Journal of Chromatography, 402 (1987) 159–172
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 614

APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TO THE ASSESSMENT OF SUBUNIT HETEROGENEITY IN PLANT 11S STORAGE GLOBULINS

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(Received April 2nd, 1987)

SUMMARY

Plant 11S storage proteins from a wide range of species have been resolved into their component subunits by ion-exchange fast protein liquid chromatography under denaturing conditions. The complex profiles obtained were reproducible and characteristic for each plant species analysed. The technique is shown to have distinct advantages over the more conventional electrophoretic approaches used to assess 11S subunit heterogeneity. The potential for the fast protein liquid chromatographic method as a simple screening system is discussed.

INTRODUCTION

The 11S globulin class of plant storage proteins is found in all legumes, *e.g.*, pea, soya, bean, and also in several non-legume species, *e.g.*, oat, rice and brazil nut[1,2]. The structure and properties of these proteins and their importance to the food industry have been well documented[1–3]. Briefly, to date all 11S globulins are hexamers of 50 000–60 000 molecular weight (M_r) subunits. Each subunit is composed of an A-chain (acidic p*I*, $M_r \approx 40\,000$) and a B-chain (basic p*I*, $M_r \approx 20\,000$) linked via a disulphide bond. Subunits are synthesized as a single polypeptide[1,4] and are proteolytically cleaved post-translationally to yield "AB pairs".

The subunits are homologous, but heterogeneous, being coded for by a multigene family (for example, eight different genes code for pea 11S globulin subunits[5]). Post-translational processing, *e.g.*, proteolysis, deamidation[6], and modifications occurring during certain purification procedures[7] also increase heterogeneity. The range of subunits is known to vary both within and between species[1,2,8,9] and additionally their proportions can also be affected by agronomic factors[10,11].

The subunits of the soyabean 11S globulin, glycinin, have been the most extensively characterised. Five of them have been sequenced[12] revealing an alternating pattern of homologous and variable regions within the primary structure. Sequence data from other plants 11S globulin subunits have shown that this homology also extends across species. Comprehensive evaluations of the available subunit sequence data have recently been reported[2,13,14].

0021-9673/87/$03.50

Attempts have been made to relate specific subunits to certain functional properties or nutritional quality[8,15], and cultivars have been bred that contain elevated proportions of advantageous subunits, *e.g.*, a higher sulphur content. Assessment of subunit heterogeneity has therefore been an important feature of 11S globulin studies, not only as a basic tool for structural characterisation but also as a means of identifying subunits of potential industrial importance. Usually an electrophoretic technique has been used, *e.g.*, sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) or isoelectric focusing (IEF), but each one has proved unsatisfactory in some aspect[16,17]. Here we describe the use of high-performance liquid chromatography (HPLC) as an alternative to electrophoresis for the assessment of 11S globulin subunit heterogeneity. HPLC analysis of proteins is a rapidly expanding technique, and has already been applied successfully to several other food protein systems[18]. A preliminary account of this work has already been published[19].

EXPERIMENTAL

Chemicals and reagents

All reagents used for HPLC analysis were of analytical-reagent grade and were obtained from either Sigma (Poole, U.K.) or BDH (Dagenham, U.K.). Solutions were prepared using distilled deionised water supplied by BDH, and were filtered through 0.22-μm membranes using a Millipore (Harrow, U.K.) filtration system. Reagents used for electrophoresis were purchased from BDH and were of Electran grade, except for Ampholines (LKB, Bromma, Sweden) and Coomassie Blue R-250 and protein molecular weight standards (both Sigma).

Peas (*Pisum sativum* c.v. Birte) were obtained from P.G.R.O. (Peterboro, U.K.). Soya meal was purchased from BDH, beans (*Vicia faba* c.v. Maris Bead) were obtained from the Tyneside Seed Stores (Gateshead, U.K.), sesame seeds (*Sesamum indicum*) were supplied by Community Foods (London, U.K.), Brazil nuts (*Bertholletia excelsa*) were obtained from J. S. Sainsbury (Norwich, U.K.) and oats (*Avena sativa*, c.v. Rhiannan) were provided by the Welsh Plant Breeding Institute, Aberystwyth, U.K.

Apparatus

HPLC analyses were performed using a fast protein liquid chromatography (FPLC) system (Pharmacia, Uppsala, Sweden) with P500 pumps and a UV-1 detection unit. Mono Q (5/5) and Mono S (5/5) columns were employed. SDS-PAGE was carried out with a GE2/4 Pharmacia electrophoresis system. IEF was performed with an LKB flat-bed Multiphor system.

Preparation of 11S globulins

Pea 11S globulin was prepared as described by Lambert *et al.*[7]. Soya 11S globulin was isolated by hydroxyapatite-Ultrogel chromatography of a cryoprecipitated soya extract[20]. Bean 11S globulin was isolated according to the method of Cully[21]. Sesame 11S globulin was prepared as detailed by Hasegawa *et al.*[22]. Brazil nut 11S globulin was isolated according to the method of Kamiya *et al.*[23]. Oat 11S globulin was prepared as described by Robert *et al.*[24]. Pumpkin 11S globulin was purchased direct from Sigma. All 11S globulin preparations were finally purified by

gel filtration using a Sephacryl S-300 column (80 cm × 2.5 cm) prior to dialysis against ammonium hydrogen carbonate and lyophilisation. Samples were stored dessicated at −20°C.

Pea 11S globulin subunits P3a and P4 were purified as described by Bacon *et al.*[16].

