

Short communication

Cross-linked *Leucaena* Seed Gum Matrix: An Affinity Chromatography Tool for Galactose-specific Lectins

Kottapalli Seshagirirao*, Chaganti Leelavathi and Vemula Sasidhar
Department of Plant Sciences, University of Hyderabad, Hyderabad 500046, India

Received 7 July 2003, Accepted 15 August 2003

A cross-linked leucaena (*Leucaena leucocephala*) seed gum (CLLSG) matrix was prepared for the isolation of galactose-specific lectins by affinity chromatography. The matrix was evaluated for affinity with a known galactose-specific lectin from the seeds of snake gourd (*Trichosanthes anguina*). The matrix preparation was simple and inexpensive when compared to commercial galactose-specific matrices (i.e. about 1.5 US\$/100 ml of matrix). The current method is also useful for the demonstration of the affinity chromatography technique in laboratories. Since leucaena seeds are abundant and inexpensive, and the matrix preparation is easy, CLLSG appears to be a promising tool for the separation of galactose-specific lectins.

Keywords: Affinity matrix, Galactose-specific lectins, *Leucaena* seed gum, Mimosaceae

Introduction

The term "gums" is used to describe a group of naturally occurring polysaccharides, which find widespread chemical and industrial use because of their ability to either form the viscous solution or gels or to stabilize emulsions and dispersions. For biochemical applications, the widely used gums are the mucilaginous polysaccharide from seaweeds, among agar, it is perhaps the best known (Smith and Montgomery, 1959; Phillips and Williams, 1993).

Lectins (heamagglutinins) are the proteins of non-immune origin that agglutinate and bind specifically and reversibly to carbohydrate moieties of glycoconjugates (Leiner, *et al.*, 1986). Lectins have served as invaluable tools in biological and medical research for the separation and characterization

of glycoconjugates and glycopeptides, histochemistry of cells and tissues, and the study of cell differentiation (Gabijs and Gabijs, 1993). Because of the importance of lectins and glycoconjugates in many biological interactions, the development of carbohydrate-based technologies (or glycotecnologies) has been intensively pursued. Due to the well-defined carbohydrate specificity, affinity chromatography has become a standard procedure for the purification of lectins in a single step. There are several commercial affinity matrices that are coupled with different ligands to isolate different lectins. The galactose-specific lectins are being isolated by affinity chromatography on Sepharose (a modified form of agar) and/or cross-linked agar with galactose/galactose-containing sugars (Leiner, *et al.*, 1986; Gabijs and Gabijs, 1993). The affinity matrices that are coupled with cyanogen bromide and homobifunctional have many disadvantages. These include low binding capacity, instability, extensive labor, and expensive preparation (Lowe, 1985; Dean *et al.*, 1985).

Nevertheless, the chemistry of other plant gums is known. The only four gum sources that are used as affinity ligands for the isolation of a few galactose-specific lectins are the Arabic gum (Fujita *et al.*, 1975), the guar gum (Goldstein *et al.*, 1976; Appakuttan *et al.*, 1977; Komath *et al.*, 1996; Cavada *et al.*, 1998; Sampaio *et al.*, 1998; Seshagirirao *et al.*, 1999), *Adenanthera pavonia* galactomannan (Moreira *et al.*, 1998), and quite recently, cashew gum (Lima *et al.*, 2002). Among them, the cross-linked guar gum matrix is the most popularized affinity matrix.

In the present study, we report a new galactose-specific affinity matrix from seeds of leucaena, *Leucaena leucocephala* (Lam.) de Wit. (Synonym *L. glauca*), a tropical fodder tree of Mimosaceae. The seeds consist of 15% galactomannan with 1.33 of mannose and galactose ratio (M/G) from the total carbohydrate content (Unrau, 1961). The cross-linked leucaena seed gum (CLLSG) matrix was prepared, and the matrix was evaluated for affinity with a known galactose-specific lectin from the seeds of snake gourd (*Trichosanthes anguina*) (Komath *et al.*, 1996; Anuradha and Bhide, 1999).

*To whom correspondence should be addressed.
Tel: 91-40-23010500 Ext. 4504; Fax: 91-40-23010120 or 23010145
E-mail: kssl@uohyd.ernet.in

Table 1. CLLSG affinity evaluation with *Trichosanthes anguina* seed lectin

Purification step	Protein (mg)	Specific activity*	Total activity ($\times 10^3$ unit)	Yield (%)
Seed kernel PBS extract	963	256	328	100
80% $(\text{NH}_4)_2\text{SO}_4$ ppt.	857	341	292	89
CLLSG affinity chromatography	134	2048	274	83.6

*Specific activity is expressed as titre; the reciprocal of the maximal dilution of protein that gives visible agglutination with 2% trypsinized rabbit erythrocytes

Materials and Methods

Materials Leucaena seeds were collected from trees that were planted at the University of Hyderabad campus. Snake gourd seeds were purchased from a local market. SDS-PAGE molecular weight markers were purchased from Bangalore Genei, India. All of the other chemicals were of analytical grade. They were manufactured in India by different firms.

