The Effects of Nucleotides on the α-Glucosidase Formation in Baker's Yeast Protoplasts*

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The few papers dealing with yeast and 3′,5′-AMP (cAMP) provide some evidence that cyclic cAMP may be involved in the metabolic control of yeast, as has already been well documented for bacteria.[2] It has been reported that the concentration of cAMP in *Schizosaccharomyces pombe*, *Saccharomyces carlsbergensis* and *Sacch. fragilis* depends on the growth conditions.[3-5] Moreover, it has been demonstrated for *Sacch. cerevisiae* protoplasts that certain nucleotides, including cAMP, can overcome the glucose repression of respiratory adaptation.[6] cAMP has been shown to have a de-repressive effect on the glucose repression of sporulation in intact baker's yeast.[7]

The effects of cAMP and some other nucleotides on the partially repressed α-glucosidase synthesis in baker's yeast protoplasts are reported in this paper.

A commercial baker's yeast strain was used in the experiments. Protoplasts were prepared from an early log-phase culture according to Nurminen *et al.*[8] by using β-mercaptoethanol and *Helix pomatia* snail gut juice. The methods used for the induction and the determination of α-glucosidase were slightly modified from those described by Burger *et al.*[9] The induction medium was 0.7 M in $MgSO_4$ and contained 0.65 % maltose, 1 % Casamino acids and approximately 0.2 % protoplasmic protein (determined according to Lowry *et al.*[10]). In some experiments 0.8 M mannitol was used as the osmotic stabilizer for the protoplasts in place of $MgSO_4$. The pH of the mixture was adjusted to 4.7 with McIlwaine's citrate-phosphate buffer. Any additions to the induction mixture are indicated. The induction was carried out by incubating 10 ml of the mixture at 30°C in a 50 ml Erlenmeyer flask in a shaker. A preparation for α-glucosidase analysis was made by centrifuging 1 – 2 ml of the induction mixture, suspending the sediment in 1 – 2 ml of distilled water and allowing it to stand with occasional shaking for at least 20 min at 0°C. During this time protoplasts were broken. The supernatant remaining after centrifugation of the broken cell suspension is hereafter referred to as lysate. 50 – 200 μl of lysate were taken for the determination of α-glucosidase activity. It was made by measuring the hydrolysis of *p*-nitrophenyl-α-D-glucoside (PNPG) at 400 nm with a spectrophotometer linked to an automatic recorder. The assay mixture for α-glucosidase was the same as described by Kloet *et al.*[11] One unit was defined as the amount of enzyme which liberated 1 nmol of *p*-nitrophenol in 1 min at 30°C with 1 ml of lysate present in the assay mixture.

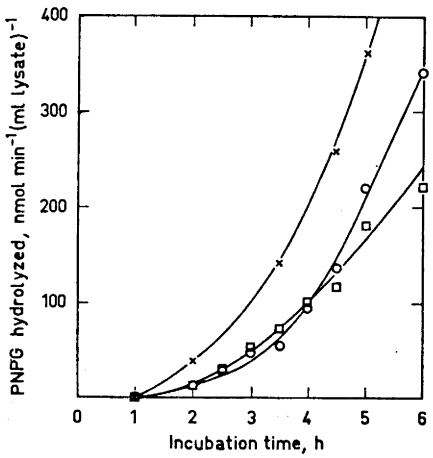

Fig. 1. Kinetics of α-glucosidase induction in baker's yeast protoplasts. The induction of α-glucosidase, the preparation of a lysate and the assay of α-glucosidase activity were carried out as described in the text. The induction medium (0.7 M $MgSO_4$ as osmotic stabilizer) was supplied with 0.25 % glucose (×), 1 % glucose (□) or 1 % glucose + 0.3 mM cAMP (○).

* Some of the results reported in this paper were presented at the Third International Specialized Symposium on Yeasts at Otaniemi/Helsinki, 7th June 1973.[1]

Table 1. α-Glucosidase activity. The effect of low Mg^{2+} and Mn^{2+} ion concentrations on the α-glucosidase induction in baker's yeast protoplasts. The effects of cAMP and ATP in the presence of Mg^{2+} and Mn^{2+} ions on the α-glucosidase induction. The induction of α-glucosidase, the preparation of lysate and the assay of α-glucosidase were carried out as described in the text. The supplements to the induction medium (0.8 M mannitol as osmotic stabilizer) are shown in the table. Activities are expressed as nmol of PNPG hydrolyzed/(min ml) lysate. The samples were taken 4 h after the start of induction.