Preparation of samples for HPLC

For analysis on the Mono Q anion-exchange column the 11S globulin (2 mg) was dissociated into component subunits by dissolving in 1 ml of 50 m*M* Tris buffer, pH 8.0, containing 5% (w/v) betaine and 6 *M* urea. Iodoacetamide (IAA) was added to 2 m*M* and the sample stored in the dark for 15 min at room temperature to block any free SH groups and thereby prevent artifactual heterogeneity produced as a result of thiol–disulphide interchange reactions. Excess IAA was removed quickly (separation time *ca.* 10 min) by gel filtration using a Pharmacia PD-10 column.

Equivalent samples to be applied to the Mono S cation-exchange column were dissociated in 1 ml 50 m*M* citrate buffer, pH 5.0, containing 6 *M* urea and 5% (w/v) betaine. These samples were not treated with IAA as thiol–disulphide interchange is unlikely to occur at this pH. Prior to HPLC analysis all samples were centrifuged in an MSE (Crawley, U.K.) Micro Centaur at full speed for 2 min. All urea-containing solutions were prepared fresh daily.

Chromatographic procedure

The Mono Q column was equilibrated with 50 m*M* Tris buffer, pH 8.0, containing 5% (w/v) betaine and 6 M urea. A 0.5-ml sample (0.5 mg protein) was injected onto the column, and unbound material eluted with the above solvent (10 min), before developing the column with a linear (60 min, 0–500 m*M*) gradient of sodium chloride. The flow-rate was 1 ml/min throughout. After each run, the column was cleaned by elution with 1 *M* sodium chloride (10 ml) and 5 × 0.5 ml injections of 50% (v/v) acetic acid before re-equilibration. Total regeneration time was *ca.* 20 min. For analysis of samples on the Mono S column, the above procedure was adopted except that the Tris buffer system was replaced by 50 m*M* citrate, pH 5.0, and 1 *M* sodium hydroxide was used to clean the column instead of acetic acid. All samples underwent a minimum of three analyses. All experiments were conducted at room temperature. Where modified procedures were employed for some experiments, these are described in the figure legends.

Electrophoretic analysis

SDS-PAGE was performed using the buffering system of Laemmli[25] in 10–20% acrylamide gradient gels (200 × 140 × 1.5 mm). Samples were dissolved in 10 m*M* Tris buffer, pH 7.8, containing 2% (w/v) SDS, 5 m*M* EDTA and 2 m*M* IAA. No reducing agent was added, nor were samples boiled prior to analysis, thus ensuring the subunits remained intact[19]. Electrophoresis was performed at 500 V and 30 mA per gel. Gels were stained for protein with Coomassie Blue R-250.

Isoelectric focusing was performed in 26 × 12 cm × 0.3 mm gels, containing 4% (w/v) acrylamide, 6 *M* urea, and pH 3.5–10 Ampholines (2% w/v). Gels were prefocused at 500 V for 30 min before applying samples. Anode buffer was 0.1 *M* sulphuric acid and the cathode buffer 0.1 M sodium hydroxide. Focusing was carried

out at 1500 V with cooling until the current reached 0.5 mA (ca. 3–4 h). Ampholines were washed out with 12% (w/v) trichloroacetic acid in ethanol–acetic acid–water (30:10:60, v/v/v), before staining for protein with 0.1% (w/v) Coomassie Blue R-250 in the above ethanol–acetic acid–water solvent.

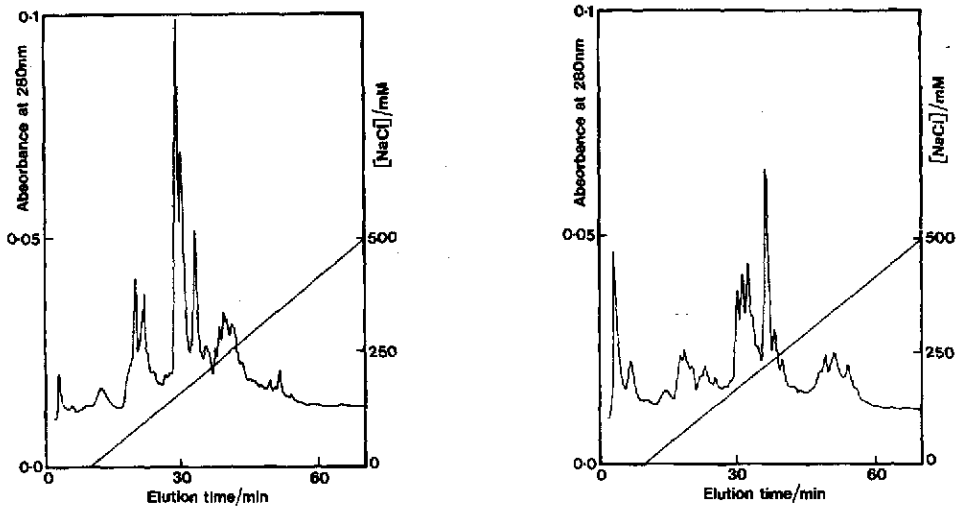


Fig. 1. FPLC analysis of pea 11S globulin subunits on a Mono Q anion-exchange column. Samples were prepared and analysed as described in the Experimental section. The straight line indicates the sodium chloride gradient.

Fig. 2. FPLC analysis of soya 11S globulin subunits. See legend to Fig. 1.

