Research article

Conformational analysis of champedak galactose-binding lectin under different urea concentrations

Nurul Iman Ahamed Kameel, Yin How Wong, Adawiyah Suriza Shuib, Saad Tayyab

Biomolecular Research Group, Biochemistry Programme, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603, Kuala Lumpur, Malaysia

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Conformational analysis of champedak galactose–binding (CGB) lectin under different urea concentrations was studied in phosphate-buffered saline (pH 7.2) using far-ultraviolet circular dichroism (far-UV CD), tryptophan (Trp) fluorescence and ANS fluorescence. In all cases, CGB lectin displayed a two-step, three-state transition. The first transition (from the native state to the intermediate state) started at ~2.0 M urea and ended at ~4.5 M urea, while the second transition (from the intermediate state to the completely denatured state) was characterized by the start- and end-points at ~5.75 M and ~7.5 M urea, respectively, when analyzed by the emission maximum of Trp fluorescence. A marked increase in the Trp fluorescence, ANS fluorescence and ~CD values at 218 nm (~CD_{218 nm}) represented the first transition, whereas a decrease in these parameters defined the second transition. On the other hand, emission maximum of the Trp fluorescence showed a continuous increase throughout the urea concentration range. Transformation of tetramer into monomer represented the first transition, whereas the second transition reflected the unfolding of monomer. Far-UV CD, Trp fluorescence and ANS fluorescence spectra were used to characterize the native, the intermediate and the completely denatured states of CGB lectin, obtained at 0.0 M, 5.0 M and 9.0 M urea, respectively. The intermediate state was characterized by the presence of higher secondary structures, increased ANS binding as well as increased Trp fluorescence intensity. A gradual decrease in the hemagglutination activity of CGB lectin was observed with increasing urea concentrations, showing complete loss at 4.0 M urea.

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1. Introduction

Protein denaturation studies have gained much interest in the pursuit of understanding the conformational stability and the structure–function relationship of proteins (Privalov, 2012; Wong et al., 2014). Out of various denaturants, chemical denaturants such as urea and guanidine hydrochloride have been found popular in protein denaturation studies in being effective to disrupt all noncovalent interactions (O’Brien et al., 2007), which are known to stabilize the native protein conformation. The denaturation of multimeric proteins is more complex than the monomeric proteins due to the presence of intersubunit interactions in addition to the intrasubunit interactions within the protein (Biswas and Kayastha, 2004; Sinha et al., 2005; Dev et al., 2006).

Lectins are ubiquitous carbohydrate-binding proteins, possessing at least one specific sugar-binding site (Goldstein, 1980; Peumans and Van Damme, 1995). In plant seeds, they constitute up to 5% of the total protein content (Van Damme, 2008). The physiological importance of lectins in plants has been accredited due to their role in plant defense against pathogens, plant development and symbiotic plant–microbe interactions (Brill et al., 2001; De Hoff et al., 2009; Yamaji et al., 2012). Due to their sugar-binding properties, plant lectins have also been harnessed as tools in the characterization of glycoconjugates, carriers in drug delivery systems and for their potential anti-tumor applications (Gabor et al., 2002; Wu et al., 2009; Fu et al., 2011).

Champedak (Artocarpus integer) is a fruit tree species of the same genus as of the jackfruit and is local to Southeast Asia. Champedak seeds are the source for champedak galactose-binding (CGB) lectin, which has been well characterized for its molecular properties, including hemagglutinating activity (Hashim et al.,
In addition to galactose, CGB lectin has also shown greater affinity towards several galactose derivatives (Hashim et al., 1991). A few human serum proteins i.e. IgA1 and C1 inhibitor have been found precipitated with CGB lectin (Hashim et al., 1993). Due to its sugar-binding specificity, CGB lectin has been used to probe early detection of endometrial, ovarian and prostate cancers (Mu et al., 2012, 2013; Jayapalan et al., 2013).

