Purification, properties and amino acid sequence of a low- M_r abundant seed protein from pea (*Pisum sativum* L.)

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The seeds of pea (*Pisum sativum* L.) contain several proteins in the albumin solubility fraction that are significant components of total cotyledonary protein (5–10%) and are accumulated in developing seeds concurrently with storage-protein synthesis. One of these proteins, of low M_r and designated 'Psa LA', has been purified, characterized and sequenced. Psa LA has an M_r of 11 000 and contains polypeptides of M_r 6000, suggesting that the protein molecules are dimeric. The amino acid sequence contains 54 residues, with a high content (10/54) of asparagine/aspartate. It has no inhibitory action towards trypsin or chymotrypsin, and is distinct from the inhibitors of those enzymes found in pea seeds, nor does it inhibit hog pancreatic α -amylase. The protein contains no methionine, but significant amounts of cysteine (four residues per polypeptide), suggesting a possible role as a sulphur storage protein. However, its sequence is not homologous with low- M_r (2S) storage proteins from castor bean (*Ricinus communis*) or rape (*Brassica napus*). Psa LA therefore represents a new type of low- M_r seed protein.

Low- M_r proteins (often referred to as '2S proteins') are found as relatively abundant components in the seeds of many plant species, including both dicotyledons and monocotyledons (Derbyshire *et al.*, 1976). Whereas in many legume seeds these proteins have been characterized as proteinase inhibitors, e.g. in soya bean (*Glycine max*) (Kunitz, 1946) and cowpea (*Vigna unguiculata*) (Gatehouse *et al.*, 1980), in other species, e.g. rapeseed (*Brassica napus*) and castor bean (*Ricinus communis*) the low- M_r proteins have no proteinase-inhibitor activity, but seem to function as storage proteins (Lonnerdal & Janson, 1972; Li *et al.*, 1977). A complete amino acid sequence of the castor-bean protein has been published (Sharief & Li, 1982), and partial amino acid sequence data predicted from cloned complementary DNA species is available for the rapeseed proteins (napins) (Crouch *et al.*, 1983).

The proteins accumulated by seeds are of interest not only in their own right as contributing to the nutritional and other properties of seeds, but

Abbreviations used: SDS, sodium dodecyl sulphate; Psa LA, *Pisum sativum* low- M_r albumin protein; dansyl, 5-dimethylaminonaphthalene-1-sulphonyl; h.p.l.c., high-pressure liquid chromatography; Psa MA, *Pisum sativum* major albumin protein.

are also of interest as developmentally regulated gene systems. The major seed storage proteins in pea (legumin, vicilin and convicilin) belong to the globulin solubility fraction and are accumulated in protein bodies in cotyledon cells, forming the main protein storage reserve of the seed. In addition to these, other proteins are also accumulated in seeds, although to a lesser extent, including the seed lectin, and at least two other proteins in the albumin solubility fraction (Murray, 1979; Croy *et al.*, 1984). One of these proteins, subunit M_r 25 000, corresponds to the abundant protein, Psa MA (*Pisum Sativum* Major Albumin; previously called 'PMA'), isolated from the albumin fraction of pea and described in a previous paper (Croy *et al.*, 1984). This protein was a homodimer of either approx. 25 000- M_r subunits or approx. M_r -24 500 subunits, the two forms being similar, but distinct. It appeared to be specific to pea and closely related species, and no functional properties could be assigned to it, nor did it behave as a storage protein on seed germination. During the course of work on Psa MA a second abundant albumin protein in pea was observed, which appeared to be a low- M_r '2S' type, and the present paper reports its purification, characterization and amino acid sequence. The nomenclature of plant seed proteins is at present

unsystematic, but in the hope that such a system might be more widely adopted, we will refer to this protein by a two-part abbreviation, one part for the species (*Pisum sativum*) and one part for the protein type (Low- M_r Albumin), viz. Psa LA, a system of nomenclature similar to that used for bacterial restriction endonucleases.

Materials and methods

Plant material

Seeds of pea (*Pisum sativum* L., var. Feltham First) were obtained from Tyneside Seed Stores, Gateshead, Tyne and Wear, U.K. For protein purification, testas were removed from the seeds and the cotyledons ground in a Janke and Kunkel (Staufen in Breisgau, Germany) water-cooled mill. After passage through a 365 μ m-pore-size sieve, seed meal was defatted with hexane (2 $\times$ 30 min extractions at 10 ml of hexane/g of meal at 4°C), filtered off and air-dried.

