

Isolation and Purification of Lectin from *Momordica balsamina* Seeds

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Abstract

Background: Lectins are multivalent proteins of non-immune origin that reversibly and non-enzymatically bind carbohydrates with high specificity for the chemical structure of the glycan array without changing their structure. Lectins have been mostly isolated from plant seeds although recently it had been known to be found in other plant tissues, prokaryotes and higher animals.

Objectives: The present study aimed to isolate and purify *Momordica balsamina* seedslectin (MbSL). **Materials and methods:** A season fresh of *Momordica balsamina* fruit seeds were brought from urban areas of Sudan (Gadrf and north Kurdofan states), then the lectin was isolated from saline extract by affinity chromatography on alpha agarose lactose matrix.

Results: The lectin content was about 1200 mg/ 100 g dry flour. MbSL agglutinated all human RBCs type with preference toward the O blood group. The lectin also agglutinated mouse, donkey and cow blood cells and showed no effect on gout erythrocytes. Lactose was the most potent inhibitor of MbSLhemagglutinating activity, (minimal inhibitory concentration) MIC = 25mM, followed by galactose, MIC=50 mM, and then arabinose, MIC= 100mM.

Conclusion: a lactose-binding lectin from seeds of *Momordica balsamina* medicinal plant shares a high degree of similarity with other Cucurbitaceae family lectins in term of their sugar specificity.

Keywords: *Momordica balsamina*; lectin; seeds; plantlectin; protein

1. Introduction

Lectins, multivalent cell-agglutinating proteins, by virtue of their exquisite sugar specificities are useful tools in widespread applications for monitoring the expression of cell-surface carbohydrates as well as for the purification and characterization of glycoconjugates (Kestwal et al., 2007). Accordingly, lectin can be described as a substance which can agglutinate cells or precipitate glycoconjugates, with a structure resembling a carbohydrate binding protein or glycoprotein and is not of immune origin (Vasconcelos et al., 2009).

Lectins have been known since the turn of the 19th century. However, for a long time they attracted little attention, especially as it was assumed that they were confined to the plant kingdom and not present in humans or other animals (Esko & Sharon, 2009). The use of plants in traditional medicine is now bringing attention of many scientists to examine the active components and to study toxicity for more safe and valuable practice. Many of traditional medicinal plants have been studied for their protein content and lectin activity. However, only about 1% of these are proved through scientific studies to have real therapeutic value when used by humans (Bhaskar et al., 2012).

Lectins have been classified according to their sugar-binding specificity to monospecific and polyspecific, the later can interact with more than one sugar (Neutsch et al., 2012).

Mature seeds are the main source of plant lectins, but they are also found in other vegetative tissues such as leaves, fruits but merely roots; (De Hoff et al., 2009). Plant lectins are secretory proteins that accumulate either in the vacuole or extracellular matrix. Most plants contain only one lectin, but in some cases, they contain two or more biologically different lectins (Hartley & Lord, 2004; Peumans & Van Damme, 1998).

M. balsamina is a plant commonly known as Balsam apple or Bitter melon, Dragon Flower in Arabic and Aeer locally in Sudan. It's a climber or trailer with annual stems attaining 4–5 meter in length, a plant of dry Savannah. Hutchinson, (1954) described the fruit as orange yellow, beaked, 2 1/2 inches in length bursting and exposing red brown seeds (Thakur et al., 2009). This species is closely related to *M. charantia* which found in areas of greater rainfall (Horejsi et al., 1980).

The leaves, fruits, seeds, and bark of the plant contains resins, alkaloids, flavonoids, glycosides, steroids, terpenes, cardiac glycoside, saponins having various medicinal importance for instance anti-HIV (Kaur et al., 2013).

2. Materials and Methods

Plant Materials

Momordica balsamina fruit seeds were brought from urban areas of Sudan (Gadrif and north Kurdofan states).

Erythrocytes

Typed human blood cells (A, AB, B and O) were obtained from healthy donors, while animal blood cells were obtained from the animal house of Sudan University of Science and Technology, Khartoum, Sudan.

Chemicals

Alpha agarose lactose affinity matrix was purchased from Sigma Chemicals. All other reagents were either of analytical grade or of highest quality available.

Protein Estimation

The protein content of the samples obtained during the purification process was determined by the method of Lowry et al. (Lowry et al., 1951), using bovine serum albumin as the standard. Readings at 280nm were also used to determine the protein content of the column elutes. (Figure 1, 2)

Preparation of Seed Extract

The extraction was carried out as described by Konozy et al., (Konozy et al., 2002). Season fresh, mature, and good quality seeds of *M. balsamina* were ground to a fine flour in a coffee grinder and the meal (100 g) was defatted with petroleum ether in a ratio of (5mL/1g powder). Ether layer was removed by filtration system. Soluble protein was precipitated with equal amount of chilled acetone (added drop wise with continuous stirring).

Extract was filtered through filtration system and further dehydrated extensively with chilled acetone to get acetone dried powder. Acetone dried powder was extracted for 4 hours with 0.145 mMNaCl at 40 C (in a ratio 5:1). The whole extract was filtered through filter paper and then centrifuged for 45 min at 6000rpm at 4° C.