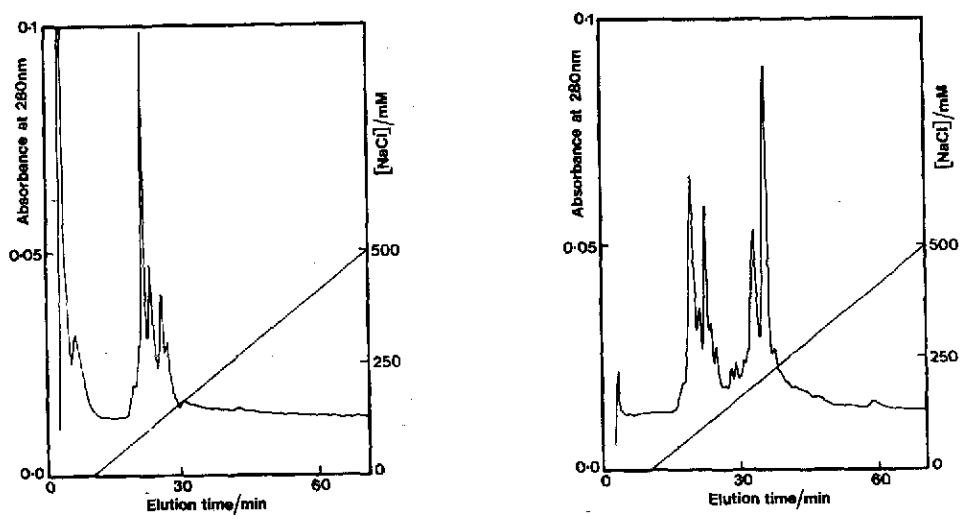


Fig. 3. FPLC analysis of sesame 11S globulin subunits. See legend to Fig. 1.

Fig. 4. FPLC analysis of pumpkin 11S globulin subunits. See legend to Fig. 1.

RESULTS AND DISCUSSION

Resolution of 11S globulin subunits by HPLC

Experimental design. 11S globulin fractions from seven plant species were dissociated into their component subunits with urea, and analysed on a Mono Q an-

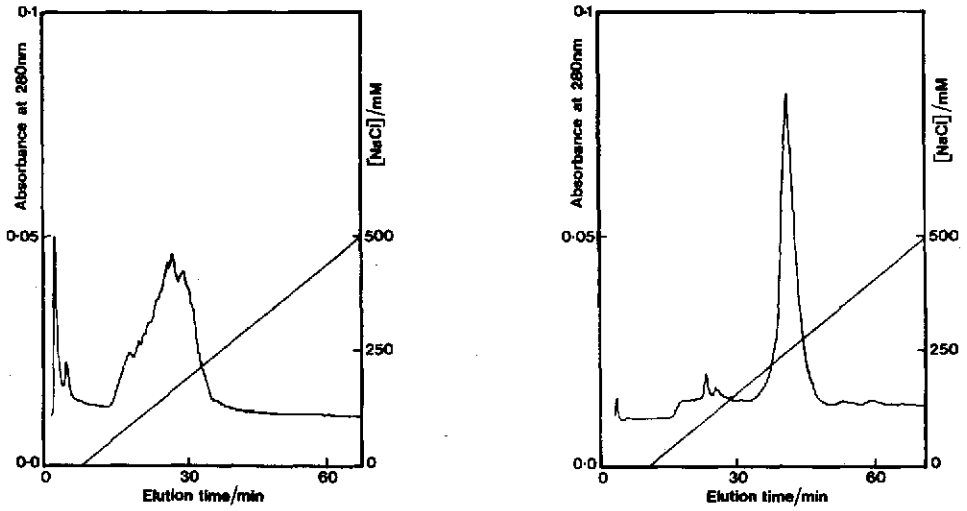


Fig. 5. FPLC analysis of oat 11S globulin subunits. See legend to Fig. 1.

Fig. 6. FPLC analysis of Brazil nut 11S globulin subunits. See legend to Fig. 1.

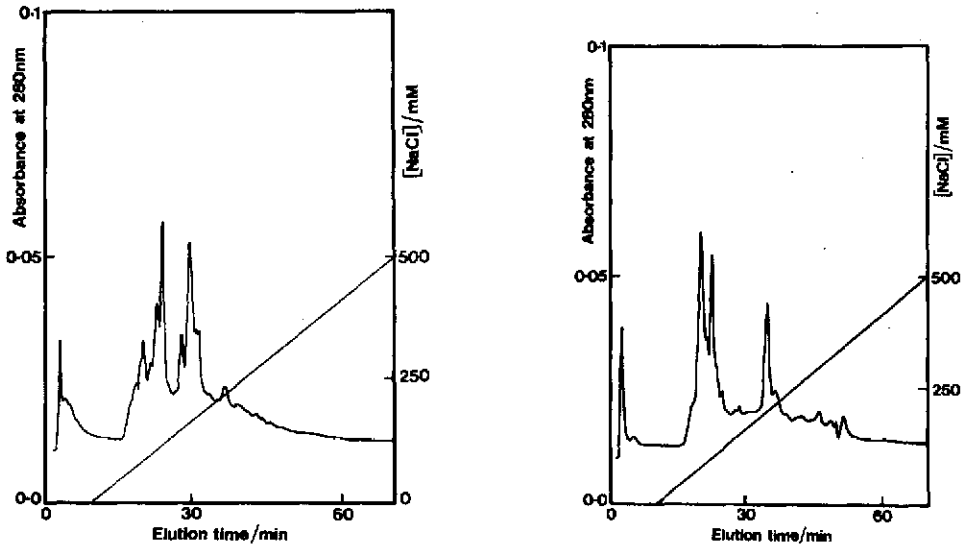


Fig. 7. FPLC analysis of bean 11S globulin subunits. See legend to Fig. 1.

