Plant Proteolytic Enzymes

Volume II

Editor

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PREFACE

Knowledge on protein degradation and on the proteolytic enzymes involved appears to be equally important for the understanding of cell metabolism and development and knowledge on protein synthesis. This is not an overstatement. It is simply concluded from the fact that protein degradation and protein synthesis depend on each other. Both processes contribute equally to the vitally important protein metabolism, both of them are equally involved in protein turnover and in all of the changes in quantity and quality of cellular protein as associated with cell differentiations and, hence, with development. Leaf senescence provides just one example demonstrating that even the marked loss of protein depends on protein synthesis. Seed germination may be mentioned as an example for the opposite case in which protein synthesis is intimately associated and even dependent on proteolysis.

Biologists have been much more interested and successful in the elucidation of protein synthesis as compared with protein degradation which has been, and to some extent still is, a rather neglected field. This is certainly due to the fact, that protein synthesis was appreciated as the key to the understanding of the connection between genome and metabolism. However, the elucidation of protein synthesis may have been easier than the work toward the understanding of protein degradation although the latter represents, in biochemical terms, nothing but simple hydrolysis.

A considerable number of plant proteolytic enzymes has been described so far, but only in rare cases have the catalysts been associated with a clear cut function. It is a truism that the number of proteases (including the number of proteases which will be discovered in the future) is much smaller than the number of protein species, i.e., the potential substrates present in plant cells. The million dollar question concerns, therefore, the explanation of specificity of protein degradation which has been observed in various instances, e.g., when turnover rates were determined for individual protein species or losses of certain proteins were followed in senescent leaves. It appears to be impossible to explain such phenomena with activities of highly specific proteolytic enzymes responsible for selective degradation of certain proteins. It rather seems that a comparatively low number of proteolytic enzymes is responsible for unspecific degradations of proteins with which they get into contact. Hence, the mechanisms responsible for the contact between proteolytic enzymes and those proteins which are destined for degradation appear to be an important aspect of protein degradation.

A hypothetical solution of this problem is subcellular compartmentation. The concept of lysosomes, originally developed for rat liver cells, was adopted for plant cells. In the past few years it has been documented convincingly that plant cells, indeed, contain a lysosomal extraplasmatic compartment, the vacuole, in which proteolytic enzymes together with other hydrolases are located. The concept of lysosomes is intelligible because proteolytic enzymes appear to be separated in a specific compartment, the cytoplasm, the truly living entity of the cell, being protected against the uncontrolled attack by digestive enzymes. Yet, the concept certainly does not explain the mechanism of selective and controlled protein degradation. It merely switches over from the specificity of proteolytic enzymes to the specificity of transport of cytoplasmic proteins into the vacuoles. In fact, the role of vacuolar porteolytic enzymes has so far been demonstrated unambiguously only in the very special case of protein bodies, in which the compartmentalization of the substrates, the reserve proteins, coincides with that of the proteolytic enzymes responsible for intracellular protein digestion during seed germination.

It is quite possible and even probable that only a fraction of proteolytic enzymes which can be assessed by using the conventional substrates has been discovered so far. It is not unlikely that proteolytic enzymes which are more directly related to the degradation of cytoplasmic proteins than are the vacuolar enzymes remained undiscovered because they

have comparatively low activities and unusual properties or specificities that do not allow the determination with the common substrates. Recent findings of proteolytic activities in chloroplasts and in mitochondria support this view. The example of the proteolytic system of yeast (not covered in the present volume) may show the prerequisites for discovering minor proteolytic enzymes. At the same time the example demonstrates the limitations of work with higher plants.

Bakers yeast cells contain two principal endopeptidases (A and B), two carboxypeptidases (Y—formerly protease C and S), and several aminopeptidases. This proteolytic system is located in the vacuoles (A, B, Y, S, and two aminopeptidases). It is particularly interesting that yeast cells also contain several proteins which specifically inhibit the proteases A, B, and C. These inhibitor proteins are located in the cytosol. Hence, the proteolytic machinery in the vacuoles is fully active with the cytoplasm being protected, not only through compartmentation, but also by virtue of protease inhibitor proteins. Assuming that this system is responsible for protein degradation, one would expect that proteinase deficient mutants are not viable. Yet, mutants lacking the two vacuolar endopeptidases are able to grow, differentiate, and even sporulate, although the rate of protein turnover is markedly lower than in normal strains. Working with normal strains, Wiemken was able to demonstrate unambiguously that vacuoles are "the sole compartments" of endopeptidases.[1]

