Serine-induced formation of aerial hyphae and conidia

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Abstract
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The present results indicate that the system studied is not repressed by the choline or DMAE, but on the contrary, that these compounds could be the inducers of K- and PMMAE-cytidylyltransferase.

Louie, S., A. Chan, and G. Soika. Serine-induced formation of aerial hyphae and conidia by a Neurospora mutant.

ser-2 (isolate #65004) is a very "leaky" serine brodytroph. It grows rapidly on minimal medium but does not form abundant aerial hyphoe or pigmented conidio unless supplemented with L-serine. It also responds to glycine, but no other amino acid, or intermediate in the serine biosynthetic pathway, can substitute for serine. The addition of serine to cultures growing on solid media causes the mutant to form aerial hyphoe and pigmented conidio at approximately the same rate as do wild type strains on minimal medio. This property was examined by comparing growth rates of ser-2 and a wild type strain (STA4), employing a variety of growth parameters.

Figure 1 shows the results of an experiment designed to compare the rate of hyphal elongation on solid Vogel's minimal medium (2% sucrose as carbon source). This method ignores penetration of hyphae into the agar and aerial hypha formation (Zolokar 1959 Am. J. Botany 46:555). Under these conditions STA4 and ser-2 show identical growth rates in the absence of serine.

When these strains are grown in Vogel's minimal liquid medium with vigorous agitation, dry weight increases logarithmically for at least 24 hours (Lucz 1963 J. Cell. Biol. 16:483). Formation of aerial hyphae and conidio is minimized in submerged culture, yet Figure 2 (which is representative of many such experiments) indicates that ser-2 grows more slowly than does STA4 under these conditions.

Growth in stationary liquid culture is essentially unrestricted and 3-dimensional (Marshall and Alexander 1960 J. Bacteriol. 80:412) and can best be expressed as the cube root of the increase in dry weight (Emerson 1950 J. Bacteriol. 60:221). After approximately two days of incubation, wild type organisms begin to form aerial hyphoe above the mycelial mot. The appearance of these structures is delayed at least one week in ser-2. The defect can be completely overcome by addition of 0.1mL serine to the growth medium. From Figure 3 it can be seen that ser-2 and STA4 on minimal and serine-supplemented medio have--lo, growth rates for approximately 2 days, while the mycelial mot is being formed across the surface of the liquid. The failure of ser-2 cultures on minimal medium to form aerial hyphoe results.

Figure 1. Hyphal elongation in solid medium. Growth tube culture at 30°C in constant darkness on Vogel's minimal medium. (STA4 open circles; ser-2 darkened circles).

Figure 2. Logarithmic growth in minimal liquid medium. Cultures grown at 30°C in constant darkness. 30ml. Vogel's minimal medium in 125 ml. Ehrlenmeyer flasks agitated at 150 rpm.
in a premature cessation of "cube root growth".

We feel that these data suggest that rer-2 is a biochemical-developmental mutant which requires additional serine only for the production of aerial hyphoe and pigmented conidia. This mutant is obviously able to synthesize sufficient serine to support normal growth of the basal mycelial mat. The conidia formed on ser-2 (either by supplementation with serine or prolonged incubation) appear to be similar to those of the wild type in size, shape and carotenoid pigment content.

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Figure 3. "Cube root growth" in stationary liquid cultures. 125 ml Erlenmeyer flasks containing 25 ml of liquid medium were incubated at 30°C. The results of the experiment were not affected qualitatively by a wide range of inoculum sizes of filtered conidia, (STA4 on minimal medium, open circles; ser-2 on minimal medium, darkened circle: -2 on 0.1 M L-serine-supplemented medium, half-darkened circle)

Kapoor, M. Pyruvate kinase of N. crassa: conformational changes induced by Tris buffer.

Pyruvate kinase (PK) was isolated from N. crassa (St. Lawrence strain 79a; FGSC#533). The inoculum was grown on Vogel's minimal medium with 2% sucrose for 30 hrs. at 28°C, in an Environmental (New Brunswick) rotatory shaker. The mycelium was harvested at -65°C. The enzyme was isolated from lyophilized mycelium in 0.05 M phosphate buffer containing 5 x 10^-4 EDTA and 10^-4 M 2-mercaptoethanol, pH 7.5, and purified to near homogeneity by the application of several fractionation steps including heat treatment, ammonium sulphate precipitation (0.40 to 0.65 saturation), ion exchange chromatography on columns of DEAE Sephadex, a second ammonium sulphate fractionation and gel filtration on a Sephadex G-200 column, followed by ammonium sulphate precipitation of the active fractions.

The enzyme preparation thus obtained was found to be approximately 95% purified. Upon electrophoresis on polyacylamide gels, one major band containing about 95% of the total protein (the pyruvate kinase band) and two minor ones were detected. The two minor components are probably contaminants although the presence of subunits produced as an artifact of electrophoresis has not been ruled out. Sedimentation in the Analytical Ultracentrifuge (Spinco model E) revealed the presence of a single symmetrical peak; no minor peaks could be discerned on sedimentation of enzyme solutions containing 2 and 4 mg protein per ml. Details of the purification procedure will be published elsewhere. The final enzyme preparation was stored in 0.2 M phosphate buffer, pH 7.5, containing 10^-4 EDTA and 2 x 10^-3 M dithiothreitol. It was stable for at least two months at -20°C. Before use the enzyme was routinely diluted to a concentration of 100 µg/ml in the same buffer, 0.1 ml being used in individual assays.

Enzyme activity was determined by measuring the pyruvate produced from phospho-enol-pyruvate (PEP) and ADP in the presence of magnesium chloride by coupling the reaction with lactate dehydrogenase (LDH) and following the disappearance of reduced NAD at 340 µm in a Beckman-DU spectrophotometer. The quantity of LDH in individual assay systems was maintained at a 100-fold excess over that of PK. The pH curve for pyruvate kinase reaction, using phosphate buffer in the assay mixture, showed a broad pH optimum between 6.8 and 8.0 with very little variation in activity within this range. A progress curve of the reaction (OD 340 vs. time) at all pH values tested (6.5 to 9.5) in phosphate buffer was linear. With Tris-HCl buffer, on the other hand, the reaction showed a pronounced initial lag, lasting for approximately 2.0 min and then a linear reaction rate was attained. The reaction velocity during the linear phase was observed to be no more than 60% of that obtained in phosphate buffer.

Thus there are two distinct aspects of the effect of Tris buffer: (a) the introduction of a lag period and (b) a diminished reaction velocity during the linear phase. These two effects of Tris buffer were observed in reactions performed at pH values ranging from 7.5 to 9.5, the log becoming more pronounced at higher pH. It would appear that the first effect is connected with a conformational change induced by iris leading to a lag in the reaction and that the attainment of linearity is a consequence of a subsequent