GIBBERELLIC ACID-INDUCED LIPASE AND α-AMYLASE FORMATION AND THEIR INHIBITION BY AFLATOXIN

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Aflatoxins are a mixture of metabolic products of the fungus Aspergillus flavus, Link ex Fries (Asao, et al., 1963). These compounds are toxic to rapidly growing young animals (turkey poults and ducklings) and can cause liver tumors in rats, trout, and other experimental animals (Lancaster, et al., 1961; Ashley, et al., 1964). Low concentrations of this toxin inhibit incorporation of amino acids into protein by liver preparations (Smith, 1963); mitosis and DNA synthesis in human lung cells (Legator and Withrow, 1964); and chlorophyll formation in seedlings of Lepidium sativum (Schoental and White, 1965). The evidence seems to be accumulating that aflatoxins are inhibitors of protein synthesis. This notion was examined further by a study of the effect of aflatoxins in the formation of enzymes in excised tissue of seeds as influenced by gibberellic acid.

Certain enzymes, notably α-amylase, develop on germination in the cotyledons of pea and aleurone layer of barley but not in excised cotyledons or in the aleurone layers (Varner and Schidlovsky, 1963; Paleg, 1960). The embryo (more specifically the axis tissue in

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dicotyledonary seed) apparently holds the key to initiation of specific enzymatic activities in the germinating seed. Although hormonal control of protein levels in the plant is not a new concept, some evidence now exists which suggests that certain enzyme activities in germinating seed are the result of de novo protein synthesis (Grabar and Daussant, 1964) and that this synthesis of new protein is influenced by gibberellic acid (GA) (Varner and Ram Chandra, 1964). This important phytohormone was shown to partially replace the effect by axis tissue on excised pea cotyledons and to initiate α-amylase activity in the distal halves of barley (Varner, Balce, and Huang, 1963; Paleg, 1960). It has been suggested that a major aspect of the passage from the quiescent to the active stage in seed germination is the formation or activation of messenger RNA (Marcus and Feeley, 1964). Gibberellic acid possibly exerts its control on certain enzymatic activities by initiating specific RNA synthesis (Varner and Ram Chandra, 1964).

Lipase activity is absent in the dry cottonseed but, under appropriate conditions for germination, increases until the third day when a peak is reached (St. Angelo and Altschul, 1964). When untreated distal halves were incubated alone, lipase activity was very low (Table 1). However, if the proximal and distal halves were incubated together, lipase activity developed to the same level as that of the whole seed. Furthermore, distal halves treated with gibberellic acid (10^{-5}M) exhibited a 2-1/2-fold increase in lipase activity. The development of GA-induced lipase activity could be completely inhibited with actinomycin D (50 μg/ml). This suggests that lipase activity in the germinating cottonseed is related to DNA-dependent RNA synthesis. The development of lipase activity could also be inhibited with aflatoxin (45 μg/ml).

When GA-treated distal halves of barley were exposed to aflatoxin

(25 μg/ml of a mixture of aflatoxins B and G), α-amylase activity in the medium was inhibited by 50%. The assay for α-amylase was that of Shuster and Gifford (1962).

Table 1. Summary of the effects of various treatments on lipase activity in the germinating cottonseed.

Treatment	Lipase activity (μ moles fatty acid/30 min.)
Dry Seed	0.34
Germinating Seed	27.18
Proximal Halves	22.02
Distal Halves	4.25
+Proximal Halves	22.67
+GA	11.18
+GA + Actinomycin D	2.31
+GA + Aflatoxin	4.20

Hulled cottonseed were halved by cutting perpendicular to the longitudinal axis. The seed halves to be tested (2 g fresh wt) were surface sterilized 15 min. with 1% sodium hypochlorite, rinsed with distilled water, and incubated in 125 ml flasks containing 2 ml of 0.01M phosphate buffer (pH 5.0) and the appropriate treatment. The incubation flasks were placed on a wrist-action shaker to assure the seed halves of adequate oxygen tension. After incubation for 3 days, 2.5 g (wet wt) of the seed halves were homogenized and strained through 2 layers of cheesecloth. Lipase activity of the homogenate was determined by the method of St. Angelo and Altschul (1964). Most of the data with aflatoxin were obtained with a partially purified preparation (50%) containing approximately equal amounts of aflatoxins B and G. The effects were verified with purified (95%) aflatoxin B. Gibberellic acid was a commercial preparation (86% pure).

There is no evidence that the formation of lipase in germinating cottonseed represents de novo synthesis. But the similarities of GA induction and actinomycin inhibition with this enzyme as compared with α-amylase would suggest that de novo synthesis is likely for the lipase as well. These data lend support to the idea that aflatoxin is an inhibitor of protein synthesis; its mode of action is being explored further with this system.

The data shown in Table 1 were obtained on several different

samples of cottonseed. Other samples of equivalent viability (over 90%) did not, however, exhibit a requirement of GA or proximal halves for lipase formation in the distal halves. In those samples of seed which did not require GA for lipase formation, aflatoxin had little or no effect on the lipase activity although chlorophyll development was visually reduced. We have no explanation for the different behavior but suspect that it arises from differences in conditions of maturation and storage. This raises a question about reports that the formation of other enzymes such as isocitric lyase (Marcus and Feeley, 1964) are not under axis control. This may be a property of the particular seed sample.

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