# CHARACTERIZATION STUDIES ON A NEW LECTIN FOUND IN SEEDS OF *VICIA ERVILIA*

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## 1. Introduction

As part of a screening program for the occurrence of lectins in leguminous plants [1,2] we have found a lectin in seeds of *Vicia ervilia,* which we have purified and partially characterized. The serological specificity of extracts from seeds of this species was reported earlier [3–5]. The compound was isolated directly from a raw extract of *V. ervilia* seeds by specific adsorption to a D-mannose-substituted Sepharose gel and subsequently desorbed with a glucose solution. The material recovered from a second adsorption-desorption treatment was found to be homogeneous on ultracentrifugation and free zone electrophoresis although with a pH-dependent tendency for aggregation. Its mol. wt of 60 000 may be accounted for by four subunits which are identical pairwise with molecular weights of 21 000 and 4700, respectively. The lectin molecule lacks carbohydrate and furthermore lacks sulphur containing amino acids and is rich in acidic and hydroxylic amino acids, which are usual features of this class of molecules [6–9].

The sugar specificity appears similar to that of Concanavalin A. The purified lectin has been submitted to other investigators for examination of its possible utility as a biospecific adsorbent, especially for virus purification.

## 2. Materials and methods

### 2.1. *Materials*

*V. ervilia* wild were purchased from Consorzio Agrario Provinciale di Roma. The biospecific adsorbent was prepared from Sepharose 6B (Pharmacia, Uppsala, Sweden) to which D-mannose (Sigma Co., St. Louis, Mo., USA) was linked via a divinyl sulphone (DVS) bridge (DVS from Labkemi, Gothenburg, Sweden). All chemicals used were reagent grade.

### 2.2. *Preparation of the biospecific adsorbent D-mannose Sepharose 6B [10]*

100 ml of packed Sepharose 6B, carefully washed with distilled water, was incubated with 100 ml 0.5 M carbonate buffer of pH 11 and 10 ml DVS. The mixture was kept at room temperature for 70 min under stirring and was subsequently transferred to a glass filter and carefully washed with distilled water. To the activated gel (100 ml) was then added 100 ml of a 20% (w/v) solution of D-mannose in 0.5 M carbonate buffer of pH 10 and the coupling reaction was allowed to take place overnight at room temperature. The resulting product was extensively washed with distilled water on a glass filter and then suspended in 0.5 M bicarbonate buffer of pH 8.5. Two ml of 2-mercaptoethanol was added to each 100 ml of suspension. After 2 hr the product was again recovered and washed carefully with distilled water.

### 2.3. *Purification of the lectin through affinity chromatography*

Finely ground seeds of *V. ervilia* were extracted overnight with phosphate buffered saline (PBS) of pH 7.0 in the proportions 1:10 (w/v). The extract was centrifuged and the clear supernatant passed through a fine glass filter prior to exposure to the specific sorbent. The D-mannose Sepharose was packed in a column through which the extract was pumped until

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saturation. Fresh PBS was subsequently pumped through the column until the eluate showed no u.v. absorption at 280 nm. Prior to desorption the conjugate was exposed to a further extensive wash on a glass filter under stirring. (The porous discs which form the top and bottom of the column were washed with particular care.) The conjugate was subsequently transferred back to the column where desorption of the lectin took place on passage of 0.5 M glucose in PBS. The desorbed material was pooled and dialysed against distilled water. After a second adsorption on to fresh D-mannose Sepharose and subsequent glucose desorption, the lectin was recovered, extensively dialysed against distilled water and lyophilized. (Dist. water pH 6–7)

2.4. *Methods for characterization*

The agglutinating activity was determined using a 2% PBS suspension of 3 times washed human red cells of A, B and O type. Inhibition of the agglutination reaction was studied using 0.1 M solutions of respective carbohydrates in PBS. After desalting on Biogel P4 in distilled water, the lectin was analysed for sugar content using the method of Dubois [11]. Ultracentrifugal analyses were carried out on a Beckman Spinco instrument equipped with Schlieren and interference optics. The molecular weight was calculated according to Yphantis [12] assuming a partial specific volume $\bar{v} = 0.72$ cm$^3$/g. The uncertainty in the determinations was estimated to 10%. Polyacrylamide gel electrophoresis in disc [13] and free zone electrophoresis [14] were carried out in the buffer systems and at the voltages indicated in the legends to fig.1 and 2. Analytical isoelectric focusing was performed with polyacrylamide as a carrier using an LKB 2117 Multipore apparatus whereas preparative isoelectric focusing was carried out on an LKB Ampholine Column Type 8101 (110 ml volume). In both cases sucrose was used as a stabilizer and the pH gradient ranged from 3.5–10. Gels filtration in 6 M guanidinium hydrochloride (GuHCl) was performed on Sepharose 6B [15] although without prior reduction and alkylation of the lectin. Fractions containing material with u.v. absorption at 280 nm were pooled separately, dialysed against distilled water and lyophilized. The molecular sieving pattern of the lectin and its pH dependence was studied on Biogel P100 from BioRad (Richmond, Calif. USA). Amino acid analysis was carried out according to Moore and Stein using a Durrum automatic analyzer. Hydrolysis times were 24 and 72 hr.

