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# Natural agglutinin from crab, *Trichopeltarion nobile*

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Haemolymph of *Trichopeltarion nobile* was strong with agglutinated rabbit erythrocytes. The specificity of this agglutinin was studied by heamagglutination inhibition with N-acetyl neuraminic acid. The optimum temperature for the haemagglutination activity for the haemolymph of *Trichopeltarion nobile* was stable between 30°C and 40°C and the optimum pH was 7 to 8. The agglutinin required calcium ions for its activity. A fraction containing the agglutinin was isolated by gel filtration chromatography Sepadex$G_{75}$.The corresponding protein molecular weight of 96kDa.

*Trichopeltarion nobile* /Biochemistry

**L**ectins are protein or glycoproteins of nonimmune origin derived from plants, animals or micro-organisms. Lectins will specifically recognize and bind to carbohydrate structures on the surface of cells, on cytoplasmic and nuclear structures, and in extracellular matrix in cells and tissues in throughout the animal and plant kingdoms. Lectins are a group of sugar-binding proteins that recognize specific carbohydrate structures and agglutinate various cells by binding to cell surface glycoconjugates. Animals produce a variety of lectins, many of which have been implicated in cell recognition phenomena. One of the role of marine invertebrate lectin is to act as humoral factors in defence mechanism, as immunoglobulins in invertebrates. This is suggested from some observation such as activation of phagocytes by the binding the lectin to foreign cells (opsonin activity) or enhancement of lectin production in body fluids after injection of foreign substances (Hatakeyama et al.1995). In 1987, Smith detected a protein variation in human serum by externally released precipitins from the blue crab, *Callinectes sapidus.* N-glycolyl sialic acid binding lectin was isolated from the haemolymph of marine crab, *Scylla serrata* (Mercy et al.1992). Murali et al. (1994) isolated a lipopolysaccaharide - binding hemagglutinin with specificity for acetylated amino sugars in the serum of the hermit crab, *Diogenes affinis*. This agglutinin involved in the immune response of crustaceans against bacterial infections. Murali et al. (1999) purified a natural agglutinin from the serum of the hermit crab, *Diogenes affinis* through a single - step affinity chromatography using N-acetylglucosamine - coupled Sepharose 6B. In this work, we performed natural agglutinin from, *Trichopeltarion nobile*.

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Biochemistry

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## Materials and Methods

### Collection of experimental animal

The sample *Trichopeltarion nobile* was collected from Chinnamuttom coast, Kanyakumari District, Tamil Nadu,Tamil nadu. The live collected animals was maintained in an aquariam (24-4$^0$C, salinity 36%) for the collection of haemolymph.

### Preparation of sample

*Trichopeltarion nobile* haemolymph was obtained from the joints of leg, using a syringe with Tris buffer saline. Then it was centrifuged at 4000rpm for 10min. Then the supernated was frozen (-20$^0$C) until use.

### Preparation of 1.5% Erythrocyte suspension

The human and animal blood samples were collected with Alsever's medium.Then the freshly collected $A^+$, $B^+$ and $O^+$ human, rabbit, cow and chicken erythrocytes were washed twice by centrifuged at 4000rpm for 10mins with Tris buffer saline. Finally 1.5% erythrocytes were suspended into Tri buffer saline.

### Haemagglutination assay

Haemagglutination assay was carried out as described by Ravindranath and Paulson, (1987).The heamagglutination assay performed on U- plates. Breifly 25µl of Tris buffer saline was taken in microtitre well plates. 25µl of sample was serially diluted in Tris buffer saline . Then 25µl of 1.5% erythrocyte was added to each well and mixed well. Then the plates were incubated at room temperature for 1hours. The agglutination titre was recorded as the reciprocal of the last dilution, giving evidence of agglutination at 1hour of incubation.

### Haemagglutination inhibition assay

The carbohydrate specificity of the lectin was investigated by competitive inhibition using the following sugars. Sorbital, inositol, melibiose, maltose, fructose, glucosamine, rhamnose, dextrose, arabinose, trehalose, rhaffinose, cellobiose, xylose, sucrose, salicin, glucose, falactose, N-acetyl neuraminic acid, D (+) galactosamine. In each well 25µl of diluted sample was added. Then 25µl of inhibitors serially diluted in diluted sample added wells. Then the plates were incubated at room temperature. After incubation period 25µl of erythrocyte was added then it was again incubated for 1hour at room temperature.

### Characterization of agglutinin

Effect of temperature on the sample was first incubated in water bath for 30min at 30$^0$C to 70$^0$C. Heamagglutination activity assay was then performed for each sample. Effect of pH on heamagglutination activitywas also determined by using the following buffers.Glycin pH (2to3),acetate buffer P$^H$(4-5),Tris buffer saline pH (7-10).

