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A New Lectin From *Bryophyllum mortagei*

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Abstract

A 210kDa L- rhamnose-binding lectin was isolated from the leaf of *Bryophyllum mortagei* by gel filtration on Sephadex G-75 column. *Bryophyllum mortagei* lectin (designated as BmL) is a 160kDa protein consisting of 36kDa subunits linked by non-covalent bonds as determined by SDS-PAGE electrophoresis in the presence of 2-mercaptoethanol. The lectin exhibited strong heamagglutinating activity towards rabbit erythrocytes. Trypsinated rabbit RBC increased and papain treated decreased the activity. Its heamagglutinating activity was stable in pH range of 7.0–10.0 and temperature was stable up to 60^{o}C and the heamagglutinating activity was lost at 80^{o}C. The metal ions did not enhance the lectin activity.

Keywords: *Bryophyllum mortagei (BmL)*, L- rhamnose, Sephadex G-$_{75}$, Red blood cells (RBC).

Introduction

Lectins are carbohydrate binding proteins which recognize and interact with specific monosaccharide or oligosaccharides unites and they agglutinate cells and / or precipitate glycoconjugates (Goldstein *et al.,* 1980). Lectins have been isolated from a diversity of organisms including flowering plants (Kang *et al.,*2007), animals (Watanabe *et al.,*2008;Watanabe *et al.,* 2007). Lectins are commonly found in the seeds of leguminous plants but they can also be obtained from other plants parts , leaves, roots and stems (Boyd,1970:Lis and Sharon,1973). Lectins have also been reported from leaves of *Bauhinia monondra* (Coelho and Silva, 2000).The lectin from leaves of jacalin cycad, *Cycasrevoluta thumb* (Yagi *et al.,*2002). It was examination of several lysylendopeptick derived peptides. *Artocarpus altilis* lectin from pinnate leaves (Pancho,1979). Many biological activities, including anti-proliferative,anti-tumor, immunopotentiating , anti-insect, antifungal and antibacterial activities, have been found in lectins (Oliverira *et al.,*2008;Abdullaev and Mejia,1997; Rubinstein *et al.,*2004; Herre *et al.,* 2004; Macedo *et al.,* 2003; Wong, 2006). Some of them would be useful for detecting tumor specific or associate antigen and developmentally regulated sugar residues (Konska *et al.,*1981). Recentely veterinary and medical research has demonstrated that the lectins involved in innate immunity play a crucial role in disease resistancen (Malhota and Sim,1995: Tizzard,1996: Turner,199). In this report describes identification and characterization of a new lectin from *Bryophyllum mortagei.*

Materials and Methods

Preparation of crude extracts

Collected the plant leaves were washed thoroughly in sodium hyphochloride and distilled water. Then it was cut into small pieces and grained with Tris buffer, pH 7.6 containing (50mM Trise base,100mM NaCl,10mM $CaCl_2$).The resulting mixture was filtered through chees cloth and centrifuge at 4000rpm for 25min at 4^{o}C to remove the leaf debris. The supernatant was collected and assay for heamagglutination activity (Ravindranath *et al.,*1985).

Heamagglutination activity and Carbohydrate inhibition assays

Heamagglutination activity of the sample was assay in a 96 well of microtitre V plate according to the two fold serial dilution procedure (Ravindranath,1951). 25µl of sample serially diluted with each well of Tris buffer. Then 25µl of 2% rabbit erythrocytes was added and gently shaked and allowed to incubated for 1hr at room

temperature. The heamagglutination activity was observed and total heamagglutination activity (titre fraction volume) was expressed in heamagglutination unite. In the studies of heamagglutination inhibition by carbohydrates, the lectin was previously incubates with carbohydrates.

Stability towards temperature

The stability of the sample towards temperature was determined by incubating 50µl of sample at temperature ranging from 30^{O}C-80^{0}C for 15min with 10^{O}C increase at each step. The heamagglutination activity was determined after every temperature rise to check the effect of temperature on the sample.

Stability towards the pH

The stability towards pH was determined by dialyzing the sample against buffers in the range of pH 7.0-10.0. The sample was later centrifuged and the pH was adjusted to 7.0 with 0.1 N HCl or 0.1 N NaOH before testing the heamagglutination activity.

Purification of the sample

Lectin was isolated from *Bryophyllum mortagei* by gel filtration chromatography. The sample was applied on the Sephadex G_{75} column (4 ×10c) equilibrated with the same buffer. The heamagglutination active fraction was stored at -20^{O}C.The molecular and the homogeneity of the purified lectin was evaluated in 7% polyacrylamide gel electrophoresis (PAGE) in the presence of 0.1% sodium dodecyl sulfate (SDS),using the Laemmli buffer system(1970), gel was stained with 0.1% Coomassie brilliant blue.

Results and Discussion

The heamagglutination may well be considered to react with specific antigen on the erythrocytes but some have specifity associated with the human ABO blood group system. D-galactose / N-acetyl –D-galactosamine specific lectin was isolated from *Erythrina cristagalli* and it agglutinated human erythrocytes of all blood types as well as rabbit erythrocytes (Iglesias *et al.,*1982). In this study the plant extract *BmL* agglutinated all human erythrocytes but animals it agglutinated particular erythrocytes cow, pig, hen, goat and rabbit erythrocytes.

Table -1: Heamagglutination activity of untreated and treated rabbit erythrocytes by *Bryophyllum mortagei*

Erythrocytes types	Titre values
Untreated erythrocytes	1:64
Papain treated erythrocytes	1:32
Trypsin treated erythrocytes	1:512

Rabbit erythrocytes was high heamagglutination activity than other erythrocytes . The lectin was found to be non-blood type specific because they agglutinate all types of human and animal erythrocytes. Then heamagglutination activity towards papain, trypsin treated and untreated rabbit erythrocytes according to this result the trypsin treated erythrocytes was increase the heamagglutination activity. *Parkia javanica beans* also depend on pH ,the optical pH value was 7,it was stable in the pH range 7-10 and more acidic and basic pHs decreased both stability and activity (Utarabhanda and Akkayanont, 1995).The optimum pH of this lectin to agglutinate rabbit erythrocytes was pH 7 to 8, the activity was reduced below 7 and above 9. Malpezzi and Freitas, 1991, demonstrated the relatively long time of incubation (even over min) at 75^{o}C did not affect the hemolytic activity of the crude venom of the *Bunodosoma caissarum*. Hemolytic activity over the temperature of this sample range of 30-90^{O}C.Hemolytic activity of this sample was found to be independent of calcium ions. A number of mono and disaccharides were tested for their ability to inhibit the agglutination reaction between the erythrocytes and the lectin. In this sample was completely inhibited by L- rhamnose. Galactose binding specificity is also characteristic of the lectin isolated from *Bauhinia purpurea* (Young *et al.,*1985). Molecular weight determined by gel filtration reported the lectin from *Artocarpus hirsute* to have a molecular weight of 45kDa (Antony *et al.,*1989). *BmL* was purified by gel filtration chromatography on Sepadex $G_{75.}$ The purified lectin was estimated 210kDa on native PAGE. By comparing the molecular masses of the native protein and its subunits, indicating that BmL was an oligomeric protein 210kDa made up from two distinct subunits160kDa and 36kDa, without any covelent disulfide bonds between the subunits.

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