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Synthesis of Lectin-Like Protein in Developing Cotyledons of Normal and Phytohemagglutinin-Deficient *Phaseolus vulgaris*[1]

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ABSTRACT

The genome of the common bean *Phaseolus vulgaris* contains a small gene family that encodes lectin and lectin-like proteins (phytohemagglutinin, arcelin, and others). One of these phytohemagglutinin-like genes was cloned by L. M. Hoffman *et al.* ([1982] Nucleic Acids Res 10: 7819–7828), but its product in bean cells has never been identified. We identified the product of this gene, referred to as lectin-like protein (LLP), as an abundant polypeptide synthesized on the endoplasmic reticulum (ER) of developing bean cotyledons. The gene product was first identified in extracts of *Xenopus* oocytes injected with either cotyledonary bean RNA or LLP-mRNA obtained by hybrid-selection with an LLP cDNA clone. A tryptic map of this protein was identical with a tryptic map of a polypeptide with the same SDS-PAGE mobility detectable in the ER of bean cotyledons pulse-labeled with either [^{3}H]glucosamine or [^{3}H]amino acids, both in a normal and in a phytohemagglutinin-deficient cultivar (cultivars Greensleeves and Pinto UI 111). Greensleeves LLP has M_r 40,000 and most probably has four asparagine-linked glycans. Pinto UI 111 LLP has M_r 38,500. Unlike phytohemagglutinin which is a tetramer, LLP appears to be a monomer by gel filtration analysis. Incorporation of [^{3}H]amino acids indicates that synthesis of LLP accounts for about 3% of the proteins synthesized on the ER, a level similar to that of phytohemagglutinin.

Carbohydrate-binding proteins called lectins are synthesized by a large variety of plants. These proteins, which might play a role in plant defense mechanisms, accumulate either in vacuoles or extracellularly (for a review, see ref. 9). It has been shown in several cases that lectins are encoded by small gene families with various degrees of sequence identity and different levels of expression in different tissues. In the legumes, for example, certain lectin genes are expressed at high levels in the developing embryos (seeds), and other genes are expressed at much lower levels in vegetative tissues or cell suspension cultures (9).

In the common bean *Phaseolus vulgaris*, the main seed lectin is called PHA,[3] protein that makes up to 10% of total protein of the seed. The two tandemly linked and highly homologous genes, *dlec1* and *dlec2*, encode the two polypeptides PHA-E and PHA-L, respectively (12). The mature protein is a tetramer consisting of these two polypeptides in all possible combinations (17). The protein is made on the rough ER and accumulates in protein storage vacuoles (protein bodies) (2, 3). Although the function of PHA has not been conclusive demonstrated, it has been suggested that it protects bean seeds from insect predation (14), and its interference with mammalian (15, 16) and insect (10) digestive systems has been investigated.

The *P. vulgaris* genome contains a number of sequences that hybridize at low stringency with PHA clones (4, 23), indicating that there may be a number of PHA-like genes. Recent work by Osborn *et al.* (18, 20) shows that some wild accessions of bean contain a PHA-like gene that encodes the seed protein arcelin; this protein is toxic to an important bean bruchid pest, *Zabrotes subfasciatus*, which is not affected by PHA. Another gene, which is expressed in developing seeds and exhibits sequence homology with respect to PHA and arcelin genes, has been characterized by Hoffman *et al.* (11, 13). Since the polypeptide coded by this gene has never been isolated, it is not known whether it has carbohydrate-binding properties, and therefore it is referred to as lectin-like protein or LLP. The LLP gene has about 65% sequence identity with the PHA-E and PHA-L genes, while the identity at the amino acid level is about 45%; small gaps have to be introduced in the LLP sequence for optimal alignment with the PHA sequences, which are slightly longer. The amino-acid sequence of LLP contains a putative N-terminal signal peptide and five potential glycosylation sites and does not have a hydrophobic region of sufficient length to constitute a membrane spanning domain. With these characteristics, the protein should enter the secretory system and may accumulate as a glycoprotein either in the protein storage vacuoles of the seeds or be secreted into the cell wall. We have attempted to identify the product of the LLP gene by labeling cotyledons with radioactive precursors, isolating the ER, and analyzing the radioactive polypeptides after SDS-PAGE. We tentatively identified the LLP protein in the ER as a M_r 40,000 glycoprotein by showing that *Xenopus* oocytes injected with hybrid-selected LLP-mRNA synthesize a glycoprotein with the same M_r (28). We wished to obtain definite proof that the polypeptide tentatively identified as LLP is indeed the product of the LLP gene. This was done by comparing LLP synthesis in two different bean cultivars: Greensleeves and Pinto UI 111 (here-

