Communication

Preferential Loss of an Abundant Storage Protein from Soybean Pods during Seed Development

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ABSTRACT

A temporary vegetative storage protein, composed of similar 25 kilodalton and 27 kilodalton subunits, was found to be abundant in soybean (Glycine max (L.) Herr. var Hobbit) leaves, stems, pods, flower petals, germinated cotyledons, and less abundant in roots, nodules and seeds. Total pod protein was highest at 3 weeks after flowering and declined by 37% within 3 weeks during seed development. During this time the vegetative storage protein declined from 18% to 1.5% of the total pod protein and accounted for 45% of the protein lost from pods. This indicates that the vegetative storage protein makes a significant contribution to the pool of nutrients mobilized from pods for transport to developing seeds.

Most studies of nutrient redistribution in plants have investigated changes in total N rather than protein content or composition. In addition to soluble molecules, such as amides and amino acids, proteins in vegetative tissues can contribute substantially to the pool of N and other nutrients which are available to developing seeds (9, 10, 14). This requires that they first be degraded to soluble compounds which can be transported via the xylem or phloem. Wittenbach (17) found that leaf protein levels in soybean declined during seed development, beginning about 4 weeks after flowering.

Though eventually a general loss of protein occurs as senescence approaches, the events preceding senescence are highly organized and certain proteins are lost earlier than others. A glycoprotein, which was localized in vacuoles of leaf bundle sheath and paraevinal mesophyll cells (3), comprised about 10% of the soybean soluble leaf protein at flowering and was preferentially lost from leaves during seed development (16). This temporary storage protein, termed VSP is composed of two similar subunits of around 25 and 27 kD, whose primary structures have been determined by analysis of cDNA clones (11).

Expression of the VSP genes is highly regulated in leaves during plant development. VSP transcript and protein levels were elevated in young leaves which import nutrients, and they declined when the leaves matured and began exporting nutrients to other tissues (12). Blockage of the export path or the removal of plant sinks (i.e., pods and seeds) greatly elevated the expression of these genes in leaves. This pattern of increased expression when amino acids or other metabolites are abundant is consistent with the temporary storage role which has previously been proposed for these proteins (16, 18).

Along with leaves and stems, pods are important contributors to the pool of reserves available to seeds. They account for up to 30% of the N that is mobilized to soybean seeds (5, 19), and pods begin losing N earlier than stems or leaves (8). Pods are a temporary sink for N, which can be stored as either soluble compounds or proteins (10). In pea there is a close correlation between proteolytic activity and protein breakdown in pods during seed development (13). The close proximity of pods to the seeds could facilitate a rapid transport of metabolites derived from proteolysis in pods.

VSP has also been noted in stems and leaf petioles (18). In order to better understand the function of VSP in soybean,

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2 Abbreviations: VSP, vegetative storage protein; WAF, weeks after flowering.
this study examined which plant organs it accumulated in, and whether it played a temporary storage role in seed pods. The results show that VSP is an important storage protein in pods. Modification of this protein by molecular techniques might enhance the availability of precursors for seed protein synthesis.

MATERIALS AND METHODS

Plant Material

Soybean (Glycine max (L.) Merr., var Hobbit) plants were grown in field plots at the University of Nebraska-Lincoln during the summer of 1988. Seed pods were harvested at midmorning, placed on ice, and then frozen at −80°C until analysis. Samples used for Figure 1 were from plants grown in a controlled environment chamber. Samples were all harvested at the time of flowering, except for pods and seeds which were obtained at midseed maturation, and cotyledons which were from 6 d old seedlings.

Protein Isolation and Analysis

Soluble protein was extracted and analyzed by SDS-PAGE and immunoblotting as described previously (12) except that 0.1 mM leupeptin (Sigma) was included in the extraction buffer to limit proteolysis (1) during isolation of proteins from pods at different developmental stages. Samples analyzed by Coomassie-stained gels were first concentrated by precipitation in 70% acetone and then dissolved in an appropriate volume of gel loading buffer. Mol wt standards were from Bethesda Research Laboratories.

Extractable protein levels were quantified with the Bio-Rad protein assay system, with albumin as the standard. Concentrations at each stage of pod development were averaged from three separate analysis of samples of at least 20 pods each. Seed protein was averaged from two analysis of at least 50 seeds for each developmental stage. Levels of VSP in pods were determined by densitometer scans (LKB Ultrascan XL) of immunoblots and comparison with a dilution series of purified VSP (12).

RESULTS AND DISCUSSION

Tissue Distribution of VSP

The presence of VSP in various plant tissues was assayed by immunological staining after transfer from SDS-PAGE gels (Fig. 1). Total protein was loaded at different levels as indicated, to enhance detection in the different samples. Purified VSP (lane 1) yielded the intensely staining bands at 25 and 27 kD. The diffuse staining material at around 55 kD was due to undissociated aggregates of the VSP polypeptides, since the individual 25 and 27 kD bands were recovered if this material was isolated from a gel and electrophoresed again (data not shown). The nature of the doublet below the VSP polypeptides is not known, but it may be the degradation products of VSP.

