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INHIBITORY EFFECT OF GLUCOSE AND ADENOSINE 3′,5′-MONOPHOSPHATE ON THE SYNTHESIS OF INDUCIBLE *N*-ACETYLGLUCOSAMINE CATABOLIC ENZYMES IN YEAST

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Summary

Glucose can block the utilization of *N*-acetylglucosamine in *Saccharomyces cerevisiae*, a facultative aerobe, but not in *Candida albicans*, an obligatory aerobe. Furthermore, glucose represses the synthesis of the enzymes of the *N*-acetylglucosamine catabolic pathway in *S. cerevisiae*, but not in *C. albicans*. The results suggest that catabolite repression is present in *S. cerevisiae*, but not in *C. albicans*. Cyclic AMP added to *S. cerevisiae* cells maintained in a glucose medium cannot bring about their release from catabolite repression. On the contrary, the synthesis of inducible enzymes of *N*-acetylglucosamine pathway was inhibited by cyclic AMP in both the yeasts. This seems to indicate that cyclic AMP can penetrate into the yeast cells. Furthermore, cyclic AMP inhibits protein synthesis, suggesting that protein synthesis in yeast is under cyclic AMP control.

Introduction

Carbon catabolite repression [1] is a regulatory mechanism of considerable importance in prokaryotic as well as in eukaryotic microorganisms. Whereas important aspects of the molecular mechanism of catabolite repression in prokaryotes are fairly well understood [2], much less is known about the analogous process in eukaryotes. *Saccharomyces cerevisiae* appears to be particularly suited for studies on catabolite repression. In this organism, the levels of a great

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number of enzymes are dependent on the availability of a readily utilizable carbon source during growth [3]. In our previous studies we also demonstrated that addition of glucose to other inducible systems present in both bacteria and yeast glucose could repress the induction of enzymes involved in the catabolism of various glucosamine (GlcNAc) derivatives in *S. cerevisiae*. However, these enzymes are not sensitive to catabolite repression in *Candida albicans*, a pathogenic strain of yeast [4].

The role of adenosine 3',5' monophosphate (cyclic AMP) in intracellular regulation in *S. cerevisiae* is not clear particularly with respect to its significance in and relevance to catabolite repression. The content of cyclic AMP of wild-type cells of *Saccharomyces* sp. is [5] is higher on lactate than 20% glucose, and in *Saccharomyces cerevisiae* grown on 2% galactose its level is increased by 70% over that found after growth on 2% glucose. But a mutant of the same strain, partially resistant to catabolite repression of several enzymes, still exhibits wild type levels of cyclic AMP on either carbon source [6]. Furthermore, we have shown that exogenous cyclic AMP, which is believed to mediate glucose derepression in yeast, [7] stimulates protein synthesis in cell-free systems [8].

In the present paper results are presented to show that unlike other catabolite sensitive systems, the induction of N-acetylglucosamine catabolic pathway in *S. cerevisiae* could not be reversed from catabolite repression by exogenous cyclic AMP. On the contrary, cyclic AMP stimulates induction of protein synthesis not only in *C. albicans*, an obligatory acetate (where glucose effects no catabolite repression is not operative) but also in *S. cerevisiae*, a facultative acetate (where catabolite repression is operative).

Materials and Methods

Materials. Agar, peptone and yeast extract were obtained from Difco. The following chemicals were purchased from Sigma Chemical Co., St. Louis, MO: N-acetylglucosamine, glucosamine-6-phosphate, cyclic AMP, ATP, ADP, bovine serum albumin, *p*-dimethylaminobenzaldehyde, phosphoglucose isomerase and glucose-6 phosphate dehydrogenase. N-[^{3}H]Acetylglucosamine (500 Ci/mol) and cyclic AMP assay kit were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Cyclic [^{3}H]AMP was purchased from New England Nuclear, Boston, MA, U.S.A. and [^{3}H]leucine (15 Ci/mmol) was obtained from Bhabha Atomic Research Centre, Bombay, India. All other chemicals were of analytical grade.

