Biochimica et Biophysica Acta 820 (1985) 324–326
Elsevier

BBA 70250

BBA Report

Identification of two forms of the maltose transport system in *Saccharomyces cerevisiae* and their regulation by catabolite inactivation

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(Received August 1st, 1985)

Key words: Maltose transport system; Catabolite inactivation; Sugar transport system; (*S. cerevisiae*)

The maltose transport system of *Saccharomyces cerevisiae* exists in two forms with K_m values of approx. 4 mM and 70 mM, respectively. The V_{max} of the high-K_m form is about 4-fold greater than the V_{max} of the low one. A rapid and irreversible inactivation of both forms is detected on protein synthesis impairment. This inactivation is stimulated by the catabolism of fermentable sugars and prevented during ethanol catabolism. It is concluded that both forms of the maltose transport system are regulated by catabolite inactivation.

A rapid and irreversible inactivation of the sugar transport systems in *Saccharomyces cerevisiae* has been detected on protein synthesis impairment [1,2]. This inactivation has been attributed to the rapid turnover of these carriers [1,2]. However, a detailed study on the glucose transport system has shown that, in this case, inactivation is due to a catabolite inactivation process [3]. In this paper the nature of the inactivation of the maltose transport system has been studied. For this purpose, maltose uptake in maltose grown cells has been measured under different experimental conditions. Using a wide range of maltose concentrations two forms of this transport system with different affinity constants have been detected. The results indicate that both forms are regulated by a catabolite inactivation process in a similar manner to the two components of the glucose transport system [3].

The enzymes and nucleotides were from Sigma Chemical Co. (St. Louis, MO). D-[U-^{14}C]Maltose was from Radiochemical Center (Amersham, International) (Amersham, U.K.). All other reagents were of analytical grade. Strain ATCC 42407 was grown aerobically in enriched medium containing 0.3% (w/v) yeast extract with 2% (w/v) maltose as carbon source. Flasks (2.5 litres) containing 250 ml of medium were incubated at 30°C in a gyratory shaker at 250 rpm. Composition of ammonium-free medium was described in Ref. 4. Cell growth was monitored by optical absorbance measurement at 640 nm or by dry weight determination. Total proteins were determined as described in Ref. 5. The activity of the maltose transport was measured as described in Ref. 6. Uptake was initiated by addition of the labeled sugar.

Identification of two forms of the maltose transport system with different affinity constants. It has been reported that the maltose transport system shows a K_m for maltose of about 4 mM [2,6]. This value was calculated from uptake experiments in which maltose was present at concentrations that did not exceed 10 mM [2,6]. At this maltose concentration, the existence of an additional component with greater K_m value, as has been recently described for the glucose transport system (K_m about 50 mM) [3,7], would not have been detected. Therefore, to check for the possible existence of multiple forms of the maltose transport system

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with different K_m values we studied the kinetics of the maltose uptake using a wide range of sugar concentrations (from 0.4 mM to 250 mM), and plotted the results according to the Eadie-Hofstee transformation [8,9]. This plot amplifies any deviation from Michaelis-Menten kinetics and is recommended for the detection of different components in mixtures of isoenzymes [7,10]. The biphasic kinetics observed (Fig. 1A) indicate that the maltose transport system exists in, at least, two forms whose apparent K_m values are 4 mM and 70 mM, respectively. They also indicate that the V_{max} of the high-K_m form is about 4-fold greater than the V_{max} of the low-K_m form (intersecting points of the respective straight lines with the ordinate-axis).

Inactivation of the two transport forms. It is known that the low-K_m form of the maltose transport system is inactivated upon protein synthesis inhibition with cycloheximide [2]. We studied the behaviour of the high-K_m transport form and found it to be also inactivated under these conditions. As shown in Fig. 1, upon addition of cycloheximide to maltose growing cells, the V_{max} of the two transport forms substantially decreased whereas their K_m values remained constant (Fig. 1B). Similar results were obtained (Fig. 1C) when synthesis of the maltose carrier was repressed by glucose addition [11].

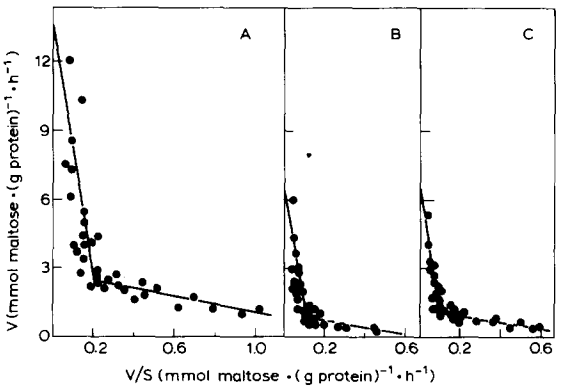

Fig. 1. Kinetic constants of the two forms of the maltose transport system. Yeast cells were grown with 2% (w/v) maltose and harvested during logarithmic growth. (A) Transport was measured using D-[U-^{14}C]maltose at concentrations ranging from 0.4 mM to 0.25 M. (B) Cells were transferred to fresh complete medium containing 2% (w/v) maltose and 10 μg of cycloheximide per ml. After incubation at 30°C for 105 min, transport was measured as above. (C) Cells were transferred to fresh complete medium containing 2% (w/v) maltose and 2% (w/v) glucose. After incubation at 30°C for 90 min, transport was measured as above.