Fig. 8. FPLC analysis of pumpkin 11S globulin subunits on a Mono Q anion-exchange column. Samples were prepared and analysed as described in the Experimental section except that samples were prepared in the absence of IAA.

ion-exchange column attached to an FPLC system, as described in the Experimental section. Each globulin produced a characteristic and reproducible elution profile (Figs. 1–7). Every precaution was taken to minimise the possibility of artifactual heterogeneity. Thiol–disulphide interchange reactions were prevented by blocking free thiols with iodoacetamide (IAA). Fig. 8 shows the effect of excluding IAA from the analysis of pumpkin 11S globulin. Comparison with Fig. 4 (*i.e.*, with IAA present) reveals that the unbound fraction is substantially larger and the fourth bound peak (elution time *ca.* 35 min) is greatly diminished when IAA is absent. It is possible that the enhanced unbound material represents free basic polypeptides generated by protein thiol groups cleaving the interdisulphide bond between A and B chains. (The free acidic polypeptides would presumably bind very tightly to the column under the conditions used, and would not be eluted even with 1 *M* sodium chloride). The addition of IAA would block these reactive protein thiol groups and thus prevent the liberation of the free basic chains. Some of the 11S globulins showed no significant differences in the absence of IAA (results not shown) presumably reflecting a variation in the potency of the denatured subunits at cleaving the inter-disulphide bond. The possibility of polypeptide modification by carbamoylation resulting from cyanate impurities in the concentrated urea solutions was also assessed. In some experiments the urea was pre-treated with Amberlite mixed-bed resin RG501-*X*8 (Bio-Rad, Watford, U.K.) to remove any impurities; in others, samples were left in the Tris–urea buffer at room temperature for 6 h before analysis to maximise any modification reactions. In neither study were the results obtained significantly different. We are confident, therefore, that the profiles presented herein reflect genuine differences in subunit population in the various 11S globulins.

As 11S globulin subunits are known to possess regions of high charge density[1,2], the zwitterion betaine was included in all buffers (5%, w/v) as recommended by Pharmacia[26] in an attempt to improve resolution and decrease aggregation. No major profile differences were found when the seven seed globulins were analysed in the absence of betaine, but the long term performance of the column was impaired as gauged by an increase in back-pressure with prolonged use. Fig. 9 shows the profile for bean 11S globulin without betaine present. In all cases the inclusion of the zwitterion slightly retarded the peaks.

For a given sample the degree of reproducibility of analyses was very high, with retention times of major peaks virtually indentical. Occasionally peak heights varied, and some of the minor peaks and shoulders were not consistent. This could in part be due to protein aggregating and irreversibly binding to the column. The recovery of protein was typically *ca.* 90% (as gauged by absorbance measurements at 280 nm); the remaining 10% or so could explain the variation in peak heights noted above. In Table I the reproducibility of peak height and retention time for the four major peaks present in the pumpkin globulin profile (Fig. 4) are shown. Good agreement was achieved between different batches of the same 11S globulin, and a five-fold increase in sample loading was not found to affect the resolution attained significantly. Figs. 1 and 10 show such analyses for pea 11S globulin subunits. The only differences are the relative heights of the double peak eluting at *ca.* 30 min, and the group of small peaks eluting at *ca.* 40 min. The use of internal reference proteins run concurrently with each sample may improve reproducibility further by avoiding possible problems arising from variations between columns and buffers.

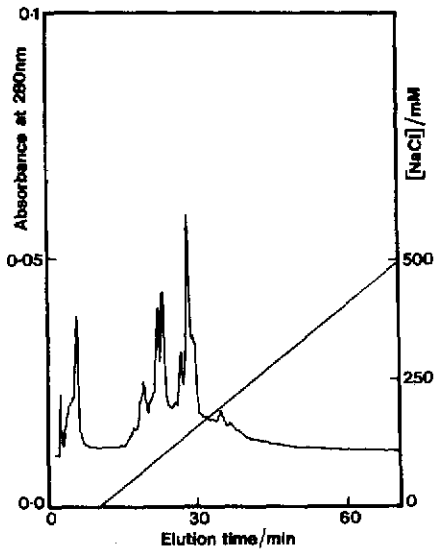


Fig. 9. FPLC analysis of bean 11S globulin subunits on a Mono Q anion-exchange column. Samples were prepared and analysed as described in the Experimental section except that betaine was omitted from all buffers.

Samples were also analysed on a Mono S cation-exchange column operating at pH 5.0. Fig. 11 shows the data obtained for pea 11S globulin. One advantage of this system is that thiol–disulphide interchange and carbamoylation reactions are unlikely to occur at pH 5.0. Unfortunately, the resolution achieved with the Mono S column was generally inferior for all samples, compared with the Mono Q column (*e.g.*, compare Fig. 11 with Fig. 1).

Alternative combinations of buffers, flow-rate, eluting ion and gradient design were explored with the two columns, and indeed some of the regimes tested gave slightly better separations than the Mono Q–Tris, pH 8.0–sodium chloride system described here, for *some* of the globulins. However, since the standard Mono Q method gave the best overall resolution for *all* the globulins studied, this was the protocol selected for this comparative study.

TABLE I

REPRODUCIBILITY OF CHROMATOGRAPHIC SEPARATIONS

Pumpkin 11S globulin subunits were resolved as described in Fig. 4. Values represent the mean ± S.E.M. of five determinations on separate occasions.

Peak elution time (min)	*Relative peak height*
19.94 ± 0.57	1.2 ± 0.27
22.33 ± 0.45	1.0
33.40 ± 0.49	0.8 ± 0.18
35.02 ± 0.34	1.6 ± 0.31

