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Bean Lectins

Part 2: Relationship Between Qualitative Lectin Variation in *Phaseolus vulgaris* L. and Previous Observations on Purified Bean Lectins

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Summary. The relationship between the polypeptide composition and the agglutination behaviour of the lectin-containing G2/albumin protein groups has allowed the identification of the active lectin polypeptides in different cultivars of *Phaseolus vulgaris* (Brown et al. accompanying paper). These results were used to ascertain the particular G2/albumin group contained in the various lectin sources used previously for the purification of lectin proteins. Many studies were found to have included lectin sources which contained the same G2/albumin pattern (T_{G2}) and this common denominator has permitted the direct comparison of the properties reported for these purified lectins. Thus, much of the extensive literature on bean lectins is concurred.

Key words: *Phaseolus vulgaris* – Lectins

Introduction

Lectins or phytohaemagglutinins (PHA) are present in the seeds of many plant species (Toms and Western 1971). They are glycoproteins consisting of a number of subunits, each of which contains specific binding sites for sugar residues. The hapten-like interactions between these carbohydrate-binding sites and the sugar residues on the surface of blood cells give rise to the agglutination and mitogenic properties of the lectins. These properties have stimulated the widespread interest in lectin proteins due to their importance in studying cell surface and immunological interactions (Reviews: Sharon and Lis 1972; Lis and Sharon 1973, 1981; Liener 1976; Goldstein and Hayes 1978).

The lectins of the common bean (*Phaseolus vulgaris* L.) are the most widely studied, possibly due to their availability. Over many years, specific agglutinating and mitogenic lectin components have been isolated and characterized from a variety of sources. The wide range of sources and the use of unspecified sources renders it difficult to relate the properties of the characterized lectins to one another and to determine the extent to which the varied observations are due to genetic or procedural differences. However, some reports, which have used different lectin sources, have described a similar pattern of five lectin proteins on non-denaturing electrophoresis (Reisfeld 1962). These five proteins represent isomeric tetramers made up of two different subunits in varying proportions, one leucoagglutinating and mitogenic, the other erythroagglutinating (Yachnin et al. 1972; Yachnin and Svenson 1972; Miller et al. 1973, 1975; Felsted et al. 1975; Leavitt et al. 1977; Manen 1978 a, b).

In order to relate the qualitative variation for bean lectins, reported in the accompanying paper (Brown et al. 1982), to the above model and to the many other observations it has been necessary to include, in this study, lectin sources comparable to those used in previous studies. The extensive literature dealing with the purification and isolation of bean lectins has made use of essentially three types of lectin sources. Firstly, lectins have been purified from specified bean cultivars and to relate the results of these studies to those presented here, the same cultivars have been included. Secondly, in other studies, unspecified lectin sources, given the more generic names of "Black kidney" bean, "Red kidney" bean, "Kidney" bean, and "Navy" bean, have been used for the purification of lectins. The 107 bean lines screened in the accompanying paper were also scored for their commercial type so that the lectin compositions of the different classes of beans could be related to the above lectin sources. Finally, commercial phytohaemagglutinin preparations, used as a starting point for lectin purification in some of the more recent studies, have also been included in this paper.

Materials and Methods

Phytohaemagglutinins

Commercially available phytohaemagglutinin was obtained from Sigma (Lot. 30C-9630), Difco (Lot. 686 328) and Well-

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come Reagents (Lot. K 1340). Purified erythroagglutinin (Lot. 99C-9890) and leucoagglutinin (Lot. 75C-3928) were obtained from Sigma. These proteins were dissolved in 0.5 M NaCl prior to dissociation and electrophoresis Brown et al. 1981 a).

Protein Extraction, Electrophoresis, Haemagglutination Tests

Protein extraction, two-dimensional electrophoresis, and haemagglutination tests were carried out as described by Brown et al. (1982).

