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Characterization of Con C, a lectin from *Canavalia cathartica* Thouars seeds

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Abstract

Con C, a lectin from *Canavalia cathartica*, a wild species of *Canavalia* was isolated and partially characterized. Mannose–agarose resin was used as an affinity matrix for purification. The lectin showed strong agglutination activity (2.34 AU/ng protein) towards rabbit erythrocytes. Con C agglutinated A, B and O groups of human blood with a preference for A and O groups. The native molecular weight of lectin was 62 kDa and the subunit molecular weight was 31 kDa. No carbohydrate moiety was found to be associated with the lectin. Con C showed a broad pH optima of pH 4–8. Total inactivation of lectin activity occurred at 70 °C when heated for 10 min. The lectin was found to be mitogenic for mouse-spleen cells (total). N-terminal analysis revealed 94% homology with *C. ensiformis* lectin (Con A). *C. cathartica* is a legume with high nutritional values and less antinutritional factors and the potential of the same is yet to be exploited.

Keywords: *Canavalia* species; *Canavalia cathartica*; Lectin; Agglutination; Mannose-specific; Antinutritional factors

1. Introduction

Lectins are a group of proteins or glycoproteins that bind carbohydrates showing distinct structure with a high degree of specificity (Correia & Coelho, 1995; Sharon & Lis, 1990; Traverse et al., 1996). Lectins are commonly found in seeds of leguminosae and accumulate during seed maturation. Though plant lectins are mainly isolated from dry seeds, its occurrence in other vegetative tissues like leaves, roots, stem and tubers has been reported (Goldstein & Hays, 1978; Sharon & Lis, 1989; Suseelan & Mitra, 2001; Suseelan, Mitra, Pandey, Sainis, & Krishna, 2002). Lectins have been reported in different callus tissues also (Del Campillo, Howard, & Shannon, 1981; Jayavardhanan, Padikkala, & Panikkar, 1996; Meimeth, Van Thant, Marcottle, Trinn, & Clarke, 1982). There are lectins with enzyme activities like α- and β-galactosidases (Dey, Naik, & Pridham, 1982; Hankins & Shannon, 1978; Murray, 1983; Suseelan, Bhatia, & Mitra, 1997a; Suseelan, Bhatia, & Mitra, 1997b). The physico-chemical characterization of lectins is essential to explain their characteristic biological properties. There are reports of toxic proteins and lectins that are frequently associated in the seeds of many plants including edible legumes (Carlini, Barcellos, Baeta-Neves, & Guimaraes, 1988; Liener, 1986).

Lectins have been characterized from a number of *Canavalia* species (Cunningham, Wanf, Waxdal, & Edelman, 1975; Hague, 1975; Jayavardhanan et al., 1996; Kojima, Ogawa, Sano, & Matsumoto, 1991; Surolia, Prakash, Bishayee, & Bachhawat, 1973; Terada, Enomoto, Hashimoto, & Kimoto, 1987). Concanavalin A (Con A) obtained from the seeds of *C. ensiformis* is the extensively studied glucose/mannose specific lectin. Importantly, exploitation of the less familiar, wild plant species is one of the possible

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measures to overcome the malnutrition of the increasing population in developing countries. Identification of such new plant species has been of worldwide interest to compliment conventional crops. Legumes are known to be an economically viable source of proteins. Although conventional legumes are a good source of proteins, the commercial exploitation of wild species have been limited, since their nutritional and antinutritional implications have not yet been evaluated.

The work reported here has been initiated keeping the above objectives as the foremost priority. *C. cathartica* Thouars and *C. maritima* (Aubl) Thouars are the most commonly found two wild legume species on the sand dunes of southwest coast of India (Arun, Beena, Raviraja, & Sridhar, 1999). The pods of these legumes are consumed by coastal dwellers. *C. maritima* and *C. cathartica* are two perennial landraces, which share a common challenge for developing as viable crops. These wild legumes are known for their fast growth, better tolerance to adverse environmental conditions and resistance to diseases (Seena & Sridhar, 2006). The nutritional evaluation of these species has been done to a greater extent by Seena and Sridhar (2004); Seena and Sridhar (2006) and they found that these legumes have great potential for their protein, carbohydrate, mineral and sulphur and essential amino acid contents. However, the antinutritional evaluation of these species has been only partially completed (Arun, Sridhar, Raviraja, Schmidt, & Jung, 2002). *C. cathartica* is found to be distributed throughout tropical Asia and Africa (Arun et al., 1999). More information on the antinutritional factors is needed to assess their utilization as a high potential food legume. Here we report the purification and partial characterization of a lectin from *C. cathartica* as part of the evaluation program of this promising, unexplored *Canavalia* species. *Canavalia* species are known for their strong lectin activity and as lectin is considered to be one of the antinutritional factors for animal species, characerization of lectin from this species is worthwhile.

