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A glucose/mannose binding lectin from litchi (*Litchi chinensis*) seeds: Biochemical and biophysical characterizations

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ABSTRACT

Background: Lectins are highly important biomolecules to study several biological processes. A novel α-D-glucose/mannose specific lectin was isolated from the seeds of litchi fruits (*Litchi chinensis*) and its various biophysical and biochemical properties were studied.

Methods: Purification was done by successive Sephadex G 100 and Con A-Sepharose 4B affinity chromatography. SDS-PAGE, Surface Plasmon Resonance (SPR), steady state absorbance, fluorescence, time-correlated single-photon counting, circular dichroism and antibiofilm activity by measuring total protein estimation and azocasein degradation assay have been performed.

Results: The purified lectin is a homodimer of molecular mass ~ 54 kDa. The amount of lectin required for hemagglutination of normal human O erythrocytes was 6.72 μg/ml. Among the saccharides tested, Man-α-(1,6)-Man was found to be the most potent inhibitor (0.01 mM) determined by hemagglutination inhibition assay. Steady state and time resolved fluorescence measurements revealed that litchi lectin formed ground state complex with maltose (K_a=4.9 ($\pm$0.2)$\times 10^4$ M^{-1}), which indicated static quenching (Stern-Volmer (SV) constant K_{sv}=4.6 ($\pm$0.2)$\times 10^4$ M^{-1}). CD measurements demonstrated that litchi lectin showed no overall conformational change during the binding process with maltose. The lectin showed antibiofilm activity against *Pseudomonus aeruginosa*.

Conclusions: A novel homodimeric lectin has been purified from the seeds of litchi fruits (*Litchi chinensis*) having specificity for α-D-glucose/mannose. The thermodynamics and conformational aspects of its interaction with maltose have been studied in detail. The antibiofilm activity of this lectin towards *Pseudomonus aeruginosa* has been explored.

General significance: The newly identified litchi lectin is highly specific for α-D-glucose/mannose with an important antibiofilm activity towards *Pseudomonus aeruginosa*.

1. Introduction

Lectins are a class proteins or glycoproteins ubiquitous in nature possessing at least one non-catalytic domain that binds reversibly to specific mono- or oligosaccharide [1]. Because of their well-defined carbohydrate specificity they serve as valuable reagents in the study of biochemistry, cell biology, hematology, immunology, glycobiology, and oncology and have enormous applications in biomedical research including cancer research [2]. Lectins differentiate between the malignant and normal cells based on the altered glycosylation on cell surface, associated with malignancy and metastasis [3,4]. They can be used for the detection of glycan changes in certain diseases, which involve fucosylation, sialylation and branching of complex carbohydrates. Recognition of these altered structural profiles of glycans by lectins provides valuable disease biomarkers [5–8].

Two lectins were isolated and partially characterized from the seeds of *Talisia esculenta* and *Koelreuteria paniculata* belonging to the Sapindaceae family [9,10]. *T. esculenta* lectin of MW 40 kDa having specificity towards D-glucose, D-mannose and *N*-acetylglucosamine inhibited the growth of the pathogenic fungi *Fusarium oxysporum*, *Colletotrichum lindemuthianum* and *Saccharomyces cerevisiae. K. paniculata* lectin with MW 66 kDa has binding specificity to *N*-acetylglucosamine, which showed insecticide activity against *Callosobruchus maculates*, *Anagasta kuehniella. Litchi*

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chinensis Sonn. (lychee), which also belongs to the family of Sapindaceae, is cultivated throughout Southeast Asia, especially in China [11]. *Litchi chinensis* extract was reported to have anti-inflammatory, antioxidant, and antidiabetic activities [12]. Recently it has been documented that *Litchi chinensis* extract showed antiplatelet, anticoagulant and thrombolytic activity and this could be a new natural source for the development of therapeutics for thrombosis and cardiovascular disease [13].

Bacterial biofilm plays crucial role in growth and pathology by supporting their stable attachment to different interfaces along with rendering physical protection against adverse environmental conditions like exposure to UV, salt concentration, pH, attack by host immune system, and antimicrobial agents etc [14]. These biofilms are generally rich in exopolysaccharides, proteins, teichoic acids, extracellular DNA and enzymes. Because of the biofilm formation, some pathogens are difficult to treat; thus, inhibition of biofilms is becoming an important area of concern in infection biology. Novel approaches to interrupt biofilm network, often by natural products, have shown high efficiency in many cases [15]. Our newly characterized litchi lectin has shown an important antibiofilm activity towards *Pseudomonas aeruginosa.*

The present study reports the purification of a glucose/mannose specific lectin from litchi seeds, *Litchi chinensis* by chromatographic techniques and its biochemical and biophysical characterization. Antibiofilm activity of litichi lectin against *P. aeruginosa* is also described herein.

2. Materials and methods

2.1. Materials

Acrylamide, *N*,*N*′-methylenebisacrylamide, ammonium persulfate, TEMED, sodium dodecyl sulfate, Tris, Pronase from *Streptomyces griseus*, BSA, D-Glc, D-Man, D-GlcNAc, D-Gal, α-Me-D-Glc, β-Me-D-Man, D-Ara, L-Rha, maltose, trehalose, cellobiose, raffinose, CNBr activated-Sepharose 4B, diaminobenzidine (DAB), concanavalin A (Con A), biotin 3-sulfo-N-hydroxysuccinamide ester, antibiotin HRP were purchased from Sigma, U. S. A. Precision Plus Protein Standards was procured from BioRad, U. S. A. The mannobiose, *viz*., Man-α-(1,2)-Man, Man-α-(1,3)-Man, Man-α-(1,6)-Man was supplied by Dr. Santanu Mandal, University of Manchester, Manchester, UK. CM5 sensor chip, surfactant P20, amine-coupling kit containing *N*-ethyl-*N*′-3-dimethylaminopropyl carbodiimide hydrochloride (EDC), N-hydroxysuccinamide (NHS), ethanolamine hydrochloride were purchased from BIACore, AB, Uppsala, Sweden. All other reagents used were of high analytical grade and obtained from commercial sources.

2.2. Purification of litchi lectin

Litchi seeds were obtained from litchi fruits and washed with saline. The dark brown shining seed coat was removed and the seeds were soaked in saline (20% w/v) and crushed in a blender. The slurry, after stirring at 277 K for 8 h was filtered through Whatman No 1 filter paper. The filtrate was centrifuged at 10,000 rpm for 1 h, dialyzed, concentrated by Amicon filter and stored at 253 K with 0.02% NaN_3 as the crude litchi extract. The litchi extract was subjected to gel filtration on Sephadex G-100 (10 × 300 mm), pre-equilibrated with 0.9% saline and the added protein was eluted with saline. After measuring the absorbance of the fractions, the hemagglutination assay was performed and the active fractions were pooled and concentrated. The concentrated protein was then loaded on Concanavalin A-Sepharose 4B column (10 × 50 mm). The selection of affinity chromatography was made by dot blot analysis of active fraction obtained after gel-filtration as described below. The column was washed with 10 mM TBS-saline buffer (10 mM Tris, 150 mM NaCl, pH 7.5) and the bound protein was eluted with 100 mM methyl-α mannoside in TBS containing $MnCl_2$, $CaCl_2$, $MgCl_2$, 1 mM each. The activity of eluted fractions was checked by hemagglutination assay and the active fractions were pooled and concentrated.

2.2.1. Dot blot analysis

Briefly, 10 μg of Sephadex G-100 column eluted active protein as well as equal amount of BSA were dotted separately onto nitrocellulose paper and kept for 1 h at room temperature. After washing, the membrane was blocked with 10 μl of 1% BSA in tris buffered saline followed by addition of 10 μl biotin-labelled Con A (1:500). After 30 min incubation, antibiotin-HRP conjugate (1:500) was added. The Con A reactive protein spot was visualized after addition of diaminobenzidine (DAB) and 0.01% H_2O_2 in sodium acetate buffer (pH 5). The reaction was stopped by washing the membrane with distilled water and the membrane was left to dry for the colorimetric study.

2.3. Analytical assay

The protein content of the purified lectin was estimated by Bradford method [16] and the total neutral sugar content was estimated colorimetrically by the phenol/H_2SO_4 method [17], using D-mannose as the standard.

