α-Amylase Synthesis in Wheat Kernels as Influenced by the Seed Coat

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ABSTRACT

The effect of seed coat removal on the synthesis of α-amylase isoenzymes in wheat was investigated. The immature wheat endosperm-aleurone (seed coat and embryo detached) produced considerably less α-amylase activity than immature whole or de-embryonated wheat kernels, when incubated under identical conditions of 18.5 °C and 99% humidity, in the presence or absence of gibberellin acid (GA3). The incubated endosperm-aleurone also exhibited unique α-amylase isoenzyme composition when compared to the isoenzyme compositions of incubated whole and de-embryonated immature and mature wheat kernels both in the presence or absence of GA3. Subsequent studies indicated that the seed coat may contain factor(s) required for normal α-amylase isoenzyme synthesis.

The deleterious effects of elevated α-amylase activity in wheat caryopses has generated an intensive study of this enzyme system. Considerable research has been directed towards characterizing the α-amylase present in germinating (3, 9, 10, 16, 18) and immature wheat (4, 8, 13, 16). This research has demonstrated that α-amylase isoenzymes exist in both germinating and immature wheat. Electrophoretic studies have indicated that germinating wheat α-amylase is comprised of two sets of isoenzymes (1, 16) with five and three isoenzymes, respectively (9). In contrast, immature wheat was found to contain only one set of three isoenzymes (8). Subsequent studies, using polyacrylamide slab-gel isoelectric focusing have indicated that up to 22 α-amylase isoenzymes, separable into three groups (GI, GII, and GIII) may be found in immature Canadian-grown wheat cultivars (15).

Recent studies have elucidated the sites of synthesis of these isoenzymes in the tissues of the immature wheat kernel (14). With dissected tissues, anomalous α-amylase isoenzyme patterns were obtained which indicated that the seed coat (inner pericarp, testa, hyaline layer) played some part in α-amylase synthesis. Here, we describe preliminary studies which were undertaken to determine the effect of the seed coat on the synthesis of α-amylase.

MATERIALS AND METHODS

Hard red spring wheat (Triticum aestivum cv. Cypress) was used throughout the study presented here. This cultivar was grown in the Canada Department of Agriculture experimental plots at Glenlea, Manitoba, during the summer of 1976. The immature wheat was harvested 39 days after flowering at the waxy-ripe stage (17) with a moisture content of 17.1% and was stored at −19 °C. The mature wheat was harvested from the same crop 45 days after flowering at the dead-ripe stage, with a moisture level of 7.0%.

Dissection and Incubation Procedure. The endosperm-aleurone was dissected from the immature kernel and was incubated at 18.5 °C and 99% moisture for 0 to 5 days as described by Marchylo et al. (14). To dissect the immature and mature wheat kernels, it was necessary to soak them in water at room temperature for about 2 h prior to dissection. The embryo was carefully removed to ensure that none of its scutellar tissue remained attached to the endosperm. Whole and de-embryonated kernels were incubated under the same conditions as the dissected tissues. All experiments were performed in duplicate.

Determination of α-Amylase Activity and Isoenzyme Composition. The α-amylase activity was assayed via an automated fluorometric procedure (12). Activity was expressed in terms of mg maltose liberated/min at 37 °C. The α-amylase isoenzymes were separated with a polyacrylamide slab-gel isoelectric focusing system and detected via a β-limit dextrin plate technique as described previously (14). Extracts were prepared in both cases as described (15).

RESULTS AND DISCUSSION

Increases in α-amylase activity in immature whole and de-embryonated wheat kernels and immature endosperm-aleurone (with embryo and seed coat removed) tissues following incubation in the presence and absence of GA3 are shown in Figure 1. In all cases, the α-amylase activity increased both in the presence and absence of GA3.

