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The Physiological Role of Storage Proteins in Seeds [and Discussion]

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Phil. Trans. R. Soc. Lond. B 1984 **304**, 275-285
doi: 10.1098/rstb.1984.0023

Phil. Trans. R. Soc. Lond. B **304**, 275–285 (1984)

Printed in Great Britain

The physiological role of storage proteins in seeds

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Seed storage proteins provide a source of amino acids and reduced N necessary for germination and early growth of the seedling. Because the long term aim of much of the current research in this area is to modify the composition of the storage protein fraction, it is of interest to ask what kinds of changes might be tolerated by the developing seed without affecting this physiological role. For example, glycosylation and many of the post-translational modifications seen in some legume storage proteins may not be essential and major alterations in the relative amounts of the component proteins in the storage protein fraction are also tolerated. Some nutrient deficiencies result in very extensive changes in this latter category and nutrient deficient plants provide a useful tool for the study of some of the cellular mechanisms that regulate the composition of the storage protein fraction. Sulphur deficiency and potassium deficiency have contrasting effects on the relative proportions of legumin and vicilin in pea seeds. These changes are mainly the result of altered levels of their respective mRNAs together with a change in the pattern of synthesis and accumulation of these two proteins during seed development.

Introduction

Seeds of crop plants provide the major proportion of the protein consumed by humans and their livestock. This fact provides the justification for much of the current research on seed storage proteins. However, these proteins have evolved to fill an entirely different role, namely, to act as a reserve of reduced N that is drawn on to supply the needs of the seedling during germination and early stages of growth. The long-range objective of much of the current research on seed storage proteins is to modify these proteins in such a way that they are better suited to the dietary needs of humans and other monogastric animals such as poultry and pigs. Monogasts must receive in their diet an adequate supply of nine or ten essential amino acids that they are unable to synthesize for themselves. Seed storage proteins of our major crops have some serious imbalances with respect to these essential amino acids. In general terms, the cereal proteins are deficient in lysine, tryptophan and threonine and legume storage proteins are deficient in the sulphur-containing amino acids, cysteine and methionine. The long-term aim of much of our current research is to increase the proportion of these limiting amino acids in seed proteins. However, any modifications that we might aim to bring about must be compatible with those properties of storage proteins that enable them to fulfil their physiological role. These properties can, at present, be defined only in fairly general terms. However, some indication of the permissible degree of variation in the composition of the storage protein fraction can be obtained from a consideration of the kinds of changes that we already know are tolerated by the developing seed. A number of such changes will be reviewed in this paper.

A storage protein can be defined as any protein which (i) accumulates in the seed in significant quantities; (ii) occurs only in the seed; and (iii) can be hydrolysed to release its

constituent amino acids that are then used as a source of reduced N by the seedling during germination and early growth. Consistent with their role as a reserve of reduced N, storage proteins commonly contain a high proportion of the amides, glutamine and/or asparagine.

In general terms, the principal storage proteins of cereals are the prolamins (rich in proline and glutamine, insoluble in water and salt solutions but soluble in aqueous alcohol) and glutelins (insoluble in water, salt solutions and aqueous alcohol, soluble in dilute acid or alkali). One exception is oats where a globulin is the major storage protein (Peterson & Smith 1976). The main storage proteins of legumes are globulins (water-insoluble, salt-soluble), although recently albumins have also been identified that fit the above definition (Bollini & Chrispeels 1978; Youle & Huang 1978; Manickam *et al.* 1980; Schroeder 1983).

There is a remarkable degree of uniformity in the physicochemical properties of storage proteins within these classes in the cereals and in the legumes, respectively. The globulins are either legumin-like or vicilin-like. Legumin type of globulins have a molecular mass of approximately 360000 (12 *S*), consisting of six similar subunits (of approximately 60000 Da molecular mass) each of which in turn contains an acidic and a basic polypeptide (approximately 40000 and 20000 Da, respectively) covalently linked through a disulphide bond. Globulins of the vicilin type have a molecular mass of approximately 180000–200000 Da (7–9 *S*) and a more complex subunit structure than legumins with no involvement of disulphide bonds. All the legume globulins studied to date (as well as oat globulin) belong to one or other of these categories. Prolamins are characterized by their low molecular mass, lack of subunit structure, their high level of proline and glutamine and low level of lysine residues.