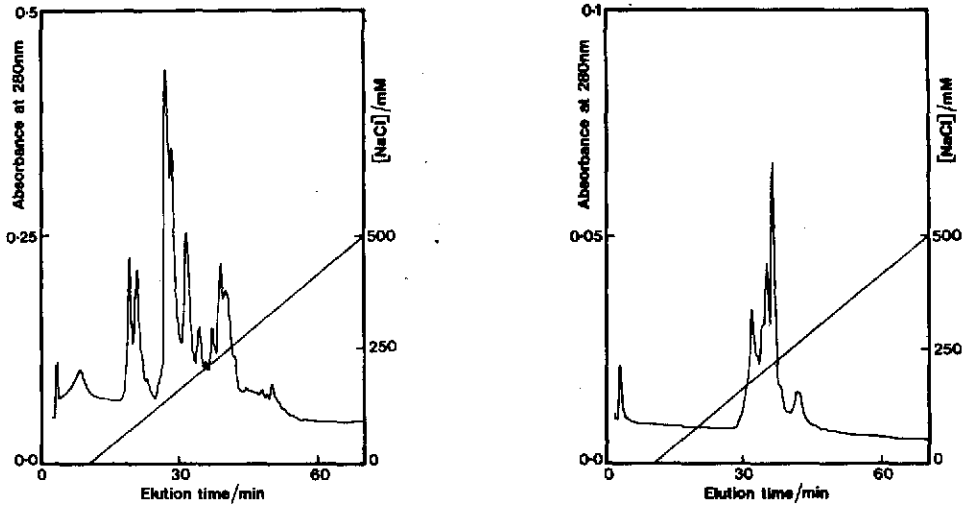
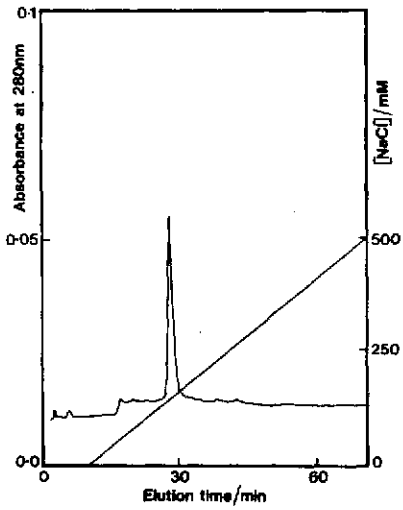


Fig. 10. FPLC analysis of pea 11S globulin subunits on a Mono Q anion-exchange column. Samples were prepared and analysed as described in the Experimental section except that five times more protein was loaded onto the column.

Fig. 11. FPLC analysis of pea 11S globulin subunits on a Mono S cation-exchange column. Samples were prepared and analysed as described in the Experimental section.

Evaluation of the Mono Q HPLC profiles. 11S globulins are known to comprise subunits derived from a pool of similar but non-identical polypeptides[1,2]. The degree of subunit heterogeneity found within seven different plant 11S globulins is demonstrated vividly by the complexities of the HPLC profiles shown in Figs. 1–7. Among the interesting features observed was the very broad, poorly-resolved peak obtained with the oat globulin (Fig. 5). No improvement in resolution was achieved with any other HPLC protocol tested, nor were the oat globulin subunits resolved satisfactorily by electrophoresis (see below). The subunits of Brazil nut 11S globulin also eluted essentially as a single peak (Fig. 6). Although much narrower than that for oat globulin, it was broader than the more typical peaks suggesting some degree of heterogeneity, a fact born out by subsequent electrophoretic analyses (see below). The peak eluted at *ca.* 40 min, considerably later than most of the other globulin subunits, implying that Brazil nut 11S globulin subunits are generally more acidic. Conversely sesame globulin subunits bound poorly to the column, all eluting before 30 min, with a substantial proportion not binding at all, suggesting that these are more basic than the other globulin subunits. The pumpkin profile (Fig. 4) was characterized by two double peaks, one eluting just prior to 30 min and one just after. Profiles for the soya and pea 11S globulins were particularly complex (Figs. 2 and 1, respectively). Nevertheless, we have begun to ascribe some of the peaks in the pea profile to subunits that we have purified and partially characterized[16]. For example, Figs. 12 and 13 demonstrate that the subunits termed P3a and P4 in the above reference, correspond to the peaks in Fig. 1 eluting at 29 min and 32 min respectively. This provides further confirmation that the peaks represent genuine subunits and not artifactual heterogeneity.



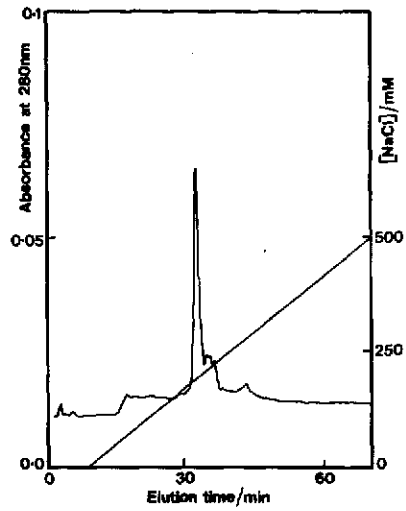


Fig. 12. FPLC analysis of pea globulin subunit P3a. See legend to Fig. 1.

Fig. 13. FPLC analysis of pea globulin subunit P4. See legend to Fig. 1.

Resolution of 11S globulin subunits: comparison of HPLC with electrophoresis

11S globulin subunit heterogeneity is conventionally assessed by electrophoretic methods, particularly SDS-PAGE or IEF. Below we compare the resolution obtained with the HPLC technique described in this report, to those methods.

SDS-PAGE. Fig. 14 shows the separation of the seven 11S globulin subunits by SDS-PAGE under non-reducing conditions. Gradient gels of 10–20% acrylamide were found to give maximal resolution. The species around M_r 50000–60000 correspond to the traditional 11S globulin subunits, while those of M_r below 45000, *e.g.*, bean and Brazil nut, represent atypical "small" subunits previously observed by others[1,2], but as yet poorly characterized. Soya and pea 11S globulins also possess "small" subunits but they only appear as faint bands at the level of loading employed in Fig. 14. It is not known whether these subunits are genuinely smaller in size, or whether they migrate anomalously during electrophoresis. Because many of the subunits possess very similar or identical molecular weights they are not well resolved by SDS-PAGE with only the "small" subunits being easily distinguished. The number of resolved components is clearly less than that obtained with the HPLC method, with the notable exception of Brazil nut 11S globulin which gave one broad peak by Mono Q analysis but three major bands and one minor band by SDS-PAGE. Interestingly, oat 11S globulin, which produced a very broad peak by HPLC, showed a very diffuse band on SDS-PAGE. Such behaviour suggests the presence of many very similar polypeptides arising from some form of variable covalent modification such as acetylation, glycosylation or exopeptidolysis. The oat in question was a "naked" variety (c.v. Rhiannan); it would be interesting to test if other varieties display similar characteristics. Three subunits of pumpkin 11S globulin were resolved, but at higher loadings only one band was discernible. Sesame 11S globulin subunits migrated as one major species with a broad, diffuse leading edge. Yuno *et al.*[27] obtained similar results and claimed that three subunits were distinguishable.