Purification of Psa LA

The albumin protein fraction of pea seeds was extracted from 40 g of seed meal by 2 $\times$ 2 h extractions with continuous stirring at 4°C with 20 mM-sodium acetate buffer, pH 5.0, at 5 ml of buffer/g of meal. Residue and supernatant were separated by centrifugation at 10000 g for 30 min at 4°C. The supernatant was fractionated by precipitation with $(\text{NH}_4)_2\text{SO}_4$ at 4°C, the fraction precipitating in the range 50–90% rel. sat. being retained; precipitates and supernatants were separated by centrifugation as described above. The 90%-rel.satn.- $(\text{NH}_4)_2\text{SO}_4$ precipitate was redissolved in 5 ml of 50 mM-Tris/HCl buffer, pH 7.5, containing 0.1 M-NaCl, and chromatographed on a column of Sephadex G-75 (Pharmacia Fine Chemicals) (1.6 cm diam. $\times$ 150 ml volume) at a flow rate of 6 ml/h. Fractions (2 ml) were collected and pooled by peaks of absorbance at 280 nm, dialysed against distilled water, freeze-dried and analysed by SDS/polyacrylamide-gel electrophoresis. Fractions from the peak containing Psa LA were further purified by chromatography on a column of DEAE-cellulose (DE 52; Whatman Biochemicals) (1.6 cm diam., 70 ml volume), equilibrated with 50 mM-Tris/HCl buffer, pH 7.5. The sample was dissolved in DEAE-cellulose column buffer (20 ml), applied to the column, washed on with 1 column volume of starting buffer, and the column was then eluted with a linear gradient (250 ml + 250 ml) of NaCl (0–0.5 M) in the column buffer at a flow rate of 15 ml/h. Fractions (5 ml) were pooled by peaks of absorbance at 280 nm, dialysed against distilled water, freeze-dried and analysed by SDS/polyacrylamide-gel electrophoresis. NaCl concentrations in the

eluted fractions were determined by refractive index.

Characterization of Psa LA

The M_r of Psa LA was determined by gel filtration on a column of Sephadex G-50 (superfine) (Pharmacia) standardized with the following proteins: β -lactoglobulin molecules (M_r 35000), soya-bean trypsin inhibitor (M_r 20100), myoglobin (M_r 17200) and cytochrome *c* (M_r 12700) (all proteins were from Sigma). The column (1.6 cm diam., 115 ml volume) was run in 50 mM-Tris/HCl buffer, pH 7.5, containing 0.1 M-NaCl. The polypeptide M_r of Psa LA was determined by SDS/polyacrylamide-gel electrophoresis in 17% (w/v) polyacrylamide gels by the method of Laemmli (1970), and by SDS/polyacrylamide-gel electrophoresis in urea-containing gels (15% polyacrylamide) using the gel system of Hashimoto *et al.* (1983). The following polypeptides were used as M_r standards; Soya-bean trypsin inhibitor, β -lactoglobulin subunits (18400 M_r), pea lectin (M_r 17700 and 5800 M_r for α - and β -chains), lysozyme (M_r 13200), cytochrome *c* and insulin (M_r 3400, α -chain). Isoelectric focusing of Psa LA was carried out in 7.5% (w/v) polyacrylamide gel slabs as described by Vesterberg (1975). For 'Western' blotting, proteins were transferred to nitrocellulose filters after SDS/polyacrylamide-gel electrophoresis by electroblotting (with a Bio-Rad Laboratories Trans-Blot cell and powerpack according to the manufacturer's protocols). The filters were allowed to react with specific antibodies (raised in rabbits as described previously; Evans *et al.*, 1979), and reacting proteins were detected by incubation with peroxidase-coupled second antibody (swine anti-rabbit; Orion Diagnostica, Helsinki, Finland) and staining with 4-chloronaphth-1-ol as described by Towbin *et al.* (1979).

Analysis of the carbohydrate content of Psa LA was carried out both on the native protein using the phenol/ H_2SO_4 method (Dubois *et al.*, 1956) and after SDS/polyacrylamide-gel electrophoresis by reaction with dansylhydrazine (Eckhardt *et al.*, 1976).

For carboxymethylation, Psa LA was dissolved in 6 M-guanidinium chloride (in 0.5 M-Tris/HCl buffer, pH 8.0, containing 2 mM-EDTA), reduced with dithiothreitol and allowed to react with iodoacetamide as described by Glazer *et al.* (1975). CNBr cleavage of the carboxymethylated protein was carried out as previously described (Croy *et al.*, 1980).

