

RESEARCH REPORT

Physico-chemical characterization of bacterial and hemagglutinins from the serum of the mud crab *Scylla serrata*SS Jayaraj¹, R Thiagarajan², M Arumugam², S Vincent¹¹Unit of Environmental Health and Biotechnology, Department of Advanced Zoology and Biotechnology, Loyola College, Chennai 600 034, India²Unit of Pathobiology, Department of Zoology, University of Madras, Guindy Campus, Chennai 600 025, India

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Abstract

A naturally occurring hemagglutinin (HA), with activity against bacteria and yeast cells were detected in the serum of *Scylla serrata* using mammalian erythrocytes (RBC), various bacteria and yeast as indicator cells. The serum gave highest HA titer with rabbit RBC, tripsinized yeast and *Vibrio fluvialis*. An analysis of the physico-chemical properties of the HA showed it to be specifically dependent on the presence of Ca²⁺ for its activity, stable between pH 7 to 9 and showed thermal stability between 10 to 30 °C. Further studies demonstrated that the HA is precipitable by ammonium sulphate and TCA. HA- inhibition assays performed with carbohydrates revealed that the serum HA was specific for non-reducing terminal glucose with 1-2 glucosidic linkages. Thus this agglutinin appears to be unique among all the known crustacean agglutinins.

Key Words: hemagglutinin; bacterial agglutinin; yeast agglutinin; serum; mud crab; *Scylla serrata***Introduction**

Mud crab *Scylla serrata* is an economically important decapod crustacean in aquaculture in India. Understanding the defence system of *S. serrata* at humoral and cellular levels is indispensable for preventing disease occurrence. Lectins are carbohydrate-binding proteins and in invertebrates, lectins are vital means for non-self recognition and clearance of invading microorganisms. In invertebrates, phagocytosis is considered to be the primary mechanism of innate defense against foreign invaders (Ratcliffe and Rowley, 1981; Coombe *et al.*, 1984; Ratcliffe *et al.*, 1985). In this process, an intimate interaction of humoral substances, particularly as recognition factors, has been implicated (Richards and Renwranz, 1991; Zelck and Becker, 1992). A variety of humoral factors, naturally occurring and/or formed after antigenic stimulation, have been detected in the serum of invertebrates and they include agglutinins (Cornick and Stewart, 1973; Renwranz, 1986; Nalini *et al.*, 1994; Murali *et al.*, 1999; Jayasree, 2001; Jayaraj *et al.*, 2008a), lysins (Osada *et al.*, 1993), antibacterial (Xylander and Neverman, 1990), and antifungal proteins (Iijima *et al.*, 1993), phenoloxidase system (Söderhäll, 1982), LPS binding protein (Jomori and Natori, 1992) and β-

1, 3 glucan binding protein (Jayaraj *et al.*, 2008b). Due to the probable functional similarities between agglutinins and vertebrate antibodies and the indications that agglutinins serve a defensive function (Ofek and Sharon, 1988), invertebrate agglutinins have been extensively studied.

Agglutinins (= lectins) are di- or multivalent carbohydrate-binding proteins with the ability to agglutinate cells with complementary carbohydrates on their surfaces (Sharon and Lis, 1972; Barondes, 1988). They are known to specifically recognize the whole sugar (Bretting and Kabat, 1976), a specific site in a sugar (Shimizu *et al.*, 1977), a sequence of sugars (Kobiler and Mirelman, 1980), or their glycosidic linkages (Koch *et al.*, 1982). The agglutinating molecules are widely distributed in microorganisms (Sasmal *et al.*, 1992), plants and animals (Gold and Balding, 1975).

The body fluid or hemolymph of almost all invertebrate species tested contains agglutinins (Yeaton, 1981; Ratcliffe *et al.*, 1985; Renwranz, 1986; Nalini *et al.*, 1994; Murali *et al.*, 1999; Jayasree, 2001; Jayaraj *et al.*, 2008a). The presence of agglutinins has also been detected in the mucus as well as in certain tissues of invertebrates (Renwranz, 1986; Mullainadhan and Renwranz, 1989; Suzuki and Mori, 1991). However, its immunological role is best understood in the hemolymph, and recent studies have shown that purified, hemolymph-derived agglutinins served as opsonin in a few insects and molluscs (Renwranz and Stahmer, 1983; Pendland

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et al., 1988; Fryer *et al.*, 1989; Richards and Renwartz, 1991; Jayaraj *et al.*, unpublished observations). Although a number of studies have demonstrated the presence of humoral agglutinins in several crustacean species, it can be noted that the immunological roles of these agglutinins remain largely unknown and that the carbohydrate specificity of serum agglutinins from crustaceans have been elucidated only in a few species (Vasta *et al.*, 1983; Smith and Chisholm, 1992; Nalini *et al.*, 1994; Kondo *et al.*, 1998; Jayasree, 2001). This study thus describes RBC and bacterial binding activities, physico-chemical properties and carbohydrate specificity of a naturally occurring agglutinin in the serum of the marine crab *S. serrata*.

