Inactivation of the galactose transport system in *Saccharomyces cerevisiae*

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The galactose transport system of *Saccharomyces cerevisiae* consists of one component which shows a K_m value of approx. 4 mM in growing cells. A rapid and irreversible inactivation of this transport is detected on impairment of protein synthesis. This inactivation shows the following characteristics: (i) it is due to changes in the K_m and V_{max} of the transport system; (ii) it follows first-order kinetics; (iii) it is an energy-dependent process and is stimulated by the presence of an exogenous carbon source; (iv) fermentable substrates stimulate inactivation more efficiently than non-fermentable substrates.

(Saccharomyces cerevisiae) *Sugar transport* *Galactose* *Transport inactivation*

1. INTRODUCTION

Transport systems in *Saccharomyces cerevisiae* are very often composed of several forms distinguishable by their different affinity constants for the respective substrates [1–7]. With respect to sugars, it is known that maltose [4] as well as glucose transport systems [5] consist of two components that, in the latter case, are controlled differently by catabolite repression [6] and by the presence of hexose kinases [7]. To our knowledge, the existence of several forms in the case of the galactose transport system has not yet been explored.

The two components of the maltose [4] as well as those of the glucose transport systems [5] are irreversibly inactivated upon protein synthesis impairment. This inactivation, which is stimulated by catabolism of fermentable substrates and prevented during ethanol catabolism [4,5], is due to a decrease in the V_{max}, whereas the K_m values remain constant [4,5].

This research attempts to establish the behaviour of the galactose transport system when protein synthesis is impaired as well as to explore the possible occurrence of various components in this system. For this purpose galactose uptake in galactose-grown cells has been measured under different metabolic conditions using a wide range of galactose concentrations. The results indicate that, in contrast to maltose and glucose transport, the galactose transport system shows only one component whose K_m and V_{max} change with time when protein synthesis is inhibited.

2. EXPERIMENTAL

D-[1-^{14}C]Galactose was from Amersham and antimycin A from Sigma (St. Louis, MO). All other reagents were of analytical grade. Strain ATCC 42407 was grown with 2% (w/v) galactose as in [4]. The composition of ammonium-free medium was described in [8]. Cell growth was monitored by optical absorbance measurement at 640 nm or by dry weight determination. Total proteins were determined as described in [9]. To measure the galactose transport system activity, samples of exponentially growing cultures were filtered and, after washing, the cells were suspended in 50 mM K_2HPO_4 (pH 6.0). Aliquots of this

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suspension corresponding to 2 mg (dry wt) were added to the labelled sugar (0.1–1 mCi·mmol^{-1}) to a final volume of 50 μl. After incubation at 20°C for 15 s, 10 ml chilled (4°C) water was added and the cells collected on glass-fiber filters and washed with 10 ml chilled water. Radioactivity incorporated by the cells was measured. Controls for unspecific sugar adsorption were run in parallel using yeast cells previously heated for 15 min at 100°C.

3. RESULTS AND DISCUSSION

3.1. *Kinetic constants for the galactose transport system*

It has been reported that the galactose transport system shows a K_m for galactose ranging from 4 to 11 mM depending on the metabolic conditions [10]. These values were obtained from experiments in which the galactose concentration did not exceed 20 mM. At this substrate concentration, the existence of an additional component with a greater K_m value, as recently reported for the glucose and maltose transport systems (K_m about 50 and 70 mM, respectively [4,5]) would not have been detected. Therefore, to check the possible existence of various components of the galactose transport system with different K_m values we studied the kinetics of galactose uptake using a wide range of sugar concentrations, from 1 to 250 mM. The results obtained were plotted according to the Eadie-Hofstee transformation [11,12]. The monophasic kinetics observed indicate that, in contrast to glucose and maltose transport, the galactose transport system apparently exists only in one form whose affinity constant during exponential growth with galactose is about 4 mM (fig.1). This value is in agreement with that reported in [10].

3.2. *Inactivation of the galactose transport system upon protein synthesis impairment*

Inhibition of protein synthesis, either by addition of cycloheximide (fig.1A) or by deprivation of a nitrogen source (fig.1B,C) from exponentially growing yeast produced a decrease in the V_{max} (intersecting points of the straight lines with the ordinate axis) as well as an increase in the K_m value (slopes of the straight lines) of the galactose uptake. Furthermore, the results indicate that these changes occur progressively upon protein synthesis inhibition. In the experiments shown in fig.1C, K_m values of 3, 5 and 8 mM were obtained for the increasing starvation periods assayed. These differences between the K_m values were statistically significant (Student's t-test: $P < 0.01$).

A detailed study of the V_{max} changes was performed using 60 mM galactose in the assay. K_m values range from 3 to 8 mM in the experimental conditions used (fig.1). Therefore, at 60 mM galactose the activity of the transport system would be quite close to the V_{max} value since at this sugar concentration the carrier would be almost saturated.

