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Isolation and characterization of lectins from kidney beans (*Phaseolus vulgaris*)

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Abstract

The bioactive properties of lectins obtained from raw and canned red kidney bean (*Phaseolus vulgaris*) were studied to determine the changes in their bioactivity during the canning process. Phytohaemagglutinin (PHA) was extracted using Affi-gel Blue gel and thyroglobulin-Sepharose and had a molecular weight of 32 kDa. Both the raw and the canned kidney beans possessed the ability to agglutinate red blood cells and inhibit α-glucosidase. The activity found in the canned beans was similar to that from the in the raw kidney beans. However, the amount of lectin that could be extracted from thyroglobulin-Sepharose was much less in the canned samples than in the raw kidney bean samples. The extracted lectin from the raw kidney beans was also subjected to a heating and cooling treatment using a differential scanning calorimeter. The lectin had a nonset denaturation temperature of 77.76 °C and it did not renature upon cooling. In this study, we demonstrated that extracts from raw red kidney bean and canned red kidney bean contain bioactive compounds capable of inhibiting HIV-1 RT *in vitro*.

Keywords: Lectin; HIV; Haemagglutination; Kidney bean; Bioactivity property

1. Introduction

Legumes, such as red kidney beans, are a major food source for both human and animals in the world, due to their nutritional benefits such as high in fiber and low in fat, as well as being a cheap source high protein content [1,2]. However, because of its anti-nutritional factors, additional processing steps such as cooking must be included in order to make the beans safe to eat [2,3]. These anti-nutritional compounds such as lectins are not only considered to be resistant to digestive enzymes, but are also partially resistant to thermal denaturation and therefore needs to be cooked for long time [4].

Legume lectins are large family of homlogous carbohydrate binding proteins. They also display a variety of biological activities including anti-tumor [5], immunomodulatory [6], anti-fungal [7], anti-human immundoficiency virus (HIV) [8], and anti-insect [9] activities. Lentins can be defined as glycoproteins of nonimmune origins that are capable in the recognition and reversible binding to carbohydrate moieties without the alteration of the covalent structure of the recognized glycosyl ligands [3,10]. There are five isoforms of the lectin consisting of different tetrameric combinations of PHA-E (MW = 34,000) that are known to agglutinate red blood cells, and PHA-L (MW = 32,000) which agglutinate leucocytes and have mitogenic activity [11]. Because of their distribution in all tissues of plants, it has been suggested that lectins provide an important role in plants as a defense mechanism and in the recognition of nitrogen fixing bacteria [3,10–12]. PHA is also used for the stimulation of cell proliferation in lymphocyte cultures.

Lectins serve many different biological functions, from the regulation of cell adhesion to glycoprotein synthesis and the control of protein levels in the blood. An accepted function for these proteins is their benefits as a defensive mechanism [3,11,12]. It has been long known that these proteins are capable of agglutinating red blood cells in mammals, including all human blood types [13,14]. Thus, the purified lectins are important in a clinical setting because they are used for blood typing. Some of the glycolipids and glycoproteins in an individual's red blood cells can be identified by lectin. Other functions are its ability to bind to the surface of the intestinal walls. They have also been associated with the appearance of

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lesions, disruptions and abnormal growth of the microvilli, as well as the malabsorption of nutrients across the membranes [11,15]. Recent studies have shown that these compounds possess anti-HIV activity [16]. Therefore, lectins are considered to be direct predecessors to the immune system and they still play great role in it, such as complement activation pathway, mannose-binding lectin, etc.

Red kidney beans contain the lectin *Phytoheamagglutinin* (PHA). PHA contains potent cell agglutinating and mitogenic activities. The inhibitory effect of PHA on human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT) has also been reported in literature [17–20]. Biological functions of PHA investigated to date are agglutination of human red (PHA-E) and white blood cells (PHA-L), mitogenic effect and a binding preference for cancer cells. It has been demonstrated in several studies that isolated lectin and lectin like proteins from kidney bean seeds (*Phaseolus vulgaris*) were able to directly inhibit HIV-1 reverse transcriptase (HIV-1 RT), one of the three enzymes essential for HIV replication [18,20,21], as well as X-glucosidase which has a role in HIV-1 envelope protein gp-120 processing [16]. Although the exact mechanism is not totally understood, there have been several hypotheses. One main method concept suggested a reduction in the activity of the glycohydrolases [21]. α-Glucosidase is found in the Golgi apparatus and has been found to be involved in the glycosylation of gp-120 for HIV-1 [22].

The difficulty of separating multiple lectin isoforms by conventional techniques has led to functional properties and biological activities for protein mixtures. While many of the naturally occurring lectin variants probably differ little in their functional properties, in other cases the differences in sequence between isoforms causes significant effects on the biological activity of the molecule. Unfortunately, there is a dearth of information in the literatures on how lactins' bioactivities change in the bean products after during thermal process. Therefore, the purpose of this study was to isolate those lectins by affinity chromatography, using Affi-gel Blue and thyroglobulin-Sepharose, from commercially canned and dry raw red kidney beans and to determine their activities for haemagglutination and glycohydrolase inhibition. A denaturation study was also to conduct on the PHA extracted samples from the raw red kidney beans using a differential scanning calorimeter (DSC). The inhibiting effect on HIV-1 reverse transcriptase of lectins from red kidney beans was also determined *in vitro*. The results obtained from the study on the bioactivity of isolated lectins from red kidney beans are very useful for providing information on alternative source of protein, as well as food processing strategies and heat processing designs.

