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PURIFICATION AND PROPERTIES OF A LECTIN FROM *LONCHOCARPUS CAPASSA* (APPLE-LEAF) SEED

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Key Word Index—*Lonchocarpus capassa*; Leguminosae; apple-leaf tree; hemagglutinating activity; characterization; amino acid sequences.

Abstract—A lectin has been purified from *L. capassa* seed by ammonium sulphate fractionation and affinity chromatography on a column of D-galactose-derivatized Sepharose. The lectin is a glycoprotein which contains 3.8% neutral carbohydrates comprised of mannose, *N*-acetylglucosamine, xylose and fucose. The subunit M_r of the lectin is 29 000, it has only alanine as N-terminal amino acid and contains 240 amino acids with a high content of acidic and hydroxy amino acids, single residues of methionine and histidine and the absence of cystine. The lectin of *L. capassa* seed is a metalloprotein in that it contains 0.8 mol Ca^{2+} and 0.4 mol Mn^{2+} per mol. It agglutinates untreated human A, O and B type erythrocytes and rabbit erythrocytes. *N*-Acetyl-D-galactosamine was the best inhibitor. D-Galactose and various carbohydrates containing this sugar inhibit the hemagglutinating activity of the lectin. The lectin is also inhibited by D-glucose. The amino-terminal sequence of the lectin from *L. capassa* seed shows a significant degree of homology with many lectins from leguminous plants and is related to concanavalin A by a circularly permuted sequence homology.

INTRODUCTION

Lectins are distributed in the plant kingdom, and seeds belonging to the Leguminosae plant family are particularly rich sources of these proteins [1, 2]. The lectins from kidney beans [3], peanuts [4] and soybeans [5] are composed of subunits similar in size to the monomer of Con A [6]. However, whereas they specifically bind galactose, Con A binds glucose and mannose. Further, the lectins of soybeans and kidney beans are glycoproteins, whereas Con A contains no covalently bound carbohydrate. The lectins from lentils [7], peas [8] and flava beans [6] have sugar-binding specificities similar to Con A but the M_rs of these lectins are only half that of Con A tetramer. Moreover these lectins are made up of two types of polypeptide chains (α and β) both smaller than the Con A monomer. In spite of these differences all of the known amino acid sequences of leguminous lectins can be aligned starting at the N-terminus, except that of Con A. However, residue 123 of Con A aligns with the first residue of the other lectins. Comparison of the complete sequence of Con A [6] with the sequences of flava bean [6], soybean [5] sainfoin [9] lentil [7] and peanut [5] lectins demonstrate an unusual circularly permuted homology [10, 11]. The structural relationships and properties of legume lectins are well described by Shannon [12, 13]. During a survey of various leguminous seeds which are native to South Africa, it was observed that extracts of the seeds of *Lonchocarpus capassa* (apple-leaf) have a potent hemagglutinating activity for human erythrocytes. Although the proteinase inhibitors from the seeds of *L. capassa* have been studied to date, nothing has been published on the lectins from this source [14]. As a contribution to studies on lectins from leguminous plants the present communication describes the purification and some properties of a lectin from *L. capassa* (apple-leaf) seeds.

RESULTS

The purification of the lectin from *L. capassa* seeds is summarized in Table 1. The affinity chromatography step on a column of galactose-derivatized Sepharose is depicted in Fig. 1. Peak C2 which was eluted with 0.2 M galactose contained 60% of the hemagglutinating activity of the seed extracts. Further fractionation of the lectin on a column of galactose-derivatized Sepharose using a linear galactose gradient (0–0.2 M over 500 ml) in 0.05 M phosphate buffer pH 7.2 in 0.9% sodium chloride, revealed one sharp and symmetrical peak. Disc electrophoresis both in the absence and presence of sodium dodecyl sulphate (SDS), showed that the lectin is homogeneous. The subunit M_r of the lectin determined by SDS gel method is 29 000. The lectin contains 0.8 mol Ca^{2+}, 0.5 mol Mg^{2+} and 0.4 mol Mn^{2+} per mol. The amino acid composition of the lectin is given in Table 2. In common with most legume lectins, it is devoid of cystine, has single residues of methionine and histidine and is rich in acidic and hydroxy amino acids [1]. The N-terminal amino acid sequence of the lectin, determined on the Beckman sequencer is given in Fig. 2 (a). Alanine is the only N-terminal amino acid observed. The lectin is a glycoprotein and contains 3.8% neutral carbohydrate as determined by the phenol–sulphuric acid method. High performance liquid chromatography of the alditol benzoate sugar

