A PARTICULATE PHOSPHOFRUCTOKINASE FROM YEAST

Zita LOBO and P. K. MAITRA
Tata Institute of Fundamental Research, Bombay 400 005, India

Received 9 December 1981

1. Introduction

The productive catabolism of glucose 6-phosphate is known to have 2 options; it can either go through the glycolytic pathway or along the hexose monophosphate shunt before the 2 sequences meet (fig.1). Mutants of *Saccharomyces cerevisiae* lacking fructose 6-phosphate kinase (*pfk1*) are known to grow on glucose [1,2]. If this is due to the use of the shunt as the alternative route, then a further block at, say the first enzyme of the pentose phosphate pathway would render *pfk1* mutants glucose-negative. We describe here results of experiments that do not bear out this prediction. On the contrary, we find that double mutants carrying lesions in phosphofructokinase and glucose 6-phosphate dehydrogenase (*zwf1*) grow on glucose, while those defective both in phosphofructokinase and phosphoglucoisomerase (*pgi1*) grow on fructose. These results have been resolved by the observation that the yeast *S. cerevisiae* contains a hitherto undescribed enzyme called phosphofructokinase II (ATP: D-fructose 6-phosphate 1-phosphotransferase) that catalyses the formation of fructose 1,6-diphosphate from fructose 6-phosphate and ATP. Preliminary characterisation of the enzyme is reported here; the enzyme is particulate.

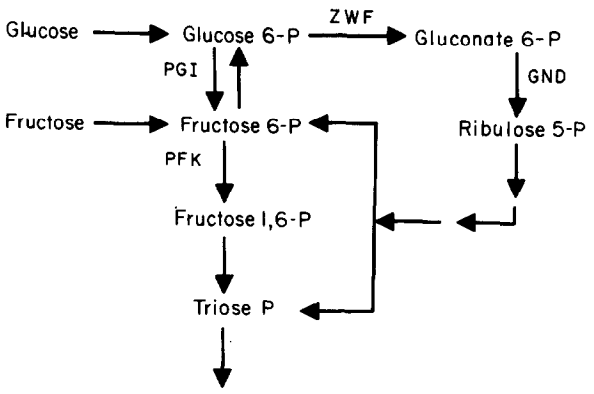

Fig.1. Pathways of glucose 6-phosphate metabolism. *Abbreviations:* P, phosphate; PGI, phosphoglucose isomerase; PFK, fructose 6-phosphate kinase; GND, gluconate 6-phosphate dehydrogenase; ZWF, glucose 6-phosphate dehydrogenase.

2. Materials and methods

2.1. *Strains*

Mutants of *S. cerevisiae* lacking phosphofructokinase and phosphoglucoisomerase have been described [3,4]. Mutants lacking glucose 6-phosphate dehydrogenase have been isolated in this laboratory. The allele *pfk1-1* is an ochre-suppressible non-sense mutation having <0.001 munit phosphofructokinase act./mg protein present in soluble supernatants, while the allele *zwf1-1* has <0.05 munit glucose 6-phosphate dehydrogenase act./mg protein in crude extracts. *pgi1-1* has detectable enzyme activity, but is completely glucose-negative. Double mutants α *pfk1-1 zwf1-1 ade1 his2* and α *pfk1-1 pgi1-1 ade1* were constructed by standard genetic procedures [4]. Strains were grown in liquid media with shaking at 30°C on rotary shakers in yeast extract and peptone containing the desired carbon sources [4].

2.2. *Enzyme assays*

These have been described [5]. Phosphofructokinase was assayed both in cell-free supernatants and in toluene lysates in a reaction mixture containing 5 mM fructose 6-phosphate, 1 mM ATP, 0.03 mM NADH, 1 unit each of aldolase and α-glycerophosphate dehydrogenase and 10 units triose phosphate isomerase. Phosphofructokinase velocity was expressed as nmol fructose, 1,6-diphosphate produced/min at 23°C (munits). For toluene lysates the results have

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$02.75

been expressed as E_{650}, absorbance measured at 650 nm in a 1 cm path.

Fructose 2,6-diphosphate was a kind gift from Dr E. van Schaftingen. All other substrates and enzymes were from either Boehringer or Sigma.

3. Results

3.1. *Properties of double mutants*

The ability of *pfk1* mutants to grow on glucose is unexpected. Whether this was due to the in vivo leakiness of the lesions [1,2] cannot be easily ruled out. Therefore, we selected a non-sense mutant allele *pfk1-1* for these experiments. Loss of this enzyme reduces the rate of growth on hexose sugars at most by a factor of 2 (table 1, lines 1,2 and 5,7). Surprisingly, the double mutants *pgi1 pfk1* (line 4) and *pfk1 zwf1* (line 8) continue to grow on fructose and glucose, respectively. Table 1 also shows the effect of the single mutations *pgi1* (line 3) and *zwf1* (line 6) on the growth rate. The double mutant *pfk1 pgi1* produces alcohol from fructose, so does *pfk1 zwf1* from glucose at a rate ⩾50% of that of the wild-type. Since *pgi1* does not grow on glucose nor can glycolyse it, these data indicate that there must be some enzyme(s) that connects fructose 6-phosphate to a step below phosphofructokinase.

