Phosphofructokinase Mutants of Yeast

BIOCHEMISTRY AND GENETICS

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Mutants of Saccharomyces cerevisiae completely lacking the soluble glycolytic enzyme fructose-6-P kinase are described. The mutations are semidominant, do not complement one another, and define a gene PFKL located 28-cm distal to rna1 on the extended right arm of chromosome XIII. Of 10 independent mutants, 3 can be suppressed by ochre suppressors. All mutants examined synthesize proteins that cross-react to the antibody against the purified yeast P-fructokinase. The enzyme in spontaneous revertants is distinguishable from the wild type enzyme with respect to thermolability and ATP inhibition. The locus PFKL thus defines the structural gene of the enzyme.

The pfkl mutants are not leaky in vivo. All the glucose consumed by a double mutant lacking both P-fructokinase and 6-P-glucuronate dehydrogenase ends up as 6-P-glucuronate, yet the pfkl mutants can glycolyze and grow on glucose in air. The cell mass produced per unit of glucose also remains unchanged. Anaerobically, however, growth does not take place, nor does glycolysis. P-fructokinase is thus a dispensable enzyme for aerobic growth, but indispensable for anaerobic growth. The properties of pfkl mutants suggest that yeast has an alternative mechanism for the aerobic metabolism of fructose-6-P, presumably through the recently reported particulate P-fructokinase (Lobo, Z., and Maitra, P. K. (1982) FEBS Lett. 137, 279-282).

Mutants lacking P-fructokinase are potentially interesting. This enzyme catalyzes an irreversible step in the Embden-Meyerhof pathway and is said to be a flux-determining enzyme in glycolysis (1). The kinetic properties of the enzyme from diverse sources are consistent with this interpretation. P-fructokinase from yeast has been implicated in particular in the Pasteur effect (2) and in the generation of glycolytic oscillations (3). A study of mutants of this enzyme is likely to shed light not only on such questions of cellular physiology but on mechanisms underlying allosteric regulation in general.

Mutants of Saccharomyces cerevisiae lacking this enzyme have been reported from several laboratories (4-7). An unexpected feature of all of these mutants is their ability to grow on glucose. The location of P-fructokinase in the glycolytic pathway suggests that the pentose phosphate pathway may act as a bypass in pfkl mutants. The growth of these mutants on glucose might also reflect their leakiness in vitro. However, the recently discovered second P-fructokinase in S. cerevisiae (8) probably allows the growth of pfkl mutants on hexose sugars.

We describe the results of some of our experiments which have a bearing on the problem of growth of pfkl mutants on glucose. Genetic characteristics of the mutation are also described.

MATERIALS AND METHODS

Strains—A large number of S. cerevisiae strains which were used for mapping and suppression experiments, will be mentioned where appropriate in the text. All of these strains were generously supplied by the Yeast Genetic Stock Center at Berkeley. Other strains have been described: wild type strain haploid S. cerevisiae (9); pyruvate kinase mutants pyk1-4 and pyk1-5 (10); 6-P-glucuronate dehydrogenase mutant gnd (11); the P-fructokinase mutant allele pfk1-1 was isolated earlier (12) as a glucose-negative mutant. Mutants lacking pyruvate decarboxylase pdc1 were isolated by H. M. Chikarmane of this laboratory. Media—The minimal medium and the YEP medium containing yeast extract, peptone, and carbon source have been described (9). The pfk1 and gnd mutants were maintained on YEP medium containing ethyl alcohol.

Chemicals—Fructose-6-P was from Sigma. It was essentially free of glucose-6-P and fructose-1,6-bisP. Other substrates and enzymes including yeast P-fructokinase were from Boehringer.

