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Relationship of Ribonucleic Acid Metabolism in Embryo and Aleurone to α-Amylase Synthesis in Barley[1,2]

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ABSTRACT

RNA metabolism of embryo and aleurone of barley grains (*Hordeum vulgare* L. cv. Himalaya) was studied to elucidate the role of these tissues in the control of α-amylase synthesis and germination. The extent of ^{3}H-uridine incorporated into various RNA classes of the embryo during the first 12 hours of germination was low but constant. Subsequently, there was a rapid increase in RNA synthesis of all fractions. In the aleurones, after 16 hours, a gradual decrease in ^{3}H-uridine incorporation was observed, and by the time the synthesis of RNA in the aleurones had stopped, α-amylase level was at its highest in the grain.

On transfer to accelerated aging conditions (43 C; 85% relative humidity), the grains lost their viability within 4 weeks. That this was due to a rapid deterioration of the embryo and not of the aleurone was apparent in studies on α-amylase formation, RNA metabolism, and ATP content in grains in various physiological states reported here. Results presented here also reveal a marked influence of the embryo and GA_3 on the quality of the newly synthesized RNAs. Aleurones which lacked the impulse of embryo or GA_3 were capable of synthesizing RNA but these RNAs were less heterodisperse than RNAs from aleurones which were under the influence of an embryo or GA_3.

GA_3 induces synthesis of α-amylase in cereal grains. Work conducted largely with barley grains showed that α-amylase is synthesized *de novo* in barley aleurone layers (7, 25) in response to GA_3. The dependence of α-amylase synthesis in the aleurone on RNA synthesis has been discussed earlier (19, 20, 26, 27, 31). Recently, Zwar and Jacobson (31) found a polydisperse profile of RNA in barley aleurone in response to GA_3.

A number of studies indicate that GA_3 is not a complete substitute for embryo or embryonic factors controlling α-amylase synthesis in the germinating grain. Thus, GA_3 is unable to restore fully the synthesis (quantitative or qualitative) of α-amylase in embryoectomized barley grain (18, 22) or in wheat grain naturally lacking an embryo (15). The GA_3-induced α-amylase synthesis in embryoless barley and wheat grains is inhibited by ABA and is not reversed by cytokinins: in the intact grains, cytokinins effectively reverse the inhibition (11, 15). An involvement of IAA in the synthesis of α-amylase in intact barley grains has been suggested (16), but in the embryoless endosperm IAA had no effect on the enzyme synthesis in presence or absence of GA_3 (6). Evidence has been presented for the control of α-amylase synthesis in the aleurone by hormonal interactions in the coleoptile (28, 29).

This work was undertaken to study the RNA metabolism of embryo and aleurone of barley grains maintained in various physiological states to further elucidate their respective roles in the control of α-amylase synthesis. Preliminary data concerning this work have been presented (23, 24).

MATERIALS AND METHODS

Plant Material and Growth Conditions. Barley grains (*Hordeum vulgare* L. cv. Himalaya, 1967 harvest) were stored at 4 C and 20% relative humidity. The grains were soaked for 10 min in Clorox (1.0% sodium hypochlorite solution) and rinsed thoroughly with water. Intact seeds were grown on sterile agar containing 0.75% Bactoagar (Difco) at 25 C in the dark, as described before (11). Embryoless endosperm segments, obtained by removing the embryo and cutting the endosperm longitudinally, were incubated with the cut surface down on agar plates containing 0.2% (w/v) $CaCl_2$, with or without 10 μM GA_3.

For the study of rapid deterioration and loss of vigor, the grains were stored for various lengths of time under accelerated aging conditions of 43 C and 85% relative humidity in large desiccators.

Extraction and Estimation of α-Amylase. Various tissues were ground with a small amount of glass powder with 0.2% (w/v) $CaCl_2$. The homogenate was centrifuged for 20 min at 10,000*g*, and α-amylase activity was determined in the supernatant according to Briggs (2, 4) using β-limit dextrin as a specific substrate in presence of an excess β-amylase (sweet potato β-amylase, Sigma). The usefulness of this method, especially in determining α-amylase has been described earlier (2). β-Limit dextrin was obtained by adding 1 volume of β-amylase solution (1.66 mg of β-amylase in 100 ml of 1.0 M NaCl) to 5 volumes of 0.1% soluble starch (Nutritional Biochemicals Corp.) solution containing 8.0 mM NaCl and 12.8 mM acetate buffer, pH 5.3. This solution was prepared at least 1 hr before use. The reaction was carried out by incubating 1.0 ml of α-amylase extract or 1.0 ml of water (blank) with 1.5 ml of β-limit dextrin at 25 C. The reaction was followed by adding, at different times, 0.2-ml samples to 10 ml of iodine reagent (0.254 g of I_2 and 4.0 g of KI in 1 liter of water) and reading the absorbance at 550 nm in a Beckman DB spectro-

