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Repression of enzymes of the pentose phosphate pathway by glucose in fission yeast

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Abstract We examine here the effect of carbon sources on the synthesis of the shunt pathway enzymes in the fission yeast *Schizosaccharomyces pombe* growing on a mixture of ethanol and glycerol. δ-Gluconolactone induces practically every one of these enzymes. Glucose in contrast tends to attenuate the synthesis of the majority of them. RNA analysis confirms that their induction and repression reflect changes in the levels of their transcripts.

Key words: Pentose phosphate pathway; Kinetics; Repression; *Schizosaccharomyces pombe*

1. Introduction

The cell cycle and cell physiology of *Schizosaccharomyces pombe* have been extensively studied [1,2]; however, not much is known about its metabolic features. Our understanding of carbon metabolism in yeasts derives in large part from the earlier studies on *Saccharomyces cerevisiae* [3]. Previous studies suggested that analogous pathways function in both yeasts [4]. Interestingly, the characterisation of a few glycolytic enzymes in *S. pombe* demonstrates distinct differences in the functioning of this major metabolic pathway in the two yeasts, e.g. pyruvate kinase from fission yeast is a dimeric protein and is not activated by fructose 1,6-bisphosphate, in contrast to the tetrameric enzyme of *S. cerevisiae* for which fructose 1,6-bisphosphate acts as a strong activator [5,6].

S. pombe grows on a rather limited number of carbon sources compared to *S. cerevisiae*. Budding yeast utilises ethanol or acetate as the sole source of carbon due to the functioning of the glyoxylate pathway [7]. However, *S. pombe* is able to utilise these carbon sources only in the presence of glucose due to the absence of the glyoxylate pathway [8–10]. However, δ-gluconolactone (δgl), a 1,4-lactone derivative of D-glucose which is a weak carbon source for *S. cerevisiae*, could serve as a sole carbon source for fission yeast. δgl is a hexose which could be exclusively metabolised through the pentose phosphate pathway. Earlier work in our laboratory has also demonstrated that the shunt pathway enzymes in *S. pombe* are induced by δgl [11].

The presence of shunt pathway enzymes and their steady-state levels in continuous cultures of fission yeast have been demonstrated earlier [12]. However, there have been no reports on the kinetics of synthesis of the shunt pathway enzymes. This paper describes the kinetics of the shunt pathway enzyme synthesis under conditions favouring induction by δgl in a wild-type strain of *S. pombe*. We also report here the effect of glucose on the activities of these enzymes.

2. Materials and methods

2.1. Strains and media

The wild-type strain of *S. pombe* Sp 972 h^- was used for all experiments. Cells were grown in YES medium (0.5% yeast extract and other supplements containing 0.01% each of uracil, adenine, leucine, lysine, and histidine) with indicated carbon sources. Yeast extract was obtained from Difco Laboratories, Detroit, MI, USA. All amino acids were from Sisco Laboratories, Mumbai, India.

2.2. Enzyme assay

Enzymes were assayed fluorimetrically in cell-free extracts as described previously [13]. All cell-free extracts were made in KEE buffer (50 mM potassium phosphate pH 7.4, 2 mM β-mercaptoethanol, 2 mM EDTA) containing 1 mM PMSF. Except for 6-phosphogluconolactonase all enzymes were assayed in 50 mM triethanolamine buffer, pH 7.4, containing 25 mM KCl and 10 mM $MgCl_2$. Gluconolactonase was assayed in lactonase buffer (50 mM MES buffer, 25 mM KCl, 10 mM $MgCl_2$, pH 6.5).

Most of the substrates were obtained from Sigma Chemicals, St. Louis, MO, USA and all the enzymes were obtained from Boehringer Mannheim, Penzberg, Germany.

2.3. Protein estimation

Quantitation of proteins was done using the methods described earlier [14,15] using bovine serum albumin as a standard.

2.4. Northern analysis

Yeast total RNA was prepared from respective strains and characterised by Northern analysis as described earlier [16]. DNA fragments labeled by random priming with an α-[^{32}P]dATP labeling kit from Boehringer Mannheim, Germany, were used as probes. Hybridisation was performed at 60°C and radioactive bands were detected by autoradiography.