Enzyme Assays—P-fructokinase activity was assayed fluorometrically in either cell-free extracts or Toluene lysates as before (12) in a reaction mixture containing 5 mM fructose-6-P, 1 mM ATP, 0.03 mM NADH, 1 unit each of aldolase and α-glycerophosphate dehydrogenase, and 10 units of triose P isomerase. To avoid interference by the particulate enzyme activity which was present only in cultures grown on glucose but not on alcohol (8), glucose-grown cultures were examined only in cell-free supernatants for the soluble enzyme. Alcohol-grown cultures were assayed either in Toluene lysates or in centrifuged cell extracts.

The segregation of P-fructokinase in tetrads was followed in Toluene lysates (9) in experiments to isolate the gene with an ethyl alcohol selecting medium. The pfkl mutants were examined in spontaneous revertants and in crosses to wild type strains. The marker pfkl segregated independently of pykl-4 and pykl-5 (10), and pfkl was isolated as a glucose-negative mutant. Other strains have been described (10).

RESULTS

Isolation of Mutants Lacking P-fructokinase—The allele pfkl-1 which has been used in most of the experiments unless mentioned otherwise was isolated accidentally as a glucose-negative mutant among clones resistant to 2-deoxyglucose (4). In crosses to wild type strains, the marker pfk1 segregated independently of 2-deoxyglucose resistance and glucose negativity.

The majority of pfkl mutants were obtained as spontaneous glucose-resistant revertants of pykl mutants (5) growing on YEP plates containing 150 mM ethanol and 50 mM glucose. The more than 120 independent pfkl mutants which have been isolated by this method have been designated pfk1-2 through pfk1-200. A third method of isolation of pfk1 mutants...
was to revert pdcl mutants on glucose media; 3 mutants (alleles pfhl-1-201 to pfhl-1-203) were used for complementation studies. The pfkl and pdcl markers were eliminated by crossing these isolates to a wild type strain; the resultant single gene mutants lacking P-fructokinase were all able to grow on glucose.

P-Fructokinase Activity in Haploids and Diploids—The activity of P-fructokinase in the pfkl mutants was generally less than 0.1% of the wild type level. A few mutants which had detectable activity (about 1%) were suppressed poorly for growth on alcohol + glucose medium. The enzyme levels in homozygous and heterozygous diploids carrying the allele pfhl-1 were examined in cell-free extracts from cultures growing exponentially on glucose. The P-fructokinase activity was as follows: PFKL, 290 nm/mg of protein; pfkl-1, <0.001 nm/mg of protein; PFKL/PFKI, 330 nm/mg of protein; PFKI/pfhl-1, 90 nm/mg of protein. In stationary cultures of glucose-suppressible SUP7/pfhl-17 (nonsense) the test isolates of pfkl mutant to examine their suppressibility in the diploid. For example, the hybrid PPI-6 (nonsense suppressor) was P-fructokinase-negative spores as the suppressor was lost. We conclude from this that the following 3 alleles are nonsense suppressors SUP7: pfkl-17 SUP7 was also enzyme-positive. One other diploid of the constitution pfkl-6 (nonsuppressible) SUP7/pfkl-1 (nonsense) +, was, however, found not to have any P-fructokinase activity. When this diploid was sporulated and the spores examined for the enzyme activity it was found that haploids carrying pfkl-1 SUP7 were in fact P-fructokinase-positive. In contrast, the enzyme-positive diploid pfkl-9 (nonsuppressible) +/pfkl-6 (nonsuppressible) SUP7 failed to produce a spore that had P-fructokinase activity. We interpret these observations as reflecting interaction of subunits in this oligomeric protein, providing an instance of negative complementation in the second case, and positive complementation in the last. We have not encountered a pfkl mutant that is fully dominant with respect to its wild type allele PFKI.

Growth Properties—One of the features of pfkl mutants that distinguish them from most other glycolytic lesions is their ability to grow on glucose, fructose, and mannose much as the wild type yeast. Results in Table I illustrate this with respect to the nonsense allele pfkl-1 growing, respectively, on alcohol and glucose as carbon sources. Loss of P-fructokinase reduced the rate of growth on alcohol only marginally, while on glucose it was halved. Restoration of enzyme activity by suppression of the nonsense mutation either by the unlinked tRNA suppressor SUP7 or by an intragenic suppressor R5 improved the rate of growth on glucose. However, the absence of any detectable enzyme activity in the soluble supernatants did not prevent growth.

