

Identification, Purification and Properties of the Precursor of Conglutin β , The 7S Storage Globulin of *Lupinus albus* L. Seeds

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ABSTRACT

Lupinus albus L. developing cotyledons 35 d after flowering contained a major polypeptide of average M_r 64 000, immunologically related to conglutin β , the 7S storage globulin of this seed. This polypeptide decreased during seed maturation, without completely disappearing in the mature seed. This drop was accompanied by the formation of polypeptide fragments typical of the mature conglutin β . The 64 000 polypeptide has been identified as the precursor polypeptide of conglutin β .

Undenatured conglutin β precursor, purified by ion exchange chromatography and size exclusion chromatography, showed surface and association properties identical to the mature conglutin β molecule. The precursor oligomer, of M_r 190 000, consisted of an association of three 64 000 subunits. They strongly reacted with concanavalin A indicating the presence of covalently linked carbohydrate.

Tryptic treatment of the undenatured conglutin β precursor led to the accumulation of a relatively stable 59 000 polypeptide which was cleaved later on and produced three large polypeptide fragments differing from the mature conglutin β polypeptides.

Key words: Conglutin β , precursor, developing seeds, *Lupinus albus* L.

INTRODUCTION

Legume storage proteins are synthesized during seed development as precursor polypeptides which undergo proteolytic processing similar to typical secretory proteins (for a review see Müntz, Bassüner, Lichtenfeld, Scholz, and Weber, 1985). The first step of the process is the co-translational removal of the pre-pro-polypeptide leader sequence.

A number of post-translational proteolytic events, which usually occur after protein deposition within the protein bodies, converts the pro-protein into its mature form. Proteolysis is generally limited and highly specific (Wright, 1988), although no strict structural requirement has been demonstrated for a storage protein to fulfill its role so far.

The 7S storage proteins are present in most legume seeds and consist of trimeric associations of 40 000–75 000 polypeptides which are often nicked to various extents (reviewed by Casey, Domoney, and Ellis, 1986). The model

for derivation of the polypeptide fragments from the 50 000 pro-polypeptide in pea has been made possible by comparing amino acid with cDNA sequence data (Gatehouse, Lycett, Delauney, Croy, and Boulter, 1983; Lycett, Delauney, Gatehouse, Gilroy, Croy, and Boulter, 1983).

In developing lupin seeds conglutins β and α , which belong to the 7S and 11S classes of storage proteins, respectively, also named vicilin- and legumin-like proteins, are synthesized as precursors which undergo proteolytic processing (Gayler, Boadle, Snook, and Johnson, 1984; Johnson, Knight, and Gayler, 1985; Deckert and Gwozdz, 1991; Duranti, Guerrieri, Cerletti, and Vecchio, 1992). However, a delayed conversion of the precursors leads to their transient accumulation in the developing cotyledons (Gayler *et al.*, 1984). This has allowed the purification and characterization of conglutin α precursors from the developing seeds of *Lupinus albus* L. (Duranti *et al.*, 1992).

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Abbreviations: SDS-PAGE: sodium dodecyl sulphate polyacrylamide gel electrophoresis; PMSF: phenyl methyl sulphonyl fluoride; DEAE: diethyl amino ethyl; DAF: days after flowering; Con A: concanavalin A.

The precursors of conglutin β have been found to consist of polypeptides ranging in M_r from 66 000 to 72 000 in *Lupinus angustifolius* (Gayler *et al.*, 1984) and from 43 000 to 68 000 in *Lupinus luteus* (Deckert and Gwozdz, 1991). These pro-polypeptides have been shown to assemble into multimeric forms of M_r around 190 000 (Gayler *et al.*, 1984). However, a detailed molecular characterization of the lupin conglutin β precursor molecule is still lacking.

The aim of this work was to purify and characterize the conglutin β precursor in white lupin seed. The subunit composition, the presence of covalently linked carbohydrate on the conglutin β precursor polypeptides, their oligomeric assembly and their susceptibility to trypsin have been established. These properties have been compared with those of the corresponding mature protein in order to contribute to the comprehension of the phenomena underlying the proteolytic processing of legume storage protein precursors.