Organisms and culture conditions. The wild type yeast strains, non-pathogenic *S. cerevisiae* 3059 and pathogenic *C. albicans* 3100 were obtained from the National Chemical Laboratory, Pune, India. The conditions for growth, enzyme induction and preparation of crude extract have been described previously [9,10]. Utilization of sugars was measured by determining the amount of sugar that had disappeared from the culture medium. Aliquots (1–2 ml) were taken out at the desired times and filtered in bulk and the cells were removed by centrifugation at 2000 × *g* for 5 min. In the supernatant N-acetylglucosamine [11] and glucose [12] were estimated according to the published procedures.

Cyclic [^{3}H]AMP uptake studies. Cyclic [^{3}H]AMP accumulation was measured by the following method. Cells of *C. albicans* harvested at mid-log phase were washed and resuspended in water. Cyclic [^{3}H]AMP (2.5 μCi/ml)

was then added to give a final concentration of 0.1 mM. At various times after addition of cyclic AMP, 0.5 ml aliquots were immediately filtered with glass-fibre filters (GF/C, Whatman) and washed twice with ice-cold water. The filters were dried and the radioactivity was determined by scintillation counting. A sample taken immediately after addition of labelled cyclic AMP allowed the measurement of any radioactivity remaining trapped in intracellular fluid after washing. This 'blank' value was subtracted from all cellular radioactivity estimations.

Enzyme assays. *N*-Acetylglucosamine kinase [13], *N*-acetylglucosamine permease (high-affinity uptake system) [4,14] and glucosamine-6-phosphate deaminase [15] were assayed according to the published procedures. For the measurement of *N*-acetylglucosamine permease (high-affinity uptake system), yeast cells ((1—2) · 10^6 cells/ml were suspended in water and preincubated for 5 min at 30°C. *N*-[^{3}H]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 ice-cold water. The filters were dried, and their radioactivity was determined in 10 ml of a toluene-based scintillation mixture in a Packard Tri-carb liquid scintillation spectrometer.

Measurement of protein synthesis. Cells ((8—10) · 10^6 cells/ml) harvested at mid-log phase were washed and resuspended in *N*-acetylglucosamine/phosphate medium as previously described [16]. At zero time, cells were divided into two portions; one sample (control) remained untreated whilst the other received 3 mM cyclic AMP. At various times, 1 ml suspension was taken out and then labelled with [^{3}H]leucine (2 μCi/ml) for 5 min. Radioactivity was determined by precipitating with cold 5% trichloroacetic acid, plating on glass-fibre filters, drying and counting in a toluene base scintillation mixture.

Protein was determined by the method of Lowry et al. [17] using bovine serum albumin as standard. Cyclic AMP assay was performed according to the procedure of Gilman [18].

Results

Effect of glucose on N-acetylglucosamine utilization by yeast

As shown in Fig. 1, both *S. cerevisiae* and *C. albicans* utilize *N*-acetylglucosamine as a carbon source. Like glucose, *N*-acetylglucosamine is taken up readily as the cells start to multiply after 2—3 h lag. The following steps are involved in GlcNAc catabolic pathway [19,20].

$$\text{GlcNAc} \xrightarrow{\text{kinase}} \text{GlcNAc-6-}P \xrightarrow{\text{deacetylase}} \text{GlcN-6-}P \xrightarrow{\text{deaminase}} \text{Fru-6-}P$$

GlcNAc kinase (EC 2.7.1.59) is absent in glucose-grown cells but is synthesized in presence of GlcNAc in *C. albicans*. However, this enzyme could not be detected in *S. cerevisiae* or any other yeast tested so far in our laboratory [14]. A high-affinity uptake system (permease) for GlcNAc is induced in both the yeasts and GlcNAc enters the cells as phosphorylated derivative [14]. Furthermore, glucosamine-6-phosphate deaminase (EC 5.3.1.10) is present in glucose-

