ABSTRACT

Lectins belong to a group of proteins that bind free sugar or sugar residues of polysaccharides, glycoproteins, or glycolipids, which exist in free or bound form (as in cell membranes). Plant lectins have been attracting much attention because of their ease of isolation and their usefulness as reagents for detecting glycoconjugates in solution and on cell surfaces. The recognition of carbohydrate moieties by lectins has important implications to a number of biological processes such as in cell-cell interactions, signal transduction and in cell growth and differentiation. A large number of lectins have been reported from many plant species but correlation between structure, conformation and stability has been studied only for a few of them.

Cucurbitaceae lectins have been purified and characterized in some detail from plants *Momordica charantia* (bitter gourd), *Trichosanthes anguina* (snake gourd), *Trichosanthes kirilowii* (Chinese Snakegourd) and *Trichosanthe cucumerina*. Ribosome Inactivating Proteins (RIP's) belong to two different classes (RIP type-I & II) and are found ubiquitously in plant kingdom. The cucurbitaceae lectins from *Trichosanthes dioica* and *Trichosanthes anguina* have been assumed to belong to type-II RIP family with respect to their secondary structural elements, biophysical characteristics, carbohydrate specificity as well as subunit composition. In the study reported here these lectins were biophysically characterized with respect to thermal, chemical and pH stability. One of them, the lectin from *T. dioica* has been structurally characterized.
Another lectin that was taken up for structural studies is Galactose / N-acetyl Galactosamine specific lectin from *Erythrina indica*. Many Galactose / N-acetyl Galactosamine specific lectins have been isolated from various species of the genus *Erythrina* belonging to sub-family Fabaceae of Leguminoseae. They include lectins from *E. cristagalli*, *E. indica*, *E. costarensis*, *E. variegata*, *E. arborescens*, *E. lithosperma* and *E. suberosa*, *E. speciosa*, and *E. velutina*. Studies on lectin from the seeds of *Erythrina corallodendron* has been reported in the literature. The *Erythrina indica* seed lectin was obtained in pure form for structure determination through collaboration. Thus, lectins mainly from *Trichosanthes dioica*, and *Erythrina indica* were selected for structural investigation for their importance as being present in commonly used edible fruits or stems.

In the past few years, araceae has been identified as a lectin-rich plant family with lectins constituting 70-80% of storage proteins in their tubers. Although, extensive studies on three-dimensional structures of lectins and lectin-carbohydrate interactions have been carried out, till date no report of folding-unfolding as well as fluorescent spectroscopic characterization of monocot lectins belonging to araceae are available. Here we report biophysical characterization of the *Sauromatum guttatum* (Voodoo lily) *Arisaema tortosum* (Himalayan cobra lily) lectins by using steady-state and time resolved fluorescence and CD spectroscopy.

Organization of the chapters in this thesis is as follows:

Chapter 1: General Introduction

Chapter 2: Materials and Methods
Chapter 3: Crystallization and X-ray crystallographic studies of a lectin from *Trichosanthes dioica*.

Chapter 4: Biophysical and stability studies on two *Trichosanthes* lectins.

Chapter 5: Crystallization and X-ray crystallographic studies of a Galactose/N-acetyl Galactosamine specific lectin from *Erythrina indica*.

Chapter 6: Comparative biophysical studies on two araceae lectins from *Sauromatum guttatum* and *Arisaema tortosum*, by fluorimetric methods and circular dichroism (CD).

Chapter 7: Comparisons of plant lectin characteristics studied and conclusions.

**Chapter 1: General Introduction**

This introductory chapter of the thesis describes the studies on various lectins from plant, animal and microbial sources with respect to their biomedical application, their sugar specificity and three-dimensional structures. Besides this general overview, it also provides a brief outline of the three plant lectin families from which five lectins have been chosen for detailed study, through structural investigations as well as comparative biophysical studies described in the thesis.

**Chapter 2: Materials & Methods**

This chapter presents the details of the materials and various methods used for the purification of *Trichosanthes dioica* lectin to obtain good diffraction quality crystals. It also includes the methodology followed for crystallization, X-ray data collection, data processing, structure determination, structure refinement and analysis of the refined structure of both the *Trichosanthes dioica* and *Erythrina indica* lectins. The biophysical
experiments conducted for the comparison of *T. dioica* and *T. anguina* lectins as well as for lectins from *S. guttatum* and *A. tortosum* have been described.

**Chapter 3: Crystallization and X-ray crystallographic studies of a lectin from *Trichosanthes dioica***

The lectin from *T. dioica* seeds has previously shown to be similar to type-II RIPs on the basis of its biochemical characteristics. Another lectin from the cucurbitaceae family, the *T. kirilowii* lectin (TKL-1) has been structurally characterized and it has also been placed in this plant lectin family. The structure of TDSL has been determined at 2.8Å, and its structure has been solved by molecular replacement using the coordinates of TKL-1 (PDB code: 1GGP). The structure has been refined using the same model, but better values of $R_{factor}$ and $R_{free}$ have not been obtained in the absence of sequence information. Hence a well refined structure could not be obtained. The structure shows close similarity with type-II RIPs such as abrin-a and ricin, but like TKL-1 it lacks the residue Tyr74 (of abrin-a) that is essential for adenine binding and thus the RIP activity. While the active site residues Glu164 and Arg167 are also observed in TDSL. All other active site residues and the invariant residues that have been described in all other type-II RIPs are also conserved in TDSL. On the basis of these structural features, TDSL has been categorized as type-II RIP.

