



## Research paper

# Interactions between indole-3-acetic acid (IAA) with a lectin from *Canavalia maritima* seeds reveal a new function for lectins in plant physiology



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## ABSTRACT

Indole-3-acetic acid (IAA) bound is considered a storage molecule and is inactive. However, some studies have proposed an additional possible regulatory mechanism based on the ability of lectins to form complexes with IAA. We report the first crystal structure of ConM in complex with IAA at 2.15 Å resolution. Based on a tetrameric model of the complex, we hypothesize how the lectin controls the availability of IAA during the early seedling stages, indicating a possible new physiological role for these proteins. A free indole group is also bound to the protein. The ConM interaction with different forms of IAA is a strategy to render the phytohormone unavailable to the cell. Thus, this new physiological role proposed for legume lectins might be a novel mechanism by which IAA levels are decreased in addition to the destruction and formation of new complexes in the later stages of seed germination.

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## 1. Introduction

Auxin is a term used for substances that act like indole-3-acetic acid (IAA) in the processes of photo- and gravitropism [1], inhibit the growth of primary roots and stimulate the development of lateral roots [2]. IAA was the first auxin described and is the most abundant form of this phytohormone [3]. In addition to the activities listed above, IAA is involved in cell division, growth and differentiation [4,5], pollen development [6], leafy formation [7] and embryogenesis [8,9]. All of these functions are regulated by a gradient established by the balance between IAA biosynthesis and its directional transport. IAA biosynthesis may be dependent on [10] or independent of [11,12] tryptophan. Furthermore, the properties of IAA can be understood by its ability to induct gene

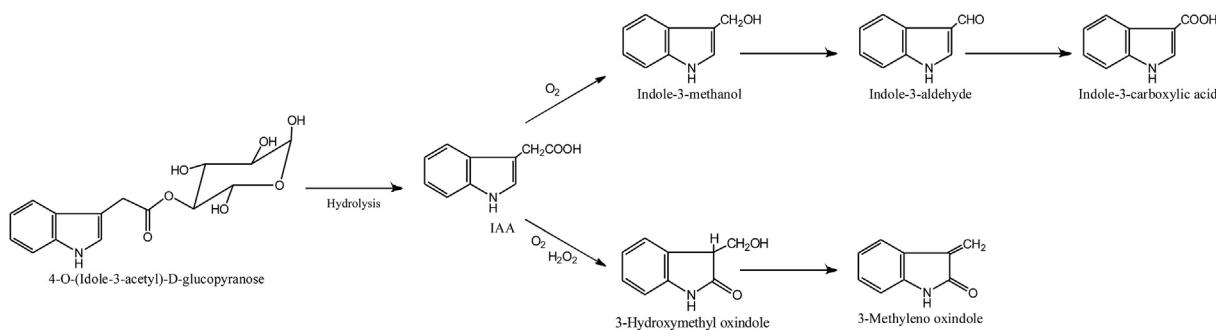
expression [13], which occurs through the nuclear auxin receptor F-box protein TIR1 [14,15]. However, other studies indicate that the activity of IAA might occur without a change in gene expression, such as via the activation of H<sup>+</sup>-ATPases, the elevation of cytosolic calcium levels [16,17] due the fast response stimulated by the hormone [18,19].

IAA exists in a variety of chemical states. Free auxins are the most active form of IAA, whereas bound auxin is considered a storage molecule and is inactive (Fig. 1). The most common inactive states are auxin glycosyl esters. This is the major form of auxin found in seeds and storage organs [20]. These conjugates are formed through the activity of UDP-glucosyltransferases, which link the IAA carboxyl group to mono- or disaccharides, generating low molecular weight compounds, or to polysaccharides or carbohydrate components of glycoproteins to generate high molecular weight compounds [21,22]. Although there is evidence for the hydrolysis of the compounds [23,24], this process is less understood. Some studies indicate that free endogenous IAA levels in diverse tissues and organs, such as seeds, are very low [25], a characteristic that is explained by the covalent conjugation of IAA with sugars, amino acids and peptides [26]. In some studies with bean seeds, for example, IAA is conjugated with amides before germination [27]. At

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**Fig. 1.** IAA chemical states. Hydrolysis of glycosylated IAA and the oxidative pathway of IAA in the presence or absence of hydrogen peroxide.

the early stages of development, IAA levels increase rapidly, which could be interpreted as the consequence of the enzymatic breakdown of this compound [28].

Additionally, IAA can be regulated by oxidative pathways. There are two basic oxidative processes: (1) enzymatic reactions and (2) photooxidation. Enzymatic destruction, which is catalyzed by IAA oxidase, is the more important process (Fig. 1). There are three primary mechanisms by which the level of free auxin in tissues is regulated: synthesis, glycosylation and oxidation. Some studies have suggested an additional possible regulatory mechanism based on the ability of lectins to form complexes with IAA [29].

Lectins are a class of proteins characterized by their capability to interact specifically and reversibly to mono- and oligosaccharides [30,31]. The biological and physiological functions of plant lectins are related to the ability of these proteins to decipher the glycocodes on the surface of cells, once lectin properties are completely abolished by specific sugars [30,32–34]. However, lectins from leguminous and other plant families, as well as other organisms [35], might also interact with other molecules, such as nonprotein amino acids [36] and hydrophobic compounds [29,37–39]. In the latter case, studies have been performed with the fluorescent probes ANS (8-anilinonaphthalene-1-sulfonate) and TNS (2- $\rho$ -toluidinonaphthalene-6-sulfonate) [37,40], adenine [41–43], hydrophobic sugar derivatives [44,45] and the auxin IAA [29].

