The binding of auxin to the *Arabidopsis* auxin influx transporter, AUX1

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Keywords

Auxin; AUX1; polar auxin transport; auxin transport inhibitor; baculovirus; amino acid auxin permease

Abbreviations

IAA – indole-3-acetic acid; 2,4-D - 2,4-dichlorophenoxyacetic acid; 1-NOA - 1-naphthoxyacetic acid; NAA - naphthylacetic acid; CHPAA - 3-chloro-4-hydroxy-phenylacetic acid; NPA – naphthylphthalamic acid.

Abstract

The cellular import of the hormone auxin is a fundamental requirement for the generation of auxin gradients which control a multitude of plant developmental processes. The AUX/LAX family of auxin importers, exemplified by AUX1 from *Arabidopsis thaliana,* has been shown to mediate auxin import when expressed heterologously. The quantitative nature of the interaction between AUX1 and its transport substrate indole-3-acetic acid (IAA) is incompletely understood and we sought to address this in the present investigation. We expressed AUX1 to high levels in a baculovirus expression system and prepared membrane fragments from baculovirus-infected insect cells. These membranes proved suitable for determination of the binding of IAA to AUX1 and enabled us to determine a Kd of 2.6 μM, comparable with estimates for the Km for IAA transport. The efficacy of a number of auxin analogues and auxin transport inhibitors to displace IAA binding from AUX1 has also been determined and can be rationalized in terms of their physiological effects. Determination of the parameters describing the initial interaction between a plant transporter and its hormone ligand provides novel quantitative data for modelling auxin fluxes.

Introduction

A plethora of plant developmental processes is controlled by auxins, including tropic responses to light and gravity, tissue differentiation, development and senescence (Delker et al., 2008). The most naturally abundant auxin is indole-3-acetic acid, IAA (Estelle, 1996; Perrot-Rechenmann and Napier, 2005), gradients of which are dependent on the directional import and export of the hormone into and out of cells. The chemiosmotic hypothesis of auxin transport (Rubery and Sheldrake, 1974; Raven, 1975; Goldsmith, 1977) states that the weak organic acid IAA (pKa of 4.8) is taken up by cells by a combination of carrier-mediated uptake (presumably of the IAA^- anion) and diffusion of the undissociated lipophilic acid (IAAH). Within the neutral cytoplasm auxin will exist almost entirely as IAA^-, effectively becoming trapped, and requiring an IAA^- transporter to efflux it from the cell. The coordinated actions of influx and efflux carriers of IAA are necessary to drive polar auxin transport and to generate the hormone gradients which regulate cellular events. Despite their importance there is still relatively little biochemical characterization of these transport proteins (Estelle, 1996; Friml, 2003; Blakeslee et al., 2005; Kerr and Bennett, 2007).

In recent years the proteins responsible for auxin influx (AUX/LAX) and efflux (PINs and multidrug resistance type ATP binding cassette transporters) have been identified in genetic screens (Palme and Galweiler, 1999; Noh et al., 2001). AUX1, from *Arabidopsis thaliana* (Bennett et al., 1996), represents the paradigm for auxin influx carrier proteins (Kerr and Bennett, 2007), which are represented in all plant species analysed to date. The protein is believed to act as a proton:auxin symporter and shares a high degree of sequence homology to the amino acid auxin permease (AAAP) family of transporters (Saier, 2000) and indeed may be part of the general amino acid polyamine cation transport family (Jack et al., 2000).

Recent characterization of AUX/LAX (and PIN) family members has shifted from *in planta* assays to *in vitro* assays following expression of the proteins in heterologous systems (Petrasek et al., 2006; Yang et al., 2006; Kerr and Bennett, 2007). The most notable achievement of this research has been the demonstration that AUX1 is a high affinity IAA importer when expressed heterologously in *Xenopus* oocytes (Yang et al., 2006). Importantly, this research was able to demonstrate that AUX1 is likely to function as a transporter of the anionic form of IAA (IAA^-) which co-exists with uncharged IAA (IAAH) at the mildly acidic pH (ca. 5.2 to 5.5) of the apoplast (Yang et al., 2006). The Km for IAA uptake into *Xenopus* oocytes was determined to be 800 nM, compared to previous estimates obtained in studies of crown gall suspension cells of 1-5 μM (Rubery and Sheldrake, 1974).

Understanding the transport of IAA by AUX1 requires quantitative characterization of a multi-step process involving ligand recognition at the extracellular face, protein conformational changes and ligand release into the cytoplasm. In the current manuscript we have investigated the initial step of auxin transport, namely the interaction of AUX1 with IAA. Determination of AUX1:IAA interactions has been achieved using a radio-ligand binding assay, having expressed the protein to high levels in a baculovirus-infected insect cell system. We have determined that AUX1 is able to bind IAA and that the affinity (K_d) of this binding is comparable with the Km obtained for transport (Rubery and Sheldrake, 1974; Yang et al., 2006). A range of auxin analogues has also been investigated for their relative affinity for the AUX1 transporter and this data can be reconciled with *in vivo* effects of these compounds.

Materials and Methods

All reagents were of the highest grade and were obtained from Sigma or Fisher. Molecular biology reagents were from New England Biolabs, Invitrogen or Fermentas. [^{3}H]-IAA, specific activity 962 GBq/mmol was obtained from GE Healthcare.