Working with mutants deficient in the vacuolar proteases, Wolf has not only discovered several novel proteolytic enzymes the activity of which in normal strains is completely masked; he also showed that "vacuoles are not the sole compartment of proteolytic enzymes in yeast";[2] the newly discovered proteinases D and E were found to be located outside the vacuoles. These extravacuolar enzymes may play essential roles in proteolysis and it is even feasible that the functions will be elucidated should selection for corresponding mutants be practicable. In any case, the researcher dealing with yeast is in a much more promising situation that his colleague working with higher plants who will have to wait for the availability of corresponding genetial research tools.

The present volume is undoubtedly a most valuable source of information about plant proteolytic enzymes. It covers not only the enzymological aspects, but also the various functions including those which are hypthetical at the moment and probably also difficult to prove in the future. Hopefully, the book will stimulate plant physiologists to step into the fascinating field of protein degradation and help to overcome the difficulties in understanding how the proteases are integrated in the metabolism of the living plant cells.

Michael J. Dalling

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Chapter 1

MOBILIZATION OF MONOCOT PROTEIN RESERVES DURING GERMINATION

K. R. Preston and J. E. Kruger

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I. INTRODUCTION

In germinating seeds, the mobilization of storage proteins during germination provides a primary source of amino acids: nitrogen and carbon skeletons to the growing plant. In order to obtain a better understanding of this process, three basic ingredients are necessary. First, an understanding of the properties of storage proteins including their mode of deposition and their physical and chemical properties is required. Second, an understanding of the pattern of breakdown of these proteins during germination is necessary. And last, an understanding of the proteolytic enzyme systems responsible in terms of their mode of action is required. In the present chapter, the details of this process in terms of the above basic ingredients will be discussed for monocot seeds. Of necessity, due to the information available, this discussion has been restricted to monocot cereals.

II. NATURE OF STORAGE PROTEINS

A. Formation of Storage Protein Bodies During Maturation

The major sites of storage protein deposition in resting seeds occur in the form of protein bodies. In monocot seeds the bulk of the storage protein is deposited in the form of protein bodies in the endosperm, and to a lesser extent in the aleurone, during seed maturation. At present there is some confusion as to the mechanism involved in the formation of these protein bodies. In the aleurone of cereals such as maize, protein bodies appear to have a vacuolar origin.[1] However, in cereal endosperm tissue, evidence for both the formation of protein bodies in vacuoles and from the enlargement of regions of the rough endoplasmic reticulum (RER) has been presented. In maize, strong evidence suggests that storage proteins are synthesized on the RER and deposited in the lumen in the form of protein bodies surrounded by single membranes.[1-5] Formation of protein bodies in rice also appears to occur by a similar process.[6] However, in wheat and barley the situation isn't as clear.

Although it is now generally agreed that storage protein synthesis of wheat and barley storage proteins occurs on RER, the manner in which the storage proteins are deposited has been a subject of controversy. Studies by Miflin et al.[5] based upon the distribution of "marker" enzymes in isolated wheat and barley storage protein bodies suggests deposition of these bodies in the lumen of RER following synthesis. However, studies by Cameron-Mills and von Wettstein[7] suggest a vacuolar origin for protein bodies in barley. Campbell et al.[8] studied the development of intact plants and detached ears of wheat in culture by light and electron microscopy. They found accumulation of protein inside the cistern that led to the formation of protein bodies in the lumen of RER similar to those of maize.[3] However, protein bodies, though in smaller amounts, were also found in vacuolar structures. This led to a postulate that protein synthesis was carried out by polyribosomes attached to the RER and that proteins either accumulated in the lumen or were transported to vacuoles. The process appeared to occur preferentially during the early stages of development, while the latter process occurs preferentially during the later stage of development. Studies by Buttrose[9] are consistent with this interpretation. Recent studies by Bechtel et al.[10] suggest that the major site of protein accumulation in developing wheat is in the vacuoles via a soluble mode of transport.

