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A profile of protein-protein interaction: Crystal structure of a lectin-lectin complex

Sukumaran Surya, Joseph Abhilash, Krishnan Geethanandan, Chittalakkottu Sadasivan, Madhathilkovilakathu Haridas*

Inter University Centre for Bioscience and Department of Biotechnology and Microbiology, Kannur University, Thalassery Campus, Kannur 670661, India

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ABSTRACT

Proteins may utilize complex networks of interactions to create/proceed signaling pathways of highly adaptive responses such as programmed cell death. Direct binary interactions study of proteins may help propose models for protein-protein interaction. Towards this goal we applied a combination of thermodynamic kinetics and crystal structure analyses to elucidate the complexity and diversity in such interactions. By determining the heat change on the association of two galactose-specific legume lectins from *Butea monosperma* (BML) and *Spatholobus parviflorus* (SPL) belonging to *Fabaceae* family helped to compute the binding equilibrium. It was extended further by X-ray structural analysis of BML-SPL binary complex. In order to chart the proteins interacting mainly through their interfaces, identification of the nature of forces which stabilized the association of the lectin-lectin complex was examined. Comprehensive analysis of the BMLSPL complex by isothermal titration calorimetry and X-ray crystal structure threw new light on the lectin-lectin interactions suggesting of their use in diverse areas of glycobiology.

1. Introduction

A protein complex is a group of two or more proteins formed by dynamic interactions that are stable over time and usually that corresponds to a protein-protein interaction network (PPIN). Functioning of many proteins was associated with the nature and state of oligomerization or quaternary associations within the proteins [1]. The protein-protein interaction may provide a clear picture concerned with cellular functioning and biological processes, where some proteins are highly connected with five or more interactions or links, and they are called hub proteins [2].

Some others may be poorly connected with only one or two proteins [3]. Proteins interact through the binding sites which determine the specificity and strength of the interactions known as the protein interfaces [4,5]. The structural features of protein interfaces along with the principles of protein-protein associations are elucidated by different methods [6] including the accessible surface area calculations [7,8], type and nature of amino acid interactions constituting the protein interfaces [9,10], conservation of amino acid residues at the interfaces [11,12], docking of one monomer on to the other and the prediction of protein-protein interactions by computational design [13–17]. In order to investigate protein-protein interactions, lectins, belonging to the group of glycoproteins of non immune origin which bind reversibly to a specific monosaccharide or oligosaccharide [18–20], may serve as an excellent model system. They have similar tertiary structures characterized by the 'jellyroll' fold, different modes of quaternary associations, high sequence similarities, and similar other biophysical properties. The jelly-roll structure consists of three sets of anti-parallel β-sheets. There is a six-stranded flat 'back' sheet, a curved seven-stranded 'front' sheet and a short five-member sheet. The sheets are connected by loops [21,22]. The structural classification of lectin super family has been done by SCOP [23]. It comprises of 15 families, including the legume lectins. They exhibit high level of primary structure similarity and show remarkable variations in carbohydrate binding [24,25]. The carbohydrate-binding and metal-binding sites are found to be structurally overlapping in several legume lectins [26,27].

The interaction of carbohydrates with proteins plays a key role in many biological processes such as cell-cell recognition, growth and differentiation, bacterial and viral adhesion, infection, metastasis and inflammation [28]. The molecular, cellular and the evolutionary arguments suggest that lectins play a major role in plant defense [29]. The well known potential of legume lectins to

* Corresponding author.
E-mail addresses: suryasukumaran089@gmail.com (S. Surya), abhibio@gmail.com (J. Abhilash), kgixora@gmail.com (K. Geethanandan), csadasivan@rediffmail.com (C. Sadasivan), mharidasm@rediffmail.com (M. Haridas).

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agglutinate cells and to precipitate multi-valent carbohydrates is due to the fact that these proteins are oligomeric and can form either dimers or tetramers and networks of such interactions. The strongly conserved monomeric unit can oligomerise in different ways, making these proteins as tools for glycobiological research and help analyze the details of lectin-lectin interactions [30]. The broad spectrum of carbohydrate-binding specificities of lectins may be viewed as a signal of the plants thriving for development of recognizing molecules against different types of sugar-containing receptors [31]. Legume lectins can be easily purified in large quantities by exploiting their sugar specificities. This may helps us to study the molecular basis of protein carbohydrate interaction. They also exhibit a wide variety of carbohydrate specificities despite strong sequence conservation [32].

Lectin-lectin interaction network is an indisputable approach for identifying functional modules [33]. Accurate prediction of protein-protein interactions directly from amino-acid sequences is one of the immense challenges faced by the computational biology. Previously, detection of protein-protein interactions were strictly limited to proteins, whose three- dimensional structures were known and the predictions were based on structural context; however, currently genomic context also was possible [34,35]. Interactions were determined with the aid of labor-intensive techniques such as co-immunoprecipitation, pull down assays or affinity chromatography, but nowadays they are replaced by High-throughput experimental techniques such as yeast two-hybrid protein chip technologies and mass spectrometry [36–38]. The computational prediction of protein-protein interactions involves (i) determining whether the proteins may interact or not (ii) identification of the residues which are involved in protein-protein interactions (iii) evolutionary or genomic context to predict interaction [39]. The proteins engage themselves with this structural diversity formed due to the presence of the amino-acid side chains that protrude out from their body, producing binding pockets and recognition sites [40]. Targeting lectin-lectin interactions is far more challenging as compared to the traditional approaches for the identification of small-molecule inhibitors or ligands of proteins. Obtaining high-resolution information and further validation of lectin-lectin interaction through X-Ray crystallography can also be challenging in many instances. In this context, we have produced lectin-lectin complex crystal structure and kinetics of their interactions; with *Butea monosperma* and *Spatholobus parviflorus* seed lectins.

