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Structural–functional insights and studies on saccharide binding of *Sophora japonica* seed lectin

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ABSTRACT

Functional and conformational transitions of the *Sophora japonica* seed lectin (SJL) were studied in detail using bioinformatics and biophysical tools. Homology model of the lectin displayed all the characteristics of the legume lectin monomer and the experimental observations correlated well with the structural information. *In silico* studies were performed by protein-ligand docking, calculating the respective binding energies and the residues involved in the interactions were derived from LigPlot$^+$ analysis. Fluorescence titrations showed three times higher affinity of T-antigen disaccharide than *N*-acetyl galactosamine (GalNAc) towards SJL indicating extended sugar binding site of the lectin. Thermodynamic parameters of T-antigen binding to SJL indicated the process to be endothermic and entropically driven while those of GalNAc showed biphasic process. SDS-PAGE showed post-translationally modified homotetrameric species of the lectin under native conditions. In presence of guanidine hydrochloride (0.5–5.0 M), the tetramer first dissociated into dimers followed by unfolding of the protein as indicated by size exclusion chromatography, fluorescence and CD spectroscopy. Different structural rearrangements were observed during thermal denaturation of SJL at physiological pH 7.2, native pH 8.5 and molten globule inducing pH 1.0. Topological information revealed by solute quenching studies at respective pH indicated differential hydrophobic environment and charge density around tryptophan residues.

1. Introduction

Protein molecules represent a remarkable relationship between structure and function at molecular level. The equilibrium between the folded and the unfolded states can be perturbed by changing the thermodynamic state of the system like temperature, pH, pressure and co-solvents [1,2]. Protein stability is majorly dictated by forces that help in maintaining the native structure of a protein which include disulfide bonds and the non-covalent interactions. The biochemical stability of a protein is related to its changes in its activity or structure when subjected to stress conditions.

Lectins are the proteins with unusual ability to specifically recognize carbohydrate structures have enormous applications in cell biology, biochemistry and medical field especially in the detection of cell surface changes during malignancy [3–6]. The binding properties of lectins have been used to study the structural and functional role of cell surface carbohydrates, to detect sugar moieties on normal and tumor cell surfaces and to isolate and characterize glycoconjugates [7–10]. Lectins have been the topic of immense interest of scientists in last few decades for studying the folding process of oligomeric proteins. Legume lectins have been extensively studied and a great deal of information including crystal structure of many of them is available [11,12]. Several legume lectins have been characterized for their unfolding behavior in presence of denaturing agents like temperature, urea or guanidine hydrochloride [13–16].

Bioinformatics concerns the development of new software and tools for the analysis of molecular biological data. In recent years, the computational studies on protein-carbohydrate interactions have added the advantages to the drug discovery process. Lectins have been used in pharmaceutical applications as a system for drug targeting and drug delivery [17]. For example, the *Bacillus anthracis* tetrasaccharide has been used as a promising lead for an anthrax vaccine [18].

Abbreviations: ANS, 8-anilino-1-napthalenesulphonate; βME, beta mercaptoethanol; GalNAc, *N*-acetyl-D-galactosamine; GdnHCl, guanidine hydrochloride; SJL, *Sophora japonica* lectin.

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Homology modeling is the simple approach to three-dimensional structure prediction. The main aim of protein modeling is to predict a structure from its sequence with an accuracy that is comparable to the results achieved experimentally [19]. This will help in the use of rapidly generated *in silico* protein models such as structure-based drug design, analysis of protein function and interactions and rational design of proteins with increased stability or novel functions.

The docking process predicts the ligand conformation and orientation within a targeted binding site. The molecular docking comprises the process of generating a model of a complex based on the known 3D structures of its components, free or complexed with other species [20]. For protein–ligand docking methods, the docking problem allows the search for the precise ligand conformations and orientations (commonly referred to as 'posing') within a given targeted protein. The binding affinity prediction addresses the question of how well the ligands bind to the protein (scoring).

Sophora japonica (Family: Fabaceae), is a leguminous plant commonly called as Chinese Scholar Tree or Japanese Pagoda Tree. It is used in the treatment of haemorrhoids, uterine bleeding, constipation, stuffy sensation in the chest, and several other health disorders [21]. The seed lectin from the plant is a tetrameric protein with molecular mass of 120-kDa, shows maximum stability at pH 8.5 with Ca^{2+} ions and specificity towards *N*-acetyl D-galactosamine (GalNAc) [22,23]. Besides this, there is very little information available on kinetics of sugar binding [24] or conformational transitions of the protein. Immunological studies and molecular cloning of the lectin have been reported earlier [25,26]. Van Damme et al. suggested that the cloning studies of seed lectin genes showed the seed lectin is only a GalNAc-specific lectin which is highly homologous to but not identical with the bark lectin [26]. In the present paper, we report homology model, protein ligand docking, thermodynamic parameters of GalNac and T-antigen disaccharide (Galβ1-3GalNAcαO-Me) binding and thermally and chemically induced conformational and functional transitions of *Sophora japonica* seed lectin (SJL).