Purification of Lectin on Alpha Agarose Lactose Matrix:

This was carried out essentially as described by Konozy et al.,(Konozy et al., 2003) In a syringe of 10mL capacity, 2mL of alpha agarose lactose were loaded; column was initially washed with 100 mL of 100 mM acetate buffer pH 5, equilibrated with 0.145M NaCl. Protein was loaded into the column; recycled for several times to ensure maximum retention of lectin on matrix. Unbound proteins were washed off with equilibration saline till reading at OD280nm dropped to ≤ 0.2 . Elution of bound lectins was done by 200 mM lactose; 3 ml fractions were collected at reduced flow rate of 3mL/min. Fractions were read for protein content by spectrophotometer at 280 nm. Fractions that exhibited ODs above 0.06 were pooled, precipitated by ammonium sulphate 100%, then dialyzed against distilled water and tested for lectin activity.

Hemagglutination Inhibition

Hemagglutination test was conducted in a microtiter plates, in a final volume of 100 μ l. Each well contained 50 μ l of lectin solution and 50 μ l of 4% (v/v) suspension of either untrypsinized or trypsinized erythrocytes. Agglutination was assessed after incubation for 30 minutes at room temperature. Hemagglutinating activity was expressed titer, namely, the reciprocal of the highest dilution that gave a positive result (Konozy et al., 2003). The specific hemagglutinating activity was expressed as titer (per mg lectin). Type O blood group was used throughout out this study.

Carbohydrate Content:

The neutral sugar content of the lectin was estimated by the phenol-sulfuric acid method using glucose as standard.(Dubois et al.,1951). (Figure3).

Lectin sugar Specificity:

The sugar specificity of the lectin was tested as described by Konozy et al.,(Konozy et al., 2002) by inhibiting the hemagglutinating activity using 200mM of 7 different sugars (Arabinose, Glucose, Galactose, Mannose, Maltose, Sucrose and Lactose). Results were expressed as the minimal concentration of sugar which effectively inhibited hemagglutinating units of lectin.

3. Results

Blood Group Specificity and Sugar Inhibition:

Crude extract of *Momordica balsamina* seeds contains a hemagglutinating activity that agglutinated some animal red blood cells (RBCs) and all human red blood cells which was greater towards O blood type as shown in Table.1.

This hemagglutinating activity was inhibited by haptenic sugar D-galactose and lactose, with lactose showing strong effect as shown in Table.2.

MomordicabalsaminaSeeds Lectin Purification:

The purification procedures of this lectin from seed extract of *Momordica balsamina* are summarized in Table.3. The lectin was extracted from the ground meal of seeds and purified in a single step by affinity chromatography on alpha agarose lactose matrix.

4. Discussion

The crude protein extracts of *Momordica Balsamina* Seeds revealed high hemagglutinating activity when tested against human and animals RBCs and showed more activity towards the O blood type (as shown in Table 3.1) which could be inhibited by many sugars (as shown in Table 3.2). It is known from other researchers in Cucurbitaceae family lectins that they tend to be galactose specific as was found for lectins from closely related species *Momordicacharantia* (Huang et al., 2008)and lectin from the seeds of *Trichosnthesdioica* (Sultan et al., 2004). Since lactose was a stronger inhibitor than galactose, the purification was done in a single step by affinity chromatography on alpha agarose lactose matrix.

InConclusion: MbSL shares a high degree of similarity with other Cucurbitaceae family lectins in sugar specificity.

Table 1: Hemagglutinating Activity of *Momordica balsamina* Seed Lectin against Different Blood Cells

| Erythrocytes type | Trypsin untreated (Specific activity) | Trypsin treated (Specific activity) |
|-------------------|--|--|
| Human | | |
| 1) A | 16 | 32 |
| 2) B | 8 | 8 |
| 3) AB | 4 | 130 |
| 4) O | 16 | 130 |
| Animal | | |
| 1) Mice | 0.08 | 0.02 |
| 2) Donkey | NAD | NAD |
| 3) Cow | 0.8 | NAD |
| 4) Goat | NAD | NAD |

NAD: No agglutination detected

Table 2: Carbohydrate Inhibition of Agglutination by *Momordica balsamina* Seed Lectin

| | MIC (mM) |
|-----------|----------|
| Arabinose | 100 |
| Glucose | NI |
| Galactose | 50 |
| Mannose | NI |
| Maltose | NI |
| Sucrose | NI |
| Lactose | 25 |

MIC: minimum inhibitory concentration, NI: sugar not inhibitory up to a concentration of 200mM.

| Stage of purification | volume (ml) | Protein conc. (mg/ml) | Total Protein conc. (mg) | Total CHO (mg) | Lectin Activity (U/ml) | Total Activity (U/ml) | Specific Activity (U/mg) | Fold purification | % Yield |
|-------------------------|-------------|-----------------------|--------------------------|----------------|------------------------|-----------------------|--------------------------|-------------------|---------|
| Saline extract | 240 | 27 | 6480 | 26 | 216 | 51840 | 8 | 1 | 100 |
| Affinity Chromatography | 15 | 1.2 | 18 | 0 | 2048 | 30720 | 1707 | 213 | 59 |

