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# Catabolite Repression of Enzyme Synthesis Does Not Prevent Sporulation

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In the presence of excess glucose, a decrease of guanine nucleotides in *Bacillus subtilis* initiated sporulation but did not prevent catabolite repression of three enzymes. Therefore, the ultimate mechanism(s) repressing enzyme synthesis differs from that suppressing sporulation.

Sporulation of *Bacillus subtilis* remains suppressed in the presence of ammonium ions, phosphate, and carbohydrates such as glucose. Similarly, the synthesis of many catabolic enzymes remains repressed in the presence of glucose and other carbohydrates, a phenomenon that has been called catabolite repression (8). Owing to the similarity of these effects of carbohydrates, it appeared possible that a simple mechanism controls both sporulation and catabolite repression (10). Several correlations suggesting this possibility have been published. For example, the enzymes of the citric acid cycle are repressed during growth in media containing glucose and amino acids, and they increase during the early stages of sporulation (2). A good correlation exists between the capability of different sugars to repress acetoin dehydrogenase and to suppress sporulation (7).

It has been shown in our laboratory that sporulation can be initiated in the presence of excess glucose, ammonia, phosphate, and other nutrients by conditions causing a partial deprivation in the synthesis of purine and in particular of guanine nucleotides (1, 1a, 3, 9). Under these and also all usual sporulation conditions, the concentrations of guanosine di- and triphosphates decrease, whereas those of the other nucleotides decrease in some and increase in other cases (5). The possibility of initiating sporulation in the presence of glucose allows a novel approach to study the relationship between the control of catabolic enzymes and sporulation.

We have here investigated the induction of three catabolic enzymes (inositol dehydrogenase, acetoin dehydrogenase, and sorbitol dehydrogenase) in a medium containing amino acids. We show that the synthesis of these enzymes remains repressed in the presence of glucose even when sporulation is initiated by a deficiency in GMP synthesis.

The strains of *B. subtilis* used were 60015 (*trpC2 metC7*) and 61676 (*guaA3 metC7 purH1 trpC2*) (1).

Synthetic medium contained 10 mM ammonium sulfate; 5 mM potassium phosphate; 100 mM morpholinopropane sulfonate, adjusted to pH 7.0 with KOH; 2 mM $MgCl_2$; 0.7 mM CaCl; 50 μg of $MnCl_2$; 1 μM $ZnCl_2$; 5 μM $FeCl_3$; 2 μM thiamine-hydrochloride; and the following amino acids (in μg/ml): L-cystine, 40; L-arginine, 400; L-isoleucine, L-leucine, and L-valine, 200; L-glutamic acid, 800; L-histidine, L-lysine, L-phenylalanine, L-proline, and L-threonine, 100; L-aspartic acid, 665; L-alanine, 445; L-glycine, 375; L-serine, 525; L-tryptophan, 150; and L-methionine, 160. Strain 61676 was also supplied with 1 mM guanosine and 0.75 mM adenosine. Decoyinine was a gift from G. B. Whitfield of Upjohn Co., Kalamazoo, Mich.

Cells were inoculated from an overnight culture on tryptose blood agar base (Difco) plates into synthetic medium at an initial absorbancy at 600 nm ($A_{600}$) of 0.05. Growth was measured by the increase of $A_{600}$. Ten hours after addition of decoyinine or removal of guanosine, the viable cell titer was measured by diluting the cultures in 0.1 M potassium phosphate buffer, pH 6.5, plus 1 mM $MgCl_2$ and plating on tryptose blood agar base plates; the titer of heat-resistant spores was determined by heating the dilution tubes for 15 min at 75°C and plating.

For the assay of acetoin dehydrogenase, the bacteria of a 10-ml culture were harvested by centrifugation, washed twice with 0.5 M potassium maleate buffer (pH 6.5), and resuspended in 1 ml of the same buffer. The enzyme was assayed as described elsewhere (6).

For the assay of the other enzymes the bacteria of 10 to 20 ml of culture were harvested by centrifugation and washed twice with ice-cold 0.05 M potassium phosphate buffer plus 0.15 M NaCl, pH 8.0, and then resuspended in 0.9 ml of the same solution. After addition of 0.1 ml of 1 mg of lysozyme per ml, the suspension was incubated for 20 min at 37°C and then centrifuged

for 20 min at 48,000 × $g$. The supernatant was used for the enzyme assays. For inositol dehydrogenase, the assay mixture contained 50 mM inositol. NAD (0.5 mM), 100 mM Tris-chloride (pH 9.0), and cell-free extract (about 0.2 mg of protein per ml). The increase of the $A_{340}$ was recorded. Sorbitol dehydrogenase was determined similarly except that 50 mM D-sorbitol replaced the inositol. Protein was determined according to Kalb and Bernlohr (4) by measuring the $A_{230}$ and $A_{260}$.