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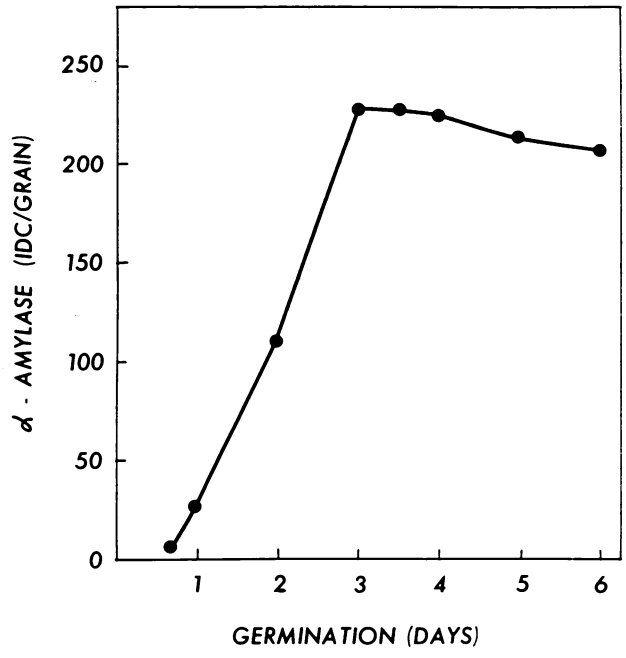

FIG. 1. α-Amylase synthesis during germination of intact barley grains. At various times of germination 10 grains were extracted in 10 ml of 0.2% $CaCl_2$ and α-amylase activity was expressed in IDC units per grain.

photometer. The enzyme activity was expressed in iodine-dextrine-color units (I.D.C. units) (2, 4).

Extraction and Measurement of ATP Content. The ATP content of the embryo and the embryoless endosperm was determined by the luciferin-luciferase assay system of St. John (21) and as adapted for studying ATP levels in seeds (Tao and Khan, unpublished results). Firefly extract (code FFX) was purchased from Worthington Biochemicals Corp. Intact grains were imbibed for 6 hr in water after which the embryo was separated from the endosperm. ATP was extracted from each tissue for 10 min in 5 ml of boiling water. The reaction was started by adding 0.1 ml of ATP extract to a mixture of 0.1 ml of firefly extract (50 mg of firefly extract in 5 ml of H_2O), 4.8 ml of 25 mM HEPES buffer, pH 7.5, and 25 mM $MgSO_4 \cdot 7 H_2O$. The light emission was measured by integrating counts for 0.4 min in the tritium channel of a Nuclear Chicago unilux II scintillation system. ATP levels were calculated in pmole ATP per embryo or embryoless endosperm.

Labeling, Extraction, and Fractionation of RNA. At appropriate times of incubation, 10 embryos were excised and transferred to 2.0 ml of sterile water containing 8.0 μc of 5-H^3-uridine (0.008 mg/μc). Aleurone layers were normally isolated with a glass homogenizer, carefully removing all starchy endosperm. Aleurones from 50 barley grains were transferred to 3.0 ml of sterile water containing 12.0 μc of 5-^{3}H-uridine. Incubation was for 1 hr at 25 C on a metabolic shaker. Total nucleic acids were extracted by phenol-sodium dodecyl sulfate-bentonite method, purified, and fractionated essentially

