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Precursors of storage proteins in *Lupinus angustifolius*

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The proteins that are synthesized during differentiation and development in the cotyledons of *Lupinus angustifolius* L. were characterized both *in situ* and after purification. The proteins present *in situ* were separated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and subjected to 'Western'-blot analysis to identify immunologically related polypeptides. The major storage proteins of the lupin, conglutins α and β, were both present in juvenile tissue only as higher M_r precursors. For conglutin β, a family of at least three polypeptides of M_r 66000–72000 accumulated during the earliest phases of protein synthesis in the developing cotyledon (20–28 days after flowering). Later in development each of these polypeptides disappeared and there was the concurrent appearance in the cotyledon of the lower-M_r fragments characteristic of mature conglutin β. For conglutin α, an equivalent family of precursor polypeptides of M_r 60000–83000 was detected. Multiple internal sites for proteolytic cleavage of all these precursors appeared to be present. However, processing of the precursors was sufficiently slow to allow them to accumulate to over 50% of total soluble protein in juvenile tissue. The precursors were purified by column chromatography under non-dissociating conditions and shown by ultracentrifugation to be multimeric proteins with M_r values in the range 150000–200000.

The storage proteins of most legumes have been divided into two major types, legumin-like 11S proteins and vicilin-like 7S proteins. For each of these types relatively simple models of subunit structure have been proposed. The 7S storage proteins of *Phaseolus vulgaris* (common bean) (Pusztai & Stewart, 1980), *Vigna unguiculata* (cowpea) (Khan *et al.*, 1980) and *Glycine max* (soya bean) (Thanh & Shibasaki, 1978; Sykes & Gayler, 1981; Yamauchi *et al.*, 1981) all consist of simple trimeric combinations of subunits of only one, two, three or four types. In general these subunits have M_r values in the range 40000–75000. The 11S storage proteins of both soya bean and peas similarly contain only two major types of subunits, although in these cases each subunit type consists of a family of several similar polypeptides (Thomson *et al.*, 1978; Moreira *et al.*, 1979).

The M_r values of these two major families of subunits are approx. 40000 and 20000 respectively. In soya bean these have been shown to be assembled into double hexameric arrays *in vivo* (Badley *et al.*, 1975) and this model has been suggested as a universal model for legumin-like proteins (Derbyshire *et al.*, 1976).

By contrast with the relatively simple structures of these storage globulins, the storage proteins in *Lupinus angustifolius* and related lupin species appear to be substantially more complex. In lupins the two major storage globulins, which form aggregates that behave as 11S and 7S proteins, are respectively conglutins α and β. However, both dissociate into a number of different subunits, and in each case these subunits show a range of M_r from approx. 15000 to 65000 (Blagrove & Gillespie, 1975; Restani *et al.*, 1981). There are in addition extensive differences in the subunits in different *Lupinus* cultivars and species (Gillespie & Blagrove, 1975). Therefore, rather than resembling either the simple subunit structures of the 11S-legumin-like proteins or the simple trimeric structure of the above 7S proteins, both conglutin α and conglutin β more closely parallel the more complex subunit structure of vicilin, the 7S protein of *Pisum*

Abbreviations used: DAF, days after flowering; SDS, sodium dodecyl sulphate.

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sativum. Polypeptides of M_r 12000–34000 are present in the vicilin from peas, in addition to the more conventional polypeptides of M_r 49000–75000 (Thomson *et al.*, 1978, 1980). All the subunits of vicilin are, however, synthesized initially as a range of polypeptides of M_r >49000, and it has been demonstrated both by pulse–chase-labelling and by sequence analysis that the polypeptides of M_r <49000 are produced by proteolytic cleavage of these higher-M_r precursors (Gatehouse *et al.*, 1981, 1982*b*; Chrispeels *et al.*, 1982).

In the present paper we have established that there is an equivalent precursor–product relationship between the complex polypeptides in mature lupin seeds and those polypeptides first synthesized in developing seeds during differentiation. We have in addition compared the synthesis of conglutins α and β with that of a third globulin in the lupin, conglutin γ, as this is a quite distinct protein with only two small subunits, M_r 28000 and 16500, and an entirely different amino acid composition (Blagrove & Gillespie, 1975; Blagrove *et al.*, 1980).

Our results overall indicate that, when redefined in terms of those polypeptides that appear to be primary gene products, the structures of the storage globulins from lupins far more readily fit the simple models proposed for conventional storage proteins.

