Research Article

Purification and Characterization of a Lectin from *Phaseolus vulgaris cv.* (Anasazi Beans)

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A lectin has been isolated from seeds of the *Phaseolus vulgaris cv.* "Anasazi beans" using a procedure that involved affinity chromatography on Affi-gel blue gel, fast protein liquid chromatography (FPLC)-ion exchange chromatography on Mono S, and FPLC-gel filtration on Superdex 200. The lectin was comprised of two 30-kDa subunits with substantial N-terminal sequence similarity to other *Phaseolus* lectins. The hemagglutinating activity of the lectin was stable within the pH range of 1–14 and the temperature range of 0–80°C. The lectin potently suppressed proliferation of MCF-7 (breast cancer) cells with an IC_{50} of 1.3 μM, and inhibited the activity of HIV-1 reverse transcriptase with an IC_{50} of 7.6 μM. The lectin evoked a mitogenic response from murine splenocytes as evidenced by an increase in [^{3}H-methyl]-thymidine incorporation. The lectin had no antifungal activity. It did not stimulate nitric oxide production by murine peritoneal macrophages. Chemical modification results indicated that tryptophan was crucial for the hemagglutinating activity of the lectin.

1. Introduction

Lectins are defined as proteins/glycoproteins possessing at least one non-catalytic domain which binds reversibly to a specific mono- or oligosaccharide [1]. 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 [2, 3], immunomodulatory and anti-insect [4], antifungal [5], antibacterial [6], anti-HIV [2, 5, 7], and mitogenic [8] 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 [9, 10], purification of glycoconjugates [11], and coating of drugs to enhance their gastrointestinal tract absorption [12, 13]. Further, specific amino acid residues are essential for maintaining the carbohydrate binding and hemagglutinating activities of lectins [14–16]. 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 [14, 16]. 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 [9, 10]. 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 Anasazi cultivar of *Phaseolus vulgaris*. To date, the isolation of a lectin from the Anasazi 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 Anasazi beans. It was assayed for the various afore-mentioned 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.

2. Materials and Methods

2.1. Materials. Anasazi beans, a product of Western Family Foods Inc., Portland, Ore, USA, were purchased from a local supermarket. Two hundred grams of Anasazi beans were soaked in 700 mL distilled water at room temperature for 4 hours to soften the beans. Then they were blended, after addition of 700 mL of distilled water in a Waring blender into a slurry. Subsequently, the mixture was centrifuged at 14000 rpm for 30 minutes at 4°C. Tris-HCl buffer (1 M) at pH 7.3 was added to the supernatant collected after the centrifugation until the final Tris concentration reached 10 mM. The supernatant was loaded on an Affi-gel blue gel (Bio-Rad, Calif, USA) column (5×18 cm) which had been equilibrated with the same buffer. After the unbound proteins had been eluted, bound proteins with hemagglutinating activity were eluted with 1 M NaCl in 10 mM Tris-HCl buffer (pH 7.3), dialyzed, lyophilized, and then subjected to ion exchange chromatography by fast protein liquid chromatography (FPLC) using an AKTA purifier (GE Healthcare, Hong Kong, China) on a 1 mL Mono S column (GE Healthcare, Hong Kong) in 20 mM Tris-HCl buffer (pH 7.3). The adsorbed fraction was further purified by FPLC-gel filtration on a Superdex 200 column (GE Healthcare) in 20 mM Tris-HCl buffer (pH 7.3).

2.2. Molecular Mass Determination by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and by FPLC-Gel Filtration. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDSPAGE) was carried out in accordance with the procedure of Laemmli and Favre [17], using a 12% resolving gel and a 5% stacking gel. At the end of electrophoresis, the gel was stained with Coomassie brilliant blue. After destaining, the electrophoretic mobilities of the marker proteins and the purified lectin were determined. The molecular mass of the lectin was estimated from the standard curve plotting electrophoretic mobility against molecular mass. FPLC-gel filtration was carried out using a Superdex 200 HR 10/30 column (GE Healthcare) that had been calibrated with molecular-mass standards (GE Healthcare) to estimate the molecular mass of the purified lectin.

2.3. Analysis of N-Terminal Amino Acid Sequence. Amino acid sequence analysis was carried out using a Hewlett Packard (HP) G1000A Edman degradation unit and an HP 1000 HPLC system.

2.4. Assay of Hemagglutinating Activity. In the assay for lectin (hemagglutinating) activity, a serial twofold dilution of the lectin solution in microtiter U-plates (50 μL) was mixed with 50 μL of a 2% suspension of rabbit red blood cells in phosphate-buffered saline (pH 7.2) at 20°C. The results were read after about 1 hour, when the blank had fully sedimented. The hemagglutination titer, defined as the reciprocal of the highest dilution exhibiting hemagglutination, is reckoned as one hemagglutination unit. Specific activity is the number of hemagglutination units per mg protein [18].

