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Sialic acid binding lectins (SABL) from Mollusca, a review and insilico study of SABL from *Solen grandis* and *Limax flavus*

Shyamasree Ghosh

Abstract
Members of Phylum Mollusca with aquatic existence are exposed to pathogens. Their innate immune system comprises of both cellular and humoral immune responses. Many members are known to synthesise lectins that confer innate immunity. Sialic acid binding lectins (SABLs) show high specificity towards *N*-acetylneuraminic acid (NeuNAc), *N*-Glycolylneuraminic acid (Neu5Gc) and Nacetyl-9-*0*-acetylneuraminic acid (Neu5, 9Ac2). No study on their crystal structure of SABLs from Mollusca exists and therefore we have analysed the only complete sequences available from the genebank and performed insilico analysis to understand the 3D structures. We discuss in this article structural peculiarities in SABL from *Solen grandis* and *Limax flavus* through insilico approaches. While all the SABLs with complete sequences had a conserved C1q complement domain, which may play role in bacterial recognition, *Solen grandis* SABL has an additional domain with similarity to Prefoldin with a probable molecular chaperon like activity and *Limax flavus* has domains bearing homology to fibrinogen-related domains (Fred) superfamily with probable role in blood clotting and are unique amongst molluscan SABLs. Their characterisation remains the future scope of the study.

Keywords: Mollusca, sialic acid binding lectin (SABL), innate immunity, *Solen grandis*, *Limax flavus*

Introduction
Phylum Mollusca includes a highly diverse group of organism, mostly marine invertebrate species with about 85,000 extant species and about 23% of marine organisms, also few freshwater and terrestrial forms with body bilaterally symmetrical or with lost symmetry secondarily. They are protostomes with their mouth arising from the embryonic blastopore which is the opening of the primitive gut that appears during gastrulation. Of the known classes, two are entirely extinct. The existing Classes of Phylum Mollusca include Class Aplacophora, including shell less worm like animals with rudimentary body plan, Class Monoplacophora, comprising of single shelled organisms, Class Polyplacophora with a ventral foot and dorsal shell made up of eight hard plates, Class Bivalvia with body encased in two shells, Class Gastropoda, with asymmetric body plan, conispiral or planospiral shell, torsion observed during development and Class Scaphoda with a single conical shell with a protruding head and tentacle like foot.
The body of the living members is encased in a calcareous shell represented by a visceral mass encased within a sac like structure called the mantle with a head and foot. Except the cephalopods, the members have an open type of circulatory system. Radula a structure for rasping with chitinous teeth, used in feeding. Body cavity is a hemocoel with circulating blood and coelomic fluid that flows through internal organs and the body releases components of humoral immunity in circulation. They develop from the trocophore larva. Molluscs are an important group to study since they have food value and are of economic importance. But due to their habitat they are exposed to a number of pathogens and it is interesting to study how they overcome such challenges for existence.
The main physical barrier to infection by the pathogen is the hard shell and mucus that cover the soft body of molluscs. Blood clotting and wound healing are involved in case of tissue injury. In Mollusca, the innate cellular defence mechanisms encompasses phagocytosis, encapsulation of pathogens, nodule and pearl formation, tissue atrophy, necrosis and liquefaction.

While granular hemocyte cells are predominant in occurrence and involved in cellular defense action, lysozyme activity, lectins and the phenyloxidase system comprise the key players of the humoral immunity in Mollusca. Size of the pathogen controls the fate of the pathogen response by the host. Lectins and activation of prophenyloxidase are required for their removal [1]. Lectins are protein molecules that recognise and have a binding specificity to carbohydrates. They act as pattern recognition molecules and play role in innate immune responses against pathogen in Mollusca as they lack adaptive immunity [1]. The smaller in size pathogens undergo removal from the host body by phagocytosis and the larger ones are eliminated by activation of either cellular or humoral responses by nodule formation or encapsulation. Other components of the humoral immune system including mercenenes which are known for their antimitotic roles, paolins known for their antiviral activity, acute phase reactants, alpha 2-macroglobulins, which are large sized protein with their anti-protease activity manifested by entrapping different proteinases with different specificities and catalytic properties although identified in molluscs suffers from the lack of signalling pathways controlling their role in conferring molluscan immunity. Lectins and antimicrobial peptides (AMPs) play a dominant role in cellular and humoral immune responses in Mollusca and find importance in understanding Molluscan immunity [2].

Amongst the different types of carbohydrate binding lectins in animals including C-type lectins, S-type lectins, P-type lectins, I-type lectins, L-type lectins R-type Lectins, C type lectins are known from molluscs. The Molluscan C type lectin has a common bi-looped structure with two significantly conserved disulfide linkages at the loop base with a Ca^{2+}-dependent carbohydrate binding ability with broad specificity towards mannose, galactose or similar carbohydrates in a manner playing role in pathogen recognition [1, 3] and is the most studied. We refer the readers to [1] for a broader review on the role of C type lectins in Mollusca.

Sialic acid binding lectins (SABL) are a specific group of lectins with affinity towards specific *N*-acetylnuraminic acid (NeuNAc), *N*-acetyl-D-galactosamine (GalNAc), *N*-acetyl-D-glucosamine (GlcNAc) and *N*-acetyl-D-mannosamine (ManNAc) glycan residues reported from Mollusca and are less studied. It is interesting to note that in such primitive animals like the Mollusca, lectins with the binding specificity to specific glycans have evolved. They are however not reported from all the classes under Phylum Mollusca but only reported from the class Bivalvia (Pelycypoda) including sea mussel and Pacific oyster and Gastropoda, including snails and sea slugs [4]. It would be interesting to understand the structural peculiarities of such lectins across the different Classes of Phylum Mollusca. Therefore there exists a need of a comprehensive article focussing on the different sialic acid binding lectins and their importance in molluscs. SABLs from *Solen grandis, Ruditapes philippinarum, Cepaea hortensis Crassostrea hongkongensis, Haliotis madak, Haliotis discus discus* and *Limax flavus* with complete peptide sequence in genebank were included in the study. Putative sialic acid binding lectin, partial (*Mytilus edulis*) were not included in the study.

Although all the Molluscan SABLs show homology to C1q of the complement complex belonging to the protein family PF 00386 with the exception of the lectin from *Solen grandis* and *Limax flavus* which show structural peculiarities and different domains which are highlighted in this article. We discuss in this article (i) the different sialic acid binding lectins released by the (ii) structural peculiarities in the SABLs through protein disorder studies (iii) SABL from *Solen grandis and Limax flavus* from conserved domains and 3D model structure and predicted protein disorder through insilico analysis.

Materials and methods

All published literature including original articles and review papers were searched from PUBMED using the keywords, Sialic acid binding lectins (SABL), molluscs. Inorder to understand the synthesis of sialic acid binding lectins by molluscs, we have searched all published articles with the keywords of sialic acid binding lectins in Mollusca.