Recently, the three-dimensional structure of CGB lectin has been elucidated by X-ray crystallography (Gabrielsen et al., 2014). It has a homotetrameric structure, where each monomer is comprised of a 133 residue-long a-chain (molecular mass, 13 000 Da) along with a noncovalently-linked shorter b-chain (21 residues; molecular mass, 2100 Da). Noncovalent interactions between the monomers stabilize the tetramer. Presence of a single tryptophan (Trp) residue in each a- and b-chains has characterized CGB lectin as Class B protein. Whereas the C-terminal half of b-chain is rich in hydrophobic residues, relatively even distribution of hydrophobic residues has been found in the a-chain. However, a six residue-long hydrophobic cluster is located near the C-terminal of the a-chain (Gabrielsen et al., 2014).

CGB lectin has been shown to possess a high degree of sequence homology to jacalin, a galactose-binding lectin, isolated from jackfruit seeds (Gabrielsen et al., 2014). The three-dimensional structures of jacalin-related lectins are characterized by the presence of a b-prism structure in each monomer, formed by the noncovalent association of three four-stranded antiparallel b-sheets (Sankaranarayanan et al., 1996; Barre et al., 2001). CGB lectin has been found similar to jacalin-related lectins in possessing the b-prism structure (Gabrielsen et al., 2014). In CGB lectin, out of the twelve b-strands involved in the formation of b-prism structure, eleven b-strands are contributed by the a-chain while b-chain forms the 12th b-strand (Gabrielsen et al., 2014). Both side chain and main chain N and O atoms of the a-chain of CGB lectin are involved in the binding of galactose through hydrogen bonds (Gabrielsen et al., 2014).

Since a protein’s function is controlled by its three-dimensional conformation, it would be of interest to study the effect of urea on the conformation and hemagglutinating activity of CGB lectin. Chemical denaturation studies of CGB lectin have not been performed to date to the best of our knowledge. Here, we describe conformational analysis of CGB lectin under different urea concentrations using various spectroscopic probes as well as the effect of urea on the hemagglutinating activity of CGB lectin.

2. Materials and methods

2.1. Materials

Urea BioXtra (type U0631), 1-anilinonaphthalene-8-sulfonate (ANS) (type A3125) and D-galactose (type G0625) were purchased from Sigma–Aldrich Inc., USA. Bio-Rad QuickStart™ Bradford 1 × Dye Reagent (catalogue #500-0205) and Bio-Rad QuickStart™ Bovine Serum Albumin Standard Set (catalogue #500-0207) were procured from Bio-Rad Laboratories, USA. HiPrep 26/60 Sephacryl S-100 HR column was supplied by GE Healthcare, UK. Champedak fruits were purchased from the local market. All the reagents used were of analytical grade purity.

2.2. Analytical procedures

Protein concentration was determined according to the Bradford method using BSA as the standard on a BioTek Synergy H1 Multi-Mode Reader (BioTek Instruments Inc., USA). ANS concentration was determined spectrophotometrically on a Shimadzu UV-2450 UV–Vis spectrophotometer (Shimadzu Corp., Japan) using a molar absorption coefficient of 5000 M⁻¹ cm⁻¹ at 350 nm.

The stock urea solution (~11 M) was prepared in the same way as described by Pace and Schultz (1997).

All experiments were carried out in triplicates at 25 °C (unless otherwise stated) in phosphate-buffered saline (PBS, pH 7.2) containing 10 mM di-sodium hydrogen phosphate, 1.5 mM potassium dihydrogen phosphate, 137 mM sodium chloride and 2.7 mM potassium chloride.