2.5. Inhibition of Lectin-Induced Hemagglutination by Carbohydrates. The hemagglutinating inhibition tests to investigate inhibition of lectin-induced hemagglutination by various carbohydrates were performed in a manner analogous to the hemagglutination test. A serial twofold dilution of each sugar samples was prepared in phosphate-buffered saline. All of the dilutions were mixed with an equal volume (25 μL) of a solution of the lectin with 16 hemagglutination units. The mixture was allowed to stand for 30 minutes at room temperature and then mixed with 50 μL of a 2% rabbit erythrocyte suspension. The minimum concentration of the sugar in the final reaction mixture which completely inhibited 16 hemagglutination units of the lectin preparation was calculated [18].

2.6. Effect of Temperature on Lectin-Induced Hemagglutination. The effect of temperature on hemagglutinating activity of the lectin was examined as previously described [19]. A solution of the lectin with 16 hemagglutination units was incubated at various temperatures for 30 minutes: 0°C, 10°C, 20°C, 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C, and 100°C. The tubes were then put on ice, and assay of hemagglutinating activity was then carried out.

2.7. Effect of pH on 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 1.0–14.0. for 60 minutes. The pH of the lectin solution was adjusted to 7.0 by the addition of 0.1 N HCl or 0.1 N NaOH before hemagglutination activity was determined.

2.8. Assay of Antiproliferative Activity on Tumor Cell Lines. Breast cancer MCF-7 cells or hepatoma HepG2 cells were suspended in RPMI medium and adjusted to a cell density of 5×10^4 cells/mL. A 100 μL aliquot of this cell suspension was seeded to a well of a 96-well plate, followed by incubation for 24 hours. Different concentrations of the lectin in 100 μL complete RPMI medium were then added to the wells followed by an incubation for 24 hours. After 24 hours, 50 μL of a 5 mg/mL solution of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) in phosphate buffered saline was spiked into each well, and the plates were incubated for 2 hours. The plates were then centrifuged at 2500 rpm for 5 minutes. The supernatant was carefully removed, and 150 μL of dimethyl sulfoxide was added in each well to dissolve the MTT (formazan) at the bottom of the wells. The absorbance at 590 nm was then measured with a microplate reader within 10 minuets.

2.9. Assay of HIV Reverse Transcriptase Inhibitory Activity. The assay of Anasazi bean lectin for ability to inhibit HIV-1 reverse transcriptase was carried out by using an enzyme-linked immunosorbent assay kit from Boehringer Mannheim

(Germany) as described by Collins et al. [20]. The assay takes advantage of the ability of reverse transcriptase to synthesize DNA, starting from the template/primer hybrid poly(a)·oligo(dT) 15. Instead of radio-labeled nucleotides, digoxigenin and biotin-labeled nucleotides in an optimized ratio are incorporated into one and the same DNA molecule, which is freshly synthesized by the reverse transcriptase (RT). The detection and quantification of synthesized DNA as a parameter for RT activity follows a sandwich enzyme-linked immunosorbent assay protocol. Biotin-labeled DNA binds to the surface of microtiter plate modules that have been precoated with streptavidin. In the next step, an antibody to digoxigenin, conjugated to peroxidase (anti-DIG-POD), binds to the digoxigenin-labeled DNA. In the final step, the peroxidase substrate is added. The peroxidase enzyme catalyzes the cleavage of the substrate, producing a colored reaction product. The absorbance of the samples at 405 nm can be read using a microtiter plate (ELISA) reader and is directly proportional to the level of RT activity. A fixed amount (4–6 ng) of recombinant HIV-1 reverse transcriptase was used. The inhibitory activity of the purified protein was calculated as percent inhibition as compared to a control without the lectin [20].

2.10. Assay of Nitric Oxide Production by Murine Peritoneal Macrophages. The assay was conducted as described by Wong and Ng, 2006 [21]. Macrophages were collected from the peritoneal cavity of mice after an intraperitoneal injection of a 3% thioglycolate solution. The cells were washed and resuspended in RPMI medium containing 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin, and 100 mg/mL streptomycin. Cells (2×10^5 cells/well) were seeded in a 96-well culture plate for 1 hour, before incubation with the Anasazi bean lectin for 24 hours. The amount of nitric oxide in the culture medium was determined by a colorimetric method. In the assay, a $100\,\mu$L aliquot of cell-free culture medium from each culture well was allowed to react with $50\,\mu$L of Griess reagent (1% sulfanilamide in 5% H_3PO_4-0.1% naphthalene-ethylenediamine dihydrochloride) for 10 minutes before the absorbance was read at 540 nm using a microplate reader. Lipopolysaccharide was used as a positive control in this assay. Dexamethasone ($5\,\mu$M) was used as inducible nitric oxide synthase (iNOS) inhibitor in this assay.