MATERIALS AND METHODS

All chemicals were the purest available from Merck, Sigma and BioRAD.

Plant material

Sweet *Lupinus albus* L. cv. Multitalia seeds were a gift of Professor L. Postiglione. The plants were grown in pots at the Faculty of Agriculture of the University of Milan during the summer months as described by Duranti, Cerletti, Postiglione, and Lanza (1990). Primary flowering was recorded and developing seeds were taken at selected days after flowering. Mature seeds were collected at about 75 DAF.

Seed coats and embryonic axes were removed and the cotyledons were frozen in liquid nitrogen and stored at -30°C prior to use.

Total protein extraction

Total proteins were extracted with 60 mM Tris-HCl buffer, pH 6.8 containing 2% SDS and 2% 2-mercaptoethanol in the ratio 3:1 (v:w) for developing seeds and 10:1 (v:w) for mature seeds. Glycerol (10%) was added before SDS-PAGE analysis.

Purification of precursor and mature conglutin β

Conglutin β precursor was purified as follows. Dehulled lupin seeds at 35 DAF were homogenized in a coffee grinder in the presence of dry ice. The powder was resuspended in distilled water (1/3 w/v) and the suspension brought to pH 5.0. After 2 h stirring in an ice bath the suspension was centrifuged at $10\,000 \times g$ for 20 min at 4°C . The pellet (5 mg), representing the globulin fraction, extracted with 0.05 M sodium phosphate (NaPi) buffer, pH 7.5 containing 1 M NaCl was desalted on a 10×2 cm column of Sephadex G 25 equilibrated with 0.05 M NaPi buffer, pH 7.5. The desalted globulin fraction was loaded on a TSK DEAE 5PW column (7.5×0.8 cm) of a FPLC apparatus by LKB-Pharmacia. Flow rate was $1\text{ cm}^3\text{ min}^{-1}$. Elution was monitored at 280 nm. After elution of a non-retained peak with the same buffer as above, a discontinuous NaCl gradient from 0.0 to 0.5 M was applied. Two composite fractions were eluted with 0.18 and 0.25 M NaCl, respectively. The use of specific antibodies (see a further section) allowed

their identification as the conglutin β and α precursors, respectively.

Aliquots of the major peak from TSK DEAE 5PW corresponding to the conglutin β precursor were loaded on a TSK G4000 SW column of a FPLC apparatus and eluted with 50 mM NaPi buffer, pH 6.8 containing 100 mM NaCl at a flow rate of $0.5\text{ cm}^3\text{ min}^{-1}$. The eluate was continuously monitored at 280 nm. The column was calibrated for apparent M_r determination with the following protein markers by Sigma: apoferritin (M_r 440 000), β -amylase (M_r 200 000), glucose oxidase (M_r 180 000), transferrin (M_r 76 000), egg albumin (M_r 45 000), soybean trypsin inhibitor (M_r 20 100) and α -lactalbumin (M_r 14 400). The regression line obtained had a correlation coefficient of -0.996 .

All the extraction and purification procedures, except HPLC runs, were carried out at 4°C or in an ice bath.

The purification of mature conglutin β from dry lupin seeds followed an identical procedure.

Two protease inhibitors, PMSF and leupeptin to a final concentration of 250 and $100\text{ }\mu\text{M}$, respectively, were added to one preparation to check if proteolysis had occurred during purification. No change either with or without the addition was detected.

Protein concentration of the pooled fractions was determined by using Bradford's method (1976) or by absorbance at 280 nm.

Production and purification of conglutin β antiserum

Antiserum to conglutin β was raised in rabbits as described by Duranti, Faoro, and Harris (1991). Purification of conglutin β antibodies by immuno-affinity chromatography was carried out essentially as described by Casey (1979).

