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# CATABOLITE INACTIVATION OF FRUCTOSE 1,6-BISPHOSPHATASE AND CYTOPLASMIC MALATE DEHYDROGENASE IN YEAST

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Summary: Catabolite inactivation of fructose 1,6-bisphosphatase and cytoplasmic malate dehydrogenase was studied using the protease-deficient and vacuole-defective yeast strain pep4-3. The catabolite inactivation of fructose 1,6-bisphosphatase in pep4-3 was found to have a normal first inactivation step but with a defective second proteolytic step. In contrast, catabolite inactivation of cytoplasmic malate dehydrogenase was normal in pep4-3. These results suggest that the proteolytic pathways utilized in the hydrolysis of the two enzymes may be different and that proteolysis of fructose 1,6-bisphosphatase may require functional vacuoles while proteolysis of cytoplasmic malate dehydrogenase may not. 

Catabolite inactivation in yeast is the glucose induced rapid loss of certain enzymic activities through proteolysis (1). The inactivation of fructose 1,6-bisphosphatase, one of the key enzymes in gluconeogenic pathway, has been most extensively studied as a model for the understanding of the mechanism of catabolite inactivation. In vivo studies have revealed that the inactivation of fructose 1,6-bisphosphatase involves a two-step process (2, 3). The first step is a rapid but reversible loss of activity immediately after glucose addition which is coincident with a transient raise in cAMP concentration and with the phosphorylation of fructose 1,6-bisphosphase (4, 5, 6). The covalent modification of fructose 1,6-bisphosphase by cAMP-dependent phosphorylation resulting in a loss of enzymic activity was postulated as the primary triggering event in catabolite inactivation (6). In the second step of catabolite inactivation, the irreversible loss of enzymic activity is parallel with the simultaneous

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loss of protein antigenecity (3, 7) and, therefore, a proteolytic mechanism is inferred. Catabolite inactivation studies on another enzyme cytoplasmic malate dehydrogenase showed that it is irreversibly inactivated without the initial rapid reversible step (8). Further study using mutants defective in variuos glycolytic enzymes leads to the conclusion that, although fructose 1,6-bisphosphatase and cytoplasmic malate dehydrogenase may differ in their initial inactivation step, they may share a common unknown proteolytic pathway (8).

In this report, we have studied catabolite inactivation of fructose 1,6-bisphosphatase and cytoplasmic malate dehydrogenase in the protease-deficient pep4-3 strain which contains defective vacuoles, due to its inability to process vacuolar hydrolytic pro-enzymes (9).

## MATERIALS AND METHODS

Saccharomyces cerevisiae X2180-1B (ATCC 26787) was obtained from American Type Culture Collection. S. cerevisiae pep4-3 ( $\alpha$ pep4-3 trp) was obtained from Yeast Genetic Stock Center. Fructose 1,6-bisphosphate and oxalacetate were obtained from Sigma. Protein concentrations were measured by the dye-binding method (10).

Catabolite inactivation. Yeasts were grown in 1% yeast extract, 2% peptone, 1% glucose and 20 mg/l tryptophan (YPG) for 16 hr at 30$^{o}$ and harvested at 4500xg for 3 min. The cells were washed once with sterile water, resuspended in 1% yeast extract, 2% peptone, 0.5% sodium acetate, 20 mg/l tryptophan and were further grown for 5 hr. The culture was centrifuged at 4500xg for 3 min, and the cells were washed twice with 0.1 M potassium phosphate (pH 6.0) and resuspended in the same buffer containing tryptophan (20 mg/l) to a cell density of 15 mg wet weight/ml. After a 10 min preincubation at 30$^{o}$, glucose or an equal volume of water was added to different aliquots of cell suspensions.

Preparation of cell extracts. Aliquots of 20 ml were withdrawn at various times during catabolite inactivation and the cells were harvested and washed twice with water by centrifugation. The washed cells were resuspended in 0.5 ml of 0.1M potassium phosphate (pH 7.0) buffer and were broken by vigorous shaking with 1 gm of glass beads in a vortex mixer for 10 periods of 1 min with 1 min intervals of cooling on ice. The homogenates were centrifuged at 10000xg for 20 min and the supernatants were used for enzymatic assays.

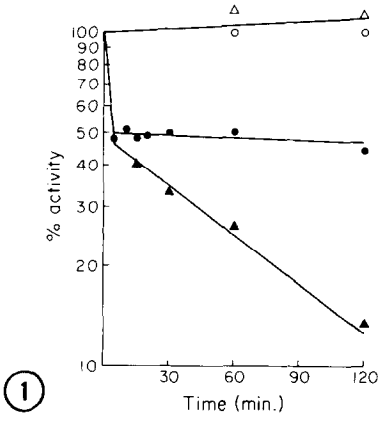
Enzymatic assays. Fructose 1,6-bisphosphatase was assayed as described previously (11). Malate dehydrogenase was assayed as described by Murphy et al. (12).

ATP determination. Yeast cell culture aliquots of 10 ml were withdrawn at various time periods of catabolite inactivation. The cells were immediately collected on chilled Millipore membrane. Extraction and determination of ATP concentration were as described by Ciriacy and Breitenbach (13).

