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ENDOSPERM DEGRADATION IN BARLEY KERNELS THAT SYNTHESIZE α-AMYLASE IN THE ABSENCE OF EMBRYOS AND EXOGENOUS GIBBERELLIC ACID

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Abstract

During germination at 16°C, whole seeds and distal half-seeds of Klages barley and two types of Clipper barley (Types A and B) were analyzed for α-amylase. Structural changes in the endosperms of these seeds and half-seeds were examined by scanning electron microscopy. In Clipper B half-seeds, α-amylase activity increased significantly, there was a detectable amount of starch granule hydrolysis and endosperm structure was markedly degraded. No starch hydrolysis and only trace amounts of α-amylase and endosperm degradation were detected in Clipper A and Klages half-seeds. There was significant α-amylase synthesis, starch hydrolysis and endosperm degradation in germinated whole seeds of all three barley cultivars. Changes were most pronounced in Clipper B. Starch degradation appeared to start in areas of the endosperm close to the embryo.

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Introduction

During germination, the starchy endosperm of barley kernels is degraded by a complex series of enzymic reactions (MacLeod et al 1964, Palmer and Bathgate 1976). Endosperm cell walls, consisting largely of β-glucan, are degraded by β-glucanases and, probably other enzymes; the protein matrix is solubilized by proteases and starch granules are hydrolyzed by α-amylase. These enzymes develop in the aleurone layer during germination and are secreted into the endosperm (MacLeod and Miller 1962, MacLeod et al 1964). α-Amylase has been the most widely studied of these hydrolytic enzymes. There is abundant evidence to show that aleurone layers treated with gibberellic acid synthesize and excrete α-amylase (Briggs 1963, 1964; MacLeod et al 1964, Varner 1964) and that gibberellic acid is synthesized in germinating barley embryos (Cohen and Paleg 1967, MacLeod and Palmer 1967, Radley 1967). These results support the attractive idea that, during germination, gibberellic acid moves from the embryo to the aleurone layer and there triggers the synthesis of hydrolytic enzymes such as α-amylase. These enzymes are then excreted into the endosperm (Palmer and Bathgate 1976).

There is also compelling evidence that some hydrolytic enzymes originate in the embryo and move into the endosperm via the scutellar epithelium (Briggs 1964, Gibbons 1979, MacGregor 1980, Okamoto et al 1980).

By manipulating environmental conditions during grain growth, Nicholls (1982, 1983) has produced two types of barley kernel, designated A and B, that differed markedly in their response to gibberellic acid. Distal half-seeds of type A, like those of normal barley, produced α-amylase only when incubated in the presence of gibberellic acid. Type B half-seeds, however, produced copious amounts of α-amylase after incubation in water. These kernels did not appear to contain above normal amounts of gibberellin-like material. Therefore, the growing conditions used appear to have pre-sensitized the aleurone layers of type B kernels so that they no longer required stimulation by gibberellic acid before producing α-amylase.

This paper describes part of a project that was initiated to examine the physiology of these

two types of barley kernel in more detail. Answers to two main questions were sought. Do type B half-seeds, incubated in the absence of gibberellic acid, readily produce endosperm cell wall and protein degrading enzymes in addition to α-amylase? Do hydrolytic enzymes from the aleurone layer of type B kernels play a more important role in endosperm degradation than do similar enzymes in normal barley? To answer these questions a scanning electron microscope (SEM) study was made of endosperms of germinated whole and half-seeds of types A and B Clipper barley and of normal Klages barley.

Materials and Methods

Barley Samples

The two-rowed barley cultivars, Klages and Clipper were used in this study. Klages was grown in test plots in Brandon, Manitoba in 1976; Clipper A was grown in South Australia in 1979/80 and Clipper B was grown under controlled environmental conditions as described elsewhere (Nicholls 1983).

Barley kernels were dehusked by steeping in 50% (v/v) sulphuric acid for 3 to 4 hrs. (Coombe et al 1967) followed by thorough rinsing with sterile, distilled water. Some kernels from each sample were cut transversely and the distal halves were placed in moist sand with the cut faces uppermost. Two μL of calcium chloride solution (0.01 M) were added to each half-seed to satisfy the calcium requirement of α-amylase. Whole kernels were placed, crease down, in moist sand. All kernels and half-kernels were maintained in a humid atmosphere at 16°C and samples were removed at intervals of 24 hrs. and frozen at -20°C until analyzed. Samples were extracted and assayed for α-amylase activity as described previously (MacGregor 1976).

Kernels and half-kernels were freeze-dried at -20°C prior to scanning electron microscopy examination. Whole kernels were cracked open longitudinally through the crease and these longitudinal slices, as well as distal half-kernels, were fixed to microscope stubs with Dotite silver paint (Fujikura Kasei Co. Ltd., Tokyo) and coated with gold. Samples were analyzed on a JEOL 35C scanning electron microscope at an accelerating voltage of 10 kV. Photomicrographs were taken on Plus-X Pan Kodak film.

