Auxin Transport and the Interaction of Phytotropins

Probing the Properties of a Phytotropin Binding Protein

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ABSTRACT

We have described the inhibition of polar auxin transport by several phytotropins including 1-N-naphthylphthalamic acid (NPA) and quercetin. Semicarbazones (substituted phenylsemicarbazones of 2-acetylarylcarboxylic acids) are inhibitors consistent with previously predicted general structural requirements for auxin transport inhibitors. The best semicarbazone derivative tested to date, hereafter called SCB-I, binds to the NPA binding protein with high affinity, K_b = 4 nanomolar. Quantification of the binding of various phytotropins allows us to make some general statements concerning the structure/properties of the NPA binding protein. The data suggest that the ligand binding region of this protein is multifaceted, a conclusion supported by the chemical predictions of Katekar and Geissler ([1977] Plant Physiol 60: 826–829). Although the data do not allow us to make specific conclusions on the structure of the binding site, they do show that both NPA and SCB-I could each occupy two regions of the protein. At least one of these binding regions appears to be common for both inhibitors of auxin transport. We suggest that the diversity of the binding site structure reflects the possible existence of more than one type of natural ligand controlling the process of auxin transport.

Auxins, both natural and synthetic, are a group of organic acids that have dramatic and diverse effects on plant growth and development (7). In common with other hormones, auxins can be transported from a site of synthesis to a site of action. Hormone transport systems, although easily recognizable in animals, remain largely obscure in plants. Information on the transport of auxin has been obtained, in part, because of the availability of chemical inhibitors of the process. In this publication, we describe the properties of a new class of inhibitors of auxin transport, and use the binding data obtained in this study to characterize the site involved in regulating cellular auxin efflux.

The transport of IAA, the most common natural auxin, is an essentially unidirectional polar event (25). In shoots, the free IAA pool in the apex is moved to a more basal site of action (25), thus the polarity of IAA transport is basipetal. In roots, the situation is less clear; according to Rubery (25), transport of IAA is in the same direction, now termed acropetal, but a more recent report suggests that root transport is

away from the tip, i.e. basipetal (4). This polar transport, unique to plants, is believed to occur in peas (Pisum sativum) through a limited population of vascular cells via a chemiosmotic process (15). The chemiosmotic transport of IAA was first proposed by Rubery and Sheldrake (24) and has been supported by studies with seedling sections (6) and isolated PM[2] vesicles (12). With very few exceptions (8), the results of research in the field of PAT are consistent with the original chemiosmotic proposal. In summary, chemiosmotic PAT is dependent on a ΔpH across the PM (11, 25) and the weak acidic property of IAA (pK_a = 4.7). The process of PAT can be divided into two steps. Uptake of undissociated auxin (IAAH) from the cell wall is by diffusion and/or a saturable H^+/IAA^- symport carrier (10, 19). The second step, efflux of dissociated acid (IAA$^-$) from the cytoplasm occurs via a saturable protein site (10, 25), distributed asymmetrically at the basal end of transporting cells (15, 20). This efflux carrier was originally characterized as site III by Jacobs and Hertel (14).

A number of both natural (16) and synthetic (5, 17) compounds have been observed to inhibit the process of PAT, with the characteristic result of ageotropic root growth in whole plants (17). Several of these inhibitory compounds, referred to as phytotropins, appear to act by a common mechanism (17). The most extensively investigated of these is NPA (11, 17, 25, 26), which has been shown to inhibit auxin efflux from PM vesicles (25) by binding to a protein on the cell wall face of the PM (15, 18, 20, 25, 28). Although the presence and properties of the NPA binding protein are well documented, its relationship to the process of PAT is unclear. Hertel (10) has suggested that NPA might bind to a protein that is the auxin efflux carrier, possibly existing in a different conformational state. More recently, Rubery (25) has presented information supporting the idea that the NPA binding protein is a regulatory subunit of the auxin efflux carrier. These models are as yet unproven. Perhaps more intriguing is the physiological role of the NPA binding site and the probable existence of natural phytotropins. It has recently been proposed (16) that the NPA receptor site exists, at least in part, to bind naturally occurring flavonoids. Although these

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[2] Abbreviations: PM, plasmalemma; TIBA, 2,3,5-triiodobenzoic acid; NPA, 1-N-naphthylphthalamic acid; PAT, polar auxin transport; CHAPS, (3,[(3-cholamidopropyl)-dimethylammine]-1-propanesulfonate); SCB-I, semicarbazone derivative I; I_{50}, concentration of inhibitor necessary for 50% inhibition of growth.

observations might not complete the list of natural inhibitors of PAT, they do suggest a physiological control for the process.

