SHORT COMMUNICATION

Gratuitous Catabolite Repression by Glucosamine of Maltose Utilization in *Saccharomyces cerevisiae*

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Glucosamine acted as a gratuitous catabolite repressor of maltose utilization in *Saccharomyces cerevisiae*. This repression was relieved in a linear manner as the maltose concentration was increased. Three mutants were isolated in which maltose utilization was no longer repressed by glucosamine. One of these mutants may be generally deficient in catabolite repression since it was not defective in glucosamine transport but was insensitive to glucosamine repression on all catabolite repressible carbon sources tested. Each mutant possessed a maltose uptake system which was notably less sensitive to glucose repression than that of the wild-type strain. These results are discussed in terms of a proposed model for regulation of maltose utilization in *S. cerevisiae*.

INTRODUCTION

Seven unlinked polymeric genes for the utilization of maltose have been found in various strains of *Saccharomyces*. In all probability all of these genes are regulatory rather than structural, and the presence of any one of them renders the cell capable of the inducible synthesis of maltose permease and maltase (α -glucosidase) (Khan & Eaton, 1971). All genetical evidence is consistent with these genes being regulators in a positive control system. Thus, recessive *mal6* mutations in *S. uvarum* (Ten Berge *et al.*, 1973) and recessive *mal2* mutations in *S. cerevisiae* (Zimmermann & Eaton, 1974) resulted in non-inducible phenotypes, whilst dominant *MAL4* mutations in *S. cerevisiae* (Khan & Eaton, 1971) and dominant *MAL2* mutations (Zimmermann & Eaton, 1974) resulted in constitutive phenotypes.

Maltose utilization in many strains of *Saccharomyces* is also subject to control by catabolite repression, such that even in the presence of maltose the maltose-utilizing system may be inactive in the presence of high concentrations of glucose. The mechanism of catabolite repression in yeast is obscure but appears to involve both inactivation of existing maltose permease (Görts, 1969; Van Rijn & Van Wijk, 1972) and inhibition of transcription of the structural gene for α -glucosidase (Van Wijk *et al.*, 1969). Mutants constitutive for *MAL* expression may be sensitive or insensitive to catabolite repression (Zimmermann & Eaton, 1974).

Recently, Furst & Michels (1977) have found glucosamine to be a gratuitous catabolite repressor in *S. carlsbergensis*. The present study was aimed at using this compound to probe the control of maltose utilization in the commercially important yeast, *S. cerevisiae* NCYC 1026.

METHODS

Yeast and growth. *Saccharomyces cerevisiae* NCYC 1026 was grown on either complete medium [containing (g l^{-1} in distilled water): peptone, 3; yeast extract, 5; casein hydrolysate, 5; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3] or minimal medium [containing Bacto Yeast Nitrogen Base without amino acids (11.7 g l^{-1} in distilled water)]. Principal carbon sources were routinely added after autoclaving to give 10 g l^{-1} . For growth under repressing conditions, glucose (100 g l^{-1}) was used as carbon source. When necessary, media were solidified with 1.5 % (w/v) agar (Lab M). Cultures were grown at 27°C and $200 \text{ rev. min}^{-1}$ in an orbital shaker. Growth was determined spectrophotometrically by measuring A_{660} . Yeast dry weight was calculated by reference to a calibration curve.

Isolation of glucosamine-resistant strains. Spontaneously arising glucosamine-resistant strains were isolated by inoculating 200 ml complete medium containing maltose and supplemented with glucosamine (1.5 g l^{-1}) with 10 ml cultures of strain NCYC 1026 grown for 24 h under repressing conditions. After 14 d at 17°C cultures which were well grown were streaked on to plates of the same medium, where large and small colony types were segregated. The large colony types were purified by successive growth in liquid medium and selection of large colonies from streak plates. After four passages, pure glucosamine-resistant strains were isolated and termed Gam-1, Gam-2 and Gam-3.

Uptake of glucosamine. To ensure that resistant strains were not glucosamine uptake mutants, complete medium containing maltose and supplemented with D-[6- ^3H]glucosamine (1.5 g l^{-1} , specific activity 54 GBq mol^{-1}) was inoculated with saline-washed wild-type NCYC 1026 or mutants Gam-1 or Gam-3, grown for 24 h in complete medium containing maltose, and incubated at 30°C . After 24 h, uptake of D-[6- ^3H]glucosamine was determined as previously described (Scott & Hockney, 1979). For competition experiments between maltose and glucosamine the maltose concentration in complete medium was varied from 10 to 100 g l^{-1} .

Maltose uptake. The concentration of maltose in culture media, and hence maltose uptake, was determined using a Waters Associates high-pressure liquid chromatograph model 201 with Spherisorb 5NH_2 ($250 \times 4.5 \text{ mm}$) as stationary phase and acetonitrile/water (70:30, v/v) as mobile phase. Flow rate was 1 ml min^{-1} at a pressure of 900 lbf in^{-2} (6.2 MPa). Samples were internally standardized with melezitose (10 g l^{-1}) and the equipment was calibrated with maltose solutions of known concentration. Column effluent was monitored with a Waters Associates differential refractometer model R401.