- Saline extract started from 100g dry floor of *Momordica Balsamina* seeds.
- Human blood group O erythrocytes were used for the assay.
- Total activity (Hemagglutination unit) is defined as the lectin activity multiplied by the total volume.
- Specific activity is defined as the hemagglutination unit divided by the total protein concentration.
- Fold purification is defined as the specific activity of affinity chromatography fraction divided by the specific activity of the saline extract.
- % Yield is defined as the total activity of affinity chromatography fraction divided by the total activity of the saline extract multiplied by 100.

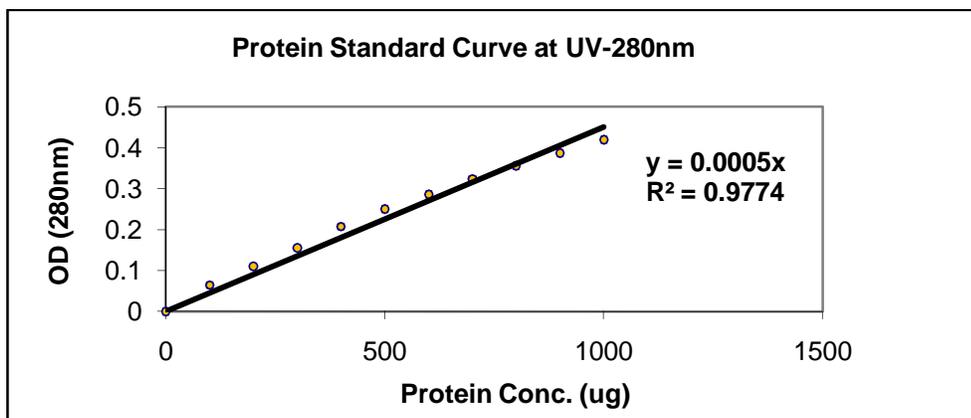


Figure 1: Protein Standard Curve at 280nm for Protein Estimation

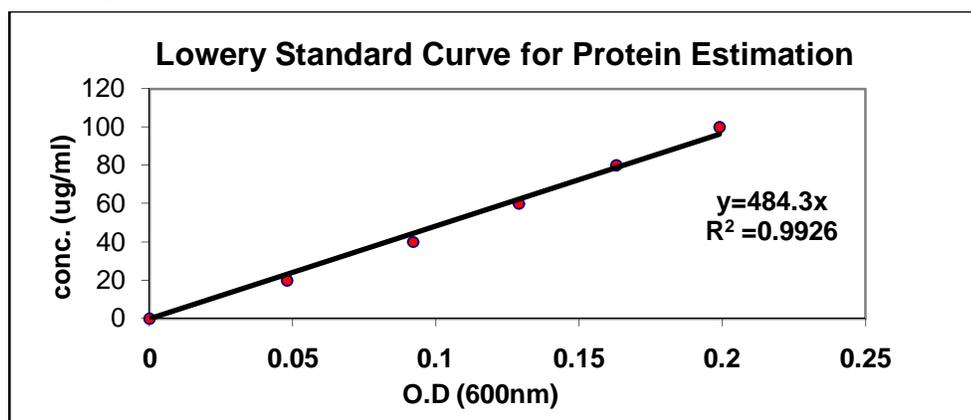


Figure 2: Lowery Standard Curve at 600nm for Protein Estimation

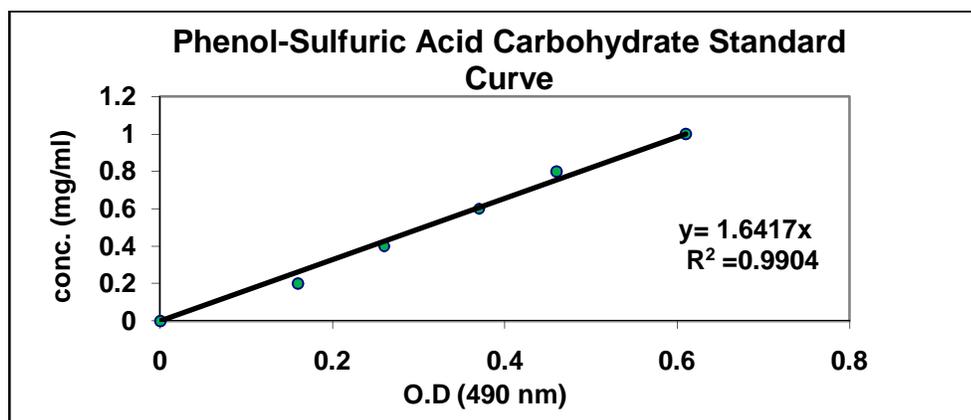


Figure 3: Carbohydrate Phenol-Sulfuric acid Standard Curve for carbohydrates estimation

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