In a number of cases this interspecific homology within a particular class of storage protein extends down to the sequence level. The N-terminal sequences of two prolamins, C-hordein from barley and ω-gliadin from wheat show a high level of conservation (Shewry *et al.* 1980) as do the basic subunits of the 12 *S* globulins from peas, broad bean, soybean and pumpkin (Gilroy *et al.* 1979; Hara *et al.* 1978; Casey *et al.* 1981).

The cell biology of storage protein synthesis

In view of the widespread similarity of the physicochemical properties of storage proteins within the cereals and within the legumes that was referred to above, it is reasonable to conclude that these proteins possess certain essential properties that enable them to fulfil their physiological role. Perhaps the most critical property of a storage protein is its characteristic of being synthesized and accumulated within the cell of a developing seed without being subjected to the normal cycle of breakdown and re-synthesis that metabolic proteins such as enzymes and membrane proteins undergo. During germination, these stored proteins must then be able to be broken down to their constituent amino acids.

The strategy which has evolved to meet these requirements is the sequestration of storage proteins in protein bodies where they are not exposed to the proteinases responsible for the breakdown of metabolic proteins. In legumes, the protein bodies arise by progressive deformation and gradual breaking up of the large central vacuole. Both legumin and vicilin are found in the same protein bodies in peas (Craig *et al.* 1979; 1980*a*, *b*). In the cereals maize, wheat and rice, the protein bodies arise from dilations of the endoplasmic reticulum (Larkins & Hurkman 1978; Campbell *et al.* 1981; Oparka & Harris 1982). Rice has two types of protein bodies, one containing prolamin and the other containing glutelin and globulin (Tanaka *et al.* 1980).

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The cell strategy for re-mobilization of storage proteins during germination differs between legumes and cereals. This reflects the fact that the cells of the endosperm and those of the cotyledon follow very different paths during seed development. On imbibition in legumes, the cotyledon cells return to an active anabolic phase. Newly formed hydrolases are secreted into the protein bodies and storage proteins are broken down and, as this process progresses, the small protein bodies coalesce to re-form a large central vacuole (Van der Wilden *et al.* 1980). In cereals, the endosperm at seed maturity is essentially dead tissue. On germination, hydrolases, which are synthesized in the cells of the surrounding aleurone layer, diffuse into the endosperm. The storage proteins are degraded and the resultant amino acids diffuse across the scutellum to the embryo (Marcus & Rodaway 1982). This cycle of selective accumulation of storage proteins during seed formation and selective breakdown during germination is central to the proper physiological functioning of a storage protein.

All the evidence to date is consistent with the notion that most major storage proteins are synthesized on the endoplasmic reticulum and transported via the lumen of the endoplasmic reticulum (reviewed in Higgins & Spencer 1981), and in some cases via the Golgi apparatus (Chrispeels 1983), to their site of deposition in the protein bodies or vacuolar protein bodies. In the case of the storage proteins of peas, the assembly of the newly synthesized polypeptides into oligomers occurs in the lumen of the endoplasmic reticulum before their transport to the protein bodies (Chrispeels *et al.* 1982*b*). The structure of storage proteins must contain the specifications that determine their selective transport from the site of synthesis to the site of accumulation. For example, all storage protein polypeptides studied to date are initially synthesized with a typical leader sequence that facilitates the transport of the nascent polypeptide through the membrane of the endoplasmic reticulum and into the lumen. We understand very little of the sequence requirements that specify the subsequent steps in the transport of storage proteins to, and their deposition in, the protein bodies. A comparison of the deduced amino acid sequence of a number of closely related members of a family of β-conglycinin subunits from soybean, showed that non-homology was restricted to very specific regions. These variable regions were thought to be in those parts of the sequence that would not affect the ability of the polypeptide to form α-helix and β-pleated sheet structures (Schuler *et al.* 1982). A similar comparison of the nucleotide and deduced amino acid sequences of three members of the major pea vicilin subunit family (50 kDa) also showed a high level of sequence conservation (Chandler *et al.* 1984). This is further evidence of selective pressure on conservation of the amino acid sequences in storage proteins.

Modifications to storage protein composition during biosynthesis

There are in theory at least three ways in which we can modify the storage protein fraction of a seed. One way is to change the actual amino acid composition of specific storage protein polypeptides, another is to introduce a storage protein gene from one species into another species and a third way is to make use of the heterogeneous nature of most storage protein fractions and somehow to enhance the proportion of those component polypeptides that are more desirable for our own end uses. As a guide to what changes are tolerable to the developing seed, I will review briefly some of the changes in storage protein composition that have been observed to date. These include chemical changes to the individual polypeptides and extensive changes in the composition of the storage protein fraction.

