A NOTE ON THE FORMATION OF α-AMYLASE IN DE-EMBRYONATED BARLEY KERNELS[1]

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The biosynthesis of α-amylase in barley kernels has been investigated by many workers (1–5). There is conclusive evidence that, during early stages of barley germination, gibberellic acid (GA_3) is released from the embryo and moves to the aleurone layer, where it stimulates aleurone cells to synthesize α-amylase (2,4). In addition, it has been demonstrated that α-amylase can be synthesized in barley distal half-seeds and isolated aleurone layers in response to exogenously applied GA_3 (4).

Although the importance of GA_3 in promoting α-amylase synthesis has been well documented, there is only limited information available on the effect of GA_3 on the synthesis of individual α-amylase enzymes in barley kernels and isolated tissues (6). This report describes preliminary findings on the formation of α-amylase in embryo-less barley half-seeds which were not treated with GA_3 or any other growth-promoting agent.

MATERIALS AND METHODS

The six-rowed barley cultivar, Conquest, was used. All kernels were soaked for 20 min in sodium hypochlorite solution (1.5%) and rinsed thoroughly with deionized, sterile water. Kernels were cut transversely, using a Grobecker farinator.

Incubations were carried out at 21°C in sterile petri dishes, each containing two pieces of 9-cm Whatman No. 29 black, qualitative filter paper and 3.5 ml of either acetate buffer (0.001 *M*, pH 4.8, 0.01 *M* calcium chloride) or acetate buffer containing 0.0001 *M* GA_3.

Samples (two kernels or four distal half-kernels) were removed from the petri

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dishes after 24, 48, 78, and 112 hr and ground in a mortar with fine sand and calcium chloride solution (2 ml, 0.001*M*, pH 5.0). Extracts were centrifuged (15,000 × *g*; 10 min) and appropriate aliquots of the supernatant solutions were used for α-amylase assays and isoelectricfocusing experiments.

α-Amylase activity was determined as described previously (7).

The LKB multiphor system was used for isoelectricfocusing experiments. The gel mixture, containing acrylamide (10 ml, 40%), N,N′-methylene-bisacrylamide (10 ml, 0.9%), ampholine (1.5 ml, pH 4–6, and 1.5 ml, pH 6–8), and sucrose (7.5 g in 36.6 ml of water), was deaerated, mixed with riboflavin (0.4 ml, 0.004%), sandwiched between two glass plates (125 × 260 mm), and polymerized overnight under a fluorescent lamp. Samples were applied to the gel on small pieces of Whatman No. 1 filter paper (5 × 3 mm). The gel was placed on a cooling block at 4°C and a current of 14 mA was maintained through the gel until the voltage reached 600 V (about 70 min). The voltage was left at this value for 3 hr, by which time the current had fallen to 6 mA.

α-Amylase isoenzymes were detected on polyacrylamide gels using the amylopectin β-limit dextrin plate technique described previously (8). This is specific for α-amylases.

RESULTS AND DISCUSSION

Synthesis of α-amylase in untreated and GA_3-treated barley kernels proceeded at similar rates during the first 72 hr of germination (Fig. 1). Activity in untreated

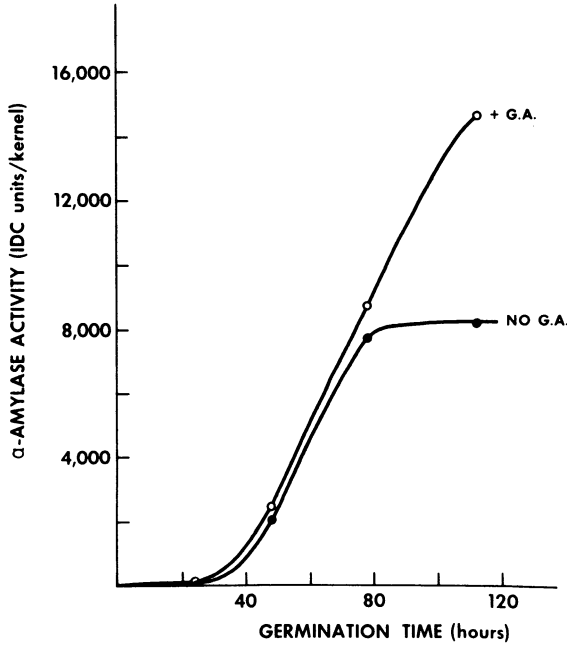

Fig. 1. Changes in the α-amylase content of barley kernels during germination in the presence and absence of gibberellic acid (GA_3).