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[3] Abbreviations: PHA, phytohemagglutinin; LLP, lectin-like protein.

after called Pinto). The two cultivars have similar levels of LLP mRNA, but Pinto has very low levels of PHA mRNA, making it easier to study LLP synthesis. The finding that LLP shows cultivar polymorphism on SDS-PAGE and the results of comparisons of peptide maps of putative LLP made in beans and of LLP made in RNA-injected oocytes allowed us to identify the LLP protein in bean.

MATERIALS AND METHODS

Labeling of Cotyledons

Midmaturation seeds were collected from plants of *Phaseolus vulgaris* cv Greensleeves or Pinto UI 111 grown in a growth chamber; excised cotyledons were placed, flat side down, on 10 μL/cotyledon of high specific activity [^{3}H]amino acid mixture (Amersham International) or D-[6-^{3}H]glucosamine hydrochloride (814 GBq/mmol, Amersham International) and incubated for 2 h at room temperature. Cotyledons were homogenized and an ER-enriched organelle fraction isolated as described (26). To inhibit *N*-glycosylation, cotyledons were preincubated for 3 h with 20 μL of tunicamycin (500 μg/mL in 1 mM NaOH, Sigma), before labeling with [^{3}H]amino acids as described above, in the presence of the same concentration of tinicamycin. Proteins were analyzed by 15% acrylamide SDS-PAGE and fluorography as described (26). Microdensitometry tracing of fluorographs was performed using a Camag TLC Scanner II (Camag, Muttenz, Switzerland) connected with a Merck-Hitachi D-2000 Cromato-Integrator (Merck).

RNA Isolation, Oocyte Injection and Labeling

RNA isolation from membrane-bound polysomes of developing cotyledons and *in vitro* translation in the wheat germ system were performed as described by Bollini *et al.* (5). The *in vitro* translation mixture was supplemented with L-[4,5-^{3}H] leucine (5.85 TBq/mmol, Amersham International). Hybrid-selection of LLP mRNA was performed as described (27), using the recombinant plasmid pPVL134 (13). Isolation, microinjection, culturing, and homogenization of *Xenopus* oocytes were performed as described (27). Oocytes were incubated in modified Barth medium (3 μL per oocyte) supplemented with 111 MBq/mL of D-[2-^{3}H]mannose (740 GBq/mmol, Amersham International). After 24 h labeling, the radioactivity was chased by transferring the oocytes to modified Barth medium and, after 6 h, to medium containing 10 mM unlabeled mannose; the incubation was continued for an additional 18 h before oocyte homogenization.

Peptide Mapping after Partial Tryptic Digestion

This was a modification of the method of Bordier and Crettol-Järvinen (6). Samples were subjected to SDS-PAGE and the gel stained with Coomassie brilliant blue to identify the bands. For each lane, a gel slice containing LLP and PHA was obtained, rinsed in water, and dried under vacuum. Each sample was soaked at room temperature in 5 mL of 150 mM Tris-Cl (pH 6.8), 0.1% SDS, containing 3.5 mg/mL albumin fraction V (Boehringer), and 100 μg/mL trypsin (Worthington). After 2 h the slices were run, perpendicular to the direction of the first electrophoresis, on a second SDS-PAGE which was processed for fluorography.

Gel Filtration

One hundred μL of an ER-enriched subcellular fraction isolated from cotyledons labeled for 2 h with [^{3}H]glucosamine were adjusted to 0.1% Triton X-100 and loaded on a Superose 12HR 10/30 column (Pharmacia, Uppsala, Sweden) connected with a Pharmacia FPLC system. Elution was with 0.05 M Na-phosphate (pH 7.0), 0.15 M NaCl (PBS) at a flow rate of 1 mL/min. Fractions of 500 μL were collected, proteins were precipitated by adding 750 μL of methanol and 7.5 μL of acetic acid, and analyzed by SDS-PAGE and fluorography. Nonradioactive mol wt markers, detected at 280 nm, were catalase (232,000), aldolase (158,000), BSA (68,000), ovalbumin (46,000), and Cyt *c* (12,400).