2. Materials and methods

2.1. Materials

C. cathartica seeds were collected from the sand dunes of the West coast of Mangalore. All the chemicals and reagents used were of analytical grade and were procured locally. Affinity matrix mannose–agarose, molecular weight markers for gel filtration and other fine chemicals were obtained from Sigma Chemical Company (St. Louis, MO, USA). Ultrogel (AcA44), DEAE-Sephacel and molecular weight markers for SDS–PAGE were procured from Pharmacia Biotech (Uppsala, Sweden).

2.2. Extraction of lectin

C. cathartica dry seed powder (20 g) was suspended in 500 ml of 0.05 M Tris–HCl, pH 8.5 containing 0.5 M NaCl and stirred for 4–5 h at room temperature (RT-25 °C). The slurry was centrifuged at 17,500*g* for 20 min in a refrigerated centrifuge (Kubota). The supernatant (crude extract) was used for further analyses.

2.3. Affinity chromatography

A mannose–agarose column (5 ml) was regenerated by washing with 20 volumes of 0.1 M Na-acetate buffer, pH 4.5, containing 0.5 M NaCl followed by 20 volumes of 0.1 M Tris–HCl, pH 8.5 containing 0.5 M NaCl. The column was then equilibrated with 50 mM Tris–HCl, pH 8.5 containing 0.5 M NaCl. The crude extract was passed directly through the column. The unbound protein was washed off with the equilibration buffer. The bound protein was eluted with 0.2 M mannose in 50 mM Tris–HCl, pH 8.5 containing 0.5 M NaCl. The eluted protein was precipitated with $(NH_4)_2SO_4$ (85% saturation) at 6 °C. The precipitate was dissolved in 20 mM Tris–HCl, pH 8 and dialyzed against the same.

2.4. Gel filtration on AcA44

The affinity purified protein was initially subjected to gel filtration on AcA44 column (55 × 1.2 cm) pre-equilibrated with 20 mM Tris–HCl, pH 8 containing 0.5 M NaCl. The eluted fractions (1 ml) were checked for agglutination activity and absorption at 280 nm (A_{280}). The active fractions were pooled and protein precipitated with $(NH_4)_2SO_4$ at 85% saturation. The protein after centrifugation was dissolved in 10 mM Tris–HCl, pH 7.4 containing 0.5 M NaCl and 0.02% NaN_3 and dialyzed against the same.

2.5. Gel filtration using FPLC

The protein after gel filtration on AcA44 column was further subjected to gel filtration by FPLC using Superose-12 column. The column was equilibrated with 10 mM Tris–HCl, pH 7.4 containing 0.5 M NaCl and 0.02% NaN_3. The protein was eluted with the same buffer containing 10 mM D-mannose in order to avoid the non-specific binding to the matrix. Fractions (0.5 ml) of the main peak were pooled and the protein precipitated with $(NH_4)_2SO_4$ (85% saturation) and the precipitate was dissolved and dialyzed against the equilibration buffer.

2.6. Protein estimation

Protein was estimated by the biuret method (Layne, 1957). A standard graph was prepared using lipid-free BSA (fraction V).

2.7. Hemagglutination assay

Twofold serial dilution method was used for hemagglutination assay (Nakagawa, Yasokawa, Ikeda, & Nagashima, 1996). Fifty microlitres of protein (50 μg) was

incubated with 50 μl of 2% suspension of trypsinised rabbit erythrocytes. After 1 h of incubation the agglutination was checked under a microscope. The agglutination activity unit (AU) or titre value was determined as the reciprocal of the last dilution that showed agglutination. The specific activity of the lectin was determined as the agglutination activity units per mg protein.

2.8. Blood group specificity

Human blood of groups A, B and O collected from three donors were used for checking the blood group specificity of the lectin. The agglutination assay was carried out as described earlier using both untreated and trypsin-treated erythrocytes.

