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The purification and characterization of a third storage protein (convicilin) from the seeds of pea (*Pisum sativum* L.)

Ronald R. D. CROY, John A. GATEHOUSE, Mariete TYLER and Donald BOULTER
Department of Botany, University of Durham, South Road, Durham DH1 3LE, U.K.

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A third storage protein, distinct from legumin and vicilin, has been purified from the seeds of pea (*Pisum sativum* L.). This protein has been named 'convicilin' and is present in protein bodies isolated from pea seeds. Convicilin has a subunit mol.wt. of 71000 and a mol.wt. in its native form of 290000. Convicilin is antigenically dissimilar to legumin, but gives a reaction of identity with vicilin when tested against antibodies raised against both proteins. However, convicilin contains no vicilin subunits and may be clearly separated from vicilin by non-dissociating techniques. Unlike vicilin, convicilin does not interact with concanavalin A, and contains insignificant amounts of carbohydrates. Limited heterogeneity, as shown by isoelectric focusing, *N*-terminal analysis, and CNBr cleavage, is present in convicilin isolated from a single pea variety; genetic variation of the protein between pea lines has also been observed.

The storage proteins of the seeds of pea (*Pisum sativum* L.) have been divided into two fractions on the basis of solubility (Osborne & Campbell, 1898). Although both fractions have been shown to be heterogeneous (Derbyshire *et al.*, 1976), the legumin fraction contains a major component, legumin, which has been purified by several workers and has been shown to be an essentially homogeneous protein that displays subunit heterogeneity (Krishna *et al.*, 1979). The other fraction, the vicilin fraction, is not so simple in its composition, and a recent review (Derbyshire *et al.*, 1976) has presented some evidence that it contains more than one major protein. Millerd (1975) has claimed that a third storage protein is also present in peas, although it is as yet uncharacterized.

In the course of investigations into the synthesis of pea seed storage proteins, it became necessary to define the protein species present more closely than in previous work. To this end the present paper describes the purification and characterization of a homogeneous protein from pea seeds that is considered to be a third storage protein. In view of the lack of a systematic nomenclature for seed storage proteins, and for reasons to be described, it has been named 'convicilin'.

Experimental

Materials

Pea (*Pisum sativum* L.) seeds, variety Feltham First, were obtained from Suttons Seeds Ltd., Torquay, Devon TQ2 7QJ, U.K. Other genetic lines of peas were kindly given by Dr. S. Blixt, Weibullsholm, Sweden. Air-dried mature pea seeds were used. Ultrogel AcA 22 was obtained from LKB Instruments Ltd., South Croydon, Surrey CR2 8YD, U.K.; hydroxyapatite (Bio-Gel HT grade) was from Bio-Rad Laboratories Ltd., Bromley, Kent, U.K. Sephadex G-200 and G-75 (Superfine grade), Blue Dextran and Pharmalyte Ampholines (pH ranges 3.5–10 and 4–6) were purchased from Pharmacia (G.B.) Ltd., London W5 5SS, U.K. The marker proteins used were from Sigma (London) Chemical Co., Poole, Dorset BH17 7NH, U.K., and were the purest grade available. All other chemicals were obtained from BDH Chemicals, Poole, Dorset BH12 4NN, U.K., and were of AnalaR grade, or the best available.

Abbreviations used: SDS, sodium dodecyl sulphate.

Methods

Buffers. Buffers used in the present work contained 0.05% sodium azide unless otherwise stated. Phosphate buffers were sterile-filtered through a 0.22 μM-pore-size cellulose acetate (Oxoid) membrane filter before use and their concentration determined by measurement of their refractive index at 20°C.

Purification of convicilin (see Fig. 1 below). Seeds were dried under vacuum for 1 h before being milled for 30 s in a ball mill. The flour was sieved through a 365 μm mesh and material not passing through was discarded. The meal was extracted twice with hexane

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at 0°C for 30min (10ml of hexane/g of meal) and each time the hexane was decanted after centrifugation. The pea meal was then dried under vacuum. A portion (40g) of defatted meal was extracted with 500ml of 0.1M-potassium phosphate buffer, pH 8.0, containing 0.4M-NaCl at 4°C for 1h, centrifuged at 23000*g* for 20min and the precipitate discarded. The protein solution was subsequently fractionated by $(NH_4)_2SO_4$ precipitation at 4°C and the material precipitating in the range 50–80% relative saturation was collected by centrifugation at 23000*g* for 20min and retained. This material was redissolved in 20ml of 50mM-sodium borate buffer, pH 8.0, and loaded on to a column (5.5cm diam., 950ml vol.) of Sephadex G-200, equilibrated, and eluted with 0.1M-potassium phosphate buffer, pH 8.0, at a flow rate of 50ml/h; 10ml fractions were collected. Fractions containing convicilin were pooled and applied directly to a column (3.5cm diam., 140ml vol.) of hydroxyapatite that had been equilibrated with 0.1M-potassium phosphate buffer, pH 8.0. The hydroxyapatite column was washed, then eluted with a linear concentration gradient of potassium phosphate buffer, pH 8.0 (0.1M–0.75M, 300ml + 300ml) at a flow rate of 40ml/h, 10ml fractions being collected. Fractions containing convicilin were pooled and either concentrated by ultrafiltration or dialysed against 50mM-sodium borate buffer, pH 8.0, or distilled water, and then freeze-dried.