Materials and methods

Plant materials

L. angustifolius L. cultivar Unicrop was grown in 15cm pots in sandy loam and fertilized weekly with a complete nutrient solution (Blagrove *et al.*, 1976). Plants were grown either in a controlled environment cabinet (12h day/night; 24°C day/19°C night; illumination 850 μeinstein·m^{-2}·s^{-1} from metal arc lamps; photoperiod 12h) or under field conditions in the spring and early summer months. Individual flowers from the terminal rachis only were tagged for subsequent harvest at intervals expressed as days after flowering (DAF). At harvest, both the seed coat and embryo were removed, and the cotyledons were snap-frozen.

Protein extraction

Cotyledons were finely ground in glass-distilled water (10vol.) and freeze-dried to improve subsequent extraction (Millerd *et al.*, 1978). Protein was extracted from the freeze-dried powder either by stirring for 2.5h with 5 vol. (v/w) of 0.1M-Tris/HCl buffer, pH8.0, containing 10% (w/v) NaCl, 0.02% (w/v) NaN_3 and 10mM-2-mercaptoethanol at room temperature or by heating with 5 vol. (v/w) 2% (w/v) SDS in 50mM-sodium phosphate buffer, pH7.0, at 75°C for 15min. Insoluble material was removed by centrifugation for 5min in a Beckman Microfuge or by centrifugation at 18000*g* for 30min at 15°C. The patterns of subunits present in such extracts were independent of the extraction procedure used. Mature meal was defatted by repeated extraction with n-hexane.

Gel electrophoresis

Electrophoresis was carried out on linear-gradient (8–20%, w/v) polyacrylamide gels essentially as described by Spencer *et al.* (1980), after samples containing a maximum of 1mg of protein/ml were heated in 2% SDS/0.15M-2-mercaptoethanol/60mM-Tris/HCl buffer, pH7.5, at 80°C for 2min. The proteins were fixed and stained as described by Gayler & Sykes (1981).

Protein purification

Authentic samples of the globulins conglutin α, conglutin β and conglutin γ that had been purified from mature seeds by the procedure of Blagrove & Gillespie (1975) were used as the primary standards and for antiserum production. Globulins from both immature and mature seeds were also purified by a more rapid procedure designed to minimize the possibility of proteolytic degradation. Protein was extracted from cotyledons as described above, dialysed against 0.15M-sodium phosphate buffer, pH7.2, overnight at 4°C and then fractionated with solid $(NH_4)_2SO_4$. This and all subsequent steps were at 4°C. The fraction precipitated between 50% and 90% saturation was adjusted to 20mg of protein/ml, and 10ml was chromatographed on a column (1.6cm × 30cm) of DEAE-Sephacel equilibrated with 0.05M-sodium phosphate buffer, pH7.5. The column was washed with 100ml of the same buffer before development with a linear gradient of 0–0.5M-NaCl in 560ml of the equilibration buffer. The flow rate was 30ml/h, and 3ml fractions were collected. Fractions containing conglutins α and β were dialysed against 50mM-sodium phosphate buffer, pH7.5, containing 0.4M-NaCl and 0.02% NaN_3, concentrated by ultrafiltration to 30mg of protein/ml content and separately purified (3ml) by gel filtration on a column (1.6cm × 60cm) of Sepharose 6B equilibrated with the same buffer.

Labelling of proteins in detached cotyledons in vivo

Incorporation of [^{3}H]leucine into proteins in detached cotyledons was performed by the procedure of Spencer *et al.* (1980). Cotyledons were placed on 20 μl drops of L-[4,5-^{3}H]leucine (10 μCi) in sterile humidified Petri dishes and incubated under a 15W fluorescent lamp at room temperature. Uptake of [^{3}H]leucine into protein was linear with time for at least 6h. After incubation a 1mm slice of the labelled tissue was extracted in 0.1M-

Tris/HCl buffer, pH8.0, containing 10% NaCl, 0.02% NaN_3 and 10mM-2-mercaptoethanol as above, the extract supernatant was diluted to a final concentration of 0.4M-NaCl in 0.1M-Tris/HCl buffer (pH8.0)/0.02% NaN_3, and particular proteins were precipitated by the addition of affinity-purified antibodies. Immunoprecipitates were washed four times in the same buffer and subjected to SDS/polyacrylamide-gel electrophoresis. ^{3}H-labelled proteins were detected by fluorography (Laskey & Mills, 1975).