This analysis revealed that VSP is a ubiquitous protein in soybean, which accumulates not only in all green tissues examined but also in flower petals, roots, and nodules. The amount relative to total soluble protein varied considerably, and the ratio of the two VSP polypeptides differed. Highest VSP levels were found in cotyledons after germination and in stems. Based on the dilution factor relative to purified VSP, this protein was about 10% of the soluble protein in germinated cotyledons. The abundance of VSP 25 was greater than that of VSP 27 in flower petals, pods, and leaves, though the ratio in leaves changed during development (12). The relative abundance of the VSP also changed dramatically at different stages of development in leaves (12) and in pods (see also below).

Low levels of VSP were also present in roots, and VSP 27 but not VSP 25 was detected in nodules. Also evident in the leaf, root, and nodule samples was a crossreacting band at about 29 kD. This may be the product of another VSP gene or may result from differential or incomplete processing of the VSP precursors (11).

Contrary to a previous report (16), VSP was detected in seeds, albeit at relatively low levels. This was not due to contamination with sample from another lane, since increased levels of seed protein loaded in adjacent lanes (data not shown) resulted in a corresponding increase in the intensity of the detected band. The different results may be because the antigen used to elicit antibody production for the present study was deglycosylated and denatured (12) rather than the native protein which was used by the other workers (16). This probably resulted in a more sensitive probe for detecting VSP after blotting, since the gel samples were also denatured.

Figure 1. Western blot analysis of VSP in different plant tissues. Total protein was loaded at the levels indicated. Mol wt are shown on the left. Purified VSP was loaded in lane 1. Lane 2 contained protein from cotyledons 6 d after germination.
Additionally, antibody to glycosylated VSP was found to be much less specific (PE Staswick, unpublished results), presumably due to the presence of similar carbohydrate containing epitopes on other glycosylated plant proteins.

**Changes in Pod Proteins during Seed Development**

Pod proteins were characterized by SDS-PAGE to monitor major qualitative or quantitative differences during seed development. Figure 2A shows that the most striking change was the decline of a very abundant protein of 25 kD. This corresponded in size with the small VSP polypeptide. To verify that this was a VSP polypeptide and to quantify the changes, Western blots (Fig. 2B) of the same samples were scanned by densitometry. Though not so obvious here, differences in staining intensity among the lanes were visually more evident on the original blot. VSP accumulated to 18% of the soluble pod protein at 3 WAF and then declined to 1.5% within 3 weeks. These results are summarized by the dashed line in Figure 3. Based on the densitometric scans, the 27 kD VSP polypeptide accounted for less than 10% of the total VSP at all stages examined.

Certain other proteins were also preferentially lost from pods during this time. The most notable were about 78 and 90 kD. The identity of these less abundant proteins which exhibited a preferential loss similar to VSP is not known. However, the 90 kD protein is the same size as one which accumulates, along with VSP, in the leaves of depodded plants (12). The reason for its transient loss from pods at 3 WAF is not known, but could be due to environmental effects, since the plants were grown in the field. The relative abundance of most other proteins detected here were altered to a lesser extent or not at all during seed maturation.

If pod proteins contribute to the nutrient needs of seeds, then total pod protein levels should decline during seed development. The level of soluble protein extracted from pods and seeds beginning 2 and 3 WAF, respectively, was determined by a standard dye-binding assay and these results are also shown in Figure 3. At 2 WAF seeds were less than 10 mg in fresh weight and by 7 WAF seeds and pods were beginning to turn yellow. Protein levels in pods reached a maximum at 3 WAF and then declined by about 37% between weeks 3 and 6. This was the period when a majority of the seed protein accumulated.

The abundance and preferential loss of VSP from pods (this study) and leaves (12, 16) when nutrients are needed by other developing tissues affirms its role as a temporary storage protein. Since VSP accounted for 45% of the pod protein which was lost between 3 and 6 WAF, it clearly plays a major role in pod protein turnover.
role in supplying the needs of developing seeds. The relatively high abundance of VSP in several vegetative tissues when compared with seeds, supports the use of the term VSP to distinguish this protein from the abundant seed storage proteins, which are unique to the seeds.

The high level of expression and preferential loss of the VSP suggest that plant productivity or seed quality might be improved if the VSP could be engineered by molecular techniques. Enriching the amide-amino acid content of VSP might provide more efficient temporary N storage capacity in soybean, which may permit improved seed yield or higher seed protein content to be attained.

Another possible strategy relates to the sulfur amino acids. The availability of reduced sulfur for developing seeds appears to be less than optimal since methionine supplementation of in vitro cultured soybean cotyledons (15) or whole plants during seed development (4) resulted in a marked increase in the sulfur rich relative to sulfur poor seed storage proteins. VSP is relatively deficient in methionine and cysteine (11). Therefore, enriching the VSP content of these amino acids by genetic engineering techniques may permit greater assimilation and storage of sulfur which could then be available to seeds during their development. The low VSP gene copy number (11) and high expression level seen here would facilitate such an approach.

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LITERATURE CITED