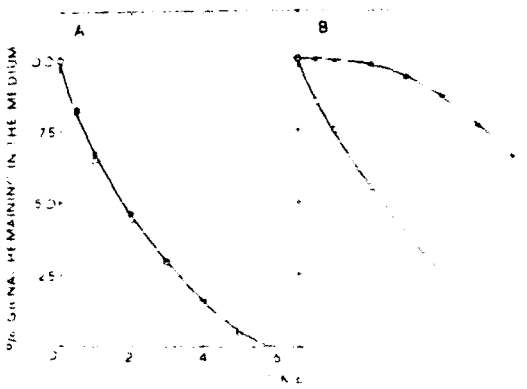


Fig. 1. Effect of glucose on the ... exponential phase of growth were ... GlcNAc, concentration of the inducer ... after centrifugation to remove the ... at zero time during induction. Control ● ... A; ... B.

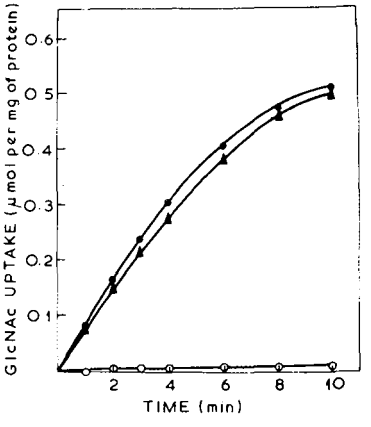
grown cells of both the yeasts ... when cells are transferred to GlcNAc medium [10].

In order to show that the repression caused by D-glucose resulted from the phenomena of catabolite repression or inhibition, utilization of N-acetylglucosamine was compared to the presence and absence of glucose. When glucose was added along with the inducer GlcNAc, the utilization of GlcNAc was severely affected in *S. cerevisiae*, but, on the contrary, in *C. albicans*, GlcNAc utilization remained almost unaffected by glucose (Fig. 1). Similarly glucose had an inhibitory effect on the synthesis of inducible N-acetylglucosamine catabolic enzymes in *S. cerevisiae* but in *C. albicans* no inhibition in the utilization of N-acetylglucosamine in *S. cerevisiae* (Fig. 2) could be due to the inhibition of the transport of N-acetylglucosamine into the cells. If this were the case, the catabolite enzymes would probably be switched off due to an apparent lack of N-acetylglucosamine. However, Fig. 2 shows that this was not the case. When uptake of N-acetylglucosamine was studied in induced cells in presence of a high concentration of glucose (100 times higher than that of N-acetylglucosamine) there was no significant inhibitory effect on uptake. Furthermore, glucose does not affect the specific binding of N-acetylglucosamine to its partially purified carrier (Singh, B. Biswas, M. and Datta, A., unpublished observations). Therefore the results obtained in this investigation show that inducible enzymes of *S. cerevisiae* are sensitive to catabolite repression whereas it is absent in *C. albicans*.

Effect of cyclic AMP on ... enzyme synthesis

Cyclic AMP is taken up by ... *S. cerevisiae* and *C. albicans* readily and the rates are comparable (Fig. 3). ... cells (8–10 × 10⁷ cells/ml) exposed to labelled cyclic AMP (total concentration ... mM) are able to accumulate the nucleotide, at an initial (no ... linear rate of ... pmol/min per mg cell protein.

