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# α-GLUCOSIDASE SYNTHESIS IN YEAST CELLS DEPLETED OF INTRAMITOCHONDRIAL ATP

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## Summary

We have studied the dependence on mitochondrial ATP of the expression of MAL genes specifying maltose utilization in yeast. It was found that bongkrekic acid does not prevent the maltose induced synthesis of α-glucosidase in derepressed cells of the wild-type and corresponding respiratory-deficient mutant of *Saccharomyces cerevisiae*. The results suggest that expression of nuclear genes specifying α-glucosidase and maltose catabolism in yeast is apparently not dependent on the proper function of mitochondrial adenine nucleotide translocase and does not even require the presence of normal levels of ATP in mitochondria.

## Introduction

The growth of the yeast *Saccharomyces cerevisiae* on maltose is dependent on the activities of α-glucoside permeases and intracellular α-glucosidase [1,2]. The synthesis of these enzymes can be induced by maltose [3—6] and involves DNA transcription and de novo synthesis of the protein, at least in the case of α-glucosidase [7]. In the presence of higher glucose concentrations, synthesis of maltose permease and α-glucosidase is repressed [3,6,8] and the former enzyme is rapidly inactivated [3,9].

The presence of any one of seven, unlinked MAL genes is necessary for maltose fermentation in yeast [10—12]. Recently, evidence has been accumulated indicating that expression of these genes bears some relation to the functional state of mitochondria [13—16]. Although mitochondrial genes apparently play no direct role in determining the ability to catabolize maltose, some function elaborated or controlled by mitochondria affects the expression of nuclear genes or their products responsible for sugar catabolism [16]. Whether this

function is related to mitochondrial ATP, generated either by oxidative phosphorylation or transported into mitochondria from cytosol, has not yet been established. Nevertheless, intra-mitochondrial ATP was found to be required not only for normal replication of mitochondrial genes [17] but also for continual growth of yeast cells, even on glucose as substrate [18,19].

In this paper, we have studied the role of the mitochondrial energy state in the regulation of maltose-induced α-glucosidase synthesis in *S. cerevisiae*.

## Materials and Methods

Cells of the prototrophic diploid laboratory wild-type strain, *Saccharomyces cerevisiae* DT XII and corresponding neutral, cytoplasmic peptite strain DT XII A were grown in semi-synthetic medium containing in 1 l, 5—20 g glucose/5 g peptone/5 g yeast extract and mixture of inorganic salts as reported previously [17]. For induction cells were incubated aerobically in medium containing 1% maltose/0.2% glucose/1% casamino acids (Difco)/50 mM phosphate buffer (pH 6.2) and yeast cells at final concentration $2 \cdot 10^8$ cells/ml. α-Glucosidase activity in cell-free extracts was determined using *p*-nitrophenyl-α-D-glucopyranoside as substrate [20]. Protein was determined by the micro-biuret method [21] using bovine serum albumin as standard.

## Results and Discussion

Cells of wild-type *S. cerevisiae* grown on glucose as substrate contained low levels of intracellular α-glucosidase, which were, however, substantially increased during subsequent incubation of the cells with maltose. When induction of the enzyme was done in the presence of bongkrekic acid, effectively