ConM is a lectin isolated from the seeds of the leguminous plant *Canavalia maritima*, which is commonly known as the bay bean, sand bean, beach bean or MacKenzie bean. ConM is a 25.5 kDa protein with 237 amino acid residues per monomer. This lectin shares a 98% identity with the lectin from *Canavalia ensiformis* (ConA), which is the most commonly studied sugar-binding protein from leguminous plants [46]. Supporting the fact that ConM is a ConA-like lectin, Ramos and collaborators [47] reported that ConM has an affinity for several monosaccharides, especially mannose and glucose, although the affinity for oligosaccharides is different between ConM and ConA.

Although the biological properties of lectins are well characterized [46,48], the contribution of these proteins to plant physiology is not fully understood. In this work, we report the first crystal structure of ConM in complex with IAA, demonstrating that this phytohormone binds in a similar manner to other hydrophobic compounds shown to bind to ConA [39]. Based on a tetrameric model of the complex, we hypothesize how lectin controls the availability of IAA during early seedling stages, indicating a possible new physiological role for these proteins.

## 2. Material and methods

### 2.1. Purification and haemagglutinating activity of ConM

Seeds from *C. maritima* were ground to a fine powder in a coffee mill and the soluble proteins were extracted at 298 K by continuous

stirring with 0.15 M NaCl [1:10 (w:v)] for 4 h, followed by centrifugation at 10,000  $\times$  g at 277 K for 20 min. Protein purification was carried by the affinity chromatography protocol previously described by Ramos and co-workers [47] using Sephadex G-50 column (10  $\times$  50 cm). The ConM was complexed with IAA in presence of light for 30 min at 310 K and haemagglutinating activity (HA) was done to confirming that indole binding does not alter the carbohydrate recognition. HA was determined in plates by double serial dilutions. Each well received 25  $\mu$ L 0.1 M Tris–HCl buffer pH 7.6 containing 0.15 M NaCl and 5 mM CaCl<sub>2</sub> and 5 mM MgCl<sub>2</sub>. A 100  $\mu$ L aliquot of supernatant was added to the first well of the column. Subsequently, 200  $\mu$ L suspension of 2% rabbit erythrocytes suspension containing 0.15 M NaCl was added to each well. HA was measured after 30 min of incubation at 310 K and 30 min of incubation at room temperature.

### 2.2. Crystallization and data collection

Lyophilized ConM was dissolved to a concentration of 10 mg ml<sup>-1</sup> in 20 mM Tris–HCl pH 7.6 containing 0.5 mM IAA. Co-crystals from the ConM–IAA complex suitable for X-ray diffraction experiments were obtained according to the protocol described by Gadelha and collaborators [49]. X-ray data were collected at a wavelength of 1.433 Å using a synchrotron radiation source (MX1 station, Brazilian Synchrotron Light Laboratory – LNLS, Campinas, Brazil). A complete data set was obtained using a MARCCD 165 detector (MAR Research) in 120 frames with an oscillation range of 1°. The diffraction data were indexed, integrated and scaled using MOSFLM and SCALA [50]. The crystal belongs to the orthorhombic space-group I222.

### 2.3. Molecular replacement and refinement

The crystal structure was determined by molecular replacement method using MolRep [51]. The atomic coordinates used as a model were obtained from ConM complexed with trehalose and maltose (PDB code 2CYF) [52], which had, after rotation and translation function calculations, a final correlation coefficient of 72.9% and an  $R_{\text{factor}}$  of 39.3%.

The initial structure was submitted as a rigid body and refined using REFMAC5 [50]. The  $R_{\text{factor}}$  and  $R_{\text{free}}$  converged to 23.41% and 25.59%, respectively. The structure was modeled using COOT [53]. The IAA molecule was added to the structure using COOT [53]. A poor electronic density was observed in the region between residues 117 and 122, and it was not possible to add the corresponding residues (Ser117, Asn118, Thr119, Ser120, His121 and Glu122) to the density, which has been reported in several other studies of this class of proteins [36,54]. A total of 60 water molecules were added using COOT [53], and a second restrained refinement was performed resulting in a  $R_{\text{factor}}$  of 20.63% and an  $R_{\text{free}}$  of 22.54%.

## 2.4. Molecular docking

Glycosylated IAA was used to verify the carbohydrate binding properties of the ConM–IAA/indole structure. Molecular docking was performed with HEX 6.3 version Molecular Graphics Program. The Hex is a fast molecular docking program for calculating and displaying interactions and feasible docking modes of pairs of protein and DNA molecules. It can superpose pairs of molecules using only knowledge of their 3D shapes and calculate protein–ligand interactions using spherical polar Fourier (SPF) correlations to accelerate the docking calculations [55].

## 3. Results

### 3.1. Purification and haemagglutinating activity

ConM was purified by affinity chromatography in single step purification and the hemagglutination assay permitted to determine the hemagglutination titer of 256 HU. ConM complexed with IAA and indole group shows haemagglutinating activity against native rabbit erythrocytes confirming that indole group binding does not alter the carbohydrate recognition domain (CRD) configuration or inhibit the carbohydrate binding, maintaining the same titer.

### 3.2. Overall structure

The unit cell parameters for the orthorhombic crystal structure of the ConM complexed with IAA were as follows:  $a = 67.1 \text{ \AA}$ ,  $b = 70.7 \text{ \AA}$  and  $c = 97.7 \text{ \AA}$ , and the Matthews coefficient was  $2.32 \text{ \AA}^3 \text{ Da}^{-1}$ , indicating the presence of a monomer in the asymmetric unit. The final refinement statistics and structure analysis are shown in Table 1.

The refined structure of ConM–IAA complex at a  $2.15 \text{ \AA}$  is a tetramer in biological assemble consisting of two “canonical” dimers linked by salt bridges between  $\beta$  strands and by IAA–protein interactions (Fig. 2). The atomic coordinates for the structure were deposited in the Protein Data Bank (PDB) with the access code 3SNM.

