Effect of Decoyinine on the Regulation of α -Amylase Synthesis in *Bacillus subtilis*†

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Decoyinine, an inhibitor of GMP synthetase, allows sporulation in *Bacillus subtilis* to initiate and proceed under otherwise catabolite-repressing conditions. The effect of decoyinine on α -amylase synthesis in *B. subtilis*, an event which exhibits regulatory features resembling sporulation initiation, was examined. Decoyinine did not overcome catabolite repression of α -amylase synthesis in a wild-type strain of *B. subtilis* but did cause premature and enhanced synthesis in a mutant strain specifically blocked in catabolite repression of α -amylase synthesis. Decoyinine had no effect on α -amylase enzymatic activity. Thus, it appears that the catabolite control mechanisms governing α -amylase synthesis and sporulation in *B. subtilis* differ in their responses to decoyinine and hence must consist at least partially of separate components.

In the gram-positive microorganism *Bacillus subtilis*, the dependent sequence of morphogenetic and biochemical events associated with endosporegenesis is normally repressed during rapid growth in the presence of excess nutrients (14). Decrease in the cellular growth rate at the onset of the stationary phase caused by limitation in the concentration of carbon, nitrogen, or phosphate has long been considered to trigger sporulation (for a review, see reference 12). While the mechanism controlling this switch from the pattern of gene expression associated with vegetative cells to that of sporulating cells remains to be elucidated, evidence exists indicating that the initiation of sporulation is accompanied by a decrease in the intracellular GTP pool (5). In addition, a variety of treatments, each of which causes a decrease in the intracellular level of GTP, can induce sporulation of *B. subtilis* even in the presence of excess nutrients (3, 4, 10, 11, 17).

The synthesis of the extracellular enzyme α -amylase by *B. subtilis* is also activated early in the stationary phase, temporally coincident with the initiation of sporulation, and it too is repressed by excess glucose (for a review, see reference 13). Modulation of *amyE*-encoded α -amylase synthesis in wild-type *B. subtilis* is due in part to regulated transcription by RNA polymerase σ^{43} from the *amyR* promoter/*cis*-regulatory region (W. L. Nicholson, Y.-K. Park, T. M. Henkin, M. Won, M. J. Weickert, J. A. Gaskell, and G. H. Chambliss, *J. Mol. Biol.*, in press). The temporal activation and glucose-mediated repression mechanisms can be decoupled by *gra-10*, a *cis*-acting mutation within *amyR* which abolishes glucose repression while leaving temporal activation intact even in the presence of excess glucose (8, 9; Nicholson et al., submitted). The following experiments were done to determine how a chemical treatment which triggers sporulation under otherwise catabolite-repressing conditions simultaneously affects expression of the α -amylase gene.

The isogenic strains WLN-4 (*sacA321 amyR1-amyE*⁺) and WLN-11 (*sacA321 gra-10-amyE*⁺) were inoculated into minimal S7 medium (4), containing 2% (wt/vol) glucose, from washed exponential-phase seed cultures grown in the

same medium. At mid-logarithmic growth phase (50 Klett units, no. 66 red filter), each culture was evenly subdivided into two flasks containing either 1/10 the culture volume of fresh S7 medium or a 1/10 volume of filter-sterilized S7 medium to which 2.5 mg of decoyinine per ml had previously been dissolved (final decoyinine concentration, 250 μ g/ml). Decoyinine (a generous gift from E. Freese) is an analog of adenosine which causes a decrease in intracellular GTP levels by inhibition of GMP synthetase (7). At regular intervals before and after the decoyinine addition, samples were removed from the cultures and the culture supernatants were assayed for α -amylase as described previously (8). At

FIG. 1. α -Amylase synthesis in strains WLN-4 (●, ○) and WLN-11 (▲, △) grown in S7 medium with (open symbols) or without (closed symbols) 250 μ g of decoyinine per ml, which was added at the time indicated by the heavy arrow. — — —, Growth of the strains in the presence (+) or absence (–) of decoyinine. The end of exponential growth in the absence of decoyinine is denoted by t_0 . The data points represent the average of two experiments.