Expression of HA-tagged AUX1 in Sf9 insect cells

Spodoptera frugiperda (Sf9) cells were grown as orbital cultures at 27-28ºC in InsectXpress medium (Lonza) supplemented with 10% fetal calf serum and 50 units/ml penicillin and streptomycin. AUX1 was expressed as an N-terminally haemagluttinin (HA)-tagged construct, HA-AUX1, in Sf9 cells following viral infection. Recombinant baculoviral DNA (bacmid DNA) was generated using Bac-2-Bac technology (Invitrogen), following manufacturer's instructions. Bacmid DNA was screened by PCR to ensure integration of the HA-AUX1 cDNA, and recombinant virus then produced by Cellfectin-mediated transfection of Sf9 cell monolayers. Viral titres were determined and amplified as previously described (King and Possee, 1992). HA-AUX1 expression was routinely induced by infecting Sf9 cells in suspension culture (2.0×10^6/ml) with a multiplicity of infection (MOI) of 1.0, for 72 hours.

Cells were harvested by centrifugation (500g, 5 minutes, 4ºC) and resuspended in approximately 10 times the pellet volume in 10mM Tris pH 7.4, 250mM sucrose, 0.2mM $CaCl_2$ with protease inhibitors (Complete EDTA-free Protease Inhibitor, Roche) then passed twice through a pressure disruptor (Constant Systems) at 5 kpsi. Cellular debris were removed by centrifugation at 300g for 15 minutes at 4ºC and total cell microsomal membranes then pelleted by centrifugation at 100,000g for 1 hour at 4ºC. The membrane pellet was re-suspended by shearing 10-20 times through a 27.5G needle, to a protein

concentration of 10-20 mg/ml in citrate-phosphate buffer pH 6.0, aliquotted and frozen at -80ºC.

Binding of [^{3}H]-IAA to HA-AUX1 containing cell membranes

Saturation and competition radioligand binding assays were performed on HA-AUX1 membrane preparations. Membrane proteins (300µg) were incubated with varying concentrations of [^{3}H]-IAA for 90 minutes at 20-22ºC in a total volume of 100µl in citrate-phosphate buffer, pH 6. Non-specific binding was determined in the presence of excess (100 µM) unlabelled IAA. Unbound ligand was separated from bound ligand by centrifugation at 20,000g for 5 min, 4ºC, and the supernatant removed. The supernatant accounted for over 95% of the radioactivity added, ruling out ligand depletion. The pellet was then washed twice in ice-cold citrate-phosphate buffer, with recentrifugation, and radioactivitity associated with the membrane pellet was then determined by liquid scintillation counting. Competition assays were performed identically, except that [^{3}H]-IAA was maintained at a fixed concentration (500 nM) and membranes were co-incubated with increasing concentrations of analogue/inhibitor (3 nM to 1 mM in semi-log intervals).

Saturation binding data was fitted using non-linear least squares regression of the equation $B = (B_{\max} \bullet [S])/(K_d + [S])$, where B is the specific binding occurring at an IAA concentration, S. Statistical comparison of the fit of this equation compared to equations representing multiple binding sites were performed with an F-test. In subsequent competition binding experiments, data was fitted to the general dose response equation of the form $f = f_{\min} + (f_{\max} - f_{\min}/(1+10^{\log IC_{50}-[S]})$, where f is the fractional IAA binding observed in the presence of concentration, S, of a competing compound. The IC_{50} represents the concentration of competing ligand required to displace 50% of IAA specifically bound to

AUX1 containing membranes. All data fitting was performed using GraphPad Prism 4.0, and each data point shown is the result of at least triplicate determinations within each experiment, and multiple, independent membrane preparations were employed.

Results

HA-AUX1 expression in insect cells

In order to investigate the binding of IAA and related auxins and auxin-like compounds to the auxin influx transporter AUX1, we expressed an epitope-tagged version of AUX1 in Sf9 insect cells. The N-terminal tagging of AUX1 with the HA tag (amino acid sequence YPYDVPDY) does not affect the localization or function of the protein, and indeed an HA-AUX1 transgene is able to rescue the agravitropic phenotype of an *aux1-22* phenotype in plants (Swarup et al., 2001). The presence of the epitope tag enables easy identification of the protein following heterologous expression. Optimum expression conditions were determined (Figure 1) and, routinely, cells were harvested 72 hours post infection. Comparable expression data in several different prokaryotic and eukaryotic expression systems indicated that the baculovirus expression system was most suited to production of large quantities of HA-AUX1-containing membranes (Carrier et al in preparation).

IAA binds specifically to AUX1 with low micromolar affinity

Initial attempts to establish a robust binding assay to measure the AUX1:IAA interaction tested the separation of free IAA from bound IAA by rapid filtration through a vacuum manifold. Despite employing numerous different filters (and combining these with protein precipitation methods) we were unable to show linear dependence of non-specific binding on protein load (Figure 2). Consequently we tested centrifugation and determined that a brief (5 minutes) centrifugation (20,000g) of Sf9 membranes was sufficient to pellet in excess of 70%

of the protein (data not shown), and give a linear dependence of non-specific binding on both sample size and IAA concentration, affording confidence in the assay's ability to measure specific AUX1:IAA interactions (Figure 3B).