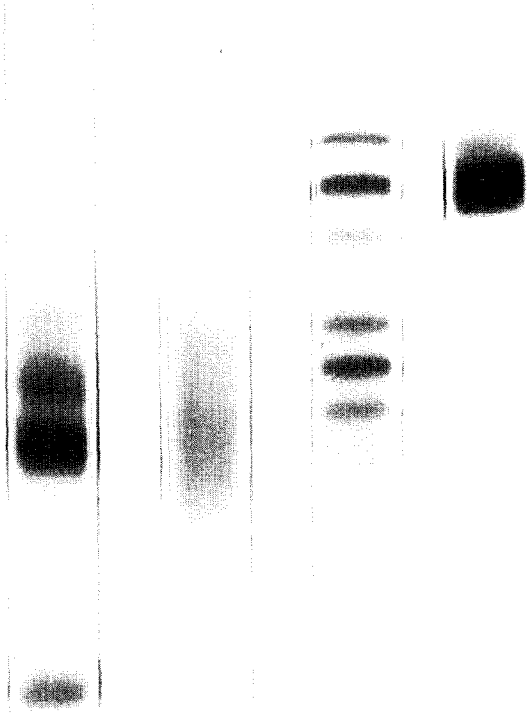

Fig.1. Polyacrylamide disc gel electrophoresis of *V. ervilia* lectin (at pH 2.4, 4.0, 8.2 and 10.0) run for 90 min. at a current of 2 mA per disc of gel composition $T_6C_5$.

## 3. Results and discussion

Raw extract from *V. ervilia* seeds has the ability to agglutinate human red cells of A, B and O type. The activity is intact at pH 3–10. The agglutination is inhibited by the presence of glucose, fructose, D-mannose, α-methylmannoside, *N*-acetyl glucoseamine, sucrose, maltose, melezitose and trehalose; mannose and trehalose are the strongest inhibitors. No inhibition was caused by galactose, galactoseamine, *N*-acetyl galactoseamine, fucose, *N*-acetyl mannoseamine, cellobiose, lactose or sialic acid.

The biospecific adsorbent, D-mannose-Sepharose 6B, the preparation of which is described in section