Conglutin β -linked Sepharose 4B column was prepared by coupling 19 mg of a solution of mature protein (7 mg cm^{-3}) to 3 g of CNBr-Sepharose 4B (Pharmacia) by following the manufacturer's recommendations. A column (3×1 cm) was set up and the antiserum was passed through it. The eluate with 0.2 M glycine, pH 2.8 containing 0.5 M NaCl was checked for its reactivity to the major storage proteins of lupin by Western blotting analysis (see a further section). The immuno-affinity purified antisera reacted only with conglutin β polypeptides. They were used immediately or frozen at -30°C .

Electrophoretic analysis

SDS-PAGE was carried out on 15% polyacrylamide gels essentially as described by Laemmli (1970). The protein bands were stained with Coomassie Brilliant blue R250.

Densitometric scannings of the Coomassie blue stained gels were performed on a Laser Densitometer by LKB at 637 nm.

Apparent molecular weights were determined by comparing the electrophoretic mobility of each band with standard protein markers by Pharmacia-LKB. The molecular weights assigned represent the average of at least four independent analyses. Variability coefficients of the means never exceeded 4%.

Quantitative data on conglutin β precursor polypeptides at various DAF were related to a normalized total peak area.

Western blotting analysis

Western blotting analysis was carried out essentially as described by Towbin, Staehelin, and Gordon (1979). After SDS-PAGE, gels were blotted onto membranes of nitrocellulose (NC) by BioRAD with a semi-dry Sartorius blotting apparatus at a constant current of 0.8 mA per square cm for 45 min at room temperature.

Immunodetection was carried out with 1:500 diluted conglu-

tin β antiserum as primary antiserum and with horseradish peroxidase-goat antirabbit antiserum and 4-chloro-1 naphthol by Sigma.

Detection of glycosylated polypeptides reacting with concanavalin A on the blotted NC sheets was carried out according to Hawkes (1982) by using peroxidase and 4-chloro-1 naphthol.

Incubation of conglutin β precursor with trypsin

Aliquots of the peak from TSK G4000 corresponding to conglutin β precursors (0.2 mg cm^{-3} , 0.5 cm^3) were incubated with trypsin (Sigma) as indicated in Fig. 4 in the same elution buffer adjusted to pH 7.5 with an enzyme/protein ratio of 1/1000 (w/w). At given times, from 50 to 100 mm^3 of the solution were withdrawn, denatured with $10\times$ denaturing buffer by heating at 100°C for 5 min and analysed by SDS-PAGE. Mature conglutin β was submitted to the same treatment.

RESULTS

Detection with specific antibodies of conglutin β -related polypeptides in total protein extracts from developing lupin seeds and densitometric scannings of the corresponding Coomassie blue polyacrylamide gel allowed the identification and quantification of a major polypeptide band of average M_r 64 000. Its relative amount at various stages of development is shown in Fig. 1A. This polypeptide band was already present at 29 DAF; it increased until 35 DAF where it reached its highest amount in the cotyledons, and then decreased with the concomitant formation of polypeptides of M_r ranging from about 14 000 to 59 000 which corresponded to the mature conglutin β polypeptides (Fig. 1B). The 64 000 polypeptide was tentatively identified as the conglutin β precursor and is referred to as such henceforth. The developing seeds at 35 DAF were taken as the source for the purification of the 64 000 conglutin β polypeptide. However, sizeable amounts of this polypeptide were still detectable in the mature seed (Fig. 1).

Ion exchange chromatography of the globulin fraction of developing cotyledons separated two peaks in the approximate ratio of 80 to 20 (w:w) with the same NaCl concentration as the mature conglutin β (not shown). The SDS-PAGE pattern of the two protein fractions did not vary significantly (not shown). Therefore, further analyses were carried out on the most prominent peak. When the protein in this peak was submitted to SDS-PAGE, the Coomassie blue stain revealed the presence of other minor bands, which did not react with conglutin β antibodies. These contaminant proteins were removed by size exclusion chromatography on TSK G4000 (Figs 2, 3).

The same column calibrated for M_r determination allowed us to assign a M_r of $190\,000 \pm 2000$ ($n=5$) for the undenatured conglutin β precursor, that suggests a trimeric association of the constituent subunits. The mature conglutin β , also shown in Fig. 2 for comparison, had the same elution volume as its precursor. Occasionally, some protein material of higher M_r was also present in the preparation of mature conglutin β .