Auxin binding to IAA was not fully saturable within the constraints of the specific activity of the commercially available ligand. Thus, to determine a K_d for IAA binding to AUX1 we effectively carried out a homologous displacement assay (see e.g. (Martin et al., 2001)) whereby a fixed concentration of [^{3}H]-IAA was employed together with increasing concentrations of unlabelled IAA. Correction of the measured binding for this isotope dilution enabled the quantification of the AUX1:IAA interaction at concentrations up to 8 μM IAA. A composite saturation binding isotherm of data obtained from multiple independent experiments, each with triplicate determinations of specific binding is displayed in Figure 3. Non-linear regression of the data was best fitted to a single site saturation isotherm (see Methods) and yielded a K_d of 2.6 μM and a maximal binding of 11,800 fmoles IAA/mg membrane protein. Under identical conditions we determined that specific binding of IAA to Sf9 cell membranes lacking AUX1 was less than 1% of HA-AUX1 membranes (data not shown), ruling out anion trapping as an explanation for our observations.

To determine a K_d for the AUX1:IAA interaction by kinetic means (i.e. $K_d = k_{off}/k_{on}$ where k_{off} and k_{on} are the measured rates of dissociation and association respectively) we investigated the association rate for IAA at 21ºC and 2ºC (Figure 4A,B). We estimate from these data that the half-lives for auxin binding to IAA are less than 10s, and thus the process is essentially complete within the 5 minutes required to pellet the membranes and separate bound from free ligand, even at 2ºC. This rapid association precludes determination of K_d from k_{on} and k_{off} values..

AUX1 interacts primarily with IAA between pH 5.0 and 6.0

The pH of IAA:AUX1 interaction was determined over the range 4.5 to 7.5. At each pH, membranes were resuspended in a citrate:phosphate buffers of the desired pH prior to incubation with ligand. The binding showed a clear pH dependence with maximal specific binding occurring between pH 5 and 6 (Figure 5, solid line, filled squares). The reduction in specific binding at pH > 7.0 is discussed below. Within the apoplastic space (pH 5.2), and assuming a pKa of 4.8 for IAA, this is consistent with an interaction with primarily the anionic (IAA^-) rather than the protonated (IAAH) form of the hormone (Figure 5, broken line). This is also consistent with structure:activity analyses of indole-3-acetic acid and related compounds that proposed a key stereo-chemical role for the acetic acid group in determination of auxin specificity (Katekar, 1979).

Auxin transport inhibitors displace IAA binding from AUX1

The displacement of IAA from AUX1 was investigated with a series of auxin analogues and auxin transport inhibitors. All compounds tested were able to completely displace (>95%) the binding of IAA to AUX1 (Figure 6A-E). The specificity of AUX1:competitor interactions was substantiated by the failure of a structurally unrelated weak organic acid (benzoic acid) to displace binding in the same concentration range (Figure 6F). By determining full dose-response curves for the displacement of IAA binding to AUX1 we were able to determine IC_{50} values (i.e. the concentration required to reduce the specific binding of IAA to AUX1 by 50%) for 5 selected compounds (Figure 6A-E, Table 1). IC_{50} values represent a measure of the relative affinity of each compound for IAA binding site on AUX1. The synthetic auxin 2,4-D, which requires a carrier for uptake into cells (Delbarre et al., 1996) inhibits IAA biding to AUX1 at pH 6.4 (at which value essentially all of the IAA and 2,4-D exist in the

anionic form) with an IC_{50} of 40 μM. The freely membrane permeable auxin 1-naphthylacetic acid (1-NAA; (Delbarre et al., 1996)) also inhibits binding of IAA to AUX1 with a comparable IC_{50} (Figure 6b). The IC_{50}s of the auxin influx inhibitors 1-naphthoxyacetic acid (1-NOA) and 3-chloro-4-hydroxyphenoxyacetic acid (CHPAA) are not significantly different to this those of 1-NAA and 2,4-D ($P > 0.1$), suggesting they have comparable affinities for AUX1. In contrast, the auxin influx inhibitor 2-naphthylacetic acid (2-NAA has an IC_{50} of 3.6 μM suggesting an approximate 10-fold more effective displacement of IAA in our assay conditions.

Discussion

The transport of auxin into plant cells occurs down both a proton and auxin concentration gradient (Lomax et al., 1995). Members of the AAAP family of membrane transporters (Saier, 2000) are proposed to be the primary auxin influx carriers in addition to which there is evidence of auxin import facilitated by the ABC transporter, AtABCB4 (previously known as AtPgp4) (Santelia et al., 2005; Terasaka et al., 2005; Cho et al., 2007; Verrier et al., 2008). The AUX/LAX family of membrane transporters, conserved in all higher plant species, are believed to act as $IAA:H^+$ symporters. In the current investigation we provide the first direct determination of the affinity of an auxin importer for its cognate ligand, although a preliminary estimate for the Kd of naphthylphthalamic acid binding to AtABCB1 (an auxin *efflux* pump previously known as AtMDR1 (Verrier et al., 2008)) expressed in yeast membranes has been obtained (Noh et al., 2001).

We believe that we are directly measuring IAA binding for three primary reasons. Firstly, we see essentially no specific interaction of IAA with Sf9 membranes isolated from non-AUX1 expressing cells. This suggests that anion trapping is not occurring in our experiments.

