Characterization of Lectin from Colpomenia Sinuosa and Effect of Physico Chemical Parameters on Haemagglutination Activity

Malini.M.M, Jansi.M, Sini Margret.M, Anooj.E.S, Lekshmi Gangadhar

Abstract:* Lectin is a protein which has the ability to bind carbohydrates and named as haemagglutinin. Lectins with specific carbohydrate specificity have been purified from various plant tissues and other organisms and exploited extensively in many aspects of biochemistry and biomedicine. Similar to land plants, lectins from marine algae appear to be useful in some biological applications. Although several studies on lectins from marine algae have been reported till date, few lectins from algae have been characterized in detail. The present study was focused on the lectin isolated from C.sinuosa. The algal lectin has high sugar specificity with N-acetylglucosamine and higher enzyme activity with trypsin. This lectin was identified as $CaCl_2$ dependent – 'C' type lectin and was sensitive to EDTA. Higher H.A titre value was observed with $CaCl_2$ and the lower with $MnCl_2$ and $ZnCl_2$. Significant lectin activity was observed between pH 7 to 8 and temperature between 20 to 40 ^{O} C.

Keywords: Lectin, algae, haemagglutinin, biochemistry

I. INTRODUCTION

"Lectins" has been derived from the Latin word "legere" which has the ability to bind carbohydrates and named as haemagglutinin. Lectins from terrestrial plants and animals have been isolated, characterized and exploited extensively in many aspects of biochemistry and biomedicine. Compared with land plant lectins, the occurrence of lectins from marine algae was first reported by Boyd *et al.* (1966). The first agglutinin from marine algae was isolated and characterized by Rogers *et al*. (1977). Similar to land plants, lectins from marine alga reveal a proteinaceous nature but differ in some of their properties. They generally have lower molecular masses than most land plant lectins and are more specific for complex oligosaccharides or glycoproteins. Further, most of marine algal lectins do not require divalent cations for their biological activity. They occur mainly in monomeric form and have a high content of acidic amino acid, with isoelectric points from 4 to 6 (Rogers & Hori, 1993). Although several studies on lectins from marine algae have been reported till date, few lectins from algae have been characterized in detail. Many important biological molecules posess ionisable groups and exist in solution as electrically charged species either as cations or anions at any pH. Under the influence of an electric field these charged particles migrate either to the cathode or to the anode, depending on the nature of their net charge (Janson and Ryden, 1989).

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II. MATERIALS AND METHODS

Protein purification and characterizations are generally a multi-step process exploiting a wide range of biochemical and biophysical characteristics of the target protein. Each protein offers its own unique set of physicochemical characteristics which are considered to be important in its application. This paper described the biochemical characterization in terms of physico- chemical properties of the agglutinin extracted from *C. sinuosa* was determined.

Description of the algae

Figure 1. Plate .1 *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier

Systematics and Description

Empire : Eukaryota
Kingdom : Chromista
Phylum : Ochrophyta
Class : Phaeophyceae
Subclass **:** Fucophycidae
Order : Ectocarpales
Family :

Scytosiphonaceae

Genus **:** *Colpomenia*

Species : *sinuosa*

Plants smooth, hemispherical, irregularly lobed, golden brown hollow - 30 cm diameter and 10 cm high, with multiple attachments to substrate. Often covered with fine colourless hairs. Reproductive sori as dark raised patches on surface. Thallus membranaceous, 300-500 cm thick, 4-6 cell layers. Plurilocular sporangia cylindrical to club-shaped, 3.7-8 cm diameter, 18-30 cm long, in dense clusters, scattered on surface. Lower intertidal to 15 m deep. Firmly attached to hard surfaces or epiphytic on other organisms (Plate.1).

Enzyme induced haemagglutination assay

Enzymes that induce and increase the haemagglutination assay were tested with enzymes such as trypsin, neuraminidase and papain following the method described by Maheswari *et al*. (1997), saline washed rabbit and human erythrocytes were resuspended in the TBS containing 5mg/ ml^{-1} trypsin, neuraminidase and papain separately and incubated for 1hour at 37^0C with occasional shaking. The enzyme treated red blood cells were washed with 0.9% saline by centrifuging at 4000 rpm for 5 minutes at room temperature. Finally the RBC's were fixed by suspending it in TBS (pH 7.6) containing 10% formaldehyde for 24 hours at 10^0C. Enzyme fixed red blood cells were extensively washed in 0.9% saline or TBS. Enzyme induced haemagglutination assay was performed with lectin sample separately for different enzymes treated RBC's and the activity was recorded.

