CATABOLITE INACTIVATION OF YEAST CYTOPLASMIC MALATE DEHYDROGENASE

A process independent of protein synthesis

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Received 22 November 1977

1. Introduction

In the yeast *Saccharomyces cerevisiae* cytoplasmic malate dehydrogenase is only expressed when the cells grow on a sugar free medium [1]. After addition of a fermentable sugar the enzyme is rapidly and irreversibly inactivated [1–3]. Since several other yeast enzymes [4–6] show the same behavior, the process was termed 'catabolite inactivation' [7]. The mechanism of this apparently new regulatory principle is not known.

After experiments with cycloheximide it was postulated that glucose induces the synthesis of a specific inactivating system. The drug inhibited the inactivation [2]. Contradictory results were obtained with a tryptophan auxotrophic mutant [3] and in starvation experiments [8], where normal inactivation rates were observed. In order to exclude a glucose inducible inactivating system we used the temperature-sensitive mutant XD 154-S3, which is essentially defective in protein synthesis at elevated temperature. In this mutant a normal catabolite inactivation takes place also at nonpermissive temperature. This shows that glucose does not induce the synthesis of a specific inactivating system.

2. Materials and methods

The prototrophic, temperature-sensitive strain XD 154-S3 was derived from a cross of temperature-sensitive mutant ts 187 (a ade1 ade2 ura1 his7 lys2 tyr1 gal1) [10] with DT 29 (α tryp 5–1). It was constructed by Professor W. Duntze, Bochum, FRG. The genotype is α prot 1–1. All growth and derepression steps were performed at 23°C (permissive temperature). For growth an optimal medium containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 3% glucose was used. The derepression medium contained 6.7 g/lit yeast nitrogen base without amino acids, 0.3% yeast extract, 0.3% sodium acetate and 20 μg/ml tetracycline to prevent bacterial growth at 36°C. In this medium 5 g wet glucose-grown cells were suspended for derepression.

Inactivation experiments were performed by addition of 2% glucose to this medium. The cells were harvested and broken as in [3]. The malate dehydrogenase activity of the crude extracts was assayed as in [1]. Protein was determined by the method in [9]. Labeling experiments were performed by addition of 0.3 μCi/ml [^{14}C]leucine to the derepression medium. The radioactive amino acid was added 30 min before the malate dehydrogenase inactivation was initiated by addition of glucose. Incorporation rate was measured at an incubation temperature of 23°C and 36°C, respectively. Aliquots, 0.5 ml, were taken at different times from the cell suspensions and stopped in 0.5 ml 20% trichloroacetic acid. The mixture was incubated in a boiling water bath for 30 min. The pellets were collected on a Millipore filter type HAWP 02500 and washed with several volumes of cold 16% trichloroacetic acid and distilled water. The filters were dissolved in 1 ml methoxyethanol and counted in a liquid scintillation counter in a usual toluene scintillator.

All nutrients were purchased from Difco labora-

tories, Detroit, MI. Radioactive leucine was a product of Amersham Buchler, Braunschweig. All other chemicals were from commercial sources and of analytical grade.

3. Results

The temperature-sensitive haploid mutant XD 154-S3 grows on an optimal medium at 23°C with a doubling time of approx. 3 h. At the restrictive temperature of 36°C no cell growth can be observed. If an aliquot of the 23°C culture is shifted to 36°C an effect on the growth rate is immediately visible. Growth ceases completely after approx. 1 h. A shift back to 23°C restores normal growth. This shows that the temperature effect is reversible. The results, shown in fig.1, indicate that the prototrophic mutant retained the phenotypic properties of Hartwell's auxotrophic temperature-sensitive strain ts 187. For this strain it has been shown that it is essentially defective in the initiation of protein synthesis at the nonpermissive temperature of 36°C [10]. When glucose-repressed mutant cells are incubated at 23°C in the derepression medium, an approx. 6-fold increase of malate dehydrogenase activity occurs within 10 h. This derepression can be blocked by incubation at 36°C. By reversed ammonium sulfate chromatography (not shown) one can show that this incease of malate