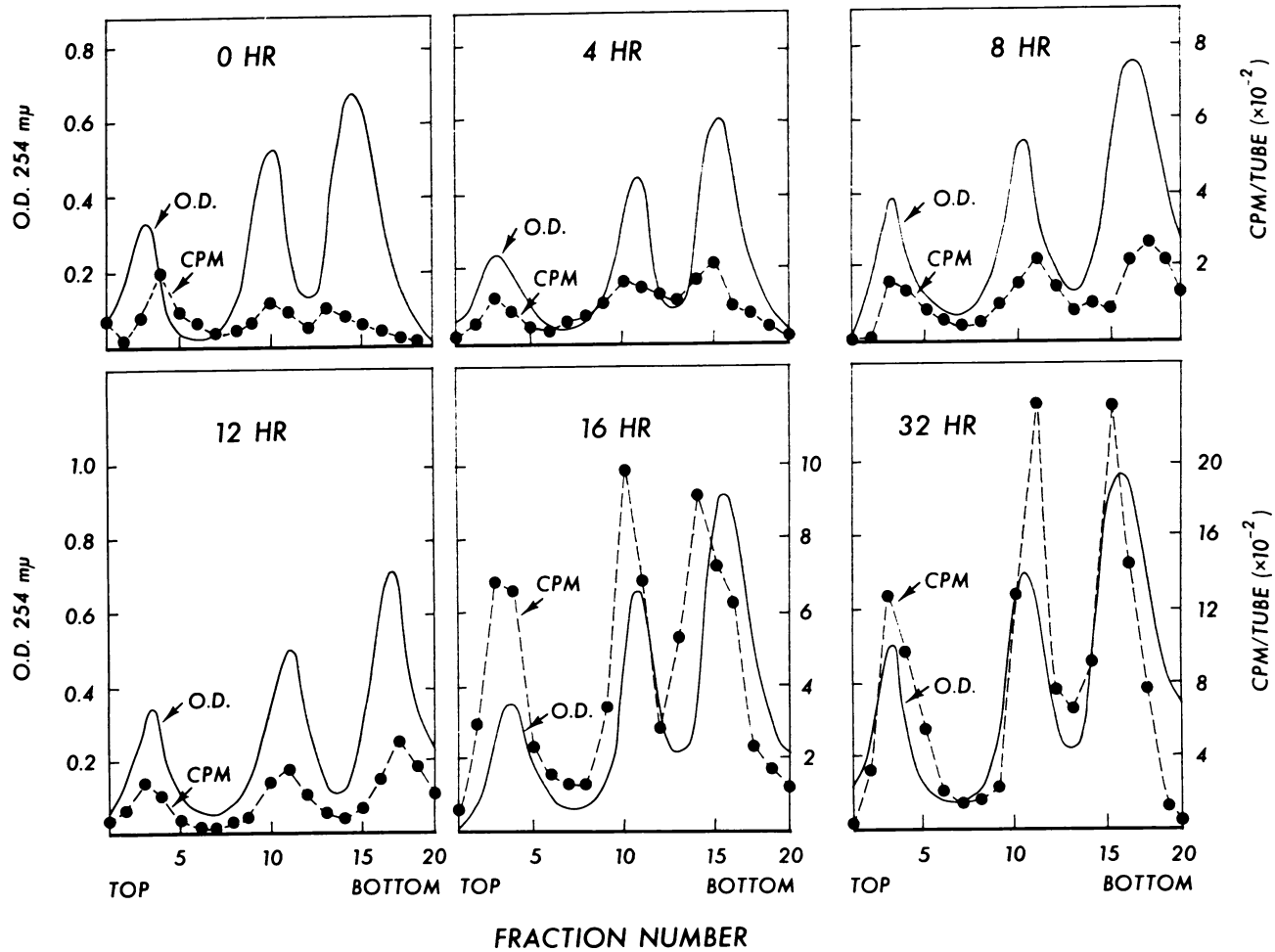

FIG. 2. Sucrose density gradient profiles of bulk and rapidly labeled RNAs extracted from barley embryos. At different times of germination 10 embryos were isolated and incubated for 1 hr in 2 ml of 5-^{3}H-uridine (4 μc/ml).

as described before (12, 14). Fractions (0.4 ml) were collected on an ISCO density gradient fractionator, Model 640. Absorption at 254 nm was continuously recorded and radioactivity was measured after trichloroacetic acid precipitation in a Nuclear Chicago unilux II scintillation system.

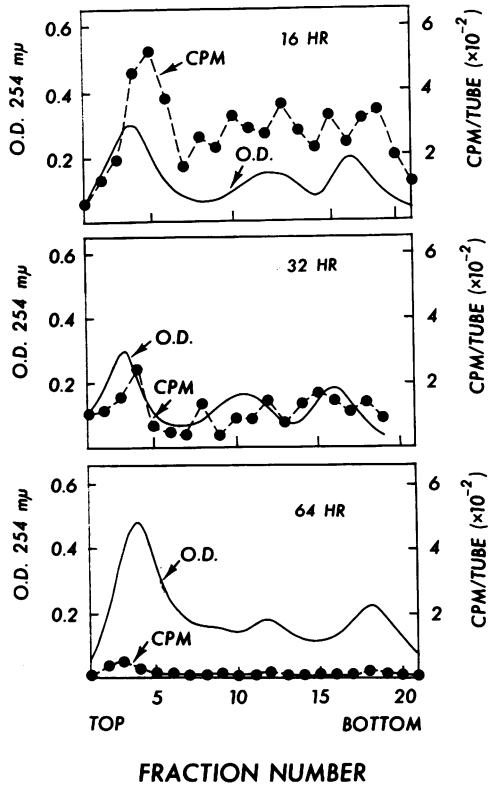

FIG. 3. Sucrose density gradient profiles of bulk and rapidly labeled RNAs extracted from barley aleurones. At various times of germination aleurones of 50 grains were isolated and incubated for 1 hr in 3 ml of 5-^{3}H-uridine (4 μc/ml).

