0273–2289/84/1012–0475$02.00

α-Glucosidase Synthesis in Batch and Continuous Culture of *Saccharomyces cerevisiae*

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Received August 23, 1983; Accepted March 19, 1984

ABSTRACT

Glucose prevents maltose utilization by *Saccharomyces cerevisiae* in batch culture, whereas in a mixed carbohydrate-limited chemostat, maltose and glucose were consumed simultaneously. The specific activity of α-glucosidase was dependent on the dilution rate as well as the proportion of maltose in the mixture. Maximum specific activities in the batch and chemostat cultures on mixtures of maltose and glucose were lower than corresponding values observed on maltose alone.

Index Entries: *Saccharomyces cerevisiae*, α-glucosidase in; α-glucosidase, effect of glucose limitation on; α-glucosidase activity, in batch and continuous cultures; carbohydrate substrate, for α-glucosidase production; yeast, induction and repression of α-glucosidase in.

INTRODUCTION

The intracellular enzyme α-glucosidase (α-D-glucohydrolase E.C.3.2.1.20) in *Saccharomyces cerevisiae* is induced by maltose or some metabolic derivative of maltose. It has been reported that the induction of the enzyme is prevented by glucose-mediated catabolite repression (*1*). α-Glucosidase from *S. cerevisiae* is not inactivated when cells are ex-

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posed to glucose (20 g L^{-1}) or the ethanol formed (*2*). In addition maltose permease that is involved in the transport of maltose is inactivated and repressed by glucose (*3*). Ethanol (20 g L^{-1}) does not affect the activity of the permease (*2,4*).

The present investigation was carried out to evaluate the possibility of using the more readily available and less expensive glucose with only small additions of maltose as the inducer for the production of α-glucosidase instead of large amounts of maltose. The chemostat provides a way of reaching the low residual concentrations of glucose in the broth that are necessary to release catabolite repression and permit maltose induction of α-glucosidase. In this paper, we report results from studies of α-glucosidase production in batch and chemostat cultures of *S. cerevisiae* on both single and mixed carbohydrate substrates.

MATERIALS AND METHODS

Organism

Saccharomyces cerevisiae Y1 was obtained from Prof. A. L. Demain (MIT, Cambridge, MA, USA).

Media

The media used in these studies had the following composition: YEP: 2% peptone (Difco) plus 1% yeast extract (Difco); YEPG: YEP medium with 1% glucose; YEPM: YEP medium with 1% maltose; YNB: 6.5 g L^{-1} of yeast nitrogen base (Difco); YNBB: YNB medium with Na_2HPO_4, 2.7 g L^{-1} and $NaH_2PO_4 \cdot H_2O$, 4.3 g L^{-1}. YEPG and YEPM were used for inoculum preparation and for storing the organism. YNBB was used in the batch and continuous fermentations.

Cultivation Conditions

The batch cultures were carried out in baffled shake flasks using the YNBB medium supplemented with either glucose or maltose, as indicated in the text. Continuous culture studies were conducted in a 1-L chemostat. The pH and the temperature were controlled at 5.5 and 30°C, respectively. Dissolved oxygen was measured using a galvanic oxygen electrode and was kept above 50% of air saturation. For liquid level control, a thermistor-type level controller was employed to activate the exit pump.

Analytical Procedures

Growth was followed by measuring culture turbidity with a Klett-Summerson colorimeter using a red filter (660 nm). Dry cell weight was determined by centrifuging a defined volume, washing and drying the

