Navy (Haricot)-Bean (*Phaseolus vulgaris*) Lectin

ISOLATION AND CHARACTERIZATION OF TWO COMPONENTS FROM A TOXIC AGGLUTINATING EXTRACT

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Two protein components were isolated in a highly purified state from a toxic fraction from the navy (haricot) bean (*Phaseolus vulgaris*). One, a lectin, strongly agglutinated horse erythrocytes and leucocytes, agglutination being readily observable with both types of cell at a lectin concentration of 4 μ g/ml. The other component (component 1), although possessing some similarity in composition, was thought to be non-agglutinating or, at most, only very weakly so. Component 1 had a mol.wt. of about 143 000 and a sub-unit mol.wt. of about 37 000, suggesting a tetrameric structure probably with identical subunits. Alanine was the only *N*-terminal amino acid identified and the molecule was notable in being devoid of tryptophan and cysteine, low in methionine and high in leucine, glutamic acid and aspartic acid. The lectin was somewhat smaller (mol.wt. about 114 000) and apparently also composed of four identical subunits of mol.wt. about 30 000. Dansylation showed that arginine occupied the *N*-terminus of the polypeptide chain. Aspartic acid, serine, threonine and leucine were the predominant amino acids of the lectin, and the sulphur-containing amino acids were entirely absent. Both constituents were glycoproteins and the compositions of the carbohydrate portions (4.9% for component 1 and 8.1% for the lectin) were generally similar, consisting of mannose and glucosamine with smaller amounts of glucose and traces of xylose and arabinose.

The legumes are an especially rich source of lectins that possess many properties of considerable biological and biochemical interest and have been the subject of recent reviews (Boyd, 1963; Dechary, 1968; Jaffé, 1969; Toms & Western, 1971; Sharon & Lis, 1972; Lis & Sharon, 1973).

Lectins have been purified from comparatively few legumes, but among those that have been studied are some from various types of *Phaseolus vulgaris*, namely the red kidney bean (Rigas & Johnson, 1964), the wax bean (Takahashi *et al.*, 1967) and the black bean (Jaffé & Hannig, 1965). These form a group differing in many respects from lectins from other sources and belong to the class of non-specific agglutinins (Toms & Western, 1971) that are capable of interacting with the erythrocytes of many species, and that cannot be inhibited readily by the addition of simple sugars. A further property of interest is the possible role of lectins in the toxicity of raw legumes when incorporated into animal diets (Liener, 1969). The study of purified lectins is clearly crucial to an understanding of their biological properties. The present paper reports the preparation and characterization of two components of a toxic agglutinating extract obtained from the navy (haricot) bean (*Phaseolus vulgaris* L.) and compares them with the lectins from other varieties of *Phaseolus vulgaris*.

Materials and Methods

Initial extract

A saline extract of navy-bean meal (Jayne-Williams & Burgess, 1974) was kindly prepared and provided by Dr. D. J. Jayne-Williams.

DEAE-cellulose chromatography

A column (7.5 cm $\times$ 27 cm) of DEAE-cellulose (Whatman DE-52, approx. capacity 1.0 mequiv./g; Reeve Angel and Co., London E.C.4, U.K.) was equilibrated with 0.01 M-sodium phosphate buffer (pH 6.8). The sample (containing about 14 g of protein) was applied in a volume of approx. 500 ml of the same buffer, and a further 500 ml of the buffer was passed through the column. Elution at room temperature (approx. 20°C) was then continued by application to the column of a salt gradient of the 0.01 M buffer (2 litres) to the same buffer containing 0.5 M-NaCl (2 litres). Fractions (25 ml) were collected and portions (0.1 ml) withdrawn for assay of erythroagglutinating activity.