Immunochemical techniques

Antisera to conglutins α, β and γ were raised in rabbits and purified by affinity chromatography. To prepare the affinity columns pure conglutins α, β and γ (50mg) in 6ml of 0.5M-NaCl/0.1M-$NaHCO_3$, pH8.3, were each coupled to CNBr-activated Sepharose 4B (2.5g) as described by Casey (1979). Crude antiserum (6ml) was applied to a column (1cm × 10cm) of the relevant conglutin–Sepharose that had been previously equilibrated with 0.5M-NaCl in 0.1M-Tris/HCl buffer, pH8.0. Bound anti-conglutin antibodies were eluted with 0.5M-NaCl in 0.2M-glycine/HCl buffer, pH2.8. The effluent was cooled on ice and neutralized with Na_2HPO_4 during collection. Immunoglobulins were precipitated from the effluent by adding solid $(NH_4)_2SO_4$ to 50% saturation and stored at −20°C in 40mM-sodium phosphate buffer, pH7.0, containing 0.15M-NaCl and 0.02% NaN_3. Affinity-purified antibodies prepared in this way for each of the conglutins α, β and γ showed no cross-reactivity between the different conglutins when tested by Ouchterlony double-gel immunodiffusion or precipitin tests.

'Western'-blot analyses

Proteins initially separated on SDS/polyacrylamide gels were electrophoretically transferred to cellulose nitrate sheets (Schleicher und Schüll, BA85, 0.45μm pore size) by the method of Towbin *et al.* (1979). Transfer was run for 20h at 10V/cm in 25mM-Tris/192mM-glycine/20% (v/v) ethanol at pH8.3 to achieve essentially complete transfer. Immunological detection of transferred proteins on the nitrocellulose was by a modification of the procedure of Burnette (1981). After transfer and staining (15s in Amido-Schwarz), nitrocellulose sheets were shaken at 37°C sequentially in 10mM-Tris/HCl buffer, pH7.4, containing 3% (w/v) bovine serum albumin and 0.15M-NaCl for 1h, and then in the same buffer containing affinity-purified antibodies (30μg/ml) for 2h. Unbound antibodies were removed by washing five times in 10mM-Tris/HCl buffer, pH7.4, containing 0.15M-NaCl. Bound antibodies were located by incubation for 2h in the same buffer containing 0.3μCi of ^{125}I-labelled *Staphylococcus* protein A/ml. Unbound ^{125}I-labelled protein A was removed by extensive washing, and the dried nitrocellulose sheets were exposed to Kodak X-OMAT XRP-5 X-ray film that had been pre-exposed (Laskey & Mills, 1975).

Immunoelectrophoresis

Rocket immunoelectrophoresis was performed as described by Weeke (1973) in 0.5M-Tris/10mM-EDTA adjusted to pH8.6 with solid H_3BO_3 on 0.75%-agarose gels containing 10% (v/v) antiserum or 0.5mg of affinity-purified antibodies/ml. Electrophoresis was at 4V/cm for 48h. Plates were washed in 0.1M-NaCl and stained with 0.1% Coomassie Brilliant Blue R in ethanol/acetic acid/water (5:1:4, by vol.).

Results

Polypeptides in situ in developing cotyledons

The origins of the complex polypeptides present as subunits in conglutins α and β were initially investigated by studying their accumulation *in situ* in developing pods. To avoid complications due to aggregation, all proteins were first heated in excess SDS in the presence of β-mercaptoethanol to reduce disulphide bridges and dissociate all subunit complexes.

The proteins present in total salt-soluble extracts of cotyledons changed continuously throughout the development of cotyledons in pods still attached to the plant (Fig. 1). The development sequence shown in Fig. 1 can be divided into three stages: stage 1, less than 23 DAF, which is before the major burst of protein accumulation in cotyledons; stage 2, 23–40 DAF, when the protein content of seeds increases most rapidly (Fig. 2); stage 3, 40 DAF to maturity (80 DAF), when the rate of accumulation of protein is greatly decreased.

The polypeptides that accumulate in cotyledons during stage 2 are totally different in size from the proteins in the extracts of mature tissue. Densitometric scans of the stained gels show that, at 28 DAF, 75% of the protein had an apparent M_r between 60000 and 83000, whereas in the material taken at 80 DAF less than 15% of protein remained with such M_r values.

Identification of precursor polypeptides

The identity of the high-M_r polypeptides in juvenile tissue was established immunologically. The polypeptides related antigenically to particular mature proteins were identified in the different aged cotyledons by Western-blot analyses with antisera raised against samples of conglutins α, β and γ from mature seed. Fig. 3(*a*) shows the polypeptides detected in this way with anti-(conglutin