2.11. Assay of Antifungal Activity. The antifungal activity of Anasazi bean lectin toward *Botrytis cinerea, Mycosphaerella arachidicola,* and *Fusarium oxysporum* was examined using 90×15 mm Petri plates that contained 10 mL of potato dextrose agar, in view of the report that some lectins displayed antifungal activity [4]. After the mycelial colony had developed, sterile blank paper disks (0.625 cm in diameter) were placed at a distance of 0.5 cm away from the rim of the mycelial colony. An aliquot of a solution of Anasazi bean lectin was added to a disk. The plates were kept at 25°C for 72 hours until mycelial growth had enveloped disks containing the control and crescents of inhibition had formed around disks containing samples with antifungal activity.

2.12. Assay of Mitogenic Activity. Four C57BL/6 mice (20–25 g) were killed by cervical dislocation, and the spleens were aseptically removed. Spleen cells were isolated by pressing the tissue through a sterilized 100-mesh stainless steel sieve and resuspended to 5×10^6 cells/mL in RPMI 1640 culture medium supplemented with 10% fetal bovine serum, 100 units penicillin/mL, and $100\,\mu$g streptomycin/mL. The cells (7×10^5 cells/100$\,\mu$L/well) were seeded into a 96-well culture plate, and serial dilutions of a solution of Anasazi bean lectin in $100\,\mu$L medium were added. After incubation of the cells at 37°C in a humidified atmosphere of 5% CO_2 for 24 hours, $10\,\mu$L methyl [^{3}H]thymidine (0.25 μCi, GE Healthcare) was added, and the cells were incubated for further 6 hours under the same conditions. The cells were then harvested with an automated cell harvester onto a glass fiber filter and the radioactivity was measured with a Beckman model LS 6000SC scintillation counter. All reported values are means of triplicate samples [22].

2.13. Effect of Chemical Modification of Amino Acid Residues on Hemagglutinating Activity. For serine modification, the lectin ($100\,\mu$g) in 0.1 mL of 50 mM Tris-HCl buffer (pH 7.4) was incubated with 5 mM phenylmethylsulfonyl fluoride (PMSF) at 27°C for 1 hour [23]. Aliquots were removed at 15 minutes intervals. Excess reagent was removed by ultrafiltration, followed by determination of residual hemagglutinating activity. Lectin incubated without PMSF served as a control.

Reduction of the thiol groups of Anasazi bean lectin was carried out by incubating the lectin ($100\,\mu$g) in 0.1 mL of 50 mM phosphate buffer (pH 8.0) with 0.1 mM 5, 5′-dithiobis-(2-nitrobenzoic acid (DTNB) at 27°C for 1 hour. Aliquots were removed at different time intervals. Excess reagent was removed by ultrafiltration, followed by determination of residual hemagglutinating activity. Lectin incubated in the absence of DTNB served as a control [24].

For lysine modification, 0.5 mg of $NaBH_4$ was added to the lectin (5 mg) in 2 mL of 0.2 M sodium borate buffer (pH 9.0) at 4°C, followed by six aliquots ($5\,\mu$L each) of 3.5% formaldehyde at 10 minutes intervals. Excess reagent was removed by ultrafiltration, followed by determination of residual hemagglutinating activity. Lectin incubated in the absence of sodium borohydride ($NaBH_4$) served as a control [25].

Modification of tryptophan residues was carried out according to the method of Spande and Witkop [26]. The lectin was dissolved in NaOAc buffer (0.1 M, pH 5.0) to 1 mg/mL. The modification was carried out at 20°C. N-bromosuccinimide (NBS) ($10\,\mu$L, 10 mM) was added every 5 minutes. The number of tryptophan residues was calculated as described by Spande and Witkop [26]. The samples were then desalted by ultrafiltration to remove excess reagent, and then assay of hemagglutinating activity was carried out. Lectin incubated in the absence of NBS served as a control.