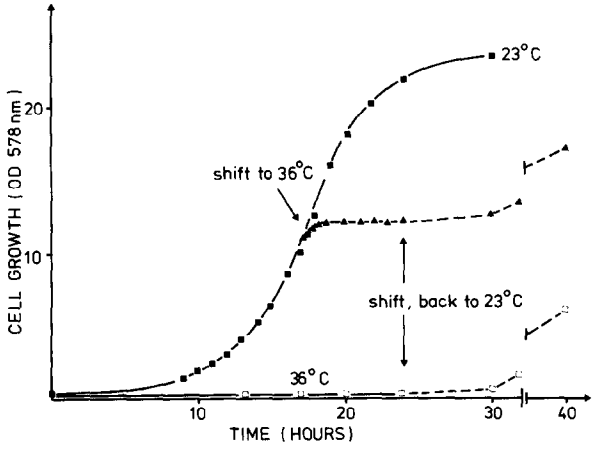

Fig.1. Growth of the temperature-sensitive yeast mutant XD 154-S3 at permissive (23°C) and nonpermissive (36°C) temperature.

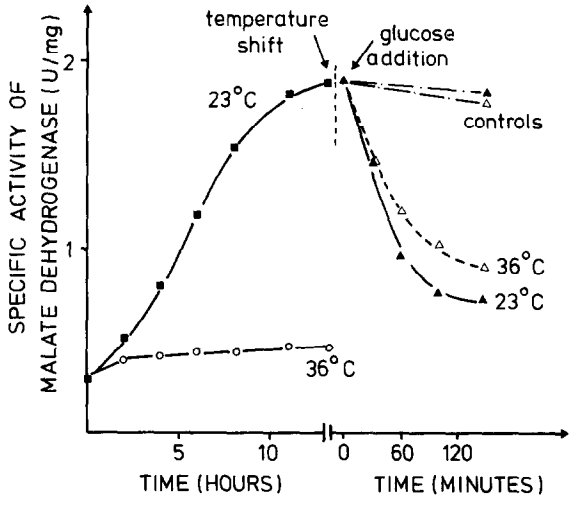

Fig.2. Derepression and inactivation of cytoplasmic malate dehydrogenase in the temperature sensitive mutant at 23°C and at 36°C. In glucose repressed mutant cells the cytoplasmic malate dehydrogenase was derepressed on a derepression medium at 23°C and 36°C, respectively. The 23°C suspension was divided into four parts. Two were further incubated at 23°C (▲). In one of these the inactivation of cytoplasmic malate dehydrogenase was initiated by addition of solid glucose to a final concentration of 2%. The other two cell suspensions were incubated at 36°C for 30 min (△) then treated as described for the 23°C cultures.

dehydrogenase activity is due to the appearence of the cytoplasmic malate dehydrogenase isoenzyme [13]. After derepression the enzyme could be inactivated in the mutant by addition of glucose to the culture medium at 23°C as well as at 36°C (nonpermissive temperature) (see fig.2).

For the initiation of this reaction it was not essential whether the cells were preincubated for 30 min or for 20 h at the nonpermissive temperature. It is noteworthy that the slower inactivation rates at 36°C were also obtained in the wild type strain M_1 which is normally used in the investigations on malate dehydrogenase inactivation [8]. This demonstrates that the slowdown of the inactivation rate is not due to temperature sensitivity.

In order to prove whether protein synthesis is really blocked in the newly constructed mutant during the glucose-dependent inactivation at 36°C, labeling experiments were performed. In the presence of [^{14}C]leucine during the glucose-dependent inactiva-