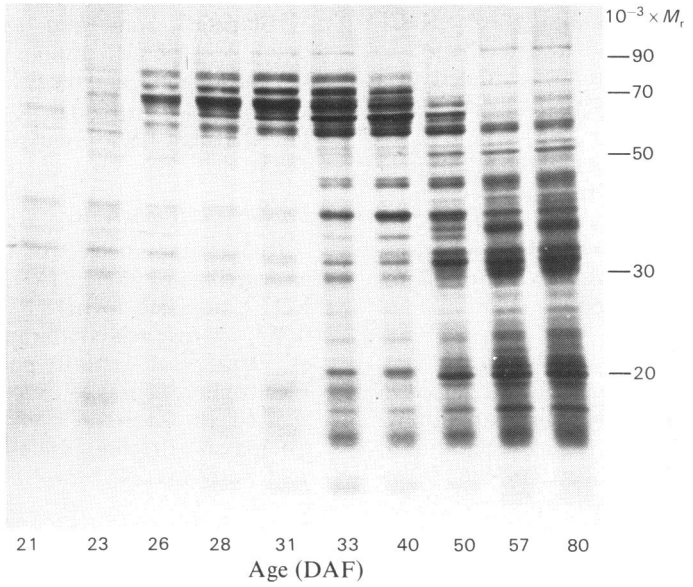

Fig. 1. *Polypeptides from seeds developing on lupins*
The total protein was extracted in 0.1 M-Tris/HCl buffer, pH 8.0, containing 10% NaCl, 0.02% NaN_3 and 10 mM-2-mercaptoethanol, from cotyledons ranging in age from 21 to 80 DAF, and 50 μg of protein/track was subjected to electrophoresis on polyacrylamide gels in the presence of SDS after reduction in 2-mercaptoethanol. Proteins were detected by staining with Coomassie Blue. The scale of apparent M_r used in all Figures was determined with standard proteins.

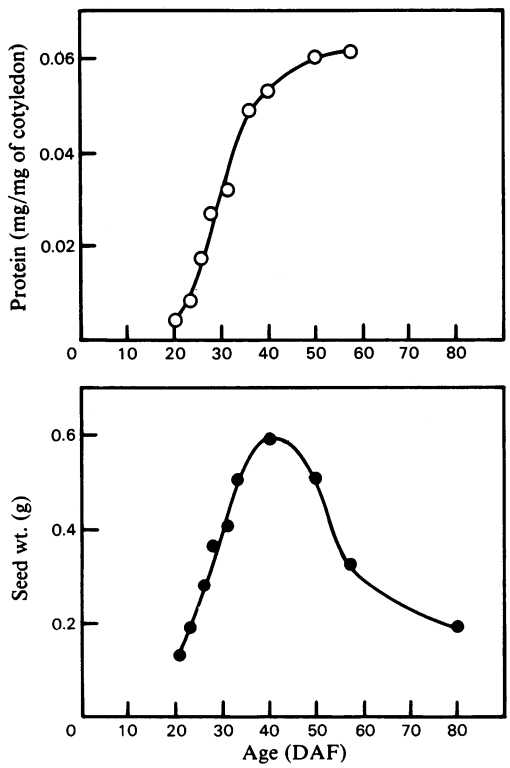

Fig. 2. *Development of lupin seeds*
Changes in fresh weight (●) and protein content (○) of seeds were measured from 21 to 80 DAF.

β) antibodies. In tissue at 80 DAF the expected polypeptides with M_r 15000–54000, which are typical of the subunits of conglutin β from mature seed tissue, were the polypeptides detected in such a Western blot. However, in tissue taken at less than 28 DAF, the major polypeptide recognized by the anti-(conglutin β) antibodies behaved with an apparent M_r of approx. 70000. In subsequent developmental stages (28–33 DAF), second and third major polypeptides also reactive with the antiserum and with M_r 74000 and 68000 respectively also appeared. This complement of high-M_r polypeptides were all produced during stage 2 of development, the period of rapid protein synthesis. The subsequent disappearance of all three of these higher-M_r polypeptides and the concurrent appearance of the expected mature polypeptides in the later stages of pod development suggests a precursor-product relationship between the juvenile and the mature polypeptides detected by their common antigenicity. These larger polypeptides detected by anti-(conglutin β) antibodies in juvenile tissue, but not in mature tissue, were therefore tentatively identified as β-conglutin precursors, and subsequently referred to as such in this paper. Further evidence of their status as precursors is presented below.