Material and Methods

Experimental animals and laboratory maintenance

The marine crab *Scylla serrata* weighing 150 to 200 g were obtained from Muttukadu estuary, Chennai. In the laboratory, these crabs were maintained in plastic tanks (90 x 45 x 60 cm) containing aerated natural seawater and the medium was changed every day. The crabs were fed with *Donax spp.* during the period of acclimation (24 h) and only male crabs were used.

Preparation of serum

Hemolymph samples (1 to 2 ml) from individual crabs were collected from the cut end of the dactylus region of the walking leg. The samples were collected in clean polystyrene plastic tubes held on ice and allowed to clot at room temperature (RT: 28 ± 2 °C for 20 min). Serum was separated by centrifugation (400xg, 10 min, RT) and the resulting clear supernatant (=serum) was used immediately.

Preparation of erythrocyte (RBC) suspension

Human and other mammalian blood samples were obtained by venous or cardiac puncture and collected in sterile Alsever's solution (Garvey *et al.*, 1979) containing 10 µg/ml of streptomycin. Prior to use, the RBCs were washed thrice with 0.9% saline and once with TBS-I (50 mM tris-HCl, 115 mM NaCl, 10 mM CaCl₂, 300 mOsm) by centrifugation (400xg, 5 min, RT). Unless specified, the RBC pellet was finally resuspended in TBS-I as 1.5% suspension (v/v).

Preparation of yeast cell suspension

A hundred mg commercial grade baker's yeast (*Saccharomyces cerevisiae*) purchased from local market were suspended in 10 ml of 0.9 % saline, washed extensively with saline by centrifugation (400xg, 5 min, RT) and suspended in the same medium. The yeast cell suspension was heat-inactivated by autoclaving the suspension for 15 min at 15 psi. After cooling the suspension to RT, the heat-inactivated yeast cells were washed extensively with 0.9 % saline and finally resuspended in TBS-I as 0.5 % (v/v) suspension.

Trypsinization of yeast cells

Five µl of washed yeast cells were suspended in 1 ml of TBS-I containing trypsin (0.5 %) to give a

final concentration of 0.5 % yeast. This suspension was incubated for 1 h at 37 °C with occasional gentle shaking. After incubation, the trypsinized yeast cells were washed once with TBS-I by centrifugation (400xg, 5 min, RT) and finally resuspended in TBS-I as 0.5 % (v/v) suspension.

Hemagglutination (HA) assay

HA assays were performed in V-bottom microtiter plates (Greiner, Nürtingen, Germany) by serial two-fold dilution of a 25 µl serum sample with an equal volume of TBS-I. After dilution, 25 µl RBC suspension was added to each well and incubated for 45 min at RT. The HA titers were recorded as the reciprocal of the highest dilution of the sample causing complete agglutination of RBC (Garvey *et al.*, 1979). Controls for all assays consisted of the substitution of the sample by TBS-I. All the HA assays were performed in duplicate.

Yeast agglutination assay

The agglutinating activity of serum against yeast cells was performed in V-bottom microtiter plates by serial two-fold dilution of 25 µl serum with an equal volume of TBS-I. After dilution, 25 µl of 0.5 % native or trypsinized yeast cell suspension was added to each well and incubated for 45 min at 26 °C. Control consisted of substitution of serum with TBS-I. The agglutination of yeast cells by serum was assessed under microscope (40 x) and the agglutination titers were recorded as the reciprocal of the highest dilution of the sample causing complete agglutination. The experiment was performed using duplicates.

