Synopsis of the thesis entitled
"Structure analysis of plant lectin domains"

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Lectins are multivalent carbohydrate binding proteins that specifically recognise diverse sugar structures and mediate a variety of biological processes, such as cell-cell and host-pathogen interactions, serum glycoprotein turnover and innate immune responses. Lectins have received considerable attention in recent years on account of their properties leading to wide use in research and biomedical applications. Seeds of leguminous plants are mainly rich sources of lectins, but lectins are also found in all classes and families of organisms. Legume lectins have similar tertiary structures, but exhibit a large variety of quaternary structures. The carbohydrate binding site in them is made up of four loops, the first three of which are highly conserved in all legume lectins. The fourth loop, which is variable, is implicated in conferring specificity. Legume lectins which share the same monosaccharide specificity often exhibit markedly different oligosaccharide specificities. This thesis primarily concerns with structure solution and analysis of lectins from the legume and β-prism II fold families using X-ray crystallography. Apart from having the property of specifically and reversibly binding to carbohydrates, lectins are also interesting models to study sequence-structure relationships, especially of how minor change in the sequence may bring about major changes in oligomerization and binding.

Chapter 1 gives an overview of different structural types of plant lectins and describes in detail, their carbohydrate binding features. The details of the various experimental procedures employed during the course of this research, are explained in Chapter 2.

Chapter 3 describes the crystal structure of a β-prism II fold lectin (RVL), from *Remusatia vivipara*, an epiphytic plant of traditional medicinal value, and analysis of its binding properties. This lectin was established to have distinct binding properties and has nematicidal activity against a root-knot nematode with the localization site identified as the high-mannose displaying gut-lining in the nematode. The crystal structure of RVL revealed a new quaternary association of

this homodimeric lectin, different from those of reported β-prism II lectins. Functional studies on RVL showed that it fails to bind to simple mannose moieties yet showed agglutination with rabbit blood cells (which have mannose moieties on the surface) and some high mannose containing glycoproteins like mucin and asialofetuin. Further, ELISA and glycan array experiments indicated that RVL has high affinity to *N*-glycans like trimannose pentasaccharide such as in gp120, a capsid glycoprotein of HIV virus, necessary in virus-association with the host cell. The structural basis for this N-glycan binding was revealed through structure analysis and molecular modelling, and it was demonstrated that there are two distinct binding sites per monomer, making RVL a truly multivalent lectin. Evolutionary phylogeny revealed the divergence in the β-prism II fold proteins with regards to the number of sugar-binding regions per domain, oligomerization and specificity.

Chapter 4 deals with the structural studies on a galactose-specific legume lectin (DLL-II) from *Dolichos lablab*, a leguminous plant. The lectin was found to be a planar tetramer in the crystal structures of the native and ligand bound forms, as expected from our solution studies and phylogenetic analysis. The protein is a heterotetramer with subunits differing only in the presence or absence of a C-terminal helical region at the core of the tetramer. Due to the static disorder in all the crystals, the central helix could be oriented in either direction. Structure analysis of DLL-II proved to be an interesting endeavour as static disorder compounded with twinning in the crystal made the data processing and structure solution a challenging process. Subsequent structure and sequence alignments led to the identification of an adenine-binding pocket in the hydrophobic core of the tetramer. Based on this, DLL-II lectin was co-crystallized with adenine and the structure revealed the presence of adenine at the predicted binding site.

Chapter 5 describes the identification and analysis of potential plant lectins/lectin-like domains in the genome of *Oryza sativa*, using bioinformatics approaches. This project was initiated to study the occurrence of legume-lectin like domains (a predominant dicot feature) in *O. sativa*, which is a monocot. Later, a large scale genome analysis for all types of lectin domains was carried out through exhaustive PSI-BLAST, profile matching by HMMer, CDD and MulPSSM. The final validation was carried out by assessing the carbohydrate binding potential of the domain by examining the sugar binding sites. The primary

interest in undertaking this work was to find the occurrence of association of these domains with other domains as in protein receptor kinases, where lectin is the receptor domain. Though primarily initiated as a bioinformatics project, further structural characterization was attempted by cloning, expression and purification of some of the annotated lectin proteins using prokaryotic expression systems. The protein expression was attained in reasonable amounts for a few of the annotated legume lectin homologs, however purification is yet to be achieved as the expressed proteins are insoluble.

A part of the results described in this thesis and the other related projects that the author was involved are reported in the following publications.

1) Purification, characterization and molecular cloning of a monocot mannose-binding lectin from *Remusatia vivipara* with nematicidal activity
Bhat GG, Shetty KN, Nagre NN, Neekhra VV, Lingaraju S, Bhat RS, Inamdar SR, Suguna K, Swamy BM. 2010. *Glycoconjugate J*. 27(3):309-320

2) Modification of the sugar specificity of a plant lectin: structural studies on a point mutant of *Erythrina corallodendron* lectin
Thamotharan S, Karthikeyan T, Kulkarni KA, Shetty KN, Surolia A, Vijayan M & Suguna K. 2011. *Acta Crystallographica D* 67(3):218-227

3) Crystal structure of a β-prism II lectin from *Remusatia vivipara*
Shetty KN, Bhat GG, Inamdar SR, Swamy BM, Suguna K. 2012. *Glycobiology* 22(1): 56-69.

4) Structure of a galactose binding lectin from *Dolichos lablab*
Shetty KN, Lavanyalatha V, Rao RN, SivaKumar N & Suguna K (Under review)

5) Occurrence of lectin-like domains: *Oryza sativa* genome analysis.
Shetty KN & Suguna K. (Manuscript in preparation)