2.4. Hemagglutination and hemagglutination-inhibition assays

The hemagglutinating activity of the litchi lectin determined according to Chatterjee et al. [18]. Briefly, an equal volume of 2% (v/v) normal or pronase-treated human O erythrocytes suspensions in saline was added to a 2-fold serially diluted sample (25 μl) in TBS in a 96-well U-bottomed polystyrene plate and kept for 1 h at 298 K. Hemagglutination titer was defined as the reciprocal of the highest dilution showing visible hemagglutination.

The hemagglutination-inhibition assay was performed by pre-incubating 25 μl of two hemagglutinating doses of litchi lectin with equal volume of serially diluted saccharides (200 mM) in 96-well polystyrene U-bottomed microtitre plate for 1 h at 298 K. Human O erythrocytes suspension in 150 mM NaCl (25 μl, 2% w/v) was added to each well and the results were recorded after 1 h. 25 μl TBS and 25 μl of mannose solution (100 mM) separately were treated as control. The inhibitory activity of sugar was defined as the minimum concentration required for complete inhibition of two hemagglutinating doses of the lectin.

2.5. Homogeniety and molecular mass

Polyacrylamide gel electrophoresis (PAGE) under nondenaturing condition was performed in acidic buffer system (β alanine/acetic acid, pH 4.3) according to Reisfled et al. [19]. The protein band was visualized by Amido Black followed by distaining in 5% acetic acid.

SDS-PAGE was done on 12% polyacrylamide gel in Tris/glycine buffer pH 8.3 according to the method of Laemmli et al. [20]. The sample was heated with 1% SDS in the presence or absence of 2-mercaptoethanol (2 ME) for 5 min at 373 K. The gel was stained with Coomassie brilliant blue, G-250. Molecular mass of the lectin was calculated comparing the relative mobility of protein standards.

2.6. Physiochemical property

2.6.1. Thermal and pH effect

Aliquots of litchi lectin in TBS, 200 μl each were incubated at

different temperatures, ranging from 283 to 353 K for 30 min and cooled to 298 K. The hemagglutination assay was performed with each aliquot as before.

The effect of pH on the hemagglutinating activity of the lectin was studied in the pH range from 3.5 to 10 using different buffers (50 mM) *viz*., glycine-HCl (pH 3.5), glycine-HCl (pH 4), sodium acetate (pH 5), citrate-phosphate (pH 6), sodium phosphate (pH 7), Tris-buffered saline (pH 7.5), Tris-buffered saline (pH 8) and glycine-NaOH (pH 10). The lectin was dialyzed against desired buffer at 277 K for 6 h and then the hemagglutination assay was performed as before.

2.6.2. Effect of divalent cation

To examine the requirement of metal ion for the activity of litchi lectin, it was dialyzed extensively at 277 K against TBS (pH 7.5) containing 50 mM EDTA. The hemagglutination assay was performed with the dialyzed lectin in the absence and in the presence of $CaCl_2$, $MgCl_2$, $MnCl_2$, 20 mM each separately.

2.7. UV–vis, steady state and time resolved fluorescence spectroscopy

Steady-state UV–vis absorption and fluorescence emission spectra of dilute solutions ($10^{-4}-10^{-6}$ M) of the lectin was recorded at 298 K using 1 cm path length quartz cells by means of an absorption spectrophotometer (Shimadzu UV-1800) and Cary Eclipse fluorescence spectrophotometer (Varian) respectively.

Fluorescence lifetime measurements were carried out by the time-correlated single-photon counting (TCSPC) method using a Horiba Jobin Yvon Fluorocube. For fluorescence lifetime measurements the samples were excited at 375 nm using a picosecond diode (IBH Nanoled-07). The TCSPC setup consists of an Ortec 9327 CFD (constant fraction discriminator) and a time to amplitude converter (Tennelec TC 863 TAC). The data were collected with a PCA3 card (Oxford) as a multichannel analyzer. The fluorescence decays were deconvoluted using IBH DAS6 software. The goodness of fit was assessed over the full decay including the rising edge with the help of statistical parameters $\chi 2$ and Durbin Watson (DW) parameters. All the solutions for room temperature measurements were deoxygenated by purging argon gas stream for about 30 min

2.8. Circular dichroism (CD) spectroscopy

CD spectra were recorded in a Jasco J-600 spectropolarimeter (Jasco Inc., Japan) in the far UV region (250–190 nm) at 298 K. Litchi lectin ($\sim 1.22 \times 10^{-5}$ M) in a 1-mm quartz cell was used in the experiment. The above experiment was conducted in the presence of different concentrations of maltose. The temperature dependent CD spectra of litchi lectin were investigated at 298, 313, 323, 343 and 353 K using a Peltier temperature-control unit. After heating up to 353 K, the litchi lectin was subsequently cooled to 298 K and its CD spectra were recorded. All spectra were recorded after accumulation of three runs. The data were expressed in terms of molar ellipticities (θ) in deg cm^2 $dmol^{-1}$.

2.9. Binding of litchi lectin with saccharides by surface plasmon resonance (SPR) analysis

The binding studies of litchi lectin with saccharides were carried out using BIAcore 3000 SPR apparatus, (BIAcore AB, Uppsala, Sweden) at 298 K. After equilibration with 10 mM HEPES-buffered saline (pH 7.4), the surface of the sensor chip was activated with a 1:1 mixture (100 μL) of 0.1 M NHS and 0.1 M EDC. Litchi lectin (50 μg/mL) was immobilized on CM5 chip in 10 mM sodium acetate buffer (pH 5.0) at a flow rate of 30 μL/min for 10 min and unreacted groups were blocked by 1.0 M ethanolamine (pH 8.5). The association rate constants were determined by passing the saccharides solutions (100–2500 nM) over the chip at a flow rate of 30 μL/min for 3 min After every cycle the sensor chip was regenerated by passing 50 mM HCl for 1 min Binding kinetics were calculated by BIA evaluation software version 3.0.

2.10. Microbial strain

In this study *P. aeruginosa* (MTCC 2488) has been used as target organism. For cultivating the organism, Tryptic soy broth (TSB) (Himedia, India) medium were used. TSB was prepared using pancreatic digest of casein (17 g/L), dipotassium hydrogen phosphate (2.5 g/L), glucose (2.5 g/L), sodium chloride (5 g/L) and papaic digest of soyabean meal (3 g/L). For the solid medium, 1.5% agar was added to the broth prior to autoclaving.

2.11. Antimicrobial activity

Antimicrobial activity of litchi lectin was done by agar diffusion method as described previously [21,22]. Briefly, paper discs of 4 mm in diameter soaked with litchi lectin solution (200 μg/ml, 400 μg/ml) and a standard antibiotic Tobramycin (MIC-64 μg/ml) [23] were placed separately on agar plates overlaid with soft agar (0.7%) that was inoculated with 6×10^6 CFUs of *P. aeruginosa* (the same number of CFUs have been used in all the experiments). Plates were incubated at 310 K for 48 h. The extent of inhibition was measured by the diameter of the clear zone around the disc. For the growth curve analysis inoculation was done with *P. aeruginosa* on three experimental sets; namely control (untreated), litchi lectin treated and a standard antibiotic, tobramycin sub MIC (1/4 MIC) dose. The absorbance was recorded at different time points at 600 nm.

2.12. Antibiofilm activity of litchi lectin

The antibiofilm activity of litchi lectin on *P. aeruginosa* was tested by growing the organism in sterile test tubes containing TSB at 310 K for 48 h under shaking condition. The experimental set contained increasing concentration of litchi lectin, a standard antibiotic (tobramycin) and an untreated control. After incubation tubes were washed three times with sterile water and stained with 0.1% (v/v) safranin for 10 min The excess stain was removed by washing with sterile water. Tubes were then dried for overnight at 310 K. Safranin-stained adherent *P. aeruginosa* bacteria were redissolved in 30% (v/v) glacial acetic acid and the absorbance was recorded at 492 nm [24]. Each assay was performed in triplicate. The following formula was used to calculate the percentage of biofilm inhibition of the compound against the bacteria.

Biofilm inhibition (in %)={(OD of the untreated sample)−(OD of the lectin treated sample)/ OD of the untreated sample} × 100.

