Discussion
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Lectins are proteins that recognize and bind to sugar complexes attached to proteins and lipids. They do this with very high specificity for the chemical structure of the glycan arrays (Bies et al., 2004). Invertebrate lectins may recognize a part of a sugar (Ravindranath et al., 1985), whole sugar (Bretting and Kabat, 1976), their glycosidic linkage (Shibuya et al., 1987; Wang and Cummings, 1988) or a sequence of sugars (Kobiler and Mirelman, 1980; Mauchamp, 1982). The most unique aspect of invertebrate lectins is the presence of sialic acid specific lectins.

Sialic acids play an important role as ligand in cell sociology. The unique structural features of the molecule, which includes a negative charge owing to a carboxyl group, enables it to play a role in cellular functions, such as transport of positively charged compounds, cell to cell repulsion, influencing conformation of glycoproteins on cell membranes, and even masking antigenic determinants on receptor molecules (Narayanan, 1994). These sialic acids are of widespread occurrence as components of the oligosaccharide chains of glycoconjugates present in animal cells and tissues and appear to play a role in many important biological recognition mechanisms (Schauer et al., 1984; Morell et al., 1971; Chavin and Weidner, 1984). Sialylation of glycoproteins changes under pathological conditions as well as during developmental stages, and altered sialylation often has significant implications in the physiological role of glycoproteins (Roth, 1996; Varki, 1997; Rutishauser, 1998). Sialic acids as combined molecules (glycoproteins or glycolipids) dot the outer surface of all cells and serve as cellular identification tags to the surrounding world (Nangia – Makker et al., 2002).
Probes which are capable of detecting and distinguishing those tags have proven to be valuable tools for biomedical research. Thus lectins are carbohydrate binding proteins that are able to detect subtle differences between complex carbohydrate structures.

To harness these lectins in biological research, the binding specificities toward glycoconjugates over monosaccharide or oligosaccharide sequences have to be characterized because the most frequent and irreplaceable function of the lectins is to detect specific glycotopes in glycoconjugates possessing the glycotopes from mixture.

In decapods, the specificity of lectin towards carbohydrates is mainly related to N-acetylated carbohydrates, such as NeuAc, GluNAc and GaINAc (Marques and Barracco, 2000). Although the recognition of NeuAc is a common feature among crustacean lectins, some of them exhibit a particular pattern of specificity towards O-acetylated sialic acid derivatives (Hall and Rowlands, 1974 a; Cassels et al., 1986). The report of Ravindranath et al. (1985) has shown the specificity of the marine crab Cancer antennarius hemolymph lectin to 9-O/4-O-acetyl sialic acid, whereas the hemolymph lectins of the freshwater prawn Macrobrachium rosenbergii and the marine crab Liocarcinus depurator have been shown to specifically recognize 9-O-acetyl sialic acid (Vazquez et al., 1993; Fragkiadakis and Stratakis, 1997). The agglutination of the bacterium Bacillus cereus by M. rosenbergii hemolymph lectin can be related to the recognition of these O-acetylated sugars on the bacterial cell surface (Vazquez et al., 1996). Specificity towards O-acetyl sialic acid residues was also observed in the hemolymph lectin of the freshwater crab Paratelphusa jaquemontii (Maghil Denis et al., 2003).
It is noteworthy that although crustacean lectins apparently share no common structural relation, the specificity of recognition seems to have been well conserved within this group of invertebrates. On the other hand, a sialic acid binding lectin with specificity for NeuGc was purified from the hemolymph of the marine crab *Scylla serrata* (Mercy and Ravindranath, 1993) and *Philyra pisum* (Kim *et al.*, 2006). The unique binding specificity of these lectins distinguishes it from other known sialic acid specific lectins.

Inspite of the N-acetylated carbohydrate recognition mentioned above, different sugar specificity has also been observed among crustaceans. Three galactose binding lectins have been described in the hemolymph of the acorn barnacle *M. rosa* and the recognition towards the same monosaccharide has been assigned to the lectin present in the hemolymph of a different species of barnacle *Balanus rostratus* (Toda *et al.*, 1998). However, specificity to galacturonic and glucuronic acids and also to NeuAc has been displayed by lectin occurring in a different species of barnacle, *B. balanoides* (Ogata *et al.*, 1983). On the other hand, galactose binding is not restricted to barnacles, since Fragkiadakis (2000) have also shown the presence of galactose recognizing lectin in the hemolymph of the crustacean *Potamon potamios*. Additional carbohydrate specificity might be exhibited by crustacean lectins, as seen for the serum lectin of the freshwater crab *Parathelphusa hydrodromus*, which appears to be unique among all the known crustacean agglutinins in being specific for non reducing terminal glucose with α, 1-2 glycosidic linkage (Nalini *et al.*, 1994).