3.2. *Presence of a particulate phosphofructokinase*

Seventeen independent isolates of *pfk1* allele, including 2 ochre-suppressible non-sense mutations were grown overnight on glucose and toluene lysates of washed cultures were examined for phosphofructokinase. Without exception, each of the cultures was found to possess phosphofructokinase activity ranging from 5–10 munits enzyme/E_{650}. However, the enzyme activity could not be seen in cell-free supernatants obtained by using either the French pressure cell or an ultrasonic oscillator. The highest activity of the enzyme was seen in toluene lysates; some activity was seen both in a 1200 × *g* pellet from a cell-free homogenate as also in a 10 000 × *g* pellet after removal of mitochondria. The recovery was, however, ⩽20% of the initial activity. We have been unable so far to make a soluble preparation of the enzyme.

Table 1
Growth rate of mutants

Carbon source	Strain genotype			Doubling period (h)
	pfk1	*pgi1*	*zwf1*	
Fructose	+	+	+	1.9
	–	+	+	2.8
	+	–	+	3.0
	–	–	+	3.2
Glucose	+	+	+	1.8
	+	+	–	2.2
	–	+	+	3.5
	–	+	–	3.5

3.3. *Properties of the enzyme*

The activity of the particulate phosphofructokinase from the non-sense mutant *pfk1-1* was on an average 7 munits/E_{650}, accounting for ~20% of the total phosphofructokinase activity assayed in toluene lysates of the wild-type strain. The enzyme was present in glucose-grown cultures of the *pfk1* mutant but absent when grown on ethanol. These results are shown in table 2. The particulate phosphofructokinase was therefore more inducible than the soluble enzyme.

The enzyme is cold-labile. Exposure to 0°C of a 1200 × *g* pellet off a cell-free supernatant led to complete loss of the activity in 4 h. When such a pellet was suspended in a buffer containing 25–50% glycerol the enzyme activity was stable even after 24 h either at 0°C or at 23°C.

Unlike the soluble phosphofructokinase, the particulate enzyme was not inhibited by ATP (fig.2) while the soluble enzyme was completely inhibited under these conditions (not shown). The enzyme in the toluene extract of the wild-type strain was severely, but not completely inhibited, reflecting the presence of the particulate enzyme. The particulate phosphofructokinase displayed hyperbolic saturation with

Table 2
Phosphofructokinase activity in mutant and wild-type strains of *S. cerevisiae*

Strain	Grown on	Enzyme activity in	
		Toluene lysate[a]	Cell-free extract[b]
Wild-type	Alcohol	19	230
	Glucose	35	400
pfk1 Mutant	Alcohol	0	0
	Glucose	7	0

[a] Phosphofructokinase activity is expressed as munit/E_{650}
[b] Enzyme activity expressed as munit/mg protein

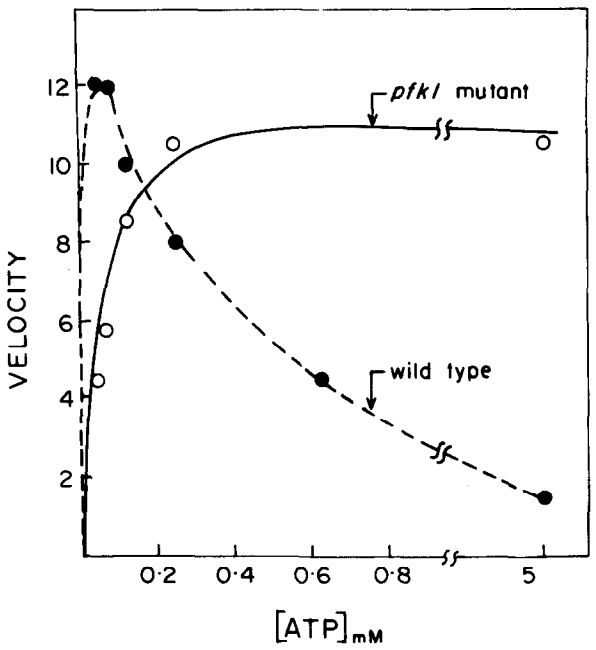

Fig.2. Effect of ATP on phosphofructokinase activity in toluene lysates of wild-type strain and in the mutant lacking the soluble enzyme. The velocity is in arbitrary units of NADH fluorescence. Fructose 6-phosphate was 1 mM.

both the substrates, fructose 6-phosphate and ATP; while the K_m for ATP was 0.1 mM at either 1 mM or 10 mM fixed concentration of the sugar phosphate substrate, the affinity for fructose 6-phosphate seemed to vary with the concentration of the fixed substrate, ATP. The K_m-values for fructose 6-phosphate at 0.1 mM and 2 mM ATP were 0.9 mM and 10 mM fructose 6-phosphate, respectively. Fructose 2,6-diphosphate which has a pronounced stimulating action on the soluble phosphofructokinase from yeast [6] had no such effect on the particulate enzyme. Further, this enzyme synthesizes, unlike the soluble phosphofructokinase 2 of rat liver [7], fructose 1,6-diphosphate and not fructose 2,6-diphosphate.

