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EXTRACTION, PURIFICATION AND CHARACTERIZATION OF LECTIN FROM *PHASEOLUS VULGARIS* L. CV. WHITE SEEDS (WHITE KIDNEY BEAN)

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Abstract

The purpose of the research was to study the purification and characterization of lectin from Phaseolus vulgaris L. cv.white seeds. The lectin was purified by sequence of steps , namly , first with ammonium sulfate precipitation followed by ion exchange (DEAE cellulose) and gel filtration (sephadex G-200) chromatographies , and finally by polyacrylamide electrophoresis (PAGE). Single band was observed in native PAGE . The lectin was shown to have molecular weight of 33 kDa in SDS PAGE and about 35 kDa in gel filtration and purified about 9.01 fold to final specific activity of 64 titer/mg of protein. The hemagglutination activity of the lectin was stable within the pH range from 4-11 and temperature range from 0°-50°C. Chemical modification results indicate that lysine and tryptophan were crucial for the hemagglutination activity of lectin. The results of carbohydrates specificity showed that the lectin was had complex sugar specifities, but not specific to xylose and mannose.

Keywords: *lactin, purification, characterization, Phaseolus vulgaris L.*

INTRODUCTION

Lectins are defined as proteins/glycoproteins possessing at least one non-catalytic domain which binds reversibly to a specific mono-or oligosaccharide (Van damme et al., 2003). Over the last few decades, lectins have become a topic of interest to a large number of researchers owing to their potentially exploitable biological properties including antitumor (Abdullaev et al., 1997; Ye et al., 2001), immunomodulatory and anti-insect (Rubinstein et al., 2004), antifungal (Barrientos et al., 2005), antibacterial (Pusztai et al., 1993), anti-HIV (Tsang et al., 2001; Barrientos et al., 2005; Pollicita et al., 2008), and mitogenic (Wimer, 1990) activities. Because of their sugar binding properties, lectins have been extensively studied and used as molecular tools for the study of carbohydrate architecture and dynamics on the cell surface, and have been exploited for such practical applications as distinguishing between normal and malignant cells (Sharon, 1993; Padma et al., 1998), purification of glycoconjugates (Yamamoto et al., 1984), and coating of drugs to enhance their gastrointestinal tract absorption (Naisbett & Woodley, 1990; Leher, 2000). Further, specific amino acid residues are essential for maintaining the carbohydrate binding and hemagglutinating activities of lectins (Bao et al., 1996; Ba˘i˘miev et al., 2007). Identification of these amino acid residues is a prerequisite for investigating the structure-function relationships of lectins. Chemical modification with group-specific modifying agents provides a general approach for identification of the amino acid residues present in the functional or active site of proteins, including lectins (Bao et al., 1996; Nadimpalli, 1999). Hence, elucidation of biological activities of lectins and amino acid residues essential to these activities is a meaningful undertaking. Although lectins are found ubiquitously in plant species, they have variable structures and specific activities according to the plants they originate from cells (Sharon, 1993; Padma et al., 1998). Thus, purification and characterization of lectins from a variety of plant species interests researchers in the field of glycobiology. The more is known about the lectins, the wider the applications of this type of proteins that can be achieved. This study reports the purification and some properties of a new lectin isolated from seeds of the *Phaseolus vulgaris* L. cv.white cultivar (common name, white kidney bean). To date, the isolation of a lectin from the *Phaseolus*

vulgaris L. bean and examining it for various potentially exploitable biological activities such as mitogenic, antitumor, immunomodulatory, and HIV-1 reverse transcriptase inhibitory activities have not been attempted. In this study, a lectin was isolated from Phaseolus vulgaris L. beans. It was assayed for the various aforementioned activities. In order to further characterize the lectin, a chemical modification study was undertaken to determine the involvement of different amino acid residues in its hemagglutinating activity.