How do pfkl mutants lacking any measurable enzyme activity of P-fructokinase in soluble supernatants continue to grow on glucose? A reasonable explanation is that the pentose phosphate pathway acts as a bypass of this enzyme. Because of the stoichiometry

$$3\text{-Glucose-6P} \rightarrow 2\text{-fructose-6-P} + \text{glyceraldehyde-3-P} \rightarrow \text{CO}_2 + \text{NADP}^+$$

operative during the use of this pathway, half the glucose ends up as CO$_2$ and is presumably lost from productive metabolism. The mutant allele pfkl-1 was grown aerobically on limiting glucose to stationary phase and the yield of cell mass, measured as $E_{OM}$ was determined. Fig. 1 shows a plot of the growth rate for several concentrations of glucose using both the YEP medium and a minimal medium. The linearity between the cell yield and glucose added showed that they were proportional at low concentrations. However, the loss of P-fructokinase made hardly any difference to the efficiency of cell synthesis at limiting concentrations of glucose. Except for a reduction in the rate of growth, pfkl mutants behaved very much like the wild type strain during aerobic growth on sugars.

Leakiness of the Nonsense Mutants—In order to examine

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<tr>
<td></td>
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<td>pfkl-1 R5</td>
<td>pfkl-1 (R5)</td>
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1 H. M. Chikarmane, personal communication.
if the pfkl mutants were leaky in vivo, the nonsense allele pfkl-I was used to construct a double mutant pfkl gnd lacking the shunt pathway enzyme, 6-P-glucuronate dehydrogenase, in addition to lacking P-fructokinase. The double mutant did not grow on glucose, but grew on alcohol, and possessed no more than 5% of the dehydrogenase activity of the wild type strains. P-fructokinase activity measured either in the cell-free extracts or in toluene lysates was undetectable since the particulate activity was not induced by growth on alcohol (8). It was grown in YEP alcohol medium to stationary phase and a washed suspension of cells was treated with glucose. Aliquots were withdrawn into acid for estimation of 6-P-gluconate, glucose-6-P, and glucose. Results are shown in Fig. 2. The utilization of glucose started rapidly but slowed down considerably as 6-P-glucuronate concentration kept on rising and that of glucose-6-P rose and fell. The total amount of glucose utilized at the end of the incubation was calculated from the extrapolated zero time value and found to be 24 µmol/g of yeast; that of 6-P-glucuronate formed was 25 µmol/g. All the glucose utilized thus ended up as 6-P-glucuronate suggesting that neither of the pfkl nor gnd blocks permitted any significant metabolic leak past these lesions.

**Aerobic Glycolysis**—Results in Fig. 3 depict a comparison of the profile of glycolytic intermediates in the pfkl mutant and the wild type yeast during aerobic metabolism. The cultures were grown in YEP glucose to stationary phase. The loss of P-fructokinase led to a nearly 3-fold drop in the rate of glucose consumption and a severe restriction in the outflow of this glucose into alcohol. As expected from a lesion in P-fructokinase, the level of fructose-1,6-bisP was much lower than in the wild type, while that of glucose-6-P was higher. Fructose-6-P levels (not shown) remained approximately at a quarter of those of glucose-6-P throughout. The kinetics of ATP indicated that the mutant strain was able to synthesize this substance, however, the turnover time was longer than in the wild type strain. The drop in the intracellular concentration of P-enolpyruvate was delayed unlike in the wild type strain mirroring a corresponding lag in the accumulation of fructose-1,6-bisP (13).