Similar precursors to α-conglutin were detected in juvenile cotyledons of lupin by using affinity-purified anti-(conglutin α) serum in both 'Western'

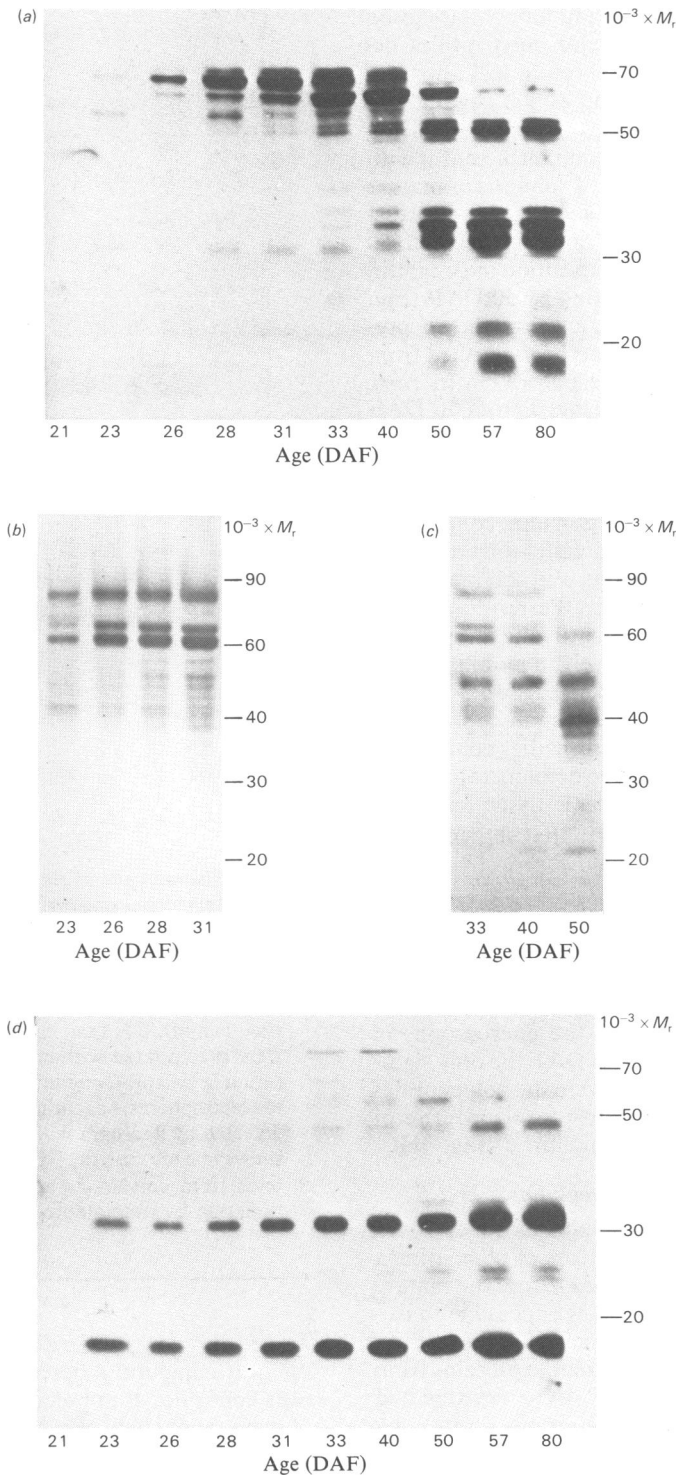

Fig. 3. *Changes in specific polypeptides in developing cotyledons: (a) conglutinin β-related polypeptides; (b) and (c) conglutin α-related polypeptides; (d) conglutin γ-related polypeptides*

Polypeptides from cotyledons of the ages DAF shown were extracted and separated by electrophoresis exactly as indicated in Fig. 1 legend, then analysed by the 'Western'-blot procedure. Proteins that bound (*a*) anti-(conglutin β) antibodies, (*b*) and (*c*) anti-(conglutin α) antibodies and (*d*) anti-(conglutin γ) antibodies were detected by autoradiography after incubation with ^{125}I-labelled *Staphylococcus* protein A.

blots (Fig. 3*b*), and by direct immunoprecipitation (Fig. 7). In this case the major polypeptides detected with anti-(conglutin α) serum had apparent M_r approx. 83000, 68000 and 62000. Again, this group of high-M_r polypeptides disappeared during the later stages of development concurrently with the appearance of the expected subunits of mature α-conglutin, of M_r 22000–50000 (Fig. 3*c*). Processing of conglutin α proceeded rapidly from 30 to 50 DAF, so that the subunit composition of conglutin α detected by 'Western' blotting by 50 DAF (Fig. 3*c*) was essentially the same as that shown previously for conglutin α that had been purified from dried seeds (Blagrove & Gillespie, 1975). Its composition then remained unchanged from 50 DAF through to maturity (80 DAF). The polypeptides of M_r 83000, 68000 and 62000 therefore also behaved as putative α-conglutin precursors.