Table 1. Summary of the purification of the lectin from *L. capassa* seed

Steps	Protein (g)	Total activity (units × 10^3)	Specific activity (units/mg × 10^3)	Yield (%)
Seed	30	75.0	2.5	100
$(NH_4)_2SO_4$ precipitation				
0–30% fraction	0.42	0.5	1.1	0.7
30–60% fraction	4.41	55.1	12.5	73.5
60–100% fraction	2.31	1.8	0.8	2.4
Affinity chromatography	0.18	45.0	250	60.0

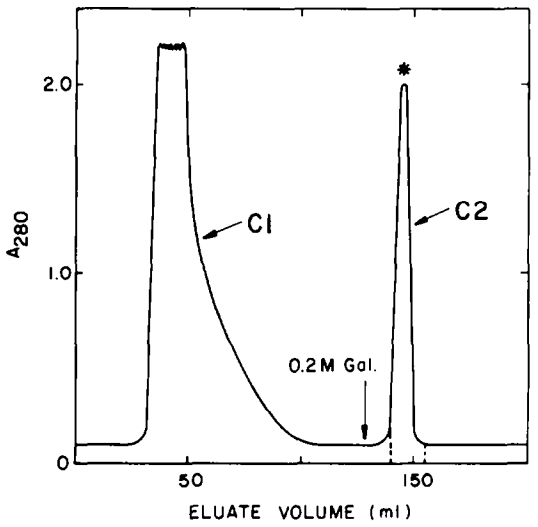

Fig. 1. Affinity chromatography of lectin from *L. capassa* seed on a column of galactose-derivatized Sepharose 6B. The 30–60% $(NH_4)_2SO_4$ fraction in 0.05 M potassium/sodium phosphate ph 7.2 in 0.9% NaCl was loaded on the column (1.9 × 15 cm). The hemagglutinating activity was eluted with 0.2 M galactose solution at the flow rate of 100 ml/hr. The column temperature was 4° and the eluate was monitored at 280 nm. * Indicates peak with hemagglutinating activity.

derivatives showed that the lectin contains mannose, *N*-acetylglucosamine, xylose and fucose in amounts corresponding to 5.5, 1.8, 1.0 and 0.5 mol per mol of M_r 29 000, respectively.

The lectin agglutinates untreated human erythrocytes of types A and O at a concentration of 50 μg per ml (Table 3). With human type B erythocytes and rabbit erythrocytes a 4–5-fold higher concentration of the lectin is required to cause agglutination, while baboon erythrocytes are not agglutinated even at 1 mg lectin per ml. Various carbohydrates were tested for inhibition of hemagglutinations by the lectin (Table 4). *N*-Acetyl-D-galactosamine is the most potent inhibitor, while galactose and carbohydrates containing this sugar exhibit various degrees of inhibitory activity. Surprisingly, inhibition by methyl-α-D-glucopyranoside and D-glucose was observed. Maltose, L-fucose and L-sorbose have no effect up to a concentration of 0.1 M.

The lectin contains a single methionine residue and was submitted to cleavage by cyanogen bromide. The two fragments was soluble in 50% acetic acid but could not be separated by gel filtration on Sephadex G-75 column. The N-terminal amino acid sequence of the mixture of unseparated fragments gave two different PTH-amino acid for most steps. Since one of each pair of results could be ascribed to the known N-terminal sequence of the uncleaved lectin, it was possible to assign the remainder to the N-terminal sequence of the fragment obtained at the methionine cleavage point.

Table 2. Amino acid composition of the lectin given as mols of residue per mol lectin

Amino acid	Residues	Amino acid	Residues
Aspartic acid	35.4 (35)	Methionine	1.1 (1)
Threonine	26.6 (27)	Isoleucine	15.9 (16)
Serine	24.8 (25)	Leucine	15.5 (16)
Glutamic acid	13.6 (14)	Tyrosine	7.0 (7)
Proline	12.1 (12)	Phenylalanine	13.1 (13)
Glycine	19.7 (20)	Lysine	9.8 (10)
Alanine	13.9 (14)	Histidine	1.0 (1)
Half-cystine	0.1 (0)	Arginine	6.3 (6)
Valine	19.0 (19)	Trytophan	3.9 (4)*
		Total	240

*Determined by the method described in ref. [24].

