ORNITHINE DECARBOXYLASE ACTIVITY IN GERMINATING CONIDIA OF *ASPERGILLUS NIDULANS*

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1. Introduction

The cellular functions of the naturally occurring polyamines, putrescine, spermidine and spermine are unknown. Several workers [1–4] have used putrescine auxotrophs of *E. coli* in order to try to establish which cell processes are impaired when the intracellular concentration of polyamines are reduced. A mutant of *Aspergillus nidulans* was reported by Sneath [5] in 1955 to have an absolute requirement for putrescine for growth. However, the observations described in this paper using a recombinant of Sneath's original auxotroph show that putrescine is required for germination of the conidia but not for subsequent hyphal growth. This transient putrescine requirement during germination of the mutant is due to a deficiency in ornithine decarboxylase at a time when there is a rapid increase in ornithine decarboxylase in a similar prototroph of *Aspergillus nidulans*. The increase in ornithine decarboxylase activity in the prototroph corresponds with the period of most rapid RNA synthesis.

2. Materials and methods

The putrescine auxotroph used was *A. nidulans* puA_i, which is a biotin prototrophic recombinant obtained from Sneath's original isolate (*bi*, w_3, pu_1), and the prototroph used was *A. nidulans* BWB 272 which is a recombinant derived from w_3, *bi*. These two strains will be referred to as the auxotroph and prototroph respectively. Both strains were grown in submerged culture as described previously [6], but with the omission of putrescine from the medium except in the instances stated below. 1 μCi[1,4-^{14}C] putrescine dihydrochloride, specific activity 63 mCi/mmol was added to each 50 ml of medium in the experiments in which putrescine uptake was measured. The inoculum used was such as to give a final conidial density of 1.25×10^7 conidia/ml. When the auxotroph was grown for subsequent ornithine decarboxylase assays 2 mg putrescine hydrochloride litre/growth medium was used. The prototroph was grown both in the presence and in the absence of putrescine when used for ornithine decarboxylase assays (see fig.3). Conidia were harvested on millipore filters, and disrupted by grinding with acid-washed sand. RNA was estimated as described by Fleck and Begg [7]. Ornithine decarboxylase was assayed by measurement of $^{14}CO_2$ released after incubation with [1-^{14}C] ornithine [8]. The incubation mixture was as follows: 50 mM Tris–HCl, pH 8.0, 0.05 mM L-ornithine, 0.031 mM Pyridoxal phosphate, 0.25 μCi DL-[1-^{14}C] ornithine monohydrochloride, 58 mCi/mmol, 0.1 ml of enzyme extract in a total volume of 0.4 ml. The enzyme was extracted from conidia or growing hyphae in 10 mM potassium phosphate, 0.1 mM EDTA, 2 mM dithiothreitol pH 7.5. The extract was centrifuged at 10 000 $g \times 20$ min. Ammonium sulphate was added to the supernatant to give 50% saturation. The precipitated protein was redissolved in the extraction buffer and dialysed against 100 vol. of the same buffer. Measurements of radioactivity were made using a liquid scintillation counter and the measurements were corrected to D.P.M. using the channels-ratio method.

3. Results and discussion

When either the prototroph is grown in submerged

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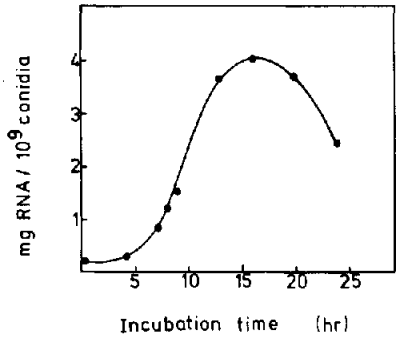

Fig.1. The change in total RNA when conidia from a prototrophic strain of *Aspergillus nidulans* germinate in submerged culture.