2. Materials and methods

2.1. *Purification of* B. monosperma *and* S. parviflorus *seed lectins*

The *B* and *S. parviflorus* seeds were dried, ground in 20 mM Phosphate buffered saline (PBS) of pH 7.4 and sonicated under sterile conditions. The sonicated slurry stirred overnight and centrifuged at 10,000 × *g* for 30 min at 4 °C and the supernatant was further fractionated by 50–70% ammonium sulphate precipitation. The precipitate obtained after centrifugation was dissolved in minimal volume of 20 mM PBS, pH 7.4 and dialyzed extensively against several changes of PBS. The clear solution was further subjected to chromatographic separation. The dialyzed ammonium sulfate fraction (50–70%) was loaded onto a CM Sephadex C-50 column pre-equilibrated with the dialysis buffer. The resin-bound protein was further eluted with a gradient buffer containing 0.2 M NaCl [41–43]. The elution profile of the column was monitored by absorbance at 280 nm. The fractions of 5 mL each were collected and all fractions were checked for haemagglutination. The yields of BML and SPL lectins were 10 mg and 12 mg respectively from 0.05 kg of dry weight of seeds. The fractions showing haemagglutination activity were pooled and dialyzed extensively against PBS for further purification.

2.1.1. *Affinity chromatography*

The dialyzed protein solution was applied onto a column of guar gum cross-linked with epichlorohydrin [44]. The column was then equilibrated with the same buffer at a flow rate of 30 mL/h. The resin bound protein was then decoupled by means of 0.2 M D-galactose in PBS, pH 7.4. Elution was carried out at a flow rate of 30 mL/h. Fractions containing pure protein were pooled and dialyzed extensively against PBS, pH 7.4 with several changes. The dialyzed protein solution was then concentrated with a Centricon tube having 10 kDa cutoff at 4 °C for further analysis.

2.2. *Isothermal titration calorimetric assay of interaction between BML and SPL*

The calorimetric titrations were performed at the temperature 298.15 K using VP-ITC isothermal titration calorimeter from Microcal (Northampton, MA, USA) to characterize the thermodynamic parameters of BML-SPL interaction. Purified BML and SPL were concentrated to 10 mg/mL approximately. About 0.005 mM solution of SPL and 0.075 mM solution of BML were prepared in 20 mM PBS, pH 7.4 for the binding experiment of BML with SPL. The control experiment was also performed by titrating BML against BML and SPL against SPL in order to compare the interaction kinetics and the non-linear least square fitting method was applied for valuable interpretation of the data. The protein samples were degassed before loading to ensure the absence of air bubbles.

A total volume of about 290 μL of BML solution was added from the rotating syringe to the cell which contains the SPL solution. The volume of the first injection was 2 μL. Five seconds was taken for each injection and a time interval of 120 s was set between two consecutive injections to allow the exothermic peak resulting from the reaction to return to the baseline. Total 30 injections were made. The reference power was set as 10 μcal and the stirring speed was adjusted to 307 rpm. The final data obtained at the end of the injections was fitted by a nonlinear least square method using ORIGIN software from Microcal. After the experiment the thermodynamic parameters ΔG, ΔH and ΔS were calculated according to $\Delta G = \Delta H - T\,\Delta S$.

2.3. *Lectin: lectin binary complex preparation and crystallization*

Purified BML and SPL were concentrated to 10 mg/mL respectively. The complex was prepared by gentle mixing of equi-molar solutions of purified BML and SPL incubated at 4 °C for about 2 h by gentle tapping. Diffraction quality crystals were obtained in 25% PEG 8000, 5% MPD in 20 mM phosphate buffer of pH 7.4, at 25 °C by hanging drop vapour diffusion method. About 3 mL protein complex solution was placed on a siliconized cover slip and 3 mL of precipitant from the well was added to it. In order to avoid the exposure, cover slip was sealed with silicon grease. Trials were made in varying crystallization conditions. The best diffraction-quality crystals were obtained at pH 7.4, in three weeks.

2.4. *X-ray data collection*

Prior to data collection, individual crystals were soaked in crystallization solution containing 25% glycerol for 1 min to achieve cryo-protection. The pre-soaked crystals were exposed to immediate flash-freezing by cold liquid nitrogen-gas stream. X-ray diffraction data were collected using a MAR 345 image plate. The X-ray beam (Cu Kα radiation, 1.5418 Å) from a Bruker Microstar rotating anode X-ray generator operating at 50 kV and 100 mA