Isolation and properties of a lectin from the seeds of the Indian bean or lablab (*Dolichos lablab* L.)*

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The lectin of the Indian bean or lablab (*Dolichos lablab* L.) was purified by affinity chromatography on two types of affinity carriers: *O*- α -D-mannopyranosyl-Separon and Separon-bound ovomucoid. The lectin is homogeneous in the ultracentrifuge: $s_{20,w} = 6.14$ S, $M_r = 110\,000$; the molecule appears to comprise two pairs of two types of subunits (M_r 16 000 and 40 000), and contains 2% neutral sugar and 0.2 Mn and 0.5 Zn atom respectively. The lectin agglutinates human erythrocytes non-specifically with regard to ABO grouping at a limit concentration of 8 μ g/ml, and this activity is inhibited most effectively by *N*-acetyl-D-glucosamine, methyl α -D-mannopyranoside and ovomucoid, but not by free D-mannose.

The presence of a haemagglutinin in seeds of the Indian bean or lablab (*Dolichos lablab* L.) has been known for a long time (Tobiška, 1964). Extracts of *D. lablab* seeds were found also to be mitogenic (Aurich *et al.*, 1971). To our knowledge, however, little attention, in comparison with other lectins, has been paid to the isolation and characterization of this lectin. Rao *et al.* (1976) used for its purification conventional methods of protein chemistry and described some of its properties.

Though the erythroagglutinating activity of the *D. lablab* lectin was inhibited most effectively by methyl α -D-mannoside, in common with many other lectins specific for D-glucose and D-mannose [e.g. concanavalin A, *Pisum sativum* (pea) and *Lens esculenta* (lentil) lectins], further inhibition studies showed that the sugar specificity of the *D. lablab* lectin is quite different. Therefore it seemed worthwhile to seek a suitable affinity carrier for isolation of the lectin and to study its properties in more detail.

Materials and methods

The seeds of *D. lablab* L. were collected at Marmaris in the southern part of Turkey.

Separon H-1000 was a product of Laboratorni

Abbreviations used: SDS, sodium dodecyl sulphate; t.l.g.c., thin-layer gel chromatography.

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přístroje n.p., Praha, Czechoslovakia; it was glycosylated with free D-mannose or *N*-acetyl-D-glucosamine in dioxan with dry HCl as catalyst as described by Filka *et al.* (1978). Ovomucoid isolated from egg white (Lineweaver & Murray, 1947) or concanavalin A were bound to periodate-oxidized D-glucopyranosyl-Separon H-1000 containing 12.4% D-glucose (Filka *et al.*, 1979). The water-soluble *O*-glycosyl-polyacrylamide copolymers used for affinity electrophoresis were prepared as described by Hořejší *et al.* (1978).

Isolation of lectin

Finely ground seeds of *D. lablab* (1 kg) were suspended in 5 litres of 0.15 M-NaCl and left at 4°C for 20 h. The seed slurry was removed by centrifugation (1500 g for 20 min). The supernatant was 80% saturated with solid $(\text{NH}_4)_2\text{SO}_4$, and the precipitate separated by centrifugation, suspended in 4 vol. of water and extensively dialysed. The precipitate formed during dialysis was removed and the supernatant freeze-dried. The yield of the crude active protein fraction was 13.3 g.

The lectin was isolated by means of affinity chromatography on two different types of affinity carriers.

(a) The crude protein fraction (1.5 g) was dissolved in 20 ml of 0.15 M-NaCl or 0.05 M-Tris/glycine buffer, pH 8.3, and the solution was applied to a column (3.8 cm $\times$ 18 cm) of *O*- α -D-mannopyranosyl-Separon H-1000 equilibrated with the corresponding solution. The column was washed

with the same solution with a flow rate of 12 ml/h until the A_{280} of the eluate decreased to 0.5. The column was then eluted with 0.4 M-borate buffer, pH 9.0. Alternatively, the affinity column was washed by the starting buffer until absorbance of the eluate was less than 0.1, and then a small portion of bound lectin was released by 0.1 M-methyl α -D-glucopyranoside.