Bacterial agglutinating activity

Frozen stock culture of bacteria were inoculated in TBS-I and incubated for 6 h. The broth cultures were then centrifuged (5,000xg, 10 min). The pellet was collected and washed 3 times by centrifugation with TBS-I. The final concentration was adjusted to 1×10^8 cells ml⁻¹ in TBS-I before use. Two-fold serial dilutions of serum samples (in duplicates) were made in TBS-I. Then, 25 µl of each serum dilution was incubated with 25 µl bacterial suspension. The reaction mixture was incubated at 20 ± 2 °C for 1 h. The appearance of clumps of bacteria was then recorded by microscopic examination (40x). Agglutination titer was defined as the reciprocal of the last dilution giving evidence of agglutination after incubation. The negative controls comprised mixed equal volumes of bacterial suspension and TBS-I.

Divalent cation dependency and EDTA sensitivity

Serum samples (each 300 µl in duplicates) were dialysed (MW exclusion limit <10,000) extensively at 20 °C against divalent cation-free TBS-II (50 mM tris-HCl, 135 mM NaCl, 300 mOsm) to examine cation dependency or in TBS-III containing 50 mM EDTA (50 mM tris-HCl, 72 mM NaCl, 40 mM CaCl₂, 300 mOsm) to test EDTA sensitivity of the agglutinating activity of serum. The samples dialysed against TBS-III were subsequently re-equilibrated by dialysis in TBS-II. All the resulting dialysates were centrifuged (400xg, 10 min, 20 °C). The supernatant was tested for hemagglutinating activity using rabbit RBC in the presence of TBS

that did or did not contain 10 mM CaCl₂, MgCl₂ (or) MnCl₂. A serum sample (300 µl) concurrently dialysed against, TBS containing 10 mM CaCl₂ (TBS-I) was also tested for the hemagglutinating activity against rabbit RBC in TBS-I.

pH and thermal stability

The stability of serum HA activity (in duplicates) at different pH was examined by dialyzing (24 h, 4 °C) 300 µl serum samples against the following buffers at pH ranging from 3 to 12 (Lillie, 1954; Pearse, 1968): 0.2 M acetate buffer (pH 3 to 6), 0.2 M tris-HCl buffer (pH 7 to 9) and 0.1 M glycine-NaOH buffer (pH 10 to 12). After dialysis, all the samples were finally re-equilibrated by dialysis against TBS-I and the HA titer was determined with rabbit RBC. In another experiment designed to study the thermal stability of HA, 300 µl serum samples were held for 30 min at temperatures ranging from 10 to 100 °C, centrifuged and tested for HA activity with rabbit RBC.

Precipitation by ammonium sulphate and trichloro acetic acid

Precipitation of HA activity from serum (in duplicates) was attempted using 25, 50 and 75 % ammonium sulphate [(NH₄)₂SO₄] solution as well as 10 % trichloroacetic acid (TCA) as described previously (Millar, 1987). The HA activity was finally measured using rabbit RBC.

HA-inhibition assays

Several carbohydrates were tested for their ability to inhibit serum HA activity. They were dissolved in TBS-III (50 mM tris-HCl, 115 mM NaCl, 50 mM EDTA, 300 mOsm) and if necessary, the pH was adjusted to 7.5 using concentrated NaOH. Serum samples were diluted with TBS-IV (50 mM tris-HCl, 5 mM NaCl, 30 mM CaCl₂, 135 mOsm) to a HA titer of 4 against rabbit RBC. The inhibitor to be tested (25 µl) was serially diluted two-fold with an equal volume of diluted sample in microtiter plates and incubated for 1 h at RT. Rabbit RBC suspension (25 µl) was added to each well and kept for 3 h at RT. The minimal concentration of carbohydrate that completely inhibited HA activity was recorded. The experiments were performed in duplicates.

Protein determination

Total protein concentration was measured using bovine serum albumin (BSA) as a standard (Lowry *et al.*, 1951).

Result

Serum HA profile

The serum of mud crab *Scylla serrata* agglutinated a variety of mammalian RBC types. Among the various RBC types tested, the highest titer of 128 was obtained with rabbit erythrocytes. The serum did not discriminate human A, B and O RBC types and agglutinated them to the same degree. Sheep and goat RBC were agglutinated at relatively low titers (Table 1). However serum did not agglutinate ox RBC and the serum showed highest agglutinating activity against trypsinized yeast cells when compare to native yeast cells (Table 2).