**Anaerobic Glycolysis**—Because of the involvement of P-fructokinase in Pasteur effect in many systems (1), the features of anaerobic glycolysis in pfkl mutant would be of interest. Our conclusion is that the mutation causes a very considerable, if not complete, reduction in the rate of anaerobic glycolysis. Results in Fig. 4 illustrate a typical experiment. Glucose was not utilized anaerobically; its addition led to a progressive loss of ATP and a monotonic accumulation of glucose-6-P. The levels of fructose-1,6-bisP (not shown) were low during the entire incubation period. The presence of oxygen on the other hand elicited a normal response. The effect of anaerobiosis was reversible. When N₂ gas was replaced with O₂, glucose utilization resumed and reached the aerobic rate. Mutants lacking P-fructokinase are thus characterized by having a negative Pasteur effect.

**Anaerobic Growth**—Due to the high affinity of cytochrome oxidase to oxygen (14) anaerobic experiments with complete exclusion of oxygen are technically difficult. We have used in these experiments three different but related methods to simulate anaerobic conditions of growth. In the first method pfkl strains were examined for growth on YEP glucose plates containing 0.3 mM sodium azide to inhibit respiration (9). Unlike the P-fructokinase-positive strains which grew on these plates within 1 day, pfkl mutants did not grow till the third day. This method was routinely used in conjunction with enzyme assay to follow the segregation of the marker pfkl in genetic crosses. In the second method the strain pfkl-I was crossed to a strain X3144-11A of the genotype a leu2 trpl rad1 arg9 ilv3 pet8 petx and spores of the genotype pfkl pet8 petx were isolated from 4-spore tetrads germinated on glucose plates. Although the viability of spores was normal, the unexpected observation was that these particular pfkl segregants could not be revived on further transfer to YEP glucose plates. Repeated transfers invariably led to the reappearance of P-fructokinase-positive clones. The third method of curing pfkl mutants of their mitochondrial genome by ethidium bromide (15) were also unsuccessful. The survivors were revertants for P-fructokinase activity. We conclude therefore that loss of P-fructokinase is incompatible with anaerobic growth.

**Revertants**—Since we were interested in revertants that were respiratory sufficient, the majority of the revertants were
isolated as clones able to form colonies on YEP glucose plates containing azide. The spontaneous reversion frequency varied from $1 \times 10^{-7}$ to $5 \times 10^{-7}$ depending on the pfhl allele. The nonsense mutant pfhl-1 yielded both extragenic and intragenic revertants. On crossing to the wild type strain the revertant R1 gave enzyme-positive as also enzyme-negative R2. The spontaneous reversion frequency varied nonsense mutant pfkl-1 yielded both extragenic and intragenic revertants. On crossing to the wild type strain the revertant R1 gave enzyme-positive as also enzyme-negative R2. For example, was not perceptibly inhibited by increasing concentration of enzyme specific activity, thermolability, and unlinked to the locus pfkl. The intragenic revertant R5, on generation of equal frequency of parental ditype and nonparental ditype ascii showed that the suppressor locus in R1 was unlinked to the locus pfkl. The intragenic revertant R5, on the other hand, yielded no enzyme-negative progeny in 17 tetrads; its suppressor could not be segregated from the pfkl locus. No extragenic suppressor was found for the nonsuppressible mutant alleles tested.

The revertants from a number of pfkl mutants covered a wide spectrum of enzyme specific activity, thermostability, and kinetic property. Results in Fig. 5 illustrate this with respect to an intragenic revertant from a nonsense allele pfkl-1 and a SUP7-suppressed derivative pfkl-1 SUP7, and intragenic revertants pfkl-7 R13 and pfkl-7 R22 obtained from the nonsuppressible allele pfkl-7. The enzyme from the revertant R22, for example, was not perceptibly inhibited by increasing concentrations of ATP unlike P-fructokinase from the revertant R13 or the wild type strain as seen in toluene lysates. When cell-free extracts were used, however, the reverted enzyme in R22 was found to be inhibited by high concentration (4 mM) of ATP, while the wild type enzyme was inhibited at a much lower concentration of ATP (0.25 mM) under these conditions. No reverted enzyme was found to be completely insensitive to inhibition by ATP. The results in Fig. 5 also indicate that the reverted or the nonsense-suppressed enzyme could be either more stable or more labile to heat compared to the wild type enzyme. The inactivation was monophasic in every case studied.

