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Ligand Specificity of a High Affinity Cytokinin-binding Protein[1]

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ABSTRACT

A soluble cytokinin-binding protein from wheat germ that has a high affinity for a range of purine cytokinins also interacts with a variety of nonpurine compounds that can affect cytokinin-modified processes in animal or plant cells or which bind to proteins known to interact with certain cytokinins. A variety of structurally disparate compounds which inhibit chloroplast photosystem II activity (including phenylurea, carbanilate, and alkylamino-2-chloro-*sym*-triazine compounds) displace kinetin from the protein in an apparently competitive fashion. However, various energy transfer inhibitors (including organotin compounds and *N,N'*-dicyclohexylcarbodiimide) also inhibit kinetin binding to the protein. N^6,2-O'-Dibutyryl-3',5'-cyclic AMP and 1-methyl-3-isobutylxanthine (the effects of which on fibroblast morphology and motility can be mimicked by cytokinins) are inhibitors of kinetin binding to the protein. A variety of compounds that can have antimitotic effects (including 1-methyl-3-isobutylxanthine and certain alkylated cyclic nucleotide, carbanilate, and tryptamine compounds) displace kinetin from the protein. However, a variety of indole derivatives also displace kinetin from the cytokinin-binding protein, which in a qualitative sense has a broad ligand specificity.

High affinity cytokinin-binding proteins are present in the ribosomal and postribosomal supernatant fractions from centrifugal fractionation of wheat germ homogenates (9, 10). Fox and Erion (10) have resolved an approximately 100,000 dalton high affinity cytokinin-binding protein (CBF-1) from the ribosomal fraction from wheat germ. They have also resolved a very similar protein (probably identical to CBF-1), an approximately 30,000 dalton high affinity cytokinin-binding protein (CBF-2) and a high mol wt low affinity cytokinin-binding protein (CBF-3) from the postribosomal supernatant fraction from wheat germ (10). Polya and Davis (25) have partially purified an approximately 180,000 dalton cytokinin-binding protein CBP[2] from wheat germ that, like CBF-1 (10), has a high affinity for a range of purine cytokinins but not for adenine derivatives lacking cytokinin activity. The CBP and CBF-1 preparations may contain identical cytokinin-binding proteins. A 5,000 dalton soluble cytokinin-binding protein has been isolated from tobacco leaves by Yoshida and Takegami (29), but this protein has a low affinity for cytokinins-the K_d for N^6-benzyladenine is 40 μM (29). In contrast Sussmann and Kende (26) have delineated a cytokinin-binding site associated with a particulate fraction from tobacco cells that has a K_d for N^6-benzyladenine of about 0.1 μM. Thus, the high affinity wheat germ binding proteins (10, 25) and the particulate tobacco site (26) are the only known plant cytokinin-binding species with affinities for cytokinins appropriate to cytokinin receptor functions.

While the tobacco particulate site is present at a concentration (about 10^{-8}mol/kg) consistent with a receptor function (26), CBF-1 and CBP are present in wheat germ at concentrations ($>10^{-6}$mol/kg) that argue against receptor functions for these proteins analogous to the amplifying functions of animal steroid hormone receptors or adenylate cyclase-modifying hormone receptors (25). Further, while *cis*- and *trans*-zeatin bind tightly to the tobacco particulate site (26), the wheat germ cytokinin-binding protein has an affinity for zeatin that is anomalously low in relation to a possible receptor function (25). As one approach to the problem of determining the function of CBP we have examined the specificity of this protein for nonpurine compounds known to have cytokinin-like effects or which can bind to sites known to bind compounds with cytokinin activity. The present paper also describes the interactions of CBP with DBcAMP and MIX, the effects of which on cultured animal cells can be mimicked by cytokinins (15).