Gel electrophoresis. Protein samples were analysed by SDS/polyacrylamide-gel electrophoresis in 17% (w/v) polyacrylamide slab gels with the system of Laemmli (1970). Subunit molecular weights were determined in 17% polyacrylamide and 10% polyacrylamide gel slabs, the following standard proteins being run on the same slabs: phosphorylase *b* (mol.wt. 100000), transferrin (76600), bovine serum albumin (68000), catalase (60000), ovalbumin (43000), carboxypeptidase A (34300), soya-bean trypsin inhibitor (21000), cytochrome *c* (12700).

Amino acid analysis. Amino acid analysis of proteins was performed by the methods of Moore & Stein (1963) on a Locarte single-column amino acid analyser after hydrolysis of the protein for various times in 6M-HCl at 105°C *in vacuo*. The sulphur amino acids methionine (as sulphone) and cysteine (as cysteic acid) were determined by analysis of performic acid-oxidized protein (Hirs, 1956).

Determination of N-terminal amino acids. The *N*-terminal amino acids of convicilin were determined by the SDS/dansyl technique of Gray (1972). The *N*-terminal dansyl-amino acid was identified by two-dimensional chromatography on polyamide thin-layer sheets (Woods & Wang, 1967).

Sugar analysis. Sugars present in a sample of convicilin that had been precipitated from solution by adding trichloroacetic acid to a final concentration of 10% (w/v) and washed extensively with 10% trichloroacetic acid were determined after hydrolysis and gas-liquid chromatography of their derivatives (Sweeley *et al.*, 1966).

Cleavage with CNBr. Convicilin dissolved in 70% (v/v) formic acid was made to react with a 100-fold molar excess of CNBr (2g/ml solution in acetonitrile) for various times (Kasper, 1970). The solutions were evaporated to dryness under vacuum and the residues were analysed by SDS/polyacrylamide-gel electrophoresis.

Molecular-weight determination. A column (2.2cm diam., 320ml vol.) of Ultrogel AcA 22 was equilibrated by upward flow (flow rate 8.0ml/h) with 0.1M-Tris/HCl buffer, pH 8.0, containing 0.2M-NaCl. The following standard proteins were chromatographed on the column and their elution volumes measured: thyroglobulin (669000mol.wt.), ferritin (440000), *Pisum* legumin (400000), catalase (240000), immunoglobulin G (160000), bovine serum albumin (68000) and myoglobin (18000). The molecular weight of convicilin was determined by comparing its elution volume with the standard proteins on a plot of V_e/V_0 against log(mol.wt.). A total protein extract (prepared as in Fig. 1) of pea seeds was chromatographed under the same conditions; 5ml fractions were collected. The fractions were made 10% with respect to trichloroacetic acid and the precipitated protein was analysed by SDS/polyacrylamide gel electrophoresis followed by staining with Coomassie Blue and densitometric scanning of the stained gel at 580nm.

Immunodiffusion. Immunodiffusion of convicilin and vicilin against antibodies raised against protein fractions and purified by $(NH_4)_2SO_4$ precipitation and chromatography on QAE (quaternary aminoethyl)-Sephadex (Livingstone, 1974; Joustra & Lundgren, 1969) was carried out by standard methods (Ouchterlony & Nilsson, 1978). Precipitin arcs formed after 48h diffusion were cut out of the gel, washed thoroughly in 50mM-sodium borate buffer, pH 8.0, containing 0.15M-NaCl to remove soluble protein, and analysed by SDS/polyacrylamide-gel electrophoresis. Samples were dissolved in SDS sample buffer containing no 2-mercaptoethanol in order to prevent immunoglobulin G heavy and light subunits interfering with the band patterns of convicilin or vicilin.

Reaction with concanavalin A. The reaction of convicilin or vicilin with concanavalin A was tested by a diffusion system analogous to that used for immunodiffusion, with concanavalin A replacing the antibodies. Positive controls of known glycoproteins and negative controls in the presence of 0.1M-α-methyl mannoside were carried out.

Isoelectric focusing. Convicilin was subjected to isoelectric focusing in polyacrylamide-gel slabs containing 8M-urea in the pH ranges 3.5–10 and

4–6 by the methods of Vesterberg (1975). The pH gradient in the slab was determined by standard methods (Vesterberg, 1975).