Results

Polypeptide Composition and Haemagglutination Activity

The polypeptide composition of the commercially available phytohaemagglutinins along with their G2/albumin and phaseolin types and haemagglutination ratios are presented in Table 1. The G2/albumin patterns and the individual polypeptides referred to are described in the accompanying paper (Brown et al. 1982). The two dimensional gels of these lectin preparations are not shown. Commercially available phytohaemagglutinin (from Sigma and Difco) showed the T_{G2} pattern upon two-dimensional SDS-PAGE and both showed a trace of phaseolin with the 'Tendergreen' (T) pattern. Purified erythroagglutinin (PHA-E; Sigma) also contained the T_{G2} pattern but the amount of polypeptide 1 of the T_{G2} pattern (Fig. 2a; accompanying paper) was consistently increased. Purified leucoagglutinin (PHA-L; Sigma) contained only polypeptides 2 and 3 of the T_{G2} pattern (Fig. 2a; accompanying paper). PHA-E also contained a trace of phaseolin while PHA-L did not (gels not shown). Phytohaemagglutinin (Wellcome) gave an electrophoretic pattern similar to but not identical to a mixture of T_{G2} and V_{G2}. There was a trace of phaseolin-like protein which did not correspond to either the 'T', 'S', or 'C' phaseolin patterns. Both these G2/albumin and phaseolin patterns have been observed in lines of *P. coccineus* (J. W. S. Brown and T. C. Osborn, unpublished data) and it therefore appears that a *P. coccineus* source has been used for this particular preparation (Table 1).

Phytohaemagglutinin (Sigma; Difco) had agglutination ratios of 0.5 consistent with their containing the T_{G2} pattern. The leucoagglutinin (PHA-L) preparation (Sigma) agglutinated trypsin-treated cow red blood cells strongly but only agglutinated rabbit red blood cells at very high concentrations, giving an agglutination ratio of 0.001. The erythroagglutinin (PHA-E) preparation (Sigma) agglutinated both types of erythrocytes with a ratio of 1.0 (Table 1).

Discussion

Similarities Among Other Bean Lectin Reports

The main physicochemical, agglutinating, and mitogenic properties of lectin preparations from numerous studies are summarized in Table 2. In general most preparations show similarities in their extraction and purification methods. Extraction buffers which have been used are saline solutions (Table 2, Refs. a, b, c, f, h, l, m, n, o, q), acidic aqueous solutions (Refs. d, g, h, l, o), and sodium borate buffers (Refs. n, p). Both ammonium sulphate precipitation (Refs. a, b, c, f, h, l, m, n, o, q) and ethanol precipitation (Refs. a, g, i) have been utilized as the purification procedure (Refs. a, c) or as a preliminary purification step prior to affinity or gel filtration chromatography (Ref. b, d–q). Other purification steps have involved sucrose density gradient centrifugation (Ref. p), and isoelectrofocusing (Refs. n, p). Lectins from commercially available preparations have been isolated by disc gel electrophoresis (Ref. r), isoelectrofocusing (Ref. w), and gel filtration chromatography (Refs. s–v, x).

In all cases where amino acid analysis of the isolated lectin fractions was performed, sulphur containing amino acids were either absent or virtually absent, and asparagine, serine, leucine, and threonine were always among the six most abundant amino acids (Refs. a, c–f, h, i, m–o, q, r, t, w). The apparent molecular weights for the native lectin tetramers were similar ranging from 85,000 to 150,000 daltons with the majority of preparations being in the range of 114,000

Table 1. Phaseolin and G2/albumin types, polypeptide compositions and agglutination ratios of commercially available lectin preparations

Lectin preparation	Phaseolin type	G2/albumin type	Polypeptide composition	Agglutination ratio
Phytohaemagglutinin (Difco)	T(trace)	T_{G2}	1 – 5	0.5
Phytohaemagglutinin (Wellcome)	*P. coccineus*	*P. coccineus*		4.0
Phytohaemagglutinin (Sigma)	T(trace)	T_{G2}	1 – 5	0.5
Erythroagglutinin (Sigma)	T(trace)	T_{G2}	1 – 5	1.0
Leucoagglutinin (Sigma)	–	T_{G2}	2, 3	0.001