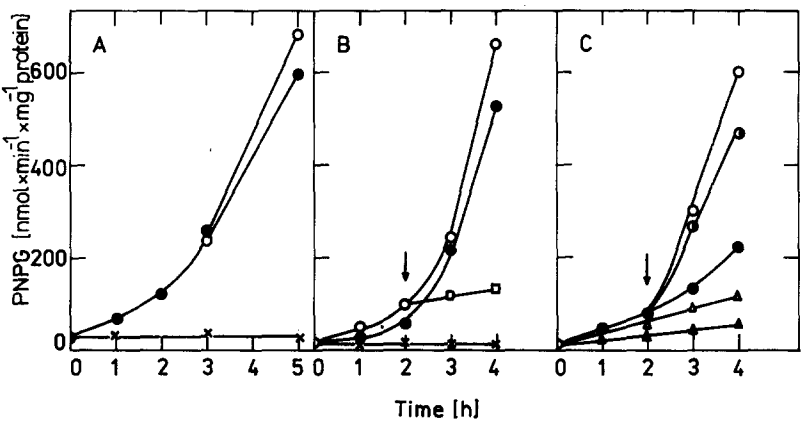


Fig. 1. Effect of bongkrekic acid on induced α-glucosidase synthesis in wild-type and respiratory-deficient mutant of *S. cerevisiae*. Cells incubated with maltose for the time indicated on the abscissa were washed and the activity of α-glucosidase was determined as described in Materials and Methods. A, wild-type grown for 24 h in 2% glucose; B, mutant grown for 24 h in 0.5% glucose; C, mutant grown for 18 h in 2% glucose (○) or 5% glucose (△). ○———○, control; ●———●, bongkrekic acid (10 μg/ml) ◑———◑, bongkrekic acid added at arrow; □———□, glucose (20 mg/ml) added at arrow; X———X, cycloheximide (200 μg/ml); △———△, control for cells pregrown in 5% glucose; ▲———▲, bongkrekic acid (10 μg/ml). PNPG, *p*-nitrophenyl-α-D-glucopyranoside.

inhibiting the translocase of adenine nucleotides in mitochondria [18], the rate of maltose-induced α-glucosidase synthesis (sensitive to cycloheximide) was very similar to that found in the control experiment (Fig. 1A). Only slight inhibition of induced α-glucosidase synthesis, determined after 4 h of induction, was observed when the synthesis of ATP in mitochondria of derepressed cells grown for 24 h in medium with 0.5% glucose was abolished by inhibition of respiration with 2 mM cyanide (16% inhibition), or also when in the presence of cyanide the influx of glycolytically-formed ATP into mitochondria was prevented by bongkrekic acid (27% inhibition). Thus, in *S. cerevisiae*, neither the connection of ATP pools between mitochondria and cytosol nor the normal level of intra-mitochondrial ATP are required for maltose-induced α-glucosidase synthesis.

This conclusion was supported also by the results obtained by studying α-glucosidase synthesis in the cytoplasmic, neutral respiratory-deficient mutant of *S. cerevisiae* lacking the mitochondrial genome and unable to synthesize proteins inside the mitochondria [22]. This mutant was able to synthesize α-glucosidase in response to maltose as in the wild-type strain. In derepressed cells, grown in medium with 0.5—2% glucose to stationary phase, the presence of bongkrekic acid in the induction medium did not prevent the synthesis of α-glucosidase. The synthesis of the latter was, however, very sensitive to glucose repression (glucose added at 2 h) and was prevented by inhibition of translation with cycloheximide (Fig. 1B). In contrast, cells harvested during the exponential phase of growth in medium containing higher concentrations of glucose (2—5%), showed a significantly lower rate of α-glucosidase synthesis in the presence of bongkrekic acid (Fig. 1C). During the first 2 h of induction the cells were more sensitive to antibiotic action, as the inhibition with bongkrekic acid added at 2 h was not as strong. This differential effect of bongkrekic acid could be ascribed to increased catabolite repression [18], which was more evident in cells grown in medium with 5% glucose. Under these conditions of growth, the initial rate of α-glucosidase synthesis in the wild-type strain was also lower and slightly sensitive to the action of bongkrekic acid. Inhibition of maltose-induced α-glucosidase synthesis by glucose as well as the differential rate of its synthesis in yeast cells grown under repressing and non-repressing conditions were reported earlier [23,24].

TABLE I

GROWTH OF RESPIRATORY-DEFICIENT MUTANT ON MALTOSE IN THE PRESENCE AND ABSENCE OF BONGKREKIC ACID

Cells pre-grown for 24 h in semisynthetic medium containing indicated carbon source were inoculated into medium containing maltose to starting concentration of $2 \cdot 10^6$ cells/ml. After 24 h of growth at 30°C the concentration of cells in culture was determined in haemocytometer and checked again in 48 h.

| Cells pregrown in | Growth yield in 2% maltose ($10^6$ cells/ml) | |
|---|---|---|
| | Without antibiotic | Bongkrekic acid (5 μg/ml) |
| 0.5% glucose | 220 | 32 |
| 2% glucose | 216 | 36 |
| 2% maltose | 206 | 43 |

When cells of the respiration-deficient mutant pre-grown in 0.5—2% glucose-containing medium were transferred to the same medium containing 2% maltose instead of glucose as carbon source, a regular growth on disacharide was observed (Table I). The addition of bongkrekic acid to maltose-containing medium did not prevent sugar utilization, although the multiplication of cells in this case was limited only to 3—4 generations, as found recently for glucose [18,19]. This indicates that even under growing conditions when the stress for glycolytically formed ATP is higher, yeast deficient in intramitochondrial ATP are still competent to synthetize α-glucosidase and thus, allowing the catabolism of maltose in cells.

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