JOURNAL OF BACTERIOLOGY, June 1985, p. 1302–1303
0021-9193/85/061302-02$02.00/0

Vol. 162, No. 3

NOTES

Induction of Penicillin-Binding Proteins under Catabolite-Repressed Conditions

CHRISTINE E. BUCHANAN

Department of Biology, Southern Methodist University, Dallas, Texas 75275

Received 31 January 1985/Accepted 20 March 1985

Decoyinine, an inhibitor of GMP synthetase, was used to induce sporulation under catabolite-repressed conditions in *Bacillus subtilis*. Sporulation-specific penicillin-binding proteins 4* and 5* were produced in abundance, and there was an increase in vegetative penicillin-binding proteins 2B and 3. These results, which were completely blocked by addition of guanosine, suggest that synthesis of penicillin-binding proteins is neither catabolite repressed nor directly dependent on the stringent response.

During sporulation of *Bacillus subtilis* there is a marked increase in the amount of two vegetative penicillin-binding proteins (PBPs) as well as synthesis of two new PBPs (2, 12). These PBPs appear at specific times during development of the spore and are probably required for normal forespore septation and cortical peptidoglycan synthesis, since formation of each of these structures is sensitive to inhibition by penicillin (3, 14).

Two techniques are commonly used to induce sporulation, nutrient exhaustion and downshift to a nutrient-deficient medium. In both methods the cells are being starved for a rapidly metabolizable source of carbon, nitrogen, or phosphorus. As a consequence, certain enzymes that are catabolite repressed during exponential growth will be induced; at least some of them are probably irrelevant to sporulation, whereas others may only be required for sporulation under starvation conditions. The aim of this study was to determine whether any PBPs fall into either of these classes of induced enzymes or whether induction of PBPs is unrelated to catabolite repression.

Freese and his colleagues (4) and Lopez et al. (5, 6) found that under most sporulation conditions described in the literature, there is a corresponding decrease in the intracellular levels of GDP and GTP. They elegantly demonstrated the importance of guanine nucleotide deprivation for the initiation of sporulation by treating cells with decoyinine, an inhibitor of GMP synthetase. Partially inhibitory concentrations of this drug induce sporulation even in the presence of excess glucose, ammonia, and phosphate (6, 8). Thus, this provides a means for distinguishing induction of sporulation-specific enzyme synthesis from catabolite derepression (7).

B. subtilis 168 *trp*$^-$ was obtained from J. H. Hageman, New Mexico State University, Las Cruces, N. Mex. Decoyinine U-7984 was a gift from J. Grady, The Upjohn Co., Kalamazoo, Mich. Guanosine was purchased from Sigma Chemical Co., St. Louis, Mo. All reagents for polyacrylamide gels were from BioRad Laboratories, Richmond, Calif. [^{3}H]benzylpenicillin (ethylpiperidine salt, 25 Ci/mmol) was obtained from Merck Sharp & Dohme Laboratories, West Point, Pa.

Cells were grown in 500 ml of S6 medium (8) containing 1% glucose in baffled 2.8-liter Fernbach flasks at 37°C in a G25 incubator shaker (New Brunswick Scientific Co., New Brunswick, N.J.) set at 400 rpm. Growth was monitored at 600 nm with a Coleman Junior II spectrophotometer. The spore titer was measured by heating a sample of each culture for 10 min at 80°C and comparing the viable count of the heated sample to that of an unheated one. In midlog phase 2 mM decoyinine was added to one flask, 2 mM decoyinine–1 mM guanosine were added to another flask, and a third flask was left untreated. Half the contents of each flask was harvested 3 h later, and the other half was harvested after 5 h. Membranes were collected by differential centrifugation after disruption of the cells by sonication (12). The PBPs were detected by incubating the membranes with [^{3}H]penicillin, separating the proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and fluorography (1, 12).

The effects of decoyinine on growth and sporulation of *B. subtilis* have previously been described in detail (6, 8); my results were essentially the same. Addition of decoyinine caused a threefold increase in the generation time of the culture, and a spore titer of 25 to 40% was obtained within 7 h. Guanosine completely blocked the effects of decoyinine; the sample treated with both decoyinine and guanosine was indistinguishable from the untreated control. The frequency of heat-resistant spores achieved in each of these two cultures was only 1% for the same time period.

The PBP changes that occurred in the decoyinine-treated sample were consistent with those changes observed in cells sporulating under other conditions (Fig. 1). Two new PBPs (4* and 5*) were produced, and there was an increase in vegetative PBPs 2B and 3. The increase in PBP 2B (the middle band of the PBP 2 triplet) is not readily apparent in Fig. 1 because the sampling time did not coincide with the peak in this protein. Decoyinine treatment did not generate any PBPs that have not also been induced under other sporulation conditions, nor were there any unexpected losses. The increase in PBP 2C during sporulation is highly variable and is apparently a strain-specific phenomenon, whereas the decline in PBP 2A is not restricted to sporulating cells but occurs whenever cells cease exponential growth (2, 9, 12). The sporulation-related changes that were observed could clearly be attributed to reduced levels of the guanine nucleotides, because none of them occurred when guanosine was added. These results indicate that the synthe-