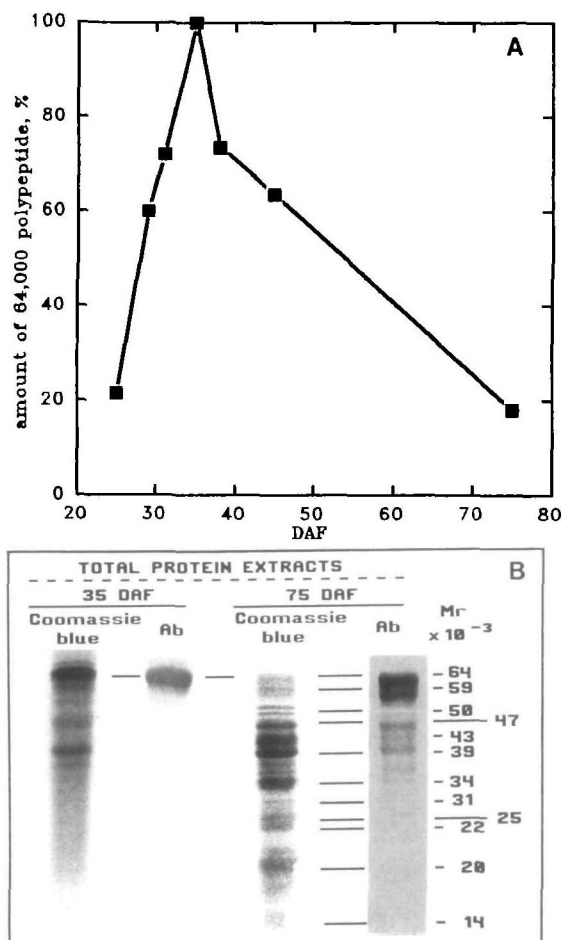


FIG. 1. Relative amounts and SDS-PAGE analysis of conglutin β polypeptides in developing and mature lupin seeds. The amount of conglutin β 64 000 polypeptide in the developing seeds at various days after flowering (DAF) is given in (A) as a percentage of its highest content. Coomassie blue stained polyacrylamide gel and Western blotting analysis with conglutin β antiserum (1:500 dilution) (Ab) of total protein extracts from developing (35 DAF) and mature (75 DAF) lupin seeds are shown in (B). About $25 \mu\text{g}$ of protein were loaded in each track. M_r 's were determined by comparison with standard protein markers. For details see Materials and Methods.

The mature conglutin β and its precursor obtained from size exclusion chromatography were submitted to SDS-PAGE and Western blotting analysis with Con A for carbohydrate staining (Fig. 3). Purified conglutin β at 35 DAF consists of a major polypeptide of 64 000 with a minor component of M_r 59 000, which in some preparations accounted for up to about 30% of the total conglutin β polypeptides (not shown). Both these polypeptides were also present in the mature conglutin β , where they still represented about 15% of the total band area (see also Fig. 1). Three major groups of polypeptides were detectable with Coomassie blue in the mature conglutin β ranging, respectively, from 14 000 to 31 000, from 34 000 to 50 000 and from 59 000 to 64 000. They corresponded to the conglutin β mature polypeptides described by Restani, Duranti, Cerletti, and Simonetti (1981). The