2.3. Lectin purification

Champedak galactose-binding (CGB) lectin was purified from the champedak seeds following the procedure described earlier (Hashim et al., 1991) with slight modifications. Ground champedak seeds (130 g) were stirred overnight in 1 L of PBS (pH 7.2) at 4 °C. The homogenate was filtered, followed by centrifugation at 8000 × g for 15 min to get the supernatant. The crude extract was obtained from the supernatant by precipitation with 60% ammonium sulfate at room temperature. It was then subjected to galactose-Sepharose affinity chromatography and the bound lectin was eluted using 0.8 M galactose. The isolated CGB lectin was dialyzed against PBS (pH 7.2) for two days with four changes of the buffer. It was then concentrated using Merck Amicon Ultra 15 centrifugal concentrators, 10 000 MWCO (Merck & Co., USA). The lectin was purified to homogeneity using HiPrep 26/60 Sephacryl S-100 HR column attached to AKTA avant 25 system (GE Healthcare, UK) and stored at −20 °C.

2.4. Circular dichroism spectroscopy

Circular dichroism (CD) measurements were made in the far-UV region at 25 °C on a Jasco J-815 spectropolarimeter (Jasco Int. Co. Ltd., Japan) connected to a Jasco PTC-4235/15 Peltier-type temperature controller, using a protein concentration of 0.2 μM. The samples were scanned between 190 and 260 nm with four scan accumulations using quartz cuvettes of 1 mm path length. The bandwidth and the scanning speed were fixed at 1 nm and 100 nm/min, respectively.

2.5. Fluorescence spectroscopy

Fluorescence measurements were performed on a Hitachi F-2500 spectrofluorometer (Hitachi Ltd., Japan), using a protein concentration of 0.1 μM in a 1 cm pathlength cuvette. The slit widths for the excitation and emission wavelengths were fixed at 10 nm each. For tryptophan (Trp) fluorescence measurements, the samples were excited at 295 nm and the emission spectra were recorded in the wavelength range, 300–400 nm.

For ANS fluorescence measurements, the dye (ANS) to protein molar ratio was kept as 100:1 and the fluorescence spectra were recorded in the wavelength range, 400–600 nm upon excitation at 380 nm.

The fluorescence spectra, obtained above were corrected for blank contributions, if any.

2.6. Denaturation experiments

Urea denaturation experiments were performed following the method described earlier (Muzammil et al., 2000) by titrating a fixed concentration of the protein (0.2 μM for CD measurements and 0.1 μM for fluorescence measurements) with increasing urea concentrations (0.0–9.5 M) in a total volume of 1.5 ml for CD measurements, 6 ml for intrinsic fluorescence measurements and 3 ml for ANS fluorescence measurements. The mixture was...
incubated for 14 h at room temperature before spectral measurements.

2.7. Hemagglutination assay

Rabbit red blood cells (RBC) were separated from the serum by centrifugation at 3000 × g at 25 °C, washed thrice with 0.15 M sodium chloride before being suspended in PBS (pH 7.2) to obtain 2% RBC suspension. The CGB lectin was incubated at different urea concentrations (0.0–4.0 M) for 14 h. The assay was carried out by adding 50 μL of the lectin (2.6 × 10^{-2} nM) sample and 50 μL of 2% RBC suspension into V-bottomed microtitre plates. The plates were incubated at room temperature for 3 h and the highest concentration of urea, which still permitted agglutination was recorded.

3. Results and discussion

3.1. Urea denaturation

Urea denaturation using CGB lectin was studied using three different spectral probes, namely far-UV CD, tryptophan fluorescence and ANS fluorescence measurements.

3.1.1. Far-UV CD

Being characterized as a β-structure protein (Gabrielsen et al., 2014), CD measurements were made at 218 nm to study urea denaturation of CGB lectin. As shown in Fig. 1, urea-induced denaturation of CGB lectin was characterized by a two-step, three-state transition. Although a gradual increase in the −CD_{218} nm value was noticed up to 4.5 M urea, CD measurements could not be made within the urea concentration range, 2.0–4.25 M urea due to the appearance of turbidity. Use of a relatively higher protein concentration (0.2 μM) in CD measurements seems to be responsible for visible protein aggregation. Increase in the −CD_{218} nm value up to 4.5 M urea clearly suggested reformation of the secondary structures in the protein. The tetrameric structure of CGB lectin is stabilized by two different interfaces between these subunits involving both hydrogen bonds and hydrophobic interactions (Gabrielsen et al., 2014). Loss of these interfocal contacts between different subunits in the presence of urea seems to allow the release in structural restrictions experienced by these subunits in the tetrameric form of the protein. The CD_{218} nm value remained constant between 4.5 M and 5.75 M urea, suggesting the formation of a stable conformational state of the protein. In view of the tetrameric nature of CGB lectin, it appears that all subunits might have undergone dissociation into monomers at 4.5 M urea. Similar dissociation of tetrameric proteins into structured monomers in the presence of urea has also been reported earlier in concanavalin A and soybean agglutinin (Ghosh and Mandal, 2001; Chatterjee and Mandal, 2003).