The IAA strong ( $F_{\text{o}} - F_{\text{c}}$  map –  $3 \sigma$ ) electron density was found in the polar central cavity of the ConM tetramer structure (Fig. 3A). The IAA molecule was positioned in the electron density map and refined. The omit map confirmed the presence of the ligand. Upon inspecting the structure, an electron density was observed near the carbohydrate binding site. This density could be due to a free indole group, the oxidation product of IAA photodecomposition. The indole was positioned in the site and fully embedded in the electron density map (Fig. 3B). The overall interactions are shown in Table 2.

### 3.3. Interaction with indole-3-acetic acid (IAA)

IAA interacts with the protein through hydrogen bonds (Table 3) and van der Waals forces via residues Ser108 and Asn131 and two water molecules number 26 and 31 (Fig. 3A). The OG oxygen from Ser108 formed hydrogen bonds with the O2 and O3 oxygen atoms of IAA of  $3.0$  and  $2.8 \text{ \AA}$ , respectively. The IAA O2 oxygen atom also formed two additional hydrogen bonds of  $3.4$  and  $3.3 \text{ \AA}$  lengths with water molecules 26 and 31, respectively. The OD1 hydroxyl oxygen from Asn131 and the O3 oxygen of IAA formed a hydrogen bond of  $2.3 \text{ \AA}$  in length. van der Waals interactions occurred between the O2 oxygen and C18 carbon of IAA and the Ser108 CB carbon atom, which were  $3.4$  and  $3.1 \text{ \AA}$  apart, respectively. These interactions also were formed between Asn131 OD1 oxygen and C7 carbon of IAA.

The tetrameric structure was generated by crystallographic symmetry to verify the relative position of the IAA molecule in

**Table 1**

The data collection, refinement statistics and structure analysis.

Parameter	Value
<i>Data collection</i>	
Beamline wavelength	$1.42 \text{ \AA}$
Space group	I222
Unit cell parameters ( $\text{\AA}$ )	
$a$	$67.1$
$b$	$70.7$
$c$	$97.7$
Total reflections	$83,787$
Number of unique reflections	$12,629$
Protein molecules per asymmetric unit	$1$
$R_{\text{merge}}^{\text{a}} (\%)$	$11.5 (24.4)^{\text{d}}$
Completeness (%)	$97.2$
Multiplicity	$3.2$
$I/\sigma$	$4.0 (2.8)^{\text{d}}$
<i>Refinement</i>	
Resolution range ( $\text{\AA}$ )	$24.36$ – $2.15$
$R_{\text{factor}}^{\text{b}} (\%)$	$20.6$
$R_{\text{free}}^{\text{c}} (\%)$	$22.5$
Number of residues in asymmetric unit	$236$
Number of water molecules	$60$
<i>RMS deviations from ideal values</i>	
Bond lengths ( $\text{\AA}$ )	$0.026$
Bond angles (degrees)	$2.654$
<i>Temperature factor</i>	
Average B value for whole protein chain ( $\text{\AA}^2$ )	$28.1$
<i>Ramachandran plot</i>	
Residues in most favored regions (%)	$90.75$
Residues in additional allowed regions (%)	$9.25$
Residues in generously allowed regions (%)	$0$

$$\text{a } R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I(hkl)_i - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i |I(hkl)_i|} \text{ where } I(hkl)_i \text{ is the intensity of ith measurement}$$

of reflection  $h$  and  $\langle I(hkl) \rangle$  is the mean value of the  $I(hkl)_i$  for all I measurements.

$$\text{b } R_{\text{factor}} = \frac{\sum_h |F_{\text{obs}} - |F_{\text{calc}}||}{\sum_h |F_{\text{obs}}|}$$

c Calculated with 5% of the reflections omitted from refinement.

d Values in parentheses represent the high resolution shell.

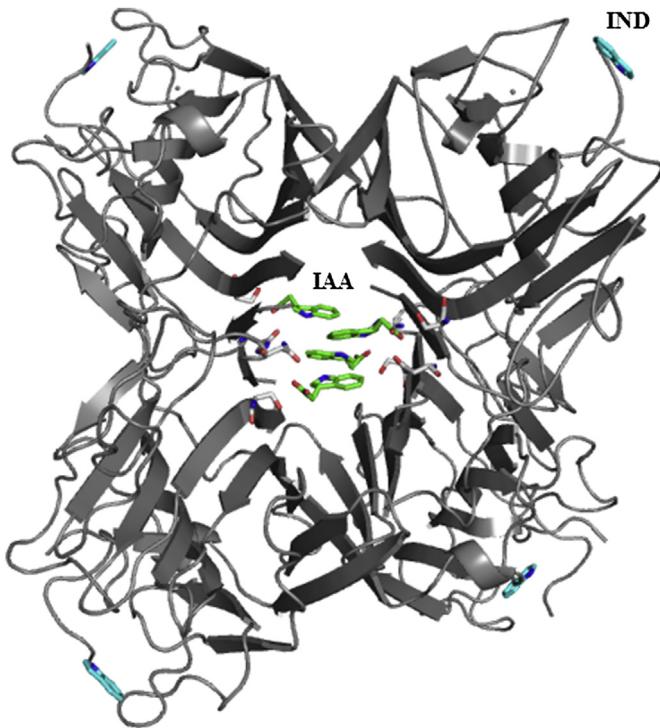
relation to the biological assembly of ConM. IAA molecules were positioned in the central cavity. The structural IAAAs were positioned in a face-to-face arrangement relative to the indole group. Stacking hydrophobic interactions between the IAA rings stabilized the tetrameric form of ConM (Fig. 4). The amino acids that stabilize the hydrophobic pocket are Ser108, Ser110, Val129, Asn131, Thr194, and Thr196.