Gel filtration

Gel filtration was performed by using columns (2.2 cm $\times$ 58 cm) of Sephadex G-200 (particle size

40–120 μ m; Pharmacia, Uppsala, Sweden) in 0.2M-sodium phosphate buffers of pH 2.7, 5.0, 6.5 or 8.7, containing in addition 0.2M-NaCl. Samples (approx. 40mg of protein) were applied in a volume of 1.0ml and the flow rate in an upward direction was adjusted to 8ml/h.

For preparative work, either a 2.7 litre column (6.4cm $\times$ 83.5cm) with samples of about 2g of protein or a 5.8-litre column (9.6cm $\times$ 90cm) with up to 12g of protein were used. Both columns were filled with Sephadex G-200 and upwards elution was used at about 100ml/h with 0.2M-sodium phosphate buffer (pH 6.8) containing 0.2M-NaCl. Fractions (25ml) were collected and portions (0.1ml) taken for erythroagglutinating-activity assays.

Agglutinating activity

Erythroagglutinating activity was measured as described by Jane-Williams (1973).

For qualitative estimation of leucoagglutinating activity 25ml of horse blood was centrifuged for 20min at 800g and the leucocyte layer withdrawn by using a Pasteur pipette. The leucocytes were resuspended in 0.15M-NaCl (25ml) and washed essentially free of erythrocytes by suspension and centrifugation three times in 25ml volumes of 0.15M-NaCl. They were finally resuspended in 0.15M-NaCl (25ml). The leucoagglutinating activity of protein samples was observed by inspection after the addition of 0.2ml portions of leucocyte suspension to 1.0ml portions of 0.15M-sodium phosphate buffer, pH 6.8, containing 0.1ml of the protein solution under investigation.

Ultracentrifuge measurements

A type An-D rotor in a Beckman Spinco model E ultracentrifuge at 59780 rev./min at 25°C was used for sedimentation-velocity analysis in 0.2M-sodium phosphate buffer, pH 6.8. Observed sedimentation coefficients were corrected for temperature and solvent density to give the $s_{20,w}$ values.

Weight-average molecular weights were determined by the high-speed equilibrium technique (Yphantis, 1964). Partial specific volumes ($\bar{v}$) were determined by pycnometry and also calculated from the amino acid and carbohydrate composition (McMeekin *et al.*, 1949; Charlwood, 1957).

Other analytical methods

Polyacrylamide-gel electrophoresis. This was carried out at pH 4.3 by using the 'small-pore' separation gel system of Reisfield *et al.* (1962) except that the acrylamide concentration was decreased to one-third to give 5% (w/v) gels and the apparatus buffer consisted of 0.35M-glycine adjusted to pH 4.3 with acetic acid. For gels run at pH 8.0 the buffer used was

0.1M-Tris-citrate and this same buffer was used in the apparatus. When appropriate, gels were made 5M with respect to urea by the incorporation of 8M-urea into suitable component solutions.

Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate was carried out by the method of Andrews & Cheeseman (1972) except that 2-mercaptoethanol (0.1M) was added to all samples and standards before treatment with sodium dodecyl sulphate. Staining of gels was with Coomassie Blue (Andrews & Cheeseman, 1972) or by a periodic acid-Schiff-staining technique (Zacharius *et al.*, 1969).

Starch-gel electrophoresis. This was performed at pH 1.9 as described by Aschaffenburg (1966) except that 2-mercaptoethanol (0.02M) was added and the urea concentration was increased to 44g/80ml of buffer (giving gels containing approx. 7M-urea).

Amino acid analysis. Portions (0.5ml) of protein solution containing 0.6–2.1mg of protein were placed in hydrolysis tubes and evaporated to dryness over NaOH pellets and P_2O_5 . Portions (0.5ml) of 0.2M- Na_2CO_3 , adjusted to pH 9.2 with $NaHCO_3$, were added to each tube and the contents freeze-dried to remove traces of NH_3 in the samples. The residues were dissolved by the addition of 1.0ml of 5.8M-HCl [AristaR-grade conc. HCl (BDH Ltd., Poole, Dorset, U.K.) diluted with an equal volume of water] or by the addition of 1.0ml of 3M-toluene-*p*-sulphonic acid reagent (Liu & Chang, 1971) and the tubes sealed under vacuum and heated at 104°C for 24, 48 and 72 h. Cysteine, cystine and methionine were measured as cysteic acid and methionine sulphone after performic acid oxidation (Hirs, 1956). Analyses were carried out on a Jeol JLC-5AH automatic amino acid analyser (Japan Electron Optics Laboratory Co. Ltd., Tokyo, Japan).