Sugar specificity of C. sinuosa lectin (Inhibition Assay)

The lectin's sugar specificity was carried out following the standard procedure of Faria *et al.* (2004) by comparing the inhibitory activity of various sugar solutions such as, N-acetyl glucosamine, N-acetyl neuraminic acid, glucosamine, mannose, lactose, fucose, sucrose, fructose, rhamnose, ribose, maltose, trehalose, dextrose, sorbitol and galactose, on hemagglutination of human O^+ erythrocytes. 25 µl of lectin dilution was placed on microtitre 'V' plates and incubated for 1 hr at 37^0C with 25 µl of sugars at different concentration. Subsequently, 25 µl of a 2% human O^+ erythrocytes suspension in TBS was added. The specificity was also determined using glycoproteins such as mucin and fetuin. The inhibitory capacity was expressed as the minimum concentration of the carbohydrates and glycoproteins which are required to inhibit haemagglutination activity. Inhibition assays were performed in triplicate. The results were expressed as the minimum concentration of the inhibitor required to terminate the agglutination completely.

Cation dependency and EDTA sensitivity

According to the method of Pandolfino and Magnuso (1980) the cation dependency and EDTA sensitivity of haemagglutination activity of *C.sinuosa* lectin was determined in TBS containing different concentration of $CaCl_2$. The lectin samples (each 500µl) were dialyzed extensively against TBS (to test divalent cation dependency) or in TBS-EDTA (to examine EDTA sensitivity) at 15^0C. The samples were dialyzed against TBS-EDTA and were subsequently re-equilibrated by dialysis in TBS. After centrifugation (4000 rpm for 5minutes at room temperature) the supernatant was used to determine the haema glutinating activity with human O^+ erythrocytes.

Effect of metal cation on haemagglutination activity of *C. sinuosa* lectin

The effect of divalent metal ions on lectin activity was assessed by extensive dialysis of *C.sinuosa* lectin sample against TBS containing different concentration of metal ions such as $CaCl_2$, $MgCl_2$, $ZnCl_2$, $MnCl_2$, $FeCl_2$ and $MnSO_4$ (pH 7.6) for 24 h at 5^0C as described by the method of Pandolfino and Magnuso (1980). The haemagglutination activity was determined with RBC suspended in TBS before and after addition of metal ions.

Table 1: Enzyme induced haemagglutination activity

	Blood group			
	A^+	B^+	O^+	R
Trypsin	256	128	1024	256
Papain	32	16	512	64
Neuraminidase	2	-	-	-

Effect of pH and thermal stability

The *C.sinuosa* sample (500µl) was dialyzed against the buffers such as acetate buffer, Tris-HCl and glycine NaOH at the pH ranging from 3.5 to 10. After dialysis, all the samples were again finally equilibrated by dialysis against TBS. The dialysates were centrifuged and the supernatant was tested for haemagglutinating activity using human O^+ erythrocytes. Thermal stability of sample was examined by holding 100 µl of samples for 30 min at temperature ranging from 10-80^0C. All the samples were centrifuged and the clear supernatant was used to determine agglutination activity with human O^+ erythrocytes.

III. RESULTS

Enzyme induced haemagglutination assay

Erythrocytes treated with enzymes were tested for HA activity and the results revealed that trypsin treated O^+ erythrocytes showed increased activity when compared with A^+,B^+ and rabbit (R) erythrocytes. The highest HA titer value was observed with trypsinized O^+ (1024) followed by trypsinized rabbit A^+erythrocytes (256). Trypsinized B^+ erythrocytes exhibited a HA titre of 128 (Table.1). Among the papain treated human erythrocytes, O^+ showed the highest titre value of 512 similar to that of trypsin treated human O^+ erythrocytes and the other erythrocytes showed a reduced titre ratio of 32 for A^+ and 16 for B^+ and 64 for rabbit (R) erythrocytes. But the neuraminidase treated human A^+ erythrocytes only showed the lowest titre ratio of 2 and the other neuraminidase treated erythrocytes did not show HA activity.