Consistent with this, our membrane preparations are formed in a low ionic strength buffer precluding the formation of vesicles with a sizeable internal volume, which we have confirmed by electron microscopy (data not shown). Finally, we show that IAA displacement is specific to auxin analogues since benzoic acid is not able to displace IAA binding. Our current data suggests that the initial event in the translocation pathway, namely the interaction of auxin with its ligand, occurs with a measured Kd of 2.6 μM (Figure 4), which compares well with the estimated auxin concentration in root tips of 1 μM (Ljung et al., 2001; Ljung et al., 2005). Similarly, our data is comparable to the Km for IAA transport measured in EYFP-AUX1 expressing *Xenopus* oocytes (0.8 μM; (Yang et al., 2006) and with earlier estimates for the Km for auxin transport in plant suspension cell culture experiments (1-5 μM; (Rubery and Sheldrake, 1974)).

Our pH profile for IAA binding to AUX1 affords further confidence that the function of AUX1 is largely preserved our heterologous system. The pH optimum for specific binding was observed between pH 5.0 and 6.0, where IAA would be expected to be 60-95% in the dissociated state. The reduction in observed specific binding at pH greater than 7.0 is consistent with a reduced interaction with IAA following transport of the hormone to the neutral cytoplasm. It is also tempting to speculate that one or more ionisable residues within AUX1 with pKas close to neutrality may be key in the transport process, as seen for conserved histidine residues in the related human proton-coupled amino acid transporter (Metzner et al., 2008).

The role of auxin in plant development has led to the development of numerous synthetic auxin analogues as specific inhibitors of the influx and efflux pathways. In the current study 2 synthetic auxins and 3 auxin influx inhibitors (Imhoff et al., 2000; Parry et al., 2001) were

analysed for their ability to displace IAA binding from AUX1. All were able to completely displace the bound IAA, and the IC_{50} values for this displacement can be compared with their relative abilities to inhibit radiolabelled 2,4-D uptake into tobacco suspension cells (Delbarre et al., 1996; Imhoff et al., 2000). Notably, this study identifies 2-NAA as having the highest relative affinity of the auxin influx inhibitors analysed, which parallels the conclusions from suspension cell studies (Delbarre et al., 1996; Imhoff et al., 2000), membrane vesicle experiments (Jacobs and Hertel, 1978; Hertel et al., 1983) and tissue transport data (Sussman and Goldsmith, 1981). There is an approximate 10-fold difference in our measured IC_{50} values (low micromolar) and the values responsible for inhibition of 2,4-D uptake (usually 10-fold higher; (Delbarre et al., 1996; Imhoff et al., 2000)). Since IC_{50} values are strictly assay dependent with variation due to, for example, protein concentration and temperature, direct comparisons of the data are precluded.

The *aux1* agravitropic phenotype in plants is to some extent mirrored by an alternative mutation, *axr4*. Further analysis of this mutation has led to the determination that the AUX1 protein requires an endoplasmic reticulum localized accessory protein, AXR4, for correct targeting to the plasma membrane (Dharmasiri et al., 2006; Hobbie, 2006). In the current study, and in heterologous system transport studies, it has now been determined that the overall Km for auxin transport and the Kd for auxin binding are similar to estimates for transport Km in plant cells (Yang et al., 2006). Since AXR4 homologues are apparently absent in insects (Adams et al., 2000) and amphibians, this reinforces the suggestion that although AUX1 requires AXR4 for targeting in plants, it is not a fundamental requirement for correct function.

The elucidation of the K_d for IAA binding to AUX1, together with the recent determination of the Km for transport (Yang et al., 2006) and the generation of a set of allelic AUX1 isoforms (Swarup et al., 2004) are all important steps towards a full biochemical understanding of the transport cycle of AUX1, which has important implications for our understanding of the generation and maintenance of physiologically relevant auxin gradients (Kramer and Bennett, 2006), and for mathematical modelling of such processes.

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Table 1. Displacement of IAA by related auxin transport inhibitors.

	2,4-D	1-NOA	1-NAA	2-NAA	CHPAA
IC_{50} (s.d.)	39.7 (14.6)	53.6 (22.0)	70.8 (21.3)	3.6* (0.6)	32.4 (11.1)

* - denotes IC_{50} (μM) for 2-NAA is significantly different ($P < 0.01$) from other values (ANOVA).

Figure Legends

Figure 1.**High level expression of HA-tagged AUX1 in insect cell membranes.** Sf9 cells were infected with recombinant HA-AUX1 expressing baculovirus at the indicated multiplicity of infection (MOI) and harvested at the indicated hours post infection (hpi). Cell lysates (10 μg) were resolved on 10% SDS-PAGE gels, electroblotted and recombinant AUX1 protein (ca. 40-45 kDa) identified by western blotting with anti-HA monoclonal antibodies.

Figure 2. **Filtration binding assays fail to display linear non-specific binding of IAA to HA-AUX1 containing membranes.** [^{3}H]-IAA was incubated with increasing quantities of HA-AUX1 membranes for 90 minutes at room temperature and bound ligand separated from free ligand by rapid vacuum filtration. Filters were washed and non-specifically bound radioactivity determined as described in the methods.