Monosaccharide analysis. For the qualitative identification of monosaccharides, samples (2–4mg of protein) were dissolved in 2M-HCl (2ml) and heated in sealed tubes at 104°C for 2h. Under these conditions recoveries of added mannose and glucosamine were greater than 90%. The hydrolysates were evaporated to dryness at room temperature over NaOH pellets and the residues dissolved in water (1ml). These solutions were desalted and freed of amino acids by passage in distilled water through a short column (0.8cm $\times$ 5.0cm) of Dowex 1 (X8; OH^- form), then through a similar column of Dowex 50 W (X8; H^+ form) and evaporated to dryness over NaOH pellets. The neutral monosaccharide residues were examined by paper chromatography as described previously (Andrews *et al.*, 1967).

The relative proportions of the neutral hexoses and pentoses in the hydrolysates were estimated after separation as borate complexes on ion-exchange columns (Smith & McAllan, 1971). Total neutral

monosaccharide was determined with mannose as standard by a modified anthrone procedure (Andrews *et al.*, 1967) and confirmed by the orcinol method (François *et al.*, 1962).

Hexosamines were eluted from the small Dowex 50 W (X 8) column referred to above with 0.5 M-HCl (5 ml) and the eluates were evaporated to dryness over NaOH pellets. Qualitative identification of the hexosamines in the residues was by paper chromatography (Andrews *et al.*, 1967) and was confirmed by data from the amino acid analyser. Quantitative assay of hexosamines was carried out by a modified Elson-Morgan procedure (Boas, 1953).

N-Terminal amino acids. *N*-Terminal amino acids were dansylated (Gray, 1967) and the dansyl derivatives identified by thin-layer chromatography on silica-gel G plates (Needleman, 1970).

Results

Preliminary studies

Gel filtration of the unfractionated extract on Sephadex G-200 gave apparently identical elution patterns (Fig. 1) at pH 2.7, 5.0, 6.5 and 8.7, and in all cases erythroagglutinating activity was found only in the first major protein peak (I), the elution position of which corresponded to an approximate mol.wt. of 125000. Peaks (II) and (III) corresponded to mol.wt. about 56000 and 28000 respectively, and peak (IV) represented small molecules at the column volume which had been incompletely removed by

Fig. 1. Gel filtration of unfractionated navy-bean extract

Freeze-dried navy-bean extract (39 mg) was run on a column (2.2 cm $\times$ 58 cm) of Sephadex G-200 in 0.2 M-sodium phosphate buffer, pH 5.0, containing 0.2 M-NaCl. Column flow rate was approx. 8 ml/h and the E_{280} of the effluent was monitored continuously with a Cecil 272 spectrophotometer (Cecil Instruments Ltd., Cambridge, U.K.) fitted with a 75 μ l stream-analysis cell. V_0 was 60 ml.

dialysis of the saline extract during preparation. Starch-gel electrophoresis showed that peak (I) consisted of two major and two minor electrophoretic components which were arbitrarily labelled components 1, 2, 3 and 4 in order of decreasing electrophoretic mobility, the two major components being 1 and 2. Component 2 possessed powerful agglutinating activity (see below) and will be referred to as navy-bean lectin, whereas component 1 was probably inactive. Low amounts of activity in component 1 fractions appeared to be related to the presence of traces of lectin and to bear no relationship to the presence of the minor components 3 and 4, suggesting that these were probably also inactive, although unequivocal evidence must await their isolation.

