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Purification and partial characterization of a lectin from the seeds of *Trichosanthes kirilowii* Maximowicz

Anna Ida Falasca*, Ada Abbondanza+, Luigi Barbieri+, Andrea Bolognesi+, Carlo Alfonso Rossi and Fiorenzo Stirpe+

Dipartimento di Biochimica and +*Dipartimento di Patologia sperimentale dell'Università di Bologna, I-40126 Bologna, Italy*

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A lectin was purified from the seeds of *Trichosanthes kirilowii*, belonging to the family Cucurbitaceae, growing in China. The lectin is a glycoprotein of 57 kDa, consists of two subunits with apparent molecular masses of 37 and 25 kDa, is specific for galactose, and is not mitogenic for human lymphocytes.

Lectin; Hemagglutinin; Affinity chromatography; (*Trichosanthes kirilowii*)

1. INTRODUCTION

Galactose-specific lectins have been purified from seeds of *Trichosanthes anguina* [1] and roots of *T. kirilowii* Maximowicz (Tianhuafen) [2,3], two Cucurbitaceae growing in China. The latter roots also contain an abortifacient protein, trichosanthin, that has been shown [4,5] to be a ribosome-inactivating protein (RIP, review [6]); a similar RIP, trichokirin, was found in the seeds of *T. kirilowii* [7]. The simultaneous presence of RIPs and of lectins with specificity for galactose-containing sugars has been observed in two other Cucurbitaceae, namely in seeds of *Momordica charantia* [8] and roots of *Bryonia dioica* [9,10].

Here, a galactose-specific lectin was purified from the seeds of *T. kirilowii*: it is a glycoprotein similar to, but not identical with, the isolectins purified from roots of the same plant.

Correspondence address: F. Stirpe, Dipartimento di Patologia sperimentale, Via S. Giacomo 14, I-40126 Bologna, Italy

* *Present address:* Istituto di Chimica biologica dell'Università di Parma, I-43100 Parma, Italy

2. EXPERIMENTAL

2.1. *Materials*

Seeds of *T. kirilowii*, originally from China, were obtained from Sanofi Recherche (Montpellier). Materials for chromatography and chromatofocusing were from Pharmacia (Uppsala). Sepharose CL 6B was acid-treated as in [11]. Sugars were from Sigma (St. Louis, MO).

2.2. *Purification of the lectin*

Seeds (350 g) were ground with an Ultra-Turrax apparatus with 2 l of 0.14 M NaCl containing 5 mM sodium phosphate buffer, pH 7.5 (phosphate-buffered saline, PBS). After overnight stirring at 4°C, the gross residues were removed with a kitchen centrifuge and the extract was centrifuged for 45 min at 10000 × *g* at 2°C. To the supernatant (crude extract) solid $(NH_4)_2SO_4$ was added to saturation. The precipitate was collected by centrifugation, redissolved in PBS and applied to a Sephadex G-25 column (100 × 10 cm), equilibrated and eluted with 5 mM sodium phosphate buffer (pH 7.0). The A_{280} of the effluent was monitored, and the absorbing fractions were applied to a CM Sepharose column, equilibrated with the same buffer, to remove an RIP, trichokirin [7] and other basic proteins. The effluent absorbing at 280 nm was applied to an acid-treated Sepharose CL 6B column (12 cm height × 10 cm diameter). After washing, the lectin was eluted with 0.2 M lactose containing 0.14 M NaCl and 5 mM sodium phosphate buffer (pH 7.5). A single peak in which the haemagglutinating activity coincided with the A_{280} was obtained. The purified lectin was dialysed extensively vs water and freeze-dried.

2.3. *Chemical determinations*

The molecular mass was determined by SDS-polyacrylamide

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Table 1

Purification of *Trichosanthes kirilowii* lectin

Preparation	Total protein (mg)	Specific activity (U/mg protein)	Total activity (U) ($\times 10^{-3}$)	Yield (%)
Crude extract	5331	250	1333	(100)
$(NH_4)_2SO_4$ precipitate	4320	280	1210	91
Sephadex G-25	3507	320	1122	84
CM Sepharose	2167	620	1345	101
Sepharose CL 6B (acid-treated)	284	3030	860	65

Experimental conditions are described in the text. Results refer to 350 g seeds

gel electrophoresis with the following standards (all from Bio-Rad): phosphorylase *b* (92.5 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa) and lysozyme (14.4 kDa), and by gel filtration on a Sephacryl S200 column (95 × 1.6 cm), with the following markers (all from Pharmacia): bovine serum albumin (67 kDa), ovalbumin (43 kDa), chymotrypsinogen A (25 kDa) and ribonuclease A (13.7 kDa).