Table 1 Hemagglutinating (HA) activity of serum from the mud crab *S. serrata* against various mammalian erythrocyte (RBC) types

RBC types tested	HA titer*
Rabbit	128
Mice	64
Rat	32
Human B	32
Buffalo	16
Human A	8
Human O	8
Horse	4
Goat	2
Sheep	2
Ox	0

* Based on 20 determinations for each RBC type

Bacterial agglutination

The serum strongly agglutinated *Vibrio fluvialis* (titer 8), weakly agglutinated *Vibrio parahaemolyticus*, *Vibrio mimicus*, *Escherichia coli*, *Pseudomonas spp.* and *Aerobacter aerogenes*. The results of bacterial agglutination was assessed using a phase-contrast microscope (Table 3).

Divalent cation dependency an EDTA sensitivity

The serum tested in TBS containing 10 mM CaCl₂ (TBS-I) gave a hemagglutination titer of 128 against rabbit RBC. When the serum was dialysed against TBS- I and then tested in the absence of divalent cation, the agglutination titer reduced to 16. But, this serum sample recovered it's HA activity only upon addition of Ca²⁺ to the reaction mixture. Further, substitution of Ca²⁺ with Mg²⁺ showed a considerable improvement in HA titer, while Mn²⁺ could not reverse the effect of EDTA treatment. The serum dialyzed against TBS-III containing 50 mM EDTA and tested in the absence of divalent cation, considerably lost its agglutinating activity against rabbit RBC (Table 4). Further the addition of Mg²⁺ or

Mn²⁺ to this sample could not restore the original HA activity and addition of Ca²⁺, rescued the activity to 64 (Table 4).

pH and thermal stability

The serum hemagglutinating activity of the marine crab *S. serrata* was tested in the pH range of 3-12. As shown in Figure 1, the hemagglutinating activity against rabbit RBC was found to be relatively stable between pH 7 and 9 reduced at pH below or above this pH range and completely lost at pH 11 and 12. The activity of the serum against rabbit RBC was unaffected only up to 30 °C but it was reduced considerably at 40 °C and 50 °C and completely inactivated at 60 °C and above (Fig. 2).

Precipitation of serum HA activity by ammonium sulphate and TCA

Serum was incubated with ammonium sulphate (25, 50 and 75 %) for 3 h. The 50 % concentration of ammonium sulphate completely precipitated HA activity from the serum, whereas ammonium sulphate concentration at 25 and 75 %, moderately precipitated the serum HA activity. By contrast 10 % TCA failed to precipitate serum HA activity (Table 5).

Carbohydrate binding specificity

Among the 24 carbohydrates tested, as many as 15 carbohydrates were found to inhibit serum hemagglutinating activity at concentrations ranging from 50 to 100 mM. All the three acetylated hexosamines (GlcNAc, GalNAc and ManNAc), but not their hexoses and hexosamine counterparts, were inhibitory at 50 or 100 mM. But the few sialic acids examined in this study and 9 other carbohydrates were not inhibitory when tested up to concentrations from 20 to 200 Mm (Table 6). Among the six different polysaccharides tested (Table7), only mannan and laminarin inhibited the HA activity at 0.25 and 0.50 mg/ml, respectively. Among all the inhibitory carbohydrates, mannan was found to be most potent.

Table 2 Agglutinating activity of *S. serrata* serum against native and trypsinized yeast cells

Yeast cells tested	HA titer*
Native	16
Trypsinized	64

* Based on 20 determinations for native and trypsinized yeast cells

Discussion

The serum of the marine mud crab *S. serrata* was found to possess naturally occurring agglutinating activity which showed the highest reactivity with rabbit RBC among the RBC types tested. These results also suggest that the RBC types agglutinated by the serum of *S. serrata* probably share a common surface receptor but with a quantitative difference in its HA binding sites. The serum agglutinated a variety of bacteria including Gram + and Gram- types and the species of *Vibrio* tested are known to be the most frequent opportunistic pathogens of aquatic crustaceans (Equidius, 1987; Vargas-Albores *et al.*, 1993) and the plasma showed highest agglutinating activity against trypsinized yeast cells (Jayaraj *et al.*, 2008b). The ability of the serum of *S. serrata* to agglutinate bacteria, particularly the potential pathogens, implicates a possible involvement of the humoral agglutinins in host defense response.