MATERIALS AND METHODS

$(NH_4)_2SO_4$ Precipitation Cytokinin-binding Assays. The soluble wheat germ CBP was isolated as described by Polya and Davis (25). The binding of kinetin to CBP was routinely determined in triplicate by the radiochemical procedure described previously (25) in which an insolubilized CBP-kinetin complex is pelleted centrifugally at 0 C through an equilibrium medium (1 ml total volume) containing 90% saturated $(NH_4)_2SO_4$. Kinetin binding was routinely determined at pH 5.7 or at pH 8.0. When unbuffered 100% saturated $(NH_4)_2SO_4$ was added to the assay as described previously (25) the final equilibrium pH was 5.7. A final assay pH of 8.0 was achieved by addition of 100% saturated $(NH_4)_2SO_4$-50 mM Tris-HCl (pH 8.0) in the standard assay. The standard assay always contained 0.1 nmol kinetin ([8-^{14}C]kinetin: 15 mCi/mmol) and assay counts were routinely corrected by subtraction of counts obtained in the same assay conducted in the presence of 10 nmol unlabeled kinetin to obtain a measure of specific kinetin binding. Specific kinetin binding was routinely about 80% of total kinetin binding and standard deviations in the binding assays were about 2.5% of the means.

Equilibrium Dialysis. Equilibrium dialysis was performed using 10-cm-long sacs of size 8/32 dialysis tubing (Visking Co., Chicago) enclosing 1 ml of a solution containing 0.5 M $(NH_4)_2SO_4$, 50 mM Tris-HCl (pH 8.0), 0.1% (v/v) 2-mercaptoethanol, and 3.6 mg CBP. Dialysis sacs were suspended in 40 ml of a solution containing 0.5 M $(NH_4)_2SO_4$, 50 mM Tris-HCl (pH 8.0), 0.1% (v/v) 2-mercaptoethanol, 0.1% (v/v) dimethylsulfoxide, 5×10^{-7} M kinetin ([8-^{14}C] kinetin: 15 mCi/mmol), and the test ligand. The equilibrium dialyses were performed in triplicate (controls, *i.e.* with no

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[2] Abbreviations: atrazine: 2-chloro-4-(isopropylamino)-6-ethylamino-*s*-triazine; CBCC: 4-chloro-2-butynyl-*N*-(3-chlorophenyl)carbamate; CBP: soluble wheat germ cytokinin-binding protein; CIPC: isopropyl-*N*-(3-chlorophenyl)carbamate; CMU: 3-(4-chlorophenyl)-1,1-dimethylurea; DBcAMP: N^6,2-O'-dibutyryl-3',5'-cyclic AMP; DCCD: *N,N'*-dicyclohexylcarbodiimide; EPC: ethyl *N*-phenylcarbamate; ipazine: 2-chloro-4-(isopropylamino)-6-diethylamino-*s*-triazine; IPC: isopropyl *N*-phenylcarbamate; MBcAMP: N^6-monobutyryl-3',5'-cyclic AMP; MIX: 1-methyl-3-isobutylxanthine; propazine: 2-chloro-4,6-bis(isopropylamino)-*s*-triazine; simazine: 2-chloro-4,6-bis(ethylamino)-*s*-triazine.

additions, with 9-fold replication) in 50-ml stoppered test tubes that were shaken gently at 4 C for 65 h before sampling and counting as described previously (25).

Chemicals. Indole-3-butyric acid and indole-3-propionic acid were obtained from Calbiochem; all other indole derivatives, purine cytokinins, CIPC, MIX, 2',3'-cyclic nucleotides, alkylated cyclic nucleotides, papaverine, theophylline, theobromine, melatonin, 5-methoxytryptamine, colcemid, colchicine, vinblastine sulfate, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, and griseofulvin were obtained from the Sigma Chemical Co. Atrazine, simazine, propazine, and ipazine were obtained from CSIRO Plant Industry, Canberra, Australia. DCMU, CMU (80%), IPC, and CBCC were obtained from ICN Pharmaceuticals; podophyllotoxin and *N,N'*-dicyclohexylurea from Aldrich Chemical Co.; DCCD and caffeine from Calbiochem; phenylurethane (EPC) from T. C. I., Japan; theophylline 7-acetic acid and 1,3,9-trimethylxanthine from Fluka AG, West Germany; trifluralin (96.6%) from Lilly Industries, Sydney, Australia; trimethyltin chloride and triphenyltin chloride from K & K Life Sciences Group, New York. [8-^{14}C]Kinetin (15 mCi/mol) was obtained from the Radiochemical Centre, Amersham, Bucks., U. K. All other chemicals were of analytical reagent grade.