Supplement	0.25 % glucose	1 % glucose	0.25 % glucose + 1 mM cAMP	0.25 % glucose + 1 mM ATP
–	134	85	142	157
5 mM Mg^{2+}	126	57	137	125
5 mM Mn^{2+}	99	43	157	169

The kinetics of α-glucosidase formation in the presence of 0.25 % glucose, 1 % glucose and 1 % glucose + 0.3 mM cAMP are presented in Fig. 1. No α-glucosidase was synthesized by protoplasts during the first hour. This period was observed to be independent of the glucose concentration and the presence of cAMP. The repressive effect of increased glucose concentration on the α-glucosidase formation was obvious. cAMP does not seem to have any positive effect and, if anything, acts as a weak repressor. The repressive effect, however, could not be observed in all the experiments. The synthesis rate of α-glucosidase began to be accelerated by cAMP about 3.5 h after the beginning of the enzyme induction. The synthesis rate of α-glucosidase in protoplasts induced in the presence of 1 % glucose + 0.3 mM cAMP did not quite reach that in protoplasts induced in the presence of 0.25 % glucose.

The effects of cAMP concentration on the α-glucosidase synthesis is shown in Fig. 2. A cAMP concentration of about 0.3 mM gave the maximum de-repressive effect, which decreased slowly at higher concentrations.

The inhibitory effect of Mg^{2+} ions on the α-glucosidase induction in baker's yeast protoplasts was reported by Burger *et al.*[8] It was now observed that, besides Mg^{2+} ions, Mn^{2+} ions have an inhibitory effect on the α-glucosidase induction (Table 1). This effect of Mn^{2+} ions was more intense than that of Mg^{2+} ions, but weaker than that of 1 % glucose. The inhibitory effect of Mg^{2+} ions could no longer be observed when $MgSO_4$ was used as an osmotic stabilizer for the protoplasts (see Ref. 1). This is possibly a consequence of $MgSO_4$ being a better osmotic stabilizer than mannitol. The results presented in Table 1 also show that cAMP and ATP nullify the repressive effect of Mn^{2+} ions, and that cAMP can overcome the repressive effect of Mg^{2+} ions as well. The presence of Mn^{2+} ions increase the de-repressive effect of these nucleotides. It is to be noted that the adenyl cyclase in baker's yeast, the only enzyme known to synthesize cAMP is manganese dependent.[12]

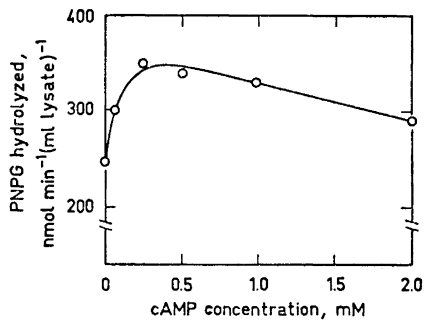

Fig. 2. Effect of cAMP concentration on the α-glucosidase induction in baker's yeast protoplasts. The induction of α-glucosidase the preparation of a lysate and the assay of α-glucosidase were carried out as described in the text. The induction medium (0.7 M $MgSO_4$ as osmotic stabilizer) was supplied with 1 % glucose and different cAMP concentrations. The samples were taken 6 h after the start of induction.

The effects of some other nucleotides on α-glucosidase formation were also studied. The results are presented in Table 2. They

Table 2. The effect of different nucleotides on the induction of α-glucosidase in baker's yeast protoplasts. The induction of α-glucosidase, the preparation of lysate and the assay of α-glucosidase were performed as described in the text. The induction medium (0.7 M $MgSO_4$ as osmotic stabilizer) was supplied with 1 % glucose and different nucletides to a final concentration of 1 mM. Activities are expressed as nmol of PNPG hydrolyzed/(min ml) lysate. The samples were taken 4.5 h after the start of induction.

Nucleotide [a]	α-Glucosidase activity
None	155
Cyclic 3′,5′-AMP	168
ATP	168
Cyclic 3′,5′-GMP	163
Cyclic 2′,3′-AMP	155
Cyclic 3′,5′-UMP	155
AMP	150
ADP	138
$O^{2'}$-MB cyclic 3′,5′-AMP	137
N^6,$O^{2'}$-DB cyclic 3′,5′-AMP	137
N^6-MB cyclic 3′,5′-AMP	121

[a] MB = monobutyryl, DB = dibutyryl.

indicate that the de-repressive effect of cAMP cannot be due to the 3′,5′-ring structure alone; cyclic 3′5′-UMP and butyryl derivatives of cAMP have no de-repressive effects. A definite stimulative effect of ATP was exhibited under these experimental conditions. The de-repressive effect of cyclic 3′5′-GMP was to be expected.

The results obtained support the view that cAMP participated in the catabolite repression of yeast.

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Microwave Spectrum of Thiete 1,1-Dioxide

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Thiete 1,1-dioxide (*cf.* Fig. 1) was first synthesized by Dittmer and Christy in 1960.[1,2] A complete structural investigation by X-ray diffraction was performed by Lowenstein [3] in 1965. Within the limits of error of the method he found a planar ring structure with the plane of the O – S – O bond perpendicular to the plane of the ring. The refinement of the structure was not carried far enough to reveal the positions of the hydrogen atoms. The results of Lowenstein are tabulated in Table 1.