Review–Hypothesis

Compartmentalization of seed reserve proteins

Proposed discriminatory factors

Khosrow Adeli and Illimar Altosaar

Department of Biochemistry, School of Medicine, University of Ottawa, Ottawa, Ontario K1N 9B4, Canada

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Two types of storage pathways are proposed for the synthesis and compartmentalization of seed reserve globulins and prolamins. A discussion of the regulatory mechanisms in reserve protein topogenesis reveals that certain factors such as solubility characteristics of the protein molecule and/or specific sorting sequences may underline the discrimination in deposition of globulins and prolamins. Finally, a hypothetical relationship between the type of storage pathway and the seed protein concentration is briefly discussed.

Reserve globulin *Reserve prolamin* *Seed* *Topogenesis* *Sorting sequence* *Compartmentalization*

1. INTRODUCTORY REMARKS

All plant cells are secretory cells, since they synthesize and discharge the proteins and carbohydrates of their cell walls. Various plant seed cells, however, produce specialized proteins for the specific purpose of storage for later use during germination. Much attention has been focused on the developing storage tissues of legumes and cereals which synthesize and deposit considerable amounts of proteins during embryogenesis [1,2]. The mechanisms of seed reserve protein deposition, however, have not yet been fully clarified and many questions remain unanswered.

Plant seed reserve proteins exhibit no enzymatic activity and have thus been classified on the basis of their physical properties into 4 solubility classes [3]. The major protein fractions in most cereal and legume seeds are the alcohol-soluble prolamins and the saline-soluble globulins, respectively [4]. Globulins which predominate in legume seeds (50% of total seed protein) seem to be synthesized via a common pathway, but unique from the biosynthetic pathway of cereal prolamins. Evidence obtained through in vivo and in vitro protein synthesis studies [5–8], electron microscopic investigations [9,10], and the injection of mRNA sequences coding for seed reserve proteins into surrogate secretory cells [11–13] all support the involvement of two distinct protein depository systems in seed storage tissues. In the present article, the compartmentalization of globulins and prolamins is discussed as two distinct model systems and attempts are made to explore the biochemical basis of this discrimination.

2. GLOBULIN COMPARTMENTALIZATION

The reserve proteins of legume seeds primarily consist of two holoproteins with sedimentation coefficients of 11–12 and 7 S [14]. These protein oligomers comprise several heterogeneous polypeptides which may have arisen as multigene families [15,16]. In pea, for example, the subunits of the 11 S (legumin) and 7 S (vicilin) reserve globulins are synthesized in vivo [17–19] and in

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vitro [17,20,21] as higher-M_r precursors. Nucleotide sequencing of cDNA molecules coding for pea reserve legumin and vicilin polypeptides and comparison with their amino acid sequences have also shown the precursor-product relationship [22,23].

Subcellular fractionation studies have demonstrated that membrane-bound polysomes are the prime biosynthetic site of legumin and vicilin precursors in the developing cotyledons of *Pisum sativum* [7,18], *Glycine max* [17] and *Phaseolus vulgaris* [19,24]. The reserve globulin precursors were shown to be transiently associated with the rough endoplasmic reticulum (RER) shortly after in vivo labeling of developing cotyledons [18,19]. The co-translational segregation of globulin precursors in the RER membrane was also demonstrated using in vitro cell-free translation systems [25,26]. The sequestration of several reserve globulins involves an N-terminal hydrophobic signal peptide. This leader sequence was initially demonstrated by an increase in the electrophoretic mobility of in vitro synthesized globulin precursors in the presence of *Vicia faba* microsomal membranes [17,26]. The leader sequences have now been directly detected by amino acid sequencing [27] and cDNA sequencing [22]. The role of these hydrophobic signal sequences in the segregation of globulin precursors was recently confirmed by Bassüner et al. [28] who demonstrated the involvement of the signal recognition particle (SRP), discovered previously in animal cells [29].