RESULTS

Interaction of Methylxanthines and Alkylated Cyclic Nucleotides with CBP. Since the effects of cytokinins can mimic the effects of MIX and DBcAMP on fibroblast morphology, motility, and adhesion (15), and since N^6-alkylated 3',5'-cyclic AMP derivatives can have cytokinin activity in plants (6, 8, 19), the effects of MIX and cyclic nucleotides on kinetin binding to CBP were examined. At pH 5.7 and pH 8.0, 0.1 mM MIX causes substantial inhibition of specific kinetin binding to CBP; except for caffeine, other methylxanthines at 0.1 mM are relatively ineffective (Table I). Theophylline does not elicit the fibroblast morphology changes induced by MIX and cytokinins (15), and is ineffective in displacing kinetin from CBP (Table I). The titration of kinetin binding to CBP by MIX is shown in Figure 1; the concentrations for half-maximal inhibition of specific kinetin binding at pH 5.7 and pH 8.0 are 10 and 13 μM, respectively. Papaverine, like MIX an inhibitor of animal cyclic nucleotide phosphodiesterase (1) and of neoplastic cell division (5), also inhibits kinetin binding (Fig. 1); the concentration for half-maximal inhibition of specific kinetin binding by papaverine at pH 5.7 or pH 8.0 is about 2 μM (Table II). Figure 2 presents a double reciprocal plot analysis showing that MIX inhibits kinetin binding to CBP in an apparently competitive fashion at pH 8.0, the apparent K_d of CBP for MIX being 5×10^{-6} M (Table III).

Table I. *Effects of Methylxanthines and 2',3'-Cyclic Nucleotides on Kinetin Binding to CBP*

Specific kinetin binding was determined in triplicate in the standard assay conditions at pH 5.7 or pH 8.0 in the presence or absence of 0.1 mM methylxanthine or 0.1 mM 2',3'-cyclic nucleotide in the final 1-ml assay volume. Inhibition of specific [8-^{14}C]kinetin binding is expressed as per cent of the effect of inclusion of 10^{-5} M unlabeled kinetin (100%).

Addition	Inhibition (% Control)	
	pH 5.7	pH 8.0
Kinetin (10^{-5} M)	100	100
MIX	86	90
Caffeine	20	15
Theobromine	2	5
Theophylline	−6	1
Theophylline-7-acetic acid	−12	0
1,3,9-Trimethylxanthine	−16	1
2',3'-cyclic UMP	54	49
2',3'-cyclic GMP	9	6
2',3'-cyclic CMP	−10	0

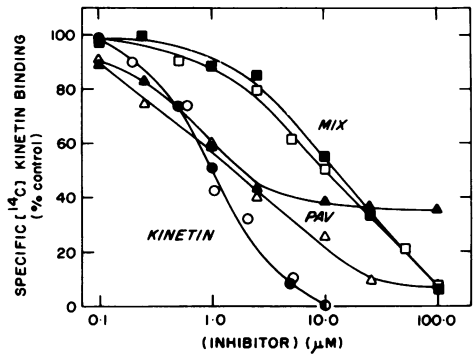

FIG. 1. Inhibition of specific [^{14}C]kinetin binding to CBP by kinetin, MIX, and papaverine. Assays were performed at pH 5.7 (open symbols) or at pH 8.0 (closed symbols); (○, ●): kinetin; (△, ▲): papaverine (PAV); (□, ■): MIX.

Table II. *Titration of the Kinetin-binding Site of CBP with Various Ligands*

Specific kinetin binding was determined in triplicate in the standard assays at pH 5.7 or pH 8.0 in the presence or absence of various concentrations of test ligands. The concentration of test ligand for 50% inhibition of specific kinetin binding (C_{50}) was estimated from plots of specific kinetin binding *versus* test ligand concentration (*cf.* Fig. 1).