Closer investigation of peak (I) revealed that the distribution of the four components across it was not uniform, component 1 accompanied by components 3 and 4 being predominant in leading fractions and the lectin predominating towards the trailing edge of the peak. This suggested that a recycling technique might be of value in further purification, and milligram amounts of the lectin have been obtained by

Fig. 2. Purification of component 1 by gel filtration

Precipitated protein material (1.5 g; see the text for details) was run on a column (6.4 cm $\times$ 83.5 cm) of Sephadex G-200 in a 0.2 M-sodium phosphate buffer, pH 6.8, containing 0.2 M-NaCl. Flow rate was approx. 100 ml/h and the 25 ml fractions collected were assayed for erythroagglutinating activity and the E_{280} values were recorded. Leading fractions were enriched in component 1 and hence were high in protein content but low in erythroagglutinating activity. These fractions were pooled as indicated by the bar and recycled through the column. ●, Fractions with relatively high erythroagglutinating activity containing lectin-impurity; ○, fractions with little or no activity.

pooling and recycling appropriate fractions three times through Sephadex G-200 columns. Component 1 could not be obtained pure in this way directly. However, if a dialysis step (see below) was used to remove the interfering components 3 and 4, such a technique provided a suitable small-scale preparative route. The large-scale preparation of quantities of material sufficient for animal-feeding experiments (Andrews & Jayne-Williams, 1974) required different fractionation procedures.

Large-scale fractionation of extract

Freeze-dried extract (60 g) derived from approx.

1.4 kg of navy-bean flour, was dissolved in 0.01 M- NaH_2PO_4 (4 litres) and dialysed (1.5 in Visking tubing) for 48 h at 4°C against 22 litres of 0.01 M- NaH_2PO_4 , with a change of external buffer after 24 h. Without permitting the solution to warm, the contents of the dialysis sacks were pooled and centrifuged at 1200g for 30 min at 4°C. Under these conditions most of component 1 was precipitated, whereas the bulk of the lectin remained in the supernatant.

Quail-feeding trials conducted at this stage (Andrews & Jayne-Williams, 1974) showed that toxicity was associated with the supernatant

Fig. 3. Fractionation of lectin on DEAE-cellulose

Material precipitated by 50–80% satd. $(\text{NH}_4)_2\text{SO}_4$ from 4 litres of extract-supernatant fraction (see the text) was applied to a column (7.5 cm $\times$ 27 cm) of DEAE-cellulose in 0.01 M-sodium phosphate buffer, pH 6.8, and eluted with a 0–0.5 M-NaCl gradient as described in the Materials and Methods section. Erythroagglutinating activity was confined to peak C and lectin fractions were pooled as indicated by the bar. ----, [NaCl].

material, so that although this was fractionated further to yield quantities of lectin sufficient for subsequent feeding trials, component 1 was purified on only a small scale for biochemical characterization. Since the above precipitate contained component 1 accompanied by some lectin and faster-moving electrophoretic components, but was free of the interfering components 3 and 4, component 1 could be purified by cycling through gel-filtration columns. The precipitate fraction was dissolved in 125 ml of 0.2M-sodium phosphate buffer (pH 6.8) containing 0.2M-NaCl and a portion (50 ml) containing approx. 1.5 g of protein was applied to the 2.7 litre gel-filtration column. Fractions at the leading edge of the protein peak (Fig. 2) were relatively high in protein content but possessed only low erythroagglutinating activity, and these were pooled as indicated (Fig. 2) to yield 0.45 g of partially purified component 1. After two further cycles component 1 (0.15 g) of high purity was obtained.

