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GLUCOSE REPRESSION OF THE INDUCIBLE CATABOLIC PATHWAY FOR N-ACETYLGLUCOSAMINE IN YEAST

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SUMMARY : In order to investigate the mechanism of glucose repression of the N-acetylglucosamine metabolic enzymes in Candida albicans, an obligatory aerobic yeast, the activities of the following inducible enzymes were assayed : the N-acetylglucosamine uptake, N-acetylglucosamine kinase and glucosamine-6-phosphate deaminase. In the presence of glucose or other sugars e.g. succinate and glycerol, synthesis of these enzymes took place at a normal rate, suggesting that the hexose produces no catabolite repression in this organism. On the contrary, strong inhibition by glucose was observed on the activities of N-acetylglucosamine uptake and deaminase in N-acetylglucosamine-grown cells of Saccharomyces cerevisiae, a facultative aerobe. From the results, it is concluded that "glucose effect" or catabolite repression is absent in Candida albicans, a pathogenic strain of yeast.

It has been shown for a number of microbial systems including yeast that the synthesis of enzymes concerned with the uptake and catabolism of energy yielding substrates is repressed by glucose and other carbon sources (1,2). The phenomenon called catabolite repression or "glucose effect" is characterized by a marked decrease in the differential rate of synthesis of inducible protein(s) of the cell when glucose is added to the culture. This effect is well established in Saccharomyces cerevisiae (3) and is expressed only during steady-state growth. Recently, we have reported the induction of N-acetylglucosamine kinase, the first enzyme in the metabolism of N-acetylglucosamine in Candida albicans(4). This inducible enzyme is absent from cells grown on glucose but

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Abbreviation : Adenosine-3',5'-monophosphate, cyclic AMP or cAMP.

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appears when N-acetylglucosamine is used as a carbon source in the growth medium. Other enzymes in this pathway e.g. N-acetylglucosamine uptake and glucosamine-6-phosphate deaminase are also inducible in both Candida albicans and Saccharomyces cerevisiae (manuscript in preparation). Since the repression of catabolic enzymes has been observed in most of the inducible enzyme systems in bacteria and yeast, one would expect glucose or other carbon sources to repress the synthesis of these enzymes. The present communication is concerned only with a comparative aspect of catabolite repression control of the same inducible enzymes in two different strains of yeast, namely pathogenic Candida albicans, and non-pathogenic Saccharomyces cerevisiae. The results presented in this paper suggest that unlike other inducible enzyme systems present in bacteria and yeast, glucose, glycerol and succinic acid could not repress the induction of enzymes involved in N-acetylglucosamine metabolism in Candida albicans. However, these enzymes are sensitive to catabolite repression in Saccharomyces cerevisiae.

MATERIALS AND METHODS : Yeast extract, agar and peptone were obtained from Difco. The following chemicals were purchased from Sigma Chemical Co., St. Louis Missouri, U.S.A. : N-acetylglucosamine, glucosamine-6-phosphate, ATP, cyclic AMP, $NADP^+$, glucose-6-phosphate dehydrogenase and phosphoglucoisomerase. [^{3}H] cAMP (16.3 Ci/mM) and N-[^{3}H] acetyl-D-glucosamine (686 mCi/mM) were obtained from Amersham/Searle Corporation.

Organism and growth conditions. The wild type yeast strains nonpathogenic Saccharomyces cerevisiae 3059 and pathogenic Candida albicans 3100 (obtained from the National Chemical Laboratory, Pune, India) were used throughout this study. The conditions for growth, enzyme induction and preparation of crude extract have been described in detail previously (4).

Enzyme assays. N-acetylglucosamine kinase (4) and glucosamine-6-phosphate deaminase (5) were assayed according to published procedures. For measurement of high affinity uptake of N-acetylglucosamine, yeast cells (1 to 2 x 10^6 cells/ml) were suspended in water and preincubated for 5 min at 30°C. [^{3}H] N-acetylglucosamine (1 µCi/ml) was then added to give a final concentration of 0.05 mM. Then 5 min after addition of N-acetylglucosamine, the cells from 0.5 ml samples of the incubation mixtures were collected on glass-fibre filters (GF/C, Whatman) and washed twice with 5 ml of ice cold water. The filters were dried, and their radioactivity was

