# CYCLIC 3′, 5′-AMP IN *SACCHAROMYCES CARLSBERGENSIS* UNDER VARIOUS CONDITIONS OF CATABOLITE REPRESSION

R. VAN WIJK
*Van't Hoff Laboratory, Utrecht, The Netherlands*

and

T.M. KONIJN
*Hubrecht Laboratory, Utrecht, The Netherlands*

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## 1. Introduction

Cyclic 3′, 5′-AMP is known to function as a mediator of hormone induced changes in the metabolism of vertebrates and invertebrates [1, 2]. It also occurs in *Escherichia coli* [3] where it plays a role in catabolite repression [4]. Cheung [5] reported the presence of low levels of this nucleotide in cells of *Saccharomyces carlsbergensis*. No data are available in the literature about the cyclic 3′, 5′-AMP level in yeast under various conditions of catabolite repression. Our results might suggest a regulatory role of this nucleotide in yeast.

## 2. Materials and methods

Using a sensitive microbiological assay [6] we measured the concentration of cyclic 3′, 5′-AMP in cells of *S. carlsbergensis* after growth in media with different sugars. The bioassay is based on the attraction of amoebae of the cellular slime mold *Distyostelium discoideum* by cyclic 3′, 5′-AMP. After purification by columm and paper chromatography [7] the attractant in yeast was identified as cyclic 3′, 5′-AMP. The yeast extracts were prepared by chilling very rapidly a culture sample of 50 ml containing 20–40 mg cell dry weight. The cell suspension was centrifuged, the pellet resuspended in 1 ml 15% perchloric acid and frozen at −50°. This part of the procedure was completed within two min. The cells were then freeze-thawed three times, centrifuged and the supernatant neutralized with KOH. After precipitation of the $KClO_4$ crystals, the cyclic 3′, 5′-AMP in the supernatant was measured with the bioassay. The various supernatants were compared with each other by dilution. In this way the activity of a sample *b* can be expressed as a function of sample *a*; for instance sample *b* is more active than a *x* fold dilution of sample *a*, but less active than a *y* fold dilution of sample *a*. *x* and *y* are successive values in a dilution range which differ by a factor of 3 or when this is not accurate enough by a factor of 2. The levels presented are the mediate values between *x* and *y*. In this way it was possible to measure the relative activity in the different yeast extracts. Their cyclic 3′, 5′-AMP levels were determined by comparing the activity of a sample with successive values (differing by a factor of 2 or 3) in a dilution range of cyclic 3′, 5′-AMP.

## 3. Results and discussion

We compared the cyclic 3′, 5′-AMP levels of yeast cultures which were grown in media containing 2% glucose or 2% galactose as their sole carbon source. It is known that the carbon metabolism is not the same under these two conditions, because the degree of catabolite repression of some enzymes, e.g. the citric acid cycle enzymes [8, 9], differs. Table 1 shows the differences in the specific activity of α-glucosidase and succinate dehydrogenase, which are both



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Table 1

| Enzymic activity | Source of carbon | |
|---|---|---|
| | Glucose | Galactose |
| Glucose-6-phosphate dehydrogenase | 100 | 100 |
| α-Glucosidase | 6 | 63 |
| Succinate dehydrogenase | 4 | 27 |
| Intracellular concentration of cyclic 3′, 5′-AMP (μM) | 0.04 | 0.25 |

Enzyme activities and the intracellular cyclic 3′, 5′-AMP concentrations of *S. carlsbergensis* cells grown in synthetic media [10] containing 2% glucose or 2% galactose as the sole carbon source and 0.1% $(NH_4)_2SO_4$ as the sole nitrogen source. The cells were harvested in the logarithmic growth phase (0.5 mg cell dry weight per ml medium). A cellular volume of 3.75 μl per mg cell protein was used for the calculation of the intracellular cyclic 3′, 5′-AMP concentration [9] and enzyme activities were expressed as nmoles substrate converted per mg protein per min.