The lectin was prepared from 4 litres of the above supernatant fraction by the addition of $(\text{NH}_4)_2\text{SO}_4$. The material precipitated between 50% and 80% saturation at 4°C was dissolved in water to give a total volume of about 500 ml, placed in 24/32 in Visking tubing and dialysed at 4°C against 10 litres of 0.01 M-sodium phosphate buffer (pH 6.8) for 48 h with a change of external buffer after 24 h. The dialysed

solution was then further fractionated on a DEAE-cellulose ion-exchange column (Fig. 3). Starch-gel electrophoresis showed that the lectin was eluted in peak C, which also contained all the erythroagglutinating activity. Suitably pooled fractions (Fig. 3) were concentrated by dialysis against polyethylene glycol (Carbowax 20 M; Union Carbide Chemicals Co., London W.1, U.K.) to about 200 ml and passed through the 5.8-litre gel-filtration column. This procedure yielded approx. 5.5 g of purified lectin. Material for biochemical characterization was subjected to a further gel-filtration cycle on the 2.7-litre column. The preparative scheme is summarized in Table 1.

Agglutinating properties

Agglutination of erythrocytes caused by the crude navy-bean extract was not inhibited by D-glucose, D-glucosamine, N-acetyl-D-glucosamine, D-galactose, D-galactosamine, N-acetyl-D-galactosamine, D-mannose, D-ribose, D-fructose, L-arabinose, L-fucose, L-rhamnose, D-xylose, L-sorbose, D-laevulose, α -D-melibiose, raffinose, lactose, sucrose, D-cellobiose, N-acetylneuraminic acid, α -methyl-D-glucoside or α -methyl-D-mannoside at concentrations in the range 1–10 mM in 0.1M-sodium phosphate buffer (pH 6.8). Likewise, mixed oligosaccharides from human milk (a gift from Dr. P. Andrews) and the glycoproteins κ -casein and glyco- α -lactalbumin (kindly provided by Dr. T. E. Barman) also failed to inhibit the agglutination reaction. These findings are similar to those of Kornfeld & Kornfeld (1970), who showed that the erythroagglutinating activity of the lectin of the red kidney bean (*Phaseolus vulgaris*) was not inhibited by monosaccharides except N-acetylglactosamine at very high concentrations. Binding of the lectin to the erythrocyte membrane probably involved not only non-reducing terminal monosaccharide residues but also the inner sugars of the oligosaccharide chain and the peptide backbone of glycoproteins on the cell surface.

A requirement for metal ions in the agglutinating or mitogenic activities of some lectins has also been reported (Tunis, 1965; Agrawal & Goldstein, 1968; Galbraith & Goldstein, 1970; Paulová *et al.*, 1971; Takahashi *et al.*, 1971) and inhibition by EDTA has been observed. In the present work erythroagglutination was unaffected by addition of disodium EDTA to saturation values in 0.1M-sodium phosphate buffer (pH 6.8), suggesting that metal ions are probably not required for activity by navy-bean lectin.

Characterization of component 1 and navy-bean lectin

Some properties of component 1 and the lectin are shown in Table 2. Classification of component 1

Table 1. *Purification of navy-bean component 1 and lectin*

This procedure started from 60 g of freeze-dried navy-bean saline extract containing about 30 g of protein in addition to salts and small diffusible molecules. Protein contents were based on E_{280} readings, taking an arbitrary value of 1.0, in 1 cm path-length cells, to be a concentration of 1 mg/ml. Sample solutions were examined for agglutinating activity after serial dilution, and the maximum dilution giving a positive response (agglutinating titre) divided by the concentration (mg/ml) was taken as the specific activity. Since this procedure is only semi-quantitative, errors may be of the order of at least $\pm 50\%$.

	Total protein content (g)	Specific activity (titre/mg)
Saline extract (60g)	30.0	70
Preliminary dialysis supernatant fraction	25.5	80
50–80%-satd.-(NH_4) ₂ SO ₄ precipitate	14.0	180
DEAE-cellulose column, peak-C material	6.5	300
Sephadex G-200 column, first cycle, lectin	5.5	400
Preliminary dialysis precipitate fraction	4.0	15
Sephadex G-200 column, third cycle, component 1	0.4	<1

as a second lectin in the navy bean, possessing weak agglutinating activity, would not appear to be justified on the basis of present evidence, since trace contamination with the major lectin component may account for the low amounts of residual activity observed (Table 2).