Amino acid composition and sequence of Psa LA

Carboxymethylated Psa LA was acid hydrolysed as described by Croy *et al.* (1980). The resulting amino acid mixture was analysed on a Varian

5060h.p.l.c. system (Varian Associates, Walnut Creek, CA, U.S.A.) using a Micropak amino acid column. Amino acid peaks were detected with a PCR-1 post-column derivatization system using *o*-phthalaldehyde. The analysis system used Pierce Pico-system Buffers (Pierce and Wariner, Chester, Cheshire, U.K.) as described in the Varian (1981) operator's manual. Peptides from Psa LA were hydrolysed and analysed similarly. Cysteine was determined as its carboxymethyl derivative.

The *N*-terminal amino acid of Psa LA was determined by the dansylation method of Gray (1972). The *N*-terminal amino acid sequence of the protein was determined as described for its component peptides, below. The *C*-terminal amino acid of Psa LA was determined by digestion with carboxypeptidase A (Sigma) as described by Narita (1970). Identification of dansylated amino acids was by two-dimensional t.l.c. on polyamide sheets (BDH) (Woods & Wang, 1967).

Peptides from Psa LA were obtained by digestion of 2mg portions of the protein with bovine trypsin (Type XI, Sigma; digestion conditions: enzyme/protein, 1:50, w/w; buffer, 0.1M- NH_4HCO_3 , pH8.0; 18h at 25°C), bovine α -chymotrypsin (Boehringer Mannheim; digestion conditions: enzyme/protein, 1:50, w/w; buffer, 0.2M-*N*-ethylmorpholine/acetate, pH8.5; 2h at 37°C) or *Staphylococcus aureus* V8 proteinase (Miles Laboratories; digestion conditions: enzyme/protein, 1:30, w/w; buffer, 50mM- NH_4HCO_3 , pH8.0; 18h at 37°C). Peptides were freeze-dried and redissolved in 0.1% trifluoroacetic acid; they were subsequently separated by h.p.l.c. on a reverse-phase column (C_{18} , 5 μm ; Micropak MCH-5) as described previously (Gatehouse *et al.*, 1982).

The amino acid sequences of peptides were determined by manual micro-sequence analysis using the diaminoazobenzoyl isothiocyanate/phenyl isothiocyanate double-coupling method of Chang *et al.* (1978).

Determination of Psa LA

The amount of Psa LA in pea seed meal was determined by comparative urea/SDS/polyacrylamide-gel electrophoresis. Weighed amounts of pea seed meal, freeze-dried pea total albumin extract and Psa LA were extracted overnight in gel sample buffer with continuous agitation, and centrifuged to remove any undissolved material. Samples of the supernatants were loaded on to the gel. After electrophoresis, staining with Coomassie Blue G-250 (Sigma) and destaining, tracks on the gel were cut out and scanned at 605nm with a Gilford 2000 spectrophotometer and densitometer attachment. The areas of peak corresponding to Psa LA were estimated by cutting out and weighing the appropriate

areas of the track. A semi-quantitative estimate of Psa LA in seed extracts and albumin proteins was obtained by comparison of peak areas of these tracks with those produced by known amounts of Psa LA.

Enzyme assays

Inhibitory activities against trypsin and chymotrypsin (bovine, both from Sigma) were assayed as described by Erlanger *et al.* (1961). Inhibitory activity against α -amylase (hog pancreas; Sigma) was assayed by radial diffusion assays on starch/agarose plates (Mestecky *et al.*, 1969).

Results and discussion

The flow diagram shown in Scheme 1 outlines the purification of Psa LA which was monitored throughout by SDS/polyacrylamide-gel electrophoresis analysis of protein fractions. An albumin extract of pea seed meal was initially fractionated by precipitation with $(\text{NH}_4)_2\text{SO}_4$. The 50–90%-rel.satn.- $(\text{NH}_4)_2\text{SO}_4$ precipitate containing essentially all the Psa LA protein was fractionated by gel filtration on Sephadex G-75 (Fig. 1); Psa LA was eluted in the third peak and its trailing shoulder.

Fig. 1. U.v.-absorbance (280nm) elution profile of albumin proteins from Sephadex G-75 column

The cross-hatched area was pooled for further purification. SDS/polyacrylamide-gel electrophoresis analysis of numbered protein peaks is shown under the elution profile (reducing conditions; approx. 20 μg of protein loaded per track).