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Sugar specificity of *C.sinuosa* (Inhibition Assay)

The sugar specificity of *C.sinuosa* lectin was assessed by haemagglutination inhibition efficiency by various sugar and glycoproteins. The result revealed that N-acetyl glucosamine produced higher inhibitory activity (32) among the used sugars, followed by N-acetyl neuraminic acid (16) and mannose (8). The other sugars such as lactose, sucrose,

Table 2: Tested results

Sugar and glycoproteins tested	Max.Concentration tested(mM)	H.A.Inhibition titre
N-acetyl glucosamine	200	32
N-acetyl neuramic acid	200	16
Glucosamine	200	-
Mannose	200	8
Lactose	200	-
Fucose	200	-
Sucrose	200	-
Fructose	200	-
Rhamnose	200	-
Ribose	200	-
Maltose	200	-
Trehalose	200	-
Dextrose	200	-
Sorbitol	200	-
Galactose	200	-
Fetuin	100	8
Mucin	200	8

fructose, ribose, maltose, trehelose, dextrose, sorbitol, galactose, rhamnose, did not show any inhibitory activity against *C.sinuosa* lectin (Table.2).

In the case of glycoproteins, fetuin and mucin were the potent inhibitors of agglutinin and had scored inhibitory efficiency with the HA titre of 8. The result revealed that the sample isolated from the alga *C.sinuosa* contain a lectin which has high specificity for N-acetyl glucosamine.

