Characterization of a lectin purified by bioadsorption method from centipede *Rhysida longipes longipes*

[1]Vargila, F, [2]Vinoliya, J, [3]Mettilda, S, [4]Rathika, R.K
[1]Research Scholar, [2]Assistant Professor, [3]Assistant Professor, [4]Research Scholar
Department of Zoology, Holy Cross College, Nagercoil
Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India.

Abstract

Centipedes are venomous arthropods that have been used in conventional medicine for hundreds of years in China. However, very little is known about the active components of centipedes signifying pharmacological importance. In this study, a natural agglutinin from the hemolymph of the centipede *Rhysida longipes longipes* was purified by bioadsorption method and was characterized. The purified lectin showed strong affinity for rabbit erythrocytes and was specific for N-Acetyl glucosamine (GluNAc). The crude hemagglutinin and the purified lectin were sensitive to pH and temperature. The addition of calcium up to 100 mM and EDTA up to 10 mM had no effect on HA activity. The purified lectin was found to be a multiple lectin of 226 kDa, 83 kDa and 59 kDa on PAGE.

Index Terms: Agglutinin, Centipede, Hemolymph, Lectin, *Rhysida longipes longipes.*

Introduction

Centipedes are an important group of arthropods controlling the harmful pests in the terrestrial ecosystem (Yadav, 1994). They are the members of the family Scolopendridae and often treated as creatures of annoyance value owing to their deadly poisonous nature and painful bite. The innate immune system of arthropods comprising both cellular and humoral responses is well studied (Tsakas and Marmaras, 2010). The humoral responses contain synthesis of antimicrobial peptides (AMPs) and activation of the phenoloxidase (PO) cascade, and can be activated by several known cascade pathways (Lee et al., 2013).

Humoral immune factors like hemagglutinins, bacterial agglutinins, precipitins, bactericidal factors, bacteriolysins, hemolysins, opsonins, clotting factors, and lysozymes play an important role in the defense mechanism of invertebrates. Hemagglutinins also known as “agglutinins” or “lectins” are proteins or glycoproteins that specifically recognize carbohydrate structures. The physical and chemical properties of a number of agglutinins have been characterized (Sharon and Lis, 1972; Gold and Balding, 1975; Goldstein and Poretz, 1986). Humoral agglutinins act as bridges between the carbohydrates in foreign cells and those on phagocytic cells (Sharon, 1984) thus involved in recognition functions and can bind a wide spectrum of foreign substances.

Lectins that bind to oligomers of GluNAc have been identified in plants and animals, and their interactions with glycoproteins from pathogens, including fungi, have been demonstrated (Peumans and Van Damme, 1995). Van Damme et al. (2004) suggested that carbohydrate-binding moieties of these lectins are involved in endogenous signaling processes. The distinctive potential to recognize specific carbohydrate structures had made lectins indispensable tools for researchers in many biomedical fields (Leiner et al., 1986) and had proved to be useful reagents for probing structural features of cell surface glycoproteins (Lakhtin, 1995; Ni and Trizald, 1996). Vinoliya (2006) studied the naturally occurring agglutinin activity in the hemolymph of centipedes by hemagglutination assay and reported the presence of hemagglutinin in *Rhysida longipes longipes* that agglutinates rabbit erythrocytes. Based on the above mentioned report this study focused on the purification of lectin from centipede *Rhysida longipes longipes* by bioadsorption method and to characterize the purified lectin.

Materials and methods

Experimental Animal

Centipedes *Rhysida longipes longipes* were collected from Holy Cross College Campus, Suchindrum, Aralvoimozhi, Friday Market, Kanyakumari District, Tamilnadu, India and brought to the laboratory.

Collection of hemolymph

Hemolymph of the centipede was collected as per the procedure described by Xylander and Nevermann (1990).

Hemagglutination Assay (HA)

Erythrocyte collection

Mammalian erythrocytes were collected for hemagglutination assay (Vinoliya, 2006). Blood for this purpose was obtained by heart puncture (rat), fore arm (man) or neck (horse, donkey) or from the slaughter house (rabbit, pig, cow, buffalo, sheep, and goat). Erythrocyte was collected directly in modified Alseivier's medium and they were suspended and washed three times with ten volumes of Tris-buffered saline and resuspended in the same as 1.5% suspension.

Hemagglutination Assay (HA)

Hemagglutination assay was carried out as described by Ravindranath and Paulson (1987). HA titer was determined as the reciprocal of the highest dilution of hemolymph giving complete agglutination after 60 minutes at room temperature (30° C ± 2).