Peptide sequences of lectins from Phylum Mollusca from Genebank

In this study we searched the NCBI, Genebank for known peptide sequences of lectins from molluscs in the database. Sequences in fasta format with Gene bank accession number are enlisted in Table 3. Sialic acid binding lectin with partial or putative sequences were not included in the study.

Conserved Domain Analysis

Conserved domain for the aforementioned lectins were searched using the Conserved Domain database (CDD). It is protein annotation resource with a collection of well-annotated multiple sequence alignment models for ancient domains and full-length proteins. Position-specific score matrices (PSSMs) enable fast identification of conserved domains in protein sequences via RPS-BLAST.

Predicting disorder in protein

DisEMBL 1.5 [5] is a public web server for predicting disorder in proteins. DisEMBL fetches the sequence and description of the polypeptide from an ExPASy server using Biopython.org software. The probability of disorder is shown graphically. The green curve is the predictions for missing coordinates, red for the hot loop network and blue for coil. The random expectation levels for the different predictors are shown on the graph as horizontal lines but should only be considered an absolute minima. Protein disorder is predicted by a two-state model in where each residue is either ordered or disordered. Three different criteria for defining disordered residues of proteins are used: Loops/coils defined by DSSP (Database of secondary structure assignment programme) in which residues are assigned belonging to one of several secondary structure types. For this definition we considered residues as alpha - helix ('H'), 3_10-helix ('G') or beta-strand ('E') as ordered, and all other states ('T', 'S', 'B', 'I', ' ') as loops or coils. Protein disorder is only found within loops. Hot loops constitute a subset of the above, consisting of high degree of mobility loops as determined from C-alpha temperature (B-)factors. Highly dynamic loops are considered as protein disorder. Missing coordinates in X-Ray structure as defined by REMARK-465 entries in PDB. Non assigned electron densities which reflect intrinsic disorder, have been used early on in disorder prediction [5].

Insilico 3D structure

Peptide sequences in FASTA format for the lectins from *Solen grandis* and *Limax flavus* showing peculiarities by CDD analysis from Genebank was submitted to the I-TASSER webserver (http://zhanglab.ccmb.med.umich.edu/I-TASSER) [6] which led to the generation of 3D protein structure and also predicted the biological functions of protein molecules from

amino acid sequence. Of the five models with an individual C-score(confidence scores) calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations, the highest C-score with greater confidence was shown.

Results

(i) Different sialic acid binding lectins (SABLs) in Molluscs

Sialic acid binding lectins (SABLs), are members of immunoglobulin family that play role in conferring host defence mediated by cell-cell interaction, in response to recognising of different types of glycans. Although they are mostly studied in human and vertebrate, few reports exists from invertebrates. In Phylum (Table 1) their occurrence has been traced within different members under classes Bivalvia and Gastropoda which we have summarised in Table 2.

Table 1: Taxonomic position of molluscs[38]

Kingdom	Animalia
Superphylum	Lophotrochozoa
Phylum	Mollusca

Table 2: Sialic acid binding lectins, their source and biological activity in Mollusca

Species	Lectin	Specificity	Structure	Function	Ref
Mollusca					
CLASS: Bivalvia					
Solen grandis	SgSABL-1 and SgSABL-2	Sialic acid, *N*-Acetylneuraminic acid (Neu5Ac)	Both SgSABL-1 and SgSABL-2 with a C1q domain	Pathogen and pattern recognition	[7]
Crassostrea hongkongensis	*Ch-salectin*	Sialic acid, *N*-Acetylneuraminic acid (Neu5Ac)	156 amino acids with a signal peptide and a C1q domain.	bactericidal	[8]
Venerupis philippinarum	VpSABL	Sialic acid, *N*-Acetylneuraminic acid (Neu5Ac)	195 amino acids with a C terminal C1q domain	Pattern recognition receptor (PRR), recognition of Gram-negative bacteria, *Vibrio anguillarum*	[9]
Ruditapes philippinarum	MCsialec	Sialic acid, *N*-Acetylneuraminic acid (Neu5Ac)	Polypeptide of 200 amino acids, 21.928 kDa	Antibacterial overexpressed during infection by *Vibrio tapetis*	[10]
Modiolus modiolus	Modiolin H and modiolin E	Sialic acid, *N*-Acetylneuraminic acid (Neu5Ac)	Three subunits with different molecular weight and isoelectric points (pI); Mr14 (pI 5.1 and 5.5), 17.5 (pI 5.5) and 20 (pI 4.9) kDa.	Antibacterial	[19]
Crassostrea gigas	Hemagglutinin Gigalin H (human) and Gigalin E (equine)	Sialic acid, *N*-Acetylneuraminic acid (Neu5Ac)	Exact structure not known	agglutination property on human and equine RBC	[26]
Crassostrea virginica	Hemagglutinin	Sialic acid, *N*-Acetylneuraminic acid (Neu5Ac)	Exact structure not known	Antibacterial; agglutination property	[27]
Mytilus edulis	Located in hemolymph	Sialic acid, *N*-Acetylneuraminic acid (Neu5Ac)	Exact structure not known		[28]
Anadara granosa	AFL	*N*-Glycolylneuraminic acid (Neu5Gc)	Native tetrameric protein (254 kDa, pI 6.65). Two subunits, 65 kDa and 62 kDa each. Aspartic acid, glutamic acid, serine and glycine. are dominant amino acid	Ca^{2+} dependent agglutination of erythrocytes	[20]
CLASS: Gastropoda					
Cepaea hortensis	Agglutinin	Nacetyl-9-*0*-acetylneuraminic acid (Neu5,9Ac2)	159 amino acids, with putative signal sequence peptide. Mature protein with141 amino acid with15,529 Da mass	Agglutinate human RBC, and bind with *Streptococcus agalactiae*	[21, 30]
Achatina fulica	Achatinin H	Nacetyl-9-*0*-acetylneuraminic acid (Neu5,9Ac2)	16 monomer units with each sugar binding site, with high beta-sheet content (46%) and a low alpha-helix content (24%).	Diagnosis, prognosis of childhood acute lymphoblastic leukemia (29-33)	[16]
Pila globosa	PAL	*N*-Glycolylneuraminic acid (Neu5Gc)	Glycoprotein with three nonidentical subunits, 190, 145, and 105 kDa, native Mw 440 kDa, 25% carbohydrate, pI 7.0.	Hemagglutination in Ca^{2+} dependant manner.	[22]
Limax flavus	LFA	Specificity towards Neu5Ac more as compared to Neu5Gc	The recombinant lectin expressed in E coli bears homology to several molecules including fibrinogen domain of human tenascin-C, C-type lectin in humans and ficolin from pigs.	Agglutination	[17, 29]

Neu5Ac is the sialic acid found in humans while Neu5Gc is not found in human [37].

