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Isolation and Characterization of *Saccharomyces cerevisiae* Glycolytic Pathway Mutants

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Yeast strains carrying recessive mutations representing four different loci that cause defects in pyruvate kinase, pyruvate decarboxylase, 3-phosphoglycerate kinase, and 3-phosphoglycerate mutase were isolated and partially characterized. Cells carrying these mutations were unable to use glucose as a carbon source as measured in turbidimetric growth experiments. Tetrad analysis indicated that these mutations were not linked to each other; one of the mutations, that affecting phosphoglycerate kinase, was located on chromosome III.

Although numerous studies have been carried out concerning mutants defective in the upper part of the glycolytic pathway in microorganisms (review references 1 and 3), mutants with lesions in the triose interconversion enzymes after glyceraldehyde 3-phosphate have been isolated only recently. Freese et al. (4) reported a *Bacillus subtilis* mutant lacking 3-phosphoglycerate kinase activity. Irani and Maitra (7) reported the isolation of mutants lacking enzyme activities of 3-phosphoglycerate kinase, glyceraldehyde 3-phosphate dehydrogenase, and enolase in *Escherichia coli*. Their rationale was that mutants defective in triose interconversion enzymes will fail to grow on glucose, glycerol, or succinate alone, but they can grow when glycerol and succinate are present together. Hillman and Fraenkel (6) also isolated glyceraldehyde 3-phosphate dehydrogenase mutants in *E. coli* which map at a different site from those isolated by Irani and Maitra. Finally, mutants lacking pyruvate kinase activity in *Aspergillus nidulans* were isolated by Payton and Roberts (12).

In this paper, we wish to report the isolation and characterization of two new classes of glycolytic mutants lacking pyruvate decarboxylase (EC 4.1.1.1) and 3-phosphoglycerate mutase (EC 5.4.2.1) and of mutants lacking pyruvate kinase (EC 2.7.1.40) and 3-phosphoglycerate kinase (EC 2.7.2.3) activities in the yeast *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Yeast strains. Two strains were used for the isolation of mutants. The pyruvate kinase mutants (*pyk*), phosphoglycerate kinase (*pgk*), and phosphoglycerate mutase (*pgm*) were isolated from the *S. cerevisiae* strain K8 (*α ade2 trp5 leu1*) obtained from E. Jones, and the pyruvate decarboxylase mutants (*pdc*) were isolated from strain K19 (*α ade1 trp1*) obtained from J. Parker.

Media. Basic complete medium (YP) contained 1% yeast extract and 2% peptone (1.5% agar was added for plates). Various carbon sources were added to the YP medium at the concentrations specified: 2% glucose (YPD); 2% galactose (YPGal); 3% glycerol (YPG); 2% ethanol (YPE); 3% glycerol and 2% ethanol (YFGE); 2% glucose, 3% glycerol, and 2% ethanol (YPDGE); and 2% galactose, 3% glycerol, and 2% ethanol (YPGalGE).

Enzyme preparations and assays. Cells from logarithmic-phase cultures were harvested by centrifugation and washed once with deionized water. The cells were then stored at −4°C until ready for use. The frozen cells were suspended in 0.5 M potassium phosphate buffer (pH 7.5; 0.5 g/ml). The suspension (8 ml) was mixed with 4 g of glass beads (diameter, 0.45 to 0.50 mm), and the mixture was shaken for 1 min in a Braun homogenizer cooled by liquid carbon dioxide and then centrifuged at 12,000 × *g* for 15 min. The supernatant was assayed for enzyme activity at 25°C by spectrophotometric methods (10). Protein concentrations were determined by the method of Lowry et al. (9).

Growth experiments. Cultures were incubated in 300-ml nephelometric flasks at 30°C in a shaking water bath. Cells grown to the logarithmic phase in YPGE were concentrated, washed with YP medium, suspended in YP, and used as inocula for growth experiments. Portions of the cell suspension (0.1 ml each) were used to inoculate 10 ml of designated medium to a turbidimetric reading of 5 to 10. Growth was monitored by measuring the turbidity in a Klett-Summerson photoelectric colorimeter equipped with a 660-nm filter.

Genetic methods. Mutagenesis was induced by methanesulfonic acid ethyl ester according to the method of Sherman et al. (15). Treated cells were plated and allowed to grow on YPGE plates for 2 days before replica plating onto YPD and YPGE media. Colonies that grew on YPGE but not on YPD were picked. For tetrad analysis, asci were dissected by the method of Johnston and Mortimer (8), and

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linkage relationships were determined by conventional tetrad analysis (13).