RESULTS

The time course of α-amylase activity in Himalaya barley grain during germination is shown in Figure 1. After a lag period of about 12 hr, there was a sharp increase in enzyme activity, which continued to increase for 3 days, and then leveled off. A similar pattern of α-amylase activity has been reported in other barley varieties (3, 30).

The sucrose density gradient profiles of the bulk and rapidly labeled RNAs from the embryo, excised at different stages of germination of the grain, is shown in Figure 2. It can be seen that dry embryos (0 time) are capable of incorporating ^{3}H-uridine in all classes of RNA during 1 hr of labeling. The level of incorporation remained nearly constant for 12 hr. Subse-

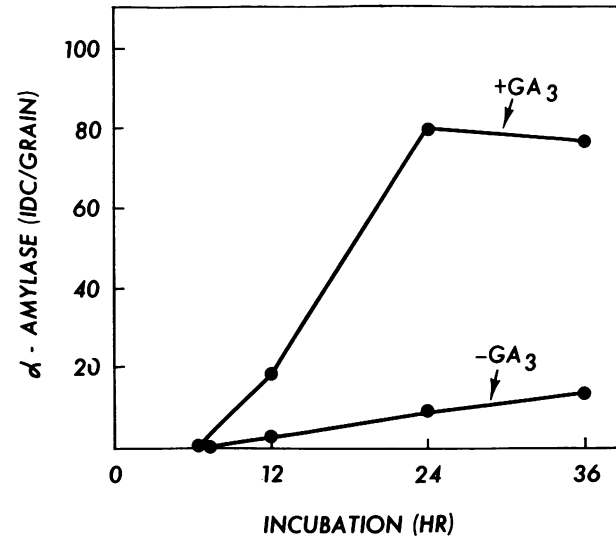

FIG. 4. α-Amylase synthesis in embryoless barley endosperm incubated in 0.2% $CaCl_2$ with or without 10 μM GA_3. Ten embryoless endosperm were extracted in 10 ml of 0.2% $CaCl_2$ and α-amylase activity was expressed in IDC units per endosperm.

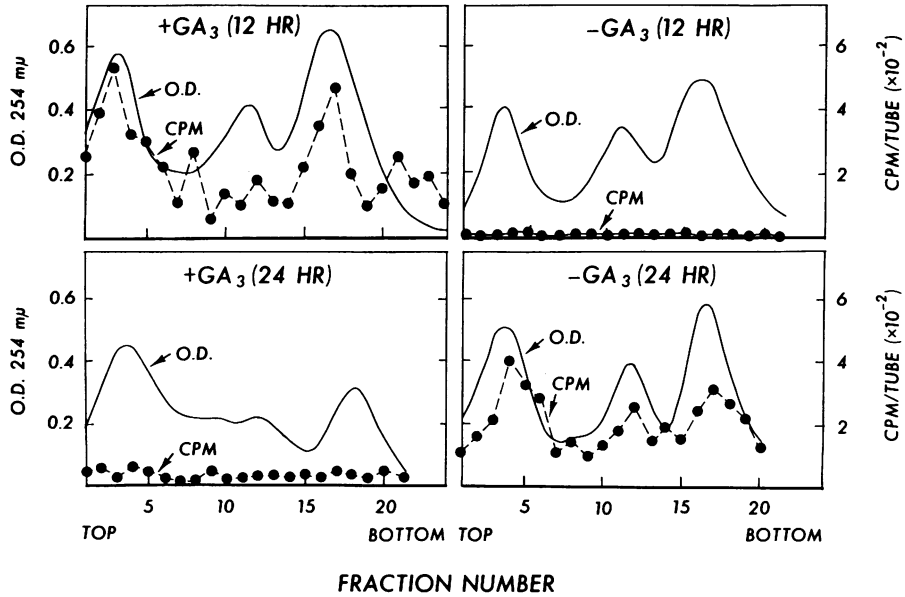

FIG. 5. Sucrose density gradient profiles of bulk and rapidly labeled RNA extracted from aleurones of embryoless endosperm. After incubation for 12 or 24 hr with or without 1 μM GA_3 aleurone of 50 embryoless endosperms were isolated and incubated for 1 hr in 3 ml of 5-^{3}H-uridine (4 μc/ml).

quently, there was a rapid and over-all increase in RNA synthesis. Whether the early incorporation of label in RNAs reflects changes during germination or is a result of the excision of the embryo was not ascertained. The onset of rapid RNA synthesis in the embryo coincided closely with the appearance of α-amylase in the endosperm (Fig. 1).