### 3.4. Indole ring interactions

Indole formed hydrogen bonds and van der Waals interactions with residues Asn14, Asp16 and Arg228, which are part of the carbohydrate recognition domain (CRD) of *Diocleinae* lectins (Table 2). The presence of indole induced changes in the structural arrangement of a part of the CRD. First, the side chain of Asp16 was rotated by  $90^\circ$  in relation to its position in the crystal structure of native carbohydrate-bound ConM. Furthermore, two water molecules that normally interact with the side chain of Arg228 were no longer within binding distance, being  $5.0$  and  $3.9 \text{ \AA}$  away from the NE and NH<sub>2</sub> atoms, respectively.

### 3.5. Molecular docking of inactive IAA form

Molecular docking was used to dock the glycosylated form of IAA onto ConM to verify the interaction between the inactive form of IAA with the CRD trough carbohydrate motif. The best results of the molecular docking showed the glycosylated IAA interacting via its carbohydrate motif in a conventional arrangement through



**Fig. 2.** Jelly-roll motif of ConM monomers displayed as tetramer. The native form of ConM with common  $\beta$  sheet bound with four IAA molecules. These IAA molecules interact themselves through stacking hydrophobic interactions. Indole (IND) is represented in blue near CRD domain and IAA in green in the center of the tetramer.

conserved hydrogen bonds with Asp208 (Fig. 5). The indole in the hydrophobic sub-site of CRD does not alter the binding of glycosylated IAA through the carbohydrate binding site as confirmed by haemagglutinating activity.

#### 4. Discussion

##### 4.1. ConM binds to active and inactive IAA forms

IAA was found near the site previously shown to bind to alpha-aminobutyric acid (ABU) in the *Canavalia gladiata* lectin (CGL) [36], but in a lateral position relative to the canonical dimer. According to the structure, IAA would be released upon dissociation of the

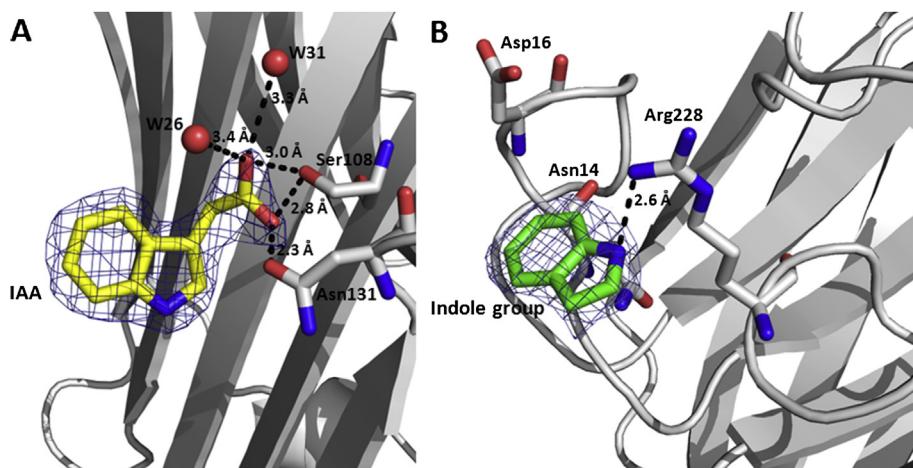
tetramer into dimers. Previous studies have shown a pH-dependent equilibrium between the dimer and tetramer: under acidic conditions ( $\sim$ pH 5.5), the dimeric form predominates, whereas under neutral conditions ( $\text{pH} \sim 7.5$ ), the tetrameric form predominates [56,57].

ConA-like structures showed poor electron density for the surface loop at residues 118–122, but when bound to alpha-aminobutyric acid (Abu), the stability of the dimer of CGL increased, allowing visualization of electron density in the loop region of 117–123 [36]. This improvement in the electron density map occurred due to the positioning of the Abu site and permitted intermonomer interactions that stabilized the canonical dimer. Similar effects were not observed with ConM bound to IAA because the positioning of the IAA binding site allowed only interconnections between the dimers, but do not present a strong capacity of stabilize the canonical dimer.

The tetrameric contacts were established by a few salt bridges and by IAA; tetramer stabilization by IAA as bridge between dimers may also involve indirect dimer stabilization, as part of the tetramer (Fig. 2). These features are different from the interaction between Abu and CGL. The IAA and ConM interactions seem to be a path to enable rapid availability of IAA without compromising the structural aspects of lectin.

Some studies have proposed the existence of two independent hydrophobic sites in lectins. For example, Yang and collaborators [40] identified a single high-affinity binding site in native ConA for a fluorescent hydrophobic probe. Edelman and Wang [29] reported that tryptophan and IAA bind with low affinity to ConA, proposing the existence of one site on each of the four subunits of ConA. Additionally, Roberts and Goldstein [37] showed a single high affinity binding site for TNS in the lima beans lectins and four low affinity sites for the same probe, one in each monomer. In summary, ConM could have a single high affinity site for hydrophobic compounds per tetramer and one low affinity site per monomer. Based on previous studies, the high affinity site or hydrophobic cavity in ConA is surrounded by following residues: Tyr54, Leu81, Leu85, Val89, Val91, Phe111, Ser113, Val179, Ile181, Phe191, Phe212 and Ile214 [58,59].

The IAA ligand site in ConM is surrounded by Ser108 and Asn131. This site is similar to that demonstrated by Hardman and Ainsworth [39] for minor peaks viewed in crystals of ConA soaked with iodobenzoic acid and phenyl phosphate. Thus, based on this and on the tetrameric model for ConM in complex with IAA, four low affinity binding sites for hydrophobic substances, which are approximately 21.9 Å (based on the distance between Tyr54 and



**Fig. 3.** Electron density maps for ligands. A) The IAA electron density map contoured  $1\sigma$  and hydrophilic interactions between IAA and the side chains of Ser108 and Asn131 and water molecules 26 and 31 by hydrogen bonds. B) The indole group electron density map contoured  $1\sigma$ .