3. Results

3.1. Search for a neutral carbon source

δ-Gluconolactone is known to induce enzymes of the oxidative part of the pentose phosphate pathway in budding yeast [17]. In *S. pombe* not only the enzymes of the oxidative part but even those of the non-oxidative part are induced by δgl [11]. There is a decrease in the levels of these enzymes when cells of fission yeast are transferred from δgl to glucose. This drop in activity could be due to lack of the inducer, δgl, or due to a repressive effect of glucose. In order to determine whether glucose plays a role in repression of synthesis of shunt pathway enzymes in fission yeast the presence of a 'neutral' carbon source is necessary. A neutral carbon source is

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Abbreviations: PPI, pentose phosphate isomerase; PPE, pentose phosphate epimerase; TAL, transaldolase; TKT, transketolase; GNK, gluconate kinase; GND, 6-phosphogluconate dehydrogenase; PGL, 6-phosphogluconolactonase

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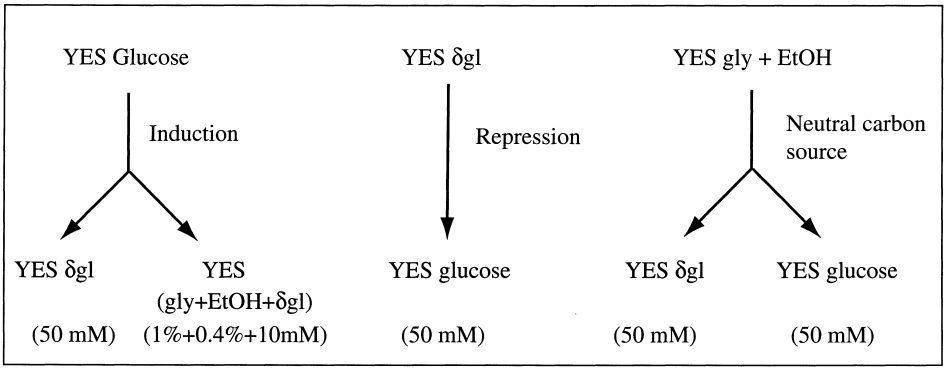

Fig. 1. Schematic representation of the experimental procedure. The wild-type strain of *S. pombe* was grown in three flasks of YES medium containing glucose, δgl and glycerol+ethanol respectively. Cells were harvested during the exponential phase, washed and reinoculated as indicated by the arrowheads. The culture density on inoculation was 4% (0.2×10^7 cells/ml) of the saturation O.D. (5×10^7 cells/ml).

one that has neither an inducing nor a repressing effect. We selected a combination of glycerol and ethanol for this purpose. The doubling time of fission yeast on 1% glycerol+0.4% ethanol is about 3.5 h. The schematic representation of the experimental procedure is depicted in Fig. 1.

The cells were initially grown in glycerol+ethanol and then transferred to δgl, glucose and glycerol+ethanol respectively. Enzyme levels were measured in cells grown in these three different carbon sources. It can be observed from data in Table 1 that pentose phosphate isomerase (PPI), pentose phosphate epimerase (PPE) and transaldolase (TAL) levels in gly+EtOH are intermediate to the levels on δgl and glucose, e.g. the level of TAL in glycerol+EtOH is almost four-fold higher than that in glucose. Thus it could be suggested that the combination of glycerol+ethanol serves as a neutral carbon source for these enzymes. However, in the case of 6-phosphogluconate dehydrogenase (GND), gluconate kinase (GNK), 6-phosphogluconolactonase (PGL) and transketolase (TKT) there is no change in the levels of these enzymes on glucose or glycerol+EtOH. Probably glycerol+ethanol does not serve as a neutral carbon source for this set of enzymes or there is no glucose repression on these enzymes, i.e. in the absence of the inducer (δgl) there is a basal rate.

3.2. Induction of shunt pathway enzymes by δgl

The kinetics of shunt pathway enzymes under conditions favouring induction by δgl was monitored in the wild-type strain of the fission yeast. Initially the cells were grown in YES medium containing glucose (50 mM) and 1% glycerol +0.4% ethanol respectively. The transfer of cultures to various carbon sources was carried out as described in Fig. 1. Two concentrations of δgl were used to determine if the induction was concentration-dependent.