In the aleurone, excised after 16, 32, and 64 hr of germination, the specific radioactivity of all classes of RNA continued to decrease (Fig. 3), and after 64 hr all ^{3}H-uridine incorporation had stopped. This disappearance of label in the aleurone after 64 hr of germination coincided with the cessation of α-amylase synthesis (Fig. 1). A heterodisperse distribution of rapidly labeled RNA was clearly apparent at 16 and 32 hr of germination.

The embryoless endosperm is nearly completely dependent on GA_3 for the production of α-amylase (Fig. 4). The synthesis of the enzyme in this system has a lag period of 6 to 8 hr in conformity with an earlier report using the same barley variety (19). The α-amylase synthesis reached a maximum level in about 24 hr of incubation.

Sucrose density gradient profiles of bulk and rapidly labeled RNA extracted from the isolated aleurones of embryoless endosperm are shown in Figure 5. In the aleurones, in the presence of GA_3, there is an active synthesis of RNA at 12 hr of incubation, whereas at 24 hr the aleurones are no longer active in RNA synthesis. This coincides with the inability to further synthesize α-amylase in response to GA_3 (Fig. 4). In aleurones, in the absence of GA_3, the pattern of incorporation is completely different. While there is no synthesis of RNA in 12 hr, label is found in all RNA fractions after 24 hr. In contrast to the heterodisperse profile in presence of GA_3, the rapidly labeled RNAs, in absence of GA_3, coincide with the absorption profiles of the bulk RNAs. These data clearly show a profound influence of GA_3 on the timing and the nature of RNAs synthesized by the aleurone.

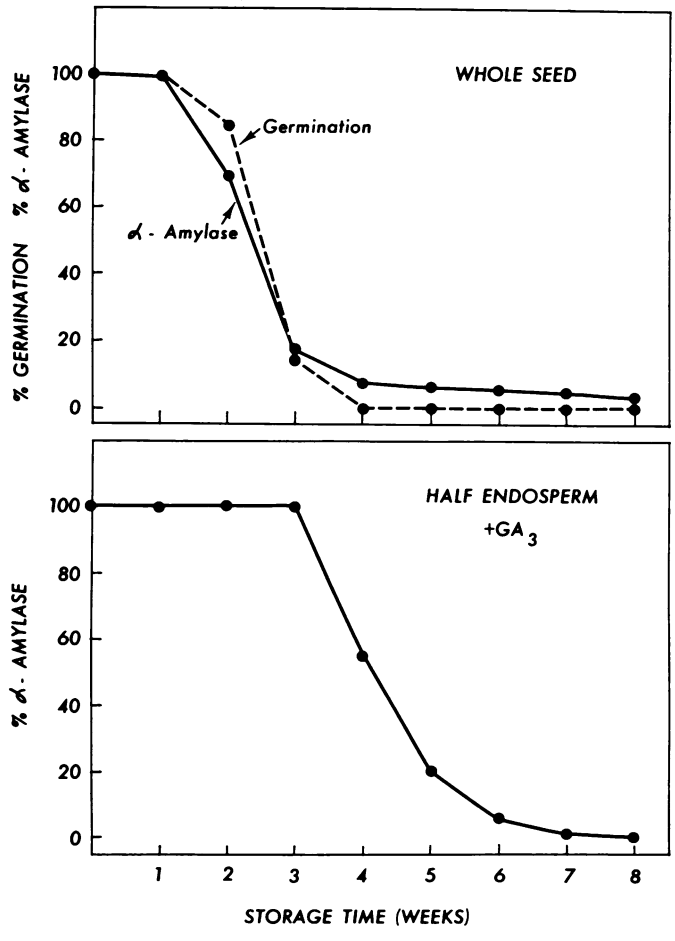

FIG. 6. Influence of accelerated aging on germination and α-amylase synthesis in barley grains. After various lengths of storage, intact grain (whole seeds) were incubated for 3 days on 0.75 per cent agar plates. Germination and α-amylase were expressed as per cent of control at 0 weeks of storage. Influence of accelerated aging on 10 μM GA_3 dependent α-amylase synthesis in embryoless endosperm (half endosperm) was expressed as percent of control at 0 weeks of storage.

Table I. *Influence of Accelerated Aging on ATP Content in Imbibed Barley Grains*

Barley grains were imbibed in water for 6 hr at 25 C. ATP was measured in embryo and embryoless endosperm separately.

Storage Time	ATP Content	
weeks	*pmole/embryo*	*pmole/endosperm*
0	370	23
1	380	25
2	110	22
3	12	19
4	6	11
10	3	4

A transfer of barley grains to a condition of high temperature and humidity (43 C and 85% relative humidity) affects the grain drastically (Fig. 6, whole seed). After 1 week of storage under these conditions the percentage of germination dropped sharply. Parallel with this drop was a decrease in α-amylase synthesis by the intact grain. After 3 to 4 weeks of storage, barley grains failed to germinate (no visible sign of growth of coleoptile or root).

