Mutations in the regulatory subunit of soluble phosphofructokinase from yeast

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Mutant alleles of the gene *PFK2* have been obtained that alter the sensitivity to ATP inhibition of the soluble yeast phosphofructokinase. One of the alleles makes the enzyme sensitive to micromolar concentrations of ATP. Intragenic revertants of *PFK2* mutants confirm that the *PFK2* gene determines not only the regulatory properties of the soluble enzyme but also the catalytic activity of particulate phosphofructokinase.

Yeast phosphofructokinase *Regulatory subunit mutant* *Hyper-allostery* PFK2 *gene product* *Particulate phosphofructokinase* *Phosphofructokinase mutant*

1. INTRODUCTION

Yeast phosphofructokinase is a soluble allosteric enzyme, regulated by a variety of effectors including ATP [1], that inhibit the enzyme in the presence of low concentrations of its substrate fructose 6-phosphate (F6P). According to biochemical evidence [2], the enzyme is an octamer composed of equal proportions of two non-identical subunits, which according to [3] and [4] might have distinct catalytic and regulatory functions. Genetic evidence reported earlier [5] indicates that there are two genes controlling soluble phosphofructokinase activity, namely *PFK1* and *PFK2*, coding for the catalytic and regulatory subunits of the enzyme, respectively. The gene *PFK2* is also required for activity of particulate phosphofructokinase [6]. This conclusion was arrived at by examining the soluble phosphofructokinase in a *pfk2* mutant lacking the particulate enzyme.

We report the isolation of new alleles of *PFK2* and an examination of mutant forms of the soluble enzyme in segregants having these alleles. Studies of soluble phosphofructokinase activity in intragenic revertants of *pfk1* and *pfk2* mutants support the conclusion that the regulatory properties of the soluble enzyme are controlled entirely by *PFK2*.

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2. MATERIALS AND METHODS

The mutation *pfk1-1* is an ochre-suppressible mutation and has been described previously [7]. *pfk2* mutations were obtained as a class that rendered *pfk1-1* glucose-negative. Two alleles of *pfk2* have been described earlier [5]. Several more alleles of *pfk2* are described here. Strains were grown on 0.3% yeast extract and 1% peptone (YEP) with glucose or ethanol as carbon source, on a rotary shaker at 30°C. Enzymes and substrates were from Boehringer or Sigma.

3. RESULTS

3.1. *Isolation of mutants*

Mutants in *pfk2* were obtained by looking for glucose-negative colonies from a *pfk1-1* strain lacking soluble phosphofructokinase activity. The appearance of $3^+:1^-$ and $4^+:0^-$ tetrads on glucose in crosses of some of these mutants with the wild-type strain, suggested that 27 of these mutants were suppressed by the wild-type allele *PFK1*. Each of the 27 mutants segregated $2^+:2^-$ on

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glucose in crosses with the parent *pfk1-1* strain. Complementation analysis of the 27 mutants showed that 20 of these belonged to a single complementation group, *PFK2*.

3.2. *Effect of* pfk2 *alleles on soluble phosphofructokinase*

Spores of the genotype *PFK1 pfk2* were obtained by crossing the glucose-negative mutants *pfk1 pfk2* with a wild-type strain *PFK1 PFK2*. Phosphofructokinase activity in spores of the genotype *PFK1 pfk2* was characteristically different from that in spores of the genotype *PFK1 PFK2*. Table 1 shows the nature of ATP inhibition for these different alleles of *pfk2* along with a wild-type strain. The first column shows phosphofructokinase activity at low fructose 6-phosphate (F6P) (1 mM) and low ATP (0.05 mM) concentrations. The second column shows PFK I activity at the same concentrations of F6P but under inhibitory concentrations of ATP (2.25 mM). The third column shows phosphofructokinase activity under high concentrations of both F6P and ATP.

The wild-type enzyme (last line) is strongly susceptible to the inhibitory action of ATP. The allele *pfk2-5*, however, is completely non-allosteric, showing an increase in activity by a concentration of ATP that strongly inhibits the wild-type enzyme. The allele *pfk2-7* is similar to the wild-type enzyme. The enzyme from allele *pfk2-4* is hardly active unless fructose 2,6-bisphosphate was present or under very high concentrations of F6P (10 mM, see later). The level of another glycolytic enzyme phosphoglucoisomerase was similar in all the spores.