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Chemical changes due to co- and post-translational modification

Removal of leader sequence

The presence and perhaps removal of the leader sequence (signal peptide) appears to be an obligatory part of the translocation of the nascent storage protein polypeptide chain through the membrane of the endoplasmic reticulum (Higgins & Spencer 1981). There appears to be a high degree of conservation for this step throughout higher organisms since microsomal preparations from either pea cotyledons or dog pancreas will effect the co-translational removal of the leader sequence and the sequestration of the storage protein polypeptide into the microsomal vesicles (Higgins & Spencer 1981). It would be of considerable interest to know whether the movement of the storage protein polypeptide into the endoplasmic reticulum and its subsequent transport to the protein bodies would be blocked if the leader sequence were not removed. Leader sequences in general are characteristically rich in methionine and cysteine, two amino acids that are very deficient in legume storage proteins. In the case of a major polypeptide of pea vicilin (50 kDa), for which the complete amino acid sequence has been deduced from a complementary DNA sequence, there are four methionine and one cysteine residues in the 27 residue long leader sequence and not one residue of either amino acid among the 432 amino acids that make up the mature polypeptide (Chandler *et al.* 1984).

Glycosylation

Legume storage proteins of the vicilin-type (vicilin, phaseolin, β-conglycinin) undergo a second chemical modification, namely glycosylation. Available evidence suggests that this occurs co-translationally and involves the formation of dolichol phosphate-sugar intermediates and the transfer of a 'core' glycosyl chain from the intermediate to the nascent polypeptide chain (Elbein 1979). The glycosyl transferases responsible for this step are localized on the endoplasmic reticulum (Nagahashi & Beevers 1978) and newly synthesized vicilin sequestered in the endoplasmic reticulum is already glycosylated (Chrispeels *et al.* 1982*a*). By analogy with animal systems, further modifications to this side chain may occur in the Golgi apparatus (Hubbard & Ivatt 1981; Chrispeels 1983). Studies on storage protein synthesis in peas, in the presence of the inhibitor tunicamycin, suggest that glycosylation is not an essential step in the synthesis and deposition of vicilin. At levels of tunicamycin that inhibited 80% of all glycosylation, there was no reduction in the level of total protein synthesis in developing cotyledons (Badenoch-Jones *et al.* 1981) and vicilin was synthesized, assembled into its oligomeric form within the endoplasmic reticulum, transported intracellularly and accumulated in the protein bodies in a normal manner (Chrispeels *et al.* 1982*a*, *b*) in spite of an absence of glycosylation. Furthermore, the non-glycosylated vicilin underwent a further series of characteristic post-translational modifications in the protein bodies to yield the range of subunits that are characteristic of the mature vicilin fraction (see below). The 14 kDa subunit of vicilin, which is normally glycosylated, was found in its non-glycosylated form of 12 kDa when synthesis occurred in the presence of tunicamycin (Davey *et al.* 1981).

Post-translational endoproteolytic processing

Some, but not all, legume storage proteins are synthesized as large polyproteins that undergo further modification involving endoproteolytic cleavage of the initial polypeptide chain. This occurs at least 1 h and up to 12 h after the polyprotein is synthesized (Chrispeels *et al.* 1982*a*, *b*).

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All proteins of the legumin type are cleaved to yield the disulphide-bonded acidic and basic polypeptides that are characteristic of the mature protein (Croy *et al.* 1980; Spencer & Higgins 1980; Sengupta *et al.* 1981; Tumer *et al.* 1981; Matlashewski *et al.* 1982). However, among the vicilin type of storage proteins, endoproteolytic processing varies from one species to another. Mature β-conglycinin from soybeans and phaseolin from French bean are made up of three subunits whose size is close to that of the primary translation product. Post-translational processing is mainly restricted to glycosylation (Sengupta *et al.* 1981). Vicilin, the analogous protein in peas, consists of at least 12 subunits, at least seven of which arise by post-translational, endoproteolytic processing of primary translation products. The initial vicilin translation products are polypeptides of 75 kDa, 70 kDa and a family of closely related polypeptides of approximately 50 kDa (Higgins & Spencer 1981; Croy *et al.* 1980). These are assembled into oligomers of the size of the mature protein (7 *S*) within the lumen of the endoplasmic reticulum and are transported to the protein bodies in this form (Chrispeels *et al.* 1982*b*). It is only after deposition of the vicilin oligomers in the protein bodies that they undergo endoproteolytic cleavage to yield the seven additional subunits characteristic of 'mature' vicilin. This processing occurs over a period 6–20 h after polypeptide synthesis (Chrispeels *et al.* 1982*a*, *b*). The additional subunits arise by endoproteolytic cleavage at either one or both of two specific sites in some, but not all, members of the 50 kDa group of polypeptides (Gatehouse *et al.* 1982; Spencer *et al.* 1983). It is probable that this cleavage is accompanied by the loss of a small number of residues at the processing site (Spencer *et al.* 1983). From the fact that the much-cleaved vicilin of peas, and the uncleaved β-conglycinin and phaseolin of soybean and French beans both perform their physiological function equally well, it would appear that this type of post-translational change is acceptable but not essential.