pellet at 105°C for 12 h; 0.67 g L^{-1} cell dry weight corresponds to 100 Klett units. Glucose concentration was measured with a hexokinase/glucose-6-phosphate dehydrogenase kit (Calbiochem, La Jolla, CA, USA). Maltose was determined on the basis of glucose released after hydrolysis with α-glucosidase from yeast (Sigma Chemical Co., Saint Louis, MO). Ethanol was measured on a Hewlett-Packard 5380A gas chromatograph equipped with a Chromosorb 101 column (5.5 ft stainless with ⅛ in. ID) and a flame ionization detector. The column temperature was 110°C, helium was the carrier gas at 30 mL/min, and methanol was used as internal standard. α-Glucosidase activity was determined using cells that were decryptified with dimethylsulfoxide (DMSO) according to the method described by Adams (*5*). A 2 mg quantity of cells were added to 4 mL of cold water, centrifuged for 15 min at 10,000*g*. The pellet was resuspended in 0.5 mL 45% DMSO, incubated at 30°C for 25 min, and then centrifuged for 15 min at 10,000*g*. The cells were washed in 3 mL 0.1*M* Na-phosphate buffer, pH 6.8, then centrifuged for 10 min at 10,000*g*, and the pellet was resuspended in 1 mL of the same buffer. The assay mixture contained 150 μmol of maltose, 0.1*M* Na-phosphate buffer, pH 6.8, and 0.1 mL of the permeabilized cell suspension in a total volume of 1 mL. The reaction was carried out at 30°C for 20 min and was stopped by the addition of 1 mL 1*M* Tris, pH 7.0, a strong inhibitor of α-glucosidase activity (*6*). Glucose release was measured by means of the coupled hexokinase–glucose-6-phosphate system. Under the assay conditions used, enzyme activity was proportional to the enzyme concentration. The specific activity of α-glucosidase was expressed in units per gram of cell dry weight (U/g-cell), where 1 unit (U) is defined as 1 μmol maltose split/min.

RESULTS

Batch Culture

When the organism is growing on glucose, the specific α-glucosidase activity is very low (10 U/g cell). A gradual increase in α-glucosidase activity is observed during growth on maltose alone and the maximum value is 850 U/g cell (Fig. 1). When the organism is grown on a mixture of maltose and glucose, there is sequential utilization of glucose and maltose as shown in Fig. 2. α-Glucosidase is synthesized while the glucose concentration is fairly high, and maltose is utilized slowly until glucose is depleted. The specific enzyme activity reaches a maximum value of 490 U/g cell. Thus, increasing the ratio of maltose to glucose results in increasing the maximum α-glucosidase activity. In these experiments the maximum specific growth rate on glucose as well as maltose is 0.5 h^{-1}. Ethanol is formed during growth on both substrates and is then metabolized when all carbohydrate is consumed. When maltose is depleted, α-glucosidase synthesis appears to stop. The observed decrease in spe

