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Isolation and Characterization of Carbohydrate Binding Protein (Lectin) from Sesame Oil Seed (*Sesamum indicum, Till*)

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Abstract: The carbohydrate binding protein (Lectin) of the sesame oil seeds have been purified from the seed homogenate by 100% ammonium sulfate precipitation followed by gel filtration on Sephadex G-75 and ion exchange chromatography on DEAE-cellulose. The purified lectin was found to be homogeneous in polyacrylamide gel electrophoresis. The protein has a molecular weight of 25 kDa. Both in the disc and SDS-PAGE analysis only a single band was observed indicating that the protein does not contain any subunit. The protein is agglutinated with erythrocytes of albino rat. The binding of the protein to erythrocytes of albino rat was inhibited by mannose and glucosamine indicating that the protein possesses two binding sites for carbohydrates. The percentage of sugar present in the glycoprotein was found.to be about 10%. The reducing sugar in the hydrolysate of the protein was identified to be galactose. The pH stability of the protein was studied. The protein showed its hemagglutination activity in the pH range 6.5 to 8.4.

Key words: Galactose-specific lectin, hemagglutination, sesame seed

Introduction

Sesame is widely cultivated in most of the countries of Asia and Africa. In Bangladesh, it is mainly cultivated in the fertile land of Chor and Bill area (alluvial deposit). It is used in confectionery and for making margarine. It can be used in the manufacture of soaps, cosmetics, perfumes, insecticides and pharmaceutical products.

The lectins are a glycoproteins, first recognized in the seeds of plants have multiple binding sites and agglutinate animal cells. Many plant lectins have been purified and their structures and binding properties have been well characterized (Smith and White, 1966). Lectins are found in Brassica oil seeds, Lin seeds, Sesame oil seeds and other plant tissues. Lectin can be involved in hemagglutination, lymphocyte transformation, inactivation of certain types of tumor cells and precipitation of certain polysaccharides and lipoproteins (Novogrodsky and Katchalski, 1971). Lectins are being used increasingly to probe the structure of carbohydrates on the surfaces of normal and malignant cells (Absar and Gunki, 1994). Lectin is also used to detect glycoprotein abnormality on membrane in the fields of tumor lectinology and oncology.

Many researchers (Nath and Giri, 1957) have studied detail in the composition of sesame oil seeds and reported that it contains a significant amount of protein. But no report is available so far regarding the nature and specific properties of proteins specially the presence of lectins in sesame seed. The present work aims to purify a lectin from sesame seed in biologically active form and characterize it. We determined the molecular weight of the protein in addition to its subunit structures, agglutinating activity, pH and temperature stability and sugar specificities.

Materials and Methods

Sesame seed (*Sesamum indicum*, *Till*) were collected from Rajshahi local market in the month of December-January. Sephadex G-75 and DEAE-cellulose were obtained from Pharmacia fine Chemicals Co. Glucose, Mannose, Galactose and Arabinose were obtained from Sigma Chemical Co., USA. Bromophenol blue, Coomassie brilliant blue R-250 were purchased from Bio-Rad Laboratories. All other reagents are of analytical grade and were used without further purification. Unless otherwise specified, all operations were performed at 4°C.

Preparation of crude extract: The seeds were finely crushed into powder using a homogenizer (model AM-5). The oily extract was then homogenized uniformly with petroleum ether (40 to 60°C) and kept in a beaker at 2 to 4°C for an hour with occasional stirring. The homogenate was then filtered through a clean muslin cloth. The filtrate was further clarified by centrifugation using refrigerated centrifuge (model-HSO2) at 8000xg for 10 minutes. The residues obtained from filtration and centrifugation were collected and air dried at room temperatures.

The fat free dry powder of sesame seed was mixed uniformly with pre-cooled 1% acetic acid and kept overnight at 4°C with occasional stirring. The suspension was then filtered through cloth in the cold room. The filtrate was collected and clarified further by centrifuging in a refrigerated centrifuge at 8000xg for 15 minutes. The clear supernatant was collected and adjusted to 100% saturation by adding solid ammonium sulfate with continuous stirring. The precipitate obtained was collected by centrifugation, dissolved in minimum volume of deionized water and dialyzed against distilled water for 24 hours at 4°C with several changes. After centrifugation the clear supernatant was used as crude extract.

