Genetical and Biochemical Studies of Glucosephosphate Isomerase Deficient Mutants in *Saccharomyces cerevisiae*

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A number of glucose-negative mutants of *Saccharomyces cerevisiae* were isolated and shown to contain very low activities of glucosephosphate isomerase. Mutants almost totally lacking this enzyme (less than 1 % of wild-type activity) grew on fructose if provided with a small quantity of glucose. Larger amounts of glucose led to the accumulation of glucose 6-phosphate and growth inhibition. These mutants did not grow on galactose. Other mutants with low enzyme activities (about 1 % of wild type) grew on fructose alone and also on galactose. The mutant characters were determined in both cases by single gene mutations which were mapped on chromosome II and, presumably, identify a structural gene locus for the enzyme.

INTRODUCTION

Glucosephosphate isomerase deficient mutants were first described in *Escherichia coli* by Fraenkel (1968*a*) but the lesion had little effect on the physiological behaviour of the cells. Since the hexose monophosphate pathway is indispensable for the synthesis of reduced NADP (Gancedo & Lagunas, 1973) and glucose 6-phosphate is required for the synthesis of products, such as glycoproteins, glycolipids and constituents of the cell wall, by other pathways, one might expect a profound reduction in glucosephosphate isomerase (EC 5.3.1.9) to be deleterious for normal cell function. However, a glucosephosphate isomerase deficient mutant in yeast, reported by Maitra (1971), did not appear to be affected in this way, and it was able to grow on galactose even though glucosephosphate isomerase catalyses an obligatory step in galactose utilization (Leloir, 1951).

We report here yeast mutants with different deficiencies in glucosephosphate isomerase and examine their effect on the metabolism of various sugars.

METHODS

Micro-organisms. The following strains of *Saccharomyces cerevisiae* were used: 196-2 (*a hi6*); 197-4D (α *hi7*), obtained from M. Luzzati, Paris, France; and K1-3D (*a ly2-1, me4-1, ty1-1, tr5-18, ac^c, can1*), from the collection of P. P. Puglisi, Parma, Italy.

Media. The complete medium (YP) contained (g l⁻¹): yeast extract, 10; peptone, 10. Additional carbon sources (glucose, fructose, galactose or glycerol) were supplied at 20 g l⁻¹.

The minimal medium was that of Galzy & Slonimski (1957). In some media, glucose was replaced by fructose. Other additions to the minimal synthetic medium were made according to the nutritional requirements of the strain. For solid medium, Oxoid agar no. 3 was added at 20 g l⁻¹.

Mutagenesis and isolation of glucose-negative mutants. Cells of the haploid wild-type strain 196-2 grown on the YP/glucose medium were treated with ethyl methanesulphonate as described by Thouvenot & Bourgeois (1971). The glucose-negative mutants were accumulated and isolated as described by Herrera

(1976). Glucose-negative mutants were selected for their ability to grow on YP/fructose medium but not on YP/glucose medium.

Yeast growth. The strains were incubated at 30 °C with shaking at approximately 90 reciprocations min⁻¹ and absorbance was read in a Klett-Summerson photocolormeter.

Genetic analysis. Formation of diploids and their sporulation was examined and tetrad analysis was done as described by Hawthorne & Mortimer (1960). Complementation tests were performed with diploid cells obtained by the prototroph selection technique of Pomper & Burkholder (1949).

Assay of enzymes. Activities of glucosephosphate isomerase (EC 5.3.1.9), glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and phosphogluconate dehydrogenase (decarboxylating) (EC 1.1.1.44) were measured in cell extracts obtained after disrupting the cells in a Braun cell homogenizer model MSK (Bronwill) for 50 s (30 s first velocity, 20 s second velocity). During disruption, the flask was cooled with a stream of CO₂. The cell extract was immediately centrifuged at 25000 g for 20 min at 4 °C, and the supernatant fluid was dialysed against 34 mM-Tris/HCl buffer (pH 7.4) at 5 °C for 18 h. Glucosephosphate isomerase activity was assayed according to Noltmann (1966) in a reaction coupled with glucose-6-phosphate dehydrogenase. Glucose-6-phosphate dehydrogenase was determined according to Kuby & Noltmann (1966). Phosphogluconate dehydrogenase was assayed according to Pontremoli & Grazi (1966). Protein was determined by the biuret method (Layne, 1957) using bovine serum albumin as standard.