MATERIALS AND METHODS

2.1. Materials

Phaseolus vulgaris L. cv.white (White kidney bean) from local source, Hilla, Babylon, Iraq, human red blood cells (healthy persons), ammonium sulfate, DEAE cellulose, sephadex G-200, PAGE, electrophoretic reagents were purchased from Sigma (USA), 5mMphenylmethylsulfonyl fluoride (PMSF), 5, 5-dithiobis- (2 nitrobenzoic acid (DTNB), NaBH4, Nbromosuccinimide (NBS) were obtained from Himedia (India), BSA, alkaline phophatase, RNase and Trypsin obtained from Biobasic .

2.2. Methods

2.2.1. Isolation of Phaseolus vulgaris lectin

By using, the soaking Method white kidney beans were ground to a powder in filtered through 80-mesh grit. The powder (5 g) was mixed with 0.15M NaCl (1:8, w/v) for 48 h at 4^0C, and filtered through 80-mesh grid. Subsequently, the filtrate was centrifuged at 9168×g for 30 minutes, and the supernatant was fractionally precipitated with ammonium sulfate at 10%-100% saturation, respectively. The four pellets were combined, dissolved in a minimal volume of water, and dialyzed against distilled water at 4°C (Yufang et al., 2010).

2.2.2. Determination of protein concentration.

Determination of Protein Concentration. Bradford′s method (Bradford, 1976) was used for protein quantification, using bovine serum albumin (BSA) as the standard.

2.2.3. Hemagglutinating activity assay.

Serial two-fold dilutions of the lectin solution in microtiter v-plates (25 µL) was mixed with 25 µL 2% human red blood cell suspension in saline (pH 7.2). Readings were recorded after about 30 minutes at room temperature, when the blank had fully sedimented. The hemagglutination titer, defined as the reciprocal of the highest dilution exhibiting hemagglutination, was treated as one hemagglutination unit. Specific activity was expressed as the number of hemagglutination units per mg protein (Yufang et al., 2010).

2.2.4. Purification of Phaseolus vulgaris L. cv.white lectin (White Kidney Bean).

Purification of white kidney beans lectin firstly at precipitation by ammonium sulfate at 10%-100% saturation rate to Crude extract from the soaking and then dialyzed against distilled water,then loaded on a DEAE cellulose column (2.6 cm × 60 cm) that had been equilibrated with the same buffer, and subjected to ion exchange chromatography. The column was washed initially with 0.1M NaCl in 0.02M Tris-HCl (pH 8.0) to remove proteins that had not specifically absorbed to the column, then washed with linear salt gradient elution. Fractions showing hemagglutinating activity were further purified by sieve chromatography on a Sephadex G-200 column in 0.15M NaCl in 0.02 M Tris-HCl (pH 8.0).

2.2.5. SDS-PAGE.

SDS-PAGE. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in accordance with the method of Laemmli (Laemmli, et al., 1973) using a 15% separating and a 10% stacking gel.

2.2.6. Molecular mass determination.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in accordance with the procedure of Laemmli and Favre (Laemmli, et al., 1973). The molecular mass of the lectin was estimated from the standard curve plotting electrophoretic mobility against molecular mass. Gel filtration was carried out using a Sephadex G-200 column that had been calibrated with molecular-mass standards proteins (BSA, Alkaline phophatase, RNase and Trypsin) to estimate the molecular mass of the purified lectin.

2.2.7. Sugar specificity.

The Sugar specificity investigate inhibition of lectin-induced hemagglutination by various carbohydrates were performed in a manner analogous to the hemagglutination test. Prepare 1-100 mM of each sugar samples was prepared

in phosphate-buffered saline. All of the sugar samples were mixed with an equal volume (25 µL) of a solution of the lectin. The mixture was allowed to stand for 30 minutes at room temperature and then mixed with 50 µL of a 2% human erythrocyte suspension. The known sugar which gives agglutination with blood suspension and no precipitate of red blood cell that means the tested lectin specific to that sugar type (Wang et al., 2000).

2.2.8. Effect of temperature on purified lectin-induced hemagglutination.

The effect of temperature on hemagglutinating activity of the purified lectin was examined as previously described (Wang et al.., 2003). A solution of the lectin was incubated at various temperatures for 30 minutes: 0°C, 10°C, 20°C, 30°C, 40°C, 50°C, 60°C and 70°C. The tubes were then put on ice, and assay of hemagglutinating activity was then carried out.