Protein-body separation. Protein bodies were prepared by a modification of the procedures described by Croy (1977). All operations were carried out at 4°C. Cotyledons (5g) from mature pea seeds before commencement of drying out were homogenized by gentle chopping in a domestic food mixer (slow speed, 60s) in 50ml of 50mM-sodium phosphate buffer, pH 7.5, containing 0.5 M-sucrose. The homogenate was centrifuged for 5min at 300*g* and the pellet discarded, and then centrifuged for 10min at 10000*g* and the supernatant discarded. The crude protein-body pellet was resuspended in 1ml of buffer as described above and purified by centrifugation through a step gradient prepared by layering 5ml of 90% (w/v) sucrose, 8ml of 70% sucrose and 8ml of 30% sucrose in buffer. The protein body sample was layered on to the gradient and centrifuged at 60000*g* for 2h. Protein bodies were removed from the 70/90% sucrose interface and subsequently analysed by SDS/polyacrylamide-gel electrophoresis.

Biosynthesis in vivo and in vitro during seed development. Seed materials have been described previously (Evans *et al.*, 1979) and methods in Croy *et al.* (1980).

Results

Purification of convicilin

Convicilin was purified from pea meal as shown in the flow diagram (Fig. 1). After extraction and $(NH_4)_2SO_4$ fractionation, the appropriate precipitate was redissolved and passed through a column of Sephadex G-200. The elution profile is shown in Fig. 2(*a*). When fractions from the large asymmetrical peak of protein were analysed by SDS/polyacrylamide-gel electrophoresis, those from the leading edge of the peak were found to contain exclusively legumin subunits (40000 and 20000mol.wt.) (Wright & Boulter, 1974), those from the middle of the peak were found to contain 71000-mol.wt. subunits in addition, whereas those from the trailing edge of the peak had a typical vicilin pattern (50000, 33000, 17000 and lower-molecular-weight subunits; Croy *et al.*, 1980). The fractions containing a high proportion of 71000-mol.wt. subunits were pooled and further purified by chromatography on hydroxyapatite. Protein was applied to the column equilibrated with 0.1 M-potassium phosphate buffer, pH 8.0, and eluted by linear concentration gradient of this salt. The elution profile is shown in Fig. 2(*b*). The initial large peak of protein eluted at 0.25 M-potassium phosphate was shown to be legumin by SDS/polyacrylamide-gel electrophoresis. The leading edge of the second peak (at 0.28–0.35 M-potassium phosphate) was convicilin, containing 71000-mol.wt. subunits with only traces of lower-molecular-weight subunits; subsequent fractions were contaminated with vicilin (shown in separate experiments to elute at 0.5 M-potassium phosphate). Analysis of the purified convicilin by SDS/polyacrylamide-gel electrophoresis is shown in Fig. 3; the presence or absence of 2-mercaptoethanol made no difference to the observed band pattern. Densitometric scanning of the stained gel showed that the convicilin was at least 90% pure.

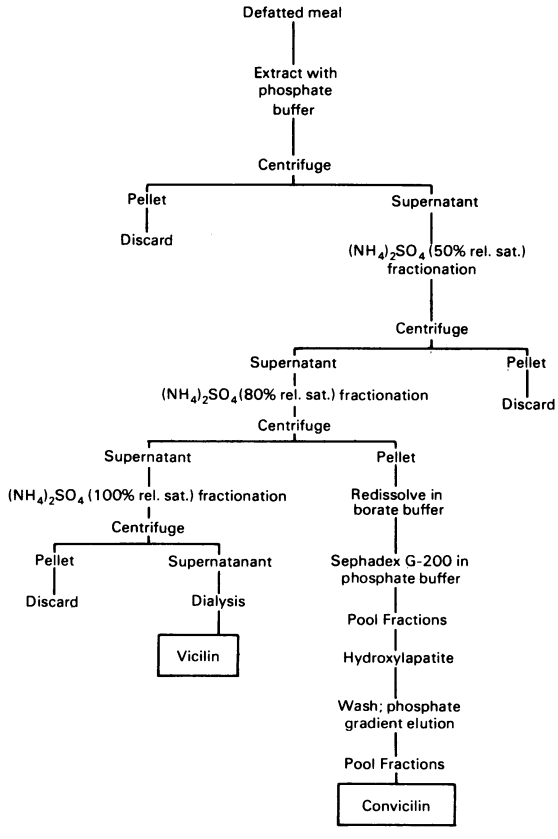

Fig. 1. *Flow diagram for the purification of convicilin*
Abbreviation used: rel. sat., relative saturation.

Properties of convicilin

Amino acid composition. The amino acid composition of convicilin is given in Table 1, and compared with vicilin and legumin from *Pisum sativum*. The amounts of methionine and lysine in convicilin correspond to 0.9 residue per 71000-mol.wt. subunit for both.