In this paper, we present the structure of a new class of synthetic phytotropin and investigate the relationship to the binding of other known inhibitors of auxin transport. We have used the presence of diverse chemical inhibitors of auxin transport to begin characterization of the phytotropin binding protein.

MATERIALS AND METHODS

Whole Plant Studies

To demonstrate the physiological (whole plant) effects of the semicarbazone, we have analyzed inhibition of root growth in tomato (*Lycopersicon esculentum*) (17, 21) together with the inhibition of [^{14}C]IAA efflux from pea (*Pisum sativum*) stem segments.

Wild-type, nonhybrid tomato seeds (VFN8 from P and S Petoseed, Davis, CA) were surface-sterilized in 20% bleach, 0.01% Triton X-100 for 20 min. The seeds were washed extensively in sterile water to remove all bleach and transferred to sterile solid medium, described below, and allowed to germinate for 3 d at 30°C. The solid medium for both germination and assay contains 1× Murishige and Skoog salts (Gibco, Bethesda, MD), 1.0 mg/L thiamine, 0.5 mg/L nicotinic acid and pyridoxine HCl, 100 mg/L inositol, 15 g/L sucrose, and 8 g/L agar at pH 6.0.

After germination, seeds with roots of approximately 1 to 2 mm were transferred to agar plates containing the medium described above with and without NPA or SCB-I. The plates were oriented vertically and roots allowed to grow for 48 h at 25°C under 24 h fluorescent light at 20 μE m^{-2}s^{-1}. The root length was measured after 2 d and was approximately 30 to 35 mm in the absence of inhibitors in all experiments analyzed. Growth as a function of -log [inhibitor] was plotted and the concentration necessary for 50% inhibition of growth was determined. Peas were grown for 7 d, in the dark; 5 mm segments (approximately 10–11 mg) were removed from between the third and fourth internode as described by Davis and Rubery (2) and incubated in 0.25 M sucrose, 1 mM MgCl$_2$, 20 mM citrate (pH 5.3) (2). The buffer contained 10^{-7} M [^{14}C] IAA and was incubated for 2 h at 22°C. Addition of NPA and SCB-1 inhibited the efflux of the [^{14}C]IAA from the segment, as determined against untreated control samples. Before counting in liquid scintillation cocktail, segments were dipped briefly in buffer and vacuum dried over two layers of Whatman No. 1 filter paper. The segments were then left overnight in aqueous cocktail scintillant and counted on 2 successive d to eliminate any quenching effect of the pigments.

Binding Studies

For this study, corn (*Zea mays* L.) and zucchini (*Curcubita pepo* L.) were grown in dark conditions at 27°C until approximately 10 cm high, 6 d for corn and 5 d for zucchini. Intact, right-side out, PM vesicles were prepared from excised corn coleoptiles and zucchini hypocotyls by phase separation techniques adapted from Hodges and Mills (13) and Widell (29). Tissue (approximately 150 g) was homogenized in a Waring blender with 0.25 M sucrose, 3.0 mM EDTA, and 0.25 mM