Although the exact mode of protein body formation in cereal endosperms is subject to controversy, it is generally agreed that the protein bodies, following formation, are surrounded by a membrane. In mature seeds of maize[11] and millet,[12] discrete protein bodies are evident, while in wheat[12] and barley[11] they are not. On the basis of this evidence and of the resistance of isolated mature maize protein bodies to hydrolysis by proteinase-k in contrast to the susceptibility of wheat and barley protein bodies, Miflin and Burgess[13] suggested that the membrane surrounding maize protein bodies is intact after maturation,

while those of barley and wheat are not. Thus the ability of proteolytic enzymes during germination to hydrolyze the storage proteins in various cereals may be, to some extent, dependent upon the state of the membrane surrounding the protein bodies.

B. Properties of Storage Proteins

Cereal proteins are usually classified by their extractability in various solutions according to procedures similar to those originally developed by Osborne.[14-16] Sequential extraction of ground seeds or endosperms with saline solution, aqueous alcohol solutions, and dilute acid or basic solutions yield four distinct protein groups including albumins (water and salt soluble), globulins (salt-soluble; water-insoluble), prolamins (aqueous alcohol-soluble), and glutelins (acid or alkali-soluble). The relative proportions of each of these protein fractions can vary widely from species to species and from cultivar to cultivar within any species. In addition, variations in extraction procedure can have a strong influence.[17]

1. Albumins and Globulins

The salt-soluble albumins and globulins of cereals are generally considered to be cytoplasmic proteins. They consist largely of enzymes and of enzyme inhibitors.[19] Inhibitors of animal, microbial, and insect alpha-amylase and protease, which probably act as protective agents, can account for a large proportion of these proteins.[19-21] Inhibitors of endogenous enzymes have also been shown to be present in these protein fractions. This topic will be discussed in more detail later in the text. Although cereal albumins and globulins are not normally considered as storage proteins, they may serve this purpose as a secondary function following germination. The rapid disappearance of protease inhibitor activity in barley during germination is suggestive of this.[22]

Although the salt-soluble albumins and globulins of cereals account for the majority of the protein nitrogen in the germ and aleurone, they generally make up a much lower proportion of the endosperm protein. In wheat, salt-soluble proteins account for approximately 15 to 25% of endosperm nitrogen of which 70 to 75% are albumins.[23-27] Rye probably has the highest proportion of albumins plus globulins of any cereal, ranging from 40 to 50%.[23,24,28] Barley salt-soluble proteins appear to account for approximately 15 to 30% of total endosperm protein,[23,24,29,30] although lower values have been reported.[14,31] Values for rice[32,33] and oats[23,24,34] appear similar to barley. In maize, albumins and globulins have been reported to account for 7 to 20% of total seed or endosperm protein,[23,24,35] while in sorghum reported values have ranged from 20 to 26%.[23,36] In most of the studies cited above, albumins accounted for a much higher proportion of the salt-soluble proteins than did globulins.

For all species, both the albumin and globulin fractions are made up of large numbers of individual components as determined by electrophoresis.[25,30,37,38] Molecular weights vary widely, although the albumins generally give lower values than the globulins.[30,39,40]

Amino acid composition of cereal albumin and globulins are given in Table 1 and Table 2. Both groups of proteins have similar patterns including a high level of glutamic acid (plus glutamine), aspartic acid (plus asparagine), glycine, and alanine. The globulins generally have higher levels of arginine and lower levels of aspartic acid (plus aspargine) compared to the albumins.

2. Prolamins

Prolamins are normally considered to be the major group of proteins in cereal endosperm with the exception of oats and rice where glutelins are predominant. Pernollet[41] has summarized data concerning the distribution of protein fractions in protein bodies of cereal endosperms. In barley, wheat, maize, and sorghum, prolamins appear to account for 80 to 100% of the protein in these bodies, while in rice glutelins appear to predominate.

Cereal prolamins can sometimes be extracted with water,[42] but have a strong tendency to

Table 1
AMINO ACID COMPOSITIONS OF CEREAL ALBUMINS (mol %)[a]