2.13. Estimation of total protein concentration in biofilm

The presence of bacterial biomass was further estimated by the checking the total protein concentration. To estimate the total extractable protein *P. aeruginosa* was inoculated into sterile test tubes containing TSB in the presence and absence of litchi lectin and a sub MIC dose of tobramycin (1/4th MIC) and incubated at 310 K for 48 h. To extract the protein from the adhering bacteria, the planktonic cells were removed out, tubes were then washed with sterile water and thereafter boiled in 0.5 N NaOH (5 ml) for 30 min The suspension was centrifuged and the supernatant was collected. Protein concentration was determined by the Lowry method [25]. The following formula was used to calculate the percentages of biofilm inhibition of the compound against the

bacteria.

Total protein in %

= {OD of the untreated sample–(OD of the lectin treated sample) / OD of the untreated sample} × 100

2.14. Determination of swarming motility of P. aeruginosa

Swarming motility was determined in small petri dishes (35 × 10 mm) containing nutrient agar (8 g/L) (Himedia, India) supplemented with glucose (5.0 g/L). An aliquot of an overnight saturated culture of *P. aeruginosa* (~10^8 CFU/mL) either treated or untreated with the highest dose of litchi lectin and a standard antibiotic tobramycin was inoculated in the centre of the plates and subsequently dried for 20 min at room temperature. The plates were then incubated at 310 K for 48 h. Swarming motility was determined by measuring the diameter of circular zone of colony growth from the point of inoculation [26].

2.15. Azocasein degrading proteolytic activity

To determine the azocasein degrading proteolytic activity, *P. aeruginosa* were grown in 24-well polystyrene plates containing 1 mL of sterile TSB in the presence and absence of litchi lectin and a standard antibiotic (tobramycin) and incubated at 310 K for 48 h. The proteolytic activity in the cell free supernatant of *P. aeruginosa* was determined according to the method of Kessler et al. with

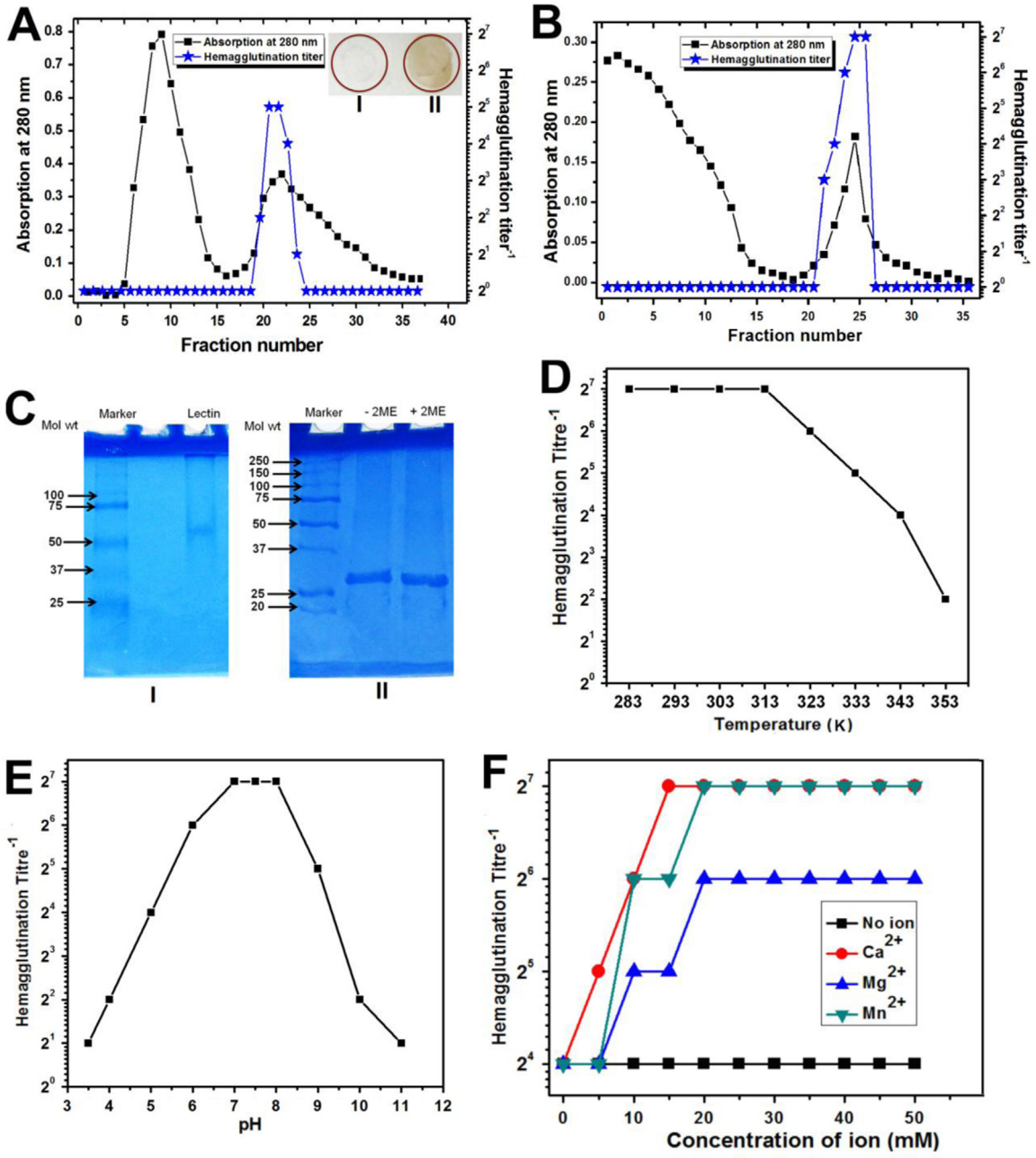

Fig. 1. (A) Elution profile of litchi seed extract from Sephadex G-100 gel filtration column. (Inset) The dot blot experiment with (I) BSA and (II) concentrated gel filtration active fraction by Con A. (B) Elution profile of the active protein fraction from Sephadex G-100 column by affinity chromatography on Con A-Sepharose column. (C) PAGE of the purified litchi lectin (I) under non-denaturing acidic condition and (II) SDS-PAGE with and without 2- mercaptoethanol (2ME) (D) Effect of temperature ranging from 283 to 373 K on the hemagglutinating activity of litchi lectin. (E) Effect of different pH ranging from pH 3.5 to pH 10 on the hemagglutinating activity of litchi lectin. (F) Effect of Ca^{2+}, Mg^{2+} and Mn^{2+} ion on the hemagglutination activity of litchi lectin.

minor modifications [27]. Briefly, cell suspensions (both treated and untreated) were separately centrifuged at 10,000 rpm for 5 min to collect the cell free extract. To 200 μL of cell-free supernatant from either treated or untreated sample, were added 50 μL 0.3% azocasein (Sigma) in 0.05 M Tris–HCl (pH 7.5) and the reaction mixture was subsequently incubated at 310 K for 1 h. The reaction was then stopped by the addition of l0% trichloroacetic acid. Thereafter, the reaction mixture was centrifuged at 10,000 rpm for 5 min and the absorbance was recorded at 400 nm [28]. The following formula was used to calculate the percentages of biofilm inhibition of the compound against the bacteria.

Azocasein degradation (in %)

$$= \{\text{OD of the treated sample / OD of the untreated sample}\} \times 100$$

Each experiment was performed in triplicate. The values were the mean of three assays ± SD. Significance was determined by using Student's *t*-test and mentioned as P value < 0.05 (noted with *) and P value < 0.005 (noted with **).

3. Results and discussion

3.1. Purification and physicochemical characterization of lectin

The purification of litchi lectin was carried out by successive two-steps: gel filtration chromatography on Sephadex G-100 and affinity chromatography on Con A-Sepharose column. The seed extract (20% w/v) by gel filtration chromatography separated into two fractions (Fig. 1(A)). The dot blot study indicated the binding of the active fractions after gel filtration with Con A and selection of Con A-sepharose in the final phase of affinity based purification was made accordingly (Fig. 1(A) inset). The second fraction having hemagglutination activity was purified by affinity column and the purification achieved was 51 fold (Fig. 1(B); Table 1).