trizma base (pH 7.2), filtered through cheesecloth, and centrifuged at 13,000g for 10 min. The supernatant was centrifuged at 80,000g for 30 min to pellet membranes; this membrane fraction was then resuspended in 0.25 M sucrose, 5.0 mM potassium phosphate, pH 7.8. Initial phase separation occurred using 0.63% dextran and 0.63% PEG (13) after centrifugation at 1000g for 10 min. The upper phase containing PM was decanted, whereas the lower phase was again treated with 0.63% PEG. The two upper phase extractions were pooled and centrifuged at 80,000g for 30 min. The pellet of PM was resuspended in pH 7.8 buffer, final volume 5 mL, for storage at −80°C. This protocol is reported to generate 95% pure PM vesicles (29), which are tight and right-side-out (13). Enzymatic confirmation of PM in these preparations was achieved by measuring glucan synthase II activity after Depta *et al.* (3). For use in binding assays and accumulation studies, frozen or fresh membrane preparations were diluted in 10 volumes of test buffer containing 0.25 M sucrose, 1 mM MgCl$_2$, 20 mM citrate, pH 5.3. The accumulation of [^{14}C]IAA by PM vesicles was measured as described by Hertel *et al.* (11). Quantitation of phytotropin binding ([^{3}H]NPA and [^{3}H] SCB-I) was adapted from the centrifugation protocol of Lützelschwab *et al.* (20). Radiolabeled ligand was prepared in test buffer (pH 5.3) to give a final concentration range of 0.25 to 5.0 nM. Total volumes were 1 mL, consisting of 50 μL ligand solution plus 950 μL membrane preparation. Membranes and ligand (± unlabeled competitor) were mixed at 4°C for 15 min before pelleting at 100,000g for 10 min. The supernatant was allowed to drain before the base of the microcentrifuge tube was removed and counted on a Beckman LS 5000 CE scintillation counter.

Solubilization of the membrane-associated phytotropin binding protein was achieved by the procedure of Thein and Michalke (27) using a 1% CHAPS and 33% (v/v) glycerol treatment of PEG phase-separated PM. Phytotropin binding to solubilized receptors was achieved by filtration (27). The structures of the chemical ligands used in this study are illustrated in Figure 1. [^{3}H]NPA (specific activity 49 Ci/ mmol) was obtained from R.P.I. Corp., Mount Prospect, IL.