To measure the induction of the three dehydrogenases, a culture of the standard strain 60015 growing in synthetic medium was divided into four equal parts to which the following compounds were added: (i) nothing, (ii) 50 mM enzyme inducer, (iii) 50 mM enzyme inducer plus 50 mM glucose, (iv) iii plus 400 μg of decoyinine per ml.

All cultures containing decoyinine sporulated well (20 to 50% spores at $t_{10}$), whereas the other glucose-containing cultures produced less than 0.005% spores (Fig. 1). Figure 1 demonstrates that inositol dehydrogenase and acetoin dehydrogenase were induced only in the cultures containing inducer but no glucose; both enzyme activities remained repressed in the glucose-containing culture, irrespective of the presence or absence of decoyinine. Sorbitol dehydrogenase was not as severely repressed by glucose as the other two enzymes; but it was synthesized in the culture containing glucose and decoyinine at no higher rate than in the culture containing glucose and no decoyinine.

The same experiments were repeated in strain 61676 (*purH guaA*), but in this case, sporulation was initiated by removal of guanosine. The strain was grown in synthetic medium containing 1 mM guanosine. When the $A_{600}$ of the culture was 0.5, 20 ml was taken for the determination of enzyme activity. The remaining cells were collected on a membrane filter, resuspended in the same volume of synthetic medium, and divided into four parts to which the compounds were added as above.

As in the experiments with decoyinine, only the cultures in which a deficiency in guanosine nucleotides had been produced contained a high titer of spores (Fig. 2). But no induction of inositol dehydrogenase and acetoin dehydrogenase could be observed in the cultures containing glucose, irrespective of the presence or absence of guanosine. For sorbitol dehydrogenase, the specific activities in the sporulating culture were