## RESULTS

The wild type parental yeast strain (X2180-1B) showed a typical two-step catabolite inactivation of fructose 1,6-bisphosphatase (Fig. 1). There was a 50% drop in activity 5 min after glucose addition followed by a more gradual enzymic loss. A half-life of approximately 60 min. was found for the second step. In sharp contrast, pep4-3 strain, although retaining the initial 50% drop in activity, showed an abnormally slow second proteolytic step (Fig. 1). Following the initial 50% drop, no further loss of activity was found after 60 min of incubation in the presence of glucose and only a slight decrease after 120 min, when the wild type contained less than 15% of the initial activity.

Previous studies using glycolytic (8) and fdp (14) mutants showed that failure in these mutants to demonstrate catabolite inactivation of fructose 1,6-bisphosphatase can be correlated with the rapid depletion of cellular ATP concentration. ATP concentrations were measured in the parental and pep4-3 strains during catabolite inactivation (Fig 2A and B) and no



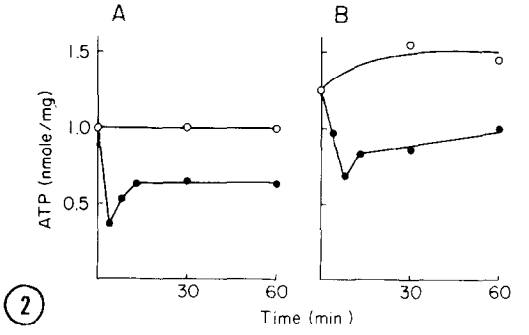




Fig. 1. Catabolite inactivation of fructose 1,6-bisphosphatase.
Catabolite inactivation was initiated by the addition of glucose (2%) at zero time and cell extracts were prepared from aliquots taken at the various times indicated as described in Methods. Fructose 1,6-bisphosphatase activities were assayed as described (11). S. cerevisiae X2180-1B, with (▲) or without (△) glucose; pep4-3, with (●) or without (○) glucose. 100% activity corresponds to a specific activity of 16.7 mU/mg for X2180-1B and 13.3 mU/mg for pep4-3.

Fig.2. ATP concentrations during catabolite inactivation.
Catabolite inactivation was initiated by the addition of glucose (2%). ATP was extracted and measured from aliquots taken at variuos time indicated as described in Methods. (A) S. cerevisiae X2180-1B, (B) pep4-3. (○) in the absence and (●) in the presence of glucose.

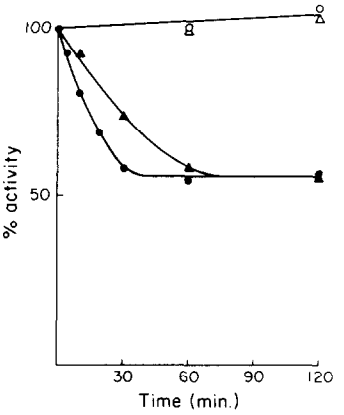



Fig.3. Catabolite inactivation of cytoplasmic malate dehydrogenase. Catabolite inactivation was initiated by the addition of glucoase (2%) at zero time and cell extracts were the same as those prepared in Fig. 1. Malate dehydrogenase activities were assayed as described (12). S. cerevisiae X2180-1B, with (▲) or without (△) glucose; pep4-3, with (●) or without (○) glucose. 100% activity corresponds to a sepecific acitivity of 4.1 U/mg for X2180-1B and 3.45 U/mg for pep4-3.

significant difference was found. There was a transient drop and a gradual recovery of ATP concentrations following glucose addition. Both yeasts maintained 60-70% of their initial ATP concentration.

Inactivation of cytoplasmic malate dehydrogenase was also studied (Fig 3). Under our growth condition, both parental and pep4-3 yeasts contained about equal amounts of the cytoplasmic and mitochondrial malate dehydrogenase isozymes. Only the cytoplasmic isozyme is catabolite inactivated (15) with a half-life of approximately 30 min in the wild type parental strain (Fig.3). In the pep4-3 strain, cytoplasmic malate dehydrogenase was catabolite inactivated at a faster rate with a half-life of approximately 15 min (Fig.3). Therefore, the protease-deficient pep4-3 strain showed a defect in the catabolite inactivation of fructose 1,6-bisphosphatase but not in cytoplasmic malate dehydrogenase.

## DISCUSSION

Several yeast proteases have been biochemically and genetically characterized (16). Proteases A, B and carboxypeptidase Y were localized in the vacuole, the lysosome-like organelle of yeast (17). The strain pep4-3 is a pleiotropic mutant containing inactive vacuolar enzymes due to its

inability to process pro-enzymes (9). Our study on the catabolite inactivation in pep4-3 showed that fructose 1,6-bisphosphatase had a normal initial inactivation step indicating the proper phosphorylation of the enzyme but had an abnormal proteolytic step (Fig 1). The presence of abundant ATP during the catabolite inactivation (Fig 2) indicates that this failure is not due to energy shortage in contrast to that found in the glycolytic or fdp mutants (8, 14). Unlike the catabolite inactivation of fructose 1,6-bisphosphatase, cytoplasmic malate dehydrogenase is catabolite inactivated at a higher rate in pep4-3 than in wild type. These results indicate that the proteolysis of fructose 1,6-bisphosphatase may require functional vacuoles (directly or indirectly) while the proteolysis of cytoplasmic malate dehydrogenase should be an extra-vacuolar event. Therefore, different proteolytic pathways maybe involved in catabolite inactivation.

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