The particulate enzyme differs from the soluble enzyme in one other respect; the former is strongly inhibited by fructose 1,6-diphosphate, while the latter is known to be stimulated [6]. In the standard assay system that measures the diphosphate continuously by removing it (section 2.2), the rate of NADH disappearance was proportional to the amount of the enzyme added. However, discontinuous assays that measure the fructose 1,6-diphosphate produced showed no such proportionality either to the amount of the enzyme or to the time of incubation. Inclusion of aldolase alone or together with all the other reactants to convert fructose 1,6-diphosphate to pyruvate improved the assay as reflected by the disappearance of fructose 6-phosphate or accumulation of pyruvate. In contrast, the soluble phosphofructokinase gave the same result in the continuous assay as in the discontinuous method.

The enzyme could not be assayed in toluene extracts by measuring the ADP produced as the lysate had ATPase activity that greatly surpassed the phosphofructokinase in velocity.

3.4. *Genetics of phosphofructokinase II*

A number of glucose-negative mutants were obtained from *pfk1* mutants by inositol starvation [6]. If their inability to grow on glucose was due to the loss of this particulate fructose 6-phosphate kinase, it should be possible to cure their growth defect by the gene *PFK1*. However, if the glucose-negative phenotype were due to the loss of any other enzyme, *PFK1* would not be able to suppress it. Ten such glucose-negative mutants were crossed to a wild-type strain and the suppression pattern examined in tetrads. Four of these mutants could be suppressed by *PFK1* (or a gene linked to it), while others could not be suppressed. Mutants which were suppressed were examined for complementation in pairwise crosses for growth on glucose whereupon 2 mutant genes *pfk2* (3 mutants) and *pfk3* (1 mutant) were identified. That is, the glucose-negative mutants from the *pfk1* strain had two possible genotypes: *pfk1 pfk2* and *pfk1 pfk3*, respectively. No particulate phosphofructokinase could be detected in these mutants on growing them for 24 h on alcohol followed by 6 h incubation in glucose-supplemented media; the parental *pfk1* mutants had a low but detectable enzyme activity under the same conditions.

Intercrosses between these 2 groups of mutants *pfk1 pfk2* and *pfk1 pfk3* yielded strains of the putative genotype *pfk1 pfk2 pfk3* which had a much tighter glucose-negative phenotype than either of the parents. Such intercrosses also generated recombinants that could grow on glucose and possessed the particulate phosphofructokinase. Six glucose-positive recombinants appeared amongst 28 viable progeny spores dissected. These results are thus consistent with 2 unlinked genes *pfk2* and *pfk3* controlling the glucose growth of *pfk1* mutants by way of the particulate phosphofructokinase.

4. Discussion

The existence of the particulate phosphofructokinase readily explains why yeast mutants lacking the soluble allosteric enzyme are able to grow on hexose sugars. Although we have not been able to solubilise the enzyme nor establish the stoichiometry in regard to ATP and ADP, the fact that it uses fructose 6-phosphate is clear. Two properties of the enzyme deserve a mention. One is its particulate nature that distinguishes it from all other glycolytic enzymes of yeast. The other is the pronounced inhibition by the product fructose 1,6-diphosphate; 0.05 mM of this substance inhibits activity by >50%. However, the enzyme, unlike the soluble phosphofructokinase, is not inhibited by ATP.

That the particulate phosphofructokinase serves a glycolytic function is shown by the following results: the growth of *pfk1* mutants on glucose; the ability of the double mutant *pfk1 pgi1* to grow on fructose; its inducibility by glucose; and the loss of glucose growth in secondary mutants from *pfk1* bearing a lesion in either of the genes *pfk2* or *pfk3*. We are studying their role in the control of yeast glycolysis.

References

[1] Clifton, D., Weinstock, S. B. and Fraenkel, D. G. (1978) Genetics 88, 1–11.
[2] Ciriacy, M. and Breitenbach, I. (1979) J. Bacteriol. 139, 152–160.
[3] Lobo, Z. and Maitra, P. K. (1977) Mol. Gen. Genet. 157, 297–300.
[4] Maitra, P. K. and Lobo, Z. (1977) Mol. Gen. Genet. 156, 55–60.
[5] Maitra, P. K. and Lobo, Z. (1971) J. Biol. Chem. 246, 475–488.
[6] Van Schaftingen, E., Jett, M.-F., Hue, L. and Hers, H.-G. (1981) Proc. Natl. Acad. Sci. USA 78, 3483–3486.
[7] Van Schaftingen, E. and Hers, H.-G. (1981) Biochem. Biophys. Res. Commun. 101, 1078–1084.
[8] Henry, S. A., Donahue, T. F. and Culbertson, M. R. (1975) Mol. Gen. Genet. 143, 5–11.