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Research Article

ADENINE BINDING CAPACITY OF A PLANT LECTIN *CONCANAVALIN A*

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Summary: Adenine binding site has been identified in the lectin *Concanavalin A (Con A)*, isolated from the legume plant *Canavalia ensiformis*. We found that, this protein bound with high affinity adenine with k_D=0.38±0.08 μM. Emission spectra showed a significant decrease of the intrinsic tryptophan fluorescence, due to the interaction. The hyperbolic shape of the titration curve indicated presence of an "adenine" binding site. Additionally, enhancement of the extrinsic ANS fluorescence and sigmoidal shape of the titration curve revealed existence of hydrophobic sites within the protein molecule.
The "adenine" binding site is suggested to have putative physiological role similar to many plant lectins.

Keywords: Adenine; binding; fluorescence; Concanavalin A; lectin.

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INTRODUCTION

Lectins are carbohydrate binding proteins, found in plants, animals, bacteria and humans. Among plant lectins, legume lectins are the largest and the most studied family.

Interestingly, they participate in different physiological processes such as: protein storage, defense, recognition, embryogenesis, development, etc. It is supposed that their role depends from their distribution, as they are found in different organs and tissues of the plants.

Although many papers are focused on revealing the physiological role of plant lectins, their actual role in plants remains unclear. Furthermore, the actual *in vivo* ligands of plant lectins are not known and what is their function remains enigmatic.

During the last decades, it has been shown that in addition to their carbohydrate-binding sites many plant legume lectins, isolated from *Dolichos biflorus* and other legume plants bind different hydrophobic ligands as: 1,8-ANS (1-Anilinonaphthalene-8-Sulfonic Acid) and 2-(p-toluidinyl) naphthalene sulfonic acid (ANS, TNS), adenine (Hamelryck et.al., 1999). The affinities towards these hydrophobic compounds were higher than their affinities for carbohydrates, which indicated that the

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interaction with hydrophobic ligands might have a putative biological role.

It was also found that some non-legume lectins as: wheat germ agglutinin and other plat lectins bind non carbohydrate ligands such as: plant hormones (Bogoeva et. al., 2004), porphyrin compounds (Komath et. al., 2006; Bogoeva et. al., 2011; Bogoeva et. al., 2012; Petrova et. al., 2013). Of special interest to us was to study the legume lectin *Concanavalin A* (Con A), isolated from the seeds of the plant *Canavalia ensiformis* (Sumner and Howell, 1936).

It is a homotetramer (with a molecular weight of the monomer 26.5 kDa). Similar to other plant lectins it interacts with fluorescent dyes (Banerjee and Kishore, 2006) and porphyrins (Bogoeva et. al., 2014). In this relation, of special interest to us is to test whether *Con A* possesses an "adenine" binding site, similar to *Dolichos biflorus* lectin (Hamelryck et.al., 1999) and Phaseolus lunatus lectin (lima bean lectin) (Roberts and Goldstein, 1983). Finding an answer of this matter may enable to clarify its putative function.

The presence of an "adenine" binding site in *Con A* suggests that this site may play an important role *in vivo*.

MATERIALS AND METHODS

Reagents

Con A, a product of Sigma (St. Louis, MO), was dissolved in PBS (20 mM phosphate buffer, 0.15 M NaCl, pH 6.7). The protein concentration was measured by the absorbance at 280 nm, using a molar extinction coefficient (ε_M) of 3.08 x 10^4 M^{-1} cm^{-1} for a Con A monomer, calculated from the aromatic amino acid content (4 Trp and 7 Tyr) (Edelman et. al., 1972). The concentration of adenine was determined from the absorbance at 260 nm ($\varepsilon_{M,\ 260}$ 13 x 10^3 M^{-1} cm^{-1}). 1-Anilinonaphthalene-8-sulfonic acid (ANS) was purchased from Sigma (St. Louis, MO, USA). The concentration of ANS was determined by its absorbance at 370 nm ($\varepsilon_{M,\ 370}$=6.8 x 10^3 M^{-1} cm^{-1}). Adenine and the fluorescent dye were dissolved in ethanol. The volume of the organic solvent did not exceed 5% of the final volume of the protein samples.

Fluorescence measurements

Fluorescence spectra were registered by a Shimadzu RF-5000 spectrofluorometer, equipped with a thermostatted cell holder. During all experiments the absorbance of the samples, at the excitation wavelength, was kept less than 0.05. All fluorescent measurements were carried out in PBS, at 20°C.