NeuAc, a sialic acid with an acetyl group on C-5, is an effective inhibitor of the agglutination activity, as seen for some other crustacean species. However, this sugar specificity is not absolute but shared by other N-acetylated aminosugars, mainly
GaINAc and GluNAc, which contain the acetyl group on C-2. Although an acetyl group seems essential for lectin-ligand interaction, the latter two monosaccharides are usually less efficient in their hemagglutination inhibitory capacity, as seen, for example, in *Penaeus monodon* (Ratanapo and Chulavatnatol, 1990), *P. schmitti* and *P. paulensis* (Marques and Barraco, 2000). This could indicate the importance of the position of this group as well as its equatorial arrangement. One exception to this behaviour is the lectin of *P. indicus*, for which GaINAc, GluNAc, ManNAc were as effective as NeuAc in inhibiting the serum agglutination activity (Maheswari *et al.*, 1997). The diverse specificity of crustacean lectins of biomedical importance is the hallmark of the phylum arthropoda.

Undoubtedly, sialic acid specific lectins of crustaceans are valuable diagnostic tools for elucidating the variety of sialic acid and sialyl linkages. The importance of these lectins is enhanced by realizing that enumerable tumor-associated antigens and pathogenic strains of bacteria containing sialic acids in their terminal residues (Doyle and Keller, 1984). Hence this investigation is undertaken to elucidate the sialic acid specificity of a crustacean hemolymph lectin.

This research was directed towards the identification, purification and characterization of the *Episesarma tetragonum* agglutinins. Two different agglutinins EtA-1 and EtA-2 were identified and purified from the crab hemolymph. The agglutinability of dog erythrocytes by EtA-1 was effectively blocked by fetuin. The noteworthy feature of this inhibition study was that the glycoproteins containing Siaα, 2-3Galβ, 1-4 GluNAc linkages such as fetuin, porcine thyroglobulin, α-acid glycoprotein and PSM inhibited the HA activity of EtA-1. After removal of sialic acid, the inhibitory effect of fetuin was reduced confirming the sialic acid affinity of
EtA-I. HA1 by galactose, GluNAc of purified EtA-1 is of considerable interest, as this confirms the specificity of EtA-1 to Siaα, 2-3Galβ, 1-4 GluNAc linkages. NeuAc was non inhibitory in contrast to NeuGc. However the inhibitory potency of NeuGc was lower than galactose and GluNAc. Therefore the most important prerequisite for EtA-1 binding to glycoconjugate seems to be Sia (NeuGc)α, 2-3Galβ, 1-4 GluNAc linkages rather than NeuGc per se. Thus EtA-1 exhibits high affinity towards sialoconjugate possessing terminal NeuGcα, 2-3Galβ, 1-4GluNAc, which are usually present in N-linked oligosaccharides.

The minor lectin EtA-2 was found be O-acetyl sialic acid specific. BSM was the only potent inhibitor of EtA-2. Sialidase treatment of BSM diminished its inhibitory potency revealing the sialic acid specificity of EtA-2. BSM contains mainly 9-O-acetyl and 8,9-di-O-acetyl NeuAc. The O-acetyl groups from sialic acid are vital for the recognition process of EtA-2, since de-O-acetylation of BSM completely abolished the recognizing process. These observations confirm the specificity of EtA-2 to O-acetyl sialic acid.

Thus two lectins namely EtA-1 and EtA-2 were identified and purified from the hemolymph of the crab *E. tetragonum*. EtA-1 was a major lectin with unique specificity towards NeuGcα, 2-3Galβ, 1-4 GluNAc linkages and, EtA-2 a minor lectin with specificity to O-acetyl sialic acid.

The inability of the crustacean tissues to synthesize sialic acid rules out any physiological role of these lectins in the biology of the crab. Obviously these lectins could function as “protectins” by binding to invading microorganisms that may carry the sialyl linkages, such as the bacterial polysaccharides and capsules of virus. By injecting different kinds of erythrocytes that express different levels of the HA
receptor, it was attempted to assess whether the crab lectins could serve such a defensive function and observed a correlation between lectin affinity and clearance. The greater the lectin receptors on the erythrocytes, the faster the clearance. There is no doubt that the mechanism involved in the clearance of erythrocytes is associated with the amount of lectin-receptors. Further verification was obtained by injecting lectin-coated erythrocytes, which were cleared faster than the untreated erythrocytes, to once again prove the interaction between the clearance mechanism and the lectins. Clearance could be a process of opsonization of the pathogen leading to efficient phagocytosis, or such opsonization may lead to hemolysis or destruction by killer cells. These concepts deserve further study.