RESULTS

Tomato root growth inhibition by auxin transport inhibitors is shown in Figure 2. The plot of growth *versus* -log [inhibitor] yields a sigmoidal curve (Fig. 2A) with a narrow range of linear response (Fig. 2B). At the highest concentrations of SCB-I (10^{-7} M), not only is root growth retarded, but the roots grew ageotropically, both against and perpendicular to gravity. At the lower concentrations (5 × 10^{-9} M), root growth appeared entirely normal. The linear range of the inhibition curves was used to determine the I$_{50}$ (Fig. 2B). The qualitative effects of NPA and SCB-I were very similar, but roots are threefold more sensitive to SCB-I. For SCB-I, the I$_{50}$ equaled 4.8 (± 0.5) × 10^{-8} M (n = 8) and for NPA the I$_{50}$ equaled 1.2 (± 0.7) × 10^{-7} M (n = 6). In addition to the higher activity, the inhibitor action of SCB-I was more prolonged than that of NPA. Inhibition of [^{14}C]IAA efflux from pea stem segments (Fig. 3) shows results entirely consistent with inhibition of auxin efflux (2). At a concentration of 10^{-6} M, NPA causes a statistically significant increase in the accumu-
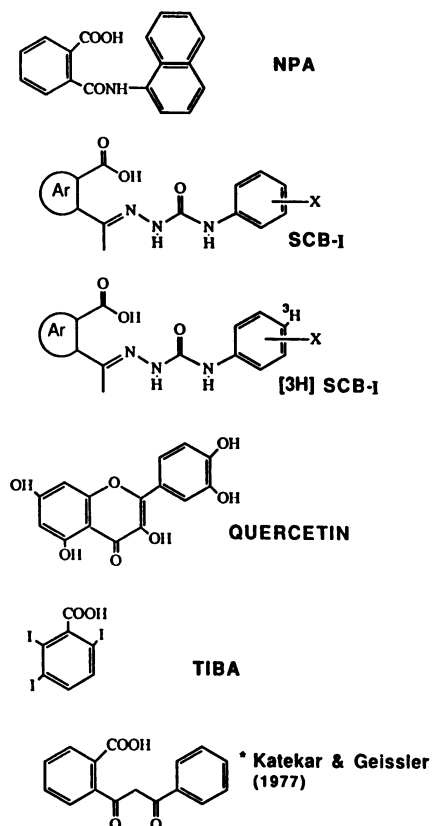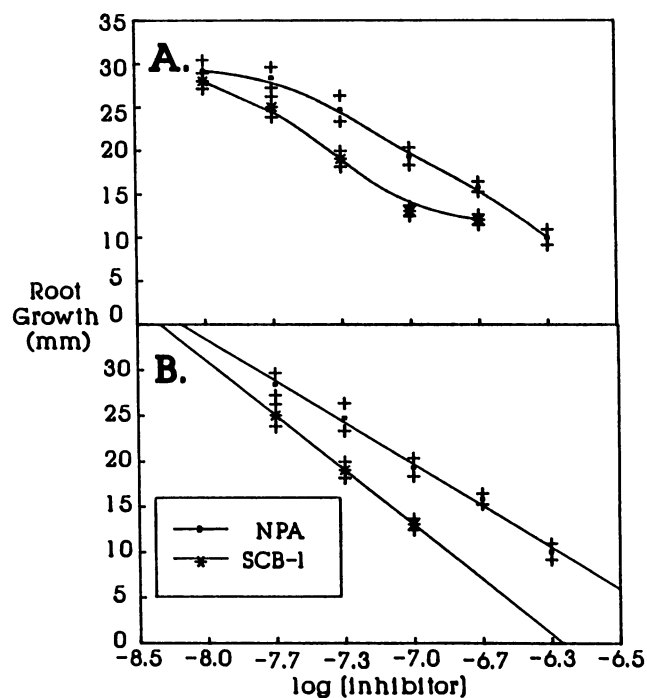
Figure 1. Chemical structures used in this study, where Ar is a five- or six-membered ring with or without a heteroatom. * Structure not used in this study, but is included for comparison of general phytotropin structure.
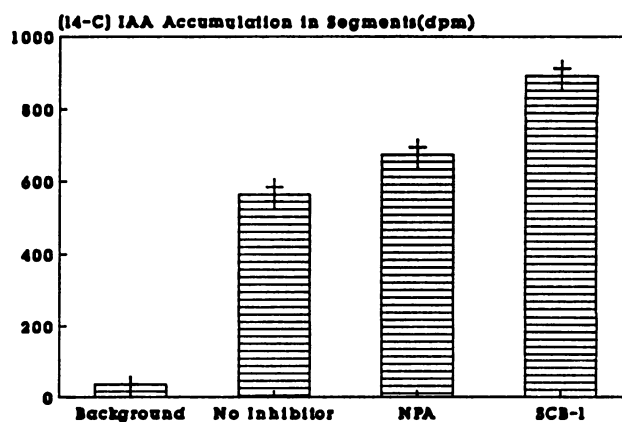

Figure 2. The effect of SCB-I and NPA on tomato root growth. Root elongation was measured on solid agar containing varying concentrations of inhibitor. The root length as a function of inhibitor concentration is plotted and the error bars represent the standard error. Each point represents analysis of 10 roots. A, Sigmoidal curve showing inhibition effects over a broad concentration range. B, Narrow concentration range, showing linear response from which I_{50} is calculated. Correlation coefficients are 0.986 and 0.992 for SCB-I and NPA, respectively.

lation of [^{14}C]IAA by pea stem segments, whereas at the same concentration SCB-I causes an even greater IAA accumulation.

Accumulation of [^{14}C]IAA by isolated PM vesicles has been used to characterize the effects of NPA and TIBA on PAT (11, 12). It is largely on the basis of these vesicle experiments that NPA and TIBA have been characterized as inhibitors of auxin efflux. The results of our experiments (Table I) are in close agreement with previous observations (11, 12) and suggest that SCB-I as well as TIBA and NPA causes accumulation of [^{14}C]IAA.