2.2.9. Effect of pH on purified lectin-induced hemagglutination.

The pH stability of the lectin was determined by incubation of the lectin (1 mg/mL) in buffers of different pH values ranging from pH 4.0–12.0 for 60 minutes. The pH of the lectin solution was adjusted to 7.0 by the addition of 0.1N HCl or 0.1N NaOH before hemagglutination activity was determined (Yufang et al., 2010).

2.2.10. Effect of chemical modification of amino acid residues on hemagglutinating activity.

For serine modification, the lectin (100 µg) in 0.1mL of 50mM Tris-HCl buffer (pH 7.4) was incubated with 5mMphenylmethylsulfonyl fluoride (PMSF) at 27°C for 1 hour (Habeeb, 1966). Aliquots were removed at 15 minutes intervals.followed by determination of residual hemagglutinating activity. Lectin incubated without PMSF served as a control.

Reduction of the thiol groups of white kidney beans lectin was carried out by incubating the lectin (100 µg) in 0.1mL of 50mM phosphate buffer (pH 8.0) with 0.1mM 5, 5-dithiobis- (2-nitrobenzoic acid (DTNB) at 27°C for 1 hour. Aliquots were removed at different time intervals, followed by determination of residual hemagglutinating activity. Lectin incubated in the absence of DTNB served as a control (Fraenkel, 1957).

For lysine modification, 0.5 mg of NaBH4 was added to the lectin (5 mg) in 2mL of 0.2M sodium borate buffer (pH 9.0) at 4°C, followed by six aliquots (5 µL each) of 3.5% formaldehyde at 10 minutes intervals. Excess reagent was removed by ultrafiltration. Lectin incubated in the absence of sodium borohydride (NaBH4) served as a control (Mean & Feeney, 1968).

Modification of tryptophan residues was carried out according to the method of Spande and Witkop (Spande & Witkop, 2006). The lectin was dissolved in NaOAc buffer (0.1 M, pH 5.0) to 1mg/mL. The modification was carried out at 20°C. Nbromosuccinimide (NBS) (10 µL, 10mM) was added every 5 minutes and then assay of hemagglutinating activity was carried out. Lectin incubated in the absence of NBS served as a control.

RESULTS AND DISCUSSIONS

Soaking Method showed spicfic activity about 7.1 titer/mg of white kidney beans. The protein concentration was calculated based on the regression equation (Figure 1), and results showed protein concentration about 2.74 mg/ml by precipitation by ammonium sulfate at 70% saturation rate. Purification of white kidney beans lectin show two fractions were obtained from ion exchange chromatography with DEAE cellulose (Figure 2) just the first peak showed hemagglutination activity about 45.5 titer/mg,so just the first peak concentrated and loaded on sephadex G-200 to gel filtration step which show only one peak with hemagglutination activity about 57.07 titer/mg (Figure 3), the volume obtained from peak of sephadex G-200 was concentrated and reloaded on sephadex G-200 to improved the purification step of lectin and the result of second loading of white kidney beans lectin on sephadex G-200 is also one peak show specific activity as 64 titer/mg (table 2). The peak of second loading of gel filtration was dialyzed and concentrated, then stored at-20°C. The purified lectin (fraction I in Figure 3) formed a single band with a molecular mass of about 33 kDa in SDS-PAGE electrophoresis and three bands was showed in results of ione exchange electrophoresis (Figure 4), which confirmed the effectiveness of the purification method used and about 35.5 kDa in gel filtration method to determination molecular mass.