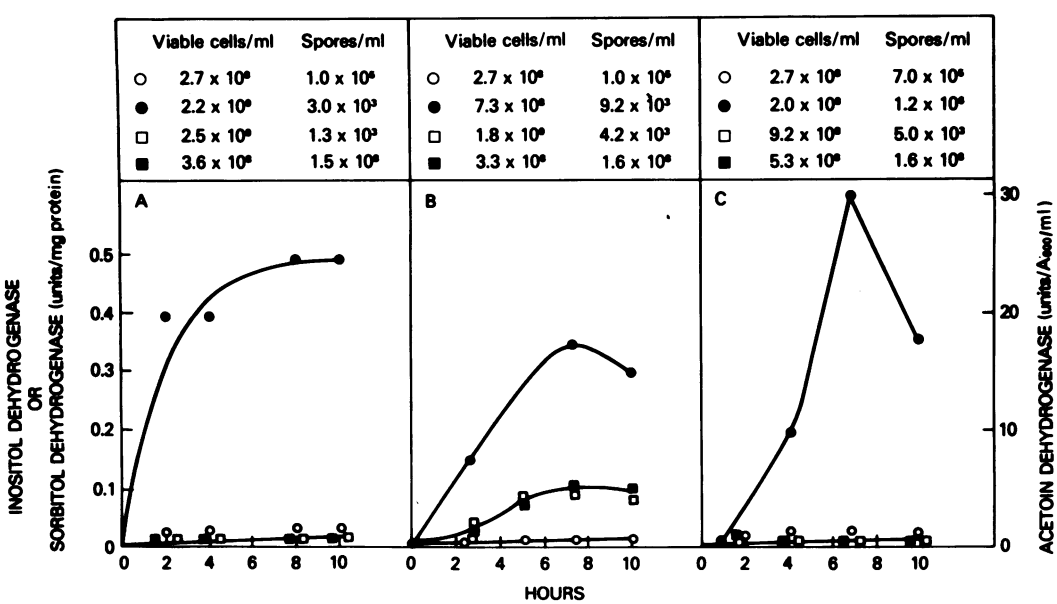


FIG. 1. *Sporulation and enzymatic activities after addition of the respective enzyme inducer and decoyinine or glucose or both. The standard strain 60015 was grown in synthetic medium to $A_{600}$ = 0.5. A 20-ml sample was taken for the determination of the enzymatic activities. The remaining cells were rapidly washed, resuspended in synthetic medium, and split into four parts containing the following additions: ○, none; ●, 50 mM enzyme inducer (i-inositol, D-sorbitol, acetoin resp.); □, 100 mM glucose plus 50 mM inducer; ■, 100 mM glucose, 50 mM inducer, and 1.8 mM decoyinine. Samples were withdrawn for enzyme assays at the times indicated. The viable and spore titers were determined 10 h after splitting the culture. (A) Inositol dehydrogenase. (B) Sorbitol dehydrogenase. (C) Acetoin dehydrogenase.*

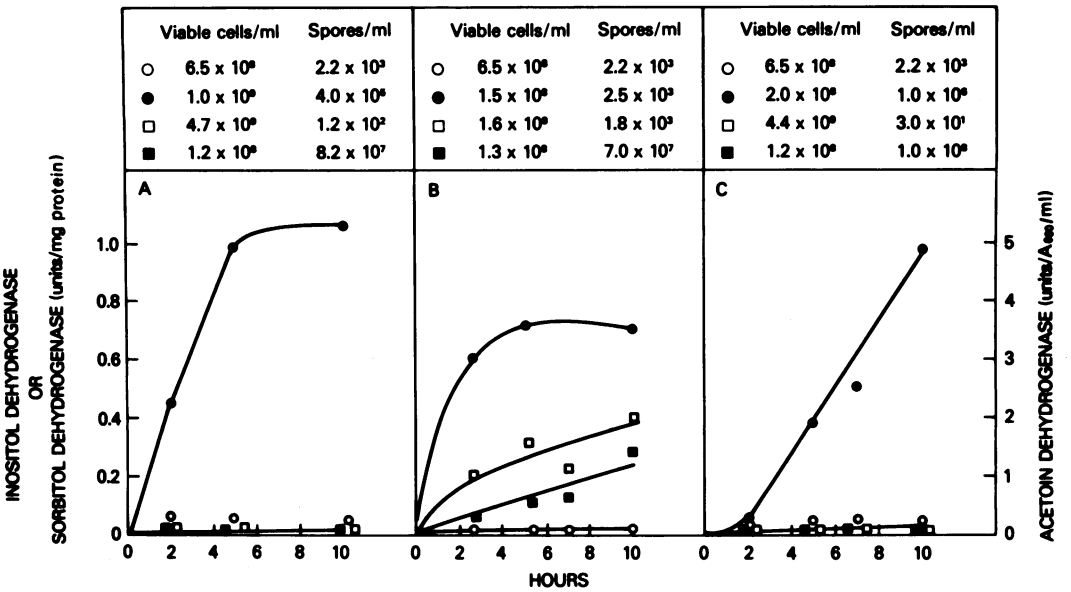


FIG. 2. *Sporulation and enzymatic activities of a gua auxotroph after addition of the respective enzyme inducer and removal of guanosine or addition of glucose or both. Strain 61676 was grown in synthetic medium containing 1 mM guanosine and 0.75 mM adenosine to $A_{600}$ = 0.5. A 20-ml amount was then taken for enzyme assays. The remaining culture was filtered through a filter (Millipore Corp.), washed with prewarmed synthetic medium, and resuspended in the same volume of synthetic medium containing 0.75 mM adenosine. This suspension was divided in four parts to which the following additions were made to give the stated final concentrations: ○, 1 mM guanosine; ●, 50 mM enzyme inducer-1 mM guanosine; □, 1 mM guanosine-50 mM inducer-100 mM glucose; ■, 50 mM inducer-100 mM glucose (but no guanosine). At the times indicated, samples were withdrawn for the determination of the enzymatic activities. (A) Inositol dehydrogenase. (B) Sorbitol dehydrogenase. (C) Acetoin dehydrogenase.*

not higher than those in the nonsporulating culture.

Our results demonstrate that the decrease in the synthesis of guanine nucleotides, which is sufficient to initiate sporulation (1, 1a, 5, 9), does not release the studied enzymes from catabolite repression by glucose.

Therefore, the ultimate mechanisms controlling sporulation and catabolite repression of enzymes differ. However, this does not rule out the possibility that some earlier step in the pathway(s) of catabolite repression might normally influence the pool of guanine nucleotides and thereby indirectly control sporulation.

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