N-Terminals. The *N*-terminal amino acids of convicilin, determined by using the dansyl/SDS technique (Gray, 1972) were aspartate and leucine in comparable amounts.

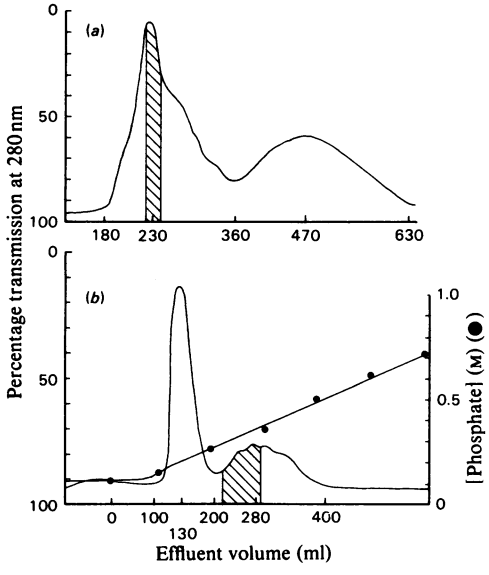

Fig. 2. *Purification of convicilin*
(*a*) Elution profile from chromatography on Sephadex G-200. (*b*) Phosphate-gradient-elution profile from chromatography on hydroxyapatite. Hatched areas show the fractions containing convicilin.

Table 1. *Amino acid composition of convicilin and vicilin*
Values are means of three or more separate determinations. The S.E.M. was less than 5% in all cases.

	Composition (residues/100 residues)		
Amino acid	Convicilin	Vicilin	Legumin‡
Asp	11.64	18.87	13.07
Thr	2.55*	2.69*	3.08
Ser	6.39*	7.67*	5.77
Glu	22.08	19.18	19.74
Pro	5.47	1.99	5.50
Gly	5.90	4.85	6.89
Ala	4.23	4.13	6.01
Val	4.46	4.84	4.97
Met	0.13†	0.00†	0.66
Ile	3.85	5.11	3.95
Leu	8.71	9.83	7.60
Tyr	2.59	1.91	3.64
Phe	3.30	4.67	2.30
His	2.22	1.61	1.75
Lys	8.18	8.10	4.24
Arg	8.15	4.57	9.99
Cys	0.13†	0.00†	1.21

* Corrected for decomposition from 22, 48 and 72h hydrolyses.
† From analysis of performic acid-oxidized protein.
‡ From Casey (1979).

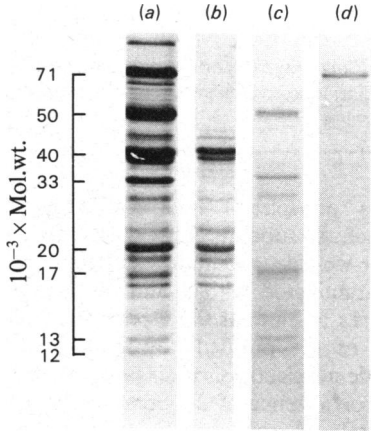

Fig. 3. *Analysis by SDS/polyacrylamide-gel electrophoresis of various protein fractions and convicilin*
(*a*) Total soluble protein fraction (see Fig. 1); (*b*) purified legumin; (*c*) purified vicilin; (*d*) purified convicilin.

Absorbance. The $A^{1\%}_{280,1\,cm}$ for convicilin was 7.3.

Sugar analysis. Small amounts of glucose, galactose and mannose were detected in convicilin, but the amounts were comparable with those found in a known non-glycoprotein (bovine serum albumin) and were significantly less than one sugar residue per 71000-mol.wt. subunit; it was therefore concluded that convicilin contains no significant amounts of carbohydrate.

Cleavage with CNBr. Two major fragments were produced on CNBr cleavage; they had approx. mol.wts. 55000 and 14000 (see Fig. 4). This indicates one methionine residue per subunit, in agreement with the amino acid analysis. However, some material remained undigested, even after 48h reaction, and there were a number of further minor cleavage products.

Subunit molecular weight. Molecular weights of 72000±3000 in 17% acrylamide gels, and 70000±2500 in 10% acrylamide gels, were determined for the convicilin subunit(s) on SDS/polyacrylamide-gel electrophoresis by comparison with standard proteins. A mean value of 71000 was adopted.

Molecular weight. Purified convicilin was eluted as a single peak after gel-filtration chromatography (see Fig. 6*b* below) A mol.wt. of 290000±40000 was determined for convicilin by comparison with standard proteins, suggesting a tetrameric molecule consisting of four 71000-mol.wt. subunits.