Results and Discussion

Klages is a good quality malting barley and germinates quickly with rapid production of hydrolytic enzymes such as α-amylase (MacGregor 1978). This is illustrated in Fig. 1 where the α-amylase content of whole kernels reached almost 4,000 units/kernel after 5 days of germination. Only small but detectable amounts of activity were present in distal half-seeds after the same period of germination. By contrast, α-amylase development in Clipper A was significantly slower and was less than 2,000 units/kernel after 5 days. This slower rate of production of α-amylase is more typical of 2-rowed malting barley cultivars (MacGregor 1978). α-Amylase levels in half seeds were also very low, as would be expected.

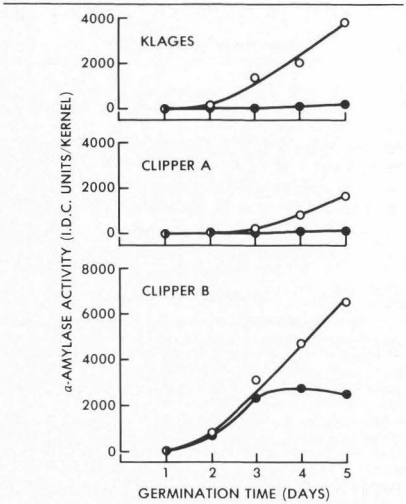

Fig. 1 Synthesis of α-amylase in whole seeds and distal half-seeds of Klages, Clipper A and Clipper B incubated on moist sand at 16°C. ○ whole seeds; ● half-seeds.

α-Amylase profiles of germinating kernels of Clipper B were quite different. Enzyme activity increased steadily after 24 hrs. of germination and after 2.5 days had reached the level attained by Clipper A after 5 days. At the end of the 5-day germination period Clipper B contained 3.8 times more α-amylase/kernel than did Clipper A and 1.7 times more than Klages. There was no sign of the lag phase in α-amylase biosynthesis apparent in both Klages and Clipper A profiles. As expected from previous results (Nicholls 1983), α-amylase formation in distal half-seeds of Clipper B was rapid and significant in amount. After 3 days these half-seeds had produced more α-amylase than had the whole seeds of Clipper A after 5 days. This rapid synthesis of α-amylase was not maintained and the enzyme level started to decrease after 4 days. Similar results have been reported for barley half-seeds germinated with gibberellic acid (MacGregor 1976) but the reason for the decline in activity is not known. Perhaps there is a feed-back mechanism whereby starch degradation products accumulating in the half-seed are able to slow down α-amylase synthesis to such an extent that natural degradation of the enzyme is greater than enzyme synthesis. In whole kernels, degradation products do not accumulate because they are translocated to and metabolized by the embryo.

Cut faces of distal half-seeds of the three barley samples were examined by scanning electron

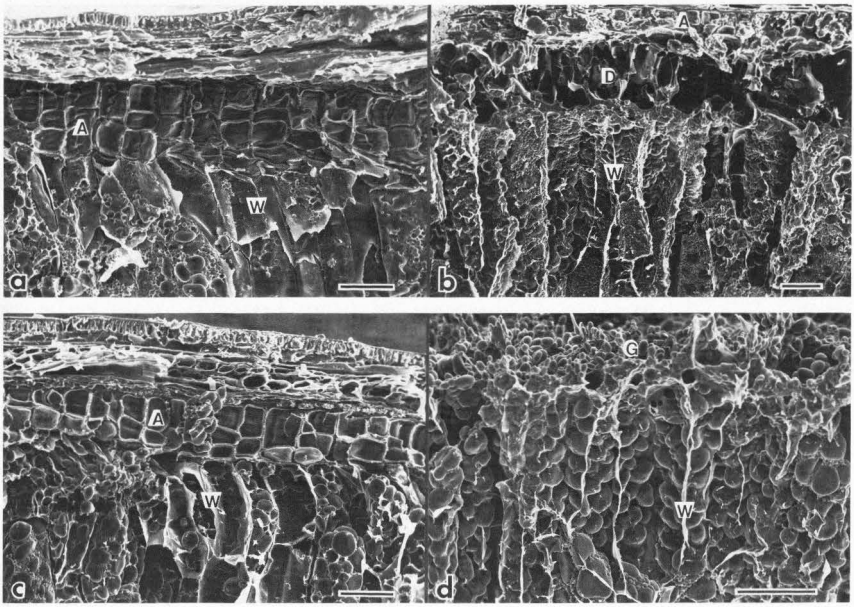

Fig. 2 Micrograph of distal half-seeds of a, sound Klages; b, 120 hr incubated Klages; c, sound Clipper A; d, 120 hr incubated Clipper A (section immediately under the aleurone layer). A - aleurone layer; W - endosperm cell walls; D - disjointed layer; G - area immediately under the aleurone; bar is 50 μm.

microscopy. Although the whole cut face was looked at, special attention was paid to endosperm areas immediately under the aleurone on the dorsal side of the kernel. It is from this region of the aleurone that hydrolytic enzymes might be expected to appear (Palmer 1975). In Klages barley the aleurone layer is 2 to 3 cells thick (Fig. 2a) and the endosperm consists of cells packed with large and small starch granules embedded in a protein matrix. All of these features have been discussed in detail elsewhere (Pomeranz and Sachs 1972, Palmer 1980, MacGregor 1980).