As seen in Fig. 2, GND, GNK and PGL are induced almost 300-fold when the culture was initially grown in glycerol +ethanol and then transferred to δgl (50 mM). Comparatively there is a decrease in the extent of induction (10-fold) when the cells are transferred from glucose to either δgl (50 mM) or glycerol+EtOH+δgl (10 mM) (Fig. 2). Probably these enzymes take more time to overcome the glucose repression.

PPI, PPE and TAL were significantly induced by δgl irrespective of its the concentration and the previous carbon source on which it had been grown (glucose or glycerol+ethanol) (Fig. 2). Only GNK displays a difference in induction with a change of concentration, i.e. in 50 mM δgl the induction is at least fourfold more than in 10 mM δgl. TKT also shows induction by δgl at both concentrations (10 and 50 mM) and even when previously grown on glucose.

3.3. Glucose represses synthesis of most of the shunt pathway enzymes

The cells were transferred from YES δgl to glucose to look for a repression effect. It was observed that there was a drop in the levels of all the shunt pathway enzyme activities (Fig. 2). To confirm that the drop in the activity is due to glucose repression it is necessary to show intermediate levels of enzymes on a neutral carbon source.

Indeed some of the enzymes do show intermediate levels

Table 1
Search for a neutral carbon source

Enzyme	Specific activity (mU/mg)		
	Glucose (50 mM)	δgl (50 mM)	Gly+EtOH
GND	162	746	196
GNK	35	294	46
PPI	10	84	70
PGL	70	210	68
PPE	35	293	99
TAL	14	102	41
TKT	20	58	21

The wild-type strain of *S. pombe* was grown in YES medium containing glycerol+ethanol (1%+0.4%) to the mid-exponential phase. The cells were harvested after 18 h and inoculated in respective media as described in Fig. 1. Enzyme activity was measured fluorimetrically in the cell-free extracts.

