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Short Communication

Studies on some HMP pathway enzymes in *gal* mutants of *Aspergillus nidulans*

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Abstract

Biochemical studies carried out with galactose non-utilising mutants of microorganisms demonstrated that the enzymic defect in these mutants is similar to the enzymic defect seen in galactosemics. In the present study, we carried out investigations on three enzymes of the HMP pathway in some *gal* mutants of *Aspergillus nidulans*. The study indicates that this pathway is disturbed in these mutants when they are cultured in the presence of galactose, which may suggest that galactose and some of its metabolites which are said to accumulate in the galactosemic condition may impair the HMP pathway in this state.

Key words : Microorganisms, galactose, *gal* mutants, genetic studies.

1. Introduction

Mutants of microorganisms unable to utilise galactose as the sole source of carbon have been isolated and some biochemical studies have been carried out with them[1–4]. Galactose non-fermenting mutants of *Aspergillus nidulans* have been produced and extensive genetic studies have been carried out with them[5,6]. The enzymic defect in these mutants parallels the enzymic lesion in the galactosemic individuals. In the present investigation we report the changes in some of the HMP pathway enzymes in some galactose non-fermenting mutants of *Aspergillus nidulans*.

It has been established that one of the important pathways through which glucose is metabolised in the lens is through glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Hence, the HMP pathway has a pivotal role to play with respect to the metabolic activities of the lens. It has also been shown that the lens is one of the main targets affected in the galactosemic condition.

Carter and Bull[7] had reported that an increase in the growth rate of the wild strain of *A. nidulans* in a medium containing glucose in both continuous and batch cultures

led to an increase in the proportion of glucose cycled through the HMP pathway concomitantly bringing about an increase in the levels of G6PD. This implicates the important role the HMP pathway plays with respect to the growth and nutrition of this fungus.

2. Materials and methods

Six galactose non-utilising mutants of *A. nidulans*, namely,

bi_1;	w_3;	gal_1	— constitutive mutant
bi_1;	w_3;	gal_9	— total mutant; kinase deficient
bi_1;	w_3;	gal_3	— slow growing mutants
bi_1;	w_3;	gal_4	— slow growing mutants
bi_1;	w_3;	gal_5	— total mutant; transferase deficient
$pyro_4$;		gal_{10}	— partial mutant

and the wild strain were used in the present study. These strains were maintained on minimal medium slants suitably supplemented by fortnightly subculture. Minimal medium was prepared according to the method of Pontecorvo *et al*[8].

Three conditions of growth were tested. Minimal medium containing

I — glucose and galactose in 1 : 1 proportion

II — galactose and glucose in 3 : 1 proportion; and

III — only galactose.

50 ml of the medium was dispensed in 250 ml Erlenmeyer flasks and these were sterilised at 15 lb per sq inch pressure for 15 min. The flasks were inoculated with a spore suspension of the various strains, containing roughly about 10^8 spores per ml of the medium. The flasks were incubated for 3 days at 37° C. At the end of the period of growth, the mycelia were harvested, washed and ground with buffer. The mycelial extract served as the enzyme source.

Three key enzymes of the HMP pathway, G6PDH (assayed by the method of Ells and Kirkman[9]), 6-phosphogluconate dehydrogenase and ribose-5-phosphate isomerase (assayed according to the methods of King[10] and Borenfreund[11] respectively) were studied.

3. Results

The changes in the activities of G6PDH, 6-phosphogluconate dehydrogenase and ribose-5-phosphate isomerase in the various strains, when grown in the different media, can be seen in Figs. 1, 2 and 3 respectively.

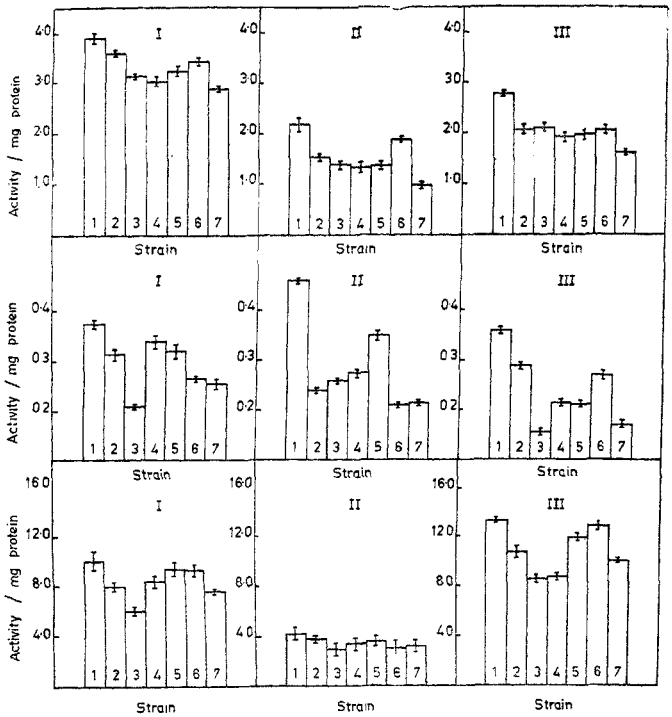

FIG. 1

FIG. 2

FIG. 3

FIG. 1. Activity of G6PDH in the mycelia of various strains grown for three days at 37° C. Activity is expressed as change in OD per min per mg protein.