Some changes were observed in the physical structure of Klages half-seeds during incubation. After 24 hrs. the aleurone cells were wrinkled and misshapen but there was no apparent degradation of endosperm cell walls or protein matrix. Portions of the sub-aleurone layer became distorted during incubation and this was most pronounced in the 5-day samples (Fig. 2b). In these areas there was a layer (D) of disjointed fragments of cell wall material between the aleurone layer and the starchy endosperm. In many half-seeds examined parts of the aleurone layer appeared to have been torn away from the endosperm. This may have taken place during drying of the kernel after incubation. Adhesion between aleurone and endosperm tissues certainly appeared to be weakened during incubation. This may explain the disjointed layer visible in Fig. 2b but there may have been some dissolution or hydrolysis of protein and cell wall material. Because starch granules were never found in these areas these distorted fragments may represent the stretched remnants of cementing material between endosperm and aleurone tissues. Below this area stretched a band of cells in which large and small starch granules are clearly visible. Much of the protein matrix had been removed from this area and the cell walls (W) were much thinner. This more open texture of the endosperm may have been caused by simple dissolution of structural components rather than by enzymic hydrolysis but more detailed studies are required to resolve this problem. There was no evidence of starch degradation.

Distal half-seeds of Clipper A appeared to behave similarly during incubation (Fig. 2c and d). The two to three cell thick aleurone layer quickly became distorted but changes occurred slowly in the endosperm. Again, aleurone and

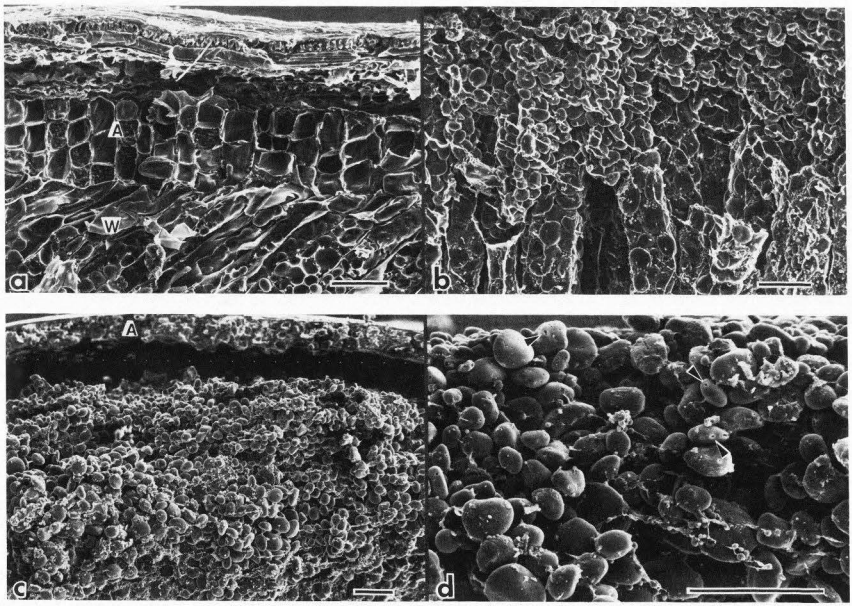

Fig. 3 Micrograph of distal half-seeds of Clipper B. a, sound; b, 48 hr incubated; c, 96 hr incubated; d, 120 hr incubated. In b and d, sections immediately under the aleurone layer are shown. A - aleurone layer; W - endosperm cell walls; arrows in d indicate degraded starch granules; bar is 50 μm.

endosperm tissues appeared to be torn apart and areas of starch granules, free of contaminating protein, were visible at the outer edge of the endosperm (G) after 5 days of incubation (Fig. 2d). These clean areas strongly suggest that some enzymic hydrolysis of protein and cell wall material had occurred.

Below this area some protein dissolution had occurred and cell walls (W) appeared to be thinner in the 5-day sample as was the case for Klages. No evidence of starch degradation was found in any of the samples examined.