The behaviour of the third globulin, conglutin γ, was in distinct contrast with conglutins α and β. The only proteins detected even in the most juvenile cotyledons examined by 'Western'-blot analysis with anti-(conglutin γ) antibodies were polypeptides corresponding precisely in mobility to the subunits of mature conglutin γ (Fig. 3*d*). Neither by this procedure nor by direct immunoprecipitation with anti-(conglutin γ) antibodies could high-M_r precursors of conglutin γ be demonstrated to accumulate and survive in juvenile tissue for 10–15 days in a manner equivalent to that shown by the precursors of conglutins α and β.

Synthesis of precursors of conglutins α and β

The nature of the primary polypeptides undergoing the most rapid synthesis in juvenile cotyledons was also determined by radiolabelling of intact cotyledons. As shown in the fluorographs in Fig. 4, in tissue taken at 20–28 DAF the only significant proteins labelled in 2 h were the polypeptides that we have concluded are the precursor forms of conglutin α (Fig. 4*a*) and conglutin β (Fig. 4*b*).

Purification of precursors of conglutins α and β

Because of the unusual length of time, 10–15 days, during which the precursors of conglutins α and β survived in the lupin pods before the mature forms began to accumulate, it was possible to isolate these juvenile proteins essentially free of their mature forms. When the salt-soluble proteins from cotyledons aged 22–28 DAF were fractionated under non-dissociating conditions first by $(NH_4)_2SO_4$ fractionation and then by chromatography on DEAE-Sephacel, precursors of conglutins α and β were separated as shown in Fig. 5. Double-diffusion immunoassay and rocket immunoelectrophoresis (Fig. 6) with antisera raised against each of the mature conglutins α, β and γ were both used to confirm that peak 'α' contained only conglutin α determinants and that peak 'β' only conglutin β determinants. On this basis it was confirmed that precursors of conglutins α and β were completely separated by the procedure.

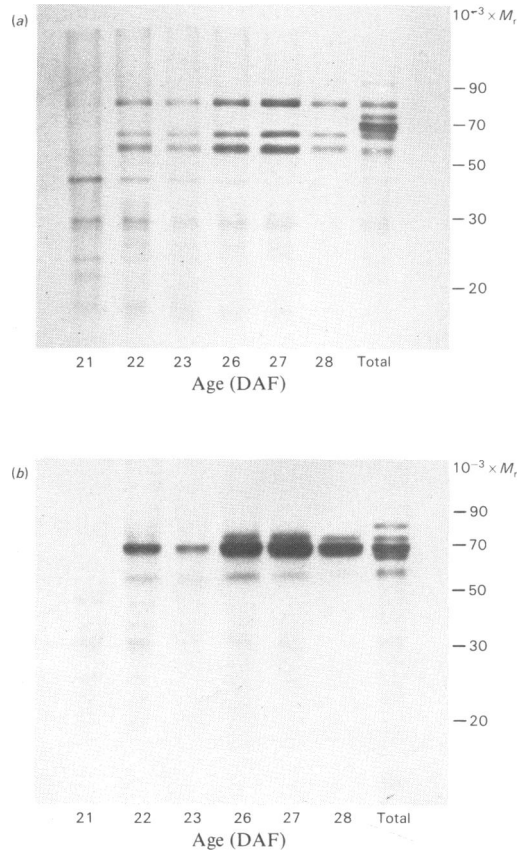

Fig. 4. *Fluorographs of radioactive storage-protein polypeptides from developing cotyledons: (a) conglutin α-related polypeptides; (b) conglutin β-related polypeptides*
Four cotyledons of each age were labelled with 10 μCi of [^{3}H]leucine for 2 h, then protein was extracted as indicated in Fig. 1 legend from a surface slice (1 mm). [^{3}H]Leucine-labelled proteins (80 μg) were precipitated with either (*a*) anti-(conglutin α) serum or (*b*) anti-(conglutin β) serum, then subjected to electrophoresis as indicated in Fig. 1 legend and detected by fluorography. The track marked 'Total' shows in each case the [^{3}H]amino acid-labelled proteins from cotyledons aged 26 DAF before fractionation by immunoprecipitation.

Despite the common antigenicities shown by the purified non-dissociated precursors and their mature equivalents in these immunological tests, both precursors again dissociated only into high-M_r subunits when reduced. Electrophoresis of the reduced proteins on polyacrylamide gels in the presence of SDS (Fig. 7) shows that each purified precursor