Table 2. Physiochemical and agglutinating properties of isolated lectins

Reference	Lectin source	Lectin fraction	Tetramer		Subunit	Agglutination activity		Mitogenic activity
			MW[a]	pI[b]	MW[a]	Leuco-cyte	Erythro-cyte	
a. Rigas and Johnson (1964)	"Red kidney bean"	PPHA	128,000	6.5	n.d.[c]	+	+	+
b. Rigas et al. (1972)								
c. Jaffé and Hannig (1965)	"Black kidney bean"	Fr. A	128,000	n.d.	n.d.	n.d.	+	n.d.
		Fr. B –	n.d.	n.d.	n.d.	n.d.	+	n.d.
d. Takahashi et al. (1967)	'Sure Crop Stringless Wax'	WBH	120,000 – 132,000	5.5	n.d.	n.d.	+	n.d.
e. Dahlgren et al. (1970)	'Blue Lake'	PHA-a''	91,000	n.d.	n.d.	n.d.	+	+
		PHA-a'	110,000	n.d.	n.d.	n.d.	+	+
f. Mialonier et al. (1973)	'Rouge'	Lectine	128,000	5.0–6.0	n.d.	n.d.	+	n.d.
g. Jaffé et al. (1974)	'Negro Nicoya'	A – α	125,000	7.9	n.d.	n.d.	+	+
		A – β	121,000	5.9	n.d.	n.d.	+	–
	'Cubagua'	B – α	104,000	7.9	n.d.	n.d.	+/–[d]	–
		B – β	85,000	5.9	n.d.	n.d.	+/–[d]	–
	'Vainica	C – α	111,000	7.9	n.d.	n.d.	–/+[e]	+
	Saavedra'	C – β	85,000	5.9	n.d.	n.d.	–/+[e]	–
	'Alabaster'	D – α	125,000	7.9	n.d.	n.d.	–	–
		D – β	85,000	5.9	n.d.	n.d.	–	–
h. Moreira and Perrone (1977)	'Rico 23'	LcPA	100,250	5.0	n.d.	n.d.	+	n.d.
i. Räsänen et al. (1973)	"Kidney bean"	Leuco-agglutinin	126,000	5.1	31,000	+	–	+
j. Leavitt et al. (1977)	"Red kidney	L_4	115,000	n.d.	33,000	+	slight	+
k. Felsted et al. (1975)	bean"	E_4	115,000	n.d.	33,000	slight	+	+
l. Sela et al. (1973)	'Brittle Wax'	PHA	125,000	n.d.	30,000	+	+	+
m. Andrews (1974)	"Navy bean"	Lectin	114,000	n.d.	30,000	+	+	n.d.
n. Pusztai and Watt (1974)	"Haricot bean"	Globulin isolectin	n.d.	n.d.	30,000	+	+	slight
		Albumin isolectins	n.d.	4.5 – 6.9	30,000	+	+	slight
o. Itoh et al. (1980)	'Tora mame'	TM-lectin	120,000	5.5	30,000	n.d.	+	+
p. Bollini and Chrispeels (1978)	'Greensleeves'	Fr. Ala	n.d.	4.6	34,000	n.d.	–	+
		Fr. Alb	n.d.	6.0	34,000; 36,000	n.d.	+	+
q. Nowakova and Kocourek (1974)	*P. coccineus* 'Scarlet Runner'	PHA-I	120,000	6.9	34,000	n.d.	+	–
		PHA-II	120,000	n.d.	34,000	n.d.	–	+
r. Oh and Conard (1971; 1972)	PHAP (Difco)	M-A	142,200	n.d.	35,000; 36,500	slight	slight	+
		M-B	142,800	n.d.	35,000; 35,500	slight	slight	+
s. Weber et al. (1972)	PHAP (Difco)	Leuco-agglutinin	140,000	5.0	36,000	+	–	+
		Erythro-agglutinin	150,000	6.5	36,000 (2)	+	+	+
t. Allen et al. (1969)	PHAP (Difco)	L-PHAP	115,000	n.d.	36,000	+	slight	+
u. Yachnin et al. (1972)		H-PHAP	n.d.	n.d.	36,000	+	+	+
v. Yachnin and Svenson (1972)								
w. Miller et al. (1973; 1975)	PHAP (Difco)	L-PHAP	n.d.	5.25	34,000	+	slight	+
		H-PHAP	n.d.	5.25; 5.95	34,000	+	+	+
x. Allan and Crumpton	PHA (Welcome)	PHA	98,000	n.d.	29,000	+	n.d.	+
					33,000	–	n.d.	–

[a] Molecular Weight (daltons); [b] Isoelectric Point (pH); [c] Not determined
[d] Agglutinates rabbit erythrocytes strongly and trypsin-treated cow erythrocytes to a lesser degree
[e] Agglutinates trypsin-treated cow erythrocytes but not rabbit erythrocytes

and 150,000 daltons. Isoelectric points varied between pH 4.5 and pH 7.9, with the majority of preparations lying between pH 5.0 and pH 6.5. The apparent molecular weights of the subunits making up the lectin tetramers showed much less variation, ranging from 29,000 to 36,500 daltons. Many studies neither distinguished between leucocyte and erythrocyte agglutinating ability nor evaluated mitogenc activity. However, some studies (Refs. j, k, i, p, q, s–w) have characterized preparations specifically as leucoagglutinins or erythroagglutinins which allows closer comparisons to be made.

