).

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