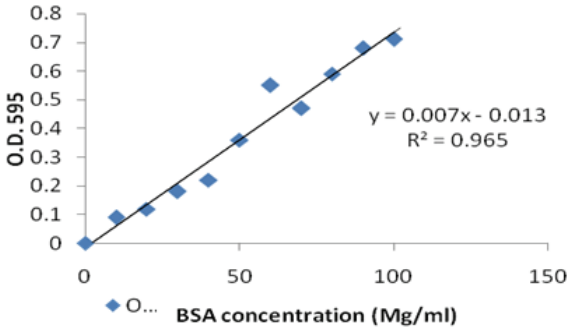


Figure 1. Standard curve of bovine serum albumin

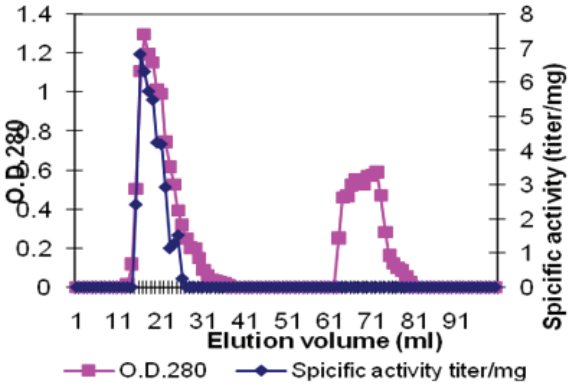


Figure 2. Fractionation of the crude extract of Phaseolus vulgaris L. cv.white bean lectin by DEAE cellulose ion-exchange chromatography equilibrated with 0.1M NaCl in 0.02M Tris-HCl (pH 8.0). The column was washed initially with the same buffer to remove proteins that were not specifically absorbed to the column (data not shown on the figure) then washed with buffer in which the NaCl concentration increased linearly from 0.1M to 1.0M. Tow peaks were obtained, among which peak I exhibited hemagglutinating activity. Column: 2.5 cm × 50 cm; flow rate: 0.3 mL/min.

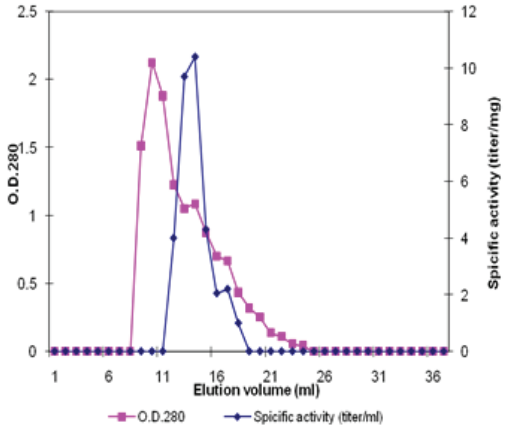


Figure 3. Fractionation of peak I on a Sephadex G-200 column with 0.15M NaCl in 0.02M Tris-HCl (pH 8.0). Column: 2 cm × 50 cm; flow rate: 0.5 mL/min. Only the major peakv exhibited hemagglutinating activity

The hemagglutinating activity of purified white kidney beans lectin could not be inhibited by many of the simple sugars tested at 1−100 mM of D (+) glucose, D (+) galactose, D (+) fructose, D (+) lactose, (+) ribose, D (+) arabinose, L (+) maltose, D (+) sucrose and N-acetylglucoseamine, but the hemagglutinating activity inhibited with D (+) xylose and D (+) mannose as show in figure (Figure 5).

The hemagglutinating activity was completely stable between 0°C and 50°C. Considerable loss in activity occurred at 60°C. Some activity was discernible at 60°C (Figure 6a). The lectin exhibited remarkable stability over the range of pH 4−10 and loss the activity at pH 11 (Figure 6b).

Residues, respectively, did not play any important role in its hemagglutinating activity. However, 85% loss of hemagglutinating activity after NBS treatment was noted, whereas no change in the control was detected. These results strongly suggest a considerable involvement of tryptophan residues in hemagglutinating activity, and stability of the lectin. NaBH4 treatment resulted in also 86% loss in hemagglutination activity suggesting partial involvement of lysine in the lectin activity. A concomitant drop in lectin activity was clearly seen upon modification of tryptophan residues.

