Biosciences, Biotechnology Research Asia Vol. **7**(1), 489-492 (2010)

Studies on lectin from *Glycine max* (L.) Merrill

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(Received: March 10, 2010; Accepted: April 16, 2010)

ABSTRACT

A plant lectin isolated in its pure state from the Indian of soya bean seeds produced single band in SDS–PAGE (30kDa) and one peak by gel filtration chromatography on Sephadex G-100, corresponding to 120 kDa. SBL is a glycoprotein bound with glucose and mannose (2 mol/mol of protein) and stabilized by 4 atoms of each of Ca^{2+} and Mn^{2+} per subunit. The soya bean lectin exhibited haemagglutinating activities in various degrees against human ABO type, rabbit and rat erythrocytes. Maximum activity was observed against rabbit and human AB blood groups. Among the various tested sugars, SBL agglutination was most inhibited by N-acetylgalactose. Hemagglutination was markedly affected by acidic pH, but was heat stable below 60ºC for 30 min. The haemagglutinating activity of the soya bean lectin was inhibited by addition of Ca^{2+}, Mg^{2+}, Mn^{2+} and EDTA. SBL is rich in hydroxyl amino acids while totally lacking sulfur amino acids.

Key words: Soya bean lectin, Hemagglutination activity

INTRODUCTION

Lectins are naturally occurring glycoproteins that bind carbohydrate residues selectively and non-covalently (Van Damme *et al*., 1998). Lectins can be found in all kingdoms of life ranging from viruses, bacteria and plants to animals (Loris, 2002). Lectins are protein that interact with cell surfaces and cause cells to agglutinate. Some lectins have been shown to have a greater capacity to agglutinate transformed cells than normal cells, and are being used to investigate the differences between the surfaces of these cells. Legume lectins are the largest and most thoroughly studied, family of the simple lectins (Sharon and Lis, 1972; Bog-Hansen, 1981. The genus *Glycine max* (family leguminosae, subfamily Paplionaceae) are grown fairly throughout in India, In this aspect, soya bean is an important legume as it has high protein content and nutritionally balanced amino acid profile. The reasonable price and steady supply are also favourable factors in soya beans emerging as an important source of protein in animal nutrition (Olguin *et al*., 2003) However, the nutritional value of soya bean meal is much lower than expected, in spite of its protein content and amino acid profile was largely attributed to antinutritional factors (Pusztai, 1991). Soya bean flour also contains several haemagglutinating isolectins. Their presence in the diet is known to reduce the growth rate of young monogastric animals (Van Damme *et al*., 1998). As parts of general programme on the study of biologically important proteins from legumes are used in the Indian dietary, the work on soya lectins has been initiated. In the present study, successful efforts have been made to remove the lectins from soya bean flour and recover these value-added products.

MATERIAL AND METHODS

Soya bean seeds were collected from various regions of Tamil Nadu and the collected

specimens were authenticated by the Department of pulses, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Human blood cells (A, AB, B and O) of healthy donors and Rabbit, rat, and sheep blood cells were obtained from the animal house of Veterinary College, Chennai.

The lectin was purified from plant materials according to Rudiger protocol (1993). The precipitate (the prepurified extract) was dissolved in a minimum amount of the buffer and extensively dialyzed against the same buffer. The prepurified extract was applied to the Sephadex G-100 column (100 h x 2.5 dia cm) at flow rate of 60 ml/h. The column was washed with the buffer at the same speed until the A280 fell down to <0.05. The buffer was exchanged by the eluting buffer (0.25 M Galactose, and 0.02% NaN_3) to desorb the lectin from the column. The fractions containing lectin were combined on the basis of A280, dialyzed against water, frozen and lyophilized.

The measurement of protein content in different fractions was described by Bradford (1976), using bovine serum albumin (BSA) as standard. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) was performed in discontinues system with 12.5% separating and 5% stacking gels according to the method of Laemmli and Favre (1973).

Serial two fold dilutions of purified soya bean lectin (10 mg/ mL) in phosphate buffer saline (PBS) (50 µl) were incubated with 50 µl of 4% erythrocyte suspension in V-shaped micro titer plates and after incubating the plate for 1h at 4°C the haemagglutination titer was scored visually. Haemagglutination-inhibition assays with the purified soya bean lectin were done using the same procedure with different concentrations of sugar solution. The haemagglutination unit (HU) was expressed as the reciprocal of the highest lectin dilution showing detectable visible erythrocyte agglutination and the specific activity was calculated as HU/mg protein.