**Chapter 4: Biophysical and stability studies on two *Trichosanthes* lectins***

In this chapter a comparative account of the biophysical characterization of lectins from *Trichosanthes dioica* and *Trichosanthes anguina* with respect to their thermal, chemical and pH stability has been described. The thermal denaturation studies of these
two lectins indicate that both these cucurbitaceae lectins show high thermal stability, although the disulphide linkages do not play any role in the stability of these proteins. Some RIPs have been shown to be highly thermostable. Since the *T.dioica* lectin resembles RIPs in molecular weight, subunit composition, size, and sugar specificity, it may belong to this family. As it has been pointed out that some *Trichosanthes* lectins have similarity with RIPs; here we show that like some RIPs, these lectins are resistant to wide range of chemical and pH denaturing conditions.

**Chapter 5: Crystallization and X-ray crystallographic studies of a Galactose/N-acetyl Galactosamine specific lectin from *Erythrina indica***

This chapter describes the structural study of the lectin from the seeds of *E.indica* (coral tree). The lectin belongs to the large family of legume lectin. The structure has been determined at 2.5 Å and solved by molecular replacement using the coordinates of EcorL (PDB code: 1FYU). **EiSL** is a homodimeric lectin and N-linked glycosylation was observed at four sites in the structure i.e. 17 and 113 in both the chains. A heptasaccharide at B17 and a hexasaccharide at B113 were traced properly in the electron density; while A-chain at both locations showed a trisaccharide comprising of Nag and Fuc residues. Structurally EiSL varies from the very closely related lectins of the same genus EcorL and ECL, with respect to a few amino acid residues. More than 90% residues have been shown to be conserved. Similar folds, conformation of loops, type of coordination bonds at the metal binding sites and residues at the sugar combining sites have been observed in EiSL as in EcorL and ECL. A galactose molecule was bound at a position that is close to the glycosylation site in a-chain (A 116 Asp) but it has been observed to be facing away from the N-linked heptasaccharide.
Chapter 6: Comparative biophysical studies on two araceae lectins from Sauromatum guttatum and Arisaema tortosum, by fluorimetric methods and circular dichroism (CD).

This chapter presents a comparative study of the structural stability and dynamics of two araceous lectins, one from Sauromatum guttatum (Voodoo lily) (SGA), and another from Arisaema tortosum (Himalayan cobra lily) (ATL). Among the Araceae lectins those from Arisaema tortosum schott, Arisaema consanguineum Schott (ACA), A. curvature Kunth (ACmA), Gonatanthus pumilus (GPA), Sauromatum guttatum Schott (SGA) and Alocasia cucullata have been purified and characterized. All these lectins show mitogenic potential for human blood lymphocytes and have complex sugar specificity, and show hemaglutination inhibition by desialylated fetuin. They are thus distinctly different from other monocot lectins in carbohydrate binding specificity.

Both the lectins chosen for the study are non-mannose binding monocot lectins isolated from the tubers of the respective araceous plants. SGA and ATL consist of a mixture of isolectins differing in charge, similar to Alocasia indica lectin, WGA and those from Amaryllidaceae and Alliaceae. Recently, SGA as well as some lectins from Arisaema sp. have been found to show antiproliferative as well as mitogenic activity.

The two monocot lectins have been characterized with respect to their tryptophan environment and secondary structure changes against chemical, pH and thermal denaturation. Transitions in the tryptophan microenvironment and secondary structure changes were studied using steady state and time resolved fluorescence and CD spectroscopy. The lectins exist as tetramers with an estimated single tryptophan residue per monomer in a polar environment. Quenching with ionic quenchers showed
predominantly electropositive environment for tryptophan residues. Acrylamide had maximum quenching effect. A decrease in KI quenching due to lectin denaturation indicated redistribution of charges as a result of possible conformational changes. The two values for lifetimes of tryptophanyl population (1.2-1.4 and 6.3-6.4 ns) reduced substantially on quenching or denaturation. Similarly, both the lectins showed a drastic loss of secondary structure in 5M Gdn-HCl or 6M Urea or at pH 2.0 and below. For the first time araceous lectins, like some of the legume lectins, are shown to bind adenine. The presence of a compact structure at highly alkaline pH (10.0 - 12.0) was observed in CD spectra.

Chapter 7: Comparisons of plant lectin characteristics studied and conclusions

This chapter outlines the comparative analysis of the structures, the sugar-binding of lectins, as well as the biophysical characteristics of lectins that have been studied in this thesis, belonging to three structurally different plant lectin families.

Comparison of the sugar binding sites of Galactose/N-acetyl Galactosamine specific lectin TDSL and EiSL was done with lectins having similar sugar specificity. The structural features of the active site of T. dioica lectin was compared with binding sites of galactose specific lectins, a type-II RIP Abrin-a and a C-type animal lectin CEL-III from Cucumaria echinata. The active site geometry and residues involved in sugar binding for EiSL have been compared with that of Jacalin, mouse galectin-9, a tunicate lectin DCL-I from Didemnum candidum and C-type lectins.

The three-dimensional structure of E. indica lectin was similar to that of lectins from E. cristagalli and E. coralloidendron in terms of the N-linked heptasaccharide,
conserved water molecules and residues in the sugar binding site. Minor structural differences have been highlighted and compared to show the higher stability of this lectin as compared to EcorL.

We have for the first time carried out structural investigations on the two araceaceous lectins from *S. guttatum* and *A. tortuosum*, to probe the tryptophan environment and secondary structure of the members of this monocot family. For both these lectins the far-UV CD spectra indicate a predominantly β sheet structure and type-III β-turns. Adenine-binding, a feature of legume lectins has been observed in members of this family for the first time. The two lectins belonging to araceae and differing from other mannose specific lectins show similarity with respect to their tryptophan environments as well as structural stability with respect to temperature, pH as well as presence of chemical denaturants.