kernels tended to level off after this period, but activity in the treated kernels continued to increase. These results are in agreement with previous findings (9).

α-Amylase was produced, also, by untreated distal half-seeds (Fig. 2). The amount was small compared to the maximum amount found in GA_3-treated

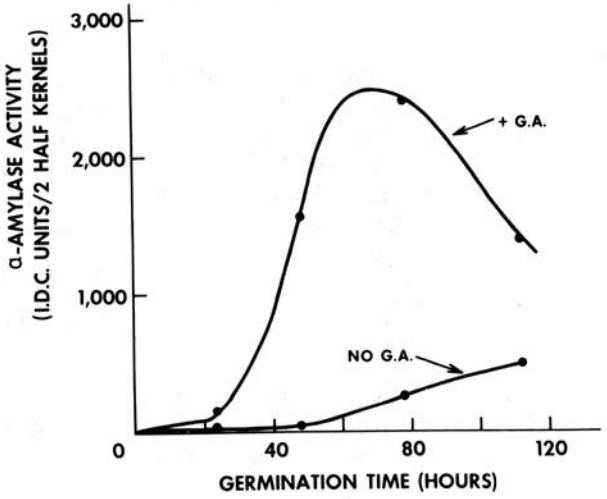

Fig. 2. Changes in the α-amylase content of barley distal half-seeds incubated in the presence and absence of GA_3.

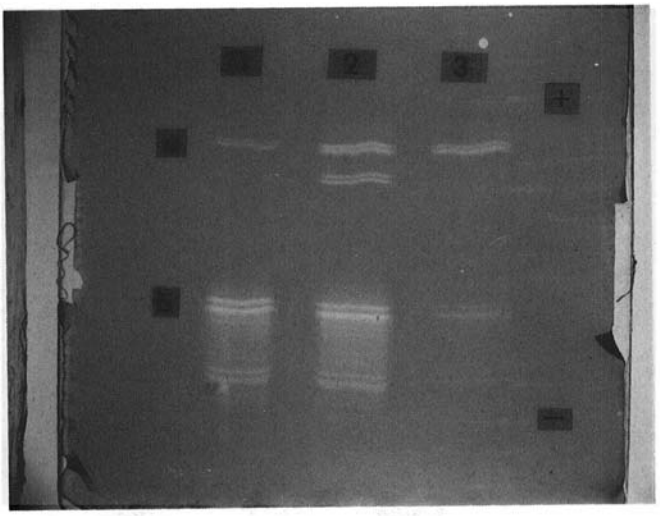

Fig. 3. α-Amylase zymogram obtained after isoelectricfocusing on a pH 4–8 gradient; 1) 78-hr germinated whole kernels; 2) distal half-seeds after 78-hr incubation with GA_3; 3) 78-hr incubated, untreated distal half-seeds. a) α-Amylase I; b) α-amylase II complex which includes the lower bands of activity.

half-seeds (Fig. 2), but after 112 hr of incubation it represented about 35% of the activity in treated half-seeds. Although several reagents other than GA_3 appear to promote α-amylase synthesis in embryo-less barley seeds (10–12) there have been no previous reports on the induction of α-amylase in untreated distal half-seeds. However, similar results for de-embryonated maize kernels have been reported in a recent publication (13).

The α-amylase content of distal half-seeds treated with GA_3 increased rapidly after 20 hr of germination, reached a maximum after 70 hr, and then declined (Fig. 2). It was noted that, after 78 hr of germination, all half-seed endosperms had been hydrolyzed extensively and large portions of kernel contents had leaked out. This probably explains the rapid decline in α-amylase activity and the large difference in maximum activities between the whole and half-seeds.