The effect of pH, temperature and EDTA, Ca^{2+} and Mn^{2+} on lectin hemagglutinating activity of the purified soya bean lectin was determined. The lectins were hydrolysed with 1 Í HCl in *vacuo* for 2 h at 110°C and analysed for amino acid and carbohydrate analysis followed by HPLC analysis.

RESULTS AND DISCUSSION

The lectin could be successfully purified in a single step by affinity chromatography on Sephadex G-100. Loading of prepurified extract on an affinity column followed by washing out the unbound proteins then eluting the bound lectin with 250 mM glucose led to increments in the specific activity up to 302 titer/mg corresponding to 72 % yield. The final lectin yield was ca. 135 mg per 100 g dry seed weight. This value is exactly the same as obtained by Lotan *et al*. (1974) and Vretblad (1993). A purified soya bean lectin (SBL) moved as a single band in SDS-page and the approximate molecular weight was found to be 30 kDa (Lis *et al*., 1966; Franco-Fraguas *et al*., 2003).

SBL showed specificity in its ability to hemagglutinate human (A, B, AB and O) erythrocytes and indiscriminately agglutinate rabbit, rat and chicken. However, hemagglutinating activity against chicken erythrocyte was comparatively the lowest one. This difference in the agglutination activity may be due to the nature of the glycoproteins protruding on the cell surface, which are weakly or not totally recognized by the lectin (Oliveria *et al.,* 2002).

Mono and disaccharides inhibited the agglutinating activity of SBL and the maximum inhibition was observed with N-acetyl- D- galactose and raffinose showed the least degree of inhibition of the agglutinating activity of SBL. The IC_{50} value of N-acetyl- D-galactose was very low as compared with other sugars. It is known that soya bean lectin i.e. SBL is specific for N-acetyl- D- galactosamine, which provide an axial 4-OH group for binding (Goldstein and Portez,1986).

The amino acid composition of the purified lectin, generally characterized by high levels of hydroxyl amino acids and low/absence of sulphur containing amino acids and high levels of hydroxyl amino acids, is in accordance with the results of Wayne Gade *et al*., (1981). SBL from soya bean is a glycoprotein consisting mostly glucose, mannose and glucosamine are present at the following

content, 5-8 moles of mannose, 1-2 mole of glucose per mole of lectin. A similar observation was also made by Lis and Nathan Sharon (1977).

Soya bean lectin showed tolerance to a wide range of pH between 5.0 and 8.0. In acidic pH, there is a loss of activity. Similar observations have been made by Damico *et al*. (2003) for the seed lectins of *Annona muricata* and by Mahmoud Sitohy *et al*., (2007) for soya bean lectins. Maximum activity for SBL was found to be at neutral pH 7. At this pH, the lectins are reported to exist as tetramer (Loris *et al*., 1998; Srinivas *et al*., 2001). The relatively lower reduction of activity, at the basic pH values may be due to some degree of base induced denaturation. Thermal denaturation studies with SBL showed its stability against high temperature for prolonged periods. The SBL remained intact without losing its hemagglutinating activity to a significant extent at 60-70°C for nearly 90 minutes. However, the lectin was significantly stable at temperatures below 60°C. Temperatures above 60°C marginally reduced the activity but not to the level of inactivation. The loss of hemagglutinating activity with increasing temperature is evidently due to heat-induced denaturation of the lectin. This denaturation may expectedly weaken the interaction between lectin and the carbohydrate ligand (Schwarz *et al*., 1993) leading consequently to attenuated agglutination activity. The (SBL) hemagglutinating activity remarkably decreased after metal removal by prolonged dialysis against 50 mM EDTA, followed by dialysis against 0.15M NaCl; however, when Ca^{2+} and Mn^{2+} (50 mM) were readded to the assay medium, the SBL activity was fully restored.

CONCLUSION

Soya bean lectin is a glycoprotein with glucose and mannose and stabilized by 4 atoms of each of Ca^{2+} and Mn^{2+} per subunit. Hence, SBL is a tetramer. SBL is rich in hydroxyl amino acids while totally lacking in sulphur amino acids. The purified soya bean lectin fractions showed antifungal activity against *Fusarium oxysporum* and *Pyricularia oryzae*. Maximum activity was observed against *Pyricularia oryzae*.

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