Effect of pH on HA assay

Hemolymph (25 µl) was serially diluted in Tris buffered saline at varying pH (5 - 9.5) and HA assay was carried out with rabbit erythrocytes.

Effect of temperature on HA assay

Hemolymph was pre-incubated for one hour at temperature ranging from 20º C to 50º C and then checked for hemagglutinin using rabbit erythrocytes.

Effect of Calcium and EDTA

To study Ca^{2+} ion dependence of hemagglutinin, HA assays were performed in TBS (pH 7.5) with and without Ca^{2+}. The hemolymph was pre-incubated at different concentrations of Ca^{2+} (0.1 to 100 mM) for 1 hour before adding erythrocyte suspension. To assess the effect of EDTA, the hemolymph was pre-incubated at concentrations (0.1 to 100 mM) of EDTA for 1 hour before adding erythrocyte suspension and analysed for HA.

Hemagglutination Inhibition Assay (HAI)

Hemagglutination Inhibition Assay was carried out using various sugars and glycoproteins as described by Ravindranath and Paulson (1987).

Purification of lectin

Hemolymph of centipede was centrifuged and the supernatant was used for purification. The purification of lectin was done by following the method of Nowak and Barondes (1975) and Vinoliya (2006).

Native Gel Electrophoresis

To find out the molecular weight of the purified lectin, the Native Gel Electrophoresis was carried out (Hames and Rickwood, 1981).

Result and discussion

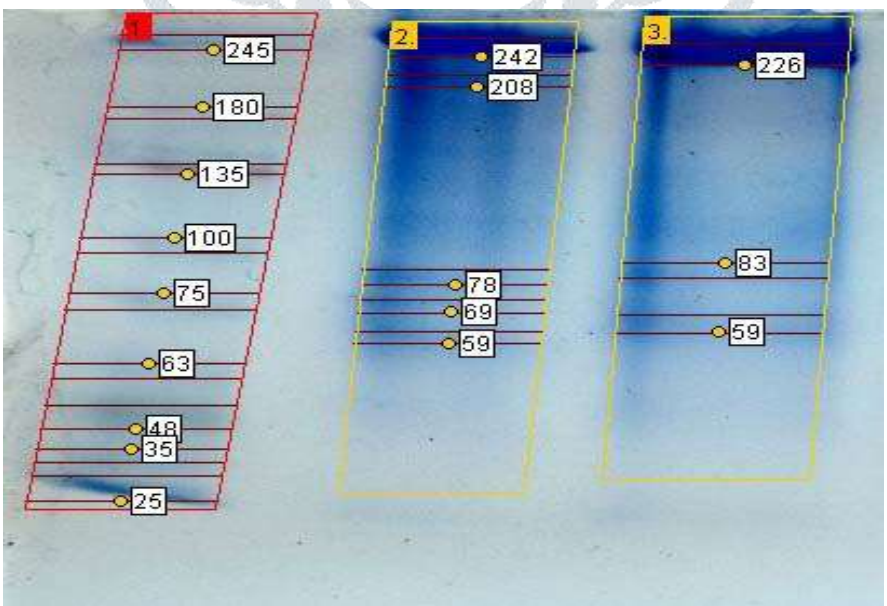
The presence of a naturally existing hemagglutinin in the serum of *R. longipes longipes* was detected using a panel of 13 mammalian erythrocyte types and it showed the highest HA titer (8192 HA titer) against rabbit erythrocyte (Table 1). *R. longipes longipes* agglutinins though capable of agglutinating a variety of erythrocytes, have specific affinity for rabbit erythrocytes that contain NeuGc and 9-O-acetyl NeuAc on its glycocalyx (Pfeil et al., 1980). The active sites on rabbit erythrocytes also conformed to a general N-acetyl glucosamine configuration as well as glucosamine configuration (Cornick and Stewart, 1973). Therefore the agglutinin binding site has common receptors for the rabbit erythrocytes and hence agglutinated strongly. Rabbit erythrocyte specific lectin was also reported in the hemolymph of millipede, *Thyropygus descriptus* (Basil Rose, 1999) and centipede, *Rhysida nuda nuda* (Vinoliya, 2006 and Vargila, 2017).