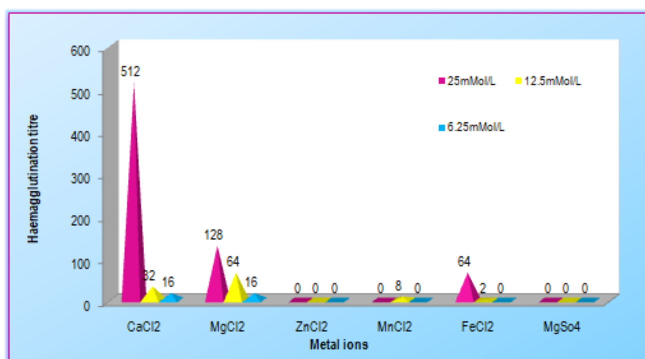

Fig.2 Effect of metal ions on haemagglutination activity

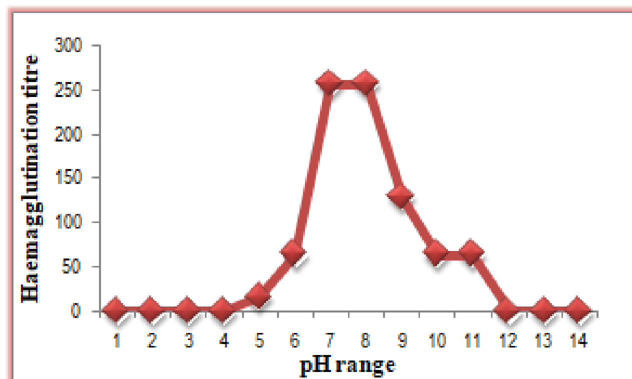

Fig.3 Effect of pH on haemagglutination activity of *C.sinuosa*

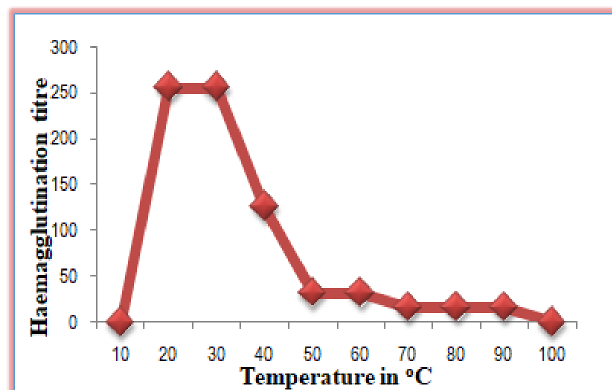

Fig.4 Effect of temperature on haemagglutination activity of *C.sinuosa* lectin

Cation dependency and EDTA sensitivity

C.sinuosa lectin was confirmed to be Ca^{2+} dependent lectin. The lectin was tested with various concentrations (6.25,12.5,25,50,100 mM) of calcium ions dialyzed in TBS or in TBS- EDTA at different concentrations. The lectin agglutinated various animal erythrocytes depending on the presence of calcium ions and the activity was abolished in the absence of $CaCl_2$ as well as in the presence of EDTA. The highest HA activity (512) of lectin was observed with $CaCl_2$ concentration of 25mM. The increase or decrease in calcium ion concentration lead to a decrease in HA activity of lectin. Thus the lectin was identified as calcium dependent or 'C' type lectin and was insensitive to EDTA.

Effect of metal cations on haemagglutinating activity of *C.sinuosa* Lectin

The lectin was tested with different concentration of divalent cations such as $CaCl_2$, $MgCl_2$, $ZnCl_2$, $MnCl_2$, $FeCl_2$ and $MgSO_4$ for the determination of HA activity. Among the experimental data , 25 mM concentration of $CaCl_2$, $MgCl_2$ and $FeCl_2$ showed higher HA activity. The haemagglutination titre value increased up to 512 for $CaCl_2$, 128 for $MgCl_2$ and 64 for $FeCl_2$ at the concentration of 25mM and a decrease in concentration expressed lower activity.

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Table :2 Sugar specificity of algal lectin (*C.sinousa*)

The other cations like $MnCl_2$ and $ZnCl_2$ showed very low haemagglutination titre value, but $MgSO_4$ did not produce any significant HA activity on lectin (Fig.1).

Effect of pH on haemagglutination Activity

Significant lectin activity was observed between pH 7.0 and 8.0 and the activity slowly reduced below pH 7.0 and above pH 8. At pH 5 and pH 12 the haemagglutination activity was completely abolished. Thus the agglutinating property of lectin to agglutinate human O^+ erythrocyte was stable between pH 7.0 and 8.0 (Fig.2).

Effect of temperature on haemagglutination activity of *C.sinuosa* lectin

The temperature range of haemagglutination activity of lectin to agglutinate human O^+ erythrocyte was stable between 20^oC and 40^oC. The activity titer was reduced above 40^0 C which leads to the loss of activity at 100^0 C and below 20^0 C. Hence the protein was suggested to be a heat sensitive protein (Fig.3).

IV. DISCUSSION

Algal lectins are in general more specific for complex oligosaccharides often glycoproteins (Rogers and Hori, 1993). Haemagglutination is referred to the presence of proteins or glycoproteins having specificity for carbohydrate binding selectively to red blood cells. These proteins are found in sap of some marine algae (Boyd *et al.,* 1966). The haemagglutination assay of crude extract of *C.sinuosa* showed a higher titre value of 512 with human O^+ erythrocytes which also agglutinated other blood types such as A, B, A^-, B^-, O^- $A1^+$ and AB^+ with low titre value. Haemagglutination assay of *C.sinuosa* with animal blood erythrocytes revealed a higher titre value of 128 with rabbit erythrocytes (R), whereas goat (G) and hen (H) erythrocytes showed very low activity. Enzyme induced haemagglutination assay of *C.sinuosa* lectin with trypsin, papain and neuraminidase revealed that the trypsin treated human O^+ erythrocytes exhibited increased HA activity of 1024. Papain treated human O^+ erythrocytes alone showed the higher activity of 512 and the other erythrocytes showed relatively less activity. Neuraminidase treated O^+ erythrocytes did not show any significant haemagglutination activity with *C.sinuosa* lectin. Trypsinization of erythrocytes increased the haemagglutination activity which may be due to the exposure of the cryptic receptor sites on the cell membrane and the removal of close interfering structure after protease digestion on the erythrocytes (Marilyn Holm *et al.,* 1987). Similar to our report, Gottschalk and Drzeniek (1972) also observed that neuraminidase treated rabbit and human erythrocytes showed lower activity. The agglutination activity of *C.sinuosa* lectin with erythrocytes of most human blood group revealed that the specificity of lectin to several sugars and glycoproteins. Sugar specificity of *C.sinuosa* lectin revealed that it has high specificity for N-acetyl glucosamine produced higher inhibitory activity (32) with *C.sinuosa* lectin. As in our report, N-acetyl glucosamine was reported to inhibit agglutination activity in *Codium giraffe* (Alvarez – Hernandez *et al.,* 1999) and *Didemnum ternatanum* (Odintsova *et al.,* 2001).