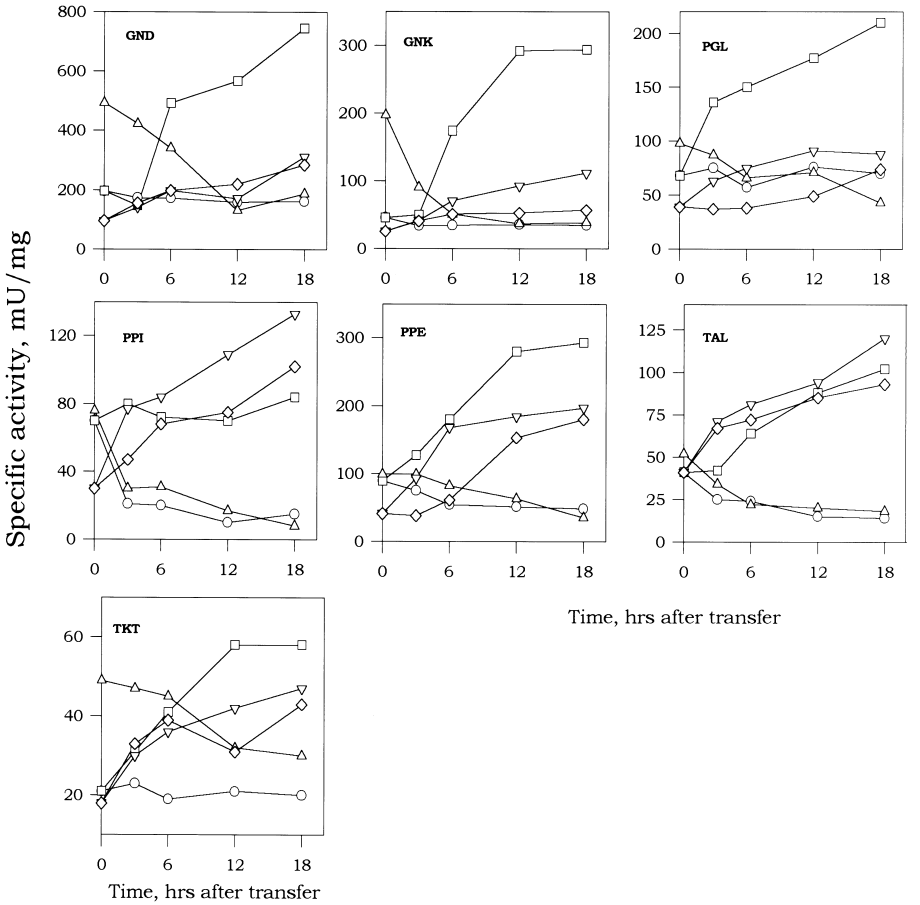

Fig. 2. Kinetics of the shunt pathway enzymes in *S. pombe*. The wild-type strain of *S. pombe* was initially grown on YES medium containing glucose (50 mM), δgl (50 mM) and glycerol+ethanol (1%+0.4%) respectively to exponential phase. The cells were harvested, washed and inoculated in the respective media containing different carbon sources. Cells grown in glycerol+ethanol were transferred to YES glucose (◯) and YES δgl (□); cells grown in YES δgl were transferred to YES glucose (△); cells grown in YES glucose were transferred to YES δgl (▽) and YES gly+EtOH+δgl (◇). Samples were collected at various time intervals and the enzyme activity was measured fluorimetrically in the cell-free extracts. The specific activity is expressed in mU/mg of protein.

when grown on the combination of glycerol and ethanol, e.g. PPI, PPE and TAL (Fig. 2). In the case of GND, GNK and PGL there was no significant decrease in the enzyme activity when transferred from glycerol+ethanol to glucose. However, when the cells were transferred from glucose to δgl there was only a 10-fold increase in the enzyme activities compared to a 300-fold increase in the case of transfer from glycerol+ethanol. It is possible that the decrease in the fold induction is because the synthesis of these enzymes is repressed by glucose. Thus, the cells have to overcome the repression.

3.4. *Induction as well as repression is at the level of transcription*

In order to investigate the induction of shunt pathway enzymes by δgl we analysed the expression of three of the shunt pathway enzymes, namely TAL, PPI and GND. The wild-type strain of fission yeast was initially grown on YES medium containing either glucose or glycerol+ethanol and then transferred to various carbon sources as shown in Fig. 1. Total RNA was prepared from these cultures and Northern blot analysis was carried out as described in Section 2.

Earlier in our laboratory we had obtained mutants affecting the structural genes of shunt pathway enzymes, viz. TAL, GND and PPI [11]. Clones complementing these mutants (*tal*, *gnd* and *ppi*) were also obtained [11] and these were used as probes to analyse the expression of these genes in the presence of various carbon sources.

The left-hand panel displays the expression pattern on different carbon sources of these three enzymes when initially grown on glucose. The right-hand panel shows the expression in cells initially grown on glycerol+ethanol and then transferred to various carbon sources. The clones complementing *tal* and *ppi* mutants show an increase in the transcript in the presence of δgl, irrespective of which carbon source they were initially grown in (lanes 3 and 4, Fig. 3). However, there was no change in the transcript level on transfer to δgl for the clone complementing the *gnd* mutant when grown initially on glucose (left-hand panel, lanes 3 and 4, Fig. 3). Interestingly, almost 3–5-fold induction was seen when it was initially grown in glycerol+ethanol and then transferred to δgl (right-hand panel, lanes 3 and 4, Fig. 3). These data are consistent with the enzyme assay data seen earlier (Fig. 2). This clearly suggests that the induction effects by δgl and glucose repression of these three shunt pathway enzymes are at the level of transcription.

