International Journal of Advanced Biotechnology and Research
ISSN 0976-2612, Online ISSN 2278–599X,
Vol5, Issue2, 2014, pp157-162
http://www.bipublication.com

PURIFICATION AND CHARACTERIZATION OF A NOVEL LECTIN FROM *GEOTRUPES STERCORARIUS*

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[Received-19/03/2014, Accepted-29/03/2014]

ABSTRACT

The present study described the purification and characterization of a novel lectin from *Geotrupes stercorarius*. The lectin was concentrated by ammoniam sulfate precipitation and purified by ion exchange chromatography using DEAE cellulose. Electrophoretic mobility of the subunit revealed apparent molecular weight of 7kDa under non reducing and reducing condition. Hemagglutination activity towards rabbit erythrocytes was not significantly modified by addition of calcium or EDTA and specifically inhibited by mannose**.** The lectin was heat stable up to 60^0C and showed stability at pH 7.6.

Keywords : Lectin, Hemagglutination activity, Erythrocytes, Ion Exchange Chromatography, Precipitation.

I.INTRODUCTION

Insects hemagglutinins are lectin or lectin like molecules that are ubiquitous, non enzymatic carbohydrate binding protein are glycoprotein's, and once bound to erythrocytes or other cells, usually cause their agglutinations also precipitate glycoconjugates (9). Lectins are widely distributed in various organisms plants, vertebrates, invertebrates and microorganisms (16). Limited number of insects lectins have been purified and characterized, including those from *Sarcophago peregrine* (10,11), *Teleogryllus commodus* (7), *Allomyrina dichotoma* (22) and *Hyalophora cecropia* (4). Insect lectins demonstrated the presence of multiple lectins with different specificities (5). Insects lectins are important for both the establishment of infection and parasitic development in the gut and hemolymph (8,17). Lectins now find most of their application in areas such as biotechnology, medicine and biology (12). In this present study deal with purification and characterization of a novel lectin from *Geotrupes stercorarius* .

II. MATERIALS AND METHOD

2.1. Collection of experimental animal and erythrocytes

The insect *Geotrupes stercorarius* was collected from the surrounding of St. Mary's college champs, Thoothukudi and were taxonomically identified Dr. Sam Manohar Das, Department Of Zoology, Scott Christian College, Nagercoil. Fresh human erythrocytes were obtained from healthy donors and animal erythrocytes were obtained from veterinary faculty's hematology laboratory.

2.2. Extraction of crude lectin

2gram of insect was homogenized in 10ml of TBS buffer. The homogenited was centrifuged at 10000 rpm for 30 min and the resulting supernatant was stored at -20^{0}C for further analysis. The clear supernatant was used as sample.

2.3. Characterization of lectin from *Geotrupes stercorarius*

2.3.1. Hemagglutinaion Assay

Hemagglutination activity of the sample was assay in a 96 well of microtiter V plate according to the two fold serial dilution procedure (6). 25µl of sample serially diluted with each well of TBS containing 50mm Tris base, 100 NaCl, 10mm $CaCl_2$, pH7.6. Then 25µl of 2% erythrocytes was added next gently shacked and allowed to incubated for 1 hr at room temperature. The hemagglutination activity was observed and total hemagglutination activity (titre x fraction volume) was expressed in hemaggluatinaton unite.

2.3.2. Blood group specificity

The blood group specificity of the lectin was established using human erythrocytes (A^{+ve}, B^{+ve} and O^{+ve}) and different animal erythrocytes (Cow goat, pig, hen and rabbit).

2.3.3. Effect of pH on hemagglutination activity

The effect of pH on the hemagglutination activity of the sample was carried out by allowing hemaglutination to occur in various buffers pH range between pH4 to pH10.

2.3.4.Effect of temperature on hemagglutinaton activity

The sample was incubated on a water bath for 20minutes at 30^{0}C to 90^{0}C. After cooling at 4^{0}C the residual hemagglutinaton activity was measured against a 2% erythrocyte suspension as described previously.