RESULTS AND DISCUSSION

Abundant Glycosylated Polypeptides with M_r 40,000 in Greensleeves and 38,500 in Pinto as putative LLP

When developing Greensleeves cotyledons are pulse-labeled with [^{3}H]glucosamine and an ER-enriched subcellular fraction (hereafter called ER) isolated, there is a major radioactive polypeptide with SDS-PAGE mobility corresponding to that of LLP synthesized in *Xenopus* oocytes injected with pure LLP mRNA (28). However, the position of that polypeptide is close to the PHA polypeptides. We therefore looked for the presence of this polypeptide in Pinto cotyledons because the Pinto cultivar of *P. vulgaris* has greatly reduced levels of PHA mRNA and protein, whereas the levels of LLP mRNA are the same in the two cultivars (24, 25). Midmaturation cotyledons from Greensleeves and Pinto were incubated for 2 h in the presence of [^{3}H]glucosamine, ER was isolated, and protein patterns were compared by SDS-PAGE and fluorography (Fig. 1). Labeling with [^{3}H]glucosamine allows only the detection of glycoproteins, thus the polypeptides of the abundant storage protein phaseolin and of PHA (vertical bars and asterisks, respectively, in Fig. 1) are heavily labeled in Greensleeves. In Pinto, PHA was detectable only as a very faint single band (asterisk in lane 2), as expected from the very reduced levels of PHA synthesis in this cultivar (25). The identity of phaseolin and PHA polypeptides was established using monospecific antibodies (not shown). The intense band marked with an arrowhead in lane 1, with M_r 40,000, represents putative LLP based on comigration with LLP synthesized by *Xenopus* oocytes injected with LLP mRNA (28): a polypeptide in the same position was not present in Pinto; however, a slightly faster migrating polypeptide (arrowhead in lane 2, M_r 38,500) was present, with the same intensity as putative Greensleeves LLP. The bands in the two cultivators could represent two electrophoretic variants of the same component, due to cultivar polymorphism, as seen for phaseolin (see Fig. 1, vertical bars).

LLP Synthesis by *Xenopus* Oocytes

We compared LLP made in *Xenopus* oocytes upon injection of LLP mRNA from either of the two cultivars, to test

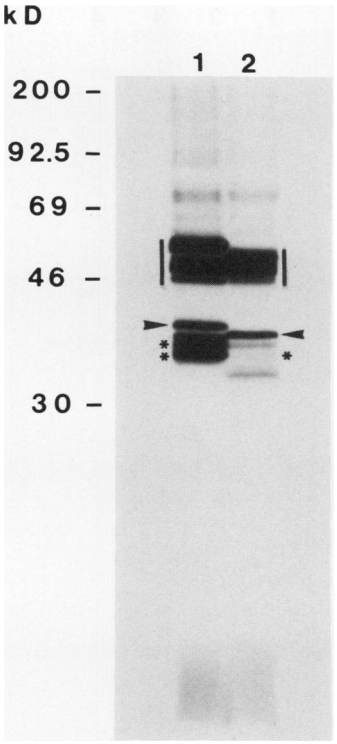

Figure 1. SDS-PAGE and fluorography of *N*-glycosylated polypeptides synthesized in developing cotyledons. Cotyledons were incubated for 2 h in the presence of [^{3}H]glucosamine before ER was isolated and analyzed. Equivalent amounts of protein were loaded on each lane. Lane 1, Greensleeves; lane 2, Pinto. In each lane, arrowhead marks putative LLP, vertical bar marks phaseolin polypeptides, and asterisks mark PHA polypeptides. Numbers on the left refer to the positions of ^{14}C methylated mol wt markers myosin, phosphorylase-b, BSA, ovalbumin, and carbonic anhydrase, from top to bottom.