SABL identified from razor clam *Solen grandis* SgSABL-1 and SgSABL-2 have been reported with sequence similarity to other invertebrates and expressed both constitutively in different tissues and inducible in response to microorganism glycan stimulation. Both the lectins have a common C1q domain complement termed as cABL-1 that play an active role in glycan recognition. This function is mediated by the ability of induction of glycan recognition when the razor clams were challenged by acetylated subunits-containing glycan lipopolysaccharide (LPS) and peptidoglycan (PGN) and β-1,3-glucan, and could stimulate expression of SgSABL-2 [7]. Ch-salectin isolated from *Crassostrea hongkongensis* [8] with an identified signal peptide and conserved C1q domain and specificity towards glycoprotein fetuin glycoprotein containing N-linked and O-linked sialylated glycans showed significant antibacterial properties and overexpressed on stimulation with *Vibrio alginolyticus*. SABL from Manila clam *Venerupis philippinarum* (VpSABL) were known to be constitutively expressed in mantle, hepatopancreas and gill, and to a lesser degree in muscle tissues and haemocytes from Manila clam *Venerupis philippinarum.* Structurally characterised with 195 amino acids with a C-terminal C1q domain bearing with homology of 10 β-strand jelly-roll folding topology common to all C1q-TNF (tumour necrosis factor) family [9]. *Vibrio anguillarum* is known to induce sialic acid binding lectin (VpSABL) expression upon exposure to bacteria and contributed to the recognition of bacterial pathogens [9].

MC-sialic acid-binding lectin (MCsialec) reported from Manila clam haemocytes infected with *Perkinsus olseni* with homology to sialic acid-specific invertebrate lectins from *Cepaea hortensis*, *Helix pomatia* and *Haliotis discus discus* [10]. C-terminal globular C1q domain isolated from plasma of surfperch *Neoditrema ransonnetii* with 212 amino acids and a signal peptide of 20 amino acids has been reported of its L-fucose-binding activity expressed in liver, stomach and intestine [11]. The protozoan parasite, *Perkinsus olseni*, and infection by Vibrio is reported to upregulate lectin synthesis in hemocytes, in Manila clams (*Ruditapes philippinarum*). MCsialec from manila clams has been reported of a polypeptide with 168 amino acid residues and molecular weight of 19.2 bearing high homology to sialic acid-binding lectin from the snail [12].

Sialic acid-specific lectin of the garden snail *Cepaea hortensis* has been cloned in *E. coli*. revealing 159 amino acids, including the putative signal sequence peptide [13].

Achatinin-H synthesised in amoebocytes in the hemolymph and albumen gland of *Achatina fulica* snails has revealed16 identical subunits of M.W. 15 kDa [14] and for its specificity towards 9-*O*-acetyl sialoglycogonjugates finds importance in detection, prediction and monitoring in childhood acute lymphoblastic leukemia [15-16]. *Limax flavus* slug expresses a sialic-acid-specific lectin gene of the multigene family on the epidermal surface and in mucous glands with significant similarity with the fibrinogen domain of human tenascin-C, with a human C-type serum lectin, and with pig ficoli [17]. A heterogeneous sialic acid-binding lectin isolated from hemolymph of the horse mussel *Modiolus modiolus* has revealed recognition and affinity towards bacterial LPS. It is reported to exhibit strong antibacterial effect against *Vibrio anguillarum*, *Vibrio salmonicida*, *Vibrio viscosus*, *Vibrio wodanis*, and *Vibrio ordalii*, with moderate antibacterial effect against infection by *Aeromonas salmonicida salmo* [18]. Modiolin H and modiolin E, both with specificity to NeuAc or sialic acid has been reported to agglutinate human and horse (equine) erythrocytes, respectively in presence of Calcium. Modiolin E revealed hemagglutination property [19]. Marine clam *Anadara granosa* produces a lectin AFL isolated from the foot muscles which reveal specificity towards N-glycolylneuraminic acid [20] *Cepaea hortensis*, snail lectin with specificity to sialic acids were discovered as early as in 1992 [21]. A N-glycolylneuraminic acid-specific lectin (PAL) from apple snail, *Pila globosa* has been purified from an albumin gland extract that could agglutinate red blood cells (RBCs) from rabbit. It is observed that presence of Ca^{2+} [22] are required for agglutination of RBC by lectin. Sialic acid binding lectin (SABL) has been identified from horseshoe crab (*Limulus polyphemus*) and the garden snail (*Helix pomatia*) [23]. The hemagglutinins from the spawn of the water snail *Biomphalaria glabrata* [24] have been reported of specificity towards sialic acids. *Helix pomatia* lectin (HPA) has specificity towards with N-glycosidic carbohydrate-peptide linkages identified by their specific binding to due to their Concanavalin A (Con A) [25].

(ii) Conserved Domain (CDD) Analysis

All sequences enlisted in Table 2 were subjected to CDD analysis. SABL proteins from *Ruditapes philippinarum*, *Cepaea hortensis*, *Crassostrea hongkongensis*, *Haliotis madaka* and *Haliotis discus discus* showed conserved domain with C1q component of complement protein of the pfam 00386 at positions 77-193, 26-148, 28-153, 27-153, 27-153 respectively (Table 2). Conserved Domain analysis of SABL from *Solen grandis* (Gene Bank accession AFA36088.1) revealed two distinct domains including (i) C1q complement domain of pfam 00386 in the region of 114-192 amino acids involved in recognition of pathogen and (ii) at region 30-110 amino acid shows homology to Prefoldin, conserved protein domain family COG1382 which has a chaperone like activity (Fig. 1).

