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Bean lectins IV: genetic variation in the non-denatured structure of lectins from different *Phaseolus vulgaris* L. cultivars

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Summary. Variation in the native conformation of bean lectins was examined using electrophoresis of non-denatured total protein extracts and purified albumin and globulin lectin. The observed variation was related to the genetic variation reported previously for lectin polypeptide composition as revealed by two-dimensional isoelectricfocusing-sodium dodecyl sulfate polyacrylamide gel electrophoresis (IEF-SDS/PAGE). When eleven cultivars with different IEF-SDS/PAGE lectin polypeptide compositions were compared, eight had unique non-denatured lectin patterns and three had identical patterns. For some cultivars differences in non-denatured lectin patterns were observed between the purified albumin and globulin lectin preparations.

Key words: *Phaseolus vulgaris* – Lectins – Albumin – Globulin

Introduction

Lectin proteins, or phytohemagglutinins, are present in large quantities in the seeds of the common bean (*Phaseolus vulgaris* L.). Their unusual properties of hemagglutination and mitogenicity and their contribution to the poor nutritional quality of raw beans have attracted much attention, resulting in numerous studies on their physical and chemical properties (Reviews: Liener 1976; Goldstein and Hayes 1978; Lis and Sharon 1981).

Several criteria have been used to characterize lectins, including agglutination of erythrocytes and leucocytes, mitogenic activity on lymphocytes, molecular weights and isoelectric points of polypeptide subunits, and the native conformation of non-denatured lectin proteins. The native conformation of lectin from "kidney beans" has been studied intensively (Miller et al. 1973; Felsted et al. 1975; Leavitt et al. 1977). Under non-denaturing conditions, it was shown to consist of five tetrameric proteins derived from all possible combinations of two polypeptide subunits, one erythrocyte-reactive, the other leucocyte-reactive.

Recently, eleven genetic variants of bean lectin were reported (Brown et al. 1982a) from the survey of 107 bean lines. These variants could be distinguished on the basis of their two-dimensional electrophoretic patterns and their agglutination behavior towards rabbit and trypsin-treated cow erythrocytes. Only one of these variants corresponded to the "kidney bean" lectin (above), and its two-dimensional electrophoretic pattern consisted of five polypeptides (Brown et al. 1982a, b), adding complexity (Osborn et al. 1983) to the model originally proposed for the native conformation. Although native conformations other than the five tetrameric type have been observed (Sela et al. 1973; Andrews 1974; Manen 1978a; Felsted et al. 1981), the lectin variants reported by Brown et al. (1982a) have not been studied for variation in non-denatured structure.

One group of cultivars contained no lectin polypeptides in the same area of the two-dimensional gels as the eleven lectin variants (above) and did not show hemagglutinating activity with rabbit and trypsin-treated cow erythrocytes (Jaffé et al. 1974; Brown et al. 1982a). However, Pusztai et al. (1981) recently isolated a lectin from 'Pinto U.I. 111' which made up only a small proportion of the seed, 0.3% (w/w). It showed a very low agglutination activity with rabbit erythrocytes but a high activity with pronase-treated rat erythrocytes. This lectin differed from previously described *P. vulgaris* lectins (Miller et al. 1973; Felsted et al. 1975; Leavitt et al. 1977) in that it was a dimer made up of subunits with molecular weights of 28,000–29,000 daltons and isoelectric points in the range of pH 4.7–5.0 (Pusztai et al. 1981).

Most studies on the properties of lectins have utilized total lectins as extracted in salt solution. Hemagglutinating activity, however, has been found in both salt soluble (globulin) and water soluble (albumin) protein fractions (Jaffé and Hannig 1965). Immunochemically related lectins were isolated from both the albumin and globulin fractions of 'haricot' kidney bean (Pusztai and Watt 1974) and the cultivar 'Processor' (Pusztai and Stewart 1978). Manen and Miège (1977) and Manen (1978b) found differences in the non-denatured structure of albumin and globulin lectins from the cultivar 'Con-

tender', and variation in the subunit composition has been reported for hemagglutinating proteins in the albumin and globulin-2 fractions from 'Sanilac' (Brown et al. 1981).

In the present study, we relate the genetic variation for bean lectins observed by two-dimensional electrophoresis to the variation in native conformation of lectins in crude protein extracts and those purified from albumin and globulin protein fractions of different bean cultivars.

Materials and methods

Plant materials

The cultivars and PI lines of *Phaseolus vulgaris* L. studied were from stocks held by Dr. F. A. Bliss (Table 1).