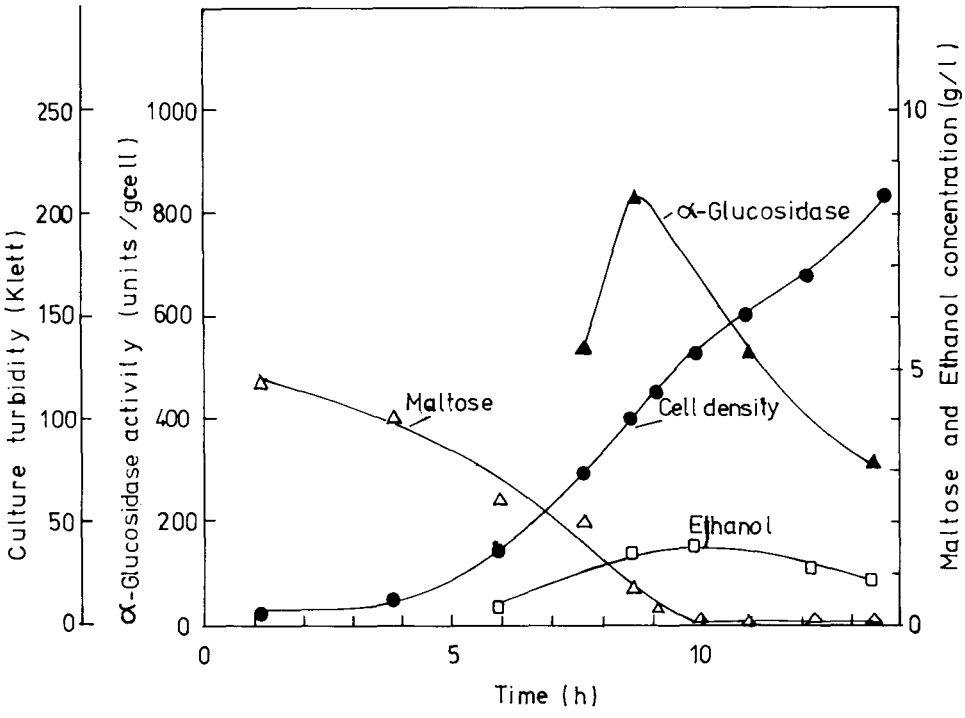

Fig. 1. Growth and specific α-glucosidase activity of *S. cerevisiae* in batch culture on maltose (5 g/L).

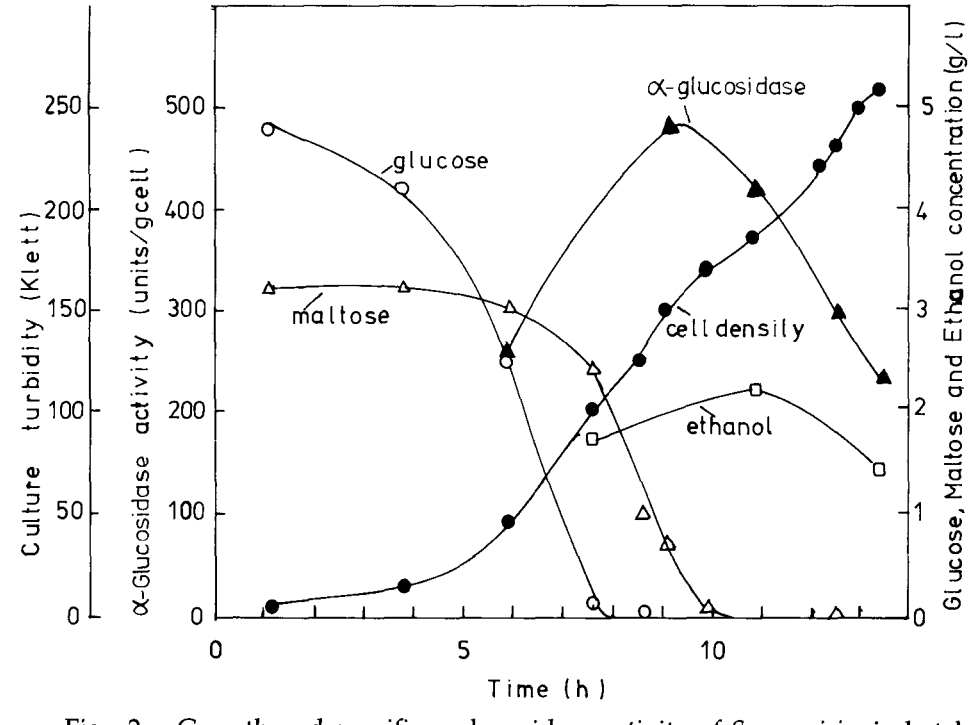

Fig. 2. Growth and specific α-glucosidase activity of *S. cerevisiae* in batch culture on a mixture of glucose (5 g/L) and maltose (3 g/L).

cific α-glucosidase activity would thus correspond to a combination of dilution that results from an increase in cell concentration as well as some degradation or inactivation (Fig. 1; Fig. 2).

Continuous Culture

In a steady-state glucose-limited chemostat there is low α-glucosidase activity that decreases from 80 to 50 U/g cell over the dilution rate (D) interval 0.09–0.30 h^{-1}. α-Glucosidase is induced in a maltose-limited chemostat and the specific activity increases with dilution rate up to a maximum value of 300 U/g cell at $D = 0.25\ h^{-1}$, as shown in Fig. 3. At higher dilution rates, α-glucosidase activity decreases and at the same time ethanol production occurs. When *S. cerevisiae* is grown on a mixture of glucose and maltose in a carbon-limited chemostat, both substrates are metabolized simultaneously (Fig. 4). Also the specific α-glucosidase activity decreases and ethanol is formed at the higher dilution rates. The specific activity, however, is only 60% of the value reached in a maltose-limited culture. At a constant dilution rate in a carbon-limited chemostat, the specific α-glucosidase activity increases with increasing ratio of malt-

