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Short Communication

Variations in arginase and OTCase levels during growth in *Aspergillus nidulans*

NEERAJA RAMAKRISHNAN AND E. R. B. SHANMUGASUNDARAM
University Biochemical Laboratory, University of Madras, Madras 600 025.

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Abstract

The variations in the enzyme levels of arginase and ornithine transcarbamylase (OTCase) during the progressive days of growth was assayed in the wild strain of *A. nidulans*. A significant increase in both the enzyme activities was seen with the onset of growth.

Key words : Arginase, OTCase levels, nitrogen sources.

1. Introduction

The importance of the Kreb's–Henseleit cycle amino acids and urea as nitrogen sources in plants and microorganisms is now well documented[1–4]. Arginine, being particularly rich in nitrogen, is recycled to form other nitrogenous compounds.

It has been established that in *Aspergillus nidulans*, a homothallic, mononucleate fungus, the pathway for arginine synthesis is similar to that observed in *E. coli*[5]. Since the enzyme arginase (L-arginine ureohydrolase, E.C. 3.5.3.1) is responsible for the catabolism of arginine, we examined (i) the induction of the enzyme by varying the concentration of arginine in the medium, (ii) the variations in the levels of enzyme activity brought about during the period of growth of the organism. The enzyme ornithine transcarbamylase (Carbamoyl phosphate : L-ornithine carbamoyl transferase E.C. 2.1.3.3), involved in the biosynthesis of arginine was simultaneously studied.

2. Materials and methods

2.1. *Strain and medium*

The wild strain of *Aspergillus nidulans*, bearing green conidia, was cultured on liquid minimal medium prepared according to the method described by Pontecorvo *et al*[6] and

incubated at 37° C. For our experimental studies, arginine was added in three different concentrations, (i) 50 mg L-arginine/litre, (ii) 100 mg L-arginine/litre, (iii) 210 mg L-arginine/litre. The mycelia used for the experiments were 24 hours to five days old.

2.2. *Preparation of extract*

The mycelial pad was carefully removed and washed with distilled water to free it of the nutrient medium. It was then blotted on a filter paper and homogenised in 0·1 M phosphate buffer, pH 6·7, in a mortar and pestle. The homogenate was centrifuged at 8,000 g for 10 min and the supernatant used in the assay of arginase and ornithine transcarbamylase. The above operations were carried out at 4° C.

2.3. *Enzyme assay*

For the assay of arginase activity, 0·1 ml of the enzymatic extract was activated at first with 20 μmoles of $CoCl_2$ in 0·1 M Tris-HCl buffer pH 7·0, at 42° C for 10 min. The addition of 20 μmoles of arginine started 1 hour of incubation at 30° C. The reaction was stopped with the addition of 0·5 ml of 10% TCA and the samples were assayed for ornithine by the method of Chinard[7]. One unit of enzyme activity is defined as the amount of enzyme required to form 1 μmole of ornithine per hour at 30° C. The specific activity is expressed as units/mg protein.

Ornithine transcarbamylase activity was estimated by measuring the citrulline produced by the procedure of Archibald[8] as modified by Ohshita *et al*[9]. One unit of the enzyme activity is defined as that amount of enzyme required to form 1 μmole of citrulline at 37° C in half an hour.

Protein was estimated by the method of Lowry *et al*[10].

3. Results and discussion

It is obvious that the onset of growth of the organism begins with a dramatic increase in the activity of arginase, catabolising arginine to ornithine and urea. The ornithine so produced may be channelled into the formation of glutamate, proline or used in polyamine synthesis. As seen from Fig. 1, even in the case of the wild strain grown on minimal medium without supplemented arginine, there is an elevation in the arginase activity initially, which then gradually falls down with progressive days of growth, showing that the catabolism of arginine is a preliminary necessity for growth. However, the enzyme being inducible by nature, there is a significant enhancement in arginase activity as the amount of exogenous arginine is increased. It is interesting to note that, as evident in the figure, the rate of the catabolism of arginine is highest during the first two days of growth in all the cases and then there is a rapid decrease.

The pattern of variation of ornithine transcarbamylase activity with the age of the mycelium provides interesting material. In the case of the wild strain grown on minimal

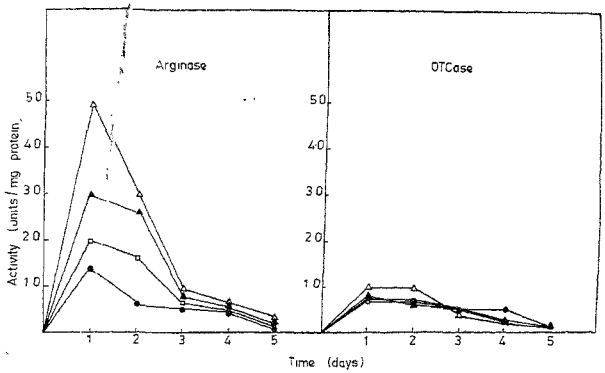

FIG. 1. Wild grown on ●—● Minimal Medium (MM)
□—□ *MM + 50 mg arginine/Litre*
▲—▲ MM + 100 mg arginine/Litre
△—△ MM + 210 mg arginine/Lire

medium alone, without added arginine, there appears an almost uniform level of activity being maintained in the first four days of growth. While, the wild cultures on medium containing 210 mg arginine/litre show a significantly higher level of ornithine transcarbamylase activity on the first and second day, the increase in activity is not so obvious in the wild strain grown on minimal medium, containing (i) 50 mg arginine/litre, (ii) 100 mg arginine/litre.

Our contention is that in the case of the wild strain grown in arginine supplemented medium, the catabolism of arginine proceeds at a rapid rate initially, providing the organism an environment with a high amount of ornithine. This causes a concomitant increase in ornithine transcarbamylase activity. This phenomena is most evident in the wild strain grown on 210 mg arginine/litre. That there is no obvious repression of ornithine transcarbamylase in the presence of arginine is significant. Data indicating that some ornithine production during the onset of arginine catabolism is reused for arginine synthesis has been reported[11].

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