Serological properties. When convicilin and vicilin (free of cross-contaminating subunits) were allowed to diffuse against an antiserum prepared against an extract containing both in an Ouch-

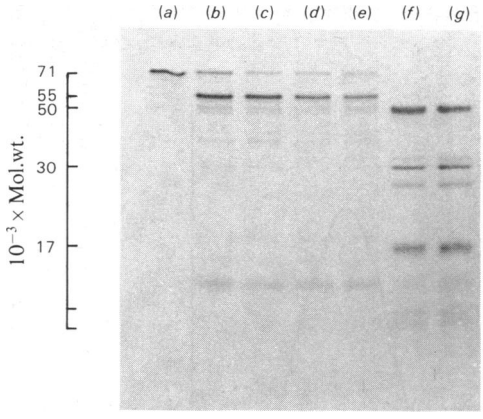

Fig. 4. *Analysis by SDS/polyacrylamide-gel electrophoresis of convicilin and vicilin before and after reaction with CNBr*
(*a–e*) Convicilin before (*a*) and after 4h (*b*), 8h (*c*), 24h (*d*), 48h (*e*) reaction. (*f* and *g*) Vicilin before (*f*) and after 48h (*g*) reaction.

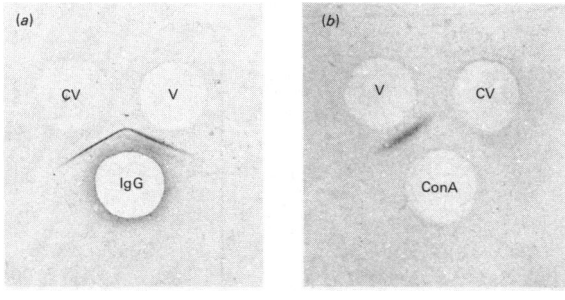

Fig. 5. *Cross-reactivity of convicilin and vicilin*
(*a*) Comparison of convicilin (CV) and vicilin (V) by double immunodiffusion against antibodies (immunoglobulin G) raised against a protein fraction containing both proteins. (*b*) Double diffusion of convicilin and vicilin against concanavalin A (Con A).

terlony double-immunodiffusion test, the two proteins gave a reaction of identity (Fig. 5*a*). The precipitin arcs produced were excised and analysed by SDS/polyacrylamide gel electrophoresis. The immunoprecipitate produced by the reaction of convicilin with the above antibodies showed only 71000 subunits, with no trace of vicilin subunits and vice versa. Convicilin gave a reaction of non-identity with legumin when tested against antiserum prepared against total pea proteins.

Reaction with concanavalin A. Concanavalin A gave no reaction with convicilin in a double-immunodiffusion experiment (Fig. 5*b*) when vicilin, known to react with concanavalin A, was included as a positive control.

Isoelectric-focusing properties. Convicilin was subjected to isoelectric focusing in a polyacrylamide-gel slab containing 8M-urea. The protein focused to a series of closely spaced slightly blurred bands, indicating limited heterogeneity, in the pI range 5.6–5.8.

Evidence that convicilin is a separate storage protein

When a total protein extract or globulin protein fraction from peas, prepared under non-dissociating conditions, was subjected to zonal isoelectric precipitation on Sephadex G-75 (Wright & Boulter, 1974), convicilin was found associated with the legumin (retarded) fraction if the ionic strength was approx. 0.15, or with the vicilin (unretarded) fraction if the ionic strength was approx. 0.25 or higher. Convicilin was also eluted from a zonal-isoelectric-precipitation column separately from vicilin and legumin by a linear salt gradient at pH5.0 (R. R. D. Croy, unpublished work). In addition, the purification procedure above employs only non-dissociating conditions throughout and as such successfully separates convicilin completely from vicilin and legumin. These results strongly support the existence of a separate and distinct protein containing only 71000-mol.wt. subunits.

Since the existence of a separate convicilin protein when purified may be argued as being due to selection of molecules containing only 71000-mol.wt. subunits from a population of molecules containing 71000-mol.wt. subunits in association with others during purification, a further experiment was carried out. A total protein extract from peas was fractionated by gel filtration on Ultrogel AcA 22 under non-dissociating conditions. Fig. 6 shows the distribution of the proteins in the elution profile from the gel-filtration column deduced from the SDS/polyacrylamide-gel-electrophoresis subunit patterns of each fraction.

The results show discrete but overlapping peaks of legumin, convicilin and vicilin in order of decreasing molecular weight. All three proteins eluted as symmetrical peaks without undue spreading; the centres of the peaks were at estimated mol.wt. values (±10%) of 380000, 280000 and 160000 respectively, indicating that convicilin in the extract has the same molecular weight as purified convicilin. The final large peak eluted from the Ultrogel column (Fig. 6*a*) represents the lower-molecular-weight proteins, including the lectin and major albumin as shown in gel 94 (Fig. 6*a*). (R. R. D. Croy, unpublished work).