General analytical methods The protein amount was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard. The affinity chromatography fractions protein profile was monitored spectrometrically at the absorbance of 280 nm. Hemagglutination assays were performed according to Lis and Sharon (1972) with trypsinized rabbit erythrocytes. SDS-PAGE was performed on a 1 mm thick vertical slab gel (10 cm \times 10 cm) with the same solutions and buffers that were described by Laemmli (1970).

Preparation of CLLSG affinity matrix In a medium steel jar of REMI, India blender, 100 g of dried leucaena seeds were blended at low-speed for few seconds. The top layer of the hulls, about 35-40 gm, was separated and the bottom settled kernel pieces, about 60-65 g, were milled to a fine powder in small powdered jar. The powder was further sieved through 1 mm mesh. Next, 60 g of kernel powder was mixed with an emulsion of 18 ml of epichlorohydrin (99%) in 180 ml of 3 M NaOH. The suspension was incubated for 24 h at 40°C and later for 6 h at 70°C (Matsumoto *et al.*, 1981). The formed gel matrix was soaked and washed several times with distilled water to remove the fine particles. Finally, about 80 ml of the gel was recovered and transferred to a 10 mM sodium phosphate buffer, pH 7.2, containing 150 mM NaCl and 0.02% sodium azide (PBS). It was used for affinity chromatography.

Evaluation of the CLLSG affinity matrix Dried snake gourd seeds, 100 g, were de-hulled and about 35 g of the kernels were recovered. The total kernels were homogenized in a REMI blender that was mixed with 350 ml of PBS. The homogenate was centrifuged at 15,000 $\times g$ for 30 min in the Sorvall RC5B Plus centrifuge. The supernatant was first filtered through muslin cloth to remove the lipid layer. It was later filtered through #640 m Qualigens (Mumbai, India) filter paper and subjected to ammonium sulfate fractionation in 0-80% saturation overnight. The fractionated solution was centrifuged at 15,000 $\times g$, and the precipitate was dissolved in PBS and thoroughly dialyzed against PBS. The dialyzed sample was loaded onto the CLLSG matrix column (2.5 \times 10 cm, ca 50 ml) that was previously equilibrated

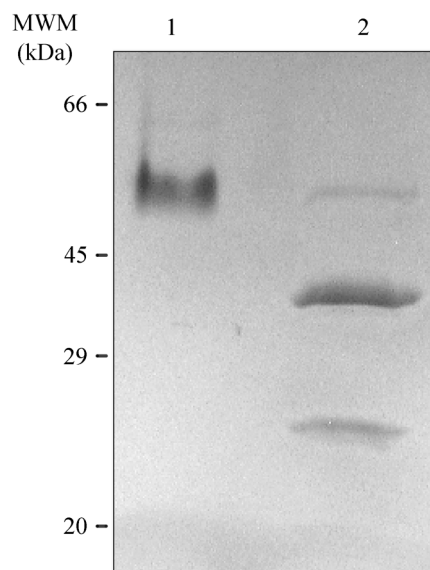


Fig. 1. SDS-PAGE analysis of Snake Gourd Seed Lectin (SGSL) isolated from the CLLSG affinity matrix. Lane 1 is SGSL in the absence of β -mercaptoethanol. Lane 2 is SGSL in the presence of β -mercaptoethanol. Lane MWM is the protein standard markers.

with PBS at the flow rate of 15 ml/hr. The column was washed with PBS until the effluent absorbance was less than 0.02 at 280 nm. The adsorbed lectin was eluted with 0.2 M lactose in PBS. The purity of the protein-rich fractions was verified on SDS-PAGE. All of the purification experiments were performed at 4°C.

Results and Discussion

In the present investigation, there was about 130 mg of snake gourd seed lectin from 35 g of kernels (i.e. ≈ 370 mg/100 g kernels) with 8-fold purification and an overall yield of 84%. This indicates that CLLSG has a higher affinity than the cross-linked guar gum affinity matrix and other galactose-specific affinity matrices that were used earlier (Appakuttan *et al.*, 1977; Komath *et al.*, 1996). The SDS-PAGE analysis also confirmed the lectin purity (Fig. 1) (Anuradha and Bhide, 1999). Although there are recent reports on cashew gum and egg-matrix affinity matrices, the cashew gum matrix was not studied critically for its affinity and commercial value (Lime *et al.*, 2002). The egg-matrix has multiple carbohydrate specificity (Zoccatelli *et al.*, 2003). The cost of the matrix

preparation is inexpensive and simple when compared to commercial galactose-specific matrices (i.e. about 1.5 US\$/100 ml CLLSG matrix). The price was calculated from the expense of seed material, chemicals, and laboratory facilities in India. The current method is also useful for the demonstration of the affinity chromatography technique in laboratories. Since leucaena seeds are abundant and inexpensive, and the matrix preparation is easy, CLLSG appears to be a promising tool for the separation of galactose-specific lectins.

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