Intracellular glucose 6-phosphate concentration. After incubation, the culture was immediately cooled to 4 °C and cells were centrifuged and washed at 4 °C with an ice-cold solution of sugars at the same concentration as used in the culture medium (to prevent changes in the concentration of metabolites during centrifugation). The flasks were then placed in a dry ice/ethanol bath. Ice-cold perchloric acid was added to yeast cells to a final concentration of 0.7 M and allowed to stand for 30 min. The mixture was centrifuged at 4 °C, the extract was neutralized with 2.5 M-K₂CO₃, and protein was precipitated with ZnSO₄ and Ba(OH)₂. Glucose 6-phosphate was determined in the supernatant by a conventional spectrophotometric assay with glucose-6-phosphate dehydrogenase and NADP (Polakis & Bartley, 1966). Contamination by glucose was determined by the glucose oxidase-peroxidase method (Bergmeyer & Bernt, 1963).

Reagents. Glucosephosphate isomerase was obtained from Sigma; glucose-6-phosphate dehydrogenase and fructose 6-phosphate were from Boehringer; glucose 6-phosphate, 6-phosphogluconate and NADP were from Koch-Light; yeast extract and peptone medium were from Oxoid; glucose, galactose, glycerol, amino acids and other nutrients for yeast growth were from BDH; fructose was from Merck.

RESULTS

Phenotypic characteristics

A total of 29 mutants were isolated by their failure to grow on glucose either in minimal medium or complex YP medium. All the mutants grew on fructose in complex medium. The mutants were separated into two groups on the basis of their response to fructose in minimal medium and galactose in YP medium: 17 mutants (group 1) grew on these media whereas the other 12 (group 2) did not. The growth of mutant strains representative of both groups and of the wild type is shown in Figs 1 and 2. To establish the nutritional requirements of the group 2 mutants which did not grow on the fructose minimal medium, different nutrients, such as purine and pyrimidine bases and amino acids, were added. None of these supplements, however, had any appreciable effect on growth on the fructose minimal medium.

Genetical and biochemical analysis

Segregation of the glucose-negative character was studied in three mutants of group 1 (7A-9, 7A-15, 7A-24) and three of group 2 (7A-21, 7A-31, 7A-32). When crossed to wild type each of the six mutants showed monogenic segregation for the glucose-negative character. In the crosses with group 2 mutants, segregants always characteristically showed inability to grow on the three sugars (galactose, fructose and glucose) in the same spore. Recombinants from the six crosses were used for a complementation test with the rest of the mutants. None of the diploids was able to grow on glucose, thus identifying the mutants as defective in the same gene function. All the diploids formed with strains bearing known mutations in the different structural genes of the galactose system (Douglas & Hawthorne, 1964) were able to grow on YP/galactose medium.

Fig. 1

Fig. 2

Fig. 1. Growth of glucose-negative mutants and of the wild-type strain on minimal medium with fructose: 10 ml minimal medium with fructose supplemented with histidine was inoculated at a concentration of 10^6 cells ml^{-1} for the mutants and 10^4 cells ml^{-1} for the wild-type strain. Wild type ($\circ$, 196-2); group 1 ($\blacksquare$, 7A-9; $\bullet$, 7A-24); group 2 ($\square$, 7A-32).

Fig. 2. Growth of glucose-negative mutants and of the wild-type strain on YP/galactose medium: 10 ml YP/galactose medium was inoculated at a concentration of 10^6 cells ml^{-1} for the mutants and 10^8 cells ml^{-1} for the wild-type strain. Wild type ($\circ$, 196-2); group 1 ($\blacksquare$, 7A-9; $\bullet$, 7A-24); group 2 ($\square$, 7A-32).