**Potential role of EtA-1 and EtA-2**

Considering the importance of sialic acids in cell sociology, lectins which specifically recognize terminal sialic acid residues are potentially useful as analytical tools in studying the biological functions of sialoglycoconjugates. These lectins, along with monoclonal antibodies raised against sialoglycoconjugates, have been used in the detection, affinity purification, cytochemical localization and quantification of such glycoconjugates (Mandal and Mandal, 1990; Varki, 1997). Lectins that recognize linkages or modifications of sialic acid are thus indispensable as reagents in biochemical research and diagnostic analysis.

Carbohydrate residues of the membrane glycoproteins can be detected using lectins due to their binding specificity to carbohydrates. Lectins, therefore have gained an importance in the field of cancer research (Sherwani et al., 2003). It is believed that an elevated level of α, 2-3-linked sialylation increases the metastatic potentials of tumor cells (Dennis et al., 1986). The increase of α, 2-3-linked sialic
acids with increased branching of glycans is considered to accompany hepatocarcinoma (Montreuil et al., 1997). Tumor associated antigen sialyl Lewis x (sLx) 1 contains a sialic acid α, (2-3) galactose moiety and has been implicated in inflammation and cancer metastasis (Tyrrell et al., 1991; Kannagi et al., 2004). Hence EtA-1 that can recognize sialic acid α, 2-3 linkages can be used in detection, quantification and study of those glycoconjugates accompanying hepatocarcinoma, inflammations and cancer metastasis. Thus there is no doubt that EtA-1 could be a valuable diagnostic tool for identifying the sialoconjugates in normal and malignant tissues.

The O-substituted sialic acid show interesting species and tissue distribution in mammals (Schauer, 1982). Moreover O-acetylation of sialic acid may change with transformation or other alteration in the environment of the cell (Varki and Kornfeld, 1980). Normally cell surfaces of human tissue contain NeuAc, but 9-O-acetyl sialic acid has been found associated with some malignant tumor cell lines (Cheresh et al., 1984; Shi et al., 1996). Human melanoma expresses O-acetyl sialic acid in GD3 and the presence of autoantibodies in the sera of melanoma patients was recognized by 9-O-acetyl sialic acid specific lectin isolated from the hemolymph of Cancer antennarius (Ravindranath et al., 1985). The sialoglycoproteins on the cell surface of leukemia erythrocytes show distinct alterations and the differentiation between several leukemia erythrocytes was marked by a 9-O-acetyl sialic acid specific lectin purified from the hemolymph of Achatina fulica snail (Basu et al., 1986; Sen et al., 1994). Hence O-acetyl sialic acid specific lectin, EtA-2 could be used as a valuable tool for the localization and assessment of the functions of glycoconjugates containing O-acetyl sialic acid.
Lectin-mediated drug targeting in future

Given fact that different cell types, both normal and diseased express different glycans arrays on their surfaces as well as demonstrated over the years by the use of lectins as histochemical tools; the idea of using lectins as targeting molecules for cell specific drug delivery is both attractive and feasible, and has generated considerable interest.

The rationale behind lectin mediated drug targeting is very simple. Most cell surface proteins and many lipids in cell membranes are glycosylated and these glycans are binding sites for lectins. The combination of a small number of sugars can produce a vast range of different chemical structures. Different cell types express different glycan arrays and in particular, diseased cells, such as transformed or cancerous cells, often express different glycans compared with their normal counterparts. Therefore lectins could be used as carrier molecules to target drugs specifically to different cells and tissues. Besides the targeting to specific cells, the lectin-sugar interaction can also be used to trigger vesicular transport into or across epithelial cells (Bies et al., 2004).

Our specific future directions are, EtA-1 and EtA-2 could be used as carrier molecule to target drugs specifically to different malformed cells. Lectins could be conjugated to nanoparticles along with suitable drug formulations might enhance drug delivery to the specific cells.

From modest beginnings as potential tools for specific drug targeting and bioadhesion applications some 20 years ago, lectins are realizing a number of important applications. Some of the problems associated with any molecular targeting system still have to be tackled. It is hoped that some of these problems
might be overcome in future by the application of biotechnology techniques to produce quantities of smaller fragments of lectins that will retain the high target specificity that these fascinating molecules possess, but will be easier to manipulate. The use of lectins in drug targeting is a fledgling subject that will surely grow in years to come.