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3′:5′-cyclic GMP in the yeast *Saccharomyces cerevisiae* at different metabolic conditions

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cGMP is characterized as undetectable in yeast [(1986) Yeast Cell Biology, UCLA Symp. Mol. Cell Biol. (Hicks, J. ed.) p.495], though in many organisms it contributes specifically to the regulation of metabolism. Here, we detected cGMP, using radioactive labeling and RIA techniques, after extraction of the cells with 1 mol/l $HClO_4$ at 37°C. The cGMP 0.015-fold cAMP, about 3-times higher with exponentially growing cells than with pressed baker's yeast, and depends on glucose and O_2 supply. The PDE inhibitors DMX and IBMX induce in growing cells an additional increase of the cGMP level, without similar effects on cAMP.

cyclic GMP; Yeast; (*Saccharomyces cerevisiae*)

1. INTRODUCTION

Though most bacteria and animal cells are provided with cGMP as well as with cAMP, no information on cGMP in yeast is available; cGMP is characterized as 'undetectable' in yeast [1]. However, in several eucaryotic organisms cGMP performs special regulatory functions (reviews [2,3]). In bacteria, cGMP takes part in processes of repression/derepression, in some cases in a direction opposite to that occurring in cAMP [4,5]. It seemed of interest, therefore, to re-examine the equipment of yeast with cGMP. We detected cGMP in extracts from *Saccharomyces cerevisiae*. The cGMP level depends on the metabolic state of the cells, and is modified when growing yeast is incubated with the PDE inhibitors 1,3-dimethylxanthine, or 3-isobutyl-1-methylxanthine, respectively.

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Abbreviations: DMX, 1,3-dimethylxanthine (theophylline); IBMX, 3-isobutyl-1-methylxanthine; PDE, 3′,5′-cyclic nucleotide phosphodiesterase (EC 3.1.4.17); RIA, radioimmunoassay; TLC, thin-layer chromatography

2. MATERIALS AND METHODS

2.1. *Yeast strains and growth conditions*

A clone formerly separated from baker's yeast ('Germania Hefe', Deutsche Hefewerke, Hamburg, FRG) was used throughout all experiments. Commercially available baker's yeast (L'Hirondelle, Lesaffre, Marc en Baroeul, France) was used additionally in some experiments. Yeast was grown aerobically at 31°C on a modified Wickerham medium [6] with vigorous aeration and stirring. In principle the medium was inoculated with an appropriate cell number and the culture grown overnight at 31°C. At 1–5 × 10^6 cells/ml, the culture was immediately taken for the experiments.

2.2. *Chemicals*

[2,8-^{3}H]Adenosine 3′,5′-cyclic phosphate ([^{3}H]cAMP, 37 MBq/ml, 1332 GBq/mmol), [8-^{3}H]guanosine 5′-triphosphate ([^{3}H]GTP, 37 MBq/ml, 396 GBq/mmol), and rabbit anti-cGMP antiserum were from Amersham-Buchler (Braunschweig, FRG). [8-^{3}H(N)]Guanosine 3′,5′-cyclic phosphate ([^{3}H]cGMP, 37 MBq/ml, 636.4 GBq/mmol) was from NEN (Dreieich, FRG). Unlabeled cyclic nucleotides, other nucleotides, PDE (EC 3.1.4.17) from bovine heart and other enzymes were from Boehringer (Mannheim, FRG). DMX and Norit A were from Serva (Heidelberg, FRG). IBMX was obtained from

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Sigma (St. Louis, MO, USA). Triethylamine was from Fluka (Buchs, Switzerland). Rabbit anti-cAMP antiserum was prepared by Dr M. Schumacher and made available by Professor Hilz (Hamburg, FRG).

2.3. *In vivo labeling and detection of cGMP*

Radioactive labeling in vivo, and separation and identification of cGMP are described in detail elsewhere [7]. In brief, exponentially growing yeast was incubated for 210 min with 4.625 kBq (1.168 mmol) [^{3}H]GTP/10^7 cells per ml, then extracted with 1 mol/l $HClO_4$, and the nucleotides separated by adsorption to Norit A. After elution, the nucleotides were chromatographed on silica gel thin-layer plates with an isopropanol/water/ammonia mixture (7:2:1), usinig 25 nmol each of cGMP and cAMP as tracer, and adenosine, guanosine, and their respective mono- and triphosphates as control markers. The UV-absorbing spots were moistened with water, scraped off, and counted in a liquid scintillation spectrometer.