The ability of the embryoless endosperm to support α-amylase synthesis at various storage times did not follow the pattern of germination and α-amylase synthesis in the intact grain (Fig. 6; half endosperm). The aleurones maintained their capacity for α-amylase synthesis for a longer time. Thus the rapid decrease in germination and α-amylase synthesis in the intact grain could be caused by the rapid deterioration of the embryo.

That embryonic tissue deteriorates at a faster rate than the aleuronic tissue was shown by other studies as well. The ATP contents of the embryo and the embryoless endosperm as a function of storage time are shown in Table I. In the embryo, ATP level, although considerably higher than in the embryoless endosperm, decreased shortly after 1 week of storage, while in the embryoless endosperm, no appreciable decrease was observed until 3 to 4 weeks of storage.

The influence of unfavorable storage conditions on RNA metabolism of the embryo and aleurone of stored grains was also studied. In the case of embryo, the label in all classes of RNA showed a continuing decrease after 1 week of storage. After 3 weeks, RNA synthesis had stopped completely, and after 4 weeks a profound qualitative change in the bulk ribosomal RNAs was observed (Fig. 7). In the case of aleurone a remarkably different pattern of label was observed (Fig. 8). In the presence of a viable embryo (0 week storage), the rapidly labeled RNA showed a heterodisperse profile. After 4 weeks of storage, when the embryo is no longer viable (Figs. 6 and 7 and Table I), there is still an active synthesis of RNA in the aleurone. In the latter case, however, the rapidly labeled RNA is less heterodisperse. After a longer storage period (8 weeks)

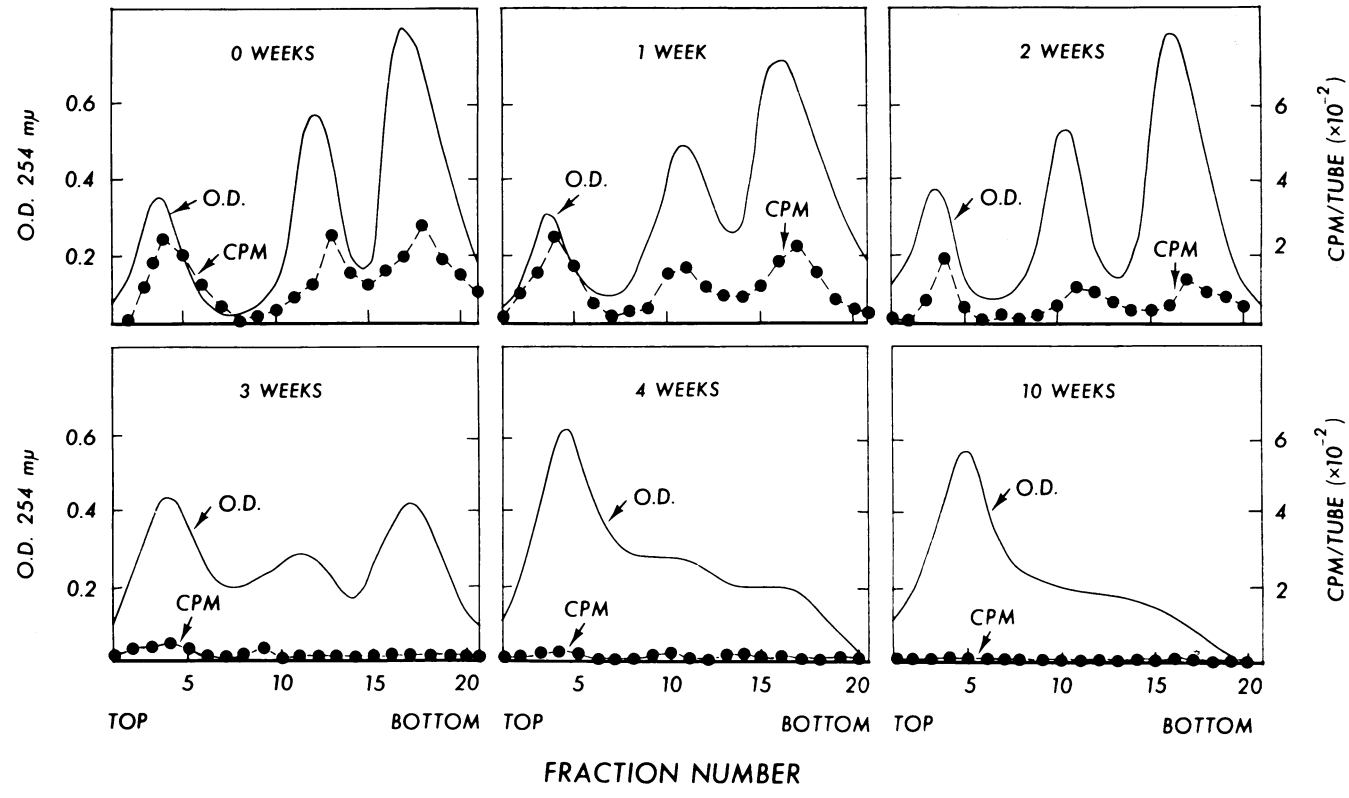

FIG. 7. Influence of accelerated aging on bulk and rapidly labeled RNA in barley embryos. After various periods of storage 10 grains were incubated for 12 hr on 0.75% agar plates. Embryos were isolated and incubated for 1 hr in 2 ml of 5-^{3}H-uridine (4 μc/ml).