Relationships Between This and Other Research

In order to relate the many and varied studies on purified bean lectins (Table 2) to the results presented here, it was necessary to ascertain the G2/albumin types of the lectin sources used in the above studies. This was possible where named cultivars were specified in other studies and these same cultivars have been included here. Also, the use of commercially available phytohaemagglutinins as lectin sources allowed direct comparisons to be made. In a few cases it was impossible to relate the lectin sources used to any of the lines studied here. In yet other studies where cultivars were not named but were referred to by more generic names, it was possible to tentatively suggest the G2/albumin type of the lectin sources.

i. Lectin Sources With Unknown G2/albumin Groups

Three cultivar lectin sources could not be related to any bean lines which we have studied: 'Rouge', 'Tora-mame', and *P. coccineus* 'Scarlet Runner' (Table 2, Refs. f, o, q). The observations for the *P. coccineus* line have been included because initial results of electrophoretic analysis of different *Phaseolus* species have shown *P. coccineus* lines to contain many of the G2/albumin polypeptides of *P. vulgaris* (T. C. Osborn and J. W. S. Brown, unpublished data). Moreira and Perrone (1977) (Ref. h) used 'Rico 23' as a lectin source. A similar line screened here, 'Ex Rico 23' had the B_{G2} G2/albumin pattern. However, without information on the subunit composition of 'Rico 23' it is difficult to relate the properties of their isolated lectin, LcPA, to the polypeptides of the B_{G2} pattern.

Andrews (1974) (Ref. m) characterized a lectin preparation from 'Navy' bean. Of the seven navy bean cultivars screened here five had either the S_{G2} or V_{G2} pattern, and it is therefore possible that the lectin source used by Andrews (1974) contained one of these two types. Pusztai and Watt (1974) (Ref. n) using "haricot" beans found a group of numerous isolectins differing in their isoelectric points on isoelectrofocusing in the absence of urea. "Haricot" beans are snap beans grown for their edible pods, and since 73% of the snap beans screened here contained the T_{G2} pattern the above lectin source may also have contained that pattern. Similarly, "Blue Lake" cultivars are snap beans and, thus, "Blue Lake" (Dahlgren et al. 1970, Ref. e) may have shown the T_{G2} pattern.

The lectin sources: 'black kidney bean' (Jaffé and Hannig 1965, Ref. c), 'red kidney beans' (Rigas and Johnson 1964, Ref. a; Rigas et al. 1972, Ref. b; Leavitt et al. 1977, Ref. j), 'Sure Crop Stringless Wax' (Takahashi et al. 1967, Ref. d), and 'Brittle Wax' (Sela et al. 1973, Ref. l) may have all contained the T_{G2} pattern. This is probable because four of the five red kidney beans, and fourteen of the sixteen wax beans screened here showed the T_{G2} pattern. Indeed, Jaffé et al. (1974) showed that the lectin, PPHA, isolated from 'red kidney bean' (Rigas et al. 1972, Ref. b) and WBH isolated from 'Sure Crop Stringless Wax', (Takahashi et al. 1967, Ref. d) both agglutinated like type 'A' cultivars consistent with their containing the T_{G2} pattern. Also, the lectins derived from 'red kidney bean' by Leavitt et al. (1977) and Felsted et al. (1975) had the same non-denaturing gel patterns of tetramers as known T_{G2}-containing sources (see below).

ii. Lectins Sources Having the T_{G2} Pattern

It is apparent from the above considerations that many of the sources used for the purification of lectins may, in fact, have contained the same lectin polypeptides, by virtue of their containing the T_{G2} G2/albumin pattern. This is further exemplified by the similarities in the non-denaturing gel electrophoretic patterns of isolectin tetramers. Manen (1978 a, b) showed five tetramers for the lectins isolated from the cultivars 'Tendergreen' and 'Contender' (T_{G2} patterns). These five tetrameric forms corresponded to the family of isolectins described by Felsted et al. (1975) and Leavitt et al. (1977) for 'red kidney beans' (Refs. j and k) and by Allen et al. (1969), Yachnin et al. (1972), Yachnin and Svenson 1972, and Miller et al. (1973, 1975) (Ref. t–w) for commercially available phytohaemagglutinin, PHAP, from Difco (T_{G2} pattern). Felsted et al. (1975) showed the same five tetramers for both PHAP (Difco) and lectin from 'red kidney bean' on non-denaturing electrophoresis (Reisfeld 1962).