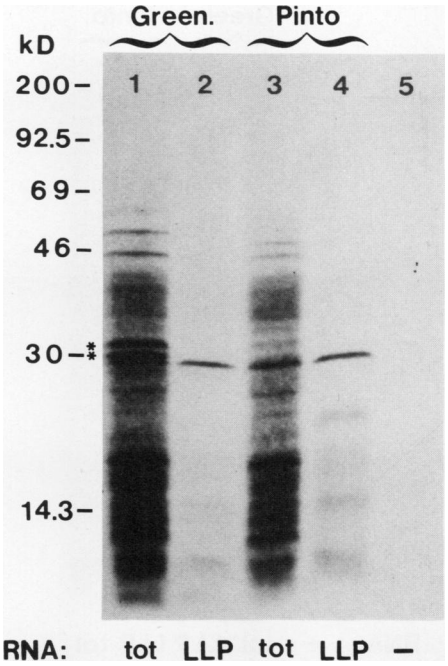

Figure 2. Polypeptides synthesized *in vitro* by mRNA hybrid-selected using an LLP cDNA clone. The plasmid pPVL134 was used to hybrid-select total RNA. Translation of the selected RNA (lanes 2 and 4) or total RNA (lanes 1 and 3) was done with the wheat germ system supplemented with [^{3}H]leucine. Products were analyzed by SDS-PAGE and fluorography. Lanes 1 and 2, Greensleeves; lanes 3 and 4, Pinto; lane 5, no RNA added. Asterisks mark the positions of PHA-E (upper band) and PHA-L (lower band). Numbers on the left refer to the positions of mol wt markers as in Figure 1, plus lysozyme (14.3 kD).

whether the same electrophoretic polymorphism was present for authentic LLP. Polysomes bound to the ER were isolated from Greensleeves or Pinto cotyledons and RNA was extracted (this will be referred to here on as total RNA, for brevity). A LLP cDNA clone (13) was used to hybrid-select the total RNA. The total or selected RNAs were first tested in the wheat germ *in vitro* translation system (Fig. 2). Hybrid-selected RNAs synthesized a single polypeptide with M_r 28,500 (Fig. 2, lanes 2 and 4). This polypeptide migrated slightly below the doublet representing PHA polypeptides (these are indicated by asterisks in lane 1 of Fig. 2). As expected, PHA is not detectable among the products of Pinto total RNA (Fig. 2, lane 3). Similar results have been obtained by Staswick and Chrispeels (24). In our stringency conditions, the hybrid-selected LLP mRNA was not contaminated by PHA mRNA (compare lanes 1 and 2 in Fig. 2).

Hybrid-selected LLP mRNA or total RNA was injected into oocytes. We were unable to obtain a satisfactory level of [^{3}H]glucosamine incorporation into oocyte-synthesized glycoproteins. Higher level of labeled precursor incorporation was obtained using [^{3}H]mannose. Since Asn-linked oligosaccharide chains always contain both *N*-acetylglucosamine and mannose, this was used as a radioactive precursor sugar in the oocytes. Injected oocytes, or uninjected control, were incubated in the presence of [^{3}H]mannose for 24 h followed by a 24 h chase before analyzing total cell homogenates by SDS-PAGE and fluorography (Fig. 3). Injection of LLP mRNA from Greensleeves resulted in the accumulation of glycosylated LLP (arrowhead in Fig. 3, lane 3; note absence in uninjected oocytes, lane 1). When LLP mRNA from Pinto was injected, LLP migrated slightly below Greensleeves LLP (arrowhead in lane 4, and compare with lane 3). LLP was easily detectable also when total RNA from either of the two cultivars was injected (lanes 2 and 5), in agreement with previous results which indicated that this polypeptide is a major *in vitro* translation product of total RNA (24, 28; and Fig. 2 here). The patterns shown in Figure 3 can, however, mislead to an overestimation of the relative level of LLP synthesis with respect to the other abundant bean seed proteins in the injected oocytes. This is due to different reasons. First, LLP carries a higher number of oligosaccharide chains with respect to PHA and phaseolin (see below), and therefore probably incorporated a higher amount of [^{3}H]mannose per polypeptide. Second, phaseolin, like most seed storage proteins, is in large part secreted by the oocytes (1), while LLP and PHA are not (27, 28). The most abundant phaseolin polypeptides were detectable in oocytes injected with total RNA (dots in lanes 2 and 5; note absence of phaseolin in lanes 1, 3, and 4; see also dots in Fig. 4); however, about 70% of total phaseolin synthesized was recovered in the oocyte