The effects of various types of chemical modifications on hemagglutinating activity of the purified lectin are summarized in Table1, DTNB, reductive methylation and PMSF treatments did not produce any alterations in the hemagglutinating activity of white kidney beans lectin, suggesting that cysteine, cystine and serine

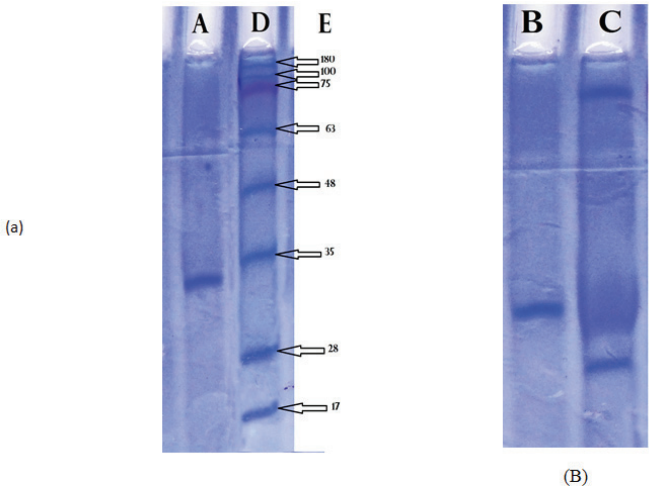


Figure 4. (a) Molecular weight determination of purified Phaseolus vulgaris L. cv.white bean lectin in SDS-PAGE electrophoresis. Line A: purified lectin. Line D: protein ladder line E: molecular weights (b) purification of lectin. Line C: crude lectin. Line B: purified lectine by gel filtration.

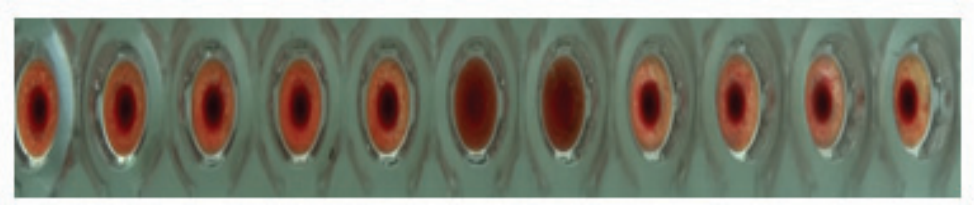
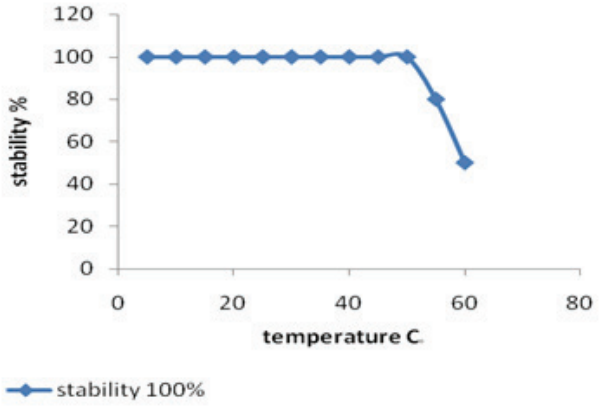


Figure 5. Sugar specificity of Phaseolus vulgaris L. cv.white bean lectin image showed the agglutination of red blood cell with (Glucose galactose lactose ribose arabinose xylose mannose fructose maltose sucrose Nacetylglucoseamine, respectively) xylose and mannose mean the tested lectine not specific for that simple sugar.



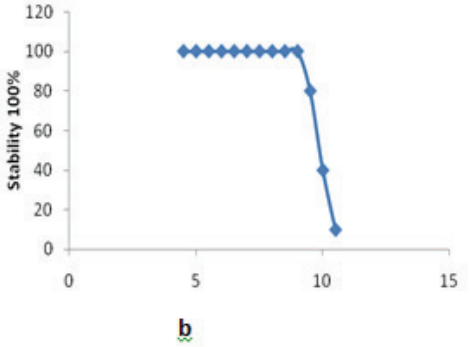


Figure 6. (a) Effect of temperature on hemagglutinating activity of Phaseolus vulgaris L. cv.white bean lectin. (b) Effect of pH on hemagglutinating activity of Phaseolus vulgaris L. cv.white bean lectin.

Treatment	Modified group/amino acid	% Hemagglutinating activity remaining
Phenylmethylsulfonyl fluoride (PMSF)	Serine	100
5, 5-Dithiobis-(2-nitrobenzoic acid(DTNB)	Thiol group	100
Reductive methylation	Lysine	14.2
N-Bromosuccinimide (NBS)	Tryptophan	14.9

Figure 7. Effect of chemical modification on hemagglutinating activity of *Phaseolus vulgaris L.* cv.white beans lectin.