Identifying a hydrophobic site by fluorescent dye ANS

The formation of the complex of ANS and Con A was studied by the extrinsic fluorescence of ANS dye upon excitation at 370 nm. The fluorescence titrations were carried out by a constant addition of ANS solution (3.4 – 82 μM) to the sample of Con A with the protein concentrations corrected for the final total volume.

Interaction of adenine with Con A

Con A (monomers), 1.8 μM, was incubated with increasing concentrations of adenine overnight, at 4°C, prior to the fluorescent measurements. Binding of adenine to Con A was studied by the

changes in the intrinsic Trp emission of the protein. Excitation was at 295 nm and the emission maximum position was registered at 338 nm. The apparent dissociation constants (K_d) and F_{max} representing the maximal decrease of the intrinsic Trp fluorescence upon saturation of all binding sites were determined by fluorescence titration data, using GraphPad Prism analysis programme.

RESULTS

Hydrophobic binding to Con A

Fluorescent dye ANS was used to identify the hydrophobic binding site of Con A. We registered increase of ANS emission due to the binding. The sigmoidal shape of the titration curve showed a cooperative interaction (Fig. 1). Titration was done by addition of small volumes of ANS to adjust the concentrations [3.4 – 82 μM]. Net fluorescence enhancement (ΔF) was calculated by subtraction of fluorescence of free ANS determined by parallel titration in the absence of protein and corrected for dilution due to the ligand addition. Binding curve with sigmoidal shape was received.

Binding of adenine to Con A

Interaction of Con A with adenine was studied by changes in the intrinsic Trp fluorescence of the protein Con A, excited at 295 nm. The emission maximum position was recorded at 338 nm. Binding of adenine caused significant decrease of the Trp fluorescence emission intensity but did not change the emission maximum position which remained constant. The fluorescent decrease after binding of adenine followed a hyperbolic trend (Fig. 2) and reached saturation at ligand concentration (approximately 2.5 μM). We established that Con A interacted with adenine with high affinity

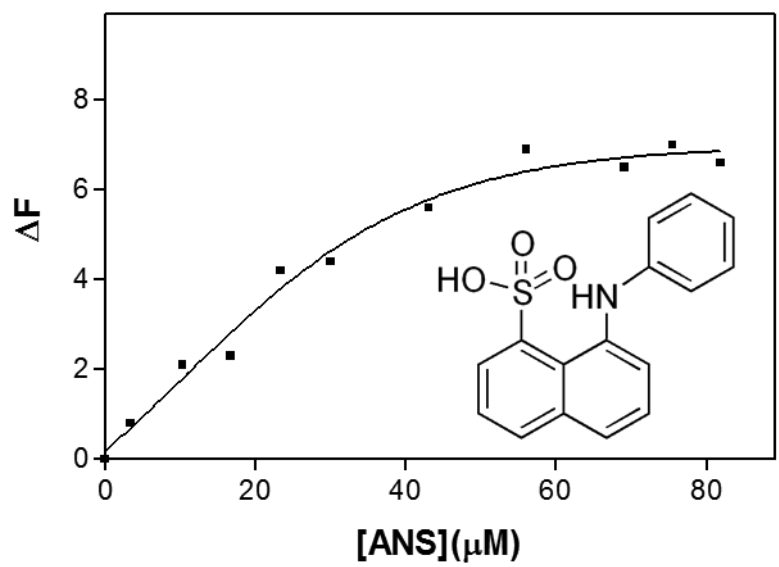

Figure 1. Enhancement of ANS fluorescence after binding of ANS to Con A (1.8 μM). The curve shows the best theoretical fit to the analyzed experimental data; (R^2=0.98).