Cyclic AMP has been ... to stimulate ... as well as yeast, from catabo-



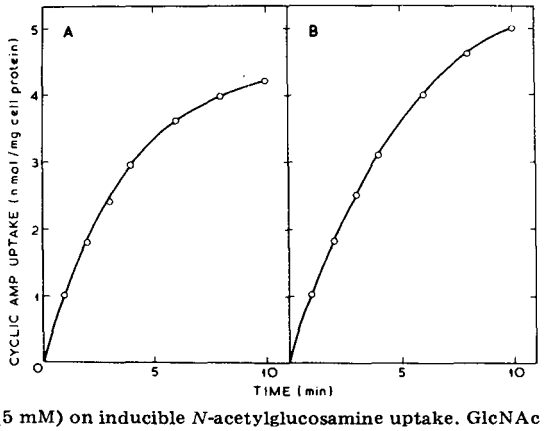


Fig. 2. Effect of high concentration of glucose (5 mM) on inducible *N*-acetylglucosamine uptake. GlcNAc uptake was assayed in 3-h induced cells except that glucose was added along with the radioactive GlCNAc (0.05 mM) wherever indicated. ○———○, uninduced cells; ●———●, 3-h induced cells; ▲———▲, induced cells plus glucose.

Fig. 3. Uptake of cyclic AMP in yeast. Uptake of [^{3}H]cyclic AMP was measured in *C. Albicans* (A) and *S. cerevisiae* (B).

lite repression. This is particularly so in the case of facultative aerobic yeasts, e.g. *S. cerevisiae* [7]. But under our experimental conditions cyclic AMP failed to release inducible GlcNAc catabolic enzymes from catabolite repression in yeast. In this connection, it would be worthwhile mentioning that at least three replicates for each experiment were performed and the pattern of results is fully reproducible. When cyclic AMP was added at different concentrations in presence of glucose, we observed varying degrees of inhibition of enzyme synthesis depending upon period of exposure to cyclic AMP (data not shown). The concentration of the nucleotide required to produce inhibition is critical. Max-

TABLE I

EFFECT OF CYCLIC AMP ON THE INDUCTION OF THE *N*-ACETYLGLUCOSAMINE UPTAKE SYSTEM IN YEAST

Cells were harvested from the exponential phase, washed and resuspended in induction medium containing 0.5% *N*-acetylglucosamine and 0.3% KH_2PO_4. In separate cultures cyclic AMP at various concentrations were added at the beginning of induction. Cells after 4 h of induction were collected and the uptake of *N*-acetylglucosamine (0.05 mM) was determined [4].

Concentration of cyclic AMP (mM)	*C. albicans*		*S. cerevisiae*	
	GlcNAc uptake (μmol/5 min per mg protein)	% of control	GlcNAc uptake (μmol/5 min per mg protein)	% of control
0	0.390	100	0.381	100
0.2	0.385	99	0.390	102
0.5	0.371	95	0.341	90
1.0	0.315	81	0.285	75
3.0	0.249	64	0.223	59
6.0	0.294	75	0.250	66
10.0	0.310	80	0.265	70

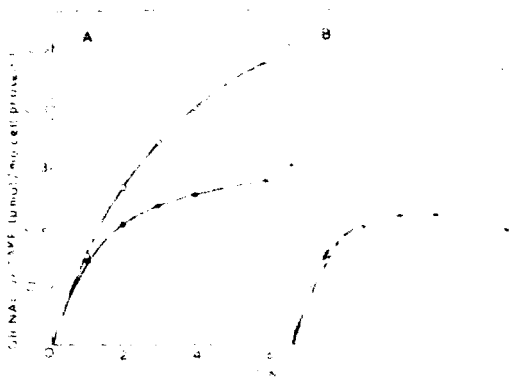


Fig. 4. Effect of cyclic AMP on the [illegible] of GlcNAc permease. [illegible] cells collected in their exponential phase of growth [illegible] in the absence (○) or presence (●) of cyclic AMP (3 mM). GlcNAc uptake [illegible] (B) at various times during induction was studied [illegible] and preincubated for 5 min at 30°C. V [^{3}H]GlcNAc [illegible] final concentration of 0.05 mM. Then 5 min after addition [illegible] samples of the incubation mixture were collected [illegible] and the filters were washed with 10 ml of ice-cold water. The filters were [illegible] toluene-based scintillation mixture.

imum inhibition of GlcNAc [illegible] cyclic AMP (35–40% inhibition) was observed at a concentration of 3 mM [illegible] with *S. cerevisiae* and *C. albicans* (Table I). [illegible] at concentrations greater than 3 mM, cyclic AMP had no significant [illegible] permease activity. This is in agreement with the published observations [illegible] that at relatively high concentrations, cyclic AMP inhibits the [illegible] dependent protein kinase. In rest of the experiments this concentration was used to study the cyclic AMP-mediated inhibition.