### Purification of Agglutinin

The protein content was concentrated with different concentration of ammonium sulphate. Seventy percentage of ammonium sulphate precipitated the agglutinin. The precipitated sample was dialyzed against Tris buffer saline.The dialyzed sample purified by gel filtration chromatography Sepadex $G_{75}$ and equilibrated with Tris buffer saline at a flow rate of 0.5m/min; 2ml fraction were collected. The optical density of each collected fraction was monitored at 280nm, and molecular masses of the protein were determined by Lammili, (1970).

### Results and Discussion

The haemolymph of many invertebrates is known to agglutinate foreign erythrocytes. The haemolymph of the *Trichopeltarion nobile* showed high haemagglutination activity to rabbit erythrocytes. Similarly, serum of the fresh water crab, *Parathelphusa jacquemontii* had a strong affinity for horse and rabbit erythrocytes (Denis et al.2003). Serum of the fresh water crab, *Parathelphusa hydrodromus* gave the highest heamagglutination activity titre with rabbit erythrocytes (Nalini,1994). However, the purified serum agglutinin from the hermit crab, *Diogenes affowed* a strong affinity for rat erythrocytes (Murali et al.1999). Two lectins isolated from *Megabalanus volcano* agglutinated human erythrocytes and also rabbit and sheep blood cells (Kamiya et al.1987). Denis et al. (2003) isolated and purified a lectin from the haemolymph of freshwater crab, *Parthelphusa jacquemontii*. N-acetyl-neuraminic acid specific. The marine crab, *Trichopeltarion nobile* was only inhibite N-acetyl-neuraminic acid (Table-1). Haemagglutinin from the amoebocytes of the horseshoe crab, *Carcinoscorpius rotundicauda* was inhibited by coagulagen, but not by sugars (Srimal et al.1985). Nowak et al. (1975) purified an agglutinin from the haemoltymph of

*Limulus polyphemus*. It′s agglutination was inhibited not only by N-acetyl neuraminic acid but also by D-glucuruonic acid.

Table-1: Inhibition of heamagglutination activity of *Trichopeltarion nobile*

| Carbohydrates | Minimum Concentration for inhibition of heamagglutination |
|---|---|
| Glucose | NI |
| Arabinose | NI |
| *Salicin* | NI |
| Fructose | NI |
| Maltose | NI |
| Galactose | NI |
| Rhamnose | NI |
| Raffinose | NI |
| *cellobiose* | NI |
| Sucrose | NI |
| *Xylose* | NI |
| Glucosamine | NI |
| N-acety1 neuraminic Acid | 100mM |
| Trehalose | NI |
| *Inositol* | NI |

NI : No Inhibition

The haemagglutination activity of *Trichopeltarion nobile* was sensitive to temperature and pH. In the present study, the optimum temperature for the haemolymph of *Trichopeltarion nobile* was 30°C to 40°C and optimum pH was 7 to 8 for heamagglutination assay. Similarly, the heamagglutinating activity of humoral defence factors of Indian river prawn, *Macrobrachium malcolmsonii* was stable over a wide range of temperatures from 30° C to 60°C and pH 3.0-7.0. A natural agglutinin from the haemolymph of the prawn, *Penaeus indicus* was purified and characterized by Jayasree, (2001). It's agglutination activity was inactivated at 85°C and maintained the activity over a wide range of pH (7-9).Here, the effect of divalent cations of agglutination activity in *Trichopeltarion nobile* was studied. Haemagglatination activity depends on $CaCl_2$, $MgCl_2$. Murali et al. (1999) purified an agglutinin from the serum of the hermit crab *Diogenes affinis.* It's haemagglutination activity (HA) was specifically dependent on $Ca^{2+}$ and reversibly sensitive to EDTA. Nalini et al. (1994) reported that the natural haemagglutination activity of the freshwater crab, *Parathelphusa hydrodromus* was specifically dependent on the presence of $Ca^{2+}$ and irreversibly sensitive to EDTA. In the previous studies, Mercy et al. (1992) purified agglutinin and characterized protein from the haemolymph of the marine crab, *Scylla serrata* by SDS polyacrylamide gel electrophoresis. This agglutinin′s molecular weight was about 55kDa. The purified agglutinin from the haemolymph of *Trichopeltarion nobile* was applied on SDS PAGE. The molecular weight was 96kDa.We conclude the isolated agglutinin was rabbit specific and addition of calcium increase the heamagglutination activity of the agglutinin.

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