Gel filtration: The crude extract, after dialysis against 50 mM borate buffer, pH 8.4 for 24 hours at 4°C was applied to a column of Sephadex G-75 preequilibrated with the same buffer. The column was washed with the same buffer at a flow rate of 25 ml/hr and 3 ml fractions of the eluate were collected using a fraction collector (Model-SF-160 Advantec, Japan). Fraction containing the proteins were collected, pooled and dialysed against distilled water.

DEAE-cellulose chromatography: The protein obtained from gel filtration was dialyzed against 10 mM Tris-HCl buffer, pH 8.4 for 24 hr at 4 °C. The protein was then applied to a DEAE-cellulose column preequilibrated with the same buffer. The proteins were eluted from the column by NaCl gradient (0.5 to 1.0 M) in the 10 mM Tris-HCl buffer, pH 8.4.

Polyacrylamide disc gel electrophoresis: Polyacrylamide disc gel electrophoresis was conducted at room

temperature, pH 8.4 on 7.5% gel as described by Ornstein (Ornstein, 1964) and 1% amido black was used as staining reagent.

Characterization of protein

Molecular weight determination: The MW of the protein was determined by SDS-polyacrylamide gel electrophoresis on 10% acrylamide gel according to Weber and Osborn (Weber and Osbarn, 1966) using lysozyme, egg albumin, α-amylase, 13-galactosidase, bovine serum albumin and trypsin inhibitor as reference protein.

Subunit structure determination: For Subunit structure determination, SDS-polyacrylamide gel electrophoresis was conducted on 10% acrylamide gel and the marker protein used were same as those used in the molecular weight determination. Dissociation and reduction of protein were performed by heating the protein for 5 minutes at 100°C in aqueous 0.1% SOS and 0.1% P-mercaptoethanol.

Hemaggiutination and hemagglutination-inhibition assay: Hemagglutination was studied using 4% albino rat red blood cells as described by Lin *et al*. (1981). Protein solution (0.2 ml) in 5 mM phosphate buffer saline, pH 7.2 was mixed with 0.2 ml of 4% rat red blood cells and incubated at 37°C for one hour. The degree of hemagglutination was observed under microscope. Hemaggutination inhibition was performed in the similar way in presence of 0-galactose, D_-glucose, D-glucosamine, D-mannose and 0-arabinose.

Protein and carbohydrate analysis: The concentration of protein was measured by the method of Lowry *et al*. (1951) using BSA as the standard. The presence of sugar in the protein was detected by Periodic acid-Schiff's method (Andrew, 1986) and total neutral carbohydrate contents of the proteins were estimated by phenol-sulfuric acid method of DuBois *et al*. (1956) with D-glucose as the standard. For identification of sugars the proteins were hydrolyzed with 1 M FICI for 4 hours at 100°C under vacuum and the sugar component was determined by the one-dimensional TLC method of Joseph and Murrell (Touchston and Dobbins, 1978) using different standard sugars. The chromatogram was developed with 3:1:1 ratio of isopropanol, acetic acid and water and the spots were identified by spraying with aniline phthalate solution.

pH stability: For pH stability, 50 mM acetate buffer of pH 5.0, 5.5 and 6.0, 50 mM phosphate buffer of pH 6.5, 7.0 and 7.5, 50 mM Tris-HCI buffer of pH 8.0 and 8.4, 50 mM borate buffer of pH 9.0 and 9.5 and 50 mM glycine buffer of pH 10.0 were used. The pH of the purified protein solution was adjusted to a particular value of the pH range 5 to 10 by using suitable buffer. The hemagglutination activity was recorded for each of these protein solution with specific pH value.

Results

The 100% ammonium sulfate saturated crude protein extract after dialysis against 50 mM borate buffer, pH 8.4 was applied to a Sephadex G-75 column at 4°C. The proteins was eluted as a two broad main peak, fraction, F-1 and fraction F-2 (Fig. 1). The active fraction, F-2 as indicated by solid line, was pooled, precipitated to 100% saturation by ammonium sulfate and further purified by DEAE-cellulose chromatography (data not shown). The homogeneity of the protein was checked by polyacrylamide gel electrophoresis (Fig. 2a). A single band was observed for the protein indicating that the protein is pure.