It is interest that the amino acid sequences of the α′ subunit of β-conglycinin (Schuler *et al.* 1982) and a 50 kDa subunit of vicilin (Chandler *et al.* 1983*b*) show close amino acid sequence homology, except in those two specific regions that we have found to be the sites of endoproteolytic cleavage in vicilin.

Changes in composition of the storage protein fraction

The total storage protein fraction of every plant studied so far consists of a number of major protein classes, for example, the prolamins and glutelins in cereals and the legumin type and vicilin type of globulins in legumes. In spite of the overall similarity of their physicochemical properties between species, the members of any one class within a single species show significant microheterogeneity. There is evidence for this in the differing mobility under various electrophoretic conditions and, in some cases, by significant variations in amino acid composition of individual members of a family of closely related polypeptides. The gliadin fraction of wheat can be resolved into 43 components by sequential electrophoresis and isoelectric focusing (Wrigley & Shepherd 1973), and soybean glycinin has been fractionated into five related subunits whose methionine content varies between one and eight residues (Staswick *et al.* 1981).

The proportion of the individual polypeptides that make up a major storage protein fraction and the proportions of the major fractions themselves in any one species are genetically determined. However these proportions are not invariable. It is of interest to examine the extent of these variations as a further indication of the degree of tolerance that exists in the composition of the storage protein fraction of seeds. Variations in storage protein composition can be

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caused by either heritable changes that arise from natural or induced mutation and selection, or by environmentally induced changes superimposed on these genetically determined characteristics.

Heritable changes can result in major alterations in the composition of the storage protein fraction. The high lysine mutants of maize, barley and sorghum show a big reduction in specific components of the prolamin fraction (Larkins 1981). In a survey of 45 lines of the genus *Pisum*, Schroeder (1982) found considerable variation in the proportions of the component polypeptides of the major protein fractions legumin, vicilin and albumin.

Environmentally induced changes in storage protein composition within a single species give a further measure of the flexibility of this system. Both high and low levels of nutrient supply have been shown to have an effect. High levels of nitrogen supply result in a preferential increase in the proportion of prolamins in both barley and wheat (Abrol *et al.* 1971; Koie *et al.* 1976). However, the most-studied situation in this category is that of plants grown under suboptimal nutritional conditions. In a survey of the effect of a range of nutrient deficiencies on the storage proteins of peas, Randall *et al.* (1979) found that deficiencies of potassium and phosphorus increased the proportion of legumin threefold, whereas a deficiency of sulphur resulted in seeds with only a trace of legumin. Deficiencies of magnesium and nitrogen had no effect on legumin levels. Similar striking effects of sulphur deficiency have been observed on the legumin-like protein in lupin (Blagrove *et al.* 1976), and on components of the prolamin (gliadin) fraction of wheat where there was an increase in low-mobility gliadins at the expense of high-mobility gliadins (Wrigley *et al.* 1980). In all these cases, sulphur deficiency results in a reduction of those storage proteins that are more rich in sulphur-amino acids and an increase in the low sulphur proteins. Although extreme sulphur deficiency in peas has a marked effect on total protein per seed, these effects on protein composition are quite marked even at less severe deficiency levels where there is little or no depression of total protein synthesis (P. J. Randall, unpublished observations).

Because of the information it could provide on the cellular mechanisms that regulate the proportions of storage proteins, we have investigated the effect of sulphur and potassium deficiencies on legumin and vicilin synthesis in peas in some detail.