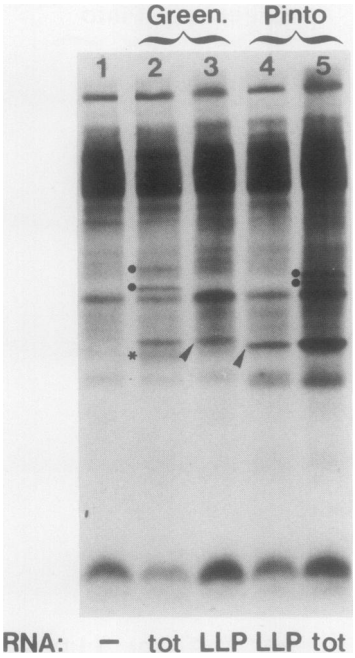

Figure 3. SDS-PAGE and fluorography of *N*-glycosylated polypeptides synthesized in *Xenopus* oocytes injected with cotyledonary RNA. Oocytes were injected and incubated for 24 h in medium containing [^{3}H]mannose, before transferring to unlabeled medium for additional 24 h. Oocytes were then homogenized in the presence of nonioinic detergent (27) and the homogenate equivalent to two oocytes analyzed in each lane. RNA injected was: lane 1, no RNA; lane 2, total RNA from Greensleeves; lanes 3 and 4, hybrid-selected LLP mRNA, from Greensleeves and Pinto respectively; lane 5, total RNA from Pinto. Arrowheads mark LLP, dots mark the major phaseolin polypeptides, and asterisk marks PHA.

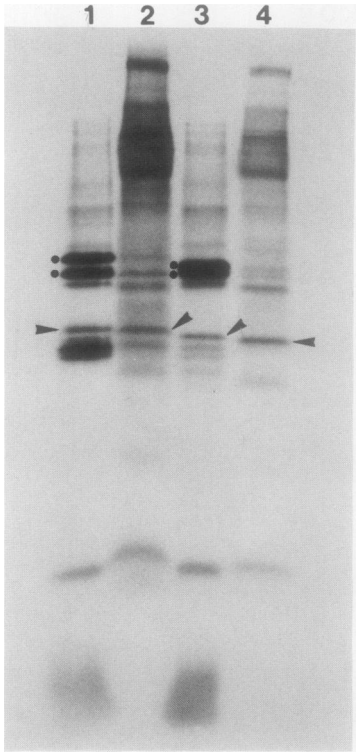

Figure 4. Comparison, by SDS-PAGE and fluorography, of *N*-glycosylated polypeptides synthesized in developing cotyledons and *Xenopus* oocytes injected with cotyledonary RNA. Labelings were as in Figures 1 and 3. Lanes 1 and 3, ER from Greensleeves and Pinto, respectively; lanes 2 and 4, homogenates of oocytes injected with total RNA from Greensleeves and Pinto, respectively. Arrowheads mark the position of LLP, dots mark the major phaseolin polypeptides.

incubation medium (not shown). Third, part of PHA was represented by the not well defined band just below LLP in lane 2 (asterisk), but the lectin has a smeared pattern when synthesized in the oocytes, due to heterogeneity of its processed oligosaccharide chain (27).

The proteins synthesized on bean ER were compared with the proteins synthesized in injected oocytes (Fig. 4): we found that for both Greensleeves (lanes 1 and 2), and Pinto (lanes 3 and 4) putative LLP present in the ER of the cotyledons had an electrophoretic mobility corresponding to oocyte-synthesized LLP (arrowheads), strongly suggesting that the polypeptide present in bean cells was indeed LLP.