After segregation in the RER, the globulin precursors are translocated into certain protein vesicles (bodies) which seem to be the final site of globulin deposition [18,19,30]. The mode of this transport is not yet known, although several electron microscopic studies [9,31,32] and one subcellular fractionation report [8] have indicated that dictyosomes mediate globulin transport. However, controversy exists about whether the translocation process operates via a vesicular system as in animal secretory cells or via tubular connections. Harris [31] suggested that reserve globulins are translocated from the RER into the Golgi apparatus via tubular ER, arguing against the existence of ER-derived vesicles. Recently, Chrispeels [8] utilized in vivo labeling and subcellular fractionation techniques to demonstrate that phytohemagglutinin (PHA) and phaseolin from *P. vulgaris* seeds, pass through dictyosomes on their way from the RER to the protein bodies. Two types of vesicles were shown to be involved in the PHA transport [8]. Although it is not clear whether ER-derived vesicles are involved, there seems to be general agreement that dictyosomes mediate globulin transport from the RER to protein bodies, a step which may be essential in the correct compartmentalization of reserve globulins.

The 11 and 7 S globulins were shown to be deposited within the same protein body by immunocytochemical localization studies using both electron and fluorescent microscopy [33–35]. The reserve globulin deposition is now known to be accompanied by post-translational proteolytic cleavage of precursors into mature subunits [17–19]. In vivo pulse-chase labeling studies have demonstrated such proteolytic processing to occur for the precursors of legumin and vicilin in pea [7,18]. The final step in this particular depository pathway seems to be the assembly of the processed polypeptides into the 11 and 7 S oligomers as demonstrated for phaseolin [19] and pea legumin [18].

The overall process of globulin deposition has been termed intracellular secretion [13] due to its remarkable homology with the secretory pathway of animal exportable proteins [36]. Direct evidence was recently provided by the injection of mRNA sequences coding for reserve globulins into *Xenopus* oocytes [13]. This experiment indicated the correct synthesis and secretion of pea (*P. sativum*) and bean (*V. faba*) legumin precursors [37] which dictate to the host cell the final destination of the newly synthesized protein.

3. PROLAMIN COMPARTMENTALIZATION

Prolamins are a group of hydrophobic, alcohol-soluble reserve proteins constituting 40–60% of total seed protein in cereals. The chemistry and molecular biology of cereal prolamins have been studied thoroughly [38–40]. Cereal prolamins such as zeins of maize and hordeins of barley are primarily synthesized on membrane-bound polysomes suggesting the existence of a signal peptide on the N-terminal end of their nascent chains

[41,42]. The presence of such N-terminal leader sequences was recently demonstrated for a maize zein polypeptide by comparison of its amino acid and cDNA sequence [43]. Subcellular fractionation studies [5] have also indicated the RER as the prime site of prolamin synthesis in specific cereals. In maize, Larkins and Hurkman [6] showed the synthesis of zein polypeptides by polysomes directly attached to the protein body membrane. This mode of synthesis and segregation, however, has only been demonstrated in maize endosperm so far. Miflin et al. [5] have discussed this aspect of reserve protein synthesis and speculated that cereal prolamins are synthesized on the RER and segregated in the ER cisternae. The newly synthesized prolamins aggregate into clumps within the ER cisternae. In barley and wheat, these aggregates form irregular deposits (protein bodies) after breakage from the RER [5]. However, in maize the proteins form spherical bodies surrounded completely by the RER membrane [5]. The exclusive detection of cytochrome *c* reductase activity (ER marker enzyme) on the membrane of maize protein bodies and not on that of any other cereal seed protein body membranes supports the above hypothesis [5,6].

No biochemical evidence exists to indicate the involvement of dictyosomes in prolamin deposition, although electron microscopic investigations [44,45] have shown the predominance of dictyosomes in wheat endosperm cells during reserve protein synthesis. Bechtel et al. [46] found protease-digestible material in these dictyosomes. Whether the dictyosomes are involved in the transport of the minor wheat globulins [47] or in the translocation of prolamins is not yet clear. Unfortunately, a detailed in vivo pulse-chase labeling study has not been performed to investigate the intracellular deposition of cereal prolamins.