Further evidence that the 71000-mol.wt. subunits

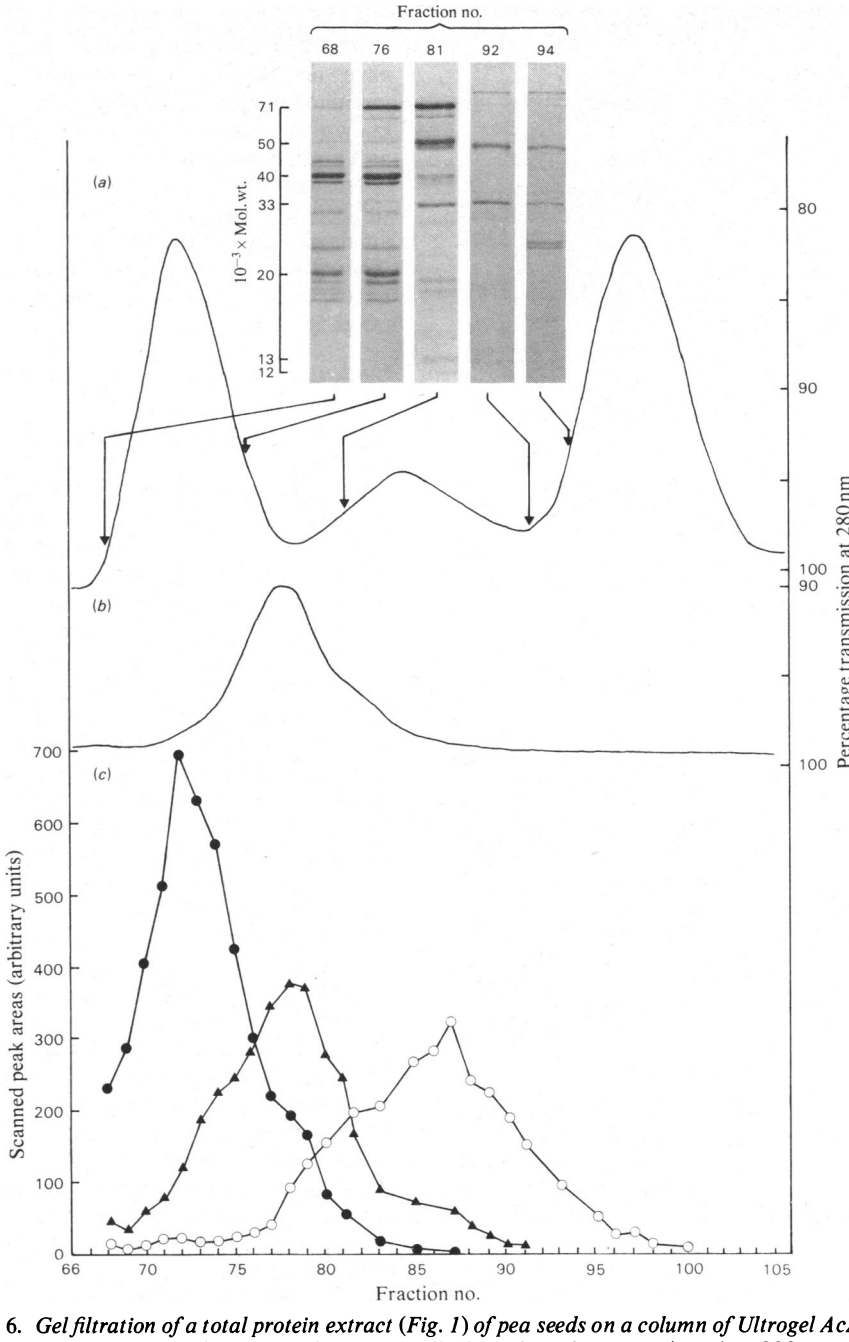

Fig. 6. *Gel filtration of a total protein extract (Fig. 1) of pea seeds on a column of Ultrogel AcA 22*
(*a*) Elution profile of a total protein extract chromatographed on the column monitored at 280nm; (*b*) the elution profile of purified convicilin chromatographed on the same column monitored at 280nm; (*c*) distribution of legumin (40000-mol.wt. subunits) (●), vicilin (50000-mol.wt. subunits) (○) and convicilin (71000-mol.wt. subunits) (▲) relative to the elution profile in (*a*), as determined from densitometric scans of SDS/polyacrylamide-gel-electrophoretic analyses of each fraction from the column. Legumin values have been plotted on half the indicated scale for clarity. Electrophoretic analyses of representative fractions are shown in (*a*).

belong to a protein distinct from vicilin (50000-mol.wt. subunits) follows from experiments in which the biosynthesis of the storage proteins has been monitored *in vivo* and *in vitro* during seed development. At an early stage in the laying down of the storage proteins during seed development, 50000-