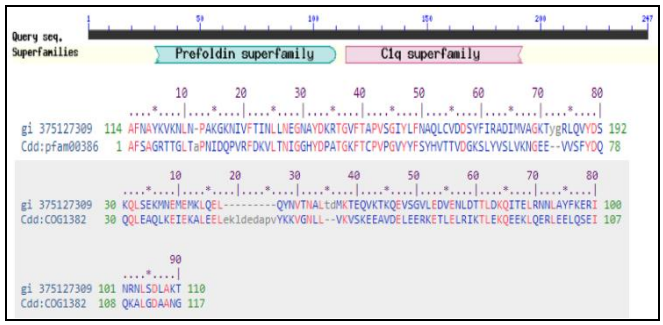

Fig 1 SABL *Solen grandis* 1A: CDD Analysis

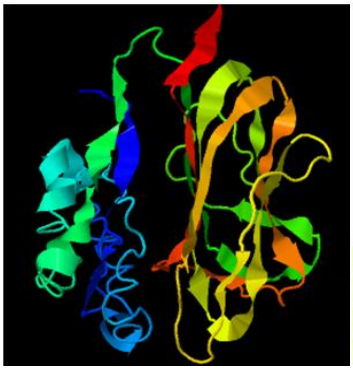

Fig. 1B: Predicted whole molecule

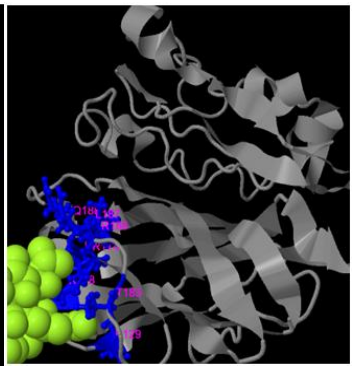

Fig 1C: Ligand binding sites

K129,I173,R174, T183,R186,L187, Q188, K218

All the three sialic acid-binding lectin from *Limax flavus* revealed conserved domains with C terminal domain of fibrinogen, belonging to the family CD00087 with Ca2+ binding, polymerisation and gamma gamma domain and probable biological role in blood clotting (Table 3, Fig. 2).

Thus the two SABL from *Solen grandis* and *Limax flavus* showing domain peculiarities as compared to other SABLs from Mollusca and were thus subjected to 3D model generation through insilico approaches.

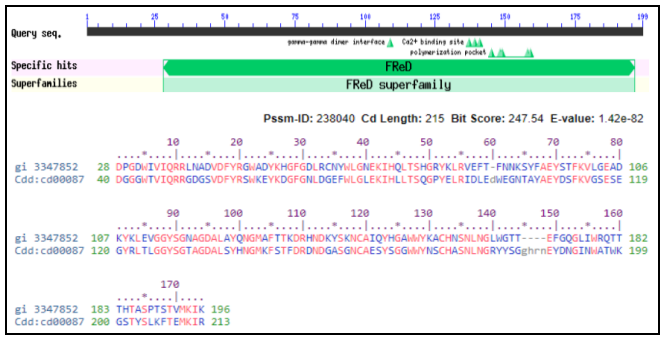

Fig. 2 SABL *Limax flavus* 2A: CDD Analysis

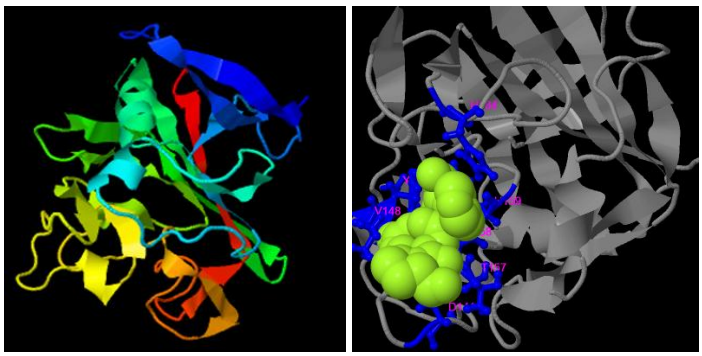

Fig. 2B: Predicted Whole molecule

Fig. 2C Ligand Binding Sites D141,V148,Y149,T157, C158,H159, H184

Table 3: Conserved Domain Analysis for lectins with complete peptide sequences

Sialic acid binding lectin	Gene Bank Source	Fasta Sequence	Conserved Domain
Solen grandis	AFA36089.1	MAFTGSLYLTMLLIIQCSMGSDFSEPRGPECSLKLVERLTRLEIDNENLKQKVAKLGSESSDVIVKFRVSKQISVANGQKIKYDVAMR NDGLAFNVEESVFKAPQDGVYIVSLTVCVDVNKWVVTEVLHEGAVTSQFLSGDNGYHTCNNEVLTQSLRKGERLWVQKLRGSAS VLFEGYGWNTFSVSLL	No conserved domain
Solen grandis	AFA36088.1	MTRVCVFTFVVCVIGGFVLVEPARSSKNYKQLSEKMNEMEMKLQELQYNVTNALTDMKTEQVKTKQEVSGVLEDVENLDTTLDKQ ITELRNNLAYFKERINRNLSDLAKTQLVAFNAYKVKNLNPAKGKNIVFTINLLNEGNAYDKRTGVFTAPVSGIYLFNAQLCVDDSYFI RADIMVAGKTYGRLQVYDSSGSEPCNAARAIVIMKSGETASVCKKDGDGRMFVDSNHEMFFNGALINNVISTTD	1. C1q complement domain: 114-192 2. Prefoldin domain: 30-110. Prefoldin is a hexameric protein of chaperone complex found in archaea and eukaryotes
Ruditapes philippinarum	ACU83226.1	MISLIDLRTMHLLDKMVRMEMKMERMEEDLSEAQTNVNVFIKKQTKILENKTAELELLEGRVDLPLIAFNAYSPIDTSPDTNEIIIWQS THLNEGNGYDTVIGIFKAPVSGLYYFAVHVCNYSSQVFQYAIVLENNNIATSYKYDNNNYDCGSMSTFTKVAAGQRVWVRCTSGST SALLWESSGRSSFIGALIHT	C1q domain 77-193
Cepaea hortensis	CAD83837.1	MTLLAGCIIFAVLGVTFGQIVEPTVAFTAVLDKNLVLENGDTLIPNKILINYGGGYNDKTGIFTAPKSGIYHLGVHAQTSLQSNLWLAL YHNDNYVFSIYGRQTEYSDGGANAAILPLKKGDKVHVKARDKSSLLGRPDNIYTTFTGFRLGPLREDDS	C1q domain 26-148
Crassostrea hongkongensis	ADW66456.1	MLLLISLLCFAIQVESYGYVYHRSPALAFTAMLYSHKTIGARAVVRYDHVVTSLGGAYRSTTGIFTAPFRGLYSFSYSLMSYPSNEVHLE MVKNGKRVFKVYFAPHTYPQSSQTLYLILNRGDTICIENSFFERKATLYEDTGGYNTFSGTLIRII	C1q domain 28-153
Haliotis madaka	ALU63755.1	MDDLFKGILFLGVTLCAAQDASQFETAFSAGLTKHLTLQAGATVIYDKVFTNIGNAYDNNTGVFTCPQTGIYVFQYHGLSMSDDTLWLE LYHNYNYVSSAYAHTNSDYASAGNSVILHLFKGDTVMVNAEPNQESNLYGVSDDVYCTFSGYLIAPVFEESVVV	C1 q domain 27-153
Haliotis discus discus	ABO26662.1	MDGVFKGILFLGVTLCAAQDVSQFETAFSAGLTTHLTLQAGATVIYDKVFTNIGNAYDNNTGVFTCPQTGIYVFQYHGLSMSDDTLWLE LYHNYNYVSSAYAHTNSDYASAGNSVILHLFKGDTVMVNAEPDQESNLYGVSDDVYCTFSGYLIAPVFEESVVVG	C1q 27-153
SABL-3 *Limax flavus*	AAC27744.1	MSVLFLAASFLLLTSFEVVAAKGCPPQDPGDWIVIQRRLNADVDFYRDWVDYKHGFGDLRCNYWLGNEKIHQLTSHGRYKLRVEVTFN NRSYFAEYSTFKILGEADKYKLEVGGYSGNAADHLAIHNGMAFTTKDRDNDADSIDCAKVYHGAWWYKTCHESNLNGLWGTTKFGQG LSWKQTTTHTASPTSTVMKIKSLD	Fred superfamily domain 28-196
SABL-1 *Limax flavus*	AAC27742.1	MSVLFLAASFLLLASFELVAAQGCPRQDPGDWIVIQRRLNADVNFYRGWADYKHGFGDLRCNFWLGNEKIHQLTSHGRYKLRVEFTFNN KSYFAEYSTFKILGEADKYKLQVGGYSGNAGDALTFHNGMAFSTNDRDNDADSIDCAKVYHGAWWYKTCHESNLNGKWGSKKYGEGL NWKAKTTFTATATSSLLKIKALK	Fred superfamily 28-178
SABL-2 *Limax flavus*	AAC27743.1	MSVLFLAASFLLLTSFELVAAAGCPPQDPGDWIVIQRRLNADVDFYRGWADYKHGFGDLRCNYWLGNEKIHQLTSHGRYKLRVEFTFNN KSYFAEYSTFKVLGEADKYKLEVGGYSGNAGDALAYQNGMAFTTKDRHNDKYSKNCAIQYHGAWWYKACHNSNLNGLWGTTEFGQG LIWRQTTTHTASPTSTVMKIKSID	Fred superfamily domain 28-196