A second transition, observed within the urea concentration range, 5.0–9.0 M urea was marked by a decrease in the −CD_{218} nm value. The start- and the end-points of the transition were observed at 5.75 M and 7.5 M urea, respectively (Fig. 1, Table 1). The second transition seems to represent the denaturation of the structured monomers into their unfolded forms due to the loss in the secondary structures as reflected from the decrease in the −CD_{218} nm value. Furthermore, this was supported by the absence of any change in the CD_{218} nm value above 7.5 M urea. These results were similar to those shown earlier with concanavalin A, representing unfolding of monomer in the second transition (Chatterjee and Mandal, 2003).

3.1.2. Tryptophan fluorescence

Fig. 2 shows the urea-induced denaturation of CGB lectin as monitored by the change in the emission maximum of tryptophan fluorescence. Native CGB lectin produced a fluorescence spectrum with an emission maximum at 333 nm due to the presence of Trp residues (Gabrielsen et al., 2014). Addition of urea to CGB lectin led to a red shift in the emission maximum, indicating change in the microenvironment of Trp residues from nonpolar to polar (Lakowicz, 2006). Such a change in the microenvironment around Trp residues may be explained in either of two ways. Surface exposure of Trp residues to aqueous environment or movement of charged groups in the vicinity of Trp residues can produce red shift in the emission maximum of a protein (Lakowicz, 2006). Such a large shift of 17 nm in the emission maximum was suggestive of protein denaturation. Tryptophan fluorescence peak usually occurs near 350 nm, when one or both faces of benzene rings are water-exposed (Eftink, 1994; Lakowicz, 2006), which is possible when protein is completely unfolded. As can be seen from the figure, the denaturation curve shows a two-step, three-state transition. No change in the emission maximum was noticed up to 2.0 M urea beyond which a gradual increase in the emission maximum was observed up to 4.5 M urea. The second transition started at 5.75 M urea and characterized by the end-point at 7.75 M urea (Fig. 2, Table 1). Movement of charged groups in the vicinity of Trp residues

<table>
<thead>
<tr>
<th>Probe</th>
<th>Urea (M)</th>
<th>First transition</th>
<th>Second transition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Start</td>
<td>End</td>
</tr>
<tr>
<td>CD_{218} nm</td>
<td>-</td>
<td>-</td>
<td>5.75</td>
</tr>
<tr>
<td>Relative fluorescence intensity at 333 nm</td>
<td>2.60</td>
<td>4.50</td>
<td>5.75</td>
</tr>
<tr>
<td>Emission maximum</td>
<td>2.00</td>
<td>4.50</td>
<td>5.75</td>
</tr>
<tr>
<td>ANS fluorescence intensity at 461 nm</td>
<td>1.50</td>
<td>4.20</td>
<td>4.20</td>
</tr>
</tbody>
</table>

Table 1: Characterization of urea denaturation curves of CGB lectin in PBS (pH 7.2), as studied by different probes.
seems to be responsible for the red shift, observed in the first transition, while surface exposure of Trp residues to aqueous environment can explain the second transition. This was supported by our CD spectral results (Fig. 1), showing reformation of secondary structures during the first transition followed by disruption of secondary structures at higher urea concentrations. Earlier studies on tetrameric lectins, i.e. concanavalin A, soybean agglutinin and peanut agglutinin have also shown a two-step, three-state transition in urea denaturation, when monitored by emission maximum (Ghosh and Mandal, 2001; Chatterjee and Mandal, 2003; Dev et al., 2006).