(b) The crude protein fraction (1.1 g) was dissolved in 20 ml of 0.15 M-NaCl and the solution was applied to a column (1 cm $\times$ 11 cm) of periodate-oxidized D-glucopyranosyl-Separon H-1000 with covalently bound ovomucoid equilibrated with 0.15 M-NaCl. The column was washed with the same solution with a flow rate of 22 ml/h until non-adsorbed proteins had been completely removed. The lectin was then released by 0.05 M-glycine/HCl buffer, pH 2.7. Fractions containing the lectin were collected, dialysed and freeze-dried.

Analytical methods

Polyacrylamide-disc-gel electrophoresis was performed in an apparatus designed by Davis (1964) in alkaline (Steward *et al.*, 1965) or acidic (Reisfeld *et al.*, 1962) buffers. Electrophoresis in alkaline medium was run for 1.5 h at a current density of 4 mA per tube (75 mm $\times$ 5 mm) or, in acidic medium, for 2 h at 7 mA per tube. Gels were stained with Amido Black 10B. Polyacrylamide-gel electrophoresis in the presence of SDS was performed as described by Weber & Osborn (1969) in a 7.5% (w/v) gel. The M_r of subunits was calculated from their electrophoretic mobilities in comparison with those of lysozyme and its covalent oligomers (Payne, 1973). Reduction of the presumed -S-S-bond was performed with 2-mercaptoethanol. Affinity electrophoresis on polyacrylamide gels was performed by the method of Hořejší *et al.* (1977), with α -D-mannosyl or *N*-acetyl- α -D-glucosaminyl ligands immobilized in the form of soluble *O*-glycosyl polyacrylamide copolymers (Hořejší *et al.*, 1978).

T.l.g.c. on Sephadex G-200 (Superfine grade) in 0.15 M-NaCl in the presence or absence of 0.2 M-methyl α -D-glucopyranoside was performed in the Pharmacia t.l.g.c. apparatus.

For amino acid analysis, lectin samples were hydrolysed at 110°C in sealed tubes under N_2 , with 6 M-HCl for 20 and 70 h respectively and analysed on an amino acid analyser (model AAA 881; Mikrotechna, Praha, Czechoslovakia). Cysteine and methionine were determined as cysteic acid or methionine sulphone respectively after oxidation of a sample with performic acid (Moore, 1963).

Total neutral-sugar content was determined by the phenol/ H_2SO_4 method (Dubois *et al.*, 1956) with D-glucose as the reference sugar. The metal content of the lectin was determined on a Varian Techtron

model AA-175 atomic absorption spectrophotometer.

Measurements of the sedimentation velocity of the lectin were performed on a MOM 3170 ultracentrifuge at a speed of 60 000 rev./min. The M_r was calculated from the sedimentation-equilibrium data (Beckman-Spinco model E ultracentrifuge) as described by Yphantis (1964), assuming a partial specific volume of 0.73.

The erythroagglutinating activity of the lectin and inhibitory activity of sugars was assayed by a test-tube serial-dilution method as described previously (Hořejší & Kocourek, 1978).

Results

For the isolation of the lectin from seeds of *D. lablab*, the use of several different types of affinity carriers was examined. Of those examined, only two could be used successfully for preparation of the lectin.

(a) *D. lablab* lectin was not bound to Sephadex G-150 under various conditions of pH.