The allele *pfk2-4* causes a severe alteration in the regulation of soluble phosphofructokinase. Fig.1 shows the effect of F6P and ATP on PFK I activity in a strain of the genotype *PFK1 pfk2-4* as compared to a wild-type strain, *PFK1 PFK2*. At an ATP concentration of 0.1 mM the mutant enzyme shows a half-saturation concentration for F6P which is 15-times that of the wild-type enzyme. Even at 10 mM F6P, the mutant enzyme is completely inhibited by 0.5 mM ATP while at 1 mM F6P, it is inhibited at all concentrations of ATP. Micromolar concentrations of fructose 2,6-bisphosphate strongly stimulated this hyper-allosteric enzyme.

In diploids heterozygous for *PFK2* the regulation of PFK I by ATP displayed characteristics intermediate between the wild-type and the mutant

Table 1

Effect of *pfk2* alleles on soluble phosphofructokinase

Spore genotype	Enzyme activity (mU/mg protein)			
	PFK I			PGI
	F6P 1 mM ATP 0.05 mM	F6P 1 mM ATP 2.25 mM	F6P 10 mM ATP 2.25 mM	
PFK1 pfk2-5	98	161	218	1392
PFK1 pfk2-7	13	3	32	1243
PFK1 pfk2-4	0	0	0(24*)	1017
PFK1 PFK2	30	0	108	1584

* Refers to F6P 10 mM and ATP 0.05 mM

Haploid spores of the genotype *PFK1 pfk2* obtained from the meiosis of (*pfk1 pfk2*)/(*PFK1 PFK2*) diploids were grown to stationary phase on YEP alcohol. Washed cells were suspended in 50 mM potassium phosphate (pH 7.4), 5 mM 2-mercaptoethanol and 2 mM EDTA and extracted with a French Press. The centrifuged supernatants were used directly as the source of the enzyme in 0.8 ml assay mixture containing F6P and ATP at the indicated concentrations, 0.03 mM NADH, aldolase, α-glycerophosphate dehydrogenase, triosephosphate isomerase and 2–10 mU phosphofructokinase in 50 mM triethanolamine buffer, 20 mM KCl and 10 mM $MgCl_2$ (pH 7.4)

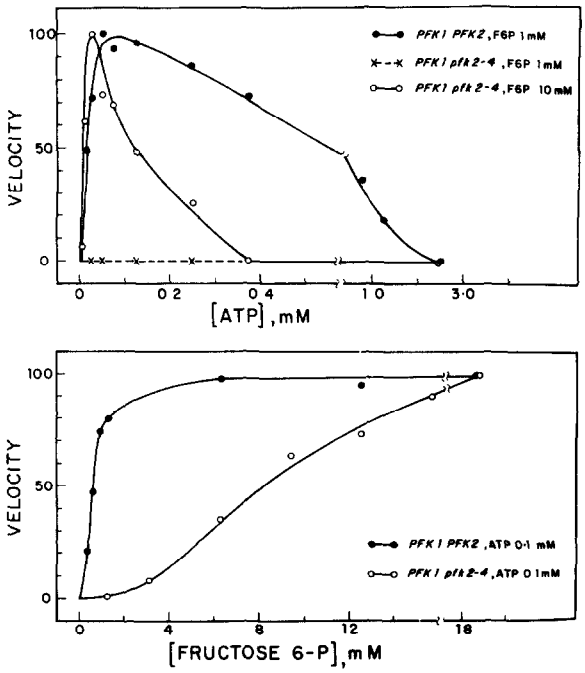

Fig.1. Effect of F6P and ATP on the velocity of phosphofructokinase from the strain *PFK1 pfk2-4* and a wild-type strain. Both strains were grown to stationary phase on 1% YEP glucose. Assays were performed using centrifuged supernatants after extraction of the cells with a French press. Experimental details were the same as in table 1.

allele (not shown). In diploids with two different alleles, e.g., *pfk2-5/pfk2-4*, the ATP inhibition pattern showed the influence of both alleles, namely, it was more active than that synthesized in presence of *pfk2-4* alone, but was strongly subject to inhibition by ATP. The enzyme reflected the influence of both alleles.

3.3. *Analysis of revertants*

Revertants of the double mutant *pfk1 pfk2* can be obtained on glucose by restoration of either the soluble or particulate phosphofructokinase activity. Out of 34 spontaneous revertants obtained from a *pfk1-1 pfk2-1* strain, 28 had restored soluble phosphofructokinase activity which was non-allosteric as determined by the resident *pfk2-1* allele. The remaining 6 were intragenic revertants in the *pfk2* locus (not shown) that restored the particulate enzyme PFK II, but not the soluble enzyme PFK I. Three of these 6 revertants have been analysed genetically by crossing each of these *pfk1 pfk2* (*sup*) strains to a tester strain *PFK1 pfk2-1*, and examining the phosphofructokinase activity of the spores in regard to ATP inhibition. Table 2 illustrates the results in respect of one such revertant R15. Spores A and D were both negative for the PFK I activity, however, spore A was glucose-positive by virtue of the particulate phosphofructokinase activity having inherited the suppressed gene *pfk2* (*sup 15*). Spore B synthesized a non-allosteric PFK I activity while the enzyme from spore C was susceptible to ATP inhibition somewhat like the wild-type enzyme (table 1, line 4). The non-allosteric allele *pfk2-1* thus changed on intragenic reversion to an allosteric variant *pfk2-1* (*sup15*). The results of analysis of the other