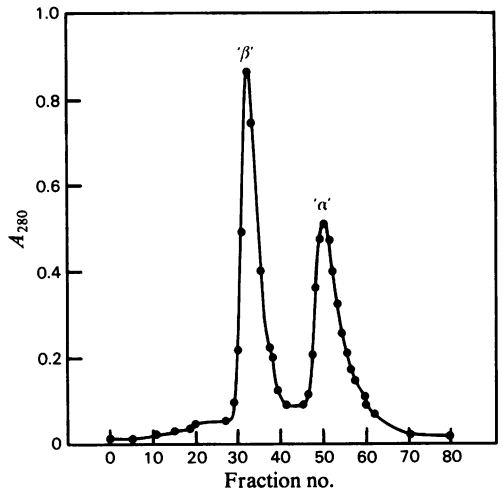

Fig. 5. *Separation of precursors of conglutins α and β on DEAE-Sephacel*

Protein extracted from a bulk sample of cotyledons aged 22–28 DAF was fractionated with $(NH_4)_2SO_4$, and 200mg of the precipitate collected between 50% and 90% saturation was applied to a DEAE-cellulose column (1.6cm × 30cm) in 10ml of 0.05M-sodium phosphate buffer, pH7.5. The column was eluted with 100ml of 0.05M-sodium phosphate buffer, pH7.5, and then with a linear 0–0.5M-NaCl gradient in the same buffer at a flow rate of 30ml/h. The eluate was collected in 3ml fractions and protein measured by A_{280}. Peaks labelled 'α' and 'β' were identified by immunoelectrophoresis as in Fig. 6.

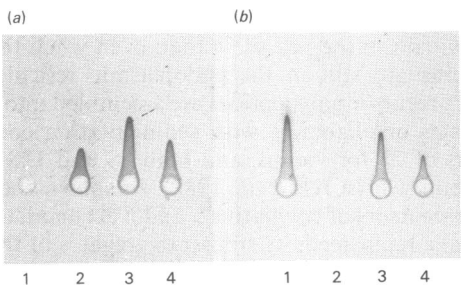

Fig. 6. *Rocket immunoelectrophoresis of precursors of conglutins α and β*

Wells 1 and 2 from left in each case contained 10μg of protein from peak 'α' and peak 'β' (Fig. 5) respectively. Wells 3 and 4 contained 10μg and 5μg respectively of authentic mature conglutin β (*a*) and authentic mature conglutins α (*b*). Gel (*a*) contained 10% (v/v) anti-(conglutin β) serum, and gel (*b*) 0.5mg of anti-(conglutin α) antibodies/ml.

contained only the expected polypeptides of M_r 60000–83000. None of the polypeptides of M_r 50000 and less, which are characteristic of both

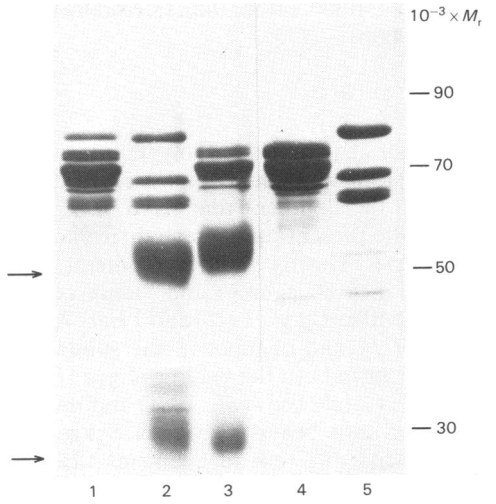

Fig. 7. *Polypeptide composition of precursors of conglutins α and β*

Proteins (50μg/track) were separated by SDS/polyacrylamide gel electrophoresis and stained as in Fig. 1. Track 1 contained the total protein extracted from cotyledons aged 22–28 DAF, tracks 2 and 3 total immunoprecipitates formed in the presence of antiserum to conglutins α and β respectively, and tracks 4 and 5 samples of peaks 'β' and 'α' as separated in Fig. 5. In tracks 2 and 3 the immunoglobulin chains that were also present in the immunoprecipitates are indicated by the arrows.

mature conglutin α and mature conglutin β (Blagrove & Gillespie, 1975), were present.

Physical characteristics of precursors of conglutins α and β

During purification, all evidence suggested that the component subunits of the precursors of conglutins α and β were present as stable oligomers or aggregates. Each of the purified precursors ran as single components during electrophoresis on cellulose acetate strips or in agarose gels run under non-dissociating conditions. Similarly, during chromatography on Sepharose 6B of the purified precursors no evidence was obtained that the subunits could move independently of one another under non-dissociating conditions. During ultracentrifugation the conglutin β precursor again behaved as a single component of $s_{20,w}$ 8.5S. The M_r of this, as determined by sedimentation-equilibrium analysis, was 187000 ± 5000.

The putative conglutin α precursor was more complex, but still showed only two components, one major component of $s_{20,w}$ 8.3S and a minor component of 12.8S. The M_r values of both these forms must also be in excess of 150000. Both precursors therefore are present as multimers or aggre-

gates, $M_r > 150000$, rather than free subunits of M_r 50000–80000.