Ligand	C_{50} (μM)	
	pH 5.7	pH 8.0
MIX	10	13
Papaverine	1.5	1.8
DCMU	0.7	0.9
CMU	4.6	2.0
EPC	9.2	19
CBCC	5.1	8.0
CIPC	1.1	1.8
IPC	2.3	
DCCD	21	7.5
Simazine	1.8	
Atrazine	0.5	3.4
Propazine	0.2	3.8
Ipazine	0.8	4.8
N-Acetyl-5-methoxytryptamine		50
L-Tryptophan ethyl ester		6.0

We have previously shown that 0.1 mM MBcAMP, 0.1 mM DBcAMP, and 0.1 mM 2',3'-cyclic AMP inhibit kinetin binding to CBP at pH 7.3 whereas 3',5'-cyclic AMP and other adenine nucleotides (5'-AMP, 3'-AMP, 2'-AMP, 5'-ADP, and 5'-ATP) are ineffective (25). 2',3'-Cyclic AMP displaces kinetin from CBP at pH 8.0 in an apparently competitive fashion (Fig. 3A), the K_d for 2',3'-cyclic AMP being 5×10^{-5} M (Table III). Of other 2',3'-cyclic nucleotides tested at pH 5.7 and pH 8.0, only 2',3'-cyclic UMP (at 0.1 mM) causes substantial displacement of kinetin from CBP (Table I). While 2',3'-cyclic AMP is an apparently competitive inhibitor (Fig. 3A), DBcAMP and MBcAMP do not appear to inhibit kinetin binding to CBP in a competitive fashion (Fig. 3B).

Interaction of PSII Inhibitors with CBP. DCMU and CMU are potent inhibitors of chloroplast PSII activity (11, 23) and these and other phenylurea compounds have been shown to have weak cytokinin activity (4). At pH 5.7 and pH 8.0, DCMU and CMU

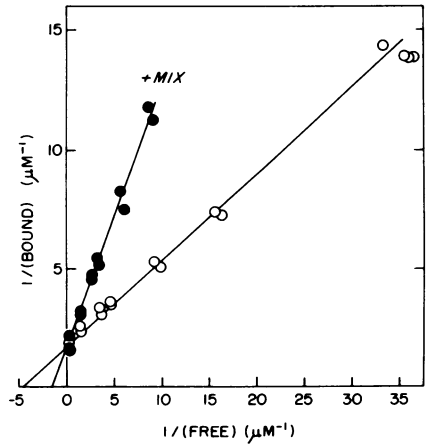

FIG. 2. Competitive inhibition of kinetin binding to CBP by MIX. Kinetin binding to CBP at a variety of kinetin concentrations was determined by the $(NH_4)_2SO_4$ precipitation procedure at pH 8.0 as described under "Materials and Methods." The concentration of bound kinetin was calculated after subtraction of background radioactivity observed in the assay conducted with 10^{-7} M kinetin ([8-^{14}C]kinetin: 15 mCi/mmol) in the absence of added CBP. The amount of [8-^{14}C]kinetin was the same in all assays and the total kinetin concentration was varied by addition of unlabeled kinetin. The reciprocal of the concentration of bound kinetin is plotted against the reciprocal of the concentration of free (*i.e.* nonbound) kinetin. All molarities refer to concentrations after addition of saturated $(NH_4)_2SO_4$ solution. (○): No inhibitor included; (●): plus 10^{-5} M MIX.

Table III. *K_d Values of CBP for Competitive Inhibitors of Kinetin Binding*

K_d values of CBP at pH 8.0 were calculated from competitive binding data of the kind shown in Figures 2 and 4 using the relation $I = I'(1 + i/K_d)$ where I and I' are intercepts on the 1/(free kinetin) axis in the absence and presence of competitor, respectively, i is the concentration of competitor, and K_d is the dissociation constant of CBP for the competitive ligand. Note that the standard deviation associated with the K_d for kinetin ($-1/I$) is 27% of the mean value (five determinations).

