EFFECT OF GLUCOSE AND CYCLIC NUCLEOTIDES ON THE TRANSCRIPTION OF α-AMYLASE mRNA IN BACILLUS SUBTILIS

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SUMMARY

Washed post-logarithmic cultures of Bacillus subtilis produce α-amylase biphasically. Phase 1 corresponds to the translation of an existing mRNA pool and phase 2 to the resumption of coupled transcription and translation. Glucose, like rifampicin, represses α-amylase synthesis at transcription. The biphasic pattern of α-amylase synthesis is abolished by cyclic AMP, cyclic GMP, AMP or ATP but not adenine. Glucose repressed transcription of α-amylase mRNA synthesis is unaffected by cyclic AMP or cyclic GMP.

INTRODUCTION

Many Bacillus spp. synthesise the extracellular enzyme α-amylase late in log-phase growth and during stationary phase (1). The rate of α-amylase synthesis is primarily regulated by catabolite repression (1,2,3). The role of cyclic AMP (cAMP) as an effector of catabolite repression is now recognised in Escherichia coli and many gram-negative bacteria (4), however, it seems likely that cAMP and the enzymes responsible for its synthesis and degradation are either absent in Bacilli (5,6,7) or present at very low levels (8). It has been suggested that cyclic GMP (cGMP) may play a regulatory role that opposes cAMP in Bacillus licheniformis (7) as it does in E. coli (9).

The aims of the present study were to determine the stage during protein synthesis at which glucose exerts a repressive action and to examine the regulatory effects, if any, that cAMP and cGMP may have on protein synthesis.

MATERIALS AND METHODS

cAMP, cGMP, AMP, ATP, adenine and rifampicin were obtained from the

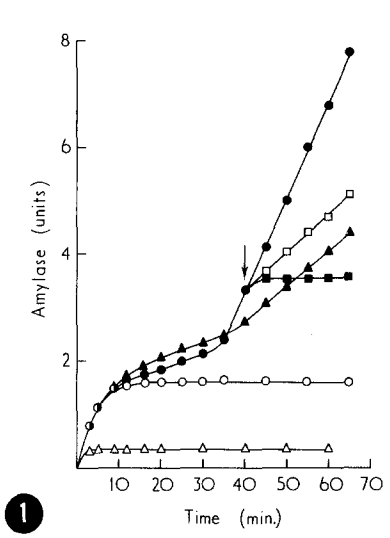
Sigma Chemical Co. Tetracycline was obtained from Cyanamid Ltd. The organism was B. subtilis B20 an industrial strain producing α-amylase. It was maintained by weekly subculture on malt extract agar (3% malt extract, 0.5% yeast extract). An inoculum from this source was grown at 37°C for 18 hours in the minimal medium of Spizizen (10) supplemented with maltose (1%) and casein hydrolysate (0.5%). The absorbance at 625 nm was 1.5-1.7. The cells were harvested at 30°C by centrifugation, washed in warm (30°C) minimal salts and resuspended in minimal salts containing 0.05% casein hydrolysate. Samples were taken at intervals, rapidly chilled to 0°C, and centrifuged. The supernatant fractions were assayed for α-amylase.

The assay was based on that of Smith and Roe (11). The reaction tube contained; phosphate buffer, 0.1 M, pH 6.0, 1 ml; NaCl, 0.5 M, 0.2 ml; starch, 0.1%, 1 ml; enzyme, 0.5 ml. The tubes were incubated at 50°C for 20 minutes and the reaction halted by the addition of iodine solution (1 ml; 0.1% KI, 0.01% I_2 in 1 N HCl). A unit is defined as that activity which reduces the absorbance at 620 nm of the starch-iodine complex by 0.5 under these conditions.

RESULTS

Washed, post-logarithmic cells of B. subtilis B20 produce α-amylase biphasically (Fig. 1). This pattern is essentially the same as that described by Gould, May and Elliot for Bacillus amyloliquefaciens. There is an initial phase (phase 1) of synthesis lasting for 25-30 minutes followed by a second phase in which α-amylase increases linearly (phase 2). If rifampicin is added to the cells at time zero, α-amylase synthesis proceeds for about 15 minutes. The amount made during this period is slightly less than in the absence of rifampicin. Addition of rifampicin at 40 minutes causes a rapid inhibition of α-amylase synthesis.

