

RAPID REVERSIBLE INACTIVATION OF FRUCTOSE-1,6-BISPHOSPHATASE IN *SACCHAROMYCES CEREVISIAE* BY GLUCOSE

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1. Introduction

Inactivation of ~90% of the activity of fructose-1,6-bisphosphatase in 1 h after addition of glucose or fructose to acetate-grown yeast cells has been mentioned [1] and described with experimental data [2]. The inactivation can be reversed by transfer of the sugar-treated inactivated cells to an acetate or ethanol containing growth medium [2]. This reappearance of fructose-1,6-bisphosphatase activity is prevented by addition of cycloheximide, a potent inhibitor of protein synthesis in yeast, and is therefore dependent on de novo protein synthesis [2]. In the course of our studies on catabolite inactivation of gluconeogenic enzymes in *Saccharomyces cerevisiae* [3,4] a rapid disappearance of 50–70% of fructose-1,6-bisphosphatase activity in 3 min after addition of glucose was observed, which was followed by a much slower disappearance of the remaining activity [5]. In contrast to the 'long term' inactivation described [2], the rapid inactivation observed after 3 min incubation with glucose is reversible after transfer of the cells to a sugar-free, acetate containing medium also in the presence of cycloheximide. Thus, in contrast to the situation after 'long term' inactivation reactivation after 'short term' inactivation is independent on the novo protein synthesis. The possibility of a rapid covalent interconversion of fructose-1,6-bisphosphatase (for definition of 'interconversion' see [6]), preceding the irreversible inactivation which can only be restored by de novo protein synthesis, is considered as an explanation for these observations.

2. Materials and methods

The diploid yeast *Saccharomyces cerevisiae* M₁

(supplied by Dr A. W. Linnane, Monash University, Clayton, Australia) was used throughout this study. Cells were grown to the stationary phase on complete medium containing 1% yeast extract, 2% peptone and 2% glucose. The cultures were aerated by shaking at 28°C for 24 h. Cells were collected by centrifugation, washed twice with 0.1 M potassium phosphate buffer (pH 6.0). Wet weight was determined on a 5000 × g, 10 min pellet. For preparation of crude extract, the washed cells were resuspended in 0.1 M potassium phosphate buffer (pH 7.0) (2% wet wt/vol.), and passed twice through a French pressure cell (Aminco, Silver Spring) with a pressure of 20 000 lb/in². The supernatant obtained after centrifugation of the homogenate at 8000 × g for 4 min was used for determination of enzyme activity and protein. Fructose-1,6-bisphosphatase activity was assayed as in [7]. Protein was determined according to [8] using bovine serum albumin as standard.

The reagents for the enzyme assay were purchased from Boehringer (Mannheim). Cycloheximide and carbonylcyanide-*m*-chlorophenylhydrazon (CCCP) was obtained from Sigma (München). All yeast media were from Difco (Detroit, MI). All other chemicals used were obtained from Merck (Darmstadt).

3. Results

Three minutes after the addition of glucose to starved yeast cells the specific activity of fructose-1,6-bisphosphatase has dropped to ~40% of the initial value (fig.1). When resuspended in a medium containing acetate instead of glucose, specific activity of fructose-1,6-bisphosphatase recovers and reaches the initial value after 2 h. Cycloheximide does not prevent