Using [^{3}H]NPA, it is possible to calculate the binding affinity (K_b) of NPA to a protein site associated with the cellular efflux of IAA (11, 25). We have demonstrated specific and competitive binding of both radiolabeled NPA and SCB-I using isolated right-side-out PM vesicles, from both corn and zucchini (Fig. 4). Kinetic binding constants (K_b and K_i) for NPA and SCB-I are summarized in Table II. The K_b values for NPA are approximately 4 nM in both corn and zucchini, a result in close agreement with the high affinity NPA binding previously reported (16). SCB-I shows almost identical K_b values to NPA in all systems. Because SCB-I and NPA compete for binding (Fig. 4), it is possible to determine reciprocal competitive binding constants (K_i). In these experiments, the assumption is that a ligand (e.g. SCB-I), at a known fixed

Figure 3. SCB-I and NPA (10^{-6} M) inhibition of [^{14}C]IAA efflux from pea stem segments measured by the method of Davis and Rubery (2). Standard errors of the mean ($n = 11$) are presented.

Table I. *Accumulation of [¹⁴C]IAA by Plasmalemma Vesicles of Corn and Zucchini (n = 15)*

Values based on the percentage increase after correction for accumulated dpm in the presence of FCCP (10 μM) determined as a control value of 100%. IAA accumulation is measured after the procedure of Hertel et al. (11) with internal vesicle pH of 7.8 and external pH of 5.3 and a [¹⁴C]IAA dose range of 0.25 to 5.00 μM. Values are averaged over the complete range of [IAA], representing five concentrations and three replicates. Data presented as mean and standard deviations.

Inhibitor	Corn		Zucchini	
	10^{-6} M	10^{-7} M	10^{-6} M	10^{-7} M
	%			
NPA	124 ± 17	101 ± 2	133 ± 12	112 ± 7
TIBA	156 ± 17	132 ± 10	118 ± 6	105 ± 9
SCB-I	146 ± 28	165 ± 21	136 ± 14	120 ± 15

concentration, competes with the radiolabeled (e.g. [³H]NPA) ligand as a function of the nonradioactive ligand's affinity. By application of Michaelis-Menten kinetics, we can calculate the binding affinity of the radiolabeled ligand (K_b) and the competing ligand (K_i). The results of these experiments are presented in Table II. If a single common binding site is responsible for all ligands, we would expect the K_i values of NPA and SCB-I determined in competition with [³H]NPA and/or [³H]SCB-I to be the same as the value of K_b determined in the absence of any competing ligand. The calculated K_i
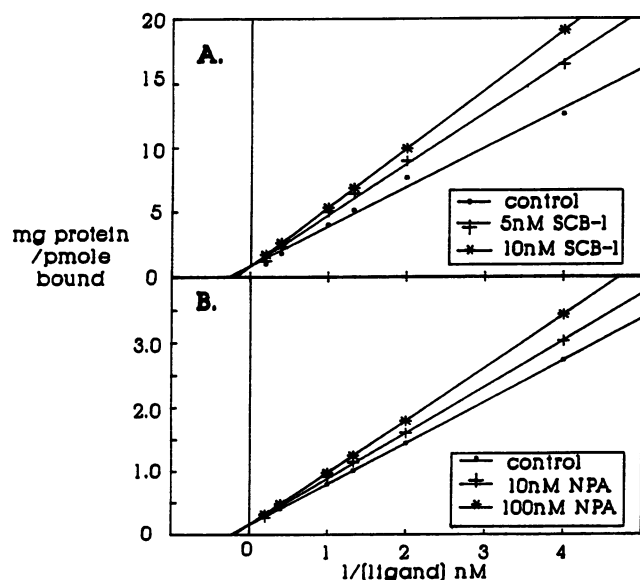
Figure 4. Double-reciprocal analysis of receptor ligand binding after Michaelis-Menten enzyme kinetics (1). From these plots, it is possible to determine binding affinities K_b and K_i: $-1/K_b$ = x axis intercept in the absence of competing ligand; K_i can be calculated from the equation $-1/K_{b(app)} = -1/K_b (1 + [I]/K_i)$, where [I] is the concentration of competing unlabeled ligand. Experimental data were generated using the procedures described by Lützelschwab et al. (20). A, [³H] NPA binding unlabeled SCB-I; B, [³H]SCB-I versus unlabeled NPA.