Protein extraction and electrophoresis

Total proteins were extracted from seeds as described previously (Manen and Miège 1977). The protein extracts and purified albumin and globulin lectins were separated by electrophoresis at pH 4.5 on 0.75 mm, 7.5% polyacrylamide slab gels according to the method of Reisfeld et al. (1962).

Lectin purification

Lectins were purified from the albumin and globulin protein fractions of 11 cultivars having different lectin subunit compositions. Bean seed flour (5 g) of each cultivar was stirred overnight in a solution (100 ml) of phosphate-buffered saline (PBS), pH 7.0 at 4 °C. The mixture was centrifuged (30,000 g, 4 °C, 30 min) and the supernatant dialysed against distilled water at 4 °C for 24 h. After centrifugation, the supernatant (albumin) was retained for affinity chromatography. The pellet was dissolved in 50 ml of 0.06 M NaCl, centrifuged and this supernatant (globulin) was also retained for affinity chromatography.

Lectins were purified from the albumin and globulin fractions using Sepharose-Thyroglobulin affinity chromatography, as described previously (Osborn et al. 1983). The albumin and globulin supernatants (50 ml aliquots) were stirred with the equilibrated affinity resin for 5–10 min and after extensive washing with PBS, pH 7.4, lectins were eluted with two 50 ml volumes of 0.5 M NaCl, 0.05 M glycine, pH 3.0. The eluants were dialysed against distilled water for 24 h at 4 °C and lyophilized. Total lectins were purified from seeds of 'Contender' as described previously (Manen and Miège 1977).

Hemagglutination tests

Hemagglutination tests were performed on lectins purified from albumin and globulin fractions of each cultivar. Serial dilutions, beginning with concentrations of 25 mg lectin per 1 ml PBS, pH 7.0, were tested for hemagglutination activity using rabbit and trypsin-treated cow erythrocytes, as described previously (Brown et al. 1981, 1982a). Commercial phytohemagglutinin (Sigma) was used as a control and it agglutinated rabbit and trypsin-treated cow erythrocytes at a minimum concentration of 1.6 and 0.8 μg/ml, respectively.

Results

Variation in non-denatured lectins

Electrophoretic patterns of non-denatured total seed protein extracts are shown in Fig. 1a. The first and last lanes (arrows) are non-denatured patterns of lectins purified from 'Contender', and the remaining lanes are non-denatured patterns of eleven cultivars containing lectins with different two-dimensional electrophoretic patterns and one lectinless cultivar, 'Pinto U.I. 111'. All cultivars contained proteins in the total extracts which migrate to the same region of the gel as the purified 'Contender' lectin. 'Bunsi', PI 310878 and PI 312041 (Fig. 1a, lanes B, 1 and 2) have indistinguishable protein patterns in the lectin-containing region of the gel. For the remaining cultivars, however, non-denatured gel patterns in the lectin region are unique. These same non-denatured lectin patterns were observed for other cultivars having lectins with corresponding two-dimensional electrophoretic patterns.

Table 1. Quantities recovered and agglutination ratios of lectins from albumin (*A*) and globulin (*G*) fractions of 12 cultivars of common bean, *Phaseolus vulgaris*

Cultivar	Fraction[a]	mg. Lectin purified[b]	Agglutination ratio[c]
'Tendergreen' (T)	A	11	0.5
	G	6	2
'Porrillo 70' (P)	A	2	0.004
	G	< 1	–
'M-1' (M)	A	14	1
	G	7	0.5
'Bunsi' (B)	A	14	0.5
	G	1	0.5
'Sanilac' (S)	A	14	16
	G	6	8
'Viva' (V)	A	6	16
	G	2	8
'Protop P-1' (Pr)	A	21	8
	G	1	4
'Pinto U.I. 111' (Pi)	A	–	–
	G	–	–
'Kerman' (K)	A	15	4
	G	1	2
PI 310878 (1)	A	17	1
	G	3	1
PI 312041 (2)	A	26	2
	G	3	2
'California Dark Red Kidney' (C)	A	6	0.004
	G	< 1	0.004

[a] *A* = albumin, *G* = globulin

[b] Quantity purified from 5 g bean flour (see "Materials and methods")

[c] Agglutination ratio = $\frac{\text{min. conc. to agglutinate trypsin-treated cow erythrocytes}}{\text{minimum concentration to agglutinate rabbit erythrocytes}}$

Differences of one step in the ratio series 0.5, 1, 2, 4, 8, etc. are not outside the range of experimental error