The isoelectric point and amino acid and neutral sugar composition of the lectin were determined as in [12].

For chromatofocusing, the freeze-dried lectin was dissolved in 25 mM imidazole-HCl buffer (pH 7.4), and applied to a chromatofocusing column of polybuffer exchanger PBE 94 (10 × 1.6 cm) equilibrated with the same buffer. A pH gradient (pH 7.4–4.0) was developed by passing through the column 230 ml of polybuffer 74 diluted 1:8 with H_2O, pH 4.0 [13]. The flow rate was 15 ml/h and 30-drop fractions were collected.

Protein was determined by the method of Lowry et al. [14] with bovine serum albumin (Sigma) as a standard, or spectrophotometrically [15].

2.4. *Haemagglutinating and mitogenic activity*

The haemagglutinating activity of the extract at the various stages of purification and of the purified lectin was determined in 96-well (U-shaped) plates. Each well contained 50 μl of serial dilutions (by doubling) of the extract or lectin solution and 50 μl of a 1% suspension, in PBS, of human or rabbit erythrocytes, either untreated or pretreated with trypsin as described [16].

Mitogenic activity of the lectin was assayed with human peripheral lymphocytes according to [17]. Positive controls with phytohaemagglutinin were run at the same time.

3. RESULTS AND DISCUSSION

Preliminary experiments showed that an extract of *T. kirilowii* seeds agglutinated human erythrocytes and that the agglutination could be prevented by galactose. This indicated that the extract contained a galactose-specific lectin, which could be easily purified in good yield by affinity chromatography on acid-treated Sepharose 6B (table 1).

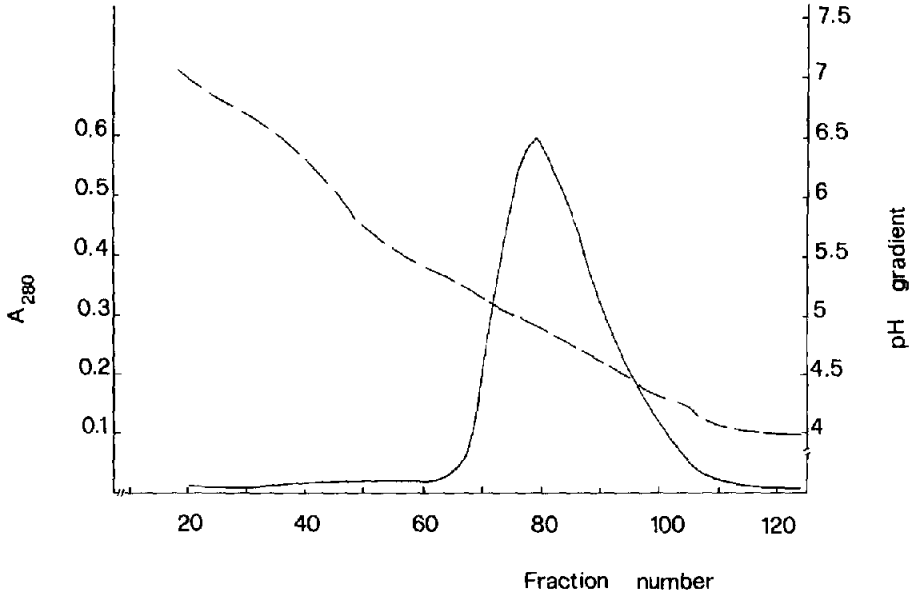

Fig.1. Chromatofocusing of *Trichosanthes kirilowii* lectin. Experimental conditions are described in the text. (——) A_{280}; (– – –) pH gradient.

The purified lectin appeared homogeneous on gel filtration on Sephadex G-200 and isoelectric focusing, and on chromatofocusing was eluted as a single peak in the range pH 5.22–4.35 (fig.1). On SDS-polyacrylamide gel electrophoresis without 2-mercaptoethanol the lectin migrated as a single band, whereas under reducing conditions it was broken down into a large subunit with apparent molecular mass 37 kDa and a smaller subunit of 25 kDa.

