THE GLUCOSE-INDUCED INACTIVATION OF AMINOPEPTIDASE I IN *SACCHAROMYCES CEREVISIAE*

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1. Introduction

The selective and irreversible inactivation of enzymes as an important regulation mechanism in microorganisms has became increasingly evident (reviewed in [1]). Several processes of this type have been detected in yeast. Since most of them are induced by glucose the term 'catabolite inactivation' has been proposed [2].

Saccharomyces cerevisiae contains 3 aminopeptidases active against a variety of amino acid and peptide derivatives [3,4]. General agreement on their nomenclature has still to be achieved. The numbering used here follows a proposal in [3] and is not the same as applied [4]. Aminopeptidase I, an enzyme with uncommonly high molecular weight, is localized in the cell vacuole. Its levels are regulated by both the carbon and the nitrogen source in the growth medium [3]. In this paper it is shown that the decrease of aminopeptidase I activity observed upon addition of glucose to stationary cultures is due to the inactivation of the enzyme rather than to the repression of its synthesis. α-Glucosidase is also inactivated under these conditions while the levels of alkaline phosphatase and those of aminopeptidase II are not markedly affected.

2. Materials and methods

The experiments were performed with *Saccharomyces cerevisiae* NCYC 366 (The Brewing Research Foundation, Nutfield, Surrey).

Cells were grown to the stationary state in a 'minimal medium' composed of 2% glucose and 0.67% yeast nitrogen base (without amino acids). They were collected by centrifugation, transferred to sterile 2% glucose and shaken for another 4 h at 30°C. At 15 min intervals samples were removed from the culture, rapidly chilled in ice and kept frozen until homogenization. Cell homogenates and $10^5 \times g$ supernatants were prepared as in [3].

The stability of enzymes in crude cell extracts was studied by incubating '$10^5 \times g$ supernatants' in the presence of antibiotics (streptomycine sulfate 50 μg/ml and penicilline G 40 IU/ml) for 4 - 7 days at 4°C. When proteinase activities were to be inhibited the homogenates were treated with 5 mM phenylmethylsulfonylfluoride and 10 μM pepstatin prior to incubation. Both compounds do not inhibit purified aminopeptidases I and II.

The assay of aminopeptidases in yeast homogenates has been described [3]. α-Glucosidase activities were measured as in [5] with *p*-nitrophenyl-α-D-glucoside (Serva, Heidelberg). Alkaline phosphatase was assayed in 0.1 M Tris/HCl (pH 8.0) with 2 mM *p*-nitrophenylphosphate (Boehringer, Mannheim). Antisera against aminopeptidase I were obtained by immunization of rabbits with the isolated 22 S form of the enzyme [6]. They were monospecific with respect to the aminopeptidase I dodecamer as checked by double-diffusion. Microcomplement fixation tests were performed with reagents from Behringwerke, Marburg, as in [7]. Amounts of aminopeptidase (5–50 ng) could be quantitatively measured by comparison of relative complement fixation with standard curves.

3. Results and discussion

3.1. *Effect of glucose on enzyme levels*

The response of some enzyme levels in yeast to a transfer of glucose-starved cells to 2% glucose is shown in fig.1. During the experiment no significant increase of cell weight took place, due to the absence of an exogenous nitrogen source. Nevertheless, the intracellular activities of α-glucosidase and aminopeptidase I fell to rather low levels within 1–2 h while the activities of aminopeptidase II and alkaline phosphatase showed little change within the same time. Both glucosidase and aminopeptidase I disappeared with half-times of about 30 min, however, the loss of aminopeptidase activity started only 30 min after addition of glucose whereas the decrease of α-glucosidase did not show such a lag phase. The constance of aminopeptidase II and phosphatase activities confirms that lower levels of aminopeptidase I and glucosidase may not be interpreted as the result of cessation of their synthesis and a subsequent dilution by denovo protein synthesis, but are caused by inactivation processes.