Figure 3. **Saturation binding of IAA to HA-AUX1**. **A.** The affinity of IAA binding to HA-AUX1 was determined by merging several independent data sets, each of which represents a homologous displacement assay using increasing concentrations of unlabelled IAA to extend the saturation isotherm, as described in the Results,. Fitting of a standard binding isotherm to the data reported a Kd of 2.6 μM. **B.** The linearity of non-specific binding of [^{3}H]-IAA to HA-AUX1 membranes with respect to the amount of protein in the assay (left panel), and the amount of ligand (right panel) is displayed.

Figure 4. **Time dependence of auxin binding**. AUX1 membranes were incubated at 21ºC (**A,** filled triangles) or 2ºC (**B,** open squares) with 50 nM [^{3}H]-IAA for the indicated time intervals. Specific binding was determined as described in the methods and is represented as

a % of the maximum specific binding observed. Data are fitted to a single-phase exponential isotherm and the $t_{1/2}$ values are 0.9s at 21ºC and 2.0s at 2ºC.

Figure 5. **IAA displays a pH-dependent binding to HA-AUX1 containing membranes.** Binding assays were performed as described with a fixed concentration of [^{3}H]-IAA (500 nM), with membranes prepared by centrifugation and re-suspension in citrate:phosphate buffers at the indicated pH. After 90 minutes at room temperature bound IAA was separated from free IAA and the % maximal specific binding (left-hand axis) is plotted as a function of pH. The % of anionic IAA (right-hand axis) at each pH is displayed as a dotted line.

Figure 6. **IAA binding is displaced by competing auxinic compounds**. IAA binding to HA-AUX1 containing membranes was allowed to come to equilibrium in the presence of increasing concentrations of the indicated competing ligands. After 90 minutes, the specific binding of IAA was determined and plotted as a function of the competing ligand concentration, and fitted to the general dose response curve to determine an IC_{50} for each compound.

Figure 1.

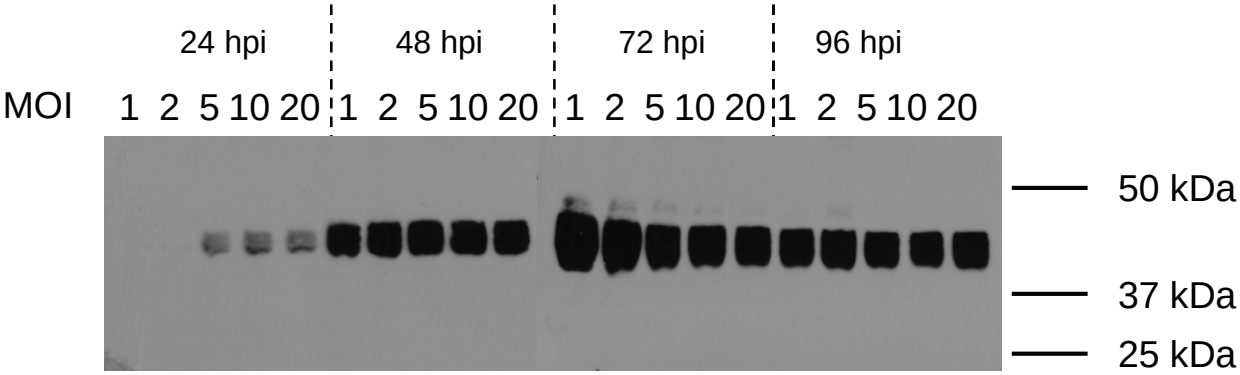


Figure 1.**High level expression of HA-tagged AUX1 in insect cell membranes.** Sf9 cells were infected with recombinant HA-AUX1 expressing baculovirus at the indicated multiplicity of infection (MOI) and harvested at the indicated hours post infection (hpi). Cell lysates (10 µg) were resolved on 10% SDS-PAGE gels, electroblotted and recombinant AUX1 protein (ca. 40-45 kDa) identified by western blotting with anti-HA monoclonal antibodies.

Figure 2.