2.3.5. Effect of divalent cations on hemagglutination activity

The sample was dialyzed overnight in various concentrations of divalent cation ($CaCl_2$, $MgCl_2$, $MnCl_2$ and EDTA). Then it was again redialyzed for 4 hrs at 4^{0}C in TBS. The hemagglutinaton activity was determined in this dialyzed sample. The sample was compared to find out the role of the metal ions.

2.3.6. Inhibition Assay

An equal volume of diluted sample was added into 25µl of two fold serially diluted inhibitor solution (Sigma, St. Louis, USA) and the plate was incubated for 1h at room temperature. Finally 25µl of 2% erythrocytes suspension was added. The minimum concentration of the inhibitors required to completely blocked agglutination was observed after 1 h of incubation at room temperature**.**

2.4. Purification of lectin from *Geotrupes stercorarius*

2.4.1.Precipitation of lectin by Ammonium sulphate

The sample was mixed with 10% ammonium sulphate. Then it was dissolved and kept in 4ºC for 4 hrs. Then it was centrifuged 4000 rpm for 10 minutes. The supernatant was transferred into 20% ammonium sulphate and is continued upto 90%. The pellet were stored at – 20ºC.

2.4.2.Ion exchange column chromatography

Purification of lectin, was carried out using DEAE cellulose anion exchange chromatography

according to the method of (21). The 70% precipitated sample was dialyzed for overnight against TBS buffer (50mm Tris base, 100 NaCl, 10mm $CaCl_2$, pH7.6). The dialyzed sample was applied to a DEAE column. The column was washed with same buffer, and it was eluted with serially increasing concentration of NaCl (0.1M - 1.2 M). The eluted all fractions were monitored by U.V. absorption spectroscopy at 280nm.

2.4.3. Molecular Weight Determination

SDS-PAGE was carried out by the method of (12). Lectin was denatured with the sample buffer containing 1% SDS by boiling for 3min at 100^0C. Treated sample was loaded into 12% SDS-PAGE on the electrophoresis set. Electrophoresis was carried out at 50vots initial current. When the stacking dye reaches the separating gel, the current was increased to 100vots. The molecular mass of the lectin was estimated by comparing its mobility with that of the following markers. Phosphorylase b (97400), Bovine Serum Albumin (66,000), Ovalbumin (43,000), Carbonic Anhydrase (29,000), Lactoglobulin (18,400), Aprotinin (6,500), Gel was silver stained by the method of (3).

2.5. Protein Concentration

Protein was determined by Lowry's method, (15) using bovine serum albumin as standard.

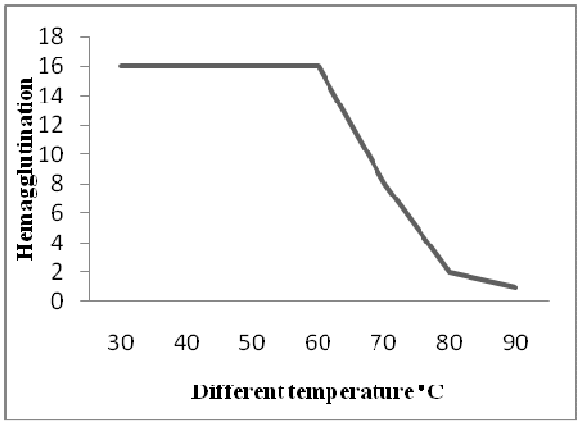
III. RESULT

3.1. Characterization of lectin from *Geotrupes stercorarius*

Crude sample of *Geotrupes stercorarius* contains a hemaggluting activity that agglutinated all human erythrocytes and animal erythrocytes with slightly more activity towards the rabbit erythrocytes at a minimum concentration of 25µg (data not showed).

The optimum pH of *Geotrupes stercorarius* lectin was agglutinated rabbit erythrocytes at pH 5.5.-10.0. The activity was reduced below pH 7.0 and above pH 8 with an activity maximum pH 7.6. The lectin hemagglutination activity over the temperature range of 30- 60^0C, but the activity decreased markedly between 65 and 90^0C, and the activity was completely abolished at 95^0C ((Fig.1).