Table 4: Prediction of disorders using DisEMBL 1.5 - Predictors of intrinsic protein disorder

Organism	Disordered by Loops/coils definition	Disordered by Hot-loops definition	Disordered by Remark-465 definition	Total
Solen grandis AFA36088.1	LOOPS 18-28, 118-130, 139-156, 163-173, 187-198, 209-231 mtrvcvftfv vcviggfVLV EPARSSKNyk qlsekmneme mklqelqynv tnaltdmkte qvktkqevsg vledvenldt tldkqitelr nnlayfkeri nrnlsdlakt qlvafnaYKV KNLNPAKGKN ivftinllNE GNAYDKRTGV FTAPVSgiyl fnAQLCVDDS YFIradimva gktygrLQVY DSSGSEPCna araivimkSG ETASVCKKDG DGRMFVDSNH Emffngalin nvisttd	HOTLOOPS 15-27, 66-83, 119-129 mtrvcvftfv vcviGGFVLV EPARSSKnyk qlsekmneme mklqelqynv tnaltdmkte qvktkQEVSG VLEDVENLDT TLDkqitelr nnlayfkeri nrnlsdlakt qlvafnayKV KNLNPAKGKn ivftinllne gnaydkrtgv ftapvsgiyl fnaqlcvdds yfiradimva gktygrlqvy dssgsepcna araivimksg etasvckkdg dgrmfvdsnh emffngalin nvisttd	REM465 59-68 mtrvcvftfv vcviggfvlv eparssknyk qlsekmneme mklqelqynv tnaltdmkTE QVKTKQEVsg vledvenldt tldkqitelr nnlayfkeri nrnlsdlakt qlvafnaykv knlnpakgkn ivftinllne gnaydkrtgv ftapvsgiyl fnaqlcvdds yfiradimva gktygrlqvy dssgsepcna araivimksg etasvckkdg dgrmfvdsnh emffngalin nvisttd	10
Ruditapes philippinarum ACU83226.1	LOOPS 59-82, 91-100, 135-161 mislidlrtm hlldkmvrme mkmermeedl seaqtnvnvf ikkqtkilen ktaelellEG RVDLPLIAFN AYSPIDTSPD TNeiiiwqst HLNEGNGYDT vigifkapvs glyyfavhvc nyssqvfqya ivleNNNIAT SYKYDNNNYD CGSMSTFTKV Aagqrvwvrc tsgstsallw essgrssfig aliht	_HOTLOOPS 1-13, 73-80, 176-195 MISLIDLRTM HLLdkmvrme mkmermeedl seaqtnvnvf ikkqtkilen ktaelelleg rvdlpliafn aySPIDTSPD tneiiiwqst hlnegngydt vigifkapvs glyyfavhvc nyssqvfqya ivlennniat sykydnnnyd cgsmstftkv aagqrvwvrc tsgstSALLW ESSGRSSFIG ALIHT	REM465 none mislidlrtm hlldkmvrme mkmermeedl seaqtnvnvf ikkqtkilen ktaelelleg rvdlpliafn ayspidtspd tneiiiwqst hlnegngydt vigifkapvs glyyfavhvc nyssqvfqya ivlennniat sykydnnnyd cgsmstftkv aagqrvwvrc tsgstsallw essgrssfig aliht	6
Cepaea hortensis CAD83837.1	LOOPS 33-79, 101-158 mtllagciif avlgvtfgqi veptvaftav ldKNLVLENG DTLIPNKILI NYGGGYNDKT GIFTAPKSGI YHLGVHAQTs lqsnlwlaly hndnyvfsiy GRQTEYSDGG ANAAILPLKK GDKVHVKARD KSSLLGRPDN IYTTFTGFRL GPLREDDS	HOTLOOPS 35-45, 116-158 mtllagciif avlgvtfgqi veptvaftav ldknLVLENG DTLIPnkili nygggyndkt giftapksgi yhlgvhaqts lqsnlwlaly hndnyvfsiy grqteysdgg anaaiLPLKK GDKVHVKARD SLLGRPDN IYTTFTGFRLGPLREDDS	REM465 none mtllagciif avlgvtfgqi veptvaftav ldknlvleng dtlipnkili nygggyndkt giftapksgi yhlgvhaqts lqsnlwlaly hndnyvfsiy grqteysdgg anaailplkk gdkvhvkard kssllgrpdn iyttftgfrl gplredds	4
Crassostrea hongkongensis ADW66456.1	LOOPS 16-25, 52-72, 79-86, 101-113, 122-132, 137-150 mlllisllcf aiqveSYGYV YHRSPalaft amlyshktig aravvrydhv vTSLGGAYRS TTGIFTAPFR GLysfsysLM SYPSNEvhle mvkngkrvfk VYFAPHTYPQ SSQtlyliln rGDTICIENS FFerkaTLYE DTGGYNTFSG tlirii	HOTLOOPS 56-67, 105-112, 137-156 mlllisllcf aiqvesygyv yhrspalaft amlyshktig aravvrydhv vtslgGAYRS TTGIFTApfr glysfsyslm sypsnevhle mvkngkrvfk vyfaPHTYPQ SSqtlyliln rgdticiens fferkaTLYE DTGGYNTFSG TLIRII	REM465 none mlllisllcf aiqvesygyv yhrspalaft amlyshktig aravvrydhv vtslggayrs ttgiftapfr glysfsyslm sypsnevhle mvkngkrvfk vyfaphtypq ssqtlyliln rgdticiens fferkatlye dtggyntfsg tlirii	9