Scheme 1. Flow diagram for Psa LA purification

These fractions were pooled and chromatographed on a DEAE-cellulose ion-exchange column; Psa LA was eluted as a single peak at a salt concentration of 0.16–0.18M (Fig. 2), the last major peak to be eluted from the column. Pooled peak fractions contained a single protein band on SDS/polyacrylamide-gel electrophoresis (Fig. 2), and all of this band was found essentially in this peak, although other peaks contained less abundant polypeptides of similar, but not identical, low M_r values.

Properties of Psa LA

M_r . The M_r of Psa LA was determined by gel filtration on a column of Sephadex G-50 (superfine grade) calibrated with standard proteins. A graph of V_e/V_0 (elution volume/void volume) against $\log M_r$ was plotted for standard proteins and used to predict Psa LA M_r . A value of 11 000 (± 1000) was estimated by extrapolation. Psa LA was eluted as a peak with a small trailing shoulder from the gel-filtration column; this suggested an interaction with the column, or possibly a small amount of contaminating molecular species of lower M_r .

The polypeptide M_r of Psa LA could not be estimated accurately from conventional SDS/polyacrylamide-gel electrophoresis as it electrophoresed as a band of $M_r < 10000$ (Fig. 2), where

Fig. 2. U.v.-absorbance (280nm) elution profile of pool from Fig. 1 from DEAE-cellulose column. Hatched area indicates fractions pooled as Psa LA. SDS/polyacrylamide-gel electrophoresis analysis of numbered protein peaks is shown under elution profile (conditions and loading as for Fig. 1); track T indicates material loaded on to column.

the gel system does not resolve polypeptides well. The M_r was thus determined by gel electrophoresis on urea-containing gels (urea/SDS/polyacrylamide-gel electrophoresis).

Psa LA as isolated ran as a series of four bands in this system even in the presence of 2-mercaptoethanol, but after carboxymethylation the protein gave a single band (Fig. 3), suggesting that the multiple bands were artefacts due to thiol-group interactions. Carboxymethylated Psa LA was thus compared with a series of carboxymethylated standard proteins; a graph of migration distance against $\log M_r$ predicted a value of 6000 (± 300) for this polypeptide. Psa LA thus seems likely to have a predominant molecular form containing two identical subunits.

Isoelectric focusing. Psa LA gave several closely spaced bands in the pI range 5.3–5.4 when subjected to isoelectric focusing under non-denaturing conditions, indicating limited heterogeneity.

Carbohydrate content. Psa 2S contains insignificant amounts of carbohydrate (less than one sugar residue per 30 000- M_r polypeptide) when assayed by the phenol/ H_2SO_4 method, and gave a negative reaction when stained for carbohydrate after SDS/polyacrylamide-gel electrophoresis with dansylhydrazine under conditions where known

Fig. 3. Urea/SDS/polyacrylamide-gel electrophoresis analysis of carboxymethylated standard proteins and Psa LA for M_r determination

Track 1, soya-bean trypsin inhibitor; track 2, β -lactoglobulin; track 3, pea lectin; track 4, lysozyme; track 5, cytochrome *c*; track 6, insulin; track 7, Psa LA.

glycoproteins stained strongly. The protein is therefore not a glycoprotein.

Antigenic properties. Psa LA did not react with antibodies raised against pea seed storage proteins, major albumin (Psa MA) or lectin either by double-diffusion tests or after electrophoretic transfer from SDS/polyacrylamide-gel electrophoresis ('Western' blotting).

Reaction with CNBr. No change in mobility of Psa LA on urea/SDS/polyacrylamide-gel electrophoresis was observed after reaction with CNBr for up to 48 h. This suggests that the protein contains no internal methionine residues.

Amino acid sequence of Psa LA

***N*- and *C*-Terminal amino acids.** The *N*-terminal amino acid of carboxymethylated Psa LA was shown by the dansylation method to be aspartic acid. No other amino acids were observed, suggesting the sample was homogeneous. The *C*-terminal amino acid determination on Psa LA with carboxypeptidase gave low yields of amino acids; however aspartic acid, asparagine and phenylalanine were released in significant amounts.

***N*-Terminal sequence.** Carboxymethylated Psa LA was directly sequenced by manual micro-sequence analysis and an *N*-terminal sequence of 12 amino acids was determined (Fig. 4).