2.4. *Phosphodiesterase assay*

The standard assay contained in a final volume of 85 μl, 0.82 mmol/l $MgCl_2$, 0.21 mmol/l Na_2EDTA, 20.5 mmol/l Tris, 50 μl Norit eluate, and 0.1 mg PDE (0.25 U/mg), pH 7.5. Control assays contained (i) an additional 5 nmol (370 Bq) [^{3}H]cGMP, (ii) 0.5 pmol (370 Bq) or 5 nmol (370 Bq) [^{3}H]cGMP instead of the Norit eluate, (iii) no enzyme. The assays were incubated for 60 min at 37°C, then 5 min at 95°C, centrifuged, and the clear supernatant was analyzed by thin-layer chromatography. About 65% of labeled cGMP was degraded when the standard assay was supplemented with [^{3}H]cGMP. In contrast, more than 90% of the labeled cGMP was recovered after incubation without PDE.

2.5. *Estimation of cGMP by RIA*

Cyclic nucleotides were estimated from yeast extracts by the RIA technique according to Steiner et al. [8] with some modifications as described in detail [7]. cGMP was estimated in the acetylated form. Yeast extracts were prepared from 1×10^8–1×10^9 cells by incubation with 2.0 ml $HClO_4$, 1 mol/l, for 60 min at 37°C and 5 min in an ice bath. After centrifugation, the supernatant was adjusted to pH 7.0–7.2 with 5 mol/l KOH and 1 mol/l potassium phosphate buffer (pH 7.4). The precipitate was centrifuged off. The clear supernatant was taken for the RIA. The yield of cyclic nucleotides increased in proportion to the number of cells extracted, up to 10^9 cells. Recovery of cyclic nucleotides added to the yeast together with $HClO_4$ was more than 95%. No cross-reactions between the antisera and the 'false' cyclic nucleotides were observed (details [7]).

3. RESULTS

3.1. *Detection of cGMP in yeast: in vivo labeling*

When an exponentially growing yeast culture (10×10^6 cells/ml) is supplemented with [^{3}H]GTP, and growth is continued for 210 min, about 5% of the incorporated radioactivity is found in the acid-soluble cell fraction. Separation of this material by TLC exhibits radioactivity comigrating with cGMP, and with cAMP, respectively, showing more label associated with cGMP than with cAMP. Incubation of the labeled acid-soluble material with PDE results in a marked loss of radioactivity from both the cGMP and the cAMP fractions (table 1).

3.2. *Detection of cGMP by RIA technique*

cGMP can also be detected in the acid-soluble cell fraction by the RIA technique. Extensive pilot tests (not shown here) revealed, however, that extraction of the cells by 1 mol/l $HClO_4$ at 37°C is an essential requirement for success. Any interaction of the anti-cGMP antiserum with cAMP can be excluded.

3.3. *Influence of glucose repression and O_2*

About three times as much cGMP as with pressed baker's yeast is found with cells from an exponentially growing culture (table 2). When exponentially grown yeast is incubated under aerobic conditions, but only with glucose and potassium phosphate as substrates, cGMP decreases to 45%, and on additional O_2-starvation to about 37%. Similar changes occur with cAMP. The ratio cGMP:cAMP thus remains nearly constant.

3.4. *Effect of PDE inhibitors*

When exponentially growing yeast is exposed to DMX (5×10^{-3} mol/l), the level of cGMP temporarily is increased significantly above the control after a lag of more than 40 min (fig.1A). Neither the level of cAMP, nor the DNA and protein contents of the cells are affected essentially, even after continued cell growth (not shown here).

With IBMX (1×10^{-3} mol/l), the level of cGMP

Table 1

Detection of cyclic nucleotides from yeast extracts by TLC

	R_f	dpm/10^9 cells	
		I	II
cGMP	0.30	18650	7180
cAMP	0.44	13600	5170

Exponentially growing yeast was incubated with [^{3}H]GTP, extracted with $HClO_4$, and the labeled nucleotides were separated by TLC. cGMP and cAMP were detected by UV-absorption, and identified by the R_f-values. I, control after incubation in a PDE assay, but without enzyme; II, after incubation with PDE. Mean from three experiments

Table 2

cGMP and cAMP in extracts from yeast kept in different metabolic states

	pmol/10^9 cells		cGMP/cAMP
	cGMP	cAMP	
(a) Pressed baker's yeast	5.9 ± 2.8	346 ± 109	0.017
(b) Exponentially growing yeast	20.6 ± 6.2	1713 ± 286	0.012
(c) With glucose + phosphate, aerated	9.3 ± 1.4	654 ± 120	0.014
(d) With glucose + phosphate, but starved of O_2	7.6 ± 2.5	580 ± 134	0.013