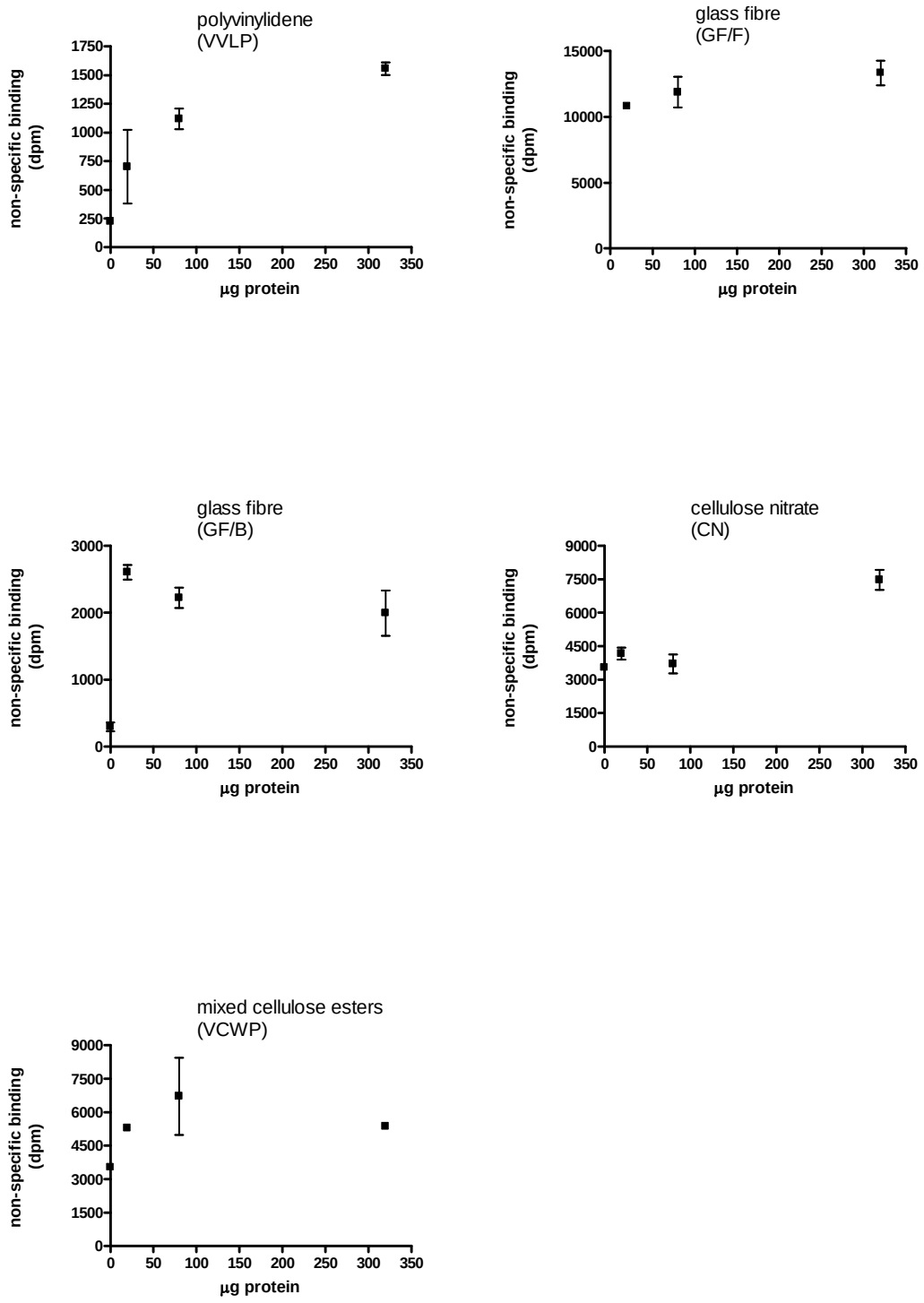
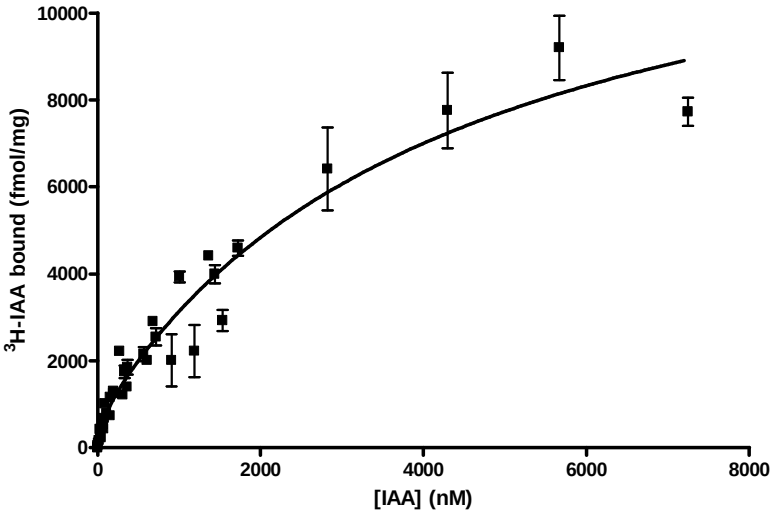

polyvinylidene
(VVLP)
glass fibre
(GF/F)
glass fibre
(GF/B)
cellulose nitrate
(CN)
mixed cellulose esters
(VCWP)
non-specific binding
(dpm)
µg protein

Figure 2. **Filtration binding assays fail to display linear non-specific binding of IAA to HA-AUX1 containing membranes.** [3H]-IAA was incubated with increasing quantities of HA-AUX1 membranes for 90 minutes at room temperature and bound ligand separated from free ligand by rapid vacuum filtration. Filters were washed and non-specifically bound radioactivity determined as described in the methods.

Figure 3.

A.



B.