**Table 2**

The overall interactions between ConM and indole group.

Indole group	Asn14				Arg228	
	ND2	C	CA	CB	NE	NH1
N1	3.5	—	—	—	3.4	2.6 <sup>a</sup>
C6	—	3.4	3.3	3.3	—	—
C7	—	—	—	3.1	—	—

<sup>a</sup> Hydrogen bonds.

Asn131 Cα carbons) from the hydrophobic cavity, were demonstrated. Additionally, we hypothesize that the absence of IAA in the hydrophobic high affinity site is due its negative charge, which might impede its binding stereochemically.

IAA is present in seeds during germination as a result of the breakdown of its conjugated forms with sugars, amino acids and peptides [26]. Lectins are abundant in seeds, especially in the protein bodies of cotyledony cells, although, like ConA, they may also be present in the cytoplasm [60]. Studies of germination in some legume seeds demonstrated that the lectin content increases during germination and early seedling stages [61,62]. Taking these data into account, as well as the facts described in the previous paragraph, we hypothesize that the ability of ConM to interact with IAA may be a strategy to render the phytohormone unavailable to the cell to protect radicles from its inhibitory activity. Thus, this new physiological role proposed for legume lectins might be a novel mechanism, in addition to the destruction and formation of new complexes, by which IAA levels can be decreased in the later stages of seed germination [63,64].

Furthermore, because abnormally high IAA levels produced by a class of pathogens known as BIPs (Bacterial Indole acetic acid Producers) might be related to the development of diseases in several plant species [65], we suggest that the ability of lectins to interact with IAA could also be a defense mechanism against these pathogens.

#### 4.2. Indole unusually binds to carbohydrate recognition domain

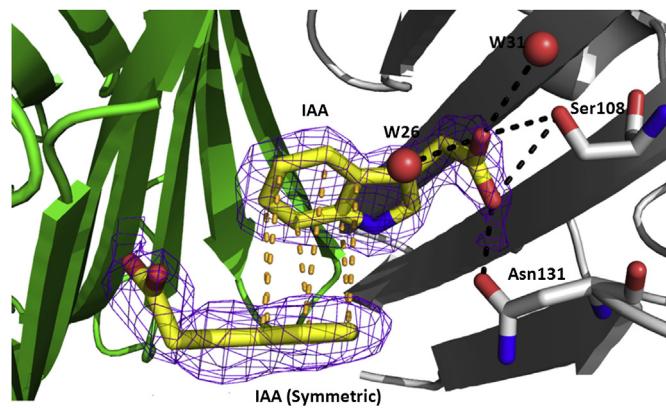
The coordination of the free indole by residues from the CRD was not expected. Although Kanellopoulos [45] has reported the coordination of 4'-nitrophenyl- $\alpha$ -D-mannopyranoside and 4'-nitrophenyl- $\alpha$ -D-glucopyranoside by the four CRDs of tetrameric ConA in which the hydrophobic element interacts with the side chains of Tyr12, Leu99 and Tyr100, the interactions observed in this study are significantly different because the indole is coordinated by polar residues; however, the conformational change of the side chain of Asp16 cannot be neglected. Previously, a study performed by Hardman and Ainsworth [39] showed that the hydrophobic sugar derivative o-iodophenyl  $\beta$ -D-glucopyranoside also binds to the ConA CRD, but the authors argued that this interaction was due to the glycan element. Thus, based on these studies and on the specificity of CRD for carbohydrates, the presence of free indole in this site is an unusual phenomenon.

To understand the plant physiological implication for this interaction, we hypothesize two possible explanations: first, the

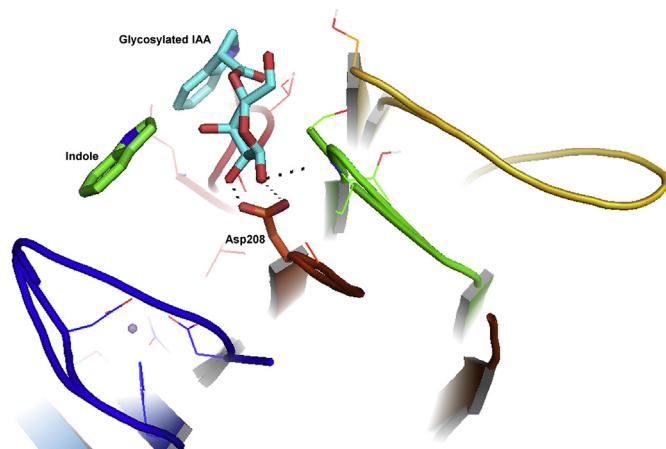
**Table 3**

The overall interactions between ConM and IAA.

IAA	Ser108		Asn131	HOH26	HOH31
	OG	CB			
O2	3.0 <sup>a</sup>	3.4	—	3.4 <sup>a</sup>	3.3 <sup>a</sup>
O3	2.8 <sup>a</sup>	—	2.3 <sup>a</sup>	—	—
C18	—	3.1	—	—	—
C7	—	—	3.4	—	—

<sup>a</sup> Hydrogen bonds.

**Fig. 4.** Interactions that stabilize ligand binding. IAA molecules positioned with a face-to-face arrangement relative to the indole group in the ConM dimeric interface. Yellow dashes represent hydrophobic interactions and black dashes displays hydrogen bonds. Red balls are water molecules.



**Fig. 5.** Molecular docking with glycosylated IAA (cyan), which interacts via its carbohydrate motif in a conventional arrangement by hydrogen bonds with Asp208 (orange). The indole (green) in the hydrophobic sub-site of the CRD does not affect the binding.

presence of indole might be indicative that glycosinolates, which are involved in plant defense [66], can be coordinated in this site; thus, lectin could act as a transporter of these compounds. Second, because free indole is a volatile chemical that several plant species produce and release in response to a herbivore attack to attract its predators [67,68], lectins could act to regulate indole availability.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.biochi.2013.05.008>.