The prolamin system differs in several aspects from the globulin system detailed above. Direct evidence for the distinct mode of the intracellular deposition of a cereal prolamin, maize zein, from that of the legume globulins was provided by the injection of zein mRNA sequences into *Xenopus* oocytes. Larkins et al. [11] demonstrated the correct synthesis of zein polypeptides and deposition in special vesicles in the oocytes; no secretion occurred. The zein-containing vesicles resembled the protein bodies found in the maize endosperm [12].

4. DISCRIMINATORY FACTORS IN GLOBULIN AND PROLAMIN TOPOGENESIS

The regulatory factors which determine whether a certain gene product should remain in the RER or be transported into protein bodies are unknown. The differences observed between the two above depository systems may be due to several phenomena. One apparent factor may be the in vivo solubility characteristics of the particular storage protein sequestered in the RER. There is an obvious difference between the in vitro solubility of globulins (salt-soluble) and prolamins (alcohol-soluble) extracted from legume and cereal seeds, respectively. There is no direct evidence, however, to support this theory. The observation that some cereal seed proteins such as thionins which are salt-soluble in nature remain in the RER cisternae after their synthesis on membrane-bound polysomes argues against the above assumption [48]. Thionins, however, are minor constituents and are not considered as reserve proteins (not found in protein bodies) [48].

The nature of the signal peptides may also be an important factor. Differences in the N-terminal sequences of the nascent chains may contribute to the differential compartmentalization of prolamin and globulin polypeptides. It is generally believed that the mRNA molecule contains the entire information for the final destination of a protein which may be coded by signal sequences or other topogenic amino acid sequences [13,37,49]. Blobel [37] has proposed a hypothesis which predicts that certain discrete segments of a polypeptide chain may determine its intracellular fate or topogenesis. A comprehensive list of signal sequences found in eukaryotic and prokaryotic proteins was recently published [50].

According to the available data, many legume globulins and cereal prolamins possess N-terminal signal peptides [17,22,26,51]. This is to be expected since both globulins and prolamins are synthesized on membrane-bound polysomes [6,7,17]. Weber et al. [52] reported the reconstitution of functional hybrid microsomes from *V. faba* stripped microsomes (smooth ER) and barley endosperm polysomes. They also demonstrated the transfer of in vitro synthesized barley hordeins across the *V. faba* microsomal membranes [52]. Based on these

results, they suggested that an evolutionarily conserved apparatus for the initial steps in the transport mechanisms may exist for legume globulins and cereal prolamins [52]. Therefore, the difference in the intracellular compartmentalization of these two types of reserve proteins may reside in their hypothetical sorting sequences.

This theory is supported by recent investigations on the secretion of plant storage proteins by *Xenopus* oocytes [11–13]. As mentioned above, the oocytes apparently retained the prolamins and secreted the globulins [11–13]. These studies revealed that the globulin mRNAs contain certain signals similar to those of the animal secretory proteins which can be decoded by the *Xenopus* oocyte secretory system; the prolamin mRNAs apparently lack such signals.

Examples of such sequences have been reported to exist in some plant proteins. Lycett et al. [22] recently showed by cDNA sequencing techniques that pea vicilin is synthesized as a preprovicilin with subsequent removal of a N-terminal signal peptide and a C-terminal peptide. The significance of this C-terminal sequence was not known. Another plant protein, thaumatin (sweet-tasting protein isolated from fruits of *Thaumatococcus daniellii*) has also been found to contain a C-terminal sequence which is removed post-translationally after the co-translational cleavage of the N-terminal signal peptide [53]. This acidic C-terminal sequence (6 amino acids long) was suggested to play a role as a topogenic signal for compartmentalization of thaumatin [53]. Recently, Crouch et al. [54] used cDNA sequencing techniques to demonstrate extensive post-translational cleavage of reserve napin precursors from rapeseed: in addition to the removal of an N-terminal leader peptide, two internal sequences which precede the two napin subunits are also post-translationally processed and deleted [54]. The significance of these internal sequences may be in effecting charge neutrality for the napin precursors (each internal sequence possesses an opposite charge to the napin subunit immediately following it). A short peptide (3 residues) was also detected at the C-terminal end of the precursors [54]. The significance of this sequence is unknown.