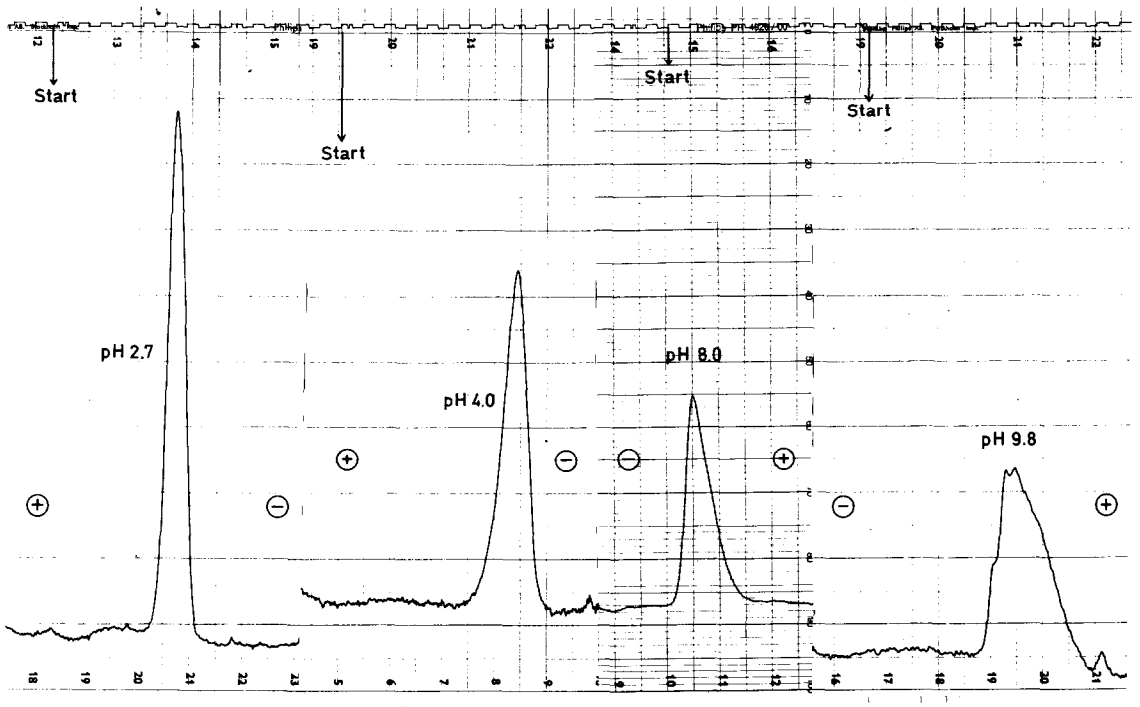


Fig.2. Free zone electrophoresis of *V. ervilia* lectin at pH 2.7 (0.2 M acetic acid, 2.5 mA, 2000 V, 15 min) at pH 4.0 (0.05 M sodium acetate, 4.5 mA, 1100 V, 135 min) at pH 8.0 (0.1 M Tris–HCl, 6 mA, 800 V, 110 min) at pH 9.8 (0.05 M glycine NaOH, 4.2 mA, 1400 V, 70 min).

Table 1
Amino acid compositions

| Amino acids | The lectin res. per mol. wt 60 000 | Sub. A res. per mol. wt 21 000 | Sub. B res. per mol. wt 4700 | The lectin | Isoelectric focusing | | Gel filtration at pH 3.9 | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Component of I.P. 6.0 | Component of I.P. 6.6 | Void peak | Main peak |
| | | | | Residues per 100 residues total amino acids | | | | |
| Asp | 65 | 30 | 1 | 13.15 | 13.40 | 13.30 | 13.33 | 13.14 |
| Thr | 55 | 21–22 | 4–5 | 11.10 | 11.45 | 11.00 | 11.11 | 10.92 |
| Ser | 43 | 14 | 8 | 8.55 | 10.19 | 10.30 | 9.27 | 9.69 |
| Glu | 37 | 12–13 | 6–7 | 7.49 | 7.99 | 7.99 | 7.89 | 7.87 |
| Pro | 19 | 7 | 2 | 3.78 | 4.51 | 4.50 | 3.83 | 3.64 |
| Gly | 32 | 13 | 3 | 6.35 | 6.98 | 6.64 | 6.66 | 6.64 |
| Ala | 36 | 14 | 4 | 7.22 | 7.30 | 7.84 | 7.42 | 7.72 |
| Val | 49 | 16–17 | 6 | 9.87 | 9.36 | 9.17 | 9.67 | 9.64 |
| Ileu | 25 | 11–12 | 1 | 4.92 | 5.08 | 5.05 | 5.05 | 5.08 |
| Leu | 27 | 10 | 4 | 5.48 | 5.40 | 5.49 | 5.67 | 5.70 |
| Tyr | 19 | 7 | 2 | 3.86 | 3.64 | 3.66 | 4.04 | 4.00 |
| Phe | 35 | 13–14 | 3 | 7.00 | 6.44 | 6.45 | 7.08 | 7.02 |
| His | 8 | 3 | 1 | 1.67 | 1.56 | 1.56 | 1.68 | 1.70 |
| Lys | 29 | 13 | 1 | 5.91 | 5.61 | 5.83 | 5.98 | 6.00 |
| Try | 11 | 2 | 2 | 2.29 | nd | nd | nd | nd |
| Arg | 7 | 2 | 1 | 1.36 | 1.16 | 1.09 | 1.33 | 1.28 |

nd = not determined.

2.2, has the capacity to adsorb 1.5 mg *V. ervilia* lectin per ml packed gel. By the specific adsorption procedure outlined in section 2.3 one obtains 700–800 mg lectin from 1 kg of seeds. The product eluted as a single symmetrical peak after gel filtration on Biogel P-100 at pH 7.0 (PBS). Ultracentrifugal analysis of the material under the same conditions showed the preparation to be homogenous with a mol. wt of 60 000.

A sugar analysis of the purified lectin showed negligable amounts of carbohydrate (about 1 mol glucose per unit of mol. wt 60 000).

Gel filtration in 6 M GuHCl on Sepharose 6B showed a splitting of the lectin molecule into subunits. The column was previously calibrated and, based on the elution vol, the mol. wts of the two units were estimated as 20 500 (subunit A) and 3600 (subunit B), respectively. Analytical ultracentrifugation of the resolved components, carried out in 6 M GuHCl, indicated mol. wts of 21 000 and 4700. The amino acid compositions of the two fractions are shown in table 1. Based on these data we suggest the *V. ervilia* lectin molecule to be composed of four subunits, two of type A and two of type B. A composite molecule of this kind would have a mol. wt of 53 000 based on the amino acid composition which is in reasonable agreement with the ultracentrifuge data.