Metal ion dependency of haemagglutination activity of *C.sinuosa* lectin was tested using various metal ions such as $CaCl_2$, $MgCl_2$, $ZnCl_2$, $MnCl_2$, $FeCl_3$, and $MgSO_4$. Among the experimental data, 25 mM concentration of $CaCl_2$ exhibited the highest HA activity with lectin and lower activity was observed with a decrease in concentration of $CaCl_2$. Marine lectins are generally identified by their metal ion requirement for their haemagglutination activity. C-type lectins are specific to carbohydrate recognition proteins which play crucial roles in the innate immunity. *C.sinuosa* lectin was considered to be calcium dependent as its activity was restored by the addition of $CaCl_2$. Similar to our report Renuka Bai (2001) and Randy Chi Fai Cheung (2015) also observed that the agglutination activity of lectin in *P.pastoris* also required Ca^{2+} ions. The lectins from red algae *Ptilota serrata* (Rogers *et al.,* 1990), *Ptilota filicina* (Sampaio 1998 b), *Enantiocladio duperreyi* (Benevides *et al.,* 1998) and from green algae *Ulva lactuca* (Sampaio *et al.,* 1998) exhibited dependence of metals such as Ca_2^+ and Mn2. Few lectins do not require any metal ions for their activity (Yang *et al*., 2007). Lectin from marine red alga, *Gracilaria cornea* is an acidic, monomeric glycoprotein that does not require divalent metal ions for its haemagglutinating activity. The lectin from the venom of the Hawaiian box jellyfish (*Carybdea alata*) showed similar result in which the HA activity was irreversibly lost after dialysis of crude venom against divalent-free, 20 mM EDTA buffer which was optimal in the presence of 10 mM Ca^{2+} or Mg^{2+} (John Chung *et al.,* 2001). Similar result was documented from lectin of starfish, *A. pectinifera* where the haemagglutination activity of lectin was completely abolished by chelating agent such as EDTA or EGTA and the activity was completely restored by the addition of $CaCl_2$ (Mari Kakiuchi *et al*., 2002). Thermal stability of *C.sinuosa* lectin with respect to its haemagglutination activity was stable at temperature range between 20^0C to 40^0C and the activity titre value has a sharp decrease above 40^0C and finally the activity was completely lost at 80^0C and below 20^0C.

V. CONCLUSION

The thermostability of proteins depend on its increased content of charged amino acid residues and revealed that the haemagglutination capacity of the lectin was not affected by exposure to a temperature of 30-70^0C for 30 minutes. The effect of pH on *C.sinuosa* lectin to agglutinate human O^+ erythrocyte indicated that the lectin was stable at pH range between 7.0 and 8.0 and the activity gradually reduced below pH 7.0 and above pH 8.0. At pH 5 and pH 10.0 the haemagglutination activity was completely abolished. At pH 8 the HA activity was greatly reduced at more than 50% of the original activity scored. But the effect of pH on *C.sertularioides* was found to be active over the range of 5.0 to 10.5. The effect of pH on the lectin revealed that the haemagglutination activity of *U.pertusa* lectin was unaltered at pH 6-8.

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The algal lectin has high sugar specificity with N-acetylglucosamine and higher enzyme activity with trypsin. This lectin was identified as $CaCl_2$ dependent – ‘C’ type lectin and was sensitive to EDTA. Higher H.A titre value was observed with $CaCl_2$ and the lower with $MnCl_2$ and $ZnCl_2$. Significant lectin activity was observed between pH 7 to 8 and temperature between 20 to 40 ^{O} C.

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