The hemagglutinating activity of the hemolymph of centipede *R. longipes longipes* was sensitive to temperature and pH. The HA activity was optimum from pH 6.5 to 8 and temperature 30 to 40°C (Table 2). In this optimum pH (6.5 – 8) the hemocyanin would have dissociated from the agglutinin resulting in a high HA titer as reported in Limulus (Printz, 1963). At extreme pH the conformational of the agglutinin might have changed resulting in the reduction of HA titer. Similar pattern was also reported in *Sarcophaga* (Komano et al., 1980), Asterias (Cooper, 1982). The decrease in HA titer at 45 to 50^0 C is due to the conformational changes that occur at the binding sites of the lectin at higher temperature which may suppress the agglutinability (Scott, 1971). Since divalent cations especially calcium are known to be important in stabilizing the structure of agglutinins (Anderson and Good, 1975), the effect of calcium on the

hemagglutinating activity was examined in the present study. It revealed that the hemagglutinin is independent on exogenous calcium for its hemagglutinability (Table 3). EDTA at higher concentrations reduced the HA but lower concentrations did not affect the HA activity. From the above results it could be suggested that the animal had sufficient calcium ions to combat the stressful environment and no external source was necessary for the HA activity. Similar results were also reported in millipedes (Basil-Rose, 1999) and centipedes (Vinoliya, 2006).

Sugar binding specificity of hemagglutinin of *R. longipes longipes* was examined by hemagglutination inhibition tests using carbohydrates and glycoproteins. Of all the sugars tested, N-acetyl glucosamine shows the highest inhibitory potency with a HA titer of 64. The HAI of sugars can be graded as N- acetyl glucosamine, melibiose, D-Fucose, sucrose, lactose, N-Acetyl mannosamine. Of the glycoproteins (transferrin and lactoferrin) tested, lactoferrin showed the highest inhibitory potency (Table 4). More or less similar glycoprotein/sugar specificity was reported in millipedes and centipedes. The hemolymph agglutinin of the millipede *Thyropygus descriptus,* was inhibited by LacNAc, Ga1NAc and lactose (Basil-Rose, 1999) and centipede *Rhysida nuda nuda* by N-acetyl glucosamine (Vinoliya, 2006). The purified lectin was found to be a multiple lectin of molecular weight 226 kDa, 83 kDa and 59 kDa (Fig 1) which agglutinated rabbit erythrocytes efficiently with a HA titer of 256 and hemagglutination inhibition titer of 64 with N-acetyl glucosamine. Thus our studies reveal the presence of multiple lectins in centipede which can be used for identifying multiple sialyl epitopes on the cell surface of microbes and malignant cells.

Fig 1: Poly Acrylamide Gel Electrophoresis of *Rhysida longipes longipes*



Lane 1 - marker, Lane 2 - Hemolymph, Lane 3 - GluNAc specific lectin

Table 1: Hemagglutination titer of hemolymph agglutinins of centipede *Rhysida longipes longipes* against different mammalian erythrocytes

Erythrocytes (n=5)	HA titer
Rabbit	8192
Mice	128
Rat	16
Human A	16
Human B	0
Dog	16
Cow	16
Pig	8
Goat	16
Buffalo	16
Human O	2
Horse	4
Donkey	0

n = No. of animals tested

Table 2: Hemagglutination titer of hemolymph of *Rhysida longipes longipes* in relationship to change in pH and temperature

pH (n=5)	HA titer	Temperature (°C) n=5	HA titer
5	2048	20	2048
5.5	2048	25	2048
6	2048	30	8192
6.5	8192	35	8192
7	8192	40	8192
7.5	8192	45	2048
8	8192	50	1024
8.5	2048		
9	2048		
9.5	1024		

n = No. of animals tested

Table 3: Effect of Calcium and EDTA on the hemagglutinating activity of naturally occurring agglutinin in the hemolymph of *Rhysida longipes longipes*

Concentration in mM (n=5)	HA titer	
	Calcium	EDTA

0	8192	8192
0.1	8192	8192
1	8192	8192
10	8192	8192
100	8192	1024

n = No. of animals tested

Table 4: Hemagglutination inhibition titer of hemolymph agglutinin of centipede *Rhysida longipes longipes* by various sugars/ glycoproteins

Sugars/ Glycoproteins (n=3)	HAI titer
N-Acetyl glucosamine	64
Melibiose	32
D-Fucose	16
Sucrose	16
Lactose	16
N-Acetyl mannosamine	8
Transferrin	0
Lactoferrin	32

n = No. of animals tested

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