The purified lectin produced a single band at ~54 kDa by non-denaturing acidic gel (12%) electrophoresis. However, litchi lectin by SDS-PAGE (12%) under denaturing condition with or without 2-ME, produced single band at ~27 kDa indicating that litchi lectin is homodimeric in nature (Fig. 1(C)).

Litchi lectin is a glycoprotein like many other lectins and contains 9.7% carbohydrate as estimated by phenol-sulphuric acid method.

The hemagglutination activity of litchi lectin was found to be equal (titer^{-1} 2^7) irrespective of O, A and B human blood groups and the titer was found to be increased (titer^{-1} 2^{10}) after pronase treatment of erythrocytes. The minimum amount of litchi lectin required for visible agglutination of normal human erythrocytes was 6.72 μg/mL.

The activity of litchi lectin was maximum between 283 and 313 K, gradually decreased with rise in temperature. After heating up to 353 K and subsequent cooling at 298 K the activity of the lectin persisted (titer^{-1} 2^2) (Fig. 1(D)). It showed activity at pH between 3.5 and 10 being maximum between pH 7 and 8 (Fig. 1 (E)).

The activity of litchi lectin is partially divalent metal ion dependent. Since after dialysis against 50 mM EDTA containing TBS, the activity of the lectin reduced from 128 to 16. Fig. 1(F) shows that Ca^{2+} and Mn^{2+} ions had more effect on the hemagglutinating activity of the litchi lectin than Mg^{2+}. *Talisia esculenta* lectin having D-Man, D-Glc and D-GlcNAc specificity from the same family (Sapindaceae) is a Ca^{2+} dependent lectin [9]. Another lectin from *Koelreuteria paniculata,* of the same family having specificity for D-GlcNAc required Mg^{2+} and Mn^{2+} for hemagglutinating activity but did not require Ca^{2+} for the same [10].

3.2. Determination of carbohydrate specificity

Inhibitory effect of various saccharides on hemagglutinating activity of litchi lectin was studied and the results are summarized in Table 2. Among the monosaccharides tested glucose and mannose were found to be very good inhibitors; of them mannose was found to be superior and was more pronounced when they are in α-glycosidic linkage, Me-α-Man (0.78 mM) > Me-α-Glc (1.56 mM). C4-OH in equatorial form is an important locus in mannose and glucose since galactose did not inhibit the hemagglutination even at 200 mM. Among the mannobiose as inhibitor the most potent was Man-α-(1,6)-Man (0.01 mM) followed by Man-α-(1,3)-Man (0.03 mM) which was found to be preferred inhibitor compared to Man-α-(1,2)-Man (0.05 mM), [Man-α-(1,6)-Man > Man-α-(1,3)-Man > Man-α-(1,2)-Man]. Such inhibitory trend of mannobiose has been observed in mannose-binding lectins from rhizomes *Ophiopogon japonicas* [29] and *Polygonatum ororatum* [30]. C6-OH is some extent hindering the interaction with the lectins, since L-rhamnose (0.39 mM) which is 6-methyl mannose showed two times more inhibitory than Me-α-Man (0.78 mM) and 8 times more inhibitory than D-mannose (3.12 mM). This result is further substantiated by inhibition with D-arabinose (0.78 mM), a five membered furanose sugar. GlcNAc is a poor inhibitor requiring high concentration (100 mM) in inhibiting hemagglutination of lectin. Among glucose disaccharides maltose [Glc-α-(1,4)-Glc] was found to be the better than cellobiose [Glc-β-(1,4)-Glc] inhibiting the interaction at a very high dose (50 mM). Therefore, litchi lectin showed its inhibitory potency in α-linkage and almost nil when glucose is β-linked. This results were further substantiated by inhibition with trehalose, [α-Glc-(1,1)-α-Glc] (0.39 mM). Raffinose, Gal-α-(1,6)-Glc-α-(1,6)-fructofuranose also inhibited the agglutinating activity of litchi lectin moderately (6.25 mM) due to α –linkage.

The sugar binding specificity of litchi lectin was further investigated by surface plasmon resonance (SPR) analyses. Litchi lectin was immobilized on the sensor chip CM-5 by amine coupling and ten saccharides were passed over it stepwise separately. The sensorgrams and the kinetic data of the binding are shown in Fig. 2A, B and Table 3, respectively. The binding of all saccharides to the immobilized lectin fitted best to a 1:1 binding model in the evaluating software. Among the saccharides tested for binding

Table 1
Purification scheme of litchi lectin.

Lectin fraction	Protein (mg/mL)	Hemagglutination titer $^{-1}$*	Specific activity (titer^{-1}/mg-protein/ml)	Purification fold	Protein recovery (%)
Crude seed extract	2.15	64	30	1	100
Gel filtration chromatography on Sephadex G-100	0.24	32	133	4.5	11
Affinity chromatography on Con A-Sepharose	0.08	128	1524	51	4

* Reciprocal of the highest dilution of the lectin showing visible hemagglutination; hemagglutination was determined with normal human O erythrocytes.

Table 2
Hemagglutination-inhibition assay by carbohydrates.

Carbohydrates	Minimum inhibitory concentration of carbohydrates* (mM)
D-mannose	3.12
Methyl-α -D-mannose	0.78
Methyl-β -D-mannose	NI**
Man-α-(1,2)-Man	0.05
Man-α-(1,3)-Man	0.03
Man-α-(1,6)-Man	0.01
D-Glucose	6.25
D-Galactose	NI**
Methyl-α-D-glucose	1.56
D-Arabinose	0.78
L-Rhamnose	0.39
N-acetyl-D-glucosamine	100
Trehalose	0.39
Maltose	0.05
Cellobiose	50
Raffinose	6.25

* Required for complete inhibition of two haemagglutinating doses of lectin against human O erythrocytes;
** NI= No inhibition.

assay maltose showed strongest binding ($K_a = 7.1 \times 10^{10}\ M^{-1}$) among the analytes. Among the tested saccharides the least binding was observed in cellobiose.

3.3. Conformational aspects of lectin-maltose interaction by fluorescence and CD

Steady-state UV–vis absorption spectra of litchi seed lectin in 150 mM NaCl, containing 10 mM $CaCl_2$ at pH 7.5 and at 298 K was measured and the effect of increasing concentration of maltose on litchi lectin was examined. With addition of maltose gradually, the entire absorption spectrum endured a hypochromic effect without any noticeable spectral shift (Fig. 3(A)). It is to be mentioned that in the absorption spectral region of the lectin, the addition of maltose with varying concentrations did not display any significant absorbance. It is noteworthy that addition of maltose beyond the concentration 6.5×10^{-5} M, the absorbance intensity was not decreased *i.e.* a saturation point was reached. It is apparent that the observed hypochromic effect in UV–vis spectra could be due to ground state complex formation between maltose and litchi lectin (Fig. 3(A)). Further, Benesi– Hildebrand (BH) plot (Fig. 3B) constructed by using Eq. (1), [31]

$$1/(\varepsilon_0 - \varepsilon_c) = 1/(\varepsilon_0 - \varepsilon_b) + 1/(\varepsilon_0 - \varepsilon_b)(1/K)(1/C). \quad (1)$$

shows clearly the linear relation between $1/(\varepsilon_0 - \varepsilon_c)$ vs 1/C, where ε_0 and ε_c are the respective molar extinction coefficients of

Table 3
Binding constants of interaction between immobilized litchi lectin and different saccharides.

Saccharide	K_a (M^{-1})	χ^2
Maltose	$7.1(\pm 0.2) \times 10^{10}$	0.29
Rhamnose	$2.2(\pm 0.3) \times 10^{9}$	0.26
Arabinose	$1.0(\pm 0.1) \times 10^{8}$	0.97
Me-α-D Man	$9.7(\pm 0.4) \times 10^{6}$	0.33
Trehalose	$8.3(\pm 0.3) \times 10^{6}$	2.0
Me-α-D Glc	$4.0(\pm 0.3) \times 10^{6}$	0.50
Cellobiose	$8.7(\pm 0.6) \times 10^{4}$	1.05
Mannose	$9.3(\pm 0.5) \times 10^{6}$	1.46
Glucose	$2.1(\pm 0.5) \times 10^{6}$	1.32
Raffinose	$1.0(\pm 0.4) \times 10^{8}$	0.68

litchi lectin in the absence and presence of maltose having the concentration C. ε_b denotes the molar extinction coefficient for the complex molecule. That the complexation process is effective even in low concentration of maltose is reflected in the linearity of the Benesi–Hildebrand double reciprocal plot (Fig. 3(B)) suggesting that the stoichiometry of the lectin- maltose complex is 1:1.