The aleurone layer (A) of Clipper B was sometimes 3 to 4 cells thick (Fig. 3a) instead of the more normal thickness of 2 to 3 cells found in Klages and Clipper A (Fig. 2). More studies on a number of barley cultivars grown under the same conditions as Clipper B would be required to determine if the particular growing conditions used caused formation of the extra aleurone cells. No published information is available on the possible variation of aleurone layer thickness with either variety or growing conditions. The structure of Clipper B endosperm appeared to be similar to that of the other two barley samples studied. After 24 hrs. of incubation many aleurone cell surfaces were wrinkled and roughened but very little change in endosperm structure was apparent. Marked changes were readily visible after 48 hrs., however (Fig. 3b). Immediately under the aleurone layer (Fig. 3b), cell walls and protein were extensively degraded and areas of free starch granules were present. These degraded areas extended only 100-200 μm into the endosperm and so they represented only a small portion of the total endosperm. There was still no evidence of starch degradation. These changes were more extensive after 72 hrs. of incubation but only after 96 hrs. was pitting of starch granules sometimes detected. By this stage a layer of starch granules virtually free of protein and cell wall material extended around the outside of the endosperm (Fig. 3c). There was little apparent increase in endosperm degradation after 120 hrs. but there was increased pitting of starch granules (Fig. 3d).

This pitting represents radial channels made by α-amylase on large starch granules as the enzyme moves into, and preferentially degrades, the central portion of these granules (Gallant et al 1972). Pitting, therefore, does not represent the full extent of starch granule degradation nor,

necessarily, the amount of α-amylase present.

These results show that aleurone cells of Clipper B are able to produce not only α-amylase but also protein and cell wall degrading enzymes in the absence of exogenous gibberellic acid. Therefore, aleurone cells of this particular barley sample have been pre-triggered so that when hydrated they produce and secrete the hydrolytic enzymes required to mobilize endosperm reserves.

Longitudinal sections of germinated whole kernels were also examined by SEM. Areas of particular interest are indicated on the diagram shown in Fig. 4. Results from previous work (MacGregor 1980, Gibbons 1980) suggested that endosperm degradation in germinating kernels started at the embryo-endosperm junction and moved out into the endosperm from there. Therefore, although the whole longitudinal section was scanned, attention was focused along the embryo-endosperm junction and all micrographs in Figs. 5-7 were taken in areas shown as A,B or C, representing the dorsal, middle and ventral areas of this junction, respectively.

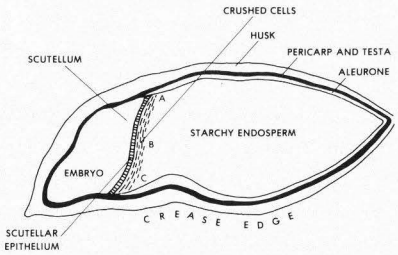

Fig. 4 Diagram of longitudinal section through the crease of a barley kernel. All micrographs shown in Figs. 5-7 were taken along the embryo-endosperm junction at areas A (dorsal edge), B (mid-point) or C (ventral crease edge).

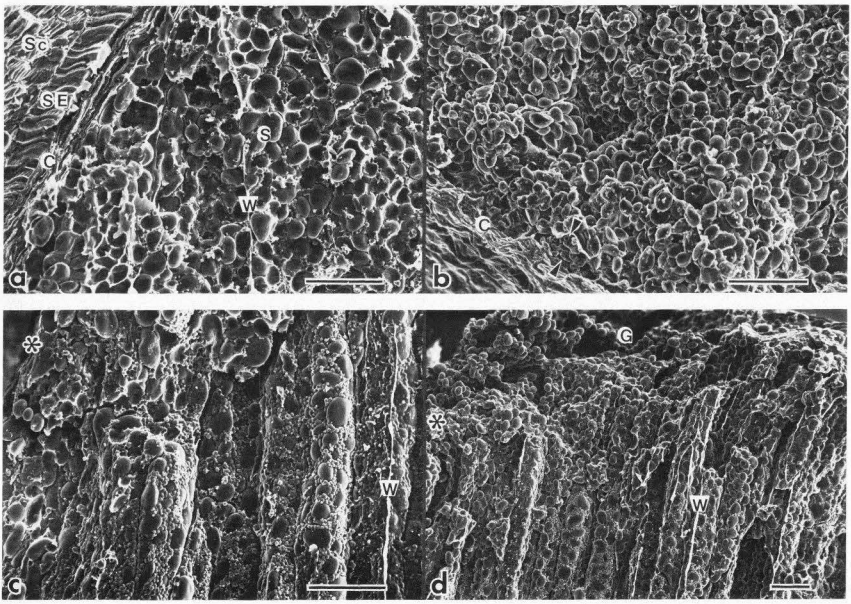

Fig. 5 Micrograph of longitudinal section of Clipper A kernels. a, sound (area B); b, 72 hr germinated (area C); c, 96 hr germinated (area B); d, 120 hr germinated (area A). Sc - scutellum; SE - scutellar epithelium; C - layer of crushed cells; S - starch granules; W - endosperm cell walls; G - area immediately under the aleurone; * - endosperm-embryo junction; arrows indicate pitted starch granules; bar is 50 μm.