Table 1 shows the tetrad segregation for the *gluc* gene with respect to standard genetic markers. As an internal control we checked that segregation of known markers showed the proportions expected according to Mortimer & Hawthorne (1966). The absence of non-parental ditype asci in *gluc* segregation with respect to *tyl* place this gene on chromosome II. According to the formula

$$\frac{(3 \text{ non-parental ditypes} + \frac{1}{2} \text{ tetratypes}) \times 100}{\text{total}}$$

the distance from *gluc* to *tyl* is 7.8 centi-morgans. Analysis of the segregation patterns for the four loci of chromosome II (*gluc*, *ly2*, *tyl* and *hi7*) in individual tetrad asci for *gluc* and *tyl* suggests that the most likely sequence is *ly2-tyl-gluc-hi7*.

Table 2 shows the specific enzyme activities in three group 1 mutants, in two group 2 mutants and in the wild-type strain 196-2. All the mutants had a marked deficiency in glucosephosphate isomerase activity: those in group 1 had about 1 % of the wild-type activity and those in group 2 about 0.25 % of the wild-type activity.

On the basis of these results, we propose the hypothesis that the group 2 mutants are unable to grow on the fructose minimal medium because their content of glucosephosphate isomerase is too low to permit a sufficient steady-state level of glucose 6-phosphate for the requirements of the hexose monophosphate pathway or biosynthetic pathways. If this hypothesis were correct, addition of a small quantity of glucose to the fructose minimal medium should restore growth of the mutants. Growth of mutant 7A-32 did, indeed, show a dependence on the glucose concentration in the medium (Fig. 3). This dependence was less for mutant 7A-24, and practically absent for the wild-type strain. External glucose concentrations higher than 0.4 mg ml^{-1} inhibited the growth of both mutants (Fig. 3).

Table 1. *Tetrad analysis of data from crosses involving the gluc1 gene and other markers*

Numbers of tetrads [parental (PD) and non-parental (NPD) ditypes
and tetratypes (T)] are shown as $\frac{\text{PD:NPD}}{\text{T}}$.

	<i>tr5</i>	<i>me4</i>	<i>ly2</i>	<i>ty1</i>	α	<i>hi7</i>
<i>gluc1</i>	$\frac{4:6}{41}$	$\frac{6:6}{40}$	$\frac{17:1}{34}$	$\frac{43:0}{8}$	$\frac{10:7}{34}$	$\frac{31:5}{55}$
<i>tr5</i>		$\frac{6:10}{37}$	$\frac{10:6}{35}$	$\frac{4:3}{36}$	$\frac{11:20}{22}$	$\frac{3:6}{12}$
<i>me4</i>			$\frac{9:11}{35}$	$\frac{7:3}{44}$	$\frac{7:8}{40}$	$\frac{3:2}{17}$
<i>ly2</i>				$\frac{19:1}{32}$	$\frac{13:7}{36}$	$\frac{3:1}{18}$
<i>ty1</i>					$\frac{10:8}{34}$	$\frac{10:1}{11}$
α						$\frac{12:18}{60}$

Table 2. *Glucosephosphate isomerase (GPI), glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) activities of the wild type and mutant strains*

Cells were grown aerobically at 30 °C in fructose medium and collected at the end of the exponential phase. Specific activities are expressed as $\mu\text{mol min}^{-1} (\text{mg protein})^{-1}$.

Strain	Specific enzyme activity		
	GPI	G6PD	6PGD
196-2 (wild type)	1500	185	65
Group 1 mutants			
7A-24	10		
	20.6	178	45
	17.0	114	
7A-9	11.2	142	45
7A-15	7.0	134	
	16.0	146	39
Group 2 mutants			
7A-31	3.6	198	54
	2.1		
7A-32	2.7	178	42
	3.4		

Figure 4 shows the intracellular concentration of glucose 6-phosphate after incubation in fructose minimal medium plus different concentrations of glucose. The intracellular concentration of the phosphorylated sugar in mutant 7A-32 had a marked dependence on the external glucose concentration. This dependence was less for mutant 7A-24, and absent for the wild-type strain.