(b) When a D-mannopyranosyl derivative of Separon was used, the lectin was only retarded on the column and was eluted on the trailing edge of the peak of non-active proteins. Only a small portion of lectin was bound to D-mannopyranosyl-Separon and could be eluted by 0.1 M-methyl α -D-glucopyranoside. When the elution with starting buffer was replaced by elution with 0.4 M-borate buffer, pH 9.0, at the moment when most of non-active proteins had been already washed out, the lectin could be eluted from the column in a sharp peak (Fig. 1). The yield of the purified lectin as obtained by elution with borate buffer was 410 mg/kg of seeds. A similar behaviour of the lectin was observed when *N*-acetyl-D-glucosaminyl-Separon was used instead of D-mannosyl-Separon. Also, an increase in the pH of the starting buffer had no effect on binding of the lectin to glycosyl-Separons.

(c) The lectin was adsorbed to Separon containing bound concanavalin A, but attempts to release the bound lectin by elution with 0.4 M-methyl α -D-glucopyranoside, 0.05 M-glycine/HCl buffer, pH 2.6, or 0.4 M-borate buffer, pH 9.0, were unsuccessful.

(d) The lectin was fully adsorbed on Separon containing bound ovomucoid. No active protein was released by elution with the starting buffer. The bound lectin could be eluted with 0.05 M-glycine/HCl buffer, pH 2.7 (Fig. 2). The yield of purified lectin in this case was 850 mg/kg of seeds.

Lectin preparations obtained from α -D-mannopyranosyl-Separon or ovomucoid-Separon yielded a single diffuse band on polyacrylamide-disc-gel electrophoresis in alkaline medium (Fig. 3, gel 2). In acidic medium the lectin yielded one heavy zone

Fig. 1. Affinity chromatography of the *D. lablab* lectin on *O*- α -D-mannopyranosyl-Separon

The crude protein fraction (1.5 g) was dissolved in 20 ml of 0.15 M-NaCl and the solution was applied to a column (3.8 cm $\times$ 18 cm) of *O*- α -D-mannopyranosyl-Separon H-1000 equilibrated with 0.15 M-NaCl. The column was washed with the same solution with a flow rate of 12 ml/h until the A_{280} of the eluate decreased to 0.5; 12 ml fractions were collected. The column was then eluted with 0.4 M-borate buffer, pH 9.0. Fractions containing lectin were collected, dialysed and freeze-dried.

Fig. 2. Affinity chromatography of the *D. lablab* lectin on Separon containing bound ovomucoid

The crude protein fraction (1.1 g) was dissolved in 20 ml of 0.15 M-NaCl and the solution was applied to a column (1 cm $\times$ 11 cm) of periodate-oxidized D-glucopyranosyl-Separon H-1000 with covalently bound ovomucoid equilibrated with 0.15 M-NaCl. The column was washed with the same solution with a flow rate of 22 ml/h until non-adsorbed proteins had been completely removed; 12 ml fractions were collected. The lectin was then released by 0.05 M-glycine/HCl buffer, pH 2.7. Fractions containing the lectin were collected, dialysed and freeze-dried.

accompanied by two less-intense bands. However, affinity electrophoresis on polyacrylamide gel containing immobilized D-mannose or *N*-acetyl-D-glucosamine residues revealed that preparations of

Fig. 3. Electrophoretic properties of the *D. lablab* lectin

1, Affinity gel containing immobilized α -D-mannopyranosyl residues (Hořejší *et al.*, 1977); 2, disc electrophoresis on polyacrylamide gel in alkaline buffer system (Davis, 1964); 3, polyacrylamide-gel electrophoresis in the presence of SDS (Weber & Osborn, 1969).

the lectin were homogeneous with respect to sugar-binding activities; at sufficiently high concentrations of immobilized D-mannose or *N*-acetyl-D-glucosamine residues [c_i (concentration of immobilized sugar) = 3.0×10^{-3} M], all material was retarded on the top of the affinity gel, and its mobility could be increased by an addition of free methyl α -D-mannopyranoside to the affinity gel (Fig. 3, gel 1).

Electrophoresis in the presence of SDS of both reduced and non-reduced samples showed two zones corresponding to M_r values of 16 000 and 40 000 respectively (Fig. 3, gel 3).