No significant differences were found in the endosperm structures of sound samples of the three types of barley kernel studied. Micrographs of area B of each type of kernel are shown in Figs. 5a, 6a and 7a. The single layer of cells of the scutellar epithelium (SE) and the layers of crushed cells (C) adjacent to the endosperm are clearly visible. Portions of cell walls (W) and intact starch granules, both large and small, can be seen embedded in a protein matrix. These structural features of mature barley endosperms are similar to those discussed previously (Pomeranz and Sachs 1972, Pomeranz 1974, Palmer and Bathgate 1976). Endosperm breakdown in Clipper A occurred very slowly and the only change detected after 48 hrs. of germination was slight degradation of cell walls and protein matrix at the ventral edge of the embryo-endosperm junction. After 72 hrs. extensive degradation of endosperm structure had occurred in area C and some pitting of starch granules was visible (Fig. 5b). Twenty-four hours later there was some endosperm degradation mid-way along the endosperm-embryo junction (Fig. 5c), but this did not extend to the dorsal edge. There was little starch pitting in area B and none was detected in area A. After 120 hrs. of germination, extensive endosperm breakdown and starch pitting were detected in areas B and C and significant structural, but no starch degradation, was found in area A (Fig. 5d). Obviously a gradient of

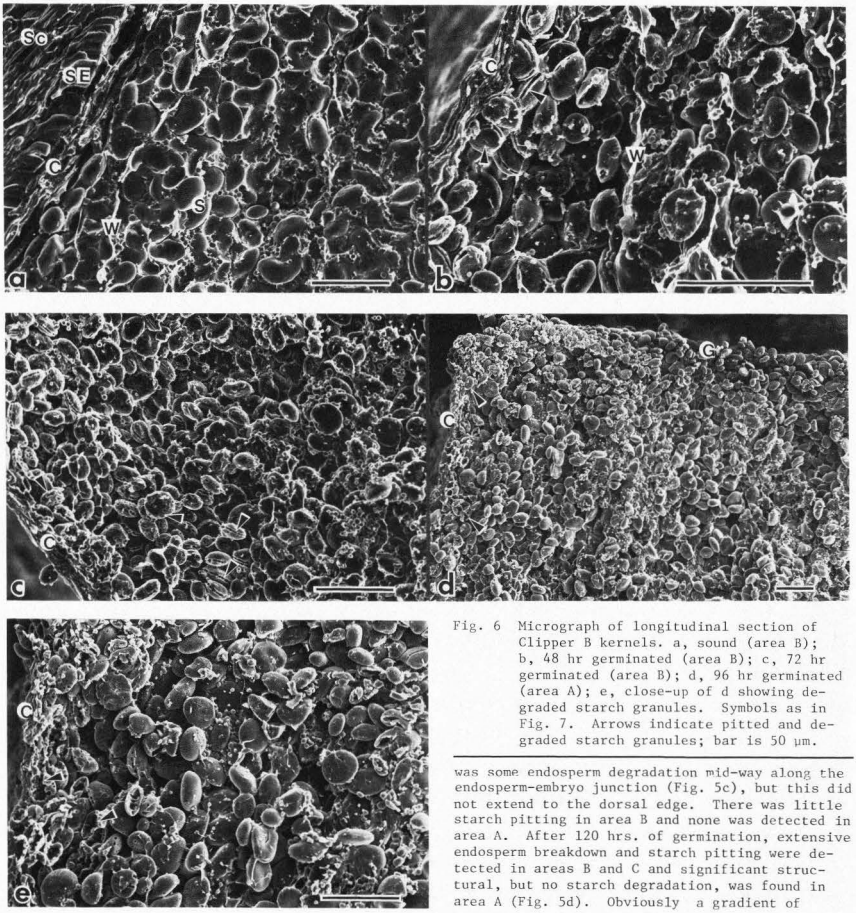

Fig. 6 Micrograph of longitudinal section of Clipper B kernels. a, sound (area B); b, 48 hr germinated (area B); c, 72 hr germinated (area B); d, 96 hr germinated (area A); e, close-up of d showing degraded starch granules. Symbols as in Fig. 7. Arrows indicate pitted and degraded starch granules; bar is 50 μm.

degradation existed in a narrow band along the embryo-endosperm junction from the ventral to the dorsal edge. Similar changes in germinating barley kernels have been described previously (MacGregor 1980).