The crude lectin was dialyzed overnight in various concentrations of divalent cation ($CaCl_2$, $MgCl_2$, $MnCl_2$ and EDTA. The hemagglutinaton activity was determined against rabbit erythrocytes. It indicated that cation were not necessary for its hemagglutination activity.



To determine the carbohydrate specificity *Geotrupes stercorarius* lectin , a competitive inhibition assay was performed using various sugar inhibitors. Hemagglutination activity against rabbit erythrocytes was inhibited by the presence of mannose and maltose (Table.1)

Table.1. Inhibition of hemagglutination activity of the *Geotrupes stercorarius* lectin by different carbohydrates.

Carbohydrates	Minimum Concentration for inhibition of hemagglutination
Glucose	NI
Arabinose	NI
Ribose	NI
Fructose	NI
Maltose	NI
Galactose	NI
Rhamnose	NI
Raffinose	NI
Lactose	NI
Sucrose	NI
Glucosamine	NI

Galatosamine	NI
N-acety1 Neuraminic Acid	NI
Trehalose	NI
Chitin	NI
Melizitose	NI
Dextrose	NI
Melibiose	25mM
Feuitn	NI
Mucin	NI
Thyroyglobulin	NI
Heparin	NI
Mannose	12.5 mM
Salicyline	NI

NI : No Inhibition

3.2. Purification of lectin from *Geotrupes stercorarius*

Crude soluble protein extract obtained from insect *Geotrupes stercorarius* was initially precipitated at 0-90% with ammoniam sulphate. The 70% was showed strong hemagglutination activity. The active percentage was then applied to a DEAE column and the retained peak was submitted to hemagglutination assay (Fig.2). The purity of the isolated lectin was verified by SDS - PAGE. SDS –PAGE gave a single band with a subunit molecular mass of **kDa** under reducing as well as non-reducing conditions (Fig.3).

IV. DISCUSSION

Very few lectins have been isolated from insects. The data presented from this study showed that the *Geotrupes stercorarius* contained a measurable amount of hemagglutinating lectin. The lectin from *Geotrupes stercorarius* agglutinated red blood cells non specifically which is typically of many lectins. The lectin was considered to be non-blood types. The lectin was agglutinate erythrocytes from both human and animal erythrocytes. The higher titre against rabbit erythrocytes. Non blood type specificity of the lectin may be due to the presence of multiple binding sites where it can recognize all the determinants for each blood type (1). *Anstephensi* midgut lectins also hemagglutination activity against rabbit erythrocytes specific (2).

The thermostability and pH stability characteristics of lectins are known to differ from lectin to lectin . The hemagglutinating activity of this lectin *Geotrupes stercorarius* was thermostable and pH sensitive. Lectins are known to be heat labile and their activity can be decreased by heat treatment (14). The finding suggests that the hemagglutination activity of lectin *Geotrupes stercorarius* was stable at the pH ranges between pH 7.0 and pH 8.0. The lectin activity reduced greatly from pH 8.0 with an activity maximum pH 7.6, an agglutinin with relatively high thermostability upto 60^0C , it is devoid of lectin activity. This may be brought about by the denaturation of the lectin that removes its agglutinating capacity. The hemagglutination was not influenced by the addition of Ca^{2+}, Mg^{2+} or the chelating agent EDTA, suggesting that any divalent cations are not essential for the hemagglutination activity.

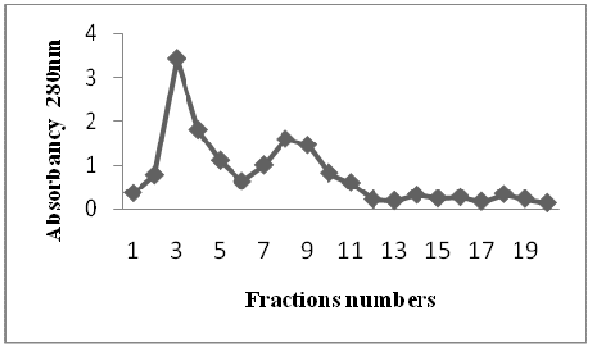


Fig : 1 : Ion exchange column chromatography of *Geotrupes stercorarius* lectin isolated on DEAE column, absorbance at 280nm.