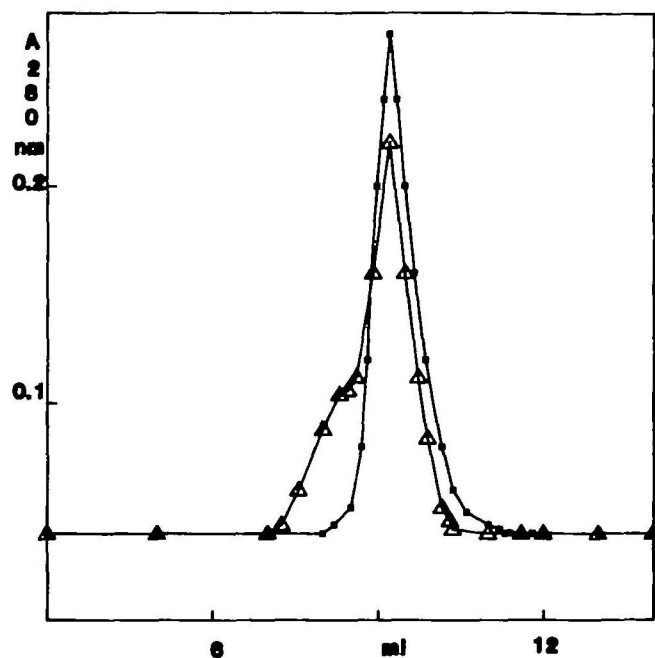


FIG. 2. Size exclusion chromatography of precursor and mature conglutin β . Size exclusion chromatography was carried out on a column of TSK G4000 as detailed in Materials and Methods. About 200 μ g of each protein preparation were loaded. (●—●) Precursor conglutin β . (Δ — Δ) Mature conglutin β .

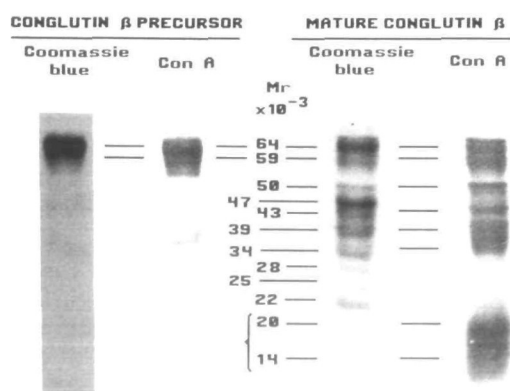


FIG. 3. SDS-PAGE pattern of purified precursor and mature conglutin β . SDS-PAGE and Western blotting analysis for carbohydrate detection with concanavalin A (Con A) of precursor and mature conglutin β were carried out as detailed in Materials and Methods. About 25 μ g of protein were loaded in each track. M_r 's were determined by comparison with standard protein markers.

molar amounts of each group were very similar. The SDS-PAGE patterns were not affected by the presence of reducing agents (not shown), due to lack of cysteine residues in the molecule (Duranti *et al.*, 1981).

Both 64 000 and 59 000 polypeptide bands in the conglutin β precursor reacted with Con A, indicating the presence of covalently linked carbohydrate, whereas in mature conglutin β the prominent polypeptide of 47 000 and those in the group from 22 000 to 34 000 did not react with

Con A. The remaining polypeptide bands, especially those of lower M_r , did react with Con A (Fig. 3).

Figure 4 shows the changes of the conglutin β precursor and the mature conglutin β polypeptides upon incubation with trypsin. The pro-polypeptide of 64 000 progressively decreased with the concomitant increase of the 59 000 polypeptide.

Quantitative analysis by laser scanning densitometry showed that the area of the disappearing band matched that of the newly formed polypeptide (not shown). The 59 000 polypeptide represented an intermediate form which completely disappeared upon more prolonged incubation giving rise to few lower size fragments. Western blotting analysis with concanavalin A showed that the 59 000 intermediate and the two subfragments of 36 000 and 34 000 were glycosylated, whereas that of 22 000 bore no carbohydrate (not shown). A similarly limited proteolysis occurred upon incubation of mature conglutin β with trypsin (Fig. 4). Here the SDS-PAGE pattern was more complex due to the presence of a larger number of polypeptides in the starting material.

DISCUSSION

In *Lupinus albus* seeds we have found a polypeptide of M_r 64 000 which is immunologically related to conglutin β and accumulates in the developing cotyledons until 35 DAF. It subsequently decreases with the concomitant formation of the mature conglutin β polypeptides. Since in developing cotyledons of *Lupinus angustifolius* conglutin β has also been shown to be synthesized as transiently accumulating precursor polypeptides (Gayler *et al.*, 1984), we came to the conclusion that lupin 64 000 polypeptide is the actual precursor of conglutin β in white lupin seeds.