2.9. Polyacrylamide gel electrophoresis

The purified lectin was subjected to electrophoretic analyses under native as well as denatured condition. Electrophoresis on 10% acid gel was performed according to the method of Reisfeld, Lewis, and Williams (1962). Electrophoresis under denaturing conditions was performed according to the method described by Laemmli (1970) using 12% polyacrylamide gel. Electrophoresis was carried out at a constant voltage (100 V) for 4–5 h in a Pharmacia electrophoretic apparatus (GE 2/4). After electrophoresis the gels were stained with 0.1% coomassie brilliant blue (CBB, R-250) prepared in destaining solution (1:3:6, acetic acid:methanol:water, v/v) and was destained in the same solution devoid of CBB.

2.10. pH stability and pH optima

Incubating the protein with buffers from pH 1 to 10 assessed the pH stability and pH optima of the lectin. The buffers used were 0.1 N HCl (pH 1.0), 50 mM glycine–HCl (pH 2–3), 50 mM Na-acetate–acetic acid (pH 4–5), 50 mM maleic acid–NaOH (pH 6.0), 50 mM Tris–HCl (pH 7–8) and 50 mM glycine–NaOH (pH 9–10). Fifty micrograms (10 μl) lectin was incubated with 90 μl of buffer in a microtitre plate and allowed to stand at 6 °C for overnight. From the above sample, a 50 μl aliquot was serially diluted with 0.3 M NaCl. Agglutination assay was performed by adding 50 μl of trypsinised rabbit erythrocytes (2%). Agglutination was checked under the microscope after incubating for 1 h at room temperature (25 °C). The agglutination units at pH 8 were considered as 100%. The experiment was performed in three replications.

2.11. Temperature optima and temperature of inactivation

Five hundred micrograms lectin in 500 μl of 20 mM Tris–HCl buffer pH 8 in a test tube was heated in a Multi-block heater from 25 to 100 °C. An aliquot of 25 μl was withdrawn at an interval of 10 min after attaining the desired temperature and the agglutination activity was assayed.

The kinetics of inactivation of the lectin was performed by heating the protein at 70 °C. Two milligrams lectin in 1 ml of 20 mM Tris–HCl buffer, pH 8 was heated in a Multi-block heater. An aliquot of 50 μl was withdrawn at every 10 min interval up to 120 min. The lectin sample at RT served as the control. Both the experiments were performed in three replications.

2.12. Effect of metal cations

The lectin solution (500 μg in 100 μl) was diluted to 500 μl with 20 mM Tris–HCl, pH 8 and 500 μl of 20 mM EDTA was added and incubated for 24 h at 6 °C. The solution was dialyzed extensively against 20 mM Tris–HCl, pH 8 with repeated changes. Aliquots of 50 μl each of the dialyzed samples was incubated individually with 50 μl of 2 mM $CaCl_2$, $MgCl_2$ and $MnCl_2$ for 2 h. The agglutination activity was assayed by serial dilution as explained earlier.

2.13. Estimation of carbohydrate content

The carbohydrate content of the lectin was determined by phenol–sulphuric acid method as described by Dubois, Gillies, Hamilton, Rebers, and Smith (1956) with D-mannose as the standard.

2.14. Inhibition of agglutination

The inhibition of agglutination was studied following the method of Kurokawa, Tsuda, and Sugino (1976). Various carbohydrates and glycoproteins were incubated with lectin and agglutination assay was carried out as described earlier.

2.15. Mitogenic activity

The mitogenicity of the lectin was tested by standard lymphocyte proliferation assay (Severinson & Larsson, 1986, chap. 63). Splenic lymphocytes (total) were obtained by dissecting spleens from 10 to 12 week-old C57BL/6 mice. Single cell suspension was prepared in RPM1 1640 medium (Cat No. R4130, Sigma), containing 15 mM HEPES buffer, 2 mM L-glutamine and 10% CPSR 2 (controlled processed serum replacement; Cat. No. 9030, Sigma). Erythrocytes were lysed by treating with 0.83 M NH_4Cl. Protein solution containing 1–100 μg lectin in 100 μl volume was added to 100 μl of cell suspension having about 2×10^5 cells and incubated at 37 °C for 48 and 72 h in 5% CO_2 atmosphere. Each sample was pulsed with 3.7×10^{-2} MBq of [^{3}H] thymidine (Sp. Act. 240 GBq/mmol.) for 16 h. The cells were harvested on glass fibre filter discs and the thymidine incorporation was quantified in a Packard liquid scintillation counter (Tri-Carb 2100TR). Three replicated experiments were carried out for both the treatments.