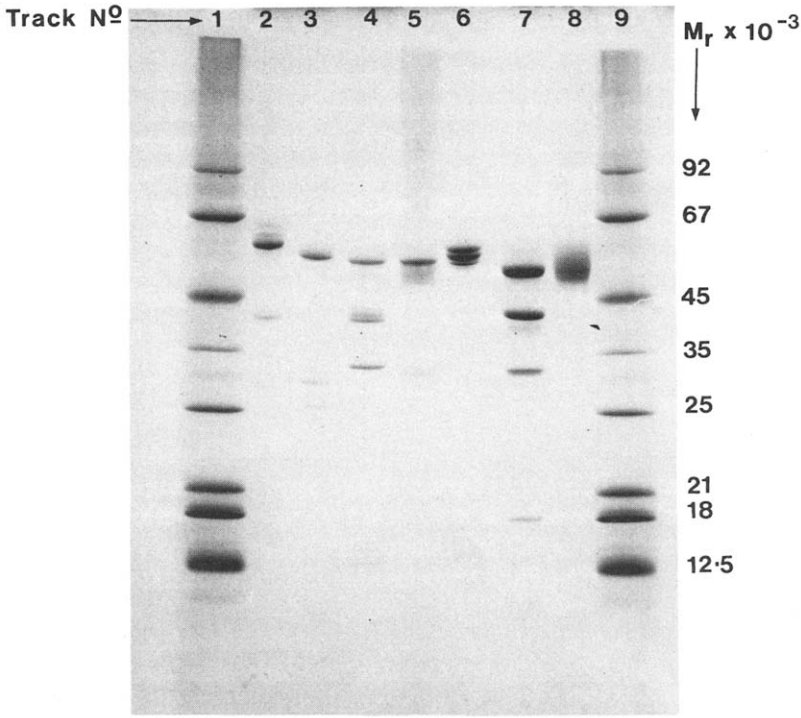


Fig. 14. SDS-PAGE analysis of 11S globulins. Samples were prepared and analysed in the absence of 2-mercaptoethanol as described in the experimental section. Tracks: 1,9 = M_r standard proteins (10 μg each of rabbit muscle phosphorylase *b*, M_r = 92 000; bovine serum albumin, M_r = 67 000; hen ovalbumin, M_r = 45 000; rabbit muscle lactate dehydrogenase, M_r = 35 000; bovine pancreas chymotrypsinogen, M_r = 25 000; soya bean trypsin inhibitor, M_r = 21 000; bovine milk β-lactoglobulin, M_r = 18 000; and horse heart cytochrome *c*, M_r = 12 500); 2 = pea (10 μg); 3 = soya (10 μg); 4 = bean (15 μg); 5 = sesame (10 μg); 6 = pumpkin (15 μg); 7 = Brazil nut (20 μg); 8 = oat (15 μg).

In the presence of 2-mercaptoethanol the subunits would be dissociated into their component A and B chains[1] but again because these are grouped into two major M_r regions (40000 and 20000, respectively) they are difficult to resolve. We have found that the percentage acrylamide and level of loading greatly affected the number of species observed (unpublished results).

IEF. Fig. 15 shows the resolution of 11S globulin subunits by flat-bed IEF under denaturing conditions. Flat-bed IEF using thin acrylamide gels is known to afford excellent resolution within a short time, especially when compared to IEF performed in cylindrical gels. Like the ion-exchange HPLC columns, IEF separates on the basis of charge, rather than M_r, and thus the resolution achieved for the 11S globulin subunits is superior to that obtained by SDS-PAGE. Fig. 15 shows that many polypeptides were resolved, but although the standard proteins were reproducibly focused, problems were encountered when analysing the globulins. Irreproducible migration patterns and diffuse bands were frequently observed. The data presented in Fig. 15 shows the most typical pattern obtained. Problems in resolving these polypeptides by IEF have been reported by other workers[17]. The position of