Ligand	K_d (M)
Kinetin	$3.0(\pm 0.8) \times 10^{-7}$
DCMU	4×10^{-7}
CMU	3×10^{-6}
Propazine	7×10^{-7}
Ipazine	7×10^{-7}
Atrazine	9×10^{-7}
Simazine	1×10^{-5}
CIPC	6×10^{-6}
IPC	6×10^{-6}
CBCC	2×10^{-5}
MIX	5×10^{-6}
2',3'-Cyclic AMP	5×10^{-5}

inhibit specific kinetin binding by 50% at concentrations of about 10^{-6} M (Table II). Since DCMU and CMU are effective ligands of CBP and are also potent inhibitors of PSII activity (11, 23), the effects of other types of PSII inhibitors on kinetin binding to CBP were examined. Kinetin binding to CBP was titrated with increasing concentrations of PSII inhibitors in the standard assays at pH 5.7 and pH 8.0 (*cf.* Fig. 1). Table II summarizes the results from these titrations. Various carbanilate esters have been shown to inhibit PSII activity (23) and the carbanilate esters CIPC, IPC, EPC, and CBCC inhibit kinetin binding to CBP, CIPC and IPC being the most effective (Table II). The alkylamino-2-chloro-*sym*-triazine derivatives propazine, atrazine, simazine, and ipazine are potent inhibitors of PSII activity (11, 23) and also inhibit kinetin binding to CBP at low concentrations (Table II). Unsubstituted *sym*-triazine (0.1 mM) does not inhibit kinetin binding at pH 8.0.

The phenylurea, carbanilate ester and alkylamino-2-chloro-*sym*-triazine compounds examined displace kinetin from CBP in an apparently competitive fashion. Representative double reciprocal plots demonstrating this mode of interaction are shown in Figure 4. K_d values were calculated from such plots and the results are summarized in Table III. With the exception of simazine (K_d 1×10^{-5} M), the alkylamino-2-chloro-*sym*-triazine and phenylurea compounds bind to CBP more tightly than the carbanilate esters examined in this way (Table III).

As a check on the specificity of CBP for PSII inhibitors we examined the effects of the energy transfer inhibitor DCCD, that, although not inhibiting basal electron flow, can inhibit coupled electron transport in chloroplasts (22, 27). Although DCCD inhibits kinetin binding to CBP at pH 5.7 and pH 8.0 (Table II) the carbodiimide derivative 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, which does not inhibit photophosphorylation (27), is ineffective at pH 5.7 or pH 8.0 at concentrations up to 10^{-4} M. The hydrolysis product of DCCD, *N,N'*-dicyclohexylurea, at 0.1 mM causes only 11 and 37% inhibition of specific kinetin binding at pH 8.0 and pH 5.7, respectively. At 10^{-4} M the organotin energy transfer inhibitors trimethyltin chloride, triphenyltin chloride, and tributyltin acetate inhibit specific kinetin binding at pH 8.0 by 64, 81, and 99%, respectively. These compounds can variously affect chloroplast electron transport in a complex fashion deriving from inhibition of the energy-coupling mechanism as well as uncoupling in appropriate conditions (28). Thus, in a qualitative sense CBP has a broad specificity for a variety of structurally disparate

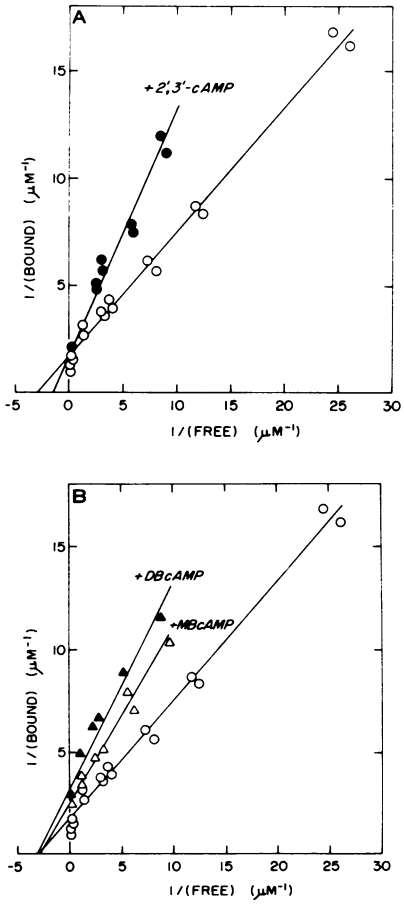

FIG. 3. Competitive and noncompetitive inhibition of kinetin binding to CBP by cyclic nucleotides. A: (○): no inhibitor included; (●): plus 5×10^{-5} M 2',3'-cyclic AMP. B: (○): no inhibitor included; (△): plus 5×10^{-5} M MBcAMP; (▲): plus 5×10^{-5} M DBcAMP; other experimental details are as in legend to Figure 2.