The molecular weight of the protein was calculated by comparing the mobility of the purified protein with that of the reference proteins. The molecular weight of the protein was estimated to be 25 kDa (Fig. 2b). When treated with 1% P-mercaptoethanol and then subjected to SDSelectrophoresis, only one slightly spread band was observed indicating that the protein does not contain subunit structure (Fig. 3). Both in the disc and SDSpolyacrylamiele gel electrophoresis, the protein gave single band. From these result, it is clear that the protein is a single strand polypeptide.

Hemagglutination and hemagglutination-inhibition assay: The hemagglutination test of the protein was performed on albino rat RBC. As shown in the Fig. 4, the protein agglutinated albino rat RBC. The minimum hemagglutination dose for the protein was determined by mixing 0.2 ml 4% washed rat RBC in 5 mM phosphate buffer saline containing 0.9% NaCI, pH 7.2 and 0.2 ml protein solution of varying concentration. Hemagglutination was observed after 1 to 2 hours incubation at 37°C. The results of the hemagglutination inhibition test of sesame oil seed proteins are presented in the Table 1.

Table 1: Hemagglutination Inhibition of the proteins from sesame oil seeds by different sugars

Sugar	Protein sample	Inhibiting hemagglutination
Glucose		N
Galactose		N
Mannose		I
Arablnose	Purified protein	N
Glucosamine		

N = No inhibitory
I = Inhibtory

It was observed that hemagglutinating activity of the protein was inhibited mainly in the presence of D-mannose and D-glucosamine. On the other hand, glucose, arabinose and galactose could not inhibit agglutination of the protein rather enhanced the agglutination of the RBC.

Carbohydrate composition: The protein showed yellow-orange colour in the presence of phenol sulfuric acid indicating that it contains sugar. The sugar composition of the protein was identified by TLC and was found to be galactose.

pH stability: As shown in the Table 2, the protein showed agglutinating activity in the pH range 6.5 to 8.4. From the table it is also clear that below pH 6.5 and above pH 9.0, the protein did not show agglutinating activity.

Table 2: Hemagglutination activity of the protein for sesame oil seeds at different pH values

Sample	pH	Hemagglutinating activity
	5.0	-
	5.5	-
	6.0	-
	6.5	+
	7.0	+
Purified protein	7.5	+
	8.0	+
	8.4	+
	9.0	-
	9.5	-
	10.0	-

(-) = No activitly
(+) = Activitly

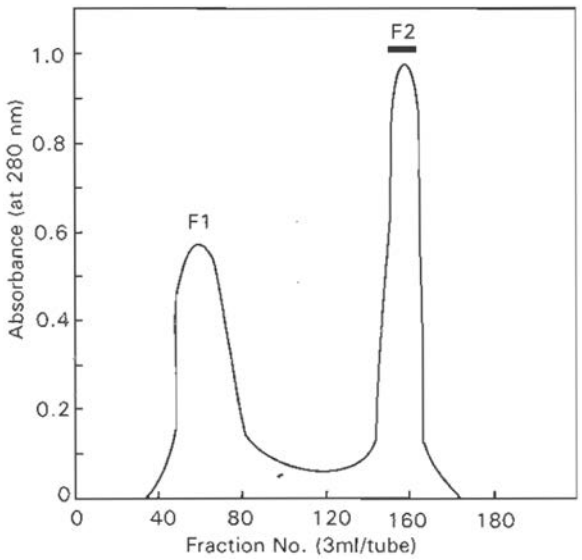

Fig. 1: Fel filtration of 100% $(NH_4)_2SO_4$ saturated crude protein extract on Sephadex G-75. The crude extract was applied to the column pre-equilibrated with 50 mM borated buffer, pH 8.4 and eluted with the same buffer. -indicates active fraction