Acetylation of the tyrosine side chain phenoxy groups of the lectin was done at room temperature by incubating $300\,\mu$g lectin in 1.0 mL of 25 mM phosphate buffer (pH 7.5) containing 60-fold molar excess (0.1–10 mM) of N-acetylimidazole. Excess reagent was removed by ultrafiltration, followed by determination of residual hemagglutinating
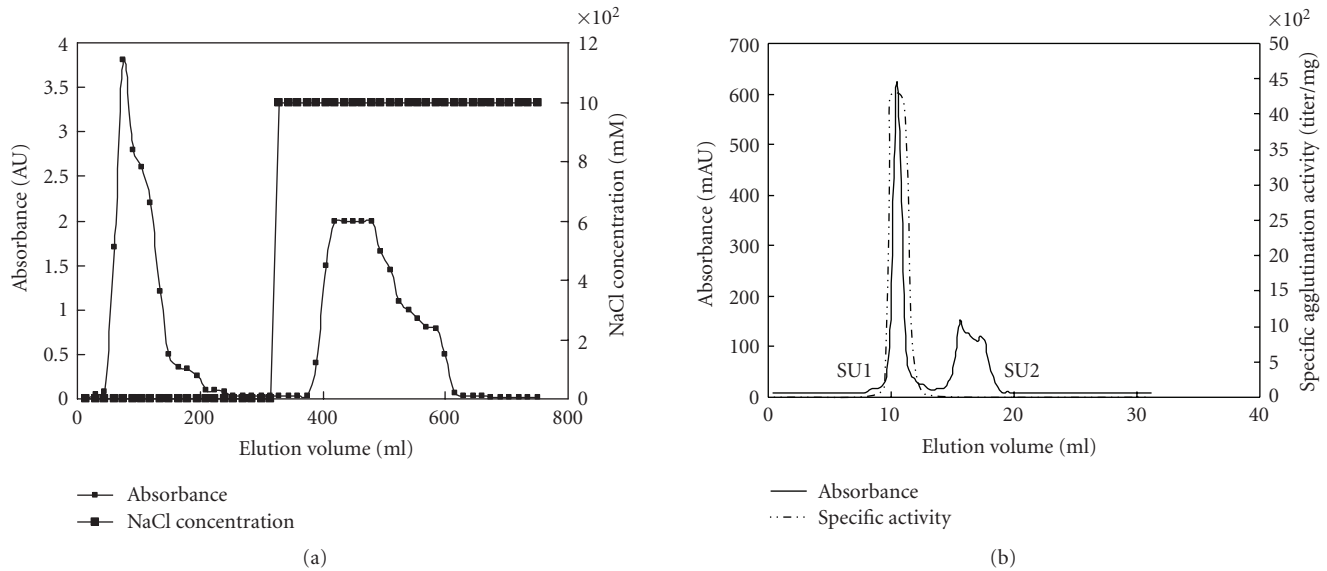
FIGURE 1: (a) Fractionation of the crude extract of Anasazi beans on an Affi-gel blue gel column equilibrated with the binding buffer (10 mM Tris-HCl, pH 7.3). The column was washed initially with the binding buffer to remove B1 and then eluted with 1000 mM NaCl in 10 mM Tris-HCl buffer, (pH 7.3) to desorb B2. (b) Superdex 200 column chromatography. Buffer 20 mM Tris-HCl buffer, (pH 7.3), flow rate: 0.5 mL/min, fraction size: 1.0 mL. Only the major peak (SU1) exhibited hemagglutinating activity. mAU = milli-absorbance units.
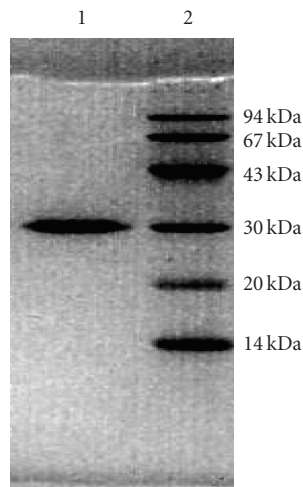

FIGURE 2: SDS-PAGE of Anasazi bean lectin. Lane 1: Anasazi bean lectin. Lane 2: molecular mass standards. From top downward, phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa), and α-lactalbumin (14 kDa).

FIGURE 3: Effect of temperature on hemagglutinating activity of Anasazi bean lectin.

activity. Lectin incubated in the absence of N-acetylimidazole served as a control [27].