![Fig. 4. Aerobic and anaerobic glycolysis in a pfhl mutant.](http://www.jbc.org/)

**Fig. 4.** Aerobic and anaerobic glycolysis in a pfhl mutant. Cells were grown on YEP glucose till the stationary phase and bubbled with a gas mixture containing 5% CO2 and 95% O2. (left) as the suspension was stirred over a magnetic stirrer in a 50-ml beaker. The anaerobic incubation was done in 50-ml test tubes with a slow stream of gas 5% N2, 5% CO2) gently agitating the suspension to minimize contamination from traces of O2 present in the gas mixture. The cell concentrations were as: aerobic, 92 mg, wet yeast/ml; anaerobic, 200 mg/ml. Details are as in Fig. 3. G6P, glucose-6-P.

**Cross-reacting Material**—Unfractionated cell-free extracts from several mutants were tested for the presence of proteins immunologically similar to P-fructokinase. Ouchterlony double-diffusion plates (16) containing rabbit antiserum raised against purified P-fructokinase from yeast were used in the central well; the peripheral wells contained either the crude extracts from the mutants, their revertants, or the wild type strain or the purified enzyme. Strong, single precipitin lines were seen in every case suggesting the presence of cross-reacting materials in the mutants, including two independent nonsense alleles of pfkl. The lines merged with one another to produce a complete hexagonal figure when a hexagonal template was used. This suggested that mutants without enzyme activity synthesized P-fructokinase-like proteins. Since the soluble P-fructokinase of yeast is known to be composed of two nonidentical polypeptide chains (17, 18), it is possible that the precipitin lines were due not to the product of the gene PFKI, but reflected the presence of the second of the two constituent subunits.

**Chromosomal Location**—The mapping of pfkI proved laborious because its segregation had to be followed by assay of enzyme activity, and secondly, it was not linked to the centromere; the second division segregation frequency with respect to trpl was 0.69 (89/129). Table II gives the linkage analysis from meiotic data using three sets of diploids heterozygous for the marker pairs pfkl-1, pfkI-SUP8, and rna1-SUP8 on chromosome XIII; SUP8 was monitored by the homozygous ochre marker lys1-1. The nonsuppressible allele pfkl-3...
was used when SUP8 was segregating; the nonsense allele pfl-l was used for the others. These results placed pfl on the extended right arm of chromosome XIII 28 centimorgans from rna1 in the order: centromere-SUP8-rna1-pfl.

**DISCUSSION**

**Aerobic and Anaerobic Growth of pfl Mutants**—The property of pfl mutants to continue to grow on sugars contrasts with that of most of the glycolytic mutants studied in yeast (19) and in Escherichia coli (20). The possibility that this was due to their leakiness in vivo can be ruled out in view of the experiment using the double mutant pfl mut described in Fig. 2. The manner of their isolation suggested that loss of P-fructokinase allowed in some unknown manner the alleviation of growth inhibition caused by glucose in pfl or pfl mutants. It is likely that growth under these conditions was permitted not so much by attenuation of metabolic flux through glycolysis (5) as by modulation of negative control such as catabolic repression. Since the pfl alleles constituted quite a tight metabolic block, the double mutant pfl pfl somehow allowed the utilization of alcohol despite the presence of glucose. However, no relief of catabolic repression or catabolite inactivation could be seen in pfl mutants with respect to isocitrate lyase and the glucose-repressible alcohol dehydrogenase (21) or of fructose-1,6-bisphosphatase or P-enolpyruvate carboxykinase. It should, however, be noted that about two-thirds of the glucose-resistant mutants from pfl strains continued to be positive for P-fructokinase activity.