2.16. Amino acid sequencing

A sample of 200 picomoles of purified lectin was used to determine the N-terminal sequence by Edman degradation method with a peptide sequencer (Applied Biosystems Procise 494, Foster City, CA). The chromatography system was calibrated with phenyl thiohydantoin (PTH) amino acid standards prior to each analysis. The PTH amino acids produced by the sequential Edman degradation of the analyzed peptides/proteins were identified on-line using the retention times of a PTH standard mixture run as reference.

3. Results

3.1. Isolation and purification

The protein extracted in 50 mM Tris–HCl, pH 8.5 containing 0.5 M NaCl revealed strong agglutination activity with both native as well as trypsinised rabbit erythrocytes. The crude extract had the sp. activity of 7.6×10^5 AU/mg protein. The crude extract was loaded onto the mannose-agarose column. The unbound fraction did not show any lectin activity. The bound protein eluted with mannose had the sp. activity of 1.1×10^7 AU/mg protein. Hence, there was 14-fold purification in one step. The affinity-purified protein was further enriched by gel filtration using Ultrogel (AcA44), where the protein separated into a major and a minor peak (Fig. 1). The major peak that showed agglutination activity (sp. act. 1.4×10^7 AU/mg protein) was subjected to gel filtration on FPLC using a Superose-12 column (Fig. 2). The major peak showing agglutination activity was pooled and protein precipitated with 85% saturation of $(NH_4)_2SO_4$. Fold purification at different stages are given in Table 1. The purified Con C showed an activity of 2.34 AU/ng protein. The molecular mass of the protein was estimated to be about 62 kDa.

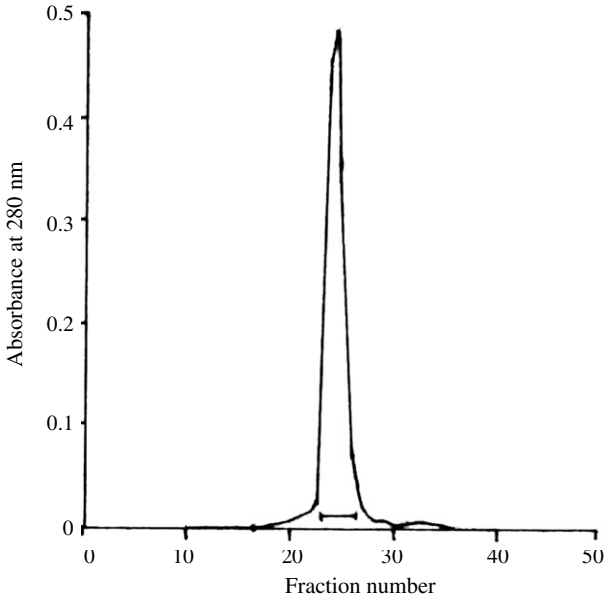

Fig. 2. Protein profile of *Canavalia cathartica* lectin on FPLC using Superose-12 column. Bar indicates the fractions pooled.

The purity of the lectin was determined by polyacrylamide gel electrophoresis (PAGE) on native gel (acid gel, pH 4.3) as well as on dissociating system (SDS–PAGE). A single band was seen on acid gel with an R_f value of 2.3 (Fig. 3). Under dissociating conditions (12% SDS–PAGE), the purified protein was resolved into a major band of 31 kDa (Fig. 4).