3. Results

Purification of the Anasazi bean seed lectin involved initial extraction in 10 mM Tris-HCl buffer (pH 7.3) and three-step chromatography including affinity chromatography on Affi-gel blue gel, ion-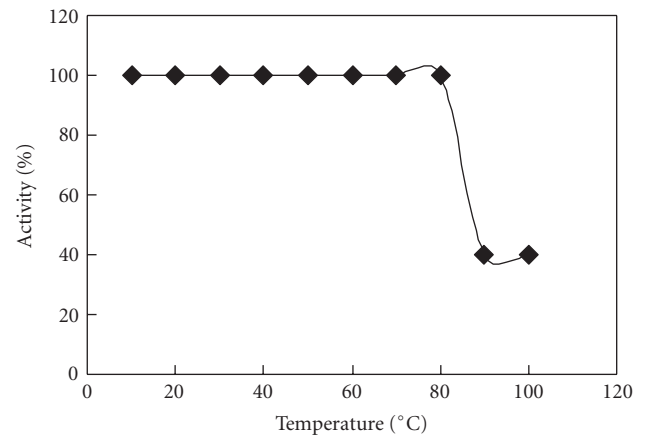exchange chromatography on Mono S, and gel filteration on Superdex 200. Fractionation of the crude seed extract using Affi-gel blue gel revealed the presence of a slightly larger unadsorbed fraction designated as B1, and a slightly smaller adsorbed fraction designated as B2 (Figure 1(a)). The latter fraction, in which hemagglutinating activity was concentrated, was subsequently fractionated on Mono S into a large unadsorbed fraction and a small adsorbed fraction (data not shown). The adsorbed fraction with hemagglutinating activity was resolved into a large peak (SU1) and a small peak (SU2) by FPLC-gel filtration on Superdex 200. Hemagglutinating activity was located in SU1 (Figure 1(b)). The purified lectin, represented by SU1, appeared as a single band with a molecular mass of 30 kDa in SDS-PAGE (Figure 2) and a 60 kDa absorbance

TABLE 1: Yields and specific hemagglutinating activities of chromatographic fractions obtained at different stages of purification of Anasazi bean lectin.

	Specific activity (titer/mg)	Total activity (titer)	Yield (mg)	Fold purification
Crude Extract	270	819200	3033	1.00
Affi-gel blue gel fraction B2	1321	737280	558	4.89
Mono S adsorbed fraction	4076	786432	192	15.09
Superdex fraction SU1 (purified lectin)	4267	114688	26	15.80

TABLE 2: Comparison of N-terminal amino acid sequence of Anasazi bean lectin with those of other *Phaseolus* lectins.

Sample name	Sequence	% Identity
Phaseolus vulgaris (cultivar Anasazi bean)	1ANQIYFNFQRFNETNLILQR 20	100
Phaseolus vulgaris (cultivar Pinto bean)	1ASETSFSFQRFVETNLILQR 20	70
Phaseolus vulgaris (cultivar Haricot bean)	1ASESYFNFQRFEETN15	73.3
Phaseolus vulgaris (cultivar Red Kidney bean)	1ASETSFSFERFNETNLILQR 20	70

Differences are underlined: 1A refers to A being the 1st residue in the lectin; R20 refers to R being the 20th residue in the lectin; N15 refers to N being the 15th residue in the lectin.
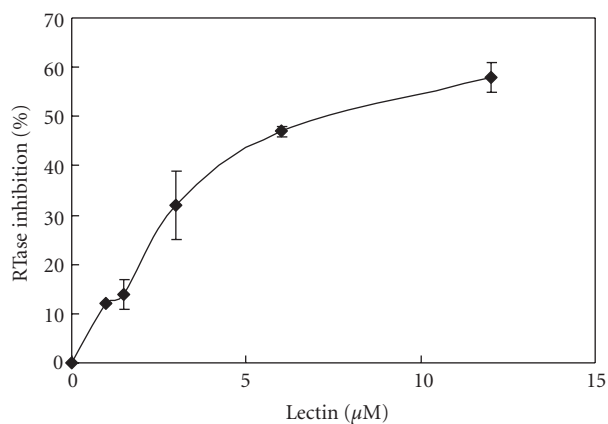

FIGURE 4: HIV-1 reverse transcriptase inhibitory activity of Anasazi bean lectin (data represent means $\pm$SD, $n = 3$). IC$_{50}$ = 7.6 μM. (Data represent means $\pm$SD, $n = 3$.)
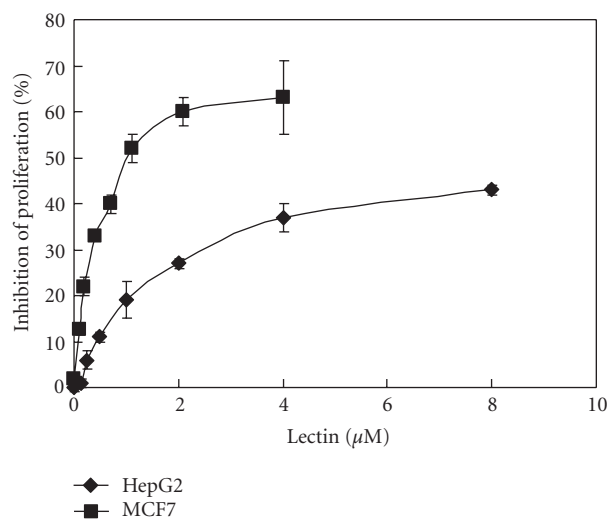