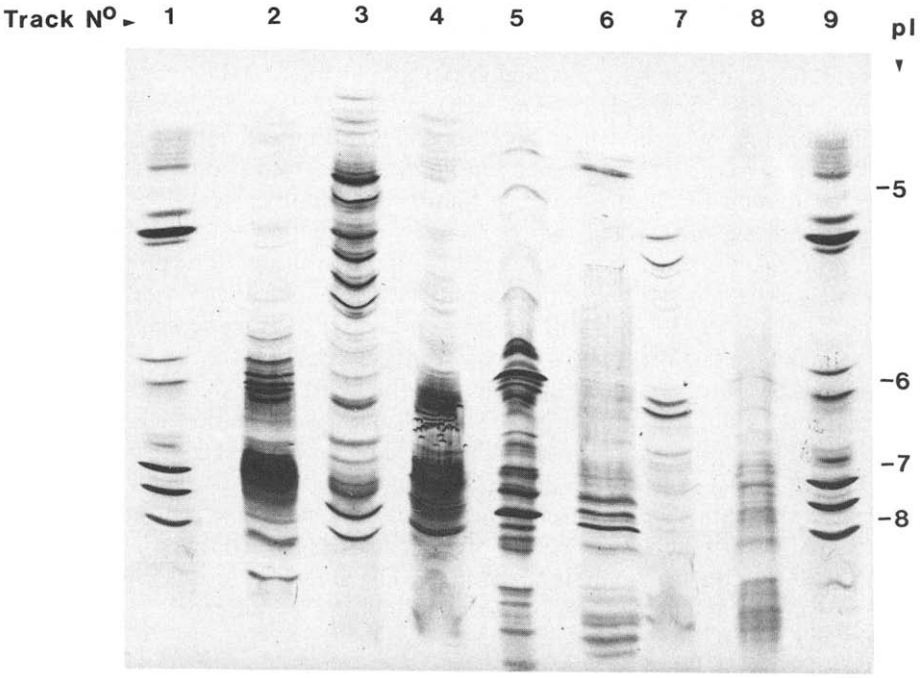


Fig. 15. Isoelectric focusing analysis of 11S globulins. Samples were prepared and analysed in 6 *M* urea as described in the experimental section. The samples were applied by laying filter paper squares (*ca.* 5 mm × 5 mm) wetted with the sample (*ca.* 50 μg protein) onto the gel surface. Pumpkin, oat and sesame samples were applied at the acidic end, all other samples were applied at the basic end. Tracks: 1,9 = Electran p*I* 4.7–10.6 marker proteins; 2 = pea; 3 = soya; 4 = bean; 5 = sesame; 6 = pumpkin; 7 = Brazil nut; 8 = oat.

sample loading was found to have a significant affect on the focusing achieved for many of the samples. Sesame, pumpkin and oat 11S globulins were resolved better by loading at the acidic end, while the others gave better results from basic-end loading. The presence of any salts greatly reduced resolution for all samples, and hence they were passed down a PD-10 gel-filtration column equilibrated in 6 *M* urea prior to analysis; pumpkin 11S globulin was surprisingly only sparingly soluble in 6 *M* urea. Most of the resolved species were located in the pH 6–8 region, with the exception of soya 11S globulin which had a significant proportion of polypeptides with a p*I* below 6. Brazil nut 11S globulin subunits, inferred to be particularly acidic by Mono Q HPLC showed no such characteristics upon IEF; similarly the sesame 11S globulin subunits believed to be generally basic by HPLC did not display this tendency upon IEF. The reasons for these discrepancies, and the poor quality of the IEF data are unclear, and currently under further investigation.

General discussion. Each of the three techniques SDS-PAGE, IEF and HPLC have their respective merits when employed to study 11S globulin subunits, and for maximum information should be used in conjunction with one another. SDS-PAGE is ideal for studying the "small" subunits, and those "normal" subunits easily resolved on the basis of size, but is otherwise inferior to the other two methods. IEF

is potentially the most powerful technique, particularly when employing recent innovations such as immobilised pH gradients and ultra-thin gels[28,29] but the problems encountered during the analysis of 11S globulin subunits need to be overcome before its full potential can be realised. The ion-exchange HPLC technique presented in this communication has been shown to give good resolution and reproducibility, the minor differences in peak heights posing insignificant problems to either manual or computer evaluation of chromatograms. Similar quantitative variations occurring upon electrophoretic analysis may not be so easily detected by visual examination of gels. The differences may be seen more readily after densitometric scanning of gels, but such analyses would give very complex patterns (*cf.* Sastry *et al.*[30] who compared densitometric traces of IEF gels with reversed-phase HPLC for the analysis of sorghum proteins). Significantly some of the basic polypeptide chains of the subunits stain very poorly with Coomassie Blue[16] and therefore band intensity would vary, making densitometric data difficult to interpret. The 280-nm extinction coefficients for the subunits would be expected to be very similar based on amino acid composition data, and thus HPLC peak areas would give less misleading quantitative data, especially if integrators and data handling techniques are used. One major advantage of the HPLC method is the ease with which resolved species can be collected and thereby made available for further analysis if required. The simplicity in scale-up for preparative purposes has enabled us to isolate several mg of pure pea 11S globulin subunits using HPLC[16].

If a high through-put of samples is required, more samples can be processed simultaneously using electrophoresis. Using the "general" HPLC method described in this report we can analyse *ca.* 6 samples per 8 h, but by employing higher flow-rates and shorter gradients adequate resolution could be achieved in 15 min for some 11S globulins, and if automatic sample loadings and data handling apparatus are available, it would be possible theoretically to analyse 50 samples per 24 h.

Applications of the HPLC method

Plant storage proteins (*e.g.*, 11S globulins) especially of legumes like soya, pea and bean are of considerable importance to the agricultural and food industries[2,3]. The screening of such crop varieties for the maintenance and testing of quality by plant breeders, traders and end-users, has routinely been performed by electrophoretic methods. For the reasons outlined above, we believe that HPLC methods similar to those described here offer a practical alternative. HPLC (both ion-exchange and reversed-phase) has for example, previously been used for the screening of sorghum, barley and wheat varieties[31–33]. The overall composition or amounts of proteins in a crop may directly influence its functional or nutritional properties, *e.g.*, the presence of specific high-molecular-weight glutenin subunits relates closely to wheat bread making quality[34], and some soya 11S globulin subunits have been related to various "gelling" properties[15]. If the identity of these "quality-promoting polypeptides" can be established it could be possible to breed improved varieties by selecting for specific proteins as "markers" of quality. For example protein extracts from many legumes have been screened for exceptional food functionality properties[35,36]; it may therefore be possible to relate 11S globulin subunit HPLC profiles to functionality. Similarly nutritionally important polypeptides could be bred into new crop varieties. For example soya and pea protein are nutritionally deficient in sulphur amino acids. Some

11S globulin subunits are higher in cysteine and methionine than others; if these could be identified in HPLC profiles they could be used as "markers" in a breeding programme. Geneticists could also use such information to follow and determine genetic interrelationships of lines.