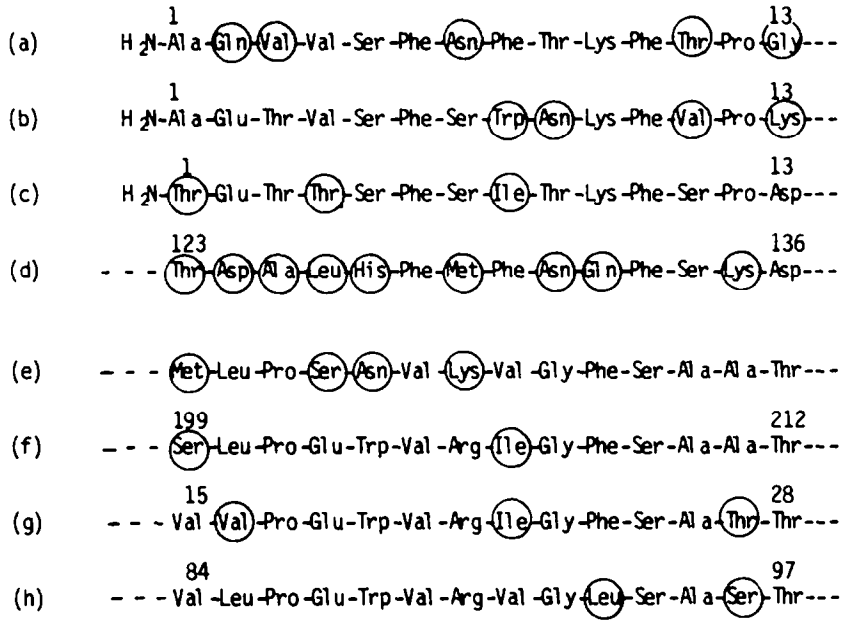

Fig. 2. Comparison of N-terminal sequences of lectins from various seeds: (a) *L. capassa* (this work); (b) soybeans [5]; (c) β-chain of lentil [7] and (d) Con A (residues 123–136) [6]. Comparison of the N-terminal sequence of the C-terminal fragment, obtained by treating the lectin of *L. capassa* seed with cyanogen bromide, with related regions in the sequence of lectins of various seeds: (e) *L. capassa* (this work); (f) soybeans (residues 199–212) [6], (g) α-chain of lentil (residues 15–28) [7] and (h) Con A (residues 84–97) [6]. The circles indicate variant amino acids.

Table 3. Minimum concentration of the lectin giving a positive hemagglutination reaction (untreated erythrocytes were used)

Erythrocyte type	Minimum conc of the lectin (μg/ml)
Human type A	50
Human type O	50
Human type B	200
Rabbit	250
Baboon	n.a*

*No agglutination at 1 mg lectin/ml.

DISCUSSION

The N-terminal sequence of the lectin from *L. capassa* seeds could be aligned with the first residue of the soybean lectin; also with the first residue of the β subunit of the two-chain lectin of lentil and residue 123 of Con A (Fig. 2). Further the N-terminal sequence of the fragment of the *L. capassa* lectin obtained at the methionine cleavage point could be aligned with residues 199 to 212 of the soybean lectin, residues 15 to 28 of the α-chain of the lentil lectin and residues 84 to 97 of Con A. What is immediately apparent is the degree of homology between the various sequences (Fig. 2).

Prior to this investigation, there have been reports on the purification and properties of lectins from various species of *Erythrina* [15–20], another legume genus. The lectins were purified by a combination of ammonia sulphate fractionation and affinity chromatography on columns of D-galactose derivatized Sepharose. They are glycoproteins and metalloproteins of M_r of *ca* 60 000 and are composed of two subunits of M_r of 28 000–30 000. The lectins agglutinate untreated human erythrocytes and D-galactose, and various D-galactosides inhibit the hemagglutinating activity of the lectins. The lectin of *L. capassa* seem to share most of the properties of *Erythrina* lectins. They have similar amino acid compositions, carbohydrate compositions, carbohydrate specificities, subunit M_rs and biological properties.