The uppermost tetramer of the five contained four identical subunits with leucoagglutinating and mitogenic properties, the lower tetramer contained four identical erythroagglutinating subunits, and the middle three tetramers represented mixtures of both subunits. Dissociation of the tetramers and fractionation on SDS-polyacrylamide gel electrophoresis showed two subunits, the higher molecular weight subunit being erythroagglutinating and the lower molecular weight subunit being leucoagglutinating (Manen 1978 a, b; Bollini and Chrispeels 1978; Oh and Conard 1972).

There is good agreement among the molecular weight estimates for the lectin subunits from the T_{G2}-containing sources. Bollini and Chrispeels (1978, Ref. p) reported values of 36,000 and 34,000 daltons for the cultivar 'Greensleeves' which has the T_{G2} pattern (Table 1). Oh and Conard (1972, Ref. r) reported molecular weights of 36,500 and 35,000 daltons for the erythroagglutinating subunit and 35,000 daltons for the leucoagglutinating subunit of PHAP (Difco). Other studies have reported the subunits to have the same molecular weight but different isoelectric points (36,000 daltons – Leavitt et al. 1977; Yachnin and Svenson 1972; Weber et al. 1969; 34,000 daltons – Miller et al. 1973, 1975) (refs j, s, v, w). Although Weber et al. (1969) found identical molecular weights for the subunits, the leucoagglutinin tetramer has a lower molecular weight than the erythroagglutinin tetramer consistent with a lower molecular weight for the leucoagglutinating subunit. Lastly, Allan and Crumpton (1971, Ref. x) using PHA (Wellcome) as a lectin source found two subunits with molecular weights of 33,000 and 29,000 daltons. The latter subunit had lymphocyte agglutinating and mitogenic properties, while the former was 'inactive'. This subunit could correspond to the erythroagglutinating subunit as erythrocyte agglutination was not estimated.

Relationship Between the T_{G2} G2/albumin Polypeptides and Isolated Lectin Subunits

Comparison of the agglutination activities and polypeptide compositions of some of the purified lectins and of commercially available phytohaemagglutinin preparations provides a means of relating the above observations to those presented in this and the accompanying paper (Brown et al. 1982). In the first case, phytohaemagglutinin from Difco and Sigma contained the T_{G2} pattern, and agglutinated rabbit and trypsin-treated cow erythrocytes. That from Sigma also agglutinated human erythrocytes, and was mitogenic (Sigma assay). Human leucocyte agglutination was not measured. Purified erythroagglutinin (PHA-E) from Sigma agglutinated both human erythrocytes and leucocytes (Sigma assay). The human erythroagglutinating activity of PHA-E was enhanced when compared to that of purified leucoagglutinin (PHA-L) (Sigma assay). The electrophoretic pattern of PHA-E contained all five polypeptides of the T_{G2} pattern but had an increased concentration of polypeptide 1. Similar erythroagglutinin preparations, purified from PHAP (Difco), were also reported to have enhanced erythroagglutination (Allen et al. 1969; Miller et al. 1973).