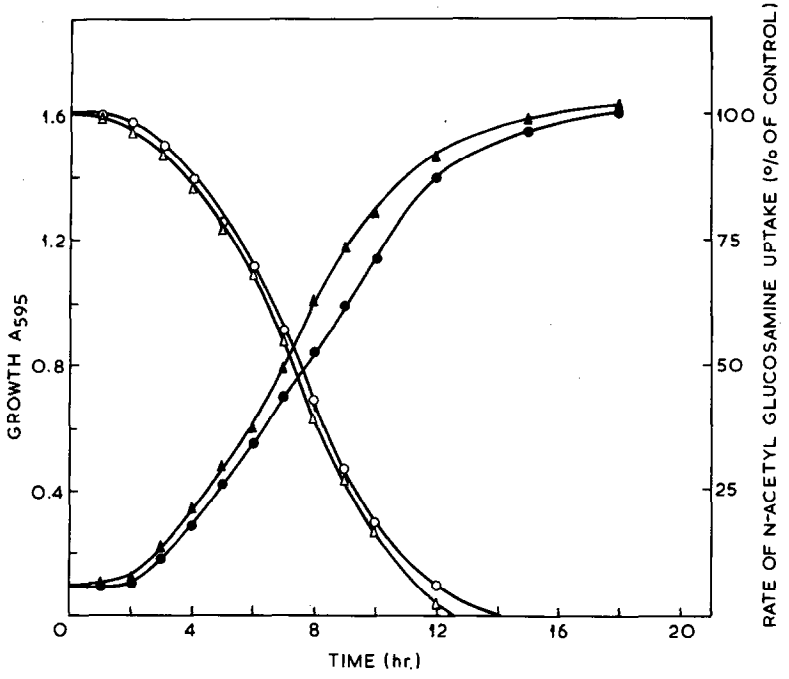


Figure 1: Utilisation of N-acetylglucosamine during the growth of C. albicans or Sacch. cerevisiae. Cells were grown in a medium containing 24 mM N-acetylglucosamine. Growth, A_{595}: ●—●, C. albicans; ▲—▲, Sacch. cerevisiae. Uptake: o—o, C. albicans; Δ—Δ, Sacch. cerevisiae.

determined in 10 ml of a toluene-based scintillation mixture in a Packard Tri-carb liquid scintillation spectrometer.

The cAMP binding assay was performed according to the procedure of Gilman (6). Protein was determined by the method of Lowry et al. (7) and N-acetylglucosamine in the medium was determined according to the method of Reissig et al. (8).

RESULTS

As shown in Fig. 1, both Candida albicans and Saccharomyces cerevisiae grew equally well on a medium containing N-acetylglucosamine as a carbon source. The utilization of N-acetylglucosamine as a carbon source is as effective as that of glucose. Like glucose, N-acetylglucosamine is taken up readily as the cells start to multiply after a 2-3 hr lag. In case of Candida albicans, addition of N-acetylglucosamine to the medium elicits an almost immediate synthesis of N-acetylglucosamine catabolic enzymes i.e.

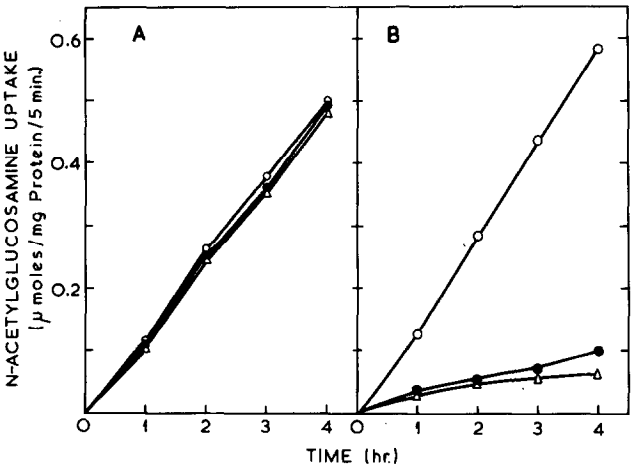
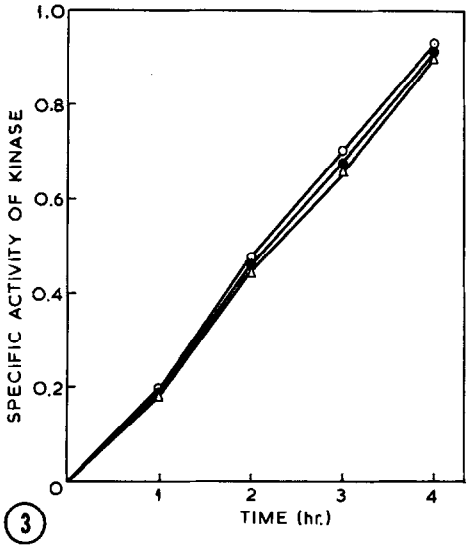
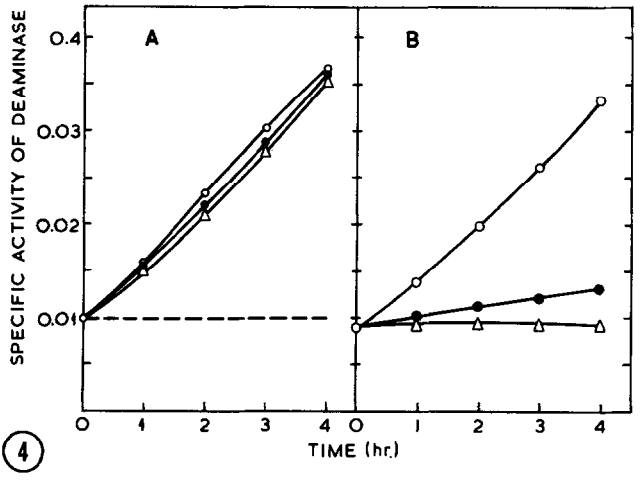