Table 3 Agglutinating activity of *S. serrata* serum against various bacterial species

Bacterial species tested	Bacterial agglutination* (O.D: 0.8)
<i>Vibrio fluvialis</i>	16
<i>Vibrio alginolyticus</i>	8
<i>Vibrio vulnificus</i>	8
<i>Vibrio anguillarum</i>	4
<i>Vibrio parahaemolyticus</i>	4
<i>Vibrio mimicus</i>	2
<i>Escherichia coli</i>	2
<i>Pseudomonas sp</i>	2
<i>Bacillus subtilis</i>	4
<i>Aerobacter aerogenes</i>	2

* The assay was repeated six times for each bacterial species with identical results using samples from different preparations

Table 4 Effect of divalent cations and EDTA on the hemagglutinating (HA) activity of serum of *S. serrata*

Serum sample tested	Cation (10 mM) in sample diluting and RBC suspension	HA titer*
Before dialysis	CaCl ₂	128
After dialysis against divalent cation free TBS (TBS-II).	None CaCl ₂ MgCl ₂ MnCl ₂	16 128 64 16
After dialysis against TBS+10 mM CaCl ₂ (TBS-I)	CaCl ₂	128
After dialysis against TBS+50 mM EDTA (TBS-III) followed by dialysis against TBS-II	None CaCl ₂ MgCl ₂ MnCl ₂	8 64 4 4

* Determination using rabbit RBC and the results based on six determinations

The serum agglutinin was heat-labile and susceptible to pH extremes. Its pH stability was comparable to that found in one earlier report (Nalini *et al.*, 1994) and the proteinaceous nature of agglutinin has been well demonstrated (Mckay and Jenkin, 1969; Acton *et al.*, 1969; Miller *et al.*, 1972).

The serum lost most of its HA activity after dialysis against cation-free TBS and when tested in the absence of cations. However, the activity in this sample completely regained only upon addition of Ca²⁺ and the HA titer of serum did not change after dialysis against TBS containing Ca²⁺. These observations demonstrated that the serum agglutinin of *S. serrata* specifically requires Ca²⁺ for its HA activity. Furthermore, the activity was sensitive to EDTA treatment, since dialysis of serum against TBS containing EDTA resulted in a significant reduction in the HA activity. None of the cations tested could restore the HA activity, albeit Ca²⁺ moderately rescued the activity in these samples, thereby indicating that the HA of *S. serrata* appears to be irreversibly sensitive to EDTA which is in contrast with other crustacean agglutinins (Hall and Rowlands, 1974a; Ravindranath *et al.*, 1985; Kamiya *et al.*, 1987).

Crustacean serum agglutinins were shown to be specific for fucose (Amirante and Basso, 1984), glucose (Umetsu *et al.*, 1991), galactose (Kamiya *et al.*, 1987; Umetsu *et al.*, 1991), GalNAc (Amirante and Basso, 1984; Vargas-Albores *et al.*, 1993), or sialic acids such as NeuAc (Hall and Rowlands, 1974b; Vasta *et al.*, 1983; Vasta and Cohen, 1984; Cassels *et al.*, 1986; Ratanapo *et al.*, 1990), 4- and 9-0-acetyl NeuAc (Ravindranath *et al.*, 1985), 9-0-

acetyl NeuAc (Vazquez *et al.*, 1993) and NeuGc (Mercy and Ravindranath, 1993). The hemagglutination-inhibition test performed in this study using different carbohydrates, encompassing several diverse, unrelated monosaccharides and their derivatives as well as di- and oligo-saccharides inhibited the serum agglutinating activity. Furthermore, all the three acetylated hexosamines tested consistently inhibited the HA activities of crab

Table 5 Ammonium sulphate and TCA precipitation of haemagglutination (HA) activity against rabbit RBC from the serum of *S. serrata*

Ammonium sulphate and TCA (%) Saturation	HA titer* (Rabbit RBC)
Untreated Sample	128
25 %	16
50 %	128
75 %	32
TCA 10 %	0

* Determination using rabbit RBC and the results based on six determinations

Table 6 Inhibition of agglutinating activity (titer = 4) of serum from the mud crab *S. serrata* by various carbohydrates