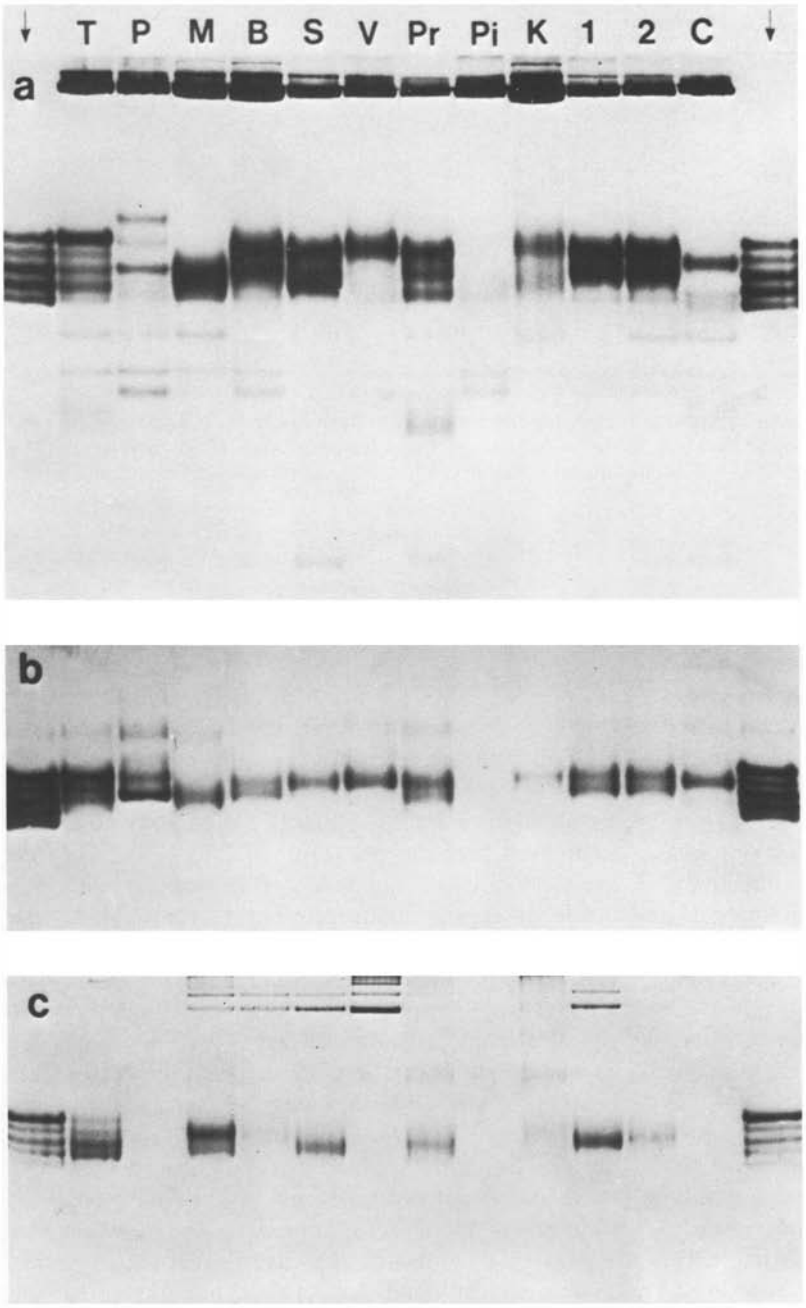

Fig. 1 a–c. Non-denatured electrophoretic patterns of seed proteins from bean cultivars. *T* 'Tendergreen'; *P* 'Porrillo 70'; *M* 'M-1'; *B* 'Bunsi'; *S* 'Sanilac'; *V* 'Viva'; *Pr* 'Protop P-1'; *Pi* 'Pinto U.I. 111'; *K* 'Kerman'; *1* PI 310878; *2* PI 312041; *C* 'California Dark Red Kidney'. **a** total protein extracts; **b** purified albumin lectins (no sample was applied to lane *Pi*); **c** purified globulin lectins (no samples were applied to lanes *P, Pi* and *C*). The first and last lanes of each gel (*arrows*) are purified 'Contender' lectin. Electrophoresis was carried out at pH 4.5 according to the method of Reisfeld et al. (1962)

Lectin purification and hemagglutination

The quantities of lectin purified by Sepharose-thyroglobulin affinity chromatography are shown in Table 1. No lectin proteins could be purified from 'Pinto U.I. 111', and for the remaining cultivars, more lectin was extracted from the albumin than from the globulin fraction.