Inhibition of protein synthesis in cells growing on galactose produced a decrease of the galactose

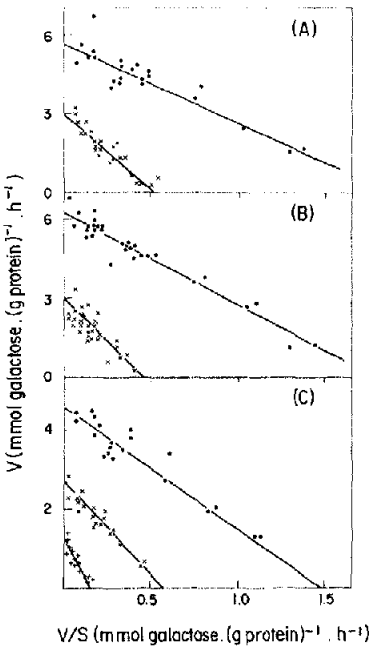

Fig.1. Kinetics of the galactose transport system. Yeast cells were harvested during exponential growth and transferred to the following media: (A) fresh complete medium containing 2% (w/v) galactose and 10 μg cycloheximide per ml. Transport was measured at zero incubation time (•) and after incubation for 2 h at 30°C (×). (B) Ammonium-free medium containing 2% (w/v) galactose. Transport was measured at zero time (•) and after 1 h 30 min incubation at 30°C (×). (C) Ammonium-free medium containing 2% (v/v) ethanol. Transport was measured at zero time (•), and after 2 h 30 min (×) or 5 h incubation at 30°C (+).

uptake V_{max} which followed first-order kinetics, indicating a half-life for the transport system under these conditions of about 1.3 h (fig.2A). A similar half-life was observed when cells were suspended in a medium without a nitrogen source in the presence of glucose (fig.2A). However, when ethanol was present a half-life as long as 3 h was found (fig.2B) and an even longer value (about 5 h) was observed when no carbon source was present (fig.2B). In this latter case, addition of glucose to the medium produced immediate inactivation of the transport system (fig.2B). The activity was recovered after suspension of the cells in a complete medium containing galactose whereas recovery did not occur when cycloheximide was present in addition to galactose (fig.2A).

Matern and Holzer [10], during their studies on the mechanism of inactivation of the galactose-fermenting system ('galactozymase'), observed

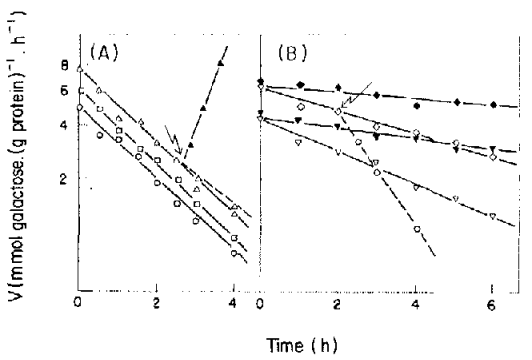

Fig.2. Characteristics of the inactivation. Yeast cells were harvested during exponential growth and transferred to the following media: (A) fresh complete medium containing 2% (w/v) galactose and 10 μg cycloheximide per ml (□). Ammonium-free medium containing 2% (w/v) galactose (△). Where indicated by the arrow, 50 mM ammonium chloride (▲—▲) and 50 mM ammonium chloride plus 10 μg cycloheximide per ml (△---△) were added to aliquots of the cell suspension. Ammonium-free medium containing 2% (w/v) glucose (○). (B) Ammonium-free medium containing 2% (v/v) ethanol in the absence (▽) and presence (▼) of 10 μg antimycin A per ml. Ammonium-free medium without carbon source in the absence (◇) and presence (◆) of 10 μg antimycin A per ml. Where indicated by the arrow glucose was added to 2% (w/v) final concentration (○). After incubation at 30°C, the galactose transport V_{max} was measured at the indicated times using 60 mM D-[U-^{14}C]galactose (0.2 mCi/mmol).

that the K_m of galactose uptake increased after addition of glucose to galactose-grown cells, whereas the V_{max} remained apparently constant. Their failure to observe changes in the V_{max} of uptake such as those shown here under similar experimental conditions (see fig.1) was most likely due to their procedure of measuring galactose uptake. These authors incubated the cells at 30°C for 5–10 min after addition of labelled galactose. However, it has been established that incubation periods as short as 5–15 s should be used to obtain true values of the yeast sugar transport systems activities [7,13].

3.3. *Inactivation depends on energy*

The relatively rapid inactivation of the galactose transport in the presence of ethanol (half-life ~3 h), contrasts with the great stability of the glucose and maltose transport systems during catabolism of this substrate (half-life >30 h) [4,5]. This difference is probably related to the distinctive respiration capacity of cells adapted to different sugars. It has been shown that under the conditions used in the present study, the ethanol respiration rate is 2–3-fold greater in cells adapted to galactose than in those adapted to glucose or maltose [5]. Accordingly, significant differences in the ATP-production rate would occur in these cells. This different rate of ATP production could affect the inactivation rate if, as suggested in the case of glucose transport [5], this process were energy-dependent. The results shown in fig.2B are in agreement with this possibility since inhibition of ethanol respiration by antimycin A decreased the inactivation rate by about 4-fold. A similar decrease was observed upon inhibition of endogenous respiration in starved cells.

The characteristics of the inactivation observed indicate that this process could be due to proteolysis of the galactose carrier. However, the involvement of other mechanisms cannot be excluded.

4. CONCLUSIONS

The present results suggest the following conclusions: (i) The yeast galactose transport system exists in only one form with a K_m value of approx. 4 mM. (ii) This transport system is irreversibly inactivated during inhibition of protein synthesis.

(iii) This inactivation is due to changes in the K_m and V_{max} of the carrier. (iv) Inactivation follows first-order kinetics, is an energy-dependent process, and is stimulated by the presence of an exogenous carbon source. (v) Fermentable substrates stimulate inactivation more efficiently than non-fermentable substrates.

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