Table 2

Analysis of an intragenic revertant of *pfk2*

Spores	Growth on glucose	PFK I activity			Genotype
		F6P 1 ATP 0.05	F6P 1 ATP 2.25	F6P 10 ATP 2.25	
A	+	0	0	0	*pfk1 pfk2-1* (*sup 15*)
B	+	65	300	400	*PFK1 pfk2-1*
C	+	39	6	60	*PFK1 pfk2-1* (*sup 15*)
D	−	0	0	0	*pfk1 pfk2-1*

The revertant R15 of the genotype *pfk1 pfk2-1* (*sup 15*) was crossed to a tester strain *PFK1 pfk2-1*. The diploid was sporulated and a tetratype tetrad analysed for PFK I activity in toluenized cells grown on YEP alcohol. Enzyme activities are expressed as arbitrary units; other details are as in table 1

Table 3

Properties of representative alleles of *PFK2*

Allele	PFK II activity	PFK I allostery	Reference
PFK2	+	+	[5]
pfk2-1	–	–	[5]
pfk2-2 23°C	+	±	[5]
pfk2-2 30°C	–	–	
pfk2-4	–	+ +	This work
pfk2-5	–	–	This work
pfk2-7	–	+	This work
pfk2-1 (sup 15)	+	+	This work

two revertants were also found to be very similar (not shown). The reversion of *pfk2* was thus associated not only with the restoration of the particulate enzyme, but also an altered allosteric control of the soluble phosphofructokinase.

4. DISCUSSION

The soluble allosteric phosphofructokinase PFK I has so far been found to be determined by only two genes, *PFK1* coding for the catalytic activity [7], and *PFK2* coding for the regulatory subunit [5]. Mutations leading to the loss of the particulate enzyme, in contrast, span at least 4 complementation groups *PFK2, PFK3, PFK4* and *PFK5* (unpublished); one of these, *PFK2*, is the common element between the two enzymes. Either of these activities is sufficient for growth on glucose as seen by reversion of the glucose-negative double mutant *pfk1 pfk2*. This explains why mutants lacking phosphofructokinase do not occur amongst glucose-negative derivatives of a wild-type strain.

Can a mutation in *PFK2* alone lead to a complete loss of the soluble phosphofructokinase activity? We think not. More than 120 mutations lacking this enzyme were at the locus PFK1 [7]. All 20 mutations in *PFK2* reported here lost the particulate enzyme; the soluble enzyme was affected only indirectly. Even in the most pertinent case of *pfk2-4* the soluble PFK I was very much present although it was detectable only at very low concentrations of ATP (table 1). However, a recent report [8] indicated that mutations in either *PFK1* or *PFK2* gene can lead to a nearly total loss of PFK I activity; of the two alleles of *pfk2* reported by these authors, *pfk2-1* had practically no activity of the soluble phosphofructokinase at 0.2 mM ATP and 5 mM fructose 6-phosphate. The second allele *pfk2-2*, in contrast, had no effect on PFK I activity. We believe that their *pfk2-1* mutation synthesizes a form of the enzyme hypersensitive to ATP, similar to our strain *PFK1 pfk2-4* and that *pfk2* mutations do not affect the soluble phosphofructokinase activity directly. Recent results from the same laboratory [9] justify this interpretation.

Although all the mutations in *PFK2* were isolated as clones lacking the particulate PFK II activity, they turned out to affect also the regulatory properties of the soluble enzyme. While in some there was no effect on this regulation, some mutations rendered the enzyme more susceptible to ATP inhibition and still others made it practically non-allosteric. Table 3 is a summary of the properties of representative alleles of *PFK2* with respect to the particulate phosphofructokinase activity and the ATP-sensitivity of the soluble enzyme. While 3 of these (*pfk2-1, pfk2-4* and *pfk2-5*) affected both these functions simultaneously, the allele *pfk2-7* altered only one of them. The allele *pfk2-2* in contrast, was temperature sensitive; at 30°C it led to the loss of both the particulate enzyme activity and the ATP sensitivity of the soluble enzyme. At 23°C however, the former function was fully restored while the latter was only partly restored [5]. The discreteness of the two functions was also borne out by the simultaneous restitution of the two activities in intragenic reversions at the *PFK2* locus.

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