FIG. 2. Activity of 6-phosphogluconate dehydrogenase in the mycelia of various strains grown for three days at 37° C. Activity is expressed as change in OD per min per mg protein.

FIG. 3. Activity of ribose-5-phosphate isomerase in the mycelia of various strains grown for three days at 37° C. Activity is expressed as mc moles of ribulose-5-phosphate formed per mg protein.

Common legends for Figs. 1, 2 and 3: Key: 1—wild; 2—gal_1; 3—gal_9; 4—gal_3; 5—gal_4; 6—gal_{10}; 7—gal_5.

I — Medium containing glucose and galactose in 1 : 1 proportion.
II — Medium containing galactose and glucose in the ratio of 3 : 1.
III — Medium containing only galactose.

From Fig. 1 it could be seen that in the medium where glucose and galactose are present in equal proportions, the activity of the enzyme is reduced in the mutant strains, maximal changes being seen in the case of gal_5 and gal_9. A further study of the results presented in the figure shows that the activity of the enzyme is further reduced in all the mutants in the medium where the proportion of galactose is still greater. Marked reductions in enzyme activity are seen in the case of gal_5 and gal_9. In a medium where only galactose is present as the carbon source, the activity of the enzyme is significantly reduced in all the mutants, the changes in gal_5 and gal_9 being particularly pronounced, the level of activity in these strains being about 45 per cent of the activity present in the wild strain.

It is observed from Fig. 2 that the activity of the enzyme 6-phosphogluconate dehydrogenase is also reduced in the *gal* mutants when grown in the various media indicated. The extent of decrease depends on the nature of the medium, the decrease becoming pronounced as the proportion of galactose in the medium increases. In a medium containing only galactose as the sole source of carbon, the enzyme activity is reduced by 40 per cent in the case of the strains gal_9 and gal_5. About 80 per cent of the control activity is expressed by strains gal_1, gal_3 and gal_4. About 84 per cent of the activity in the wild strain is present in the mutant gal_{10}. In media containing a lesser proportion of galactose, the activity of the enzyme in the mutants is higher than in a medium containing only galactose as the carbon source, but the activity in the total mutants is lower than that of other strains.

The activity of the enzyme ribose-5-phosphate isomerase in the various strains grown in the media described are presented in Fig. 3. In a medium containing equal proportions of glucose and galactose, it is seen that the enzyme activity is markedly reduced in the mutants gal_5 and gal_9 and less so in the other mutants. In a medium where the proportion of galactose is greater, the activity of the enzyme in gal_9 and gal_5 is significantly reduced. Strains gal_1, gal_3 and gal_4 record almost similar levels of activity. The activity of the enzyme in gal_{10} is in between that of the other mutants tested. In a medium containing only galactose, the ribose-5-phosphate isomerase activity decreased in the order, gal_9, $gal_5 < gal_3 < gal_1 < gal_4 < gal_{10}$.

4. Discussion

An important metabolic pathway of glucose in the lens is through glucose-6-phosphate to 6-phosphogluconate, catalysed by G6PDH. The activity of this enzyme is low in cataractous lenses from galactose fed rats. Lerman[12] has claimed that in normal lenses *in vitro*, a significant reduction in the activity of this enzyme is caused by adding galactose-1-phosphate to the culture medium. Rawal and Rao[13] studied the effect of galactose on the metabolism of rat lenses *in vitro*. They observed that lenses cultured in 20 mM concentration of galactose and harvested at various time intervals showed lower levels of G6PDH activity than the lenses maintained in a medium devoid of galactose. A similar observation was made when the lenses were treated with different concentrations

of galactose but harvested after 24 hours. Thus, their results showed that galactose has an inhibitory effect on the activity of G6PD.

Rats fed on a diet rich in galactose and thus maintained in the 'galactosemic state' have shown to have disturbances in the functioning of the HMP pathway[14]. Since the metabolism of glucose through the HMP pathway depends on the activity of G6PD, it is suggestive that the inhibitory effect of galactose on G6PD, may disturb the HMP shunt[15]. It was against this background that we undertook a study of three enzymes concerned with the HMP pathway.

From our studies we have observed a decrease in the activity of the three enzymes studied, in the galactose non-fermenting mutants, when cultured in media conaining galactose, as compared to the activity in the normal wild strain. It is seen that the activity of the enzymes depends both on the nature of the growth medium and the nature of the mutation in the strains. From our results it may be concluded that galactose toxicity affects the HMP pathway. The depression in the activity of G6PD, the key enzyme in the pathway may bring about a concomitant decrease in the other enzymes involved in the sequence of reactions.

In the galactosemic individuals and rats, as well as in the galactose negative microorganisms, the accumulation of galactose-1-phosphate has been established[16,17]. In addition, it has been shown that the accumulation of galactose itself could cause changes in the enzyme activity[18]. In our study, we have used both kinaseless and transferaseless mutants of *A. nidulans*, wherein the accumulation of galactose and galactose-1-phosphate should take place. Our studies with these mutants indicate that both these toxicity factors may interfere with the HMP pathway in these mutants.

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