Abstract

Recognition is a key event in many biological processes It is also an initiation step in various events, based on cell-cell interactions, such as fertilization, embryogenesis, organ formation, immune and microbial defense, to mention a few It is now known that almost all cells carry carbohydrate on their surfaces and that these molecules possess enormous amount of information Carbohydrates perform a diverse range of cellular functions from acting as structural components and storage molecules to playing a central role in cell-cell recognition and cellular development The discovery of carbohydrate binding proteins especially the lectins was a significant step in the study of the mechanism of cellular recognition (Halina Lis and Nathan Sharon, 1998) Lectins are multivalent sugar-binding, erythroagglutinating proteins or glycoproteins of non-immune origin Though initially discovered in plant seeds, they are ubiquitously distributed in nature A great amount of interest in them was generated when it was demonstrated that some lectins can stimulate mitogenesis of lymphocytes by binding to cell surface carbohydrates The discovery of the mitogenic properties of lectins and their ability to preferentially agglutinate malignant cells led researchers to search for new lectins and obtain them in a purified form The present thesis describes the isolation, purification, characterization and detailed carbohydrate binding specificity of a new lectin from jackfruit seeds (*Artocarpus integrifolia*) Various important aspects of protein-carbohydrate interactions with special reference to mannose binding lectins, have been reviewed in **chapter 1**

The seeds of Jackfruit (*Artocarpus integrifolia*, family Moraceae) contain two lectins, one a galactose binding lectin, Jacalin, and the other a mannose binding lectin, Artocarpin The latter displays an interesting immunological property of a potent and selective mitogenic effect on distinct T and B cell functions Mitogens have proven to be useful as tools in the study of various aspects of immune response As is well established, distinct functional populations of immunocompetent cells may be stimulated by specific mitogens, permitting one to determine their relative role in the immune response In this respect some of the most useful mitogens are, the murine and human T cell stimulators phytohemagglutinin (PHA) and concanavalin A (ConA), the latter inducing expression of

the interleukin-2 receptor, secretion of interleukin-2 and interferon-γ as a prelude to mitosis Lipopolysaccharide (LPS) and the human T cell dependent B cell activator of proliferation and antibody secretion, pokeweed mitogen (PWM) are murine B-cell mitogens Due to the important immunological property which artocarpin displays, our first objective was to isolate and purify artocarpin to homogeneity using chromatography which is described in **chapter 2** The same chapter also contains a comparative study of Artocarpin and Jacalin based on their macromolecular properties

Considering the importance of lectins as molecular probes in biology and medicine, and especially the interesting biological activity of artocarpin, we have carried out an extensive investigation into the nature of its carbohydrate specificity with a series of monosaccharides, mannooligosaccharides, and glycoproteins (S Misquith et al, 1994) **Chapter 3** provides interesting information on carbohydrate binding specificity using a sensitive enzyme linked lectin adsorbent assay (ELLA) Artocarpin belongs to the class of mannose-specific plant lectins, notable among which are ConA, pea, lentil, snowdrop and garlic lectin ConA, lentil and pea lectins are a group of mannose/glucose-specific lectins Snowdrop lectin on the other hand, recognises only mannose and mannooligosaccharides and is unable to tolerate any substitution of nonreducing terminal mannose residues in N-linked glycan chains Investigation of its carbohydrate binding specificity reveals that among monosaccharides, mannose is preferred over glucose Among mannooligosaccharides, mannotriose (Manα1-3[Manα1-6]Man) and mannopentaose are the strongest ligands followed by Manα1-3Man Extension of these ligands by GlcNAc at the reducing ends of mannooligosaccharides tested remarkably improves their inhibitory potencies, while substitution of both the α1-3 and α1-6 mannosyl residues of mannotriose and the core pentasaccharide of N-linked glycans (Manα1-3[Manα1-6]Manβ1-4GlcNAcβ1-4GlcNAc) by GlcNAc or N-acetyllactosamine in β1-2 linkage diminishes their inhibitory potencies Sialylated oligosaccharides are non-inhibitory Moreover, the substitution of either α1-3 or α1-6 linked mannosyl residues of M_5Gn or both by mannose in α1-2 linkage leads to a considerable reduction in their inhibitory power The studies suggest that artocarpin recognises best, the xylose containing heptasaccharide of

horseradish peroxidase, (HRP) which is unique among lectins reported thus far This lectin should prove to be a useful tool for the isolation and characterization of glycoconjugates displaying such structures