Purified lectins were tested for agglutinating activity toward rabbit and trypsin-treated cow erythrocytes (Table 1). When agglutination ratios were compared for albumin and globulin lectins from the same cultivar, only the 'Tendergreen' lectins had significantly different ratios.

Comparison of purified albumin and globulin lectins

The non-denatured electrophoretic patterns of lectins purified from the albumin and globulin fractions of cultivars having different lectin types are shown in

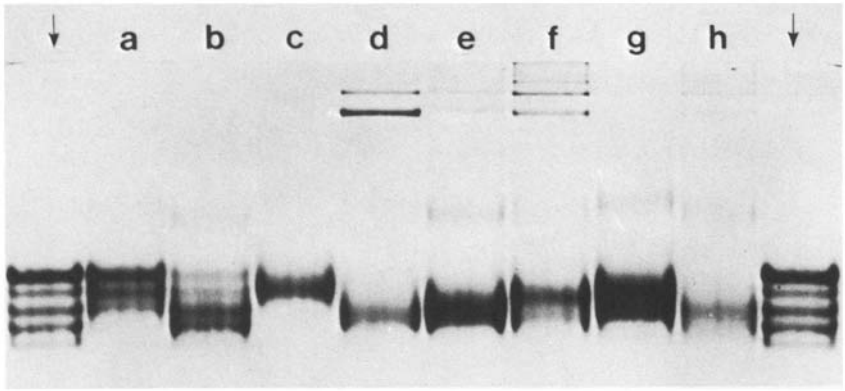

Fig. 2. Non-denatured electrophoretic patterns of purified albumin and globulin lectins from bean cultivars. *a* 'Tendergreen' albumin lectin; *b* 'Tendergreen' globulin lectin; *c* 'Sanilac' albumin lectin; *d* 'Sanilac' globulin lectin; *e* 'M-1' albumin lectin; *f* 'M-1' globulin lectin; *g* 'Protop P-1' albumin lectin; *h* 'Protop P-1' globulin lectin. The first and last lanes (*arrows*) are purified 'Contender' lectin

Fig. 1 b, c, respectively. Both the albumin and globulin lectin preparations contained mainly proteins in the lectin region of the gel. Many of the globulin lectin preparations were contaminated with phaseolin, the major globulin storage protein (top of gel). Insufficient quantities for electrophoresis were recovered from globulin fractions of 'Porrillo70' and 'California Dark Red Kidney', and the patterns obtained for 'Bunsi', 'Viva' and 'Kerman' were very faint due to the small amount of lectins in the globulin lectin preparations.

For six of the lines, 'Porrillo 70', 'Bunsi', 'Viva', PI 310878, PI 312041, and 'California Dark Red Kidney', non-denatured gel patterns of purified albumin lectins (Fig. 1 b, lanes P, B, V, 1, 2, and C) were similar to the patterns of total protein extracts in the lectin area of the gel (Fig. 1 a, lanes P, B, V, 1, 2, and C).

However, albumin lectin preparations from 'Tendergreen', 'Sanilac', 'Protop P-1' and 'Kerman' (Fig. 1 b, lanes T, S, Pr, and K) did not contain higher mobility lectins that were present in the total protein extracts (Fig. 1 a, lanes T, S, Pr, and K). These lectins were present in the globulin lectin preparations from these cultivars (Fig. 1 c, lanes T, S, Pr, and K). The albumin and globulin lectin patterns of 'M-1' (Figs. 1 b, c, lane M) were slightly different in that the former contained a single diffuse band while the latter clearly showed two bands. These differences in the mobility of albumin and globulin lectins are shown clearly in Fig. 2 for 'Tendergreen' (lanes a and b), 'Sanilac' (lanes c and d), 'M-1' (lanes e and f), and 'Protop P-1' (lanes g and h). The non-denatured gel patterns of globulin lectins purified from PI 310878 and PI 312041 contained a single band (Fig. 1 c, lanes 1 and 2) which corresponded to the band with higher mobility seen in the patterns of purified albumin lectins (Fig. 1 b, lanes 1 and 2).