Throughout ultracentrifuge, gel-filtration and electrophoretic studies both component 1 and the lectin behaved as homogeneous proteins. Likewise polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate revealed a single band staining for both protein and carbohydrate in each case, which showed that the two substances were glycoproteins and apparently homogeneous. Further evidence for homogeneity was obtained by *N*-terminal analysis, which indicated (Table 2) that each protein contained a single type of amino acid in such a location. Sedimentation-equilibrium measurements showed that by using the pycnometrically determined value for $\bar{v}$, component 1 had mol.wt. about 140 000 (Table 3). The lectin gave a value of close to 112 000

under the same conditions. Gel-filtration results were in good agreement with these (Table 3) and gave $V/V_0=1.73$ for component 1 and $V/V_0=1.88$ for the lectin. Standard proteins for calibration of gel-filtration columns were on a linear plot of log mol.wt. versus elution volume (Andrews, 1970). With V/V_0 values in parentheses, these standards were bovine γ -globulin (1.68), bovine serum albumin (2.21), ovalbumin (2.48) and chymotrypsinogen (2.88).

Under conditions of dissociation in a 6.6M-urea buffer (Table 3) gel-filtration standards of bovine serum albumin, ovalbumin, swine pepsin, bovine α_{s1} -casein, bovine β -lactoglobulin and bovine α -lactalbumin showed V/V_0 values of 1.15, 1.33, 1.45, 1.68, 1.85 and 1.96 respectively and the lectin subunit ($V/V_0=1.52$) was found to correspond to mol.wt. 32 000. In a 9M-urea solvent (Table 3) V/V_0 values for the above standards were 1.20, 1.45, 1.55, 1.93, 2.14 and 2.32 respectively, whereas component 1 gave $V/V_0=1.53$, corresponding to a subunit mol.wt. of 39 000 and the lectin again showed a value of 32 000 ($V/V_0=1.68$). Sedimentation-equilibrium experiments in the buffered 9M-urea solvent and in 6M-guanidine hydrochloride (Table 3) confirmed these gel-filtration results. Polyacrylamide-gel-electrophoresis results were also in good agreement with these determinations (Table 3). In a typical experiment component 1 gave a single band with a mobility of 10.9 cm and the lectin gave a band migrating 12.0 cm, whereas standards of bovine serum albumin, pepsin, chymotrypsinogen and β -lactoglobulin moved 8.2, 11.2, 12.8 and 14.4 cm respectively.

Among the most notable features of the amino acid compositions of the two navy-bean proteins (Table 4) was the absence from both of cysteine or cystine. Since the lectin also possessed no methionine it was devoid of sulphur-containing amino acids. The absence of tryptophan from component 1, which was confirmed

Table 2. *Some properties of component 1 and navy-bean lectin*

	Component 1	Lectin
Minimum concentration giving erythroagglutination ($\mu\text{g/ml}$)	>2000	<4
Minimum concentration giving leucoagglutination ($\mu\text{g/ml}$)	>2000	<4
Calculated $\bar{v}$	0.722	0.706
$\bar{v}$ by pycnometry	0.745	0.737
$s_{20,w}$ (protein concn. 5 mg/ml)	6.9	6.0
$s_{20,w}$ (protein concn. 2 mg/ml)	—	6.1
$E_{1\text{cm}}^{1\%}$ at 280 nm (in 0.2M- NaH_2PO_4)	5.4	10.2
<i>N</i> -Terminal amino acid	Alanine	Arginine

Table 3. *Molecular-weight data for navy-bean component 1 and lectin*

The molecular weights of the purified proteins were examined by various techniques (see the text for details).