	Wheat	Rye	Barley	Maize	Sorghum	Oats	Rice
Aspartic acid	9.9	9.0	10.5	17.0	11.2	10.5	10.1
Threonine	3.7	3.9	4.6	4.3	4.9	4.3	4.5
Serine	5.8	5.8	6.1	5.8	5.9	8.4	6.1
Glutamic acid	21.4	22.6	14.3	12.6	12.4	12.7	14.5
Proline	9.5	12.3	7.7	8.7	5.2	6.3	4.7
Glycine	7.1	6.7	10.0	9.9	10.2	12.9	10.0
Alanine	7.1	6.6	8.5	10.2	10.7	7.8	9.6
1/2 Cystine	3.3	2.4	3.9	1.8	1.4	7.0	1.9
Valine	5.7	4.9	6.0	4.5	6.1	4.5	6.0
Methionine	1.6	1.3	2.1	1.1	2.0	1.2	1.7
Isoleucine	3.2	3.3	3.2	2.9	3.2	2.6	3.5
Leucine	6.6	6.4	6.1	5.2	6.7	5.7	7.1
Tyrosine	2.9	2.4	3.5	3.9	3.1	3.3	3.2
Phenylalanine	3.2	4.0	2.7	2.0	3.2	2.8	3.3
Lysine	3.1	3.0	4.4	4.0	5.7	4.6	5.1
Histidine	1.8	1.7	1.8	2.1	2.3	1.6	2.4
Arginine	4.1	3.7	4.6	4.0	5.8	3.8	6.3

[a] Data of Wieser, H., Seilmeier, W., and Belitz, H., *Z. Lebensm. Unters. Forsch.* 170, 17, 1980. With permission.

Table 2
AMINO ACID COMPOSITIONS OF CEREAL GLOBULINS (mol %)[a]

	Wheat	Rye	Barley	Maize	Sorghum	Oats	Rice
Aspartic acid	7.9	7.0	8.8	9.3	8.0	8.1	6.7
Threonine	4.5	4.5	4.7	5.1	4.4	4.2	2.8
Serine	6.2	6.5	6.1	7.0	7.6	6.5	6.6
Glutamic acid	15.6	17.4	13.2	11.0	12.4	16.4	14.9
Proline	7.1	8.0	7.0	5.7	5.3	5.4	5.7
Glycine	8.5	8.9	9.7	10 6	9.5	9.6	10.4
Alanine	7.7	7.8	8.5	11.0	9.9	7.6	8.2
1/2 Cystine	3.7	2.1	3.1	3.3	3.6	2.4	4.2
Valine	6.4	6.0	6.4	5.9	6.0	6.1	5.8
Methionine	2.0	1.5	1.4	1.5	0.9	1.3	4.5
Isoleucine	3.6	3.7	3.0	3.9	3.4	3.9	2.5
Leucine	7.5	7.1	7.7	6.6	7.0	7.2	6.3
Tyrosine	3.0	2.3	2.8	2.7	3.1	2.8	3.8
Phenylalanine	3.2	3.7	3.4	3.3	3.4	4.1	2.9
Lysine	4.1	4.4	4.8	4.7	4.1	4.5	2.8
Histidine	2.5	2.5	2.2	2.3	3.0	2.4	2.2
Arginine	6.5	6.6	7.2	6.1	8.4	7.5	10.0

[a] Data of Wieser, H., Seilmeier, W., and Belitz, H., *Z. Lebensm. Unters. Forsch.* 170, 17, 1980. With permission.

aggregate in the presence of low levels of salt.[43] This aggregation tendency is probably associated with interprotein hydrophobic interactions.[43] Prolamins are usually extracted with aqueous alcohols such as 70% ethanol or 55% propan-2-ol following removal of salt-soluble proteins with saline solution. In some cases, a disulfide reducing agent such as 2-mercaptoethanol and/or acetic acid have been added,[25,29,44] which increases the yield of prolamins

at the expense of the glutelins. Other factors such as temperature,[17,25] alcohol concentration,[17,45] and defatting[46,47] can also have large effects upon prolamin extractability.

Wieser et al.[23] and Ewart[24] extracted the various protein fractions from a wide variety of cereals by traditional "Osborne" procedures. The results of Wieser et al.[23] indicated that maize had the highest concentration of prolamins (48%), while wheat, sorghum, and barley showed levels ranging from 25 to 34%. Levels of rye prolamins were slightly lower (21%), while oats gave a value of 14%. Rice had a very low proportion of prolamins at 2%. Results by Ewart[24] showed similar trends, although values for prolamins were much lower for each corresponding cereal.