However, before such a conclusion can be drawn it has to be ascertained whether the observed loss of

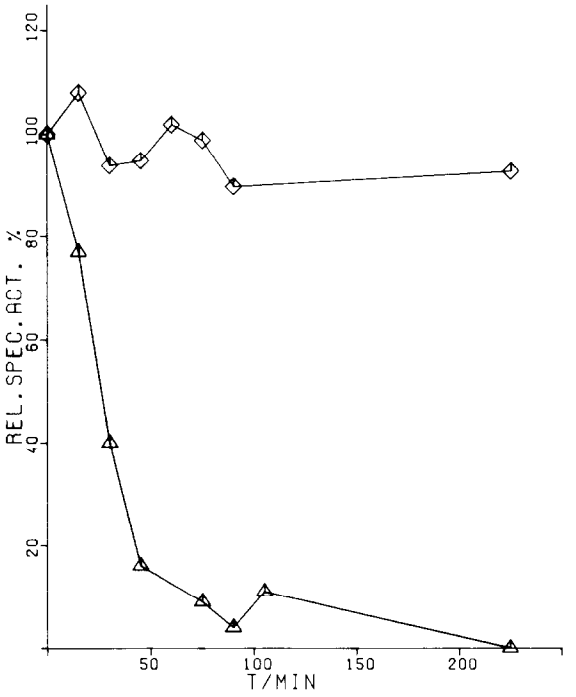
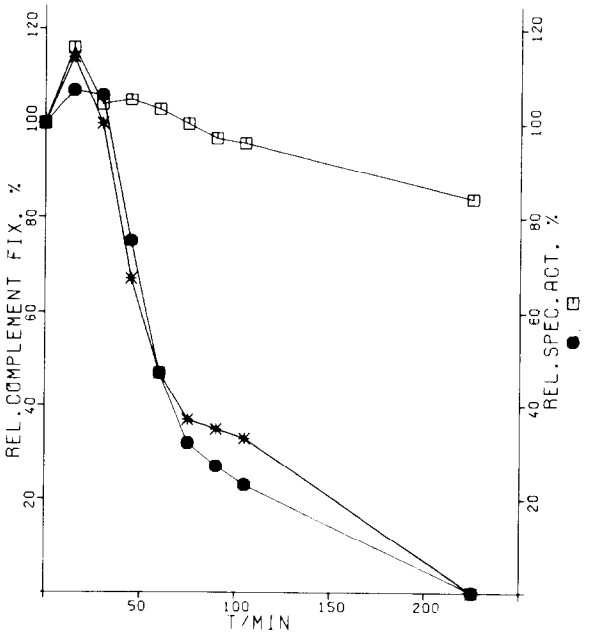


Fig.1. Response of intracellular enzyme levels to addition of glucose to starved cells. Cell extracts were prepared and enzyme activities measured as in section 2. Fig.1a shows specific activities of aminopeptidase I (•) and aminopeptidase II (□) as a function of the time elapsed after transfer of cells to glucose. In addition, the amounts of immunologically reactive aminopeptidase I protein (*) are given. Fig.1b shows data on α-glucosidase (△) and alkaline phosphatase (◇). Enzyme activities are expressed as % of the specific activity at $t = 0$, the values for aminopeptidase protein are % of the amount present at zero time (0.18 μg/mg protein).



activity indeed reflects a decrease of intracellular enzyme levels or is merely a consequence of effects that reduce the amount of active enzyme recovered in the cell extract. For instance, it is conceivable that, in the presence of glucose, the enzyme becomes increasingly attached to membranes and thus disappears from the '$10^5 \times g$ supernatant'. Alternatively, an inhibitor could be formed by the cells upon addition of glucose that would reduce the activities measured with homogenates. Finally, the observed inactivation could be the result of an artifact caused by hydrolytic enzymes to which the aminopeptidase is accessible not before the cells are homogenized.

3.2. *Immunological studies*

In order to follow the fate of the enzyme protein during inactivation, we measured the amounts of aminopeptidase I in homogenates by microcomplement fixation, using an antiserum monospecifically directed against the active 22 S enzyme molecules. The complement fixation technique, besides its sensitivity, has the advantage that the reactive protein antigen may be detected even if it is present in an insoluble form. Its application to '$10^5 \times g$ supernatants' showed a close coincidence between the amounts of aminopeptidase activity and protein present (fig.1a). Thus, it is unlikely that enzyme–inhibitor complexes are present in the supernatants since the formation of such complexes should not greatly impair the antigenic properties of the enzyme protein. During incubation with glucose the amounts of aminopeptidase I found in the '$10^5 \times g$ sediment' did not significantly increase. This contradicts the assumption that the enzyme becomes insoluble under these conditions.