Addition of urea to CGB lectin also produced a change in the fluorescence intensity at 333 nm. Fig. 3 shows the effect of urea on the fluorescence intensity of CGB lectin at 333 nm. The fluorescence intensity remained constant up to 2.6 M urea, showed a gradual increase, reaching to a maximum value at 4.5 M urea and plateaued up to 5.75 M urea. Further increase in urea concentrations led to a progressive decrease in the fluorescence intensity up to 7.5 M urea, before leveling off (Fig. 3). In other words, urea denaturation of CGB lectin was found to be a two-step transition. The first transition, marked by the increase in the fluorescence intensity was characterized by the start- and the end-points occurring at 2.6 M and 4.5 M urea, respectively (Fig. 3, Table 1). Red shift in the emission maximum of a protein is usually found upon denaturation irrespective of the change in the quantum yield/fluorescence intensity (Eftink, 1994). Such increase in the fluorescence intensity may be ascribed to the separation of quenching residues from the Trp residues (Ando et al., 2008) due to reformation of the secondary structures as shown in Fig. 1. This seems understandable as monomerization of tetramer might have relieved such quenching of Trp fluorescence. The second transition displayed a decrease in the fluorescence intensity, starting from 5.75 M urea and ending at 7.5 M urea (Table 1). The decrease in the fluorescence intensity clearly indicated the formation of the unfolded structure with surface-exposed Trp residues, which was well-supported by the results showing loss in the secondary structures at higher urea concentrations (Fig. 1). Similar increase in the fluorescence intensity at lower urea concentrations and decrease at higher urea concentrations have also been observed in the urea denaturation of concanavalin A (Chatterjee and Mandal, 2003). As evident from Figs.1–3, a stable equilibrium intermediate seems to exist within the urea concentration range, 4.5–5.75 M.

3.1.3. ANS fluorescence

ANS binding was also used as a probe to observe CGB lectin denaturation. ANS shows fluorescence upon binding to hydrophobic clusters (Horowitz and Butler, 1993), which may be more exposed in the denatured form. Fig. 4 shows the urea-induced denaturation curve of CGB lectin, as studied by ANS fluorescence measurements. There was an increase in the ANS fluorescence...
Intensity within 1.5–4.2 M urea concentration range (Table 1). Monomerization of the CGB lectin could be the determinant for such increase in ANS binding as it might have exposed the hydrophobic clusters otherwise concealed in the tetramer. For the second transition, the denaturation curve immediately plunged down from 4.2 M urea towards 6.8 M urea, showing complete abolishment of ANS binding (Fig. 4, Table 1). Loss of the ANS fluorescence intensity above 4.2 M urea could be attributed to the deterioration of the structured monomers (Varejao et al., 2011), which might have caused the hydrophobic clusters to disintegrate. Furthermore, urea might have also interrupted in ANS binding to hydrophobic clusters (Horowitz and Butler, 1993).

The denaturation curve followed by ANS binding (Fig. 4) showed slight variation in the start- and the end-points for both first and second transitions, when compared to the denaturation curves, obtained using other probes (Figs.1–3), which exhibited similar start- and end-point values. Irrespective of the smaller variation, the latter three probes displayed the formation of a stable intermediate state in a relatively wide (4.5–5.75 M) urea concentration range.

3.2. Characterization of the intermediate state of CGB lectin

In order to characterize the intermediate state of CGB lectin, spectral properties of the native state, the intermediate state at 5.0 M urea and the completely denatured state at 9.0 M urea were compared. Spectral characterization of these three states was made by analyzing their far-UV CD spectra, Trp fluorescence spectra and ANS fluorescence spectra.