The uppermost tetramer of the five described for lectin preparations from T_{G2}-containing sources was composed of four identical leucoagglutinating subunits. Felsted et al. (1975) showed the phytohaemagglutinin preparation from Wellcome to correspond to this tetramer when compared to the five tetramers obtained with PHAP (Difco) on non-denaturing gel electrophoresis. Jaffé et al. (1974) compared the agglutination behaviour of PHA (Wellcome), leucoagglutinin (from Räsänen et al. 1973, Ref. i), wax bean haemagglutinin (from Takahashi et al. 1967, Ref. d), and phytohaemagglutinin (from Rigas et al. 1972, Ref. b). The latter two preparations agglutinated like 'A' types, agglutinating both rabbit and trypsin-treated cow erythrocytes to the same extent. The PHA (Wellcome) and purified leucoagglutinin (Räsänen et al. 1973) both agglutinated like 'C' types, agglutinating trypsin-treated cow erythrocytes but not rabbit erythrocytes (Jaffé et al. 1974). Purified leucoagglutinin (PHA-L) from Sigma agglutinated trypsin-treated cow erythrocytes strongly and rabbit erythrocytes very weakly. PHA-L agglutinated human leucocytes strongly and had a slight reaction with human erythrocytes (Sigma assay). Thus, PHA-L (Sigma) was analagous to PHA (Wellcome) and leucoagglutinin (Räsänen et al. 1973) in agglutination behaviour, and, by inference, was also analogous to the upper, leucoagglutinin tetramer. PHA-L contained polypeptides 2 and 3 of the T_{G2} pattern. The presence of two polypeptide subunits was inconsistent with the leucoagglutinin tetramer containing four, identical subunits (Yachnin et al. 1972; Yachnin and Svenson 1972; Miller et al. 1973, 1975; Felsted et al. 1975; Leavitt et al. 1977; Manen 1978a, b). Recently, some heterogeneity associated with the leucoagglutinin tetramer has been observed (J.-F. Manen, personal communication) and it is possible that the SDS-PAGE systems used in the above studies were not able to resolve the two subunits reported here.

Comparison of the properties of PHA-E and PHA-L from Sigma revealed that firstly, polypeptide 1, already shown to be responsible for rabbit erythrocyte agglutination, was probably also responsible for human erythrocyte agglutination. Secondly, polypeptides 2 and/or 3, which both agglutinate trypsin-treated cow erythrocytes, also agglutinated human leucocytes. The results obtained in this study for PHA (Wellcome) do not agree with those of Jaffé et al. (1974) and Felsted et al. (1975). This discrepancy appears to be due to the use of a non-*P. vulgaris* lectin source (probably *P. coccineus*) in the preparation of the PHA lot used in this study as compared to the lots used in earlier studies.

Genetic Variation for Qualitative Lectin Content

The report of four cultivar types, A, B, C, and D of Jaffé et al. (1972, 1974) was the first suggestion of genetic variation for the lectins of *P. vulgaris*. Manen (1978a) looked at the lectins of different bean cultivars and other *Phaseolus* species by non-denaturing (Reisfeld 1962) and SDS-polyacrylamide gel electrophoresis, and demonstrated variation both between and within species. A. Pusztai (personal communication) described three lectin types, I, II, and III on the basis of one-dimensional SDS-PAGE patterns of different bean cultivars. The type I cultivars probably corresponded to those with T_{G2} patterns, the type II cultivars to those with S_{G2}, V_{G2}, or Pr_{G2} patterns, and the type III cultivars to the non-agglutinating type 'D' cultivars of Jaffé et al. (1972, 1974) (Pi_{G2}). No type 'C' (P_{G2}) cultivars were screened in this study.

Recently, Felsted et al. (1981) screened 62 cultivars by non-denaturing and SDS-polyacrylamide gel electrophoresis. It should be noted here that with one-dimensional SDS-PAGE separations of total proteins there are a number of polypeptides of the same molecular weight as the lectin polypeptides (Brown et al. 1981b, c) which may confuse the resolution of the lectin patterns. Also, the similarities in the polypeptide composition of the different G2/albumin patterns (Fig. 2) makes it difficult to distinguish the different types on a one-dimensional basis. Felsted et al. (1981) described three cultivar types, I, II, and III. Forty of the forty-three type I cultivars had non-denaturing and SDS-PAGE patterns as described above for T_{G2}-containing lectin sources. Three cultivars had a different non-denaturing pattern although the SDS-PAGE pattern was apparently the same. The non-denaturing gel patterns of these three cultivars appear similar to those of the B_{G2}-containing cultivars (Osborn and Brown, unpublished data). The type I cultivars were apparently analogous to the type 'A' cultivars of Jaffé et al. (1972, 1974) as they are highly mitogenic. All sixteen of the type II cultivars had the same SDS-PAGE patterns. However, two non-denaturing gel patterns were identified, and these differed from those of the type I cultivars. From initial observations these cultivars correspond to S_{G2}- and V_{G2}-containing cultivars (Osborn and Brown, unpublished data), and from their having low or no mitogenicity, to the type B cultivars of Jaffé et al. (1972, 1974). These type II lines also had lower leucoagglutinating activity which would correspond to the lower trypsin-treated cow erythroagglutinating activity of the S_{G2} and V_{G2} types. Only two cultivars were designated as type III cultivars. Felsted et al. (1981) suggested that one or both may be *P. coccineus* lines. This has been confirmed by the SDS-PAGE pattern of the phaseolin of both of these lines being typical of a number of *P. coccineus* lines (Osborn and Brown, unpublished data). None of the cultivars screened by Felsted et al. (1981) were type 'C' (T_{G2}) or type 'D' (Pi_{G2}) cultivars as all the cultivars screened agglutinated rabbit erythrocytes. Thus, although the vast majority of studies on bean lectins have been carried out using lectin sources containing the T_{G2} pattern, it is evident that qualitative genetic variation exists for *P. vulgaris* lectins. Felsted et al. (1981) have shown that some quantitative variation exists for agglutination of rabbit erythrocytes and mitogenicity among bean cultivars having the same lectin composition. It will now be possible to study quantitative lectin variation among bean cultivars using lines of known qualitative lectin composition.