Haliotis madaka ALU63755.1	_LOOPS 51-85, 101-112, 122-163 mddlfkgilf lgvtlcaaqd asqfetafsa gltkhltlqa atviydkvf TNIGNAYDNN TGVFTCPQTG IYVFQYHGLS MSDDTlwlel yhnynyvssa YAHTNSDYAS AGnsvilhlf kGDTVMVNAE PNQESNLYGV SDDVYCTFSG YLIAPVFEES VVV	HOTLOOPS none mddlfkgilf lgvtlcaaqd asqfetafsa gltkhltlqa gatviydkvf tnignaydnn tgvftcpqtg iyvfqyhgls msddtlwlel yhnynyvssa yahtnsdyas agnsvilhlf kgdtvmvnae pnqesnlygv sddvyctfsg yliapvfees vvv	REM465 none mddlfkgilf lgvtlcaaqd asqfetafsa gltkhltlqa gatviydkvf tnignaydnn tgvftcpqtg iyvfqyhgls msddtlwlel yhnynyvssa yahtnsdyas agnsvilhlf kgdtvmvnae pnqesnlygv sddvyctfsg yliapvfees vvv	3
Haliotis discus discus ABO26662.1	LOOPS 51-85, 101-112, 121-164 mdgvfkgilf lgvtlcaaqd vsqfetafsa gltthltlqa gatviydkvf TNIGNAYDNN TGVFTCPQTG IYVFQYHGLS MSDDTlwlel yhnynyvssa YAHTNSDYAS AGnsvilhlf KGDTVMVNAE PDQESNLYGV SDDVYCTFSG YLIAPVFEES VVVG	HOTLOOPS none mdgvfkgilf lgvtlcaaqd vsqfetafsa gltthltlqa gatviydkvf tnignaydnn tgvftcpqtg iyvfqyhgls msddtlwlel yhnynyvssa yahtnsdyas agnsvilhlf kgdtvmvnae pdqesnlygv sddvyctfsg yliapvfees vvvg	none_REM465 none mdgvfkgilf lgvtlcaaqd vsqfetafsa gltthltlqa gatviydkvf tnignaydnn tgvftcpqtg iyvfqyhgls msddtlwlel yhnynyvssa yahtnsdyas agnsvilhlf kgdtvmvnae pdqesnlygv sddvyctfsg yliapvfees vvvg	3
SABL-3 *Limax flavus* AAC27744.1	LOOPS 19-33, 40-78, 87-98, 104-121, 127-199 msvlflaasf llltsfevVA AKGCPPQDPG DWIviqrrlN ADVDFYRDWV DYKHGFGDLR CNYWLGNEKI HQLTSHGRyk lrvevtFNNR SYFAEYSTfk ilgEADKYKL EVGGYSGNAA DhlaihNGMA FTTKDRDNDA DSIDCAKVYH GAWWYKTCHE SNLNGLWGTT KFGQGLSWKQ TTTHTASPTS TVMKIKSLD	HOTLOOPS 72-79, 181-199 msvlflaasf llltsfevva akgcppqdpg dwiviqrrln advdfyrdwv dykhgfgdlr cnywlgneki hQLTSHGRYk lrvevtfnnr syfaeystfk ilgeadkykl evggysgnaa dhlaihngma fttkdrdnda dsidcakvyh gawwyktche snlnglwgtt kfgqglswkq TTTHTASPTS TVMKIKSLD	REM465 179-199 msvlflaasf llltsfevva akgcppqdpg dwiviqrrln advdfyrdwv dykhgfgdlr cnywlgneki hqltshgryk lrvevtfnnr syfaeystfk ilgeadkykl evggysgnaa dhlaihngma fttkdrdnda dsidcakvyh gawwyktche snlnglwgtt kfgqglswKQ TTTHTASPTS TVMKIKSLD	8
SABL-1 *Limax flavus* AAC27742.1	>none_LOOPS 20-33, 42-77, 86-97, 103-180 msvlflaasf lllasfelvA AQGCPRQDPG DWIviqrrln aDVNFYRGWA DYKHGFGDLR CNFWLGNEKI HQLTSHGryk lrvefTFNNK SYFAEYStfk ilGEADKYKL QVGGYSGNAG DALTFHNGMA FSTNDRDNDA DSIDCAKVYH GAWWYKTCHE SNLNGKWGSK KYGEGLNWKA kttftatats sllkikalk	HOTLOOPS 184-199 msvlflaasf lllasfelva aqgcprqdpg dwiviqrrln advnfyrgwa dykhgfgdlr cnfwlgneki hqltshgryk lrveftfnnk syfaeystfk ilgeadkykl qvggysgnag daltfhngma fstndrdnda dsidcakvyh gawwyktche snlngkwgsk kygeglnwka kttFTATATS SLLKIKALK	REM465 none msvlflaasf lllasfelva aqgcprqdpg dwiviqrrln advnfyrgwa dykhgfgdlr cnfwlgneki hqltshgryk lrveftfnnk syfaeystfk ilgeadkykl qvggysgnag daltfhngma fstndrdnda dsidcakvyh gawwyktche snlngkwgsk kygeglnwka kttftatats sllkikalk	5
SABL-2 *Limax flavus* AAC27743.	LOOPS 19-33, 40-77, 86-96, 102-148, 157-199 msvlflaasf llltsfelVA AAGCPPQDPG DWIviqrrlN ADVDFYRGWA DYKHGFGDLR CNYWLGNEKI HQLTSHGryk lrvefTFNNK SYFAEYstfk vLGEADKYKL EVGGYSGNAG DALAYQNGMA FTTKDRHNDK YSKNCAIQyh gawwykACHN SNLNGLWGTT EFGQGLIWRQ TTTHTASPTS TVMKIKSID	HOTLOOPS 182-199 msvlflaasf llltsfelva aagcppqdpg dwiviqrrln advdfyrgwa dykhgfgdlr cnywlgneki hqltshgryk lrveftfnnk syfaeystfk vlgeadkykl evggysgnag dalayqngma fttkdrhndk ysknCAIQyh gawwykachn snlnglwgtt efgqgliwrq tTTHTASPTS TVMKIKSID	none_REM465 none msvlflaasf llltsfelva aagcppqdpg dwiviqrrln advdfyrgwa dykhgfgdlr cnywlgneki hqltshgryk lrveftfnnk syfaeystfk vlgeadkykl evggysgnag dalayqngma fttkdrhndk ysknCAIQyh gawwykachn snlnglwgtt efgqgliwrq ttthtaspts tvmkiksid	6