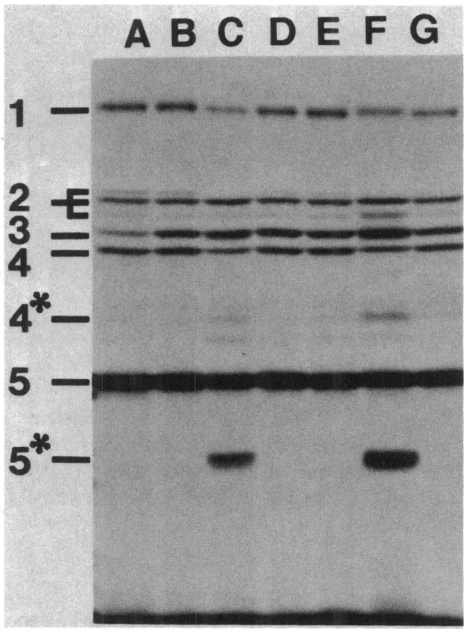

FIG. 1. Fluorograph of PBPs from membranes of *B. subtilis* 168 *trp*⁻ cells. Lanes: A, exponential phase (beginning of treatment); B, control; C, decoyinine; D, decoyinine plus guanosine (B–D, 3 h after initiation of treatment); E, control; F, decoyinine; G, decoyinine plus guanosine (E–G, 5 h after initiation of treatment). The three components of PBP 2 are referred to as PBPs 2A, 2B, and 2C, in descending order of molecular weight. Approximately the same amount of protein was loaded into each well. The bands not labeled with a number varied in repeated experiments and may reflect partial degradation of the PBPs.

sis of specific PBPs, which normally accompanies sporulation, is very likely to be an essential component of the developmental process and is not a consequence of the particular conditions of induction.

It has been suggested that decoyinine has an inhibitory effect on the terminal stages of peptidoglycan synthesis because treatment with decoyinine rapidly reduces net cell wall synthesis and prevents both autolysis and turnover of the wall (13). It is apparent from the present study that the PBPs are neither directly nor indirectly inhibited by decoyinine; indeed, it is likely that some of them must remain functional for subsequent sporulation-specific wall synthesis.

In addition to demonstrating that no PBPs are catabolite repressed, these results also indicate that induction of PBP synthesis depends more directly on reduction of GDP and GTP than on elevated levels of ppGpp or pppGpp, because these effectors of the classic stringent response do not increase during decoyinine treatment (6). This conclusion contrasts with a recent study of antibiotic production in *B. subtilis* Marburg (10). Antibiotic production is well known as an event that occurs only in sporulating cells (11). By guanosine deprivation of a guanine auxotroph, Ochi and Ohsawa (10) revealed that the mechanism for induction of sporulation is distinguishable from that for induction of antibiotic synthesis. Specifically, only sporulation is induced by a low level of GTP, whereas both events occur under conditions that evoke the stringent response.

In summary, induction of the PBPs does not reflect relief from catabolite repression nor dependence on the stringent response. This strengthens the proposal that specific PBPs are required for synthesis of spore structures and should therefore be induced under all conditions in which cells sporulate.

The technical assistance of Deanne Simmons is gratefully acknowledged.

This work was supported by Public Health Service grant AI-19829 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
2. **Buchanan, C. E., and M. O. Sowell.** 1983. Stability and synthesis of the penicillin-binding proteins during sporulation. J. Bacteriol. **156**:545–551.
3. **Dancer, B. N.** 1979. Requirement for peptidoglycan synthesis during sporulation of *Bacillus subtilis*. J. Bacteriol. **140**:786–797.
4. **Freese, E.** 1981. Initiation of bacterial sporulation, p. 1–12. *In* H. S. Levinson, A. L. Sonenshein, and D. J. Tipper (ed.), Sporulation and germination. American Society for Microbiology, Washington, D.C.
5. **Lopez, J. M., A. Dromerick, and E. Freese.** 1981. Response of guanosine 5′-triphosphate concentration to nutritional changes and its significance for *Bacillus subtilis* sporulation. J. Bacteriol. **146**:605–613.
6. **Lopez, J. M., C. L. Marks, and E. Freese.** 1979. The decrease of guanine nucleotides initiates sporulation of *Bacillus subtilis*. Biochim. Biophys. Acta **587**:238–252.
7. **Lopez, J. M., B. Uratani-Wong, and E. Freese.** 1980. Catabolite repression of enzyme synthesis does not prevent sporulation. J. Bacteriol. **141**:1447–1449.
8. **Mitani, T., J. E. Heinze, and E. Freese.** 1977. Induction of sporulation in *Bacillus subtilis* by decoyinine or hadacidin. Biochem. Biophys. Res. Commun. **77**:1118–1125.
9. **Neyman, S. L., and C. E. Buchanan.** 1985. Restoration of vegetative penicillin-binding proteins during germination and outgrowth of *Bacillus subtilis* spores: relationship of individual proteins to specific cell cycle events. J. Bacteriol. **161**:164–168.
10. **Ochi, K., and S. Ohsawa.** 1984. Initiation of antibiotic production by the stringent response of *Bacillus subtilis* Marburg. J. Gen. Microbiol. **130**:2473–2482.
11. **Schaeffer, P.** 1969. Sporulation and the production of antibiotics, exoenzymes, and exotoxins. Bacteriol. Rev. **33**:48–71.
12. **Sowell, M. O., and C. E. Buchanan.** 1983. Changes in penicillin-binding proteins during sporulation of *Bacillus subtilis*. J. Bacteriol. **153**:1331–1337.
13. **Uratani, B., J. M. Lopez, and E. Freese.** 1983. Effect of decoyinine on peptidoglycan synthesis and turnover in *Bacillus subtilis*. J. Bacteriol. **154**:261–268.
14. **Vinter, V.** 1964. Spores of microorganisms. XIV. Late stages of intrasporangial development of bacterial spores: their sensitivity to antibiotics. Folia Microbiol. **8**:58–71.