2. Materials and methods

Sophora japonica seed lectin (SJL) was procured from Vector Labs, USA. *N*-acetyl D-galactosamine (GalNac) and T-antigen disachharide (Galβ1-3GalNAcαO-Me), acrylamide, cesium chloride, potassium iodide and guanidine hydrochloride (GdnHCl) and the chemicals, reagents as well as buffers used for unfolding studies were procured from Sigma-Aldrich, USA. All the other chemicals used were of analytical grade.

2.1. Protein preparation

SJL was dissolved in Tris HCl pH 8.5 (10 mM) buffered saline (150 mM) with $CaCl_2$ (1 mM) and was stored at 4 °C until further use. Protein concentrations were determined according to the method of Lowry et al. [27] using bovine serum albumin (BSA) as standard.

2.2. Hemagglutination assay

Rabbit erythrocytes were washed with PBS (phosphate buffered saline, 20 mM potassium phosphate buffer, pH 7.2, containing 150 mM NaCl) and a 3% (v/v) suspension of the erythrocytes was prepared in the same buffer. Hemagglutination assays were performed in Tris buffer saline (mentioned above) by serial dilution method as described by Wakankar et al. [28].

2.3. Homology model construction and validation

Three-dimensional model of SJL was built by using the protein sequence from National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/protein). The models of the monomer and tetramer were built with the MODELLER 9v14 (build r10167) program [29,30] and UCSF Chimera Ver1.9 (build 39798) software [31] respectively. The model was validated by using Ramachandran plot from PROCHECK [32], ProSAII [33] programs.

2.4. Docking studies of SJL model with sachharides

The AutoDock Vina 1.1.2 docking package was used for ligand docking using the default settings [34]. The structures of all the ligands were obtained from ZINC database (http://zinc.docking.org). The PDB file of SJL generated by MODELLER was used in the format containing the information of atomic charges, atom type definitions and, for ligands, topological information. The ligand binding pocket was predicted by using I-Tasser v. 4.3–COACH (http://zhanglab.ccmb.med.umich.edu/COACH). The size of the docking grid was kept as 64 Å × 80 Å × 56 Å and the grid spacing set at 1 Å. The interactions of the ligands with protein were visualized in AutoDock Tools v. 1.5.6. (http://mgltools.scripps.edu) and PyMOL v.1.8 (www.pymol.org). The analysis of docked ligands was carried out by using LigPlot+ v.1.4.5 software [35]. The results showed the visual graphics of the hydrogen bonds and hydrophobic interactions between the carbohydrate and amino acid residues of lectin. The binding energy for each ligand was obtained and the conformation with lowest binding energy was considered as the most favorable docking pose.

2.5. Fluorescence measurements

Fluorescence measurements of SJL (70 μg/ml) were carried out on a Perkin Elmer LS-50B spectrofluorimeter. Each sample was excited at 295 nm (1.0 cm cell path length) and emission was recorded from 310 to 400 nm. Slit widths of 7 nm each were set for excitation and emission monochromators and the spectra were recorded at 100 nm/min. The baseline was corrected by subtracting the contribution of the buffer.

2.6. Thermodynamics of saccharide binding to SJL

SJL samples (70 μg/ml) in Tris HCl pH 8.5 (10 mM) buffered saline (150 mM) + $CaCl_2$ (1 mM) were placed in a quartz cuvette maintained at desired temperature (±0.1 °C) by means of a Julabo circulating water bath. For titrations, 20 mM GalNAc/10 mM T-antigen was added in 3–10 μl to SJL solution (70 μg/ml) aliquots. After mixing the solution well, the samples were excited at 295 nm and the emission spectra were recorded as mentioned above in 2.5. The fluorescence intensity at 338 nm (λ_{max} of the lectin) was considered for further analysis. Corrections were also made to compensate the dilution effect upon addition of ligand to the lectin. At the highest concentration of the ligand to lectin, volume change was less than 5% of the solution in the cuvette. Each data point was an average of three independent sets of experiments with SD less than 5%. The association constants (K_a) were calculated according to Chipman et al. [36].

The abscissa intercept of the plot of log [C] against log $\{(\Delta F)/(Fc - F\infty)\}$, where [C] is the free ligand concentration, yielded pK_a value for lectin-ligand interaction according to the relationship:

$$\log [Fo - Fc/Fc - F\infty] = \log K_a + \log [C] \quad (1)$$