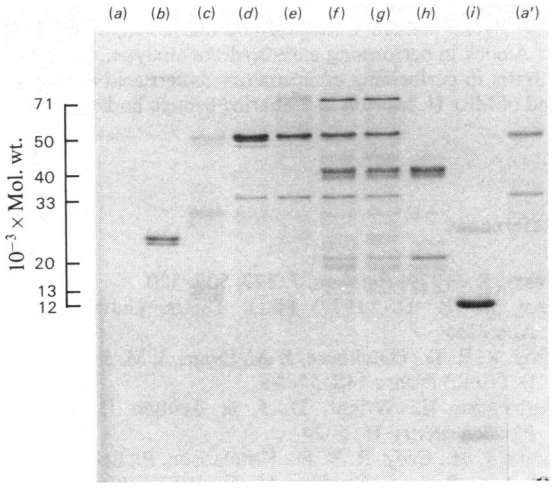

Fig. 7. *Analyses by SDS/polyacrylamide-gel electrophoresis of total soluble proteins from pea seeds harvested at different stages of development*

(*a* and *a'*) Vicilin standard (3 and 10 μg respectively). (*b*) Albumin fraction from mature seeds. (*c–g*) Total soluble proteins isolated from seeds 11 days (*c*), 13 days (*d*), 17 days (*e*), 21 days (*f*), 33 days (*g*) after flowering. (*h*) Legumin standard (10 μg). (*i*) Pea seed lectin standard (10 μg).

mol.wt. but not 71000-mol.wt. subunits are synthesized, whereas towards the end of the storage-protein-deposition stage, the 71000-mol.wt. subunits are accumulated (Fig. 7). Similar results are found in the translation products when polyribosomes at the two stages are translated in the wheat-germ and reticulocyte cell-free systems (Croy *et al.*, 1980). From the results of the protein-accumulation experiment (Fig. 7) it is noteworthy that there is a sequential accumulation of seed proteins in the order vicilin, legumin and convicilin.

Convicilin was shown to be present in the protein bodies of mature pea cotyledons by lysing isolated protein bodies and analysing their contents by SDS/polyacrylamide-gel electrophoresis. Convicilin (71000-mol.wt. subunits) was a major component of the protein-body proteins.

Discussion

A third storage protein distinct from vicilin and legumin has been identified in peas on the following evidence.

(i) Under non-dissociating conditions a protein containing only 71000-mol.wt. subunits can be separated (see Fig. 3).

(ii) The protein has a distinctive amino acid composition, particularly with respect to sulphur amino acids, having one cysteine residue and one methionine residue per subunit, whereas vicilin has no sulphur amino acids and legumin has approx. four methionine and seven cysteine residues per 60000-mol.wt. subunit. This difference is reflected in the results of CNBr cleavage; convicilin is cleaved into two fragments (approx. 55000 and 14000 mol.wt.), whereas the reagent has no apparent effect on vicilin (Fig. 4; Croy *et al.*, 1980). The glutamic acid/aspartic acid ratio in convicilin is high (approx. 2:1), distinct from vicilin (approx. 1:1) and legumin (approx. 1.5:1).

(iii) The protein gives a serological reaction of non-identity with legumin.

(iv) Experiments on biosynthesis *in vivo* and *in vitro*.

This protein has been named convicilin on the basis of its serological cross-reactivity with vicilin. Alternative names, e.g. vicilin 2, were rejected as confusing, and until such time as a systematic nomenclature for seed storage proteins is developed, we would propose the name 'convicilin' be used. However, no homology to other 'con-' proteins, e.g. concanavalin A, conglycinin or conarachin is implied. Furthermore, convicilin cannot be the third protein referred to by Millerd (1975) on the basis of the difference in subunit molecular weights and antigenic properties (Millerd *et al.*, 1978).

The serological results show that convicilin has all the vicilin determinants and therefore must be closely related to vicilin. The absence of additional determinants in convicilin shown by the immunodiffusion experiment may reflect the type of antisera used, since these were raised against a protein fraction containing a mixture of vicilin and convicilin (approx. 4:1).

Although convicilin is sometimes found as a contaminant in crude legumin preparations, as may be deduced from the results reported by Casey (1979), it is more often found in the vicilin fraction of pea globulins. Convicilin is difficult to purify from vicilin, and unless the purification procedure outlined in the present paper is used, most other procedures result in convicilin preparations heavily contaminated with vicilin. This is illustrated by the results of gel filtration of total pea seed proteins shown in Fig. 6, which clearly show vicilin contaminating many convicilin fractions. However, the subunit patterns of the earlier convicilin fractions with very high 17000-mol.wt./50000-mol.wt. subunit ratios (> 10 by densitometric scanning of gels) could not be explained by hybrid molecules containing both types of subunit, but only by vicilin contamination. Further, the normal distribution of 71000-mol.wt. subunits on elution gives no evidence for hybrid molecules, although the existence of such species cannot be ruled out on present evidence. The association of convicilin with vicilin is

implied in the results of other authors, who have commented on the heterogeneity of the vicilin fraction of pea (Derbyshire *et al.*, 1976) and by the C.S.I.R.O.'s group in Australia (Thomson *et al.*, 1978; Millerd *et al.*, 1978), who have noted repeatedly the presence of a major 75000-mol.wt. subunit in pea vicilin preparations that appears to be similar to the 71000-mol.wt. subunit described here. On the basis of crossed immunoelectrophoresis and agarose-gel electrophoresis, these authors have suggested the presence of several different vicilin types, some, but not all of which contain the 75000-mol.wt. subunit, but none of which contain only this type of subunit.