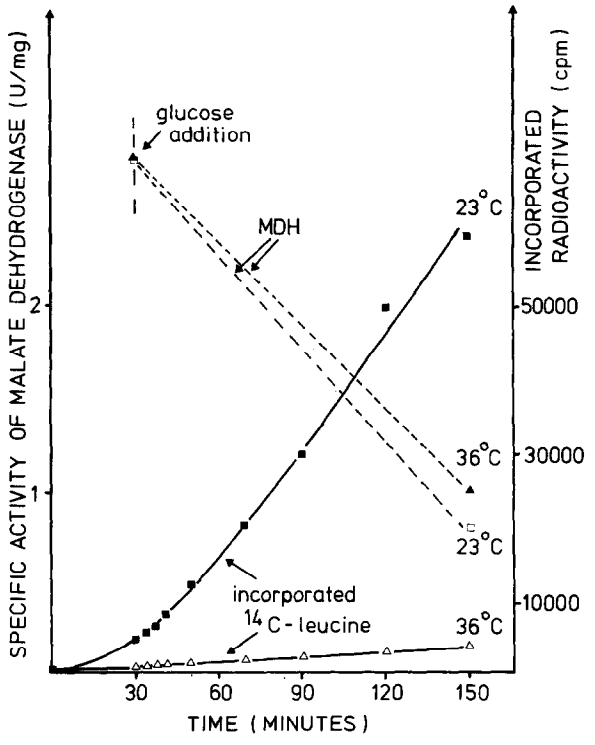

Fig.3. Incorporation of [^{14}C]leucine during the glucose-mediated inactivation of cytoplasmic malate dehydrogenase at 23°C and 36°C. Derepressing mutant cells were preincubated for 30 min at 23°C and at 36°C in a fresh derepression medium containing 0.3 μCi [^{14}C]leucine/ml. In both cell suspensions the inactivation of cytoplasmic malate dehydrogenase was initiated by addition of glucose to a final concentration of 2%. The incorporation rate of the radioactive amino acid into the hot trichloroacetic acid-precipitable fraction was measured.

tion, the incorporation rate into protein at 36°C was low but not zero (see fig.3).

It was comparable to that observed in the parental strain ts 187. However, since induction of the inactivating system must occur in the first 10 min after glucose addition [2], we would argue that at an incorporation rate below 10% control a drastic effect on the inactivation rate should be visible if protein synthesis were necessary for catabolite inactivation.

4. Discussion

The induction of a specific inactivating system for cytoplasmic malate dehydrogenase and other catabolite inactivated enzymes has been discussed after experiments with cycloheximide [2]. The drug is known to be a problematic inhibitor of protein synthesis in yeast [11] especially when used in concentrations as high as 5 μg/ml. Also it is not an especially powerful inhibitor in all cases of catabolite inactivation ([4,12], personal communications).

Experiments with the tryptophan-requiring mutant [3] and starvation experiments [8] argued against the participation of protein synthesis in the inactivation. In these experiments, however, protein synthesis from a remaining pool of amino acids was not completely excluded by incorporation experiments.

With respect to further experiments on the mechanism of catabolite inactivation it was an intriguing question to ask whether the inactivating system is already present in derepressed cells in an activatable form or whether it is newly synthesized after glucose addition. For decisive experiments Hartwell's temperature-sensitive mutant ts 187 seemed well suited [10]. However, in this haploid auxotrophic strain the derepression and inactivation of cytoplasmic malate dehydrogenase was less comparable to the wild type [8]. Therefore Duntze's prototrophic mutant [3] was used, in which the temperature-sensitive defect comes from ts 187. The growth behavior and the derepression kinetic of this haploid mutant show that it retains the useful properties of the temperature-sensitive parent and that it acquires the properties of a wild type in malate dehydrogenase regulation. The low incorporation rates in the mutant at nonpermissive temperature and the consideration that unaffected mitochondrial protein synthesis might partially account for the residual protein synthesis [14], establish that a glucose-inducible inactivating protein can not be involved in catabolite inactivation. The inactivating system must be already present in a metabolically activatable form in derepressed yeast cells.

Together with the finding that an inactive cytoplasmic malate dehydrogenase protein is immunologically undetectable after the irreversible in vivo reaction [13], the experiments support the hypothesis that the proteolytic machinery of the yeast cell might be involved in this inactivation [7].

Acknowledgements

We wish to thank Drs L. H. Hartwell and W. Duntze for the mutants ts 187 and XD 154 - S3. The work was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 76.

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