Chemicals and enzymes. Sodium pyruvate, 3-phosphoglycerate, thiamine pyrophosphate, phosphoenolpyruvate, fructose 1,6-diphosphate, and adenosine 5'-diphosphate were purchased from Sigma Chemical Co. The enzymes muscle lactate dehydrogenase, enolase, pyruvate kinase, glyceraldehyde 3-phosphate dehydrogenase, and alcohol dehydrogenase were obtained from Boehringer Mannheim Corp.

RESULTS AND DISCUSSION

Mutants were selected (see above) for their ability to grow on YPGE but not on YPD. Five independent *pyk*, two *pgm*, one *pgk*, and two *pdc* mutants were isolated. They are single recessive mutations, and mutants with the identical enzyme defect fall into the same complementation group. The tetrad data in Table 1 show that the four loci are not linked to each other since their parental ditype to nonparental ditype ratios do not deviate significantly from one.

We established the location for the *pgk* locus (Table 2) as being linked to the mating locus at a map distance of 21.8 centimorgans and to the *thr4* locus with a map distance of 45.5 centimorgans. Thus, the arrangement of the three loci on chromosome III is the following: the mating-type locus is in the middle flanked by the *thr4* locus distal to the centromere, and the *pgk* locus is proximal to the centromere (Fig. 1).

Enzyme levels in the mutants, for all four enzymes, were at least 100-fold less than that of the parental strains (Table 3). The very low levels of enzyme activities in the mutant strains as compared with those of wild type (less than 1% of wild-type level) probably reflect the high excess amount of these glycolytic enzymes present in the yeast cells so that only the nonleaky mutants show a detectable phenotype. Because four separate, single, recessive mutations caused defects in four different glycolytic enzymes, these results tend to suggest the lack of isozymes of these enzymes. We also measured the levels of all the other glycolytic

TABLE 1. *Tetrad analysis of the glycolytic mutants*

Genotype of the cross	Tetrad segregation[a]		
	PD	TT	NPD
$pdc^- pgk^+ \times pdc^+ pgk^-$	3	12	2
$pdc^- pyk^+ \times pdc^+ pyk^-$	11	24	10
$pdc^- pgm^+ \times pdc^+ pgm^-$	3	16	7
$pyk^- pgk^+ \times pyk^+ pgk^-$	13	17	14
$pyk^- pgm^+ \times pyk^- pgm^-$	3	9	4
$pgk^- pgm^+ \times pgk^+ pgm^-$	6	15	4

[a] PD, Parental ditype; TT, tetratype; and NPD, nonparental ditype.

TABLE 2. *Tetrad data indicating linkage for thr4 pgk and mating type locus on chromosome III*[a]

Gene pair	PD	TT	NPD	Centimorgan
α-pgk	71	55	0	21.8
pgk-thr4	29	66	4	45.5
α-thr4	56	40	0	20.8

[a] Map distance calculated from the equation: $x = [(TT + 6\,NPD) \times 100]/[2(PD + TT + NPD)]$. Abbreviations as in Table 1.

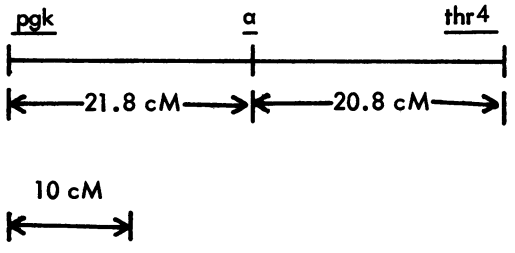

FIG. 1. *Map distance for thr4, pgk, and α on chromosome III.*

TABLE 3. *Levels of enzymes in wild-type and glycolytic pathway mutants*

Strain	Relevant genotype	Sp act (U/mg)[a]			
		Pyruvate kinase	Pyruvate decarboxylase ($\times 10^{-1}$)	Phosphoglycerate kinase	Phosphoglycerate mutase ($\times 10^{-1}$)
K8	Wild type	3.24	5.10	5.68	6.30
K19	Wild type	1.76	3.41	4.86	6.62
klg 101	pyk^-	<0.01	4.50	6.42	7.42
78-8A	pyk^-	<0.01	4.46	6.83	8.61
klg 2	pdc^-	1.71	<0.01	4.87	8.22
272-1B	pdc^-	1.38	<0.01	5.13	7.11
klg 5	pgk^-	2.80	3.86	<0.01	8.45
35-6C	pgk^-	3.16	4.52	<0.01	7.30
klg 51	pgm^-	2.53	3.74	5.43	<0.01
31-3A	pgm^-	2.91	4.92	5.53	<0.01

[a] One unit of activity = 1 μmol of the nicotinamide adenine dinucleotide reduced per min at 25°C.

enzymes in these strains, and they all showed normal and comparable activities (data not shown).