Orth and Bushuk[27] studied the "Osborne" solubility distribution of flour (endosperm) proteins in 26 cultivars of wheat. The prolamin (gliadin) fraction extracted in 70% ethanol accounted for 30 to 40% of the total flour nitrogen. However Miflin et al.[18] were able to extract over 60% of wheat nitrogen as prolamins using 50% propan-1-ol and 1% acetic acid at 60°C. Shewry et al.[48] increased the extraction of barley prolamins (hordein) to approximately 50% by direct extraction of ground seed with 50% propan-2-ol containing 2% 2-mercaptoethanol. With maize, Landry and Moureaux[35] found that, in contrast to barley and wheat, the addition of 2-mercaptoethanol to the 60% ethanol extraction solution resulted in only very small increases in prolamin extractibility. However Sodek and Wilson[49] were able to extract an additional 18% of total maize seed nitrogen as prolamin by adding 2-mercaptoethanol, although their initial extraction with 55% 2-propanol extracted less (average = 38%) than the 49% value obtained by Landry and Moureaux.[35]

Cereal prolamins generally consist of a large number of protein components as evidenced by electrophoretic studies.[50,51] Gel filtration studies indicate that prolamins of wheat, rye, and barley have structural similarities. Prolamin extracts from all three cereals can be separated into four molecular fractions of over 100,000, 60 to 85,000, 30 to 50,000, and less than 20,000.[28,51-53] In all three cereals the 30 to 50,000 fraction is predominant and consists of single polypeptide chains that are probably stabilized by intrachain disulfide bonds. In contrast, maize prolamins consist mainly of disulfide bonded polypeptide dimers with approximate molecular weights of 45,000 and polypeptide monomers with molecular weights of 19,000 and 22,000.[54] Millet and sorghum prolamins appear to have similar structural properties to maize.[55] Oat prolamins have been separated into three fractions by gel filtration.[34,56] The major fraction, which accounted for approximately 85% of the total protein nitrogen, had a molecular weight of approximately 22,000. Because of the very low concentration of prolamins in rice, few studies on these proteins have been carried out. However, Juliano and Boulter[32] found that reduced and alkylated rice prolamins had a single major subunit with a molecular weight of 23,000 using SDS-PAGE.

Amino acid compositions of cereal prolamins are characterized by high levels of glutamic acid (mainly as glutamine) and proline and low levels of basic amino acids. Wieser et al.[23] have carried out the most complete comparative study of the amino acid compositions of cereal protein fractions. Their results (Table 3), which are similar to other published data on individual cereals, show that prolamins of wheat, rye, and barley are similar. All three cereal prolamin fractions have very high proportions of glutamic acid (plus glutamine), which averages approximately 35 mol% and of proline (16.9 to 23.4%). Maize and sorghum differ from wheat, rye, and barley in that levels of glutamic acid (plus glutamine) and proline are lower, while levels of alanine and leucine are higher. Maize and sorghum also have the lowest levels of lysine. Amino acid compositions of oat and rice prolanine differ somewhat from the other groups. Oats has glutamic acid (plus glutamine) levels approaching those of wheat, barley, and rye, but less proline, while rice is similar to maize and sorghum in glutamic acid (plus glutamine), but contains less proline.

3. Glutelins

As with prolamins, glutelins are generally considered to act primarily as storage proteins,

Table 3
AMINO ACID COMPOSITION OF CEREAL PROLAMINES (mol %)[a]

	Wheat	Rye	Barley	Maize	Sorghum	Oats	Rice
Aspartic acid	2.7	2.4	1.7	5.0	7.0	2.3	7.5
Threonine	2.2	2.5	2.0	3.0	3.7	2.2	2.8
Serine	5.5	6.2	4.3	6.4	6.1	3.6	7.0
Glutamic acid	37.7	36.0	35.9	19.7	22.5	34.6	20.0
Proline	16.9	18.7	23.4	10.3	8.1	10.4	5.2
Glycine	3.0	4.6	2.3	2.6	1.5	2.7	5.9
Alanine	2.9	3.1	2.4	13.8	13.9	5.6	9.3
1/2 Cystine	2.2	2.2	1.9	1.0	1.1	3.4	0.8
Valine	4.0	4.1	3.7	3.8	6.1	7.2	6.5
Methionine	1.1	1.0	0.9	1.1	1.8	2.1	0.5
Isoleucine	3.9	2.9	3.4	3.7	5.0	3.1	4.4
Leucine	7.0	5.9	6.2	18.7	13.8	10.8	12.1
Tyrosine	2.0	1.7	2.3	3.6	2.2	1.7	6.3
Phenylalanine	4.7	4.6	5.9	5.0	5.1	5.4	4.9
Lysine	0.8	1.0	0.5	trace	0.0	1.0	0.5
Histidine	1.7	1.2	1.2	1.1	1.3	1.1	1.5
Arginine	1.7	1.9	2.0	1.2	0.8	2.8	4.8

