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Purification and some properties of a lectin from the seeds of *Trichosanthes anguina*

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Abstract

A galactose-binding lectin was isolated in electrophoretically pure form from the seeds of the snake gourd, *Trichosanthes anguina*, by affinity chromatography on an immobilised lactose column, as well as on a cross-linked *Guar Gum* column. The lectin agglutinates native erythrocytes of human A, B and 0 phenotypes and of rabbit, rat and mouse. The molecular mass of the lectin, as estimated by *Sephadex G-200* gel chromatography, is 49 kDa. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis, after reduction with β-mercaptoethanol, revealed two polypeptide chains linked by disulphide bonds in the lectin molecule. It contains no covalently linked sugars. Amino acid analysis of the lectin revealed a high content of acidic amino acids, relatively lower proportion of basic amino acids and traces of cysteine and methionine. The lectin has good thermal stability, and is inactivated when oxidised by metaperiodate.

Key words: amino acids, haemagglutinating activity, snake gourd

Introduction

Lectins constitute a class of proteins and glycoproteins, of non-immune origin, possessing specific carbohydrate binding sites, and hence, can interact with polysaccharides and glycoconjugates (Goldstein *et al.* 1980). The presence of two or more sugar-binding sites on the molecule allows lectins to agglutinate cells by binding carbohydrates of glycoprotein surface molecules. Lectins reversibly bind mono- or oligosaccharides with great specificity, in a manner comparable to antigen-antibody reaction (Sharon and Lis 1972). By virtue of their specific interaction, lectins have found significant use in membrane studies of normal and cancer cells, studies of blood group substances, histochemical and immunohistochemical

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investigations, purification of glycoproteins and polysaccharides and studies on lymphoblastogenesis (Lis and Sharon 1986). Lectins occur in a wide variety of plants, micro-organisms, invertebrates and vertebrates. Even though India is the home of a very rich tropical flora, only very few investigations have so far been undertaken to study these plants for their lectins. During our studies on the seeds of local plants for their lectins, we detected a lectin activity in the extract from the seeds of the snake gourd, *Trichosanthes anguina*. Subsequently, we isolated this lectin in pure form by affinity chromatography, and studied some of its physico-chemical properties.

Materials and methods

Trichosanthes anguina seeds were collected, dehusked, dried at 37 °C and powdered. The seed powder (25 g) was delipidated with petroleum ether and soaked in 250 cm^3 of phosphate buffered saline (PBS; 0.01 M Na_2HPO_4/KH_2PO_4 buffer of pH 7.4 containing 0.8 % NaCl) at 4 °C overnight. It was subsequently homogenised in the same buffer in a wet grinder. The resulting material was filtered through nylon-wool, and the filtrate was centrifuged for 30 min at 20 000 *g* at 4 °C. The supernatant was collected and used in the haemagglutination assay for detecting lectin activity, as well as for the isolation of the lectin.

Haemagglutination assay was performed using serial dilutions of the seed extract of pure lectin in PBS and 2 % erythrocytes (human A, B and 0 groups and mouse, goat, rat and rabbit) in PBS. After 2 to 3 h of incubation at room temperature, the wells were examined visually for haemagglutination. The highest dilution of the lectin solution causing the maximal haemagglutination was taken as the end-point. Haemagglutination inhibition assays were performed by setting up a series of wells containing twice the minimum concentration of the lectin required to cause complete haemagglutination and of the serial dilutions of the sugar under test. The microtitre plate was incubated at room temperature for 1 h and then 2 % human 0-group erythrocytes in PBS were added to the wells. The wells were examined for heamagglutination after 2 h incubation at room temperature. The lowest sugar concentration giving total inhibition of haemagglutination was recorded.

Preliminary haemagglutination inhibiton studies revealed lactose and galactose to be the best inhibitors of *T. anguina* lectin. Therefore, affinity chromatographic isolation of the lectin was tried using a *Selectin 2* column (*Pierce Chemical Company*, Rockford, USA) with immobilized lactose, and a *Gum Guar* (*Sigma Chemical Company*, St. Louis, USA) column. Soluble *Gum Guar* contains galactomannan molecules with single galactose units in $\alpha(1\rightarrow6)$ linkage (Antony *et al.* 1989). The soluble *Gum Guar* was made isoluble by cross-linking using epichlorohydrin (Appukuttan *et al.* 1977).