2. Materials and methods

2.1. Material

Both dry raw and commercial canned red kidney beans (*Phaseolus vulgaris*) were obtained from Heinz Co., Canada. The bovine serum albumin standard, α-glucosidase, *p-N*-phenyl β-D-glucopyranoside, red blood cells type B was purchased from Sigma Co., Canada. All solvents were of analytical grade.

2.2. Extraction and purification of lectins

The methods used to extract and purify lectins were taken and/or modified from Felsted et al. [23] and Ye et al. [21]. The extraction produce for red kidney bean lectin is shown schematically in Fig. 1. One hundred and fifty grams of the dry raw red kidney bean was soaked in 1 L of distilled water and homogenized using a Polytron Mixer (Kinematica, USA) for 30 s, and then stirred overnight at 4 °C. These were then adjusted with 10 mM Tris pH 7.2 and centrifuged at 9000×*g* and 4 °C for 45 min. The residue was discarded and the supernatant was adjusted to 40% saturation with ammonium sulfate. After centrifugation at 9000×*g* and 4 °C for an additional 45 min, the precipitate was discarded and the supernatant adjusted to 80% saturation with ammonium sulfate.

The precipitate was collected by centrifugation at 9000×*g* and 4 °C for 45 min and then dialyzed against 10 mM Tris 7.2 with 0.02% sodium azide to prevent growth of microorganisms. Desalting and concentrating the samples were performed at room temperature in dialysis tubing with 6–8 kDa cut off. The buffer was changed every 4 h, over a period of 2 days. The desalted sample was then lyophilized in preparation for affinity chromatography.

In affinity chromatography purification, the lyophilized sample was divided into 150 mL aliquots which were then mixed with Affi-gel Blue (Bio-Rad, Canada) for at least 4 h. Each aliquot was then transferred to a fritted glass Büchner Funnel where it was washed with 10 mM Tris–HCl buffer (pH 7.2). Unadsorbed proteins were removed and the adsorbed proteins were eluted off with 1.4 M NaCl dissolved in 10 mM Tris–HCl buffer (pH 7.2). Each resulting powder was then dissolved in a minimum amount of phosphate buffered saline (6.7 mM potassium phosphate with 0.15 M NaCl, pH 7.4) and then allowed to mix with thyroglobulin-Sepharose for at least 2 h. The mixture was first washed with the phosphate buffered saline until the absorption monitored using the Bradford assay had stabilized. This was then washed with 1 mM phosphate (pH 7.2) with 1 M NaCl, until the absorption had again stabilized. The PHA was then eluted off using 0.05 M glycine–HCL (pH 3.0) containing 0.5 M NaCl [23]. The dialysis was employed to desalt and concentrate the PHA by passing solution through a semipermeable membrane (Fisher Scientific, ON, Canada, MWCO = 3000). The PHA fractions were then freeze-dried.

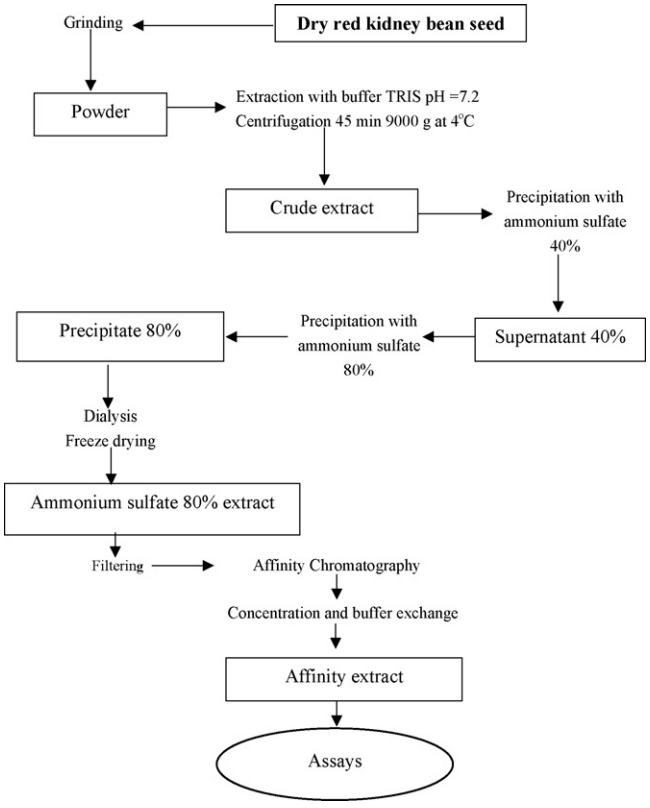

Fig. 1. Lectin isolation and purification scheme.