Table II. *Binding Affinity Values (K_b and K_i) Determined for NPA and SCB-I Using Isolated Plasmalemma from Corn and Zucchini*

All binding affinities were determined at pH 5.3 by a procedure adapted from Lützelschwab et al. (20). The values of K_b and K_i were determined by application of Lineweaver-Burk double reciprocal analysis as described in Figure 4. Data are presented as mean, ± SD. In each species, the values of K_i were significantly greater than the K_b for the same ligand (determined at the 95% confidence level).

Ligand		Corn	Zucchini
[³H]NPA	(K_b)	4.04 ± 0.2 nM (n = 19)	4.18 ± 0.7 nM (n = 23)
SCB-I	(K_i)	20.55 ± 9.8 nM (n = 3)	15.36 ± 7.2 nM (n = 2)
NPA	(K_i)	13.45 ± 4.2 nM (n = 5)	8.72 ± 4.6 nM (n = 2)
[³H]SCB-I	(K_b)	4.01 ± 0.4 nM (n = 14)	4.04 ± 0.4 nM (n = 11)
NPA	(K_i)	0.44 ± 0.3 μM (n = 2)	0.23 ± 0.1 μM (n = 2)
SCB-I	(K_i)	19.75 ± 8.4 nM (n = 2)	18.84 ± 2.2 nM (n = 2)

values are, in all cases, significantly higher than the corresponding K_b (Table II).

The procedure of Thein and Michalke (27) allows solubilization of the phytotropin binding protein while maintaining high affinity for NPA. Accordingly, we have determined the binding of NPA and SCB-I in this solubilized system. The result of these experiments are summarized in Table III, and the K_b values for SCB-I and NPA for both corn and zucchini are generally in agreement with the values obtained with the intact PM. The most significant difference in the solubilized receptor system is that the K_i value for NPA (versus [³H]SCB-I) is in the nanomolar range (cf. Table II).

Finally, we have calculated the relationship of NPA binding to other documented inhibitors of auxin transport. Table IV shows that 10^{-7} M TIBA does not compete with NPA binding in either the intact or solubilized PM of corn and zucchini at pH 5.3. This observation is consistent with published results (11, 25) and the hypothesis that TIBA acts at a site different from that of NPA. A similar lack of TIBA competition (data not shown) has been observed using [³H]SCB-I as ligand. We have also calculated the K_i for quercetin, a proposed natural phytotropin (16) in competition with [³H]NPA. The intact membranes from corn and zucchini show a low affinity quercetin binding against [³H]NPA or [³H]SCB-I (K_i approximately 1 μM) (16), but in the solubilized membrane system quercetin binds competitively with [³H]NPA (and [³H]SCB-I) to give a K_i of approximately 30 nM (Table IV).

Table III. *Binding Affinity Values Determined for NPA and SCB-I Using Detergent-Solubilized Plasmalemma*

Binding affinity values were determined by Lineweaver-Burk double reciprocal plots for NPA and SCB-I using detergent solubilized plasmalemma after Thein and Michalke (27).

Ligand		Corn	Zucchini
		nM	
[³H]NPA	(K_b)	3.93	3.63
SCB-I	(K_i)	63.3	23.2
[³H]SCB-I	(K_b)	4.36	12.6
NPA	(K_i)	31.9	18.1

Table IV. *Binding Affinity Values of Quercetin and TIBA as Determined by Competition with [³H]NPA Using Corn and Zucchini Plasmalemma*

Binding affinity values determined for quercetin and TIBA (10^{-7} M) in competition with [³H]NPA using corn and zucchini plasmalemma. N/E = no effect.

| Ligand	Corn		Zucchini	
	Intact	Solubilized	Intact	Solubilized
NPA (K_b)	4.1 nM	3.9 nM	4.3 nM	3.6 nM
TIBA (K_i)	N/E	N/E	N/E	N/E
Quercetin (K_i)	1.5 μM	34.1 nM	0.5 μM	28.6 nM