Figure 2: Effect of glucose on the induction of high affinity uptake system in yeast. Cells were harvested from the mid-log phase washed, and resuspended in induction medium containing 0.5% N-acetylglucosamine and 0.3% KH_2PO_4. At indicated times, cells were collected and uptake of N-acetylglucosamine was determined (o—o, control; see methods). In separate cultures, glucose either at a concentration of 0.5% (●—●) or 1% (Δ—Δ) was added at zero hour during induction.
Figure 2A: Candida albicans;
Figure 2B: Saccharomyces cerevisiae

a high affinity uptake system, N-acetylglucosamine kinase and glucosamine-6-phosphate deaminase without any detectable lag period (Figs. 2 to 4). These enzymes are absent from the cells grown on glucose and their synthesis requires the continuous presence of N-acetylglucosamine. However, in Saccharomyces cerevisiae only high affinity uptake system and glucosamine-6-phosphate deaminase, but not N-acetylglucosamine kinase are induced. Experiments with RNA- and protein synthesis inhibitors indicate that the appearance of these new enzyme activities are dependent on concomitant new protein synthesis and the inducer operates at a transcriptional level (manuscript in preparation).



(3)



(4)

Figure 3: Effect of glucose on the induction of N-acetylglucosamine kinase in Candida albicans. At indicated times after induction cells were collected and specific activity of N-acetylglucosamine kinase was determined in the cell free extract (o—o , control). Glucose at a concentration of either 0.5% (●—●) or 1% (Δ—Δ) was added alongwith the inducer, N-acetylglucosamine (0.5%).

Figure 4: Effect of glucosamine-6-phosphate deaminase in yeast. At indicated times after induction cells were collected and specific activity of glucosamine-6-phosphate deaminase was determined in the cell free extract (o—o , control). Glucose at a concentration of either 0.5% (●—●) or 1% (Δ—Δ) was added alongwith the inducer, N-acetylglucosamine (0.5%).
Figure 4A: Candida albicans;
Figure 4B: Saccharomyces cerevisiae.

Since glucose and other readily metabolizable substrates have been found to repress the synthesis of various inducible enzyme systems in bacteria and yeast, we have studied the effect of glucose on the induction of N-acetylglucosamine catabolic pathway. In all the experiments, we have used 0.5% (w/v) N-acetylglucosamine as inducer for induction of the enzymes. In Sacch. cerevisiae, glucose at a concentration of 0.5% represses the synthesis of N-acetylglucosamine uptake system by about 90%, whereas in C.

albicans, glucose had no significant effect even at as high concentration as 1% (Fig. 2).

Induction of N-acetylglucosamine kinase in Candida albicans offers a unique system as this enzyme is not inducible in other yeasts, tested so far in our laboratory. Results presented in Fig. 3 show that as in case of high affinity uptake system, glucose could not repress the synthesis of N-acetylglucosamine kinase in this organism.

Glucosamine-6-phosphate deaminase is a semiconstitutive enzyme in both the yeasts studied. A basal level of the activity is detected from the cells grown on glucose, however, upon addition of N-acetylglucosamine to the medium, the new deaminase activity appeared and increased steadily as long as N-acetylglucosamine was present in the medium. As shown in Fig. 4, glucose repressed the synthesis of this enzyme in Saccharomyces cerevisiae but not in Candida albicans. Other sugars e.g. glycerol and succinate at a concentration of 1% (w/v) also failed to repress the inducible synthesis of N-acetylglucosamine uptake, kinase and deaminase in Candida albicans (data not shown).

DISCUSSION

Evidence has accumulated which suggests that in yeast, glucose-derepression may be mediated through the action of cyclic adenosine 3',5'-monophosphate (cAMP) (9-13). However, in case of Candida albicans, we have reported recently (14) that cAMP produces a transient initial enhancement of protein and RNA synthesis followed by inhibition, suggesting a different mechanism of cAMP action in this organism. In this paper, we show that catabolite repression on the synthesis of the enzymes of N-acetylglucosamine catabolic pathway is absent in Candida albicans, but present in Saccharomyces cerevisiae. Furthermore, in Candida albicans, the concentration

of intracellular cAMP remained unchanged upon addition of glucose in the medium. On the basis of these results, it would, therefore, seem reasonable to conclude that regulatory mechanisms in Candida albicans may be different from other yeasts.

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