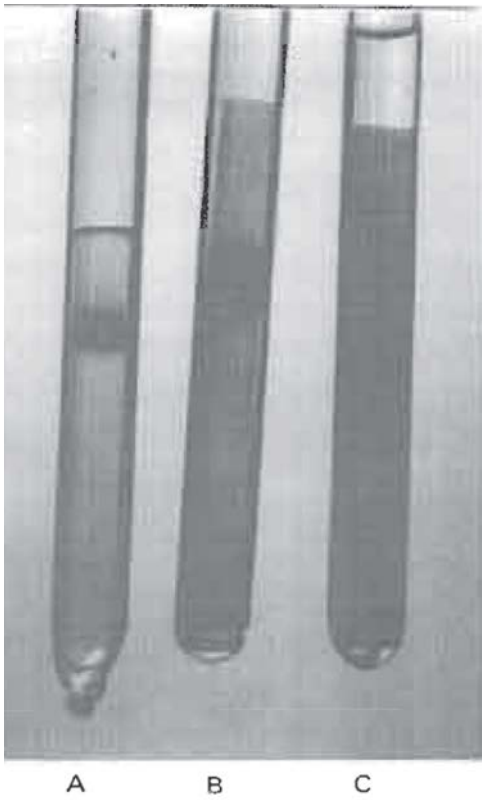

Fig. 2a: Polyacrylamide disc gel electrophoresis of the protein at room temperature on 7.5% gel (stained with 1% amido black). A = Indicates crude, B = Indicates after gel chromatography and C = Indicates after DEAE-chromatography

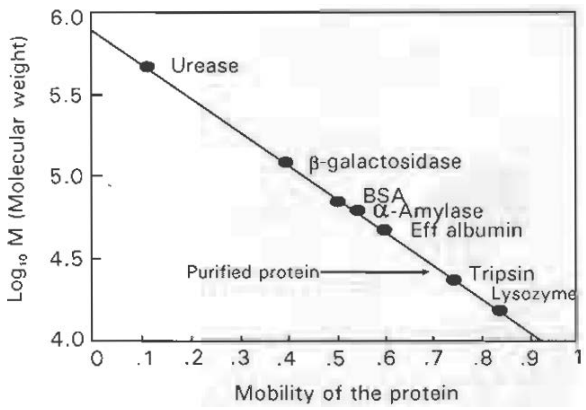

Fig. 2b: Standard cruve for the determination of molecular weight of protein by SDS polyacrylamide gel electrophoresis. The purified lectin indicated by the arrow

Fig. 3: SDS-PAGE pattern of the protein for the determination of molecular weight and submit structures

Discussion

The carbohydrate binding proteins (lectin) have been isolated and purified from the crude extract of the sesame oil seeds and the lectin are glycoprotein in nature as they gave orange yellow colour in the presence of phenol-sulfuric acid. The SDS-polyacrylamide gel electrophoresis, in presence of 13-mercaptoethanol clearly revealed that the lectin has no subunit. The agglutination of rat red blood cells by the lectin was inhibited specifically in the presence of mannose and glucosamine. On the other hand, glucose, arabinose and galactose could not inhibit agglutination of the protein rather enhanced the agglutination of the RBC.

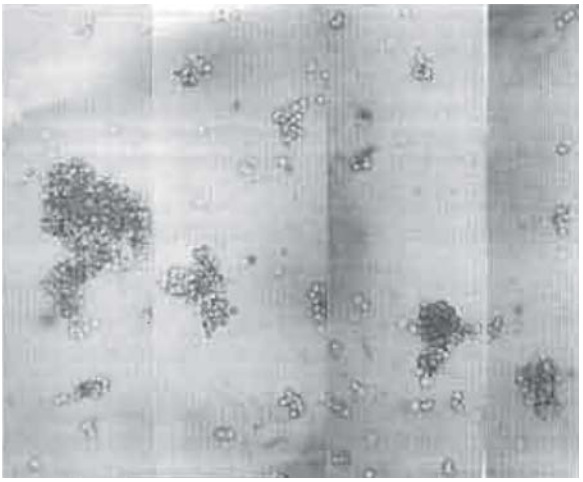

Fig. 4: Photographic representation of hemagglutination of RBC from albino rat by purified protein from sesame oil seed

The results suggest that the lectin agglutinate rat erythrocytes by binding to mannose and glucosamine containing carbohydrates on the cell surface. The pH stability of the protein was determined. The protein showed agglutinating activity in the pH range 6.5 to 8.4. The protein samples dialyzed at pH values below 6.5 and above 9.0 did not show agglutinating activity even at pH 7.0 for 24 hours. The results indicate that the activity of the protein is nat reversible.

At present we are studying about the toxicity of lectin. It should be interesting to characterize the carbohydrate binding protein (lectin) of the sesame oil seed in detail. lh our knowledge, this is the first report so far regarding the nature and specific properties of protein specially, the lectins in sesame seed.

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