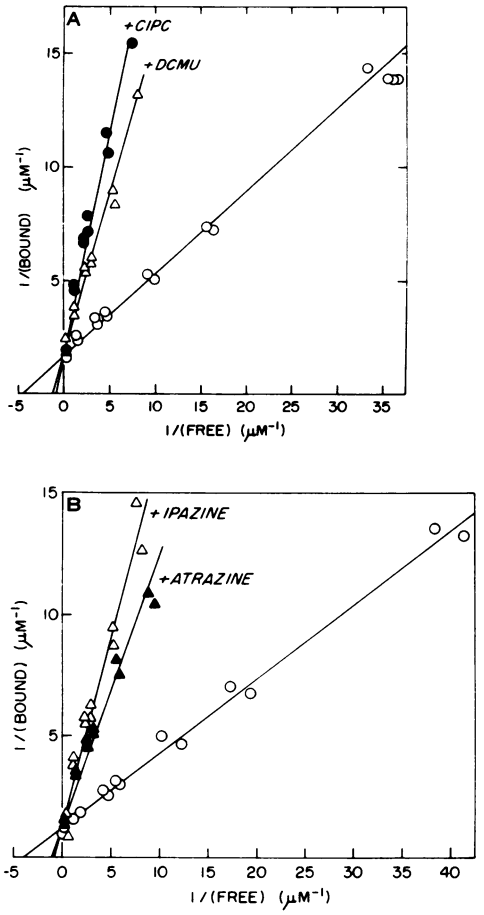

FIG. 4. Competitive inhibition of kinetin binding to CBP by PSII inhibitors. A: (○): no inhibitor included; (●): plus 2.5 × 10^{-5} M CIPC; (△): plus 10^{-6} M DCMU. B: (○): no inhibitor included; (△): plus 2.5 × 10^{-6} M ipazine; (▲): plus 2.5 × 10^{-6} M atrazine. Other experimental details are as in legend to Figure 2.

compounds that interact with different thylakoid components.

Interaction of Indole Derivatives with CBP. The carbanilate esters CIPC and IPC in addition to inhibiting PSII activity (23) are also effective antimitotic agents (13, 14, 16, 21) and alkylated cyclic nucleotides and MIX can inhibit neoplastic cell division (5, 24). A range of other antimitotic agents examined (including nocodazole, colcemid, colchicine, vinblastine, podophyllotoxin, griseofulvin, and trifluralin) do not inhibit kinetin binding when included in the standard assay at pH 5.7 at concentrations up to 0.1 mM. However *N*-acetyl-5-methoxytryptamine (melatonin) and 5-methoxytryptamine, compounds which can have microtubule-disorganizing effects in animal systems (3), displace kinetin from CBP (Table IV). As a check on the specificity of melatonin and 5-methoxytryptamine binding to CBP we examined the effects of a variety of indole derivatives on kinetin binding to the protein. A wide variety of indole derivatives at 10^{-4} M inhibit specific kinetin binding to CBP (Table IV). In general, indole derivatives tested that have a carboxylic acid function are much weaker inhibitors of specific kinetin binding than those without a carboxylic acid residue. For example, the methyl and ethyl esters of L-tryptophan at 10^{-4} M largely inhibit specific kinetin binding whereas L-tryptophan at 10^{-4} M has little inhibitory effect (Table IV). Similarly, indoxyl-β-D-glucopyranoside, which has a very hydrophilic 3-substituent, does not significantly inhibit kinetin binding (Table IV). IAA at 10^{-4} M has little effect on specific kinetin binding whereas indole-3-propionic acid and indole-3-butyric acid at 10^{-4} M cause significant displacement of kinetin from CBP (Table IV).

The kinetin-binding site of CBP was titrated at pH 8.0 with representative indole derivatives-the concentrations for 50% inhibition of specific kinetin binding by melatonin and L-tryptophan ethyl ester are 5 × 10^{-5} M and 6.0 × 10^{-6} M, respectively (Table II). While acidic indole derivatives are relatively ineffective inhibitors of kinetin binding, CBP will interact with acidic ligands. Thus, while IAA has little effect on specific kinetin binding (Table IV), the synthetic auxins 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D) at 10^{-4} M inhibit specific kinetin binding at pH 8.0 by 75 and 82%, respectively. This further demonstrates that in a qualitative sense CBP has a very broad ligand specificity.