The pro-polypeptide originates the complex array of

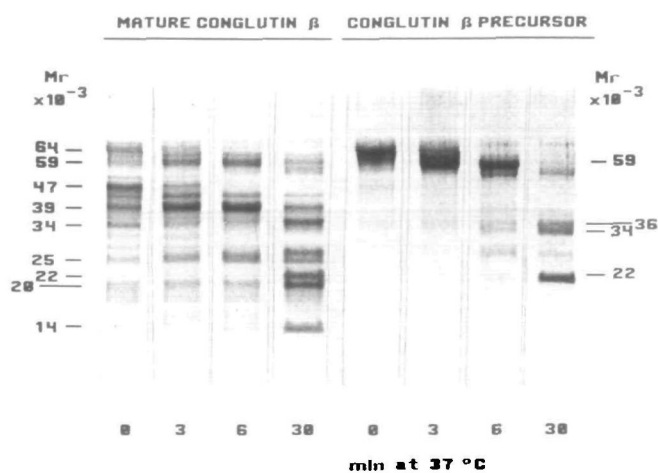


FIG. 4. SDS-PAGE analysis of trypsin-treated purified precursor and mature conglutin β . The precursor and mature conglutin β were incubated for the times indicated as described in Materials and Methods. Aliquots (10–20 μ g) were withdrawn, denatured and submitted to SDS-PAGE. M_r 's were determined by comparison with standard protein markers.

7S protein species already isolated and characterized in *Lupinus albus* (Duranti *et al.*, 1981). A similar molecular heterogeneity arising from one or few pea vicilin polypeptides has been attributed to different nicking points among the precursor(s) (Gatehouse, Croy, Morton, Tyler, and Boulter, 1981). In lupin, a larger combination of processing sites likely gives rise to the high number of 7S mature polypeptides observed. On the contrary, other 7S globulins such as phaseolin (Bollini, Vitale, and Chrispeels, 1983) and conglycinin (Beachy, Jarvis, and Barton, 1981) do not undergo marked post-translational proteolytic modifications.

The similar elution patterns of undenatured conglutin β precursor and the corresponding mature protein from ion exchange (Duranti, Restani, Poniatowska, and Cerletti, 1981), hydrophobic interaction (Sessa and Duranti, unpublished results) and size exclusion chromatography (Fig. 2) suggest that changes of the overall surface properties, the oligomeric structure and the hydrodynamic properties of the molecule has not occurred upon proteolytic conversion of the precursor. On the contrary, a single specific endoproteolytic cleavage in the processing of the precursors of conglutin α , the legumin-like protein of lupin seed, causes dramatic structural changes, resulting in remarkable modifications of the oligomeric assembly and the surface hydrophobicity of this protein (Duranti *et al.*, 1992). Apparently, less structural requirements are necessary for conglutin β than for conglutin α to be correctly deposited in the protein bodies. In addition, the presence of significant amounts of conglutin β precursors in the mature seeds, which has also been described for the corresponding protein in pea cotyledons (Chrispeels, Higgins, and Spencer, 1982), supports the conclusion that post-translational proteolysis may not be a required step in the oligomerization and deposition within the protein bodies of vicilin-like storage proteins as for the legumin-like ones.

In this work we have shown that the conglutin β precursor polypeptides are glycosylated. Whether the site(s), the extent and the type of glycosylation are the same as in the mature polypeptides still remains to be seen. Moreover, the binding of the carbohydrate could be uneven in the various polypeptides, as found to be the case in pea vicilin (Gatehouse, Lycett, Croy, and Boulter, 1982). Since a prominent mature polypeptide of 47 000 has been found to be devoid of carbohydrate, the carbohydrate moiety appears to be located in a region accounting for a quarter of the whole polypeptide, at one of the ends of the precursor polypeptide. This corresponds to what was found in pea (Gatehouse *et al.*, 1982) and broad bean (Scholz, Manteuffel, Müntz, and Rudolph, 1983) vicilins.