3.2.1. Far-UV CD spectra

Fig. 5 shows the far-UV CD spectra of the native, the intermediate and the completely denatured states of CGB lectin. The far-UV CD spectral characteristics of the native state were similar to a β-sheet protein due to the presence of a minimum at 218 nm (Greenfield, 2006). This agreed well with the presence of a high percentage of β-sheet structure in CGB lectin (Gabrielsen et al., 2014). Interestingly, the far-UV CD spectrum of the intermediate state showed ~53% increase in the CD218 nm value compared to the native state (Table 2). This seems plausible due to the loss of intersubunit interactions to form monomers at this urea concentration, which were supposed to govern the secondary structures of these subunits in the bound state. Reformation of the secondary structures in the intermediate state induced by urea has also been reported in several other studies on tetrameric and dimeric proteins (Chatteraj et al., 1996; Chatterjee and Mandal, 2003; Varejao et al., 2011; Ghosh and Mandal, 2001). On the other hand, the far-UV CD spectrum of CGB lectin in 9.0 M urea showed ~50% decrease in the CD218 nm value from that found in the native state (Table 2). Such decrease in the CD218 nm value is expected, as 9.0 M urea is sufficient to remove noncovalent interactions, required to form secondary structures (Bennion and Daggett, 2003).

3.2.2. Tryptophan fluorescence spectra

The fluorescence spectra of the native, the intermediate and the completely denatured states are shown in Fig. 6. The fluorescence spectrum of the intermediate state showed a significant increase (~44%) in the fluorescence intensity at the emission maximum along with a red shift (8 nm) in the emission maximum when compared to the fluorescence characteristics of the native state (Table 2). As discussed in Section 3.1, the fluorescence intensity of CGB lectin seems to be quenched in the native tetrameric state due to the presence of quenchers in the vicinity of Trp residues (Ando et al., 2008). Dissociation of these subunits into monomeric forms at 5.0 M urea might have distanced away the quenching residues from Trp, thus leading to an increase in the fluorescence intensity. The increase in fluorescence intensity upon urea denaturation has also been observed in lysozyme and ficin (Ong et al., 2009; Sidek et al., 2013). In contrast, at higher urea concentration (9.0 M), the emission maximum was moved to 350 nm along with a decrease in the fluorescence intensity (Fig. 6, Table 2), indicating exposure of Trp residues to the aqueous environment. In view of the occurrence of the emission maximum at 350 nm of the free Trp in aqueous buffer (Udenfriend et al., 2014), CGB lectin in 9.0 M urea is supposed to be in completely unfolded form with surface-exposed Trp residues.

3.2.3. ANS fluorescence spectra

Fig. 7 shows the ANS fluorescence spectra of the native, the intermediate and the completely denatured states of CGB lectin, observed at 0.0 M, 5.0 M and 9.0 M urea, respectively. The native state showed negligible ANS fluorescence intensity along the emission wavelength range, suggesting absence of the hydrophobic clusters in the native state. However, a marked increase in the ANS fluorescence intensity was observed with the intermediate state, showing emission maximum at 461 nm. This can be attributed to the dissociation of monomers of the CGB lectin in 5.0 M urea, thus exposing the hydrophobic clusters, which were involved in the interfacial contacts between the subunits to stabilize the quaternary structure (Wojciak et al., 2003). ANS fluorescence intensity was completely lost in the presence of 9.0 M urea, due to disintegration of the hydrophobic clusters as well as weakening of the hydrophobic interactions, which are known to be involved in ANS binding to proteins (Hawe et al., 2008). In other words, CGB lectin seems to acquire the unfolded conformation in the presence of 9.0 M urea, showing lack of ANS binding as observed with other urea-denatured proteins (Chatterjee and Mandal, 2003; Varejao et al., 2011). Since urea denaturation curve, as probed by ANS fluorescence (Fig. 4) showed the mid-point between two transitions around 4.2 M urea, ANS fluorescence characteristics of CGB lectin in 4.2 M urea was also studied. As can be seen from Fig. 7, the

![Fig. 5. Far-UV CD spectra of CGB lectin at 0.0 M, 5.0 M and 9.0 M urea concentrations in PBS (pH 7.2) at 25 °C. The spectra could not be obtained below 218 nm in the presence of urea due to high signal-to-noise ratio.](image-url)
The fluorescence spectrum was found similar to the one observed at 5.0 M urea.