The main physico-chemical properties of the lectin are listed in table 2. It has a molecular mass of 57 and acidic p*I* and is a glycoprotein, mannose and xylose being the most abundant sugars. The amino acid composition shows a relatively high content of aspartic acid, glutamic acid and serine, and the presence of the sulphur amino acids cystine and methionine.

Table 2

Physico-chemical properties of the lectin

Molecular mass (kDa)	
by gel filtration	56.7
by gel electrophoresis	57
p*I* by isoelectrofocusing	5.2

Amino acid	Composition (mol/mol)	Sugar content; total neutral sugar 2.81%	
		Sugar	Composition (mol/mol)
Lys	19.3	Fuc	1.50
His	5.3	Gal	1.02
Arg	22.0	Glc	1.12
Asx	55.8	Man	3.97
Thr[a]	30.8	Xyl	2.97
Ser[a]	53.3		
Glx	44.4		
Pro	11.0		
Gly	24.0		
Ala	25.0		
½Cys	9.3		
Val	26.3		
Met	14.9		
Ile	28.4		
Leu	38.8		
Tyr[a]	15.5		
Phe	19.1		
Trp	N.D.		

[a] Values obtained from hydrolysis at 24, 48 and 72 h were extrapolated to zero time

Experimental conditions are described in the text; N.D., not determined

Table 3

Haemagglutinating activity of *Trichosanthes kirilowii* lectin

Erythrocytes	Lowest concentration of lectin giving agglutination (μg/ml)	
	Untreated erythrocytes	Trypsinized erythrocytes
Human A	1.25 ± 0.15 (7)	0.067
Human B	1.91 ± 0.20 (5)	0.130
Human AB	0.67 (2)	0.001
Human O	1.07 ± 0.58 (6)	0.004
Rabbit	N.D.	0.087

Experimental conditions are described in the text. Values for untreated erythrocytes are means ± SE, the numbers of donors being given in parentheses; N.D., not determined

The lectin agglutinates rabbit and human erythrocytes, without significant differences between human blood groups (table 3). Agglutination is enhanced when erythrocytes are treated with trypsin and inhibited by galactose and galactose-containing sugars (table 4). The inhibitory activity

Table 4

Inhibition of haemagglutination by sugars

Sugar	Minimal concentration inhibiting agglutination (mM)
D-Galactose	12.5
α-D-(+)-Fucose	25
L-(−)-Arabinose	100
D-Galactosamine	25
N-Acetyl-D-galactosamine	100
1-*O*-Methyl-β-D-galactopyranoside	6.25
p-Aminophenyl-1-thio-β-D-galactopyranoside	4.68
p-Nitrophenyl-β-D-fucoside	2.34
Lactose	3.12
Thiodigalactoside	1.56
p-Nitrophenyl-β-D-galactopyranoside	2.34
D-(+)-Raffinose	25
2-Deoxy-D-galactose	50

Haemagglutination was determined as described in section 2, with trypsin-treated human erythrocytes, group O, Rh^+. The lectin was added at a final concentration of 0.34 μg/ml. The following sugars did not affect agglutination, at concentrations up to 100 mM: L-galactose, L-lyxose, α-L-rhamnose, L-(−)-mannose, D-(+)-glucose, L-glucose, β-D-allose, 3-*O*-methyl-β-D-galactopyranosyl-D-arabinose, 2-deoxy-D-ribose, melezitose, α-D-(+)-melibiose, lactulose, D-(+)-galacturonic acid acid

is potentiated by the presence of a β-glycosidic linkage in C-1, and is reduced if the C-2 hydroxyl group is substituted by an *N*-acetylamino group.

The lectin is not mitogenic to human peripheral blood lymphocytes (not shown).

The present results demonstrate that the lectin purified from the seeds of *T. kirilowii* has sugar specificity similar to that of the isolectins found in the roots of the same plant [2,3], but differs from them in molecular mass, p*I* and amino acid and sugar composition. This is another example of a galactose-specific lectin present in tissue from a member of the family Cucurbitaceae together with an RIP (see section 1). Whether these associations are coincidental, or have a physiological meaning remains to be ascertained.

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