Steady state fluorescence emission spectra of litchi lectin in 150 mM NaCl containing 10 mM $CaCl_2$ were recorded in the presence of different concentrations of maltose (Fig. 3(C)) at pH 7.5 at 298 K using the excitation wavelength at 280 nm. The emission band of litchi lectin was found to be quenched regularly with increasing concentration of maltose. Addition of maltose at a concentration above 6.0×10^{-5} M, showed no further decrease in fluorescence intensity which denoted a saturation point.

The similar type of fluorescence quenching was also observed at other temperatures: 308 K, 318 K and 328 K. The spectra were analyzed with the help of the Stern–Volmer (SV) relation represented by the Equation 2 (Fig. 3D) [32–35].

$$F_0 / F = 1 + K_{SV}[Q] = 1 + k_q \tau_0 [Q] \quad (2)$$

where F_0 and F denote the steady-state fluorescence emission intensities in the absence and presence of the quencher, respectively, K_{sv} is the quenching constant which was determined from the slope of the Stern–Volmer plot at lower concentrations of quencher, whereas [Q] represents molar concentration of the quencher maltose. k_q is the bimolecular rate constant of the quenching reaction, and τ_o is the average integral fluorescence life time of tryptophan which is $\sim 4.31 \times 10^{-9}$ s [36].

The linearity of SV plot with steady state fluorescence emission intensities of litchi lectin in presence of different concentration of maltose (Fig. 3(D)) was indicative of the nature of the quenching, either static or dynamic one [32]. The value of K_{SV} was calculated from the slope of the plot which was 4.6 (± 0.2) $\times 10^4\ M^{-1}$ at the maximum emission wavelength 334 nm. Further, to distinguish

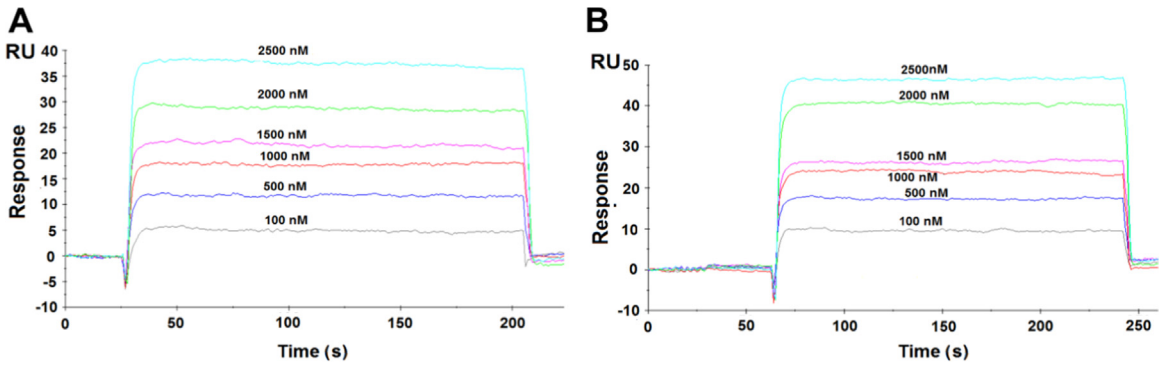

Fig. 2. Sensorgram of the interactions of immobilized litchi lectin with (**A**) Me-α-Man and (**B**) maltose by SPR. Lectin (50 μg/ml) in 10 mM Na-acetate buffer (pH 5.0) was immobilized onto the CM5 chip and the blocking was performed with 1.0 M ethanolamine hydrochloride (pH 8.5). The reference flow cell was prepared in an analogous manner without litchi lectin. Various concentrations of saccharide solutions (100, 500, 1000, 1500, 2000, 2500 nM) were injected onto lectin- immobilized sensor chip.

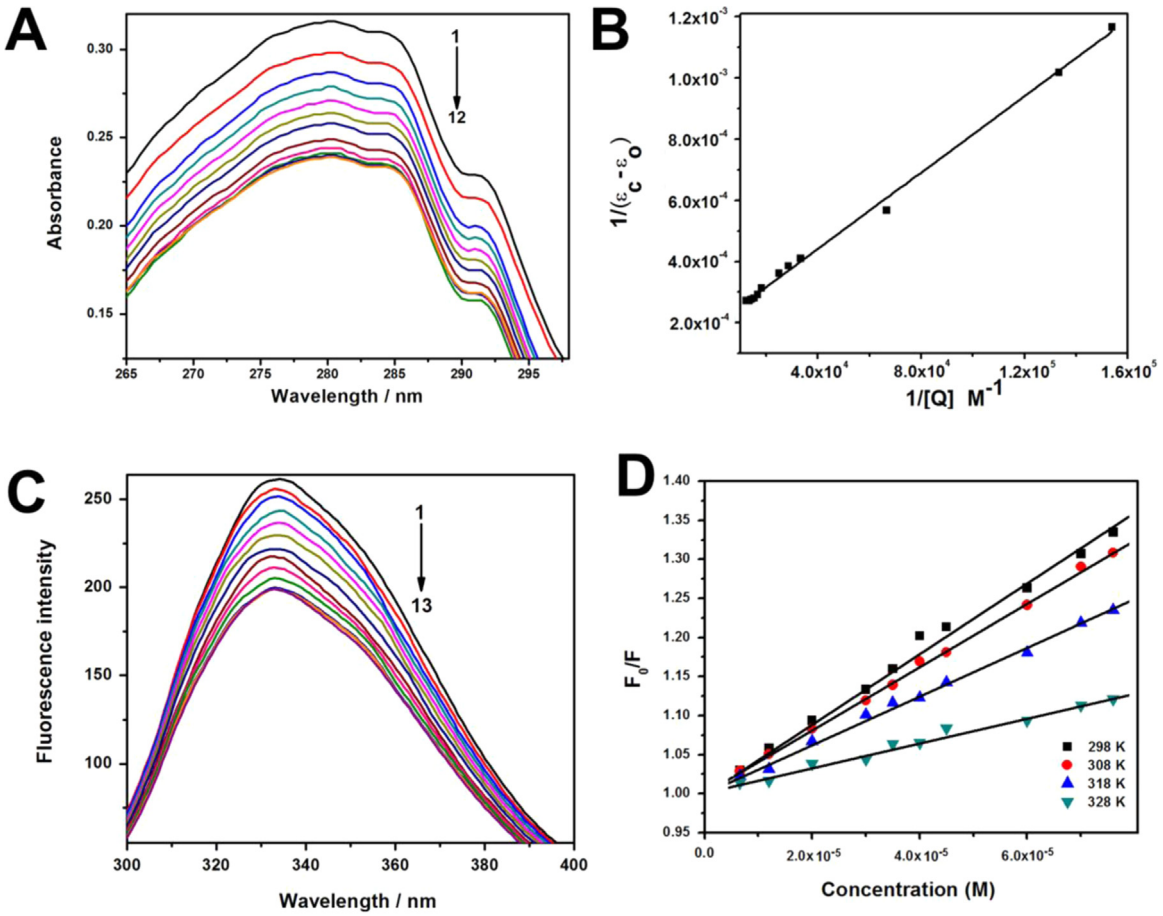

Fig. 3. (A) UV–vis absorption spectra of litchi lectin (~ 3.20×10^{-5} M) in the presence of maltose at the concentration (M): (1) 0, (2) 6.50×10^{-6}, (3) 7.50×10^{-6}, (4) 1.50×10^{-5}, (5) 3.0×10^{-5} (6) 3.50×10^{-5}, (7) 4.00×10^{-5}, (8) 5.50×10^{-5}, (9) 6.00×10^{-5}, (10) 6.50×10^{-5}, (11) 7.00×10^{-5}, (12) 7.60×10^{-5} at 298 K in 150 mM NaCl, 10 mM $CaCl_2$, pH 7.5. (B) Benesei–Hilderbrand plot for litchi lectin-maltose complex at absorption maxima 280 nm (Adj. R-Square=0.9971). (C) Fluorescence emission spectra of litchi lectin (~ 3.10×10^{-5} M) in the presence of maltose at different concentrations (M): (1) 0, (2) 6.67×10^{-6}, (3) 1.20×10^{-5}, (4) 2.00×10^{-5}, (5) 3.00×10^{-5} (6) 3.50×10^{-5}, (7) 4.00×10^{-5}, (8) 4.50×10^{-5}, (9) 5.00×10^{-5}, (10) 5.50×10^{-5} (11) 6.00×10^{-5}, (12) 7.00×10^{-5}, (13) 7.60×10^{-5} at 298 K in 150 mM NaCl containing 10 mM $CaCl_2$, pH 7.5. (D) SV plot from steady-state fluorescence emission intensity measurements of litchi lectin in presence of maltose at 298, 308, 318 and 328 K.

static and dynamic quenching modes operative in this case, the temperature dependence of K_{sv} values and the fluorescence lifetime measurements were studied.