Structural changes in endosperms of Clipper B kernels occurred much more rapidly. After only 24 hrs. of germination, endosperm degradation was detected in region C but no starch pitting was apparent. Twenty-four hours later, however, much of the cell wall and protein material had been degraded in region C and along the embryo-endosperm junction through area B (Fig. 6b). There were degraded starch granules throughout this area. Even at the dorsal edge some endosperm breakdown had occurred but starch hydrolysis had not yet started. After 72 hrs. a band of extensively degraded endosperm stretched all the way along the endosperm-embryo junction. Little intact protein or cell wall material remained in this area and starch granules were severely degraded (Fig. 6c). Close to the dorsal aleurone, however, only slight pitting of starch granules was detected. Endosperm degradation was even more extensive after 96 hrs. of germination, and by this stage very little endosperm structure was left at the dorsal edge. Large areas of clean starch granules with little contaminating protein or cell wall material were observed (Fig. 6d). This type of structure extended some distance along the dorsal edge of the seed immediately under the aleurone layer. The narrow band of severely degraded starch granules can be seen extending up to the dorsal edge (Fig. 6e). However, only limited starch degradation was detected under the aleurone layer, indicating that at this stage α-amylase was just beginning to move from the aleurone into the endosperm. It must be noted that these results do not preclude the presence of significant amounts of α-amylase in the aleurone layer. They indicate, merely, the start of α-amylase secretion from the aleurone into the endosperm. In the 5-day germinated sample much more starch degradation had taken place under the aleurone layer.

The rate of endosperm degradation in kernels of Klages barley was intermediate between those observed for the Clipper samples. After 48 hrs.

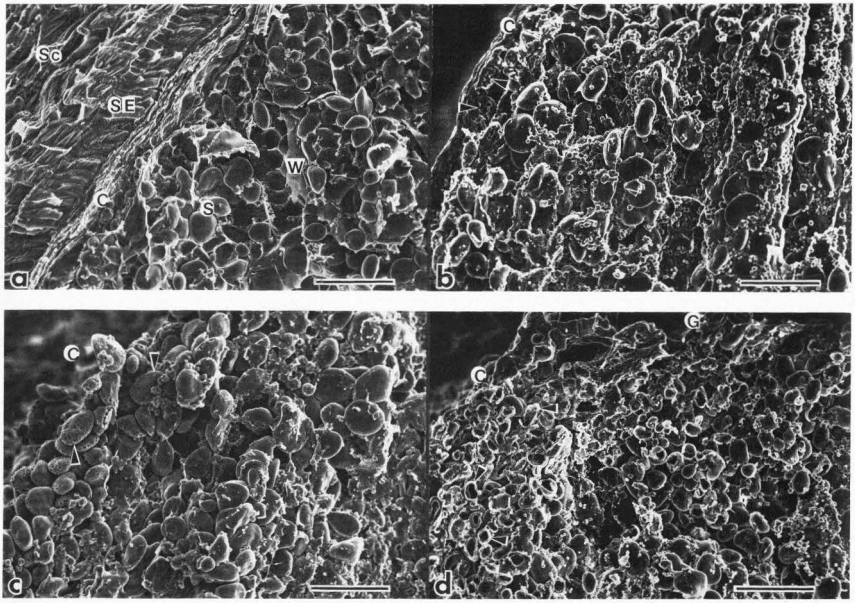

Fig. 7 Micrograph of longitudinal section of Klages kernels. a, sound (area B); b, 72 hr germinated (area B); c, 96 hr germinated (area B); d, 120 hr germinated (area A). Sc - scutellum; SE - scutellar epithelium; C - layer of crushed cells; S - starch granules; W - endosperm cell walls; G - area immediately under the aleurone; * - endosperm-embryo junction; arrows indicate pitted starch granules; bar is 50 μm.

only limited degradation was found and that was confined to area C. Degradation increased rapidly during the ensuing 24 hrs. producing large areas of clean, damaged starch granules in area C and structural breakdown with some starch degradation in area B (Fig. 7b). At this stage only limited cell wall and protein degradation were detected at the dorsal end of the embryo-endosperm junction. After 96 hrs. extensive removal of protein and cell walls had occurred in a narrow band along the junction with severe starch pitting reaching area B (Fig. 7c). After a further 24 hrs., complete destruction of the endosperm had occurred along the whole embryo-endosperm junction and starch hydrolysis was detected at the dorsal edge (Fig. 7d). Most of the starch degradation appeared to emanate from the embryo but some degraded granules were found under the aleurone layer.

Results from these SEM studies agree with the α-amylase profiles shown in Fig. 1. Only incubated half-seeds of Clipper B showed significant levels of α-amylase activity and detectable amounts of starch degradation. In whole seeds the extent of starch degradation mirrored the α-amylase profiles. Degradation of endosperm structure always preceded starch hydrolysis, indicating that cell wall and protein degrading enzymes were active in endosperms before α-amylase. These enzymes are probably synthesized and secreted into the endosperm more quickly than is α-amylase (Gibbons 1980, Okamoto et al 1980). However, more definitive studies are required to confirm this.

The high α-amylase levels observed in germinated half-seeds of Clipper B were accompanied by significant degradation of endosperm structure immediately beneath the aleurone layer. In these kernels, therefore, synthesis of the whole complement of endosperm degrading enzymes had been pre-triggered by the environmental conditions used during kernel growth.