The yeast extract employed in the YP media was subsequently found to contain 0.4 % (w/v) glucose. This might explain why the group 2 mutants grew on YP/fructose medium without addition of glucose. At any rate, best growth in the YP/fructose medium was obtained when glucose was added at 0.05 mg ml^{-1} .

DISCUSSION

Genetic analysis of the mutants showed that the lesions present in all of them were monogenic for the glucose-negative character. Therefore, the pleiotropic effect found in mutants of group 2 could be related to the low activity of glucosephosphate isomerase.

Fig. 3

Fig. 4

Fig. 3. Growth on fructose minimal medium with histidine and glucose: 10 ml 2% fructose minimal medium supplemented with histidine and different concentrations of glucose was inoculated at a concentration of 10^4 cells ml⁻¹. The incubation was stopped when no further increase in absorbance was observed. Wild type (○, 196-2); group 1 (●, 7A-24); group 2 (□, 7A-32).

Fig. 4. Effect of glucose on the intracellular concentration of glucose 6-phosphate. Strains were inoculated into minimal medium supplemented with histidine, 2% fructose and different concentrations of glucose. The inoculum level was adjusted according to the growth rate for each strain in the different media employed so that the final concentrations of cells were similar for all cultures after 24 h incubation. At the end of this period, the cells were harvested and washed by centrifugation in the cold, and then the intracellular concentration of glucose 6-phosphate was determined. Wild type (○, 196-2); group 1 (●, 7A-24); group 2 (□, 7A-32).

According to Lagunas & Gancedo (1973), *Saccharomyces cerevisiae* requires $8.7 \mu\text{mol NADPH (mg cell dry wt)}^{-1}$ to be synthesized by the hexose monophosphate pathway. Hence a yeast cell with a doubling time of about 150 min would require an isomerase activity of about $60 \mu\text{mol (g protein)}^{-1} \text{min}^{-1}$ for converting fructose 6-phosphate to glucose 6-phosphate. This analysis permits one to explain why mutants of group 2 did not grow on the minimal fructose medium since their residual glucosephosphate isomerase activity would place their doubling time at more than 50 h. Mutants such as 7A-24, with a residual activity of about $15 \mu\text{mol (g protein)}^{-1} \text{min}^{-1}$, had a doubling time of about 13 h, in good agreement with the predicted behaviour. The above considerations indicate that a sufficient steady-state level of glucose 6-phosphate is important for cell growth as seen in the experiment where glucose was added to a concentration of less than 0.4 mg ml^{-1} to the minimal fructose medium. The intracellular concentration of glucose 6-phosphate seems to be regulated fundamentally by the activity of glucosephosphate isomerase.

The observed growth inhibition by glucose in the mutants (7A-24, 7A-32) is probably related to the toxic effect of glucose 6-phosphate (Maitra, 1971; Fraenkel, 1968b). Growth inhibition caused by a reduced uptake of fructose by glucose competition seems an unlikely explanation, since the ratios of fructose/glucose concentration used in the present experiments are far from the values required to cause an exclusion of fructose by glucose either at the level of transport (Kotyk, 1967; Cirillo, 1968) or of phosphorylation (Dixon & Webb, 1964).

Glucosephosphate isomerase activity is necessary for galactose utilization (Leloir, 1951). Therefore, the inability of group 2 mutants to grow in the YP/galactose medium was

expected, since the residual glucosephosphate isomerase activities in these mutants are not sufficient to meet the requirements of the glycolytic pathway.

The glucosephosphate isomerase deficient mutants isolated by Maitra (1971) probably resemble our group 2 mutants since they were selected as colonies that grew on a fructose minimal medium but not on glucose.

Our results demonstrate that the almost total absence of glucosephosphate isomerase has a drastic effect on the normal cell function in *S. cerevisiae*, in contrast to *Escherichia coli* (Fraenkel, 1968*a*; Fraenkel & Levisohn, 1967) where the loss of this enzyme seems to have very little effect on the physiological behaviour.

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