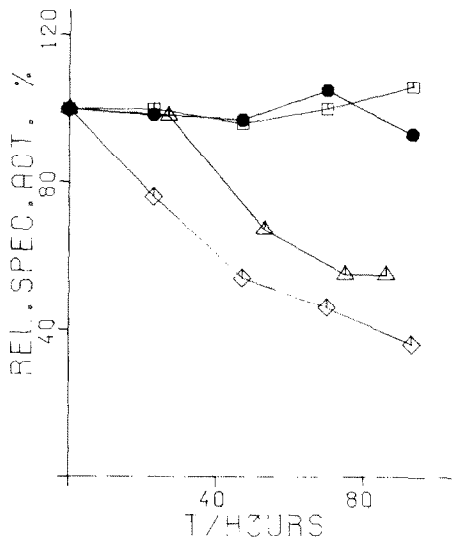
3.3. *Effect of hydrolytic activities*

To test the sensitivity of the enzymes to hydrolytic degradation in cell homogenates, we incubated '$10^5 \times g$ supernatants' for several days at 4°C without and in the presence of proteinase inhibitors. If inhibitors were present all of the activities monitored remained constant for 1 week, without PMSF and pepstatin glucosidase and alkaline phosphatase activities considerably decreased on standing. However, both aminopeptidase I and aminopeptidase II were still unaffected under these conditions (fig.2). The stabilities of either activity did not depend on the time of preincubation of the cells with glucose. Thus, it seems that the resistance of aminopeptidase I against proteolytic degradation is not impaired under conditions leading to its inactivation.

3.4. *Possible inactivation mechanisms*

Most of the yeast enzymes that are subject to catabolite control by glucose are involved in processes leading to the synthesis of glucose by gluconeogenesis or its formation from other sugars [1].

Invertase and α-glucosidase belong to this group. Their synthesis is induced by the respective disaccharide substrates and repressed by glucose [8]. Evidence has been presented indicating that the effect of glucose



Fit.2. Stability of enzyme activities in yeast homogenates. Activities of aminopeptidase I (•), aminopeptidase II (□), α-glucosidase (△) and alkaline phosphatase (◇) were measured after storage of cell-free extracts at 4°C for different times. The data shown are specific activities measured with untreated homogenates; they are expressed as % of the activities determined in extracts containing phenylmethyl sulfonylfluoride and pepstatin. In the presence of proteinase inhibitors all activities were constant during the time of the experiment.

on α-glucosidase is mediated by the lowering of intracellular cAMP levels [9]. Our results suggest that, as an additional mechanism, an inactivation process could be involved in the response of α-glucosidase to glucose.

Enzymes not participating in carbohydrate metabolism are also specifically inactivated in yeast [10,11]. However, as far as we know, yeast aminopeptidase I is the first example of a proteolytic enzyme sensitive to glucose catabolite inactivation. As to the physiological significance of this effect, one could speculate that the enzyme, the levels of which are strongly enhanced in starving cells [3], is no longer required when active growth starts again. The elevated levels of the other vacuolar proteinases are also reduced in this circumstance [12,13].

To date, our experiments do not provide clear-cut evidence as to the mechanism of the inactivation of aminopeptidase I. Proteolytic degradation seems not to be the main event in this process. On the other

hand, it is conceivable that modifications of the carbohydrate moiety of the enzyme take place as soon as glucose is available again.

Finally, the loss of enzyme activity might be the result of the dissociation of the dodecameric enzyme into hexamers. This is suggested by earlier observations that aminopeptidase I preparations from brewer's yeast regularly contain inactive hexamers [6]. However, by immunological methods, molecules of this type were not detected in homogenates of strain NCYC 366; the reason for the concomitant loss of activity and antigenic properties of the enzyme have still to be elucidated.

Acknowledgements

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