The addition of glucose at time zero has little effect on the rate of phase 1 α-amylase synthesis but it does reduce the rate of phase 2. The



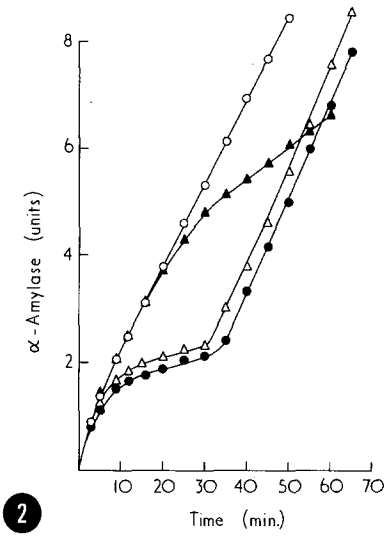


Figure 1. Production of α-amylase by a washed suspension of Bacillus subtilis B20.

The cells were harvested, washed and resuspended as described in the Methods section. The following additions were made at time zero: ●—●, nothing; ○—○, rifampicin, 0.4 μg/ml; ▲—▲, glucose, 1%; △—△, tetracycline, 100 μg/ml. At the arrow the culture was split into 4 flasks containing; ●—●, nothing; □—□, glucose, 1%; ■—■, rifampicin, 0.4 μg/ml; ■—■, tetracycline, 100 μg/ml.

Figure 2. Production of α-amylase by a washed suspension of Bacillus subtilis B20.

The cells were prepared as in Fig. 1. The following additions were made at time zero; ●—●, nothing; ○—○, cyclic AMP, cyclic GMP, AMP, or ATP, all 3 mM; ▲—▲, glucose, 1% and cyclic AMP, 3 mM, or cyclic GMP, 3 mM; ●—●, adenine, saturating concentration; △—△, cyclic AMP, 3 mM, or cyclic GMP, 3 mM, added to ●—● after the induction period.

addition of glucose at 40 minutes causes an immediate decrease in the rate of α-amylase synthesis. Tetracycline immediately halts the synthesis of α-amylase when added at time zero or 40 minutes although there is some release of preformed enzyme (Fig. 1).

The addition of cAMP (3 mM) at time zero abolishes the biphasic pattern and causes a linear increase of α-amylase at a rate similar to phase 2 synthesis (Fig. 2). The same effect is produced by cGMP, AMP or ATP (all at 3 mM) but not adenine. In the presence of glucose and either cAMP or cGMP phase 1 synthesis is extended but phase 2 adopts the glucose repressed rate

shown in Fig. 1. These nucleotides increase the reaction rate of α-amylase hence Fig. 2 includes the control experiment in which cAMP is added to the enzyme prior to the assay. This curve retains the biphasic pattern.

DISCUSSION

α-Amylase synthesis in B. subtilis B20 closely resembles that described for B. amyloliquefaciens (12) and B. subtilis 168 (13). There are pools of α-amylase and protease mRNA in B. amyloliquefaciens which support synthesis of the enzymes for approximately 40 minutes (12). However, the pool in B. subtilis B20 supports translation for only 25 minutes, possibly because it is smaller or has a shorter half-life. During this period (phase 1) α-amylase synthesis is resistant to rifampicin (an RNA polymerase inhibitor) and to glucose repression. In contrast α-amylase synthesis is sensitive to tetracycline which is an inhibitor of ribosomal function. After 25 minutes the pool is exhausted and transcription coupled with translation is resumed. This second phase of synthesis is immediately susceptible to repression by glucose, rifampicin or tetracycline. The parallel effects of glucose and rifampicin suggest that in B. subtilis glucose exerts its repressive effect at the transcriptional level as it does in E. coli (8).

The existence of phases 1 and 2 has previously been ascribed to repression of transcription due to resuspension of the culture in fresh medium (12). However, the biphasic pattern can be abolished by the addition of cAMP, cGMP, AMP or ATP but not adenine. In view of these results, a more likely explanation would be that washing and resuspending the cells in fresh medium removes their nucleotide pools and they are unable to synthesise mRNA until this deficiency has been replenished. Thus, the addition of nucleotides to the medium allows rapid reconstitution of the pools and increased mRNA and α-amylase synthesis. This correction of the biphasic pattern of α-amylase synthesis by cAMP or cGMP demonstrates that these nucleotides are

capable of entering the cell and taking part in cellular processes. However, the rate of phase 2 α-amylase synthesis in the presence of glucose is unaffected by cAMP or cGMP and these nucleotides therefore have no specific regulatory role concerning glucose repression of α-amylase transcription.

In view of these results, the inability to detect cAMP in Bacillus spp. and the inability to demonstrate either adenyl cyclase negative or cAMP receptor protein negative mutants of B. subtilis (14) it would seem probable that in these bacteria some other molecule takes the place of cAMP.

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