HepG2
MCF7

FIGURE 5: Inhibitory effect of Anasazi bean lectin on proliferation of cancer cell lines. Cell proliferation was determined by MTT assay (Data represent means $\pm$SD, $n = 3$.)

peak in gel filtration on Superdex 200 (Figure 1(b)). The protein yield and specific hemagglutinating activity of the product at each stage of the purification scheme are shown in Table 1. Anasazi bean lectin demonstrated remarkable N-terminal sequence similarity to those of *Phaseolus* lectins (Table 2). The hemagglutinating activity of purified Anasazi bean lectin could not be inhibited by any of the simple sugars tested at 1.56–100 mM, α-L-fucose, D(+)-galactose, D(+)-glucose, D(+)-glucosamine, D(+)-galactosamine, (+)-lactose, D(+)-melibiose, L(+)-mannose, D(+)-mannose, D-mannosamine, D(+)-raffinose, L-rhamnose, (+)-xylose, galacturonic acid, and N-acetyllactosamine, and also not by the glycoproteins heparin, thyroglobulin, lactoferrin, human chorionic genadotropin, and ovalbumin. The hemagglutinating activity was completely stable between 0°C and 80°C. Considerable loss in activity occurred at 90°C. Some activity was discernible at 100°C (Figure 3). The lectin exhibited remarkable stability over the entire range of pH 1–14

(data not shown). The Anasazi bean lectin inhibited HIV-1 reverse transcriptase with an IC$_{50}$ of 7.6 μM (Figure 4). The antiproliferative activity of Anasazi bean lectin toward Hep G2 and MCF-7 cells is shown in Figure 5. The lectin displayed only slight inhibition of proliferation toward HepG2 cells with an IC$_{50}$ beyond 8 μM while its IC$_{50}$ toward MCF-7 cells was 1.3 μM. The lectin stimulated the mitogenic response of mouse splenocytes with an optimal response at 1.04 μM (Figure 6). However, the hemagglutinin did not affect nitric oxide production by mouse macrophages (Figure 7). Anasazi bean lectin exhibited no effect on mycelial growth in the fungal species examined, that is, *Botrytis cinerea, Mycosphaerella arachidicola, and Fusarium oxysporum* (data not shown). The effects of various types of chemical modifications on hemagglutinating activity
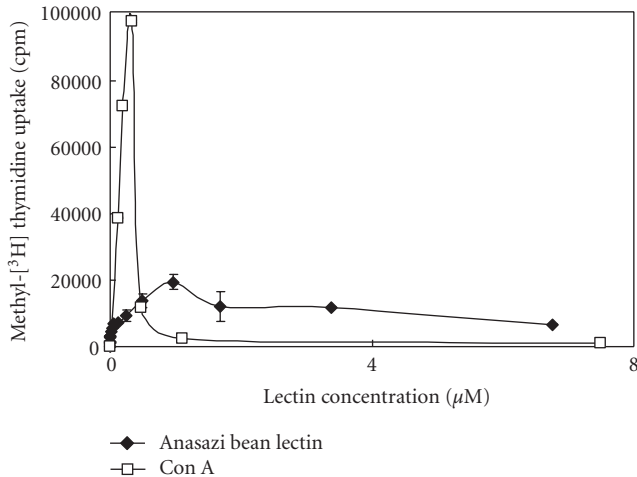
FIGURE 6: Mitogenic effect of Anasazi bean lectin and Con A toward mouse splenocytes. (Data represent means ±SD, $n = 3$.)
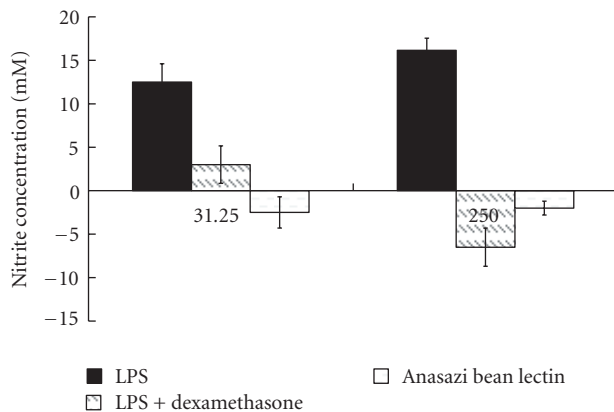

FIGURE 7: Effects of lipopolysaccharide (LPS), Anasazi bean lectin, and dexamethasone on nitric oxide production by mouse peritoneal macrophages. (Data represent means ±SD, $n = 3$.)