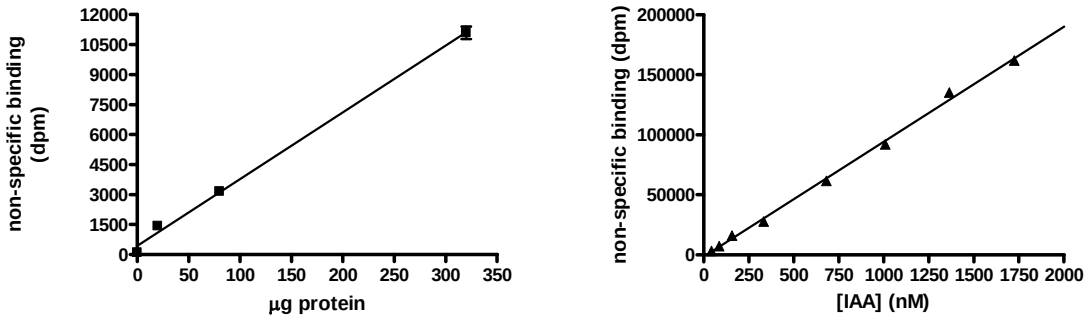
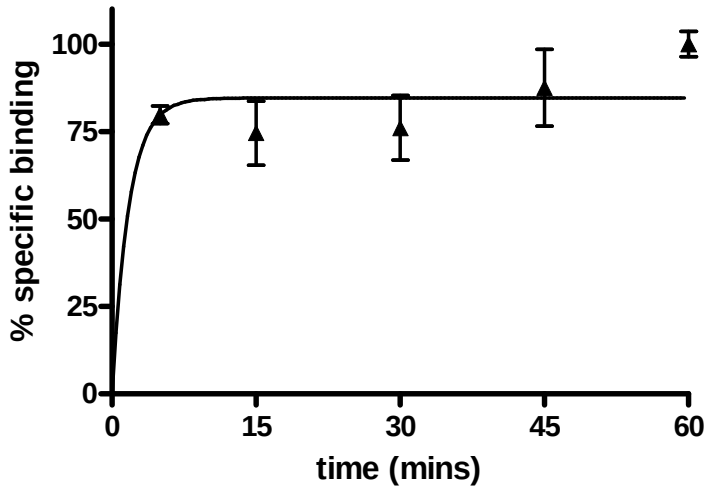


Figure 3. **Saturation binding of IAA to HA-AUX1**. **A.** The affinity of IAA binding to HA-AUX1 was determined by merging several independent data sets, each of which represents a homologous displacement assay using increasing concentrations of unlabelled IAA to extend the saturation isotherm, as described in the Results,. Fitting of a standard binding isotherm to the data reported a Kd of 2.6 µM. **B.** The linearity of non-specific binding of [3H]-IAA to HA-AUX1 membranes with respect to the amount of protein in the assay (left panel), and the amount of ligand (right panel) is displayed.

Figure 4.

A.



B.

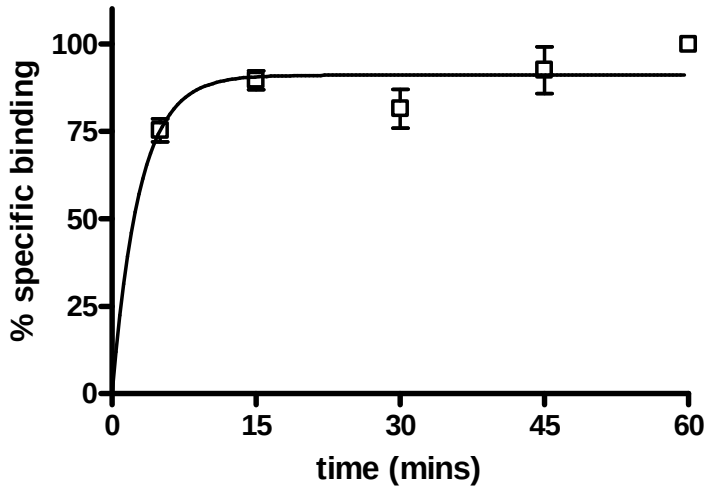


Figure 4. **Time dependence of auxin binding**. AUX1 membranes were incubated at 21ºC (**A,** filled triangles) or 2ºC (**B,** open squares) with 50 nM [3H]-IAA for the indicated time intervals. Specific binding was determined as described in the methods and is represented as a % of the maximum specific binding observed. Data are fitted to a single-phase exponential isotherm and the t1/2 values are 0.9s at 21ºC and 2.0s at 2ºC.

Figure 5.