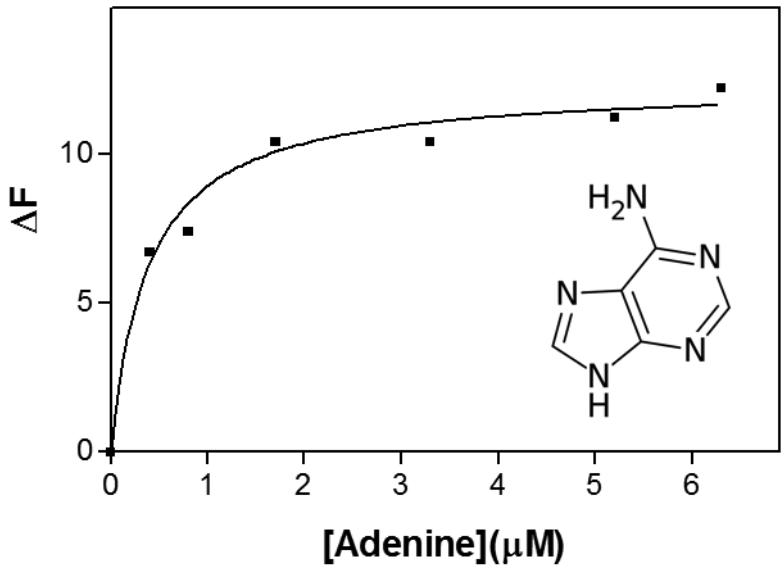

Figure 2. Binding of Con A to adenine, studied by the intrinsic protein fluorescence (λ_{exc} at 295 nm). The titration curve is the best theoretical fit to the experimental data; (R^2=0.97).

(Kd = 0.38±0.08) similar to other plant lectins as: DBL, DB 58 (*Dolichos biflorus* stem and leaf lectin), *Lima bean* lectin, Soybean agglutinin, Wheat Germ agglutinin, etc. (Bogoeva et. al., 2004; Komath et. al., 2006).

DISCUSSION

During the last decades, it has been discussed that carbohydrate binding capacity is not the only activity of the plant and animal lectins (Gabius, 1994; Kilpatrick, 2002; Komath et.al., 2006; Bogoeva and Russev, 2008).

In this study, we characterized the interaction of the protein Con A with ANS using the extrinsic fluorescence of the dye. Interestingly, the sigmoid shape of the titration (Fig. 1) showed existence of more than one class of binding sites for ANS within the lectin Con A. The binding curve revealed cooperative interactions which showed that binding of one ANS molecule facilitated the interaction of the next ANS molecules by increasing the affinity of the vacant binding sites on the lectin. The obtained results showed that the lectin Con A bound ANS. Our data are in agreement with the literature data, found by Banerjee (Banerjee and Kishore, 2006).

We also characterized the binding of the lectin Con A with adenine, using steady state fluorescence. We found that excitation at λ=295 nm of Con A showed that the Trp emission spectrum was sensitive to the interactions of the protein with adenine.

The interaction of the protein with adenine (Fig. 2) caused a fluorescence quenching of the Trp fluorescence. The conformational rearrangements within the lectin tetramer were confirmed by a small shift of the emission maximum position after titration with adenine,

similar to phycocyanin binding (Pandey et. al., 2009).

The k_D of (0.38 ± 0.08μM) for adenine showed high-affinity binding of the Con A for the ligand. We have found that Con A interacts with adenine with an affinity similar to that of WGA (wheat germ agglutinin) (Bogoeva et. al., 2004; Bogoeva et. al., 2012; Petrova et. al., 2013).

Our results have demonstrated that the plant lectin Con A possesses an "adenine" binding site.

Interestingly, the crystallographic studies on the lectin from *Canavalia gladiata* seeds (98% similar to Con A) revealed a hydrophobic pocket in it, where the non-protein amino-acid α-aminobutiric acid was bound. The residues that compose this hydrophobic pocket are highly conserved in the legume lectins. The existence of this site suggests for its possible biological activity (Delatorre et. al., 2007).

Of special interest is that, adenine binding sites were also identified in lectins, isolated from the vegetative organs of legume plant *Dolichos biflorus* (Gegg et. al., 1992) and in a non-legume lectin wheat germ agglutinin (Bogoeva et. al., 2004; Komath et. al., 2006). The exact function of this binding site is still unknown, but it is supposed that it can be involved in storage of plant hormones or plant growth regulation (Hamelryck et. al., 1999).

Etzler`s group (Gegg, 1992; Gegg and Etzler, 1994) after characterizing the adenine binding sites of *Dolichos biflorus* lectins suggested that these sites could accommodate relevant physiological ligands in plants and could perform biological role. The crystal structure of DBL-adenine complex clearly showed that four adenine molecules bind to the DBL tetramer, as well as that adenine binding site was distinct from carbohydrate one (Hamelryck et. al., 1999).

Also three legume lectins, such as: soybean agglutinin, phytohemagglutinin-L (PHA-L), and *Dolichos biflorus* lectin have been crystallized and found to have a unique tetrameric structure, in which the dimer–dimer interface creates a channel running through the center of the tetramer. Interestingly, two identical adenine-binding sites have been found at opposite ends of this channel (Varki et. al., 2009).

In conclusion, we identified an "adenine" binding site within Con A molecule, which presumed that it might have putative function in accommodation of physiological ligands.

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