Since sulphur deficiency resulted in marked reduction in the level of legumin a number of parameters were studied that might be involved in bringing about this reduction. These results are summarized in table 1. Sulphur deficiency during seed development results in an 80% reduction in the relative level of cysteine in the free amino acid pool of the cotyledons but had no effect on the level of methionine. However, in the aminoacyl-tRNA pool, which is a more direct measure of the amino acid substrates available for protein synthesis, there was no effect on either cysteine or methionine levels (Macnicol 1983). There was a 90% reduction in the relative rate of legumin synthesis in sulphur-deficient cotyledons and no detectable degradation of legumin in either control or deficient plants (Chandler *et al.* 1983*a*). Consistent with reduced legumin synthesis in sulphur deficient seeds, there was also a 90% reduction of legumin mRNA levels. This was estimated by the hybridization of total RNA from healthy and sulphur deficient cotyledons to a radioactive probe containing complementary DNA to legumin mRNA (Chandler *et al.* 1983*a*). These results indicate that the control of legumin synthesis by sulphur status is largely through control of legumin mRNA levels rather than at the level of translation or legumin stability. Whether, in this situation, legumin mRNA levels are determined by changes in its rate of transcription or in its stability after transcription is currently being investigated.

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As noted earlier, the decreased accumulation of legumin in sulphur deficient seeds is accompanied by an increased accumulation of the other major storage protein, vicilin. Increased vicilin accumulation is achieved in two ways. Firstly, there is an increased rate of vicilin synthesis in sulphur deficient cotyledons compared with healthy ones. This increased rate is reflected in an increased level of mRNA for the major vicilin polypeptide family of 50 kDa. Second, the period of vicilin synthesis is greatly extended in sulphur deficient seeds. In pea seeds, as in all other legumes studied to date, the synthesis of each storage protein polypeptide is under strict developmental control. The synthesis of the 50 kDa polypeptide family extends from approximately 12–20 d after flowering. In sulphur deficient seeds, this synthesis of vicilin continues until approximately 28 d after flowering and this is paralleled by an elevated level of mRNA for the 50 kDa polypeptides throughout this period (Chandler *et al.* 1983*b*).

TABLE 1. THE EFFECT OF SULPHUR DEFICIENCY IN DEVELOPING PEA SEEDS ON A RANGE OF PARAMETERS INVOLVED IN STORAGE PROTEIN ACCUMULATION

	free amino acid pool		amino acyl tRNA pool				
legumin accumulation	Cys	Met	Cys	Met	legumin synthesis	legumin degradation	legumin mRNA
90% reduction	80% reduction	no effect	no effect	no effect	90% reduction	no effect	90% reduction

In order to gain a better understanding of the cellular mechanisms whereby mRNA levels are controlled under sulphur deficient conditions, we tested the system for its ability to make a short-term response to improved sulphur supply. If sulphur deficient plants more than midway through seed development were supplied with adequate sulphur (as sulphate), there was a sharp increase in legumin synthesis and 2 d later legumin mRNA levels were equal to those in healthy, control plants (Chandler *et al.* 1983*a*). The control mechanisms, whatever they may be, are clearly quite reversible and rapid readjustments can be made in response to changes in nutritional status even mid-way through seed development. We do not know the nature of the controlling factor whose level is modulated by sulphur status. It is not a factor that is made elsewhere in the plant since detached pods, or even detached seeds, from sulphur deficient plants showed a similar increase in legumin mRNA when supplied with sulphate (Chandler *et al.* 1983*b*).

Sulphur deficiency also modulates the synthesis and accumulation of individual storage proteins in soybean. Under sulphur deficient conditions that only slightly reduced total protein accumulation, the level of glycinin, the 12 *S*, sulphur rich storage protein, was greatly reduced with a compensating increase in the level of β-conglycinin, the low sulphur, 7 *S* storage protein (K. R. Gayler & G. E. Sykes, personal communication). β-Conglycinin is made up of three classes of subunit, α, α′ and β, the latter having the lowest cysteine and methionine content. In healthy plants these subunits show a well defined and distinctive temporal sequence of synthesis with accumulation of the α and α′ subunits preceding that of the β-subunit by 5–7 d and their level exceeding that of the β-subunit throughout seed development (Gayler & Sykes 1981). Under sulphur deficient conditions, accumulation of the β-subunit began within 2–3 d of the α and α′ subunits and continued at an increased rate and for a longer period than usual during seed development, with the result that the β-subunit was the major component in the second half of seed development. This extra β-subunit appears to accumulate as the β3-isomer

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of β-conglycinin (K. R. Gayler & G. E. Sykes, personal communication), and unusual 7 *S* isomer detected earlier in healthy plants (Sykes & Gayler 1981). The effect of sulphur deficiency on soybeans is thus similar in many respects to its effects on pea seeds in that the synthesis of the least sulphur rich storage protein components is enhanced and the temporal developmental sequence of all storage protein components is modified.