Method	Solvent	Molecular-weight values	
		Component 1	Lectin
Gel filtration	0.2M-Sodium phosphate, pH 6.8	145 300	114 800
Sedimentation equilibrium	0.2M-Sodium phosphate, pH 6.8	140 300	112 400
Sedimentation equilibrium	6M-Guanidine hydrochloride+0.1 M-2-mercaptoethanol	—	29 300
Sedimentation equilibrium	9M-Urea+0.1 M-2-mercaptoethanol+0.2 M-NaCl+0.05M-Tris-HCl, pH 7.0	35 700	28 900
Gel filtration	9M-Urea+0.1 M-2-mercaptoethanol+0.2 M-NaCl+0.05M-Tris-HCl, pH 7.0	39 000	32 000
Gel filtration	6.6M-Urea+0.02M-2-mercaptoethanol+ 10^{-4} M-EDTA+0.02M-Tris-HCl, pH 7.0	—	32 100
Polyacrylamide-gel electrophoresis	0.05M-Sodium phosphate, pH 7.0, +0.1% sodium dodecyl sulphate	37 200	29 900

Table 4. *Amino acid composition of navy-bean component 1 and lectin*

Values based on protein dry weights were calculated as molar proportions assuming mol.wt. 143 000 for component 1 and 114 000 for the lectin. The amino acid values are the average of nine runs, with three different preparations for the lectin consisting of triplicate samples hydrolysed for 24, 48 and 72 h by the method of Liu & Chang (1971) and six runs for component 1 (24, 48 and 72 h hydrolyses in duplicate). A similar series of hydrolyses with 5.8M-HCl gave generally similar results but with slightly lower recoveries of Asp, Thr, Ser, Glu, Pro, Gly, Ala and Tyr and no Trp value.

Con- stituent	Component 1		Lectin	
	(mol/mol)	(nearest integer)	(mol/mol)	(nearest integer)
Trp	0.0	0	13.2	13
Lys	60.0	60	35.4	35
His	25.8	26	12.2	12
Arg	44.3	44	16.0	16
‡Cys*	0.0	0	Trace	0
Asp	143.0	143	116.0	116
Thr†	38.1	38	71.2	71
Ser†	93.8	94	80.4	80
Glu	167.5	168	48.2	48
Pro	39.5	40	31.2	31
Gly	67.0	67	57.7	58
Ala	54.7	55	49.7	50
Val	64.8	65	61.3	61
Met‡	8.4	8	Trace	0
Ile	55.8	56	37.2	37
Tyr	30.4	30	20.3	20
Phe	61.4	61	45.5	46
Leu	103.5	104	67.6	68
Total		1059		762

* Determined separately as cysteic acid.

† Values extrapolated to zero time.

‡ Determined separately as methionine sulphone.

by the method of Edelhoch (1967), led to a low value for the specific extinction coefficient at 280 nm (Table 2).

Although the total carbohydrate contents of component 1 (4.9%) and of the lectin (8.1%) were quite different, the compositions of the carbohydrate moieties were very similar (Table 5). The individual values for the neutral sugars given in Table 5 were calculated from the total hexose content and the relative proportions of the monosaccharides determined by ion-exchange chromatography. Both bean proteins contained glucosamine as the only amino sugar and the neutral sugars consisted predominantly of mannose with some glucose and traces of other monosaccharides.

Discussion

The occurrence of more than one lectin in a single source is not uncommon (Toms & Western, 1971), and such sources include some varieties of *Phaseolus vulgaris*, namely the black bean (Jaffé & Hannig, 1965), the red kidney bean (Allen *et al.*, 1969; Weber, 1969; Yachnin & Svenson, 1972) and the wax bean (Takahashi *et al.*, 1967). The navy bean, however, appeared to contain only one lectin. This was capable of agglutinating both horse erythrocytes and lymphocytes at similar concentrations of lectin, and there was no evidence that erythroagglutinating and leucoagglutinating activities were separable. It has been suggested that, in the red-kidney-bean lectin, these activities are associated with different types of subunit (Rigas *et al.*, 1972), and the native molecule may be made up of non-identical subunits (Rigas & Head, 1969; Yachnin & Svenson, 1972). However, the apparent homogeneity of the navy-bean lectin in the present study under all experimental conditions suggested that this lectin was probably composed of identical subunits, the native molecule being a tetramer.