Ligand Binding to Soluble CBP. The above analysis of the ligand specificity of CBP was conducted using an $(NH_4)_2SO_4$ precipitation assay in which the kinetin-insolublized CBP complex is pelleted through a solution containing the equilibrium concentration of free ligand, *i.e.* the equilibrium distribution of ligand being examined is that obtaining after addition of saturated $(NH_4)_2SO_4$ solution. The [^{14}C]kinetin bound to the insolubilized CBP is largely displaced by addition of excess unlabeled kinetin either before or after addition of saturated $(NH_4)_2SO_4$ solution and CBP is not inactivated by $(NH_4)_2SO_4$ precipitation (25). The binding of [8-^{14}C]kinetin to insolubilized CBP is reversible and the rapidity of this binding has also been previously described

Table IV. *Effects of Indole Derivatives on Kinetin Binding to CBP*

Specific kinetin 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 [8-^{14}C]kinetin binding is expressed as per cent of the effect of inclusion of 10^{-5} M unlabeled kinetin (100%).

Addition	Inhibition (% Control)
Kinetin (10^{-5} M)	100
Indoxyl-β-D-glucopyranoside	2
5-Hydroxy-D-tryptophan	3
Indole-3-acetic acid	6
L-Tryptophan	7
5-Methoxyindole-3-acetic acid	8
D-Tryptophan	13
5-Hydroxy-L-tryptophan	14
N-Acetyl-D-tryptophan	19
1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid	22
Indole-3-pyruvic acid	23
5-Hydroxyindole	30
Indole-3-butyric acid	35
Indole-3-propionic acid	37
L-Tryptophanamide	39
5-Methoxytryptamine	42
5-Methoxyindole-2-carboxylic acid ethyl ester	49
Indole-3-acetaldehyde	50
Indoxyl-1,3-diacetate	58
N-Acetyl-5-methoxytryptamine	61
Indole-3-ethanol	66
Indole-3-acetonitrile	74
6-Methylindole	75
Tryptamine	78
3-Acetoxyindole	79
Indole-3-carboxaldehyde	81
3-Acetylindole	81
5-Methoxyindole-3-carboxaldehyde	83
L-Tryptophan methyl ester	86
D-Tryptophan methyl ester	87
L-Tryptophan ethyl ester	96

(25). This type of $(NH_4)_2SO_4$ precipitation assay has been widely applied as a reliable procedure in other ligand-binding studies (*cf.* 7). Further, there is no *a priori* reason to suppose that this protein is present in a soluble rather than an insolubilized form *in vivo*. However, we have also examined the interaction of some representative ligands with CBP in a soluble state by equilibrium dialysis. The representative ligands that are active in the $(NH_4)_2SO_4$ precipitation assay all displace kinetin from soluble CBP in the conditions of the equilibrium dialysis experiments (Table V) but CBP clearly has a lower affinity for the ligands in these conditions than in the conditions of the $(NH_4)_2SO_4$ precipitation assay. This lowered affinity is observed also with kinetin—the K_d of CBP for kinetin at pH 8.0 is 3×10^{-7} M in the conditions of the $(NH_4)_2SO_4$ precipitation assay (Table III) but is 7.5×10^{-6} M in the equilibrium dialysis conditions at pH 8.0 (25).

Table V. *Inhibition of Kinetin Binding to Soluble CBP by Various Ligands*

Equilibrium dialyses were performed as described under "Materials and Methods." Test compounds were added at the concentrations indicated and per cent inhibition of [8-^{14}C]kinetin binding was calculated relative to control binding (no further additions).