Prior to affinity chromatography, the seed extract was subjected to ammonium sulphate fractionation, in which fraction precipitated in the range 40 - 60 % saturation was dissolved in a minimum quantity of PBS, and the solution was dialysed against 3 changes of PBS at 4 °C, and any precipitate formed after 24 h was

removed by centrifugation. The solution was passed through a column of immobilised lactose (*Selectin 2*) or of cross-linked *Gum Guar* pre-equilibrated with PBS. The adsorbed protein was eluted with PBS containing 50 mM lactose (for lactose column) or 150 mM galactose (for *Gum Guar* column). The protein-containing fractions were identified on a UV-spectrophotometer (*Ultrospec II, Pharmacia*, Uppsala, Sweden) at 280 nm, pooled, dialysed extensively to remove sugar and then concentrated. Protein concentration of different preparations was estimated following the method of Lowry *et al.* (1951).

The protein eluted from the affinity columns was subjected to polyacrylamide gel electrophoresis (PAGE) to determine its homogeneity. Molecular mass of the lectin was determined by sodium dodecylsulphate PAGE, using 10 % polyacrylamide gel, both in presence and absence of the reducing agent, β-mercaptoethanol, following the modified Laemmli method (Laemmli 1970, Laemmli and Favre 1973). *Sephadex G-200* (*Pharmacia,* Uppsala, Sweden) gel chromatography was also employed for molecular mass determination (Namjuntra *et al.* 1985).

The gel after PAGE was subjected to periodic acid-Schiff's reaction for detecting any carbohydrate moeity present in the molecule (according to *Pharmacia Fine Chemicals* 1983). The phenol-H_2SO_4 method after Dubois *et al.* (cited in Ashwell 1966) was also employed for carbohydrate analysis. The amino acid analysis of the lectin was performed using *LKB Alphan Plus Amino Acid Analyser* (*Pharmacia,* Uppsala, Sweden).

Thermal and pH stability of the lectin were studied at temperatures ranging between 25 °C and 95 °C and at a pH range of 4 to 10 (Chowdhury *et al.* 1987). Oxidation of the lectin by sodium metaperiodate was studied, following the method described by Chowdhury *et al.* (1987).

Results

Ammonium sulphate fractionation of the PBS extract of *T. anguina* seeds led to about 45-fold increase in lectin activity in the 60 % fraction. The affinity chromatographically isolated lectin accounted for about 80 % of the haemagglutinating activity observed in the initial seed extract. The lectin agglutinated PBS-washed erythrocytes of human A, B, 0 groups, as well as mouse, rabbit, rat and goat erythrocytes (Table 1).

Table 1. Haemagglutinating (HA) activity of an 1 mg cm^{-3} solution of *Trichosanthes anguina* lectin.

Erythrocytes	HA titre
Human (A,B and 0 groups)	4096
Mouse	256
Rat	2048
Rabbit	16384
Goat	256

In haemagglutination inhibition assay, lactose was found to be the best inhibitor, followed by galactose and N-acetyl-D-galactosamine (Table 2). No inhibition by glucose, fructose, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, D(-) arabinose, α-L-(-) fucose, mannose and xylose even at 200 mM concentration was found.

Table 2. Inhibition of agglutination of human group '0' erythrocytes by *Trichosanthes anguina* lectin by saccharides.

	Minimum inhibitory concentration [mM]
Lactose	1.56
D-galactose	4.69
N-acetyl-D galactosamine	6.25
L(+) arabinose	75.00

The lectin eluted from the affinity columns (lactose and *Gum Guar* columns) migrated as a single sharp protein band when subjected to PAGE. Gel-filtration of the lectin on a column of *Sephadex G-200* yielded a single protein peak, and the molecular mass was found to be about 49 kDa. On SDS-PAGE the lectin resolved into a single protein band of 48 kDa in the absence of β-mercaptoethanol, and two bands of 30.2 and 17.8 kDa in the presence of β-mercaptoethanol. Periodic acid-Schiff's staining of the SDS-PAGE gel and the phenol-H_2SO_4 reaction yielded negative results. The amino acid composition of the lectin is presented in Table 3.