Data of Wieser, H., Seilmeier, W., and Belitz, H., *Z. Lebensm. Unters. Forsch.* 170, 17, 1980. With permission.

although some may act as structural proteins.[51] Glutelins are generally present in protein bodies although, with the exception or rice, their concentrations are considerably less than prolamins.[41] In rice, glutelins are the major storage proteins and are the major components of the protein bodies.[57,58]

Glutelins are normally considered to be the protein fractions remaining after extraction with saline water and aqueous alcohol. Using this definition, Wieser et al.[23] found that rye had the lowest concentration of glutelins (24.5%), while rice had the highest (77.3%). Values for wheat, barley, oats, sorghum, and maize ranged from 41.8 to 53.9%. Higher values were reported for rye, oats, and maize by Ewart.[24]

Amino acid compositions of cereal glutelins from the comparative study of Wieser et al.[23] are given in Table 4. All cereal glutelins had high concentrations of glutamic acid (plus glutamine), although values were much higher in wheat compared to the other cereals. High levels of proline were also evident in wheat and barley, while much lower levels were present in oats and rice. In general, the glutelins had amino acid compositions intermediate to the prolamins and salt-solubles.

In most cereals, the glutelins appear to consist of a diverse group of proteins with high apparent molecular weights usually ascribed to interchain disulfide bonding[40] and to hydrophobic bonding.[43] In wheat,[59] barley,[51,60] and maize,[35] glutelins have been separated into four distinct fractions by sequential extraction with aqueous alcohol in the presence of 2-mercaptoethanol, pH 10.0 borate buffers with 2-mercaptoethanol and detergent (SDS, lauryl sodium sulfate, etc.) with 2-mercaptoethanol. The three extractable fractions had properties resembling prolamins, intermediate to prolamins and salt-soluble proteins, respectively. In oats the major glutelin fraction appears to be, in fact, mainly globulin in nature.[61]

III. STORAGE PROTEIN HYDROLYSIS DURING GERMINATION

When cereals germinate, the endosperm reserve proteins are degraded into their constituent

Table 4
AMINO ACID COMPOSITION OF CEREAL GLUTELINS (mol %)[a]

	Wheat	Rye	Barley	Maize	Sorghum	Oats	Rice
Aspartic acid	3.8	7.2	5.0	5.6	7.8	9.5	9.7
Threonine	3.5	4.6	4.1	4.1	5.0	4.1	4.1
Serine	6.8	6.5	6.3	5.7	5.5	6.2	6.3
Glutamic acid	30.7	20.1	24.7	16.3	17.2	19.4	15.9
Proline	12.2	9.6	14.5	11.7	8.6	5.6	5.2
Glycine	8.1	9.4	6.5	7.0	7.0	8.1	7.6
Alanine	4.5	7.4	5.7	9.6	10.3	6.7	8.1
1/2 Cystine	1.4	0.8	0.5	1.8	1.7	1.2	1.2
Valine	4.5	5.6	6.8	5.7	6.2	5.9	6.6
Methionine	1.3	1.6	1.3	2.8	1.6	1.3	2.5
Isoleucine	3.3	3.5	3.8	3.2	3.9	4.4	4.3
Leucine	7.0	7.5	7.7	11.1	9.3	8.0	8.6
Tyrosine	2.5	2.3	1.7	2.9	3.0	2.9	3.7
Phenylalanine	3.7	3.9	4.1	3.4	3.8	4.9	4.4
Lysine	2.1	4.1	2.8	2.4	3.2	3.3	3.4
Histidine	1.8	2.0	2.0	3.4	2.3	2.4	2.1
Arginine	2.8	3.9	2.5	3.3	3.6	6.1	6.3

Data of Wieser, H., Seilmeier, W., and Belitz, H., *Z. Lebensm. Unters. Forsch.* 170, 17, 1980. With permission.

peptides and amino acids by increasing levels of proteolytic enzymes. These hydrolysis products are then transferred to the scutellum where they are either directly or indirectly utilized by the growing embryo. Although in general terms, this overall process is similar in all cereals; differences in the patterns of storage protein hydrolysis are clearly evident. Cereals that are related genetically, and thus tend to have similar storage protein and proteolytic enzyme systems, tend to show much closer similarities in their hydrolysis patterns during germination, including similar rates of hydrolysis and distributions of hydrolysis products compared to those cereals that are less closely related genetically.