Peptide Mapping

A direct confirmation of the identity of bean LLP came from a comparison of the partial peptide maps of oocyte-synthesized and bean-synthesized polypeptides. An SDS-PAGE like the one shown in Figure 4 was performed. For each lane, a gel slice spanning the region were LLP and PHA migrate was treated with trypsin. The resulting peptides were separated by a second SDS-PAGE perpendicular to the direction of the first gel and a fluorograph was made (Fig. 5). Since the proteins were labeled with [^{3}H]glucosamine or [^{3}H]mannose, only the glycosylated peptides could be detected and the maps were relatively simple. Direct comparisons between putative LLP from cotyledons of each cultivar and oocyte-synthesized LLP of the same cultivar (panels a and b for Greensleeves and c and d for Pinto, solid lines), showed that for both cultivars the oocyte maps and the bean maps were virtually identical. In Greensleeves, the map generated by the two PHA polypeptides (Fig. 5, panel a, dotted lines) were almost indistinguishible from each other but completely different from the LLP map. This is in agreement with the high homology between the PHA polypeptides and the differences in sequence and glycosylation between LLP and PHA. Due to the smeared pattern of oocyte-synthesized PHA, the peptide maps of its polypeptides were not well defined (Fig. 5, dotted line in panel b). They appeared, however, to be similar to the maps of bean-synthesized PHA. The results presented in Figure 5 indicate that the bean-synthesized polypeptides with M_r 40,000 and 38,500 are indeed LLP. Different hypotheses can be formulated about the molecular basis of LLP polymorphism between Greensleeves and Pinto. Since LLPs of the two cultivars have the same SDS-PAGE mobility when synthesized *in vitro*, the difference *in vivo* must reside in some co- or posttranslational modification. The difference in mobility might be due to a different number of oligosaccharide chains, an unequal loss of mannose residues after synthesis, different interactions between oligosaccharide chains and

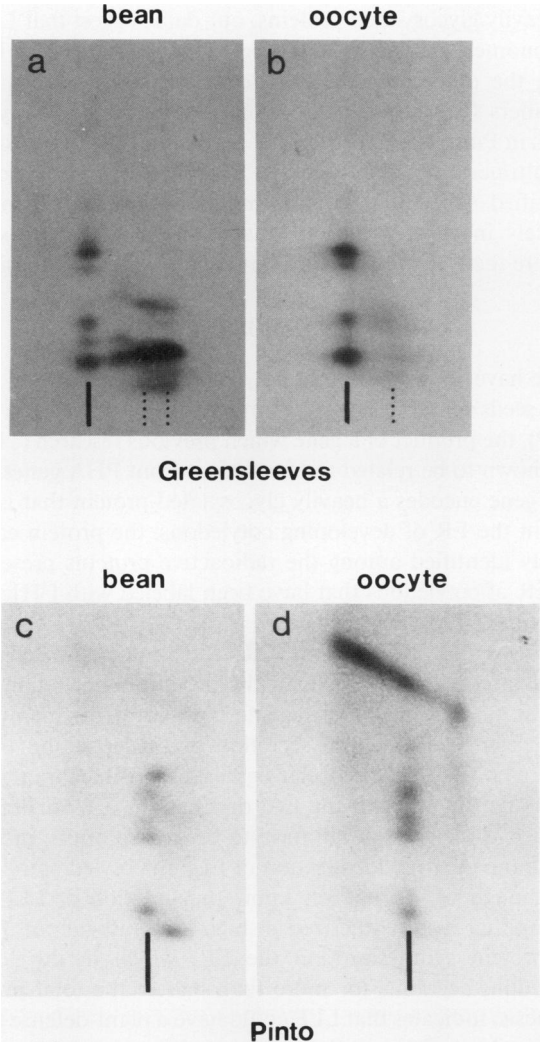

Figure 5. Partial tryptic peptide maps of LLP and PHA. SDS-PAGE with the same samples as in Figure 4 was performed, gel slices were treated with trypsin, and the peptides generated were resolved by running a second dimension SDS-PAGE which was then processed for fluorography. For each panel: first dimension is from left to right, second dimension is from top to bottom. Panels a and b, Greensleeves; c and d, Pinto. Panels a and c, maps of bean-synthesized polypeptides; b and d, maps of oocyte-synthesized polypeptides. Panels a and b and panels c and d are two independent digestions and electrophoresis. Vertical lines at the bottom mark the positions of LLP maps (solids lines) and of PHA maps (dotted lines). Diagonal smear in panel d is due to undigested material.