Carbohydrates tested	Maximum concentration tested (mM)	Minimum inhibitory concentration (mM)*
Monosaccharides		
Simple sugars		
D-mannose	200	100
L-sorbose	100	100
D-fucose	100	100
L-fucose	100	50
Deoxy sugars		
L-rhamnose	200	100
N-acetyl sugars		
N-acetyl-D-glucosamine (GlcNAc)	200	100
N-acetyl-D-galactosamine (GalNAc)	200	100
N-acetyl-D-mannosamine (ManNAc)	200	50
Disaccharides		
Trehalose (glc α 1 \rightarrow 1 glc)	200	50
Cellobiose (glc β 1 \rightarrow 4 glc)	200	100
β -gentiobiose (glc β 1 \rightarrow 6 glc)	200	50
Sucrose	200	100
Palatinose (glc α 1 \rightarrow 6 fruc)	200	50
Melibiose (gal α 1 \rightarrow 6 glc)	200	100
Lactose (gal β 1 \rightarrow 4 glc)	200	100

The following carbohydrates also did not inhibit the agglutinating activity and unless otherwise stated, all carbohydrate was tested at concentrations upto 200 mM: D-glucose, D-galactose, β -allose, D-fructose, D-glucosamine (GlcN), D-galactosamine (GalN), mannosamine (ManN), maltose (glc α 1 \rightarrow 4 glc), turanose (glc α 1 \rightarrow 3 fruc).

* The assay was repeated five times for each carbohydrate with identical results

Table 7 Agglutination–inhibition of *S. serrata* serum (agglutination titer = 4) by polysaccharides against rabbit RBC

Polysaccharides tested	Structural linkages	Maximum concentration tested (mg. ml ⁻¹)	Minimum inhibitory concentration (mg. ml ⁻¹)*
Mannan	(α 1-6 homopolymer of mannose)	1	0.25
Laminarin	(β 1-3 homopolymer of glucose)	1	0.50
Dextran T ₇₀	(α 1-6,3,2 homopolymer of glucose)	2	NI
Dextran T ₅₀₀	(α 1-6,3,2 homopolymer of glucose)	2	NI
Inulin	(α 2-6 homopolymer of fructose)	5	NI
Colominic acid	(α 2-8 homopolymer of Neu5Ac)	5	NI

* The assay was repeated three times for each polysaccharide with identical results using samples from different preparations

NI: No inhibition

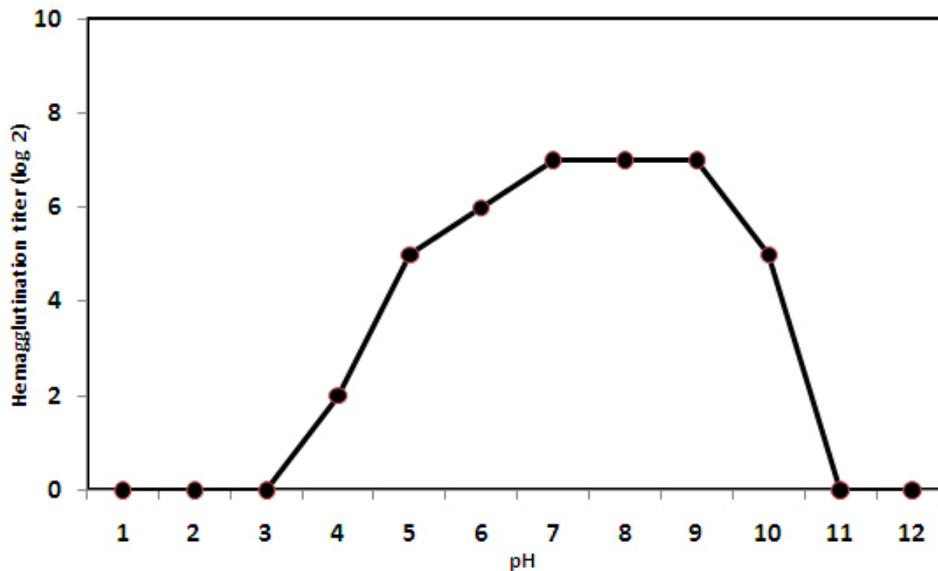


Fig. 1 pH stability of hemagglutinating activity of serum of *S. serrata* against rabbit RBC

serum and this has been earlier demonstrated (Amirante and Basso, 1984; Vargas-Albores *et al.*, 1993; Mercy and Ravindranath, 1993). The serum HA activity of *S. serrata* was not inhibited by the amino sugar tested. But it was inhibited by the simple hexoses namely mannose, L-sorbose, D-fucose and L-fucose. This finding is supported by previous studies wherein simple hexoses were shown to inhibit agglutinating activity, including fucose (Amirante and Basso, 1984), glucose (Umetsu *et al.*, 1991). The C-1 position of these

hexoses is essential for interaction with the agglutinin. The amino derivatives (GlcN, GalN and ManN) did not inhibit the HA activity. However, their N-acetyl derivatives (GlcNAc, GalNAc and ManNAc) were able to inhibit the serum HA activity. Recently, Alpuche *et al.* (2005) have shown the inhibition of purified shrimp lectin by N-acetylated sugars and this lends support to our study. The disaccharides D-maltose and turanose failed to inhibit the HA activity but all other disaccharides were inhibitory.