A. Barley, Wheat, and Rye

Barley, wheat, and rye are all members of the Gramineae family, subfamily Festucoideae, and tribe Hordeae. When these cereals germinate, there is usually a lag period of approximately 2 days after which a rapid phase of endosperm storage protein hydrolysis occurs.[62-66] After approximately 6 days, under more or less optimum conditions, the major portion of the endosperm storage proteins have been degraded.[62-64,67-69] The rapid increase in storage protein hydrolysis after 2 days is associated with large increases in proteolytic activity.[64,65,67] This latter topic will be discussed in detail in the next section.

Several studies have been published concerning changes in the protein fractions of barley, wheat, and rye during germination. Jahn-Deesbach and Schipper[66] studied changes in protein solubility distributions in whole seeds of wheat, barley, and rye during 84 hr of germination. The water-soluble albumin fraction showed large increases for all three cereals, while the salt-soluble globulin fraction showed little change during the germination period. The increase in water-soluble proteins was probably due to increases in albumin-like proteins in the embryo. The major endosperm storage proteins, consisting of alcohol-soluble prolamins and alkali-soluble glutelins, showed large decreases after approximately 48 hr. The rate of decrease in the prolamin fraction was greater than that for the glutelins, although in absolute terms the amount of glutelin degraded was similar to the amount of prolamin degraded in both wheat and barley. In rye only a small portion of the glutelins were degraded.

Folkes and Yemm[62] studied changes in the endosperm proteins of barley during germination. After germinating seed for various periods up to 10 days, endosperm was removed and extracted sequentially with salt solution, hot (80°C) 70% ethanol and ethanolic sodium hydroxide. All protein fractions showed rapid decreases after a 2-day lag period. Of the major storage protein fractions, the hot 70% ethanol soluble prolamins and the ethanolic hydroxide insoluble proteins tended to be degraded first, followed by the ethanolic hydroxide soluble glutelins. These results were in contrast to earlier studies of Bishop,[70] who found that (hordenins) glutelins were degraded faster than (hordeins) prolamins. However, these differences may have been due to differences in extraction conditions and nomenclature of the various fractions.[62]

Dell'Aquila et al.[69] studied changes in the protein fractions of three types of wheat including *Triticum aestivum*, *T. turgidum*, and *T. monococcum* during germination. Whole seeds were germinated for periods up to 6 days and then extracted sequentially with 0.5 *M* NaCl (albumins plus globulin), 70% ethanol (prolamins), and 0.1 *M* NaOH (glutelins). Both of the major storage protein fractions (prolamins and glutelins) were degraded rapidly during germination at similar rates. After 6 days, both fractions were almost completely depleted. In contrast the salt-soluble fractions, which probably included a high proportion of embryo proteins, showed slight increases during germination. Similar results were reported by Preston et al.[64] In this study, seeds of *T. aestivum* were germinated up to 5 days. Following removal of the embryo, the endosperm was extracted sequentially with salt solution and dilute acetic acid. Both the dilute acetic acid soluble proteins (prolamins and soluble glutelins) and the acid-insoluble glutelins showed rapid and similar rates of hydrolysis between 2 and 5 days of germination, while the salt-soluble fractions increased. Using a different approach, Hwang and Bushuk[65] studied gel filtration profiles of flour proteins from sprouted wheats, which were extracted in a strongly disassociating solvent (aqueous acetic acid-urea-cetyltrimethyl ammonium bromide). During an 8-day germination period, the very high-molecular-weight glutelin fraction and the major prolamin fractions disappeared and were replaced by a low-molecular-weight fraction (peptides and amino acids).

In both wheat and barley, the major products of storage protein hydrolysis present in the endosperm during germination have been shown to be small peptides and amino acids.[62,64,65,71,72] In contrast, there does not appear to be any build-up of intermediate hydrolysis products such as large polypeptides. For example, on the basis of amide content, Folkes and Yemm[62] concluded that there was no change in the properties of the various protein solubility fractions during germination of barley. Electrophoretic patterns of protein fractions in wheat also showed minimal changes during germination.[63,65,69] On the basis of these studies, it was concluded that during germination, individual proteins, when subjected to proteolytic attack, are degraded very rapidly to peptides and amino acids. However, it was shown that some differences in the rates of hydrolysis of individual storage proteins were evident. In particular, fractions of higher electrophoretic mobility were less affected during germination than those of lower electrophoretic mobility.[63]