Whole kernels of Clipper B showed increased rates of endosperm modification during germination. However, the pattern of modification did not appear to be different. Endosperm and starch degradation started at the embryo-endosperm junction and moved along the junction to the dorsal edge as well as into the endosperm. There did not appear to be a disproportionate amount of degradation under the aleurone, suggesting that in these kernels synthesis of hydrolytic enzymes had been stimulated in the embryo as well as in the aleurone. It should be remembered that in all samples examined endosperm degradation was limited to a relatively small area close to the embryo and aleurone. Even in 5-day germinated Clipper B much of the endosperm remained intact. Therefore, these studies relate only to initial stages of germination and endosperm modification.

Although endosperm degradation appeared to start, and was most severe, in areas close to the embryo during germination, it must not be assumed that the embryo rather than the aleurone is the major site of synthesis of hydrolytic enzymes. Synthesis of enzymes may be quite rapid in aleurone cells but active secretion into the endosperm may not take place immediately.

References

1. Briggs, D.E. Biochemistry of barley germination. Action of gibberellic acid on barley endosperm. J. Inst. Brewing 69:1963, 13-19.
2. Briggs, D.E. Origin and distribution of α-amylase in malt. J. Inst. Brewing 70:1964, 14-24.
3. Cohen, D. and Paleg L.G. Physiological effects of gibberellic acid. X. The release of gibberellin-like substances by germinating barley embryos. Plant Physiol. 42:1967, 1288-1296.
4. Coombe, B.G., Cohen, D. and Paleg L.G. Barley endosperm bioassay for gibberellins. I. Parameters of the response system. Plant Physiol. 42:1967, 105-112.
5. Gallant, D., Mercier, C. and Guilbot, A. Electron microscopy of starch granules modified by bacterial α-amylase. Cereal Chem. 49:1972, 354-365.
6. Gibbons, G.C. On the localization and transport of α-amylase during germination and early seedling growth of Hordeum vulgare. Carlsberg Res. Commun. 44:1979, 353-366.
7. Gibbons, G.C. On the sequential determination of α-amylase transport and cell wall breakdown in germinating seeds of Hordeum vulgare. Carlsberg Res. Commun. 45:1980, 177-184.
8. MacGregor, A.W. A note on the formation of α-amylase in de-embryonated barley kernels. Cereal Chem. 53:1976, 792-796.
9. MacGregor, A.W. Changes in α-amylase enzymes during germination. J. Amer. Soc. Brewing Chemists 36:1978, 1-5.
10. MacGregor, A.W. Action of malt α-amylases on barley starch granules. MBAA Technical Quarterly 17:1980, 215-221.
11. MacLeod, A.M. and Miller, A.S. Effects of gibberellic acid on barley endosperm. J. Inst. Brewing 68:1962, 322-331.
12. MacLeod, A.M. Duffus J.H. and Johnston, C.S. Development of hydrolytic enzymes in germinating grain. J. Inst. Brewing 70:1964, 521-528.
13. MacLeod, A.M. and Palmer, G.H. Gibberellin from barley embryos. Nature 216:1967, 1342-1343.
14. Nicholls, P.B. Influence of temperature during grain growth and ripening of barley on the subsequent response to exogenous gibberellic acid. Aust. J. Plant Physiol. 9:1982, 373-383.
15. Nicholls, P.B. Environment, developing barley grain, and subsequent production of α-amylase by the aleurone layer. Proceedings of the Third International Symposium on Preharvest Sprouting Damage in Cereals. J.E. Kruger and D.E. LaBerge (eds.), Westview Press, Boulder, Colorado. 1983.
16. Okamoto, K., Kitano, H. and Akazawa, T. Biosynthesis and excretion of hydrolases in germinating cereal seeds. Plant and Cell Physiol. 21:1980, 201-204.
17. Palmer, G.H. Direct assessment of the modification of malt. J. Inst. Brewing 81:1975, 408-409.
18. Palmer, G.H. The morphology and physiology

of malting barleys, in: Cereals for Food and Beverages, G.E. Inglett, L. Munck (eds.), Academic Press, New York, 1980, 301-338.
19. Palmer, G.H. and Bathgate, G.N. Malting and Brewing, in: Advances in Cereal Science and Technology, Y. Pomeranz (ed.), A.A.C.C. Inc. St. Paul, Minn. Vol. 1, 1976, 237-324.
20. Pomeranz, Y. A note on scanning electron microscopy of low- and high-protein barley malts. Cereal Chem. 51:1974, 545-552.
21. Pomeranz, Y. and Sachs, I.B. Determining the structure of the barley kernel by scanning electron microscopy. Cereal Chem. 49:1972, 1-4.
22. Radley, M. Site of production of gibberellin-like substances in germinating barley embryos. Planta 75:1967, 164-171.
23. Varner, J.E. Gibberellic acid controlled synthesis of α-amylase in barley endosperm. Plant Physiol. 39:1964, 413-415.