Table 3. Amino acid composition of *Trichosanthes anguina* lectin.

Amino acid	[%]*	Amino acid	[%]*
Aspartic acid	12.123	Valine	6.454
Threonine	7.145	Methionine	0.612
Serine	8.879	Isoleucine	6.029
Glutamic acid	10.110	Leucine	9.025
Proline	3.941	Tyrosine	4.196
Glycine	8.349	Phenylalanine	4.624
Alanine	7.416	Histidine	1.672
Cysteine	0.071	Lysine	4.614
		Argenine	4.738

* based on molar mass

In thermostability studies, the lectin showed optimum haemagglutinating activity between 25 °C and 45 °C. Incubation for 30 min at temperatures beyond 45 °C resulted in the decrease in haemagglutinating activity, but some activity was retained even after incubation at 95 °C. The optimum pH range was found to be 5.5 to 7.5. The titre of 1 mg cm^{-3} solution was 4096 at this pH range and decreased thereafter

reaching 128 at pH 10 and 16 at pH 4.5. The lectin lost its haemagglutinating activity when subjected to oxidation by sodium metaperiodate.

Discussion

Affinity chromatography on immobilised sugar adsorbants has been commonly employed as a relatively easy and efficient method for the isolation of lectins in pure form (Matsumoto *et al.* 1981, Goldstein and Poretz 1986). In the present study, the results of haemagglutination assays showed that the *T. anguina* lectin has good affinity for lactose and D-galactose. Therefore, immobilised lactose and cross-linked *Gum Guar* columns were used to isolate the lectin. Both the lactose and *Gum Guar* columns were found to be quite efficient in terms of purity and yield of the lectin. However, affinity chromatography on the *Gum Guar* column appears to be the relatively easy and cheap method for large-scale isolation of the lectin.

The haemagglutinating activity of *T. anguina* lectin is efficiently inhibited by lactose and D-galactose. Glucose and mannose do not inhibit the lectin. *Gum Guar* has terminal α-linked galactose, whereas in lactose terminal galactose is bound by a β-glycosidic linkage to D-glucose. The *T. anguina* lectin binds to both *Gum Guar* and lactose, revealing that the lectin can bind to α- and β-galactosides like the lectins from *Momordica carantia* and *Ricinus communis* (Goldstein and Poretz 1986). As can be expected, N-acetyl-D-galactosamine also inhibits the lectin efficiently.

On SDS-PAGE, under non-reducing condition, the lectin displayed only single protein band of 48 kDa, whereas under reducing condition two bands of 30.2 and 17.8 kDa were produced. Gel filtration of the lectin on a *Sephadex G-200* column yielded a single peak of about 49 kDa. Apparently, the lectin molecule contains two polypeptide chains linked by disulphide bonds. The negative results obtained in periodic acid-Schiff's staining and phenol-H_2SO_4 reaction reveal the absence of any carbohydrate moiety in the lectin molecule. The amino acid composition of *T. anguina* lectin is comparable to many other lectins (Namjuntra *et al.* 1985, Vijayakumar and Forrester 1986, Chowdhury *et al.* 1987) in that it has a high content of acidic amino acids, a relatively low content of basic amino acids and very small amounts of cysteine and methionine. The inactivation of the lectin caused by sodium metaperiodiate may possibly be due to the oxidation of cysteine.

Lectins have been reported from the phloem exudates of the fruits of *Cucurbita pepo, Cucumis melo, Cucumis sativus* and *Cucurbita maxima*, all belonging to the family *Cucurbitaceae* to which *T. anguina* also belong (Goldstein and Poretz 1986). These lectins bind oligosaccharides of β-(1-4)-linked N-acetyl glucosamine. No lectins were detected in the seeds of these species. The lectin isolated from the phloem exudate of *Cucurbita pepo* has been reported to be a protein consisting of a single polypeptide chain of 20 kDa. Another lectin isolated from the phloem exudate of *C. maxima* has been found to have a molecular mass of 48 kDa and is composed of two polypeptide chains linked by disulphide bonds. This lectin binds chitin oligosaccharides with a carbohydrate binding site that best accommodates

N,N',N'-triacetylchitotriose. These lectins from phloem exudates appearred to be different from the lectin from the seeds of *T. anguina.*

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