(a) Commercially available baker's yeast ('Germania Hefe', DHW, Hamburg), washed twice with water, and resuspended with water. (b) Yeast grown exponentially overnight up to 1×10^6 cells/ml (c) Yeast from an exponentially growing culture, washed twice with water, and reincubated (1×10^6 cells/ml) overnight with 0.44 mol/l glucose in 0.05 mol/l potassium phosphate buffer, pH 6.5, at vigorous aeration and stirring. (d) Yeast treated as in c, but without stirring and aeration. Means from two experiments, four analyses per experiment

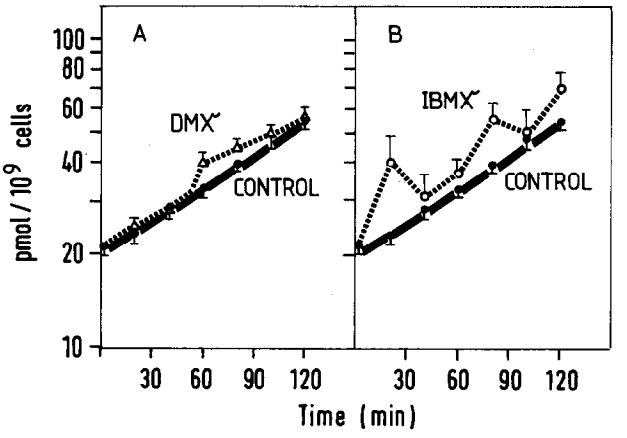

Fig.1. cGMP level in yeast growing with xanthine derivatives. Aliquots from exponentially growing cultures (about 5×10^6 cells/ml) were supplemented with 5×10^{-3} mol/l DMX (A), and with 1×10^{-3} mol/l IBMX (B), respectively, and the incubation continued. At the indicated times, 1×10^8 cells were harvested from aliquots, extracted, and cGMP was estimated. Values are means ± SD. According to Student's *t*-test, control and DMX values differ significantly ($P < 0.05$) at 60 and 80 min. With IBMX, the differences are significant ($P < 0.05$) at 20, 80 and 120 min. (●—●) Control; (△—△) DMX; (○—○) IBMX.

in growing yeast likewise is raised above the control (fig.1B). The lag is less than 20 min. When IBMX is removed by washing and reincubating the cells with fresh cold growth medium, the cGMP level is already normalized at zero time of reincubation. Again, no effects upon the levels of cAMP, DNA and protein are observed.

4. DISCUSSION

Our labeling experiments, and the studies by the RIA technique, show that the yeast *S. cerevisiae* like most other organisms is equipped not only with cAMP, but also with cGMP. In contrast to cAMP, cGMP becomes detectable, however, only after a relatively strong treatment of the cells with 1 mol/l $HClO_4$ at 37°C. Maximum values are obtained after a 60 min extraction. These conditions differ considerably from the standard extraction procedure for low-molecular-mass yeast cell constituents, e.g. NAD or nucleoside triphosphates, namely 10% trichloroacetic acid for 10 min at room temperature. The reason for this difference is not clear. However, this might explain, in part, why cGMP so far seemed to be 'undetectable' [1].

The intracellular level of cGMP depends on the metabolic conditions. The differences between pressed baker's yeast, yeast fermenting glucose aerobically or anaerobically, and cells growing exponentially, indicate that not only glucose acts as a positive effector on the cGMP level. In this regard, the situation resembles that described for *Escherichia coli* and *Bacillus licheniformis* [9]. But in contrast to *E. coli* [9,10], cGMP behaves like cAMP, the molar ratios between both cyclic nucleotides being nearly the same at all metabolic states. cAMP increases in yeast under conditions of derepression [11,12], thus modifying metabolic pathways by stimulation of protein kinase [13–16]. Our findings suggest that cGMP might play a similar role. Evidence obtained recently on a cGMP dependent protein kinase from yeast [17] supports this view.

When yeast is grown in the respective presence of DMX or IBMX, the cGMP level increases significantly above the control. This is not the case with cAMP, though we cannot exclude a quick short-lived reaction, as observed [12] at derepression by trehalose. It remains unknown whether different sensitivities to DMX and IBMX of cGMP- and cAMP-dependent yeast PDEs are responsible for these findings.

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