Discussion with Reviewers

R. Moss: In Fig. 2a there appears to be more inter-cellular cleavage of the endosperm cells (because more endosperm cell walls are visible) whereas in Fig. 2c the cleavage appears to be more intracellular (more aware of the contents of endosperm cells). The type of cleavage pattern is dependent on intrinsic grain hardness and grain moisture content. Does grain hardness influence the extent of endosperm modification in barley?
Authors: Grain hardness may well influence the extent of endosperm modification in barley but only limited information has been published on this topic. Allison and co-workers (24-26) have shown that there is a relationship between malting quality and barley milling energy (the electrical energy required to grind a given weight of barley to a flour fine enough to pass through a 1 mm sieve). Barley cultivars with good malting quality tended to have lower milling energies. However, more research is required to determine the relationship between grain hardness and rate of endosperm modification.

R. Moss: Could the authors give more information on the manner in which they produced the surfaces examined by SEM? Were the "sound" seeds frozen before fracturing, or fractured at room temperature and at what moisture content? Moisture content can markedly influence the cleavage pattern (Moss et al, SEM III, 1980, 613) and a soft wheat variety can produce cleavage patterns similar to Fig. 2d, with "clean" starch granules.
Authors: Sound seeds were fractured at room temperature and at a moisture content of ca. 10%. Both Klages and Clipper are good malting cultivars and so tend to have floury endosperms. Therefore, it is not surprising that the endosperm structure of these samples resembles that of soft wheat.

D.R. Lineback: Is the postulate of the inhibition of α-amylase synthesis by a feedback mechanism involving starch degradation products in Clipper B consonant with the observations made on starch degradation in the endosperm of these seeds? Starch degradation in Clipper B endosperm is more extensive than in the endosperms of Clipper A or Klages. If germination is allowed to proceed sufficiently long, surely more extensive degradation of the endosperm area is observed. Is the starch in the endosperm of Clipper B more rapidly degraded than in Clipper A and Klages as would be expected if a larger amount of α-amylase were present?
Authors: The suggestion that α-amylase synthesis may be inhibited by a feedback mechanism involving products of starch degradation refers to half-seeds only. In whole seeds these products do not accumulate because they are translocated to the embryo and metabolized. Starch degradation was detected only in half-seeds of Clipper B and not in Clipper A or Klages. This degradation was not extensive during the time period of the study and it is difficult to correlate the progress of degradation with changing levels of α-amylase in the half-seeds.

D.R. Lineback: Is there evidence for any particular type of attack of the germinating barley starch by α-amylase? In Fig. 6c it appears that many of the granules have been attacked around the "equator" of the granule. Large lenticular granules of wheat starch are normally attacked around the "equatorial groove" by α-amylases. Does this same type of attack occur in starch granules of germinating barley?
Authors: In general, α-amylase from malted barley preferentially attacks the equatorial region of large, lenticular starch granules from barley. The equatorial region of many of the starch granules shown in Figs. 6c and 6e have been significantly degraded. It is true that some α-amylase attack also takes place over the entire surface of particular granules (Figs. 6c and 3d). These, more uniformly pitted granules, appear to be rounder in shape and do not have a pronounced equatorial groove. Perhaps not all large starch granules from barley have the same physical structure. Whether or not there is more "equatorial groove" degradation in wheat starch granules compared to barley starch granules requires more detailed investigation.

W.J. Wolf: In reference to Fig. 2b, does it seem likely that simple dissolution of structural components would occur without prior hydrolysis? Cell walls and starch granules are not soluble in water hence it is difficult to see how they could have simply dissolved.
Authors: A portion of the protein matrix and of endosperm cell walls in barley are water soluble. Partial dissolution of components of the adhesive material between aleurone and endosperm could have weakened this adhesion and caused the break shown in Fig. 2b. However, enzymic hydrolysis of structural components in this area is indeed a more likely explanation for the disjointed layer.

H. Fuwa: During germination, does α-amylase from the aleurone layer of Type B kernels play a more dominant role in degradation of endosperm starch granules than does the same enzyme in normal barley?
Authors: The aleurone layer of Type B kernels appears to be more active than the aleurone layer

of normal kernels. However, results shown in Fig. 6 strongly suggest that in Type B kernels, as in normal kernels, enzymes from the embryo are primarily responsible for starch degradation during initial stages of germination. Therefore, the embryo as well as the aleurone of these kernels has been activated so that aleurone α-amylase is no more dominant in these kernels than in normal kernels.

Additional References

24. Allison, M.J. Amylase activity and endosperm hardness of high lysine barleys. J. Inst. Brewing 84:1978, 231-232.
25. Allison, M.J., Cowe, I.A., Borzucki, R., Bruce, F. and McHale, R. Milling energy of barley. J. Inst. Brewing 85:1979, 262-264.
26. Allison, M.J., Cowe, I.A. and McHale, R. A rapid test for the prediction of malting quality of barley. J. Inst. Brewing 82:1976, 166-167.
27. Moss, R., Stenvert, N.L., Kingswood, K., and Pointing, G. The Relationship Between Wheat Microstructure and Flourmilling. Scanning Electron Microsc. 1980; III: 613-620.