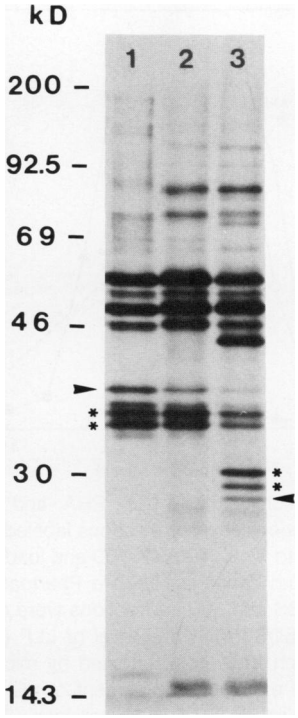

Figure 6. SDS-PAGE and fluorography of polypeptides synthesized on the ER of Greensleeves cotyledons. Lane 1, ER from cotyledons incubated for 2 h in the presence of [^{3}H]glucosamine; lanes 2 and 3, ER from cotyledons preincubated for 3 h either in the absence (lane 2) or in the presence (lane 3) of tunicamycin and then incubated for 2 h with [^{3}H]amino acid mixture. Arrowheads and asterisks mark, respectively, LLP and PHA polypeptides, in the glycosylated (left) or unglycosylated (right) forms.

polypeptide chains, which may alter SDS-PAGE mobility, or unequal removal of the signal peptide.

Level of Synthesis and Extent of Glycosylation of LLP

To gain more information about the relative level of synthesis of LLP and the number of oligosaccharide chains present on each LLP polypeptide the following experiments were performed. Greensleeves cotyledons were labeled for 2 h with [^{3}H]glucosamine or [^{3}H]amino acids, the ER was isolated and labeled proteins analyzed by SDS-PAGE (Fig. 6, lanes 1 and 2). From quantitation of the fluorograph bands by microdensitometry, [^{3}H]amino acids and [^{3}H]glucosamine incorporated into LLP represented 2.8 and 6.0%, respectively, of the total incorporation, while the values for PHA (the sum of the two bands) were 19.5% for [^{3}H]amino acids and 22.0% for [^{3}H]glucosamine. The PHA/LLP ratios for each label, therefore, were 7/1 for [^{3}H]amino acids and 3.7/1 for [^{3}H]glucosamine. The amino acid composition and mol wt of LLP and PHA polypeptides in the unglycosylated form are very similar; therefore, the ratios cited above indicate that there are about twice as many *N*-acetylglucosamine residues incorporated per polypeptide of LLP than per polypeptide of PHA. Since each PHA polypeptide in Greensleeves has two carbohydrate chains (26), there are probably four carbohydrate chains per polypeptide in LLP. This estimate fits with the relative electrophoretic mobility of glycosylated and unglycosylated LLP. Unglycosylated LLP synthesized *in vitro* migrates below unglycosylated PHA-L, (Fig. 2, lane 1, PHA-L is the faster migrating PHA polypeptide) while glycosylated LLP synthesized *in vivo* migrates well above glycosylated PHA-E (Fig. 6, lane 1). In the presence of the *N*-glycosylation inhibitor tunicamycin, developing cotyledons synthesize unglycosylated PHA polypeptides from which the signal sequence is removed (27). We therefore expected that labeling tunicamycin-treated cotyledons with [^{3}H]amino acids would have resulted also in the concomitant reduction in the amount

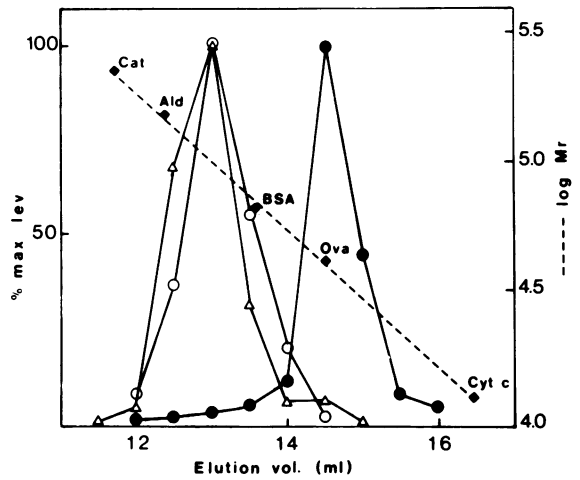

Figure 7. Gel filtration of native LLP, PHA, and phaseolin. An ER preparation from Greensleeves cotyledons labeled with [^{3}H]glucosamine was adjusted to 0.1% Triton X-100 and loaded on a Superose 12 HR 10/30 column connected with a Pharmacia FPLC system. Elution was with PBS and 500 μL fractions were collected. Proteins were analyzed by SDS-PAGE and level of LLP (●), PHA (○), and phaseolin (△) in each fraction determined by microdensitometry of the fluorograph and expressed as percent of the maximum level of each protein. Mol wt markers were catalase (232,000), aldolase (158,000), BSA (68,000), ovalbumin (46,000) and Cyt *c* (12,400).