The growth characteristics of these mutants are shown in Table 4. Strain K8, which is wild type with regard to all the glycolytic enzymes, was capable of growing on all different carbon sources. Its doubling time ranged from 1.6 h in YPD to 5.5 h in YPE medium. Strain klg 101 carrying the *pyk* lesion was able to grow on YP supplemented with both glycerol and ethanol or ethanol alone with a doubling time of 5.4 and 5.3 h, respectively. This strain, however, cannot grow on media with either glucose, glycerol, or galactose as the carbon source and, in fact, addition of glucose or galactose to a medium containing glycerol and ethanol will inhibit its normal growth. This growth pattern fits nicely with its known enzyme defect since a *pyk* mutant would not be expected to utilize any carbon source entering the glycolytic pathway before pyruvate; hence, it explains why no growth is obtained in media containing glucose, galactose, or glycerol as carbon sources. It can grow on ethanol alone because ethanol can be used as an energy source via acetate and acetyl coenzyme A which in turn is metabolized in the tricarboxylic acid cycle; also, ethanol can be utilized for gluconeogenesis via the glyoxalate cycle and for the activity of phosphoenolpyruvate carboxykinase, which will convert oxaloacetate from the glyoxalate cycle to phosphoenolpyruvate, thus bypassing the enzyme defect. This would explain why pyruvate kinase activity is unnecessary when yeast cells grow on a two-carbon substrate. The catabolite effect may involve two different mechanisms: catabolite inhibition in which the preferential uptake of glucose or of galactose inhibits the uptake of glycerol and ethanol; and catabolite repression in which the synthesis of catabolic enzymes specific for ethanol and glycerol utilization are repressed by glucose or galactose or their glycolytic conversion derivatives.

Growth experiments indicated that the *pdc* mutant strain 272-1B could grow on all the carbon sources supplied, except for glucose, and that here, again, the presence of glucose inhibited growth in media containing glycerol or ethanol as carbon sources. Since the defect in *pdc* affected only the process of fermentation but not of oxidative phosphorylation, the inability to grow on glucose and its inhibitory effect may be explained by the fact that a high concentration of glucose depresses the process of oxidative phosphorylation (Crabtree effect). This mutant can grow on ethanol because the gluconeogenic process bypasses its enzyme lesion. It also grows on galactose and glycerol (for approximately three to four generations); and furthermore, galactose shows no inhibitory effect on growth in glycerol and ethanol medium. The limited growth obtained in YPGal and YPG may be due to some unknown physiological imbalance in this mutant under the growth conditions used. Since the galactose catabolic pathway via oxidative phosphorylation is normal in this mutant, then galactose, present in the YPGalGE medium, would show no inhibitory effect if it does not accumulate significant amounts of glycolytic intermediates in the *pdc* mutant, as distinct from all the other mutants described in this communication. Because phosphoglycerate kinase and mutase activities are necessary for both glycolysis and gluconeogenesis, strains carrying these two mutations can only grow in medium containing carbon sources which will enter the glycolytic pathway on both sides of the lesion (e.g., YPGE) without causing depression of the oxidative phosphorylation process. Once again, the addition of glucose or galactose to YPGE causes just such a depression, resulting in inhibition of growth.

All four enzymes have been purified and extensively studied in yeast (2, 5, 11, 12, 14). We are presently studying the regulatory mechanism of these enzymes and using these mutations to isolate catabolite-insensitive mutants.

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TABLE 4. *Growth rate of wild-type and glycolytic pathway mutants*

Strain	Relevant genotype	Carbon source in YP medium[a]						
		GE	D	G	E	DGE	Gal	GalGE
K8	Wild type	4.2	1.6	4.1	5.5	2.3	2.4	3.8
klg 101	*pyk*$^-$	5.4	–[b]	–	5.3	–	–	–
271-1B	*pdc*$^-$	4.0	–	±4.7[c]	4.3	–	±6.0	3.6
11-7C	*pgk*$^-$	4.3	–	–	–	–	–	–
klg 51	*pgm*$^-$	4.5	–	–	–	–	–	–

[a] Expressed as doubling time per hour.
[b] –, Denotes no growth.
[c] ±, Indicates limited number of generations.

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