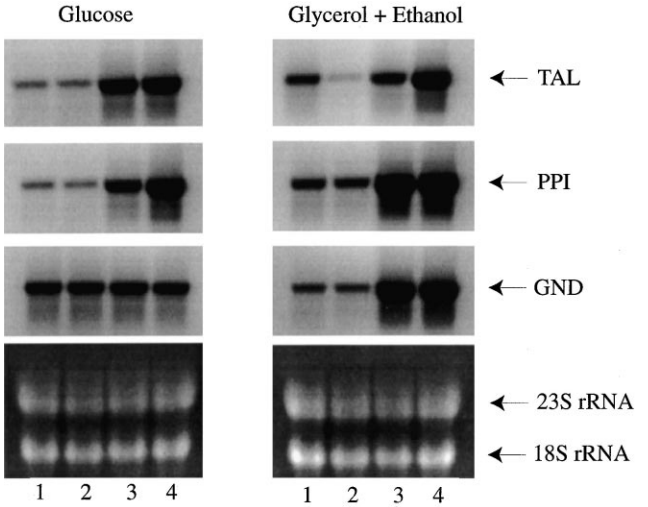

Fig. 3. Northern blot analysis of *tal*$^+$, *gnd*$^+$, and *ppi*$^+$. The wild-type *S. pombe* strain was grown in YES medium containing glucose or glycerol+ethanol respectively. The cells were washed and reinoculated in YES medium containing 1% glycerol+0.4% ethanol (lane 1), 50 mM glucose (lane 2), 10 mM δgl (lane 3) or 50 mM δgl (lane 4). Total RNA was isolated from these cells. 75 μg of RNA was electrophoresed in 1.5% formaldehyde-agarose gel and transferred to Amersham Hybond-N membrane and probed using *tal*$^+$, *gnd*$^+$ and *ppi*$^+$. 23S and 18S rRNA were used as loading control.

4. Discussion

The kinetics of enzyme synthesis of the shunt pathway in fission yeast has not been documented earlier. We have examined the kinetics of the enzyme synthesis of this pathway in *S. pombe*.

It has been known that shunt pathway enzymes are induced by δgl in budding yeast [17]. In *S. pombe* too, these enzymes are induced by δgl. This is clearly observed in Fig. 2 where all the shunt pathway enzymes show at least a fivefold induction on δgl. If cells are transferred to glucose the enzyme activity decreases (Fig. 2). This could be due to repression by glucose or decrease of activity by dilution in the absence of the inducer. In order to confirm that shunt pathway enzymes are under glucose repression it was necessary to have a 'neutral' carbon source. We used a combination of glycerol+ethanol (1%+0.4%).

It was seen that this combination could serve as a neutral carbon source for PPI, PPE and TAL (Fig. 2). This suggests that these enzymes are probably under glucose repression. However, the levels of GND, GNK, PGL and TKT remained the same on glucose and glycerol+ethanol. Does that mean that these enzymes are not under glucose repression? Interestingly, if the cells were grown on glucose prior to transfer to δgl, there was only a 10-fold induction of these enzymes, in contrast to the 300-fold induction seen in the case of glycerol +ethanol-grown cells. This probably means that these enzymes are under glucose repression and require more time to overcome the repression when initially grown on glucose. However, when grown on glycerol+ethanol there is no repression and hence the enzymes show high levels. In an experiment in which the wild-type cells were grown in the presence of equal concentrations of glucose (50 mM) and δgl (50 mM), none of the shunt pathway enzymes displayed induction by δgl in the presence of glucose (data not shown). This suggests that δgl induces all the shunt pathway enzymes and glucose represses most of them. There is no induction by δgl in the presence of glucose.

Transcript analysis correlates very well with the enzyme assay results except in the case of TAL and GND (Fig. 3). Subtle differences are observed in the results obtained from enzyme kinetics and the transcript analysis, especially in the case of TAL. In the cultures initially grown in glycerol+ethanol and then transferred to δgl (Fig. 3), there was a very distinct concentration-dependent expression, which was not as obvious in enzyme assays (Fig. 2). In contrast to this, there was a clear concentration-dependent increase in the enzyme activity of GND (Fig. 2), but the same was not observed at the transcript level. The physiological relevance and the causes for these differences are under investigation. From the above-mentioned results it can, however, be definitively said that in fission yeast shunt pathway enzymes are induced by δgl and glucose repression is observed for some of these enzymes. This is perhaps the first report on the repression of the pentose phosphate pathway by glucose.

It has been observed earlier that significant levels of shunt pathway enzymes are present in *S. pombe* and also there exists a separate transporter for δgl [18,19], which is exclusively utilised via the pentose phosphate pathway. This demonstrates the importance of this pathway in fission yeast.

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