In the absence of oxygen, however, pfl mutants are unable to grow. Their rudimentary growth in standing cultures or in the presence of azide, the spontaneous selection of enzyme-positive revertants during their growth in the petite state (both nuclear and cytoplasmic), and the inability of stationary cultures of pfl mutants to metabolize glucose in absence of oxygen justify the conclusion that the loss of this enzyme leads to impairment of anaerobic growth. If pfl mutants grew anaerobically by way of the pentose phosphate pathway and obeyed the stoichiometry shown in Reaction 1, it is immediately apparent that anaerobic growth would not have been sustained; the shunt pathway produces too many reductant molecules for too few oxidant substrates. How then did single spores of the genotype pfl pet8 pet8 germinate on glucose to produce visible colonies? Was it due to some spore-specific P-fructokinase? Examination of spore suspensions of diploids homozygous for pfl gave no indication of such an enzyme. Was it due to the diffusion of acetaldehyde as an electron acceptor from neighboring PFK1 spores? This was ruled out by the observation that germination of spores was not affected by planting them far apart from one another. It is thus difficult to escape the conclusion that there must exist a PFK1-independent mechanism that allowed petite spores lacking P-fructokinase to undergo at least 20 cell divisions to produce a visible spore colony from a single micromanipulated cell. However, this was clearly inadequate to sustain the anaerobic growth of pfl mutants. Perhaps the recently reported particulate P-fructokinase serves this purpose (8) and is also responsible for the normal growth yield of pfl mutants from glucose.

**Glycolytic Behavior**—Two shades of behavior distinguished the P-fructokinase mutant from the wild type strain in aerobic incubation, one was the delayed rise of fructose-1,6-bis-P and the attendant fall in the level of P-enolpyruvate as pyruvate dehydrogenase (21) or of fructose-1,6-bis-phosphatase or P-enolpyruvate carboxykinase. It should, however, be noted that about two-thirds of the glucose-resistant mutants from pfl strains continued to be positive for P-fructokinase activity.

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practically disappeared. Under such conditions the mutant was almost as proficient in glycolysis as the wild type reflecting again the presence of the glucose-induced particulate enzyme (8).

Anaerobically, however, the situation was very different. Glucose metabolism was negligible and the kinetics of metabolites reflected blocked glycolysis (Fig. 4). The levels of ATP, unlike the aerobic experiment, were never restored following glucose addition. Reintroduction of oxygen reversed the changes seen in nitrogen.

Nature of \textit{pfk1} Mutation—The observation that \textit{pfk1} mutants obtained by three different procedures lie in the same complementation group suggests that the catalytic activity of P-fructokinase is coded possibly by a single locus. There is ample evidence for the conclusion that this gene defines the structural determinant for this enzyme. The suppressibility of three of these mutants by tRNA suppressors showed that the gene is translated. Further, the P-fructokinase activity restored in the majority of the revertants was perceptibly different from that in the wild type strains: some were more thermolabile while some others more stable than the unmutated enzyme; some of them were altered with respect to ATP inhibition and sigmoidicity to fructose-6-P. Many of the reversion events leading to restoration of the enzyme activity mapped very close to or perhaps within the gene \textit{PFK1}.

The observation that all the mutants described here were affected in the gene \textit{PFK1} lends support to the idea that the \textit{PFK1} gene defines the structure of P-fructokinase. We have recently shown (18) that structural alterations in P-fructokinase come about by mutation in either of the genes \textit{PFK1} or \textit{PFK2} (8); lesions in the former cause loss of catalytic activity and those in the latter lead to loss of allosteric regulation (18). The observation that all the mutants described here were affected in the gene \textit{PFK1} rather than in \textit{PFK2} lends support to the idea that the former is the sole determinant that specifies the catalytic activity of P-fructokinase in \textit{S. cerevisiae}.

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REFERENCES

Phosphofructokinase mutants of yeast. Biochemistry and genetics.
Z Lobo and P K Maitra


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