EXPERIMENTAL

Materials. Lonchocarpus capassa (apple-leaf) seeds were supplied by the Transvaal Provincial Nursery, Hartbeeshoek. Sepharose 6B was obtained from Pharmacia. The sugars were obtained from Merck and Eastman Kodak Co.

Physicochemical methods. Disc electrophoresis at pH 8.9 using a 15% gel was performed according to the method of ref. [21]. SDS gel electrophoresis at pH 7.2 using a 10% gel was carried out as described in ref. [22].

Agglutination and inhibition assays. The hemagglutinating activity of the lectin was determined by the serial dilution method on microtiter plates, using 50 μl lectin soln and 50 μl of a 4% suspension of erythrocytes [19]. A unit of activity is defined as the lowest concn of the lectin giving a visible agglutination. The inhibitor activity of the sugars was measured by mixing serial dilutions of the sugar with five hemagglutinating units of the

Table 4. Inhibition of the hemagglutinating activity of the lectin by various sugars

Sugar	Minimum concentration required to inhibit hemagglutination (mM)*
N-Acetyl-D-galactosamine	0.8
Lactose	0.9
O-Nitrophenyl-β-D-galactopyranoside	1.6
Methyl-α-D-glucopyranoside	2.0
D-Galactose	3.6
Sucrose	4.0
D-Fructose	4.0
D-Glucose	4.0
D-Mannose	4.0
Raffinose	8.0
D-Mannitol	10.0
D-Glucosamine · HCl	10.0
L-Rhamnose	40.0
D-Ribose	80.0

Pooled human erythrocytes were used. The minimal inhibitory concentration is that required to inhibit completely 5 units of the lectin.

*Maltose, L-fucose and L-sorbose at 100 mM were not inhibitory to the lectin.

lectin and determining the lowest concn of the sugar giving full inhibition [18].

Chemical analysis methods. Amino acid analyses were performed with an automatic Beckman amino acid analyser. Samples were hydrolysed with 6 M HCl for 24 hr in sealed evacuated tubes; phenol was added to prevent destruction of tyrosine [23]. For the determination of tryptophan the samples were hydrolysed with 3 M *p*-toluene sulphonic acid [24]. Total neutral carbohydrate content was determined by the phenol–H_2SO_4 method [25] using mannose as reference. Individual monosaccharides were determined by HPLC after formation of alditol benzoates [26; Merrifield, E. H., unpublished results]. Metal analysis was done by atomic absorption on a Varian Techtron AA-5 spectrophotometer. The adsorbed metal ions were removed by prolonged dialysis of the lectin against metal-free H_2O.

Sequence determinations. The N-terminal sequence of the lectin and cyanogen bromide fragments were determined with a Beckman sequencer using the quadrol programme (Beckman No. 12294 mod). The phenylthiohydantoin derivatives of amino acids were identified with HPLC using a micro-Bondapak C18 column (Waters Associates Inc.).

Purification of the lectin from Lonchocarpus capassa *seed.* The procedure used was based on that described in ref. [18]. All operations were carried out in cold room (4°). Ground seed (30 g) were extracted with 300 ml 0.05 M Na/KPi buffer pH 7.2 in 0.9% NaCl (PBS) for 1 hr, the extract was filtered through cheesecloth and clarified by centrifugation at 16000 *g*. $(NH_4)_2SO_4$ (17.6 g/100 ml) was added, the ppt was removed by centrifugation, more $(NH_4)_2SO_4$ (19.8 g/100 ml) was added and the mixture kept overnight. The ppt was collected by centrifugation, suspended in H_2O (80 ml) and dialysed extensively, first against H_2O and finally against the PBS. Any ppt that formed was removed and the clear supernatant was loaded on a column (1.9 × 15 cm) of galactose-derivatized Sepharose 6B, equilibrated with PBS. The column was eluted first with the buffer and then the bound lectin was eluted with 0.2 M galactose soln. The fractions containing hemagglutinating activity were collected, dialysed against H_2O and lyophilized. The yield was 180 mg.

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