We have begun to identify and characterize some of the subunit peaks in the pea profile (see Figs. 12 and 13); further work of this nature is required if the above applications are to be fully successful. We are also screening pea varieties for total storage protein composition by HPLC and have begun screening 11S globulin subunits from different pea varieties in the hope that we might be able to correlate HPLC profiles with the functional properties exhibited by proteins from the different varieties.

ACKNOWLEDGEMENTS

This work was funded in part by the Ministry of Agriculture, Fisheries and Food. The authors wish to thank the Welsh Plant Breeding Station and the Processors and Growers Research Organisation for the generous gifts of oats and peas respectively and also wish to acknowledge the experimental expertise of M. D. Williams, S. J. Chambers and Mrs. J. Ricketts (formerly Cully) during the course of this investigation.

REFERENCES

1 R. R. D. Croy and J. A. Gatehouse, in J. H. Dodds (Editor), *Plant Genetic Engineering*, Cambridge Univ. Press, Cambridge, 1985, pp. 143–268.
2 D. J. Wright, in B. J. F. Hudson (Editor), *Developments in Food Proteins*, Vol. 5, Elsevier Applied Sci. Publ., Barking, 1987, in press.
3 D. J. Wright and M. R. Bumstead, *Phil. Trans. R. Soc. Lond.* B304 (1984) 381–393.
4 R. R. D. Croy, J. A. Gatehouse, I. M. Evans and D. Boulter, *Planta*, 148 (1980) 49–56.
5 C. Domoney and R. Casey, *Nucl. Acids. Res.*, 13 (1985) 687–699.
6 J. A. Gatehouse, R. R. D. Croy and D. Boulter, *CRC Crit. Rev. Plant Sci.*, 1 (1984) 287–314.
7 N. Lambert, S. J. Chambers, M. Phalp and D. J. Wright, *Biochem. Soc. Trans.*, 14 (1986) 1186–1188.
8 P. E. Staswick and N. C. Nielsen, *Arch. Biochem. Biophys.*, 223 (1983) 1–8.
9 R. Casey, *Heredity*, 43 (1979) 265–272.
10 P. J. Randall, J. A. Thomson and H. E. Schroeder, *Aust. J. Plant Physiol.*, 6 (1979) 11–24.
11 L. P. Holowach, J. F. Thompson and J. T. Madison, *Plant Physiol.*, 74 (1984) 576–583.
12 N. C. Nielsen, *J. Am. Oil Chem. Soc.*, 62 (1985) 1680–1686.
13 K. Borroto and L. Dure, *Plant Mol. Biol.*, 8 (1987) 113–131.
14 H. J. Carr and D. J. Wright, *Biol. Chem. Hoppe-Seyler.*, 367 (1986) 281.
15 S. Utsumi, T. Nakamura and T. Mori, *J. Agric. Food Chem.*, 31 (1983) 503–506.
16 J. R. Bacon, N. Lambert, M. Phalp, G. W. Plumb and D. J. Wright, *Anal. Biochem.*, 160 (1987) 202–210.
17 J. A. Gatehouse, R. R. D. Croy and D. Boulter, *Biochem. J.*, 185 (1980) 497–503.
18 *FPLC Application File: Analysis of Food Proteins*, Pharmacia, Uppsala, 1985.
19 N. Lambert, G. W. Plumb and D. J. Wright, *Biochem. Soc. Trans.*, in press.
20 A. C. Eldridge and W. J. Wolf, *Cereal Chem.*, 44 (1967) 645–668.
21 J. Cully, *Ph. D. Thesis*, University of Durham, Durham, 1987.
22 K. Hasegawa, M. Murata and S. Funjino, *Agric. Biol. Chem.*, 42 (1978) 2291–2297.
23 N. Kamiya, K. Sakabe, N. Sakabe, K. Sasaki, M. Sakakibara and H. Noguchi, *Agric. Biol. Chem.*, 47 (1983) 2091–2098.
24 L. S. Robert, C. Nozzolillo, A. Cudjoe and I. Altosaar, *Can. Inst. Food Sci. Technol. J.*, 16 (1983) 196–200.

25 U. K. Laemmli, *Nature (London)*, 227 (1970) 680–685.
26 *FPLC Ion Exchange and Chromatofocusing*, Pharmacia Laboratory Separation Division, Uppsala, 1985.
27 N. Yuno, T. Matoba, M. Hirose and K. Hasegawa, *Agric. Biol. Chem.*, 50 (1986) 983–988.
28 P. G. Righetti, C. Gelfi and E. Gianazza, *Chim. Oggi*, 3 (1986) 55–60.
29 G. B. Divall, *Methods Mol. Biol.*, 1 (1984) 105–111.
30 L. V. S. Sastry, J. W. Paulis, J. A. Bietz and J. S. Wall, *Cereal Chem.*, 63 (1986) 420–27.
31 C. E. Wingard, M. Iqbal and M. Griffin, *Chromatographia*, 21 (1986) 49–54.
32 J. A. Bietz, *Cereal Chem.*, 62 (1985) 201–212.
33 I. L. Batey, *J. Cereal Sci.*, 2 (1984) 241–248.
34 P. I. Payne, K. G. Corfield and J. A. Blackman, *Theor. Appl. Genet.*, 55 (1979) 153–159.
35 K. D. Schwenke, L. Prahl, E. Rauschal, S. Gwiazda, K. Dabrowski and A. Rutkowski, *Nahrung*, 25 (1981) 59–69.
36 E-S. M. Abdel-Aal, A. A. Shehata, A. R. El-Mahdy, M. M. Youssef, *J. Sci. Food Agric.*, 37 (1986) 553–559.