The apparent molecular weight found for the undenatured conglutin β precursors (M_r 190 000) is consistent with a trimeric association of 64 000 pro-polypeptides.

The formation of a trimeric oligomer in a number of legume seed storage protein precursors (Ceriotti, Pedrazzini, Fabbrini, Zoppe, Bollini, and Vitale, 1991; Chrispeels *et al.*, 1982; Dickinson, Floener, Lilley, and Nielsen, 1987; Duranti *et al.*, 1992) strongly suggests that a trimer is the translocation-competent general form for the storage proteins to be deposited into the protein bodies.

Although tryptic treatment of the lupin conglutin β precursor does not mimic the *in vivo* formation of the mature polypeptides, the limited breakdown observed allowed some conclusions on the structural properties of this protein to be drawn. Indeed, tryptic cleavage determines the temporary accumulation of a glycosylated 59 000 polypeptide and, later on, the generation of a small number of large-sized polypeptide fragments. Therefore, we conclude that after the removal of a protruding non-glycosylated C- or N-terminal tail of about 50 amino acid residues in the precursor polypeptide, fewer cleavage sites on the molecule become accessible to trypsin. A similar pattern of degradation has been observed in the incubation with trypsin of mature conglutin β , the only difference being that the starting material consisted of already nicked polypeptides. A greater susceptibility to proteolysis of a C terminus 'truncated' phaseolin has been attributed to its lost oligomerization capacity (Ceriotti *et al.*, 1991).

The formation of a relatively stable high- M_r product upon tryptic hydrolysis of soybean glycinin has been found to be due to a zipper-type proteolysis bringing about the most essential changes of glycinin conformation for further proteolysis (Shutov, Pineda, Senyuk, Reva, and Vaintraub, 1991). This is consistent with the currently accepted view of a finely tuned proteolysis which, from a mature storage protein, leads to peptides or free amino acids via the production of progressively more susceptible intermediate polypeptides (Shutov and Vaintraub, 1987).

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LITERATURE CITED

- BEACHY RN, JARVIS NP, BARTON K. 1981. Biosynthesis of subunits of the soybean 7S storage protein. *Journal of Molecular and Applied Genetics* 1, 19–27.
- BOLLINI R, VITALE A, CHRISPEELS MJ. 1983. *In vivo* and *in vitro* processing of seed reserve protein in the endoplasmic reticulum: evidence of two glycosylation steps. *Journal of Cell Biology* 96, 999–1007.
- BRADFORD MM. 1976. A rapid sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72, 248–54.
- CASEY R. 1979. Immunoaffinity chromatography as a means of purifying legumin from *Pisum* (pea) seeds. *Biochemical Journal* 177, 509–20.