(iii) Predicting protein Disorder

Using DisEMBL 1.5, public web server for predicting disorder in proteins it was observed that SABL of *Solen grandis* followed by that of *Crassostrea hongkongensis* has a larger number of loops or coils indicative of predicted disordered regions as compared to the other SABLs (Table 4). Protein Disorder studies reveal a comparatively stable structure of SABL of *Haliotis madaka* and *Haliotis discus discus* followed by that of SABL-1 *Limax flavus*. SABLs of *Limax flavus* show microheterogeneity differences with SABL 3 showing the most disorder (Table 4) as compared to SABL 2 and SABL 1 from *Limax flavus.*

(iv) 3D model Structure of *Solen grandis* and *Limax flavus* SABL

We observed that none of the sialic acid binding proteins from Mollusca has been crystallised and no crystal structure exists. Therefore, we submitted the sequences of *Solen grandis* and *Limax flavus* SABL with structural peculiarities found from CDD analysis (Table 3, Fig 1-2) to the I-TASSER Server Suite for generating 3D structures of these two lectins. We observed distinct differences in the two lectins which are unique to them as compared to other SABLs. These two lectins also show distinct differences in ligand binding site.

Discussion

Lectin synthesis and its role in innate immunity are recorded from different invertebrates and vertebrate organisms. Lectins in human have a distinct biological role in cell-cell interaction, signal transduction, innate immune responses. However the synthesis, location and role of lectins in molluscs show diversity and are distinctly different from human lectins. What is interesting to understand is the evolution of mechanism of synthesis of lectins evolved in the primitive organism like molluscs. Although human beings are incapable of synthesising the Neu5Gc, it is interesting to note that Molluscan members can synthesise the different sialic acid binding lectins with specificity towards *N*-acetylnuraminic acid (Neu5Ac, *N*-Glycolylneuraminic acid (Neu5Gc) and Nacetyl-9-*0*-acetylneuraminic acid (Neu5,9Ac2). We in this study report the diversity of sialic acid binding lectins in different Molluscan members and their specificity towards different glycans (Table 2) and the differences in the protein structure measured by the protein disorder studies (Table 4). While all SABLs studied here has structural homology to C1q component of the complement system instrumental in mediating the innate immune reactions in antibacterial host responses the SABL from *Solen grandis* has both a C1q conserved domain and a conserved domain of Prefoldin with a probable molecular chaperon like activity of this lectin (Fig 1, 2). *Limax flavus* SABL shows distinctly different structural homology to the fibrinogen C terminal globular domain that may play role in blood clotting which is unique to the SABLs of *Limax flavus* and is not seen in any of the other SABLs of Phylum Mollusca so far. We also report for the first time a structural study of SABLs from *Solen grandis* and *Limax flavus* showing peculiarity as compared to other known SABLs in Mollusca with complete protein sequence through insilico approaches (Fig. 1-2).

Basic questions regarding the specificity of lectins and their affinity towards sialic acid that have been evolved in primitive organism like molluscs is not well understood. It is also not known that amongst the existing 7 classes of Mollusca, why and how only two classes including Bivalvia and Gastropods developed such specialised lectins in controlling their innate immune system.

Further to our findings it remains a major question as to the differences of structure and functions of SABLs although belonging to the Class Bivalvia under Phylum Mollusca. Crystal structure of these SABLs does not exist. Purification and characterization of these SABLs may lead to better understanding of their biological function which also remains the scope of the future study.

Conclusion

We have reviewed in this manuscript, sialic acid lectins in Mollusca, their diversity and their specificity towards different glycans (Table 2) and the differences in the protein structure studied through insilico approaches. We report that while all the Molluscan SABLs with complete sequences in genebank had a conserved C1q complement domain, with important role in bacterial recognition, *Solen grandis* SABL has an additional domain with similarity to Prefoldin with a probable molecular chaperon like activity and *Limax flavus* has domains bearing homology to fibrinogen-related domains (Fred) superfamily with probable role in blood clotting and are unique amongst Molluscan SABLs.

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References

1. Lehmanna F, Tiralongob E, Tiralongoa J. Sialic acid-specific lectins: occurrence, specificity and function, Cellular and Molecular Life Science. 2006; 63:1331-1354.
2. Gliński Z, Jarosz J. n immune defenses. Archivum Immunologiae et Therapiae Experimentalis (Warsz). 1997; 45:149-55.
3. Devi RV, Basilrose MR, Mercy PD. Prospect for lectins in arthropods. Italian Journal of Zoology. 2010; 77:254-260.
4. Mandal C, Mandal C. Sialic acid binding lectins Experientia. 1990; 46:433-441.
5. Linding R, Jensen LJ, Diella F, Bork P, Gibson TJ, Russell RB. Protein disorder prediction: implications for structural proteomics. Structure. 2003; 11:1453-1459.
6. Zhang Y. I-TASSER server for proteins 3D structure prediction. BMC Bioinformatics, 2008; 9:40.
7. Yang J, Wei X, Liu X, Xu J, Yang D, Yang J, *et al.* Cloning and transcriptional analysis of two sialic acid-binding lectins (SABLs) from razor clam Solen grandis. Fish and Shellfish Immunology. 2012; 32:578-85.
8. He X, Zhang Y, Yu F, Yu Z. A novel sialic acid binding lectin with anti-bacterial activity from the Hong Kong oyster (*Crassostrea hongkongensis*). Fish and Shellfish Immunology. 2011; 31:1247-50.
9. Li C, Yu S, Zhao J, Su X, Li T. Cloning and characterization of a sialic acid binding lectins (SABL) from Manila clam Venerupis philippinarum. Fish and Shellfish Immunology. 2011; 30:1202-6.
10. Adhya M, Choi KS, Yu Y, Cho M. Expression and localization of MCsialec, a sialic acid-specific lectin in the marine bivalve Manila clam, Ruditapes philppinarum. Journal of Fish Diseases. 2010; 33:889-99.
11. Nakamura O, Wada Y, Namai F, Saito E, Araki K, Yamamoto A, *et al*. A novel C1q family member with