The influence of sulphur nutrition on seed protein accumulation has also been studied in detail in barley (Shewry *et al.* 1983; B. J. Miflin *et al.*, this symposium). Here too the synthesis and accumulation of the sulphur poor gliadins (C-hordeins) is greatly increased relative to the more sulphur rich B- and D-hordeins. These changes in protein synthesis and accumulation under sulphur deficient conditions were paralleled by changes in the relative abundance of the corresponding mRNAs, although there was, in addition, some indication of regulation at the level of translation.

As noted earlier, the potassium status of the pea plant during seed development also has a marked effect on storage protein composition, in this case resulting in a higher proportion of legumin in the deficient plants (Randall *et al.* 1979) and the perturbation of the normal pattern of storage protein accumulation during seed development. The total period of seed development is significantly extended under potassium deficiency and legumin synthesis, which is the major activity in the latter part of seed formation, continues for much longer than in the control plants (D. Spencer, unpublished data). We have also investigated mRNA levels under these conditions and found that this increased level of legumin accumulation is accompanied by elevated levels of legumin mRNA. This ability of the developing seed to accommodate nutritional stresses by the alteration of the normal developmentally regulated pattern of synthesis of the individual storage proteins, and to respond rapidly with altered rates of synthesis of particular proteins when the deficiency is relieved, are further examples of the flexibility of this system.

Plant growth regulators may play a role in the regulation of storage protein synthesis. Although abscisic acid did not appear to be involved in the regulation of storage protein synthesis in developing cotton seeds (Dure & Galau 1981), there was a marked increase in the rate of storage protein synthesis in response to the exogenous addition of abscisic acid to excised embryos from both *Phaseolus vulgaris* (Sussex & Dale 1979) and *Brassica napus* (Crouch & Sussex 1981). Exogenous application of naphthalene acetic acid and benzylaminopurine to pea seeds that develop on the intact plant resulted in increases in both the proportion and in the absolute amounts of legumin in the mature seed. Exogenous application of abscisic acid, on the other hand, caused an increase in the level of vicilin (Schroeder 1983).

Conclusion

The composition of the storage protein fraction can vary between wide limits without preventing seed formation. Chemical changes such as glycosylation and endoproteolytic post-translational processing, changes caused by mutations of the gene and by environmental influences such as nutritional stress and exogenous hormone application, all produce major changes in seed protein composition that are tolerated by the developing seed. In the case of nutritional stress, the changes are accompanied by marked alterations of the normal temporal developmental sequence in which the component storage protein polypeptides are synthesized.

The ability of the developing seed to accommodate these changes and to respond rapidly to altered conditions as late as midway through seed development, as in the case of recovery

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from nutrient deficiency stress, all auger well for our attempts to modify seed proteins to suit our own requirements. In particular, they indicate that major changes are tolerable in the relative amounts of the proteins that make up the total storage protein fraction of the seed.

On the other hand, the degree of sequence conservation among equivalent storage proteins both within and between species points to fairly strict sequence requirements characteristic of each class of protein and essential for its proper physiological function. This in turn suggests that modification of the amino acid composition of specific storage protein polypeptides may not be compatible with their physiological role. However, a more detailed knowledge of the 'variable' and 'constant' regions of homologous storage protein polypeptides may suggest specific regions that would be amenable to chemical modification when and if the technical means are developed to bring about such changes. The greatly increased amount of information now becoming available from amino acid sequencing and recombinant DNA methodology, together with information on the three dimensional structure of storage proteins from X-ray crystallographic analysis, all offer encouragement that a clearer understanding of these essential and variable sequences is at hand.

I am grateful to Dr T. J. V. Higgins for help in the preparation of this manuscript and to all my colleagues in the Seed Protein Group whose work, both published and unpublished, is discussed. Thanks are also due to Dr M. J. Chrispeels, Dr B. J. Miflin, Dr K. R. Gayler and Mr G. E. Sykes for making available their results before publication.

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Discussion

D. Boulter (*Botany Department, Durham University, U.K.*). There is good evidence that a legumin-type storage protein is present in oats (globulin) and rice (glutelin). Alcohol soluble, prolamin-type proteins generally occur in small amounts in legume seeds, but there is little information on their characteristics and they could well be structural proteins.

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