sensitive to catabolite repression. Also the intracellular concentration of cyclic 3′, 5′-AMP in yeast cells grown on the different carbon sources is significantly different (table 1). Previously [9] we found that the degree of repression during growth on maltose and that during growth on galactose were similar. The intracellular concentration of cyclic 3′, 5′-AMP in cells grown in a medium containing 2% maltose was compared to that in cells grown in a medium containing 2% galactose as the sole carbon source. The cyclic 3′, 5′-AMP concentrations were similar. The different cyclic 3′, 5′-AMP levels during growth on glucose and galactose (table 1) suggest a change in the amount of this nucleotide when cells previously grown on glucose are adapted to a medium containing galactose or maltose. This kind of adaptation was studied extensively at our laboratory using maltose as the carbon source. Therefore we measured the intracellular cyclic 3′, 5′-AMP concentrations during adaptation to maltose. The adaptation to maltose depends on the induction of a maltose permease system [11] and an α-glucosidase [12]. The ability of yeast cells to ferment maltose and the accompanying formation of α-glucosidase has been shown in fig. 1. The change in the degree of repression can be followed from the data on the succinate dehydrogenase specific activity. The cells were pregrown on a medium containing 2% glucose and incubated in a medium containing 2% maltose, 0.1% glucose, 1% casamino acids and 0.05 M sodium/potassium phosphate buffer pH 6.2. Table 2 exemplifies the increase of cyclic 3′, 5′-AMP in the yeast cells during their adaptation to maltose. We may conclude that the intracellular amount of cyclic 3′, 5′-AMP is higher in cells which show a lower degree of catabolite repression. Whether it is justified

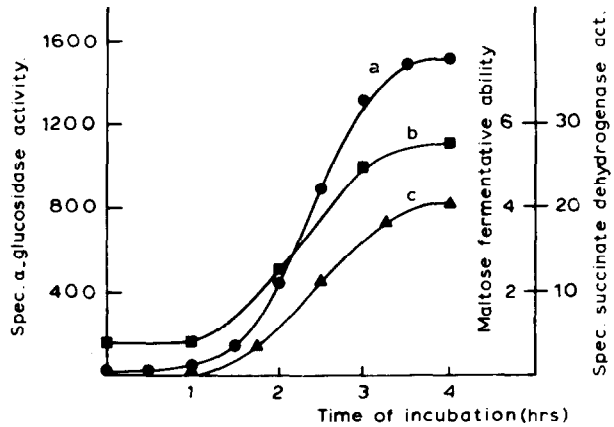


Fig. 1. Specific α-glucosidase activity and the fermentation of maltose during adaptation of cells, pregrown on glucose [10], to maltose. The maltose fermentative ability was determined manometrically. The initial rate of $CO_2$ production of rapidly washed cells was measured anaerobically. The cells were incubated in 1 ml of a medium containing 0.067 M $KH_2PO_4$ and 5 μmoles maltose.
a) specific α-glucosidase activity expressed as nmoles PNPG split $min^{-1}$ $mg^{-1}$ protein
b) specific succinate dehydrogenase activity expressed as nmoles cytochrome *c* reduced $min^{-1}$ $mg^{-1}$ protein
c) maltose fermentative ability expressed as μl $CO_2$ produced $min^{-1}$ $mg^{-1}$ dry weight of cells.

Table 2
Intracellular concentration of cyclic 3′, 5′-AMP during adaptation of *S. carlsbergensis* cells to maltose at various times of incubation (see fig. 1).

| Time of incubation (min) | Intracellular concentration of cyclic 3′, 5′-AMP (μM) |
|---|---|
| 0 | 0.04 |
| 60 | 0.04 |
| 120 | 0.18 |
| 180 | 0.32 |
| 210 | 0.32 |

to conclude that cyclic 3′, 5′-AMP plays a role in the enzymatic regulatory effects is under investigation.

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