Purification and properties of a lectin from *Lathyrus tingitanus* seeds

Pierre Rougé and Philippe Chabert

Laboratoire de Biologie Cellulaire, Faculté des Sciences Pharmaceutiques, 35 Chemin des Maraîchers, 31 062 Toulouse, France

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A lectin has been isolated from the seeds of *Lathyrus tingitanus* by ammonium sulfate precipitation, affinity chromatography on Sephadex G-100 and subsequent chromatofocusing. This lectin has a relative molecular mass about 50000 and consists of light (M_r 5000) and heavy subunits (M_r 20000). The amino acid composition, N-terminal amino acids, carbohydrate and metal content of both the lectin and its subunits are given. This lectin is non-specific in agglutination of human erythrocytes and is inhibited by D-mannose, D-glucose and their α-methylglucosides derivatives. Antibodies against this lectin crossreact with other purified *Lathyrus* lectins and other lectins from species belonging to the *Vicieae* tribe.

Lectin *Physicochemical property* *Biological property* Lathyrus tingitanus *Immunological cross reaction*

1. INTRODUCTION

Various *Lathyrus* lectins have been recently isolated [1–4] and their properties compared with the well-known pea (*Pisum sativum*) and lentil (*Lens culinaris*) lectins belonging to the same tribe of *Vicieae*. However, some discrepancies concerning their structure have been reported since Kolberg et al. [1,2] and Ticha et al. [3] have found that the lectins extracted from *L. odoratus, L. sativus* and *L. silvestris* are composed of two different chains (α and β) and possess an $\alpha_2\beta_2$ structure while Gupta et al. [4] have described a lectin from *L. sativus* which consists of two identical subunits with an M_r of 21000. We describe the isolation and main properties of a lectin isolated from *L. tingitanus* (Lath-T) which possesses an $\alpha_2\beta_2$ structure.

Abbreviations: Lath-T, *Lathyrus tingitanus*; Con-A, concanavalin A; SDS, sodium dodecyl sulfate; Na_2EDTA, ethylene diamine tetraacetic acid disodium salt; PBE, 0.1 M phosphate-buffered saline (pH 7.2)

2. MATERIALS AND METHODS

2.1. *Isolation of the Lath-T lectin and its subunits*

The seeds of Lath-T were obtained from plants cultivated in field conditions. The seed flour was extracted with 0.05 M Tris (pH 7.6), 0.15 M NaCl and the protein precipitated at 30–60% $(NH_4)_2SO_4$ were filtered through a column of Sephadex G-100 equilibrated with the same buffer. The retained whole Lath-T lectin was eluted from the column with 0.1 M glucose.

By chromatofocusing of the whole Lath-T lectin on a PBE 94 (Pharmacia) column over pH 7.4–4.0, three protein fractions were obtained. The first main fraction, rechromatographed in the same conditions, corresponds to the isolated Lath-T lectin.

The subunits of the isolated Lath-T lectin were separated by gel filtration on a Bio-Gel P60 column in 6 M guanidine–HCl [5].

2.2. *Isolation of other lectins*

Con-A, lentil and pea lectins were isolated from

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seed flour by affinity chromatography on Sephadex G-100 [6].

2.3. *Gel electrophoresis*

Electrophoresis was carried out in 7% polyacrylamide gel rods either at basic [7] or acidic pH [8]. Protein fractions were fixed and stained according to [9].

2.4. *Isoelectrofocusing*

Preparative isoelectrofocusing was conducted in an LKB 110 ml column with 1% Ampholine (pH 5.0–8.0) according to [10]. The electrofocusing was carried out for 48 h at a constant 900 V at room temperature. After fractionation, absorbance at 280 nm and pH of each fraction were measured. Analytical isoelectrofocusing was performed with ready-made LKB Ampholine PAG-plates (pH 3.5–9.5).

2.5. *Relative molecular mass estimation*

The M_r of the Lath-T lectin was estimated by gel filtration on a Bio-Gel P100 column equilibrated with 0.1 M phosphate buffer (pH 7.2). The M_r of the Lath-T subunits was estimated by SDS–polyacrylamide gel electrophoresis on gradient (4–30% acrylamide) gel slabs using a 0.09 M Tris, 0.08 M boric acid (pH 8.4) buffer containing 0.1% Na_2EDTA for electrophoresis.