FIGURE 8: Effect of the modification of tryptophan residues on the hemagglutinating activity of Anasazi bean lectin. (Data represent means ±SD, $n = 3$.)

4. Discussion

In this study, a plant lectin has been purified by three-step chromatography from seeds of the Anasazi bean. Similar to pinto bean lectin [28], Anasazi bean lectin is adsorbed on Affi-gel blue gel. However, unlike the former lectin, the latter is adsorbed on Mono S as well. The homogeneity of the Anasazi bean 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 dimer of two subunits. The molecular mass and dimeric nature of Anasazi bean lectin are similar to those of pinto bean lectin and most of the other *Phaseolus* lectins [2, 28]. On the other hand, it differs from a tetrameric 115–120 kDa lectin from tepary bean (*Phaseolus acutifolius*) [29], *Phaseolus acutifolius* var. latifolius lectin from which consists of four subunits of 21 kDa molecular mass [30], and a tetrameric 94 kDa immunosuppressive lectin isolated from seeds of *Phaseolus vulgaris* cv Cacahuate [31]. Lectins from some cultivars of *Phaseolus vulgaris* are oligomeric [32], whereas Anasazi bean lectin is dimeric. The subunit molecular weight of the oligomeric lectins is similar to that of Anasazi bean lectin. Isolectins are absent in Anasazi beans but present in some cultivars of *P. vulgaris* such as red kidney bean [32–34]. The isolectins differ from one another by the number of erythrocyte-reactive (E) subunits and lymphotcyte-reactive (L) subunits that they possess. There are five such isolectins: L4, L3E1, L2E2, L1E3, and E4. Although there is striking homology between Anasazi bean lectin and other *Phaseolus* lectins in N-terminal sequence, Anasazi bean lectin exhibits absence of simple sugar specificity. Simple sugars, the N-acetyl sugar N-acetyllactosamine and the glycoproteins heparin thyroglobulin, lactoferrin, human chorionic gonadotropin, and ovalbumin are not able to inhibit the hemagglutinating activity of Anasazi bean. In this aspect, Anasazi bean lectin is dissimilar from arcelin-1, a lectin-like protein from wild varieties of kidney bean which interacts with complex glycans [35], and also from lectins from many other *P. vulgaris* cultivars such as the galactose specific pinto bean lectin [28]. Similar to pinto bean lectin [28], Anasazi bean lectin is fairly thermostable because its hemagglutinating

of the purified lectin are summarized in Table 3. DTNB, reductive methylation and N-acetylimidazole treatments did not produce any alterations in the hemagglutinating activity of Anasazi bean lectin, suggesting that cysteine, lysine and tyrosine residues, respectively, did not play any important role in its hemagglutinating activity. However, 67% 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. PMSF treatment resulted in 43% loss in hemagglutination activity suggesting partial involvement of serine in the lectin activity. Spectrophotometric monitoring revealed that the controlled addition of 10 μL of 10 mM NBS under mildly acidic conditions (pH 5.0) led to a progressive decrease in absorption at 280 nm, indicative of oxidation of tryptophan residues. A concomitant drop in lectin activity was clearly seen upon modification of tryptophan residues (Figure 8).
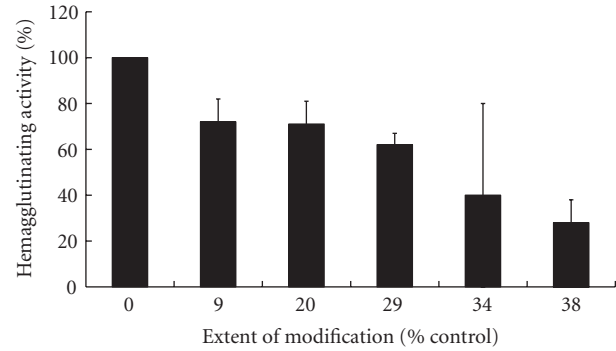
TABLE 3: Effect of chemical modification on hemagglutinating activity of Anasazi bean lectin.