Fig. 4 shows the effect [illegible] cyclic AMP on the induction of GlcNAc permease in both the yeasts. Initially cyclic AMP was without any effect for about 1 h,

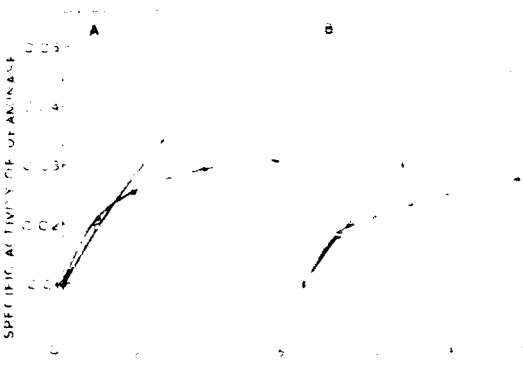


Fig. 5. Effect of [illegible] deaminase. Cells were harvested from the mid-[illegible] phase [illegible] in the absence (●) or presence (○) of cyclic AMP (3 mM). At indicated [illegible] phosphate deaminase (μmol substrate deaminated [illegible] free extract. A, *C. albicans*; B, *S. cerevisiae*.

TABLE II

EFFECT OF CYCLIC AMP ON PULSE-LABELLED PROTEIN SYNTHESIS DURING INDUCTION

S. cerevisiae cells were harvested from the exponential phase cells, washed and resuspended in induction medium to give (8—10) · 10^6 cells/ml. At zero time, cells were divided into two portions: one sample (control) remained untreated whilst the other received 3 mM cyclic AMP. At different times, 1 ml suspension was taken out and then labelled with 2 μCi [^{3}H]leucine for 5 min. Radioactivity in each aliquot was determined according to the procedure described in Materials and Methods.

Time of induction (h)	% [^{3}H]leucine incorporation compared to control as 100%
1	108
2	90
3	80
4	57
5	48

* 100% value of control sample is 1920 cpm (average).

after which a marked inhibition of permease synthesis was observed. However, the reason for the delayed action of cyclic AMP is not known.

Glucosamine-6-phosphate deaminase, which is a semiconstitutive enzyme present in both the yeasts [10], provides an ideal system for the present study. As shown in Fig. 5, the inducible synthesis of deaminase was affected in a manner similar to GlcNAc permease in both *S. cerevisiae* (catabolite repression positive) and *C. albicans* (catabolite repression negative). This could suggest that cyclic AMP-mediated inhibition is independent of glucose effect.

To examine whether cyclic AMP-mediated inhibition is limited only to inducible enzyme synthesis or is of a general nature, the cyclic AMP effect on the rate of protein synthesis during induction was studied. The results (Table II) clearly suggest that the rate of cellular protein synthesis comes down to the same extent as that of inducible enzymes. The fact that cyclic AMP inhibits protein synthesis in both the yeasts but glucose inhibits inducible enzyme synthesis only in *S. cerevisiae*, and not in *C. albicans*, indicates that the two inhibitory mechanisms could be different. Moreover, the level of intracellular cyclic

TABLE III

INTRACELLULAR CONCENTRATION OF CYCLIC AMP UNDER VARIOUS CONDITIONS

Cells from exponential phase of growth were collected washed and resuspended in 0.3% KH_2PO_4 containing the indicated sugar. After incubating for 3 h, cell-free extracts were prepared and cyclic AMP concentration in this extract was determined.

Growth conditions	Cyclic AMP concentration (pmol per mg cell protein)	
	C. albicans	*S. cerevisiae*
Glucose grown mid log phase cells	32	29
Washed cells suspended in:		
GlcNAc (0.5%)	30	31
Glucose (0.5%)	31	27
Glucose (1%)	30	28

AMP remains unchanged under various conditions employed in the present study (Table III).