## References

- [1] F. Went, On growth-accelerating substances in the coleoptile of *Avena sativa*, Proc. K. Ned. Akad. Wet. 30 (1926) 10–19.
- [2] I. Casimiro, A. Marchant, R.P. Bhalerao, T. Beekman, S. Dhooge, R. Swarup, N. Graham, D. Inzé, G. Sandberg, P.J. Casero, M. Bennett, Auxin transport promotes *Arabidopsis* lateral root initiation, Plant Cell. 13 (2001) 843–852.
- [3] C. Delker, A. Raschk, M. Quint, Auxin dynamics: the dazzling complexity of a small molecule's message, Planta 227 (2008) 929–941.
- [4] B. Commeron, D. Mazia, The mechanism of auxin action, Plant Physiol. 17 (1942) 682–685.
- [5] S. Vanneste, J. Friml, Auxin: a trigger for change in plant development, Cell 136 (2009) 1005–1016.
- [6] D.A. Ni, X.H. Yu, L.J. Wang, Z.H. Xu, Aberrant development of pollen in transgenic tobacco expressing bacterial *iaaM* gene driven by pollen- and tapetum-specific promoters, Acta Exp. Sin. 35 (2002) 1–6.
- [7] Y.K. He, W.X. Xue, Y.D. Sun, X.H. Yu, P.L. Liu, Leafy head formation of the progenies of transgenic plants of Chinese cabbage with exogenous auxin genes, Cell Res. 10 (2000) 151–162.
- [8] N.D. An, L.J. Wang, C.H. Ding, Z.H. Xu, Auxin distribution and transport during embryogenesis and seed germination of *Arabidopsis*, Cell Res. 11 (2001) 273–278.
- [9] C. Fisher-Iglesias, B. Sundberg, G. Neuhaus, A.M. Jones, Auxin distribution and transport during embryonic pattern formation in wheat, Plant J. 26 (2001) 115–129.
- [10] S.A. Gordon, Intracellular localization of the tryptophan-indoleacetate enzyme system, Plant Physiol. 33 (1958) 23–27.
- [11] A.D. Wright, M.B. Sampson, M.G. Neuffer, L. Michalczuk, J.P. Slovin, J.D. Cohen, Indole-3-acetic acid biosynthesis in the mutant maize orange pericarp, a tryptophan auxotroph, Science 254 (1991) 998–1000.
- [12] J. Normanly, J. Cohen, G. Fink, *Arabidopsis thaliana* auxotrophs reveal a tryptophan-independent biosynthetic pathway for indole-3-acetic acid, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 10355–10359.
- [13] I.A. Paponov, M. Paponov, W. Teale, M. Menges, S. Chakrabortee, J.A.H. Murray, K. Palme, Comprehensive transcriptome analysis of auxin responses in *Arabidopsis*, Mol. Plant 1 (2008) 321–337.
- [14] N. Dharmasiri, S. Dharmasiri, M. Stelle, The F-box protein TIR1 is an auxin receptor, Nature 435 (2005) 441–445.
- [15] K. Mockaitis, M. Stelle, Auxin receptors and plant development: a new signaling paradigm, Annu. Rev. Cell Dev. Biol. 24 (2008) 55–80.
- [16] M. Shishová, S. Lindberg, Auxin induces an increase in  $\text{Ca}^{2+}$  concentration in the cytosol of wheat leaf protoplasts, J. Plant Physiol. 161 (2004) 937–945.
- [17] T. Paciorek, E. Zazimalová, N. Ruthardt, J. Petrásek, Y.D. Stierhof, J. Kleine-Vehn, D.A. Morris, N. Emans, G. Jürgens, N. Geldner, J. Friml, Auxin inhibits endocytosis and promotes its own efflux from cells, Nature 435 (2005) 1251–1256.
- [18] A. Hager, G. Debus, H.G. Edel, H. Stransky, R. Serrano, Auxin induces exocytosis and the rapid synthesis of a high-turnover pool of plasma membrane  $\text{H}^{+}$ -ATPase, Planta 185 (1991) 527–537.
- [19] A. Hager, Role of the plasma membrane  $\text{H}^{+}$ -ATPase in auxin-induced elongation growth: historical and new aspects, J. Plant Res. 116 (2003) 483–505.
- [20] T.C. Moore, Biochemistry and Physiology of Plant Hormones, second ed., Springer-Verlag, New York, 1989.
- [21] A. Bajguz, A. Piotrowska, Conjugates of auxin and cytokinin, Phytochemistry 70 (2009) 957–969.
- [22] J. Ludwig-Müller, Auxin conjugates: their role for plant development and in the evolution of land plants, J. Exp. Bot. 62 (2011) 1757–1773.
- [23] A. Jakubowska, S. Kowalczyk, A.J. Leznicki, Enzymatic hydrolysis of 4-O and 6-O-indol-3-yiacetyl- $\beta$ -D-glucose in plant tissues, J. Plant Physiol. 142 (1993) 61–66.
- [24] A. Jakubowska, S. Kowalczyk, A specific enzyme hydrolyzing 6-O(4-O)-indole-3-yiacetyl- $\beta$ -D-glucose in immature kernels of *Zea mays*, J. Plant Physiol. 162 (2005) 207–213.
- [25] N.E. Good, W.A. Andrae, M.H.H. van Ysselstein, Studies on 3-indoleacetic acid metabolism. II. Some products of the metabolism of exogenous indoleacetic acid in plant tissues, Plant Physiol. 31 (1956) 231–235.
- [26] J. Normanly, Approaching cellular and molecular resolution of auxin biosynthesis and metabolism, Cold Spring Harbor Perspect. Biol. 2 (2010) a001594.
- [27] D. Benedićić, M. Ravnikar, M. Kovačcaron, Auxins in bean (*Phaseolus vulgaris* L. cv. Zorin) seedlings, Phyton 39 (1999) 79–84.
- [28] R.A. Rampey, S. Leclerc, M. Kowalczyk, K. Ljung, G. Sandberg, B. Bartel, A family of auxin-conjugate hydrolases that contributes to free indole-3-acetic acid levels during *Arabidopsis* germination, Plant Physiol. 135 (2004) 978–988.
- [29] G.M. Edelman, J.L. Wang, Binding and functional properties of Concanavalin A and its derivatives: interactions with indoleacetic acid and other hydrophobic ligands, J. Biol. Chem. 253 (1978) 3016–3022.
- [30] W.J. Peumans, J.M. van Damme, Lectins as plant defense proteins, Plant Physiol. 109 (1995) 347–352.
- [31] H. Lis, N. Sharon, Lectins: carbohydrate-specific proteins that mediate cellular recognition, Chem. Rev. 98 (1998) 637–674.
- [32] A.M. Hirsch, Role of lectins (and rhizobialepolysaccharides) in legume nodulation, Curr. Opin. Plant Biol. 2 (1999) 320–326.
- [33] T. Barbosa, S. Arruda, B.S. Cavada, T.B. Grangeiro, L.A. de Freitas, M. Barral-Neto, *In vivo* lymphocyte activation and apoptosis by lectins of the Diocleinae subtribe, Mem. Inst. Oswaldo Cruz 96 (2001) 673–678.
- [34] L.L. Murdock, R.E. Shade, Lectins and protease inhibitors as plant defenses against insects, J. Agric. Food Chem. 50 (2002) 6605–6611.