culture in the presence or absence of putrescine, or the auxotroph is grown in the presence of putrescine, approximately 80% of the conidia germinate between 5 and 8 h of incubation. If the auxotroph is incubated in the presence of putrescine for at least 4 h and it is then transferred to putrescine-free medium it will continue growing normally for a further 24 h. (These experiments cannot be usefully extended beyond about 30 h in batch culture as the organisms cannot be satisfactorily maintained in log phase.) This period for which putrescine is required by the auxotroph precedes a period in which rapid RNA synthesis occurs (fig.1). If the prototroph and the auxotroph are incubated initially in medium lacking putrescine, and then subsequently [^{14}C]-putrescine is added and its rate of uptake is measured, it can be seen that uptake is most rapid in both prototroph and auxotroph at 5 h incubation (fig.2). However the rate of uptake is much greater at this time in the case of auxotroph. Ornithine decarboxylase activity (fig.3) can be detected in the conidia from the prototroph before germination and there is a peak of activity at 8 h when putrescine is absent from the medium. When putrescine is present in the growth medium the peak activity is delayed. In contrast only very low levels of ornithine decarboxylase can be detected in the prototroph with no obvious peak times of activity.

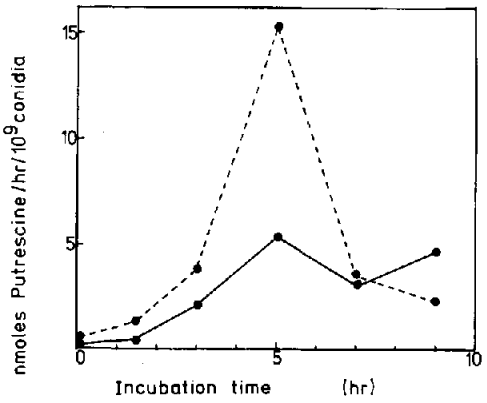

Fig.2. The rate of uptake of putrescine by germinating conidia from a prototrophic (——) strain and an auxotrophic (– – – –) strain of *Aspergillus nidulans*.

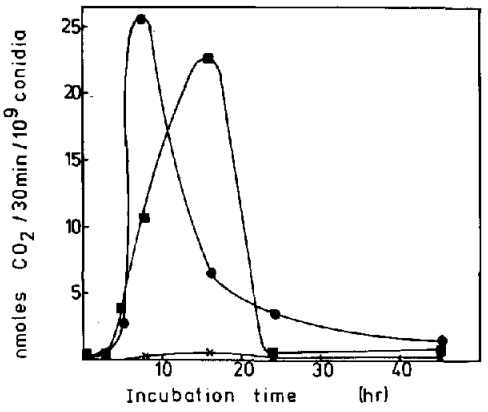

Fig.3. The ornithine decarboxylase activity of conidia: (a) from the prototrophic strain germinating in the absence of putrescine (–•–) (b) from the prototrophic strain germinating in the presence of putrescine (–■–), and (c) from the auxotrophic strain germinating in the presence of putrescine (–x–). Putrescine dehydrochloride concentration in the medium in (b) and (c) 2 mg/litre.

There is much evidence in other organisms to suggest a temporal correlation between the onset of rapid RNA synthesis, polyamine synthesis, and rapid growth [9–11]. Putrescine, the precursor of spermidine and of spermine is formed by decarboxylation of ornithine in eucaryotes. A second pathway leading to putrescine production exists in procaryotes utilising arginine decarboxylase and agmatine ureohydrolase [12]. This second pathway has not been detected in higher eucaryotes and I have been unable to detect it in *A. nidulans*. Ornithine decarboxylase is the first enzyme in the pathway leading to spermidine and spermine synthesis. The sharp increase in ornithine decarboxylase

activity in the *A. nidulans* prototroph occurs at the time of onset of rapid growth. It appears from these results that a certain basal level of ornithine decarboxylase is maintained throughout normal vegetative growth which involves mainly apical extension of hyphae but that a much higher level is required at the onset of germination of conidia. This idea is supported by the finding that when *A. nidulans* is maintained in steady state vegetative growth that the combined levels of Mg^{2+} and polyamines are maintained in a constant ratio to RNA [13]. The sharp fall in ornithine decarboxylase activity after germination is suggestive of an enzyme having a short half life. This has been shown to be the case with ornithine decarboxylase from several other sources [14–16]. It will now be of interest to discover which biochemical events require the transient high level of ornithine decarboxylase during germination.

Acknowledgement

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