T.l.g.c. on Sephadex G-200 (Superfine grade) revealed the presence of a single component with an elution volume corresponding to an M_r of 90 000. When chromatography was performed in the presence of 0.2 M-methyl α -D-glucopyranoside, the mobility of the lectin corresponded to an M_r of 120 000.

On centrifugation the lectin yielded a single symmetrical peak; its sedimentation coefficient ($s_{20,w}$) was 6.14 S (in 0.05 M-Tris/HCl buffer, pH 7.6); similar results were obtained in 0.15 M-NaCl or 0.05 M- β -alanine/acetate buffer, pH 4.3. The sedimentation-equilibrium method yielded an M_r of 110 000.

Preparations of the lectin obtained from D-mannopyranosyl-Separon by elution with methyl α -D-glucopyranoside or 0.4 M-borate buffer and the preparation obtained by affinity chromatography on

Table 1. *Amino acid composition of the D. lablab lectin*

Amino acid	Composition	
	Residues/molecule of M_r 110 000	Mol/100 mol
Lysine	50.3	4.60
Histidine	26.2	2.40
Arginine	32.6	2.98
Aspartic acid	132.6	12.13
Threonine	85.3	7.80
Serine	98.4	9.00
Glutamic acid	74.0	6.77
Proline	53.3	4.88
Glycine	133.4	12.20
Alanine	86.7	7.93
Half-cystine	0	0
Valine	78.1	7.15
Methionine	Traces	
Isoleucine	58.1	5.32
Leucine	88.3	8.08
Tyrosine	41.4	3.79
Phenylalanine	53.6	4.90

Table 2. *Inhibition of the erythroagglutinating activity of the D. lablab lectin*

The inhibitory activity of sugars is expressed as the minimum concentration of sugars (mM) or glycoproteins and polysaccharides (mg/ml) inhibiting erythroagglutination by 0.03% lectin solution. Type O erythrocytes were used. —, No inhibition was observed (the highest concentration used was 0.25 M or 4 mg/ml).

Sugar, glycoprotein or polysaccharide	Inhibition	
	mM	mg/ml
N-Acetyl-D-glucosamine	23	
Methyl α -D-mannopyranoside	26	
Methyl α -D-glucopyranoside	104	
D-Mannose	—	
D-Glucose	—	
Trehalose	—	
Ovomucoid		0.06
Acid α_1 -glycoprotein		0.5
Mannan		1
Glycogen		—
Fetuin		—
Dextran T-500		—
Blue Dextran		0.008

Separon containing bound ovomucoid did not differ in electrophoretic mobility, subunit composition, electrophoretic behaviour on affinity polyacrylamide gels, sedimentation coefficients or behaviour on Sephadex G-200 t.l.g.c.

The amino acid composition of the lectin is shown in Table 1. The lectin is rich in glycine, aspartic acid, glutamic acid, serine and threonine, and it contains also 2% neutral sugar. Atomic-absorption spectroscopy revealed the presence of 0.2 Mn and 0.5 Zn atom per molecule of the lectin (of presumed M_r 110 000). Cd, Cu, Fe and Co were not detected.

The lectin isolated from *D. lablab* seeds agglutinated human erythrocytes non-specifically at the minimum concentration of 8 μ g/ml. The erythroagglutinating activity was most effectively inhibited by methyl α -D-mannopyranoside, N-acetyl-D-glucosamine and ovomucoid. Values characterizing inhibitory potency of several sugars, glycoproteins and polysaccharides are given in Table 2. Erythroagglutinating activity of the *D. lablab* lectin was not enhanced in the presence of Mn^{2+} or Zn^{2+} .

Affinity electrophoresis on polyacrylamide gel containing immobilized D-mannose or N-acetyl-D-glucosamine residues showed that the lectin interacted with immobilized sugars only in alkaline buffer solution. The value of K_i (dissociation constant of the lectin-immobilized-sugar complex) for D-mannose was 1.2×10^{-3} M. In acidic medium the lectin strongly interacted only with Blue Dextran immobilized in polyacrylamide gel.