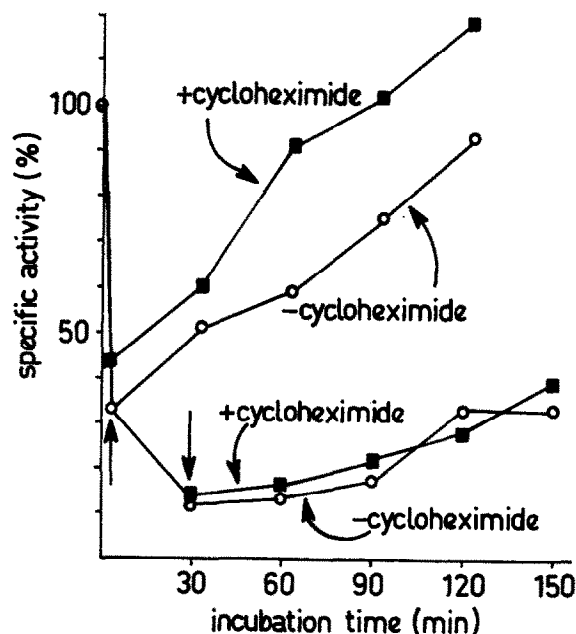


Fig.1. Reactivation of fructose-1,6-bisphosphatase activity after inactivation by glucose. Yeast cells from stationary phase (2% wet wt/vol.) were suspended in 0.1 M potassium phosphate buffer (pH 6.0) and preincubated for 5 min at 30°C. At zero time, before addition of glucose, and 3 and 30 min after treatment of cells with 2% (w/v) glucose, samples were taken, collected on Millipore filters HAWP 04700 and washed twice with 0.1 M potassium phosphate buffer (pH 6.0). The cells of the samples taken after 3 and 30 min were resuspended (2% wet wt/vol.) for reactivation in 0.1 M potassium phosphate buffer (pH 6.0) containing 2% (w/v) sodium acetate. (↑, ↓) Indicate time of resuspension of cells in glucose-free acetate medium. (○—○) Resuspension without cycloheximide; (■—■) resuspension with addition of 100 µg cycloheximide/ml. Samples were taken at the times indicated, collected and washed on millipore filters as above. Cell pellets were resuspended in 0.1 M potassium phosphate buffer (pH 7.0) (2% wet wt/vol.), extracted and assayed for fructose-1,6-bisphosphatase as in section 2. Specific activity of fructose-1,6-bisphosphatase at zero time (before addition of glucose) was set 100%.

reactivation of fructose-1,6-bisphosphatase. In the presence of the drug even a somewhat higher specific activity is observed. In contrast, samples inactivated by incubation with glucose for 30 min do not exhibit considerable reactivation of fructose-1,6-bisphosphatase activity after incubation in a glucose-free medium containing acetate (cf. fig.1). This latter observation agrees with [2] in that reactivation after 'long term' inactivation is dependent on de novo protein synthesis. Final proof, that reactivation after

'short term' inactivation of fructose-1,6-bisphosphatase is independent from protein synthesis came from a control experiment (data not shown) demonstrating that cycloheximide completely prevented incorporation of [³H]leucine into trichloroacetic acid insoluble material under the conditions used in the experiments shown in fig.1 [5].

When reactivation of fructose-1,6-bisphosphatase in 3 min inactivated cells was done only in phosphate buffer without acetate, increase in activity was slower and reached only ~50–60% of the initial value [5]. We therefore checked if the energy yielding metabolism of acetate is necessary for reactivation of fructose-1,6-bisphosphatase. As shown in table 1, reactivation is completely insensitive to 100 µg/ml cycloheximide. Fluoride partially inhibited the reappearance of enzyme activity and cyanide as well as the respiratory chain uncoupling agent CCCP almost completely prevented reactivation. These findings suggest metabolic ATP-production to be necessary for the cycloheximide insensitive reactivation of fructose-1,6-bisphosphatase.

One plausible explanation for rapid inactivation of an enzyme which can be reactivated in an ATP-dependent process without de novo synthesis of the enzyme protein is interconversion by enzyme catalyzed covalent modification [9]. In this case one should expect different properties of the enzyme before and after conversion. Crude extracts from cells derepressed in fructose-1,6-bisphosphatase (zero time in fig.1) and from cells incubated for 3 min with glucose and thus partly inactivated in fructose-1,6-bisphosphatase activity were analyzed for dependency of fructose-1,6-bisphosphatase activity on the following parameters: (i) pH 5.5–10.0; (ii) the concentration of Mg²⁺; (iii) inhibition by AMP and P_i. No differences in the enzyme activity between the two types of extracts were found [5]. However, when measured in the presence of Mn²⁺ fructose-1,6-bisphosphatase activity showed a significant difference in the two types of extracts as can be seen from table 2. When measured in the standard assay in the presence of 10 mM Mg²⁺ ~50% loss of the initial activity of fructose-1,6-bisphosphatase after 3 min incubation of the cells with glucose was observed. However, when assayed in the presence of 2 mM Mn²⁺ only ~10% inactivation of fructose-1,6-bisphosphatase was measurable (0.27 versus 0.24 U/ml). Thus, the enzyme activities in the two types of crude extracts show a clearly distinguishable response to the cations Mg²⁺