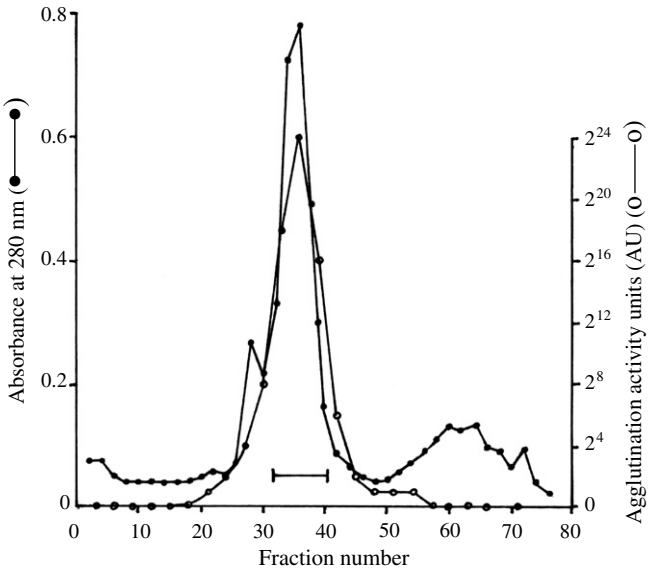

Fig. 1. Gel filtration chromatography profile of *Canavalia cathartica* lectin on AcA44 column. (●—●) Absorbance of protein at 280 nm. (○—○) Agglutination activity of protein fractions. Bar indicates the fractions pooled.

3.2. Hemagglutination inhibition assay

The results of inhibition of hemagglutination by various carbohydrates are summarized in Table 2. An initial concentration of 200 mM for various sugars and 25–200 mg/ml of glycoproteins were used for the inhibition studies. The inhibitory concentration of D-mannose and methyl-D-glucopyranoside were 100 mM. All other sugars used did not inhibit agglutination up to 200 mM.

For glycoproteins ovalbumin, and fetuin showed inhibition at 6.25 mg/ml concentration. The inhibitory concentration of mucin and asialofetuin was 3.13 mg/ml. Thyroglobulin showed the lowest inhibitory concentration of 0.195 mg/ml among the glycoproteins tested.

3.3. Blood group specificity

The purified Con C agglutinated both native as well as trypsinised rabbit erythrocytes. The lectin also agglutinated human blood of groups A, B and O. At pH 7.2 (PBS), B group blood showed a minimum agglutination of 64 units (AU) where as, groups A and O showed 256 AU under the

Table 1
Purification of *Canavalia cathartica* lectin (Con C)

Purification step	Protein (mg)	Total agglutination activity (AU)	Specific act. (AU/mg Protein)	Purification fold
Crude extract	4104	3.1×10^9	7.6×10^5	1
Mannose–agarose column	880	9.5×10^9	1.1×10^7	14.5
Gel filtration on AcA44	610	8.5×10^9	1.4×10^7	18.4
Gel filtration on FPLC	148	8.1×10^9	5.5×10^7	72.4

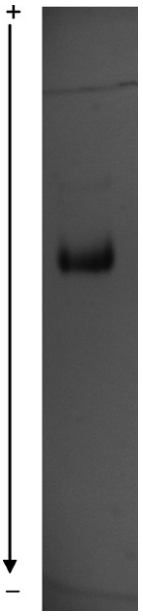

Fig. 3. Electrophoretic pattern of purified *Canavalia cathartica* lectin on acid gel (10% Native-PAGE). About 25 μg of purified lectin was loaded on the gel.

same condition. The same trend was shown at pH 8.5, where B group showed 512 AU and blood groups A and O showed 1024 AU.

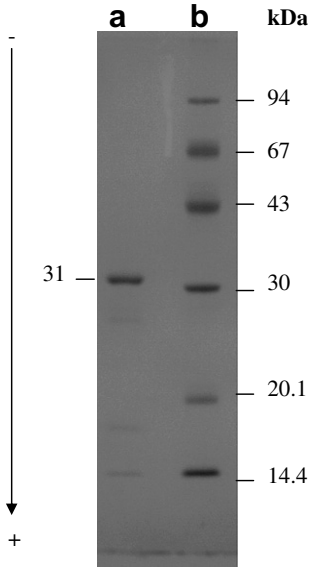

Fig. 4. Electrophoretic pattern of purified *Canavalia cathartica* lectin on 12% SDS–PAGE: (a) purified *C. cathartica* lectin. About 25 μg of protein was loaded. (b) Low molecular weight Marker proteins (Pharmacia).