An analytical isoelectric focusing of the preparation revealed a multitude of bands located in the pH interval 5.8–7.8 (see fig.3). A similar experiment on a preparative scale also indicated the presence of several components, with isoelectric points ranging from pH 5 to 6.6. Two major components from the latter run, showing isoelectric points of 6.0 and 6.6 respectively, were recovered and dialyzed at their focusing pH. Gel filtration on Biogel P-100 in the respective buffers gave the same elution volume for both components and which was equal to that for the lectin. Amino acid compositions of the two components and the lectin, shown in table 1, were identical except for some minor deviations.

In an attempt to elucidate whether the multiplicity in the isoelectric focusing pattern was due to the presence of isolectins, as has been suggested for other lectins [16–20], or whether the result could, at least in part, be explained by the, often visually manifested, tendency for aggregation we decided to study the pH-dependence of the molecular sieving and electrophoretic migration properties of the original lectin preparation. Electrophoresis on polyacrylamide gel showed several resolvable components (see fig.1). In order to eliminate possible effects of molecular sieving the electrophoretic migrations were subsequently studied by means of free zone electrophoresis. Fig.2 suggests that the lectin is electrophoretically homogeneous.

Fig.3. Analytical isoelectric focusing of the *V. ervilia* lectin. The pH gradient (from top to bottom) ranged from 3.5 to 10.

Fig.4 illustrates the molecular sieving properties of the lectin and the variation with pH. (It should be mentioned that NaCl in buffer at low and high pH causes the lectin aggregate to precipitate and thus has to be avoided). The high molecular weight material, appearing as void fraction at pH 3.9, was subjected to an ultracentrifuge molecular weight determination and gave a value of mol. wt 250 000. The amino acid composition of this material was identical to the mol. wt 60 000 unit as seen in table 1. A sedimentation study of the purified lectin at pH 4 also revealed the presence

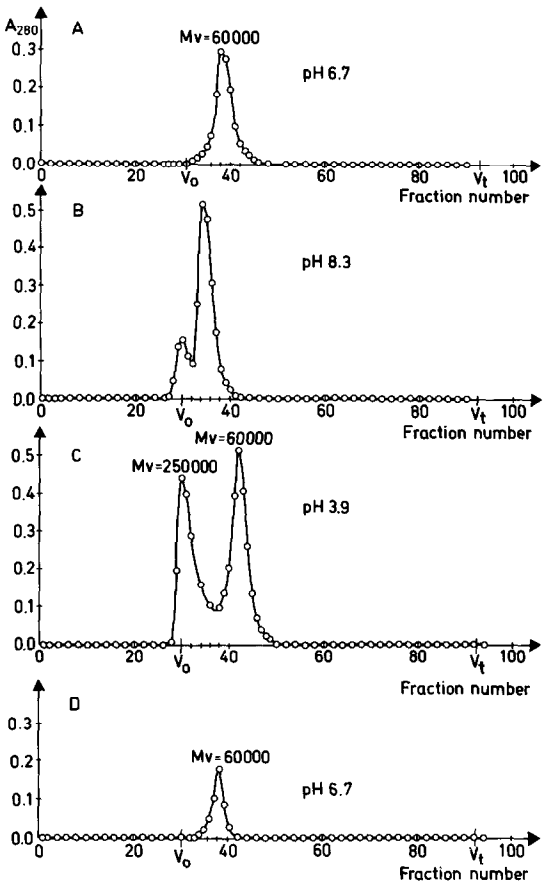


Fig.4. pH-Dependence of the elution pattern from Biogel P100. (A) 0.025 M ammonium acetate, pH 6.7. (B) 0.05 M Tris-HCl, 0.15 M NaCl, pH 8.3. (C) 0.025 M ammonium formiate, pH 3.9. (D) 0.025 M ammonium acetate, pH 6.7. Rechromatography of peak 2 from C.

of a major component (ca 60%) of $S_{20,w} = 3.75$ and minor (approx. 40%) of $S_{20,w} = 6.77$.

These results suggest that the material recovered from the biospecific adsorption step is homogenous at neutral pH. The strong tendency for aggregation in acidic and alkaline media could at least partly explain the occurrence of multiple bands. However, until the minor components from preparative isoelectric focusing have been investigated, the presence of isolectins cannot be ruled out.

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