DISCUSSION

Katekar and Geissler (17) have analyzed the apparent chemical requirements for inhibition of PAT. In summary, these requirements are a carboxylic acid function attached to an aromatic ring that is linked to a second aromatic ring, through the *ortho*-position (see Fig. 1). Similarities between this general structure type and SCB-I are apparent. Data presented in this paper confirm that SCB-I is a potent inhibitor of PAT. In addition to the inhibition of root growth (Fig. 2) and auxin efflux from pea stem segments (Fig. 3) and stimulation of IAA accumulation by PM vesicles (Table I), inhibition of IAA transport has been demonstrated with agar blocks (6), ageotropic root growth (17), and development of adventitious roots from excised seedling stems (21). SCB-I has proven active in all these assays and its effects on root growth will be addressed in a subsequent paper. The binding data of Tables II and III indicates that SCB-I and NPA act at the same protein site, a site that is structurally or functionally linked to the efflux of IAA. This observation is consistent with previous speculation that many compounds, of this general structure, act at a common site (17). For the purpose

of this discussion, we have termed all compounds, natural and synthetic, which act at this site, phytotropins.

The presented data indicate that NPA and SCB-I bind competitively (Fig. 4); the high affinity (K_b = 4 nM) of both ligands in corn and zucchini suggests similarity in this site between monocots and dicots (Tables II and III). However, the competitive binding of NPA and SCB-I should not be interpreted to mean that these phytotropins bind to the same site on the protein. A more accurate interpretation of the experimental phenomena is that phytotropins bind to the protein in a manner that precludes the binding of a second ligand. Because the site must accommodate structures at least as diverse as NPA, SCB-I, and quercetin, we might predict the possibility of a rather complex multifaceted binding site (Fig. 5A). Structural requirements for phytotropins proposed by Katekar and Geissler (17) suggest that two clearly separated binding regions might exist, one for the aromatic carboxyl and a second for the remaining aromatic (Fig. 1). At the protein level, these might relate to something as simple as separate ionic and hydrophobic binding sites, respectively. The relative affinities of the different ligands for these site(s) within the protein could affect our ability to calculate binding constants by competition with a second radiolabeled ligand. The quantification of ligand binding affinity (K_b and K_i) used in this study is derived from Michaelis-Menten kinetics (1). To apply these principles, we assume a single ligand/receptor binding site. Although, as will be discussed later, this assumption might not be entirely valid, the approach has been used in the study of PAT inhibitors (16, 27) and inhibitors of photosynthetic electron transport (9, 22).

When determining the affinity of an unlabeled ligand in competition with a labeled ligand, we operate under the assumption that only one of the two ligands can occupy the site at a given time. With this on/off assumption, the equilibrium binding of radiolabel is a function of the binding affinity (relative rates of association/dissociation) and concentrations
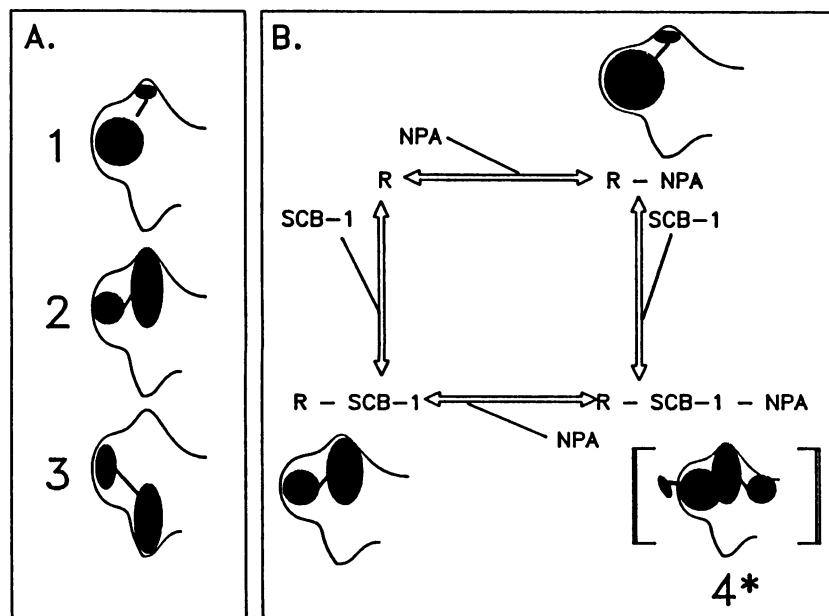
Figure 5. A, Schematic presentation of possible binding site structure that could accommodate multiple competing ligands in a single protein. Binding domains could be separate chemical interactions, *e.g.* hydrophobic versus ionic or sites limited by steric hindrance. 1 = bound NPA; 2 = bound SCB-I; 3 = bound quercetin. B, Possible formation of ternary receptor ligand complex (SCB-I-R-NPA) 4* based on the discussion of Presecan *et al.* (23). Such a binding scheme would explain the routine overestimation of K_i values compared with K_b and is consistent with the binding site structure of Figure 5A and the chemical models of Katekar and Geissler (17).