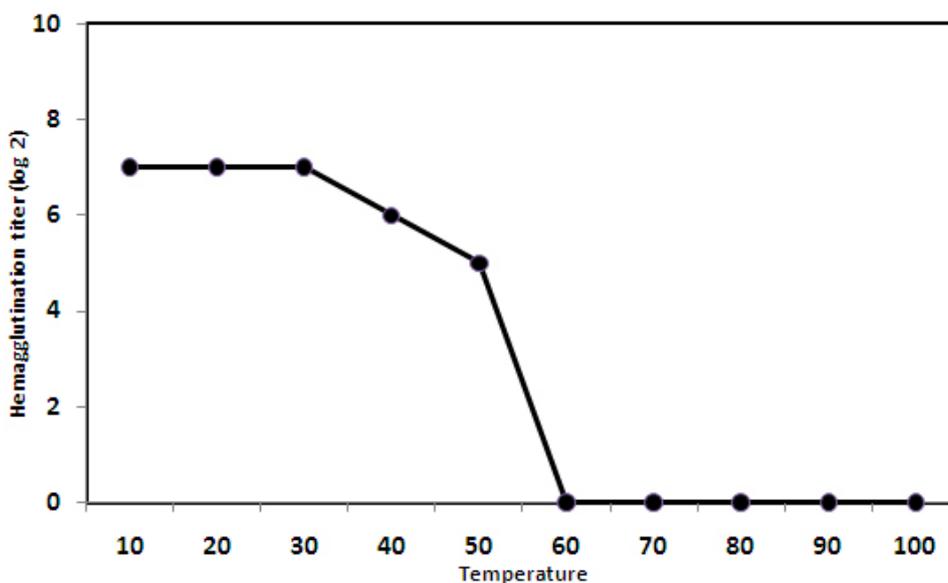


Fig. 2 Thermal stability of hemagglutinating activity of serum of *S. serrata* against rabbit RBC

All these observations clearly demonstrate that the presence of acetyl group at C-2 position of hexosamines does not favour the interaction with agglutinin whereas this position with a free hydroxyl group or its substitution with amino group is essential for the interaction. HA inhibition tests employing polysaccharides indicated that only laminarin and mannan inhibited the serum agglutinating activity. This indicates that the agglutinin molecules in crab serum tend to exhibit affinity for extended structures particularly for polysaccharides with β -linked hexoses. This is significant and shows the ability of the serum agglutinating activity to recognize a wide range of carbohydrates that will potentially help the animal to recognize a variety of pathogens based on their surface molecules (Zhang *et al.*, 2009). Thus, all the results obtained from the inhibitory effects of various carbohydrates and glycoproteins taken together clearly indicate that the agglutinins present in the serum of *S. serrata* interact with a wide range of carbohydrates including acetylated hexosamines, acetylated or non-acetylated sialic acids and several other carbohydrates, though their preference for a specific carbohydrate structure could not be ascertained. However, these findings in turn strongly suggest the natural occurrence of multiple agglutinins in the serum of this crab. The agglutinins in several crustaceans have been characterized (Cornick and Stewart, 1968; McKay and Jenkin, 1969, 1970; Huang *et al.*, 1981; Vasta *et al.*, 1983; Ratanapo *et al.*, 1990; Adams, 1991; Nalini *et al.*, 1994; Jayasree, 2001). This agglutinin appears to be unique among all the known crustacean agglutinins. Thus, based on the hemagglutination and bacterial agglutinating activity of the serum agglutinin, it is possible that this component of the crab is probably involved in non-self recognition and eliciting immune response in the mud crab *S. serrata*, against invading pathogens. The identification of immune effectors like agglutinin and the understanding of their regulation in response to infection will open the way to the selection of pathogen resistant animals. This can be achieved through the characterization and purification of this novel agglutinin of *S. serrata* as a prerequisite to elucidate the immunological roles of crustacean agglutinins.

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