Chapter 4 describes the titration calorimetric studies on the binding of various saccharides to artocarpin in order to elucidate directly the stoichiometry, the binding constant, the Gibbs binding energy change (ΔG_b), the binding enthalpy change (ΔH_b), the binding entropy change (ΔS_b) and the heat capacity change (ΔC_p) accompanying the binding reaction In fact, it permits not only the detection of these thermal effects associated with the binding reaction but also the mechanism involved therein The thermodynamics of binding of monosaccharides and mannooligosaccharides to artocarpin were determined by Isothermal Titration Calorimetry (ITC) technique at 280 K to 293 K The binding enthalpies, ΔH_b, are the same at both the temperatures and the values range from -10 94 to -47 11 kJ mol-1 The affinities of the lectin as obtained from ITC are in reasonable agreement with the results obtained by ELLA, which are based on the minimum amount of ligand required to inhibit HRP binding to artocarpin in ELLA Binding reactions are essentially enthalpically driven There is a very little change in the heat capacity on binding Enthalpy-entropy compensation observed in artocarpin-sugar interaction shows the importance of solvent reorganization being one of the principle determinants in protein-sugar interaction as has been noted in several other systems

Chapter 5 describes the ITC studies of the binding of Gal, Glc-analogues and monodeoxy-analogues of trimannoside The binding constant determined by ITC for Glc3, Gal3 and Gal6 analogues show two fold lower affinity than that of MeαMan, while that for Glc6-analogue binding constant is twice that of MeαMan The ΔH_b values for Glc3 and Gal6 analogues are -7 to -8 kJ mol^{-1} lower whereas Gal3 and Glc6 analogues show -16 to -12 kJ mol^{-1} lower enthalpy than MeαMan Analogues of mannotriose containing either Glc or Gal substituted on the α(1-3) or α(1-6) arms possessed very low affinities for artocarpin like ConA Substitution of Glc on the α(1-3) arm results in about 18-19 fold loss of binding while substitution of Glc on the α(1-6) arm leads to a 14-18 fold loss of

binding affinity relative to mannotriose Substitution of Gal on either arm α(1-3) or α(1-6) results in a substantial loss in affinity for artocarpin since Gal does not bind well to the monosaccharide site of Artocarpin as in the case of ConA. The ΔH_b values for Glc3, Glc6, Gal3 and Gal6 analogues are in the range of -23 kJ mol^{-1} to 32 kJ mol^{-1} lower than that of mannotriose Monodeoxy derivatives of the mannotriose 6d 3 arm show complete loss of binding to the lectin suggesting that the 6 hydroxyl group on the 3 arm is involved in binding process In summary the thermodynamic data obtained from ITC studies show that the ΔH_b values for deoxy analogues are nonlinear, indicating other contributions to these terms such as solvent and /or protein effects The magnitude of the $\Delta\Delta H_b$ and $\Delta\Delta G_b$ values represents not only the loss of the H-bond(s) involved, but also differences in the solvent and protein contributions to the binding of mannotriose and the deoxy-analogues

The primary structure of artocarpin has been determined and reported in the **chapter 6** The general methodology for the fragmentation of the protein and purification as well as characterization of the fragmented peptides is described therein

The last **chapter** 7 is a general discussion, which summarizes the results of this thesis

A part of the results described in this thesis have been reported in the following publications

Carbohydrate binding specificity of the B-cell maturation mitogen from *Artocarpus integrifolia* seeds Sandra Misquith, **P.Geetha Rani** and Avadhesha Surolia (1994) *J Biol Chem* **269**, 30393-30401

Homology between jacalin and artocarpin from jack fruit Artocarpus integrifolia seeds Partial sequence and preliminary crystallographic studies of artocarpin A Stephen Suresh, **P.Geetha Rani**, J Venkatesh Pratap, R Sankaranarayanan, A Surolia and M Vijayan (1997) *Acta Cryst* D **53** Part 4, 469-471

Garlic(*Allium Sativum*) lectins bind to high mannose oligosaccharide chains (1998) Tarun K Dam, K Bachhawat, **Rani,P.G** and A Surolia *J Biol Chem* **273**, No 10 , 5528-5535

Thermodynamic studies of saccharide binding to Artocarpin, a B-cell mitogen **P Geetha Rani** , Sandra Misquith, S Oscarson and Avadhesha Surolia (manuscript under preperation)