2.6. *Quantitative amino acid analysis*

Samples were hydrolyzed in twice glass-distilled 6 M HCl at 110°C for 24 h and amino acids were analyzed on a Beckman model 119 BL amino acid analyzer.

2.7. *N-Terminal amino acid analysis*

N-Terminal amino acids were determined by labelling Lath-T lectin and its subunits with 2,4-dinitrofluorobenzene [11]. The dinitrophenyl-derivatives of amino acids were identified by two-dimensional co-chromatography with standard labelled amino acids (Serva) on micropolyamide sheets. The sheets were developed in benzene–acetic acid (8:2, v/v) and formic acid–water (5:5, v/v) [12] and spots were detected under an ultraviolet light at 365 nm.

2.8. *Protein and carbohydrate analysis*

Protein was estimated according to Goa [13] with bovine serum albumin as standard. The carbohydrate content of the Lath-T lectin was determined by the phenol–H_2SO_4 method [14] using glucose as reference.

2.9. *Metal analysis*

The content of manganese, calcium and zinc in the Lath-T lectin was determined by atomic absorption.

2.10. *Haemagglutination assays*

The haemagglutinating activity of the Lath-T lectin was determined by two-fold serial dilution in phosphate-buffered saline (PBS) pH 7.2 on standard micro-titration plates. To each lectin solution (50 μl) were added 200 μl of a 1% solution of thrice-washed human erythrocytes in PBS. Agglutination was estimated 12 h later.

Inhibition of haemagglutination by sugars was tested by two-fold serial dilution in 50 μl. To each sugar dilution 50 μl PBS containing 37.5 μg/ml lectin were added. After a 1 h incubation, 200 μl of a 1% solution of thrice-washed human O Rh^+ erythrocytes in PBS were added and agglutination was estimated 12 h later.

2.11. *Immunological techniques*

Antibodies against Lath-T lectin were raised in rabbit according to an immunisation schedule described in [15] using Freund's complete adjuvant. Immunodiffusion was performed on agarose as in [16] and the precipitin lines were stained with amido black 10B [17].

3. RESULTS AND DISCUSSION

The whole Lath-T lectin obtained by affinity chromatography on Sephadex G-100 shows 3 main protein fractions in polyacrylamide gel electrophoresis at basic pH (fig.1a). The Lath-T lectin subsequently isolated by chromatofocusing corresponds to the first protein fraction separated by electrophoresis (fig.1b). Its isoelectric point is near pH 6.3 as estimated by preparative and analytical isoelectrofocusing. However, either the whole or isolated Lath-T lectins migrate as one single broad diffuse band by gel electrophoresis at acidic pH (fig.1c).

The M_r of the Lath-T lectin estimated by gel filtration is about 47000. However, its subunits,

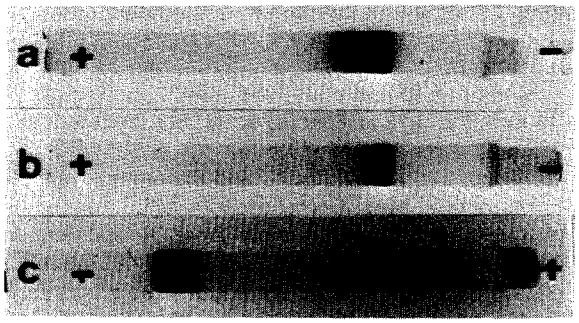

Fig.1. Polyacrylamide gel electrophoresis of (a) the whole Lath-T lectin and (b) the isolated Lath-T lectin at basic pH; run for 1.5 h at 2 mA/gel. Polyacrylamide gel electrophoresis of (c) the isolated Lath-T lectin at acidic pH; run for 3.5 h at 3 mA/gel.

which are readily separated in 6 M guanidine–HCl, have M_r values of about 5000 for the light or α-chain and 20000 for the heavy of β-chain, as determined by SDS–polyacrylamide gel electrophoresis. In the same conditions, pea and lentil lectins exhibit a very similar pattern. Assuming an $\alpha_2\beta_2$ structure for the Lath-T lectin by comparison with pea and lentil lectins, the calculated M_r (50000) is in agreement with the above experimental value. M_r-values ranging from 42800–53000 have been reported for other *Lathyrus* lectins [1–3].