Table 2
Inhibition of agglutination of Con C by carbohydrates

Carbohydrate	Inhibitory concentration[a]
D-Mannose	<100 mM
D-Glucose	>1000 mM
D-Galactose	>1000 mM
Stachyose	>200 mM
Lactose	>200 mM
D-Xylose	>200 mM
N-acetyl-D-glucosamine	>200 mM
N-acetyl-D-galactosamine	>200 mM
α-D-(+)Fucose	>200 mM
L-Glucose	>200 mM
D-Raffinose	>200 mM
D(+)glucosamine	>200 mM
D(+)galactosamine	>200 mM
Methyl-α-D-glucopyranoside	<100 mM
Methyl-β-D-glucopyranoside	>200 mM
Glycerol	>200 mM
BSA	>100 mg/ml
Ovalbumin	<6.25 mg/ml
Mucin	<3.125 mg/ml
Fetuin	<6.25 mg/ml
Asialofetuin	<3.13 mg/ml
Thyroglobulin	<0.195 mg/ml

[a] In all the cases 250 ng lectin in 25 μl volume was used for incubation.

3.4. pH profile

The pH sensitivity profile of Con C is shown in Fig. 5. The Con C exhibited a broad pH optima between pH 4 and 8. The lectin lost 50% agglutination activity at pH 8.5.

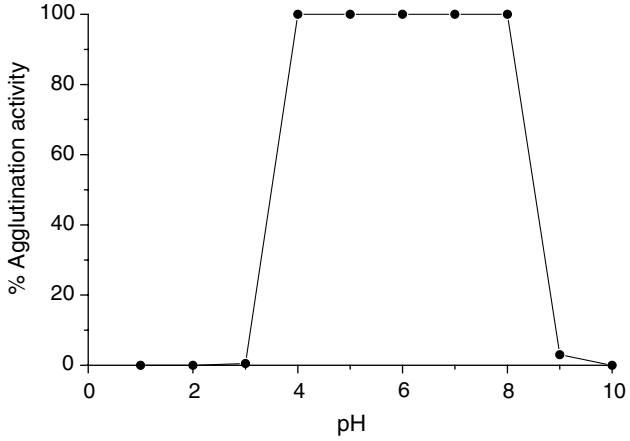

Fig. 5. pH stability and pH optima of *Canavalia cathartica* lectin.

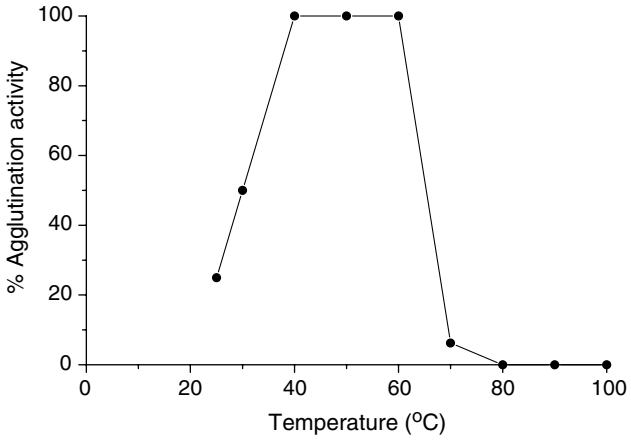

Fig. 6. Temperature optima of *Canavalia cathartica* lectin.

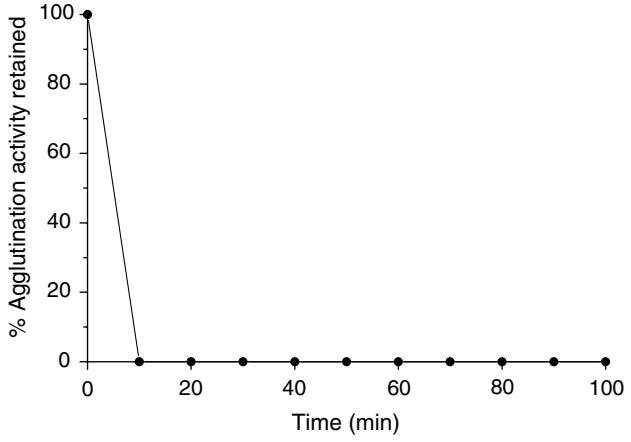

Fig. 7. Thermal inactivation of *Canavalia cathartica* lectin at 70 °C.