Although convicilin is homogeneous by SDS/polyacrylamide-gel electrophoresis, heterogeneity must be present in the protein, since it does not give a single band on isoelectric focusing and it has two *N*-terminal amino acids. Also, the results of cleavage with CNBr indicate that sequence heterogeneity may be present, since although most of the 70000-mol.wt. subunit is cleaved rapidly (within 4h), a significant proportion remains uncleaved even after prolonged (48h) reaction. This resistant fraction of convicilin may have another amino acid substituted for methionine. As well as heterogeneity in a single variety, genetic variation in convicilin has been observed among certain pea lines, e.g. in line 1552 (provided Dr. S. Blixt); the convicilin band appears to be of significantly lower molecular weight on SDS/polyacrylamide-gel electrophoresis (approx. 65000) (result not shown). Others have also presented evidence showing qualitative and quantitative variations in convicilin subunits (Przybylska *et al.*, 1978).

An equivalent protein to *Pisum* convicilin has also been isolated from field bean (*Vicia faba minor*) (variety Maris Bead) by the purification technique described in the present paper. Its molecular-weight on SDS/polyacrylamide-gel electrophoresis appears to be slightly lower than the *Pisum* protein subunit (approx. 68000) (R. R. D. Croy & M. Tyler, unpublished work).

Although no experiments designed to test the function of convicilin have been carried out, it is considered to be a storage protein, since it is not only a major seed protein but also a major component of protein bodies. As previously stated, convicilin contains sulphur amino acids, whereas vicilin does not, and is thus a component of pea storage proteins of greater nutritional value than vicilin.

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References

Casey, R. (1979) *Biochem. J.* **177**, 509–520
Croy, R. R. D. (1977) Ph.D. Thesis, University of Aberdeen
Croy, R. R. D., Gatehouse, J. A., Evans, I. M. & Boulter, D. (1980) *Planta* **148**, 57–63
Derbyshire, E., Wright, D. J. & Boulter, D. (1976) *Phytochemistry* **15**, 3–24
Evans, I. M., Croy, R. R. D., Hutchinson, P., Boulter, D., Payne, P. I. & Gordon, M. E. (1979) *Planta* **144**, 455–462
Gray, W. R. (1972) *Methods Enzymol.* **25**, 121–138
Hirs, C. H. W. (1956) *J. Biol. Chem.* **219**, 611–621
Joustra, M. & Lundgren, H. (1969) *Protides Biol. Fluids* **17**, 511–515
Kasper, C. B. (1970) in *Protein Sequence Determination* (Needleham, S. B., ed.), pp. 137–140, Chapman and Hall, London
Krishna, T. G., Croy, R. R. D. & Boulter, D. (1979) *Phytochemistry* **18**, 1879–1880
Laemmli, U. K. (1970) *Nature (London)* **237**, 680–685
Livingstone, D. M. (1974) *Methods Enzymol.* **34**, 723–731
Millerd, A. (1975) *Annu. Rev. Plant Physiol.* **26**, 53–72
Millerd, A., Thomson, J. A. & Schroeder, H. E. (1978) *Aust. J. Plant Physiol.* **5**, 519–534
Moore, S. & Stein, W. H. (1963) *Methods Enzymol.* **6**, 819–831
Osborne, T. B. & Campbell, G. F. (1898) *J. Am. Chem. Soc.* **20**, 348–362, 362–375, 393–405
Ouchterlony, Ö. & Nilsson, L.-Å. (1978) in *Handbook of Experimental Immunology* (Weir, D. M., ed.), 3rd edn., vol. 1, pp. 19.1–19.44, Blackwell Scientific Publications, Oxford
Przybylska, J., Hurich, J. & Zimniak-Przybylska, A. (1978) *Pisum Newsl.* **10**, 66–67
Sweeley, C. C., Wells, W. W. & Bentley, R. (1966) *Methods Enzymol.* **11**, 93–108
Thomson, J. A., Schroeder, H. E. & Dudman, W. F. (1978) *Aust. J. Plant Physiol.* **5**, 263–280
Vesterberg, O. (1975) in *Isoelectric Focusing* (Arbuthnott, J. P. & Beeley, J. A., eds.), pp. 78–96, Butterworths, London
Woods, K. R. & Wang, K.-T. (1967) *Biochim. Biophys. Acta* **133**, 369–370
Wright, D. J. & Boulter, D. (1974) *Biochem. J.* **141**, 413–418