Discussion

Variation in non-denatured lectins

When protein extracts from the twelve cultivars (representing the eleven lectin variants and the absence of lectin) were separated by non-denatured electrophoresis, different banding patterns were observed in the lectin region of the gel (Fig. 1 a). 'Pinto U.I. 111', the lectinless cultivar, contained lightly staining proteins in the lectin region of the gel (Fig. 1 a, lane Pi). Similar proteins were also observed in lectin-containing cultivars (Fig. 1 a, lanes P, B, V, K, 1, 2, and C). Since these proteins were not observed in the purified albumin and globulin lectin preparations (Fig. 1 b, c), they may not be lectins. However, due to their presence in 'Pinto U.I. 111' it is possible that they may represent the new type of lectin described by Pusztai et al. (1981). Also, this new lectin did not bind thyroglobulin (Pusztai et al. 1981) and therefore, would not appear in the purified albumin and globulin lectin preparations here. The new lectin type may also be present in other cultivars containing conventional lectins but has remained undetected due to its different properties and low abundance.

Of the eleven cultivars with different lectin proteins, only three ('Bunsi', PI 310878 and PI 312041, Fig. 1 a, lanes B, 1 and 2) had lectins that could not be distinguished by non-denatured electrophoresis. Those lectins differed by only one polypeptide in their two-dimensional polypeptide composition (Brown et al. 1982 a). The lectins from the remaining cultivars had unique banding patterns that could be distinguished easily on non-denatured gels. These same non-denatured lectin patterns were observed when protein extracts from other cultivars with corresponding lectin

types were electrophoresed. Therefore, for most lectin types, genetic variation in polypeptide composition was also reflected by variation in non-denatured structure.

The range of variation in non-denatured structure reported here is broader than that reported previously. In a survey of lectins from eleven *P. vulgaris* cultivars (Manen 1978a), three were found to have the five tetrameric structure, one had four of the five tetramers and the remaining seven had a single major band with the same mobility on non-denatured gels. Two of those cultivars, 'Tendergreen' and 'Contender' were used in this study and both had five banded patterns as reported previously. More recently, Felsted et al. (1981) screened total protein extracts from 62 cultivars of *P. vulgaris* for differences in agglutinating activity and mitogenicity as well as SDS and non-denatured electrophoretic patterns of lectins. They could distinguish six different lectin types by non-denatured gel patterns and four by one-dimensional SDS-PAGE patterns. Two of those cultivars with unique lectin types appeared to be *Phaseolus coccineus* by virtue of their phaseolin SDS-PAGE pattern (Brown and Osborn, unpublished data). The four *P. vulgaris* lectin types distinguishable by non-denatured gels appeared to correspond to four of the lectin types reported here (T, S, B and V), which are the four most common lectin types found in cultivated *P. vulgaris* (Brown et al. 1982a). Cultivars containing other lectin types were not reported in Felsted's survey, although cultivars with lectins like those from 'Bunsi', PI 312041 or PI 310878 could not have been distinguished by one-dimensional SDS or non-denatured electrophoresis.

Comparison of albumin and globulin lectins

In the purification of lectins from albumin and globulin fractions, higher yields were obtained from the albumin fractions of all cultivars. Lectin quantification by rocket immunoelectrophoresis also showed more lectin in the albumin fraction (Osborn and Brown, unpublished data). For four cultivars, 'Tendergreen', 'Sanilac', 'Protop P-1' and 'Kerman', lectin bands with higher mobility that were visible in total extracts were absent in the purified albumin lectin preparations but present in the purified globulin lectin preparations. In addition, the cultivar 'Tendergreen' showed significant differences in the agglutination ratios of albumin and globulin lectins. The presence of higher mobility tetramers in the globulin lectin and the higher cow : rabbit agglutination ratio reported here are consistent with previous results since these tetramers contain higher proportions of the rabbit erythrocyte agglutinating polypeptide (Osborn et al. 1983). For 'Sanilac', differences in the polypeptide composition of albumin and globulin lectins reported by Brown et al. (1981) are also reflected by differences in the mobility of non-denatured lectins from these fractions (Fig. 2, lanes c and d). Two other cultivars ('Protop P-1' and 'Kerman') with higher mobility globulin lectins absent in the albumin fraction had different polypeptide compositions for albumin and globulin lectins similar to those reported for 'Sanilac' (gels not shown).

In previous studies on lectins purified from 'Contender', Manen and Miège (1977) and Manen (1978a) found that albumin and globulin lectins contained different proportions of the five bands seen on non-denatured gels. Solubility studies on the globulin lectins revealed that their insolubility in water was due to interactions with other globulin proteins (Manen 1978a). The differences in albumin and globulin lectins reported here for some cultivars and not others may be due to different types of interactions between lectin variants and other globulin proteins.

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