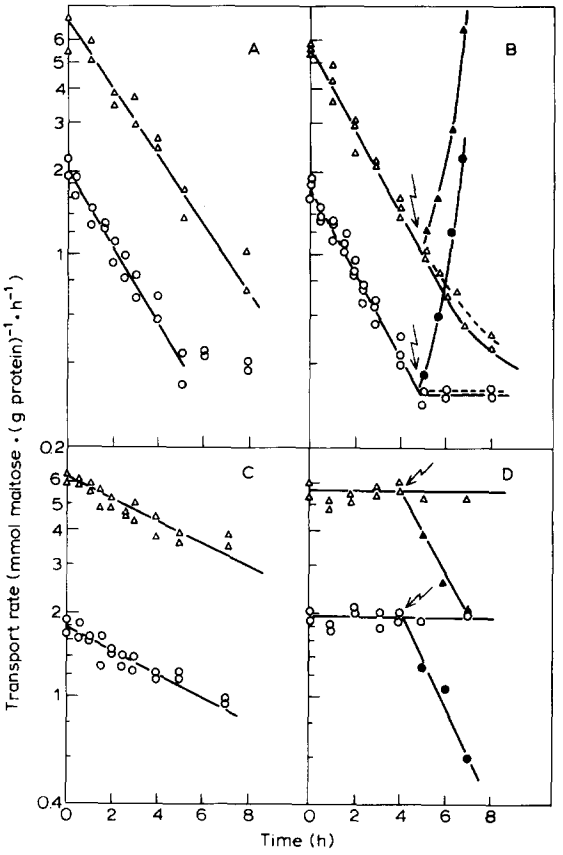

Fig. 2. Characteristics of the inactivation of the two forms of the maltose transport system. Yeast cells were grown with 2% (w/v) maltose and harvested during logarithmic growth. They were transferred to the initial volume of the following media: (A) Fresh complete medium containing 2% (w/v) maltose and 10 μg of cycloheximide per ml. (B) Ammonium-free medium containing 2% (w/v) glucose. When indicated by arrows the cells were harvested by centrifugation and suspended in: ●———● and ▲———▲, fresh complete medium with 2% (w/v) maltose; ○---○ and △---△, fresh complete medium with 2% (w/v) maltose and 10 μg of cycloheximide per ml. (C) Ammonium-free medium without a carbon source. (D) Ammonium-free medium containing 2% (v/v) ethanol. When indicated by arrows, glucose was added to 2% (w/v) final concentration. After incubation at 30°C the maltose transport was measured at the indicated times using: ○ and ●, 4 mM D-[U-^{14}C]maltose (0.5 mCi/mmol); △ and ▲, 80 mM D[U-^{14}C]maltose (0.1 mCi/mmol). Results of two experiments are shown.

Characteristics of the inactivation. The inactivation kinetics of the two transport forms were studied separately using 4 mM and 80 mM maltose as substrate. At 4 mM, about 70% of the total sugar taken up would be transported by the low-K_m form whereas at 80 mM maltose about 70% would be transported by the high-K_m form. These values are calculated by substituting the kinetic constants of both forms (Fig. 1) in the Michaelis-Menten equation. The results obtained indicate that the two transport forms are inactivated at similar rates following first-order kinetics (Fig. 2). In maltose or glucose consuming cells, half-lives of about 1.3 h for the two transport forms were observed (Figs. 2A and 2B). However, in cells deprived of a carbon source half-lives as long as 6 h were calculated (Fig. 2C) and still greater values ($\geq$ 30 h) occurred in ethanol consuming cells (Fig. 2D). In the later case addition of glucose to the medium produced immediate inactivation of both transport forms (Fig. 2D). The activities were recovered after suspension of the cells in a complete medium containing maltose as the carbon source whereas the recovery did not occur when cycloheximide was present in addition to maltose (Fig. 2B).

These results indicate that the two forms of the maltose transport system are irreversibly inactivated, the inactivation rates being dependent on the nature of the carbon source present in the medium. Indeed inactivation is stimulated during the catabolism of fermentable substrates whereas it is prevented during catabolism of a non-fermentable substrate such as ethanol. It can thus be concluded that the two forms of the maltose transport system in *S. cerevisiae* are regulated by a catabolite inactivation process, as has been described for the two components of the glucose transport system [3].

We are grateful to Drs. C. Gancedo and I. Outschoorn for critical reading of this manuscript. This work was supported by Comisión Asesora para la Investigación Científica y Técnica. A.B. had a fellowship of Gobierno Vasco, Departamento de Universidades e Investigación.

References

1 Alonso, A. and Kotyk, A. (1978) Folia Microbiol. 23, 118–125
2 Lagunas, R., Dominguez, C., Busturia. A. and Sâez. M.J. (1982) J. Bacteriol. 152, 19–25
3 Busturia, A. and Lagunas, R. (1985) J. Gen. Microbiol., submitted
4 Lagunas, R. (1976) Biochim. Biophys. Acta 440, 661–674
5 Jayaraman, J., Cotman, C., Mahler, H.M. and Sharp, C.V. (1966) Arch. Biochem. Biophys. 116, 224–252
6 Serrano, R. (1977) Eur. J. Biochem. 80, 97–102
7 Bisson, L.F. and Fraenkel, D.G. (1983) Proc. Natl. Acad. Sci. USA 80, 1730–1734
8 Hofstee, J.J. (1952) Science 116, 329–331
9 Eadie, G.S. (1952) Science 116, 688
10 Gancedo, J.M. (1974) Biochem. Educ. 2, 23
11 Van Wijk, R., Ouwehand, J., Van den Bos, T. and Koningsberger, V.V. (1969) Biochim, Biophys. Acta 186, 178–191