- [35] S.A. Stoitova, R.N. Boteva, R.J. Doyle, Binding of hydrophobic ligands by *Pseudomonas aeruginosa* PA-I lectin, Biochim. Biophys. Acta 1619 (2003) 213–219.
- [36] P. Delatorre, B.A.M. Rocha, E.P. Souza, T.M. Oliveira, G.A. Bezerra, F.B.M.B. Moreno, B.T. Freitas, T. Santi-Gadelha, A.H. Sampaio, W.F. Azevedo, B.S. Cavada, Structure of a lectin from *Canavalia gladiata* seeds: new structural insights for old molecules, BMC Struct. Biol. 7 (2007) 52–60.
- [37] D.D. Roberts, I.J. Goldstein, Hydrophobic binding properties of the lectin from lima beans (*Phaseolus lunatus*), J. Biol. Chem. 257 (1982) 11274–11277.
- [38] D.D. Roberts, I.J. Goldstein, Binding of hydrophobic ligands to plant lectins: titration with arylaminonaphthalenesulphonates, Arch. Biochem. Biophys. 224 (1983) 479–484.
- [39] K.D. Hardman, C.F. Ainsworth, Binding of nonpolar molecules by crystalline Concanavalin A, Biochemistry 12 (1976) 4442–4448.
- [40] D.C.H. Yang, W.E. Gall, G.M. Edelman, Rotational correlation time of Concanavalin A after interaction with a fluorescent probe, J. Biol. Chem. 249 (1974) 7018–7023.
- [41] D.D. Roberts, P. Arjunan, L.B. Townsend, I.J. Goldstein, Specificity of adenine binding to lima bean lectin, Phytochemistry 25 (1986) 589–593.
- [42] C.V. Gegg, D.D. Roberts, I.H. Segel, M.E. Etzler, Characterization of the adenine binding sites of two *Dolichos biflorus* lectins, Biochemistry 31 (1992) 6938–6942.
- [43] C.V. Gegg, M.E. Etzler, Photoaffinity labeling of the adenine binding sites of two *Dolichos biflorus* lectins, J. Biol. Chem. 269 (1994) 5687–5692.
- [44] H. de Boeck, F.G. Loontiens, H. Lis, N. Sharon, Binding of simple carbohydrates and some N-acetyllactosamine-containing oligosaccharides to *Erythrina cristagalli* agglutinin as followed with a fluorescent indicator ligand, Arch. Biochem. Biophys. 234 (1984) 297–304.
- [45] P.N. Kanellopoulos, The crystal structure of the complexes of Concanavalin A with 4'-nitrophenil- $\alpha$ -D-mannopyranoside and 4'-nitrophenil- $\alpha$ -D-glucopyranoside, J. Struct. Biol. 116 (1996) 345–355.
- [46] C.A.A. Gadelha, F.B.M.B. Moreno, T. Santi-Gadelha, J.B. Cajazeiras, B.A.M. Rocha, A.M.S. Assreuy, M.R.L. Mota, N.V. Pinto, A.V.P. Meireles, J.C. Borges, B.T. Freitas, F. Canduri, E.P. Souza, P. Delatorre, D.N. Criddle, W.F. Azevedo Jr., B.S. Cavada, Native crystal structure of a nitric oxide-releasing lectin from the seeds of *Canavalia maritima*, J. Struct. Biol. 152 (2005) 185–194.
- [47] M.V. Ramos, R.A. Moreira, J.T.A. Oliveira, B.S. Cavada, P. Rougé, The carbohydrate-binding specificity and molecular modelling of *Canavalia maritima* and *Dioclea grandiflora* lectins, Mem. Inst. Oswaldo Cruz 91 (1996) 761–766.
- [48] J.C. Gomes, R.R. Ferreira, B.S. Cavada, R.A. Moreira, J.T.A. Oliveira, Histamine release induced by glucose (mannose)-specific lectins isolated from Brazilian beans. Comparison with Concanavalin A, Agents Actions 41 (1994) 132–135.
- [49] C.A.A. Gadelha, F.B.M.B. Moreno, T. Santi-Gadelha, J.B. Cajazeiras, B.A.M. Rocha, J.K.R. Rustiguel, B.T. Freitas, F. Canduri, P. Delatorre, W.F. Azevedo Jr., B.S. Cavada, Crystallization and preliminary X-ray diffraction analysis of a lectin from *Canavalia maritima* seeds, Acta Crystallogr. F Struct. Biol. Cryst. Commun. 61 (2005) 87–89.
- [50] Collaborative Computational Project Number 4, The CCP4 suite: programs for protein crystallography, Acta Crystallogr. D Biol. Crystallogr. 50 (1994) 760–763.
- [51] A. Vargin, A. Teplyakov, MOLREP: an automated program for molecular replacement, J. Appl. Cryst. 30 (1997) 1022–1025.
- [52] P. Delatorre, B.A.M. Rocha, C.A.A. Gadelha, T. Santi-Gadelha, J.B. Cajazeiras, E.P. Souza, K.S. Nascimento, V.N. Freire, A.H. Sampaio, W.F. Azevedo Jr., B.S. Cavada, Crystal structure of a lectin from *Canavalia maritima* (ConM) in complex with trehalose and maltose reveals relevant mutation in ConA-like lectins, J. Struct. Biol. 154 (2006) 280–286.
- [53] P. Emsley, B. Lohkamp, W. Scoot, K. Cowtan, Features and development of Coot, Acta Crystallogr. D Biol. Crystallogr. 66 (2010) 486–501.
- [54] R.B. da Nóbrega, B.A.M. Rocha, C.A.A. Gadelha, T. Santi-Gadelha, A.F. Pires, A.M.S. Assreuy, K.R. Nascimento, C.S. Nagano, A.H. Sampaio, B.S. Cavada, P. Delatorre, Structure of *Dioclea virgata* lectin: relation between carbohydrate binding site and nitric oxide production, Biochimie 94 (2012) 900–906.
- [55] D.W. Ritchie, G.J.L. Kemp, Protein docking using spherical polar Fourier correlations, Proteins: Struct. Funct. Genet. 39 (2000) 178–194.
- [56] T.M. Oliveira, P. Delatorre, B.A.M. Rocha, E.P. Souza, K.S. Nascimento, G.A. Bezerra, T.R. Moura, R.G. Benevides, E.H.S. Bezerra, F.B.M.B. Moreno, V.N. Freire, W.F. Azevedo Jr., B.S. Cavada, Crystal structure of *Dioclea rostrata* lectin: insights into understanding the pH-dependent dimer-tetramer equilibrium and the structural basis for carbohydrate recognition in Diocleinae lectins, J. Struct. Biol. 164 (2008) 177–182.
- [57] C.S. Nagano, J.J. Calvete, D. Baretino, A. Pérez, B.S. Cavada, L. Sanz, Insights into the structural basis of the pH-dependent dimer-tetramer equilibrium through crystallographic analysis of recombinant Diocleinae lectins, Biochem. J. 409 (2008) 417–428.
- [58] G.M. Edelman, B.A. Cunningham, G.N. Reeke Jr., J.W. Becker, M.J. Waxdal, J.L. Wang, The covalent and three-dimensional structure of Concanavalin A, Proc. Natl. Acad. Sci. U. S. A. 69 (1972) 2580–2584.
- [59] J.W. Becker, G.N. Reeke Jr., J.L. Wang, B.A. Cunningham, G.M. Edelman, The covalent and three-dimensional structure of Concanavalin A. III. Structure of