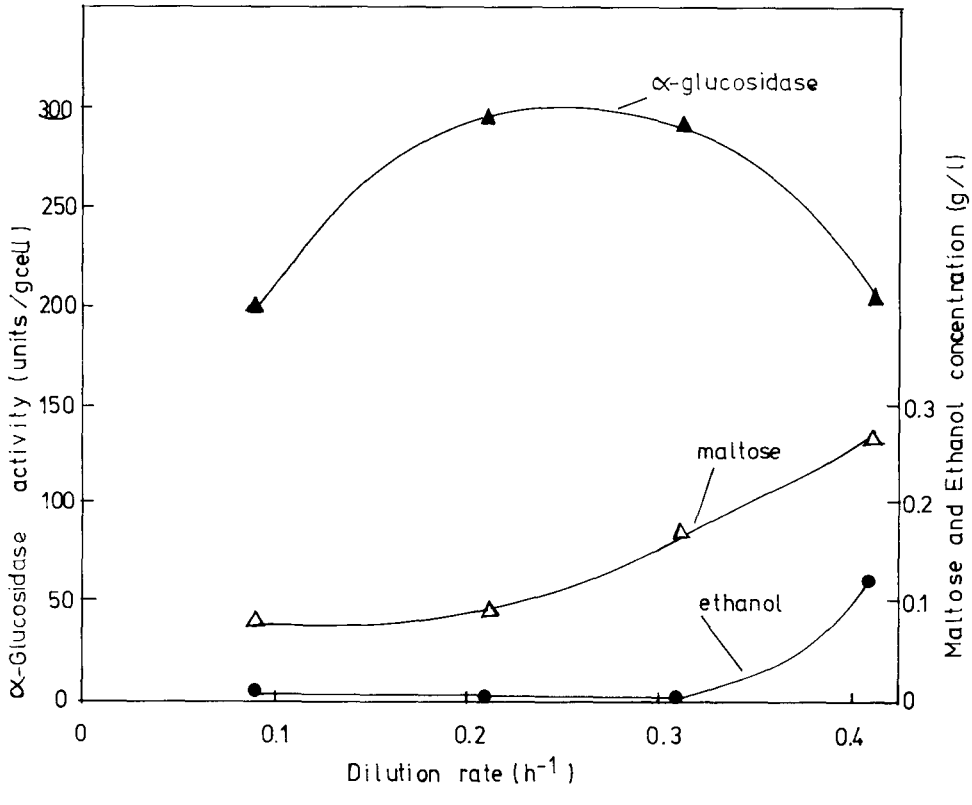

Fig. 3. α-Glucosidase specific activity, ethanol concentration, and residual maltose concentration in a maltose-limited chemostat of *S. cerevisiae* at steady state. Maltose concentration in the feed is 2.5 g/L.

