New supplements for the formate mutant of Neurospora. A possible role for ascorbic acid?

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New supplements for the formate mutant of Neurospora. A possible role for ascorbic acid?

Abstract
Recently we found what appeared to be a new auxotroph in one of our stocks. It was eventually traced to the formate (for) locus.
Identification and visualization of cellulase activities from Neurospora crassa

To elucidate the nature of cellulase activities found in Neurospora crassa, we analyzed the "induced" spent culture medium for the components of the cellulase enzyme complex. This was the prerequisite of work on the isolation of the enzymes, their purification and N-terminal sequencing.

Supernatant from a 3-4 day culture grown on Vogel's sucrose minimal medium overnight was subjected to PAGE (10% polyacrylamide). Using replicate gels, one was stained in Coomassie blue, and the other was overlaid on an agar gel containing 0.1% carboxymethyl cellulose at 25°C overnight. The exposed CMC agar gel was then stained with 0.1% Congo red, which stains the undigested CMC. Volumes of 1 ul to 50 ul of supernatant containing less than 1 ug of protein gave detectable zones of clearing of CMC, detected visually on a light box after differential destaining with 1M sodium chloride.

With the cell-1 (T11, FGSC# 4335 and 4336) mutant or wild type (74-OR23-IA), three zones of CMC clearing were visible after exposure to filter paper-induced but not uninduced supernate. The major band of activity was stable in SDS-PAGE, and its Mr was between 60,000 and 70,000. The band migrated to the same position in PAGE without SDS, suggesting that the active enzyme is a simple monomer. Two other bands of clearing were circa 50,000 (the weakest) and 30,000. With wild type (74-OR23-IA), only the 30,000 form of the enzyme was produced in sufficient quantity to be detectable when induced with cellobiose rather than filter paper.

A rapid method for DNA extraction is described. It is equally efficient with small or large quantities of mycelium, produces readily restricted DNA, and is comparable with that produced by the method of Case et al. (1979, PNAS 76:5259) in concentration of DNA obtained and average fragment size.

Mycelium from an overnight culture was harvested through a Whatman no. 1 filter, washed and freeze-dried. The mycelium (~50-100 mg) was placed in a 50 ml Sorvall tube, and an equal volume of 6M urea containing 2% SDS was added. The mixture was left on ice for 10-15 minutes, after which it was centrifuged at 10,000 rpm. The supernatant was transferred and extracted with phenol 2-3x. The DNA was precipitated from the aqueous phase by the addition of 2 vol of cold ethanol, collected by centrifugation, and dissolved in 100-300 ul of TE buffer, pH 8. RNA may be removed at this stage with RNase.

This method appears to be generally applicable to unicells, mycelium and other filamentous organisms. It has been successfully used also on the moss Physcomitrella patens.

Recently we found what appeared to be a new auxotroph in one of our stocks. It was eventually traced to the formate (for) locus. Later we found that it was not a new allele, but was the original C24 for allele which had gotten into the stock by an ancient error in stockkeeping.

In the course of determining the auxotroph's growth requirements, we found that it responded strongly to three combinations of supplements not previously reported for for. The for mutant had previously been reported to grow weakly on adenine alone, strongly on adenine plus methionine, and strongly on formate or formaldehyde. On our auxanograms the response to adenine alone is very weak, but the response to adenine combined with histidine, tryptophan or ascorbic acid is very strong. Neither histidine nor tryptophan is effective without adenine. Ascorbic acid gives a definite response without adenine but gives a stronger response when adenine is present.

All tests were done auxanographically. The supposed new auxotroph and a standard stock of for (an f1 of FGSC 133) behaved identically in all tests, and both grew well on formate.
The for mutant lacks cytosolic serine hydroxymethyl transferase, which catalyzes the reaction serine + tetrahydrofolate $\rightleftharpoons$ glycine + methylene tetrahydrofolate (see Cossins and Pang 1980 Experientia 36:289-290). This is the chief reaction that generates the transferrable C1 units of the various folate coenzymes which are needed for the synthesis of purines, methionine, thymidylate, etc. Formate supports growth of the mutant by the formation of formyl tetrahydrofolate, which can be converted to the other folate coenzymes.

We can only speculate about how the new supplements work. Tryptophan degradation is known to produce formate. Histidine is known to stimulate the growth of post-purple adenine in the presence of adenine (M. Case, cited in Perkins et al. 1982 Microbiol. Rev. 46:426-570), presumably because of the connections between histidine and adenine synthesis, but the response in our case is so great as to suggest that there may be an alternate explanation. Possibly histidine is degraded to yield 5-formiminotetrahydrofolate as in mammals, but to the best of our knowledge only the first step of this pathway has so far been demonstrated in Neurospora.

Ascorbic acid does not work simply by lowering the pH, because a neutralized solution of ascorbic acid was also effective. Several oxidation/reduction reactions occur in the synthesis of the various folate coenzymes, but none of them is a hydroxylation of the type that ascorbic acid is known to catalyze. Conceivably ascorbate is degraded to generate a formyl group. Two possible mechanisms for this have been suggested.

First, C$^{14}$ ascorbate produces C$^{14}$ oxalate in man (E.L. Smith et al. 1983 Principles of Biochemistry-Mammalian Biochemistry, 7th ed., p. 667), and pea seeds have an enzyme system that converts oxalate to formate (Giovanelli and Tobin 1964 Plant Physiol. 39:139-145). We therefore tested oxalate on for. In single auxanograms on plates of minimal with and without adenine, each of two for isolates gave definite growth responses to potassium oxalate, although only in the presence of adenine. These responses might suggest that oxalate is converted to formate, although we have no other evidence for it. nor any evidence that Neurospora converts ascorbate to oxalate.

Second, Dumbrava and Pall (1987 Biochim. Biophys. Acta 996:331-338) have found that Neurospora lacks detectable ascorbic acid but has a pool of erythroascorbic acid. Erythroescorbic acid resembles ascorbic in structure except that it is one methylene group smaller. Conceivably ascorbic is converted to erythroascorbic, producing a formyl group in the process.

If Neurospora contains no ascorbic acid when grown in its absence, any physiological role for ascorbic must be limited to situations in which exogenous ascorbic is present. This may frequently may be the case in nature, since Neurospora normally grows on plant materials.

We thank Edwin Cossins and Martin Pall for directing us to the Giovanelli and Tobin and the Dumbrava and Pall papers. - - - Department of Biological Sciences, Stanford University, Stanford CA 94305

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Growth temperature and permeability of Neurospora crassa

Whereas fungi are very efficient at accumulating compounds from the medium by active transport, compounds lacking transport mechanisms often show very low permeability. Consequently, nontransported growth supplements, antibiotics and other potential effectors may be ineffective in fungi.

In Neurospora actinomycin D has been found by Totten and Howe (1971, Biochem. Genet. 5:521-532) to be much more effective when used in cultures grown at elevated (30-35$^\circ$) temperature than when used in cultures grown at 25$^\circ$. Similarly, exogenous cyclic AMP is much more effective in stimulating cyclic AMP-regulated processes in cultures grown at elevated temperatures (30-37$^\circ$) than in cultures grown at lower (25$^\circ$) temperatures (Dumbrava and Pall, 1987, Biochem. Biophys. Acta 926:331-338). These results suggest that Neurospora is more permeable to both of those agents when grown at elevated temperatures. Consequently, growth at elevated temperatures may be a useful stratagem for experiments attempting to overcome low permeability of Neurospora to exogenous compounds. (Supported by NIH grant GM24368). - - - Programs in Genetics and Cell Biology and Biochemistry/Biophysics, Washington State University, Pullman, WA 99164-4350.