- the monomer and its interactions with metals and saccharides, *J. Biol. Chem.* 250 (1975) 1513–1524.
- [60] E. Tillberg, Indoleacetic acid levels in *Phaseolus*, *Zea*, and *Pinus* during seed germination, *Plant Physiol.* 60 (1977) 317–319.
- [61] B. Kucera, M.A. Cohn, G. Leubner-Metzger, Plant hormone interactions during seed dormancy release and germination, *Seed Sci. Res.* 15 (2005) 281–307.
- [62] A.E. Clarke, R.B. Nox, Localization of lectins in legume cotyledons, *J. Cell Sci.* 19 (1975) 157–167.
- [63] C.L. Diaz, M. Hosselet, G.J.J. Logman, E. van Driessche, B.J.J. Lugtenberg, J.W. Kijne, Distribution of glucose/mannose-specific isolectins in pea (*Pisum sativum* L.) seedlings, *Planta* 181 (1990) 451–461.
- [64] B.S. Cavada, T.B. Grangeiro, M.V. Ramos, C.V. Crisostomo, L.M. Silva, R.A. Moreira, J.T.A. Oliveira, Lectin from *Dioclea guianensis* var. *lasiophylla* Duke. Seed mobilization during germination and seedling growth in the dark, *Rev. Bras. Fisiol. Veg.* 6 (1994) 21–25.
- [65] S. Manulis, I. Barash, *Pantoea agglomerans* pvs. *gypsophilae* and *betae*, recently evolved pathogens? *Mol. Plant Pathol.* 4 (2003) 307–314.
- [66] G. Brader, É. Tas, E.T. Palva, Jasmonate-dependent induction of indole glucosinolates in *Arabidopsis* by culture filtrates of the nonspecific pathogen *Erwinia carotovora*, *Plant Physiol.* 126 (2001) 849–860.
- [67] M. Frey, C. Stettner, P.W. Paré, E.A. Schmelz, J.H. Tumlinson, A. Gierl, An herbivore elicitor activates the gene for indole emission in maize, *Proc. Natl. Acad. Sci. U. S. A.* 97 (2000) 14801–14806.
- [68] X. Zhuang, A. Fießelmann, N. Zhao, H. Chen, M. Frey, F. Chen, Biosynthesis and emission of insect herbivory-induced volatile indole in rice, *Phytochemistry* 73 (2012) 15–22.