Discussion

The major changes in the polypeptide composition of the storage-protein complement of the lupin during pod development that have been demonstrated in the present paper have their parallels in other legumes. During pod development, sequential appearance of legumin and vicilin occurs in both peas (Millerd *et al.*, 1978) and *Vicia* (Wright & Boulter, 1972), and changes in the subunit composition occur in both the vicilins of peas (Millerd *et al.*, 1978; Gatehouse *et al.*, 1981) and the β-conglycinins of soya beans (Gayler & Sykes, 1981). Two distinctly different mechanisms that might account for these changes have been established in other legumes. Where each polypeptide is coded for and produced by translation of a separate and specific mRNA, sequential changes in the proteins being synthesized can be the result of changes in the mRNA population present. This mechanism of control appears to apply in at least two cases. Firstly, the changes in the proportions of legumin and vicilin that occur in seeds when peas are grown on a suboptimal sulphur supply have been linked to changes in the amounts of particular mRNA species (Chandler *et al.*, 1983). Secondly, it has likewise been shown that the amounts of the particular mRNA species that code for the 7S and 11S proteins in both peas and soya beans correlate with the patterns of synthesis of these proteins during development of these seeds (Meinke *et al.*, 1981; Gatehouse *et al.*, 1982*a*). For this mechanism to be responsible for the changes in proteins seen in lupins in the present work, large numbers of different mRNA species, each under separate control, would be required. The evidence in the present paper suggests that this is not so. Rather it appears that the complex mixtures of small subunits in the mature seeds are produced by post-translational modifications of a limited number of precursor polypeptides that are in turn the products of a limited number of mRNA species.

Extensive modification in fact takes place with most of the proteins stored in legume seeds, once translation has been completed. Proteolysis of at least one internal cleavage site is necessary for the production of the low-M_r subunits of glycinin, legumin and vicilin from their higher-M_r precursors (Spencer & Higgins, 1980; Gatehouse *et al.*, 1981, 1983; Chrispeels *et al.*, 1982; Tumer *et al.*, 1982). For legumin and glycinin, the proteolysis at the single internal cleavage site in the precursor (M_r 60000–65000) is so rapid that only the processed subunits, of M_r approx. 40000 and 20000, accumulate as detectable proteins in the seed. The half-lives for these legumin and glycinin precursors were established by pulse–chase-labelling as less than 3h (Chrispeels *et al.*, 1982; Barton *et al.*, 1982). This is much shorter than the survival time of the putative precursors for conglutins α and β, which became labelled in equivalent experiments in lupins (Fig. 4).

By contrast with the very rapid cleavage of the precursors of legumin and glycinin, the proteolytic processing of vicilin from its higher-M_r precursors to the many smaller subunits has been shown to require between 6 and 20h (Chrispeels *et al.*, 1982). This lower rate of processing does allow some of the precursors to accumulate in the tissue. It appears that conglutins α and β are produced in the lupin in much the same way. Like the vicilin polypeptides, both the precursor and the processed fragments of the lupin globulins accumulate in the seed. In addition, the accumulation is transient. The precursors largely disappear at later stages of development and there is concurrent appearance of smaller fragments (Figs. 1 and 3). The unusual feature of the lupin is simply that the commencement of the processing is delayed for up to 10 days after the commencement of synthesis, so that in juvenile tissue the undegraded precursors accumulate. Although precursor proteins with such stability are unusual in legumes, equivalent long-lived precursors of the storage protein cucurbitin have however been observed in juvenile water-melon (*Citrullus lanatus* Th.) seeds (Blowers & Wilson, 1983).

The precursors of lupin globulins also resemble those of other legume proteins in one other important way. Studies on the molecular size of legumin and vicilin in the pea show that, even when these proteins are still in the endoplasmic reticulum, their precursor polypeptides are assembled into aggregates or oligomers with sedimentation coefficients of 7S for vicilin, and both 8S and 12S for legumin (Chrispeels *et al.*, 1982). As shown above, the precursors of conglutins α and β accumulate in juvenile lupin seeds as similar aggregates of three or four subunits. Superficially such aggregates also resemble the trimeric structures of the simplest of the mature storage proteins, glycoprotein II (Pusztai & Stewart, 1980) and β-conglycinin (Thanh & Shibasaki, 1978; Sykes & Gayler, 1981). In future studies it will be of interest to determine whether these structural similarities are simply coincidental or whether they are the result of underlying homologies in amino acid sequence equivalent to those recently shown for the above vicilin-like proteins (Lycett *et al.*, 1983).

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