Measurements of the molecular weights of glycoproteins by gel filtration may be subject to some uncertainty owing to their expanded structures (Andrews, 1965; Ward & Arnott, 1965), but in practice glycoproteins with a low carbohydrate content give satisfactory results (Andrews, 1970). Likewise glycoproteins may give erroneous molecular-weight values when studied by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate, but in this case deviations do not appear to be correlated with the carbohydrate content (Glossman & Neville, 1971). These authors concluded, however, that in most cases good estimates were possible by this technique. In the present investigation, although both component 1 and navy-bean lectin were glycoproteins, the carbohydrate contents were quite low and the close agreement (Table 3) between gel-

Table 5. *Carbohydrate composition of navy-bean component 1 and lectin*

Sugar constituent	Component 1		Lectin	
	(% dry wt.)	(mol/ mol)	(% dry wt.)	(mol/ mol)
Total hexose (anthrone)	3.90	—	6.49	—
Mannose	3.35	26.7	4.44	27.1
Glucose	0.42	3.2	1.45	9.3
Xylose	0.08	0.8	0.34	2.5
Arabinose	0.05	0.5	0.26	2.0
Galactose	0	0	Trace	0
Total hexosamine	0.95	8.5	1.64	10.4
Glucosamine	0.95	8.5	1.64	10.4

filtration data in various solvents, electrophoresis results and sedimentation-equilibrium measurements support the contention that all three methods were capable of giving reliable molecular-weight values in this instance.

A comparison of the data in Table 3 with published values showed that the navy-bean lectin was somewhat smaller than the molecular-weight range of 121 000–138 000 reported for the lectins of other varieties of *Phaseolus vulgaris* (Jaffé & Hannig, 1965; Takahashi *et al.*, 1967; Jaffé, 1969; Weber, 1969; Rigas *et al.*, 1972). The significance of these values is difficult to assess, however, and apparent differences may result in part from variations in behaviour during gel filtration or electrophoresis as discussed above, or from the use of different methods of measuring $\bar{v}$ for ultracentrifuge calculations. The experimentally determined value of $\bar{v}$ for navy-bean lectin (0.737) was close to the range of 0.745–0.758 determined by Weber (1969) for red-kidney-bean leucoagglutinin and to the value of 0.738 found by pycnometry for red-kidney-bean lectin (Rigas *et al.*, 1972). As with the navy-bean lectin (Table 2), the composition of red-kidney-bean-lectin mitogenic components led to calculated $\bar{v}$ values of 0.72 (Oh & Conard, 1972) and 0.73 (Allen *et al.*, 1969), which were lower than the above experimental values for similar material.

In terms of overall composition (Tables 4 and 5) navy-bean lectin was similar to the lectins of other varieties of *Phaseolus vulgaris* (Rigas *et al.*, 1966; Jaffé & Hannig, 1965; Takahashi *et al.*, 1967; Allen *et al.*, 1969; Oh & Conard, 1972), although there were some significant differences in both amino acid and carbohydrate constituents.

The purified navy-bean lectin was toxic when fed to quail chicks (Andrews & Jayne-Williams, 1974). Evans *et al.* (1973) have reported the separation from navy beans of a fraction that causes growth inhibition in rats. Their toxic fraction, which contained all the erythroagglutinating activity, appears to correspond precisely to that material eluted during gel filtration as peak (I) (Fig. 1), and which contains four protein components. Evans *et al.* (1973) did not fractionate their material further but did suggest the possibility that it was a mixture of proteins. Thus on the basis of current evidence it would appear probable that the protein factor in navy beans responsible for growth inhibition in rats is the quail-toxic lectin isolated in the present studies.

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