Production, separation and sequencing of peptides. Carboxymethylated Psa LA was separately digested with trypsin, chymotrypsin and *Staphylococcus aureus* V8 proteinase and the resulting peptides were separated and sequenced. Complete sets of overlapping peptides were obtained that enabled

the sequence of Psa LA to be deduced as shown in Fig. 4. Amino acid compositions of all peptides were determined semi-quantitatively as a check for the sequence assignments, and quantitative amino acid compositions were made on the *C*-terminal peptides and other peptides as necessary to confirm the sequences (results not presented). The proteinases all showed their expected specificities in cleaving the Psa LA polypeptide. The deduced sequence is 54 amino acids in length, in agreement with the M_r deduced by urea/SDS/polyacrylamide-gel electrophoresis. It contains two positions of heterogeneity: at amino acid 45 both phenylalanine and valine were found in peptides apparently not separable by h.p.l.c. Valine was a comparatively minor amino acid at this position (about 25% that of phenylalanine), but there does appear to be real heterogeneity at this position. Heterogeneity was also observed at the *C*-terminal amino acid, where separate peptides containing aspartic acid or asparagine as *C*-terminal amino acids were isolated in approximately equal proportion for all three enzyme digests. This heterogeneity is in agreement with the direct *C*-terminal determination on the protein, apart from the presence of phenylalanine in the latter, which was thus considered to be an artefact. The *C*-terminal heterogeneity may be due to post-translational deamidation of an asparagine residue, or may be encoded. These results may account for the heterogeneity of Psa LA seen on isoelectric focusing. The amino acid composition determined for Psa LA (Table 1) was in reasonable agreement with that predicted by the deduced sequence.

Secondary structure and hydrophilicity profile. The amino acid sequence determined for Psa LA was used to produce a hydrophilicity profile for the protein, using the procedure of Hopp & Woods (1981), and to produce a secondary-structure prediction using the computer program of Garnier *et al.* (1978). The results are shown in Fig. 5. Psa LA has hydrophilic regions near the *N*- and *C*-termini, and a hydrophobic region around the central point of the polypeptide; corresponding to this, the *N*- and *C*-terminal regions are high in α -helix content, whereas the central region has a high content of β -turns. There is no β -sheet conformation predicted for any region in the protein, but it seems likely that the central region of the polypeptide could form a β -sheet structure rather than continuous β -turns. The presence of four cysteine residues in the Psa LA polypeptide chain introduces a complication in that the nature and location of disulphide cross-links was not determined, and this may dictate the folding of the polypeptide chain into β -turns rather than a β -sheet structure. The *N*-terminal α -helix region has two cysteine residues in it, which could provide a

Fig. 4. Amino acid sequence of Psa LA

Continuous lines indicate expected peptides, short arrows indicate determined residues, and dotted arrows indicate residues deduced from amino acid compositions.

means of disulphide-linking the subunits of the dimeric molecules together, although the results of gel electrophoresis suggested that the subunits were not covalently linked.

Determination of Psa LA and functional properties

Semi-quantitative determination of Psa LA in seed meal. By comparison of the intensities of bands on a urea/SDS/polyacrylamide-gel electrophoresis gel

by densitometric scanning, known amounts of Psa LA were used to give a calibration curve from which Psa LA in seed-meal extracts run on the same gel could be estimated. The values obtained are only semi-quantitative, owing to the presence of other, minor bands in this region in the seed extracts. Psa LA was estimated as being present at a level of about 1.0% of total seed meal. This level is approximately half the content of the pea major

albumin protein, Psa MA (Croy *et al.*, 1984), but nevertheless suggests that Psa LA is a significant component (approx. 5%) of total cotyledonary protein.

Functional properties. Although Psa LA showed no significant inhibitory activity when tested against bovine trypsin or chymotrypsin, or hog pancreatic α -amylase, pea is known to contain proteinase enzyme inhibitors of low *M_r* (Tomé *et*

Table 1. Amino acid composition of Psa 2S compared with predicted composition from the amino acid sequence
Abbreviation used: CM-Cys, carboxymethyl-cysteine.