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TABLE 1. Stimulation of sporulation by decoyinine^a

Strain (genotype)	Decoyinine addition	Heat ^b shock	CFU/ml	S/V ^c	Fold stimulation by decoyinine
WLN-4 (<i>amyR1-amyE</i> ⁺)	—	—	7.07×10^8		
	—	+	$<5.5 \times 10^1$	$<7.8 \times 10^{-8}$	
	+	—	1.72×10^9		$>3.67 \times 10^5$
	+	+	4.92×10^7	2.86×10^{-2}	
WLN-11 (<i>gra-10-amyE</i> ⁺)	—	—	1.11×10^9		
	—	+	$<5.5 \times 10^1$	$<4.95 \times 10^{-8}$	
	+	—	2.12×10^9		$>5.37 \times 10^5$
	+	+	5.63×10^7	2.66×10^{-2}	

^a Values are averages of two experiments.^b 80°C, 15 min.^c Ratio of heat-resistant spores to total viable cells.

16 h after the decoyinine addition, the frequency of heat-resistant spores in each culture was determined.

Decoyinine treatment stimulated sporulation by greater than 5 orders of magnitude in both the wild-type strain, WLN-4, and the *gra-10*-bearing strain, WLN-11 (Table 1), indicating that decoyinine could overcome catabolite repression of sporulation under these conditions. When the α -amylase activity produced by the two strains was examined, however, it was observed that the decoyinine addition did not significantly relieve glucose-mediated repression of α -amylase synthesis in the wild-type strain, WLN-4 (Fig. 1). In the *gra-10*-bearing strain, WLN-11, α -amylase synthesis was resistant to glucose repression in the absence of decoyinine and temporally activated, as was expected. The addition of decoyinine to strain WLN-11 caused an immediate activation of α -amylase synthesis, and α -amylase accumulated at approximately two- to threefold the rate that it accumulated in the WLN-11 culture grown without decoyinine (Fig. 1). Hence, decoyinine exerted a stimulatory effect on α -amylase synthesis only in the strain in which the catabolite repression component of α -amylase regulation had been rendered inoperable by the *gra-10* mutation.

The apparent stimulation of α -amylase activity in WLN-11 by decoyinine was not an artifactual effect exerted at the level of enzyme activity itself (Table 2). Taniguchi et al. (15) observed that purine nucleotides, most notably GMP, exhibited a dramatic inhibitory effect on the activity of *Bacillus circulans* F-2 amylase and a less-pronounced inhibitory effect on *B. subtilis* α -amylase. At the concentration used in the experiments (1.07 mM), decoyinine had no effect in vitro on *B. subtilis* WLN-11 α -amylase activity (Table 2). At the same concentration, GMP exerted a slight inhibitory effect on α -amylase activity (Table 2), consistent with the findings of Taniguchi et al. (15).

Earlier studies showed that triggering sporulation in the presence of excess glucose by decoyinine addition could induce the expression of some catabolite-repressed genes (e.g., those encoding aconitase, citrate synthase, and α -ketoglutarate dehydrogenase [16]) but not others (e.g.,

inositol dehydrogenase, acetoin dehydrogenase, and sorbitol dehydrogenase [6]). On the basis of the data presented in this report, we suggest that the catabolite repression mechanisms regulating α -amylase synthesis and sporulation in wild-type *B. subtilis* more closely resembles the latter class of enzymes mentioned above.

The isolation of the *gra-10* mutation (8) afforded a unique opportunity to examine the effect of decoyinine addition on the regulation of a postexponentially expressed gene whose catabolite repression component had been inactivated. The finding that α -amylase synthesis could be stimulated by decoyinine only when the catabolite repression mechanism had been inactivated by the *gra-10* mutation suggests that the drop in the GTP level associated with the end of exponential growth may play a role in temporally regulated gene activation and not necessarily in the relief of catabolite repression.

In addition to our findings with α -amylase synthesis, decoyinine has been shown to elicit the expression of *spo0H*(2), *spoVG*(18), and *citB*(1). Each of these genes is subject to temporal regulation. It is intriguing to speculate that mechanisms regulating the expression of these genes may share a common feature with the mechanism that regulates the temporal expression of α -amylase synthesis even though one of the genes, *spoVG*, is transcribed by a different form of RNA polymerase.

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TABLE 2. Effect of decoyinine, guanosine, and GMP on α -amylase activity in vitro

Compound added ^a	Amylase sp act	% Inhibition
None	83.6	
Decoyinine	83.6	0
Guanosine	83.6	0
GMP	79.9	4.4

^a All compounds were added at a concentration of 1.07 mM.

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