Sample	H. U.	Total protein mg	Total activity titer	Specific activity Titer/mg	Fold	Yield %
Crude extract	6	27.4	192	7.1	1	100
Precipitation by ammonium sulfate at 70% saturation rate	12	65.3	24567	37.61	5.29	128
Ion exchange purification step	8	22.5	1024	45.5	6.41	5.3
Gel filtration purification step1	7	7.85	448	57.07	8.03	2.3
Gel filtration purification step2	7	7	448	64	9.01	2.3

Figure 8. Purification table

Lectins possess many bioactivities that have important practical applications, but the high price resulting from the low extraction rate restricts practical application of lectins. In this study, a plant lectin has been purified by three-step chromatography from seeds of the white kidney beans. The homogeneity of the white kidney beans lectin preparation was evidenced by the presence of a single band in SDS-PAGE. The results of SDS-PAGE and gel filtration chromatography together revealed that the lectin exists as a monomer of one subunit. The molecularmass and monomeric nature of Phaseolus vulgaris L. cv.white lectin are similar to those of Anasazi bean lectin and most of the other Phaseolus lectins (Tsang et al., 2001; Reynoso et al., 2003). On the other hand, it differs from a tetrameric 115–120 kDa lectin from tepary bean (Phaseolus acutifolius) (Reynoso et al., 2003). Phaseolus acutifolius var. latifolius lectin from which consists of four subunits of 21 kDa molecular mass (Vargas et al., 1987), and a tetrameric 94 kDa immunosuppressive lectin isolated from seeds of Phaseolus vulgaris L. cv. Cacahuate. (Vargas et al., 1993).

Lectins from some cultivars of Phaseolus vulgaris are oligomeric (Felsted et al., 1977), whereas Phaseolus vulgaris bean lectin is dimeric. Isolectins are absent in Phaseolus vulgaris L. cv.white beans but present in some cultivars of P. vulgaris such as red kidney bean (Felsted et al., 1977; Leavitt et al., 1977). The isolectins differ from one another by the number of erythrocytereactive (E) subunits and lymphotcyte-reactive (L) subunits that they possess. There are five such isolectins: L4, L3E1, L2E2, L1E3, and E4 (Yufang et al., 2010).

Although there is striking homology between white kidney beans lectin and other Phaseolus lectins in N-terminal sequence, white kidney beans lectin exhibit of some simple sugar specificity. Simple sugars as D (+) glucose, D (+) galactose, D (+) fructose, D (+) lactose, (+) ribose, D (+) arabinose, L (+) maltose, D (+) sucrose and N-acetylglucoseamine are not able to inhibit the hemagglutinating activity of white kidney beans.

White kidney beans lectin is fairly thermostable because its hemagglutinating activity is stable at temperatures up to 40°C, and is reduced only at 50°C. Interestingly, some activity remained even after heating at 60°C for 30 minutes. However, have been shown to lose activity beyond 50°C in a temperature-dependent manner (Laemmli, et al., 1973; Wang et al., 2003). The lectin shows remarkable pH stability, its activity being unaffected throughout the entire range of pH from 4 to 10. This is in contrast to lectin from Parkia javanica beans which is stable in pH 7–10 (Utarabhand & Akkayanont, 1995).

Chemical modification studies were carried out to investigate the role of specific amino acids in the hemagglutinating activity of white kidney beans lectin. The results disclose that tryptophan and lysine are important to the hemagglutinating activity, the contribution of tryptophan being more important. Previous studies have reported that lysine, tyrosine, and tryptophan (e.g., in Dolichos lab-lab bean) (Nadimpalli, 1999), or tryptophan alone are indispensable for the hemagglutinating activity of some legume lectins (Das, 1995). Specific amino acids may be involved in either direct interaction with the sugar or may have a role in maintaining conformation of the sugar binding pocket, and hence contribute to the hemagglutinating activity of lectins (Ba? i? imiev et al., 2007).

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