Static and dynamic quenching are mechanistically distinct although both of them require direct contact between fluorophore and the quencher. In static quenching formation of non-fluorogenic complex leads the way to decrease in total fluorescence intensity, whereas dynamic quenching is a collisional phenomena, where quenchers provide a nonfluorescent way of decay to the fluorophore, when they make collisions in the excited state. Therefore, temperature has a very different effect on static and dynamic quenching processes. For static quenching the strength of complexation decreases with increase in temperature and in higher temperature quenching becomes less pronounced and thus for static quenching value of K_{sv} should decrease with increase in temperature whereas dynamic quenching depends on diffusion rate of the quenchers. More the diffussion rate of quencher, more efficient will be the quenching process. Therefore, at higher temperature, with the higher rate of diffusion, the over all quenching should increase if the quenching is of purely dynamic in nature. Thus with increase in temperature, K_{sv} should increase for dynamic quenching. In the present case of the litchi lectin-maltose interactions, both K_{sv} and k_q decreased with increase in temperature (Table 4). This indicates that the type of quenching here should primarily be of static in nature and to validate the static nature of quenching in the binding phenomenon of litchi lectin with maltose, fluorescence lifetime measurements were carried using time correlated single photon counting (TCSPC) method.

Table 4
K_{sv} and kq values for litchi lectin-maltose system at the different temperatures.

Temp (K)	K_{sv} (M^{-1})	k_q ($M^{-1}s^{-1}$)
298	$4.6(\pm 0.2) \times 10^4$	$1.1\ (\pm 0.2) \times 10^{13}$
308	$4.1(\pm 0.2) \times 10^4$	$9.5\ (\pm 0.2) \times 10^{12}$
318	$2.9(\pm 0.2) \times 10^4$	$6.7\ (\pm 0.3) \times 10^{12}$
328	$1.3(\pm 0.2) \times 10^4$	$3.0\ (\pm 0.3) \times 10^{12}$

K_{SV} and k_q calculated by Eq. (2). These experiments were performed in duplicate and data presented are the mean values.

The fluorescence lifetime data shows the exponential fittings of fluorescence decay of the litchi lectin in the absence and presence of maltose (Fig. 4(A), Table 5). It is also apparent that best fit exponential decay was obtained as implicated by the observed values of χ^2. The data for lectin with or without maltose (see Table 5, Fig. 4(A)) indicated three values for life times and for free lectin they were ~ 1.7 ns, ~0.6 ns, ~4.3 ns. The unperturbed values of the fluorescence lifetimes of litchi lectin, even in the presence of three different amount of maltose further confirmed that the quenching was static in nature which is probably due to the formation of ground state complex between the lectin and maltose, as previously assumed by the nature of temperature dependence of K_{sv}. Next attempt was made to determine the binding constant

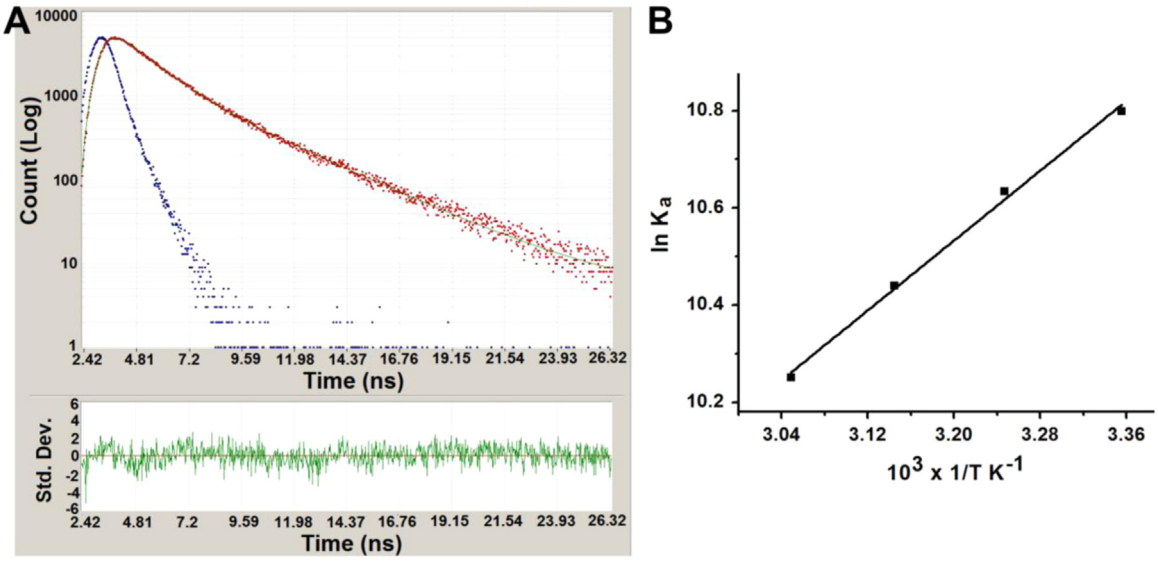

Fig. 4. (A) Fluorescence decay of the litchi lectin (red) in the absence and presence of maltose (no change in the decay pattern) along with the impulse response (faster component shown by the blue line). The residual is also shown. (B) ln K_A vs 1/T plot for the interaction of litchi lectin and maltose. Adj. R-Square =0.981.

Table 5
Fluorescence lifetimes and associated fractional contributions (fi) of litchi lectin (~ 1.42×10^{-5} M) (λ_{ex} ~280 nm, λ_{em} ~334 nm) in the presence of different concentrations of maltose.

Maltose conc (M)	f_1	τ_1 (ns)	f_2	τ_2 (ns)	f_3	τ_3 (ns)	χ^2
0	0.50	1.72	0.11	0.55	0.39	4.27	1.02
8.0×10^{-6}	0.48	2.00	0.18	0.77	0.34	4.36	0.95
1.5×10^{-5}	0.50	1.76	0.11	0.57	0.39	3.00	0.96
3.2×10^{-5}	0.48	1.87	0.15	0.66	0.37	4.33	0.96

(K_a) and number of binding stoichiometry, n of the complex.

The quantitative evaluation of the binding constant (K_a) and binding stoichiometry (n) for the litchi lectin-maltose interaction process is based on an analysis of the fluorescence quenching data by the following Equation:

$$\log\left[(f_0 - f)/f \right] = \log K_a + n \log [Q] \quad (3)$$

in which K_a is the binding constant and n is the binding stoichiometry.

From the plots of log $[(f_0 - f)/f]$ vs log[Q] at the different temperatures for the litchi lectin-maltose systems, the binding constant K_a and n values were determined as shown in Table 6. However the association constants derived from the SPR analysis are of several orders of magnitude higher (10^6 times higher in case of litchi lectin-maltose, Table 3) than those obtained by fluorescence measurements (Table 6), is due to the fact that in SPR analysis the lectin is covalently attached to the chip whereas in fluorescence measurements the lectin is free in solution.

As it is expected for static quenching phenomena, the values of K_a and n are found to decrease with increase in temperature. Thus, the unstable ground state complex could be dissociated with rise in temperature. It is important to mention here that we observed neither any additional absorption band nor any substantial deformity in the absorption spectral pattern of the lectin–maltose system, but only the hypochromic effect was seen after addition of maltose (Fig. 3(A)). Furthermore, the fluorescence spectrum of the above system did not show any additional new band other than the characteristic emission band of lectin (Fig. 3(C)). These factors taken together evidenced that there was hardly any dissociation in the ground-state complex between the lectin-maltose pair in the solution.

