NOTES

Induction of Enzymes in Dormant Spores of *Aspergillus oryzae*

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In dormant conidia of *Aspergillus oryzae*, α-amylase, invertase, and glucose dehydrogenase were induced by their respective inducers. Neither germination nor swelling occurred during this period.

When suspended in water, conidia of *Aspergillus oryzae* do not germinate or swell for months, but they begin to germinate soon after the addition of nutrients at any time during this period (10). In spite of extensive efforts, the biochemical events responsible for the initiation of fungal spore germination are poorly understood (9). Recently, dormant conidia of *A. oryzae* have been shown to contain a complete protein-synthesizing apparatus, although its activity is much lower than that of germinating conidia (4). The ribosomal fraction appears to contain a factor limiting the rate of protein synthesis during dormancy (4). Virtually nothing is known, however, of the inducibility of enzymes in dormant conidia. If the enzymes are inducible, it is possible that germination may be triggered by the induction of certain enzymes which are lacking in dormant conidia, the germinants acting as inducers. This possibility has not yet been tested. The present study was undertaken to investigate the inducibility of three enzymes, i.e., α-amylase (EC 3.2.1.1), invertase (EC 3.2.1.26), and glucose dehydrogenase (EC class 1.1.99), in dormant conidia. These enzymes have been demonstrated to be inducible in mycelium (8, 11, and unpublished data).

*A. oryzae* strain U was grown for 10 days on a glycerol-peptone medium containing (g/liter): glycerol, 30; peptone, 3; NaNO₃, 3; K₂HPO₄, 1; KCl, 0.5; MgSO₄·7H₂O, 0.5; and agar, 15. Conidia were harvested and freed from mycelial fragments as described by van Etten (12). Conidia of *A. oryzae* were shown to synthesize very limited quantities of ribonucleic acid (RNA), which was mainly of transfer and ribosomal type even when suspended in water or phosphate buffer; this RNA synthesis continued for several hours and then leveled off (6). To avoid possible effects of inducers on this unspecific process, the conidia were preincubated at 30° C for 17 hr in 20 mm phosphate buffer (pH 6.8) which contained chloramphenicol (100 μg/ml) to prevent bacterial growth, and then various inducers were added.

Figure 1 shows that the three enzymes studied were induced specifically by their respective inducers. Amylase was induced only by maltose or soluble starch, and invertase was induced only by sucrose. Other sugars tested, i.e., glucose, galactose, mannose, fructose, xylose, lactose, and raffinose, were inactive as inducers. On the other hand, glucose dehydrogenase was induced, as in the mycelium (8), by the simultaneous presence of glucose and hydroquinone. The induction of these enzymes was completely blocked by cycloheximide (20 μg/ml) or phenethyl alcohol (4 mg/ml) but was not affected by actinomycin D (80 μg/ml) or puromycin (100 μg/ml), possibly because the latter two inhibitors failed to penetrate the conidia. Some fungi, such as *A. parasiticus* (5) and *Peronospora tabacina* (3), are known to be impermeable to both actinomycin D and puromycin. The increase in enzyme activities after addition of inducer would seem to indicate de novo synthesis of enzymes rather than the specific activation of latent enzymes by their respective inducers, since (i) the increase was blocked by cycloheximide or phenethyl alcohol (7); (ii) leucine-1-¹⁴C incorporation into hot trichloroacetic acid-insoluble fraction was increased by the inducers (Fig. 2); and (iii) when the inducer was removed by filtration and the spores were suspended in the medium without inducer, enzyme-forming capacity decayed exponentially with a half-life of 20 min for the three enzymes studied. When the conidia were ex-
amined microscopically 7 hr after the addition of inducer, neither the emergence of germ tube nor swelling was observed. This was further substantiated by the finding that the packed cell volume was not increased when estimated by the microhematocrit method (2). These results are in striking contrast to the fact that, under similar conditions, almost all conidia germinated within 5 hr in a germination medium (10). It is suggested that some enzymes are inducible even in dormant conidia, although further work is needed to relate this phenomenon to the triggering mechanism of germination.

Fig. 1. Kinetics of enzyme induction in dormant conidia of A. oryzae. Conidia (10⁷/ml) were preincubated as explained in the text. Samples (50 ml) were taken at intervals after the addition of inducer; the conidia were collected by filtration, washed with water, mixed with 3 g of quartz sand (150 to 200 mesh), and kept overnight at -20 C. The frozen spores were ground in a prechilled mortar, extracted with 5 ml of 20 mM phosphate buffer (pH 6.8), and centrifuged at 10,000 X g for 15 min. The clear supernatant fluid was used as an enzyme solution. Amylase was assayed by the method of Bernfeld (1), except that assays were made at pH 5.0 and 30 C for 10 min. Invertase was assayed similarly with 75 mM sucrose as substrate. Specific activities for amylase are expressed as micromoles of reducing groups (calculated as glucose) liberated per minute per milligram of protein, and for invertase are expressed as micromoles of sucrose hydrolyzed per minute per milligram of protein. Glucose dehydrogenase was determined and its specific activity was defined as described previously (8). (A) Glucose dehydrogenase: ●, glucose + hydroquinone; ■, glucose; △, hydroquinone. (B) Invertase: ●, sucrose; △, no addition or addition of any one of the following sugars, D-glucose, D-mannose, D-galactose, D-fructose, D-xylose, lactose, maltose, and raffinose. (C) α-Amylase: ●, maltose; ○, soluble starch; △, the same as those represented by this symbol in (B), except that maltose was replaced by sucrose. The final concentrations of all of the sugars and hydroquinone were 0.5% (w/v) and 0.1% (w/v), respectively.

Fig. 2. Incorporation of leucine-14C into hot trichloroacetic acid-insoluble fraction of A. oryzae conidia during the enzyme induction. Conidia (10⁷/ml) were preincubated as described in the text and were then incubated from -120 min with various inducers. At zero time, L-leucine-14C (25.1 μC/mole, New England Nuclear Corp. Boston, Mass.) was added to a final activity of 0.1 μC/ml. At intervals, 1-ml samples were withdrawn and the radioactivity of hot trichloroacetic acid-insoluble fraction was determined as described by Horikoshi and Ikeda (4). Symbols: X, glucose + hydroquinone; ●, sucrose; ○, maltose; ■, no addition. The final concentration of the inducers was the same as in Fig. 1.

LITERATURE CITED