The C-terminal peptides found in pea vicilin [22] and rapeseed napin [54], as well as the internal sequences reported for napin precursors, may represent sorting sequences. However, no direct evidence is available to support this assumption. It may be worth noting that so far among seed reserve proteins, C-terminal sequences which are removed by post-translational processing have been reported only for globulins, vicilin and napin. The cDNA sequence analysis of a typical prolamin (maize zein) failed to demonstrate the presence of similar extra C-terminal peptides [43]. Further searches in other globulins and prolamins are essential before attributing a role for these C-terminal sequences in differentiating between globulin and prolamin compartmentalization.

Differential compartmentalization of reserve proteins has now actually been observed to occur simultaneously in the same seed storage tissue. Developing rice endosperm cells synthesize reserve prolamins and glutelins (globulin homologues, see [55]) and deposit them in different sets of protein bodies [56,57]. Recent studies have indicated that mechanisms for development of these two types of protein bodies are distinctly different [57]. Large and small spherical protein bodies (prolamin-containing) are developed within the RER cisternae whereas crystalline protein bodies (glutelin-containing) although initiated in the RER, are deposited within intracellular vacuoles [58]. Complete amino acid sequences of these proteins together with their corresponding nucleic acid sequences may eventually reveal whether topogenic domains are involved in this discrimination.

Finally, the differential glycosylation of the newly synthesized polypeptides may also play a role in compartmentalization of seed reserve proteins. In animal cells, the glycosylation of lysosomal proteins can result in their secretion, instead of their segregation into the intracellular lysosomes [59]. Several subunits of the legume 7 S holoproteins [14] as well as maize zein [60] are known to be glycosylated. Reserve protein glycosylation could be a co-translational [60] or a post-translational event [8]. The evidence, however, provided by Badenoch-Jones et al. [61] argues against the role of glycosylation in reserve protein deposition. The inhibition of in vivo protein glycosylation by tunicamycin did not alter the correct synthesis, cleavage and assembly of pea vicilin polypeptides [61].

5. IMPLICATIONS FOR SEED PROTEIN CONCENTRATION

In spite of evolutionary divergence of reserve protein gene expression in legume and cereal seeds, two common depository machineries seem to have been conserved. This conservatism may concern the sorting sequences essential for correct compartmentalization of globulins in vacuolar protein bodies and prolamins in the RER. Significant sequence homology of legume and cereal globulins [55,63] might be an indication of the maintenance of certain portions of the primary amino acid sequences of these reserve proteins.

The significance of conserving two distinct depository systems may be related to the differences in the nature of cereal and legume seeds as storage tissues. The prolamin system seems to operate in seeds which accumulate relatively low levels of protein (cereal seeds) whereas the globulin system is seen to be utilized by seeds which deposit protein abundantly (legume seeds). Recent investigations on the chemistry and biosynthesis of oat reserve proteins [62–69] may raise interest in the above hypothesis. Oat endosperm (*Avena sativa* L.), contrary to other cereal seeds, contains salt-soluble globulin as the major protein fraction (75% of total seed protein) [64,65]. The presence of a legume-like globulin system in oats may have contributed to a higher protein content; oat endosperm is known to have the highest protein content among cereal seeds [70].

Obviously many other molecular or physiological mechanisms might also affect final levels of protein concentration observed in mature grain. These may include, for example, control at the level of transcription, as proposed in wheat [71] or at the level of mRNA translation, as observed in oats [72].

Knowledge of the molecular signals required for the synthesis, transport and deposition of specific storage proteins may aid the plant breeder in changing grain quality for specific characteristics. Recent developments in gene transfer techniques in plants together with protoplast fusion technology are encouraging, leading to the prospect of engineering certain signal sequences which may allow, for example, higher levels of globulins in wheat or higher levels of gluten in oats and legumes.

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