To gain an idea about the forces involved in lectin-maltose interactions, thermodynamic parameters were calculated from the binding constant data determined at various temperatures [37]. Under the assumption of no significant variation of the enthalpy change (ΔH) within the range of temperature studied both the enthalpy change (ΔH) and the entropy change (ΔS) can be evaluated from the van't Hoff equation:

$$\ln K_a = -\Delta H/RT + \Delta S/R \quad (4)$$

where R is the universal gas constant, T is the absolute temperature in Kelvin.

The free energy change (ΔG) of the process is then estimated from the following relationship:

$$\Delta G = \Delta H - T\Delta S \quad (5)$$

From ln K_a versus 1/T plot (Fig. 4(B)), the values of ΔH and ΔS were determined. Both ΔH and ΔS were found to be negative and same at all temperatures [$\Delta H = -30.2(\pm 0.2)$ (kJ/mol), $\Delta S = -11.5(\pm 0.2)$ (J/mol/K)] as well as the value of K_a was low which lowered rapidly with increase of temperature for the lectin-maltose system. This suggests that van der Waals and hydrogen bonding interactions predominate between lectin and maltose.

Table 6
Thermodynamic parameters for litchi lectin-maltose interactions at different temperatures.

T (K)	n (Binding stoichiometry)	$K_a/(10^4)$ (M^{-1})	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/mol/K)	$T\Delta S$ (kJ/mol)
298	0.9	4.9 (±0.2)	−26.8 (±0.2)	−30.2 (±0.2)	−11.5 (±0.2)	−3.4 (±0.2)
308	0.9	3.3 (±0.2)	−26.6 (±0.2)			−3.6 (±0.2)
318	0.8	2.2 (±0.2)	−26.5 (±0.2)			−3.7 (±0.2)
328	0.8	1.6 (±0.3)	−26.4 (±0.2)			−3.8 (±0.2)

These experiments were performed in duplicate and data presented are the mean values.

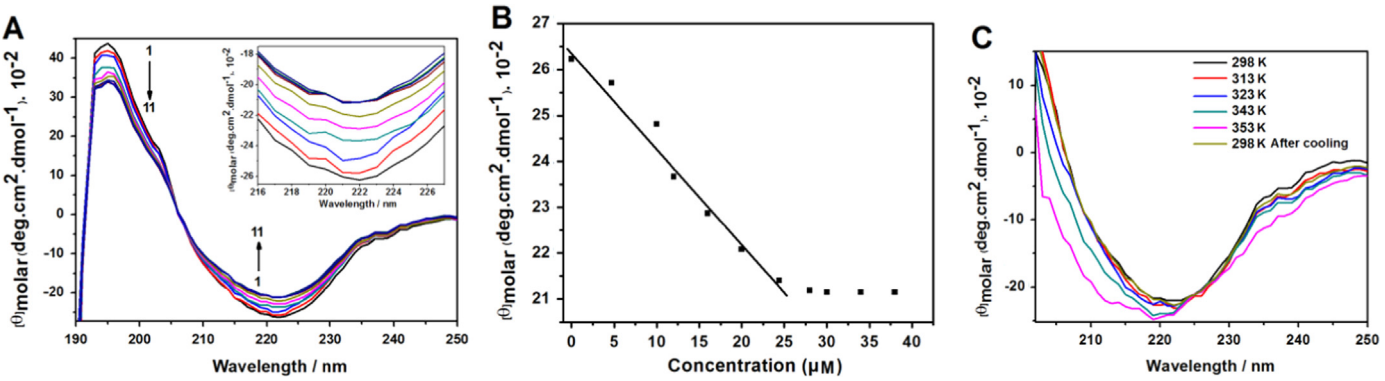

Fig. 5. (A) CD spectra of the litchi lectin-maltose system in 150 mM NaCl containing 10 mM $CaCl_2$, pH 7.5 with litchi ($\sim 1.22 \times 10^{-5}$ M) and increasing of maltose concentration (M) in (1) 0, (2) 5.00×10^{-6}, (3) 1.00×10^{-5}, (4) 1.20×10^{-5}, (5) 1.60×10^{-5}, (6) 2.00×10^{-5}, (7) 2.40×10^{-5}, (8) 2.80×10^{-5}, (9) 3.00×10^{-5}, (10) 3.40×10^{-5} and (11) 3.80×10^{-5}. (Inset) saturation was reached at a concentration $\sim 2.80 \times 10^{-5}$ M (B) Plot of molar ellipticity at 222 nm of the litchi lectin-maltose system with gradual increase in maltose concentration. Adj. R-Square = 0.932 (C) Temperature dependent secondary structural analysis of litchi lectin by CD.

Plant lectins possess diverse three dimensional structures with some common structural features that are crucial for their sugar binding specificity. The occurrence of β-sheets in lectin's three dimensional organization is predominant. These β-sheets connected by turns or loops provide a rigid concave scaffold in lectin's structures which forms the carbohydrate-binding sites specific for different sugars [38]. The sugar binding affinity depends on the different amino acid compositions in different relative stereochemical arrangements in the carbohydrate binding site [39–42].

The circular dichroism spectra of litchi lectin in the absence and presence of maltose measured at 298 K are presented in Fig. 5 (A) in the far UV region at 190–250 nm providing information about the secondary structure of lectin. The CD results are expressed in terms of mean residue ellipticity (MRE) in degree cm^2 $dmol^{-1}$ according to the following Eq. (6), [43]

$$[\theta]_\lambda = \theta / (10nlc) \quad (6)$$

where c is the molar concentration of the protein (mole/L), θ is observed rotation in millidegree (mdeg), l is the path length in cm, and n is the number of amino acid residues of protein. From Fig. 5 (A) it is observed that litchi native spectrum presents a minimum negative peak at 222 nm and a positive peak around 196 nm which suggest a high content of beta elements. With the gradual addition of maltose from 5 to 38 μM, it is clear that the spectra shapes were similar to the native one, but the intensity was decreasing gradually. The addition of maltose did not affect the position of positive maxima and negative minima which suggest that maltose did not show overall distortion effect to the secondary structure of the lectin. When higher concentration of maltose was added (> 28 μM) there was no drop of intensity suggesting that saturation was achieved that corroborates with the saturation data obtained from UV–vis and fluorescence study.

Further to study the trend of secondary structural change of the litchi lectin-maltose system with gradual addition of maltose, we plotted the molar elipticity at 222 nm with concentration of maltose (Fig. 5(B)). It was observed that the molar ellipticity progressively decreased with the rise of maltose concentration depicting small changes in the relative orientation within global structure of litchi lectin.

Fig. 1(D) demonstrated the partial retention of activity of litchi lectin even at high temperature (353 K) which is quite unusual for plant lectin. Temperature dependent secondary structural analysis suggested the degeneration of secondary structure with increasing temperature (Fig. 5(C)). However, after heating up to 353 K and subsequent cooling at 298 K the activity of the lectin persisted which supported our observation (Fig. 1(D)) as we checked the hemagglutination activity of lectin at different temperatures keeping the assay temperature at 298 K.

3.4. Anti biofilm effect of litchi lectin on P. aeruginosa

Disc diffusion assay was done to check the bactericidal effect of litchi lectin. 200 and 400 μg/ml litchi lectin and MIC (64 μg/ml) of tobramycin was used on the disc to check the antibacterial effect on TSA plate. Litchi lectin showed no antimicrobial activity against *P. aeruginosa* as evidenced by the absence of a hollow region around the disc. Conversely, a hollow zone was observed around the disc with tobramycin. The growth curve analysis of the bacteria with lectin treated set also showed no bactericidal effect on the growth of the organism (Fig. 6(A)). Sub MIC dose of tobramycin also did not inhibit bacterial growth.