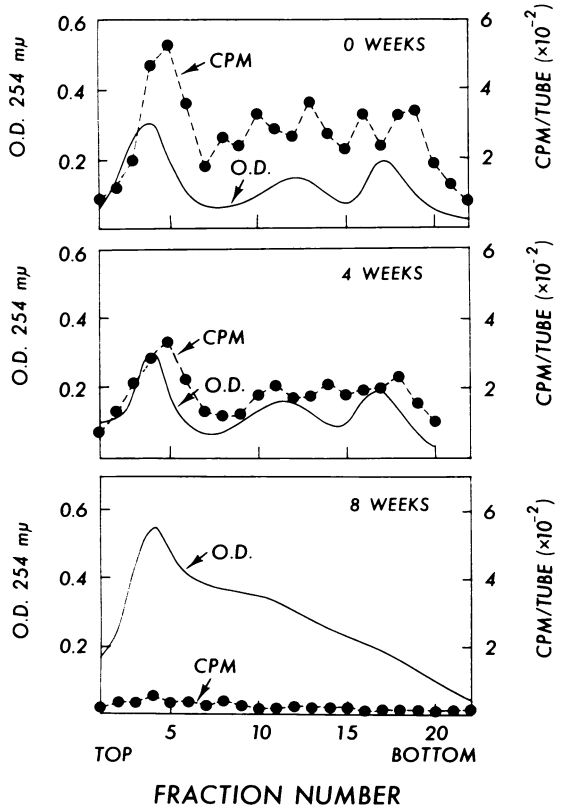

FIG. 8. Influence of accelerated aging on bulk and rapidly labeled RNA in barley aleurones. After various periods of storage, 50 grains were incubated for 16 hr on 0.75% agar. Aleurones were isolated and incubated for 1 hr in 3 ml of 5-^{3}H-uridine (4 μc/ml).

RNA synthesis stopped completely and the bulk ribosomal RNAs no longer could be defined into two different classes.

The synthesis of RNA in aleurone of embryoless endosperm (Fig. 9) followed the same pattern as in the aleurone of intact grains. As long as the aleurone was viable there was an active synthesis of RNA, and again the profile of the rapidly labeled RNA was more heterodisperse in presence of GA_3 than in its absence.

DISCUSSION

The embryo and aleurone of cereal grains are highly specialized tissues with defined physiological roles. The synthesis of α-amylase and other hydrolytic enzymes takes place in the aleurones in response to gibberellin. A number of studies indicate, however, that interactions in the embryo of hormones other than gibberellins may modulate the synthesis of α-amylase by the grains (6, 11, 13, 15, 16, 18, 22, 28, 29).

The time course of the rapidly labeled RNA synthesis in the embryo and aleurone during germination of barley grains followed different patterns. In the case of the embryo (Fig. 2) the rate of RNA synthesis was rather slow and constant during the first 12 to 16 hr of germination. The rapid increase in RNA synthesis observed after that period corresponded with the onset of α-amylase synthesis (Fig. 1) and the visible growth and development of the embryo. More experiments will, undoubtedly, be needed to determine the exact significance of increased RNA synthesis in the embryo to α-amylase synthesis in the aleurone. In the case of aleurones, a gradual decrease in RNA synthesis was observed, and after 64 hr of germination RNA synthesis stopped completely. At that time α-amylase reached its maximum level (Fig. 1), and the specialized functions of the aleurones, which are called into play early in germination, seemed to be completed.