RESULTS

When *S. cerevisiae* NCYC 1026 was grown on various carbon sources in complete medium supplemented with different concentrations of glucosamine, the results obtained (Table 1) were almost identical to those obtained previously with *S. carlsbergensis* (Furst & Michels, 1977), suggesting that glucosamine is also a gratuitous catabolite repressor in *S. cerevisiae*.

The inhibition by glucosamine (1.5 g l^{-1}) of growth of strain NCYC 1026 in complete medium with maltose (10 g l^{-1}) as carbon source was reversed in a linear manner as the maltose concentration was increased. Complete reversal was achieved at a maltose concentration of 100 g l^{-1} . Increasing the maltose concentration did not significantly affect the uptake of D-[6- ^3H]glucosamine.

Further information on the mechanism of repression by glucosamine was obtained by isolation of three mutant strains which were capable of growth on maltose (10 g l^{-1}) in the presence of glucosamine (1.5 g l^{-1}). When tested on other carbon sources (10 g l^{-1}) two of these strains, Gam-2 and Gam-3, were identical and retained sensitivity to glucosamine when grown on acetate, ethanol, glycerol and sucrose. The third strain, Gam-1, was resistant to glucosamine on all carbon sources tested, and may therefore be generally deficient in catabolite repression. All three strains and the wild-type NCYC 1026 were insensitive to glucosamine when grown on galactose. None of the strains could utilize glucosamine as a carbon source for growth.

A possible explanation of the phenotype of Gam-1 was that this strain was unable to take up glucosamine. However, after 24 h incubation in complete medium containing maltose and supplemented with D-[6- ^3H]glucosamine, the amounts of glucosamine taken

Table 1. *Effect of glucosamine on growth of S. cerevisiae* NCYC 1026 on various carbon sources in complete medium

Washed suspensions of the yeast were inoculated into complete medium containing various carbon sources and glucosamine as shown. After 72 h at 27 °C, growth was scored non-rigorously on a scale of 0 (no growth) to 4 (maximum growth).

Carbon source (10 g l ⁻¹)	Growth		
	Glucosamine concn (g l ⁻¹)		
	0	0.15	1.5
None	1	1	0
Glucose	4	4	4
Maltose	4	4	0
Pyruvate (Na)	3	0	0

Table 2. *Glucose repression of maltose uptake in wild-type and mutant strains of S. cerevisiae* NCYC 1026

Washed suspensions of the wild-type and mutants Gam-1 and Gam-3 were inoculated into minimal medium containing maltose (10 g l⁻¹) in the presence or absence of glucose (50 g l⁻¹). After 24 h at 27 °C, growth and maltose uptake were determined.

	Wild-type		Mutant Gam-1		Mutant Gam-3	
	Without glucose	With glucose	Without glucose	With glucose	Without glucose	With glucose
Maltose uptake (mg ml ⁻¹)	1.93	0.43	0.66	1.07	1.69	1.09
Growth (mg dry wt ml ⁻¹)	6.55	7.00	0.95	2.55	5.50	6.10
Maltose uptake [μ g (mg dry wt) ⁻¹]	295	61	695	420	307	179
Inhibition (%)		79		40		42

up by Gam-1, Gam-3 and the wild-type were 9.9, 1.3 and 1.4 nmol (mg dry wt)⁻¹, respectively.

That the mutant strains were genuinely altered in catabolite repression was established by comparing their maltose uptake over a 24 h period in minimal medium containing maltose (10 g l⁻¹) and glucose (50 g l⁻¹) with that in medium containing maltose alone (Table 2). It was noticeable that mutant Gam-1 had a much reduced growth rate and elevated maltose uptake compared with the wild-type. Mutant Gam-2, which was not included in this experiment, had a growth rate on maltose identical to that of the wild-type.

DISCUSSION

The *MAL* gene product is a positive regulatory protein which, in combination with maltose or with a maltose-dependent effector, activates the structural genes for α -glucosidase and maltose permease (Zimmermann & Eaton, 1974). This activation is prevented under conditions of catabolite repression. It is proposed that the presence of glucose or glucosamine in the medium elicits synthesis of a catabolite repression effector which in some way blocks induction by maltose. The simplest model consistent with the observation that glucosamine repression was overcome in a linear manner as the concentration of maltose was increased is that the effector of catabolite repression and maltose compete for binding to the *MAL* gene product. The phenotypes of the three glucosamine-resistant mutants would be explained by this hypothesis if glucosamine was unable to elicit synthesis of the catabolite repression effector in Gam-1, and if the *MAL* gene product synthesized in Gam-2 and Gam-3 was unable to bind such an effector. No reasonable explanation can be offered to explain the

elevated glucosamine and maltose uptakes in Gam-1 compared with Gam-3 and the wild-type strain, except in so far as the lack of any detectable catabolite repression in this strain might lead to dramatic metabolic perturbations within the cell. α -Glucosidase and maltose permease are separately regulable in yeast since the former is responsible for the inducible hydrolysis of maltose and of maltotriose which are transported by two independent permeases (Stewart *et al.*, 1979). Furthermore, the activity of maltose permease, but not α -glucosidase, is also regulated by catabolite inactivation (Görts, 1969; Van Rijn & Van Wijk, 1972). Continued operation of this control system may explain the incomplete relief from glucose inhibition in the mutants isolated.

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