fucose-binding activity from surfperch, Neoditrema ransonnetii (Perciformes, Embiotocidae). Fish and Shellfish Immunology. 2009; 27:714-720.
12. Kang YS, Kim YM, Park KI, Kim Cho S, Choi KS, Cho M. Analysis of EST and lectin expressions in hemocytes of Manila clams (Ruditapes philippinarum) (Bivalvia:) infected with Perkinsus olseni. Developmental & Comparative Immunology. 2006; 30:1119-1131.
13. Gerlach D, Schlott B, Schmidt KH. Cloning and expression of a sialic acid-binding lectin from the snail Cepaea hortensis. FEMS Immunology and Medical Microbiology. 2004; 40:215-221.
14. Biswas C, Sinha D, Mandal C. Investigation on interaction of Achatinin, a 9-O-acetyl sialic acid-binding lectin, with lipopolysaccharide in the innate immunity of Achatina fulica snails. Molecular Immunology. 2000; 37:745-754.
15. Sinha D, Mandal C, Bhattacharya DK. Identification of 9-O acetyl sialoglycoconjugates (9-OAcSGs) as biomarkers in childhood acute lymphoblastic leukemia using a lectin, Achatinin H, as a probe, Leukemia. 1999; 13:119-125.
16. Sen G, Mandal C. The specificity of the binding site of AchatininH, a sialic acid-binding lectin from Achatina fulica. Carbohydrate Research. 1995; 268:115-125.
17. Kurachi S, Song Z, Takagaki M, Yang Q, Winter HC, Kurachi K, *et al*. Sialic-acid-binding lectin from the slug Limax flavus--cloning, expression of the polypeptide, and tissue localization. European Journal of Biochemistry. 1998; 254:217-222.
18. Tunkijjanukij S, Olafsen JA. Sialic acid-binding lectin with antibacterial activity from the horse mussel: further characterization and immunolocalization. Dev Comp Immunol Developmental & Comparative Immunology. 1998; 22:139-150.
19. Tunkijjanukij S, Mikkelsen HV, Olafsen JA. A heterogeneous sialic acid-binding lectin with affinity for bacterial LPS from horse mussel (Modiolus modiolus) hemolymph. Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology. 1997; 117:273-286.
20. Dam TK, Sarkar M, Ghosal J, Choudhury A. Purification and partial characterization of a N-glycolylneuraminic acid specific lectin from the clam *Anadara granosa* (L). Biochemical and Biophysical Research Communications. 1993; 196:422-429.
21. Brossmer R, Wagner M, Fischer E. Specificity of the sialic acid-binding lectin from the snail Cepaea hortensis. The Journal of Biological Chemistry. 1992; 267:8752-8756.
22. Swarnakar S, Chowdhury PS, Sarkar M. N-glycolylneuraminic acid specific lectin from Pila globosa snail. Biochemical and Biophysical Research Communications. 991; 178:85-94.
23. Ng TB, Wong CM, Li WW, Yeung HW. Horseshoe crab (Limulus polyphemus) lectin but not garden snail (Helix pomatia) lectin elicits insulin-like activities in vitro. Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology. 1985; 81:503-504.
24. Bretting H, Stanislawski E, Jacobs G, Becker W. Isolation and characterization of a lectin from the snail Biomphalaria glabrata and a study of its combining site. Biochimica et Biophysica Acta (BBA). 1983; 749:143-52.
25. Olofsson S, Norrild B, Andersen AB, Pereira L, Jeansson S, Lycke E. Populations of herpes simplex virus glycoprotein gC with and without affinity for the N-acetyl-galactosamine specific lectin of Helix pomatia. Archives of Virology. 1983; 76:25-38.
26. Hardy SW, Grant PT, Fletcher TC. A haemagglutinin in the tissue fluid of the Pacific oyster, Crassostrea gigas, with specificity for sialic acid residues in glycoproteins. Experientia. 1977; 33:767-769.
27. Tamplin ML, Fisher WS. Occurrence and characteristics of agglutination of Vibrio cholerae by serum from the eastern oyster, Crassostrea virginica. Applied and Environmental Microbiology. 1989; 55:2882-2887.
28. Hardy SW, Fletcher TC, Gerrie LM. Factors in haemolymph of the mussel, *Mytilus edulis* L., of possible significance as defence mechanisms. Biochemical Society Transactions. 1976; 4:473-475.
29. Knibbs RN, Osborne SE, Glick GD, Goldstein IJ. Binding determinants of the sialic acid-specific lectin from the slug Limax flavus. The Journal of Biological Chemistry. 1993; 268:18524-18531.
30. Gerlach D, Schlott B, Schmidt KH. Cloning and expression of a sialic acid-binding lectin from the snail *Cepaea hortensis* FEMS Immunology & Medical Microbiology. 2004; 40:215-221.
31. Ghosh S, Bandyopadhyay S, Mukherjee K, Mallick A, Pal S, Mandal C, *et al*. O-acetylation of sialic acids is required for the survival of lymphoblasts in childhood acute lymphoblastic leukemia (ALL).Glycoconjugate Journal. 2007; 24:17-24.
32. Ghosh S, Bandyopadhyay S, Mallick A, Pal S, Vlasak R, Bhattacharya DK, *et al*. Interferon gamma promotes survival of lymphoblasts overexpressing 9-O-acetylated sialoglycoconjugates in childhood acute lymphoblastic leukaemia (ALL). Journal of Cellular Biochemistry. 2005; 95:206-16.
33. Ghosh S, Bandyopadhyay S, Pal S, Das B, Bhattacharya DK, Mandal C. Increased interferon gamma production by peripheral blood mononuclear cells in response to stimulation of overexpressed disease-specific 9-O-acetylated sialoglycoconjugates in children suffering from acute lymphoblastic leukaemia. British Journal of Haematology. 2005; 128:35-41.
34. Ghosh S, Bandyopadhyay S, Bhattacharya DK, Mandal C. Altered erythrocyte membrane characteristics during anemia in childhood acute lymphoblastic leukemia. Annals of Hematology. 2005; 84:76-84.
35. Pal S, Ghosh S, Bandyopadhyay S, Mandal C, Bandhyopadhyay S, Kumar Bhattacharya D, *et al*. Differential expression of 9-O-acetylated sialoglycoconjugates on leukemic blasts: a potential tool for long-term monitoring of children with acute lymphoblastic leukemia. International Journal of Cancer. 2004; 111:270-277.
36. Pal S, Ghosh S, Mandal C, Kohla G, Brossmer R, Isecke R, *et al*. Purification and characterization of 9-O-acetylated sialoglycoproteins from leukemic cells and their potential as immunological tool for monitoring childhood acute lymphoblastic leukemia. Glycobiology. 2004; 14:859-70.
37. Varki A. Loss of N-Glycolylneuraminic Acid in Humans: Mechanisms, Consequences, and Implications for Hominid Evolution. Yearbook of Physical Anthropology. 2001; 44:54-69.
38. Halanych, K.M., Bacheller, J., Liva, S., Aguinaldo, A. A., Hillis, D.M. and Lake, J.A. 18S rDNA evidence that

the Lophophorates are Protostome
Animal. Science.1995; 267: 1641–1643