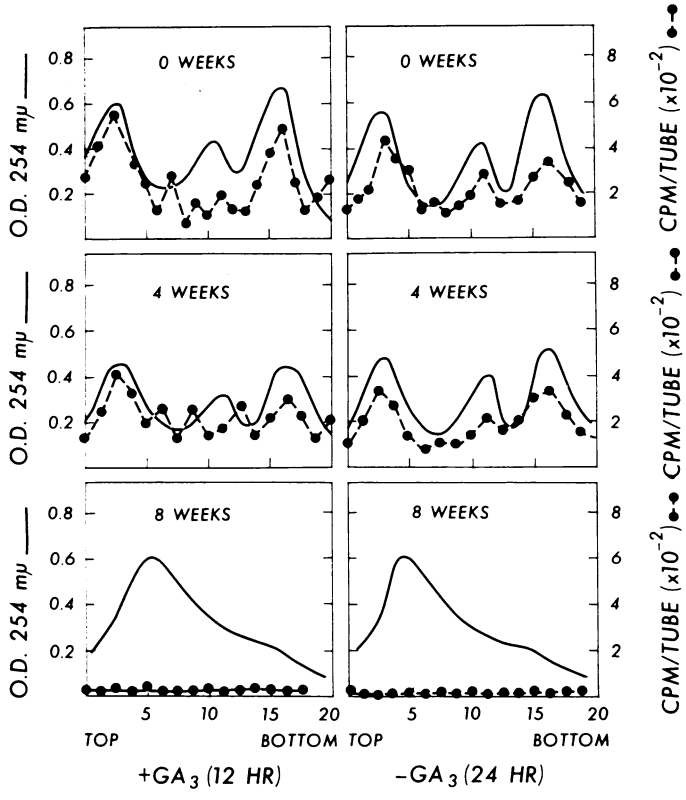

FIG. 9. Influence of accelerated aging on bulk and rapidly labeled RNA in aleurone of embryoless barley endosperm. After various periods of storage, 50 barley endosperm halves were incubated in 0.2% $CaCl_2$ for 12 hr with 10μM GA_3 and for 24 hr without GA_3. Aleurones were isolated and incubated for 1 hr in 3 ml of 5-^{3}H-uridine (4 μc/ml).

Subjecting the barley grains to an adverse storage condition affected the grains drastically (Fig. 6). The results indicated that the rapid loss in germination was due to the rapid deterioration of the embryo. For instance, after 3 to 4 weeks of storage, intact grains were unable to germinate or to synthesize α-amylase. Aleurones of these grains, however, kept their full capacity to synthesize α-amylase in response to GA_3. This could mean that in intact seeds the embryo has become unable to provide the necessary impulse to start α-amylase synthesis in the aleurones. These results indicate that under unfavorable storage conditions embryo and aleurones deteriorate independently and at a different rate. Aspinall and Paleg (1) reported an independent deterioration of wheat embryo and endosperm function with age, but under their storage conditions both tissues deteriorated at the same rate. Further evidence that embryo deteriorates at a faster rate than the aleurone was provided with studies on the ATP content and RNA metabolism of the two tissues. Ching (5) has shown a correlation between ATP level and seed vigor.

RNA metabolism studies of the embryo and aleurones under adverse conditions provided some interesting insights into the influence of embryo on aleurones. After 4 weeks, the embryonic tissue passes into a nonviable state reflected by the decomposition of ribosomal RNA (Fig. 7). The fragmentation of ribosomal RNAs during storage of rye grains has been mentioned by Hallam *et al.* (8). In aleurones, in the presence of a viable embryo (0 weeks storage), the rapidly labeled RNA shows a heterodisperse profile (Fig. 8). After 4 weeks of storage, when the embryo is dead, there is still RNA synthesis in the aleurones; however, the profile of the rapidly labeled RNA now follows the distribution of the bulk RNAs. This indicates that a healthy embryo influences the quality of RNA synthesized in the aleurones. After a longer storage period, the aleurone also passes into a nonviable state, again characterized by the decomposed ribosomal RNA.

Our studies also revealed a marked influence of GA_3 on RNA synthesis. Chandra and Varner (19) observed previously that GA_3 induced a rapid incorporation of labeled precursors into the total salt-soluble RNA of barley aleurones. With increasing time of incubation, this RNA synthesis declined considerably as compared with the control aleurones incubated with GA_3. Results presented here (Fig. 5) show an even greater effect of GA_3 on RNA synthesis in barley aleurones. The active RNA synthesis, after 12 hr of incubation in GA_3, completely disappeared after 24 hr. A similar but somewhat delayed effect was observed during germination of intact grains (Fig. 3). In that case, all RNA synthesis in the aleurones stopped after 64 hr of germination. Thus, it seems that although both GA_3 or embryo cause the aleurones to lose their capacity to synthesize RNA rather rapidly, the embryo appears to have a moderating effect on initiation and termination of RNA synthesis in the aleurones. The early action of GA_3 on the RNA synthesis could be due to a direct action of GA_3 on the DNA-bound RNA polymerase (9, 10, 12, 17). The rapid decline in RNA synthesis could be caused by the hydrolases produced in the aleurones. GA_3 not only affects the rate of RNA synthesis but also the quality of RNA synthesized (26). Further work is in progress to define the nature of the RNA, synthesized in barley aleurones under the influence of embryo or GA_3.

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