B. Oats

Oats is a member of the Gramineae family, subfamily Festucoideae, and tribe Hordeae. As in barley and wheat, the hydrolysis of oat storage proteins during germination shows an initial lag period of approximately 2 days, followed by a rapid depletion of the major endosperm reserves.[56,66,73,74] This rapid phase of storage protein hydrolysis has been associated with large increases in the levels of proteolytic activity.[73] After approximately 5 to 7 days, the bulk of the endosperm storage protein has been hydrolyzed.[56]

Jahn-Deesbach and Schipper,[66] using an "Osborne"-like fractionation procedure, found that during 84 hr of germination there was a decrease in the globulin, prolamin and glutelin fractions in whole oats and an increase in the water-soluble fraction. However, the decreases

in the former protein fractions during this period were less than that obtained with wheat, barley, and rye. Similar comparative results for the prolamin fractions of wheat, barley, rye, and oats during germination were obtained by Dalby and Tsai.[75]

Kim et al.[56] studied changes in the protein solubility fractions and electrophoretic properties of oat endosperm during germination. The nonprotein nitrogen fraction (amino acids and peptides) showed large increases up to 3 days after germination, and then decreased. The salt-soluble ablumin and globulin fractions and the 45% ethanol-soluble prolamin fraction showed decreases during germination as did the predominant 0.1 *M* acetic acid-soluble and insoluble glutelins. However, the hydrolysis rates varied for each of these fractions as well as for the various proteins within groups as determined by electrophoresis. In contrast to wheat and rye, electrophoresis revealed the formation of new bands in all fractions except the glutelins.

C. Rice

Rice is a member of the Gramineae family, subfamily Orzoideae, and tribe Oryzeae. During germination of rice, several studies have shown decreases in endosperm protein nitrogen and concommitant increases in the levels of proteolytic enzymes.[78-80] Horiguchi and Kitagishi[77,78] showed that the decrease in endosperm protein nitrogen was due almost entirely to the hydrolysis of the glutelin fraction, which forms the major protein reserves in rice. The minor globulin and prolamin fractions showed little change during 10 days of germination, while the albumin and nonprotein nitrogen fractions increased. Evidence with protein synthesis inhibitors indicated that the increase in the albumin fraction was not due to protein synthesis, but were the hydrolysis products from the degradation of glutelins. Thus the intermediate hydrolysis products of rice, i.e., albumin-like polypeptides, appear to be hydrolyzed less rapidly than in wheat, barley, and oats during germination. Also the germination time required to deplete the major storage protein fraction in rice is longer than that required by the above mentioned cereals. After 10 days of germination, approximately one third of the endosperm glutelin was still undegraded.[78-80]

D. Maize and Sorghum

Maize and sorghum are members of the Gramineae family, subfamily Panicoideae, and tribe Andropoganeae. During germination of these cereals, there is an initial lag period of 1 to 3 days after which the endosperm storage proteins are rapidly degraded.[81-93] This rapid degradation appears to occur in response to increasing levels of proteolytic enzymes.[87-90,93]

In sorghum and maize, the germination period required for the hydrolysis of the endosperm storage proteins appears to be longer than that required in barley, wheat, rye, and oats. Wu and Wall[81] found that in sorghum, approximately 9 days of germination was required for the hydrolysis of the prolamin (kafirin) and cross-linked prolamin fractions. In the same time period, little change had occurred in the glutelin fraction. Dure[82] showed that in germinating maize there was a steady loss of endosperm nitrogen from 3 to 10 days. By day 10, maize endosperm had lost 71% of its original nitrogen content. After 8 days of germination, Harvey and Oaks[87] found that approximately 65% of maize endosperm nitrogen had been depleted. This loss of nitrogen corresponded to losses in the major storage protein fractions (prolamins and glutelins) that were almost completely degraded. In contrast, the albumin plus globulin plus dializable-nitrogen fraction showed increased levels in the endosperm up to 80 hr after inhibitor, and then decreased. The degradation of the major storage protein fractions between 3 and 8 days coincided with the appearance of a protease with an acid pH optimum.

Moureaux[91] studied changes in the endosperm protein fractions of germinating maize over a 7-day period. No major changes in total nitrogen, protein nitrogen, or nonprotein nitrogen occurred during the first 2 days. Following this lag period, there was a rapid loss of the