- CASEY R, DOMONEY C, ELLIS N. 1986. Legume storage proteins and their genes. *Oxford Surveys of Plant Molecular and Cell Biology* 3, 1–95.
- CERIOTTI A, PEDRAZZINI E, FABBRINI MS, ZOPPE M, BOLLINI R, VITALE A. 1991. Expression of the wild-type and mutated vacuolar storage protein phaseolin in *Xenopus* oocytes reveals relationships between assembly and intracellular transport. *European Journal of Biochemistry* 202, 959–68.
- CHRISPEELS MJ, HIGGINS TJV, SPENCER D. 1982. Assembly and storage protein oligomers in the endoplasmic reticulum and processing of the polypeptides in the protein bodies of developing pea cotyledons. *The Journal of Cell Biology* 93, 306–13.
- DECKERT JE, GWOZDZ EA. 1991. Storage globulins synthesized *in vitro* by polyribosomes and mRNA from developing seeds of *Lupinus luteus*. *Plant Physiology and Biochemistry* 29, 269–80.
- DICKINSON CD, FLOENER LA, LILLEY GC, NIELSEN NC. 1987. Self-assembly of proglycinin and hybrid proglycinin synthesized *in vitro* from cDNA. *Proceedings of the National Academy of Sciences USA* 84, 5525–9.
- DURANTI M, CERLETTI P, POSTIGLIONE L, LANZA AMR. 1990. Synthesis of storage proteins and seed development in *Lupinus albus* under different growth conditions. *Agricoltura Mediterranea* 120, 262–7.
- DURANTI M, FAORO F, HARRIS N. 1991. Immunocytochemical localization of conglutin γ and legumin-like globulin in developing and mature seeds of *Lupinus albus*. *Protoplasma* 161, 104–10.
- DURANTI M, GUERRIERI N, CERLETTI P, VECCHIO G. 1992. The legumin precursor from white lupin seed: identity of the subunits, assembly and proteolysis. *European Journal of Biochemistry* 206, 941–7.
- DURANTI M, RESTANI P, PONIATOWSKA M, CERLETTI P. 1981. The seed globulins of *Lupinus albus*. *Phytochemistry* 20, 2071–5.
- GATEHOUSE JA, CROY RRD, MORTON H, TYLER M, BOULTER D. 1981. Characterization and subunit structure of the vicilin storage proteins of pea (*Pisum sativum* L.). *European Journal of Biochemistry* 118, 627–33.
- GATEHOUSE JA, LYCETT GW, CROY RRD, BOULTER D. 1982. The post-translational proteolysis of the subunits of vicilin from pea (*Pisum sativum* L.). *Biochemical Journal* 207, 629–32.
- GATEHOUSE JA, LYCETT GW, DELAUNEY AJ, CROY RRD, BOULTER D. 1983. Sequence specificity of the post-translational proteolytic cleavage of vicilin, a seed storage protein of pea (*Pisum sativum* L.). *Biochemical Journal* 212, 427–32.
- GAYLER KR, BOADLE BG, SNOOK M, JOHNSON ED. 1984. Precursors of storage proteins in *Lupinus angustifolius*. *Biochemical Journal* 221, 333–41.
- HAWKES R. 1982. Identification of concanavalin A-binding proteins after sodium dodecyl sulphate-gel electrophoresis and protein blotting. *Analytical Biochemistry* 123, 143–6.
- JOHNSON ED, KNIGHT J, GAYLER KR. 1985. Biosynthesis and processing of legumin-like storage proteins in *Lupinus angustifolius* (lupin). *Biochemical Journal* 232, 673–9.
- LAEMMLI UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–85.
- LYCETT GW, DELAUNEY AH, GATEHOUSE JA, GILROY J, CROY RRD, BOULTER D. 1983. The vicilin gene family of pea (*Pisum sativum* L.): a complete cDNA coding sequence for preprovicilin. *Nucleic Acid Research* 11, 2367–80.
- MÜNTZ K, BASSÜNER R, LICHTENFELD C, SCHOLZ G, WEBER E. 1985. Proteolytic cleavage of storage proteins during embryogenesis and germination of legume seeds. *Physiologie Végétale* 23, 75–94.
- RESTANI P, DURANTI M, CERLETTI P, SIMONETTI P. 1981. Subunit composition of seed globulins of *Lupinus albus*. *Phytochemistry* 20, 2077–83.
- SCHOLZ G, MANTEUFFEL R, MÜNTZ K, RUDOLPH A. 1983. Low-molecular-weight polypeptides of vicilin from *Vicia faba* L. are products of proteolytic breakdown. *European Journal of Biochemistry* 132, 103–7.
- SHUTOV AD, PINEDA J, SENYUK VI, REVA AV, VAINTRAUB IA. 1991. Action of trypsin on glycinin. *European Journal of Biochemistry* 199, 539–43.
- SHUTOV AD, VAINTRAUB IA. 1987. Degradation of storage proteins in germinating seeds. *Phytochemistry* 26, 1557–66.
- TOWBIN H, STAHELIN T, GORDON J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proceedings of the National Academy of Sciences USA* 76, 4350–4.
- WRIGHT DJ. 1988. The seed globulins. In: Hudson BGF, ed. *Development in food proteins-6* London and New York: Elsevier Applied Science, 119–77.