Both the whole and isolated Lath-T lectins are relatively rich in Asp, Thr and Ser but contain only traces of sulfur-containing amino acids (table 1). The amino acid composition of the two Lath-T subunits is very different since the light chain is rich in Ser, Glu, Ala, Val, Leu and His while the heavy chain contains more Asp, Gly, Ile and Tyr. The N-terminal amino acids of the light and heavy subunits are Val and Thr, respectively.

Lath-T lectin appears as a metalloprotein con-

Table 1

Amino acid composition of the Lath-T lectins and the isolated subunits[a]

Amino acid	Whole Lath-T	Isolated Lath-T	Light chain	Heavy chain
Asp	16.4	15.5	5.5	17.2
Thr	9.3	10.0	9.3	10.3
Ser	5.2	6.3	11.3	6.1
Glu	9.3	8.7	14.8	7.8
Pro	3.7	3.3	3.6	3.7
Gly	5.8	5.4	4.1	6.1
Ala	9.0	8.6	10.4	8.9
Val	9.9	9.7	11.2	8.7
Ile	5.7	5.8	3.7	6.0
Leu	6.3	6.4	8.0	5.6
Tyr	2.2	3.2	1.6	3.1
Phe	7.5	7.5	6.0	7.6
Lys	5.8	5.7	4.5	6.0
His	1.8	1.8	3.7	1.2
Arg	2.2	2.3	1.9	2.3

[a] Expressed as residue/100 residues

Table 2

Minimum concentration (mM) of sugar giving complete inhibition of haemagglutination

Sugar	Whole Lath-T lectin	Isolated Lath-T lectin	Pea lectin	Lentil lectin	Con-A
D-Mannose	6.25	6.25	3.12	12.5	3.12
D-Glucose	12.5	12.5	12.5	25	12.5
D-Fructose	25	25	25	50	6.25
D-Glucosamine	50	50	25	100	25
α-Methyl-D-mannoside	1.56	1.56	1.56	6.25	0.39
α-Methyl-D-glucoside	12.5	12.5	6.25	12.5	1.56
N-Acetyl-D-glucosamine	12.5	12.5	12.5	25	12.5
Sucrose	12.5	12.5	12.5	25	12.5

The following sugars were not inhibitory at final concentration of 200 mM: D-arabinose, L-fucose, D-galactose, D-galactosamine, *N*-acetyl-D-galactosamine, α-methyl-D-galactoside, β-methyl-D-galactoside, β-methyl-D-mannoside, L-rhamnose, D-ribose. The lectin concentrations used were 37.5 μg/ml, these concentrations being 4–8-times higher than that producing complete haemagglutination in the last 2-fold dilution

taining 3.0 atoms Ca^{2+}, 2.0 atoms Mn^{2+} and 0.5 atom Zn^{2+} per mole, on the basis of a calculated M_r of 50000. The phenol–H_2SO_4 method of Dubois et al. [14] indicates a carbohydrate content $<0.25\%$, by weight.

The Lath-T lectin is non-specific and agglutinates the human erythrocytes of different ABO groups equally well. It is inhibited best by D-mannose, D-glucose and their α-methylglucosides derivatives (table 2). Similar results were obtained with Con-A, pea and lentil lectins and with other *Lathyrus* lectins [1–3].

By immunodiffusion, antibodies against Lath-T lectin crossreact with other *Lathyrus* lectins (*L. aphaca, L. articulatus, L. cicera, L. hirsutus, L. ochrus, L. odoratus, L. silvestris, L. vernus*) and with other lectins from various species (*Pisum sativum, Lens culinaris, Vicia faba, Vicia sativa*) belonging to the tribe of *Vicieae*. These results indicate that the lectins bear some common antigenic determinants which agree with the extensive amino acid sequence homologies evidenced between various lectins from the *Vicieae* tribe [1,2,18].

Two other lectins with isoelectric points of about pH 5.8 and pH 5.3 are present in lower amounts in Lath-T seeds. The results obtained by gel electrophoresis of the whole Lath-T lectin at basic and acidic pH suggest that they could correspond to closely related isolectins.

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