Table 1
Effect of inhibitors on reactivation of fructose-1,6-bisphosphatase

Addition to reactivation medium	Activity (%) ^a		Reactivation in % of the value without addition of inhibitor
	After 3 min inactivation with glucose	After 120 min reactivation	
—	37	91	100
100 µg Cycloheximide/ml	41	94	98
20 mM Fluoride	37	65	52
10 mM Cyanide	38	48	19
0.01 mM Carbon-ylcyanide- <i>m</i> -chlorophenylhydrazon (CCCP)	42	42	0

^a Fructose-1,6-bisphosphatase activity prior to addition of glucose was set 100%

Experimental conditions for reactivation see legend to fig.1. Fructose-1,6-bisphosphatase was assayed as in section 2

and Mn^{2+} leading to the calculation of different activity ratios R when measured with 10 mM Mg^{2+} and with 2 mM Mn^{2+} (table 2).

Fructose-1,6-bisphosphatase was purified from derepressed cells and from cells incubated 3 min with glucose as in [10] with slight modifications [5]. As expected, the activity of the purified enzyme preparations showed no differences in their dependence on

pH, the concentrations of Mg^{2+} , AMP or P_i . Quite unexpectedly, after purification the differences of the enzyme activities observed in the crude extracts when measured with 10 mM Mg^{2+} and 2 mM Mn^{2+} , respectively (cf. table 2) were no more detectable.

4. Discussion

The inactivation of fructose-1,6-bisphosphatase observed after incubation of derepressed yeast cells with glucose or fructose for 1 h [2] is very probably the result of proteolytic degradation of the enzyme [2,11]. The main finding which favours this interpretation is the inhibition of the reactivation of fructose-1,6-bisphosphatase after transfer to a sugar-free medium by cycloheximide, i.e., the necessity of de novo protein synthesis for reactivation [2]. The cycloheximide-insensitive reactivation of fructose-1,6-bisphosphatase after very short (3 min) inactivation with glucose, shown here points to a non-proteolytic, reversible inactivation of the enzyme (probably an enzyme catalyzed covalent interconversion), which precedes and perhaps initiates the irreversible proteolytic inactivation.

We show that the Mn^{2+} -dependent activity of fructose-1,6-bisphosphatase is not inactivated after 3 min treatment of the derepressed yeast cells with glucose, whereas the Mg^{2+} -dependent activity is. A

Table 2
Effect of Mg^{2+} and Mn^{2+} on the activity of fructose-1,6-bisphosphatase in crude extracts from stationary (derepressed) cells and cells incubated for 3 min with glucose

	Fructose-1,6-bisphosphatase activity (U/ml)	
	Stationary (derepressed) cells	Cells incubated for 3 min with glucose
10 mM Mg^{2+}	0.46	0.24
0.5 mM Mn^{2+}	<0.01	<0.01
2 mM Mn^{2+}	0.27	0.24
R	1.7	1

$$R = \frac{\text{U/ml with 10 mM } Mg^{2+}}{\text{U/ml with 2 mM } Mn^{2+}}$$

Fructose-1,6-bisphosphatase activity was measured as in section 2 with the indicated concentrations of Mg^{2+} and Mn^{2+}

similar observation was made earlier with glutamine synthetase from *Escherichia coli*. The Mg^{2+} -dependent synthetase activity of this enzyme is rapidly inactivated by enzyme-catalyzed adenylation after addition of NH_4^+ to cells grown on other nitrogen sources, whereas the Mn^{2+} -dependent γ -glutamyltransferase activity of the enzyme is not, or only slightly, inactivated [12–14]. Further characterization of the presumptive interconvertible forms of fructose-1,6-bisphosphatase and search for interconverting enzymes are in progress.

In yeast cells growing in a medium with acetate or ethanol as carbon source, gluconeogenesis and therefore high activity of fructose-1,6-bisphosphatase is necessary. Transfer of such cells to a glucose-containing medium leads to the start of the phosphofructokinase reaction. This event would generate an ATP-splitting, i.e., energy wasting, 'futile cycle' by cyclic action of phosphofructokinase and fructose-1,6-bisphosphatase. The resulting ATP-depletion could kill the yeast cells as demonstrated for the sulfite-induced ATP depletion of yeast cells [15,16]. Inactivation of fructose-1,6-bisphosphatase by proteolytic degradation might be too slow to prevent a harmful depletion of ATP. Thus, the rapid inactivation of fructose-1,6-bisphosphatase by enzyme catalyzed conversion after addition of glucose might be a necessary life-saving device of the cells.

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