of glycosylated LLP synthesized and appearance of a new polypeptide migrating below L-PHA. This is what we found (Fig. 6, lane 3, arrowhead, and compare the relative intensities of glycosylated LLP in lanes 2 and 3). The new unglycosylated polypeptide had M_r 27,000, in good agreement with that expected for unglycosylated LLP without the signal peptide. It should be pointed out, however, that at this stage we have no direct proof that the M_r 27,000 polypeptide really represents unglycosylated LLP.

Native Structure of LLP

To determine the degree of oligomerization of LLP, the [^{3}H]Glc*N*-labeled proteins of the bean ER preparation were fractionated by gel filtration on Superose-12 (Fig. 7). LLP eluted from the column in a position corresponding to M_r about 45,000, slightly smaller than ovalbumin. Both PHA and phaseolin eluted in positions around M_r 130,000, as expected for tetramers of PHA polypeptides and trimers of phaseolin polypeptides. Nonradioactive PHA and phaseolin purified from cotyledonary protein bodies eluted in the same positions (not shown). The LLP that eluted from the column was brought to 6 M urea, 0.01% SDS, 0.1% 2-mercaptoethanol, 25 mM NaCl, 0.1 M phosphate buffer (pH 7.0) (denaturing buffer), and heated at 50°C for 30 min before being run again on the same column equilibrated in denaturing buffer. The elution position of LLP was not affected by this treatment. The position of ovalbumin, a monomeric glycoprotein, was also not affected while PHA in denaturing buffer peaked at an elution volume of 15.2 mL, corresponding to M_r around 29,000 which is in good agreement with that expected for PHA monomers (not shown).

Although care should be taken in interpreting the behavior of heavily glycosylated proteins, our data suggest that LLP is a monomer, despite its homology with the tetrameric PHA. Also, the other related lectins of legume seeds are usually tetramers (9), although the lectin synthesized at very low levels in Pinto is a dimer (21). It is possible that LLP acquires a multimeric structure later on after synthesis, in a not yet identified subcellular compartment; however, this appears unlikely in view of the fact that both PHA and phaseolin acquire their native structure immediately after synthesis.

CONCLUSION

We have shown here that cotyledonary cells of developing bean seeds synthesize discrete amounts of a lectin-like protein (LLP), the product of a gene which previous research (11–13) has shown to be related to but distinct from PHA genes. The LLP gene encodes a heavily glycosylated protein that is present in the ER of developing cotyledons; the protein can be readily identified among the radioactive proteins present in the ER of cotyledons that have been labeled with [^{3}H]glucosamine or [^{3}H]amino acids.

Being synthesized on the ER, LLP enters the secretory traffic of cotyledonary cells. If the protein is not retained in the ER or in some intermediate compartment, membrane traffic can lead either to secretion or to deposition in the protein bodies. The molecular signals responsible for targeting to one or the other of the two destinations are not known. Further investigations will have to be carried out in order to determine the final localization of LLP in cotyledonary cells.

Although we do not yet know the function of LLP, the finding that it is synthesized in high amounts (about 3% of the protein synthesized on the ER, which in developing cotyledons accounts for more than half of the total protein synthesis) indicates that LLP could have a plant-defense function similar to PHA and arcelin (14, 18), the other proteins of this gene family. The genes that code for these proteins could have arisen by duplication and divergence from a common ancestor (12, 18). PHA is expressed in about 90% of the cultivars and wild accessions (7, 8), while arcelin occurs in some Mexican wild accessions (19, 22) but has not been so far detected in cultivated bean. The results presented here and elsewhere (24) indicate that LLP is synthesized at similar levels in a normal and a PHA-deficient cultivar. It would be interesting to investigate whether LLP-deficient cultivars or wild accessions can be found. This could provide additional insights into both the evolution of lectin genes and the function of LLP.

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