METABOLITE REPRESSION OF FRUCTOSE 1,6-DIPHOSPHATASE IN YEAST

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Fructose 1,6-diphosphatase (FDPase), a key enzyme in gluconeogenesis, has been found to be adaptive in yeast (Gancedo et al., 1965). Evidence reported here is consistent with the hypothesis that the synthesis of FDPase in yeast is regulated by catabolite repression, and that the metabolic repressor is probably glucose 6-phosphate.

Results and Discussion

It has been reported previously that FDPase is present in Saccharomyces cerevisiae if grown on ethanol, but not if grown on glucose (Gancedo et al., 1965). S. cerevisiae can grow on galactose at a slower rate (about one half) of that on glucose. As shown in Table I, very significant concentrations of FDPase have been found in galactose-grown yeast. This observation seems to rule out a dependance on precursor induction for the synthesis of FDPase. Consistent with this conclusion is the fact that the concentration of FDP, one of the more likely candidates as metabolic inducer if induction were involved, is much smaller in yeast growing on ethanol that when growing on glucose (Gancedo et al., 1967). The absence of FDPase in glucose-grown yeast is then likely to be due to repression.

Glucose itself is not likely to be the corepressor, since its intracellular concentration in yeast is kept very low by the combined

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effect of a regulated transport and excess of a low Km hexokinase (see Sols, 1966). Moreover, fructose can be substituted for glucose as sole carbon source without derepression of FDPase. If not free glucose, the metabolic repressor of FDPase could likely be glucose 6-phosphate or fructose 6-phosphate, the former a major metabolic crossroad and the latter the primary product of FDPase activity (and both near equilibrium because of the very active glucosephosphate isomerase). Indeed it has been found that the concentration of these hexose monophosphates in growing yeast is markedly affected by the carbon source, increasing in the order ethanol < galactose < glucose (Table I). The concentration of FDPase in these cases is inversely proportional to that of hexose monophosphates.

2-Deoxyglucose (2DG) can be readily taken up by yeast leading to formation of virtually non-metabolizable 2DG 6-P (Heredia et al., 1964). At appropriate ratios of 2DG to hexose, it is possible to maintain substantial concentrations of 2DG 6-P in yeast while still permitting sustained, although inhibited, growth. 0.01% 2DG added to 2% galactose in the growth medium leads to fairly high concentrations of 2DG 6-P and very marked decrease in the concentration of FDPase in yeast (Table I). This result is consistent with the hypothesis that 2DG 6-P can repress FDPase, presumably as analogue of glucose 6-P. Another possible mechanism, alternative or additional to a decrease in the rate of synthesis of the enzyme, could be stimulation of the rate of degradation of FDPase by glucose 6-P with or without constitutive synthesis of the enzyme. Glucose 6-P is known to be involved in several regulatory events in yeast: activation of glycogen synthetase (Rothman and Cabib, 1966) and inhibition of hexose transport (Sols, 1966); it may also be involved in the repression of certain enzymes potentially implicated in gluconeogenesis (Witt et al., 1966). In relation with gluconeogenesis it may well be regarded as the endproduct of the pathway of which FDPase is the irreversible step.

In conclusion it appears that the regulation of the concentration of FDPase in yeast involves feedback repression by glucose 6-P, without requirement for metabolic induction.

Table I

Concentrations of FDPase, glucose 6-P plus fructose 6-P, and 2DG 6-P in yeast grown on different carbon sources

Carbon source	FDPase	Glucose 6-P + fructose 6-P	2DG 6-P
	milliunits/mg protein	μmoles/g fresh yeast	
Glucose	< 1 (4)	0.7 (2)	-
Fructose	< 1 (2)	-	-
Ethanol	36 (4)	< 0.05 (2)	-
Galactose	9 (4)	0.15 (3)	-
Galactose + 2DG	1 (4)	0.14 (2)	1.5 (3)

S. cerevisiae (strain 1724-14A) was grown (at 27° in a synthetic medium, with carbon sources at 2% concentration) and extracted as described by Gancedo et al. (1965). 2-Deoxyglucose, when indicated, was used at a concentration of 0.01%. The cultures were harvested in the exponential phase. FDPase was assayed as described by Salas et. al. (1964). Determination of hexose monophosphates was performed as follows: actively growing yeast was rapidly centrifuged and the sediment resuspended with about twenty volumes of fresh culture medium of the same composition. After 15 min shaking at 27° aliquots of 4.5 ml were rapidly poured with vigorous stirring on 5 ml cold 10% perchloric acid. The mixture was allowed to stand 15 min at room temperature with occasional stirring and then centrifuged. The supernatant was neutralized with solid $KHCO_3$, centrifuged, and the precipitate discarded. Hexose monophosphates were determined in the supernatant by a conventional spectrophotometric assay with glucosephosphate isomerase, glucose-6-phosphate dehydrogenase and NADP. In the case of yeast grown in the presence of 2DG another method was employed since 2DG-P would interfere at the level of the glucose-6-P dehydrogenase (Salas et al., 1965). The assay mixture was as follows: 0.1 M phosphate buffer pH 6.5, 5 mM $MgCl_2$, 5 mM ethanethiol, 0.1 mM ATP-Mg, 0.2 mM NADH and about 0.5 units of glucosephosphate isomerase, phosphofructokinase, aldolase, triosephosphate isomerase and phosphoglyceraldehyde dehydrogenase. For the determination of the intracellular 2DG 6-P the yeast was treated as described by Heredia et al. (1964); 2DG 6-P was estimated by the method of Waravdekar and Saslaw (1959). Metabolites were determined in duplicate samples. Figures in brackets indicate number of experiments. Values of FDPase activities for glucose and ethanol grown yeast are taken from Gancedo et al. (1965).

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