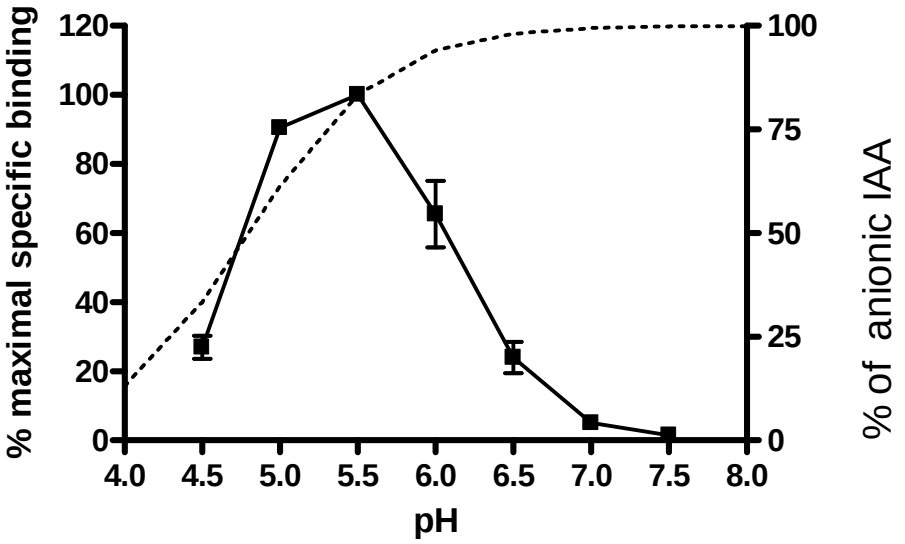


Figure 5. **IAA displays a pH-dependent binding to HA-AUX1 containing membranes.** Binding assays were performed as described with a fixed concentration of [3H]-IAA (500 nM), with membranes prepared by centrifugation and re-suspension in citrate:phosphate buffers at the indicated pH. After 90 minutes at room temperature bound IAA was separated from free IAA and the % maximal specific binding (left-hand axis) is plotted as a function of pH. The % of anionic IAA (right-hand axis) at each pH is displayed as a dotted line.

Figure 6.

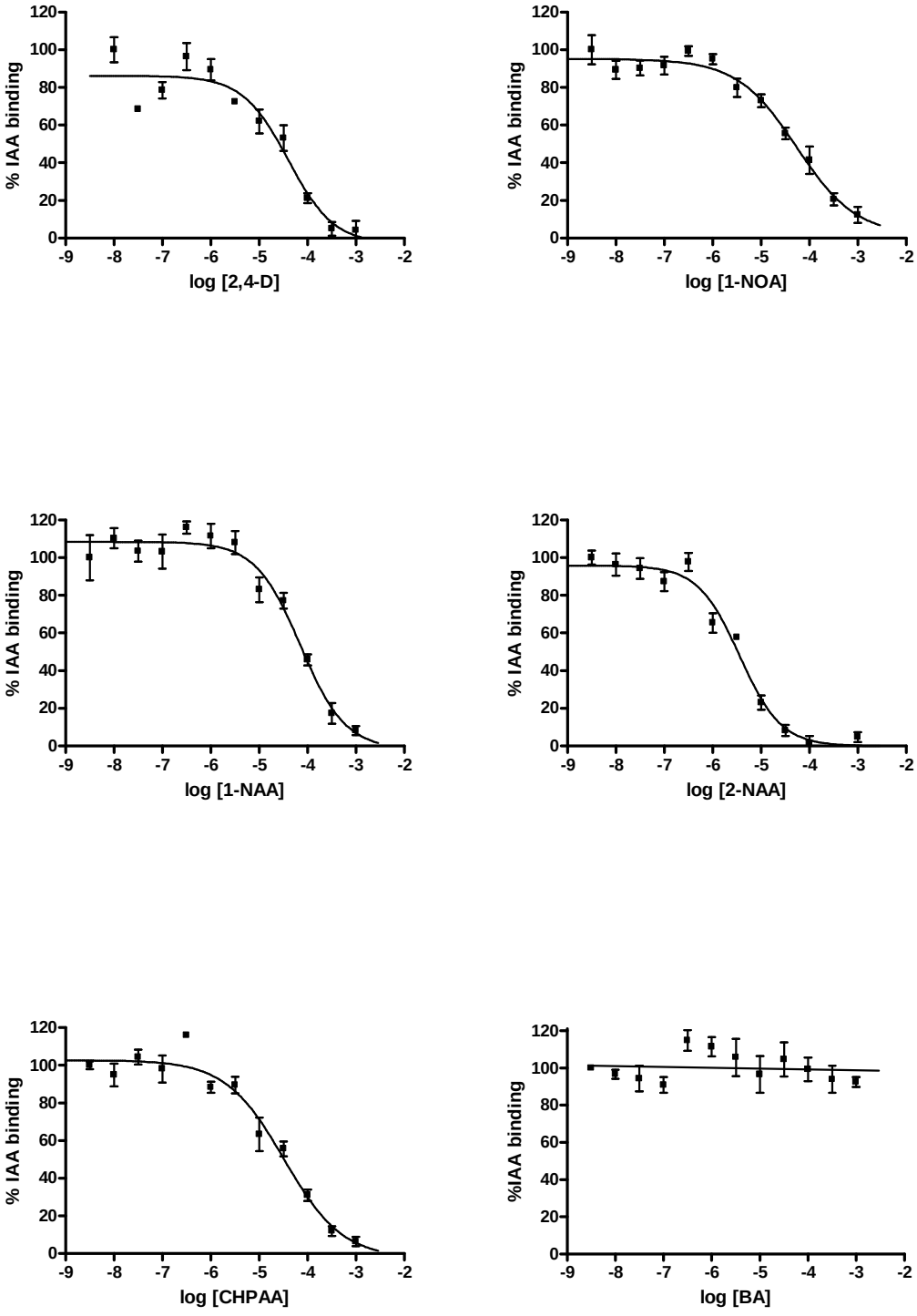

% IAA binding
log [2,4-D]
log [1-NOA]
log [1-NAA]
log [2-NAA]
log [CHPAA]
%IAA binding
log [BA]

Figure 6. **IAA binding is displaced by competing auxinic compounds**. IAA binding to HA-AUX1 containing membranes was allowed to come to equilibrium in the presence of increasing concentrations of the indicated competing ligands. After 90 minutes, the specific binding of IAA was determined and plotted as a function of the competing ligand concentration, and fitted to the general dose response curve to determine an IC50 for each compound.