### 3.3. Hemagglutination activity

Hemagglutination assay was carried out with CGB lectin in the presence of urea (0.5–4.0 M) to determine the point of denaturation where the lectin loses its binding activity towards its ligand. Fig. 8 shows rabbit RBC suspension incubated with CGB lectin, which had earlier been incubated with increasing concentrations of urea (0.0–4.0 M) for 14 h. It is important to note that RBCs settle down at the bottom of the well in the absence of any agglutination. On the other hand, agglutinated RBCs remain in suspension in the well. This can be clearly seen from the Fig. 8, where the lectin showed agglutinating ability in the native form as well as up to 2.5 M urea-treatment. Up to this urea concentration, the lectin binding sites were conserved and retained their binding affinity towards the galactose residues present on the RBC surface. At 3.0 M urea, the lectin showed reduced ability to agglutinate the RBCs and the agglutinating activity was totally lost at 4.0 M urea. Since this urea concentration falls in the range of the first transition, involving monomerization of the tetrameric lectin, absence of agglutination in the presence of 4.0 M urea seems justifiable, as most of the tetrameric lectin would have been converted into monomeric form at this urea concentration.

### 4. Conclusions

In summary, urea-induced denaturation of CGB lectin was found to be a two-step, three-state transition, involving a structured monomeric intermediate. The intermediate was characterized by the presence of higher secondary structures, increased ANS binding and higher Trp fluorescence. The hemagglutination activity of the lectin was gradually decreased with increasing urea concentrations and was completely lost ~4.0 M urea as the lectin was converted into the monomeric form.

### Contributions

NIAK, ASS: Lectin purification.
NIAK, YHW, ASS, ST: Design and analysis of experiments.
NIAK: Conducted the experiments.

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**Table 2**

Spectral characteristics of CGB lectin in PBS (pH 7.2) under various experimental conditions, as monitored by different probes.

<table>
<thead>
<tr>
<th>Probe</th>
<th>CGB lectin</th>
</tr>
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<tbody>
<tr>
<td>CD values (mdeg) at 218 nm</td>
<td>−6.89</td>
</tr>
<tr>
<td>Trp fluorescence intensity at emission maximum</td>
<td>1022</td>
</tr>
<tr>
<td>Emission maximum (nm)</td>
<td>333</td>
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<tr>
<td>ANS fluorescence intensity at 461 nm</td>
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</tr>
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<td>+5.0 M urea</td>
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<tr>
<td></td>
<td>1468</td>
</tr>
<tr>
<td></td>
<td>341</td>
</tr>
<tr>
<td></td>
<td>2706</td>
</tr>
<tr>
<td>+9.0 M urea</td>
<td>−3.48</td>
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<tr>
<td></td>
<td>1122</td>
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<tr>
<td></td>
<td>350</td>
</tr>
<tr>
<td></td>
<td>13</td>
</tr>
</tbody>
</table>

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![Fig. 6. Tryptophan fluorescence spectra of CGB lectin (0.1 μM) at 0.0 M, 5.0 M and 9.0 M urea concentrations in PBS (pH 7.2) at 25 °C.](image1)

![Fig. 7. ANS fluorescence spectra of CGB lectin (0.1 μM) at 0.0 M, 4.2 M, 5.0 M and 9.0 M urea concentrations in PBS (pH 7.2) at 25 °C.](image2)

![Fig. 8. Hemagglutination assay of CGB lectin in the absence (0.0 M urea) and the presence of 0.5–4.0 M urea. The control (C), representing the RBC suspension in PBS, has been shown at the extreme left. The assay was carried out in triplicate.](image3)
Acknowledgments

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