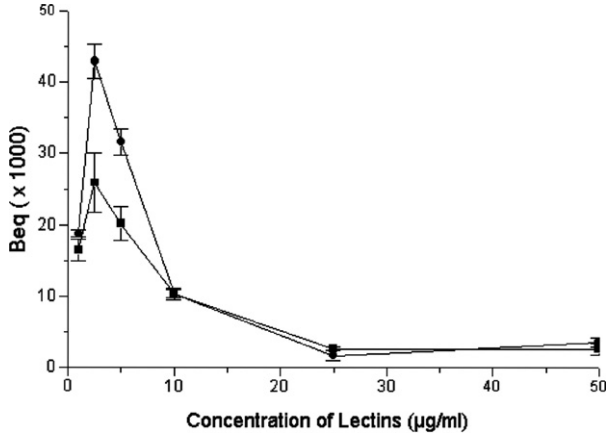

Fig. 8. Mitogenic activity of *Canavalia cathartica* lectin. (—) Mouse spleen cells (total) treated with purified *C. cathartica* lectin for 48 h. (•—•) Mouse spleen cells (total) treated with purified *C. cathartica* lectin for 72 h.

3.5. Kinetics of heat inactivation

On heating the lectin from room temperature (25 °C) to 100 °C, 100% agglutination activity was present up to 60 °C. However, 97% agglutination activity was lost when heated to 70 °C for 10 min (Fig. 6). The kinetics of heat inactivation studied at 70 °C showed loss of 50% agglutination activity by heating for 5 min and total loss of activity was observed after 10 min (Fig. 7).

3.6. Effect of metal cations

The incubation of Con C with EDTA did not result in loss of agglutination activity and also there was no change in activity when EDTA-treated Con C was incubated with Ca^{2+}, Mn^{2+} and Mg^{2+}.

3.7. Carbohydrate content

The estimation of carbohydrate content of the lectin by phenol–sulphuric acid method revealed that Con C contained no carbohydrate moiety.

3.8. Mitogenic activity

The lectin Con C was mitogenic to total mouse spleen cells of C57BL/6 mice. The highest mitogenicity was observed at 2.5 µg/ml concentration of Con C at 48 h and 72 h of treatment (Fig. 8).

3.9. N-terminal sequence analysis

The N-terminal sequence of 17 amino acids of the purified Con C was determined. The analysis showed the sequence as Ala-Asp-Thr-Lys-Val-Ala-Val-Glu-Leu-Asp-Thr-Tyr-Pro-Asn-Thr-Asp-Ile (Table 3).

4. Discussion

A lectin was purified from *C. cathartica*, a wild species of *Canavalia*, utilising the conventional purification methods like ammonium sulphate precipitation, gel filtration chromatography and affinity chromatography. Mannose–agarose was used as the matrix for affinity chromatography, since the lectin was shown to be mannose-specific.

C. cathartica Thouars is one among the wild legume species, an under exploited plant genetic resource, which is under threat (Arun et al., 2002; Rajaram & Janardhanan, 1992). The pods of this wild legume grown in the coastal

Table 3
Alignment of N-terminal amino acid sequence of *Canavalia cathartica* with other lectins

Canavalia ensiformis	A	D	T	I	V	A	V	E	L	D	T	Y	P	N	T	D	I
Canavalia maritima	A	D	T	I	V	A	V	E	L	D	T	Y	P	N	T	D	V
Canavalia cathartica	A	D	T	K	V	A	V	E	L	D	T	Y	P	N	T	D	I
Dioclea grandiflora	A	D	T	I	V	A	V	E	V	N	S	Y	P	N	T	D	I
Dioclea lehmanni	A	D	T	I	V	A	V	E	L	D	S	Y	P	N	T	D	I

sand dunes are used as food by coastal dwellers. The nutritional evaluation of some of the *Canavalia* species has been done earlier (Bressani, Brenes, Gracia, & Elias, 1987; Rajaram & Janardhanan, 1992). *C. cathartica* contains about 36% crude protein which is more than the commonly consumed pulses of India and the carbohydrate content is on par with other *Canavalia* species (Arun et al., 2002). *C. cathartica* also contained higher levels of essential amino acids including lysine. *C. cathartica* does not contain trypsin inhibitor activity (data not shown).

The *C. cathartica* lectin (Con C) activity was found to be destroyed on heating at 70 °C for 10 min. Concanavalin A (Con A), the major antinutritional factor of *C. ensiformis* seeds takes at least 3 h of cooking at 96 °C to get inactivated (Udedibie & Carlini, 1998).