Comparison of immature whole and de-embryonated wheat kernels indicated that excision of the embryo influenced the production of α-amylase. Thus, the GA3-treated de-embryonated immature wheat kernel exhibited a peak in α-amylase production after 3 days incubation and then gradually decreased, whereas production of α-amylase in the whole kernel was slower; the amount formed after 5 days approximating that of the de-embryonated kernel after 3 days. Similarly, in the absence of GA3, the de-embryonated kernel displayed a greater rate of kernel α-amylase production than did the whole kernel over the 5-day incubation period. Levels of α-amylase inducible in half-kernels of Australian wheat after 24 h germination in the presence and absence of GA3 have been shown by King (7) to depend upon the stage of kernel maturation. To investigate this, mature whole and de-embryonated kernels were also germinated in the presence and absence of GA3. With the de-embryonated mature wheat in the presence of GA3, a peak in α-amylase production, similar to that with the immature de-embryonated wheat, occurred (Fig. 2). Rates of production of α-amylase both in the presence and absence of GA3 were faster, however, with whole kernels. In this respect, the wheat α-amylase system behaves similarly to that present in mature whole and de-embryonated barley (11).
The most interesting finding (Fig. 1) was the effect of removing the seed coat from the de-embryonated wheat kernels. The resulting immature endosperm-aleurone tissue exhibited a minimal increase in α-amylase activity upon incubation (Fig. 1). In addition, activity profiles in the presence and absence of GA3 were the same for the 5-day incubation period. Thus, seed coat removal significantly affects the production of α-amylase beyond the effect attributable to embryo excision.

To elaborate upon the effect of seed coat removal, the α-amylase isoenzyme compositions of the incubated immature and mature whole and de-embryonated wheat kernels were compared (Fig. 3). The presence or absence of GA3 had no effect upon isoenzyme composition. In this respect, de-embryonated wheat appears to be similar to embryoless wheat (6). For simplification, isoenzymes were divided into three families. Isoenzymes with pI values closest to the anode were designated GI, those closest to the cathode were designated GIII and the intermediate bands were classed as GII (14).

The α-amylase isoenzyme composition of the incubated mature and immature wheat kernels were very similar (Fig. 3, A and D). In both cases, most of the visible activity was contributed by the GIII isoenzymes with lesser activity contributed by the GI isoensymes. Insignificant observed activity was contributed by the GII isoenzymes.

Comparison of the α-amylase isoenzyme compositions of the incubated de-embryonated immature and mature wheat indicated that they also were very similar with only few minor variations in isoenzymes (Fig. 3, C and E). Unlike the incubated whole kernels, the GII isoenzymes in the de-embryonated kernels contributed a substantial portion of the visible isoenzyme activity. In addition, the GII isoenzymes were much more prominent. It would appear, therefore, that the embryo is responsible for inhibition of synthesis of the GI and GII isoenzymes by the aleurone. This is surprising since it has been shown in barley that the embryo is the source of gibberellins which are known to promote α-amylase synthesis (2) by the aleurone (5).

In contrast, the α-amylase isoenzyme composition of the incubated immature endosperm-aleurone (Fig. 3B) bore little resemblance to the isoenzyme composition of the incubated immature and mature whole or de-embryonated wheat kernels (Fig. 3, A, C, D, and E). The majority of the visible α-amylase activity in the endosperm-aleurone was present at a very broad band of activity located in the high pH regions of the isoelectric focusing gel. Little visible activity was contributed by the GI, GII, and GIII families of isoenzymes. This anomalous isoenzyme composition of the incubated immature endosperm-aleurone indicates that seed coat removal somehow affects α-amylase synthesis. To elaborate further upon this phenomenon, de-embryonated immature and mature wheat kernels were incubated in the presence, absence, and partial absence of the seed coat (Table I). In the presence of the seed coat, both the mature and immature de-embryonated kernels produced large quantities of α-amylase (Table I, procedure I). In contrast, in the absence of the seed coat, the α-amylase activity

![FIG. 1. Changes in the α-amylase activity during incubation of the whole kernel, de-embryonated kernel, and endosperm-aleurone in the presence and absence of GA3.](image1)

![FIG. 2. Changes in the α-amylase activity during germination of the whole and de-embryonated kernel in the presence and absence of GA3.](image2)