In many cases, it was found that biofilm formation is a very important factor for microbial virulence and survival [14]. Even though litchi lectin had no antibacterial activity, it was necessary to check its potential antibiofilm activity. Disease progression can be managed if biofilm formation can be targeted and reduced. Antibiofilm activity assay of *P. aeruginosa* with litchi lectin showed very promising results. The result showed that litichi lectin at 400 μg/ml exhibited maximum antibiofilm activity among all the tested doses (Fig. 6(B)). From the results of the antibiofilm assay, it was also observed that the lectin showed antibiofilm activity in a dose dependent manner. With increasing doses of the lectin from 200 μg onwards, the antibiofilm activity also increased proportionately.

Estimation of total protein is also very helpful to estimate the presence of biomass on a microbial biofilm. Therefore, we have tried to estimate the bacterial biomass by calculating the presence of total protein. Here also, in consistent with the previous result of safranin staining assay, 400 μg/ml lectin showed the lowest amount of total protein (Fig. 6(D)).

To understand the viability of bacteria and their metabolic activity in a biofilm, we have measured the amount of azocasein degrading proteolytic activity between both the treated and untreated experimental sets. Viable bacteria secrete a large array of hydrolytic enzymes including proteases which cleave azocasein efficiently [28]. The result showed that with the increasing concentrations of litchi lectin (from 150 μg/ml to 400 μg/ml) the azocasein degrading proteolytic activity of the bacteria also decreased with respect to the control (Fig. 6(C)).

Bacterial motility is one of the major factors for bacterial colonisation and biofilm formation. To understand the mechanism of antibiofilm activity of litchi lectin, we have tested the swarming motility of *P. aeruginosa.* It was observed that 400 μg/ml litchi lectin significantly reduced the swarming motility (a diameter of 4 ± 1 mm)* of the bacteria as compared to the untreated control (72 ± 1 mm)*. The diameter of the motility of the bacteria treated with 400 μg/ml of litchi lectin was found to be less than the

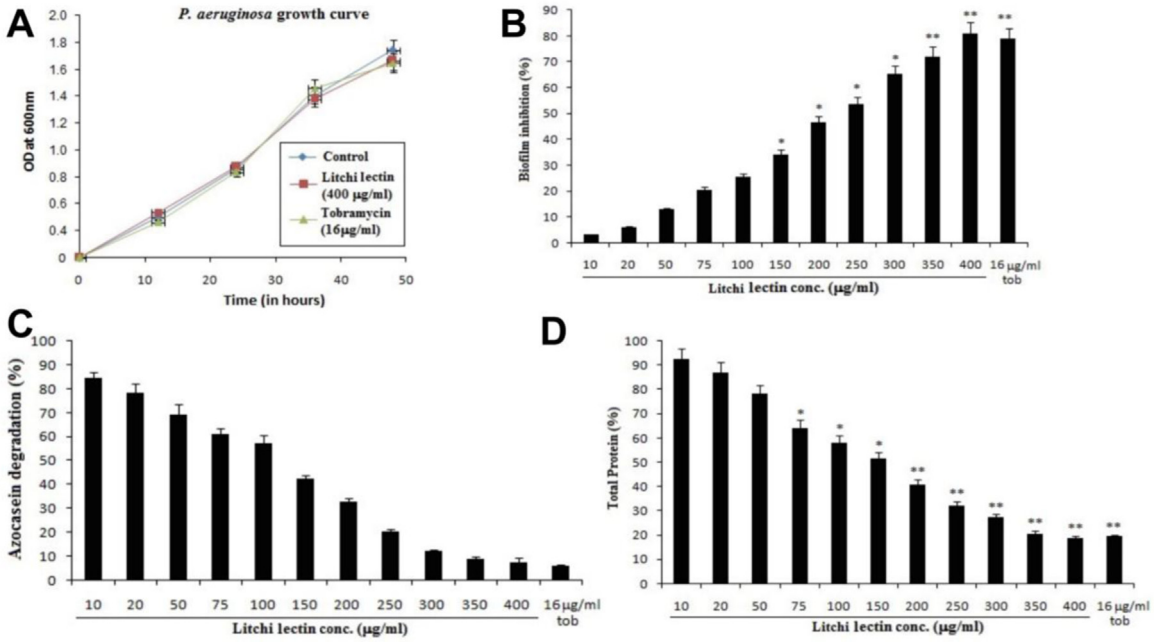

Fig. 6. (A) Growth curve analysis of *Pseudomonas aeruginosa* after treatment with litchi lectin. Cells were grown in TSB treated with litchi lectin and standard antibiotics and then OD values were taken at different time points. (B) Antibiofilm activity of litchi lectin against *Pseudomonas aeruginosa. P. aeruginosa* was grown in sterile test tubes containing TSB in the presence and absence of lectin and a standard antibiotic tobramycin. After the incubation, tubes were washed and stained with safranin and thereafter re-dissolved in 30% glacial acetic acid. Absorbance was recorded at 492 nm. (C) Azocasein degrading proteolytic activity assay of *Pseudomonas aeruginosa* after treatment with litchi lectin and tobramycin. After the treatment and incubation of 48 h, proteolytic activity of the cell free supernatant of *P. aeruginosa* was determined using azocasein as the protein substrate and then expressed as % with respect to control. (D) Protein extraction assay. *P. aeruginosa* was grown in sterile test tubes containing TSB in the presence and absence of lectin doses and a standard antibiotic tobramycin. After the incubation, the adhered microbial population was quantified as described in Material and Methods and the graph has been expressed in % with respect to control. Three replicates have been used for each type of experimental set. Error bars indicate standard deviation ($\pm$ SD). Statistical analysis were done using Student's *t*-test and the the P value < 0.05 (noted with *), the P value < 0.005 (noted with **) and the P value < 0.0005 (noted with ***) are considered to express significance level as compared to the untreated control (n=3).

diameter for the set treated with 16 µg/ml Tobramycin (5 ± 1 mm)*. So, inhibition of biofilm formation by the lectin can be attributed to the reduction in bacterial motility. [*mean $\pm$ Standard deviation (SD)].

Biofilms are communities of microbial population which attach and grow on living or nonliving surfaces. Biofilm associated infections are very often difficult to treat due to its high tolerance against various drugs [44]. For this reason, it is necessary to look for natural compounds which can efficiently attenuate biofilm formation. Inhibition of biofilm formation is the first line of defence mechanism to control surface adhered bacterial growth. Since plant lectins are known to have antibiofilm effect on various bacteria [15], we have used litchi lectin and assessed its antibiofilm activity on *P. aeruginosa*. The lectin did not have any antimicrobial activity on *P. aeruginosa* as was evident from disc diffusion assay and growth curve analysis. Litchi lectin has showed significant antibiofilm activity at a high dose of 400 µg/ml against the studied organism. It has been reported that lectins can rearrange the exopolysaccharide matrix of bacterial biofilms [15]. Therefore, we can infer that litchi lectin does indeed have antibiofilm activity against bacteria. To further check the effect of lectin on biofilm phenotypes, total protein assay was done which is a quantitative method to check bacterial biomass within a biofilm. Proteases are hydrolytic enzymes that hydrolyze the proteins of the host cells near the infected centre which thus enhances the microbial invasion and pathogenesis. In this direction, we measured the azocasein degrading protease activity of microorganisms in the presence and absence of litchi lectin. In order to understand the underlying mechanism of antibiofilm effect of lectin, we performed the motility assay of *P. aeruginosa.* From the above results, we can infer that litchi lectin could significantly reduce the biofilm load of *P. aeruginosa* along with its all associated phenotypes, but at a relatively high dose. This aspect of litchi lectin can be further exploited by use as a food preservative, or a coating agent for surgical instruments. Therefore, we can conclude that litchi lectin can be used as an antibiofilm agent for various purposes.

In conclusion, a new glucose/mannose specific lectins (~54 kDa) has been isolated from litchi (*Litchi chinensis*) seeds which is homodimeric in nature and binds to mannobiose with high potency. This specificity of lectin can be utilised for several biochemical studies *viz.* purification of mannose containing glycoproteins. It shows antibiofilm activity towards *P. aeruginosa.* From spectroscopic studies it has been observed that litchi lectin forms a ground state complex with maltose and the nature of quenching is static in nature. CD measurements of the lectin–maltose interactions show that during the binding process with maltose, no appreciable conformational change of lectin has been noticed.

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Appendix A. Transperancy document

Transperancy document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2016.05.001.

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