The elution profile of Con C by gel filtration on AcA44 column (Fig. 1) and FPLC (Fig. 2) gave a major protein peak with agglutination activity. The yield of Con C was 3.6% that is comparable to the yield of Con A isolated from *C. ensiformis* and *C. gladiata* (Surolia et al., 1973). The SDS–PAGE of Con C showed a major polypeptide of 31 kDa indicating that the native lectin is a dimer consisting of two identical subunits. Similar SDS–PAGE patterns were reported by Surolia et al. (1973) for Con A from *C. ensiformis* and *C. gladiata*, but with different molecular weights. The Con C did not show the presence of carbohydrate content.

Con C was found to be mitogenic for mouse spleen (total) cells. The maximum stimulation was shown at 2.5 μg/ml concentration of the lectin (Fig. 8). The dose response may be due to toxicity of Con C above 10 μg/ml. The result reveals that the Con C was more potent mitogen than Con A for the two treatments at 48 and 72 h (data not shown).

Con C had a single N-terminal amino acid sequence determined on an automated Edman degradation amino acid sequencer. The N-terminal sequence of 17 amino acid residues of Con C was determined and has shown homology of 94% with *C. ensiformis* lectin Con A and *C. brasiliensis* (Con Br, reported elsewhere) and 88% homology with *C. maritima*. The Con C also showed a homology of 76% with *Dioclea grandiflora* and 82% with *D. lehmanni* (Table 3). There are reports of difference in the biological activities of lectins despite the fact that lectins are structurally close (Perez, Perez, Sousa-cavad, Moreira, & Richardson, 1991).

The hemagglutination assay showed a preferential agglutination towards A and O groups of human blood. Blood group B showed 25% agglutination activity at pH 7.2 as compared to blood groups A and O. This sort of differential agglutination was not reported for lectins from any of the *Canavalia* species. Similar differential specificity was shown by *Crotalaria pallida* lectin that showed partial inhibition of agglutination for human blood group A (Regoe, deCarvalho, Marangoni, de Oliveira, & Novello, 2002). Con C did not require divalent metal cations like Ca^{2+}, Mn^{2+} and Mg^{2+} for activity, unlike Con A (Hemperly & Cunningham, 1983).

The hemagglutination activity of Con C against erythrocytes was inhibited by D-mannose. The minimum inhibitory concentration was 100 mM for 0.25 μg lectin (0.4 mM D-mannose/ng Con C). The same concentration was also shown for methyl-α-D-glucopyrannoside (Table 2). The other sugars showed inhibitory concentrations greater than 200 mM for the same concentration of Con C. The glycoproteins that showed inhibition of agglutination were ovalbumin (2.5 μg/ng Con C), fetuin (2.5 μg/ng Con C), aisalofetuin (0.625 μg/ng Con C) and thyroglobulin (0.02 μg /ng Con C).

Though the agglutination activity of Con C was found to be stable up to 60 °C, the lectin lost 50% agglutination activity in 5 min when heated at 70 °C and was totally inactivated in 10 min (Fig. 7). Also, the agglutination activity was retained over a broad pH range from 4 to 8 (Fig. 5). The broad pH range for agglutination activity is not uncommon for seed lectins (Coffey, Uebersax, Hosfield, & Bennink, 1993).

Agbede and Aletor (2005) evaluated the potentials of *C. ensiformis* and *Mucuna pruriens* and compared favourably with those reported for several other conventional edible legumes and leaf vegetables for their proximate contents. But the poor quality proteins make the legume seeds unsuitable for human nutrition. The nutritional evaluation of *C. cathartica* reveals that these seeds are a promising food source due to their higher contents of protein, carbohydrate, calorific value, essential amino acids and low saturated fatty acids and antinutritional factors (Arun et al., 2002). The lectin activity (the antinutritional factor) was found to be inactivated when the protein was heated at 70 °C (Fig. 7) and no trypsin inhibitor activity could be found in this legume (data not shown). Adaptation of *C. cathartica* as an alternative source of protein is a worthwhile effort, as this legume seems to have tremendous potential for further crop improvement by mutation breeding.

5. Conclusion

C. cathartica is an under-exploited wild species of *Canavalia* with high potential with respect to its nutritional qualities. The associated strong lectin activity, an antinutritional factor, gets inactivated at 70 °C. The dimeric lectin showed preferential agglutination of group A and O of human blood groups. The lectin is mitogenic for mouse spleen cells (total). The N-terminal analysis showed 94% homology with Con A. Con C is a new lectin that needs extensive studies for its biochemical properties and their utilities.

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