Discussion

D. lablab seeds contain a lectin that non-specifically agglutinates human erythrocytes with respect to the ABO system; this activity is inhibited most effectively by methyl α -D-mannopyranoside and N-acetyl-D-glucosamine. These properties are similar to those of D-glucose- and D-mannose-binding lectins, such as concanavalin A, lectins from *Pisum sativum*, *Lens esculenta*, *Lathyrus sativus* (chickling vetch or bitter vetch) etc. However, these lectins are also inhibited by D-glucose and D-mannose, whereas the *D. lablab* lectin does not interact with these sugars at all, or only very poorly. Thus appears that the presence of a hydrophobic group at C-1 or C-2 (methyl glycosides, N-acetyl group) favours the binding of these sugars by the lectin.

D-Glucose-binding lectins interact with Sephadex, which is conveniently used for their isolation. Our results have shown that the *D. lablab* lectin interacts with dextran gel very weakly, which is observable in t.l.g.c. on Sephadex G-200. This weak interaction is not sufficient to be used for the isolation on Sephadex as an affinity carrier.

It is interesting to compare results of affinity chromatography on Separon containing bound D-mannopyranosyl or N-acetyl-D-glucosamine residues and results of affinity electrophoresis on polyacrylamide gel containing the same immobilized sugar residues. In affinity-electrophoresis experiments carried out in the alkaline-buffer sys-

tem, the lectin interacted with immobilized sugar residues relatively strongly; the value of K_1 obtained is comparable with the value obtained for the *Lens esculenta* lectin and little higher in comparison with the *Pisum sativum* and *Lathyrus sativus* lectins and concanavalin A. Whereas in affinity-chromatography experiments the *Lens esculenta* lectin, in common with other D-glucose-binding lectins, is bound to Sephadex gel and can be isolated on D-mannopyranosyl-Separon, the *D. lablab* lectin behaves quite differently. On D-mannosyl-Separon the *D. lablab* lectin is only retarded and can be eluted by starting buffer, even though the buffer system used in electrophoretic experiments is used for affinity chromatography as starting buffer. This difference can be explained by different nature of the matrices used for immobilization of D-mannose residues.

There are also differences in the behaviour on affinity electrophoresis between D-glucose-binding lectins and the lectin from *D. lablab* seeds. Whereas D-glucose-binding lectins also interact with immobilized sugar residues in acidic buffer systems, no interaction was observed under these conditions in the case of the *D. lablab* lectin.

Our results have shown that the molecule of the *D. lablab* lectin is probably composed of four subunits of two types (with M_r 16000 and 40000 respectively) bound non-covalently. Rao *et al.* (1976) described the presence of six components on polyacrylamide-gel electrophoresis in the presence of SDS, with M_r values of 10000–35000. The subunit composition of the *D. lablab* lectin is similar to that of the *Pisum sativum*, *Lens esculenta* and *Lathyrus sativus* lectins, but the M_r values for the subunits are significantly higher than those of the above-mentioned D-glucose-binding lectins. The same is also true for total M_r .

Our present results do not allow for any satisfactory explanation for heterogeneity of the lectin in electrophoresis in acid buffer; the sedimentation analysis shows that no dissociation of the lectin

occurs at pH of the buffer applied in the electrophoresis.

In comparison with other D-glucose-binding lectins the *D. lablab* lectin contains smaller amounts of metals. In agreement with the results of Rao *et al.* (1976), the erythroagglutinating activity is not enhanced in the presence of Mn^{2+} or Zn^{2+} .

In summary, the results have shown that the lectin from *D. lablab* seeds does not resemble in its physico-chemical properties the other lectins isolated from seeds of members of the family Fabaceae and displays unique sugar-binding properties.

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