Discussion

The involvement of [illegible] in glucose catabolite repression seems to be a rather general phenomenon but the details of the mutual relationship between these two systems remain obscure. Though a correlation between concentration of glucose in the medium and intracellular level of cyclic AMP in yeast has been reported [5,22], it does not conform to the details of the time course and specificity of this effect. A recent report has shown that catabolite repression of certain enzymes in *S. cerevisiae* cannot be rescued by cyclic AMP [2]. In this study, we have also shown that the overall cyclic AMP content of the glucose grown cells was the same as that in GlcNAc-grown cells (Table III). Furthermore, not only exogenous cAMP did not release the GlcNAc catabolic enzymes from catabolite repression in *S. cerevisiae*, but also it inhibited protein synthesis in case of *C. albicans*.

In the case of *Xenopus* oocytes there is a recent report [24] which supports the observations of Datta et al. [19] on the existence of a cyclic AMP-dependent protein kinase which activates a cyclic AMP independent protein kinase which in turn inhibits ribosomal protein synthesis. In this report, Bravo et al. [24] have shown that maturation of the oocytes by cyclic AMP inhibits the incorporation of amino acids into proteins and delays the onset of maturation. In this light, the observations reported in this paper provide further support for the view that protein synthesis in lower eukaryotes is also under cyclic AMP control. Furthermore, cyclic AMP independent protein kinases are present in both *C. albicans* (unpublished data) and *Saccharomyces* [20,21].

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References

1 Majumdar, B. (1965) [illegible]
2 Pastan, I. and Adhya, S. [illegible] 52–53
3 Perlman, P. S. and Mat[illegible] [illegible]
4 Singh, B. R. and Datta, A. [illegible]
5 Sy, J. and Richter, L. [illegible] 2, 88, 2[illegible]
6 Schamhart, D. H. J. [illegible] 241, 247–252
7 Mahler, H. R. and Lin[illegible] 1047
8 Bhattacharya, A. and Ba[illegible] 438–444
9 Bhattacharya, A. Bas[illegible] 38–391
10 Singh, B. and Datta, A. [illegible] 58, 25[illegible]
11 Reissig, J. L. Strominger, J. [illegible] 8, 2, 959–963
12 Nelson, N. (1944) [illegible]

13 Datta, A. (1970) Biochim. Biophys. Acta 220, 51—60
14 Singh, B. and Datta, A. (1979) Biochim. Biophys. Acta 557, 248—258
15 White, R.J. and Pasternak, C.A. (1975) Methods Enzymol. 41, 497—502
16 Bhattacharya, A., Puri, M. and Datta, A. (1974) Biochem. J. 141, 593—595
17 Lowry, O.H., Rosebrough, N.J., Farr, A.H. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
18 Gilman, A.G. (1970) Proc. Natl. Acad. Sci. U.S.A. 67, 305—312
19 Singh, B. and Datta, A. (1979) Biochem. J. 178, 427—431
20 Biswas, M., Singh, B. and Datta, A. (1979) Biochim. Biophys. Acta 585, 535—542
21 Rubin, C.S. and Rosen, O.M. (1975) Annu. Rev. Biochem., 831—887
22 Schlanderer, G. and Dellweg, H. (1974) Eur. J. Biochem. 49, 305—316
23 Foy, J.J. and Bhattacharjee, J.K. (1978) J. Bacteriol. 136, 647—656
24 Bravo, R., Otero, C., Allende, C.C. and Allende, J.E. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 1242—1246
25 Datta, A., de Haro, C., Sierra, J.M. and Ochoa, S. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 1463—1467
26 Takai, Y., Yamamura, H. and Nishijuka, Y. (1974) J. Biol. Chem. 249, 530—535
27 Kudlicki, W., Grankowski, N. and Gasior, E. (1978) Eur. J. Biochem. 84, 493—498