Treatment	Modified group/amino acid	% Hemagglutinating activity remaining
Phenylmethylsulfonyl fluoride (PMSF)	Serine	57
5, 5′-Dithiobis-(2-nitrobenzoic acid (DTNB)	Thiol group	100
Reductive methylation	Lysine	100
N-Bromosuccinimide (NBS)	Tryptophan	33
N-Acetylimidazole	Tyrosine	100

activity is stable at temperatures up to 80°C, and is reduced only at 90°C. Interestingly, some activity remained even after heating at 100°C for 30 minutes. Haricot bean and ground bean lectins, however, have been shown to lose activity beyond 40°C in a temperature-dependent manner [19, 36]. The lectin shows remarkable pH stability, its activity being unaffected throughout the entire range of pH from 1 to 14. This is in contrast to lectin from *Parkia javanica* beans which is stable in pH 7–10 [37]. Like many lectins such as mushroom lectins, haricot bean lectin, and others [2, 3, 31], Anasazi bean lectin exhibits an antiproliferative activity toward tumor cell lines. Interestingly, the Anasazi bean lectin exhibits only slight antiproliferative activity toward Hep G2 cells while it has been shown to have a significant antiproliferative effect against MCF-7 cells. Only a very small number of lectins have been shown to have antifungal activity [2]. Like most other lectins, Anasazi bean lectin does not display antifungal activity. Anasazi bean lectin inhibits HIV-1 reverse transcriptase with an IC$_{50}$ below those of some anti-HIV natural products [38]. The IC$_{50}$ is almost twice that of pinto bean lectin [28]. However, it is almost one-tenth of that of ground bean lectin [19]. The mechanism of inhibition probably involves protein-protein interaction. It is known that HIV-1 protease inhibits HIV-1 reverse transcriptase with a similar mechanism [39]. Antifungal proteins [40–42], ribosome inactivating proteins [43–45], and lectins [2, 5, 7, 21, 46] of plant origin have been shown to inhibit HIV-1 reverse transcriptase. The ability of Anasazi bean lectin to inhibit HIV-1 reverse transcriptase is interesting in view of the inhibition of DNA polymerase alpha activity by lectins including Concanavalin A and ricin [47, 48] and DNA polymerase beta activity by red kidney bean agglutinin [47]. Induction of mitogenicity is a common feature elicited by most lectins of *Phaseolus* genus, such as pinto bean lectin, haricot bean agglutinin [32], and PHA-L4, a lectin from kidney beans [8]. However, GNL-2, a lectin from great northern beans [49], is devoid of this activity. Other lectins may also have mitogenic activity [50]. Anasazi bean lectin is capable of eliciting a mitogenic response from mouse splenocytes. The magnitude of the maximal response is about one-fourth of that of Con A; the lectin concentration needed to bring about the maximal response is 64 times less than that of Con A. Nitric oxide (NO) is known to play an important role in immunomodulation. Proinflammatory cytokines, for example, tumor necrosis factor-α (TNF-α) and bacterial products, for example, lipopolysaccharide (LPS) are well established NO stimulators. The steroid,

dexamethasone, is an inhibitor of NO production. These agents up- or downregulate the activity of the inducible form of nitric oxide synthase [51]. NO is also involved in cytotoxity against tumor cells [52], and it is possible that NO is implicated in tumor lysis induced by lectins with an antitumoral effect [53–55]. Previously, emperor banana lectin [21] has been reported to induce nitric oxide production by mouse macrophages. Anasazi bean lectin, in the present report, is not capable of augmenting nitric oxide production by mouse macrophages. Chemical modification studies were carried out to investigate the role of specific amino acids in the hemagglutinating activity of Anasazi bean lectin. The results disclose that tryptophan and serine 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 [16]) or tryptophan alone are indespensable for the hemagglutinating activity of some legume lectins [56]. 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 [14]. The application of three successive chromatographic steps, namely, affinity chromatography on Affi-gel blue gel, ion exchange chromatography on Mono S, and gel filtration on Superdex 200, is effective in isolating Anasazi bean lectin, with an approximately 15-fold purification. It is known that the sugar snap produces an antifungal protein, miraculin [57], whereas the garden pea produces a ribosome inactivating protein, pisavin, instead [58], although they are two cultivars of the same species, *Pisum sativum*. Thus different cultivars of the same species may produce different proteins. Anasazi bean lectin differs from pinto bean lectin, its counterpart from another cultivar of the same species, in the presence/absence of some features, such as sugar specificity, antiproliferative activity, HIV-1 reverse transcriptase inhibitory activity, although the two beans are two different cultivars of the same species (*Phaseolus vulgaris*). The present study on Anasazi bean lectin has revealed that tryptophan and serine residues are involved in the activity of the lectin. The involvement of other amino acid residues and groups in maintaining the conformation of the lectin could be further investigated by circular dichroism analysis [14]. The interesting features of Anasazi bean lectin comprise the following: absence of simple sugar specificity which is dissimilar to phytohemagglutinins from many other *P. vulgaris* cultivars, antiproliferative activity against tumor

cells, potent HIV-1 reverse transcriptase inhibitory activity, mitogenic activity in mouse splenocytes, and remarkable pH stability and thermostability.

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