Isoelectricfocusing studies on α-amylase synthesized by germinating kernels and GA_3-treated half-seeds showed that the enzyme consisted of three main components, each of which was heterogeneous (Fig. 3). In a previous report (14), the most acidic component was designated α-amylase I and the other two components were combined as α-amylase II.

Germinated kernels and GA_3-treated half-seeds had similar α-amylase II patterns, but there were differences in α-amylase I. Whole seeds had two strong, closely spaced bands of activity with two lower, much weaker bands, but in the half-seeds both sets of bands appeared to be equally strong. This suggests that the addition of GA_3 to germinating barley preferentially enhanced the formation of two of the α-amylase I components.

α-Amylase synthesized by untreated distal half-seeds consisted, almost entirely, of α-amylase I. More specifically, only the upper α-amylase I doublet was present, resembling the α-amylase I pattern in germinated whole kernels. Only small traces of α-amylase II, the major α-amylase component of malted barley (14), were detected.

These results suggest that the formation of a portion of the α-amylase I complex, unlike that of α-amylase II, is independent of both the embryo and an exogenous supply of GA_3. The two enzyme systems may be under different genetic control and may be synthesized by different tissues in germinating barley.

Literature Cited

1. BROWN, H. T., and ESCOMBE, F. On the depletion of the endosperm of *Hordeum vulgare* during germination. Proc. Roy. Soc. 63: 3 (1898).
2. MacLEOD, A. M., and MILLAR, A. S. Effects of gibberellic acid on barley endosperm. J. Inst. Brew. 68: 322 (1962).
3. PALEG, L. Physiological effects of gibberellic acid. III. Observations on its mode of action on barley endosperm. Plant Physiol. 36: 829 (1961).
4. VARNER, J. E. Gibberellic acid controlled synthesis of α-amylase in barley endosperm. Plant Physiol. 39: 413 (1964).
5. YOMO, H. Studies on the amylase activating substance. IV. On the amylase activating action of gibberellin. Hakko Kyokaishi 18: 600 (1960).
6. van ONCKELEN, H. A., and VERBEEK, R. Formation of α-amylase isoenzymes during germination of barley. Planta 88: 255 (1969).
7. MacGREGOR, A. W., LaBERGE, D. E., and MEREDITH, W. O. S. Changes in barley kernels during growth and maturation. Cereal Chem. 48: 255 (1971).
8. MacGREGOR, A. W., THOMPSON, R. G., and MEREDITH, W. O. S. α-Amylase from immature barley: purification and properties. J. Inst. Brew. 80: 181 (1974).
9. VERBEEK, R., van ONCKELEN, H. A., and GASPAR, T. Effets de l'acide gibberellique et de

la kinetine sur le developpement de l'activite α-amylasique durant la croissance de l'orge. Physiol. Plant. 22: 1192 (1969).
10. GALSKY, A. G., and LIPPINCOTT, J. A. Promotion and inhibition of α-amylase production in barley endosperm by cyclic 3′, 5′-adenosine monophosphate and adenosine diphosphate. Plant Cell Physiol. 10: 607 (1969).
11. GALSKY, A. G., and LIPPINCOTT, J. A. Induction of α-amylase in barley endosperm by substrate levels of glutamate and aspartate. Plant Physiol. 47: 551 (1971).
12. WHITE, G. A. Barbiturate-induced α-amylase synthesis in barley endosperm. Can. J. Bot. 48: 1981 (1970).
13. GOLDSTEIN, L. D., and JENNINGS, P. H. The occurrence and development of amylase enzymes in incubated, de-embryonated maize kernels. Plant Physiol. 55: 893 (1975).
14. MacGREGOR, A. W., LaBERGE, D. E., and MEREDITH, W. O. S. Separation of α- and β-amylase enzymes from barley malt by ion-exchange chromatography. Cereal Chem. 48: 490 (1971).

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