Amino acid	Residues/polypeptide of 54 amino acids	
	Found	Predicted
CM-Cys	3.2	4
Asp	8.8	10
Thr*	0.8	1
Ser*	5.4	5
Glu	5.7	5
Gly	4.5	4
Ala	3.9	3
Val	0.3	0
Met	0.0	0
Ile	2.0	2
Leu	1.3	1
Tyr	2.1	2
Phe	3.5	4
Lys	5.0	5
His	2.0	2
Arg	1.4	1
Pro	ND	4
Trp	ND	1

* Corrected for decomposition.

al., 1981). A total albumin extract of pea seeds was therefore chromatographed on DEAE-cellulose to estimate the distribution of proteinase inhibitors in the eluted fractions relative to Psa LA. Eluted fractions were assayed for inhibitory activity against trypsin and chymotrypsin, and were assayed for protein composition by urea/SDS/polyacrylamide-gel electrophoresis. The results are summarized in Fig. 6. Three major peaks of proteinase-inhibitor activity were eluted from the ion-exchange column, the last one of which eluted immediately before the Psa LA peak, and contained inhibitory activity against both trypsin and chymotrypsin. However, the Psa LA peak was clearly separated from the inhibitors, and thus this protein does not appear to be a proteinase inhibitor of the type normally found in legume seeds. Fractions containing high proteinase-inhibitor activities contained polypeptides of low *M_r*, similar to Psa LA. These polypeptides were observed in the earlier purification and are tentatively now identified as pea seed proteinase inhibitors.

The role of Psa LA

The presence of a low-*M_r* relatively abundant protein in pea seeds is not surprising, given that other legume seeds contain significant amounts of low-*M_r* proteins (Derbyshire *et al.*, 1976). However, Psa LA differs from other apparently similar legume seed proteins in that it is not an inhibitor of trypsin or chymotrypsin. Psa LA has no significant sequence homology with either the Kunitz soybean trypsin inhibitor or the Bowman Birk-type trypsin inhibitors, or to other proteinase inhibitors, when tested by a diagonal dot-matrix comparison test (Staden, 1982). If Psa LA is not a proteinase-

Fig. 5. Hydrophilicity profile and secondary-structure prediction for Psa LA
Predicted conformations: α , α -helix; β , β -turn; ---, coil (irregular).

Fig. 6. U.v. absorbance (280nm) elution profile of total pea albumin proteins from a DEAE-cellulose column. Bar and cross-hatching indicate fractions containing Psa LA. The inhibitory activity of a portion of each fraction (50 μ l) against trypsin (5 μ g; $\blacktriangle$) and chymotrypsin (50 μ g; $\bullet$) is also shown.

inhibitor type of protein, it may be homologous with other low M_r storage proteins found in seeds of other genera; however, no homology with the 2S storage protein of *Ricinus communis* could be detected by the above method. The Psa LA protein therefore appears to represent a new sequence family.

The role of Psa LA is not as yet clear. Like Psa MA, the other major albumin seed protein, it lacks detectable functional properties. It is a relatively sulphur-rich protein (four sulphur atoms/54 amino acid residues, c.f. legumin, which has nine sulphur atoms/525 amino acid residues; Lycett *et al.*, 1984); it may therefore act as a sulphur storage protein in a similar way to the role proposed for trypsin inhibitors (Pusztai, 1967). Psa LA is synthesized during seed development over a similar period to that of the synthesis of other storage proteins (results not shown).

Psa LA appears to be identical with a protein described as '13000 M_r ' by P. M. Chandler (personal communication) and also described by Schroeder (1984). The former authors describe the accumulation and decline of the mRNA for this polypeptide during seed development, which occurs in a manner very similar to that shown by storage-protein mRNA species. Schroeder (1984) lends support to the hypothesis that Psa LA functions as a sulphur storage protein, since its synthesis is markedly depressed in a low-sulphur environment. Careful analysis of the behaviour of this protein on seed germination will be required to confirm or deny this hypothesis, since preliminary data (not presented) suggest that the protein is not rapidly degraded on seed germination, and persists

in the cotyledons after the major storage proteins have been degraded. Psa LA may play a role as an anti-metabolite for seed-attacking pests, like some legume trypsin inhibitors (Gatehouse *et al.*, 1979), but its lack of functional properties does not fit in with this hypothesis. Taken together with the failure to find any role for Psa MA (Croy *et al.*, 1984), the present results suggest the conclusion that pea seeds have a synthetic capacity for proteins in excess of their requirements. In the case of the seed lectin and Psa MA, these proteins may well be redundant, since pea lines where the seeds contain no detectable level of one or other are known (Guldager, 1978; J. A. Gatehouse, J. Gilroy, M. S. Hoque & R. R. D. Croy, unpublished work), but Psa LA may possibly have a vital role, since a survey of over 200 pea lines undertaken in conjunction with this work did not reveal any line lacking in this protein (results not shown).

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