Hemaglutinins are involved in carbohydrate binding specifities, because it is considered that they could exert an influence on host- parasite interaction in the appropriate vectors (5). Therefore the diverse specificities of lectins within culicid may be related to the physiological function of these molecules components based on lectin - carbohydrate interactions. Sugar specificity of the lectin

Geotrupes stercorarius was examined by competitive inhibition of various sugars and glycoproteins against rabbit erythrocytes. The activity of the lectin was completely inhibited by mannose and maltose with minimum inhibitory concentration of 12.5mM and 25Mm respectively (Table.1). Remarkably, it showed no inhibition by glucose, but was inhibited by maltose, a disaccharide composed of two glucose units. It may be that the lectin reacts with a more extended structure than the monosaccharides unit. It has been reported that larger and more complex polysaccharides interact with secondary sites on the lectin surfaces as well as with the primary binding sites (18).

Known molecular weight of all lectins of invertebrates vary from 26,000 to 1,500,000. These variation in molecular weight may be due to different invertebrate species, methods of purification and analysis of agglutinins , and varied protocols undertaken by different laboratories.

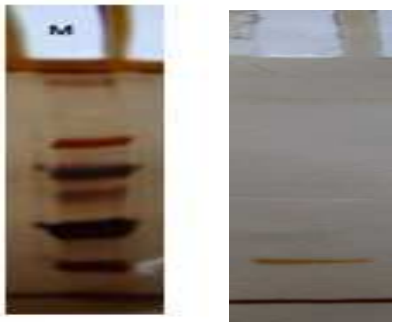


Fig : 2 : SDS-polyacrylamide gel electrophoresis of the purified *Geotrupes stercorarius*.lectin in the presence of β-mercaptoethanol. Lane :1 : Standard non-denatured molecular weight marker protein, Lane : 2 : Purified protein.

Ammonium sulphate was chosen as precipitating agent in this study due to its high solubility in water and produces high inonic strength. Increase in ionic strength decreases the protein solubility (19). Ammonium sulphate saturation (0-90%) was employed to ensure that most proteins will be precipitated. Ammonium sulphate was removed from the protein. Since it may cause false positive agglutination. In our present study the lectin was precipitated ammonium sulphate fractionation of 70% saturation. The precipitate was dialyzed against Tris buffer pH.7.6 at 4^0C for 12h. After removal of insoluble materials, the clear supernatant was applied the DEAE cellulose column. The lectin was eluted by linear gradient of NaCl from 0.1 to 1.2 mM (Fig.2). Two peaks were visible after reading the absorbance (280 nm) of the 40 fraction collected (2ml per tube) but only one fraction was showed agglutination, which falls on the first peak and its purity was evaluated only by SDS-PAGE. In the presence of SDS-PAGE the lectin migrated as a single protein band of 7kDa molecular weight (Fig.3). This state shows that purified lectin did not separated into subunits despite reducing β-mercaptoethanol. The molecular weight of the lectin purified from *Periplaneta americana* was detected to be 1,500,000 as well as 30,000 of molecular weight subunit on SDS-PADE. This is the largest in molecular weight of other insect species.

V. CONCLUSION

Mannose is an important cell surface sugar present in glycoconjugates that are implicated in a wide variety of important receptor mediated process, *Geotrupes stercorarius* lectin with exclusive specificity towards mannose has provided an interesting line of investigations with respect to lectin dependent processes and further studies must be done for further researches with some therapeutic application.

ACKNOWLEDGEMENT

We are grateful thank to the principal ST. Mary's College (Autonomous) for providing all laboratory facilities to carry out this research work.

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