of each ligand. It is reasonable to expect that the calculated value of K_i for a given ligand would be the same as K_b for that ligand (9). However, the NPA K_i value calculated in competition with [^{3}H]NPA is significantly greater than K_b (Table II) and up to 100-fold greater when calculated against [^{3}H]SCB-I. The simplest explanation for this observation is that Lineweaver-Burk analysis is subject to errors that restrict its use for analysis of receptor/ligand binding, particularly when the receptor population is low. An alternative explanation, recently discussed by Presecan et al. (23), would be that the simplified assumptions mentioned above are not valid if the possibility of forming a ternary ligand/receptor complex exists (Fig. 5B). The transient formation of a {ligand/receptor/ligand} complex will inevitably result in the overestimation of K_i; comparison of values of K_b and K_i for both NPA and SCB-I presented in Tables II and III are consistent with a systematic overestimation of K_i (*i.e.* $K_i > K_b$). The formation of a ternary complex is reconcilable with the proposals of Katekar and Geissler (17), suggesting the requirement for two separate binding regions. In this manner, we can envision the transient binding of more than one ligand at a multifaceted binding site. This phenomena is illustrated in Figure 5B and could occur with almost any combination of unlabeled and labeled ligands used in this study. Although this explanation does have some merit, and likely reflects aspects of the complexity of the phytotropin binding protein, it does not encompass all our experimental results, most notably, the difference in K_i values obtained for NPA *versus* [^{3}H]SCB-I and SCB-I *versus* [^{3}H]NPA (Table II). Although the value of SCB-I K_i is statistically greater than [^{3}H]SCB-I K_b, it is not the two orders of magnitudes we see in the reciprocal experiment of NPA *versus* [^{3}H]SCB-I. If the formation of {SCB-I/receptor/NPA} (Fig. 5) complex is responsible for 100-fold overestimation of K_i for NPA, why would we not see the same magnitude of overestimation for SCB-I K_i (*versus* [^{3}H]NPA)? One explanation is that the formation of the ternary complex ultimately favors bound SCB-I over bound NPA, but based on the similar values for K_b, this seems unlikely. A more plausible explanation appears to be a kinetic difference in the binding of the two ligands. If the binding of SCB-I is analogous to that of a slow tight binding inhibitor (30), the equilibrium, after 15 min, might strongly favor the formation of the SCB-I/receptor complex, and thus the calculated value of NPA K_i would be sizably overestimated. The use of different labeled ligands, *e.g.* [^{14}C]SCB-I and [^{3}H]NPA, simultaneously might resolve this paradox.

No matter which explanation is true, the results obtained with the solubilized membranes of both corn and zucchini (Table III) strongly suggest that membrane lipids play a role in the binding of at least some ligands. Although the K_b values of [^{3}H]NPA and [^{3}H]SCB-I are consistent in both the intact and solubilized systems, the apparent K_i of NPA (*versus* [^{3}H] SCB-I) is much lower than the equivalent value in the intact membranes. This indicates that ligand binding is subtly altered in solubilized membranes even though no effect on K_b for NPA or SCB-I is apparent. Compare the quercetin binding (K_i) constant in the intact system (approximately 1 μM) and the solubilized membrane (approximately 30 nM) (Table IV). Although some effect of the quercetin solubility might affect these results (16), this result again suggests that the membrane

integrity of this protein plays an important role in describing its binding properties.

With the current data, it is not possible to make a dramatic conclusion about the phytotropin receptor site(s) and its structural relationship to the process of auxin efflux and PAT. However, it seems certain that the binding region(s) involved with the reported binding is more complex than was initially proposed (10).

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