![FIG. 3. The α-amylase isoenzyme composition of wheat kernels incubated in the presence of GA3. A, immature whole kernel; B, immature endosperm-aleurone; C, immature de-embryonated kernel; D, mature whole kernel; E, mature de-embryonated kernel.](image3)

Table I: Incubation of De-embryonated Wheat in Presence and Absence of Seed Coat

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Immature Wheat (39 Days After Flowering)</th>
<th>Mature Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg maltose/min-kernel × 10^{-1}</td>
<td></td>
</tr>
<tr>
<td>Seed coat present*a</td>
<td>5.22</td>
<td>11.21</td>
</tr>
<tr>
<td>Seed coat absent*b</td>
<td>0.025</td>
<td>0.26</td>
</tr>
<tr>
<td>Seed coat half removedc</td>
<td>0.26</td>
<td>0.58</td>
</tr>
</tbody>
</table>

*a Incubated 4 days.

*b Seed coat removed after 1 day incubation and remaining endosperm-aleurone incubated 3 more days.

*c Half of seed coat removed after 1 day incubation and endosperm-aleurone and remaining seed coat incubated 3 more days.
was drastically reduced in both the mature and immature samples (Table I, procedure 2). The partial removal of the seed coat also decreased α-amylase production (Table I, procedure 3), but to a lesser extent than was demonstrated by complete seed coat removal. Examination of the α-amylase isoenzyme compositions (Fig. 4) indicated that the de-embryonated immature and mature wheat in the absence or partial absence of the seed coat (Fig. 4, A, C, E, and F) were comparable to those of the control samples (Fig. 4, B and D) with attached seed coats. In this instance, the isoenzyme pattern found in the incubated immature endosperm-aleurone (Fig. 3B) was not displayed, concomitant with seed coat removal. However, it must be realized that the de-embryonated kernels were incubated for 1 day prior to the complete or partial removal of the seed coat as noted in procedures 2 and 3 (Table I). It seems that, if the seed coat remains attached to the endosperm-aleurone for 1 day incubation prior to complete or partial removal, a normal α-amylase isoenzyme pattern is obtained. The implication of this result is that the seed coat may provide factor(s) required for normal α-amylase synthesis. However, a seed coat homogenate (five seed coats extracted with 0.5 ml 1 mM CaCl₂ with and without 0.1 mM GA₃) added back to the incubating immature endosperm-aleurone did not result in further increases in α-amylase activity or changes in the isoenzyme composition (Fig. 3B) of the incubated immature endosperm-aleurone. This does not mean that a factor is not present since the extraction may destroy it or alter its concentration to an ineffective level. An alternate explanation of the observed phenomena is that physical attachment of the seed coat to the aleurone layer is required or that the removal in some way damages the aleurone tissue so that α-amylase synthesis cannot occur.

CONCLUSIONS

These studies indicate that removal of the seed coat from an immature wheat kernel can significantly impair and alter normal α-amylase isoenzyme synthesis. Seed coat removal, in tandem with embryo excision, markedly decreased the production of α-amylase and significantly altered the α-amylase isoenzyme composition of wheat kernels incubated under typical germination conditions. Further work will be necessary to delineate precisely the mechanism whereby the seed coat affects α-amylase isoenzyme synthesis.

LITERATURE CITED

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Fig. 4. The α-amylase isoenzyme composition of wheat kernels incubated in the presence of GA₃: A, immature de-embryonated kernel without the seed coat (as noted in Table I, procedure b); B, immature de-embryonated kernel with the seed coat (as noted in Table I, procedure a); C, mature de-embryonated kernel in the absence of the seed coat (as noted in Table I, procedure b); D, mature de-embryonated kernel in the presence of the seed coat (as noted in Table I, procedure a); E, mature de-embryonated kernel with the seed coat partially removed (as noted in Table I, procedure c); and F, immature de-embryonated kernel with the seed coat partially removed (as noted in Table I, procedure c).