Conclusions

1. The abundance of G2/albumin polypeptides in commercially available phytohaemagglutinin preparations reinforced the earlier correlation of these proteins with lectin proteins (Brown et al. 1981 b). Furthermore, there was an exact correlation between G2/albumin polypeptide composition and agglutination behaviour in individual cultivars and in F_1 progeny (Brown et al. accompanying paper). This correlation and the extraction of lectin polypeptides from agglutinated erythrocytes allowed the identification of lectin polypeptides responsible for agglutinating rabbit and trypsin-treated cow erythrocytes.

2. Qualitative genetic variation for lectin content of different cultivars has been described in the accompanying paper (Brown et al. 1982). The different G2/albumin types were correlated with the A, B, C, and D cultivar types of Jaffé et al. (1972; 1974). In the eight G2/albumin groups described, only six agglutinating polypeptides were identified: 1 and 9 (36,500 daltons), 2, 10, and 12 (35,500 daltons) and 3 (33,500 daltons). Cultivars with the Pi_{G2} pattern (type D) contained no lectin polypeptides which would agglutinate rabbit or trypsin-treated cow erythrocytes. Cultivars with the P_{G2} pattern (type C) contained polypeptides 2 and 12 which agglutinated trypsin-treated cow erythrocytes. The lectin polypeptides of cultivars with the S_{G2}, V_{G2}, or Pr_{G2} patterns (type B) were polypeptides 9 and 10. Both polypeptides appeared to agglutinate rabbit and trypsin-treated cow erythrocytes. Cultivars with the B_{G2} and M_{G2} patterns (type A) contained agglutinating polypeptides 2, 9, 10, and 12 (above), while those with the T_{G2} pattern contained a rabbit erythrocyte specific agglutinating polypeptide (polypeptide 1) and two trypsin-treated cow erythrocyte agglutinating polypeptides (polypeptides 2 and 3).

Jaffé et al. (1972; 1974) made the observation that the properties of type 'A' cultivars were the same as the combined properties of the type 'B' and 'C' cultivars. The active lectin polypeptides of type 'B' cultivars (S_{G2}, V_{G2}, Pr_{G2}) are polypeptides 9 and 10, those of type 'C' cultivars (P_{G2}) are polypeptides 2 and 12, and those of the B_{G2} and M_{G2} type 'A' cultivars are 2, 9, 10, and 12, consistent with the observation of these authors.

The lectin polypeptides 1, 2, 3, 9, 10, and 12 are described with reference to their agglutination of rabbit and trypsin-treated cow erythrocytes. It is possible that other polypeptides in the G2/albumin patterns may agglutinate erythrocytes from other sources, and indeed, the lectins described above may also show such activity (viz. reactivity of polypeptide 1 with human erythrocytes and 2 and/or 3 with human leucocytes). The lack of specificity of polypeptides 9 and 10 compared to the specific nature of polypeptides 1 (rabbit) and 2, 3, and 12 (trypsin-treated cow) underlines the possible variation in blood cell specificity.

3. The variation in the properties of *P. vulgaris* lectins that have been reported previously appears to be due largely due to procedural differences, since many studies have probably used lectin sources with the same lectin-containing G2/albumin group (T_{G2}). It was difficult, in some cases, to relate lectin properties due to the use of non-specified bean cultivars as lectin sources. Further lectin studies should clearly specify their lectin sources and by checking the homogeneity of the seed of a particular cultivar, use pure seed samples.

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