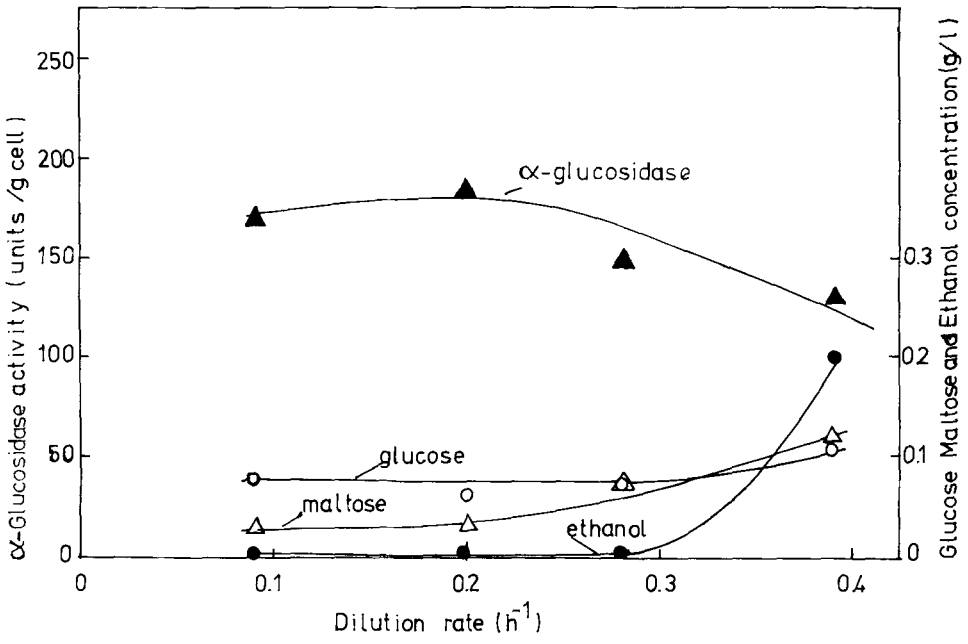

Fig. 4. α-Glucosidase activity, ethanol, residual maltose, and glucose concentrations in a maltose plus glucose-limited chemostat of *S. cerevisiae* at steady state. Concentrations of glucose and maltose in the feed are 2.0 and 1.0 g/L, respectively.

ose to total amount of carbohydrate in the glucose–maltose mixture (Table 1).

DISCUSSION

The maximum specific α-glucosidase activity in a maltose-limited chemostat culture is only 35% of the activity in batch culture on maltose alone (Fig. 1; Fig. 3). These results suggest that the maltose concentration may be important for the induction of α-glucosidase in *S. cerevisiae* since steady-state levels of maltose are much lower than those that occur in batch culture.

Our results show that glucose to a certain degree represses the α-glucosidase synthesis, but also that α-glucosidase induction is initiated in the presence of high concentrations of glucose in batch culture (Fig. 2). The maltose utilization, however, does not start until later. The derepression of maltose permease has been reported to occur in the presence of 3.6 g/L glucose (*3*), thus allowing the inducer maltose to enter the cell. These results are consistent with the observations here. In the presence of glucose (20 g/L), however, the maltose permease was also reported to dramatically increase its k_m value for maltose to 17 g/L, which may explain why rapid maltose utilization is delayed until glucose is depleted (*2,4*).

TABLE 1
Specific α-Glucosidase Activity in *S. cerevisiae* Grown in a Carbon-Limited Chemostat at a Dilution Rate of 0.17 h^{-1} with Different Weight Ratios of Maltose to Total Amount of Carbohydrate (Maltose and Glucose)

Maltose / Glucose + Maltose (%)	α-Glucosidase activity, U/g cell
100	280
83	200
33	180
17	110

In maltose as well as maltose plus glucose-limited chemostats, α-glucosidase activity increases with dilution rate until a maximum level is reached; then at higher dilution rates the enzyme activity decreases. At the same time there is a partial shift from oxidative to fermentative metabolism, as indicated by ethanol formation. These results support the hypothesis that a similar regulatory mechanism may be involved in the two events. Similar results have recently also been reported for *S. italicus* (*7*). Enzymes in the citric acid cycle and the respiratory chain have been reported to be repressed and ethanol is formed when cells are grown in batch culture on glucose (*8,9*). Induction would be expected to cause an increase in α-glucosidase activity with increasing dilution rate, whereas catabolite repression mediated by glucose would increase with dilution rate thus counteracting the effect of induction. Maltose is split intracellularily to yield 2 mol of glucose and can thus exert catabolite repression. These two counteracting effects may also be expected to operate in batch culture.

In addition an increased ratio of maltose to total amount of glucose plus maltose increased the α-glucosidase activity (Table 1; Figs. 1 and 2.). The rate of catabolism (specific maltose utilization rate) as well as the maltose concentration present thus appear to regulate the synthesis of the catabolic enzyme α-glucosidase in *S. cerevisiae*.

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