Addition	Total Protein-bound Radioactivity (cpm ± SD) × 10^{-3}	% Inhibition
No addition	60.1 ± 2.5	
Adenine (20 μM)	61.8 ± 0.3	−3
N^6-Benzyladenine (20 μM)	16.9 ± 0.4	72
Kinetin (20 μM)	14.2 ± 0.6	76
N^6-Dimethylallyladenine (20 μM)	9.0 ± 0.6	85
MIX (100 μM)	49.7 ± 0.1	17
Propazine (100 μM)	42.2 ± 1.6	30
CMU (100 μM)	28.3 ± 1.1	53
CIPC (100 μM)	27.3 ± 1.3	55
DCMU (100 μM)	8.9 ± 0.6	85

DISCUSSION

Although it is difficult to rationalize the relative affinities of the CBP ligands demonstrated here in terms of chemical structural determinants, the set of compounds interacting with CBP overlaps sets of compounds known to interact with other cytokinin-binding sites. Thus, the phenylurea compounds DCMU and CMU are weak cytokinins (4) and potent inhibitors of PSII activity (11, 23). Carbanilate esters (23) and alkylamino-2-chloro-*sym*-triazines (11, 23) also inhibit PSII activity and, like the phenylurea compounds tested here, bind to CBP (Tables II and III). The specificity of this set of interactions with chloroplast electron transport inhibitors is arguable since structurally disparate energy transfer inhibitors (DCCD and organotin compounds) are also CBP ligands. Certain cytokinins have been shown to inhibit competitively mammalian 3′,5′-cyclic AMP phosphodiesterase (12), and MIX, papaverine, 2′,3′-cyclic AMP, and alkylated 3′,5′-cyclic AMP derivatives (all CBP ligands) also bind to this enzyme (1). The K_d of CBP for MIX (5×10^{-6} M) is similar to that of beef heart 3′,5′-cyclic AMP phosphodiesterase for this compound (2), MIX being one of the more effective inhibitors of the enzyme. CBP binds a variety of nonacidic indole compounds (Table IV), and mammalian cyclic nucleotide phosphodiesterase is also inhibited by various hydrophobic indole derivatives (1). However, as with CBP, the sheer structural diversity of mammalian cyclic nucleotide phosphodiesterase inhibitors is such that an explicit model for the structural requirements for such inhibitors has yet to be defined (1). A further cytokinin-binding system that has been resolved is a glycoprotein isolated from the water mold *Achlya* by Le John and Cameron (18). This protein has a much lower affinity for cytokinins than CBP but it also binds certain indole derivatives (17), albeit indole compounds (IAA and tryptophan) that are very poor ligands for CBP (Table IV).

Cytokinin binding by the wheat germ CBP, assayed by the $(NH_4)_2SO_4$ precipitation method, is not useful as a quantitative *in vitro* cytokin detection system because of its broad ligand specificity. However, it may serve as a qualitative general model system for cytokinin-binding proteins (including the cytokinin receptor) since it binds all purine and nonpurine cytokinins tested as well as a structurally diverse range of ligands related to those recognized by other cytokinin-binding proteins. For example, the carbanilate herbicides such as CIPC can delay senescent degreening (20) and are antimitotic agents (13, 14, 16, 21) that can inhibit cytokinin-induced cell division (16). The binding of the carbanilate esters tested to CBP (Table II and III) is consistent with the hypothesis that these antimitotics act antagonistically at the level of the actual cytokinin receptor, inasmuch as the receptor and CBP clearly have overlapping ligand specificities.

The present data, while demonstrating that CBP can have a high affinity for certain nonpurine cytokinins (DCMU and CMU), also show that CBP has a broad ligand specificity in our experimental conditions. It was hoped that correlation of specific physiological effects of certain compounds with binding to CBP might provide evidence for the function of this protein but the broad ligand specificity of CBP makes the significance of such binding equivocal. The structural diversity of the CBP ligands demonstrated here would appear to argue further against a specific cytokinin receptor function for this protein (*cf.* 25) since one would expect a hormone receptor to recognize a very limited set of chemical structural determinants. In addition to possible catalytic functions, a cytokinin "buffering" or sequestering function for CBP is more plausible. This latter suggestion (as opposed to a receptor proposal) would be consistent with the high incidence of CBP and the low affinity of CBP for zeatin (25) *i.e.* the good biological activity of a cytokinin could derive from its low affinity for a high incidence, nontransducing, sequestering protein in addition to its requisite high affinity for the cytokinin receptor.

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