SUMMARY

Lectins are mono-or multivalent, proteins or glycoproteins, of non-immune origin, which recognize diverse sugar structures reversibly with a high degree of stereospecificity in a non-catalytic manner. The unique and exquisite specificity of lectins towards complex carbohydrate moieties has made them valuable molecular tools for probing the distribution and functions of glycoconjugates on the cell surface and hence they are aptly described as “deciphers of glycocode”. Lectins are ubiquitous and are being intensively studied from plants, animals and bacteria, but it is only in recent years studies on fungal lectins have the gathered greater momentum because of the interesting biological activities exhibited by them like antiproliferative, antitumor, immunomodulatory and insecticidal activity. In the past four years crystal structures of nine fungal lectins are reported, a fact that reflects the wider attention and interest of investigators with these molecules. X ray crystallographic studies have revealed unique structural folds in these lectins, which are not reported for any of the previously described lectins.

In this laboratory, fungal lectins are being investigated intensively to understand their structure and function relationship, biological function and their applications in cancer biology. Earlier in this laboratory a novel TF antigen binding lectin has been purified in crystalline form and characterized, also its functional role in development and morphogenesis of the fungi was conclusively established. With an aim to elucidate structure-function relationship, recently the crystal structure of SRL, both in free form at a very high resolution of 1.1 Å as well as in complex with N-acetyl-D-galactosamine and N-acetyl-D-glucosamine at 2.0 Å and 1.7 Å resolutions respectively has been solved. The X ray data of the free SRL are of the highest resolution obtained for any protein of this family. The protein structure is composed of two β-sheets, which consist of four and six β-strands, connected by two α-helices. Sequence and structural comparisons
reveal that SRL is the third member of a newly identified family of fungal lectins, which include lectins from *Agaricus bisporus* (ABL) and *Xerocomus chrysenteron* (XCL) that share a high degree of structural similarity and carbohydrate specificity. Although the high resolution X-ray diffraction data and the parallel independent refinement of several structures allowed to deduce the amino acid sequence of SRL directly from the electron density maps, but ambiguities remained in assigning some of the amino acids, especially Asn / Asp and Gln / Glu. Hence it was necessary to obtain the sequence information from any other reliable method. Since the cDNA sequence for SRL was not available, and our attempts to sequence by Edman’s method proved futile as the N-terminal is acetylated, we opted for sequencing by matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) mass spectrometry techniques. Of the total 15 theoretically expected tryptic peptides of SRL with 141 residues, 13 tryptic peptides were resolved and detected by LC-ESI-MS which varied in size with a minimum of single amino acid to 21 residues. Out of 13 tryptic peptides 9 peptides were sequenced successfully by LC-ESI-MS/MS, where as the sequence of remaining four were deduced solely on their molecular masses because of their poor quality of the fragmentation spectra. The sequence for two larger peptides from the tryptic digest, which were not resolved on LC, were sequenced by taking advantage of chymotryptic digestion of SRL. Out of 15 chymotryptic peptides sequence of 14 were established unambiguously by their CID spectra, while the one was assigned by its molecular mass. From the mass spectrometry analysis of tryptic and chymotryptic peptides of SRL, we could confirm and correct the sequence at five locations with respect to Asn / Asp and Gln / Glu. Analysis data also confirmed the positions of Ile / Leu, Gln / Lys residues and the sequence covering 118 of the total 141 residues accounting to 83.68% of the earlier deduced sequence.
Considering the structural, sequence and carbohydrate binding similarities of SRL with ABL and XCL, it was intriguing to note differences in the oligomeric structure. Earlier physico-chemical characterization studies of SRL showed its pH dependent oligomerization by existing as monomer at acidic pH and as dimer at basic pH, unlike ABL and XCL, which are tetramers. Although our recent X-ray crystal structure of SRL determined at 1.1 Å resolution, conclusively demonstrated the dimeric nature of SRL, there were arguments in the light of reported oligomeric state of other related fungal lectins. Considering the importance of relation between quaternary structures of lectins with their function our objective of the study was to investigate the oligomeric structure and also the pH dependent aggregation of SRL. To address this question we employed advanced techniques, MALDI-TOF-MS and Nanospray-ESI-MS.

The pH dependent oligomerization studies of SRL carried out using MALDI mass spectrometry appears to be very complex. Indeed it supported our earlier findings of SRL’s pH dependent oligomerization. But the results did not provide conclusive evidence for the monomeric state at acidic pH and dimeric state at neutral or basic pH. Nevertheless the data confirms existence of only monomer and dimer in a dynamic equilibrium state. However, the data indicated the occurrence of tetramer and other higher aggregates at pH 7.8, which explains our earlier observation that SRL undergoes irreversible aggregation, forming an insoluble precipitate on storage.

Nanospray-ESI-MS analyses infer that SRL behaves differently at different pH values. At pH 4.5 SRL predominantly exist as dimer and only monomer ions were observed at pH 6.8, presumably reflecting the complete dissociation of dimer to monomer. But at pH 7.8 the ESI mass spectrum of SRL shows multiply charged ions corresponding to the tetramer only suggesting that the monomeric species above pH 6.8 reassociates to form tetramer. The results of MALDI and nanospray ESI-MS obtained for SRL
indicate its complex oligomeric behaviour. Although these findings are intriguing and puzzling yet shed light on the complex dynamic equilibrium that exists with SRL's oligomerization warranting for further investigations.

Considering its exquisite sugar specificity of SRL towards Thomsen-Friedenreich (TF) antigen (DGalβ1→3 GalNAc-α-O-ser/Thr), an oncofetal antigen, it was interesting to investigate SRL's binding to altered glycoconjugates on cancer cell surfaces. Such investigations could open new vistas not only in understanding the glycosylation changes but also eventually lead to develop carbohydrate based vaccines. Investigation of SRL interaction with human cervical cancer lines; HeLa and SiHa, carried out in comparison with other known TF antigen binding lectins, by employing flow cytometry, confocal microscopy and lectin blot assay. Flow cytometry analyses revealed remarkable differences in the binding of SRL, PNA, ABL and ACA. Comparison of the % binding and mean fluorescence intensity (MFI) values infer that SRL showing highest binding and also large number of lectin binding sites on HeLa and SiHa cells compared to PNA, ABL and ACA. These results were further confirmed by ultrastructural localization studies using confocal microscopy. In order to identify and compare the lectin binding receptors on HeLa and SiHa cells, lectin blot profiling was carried out using biotinylated SRL, PNA, ABL and ACA. Lectin blot analysis of the membrane glycoprotein of HeLa and SiHa cells demonstrated some significant differences. In addition several common receptor bands were seen. Also, significant quantitative differences in the expression of several lectin-binding receptors were found.

*These findings indicating subtle differences in sugar binding properties of SRL and other lectins would open up new horizons to identify and characterize novel tumor associated antigens.*