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Significance of HPr in Catabolite Repression of α-Amylase

MARTIN I. VOSKUIL AND GLENN H. CHAMBLISS*

Department of Bacteriology, University of Wisconsin-Madison, E. B. Fred Hall, Madison, Wisconsin 53706

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CcpA and HPr are presently the only two proteins implicated in *Bacillus subtilis* global carbon source catabolite repression, and the *ptsH1* mutation in the gene for the HPr protein was reported to relieve catabolite repression of several genes. However, α-amylase synthesis by *B. subtilis* SA003 containing the *ptsH1* mutation was repressed by glucose. Our results suggest HPr(Ser-P) may be involved in but is not required for catabolite repression of α-amylase, indicating that HPr(Ser-P) is not the sole signaling molecule for CcpA-mediated catabolite repression in *B. subtilis*.

***Bacillus subtilis* catabolite repression.** Control of the *B. subtilis* α-amylase gene (*amyE*) by rapidly metabolized carbon sources is mediated by a mechanism of transcriptional catabolite repression (12). A disruption of the *ccpA* gene relieves carbon catabolite repression of *amyE* and several other genes (1, 7, 8). The *ccpA* gene encodes catabolite control protein A, CcpA, which has been shown to interact specifically with a catabolite responsive element (*cre*) (9, 13). Although the repressor protein for catabolite repression, CcpA, along with its operator, *cre*, has been found, the signaling mechanism by which CcpA responds to changing levels of rapidly metabolized carbon sources has remained elusive. A second *trans*-acting mutation, *ptsH1*, has been implicated in the catabolite repression of gluconate kinase, glucitol dehydrogenase, mannitol-1-P dehydrogenase, the mannitol-specific phosphotransferase system permease, inositol dehydrogenase (4), and the levanase (10) and *xylA* (2) operons. The *ptsH1* mutation prevents the ATP-dependent phosphorylation of the Ser-46 residue of the phosphotransferase system phosphocarrier protein HPr (5) by changing residue Ser-46 to Ala-46 (14). Phosphorylation of HPr(His-15), required for phosphotransferase system-mediated sugar uptake, is unaffected in the *ptsH1* mutant (5). Both the *ptsH1* and *ccpA*::Tn*917* strains appear to be relieved of catabolite repression of several of the same genes (4). However, glycerol kinase and α-glucosidase synthesis remained sensitive to catabolite repression in both a *ptsH1* strain and a *ccpA*::Tn*917* strain (4). Due to the similarity in phenotypes, a connection between activation of CcpA and HPr(Ser-P) was inferred (4), and recent studies report that HPr(Ser-P) interacts specifically with CcpA (3, 6). The apparent phenotypic similarity of *ptsH1* and *ccpA* along with biochemical evidence has led to the conclusion that HPr(Ser-P) is an effector of CcpA. However, this has not been proven conclusively and certainly not for all genes subject to regulation by CcpA.

Bacterial strains, growth conditions, and assays. The *B. subtilis* strains used in this study were wild-type *B. subtilis* 168 (*trpC2*; laboratory stock) and HPr mutant strain SA003 (*sacB′-lacZ trpC2 ptsH1*) (4). Strains were grown in nutrient sporulation medium (15), and growth was monitored using a Klett-Summerson colorimeter (no. 66 red filter). The cultures were divided into two flasks during midexponential growth. A total of 9.6 ml of culture was added to each flask, one containing 0.4 ml of H_2O and the other containing 0.4 ml of 50% glucose for a final concentration of 2% glucose. Samples were removed for α-amylase assays at 30 min before to 5 h after the cultures were divided. Culture supernatants were assayed for α-amylase activity as described previously (11).

Effect of HPr on catabolite repression of α-amylase. It has previously been demonstrated that the α-amylase *cre* and CcpA are essential for glucose repression of α-amylase production. However, the HPr *ptsH1* mutation did not prevent glucose repression of α-amylase production. As seen in Fig. 1, the addition of 2% glucose caused repression of α-amylase production in both *B. subtilis* 168 and in the HPr mutant strain. A lower concentration of glucose (0.5%) was also used with identical results to those obtained with 2% glucose (data not shown). The inability of the *ptsH1* mutation to relieve catabo-

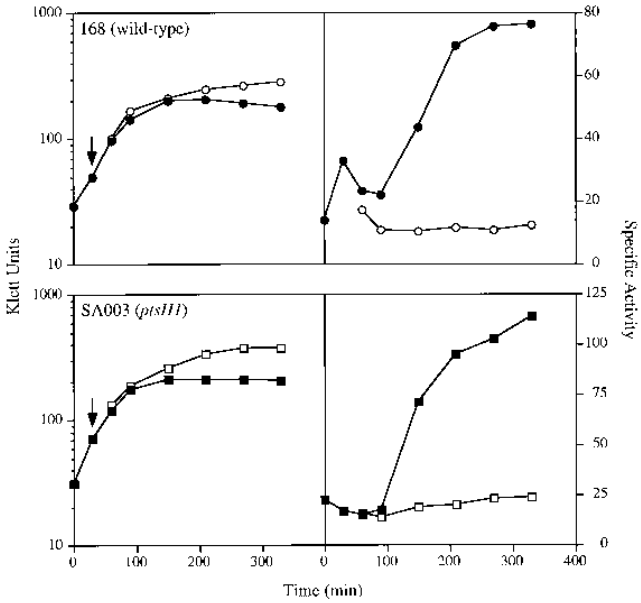

FIG. 1. Catabolite repression of α-amylase synthesis by glucose in *B. subtilis* 168 (circles) and SA003 (squares) grown in nutrient sporulation medium. *B. subtilis* cultures were grown to midexponential growth, indicated by arrows, and split into two flasks. The flasks contained either 0.4 ml of H_2O as a control (filled symbols) or 0.4 ml of 50% glucose to a final concentration of 2% (open symbols). The left panels are growth of the cultures reported in Klett units and the right panels are time courses of α-amylase specific activity. The data are averages of 12 α-amylase assays performed on culture supernatants from two independent growth curves.

* Corresponding author. Mailing address: Department of Bacteriology, University of Wisconsin-Madison, E. B. Fred Hall, Madison, Wisconsin 53706. Phone: (608) 263-5058. Fax: (608) 262-9865. Electronic mail address: ghchambl@facstaff.wisc.edu.

lite repression of the α-amylase gene, a gene repressed by CcpA at a *cre* element, indicates that another mechanism for activation of CcpA is necessary.

Models of catabolite repression. The ability of CcpA to repress a host of genes appears to be more complicated than can be explained by a model involving only one effector molecule. Control of CcpA DNA-binding activity by more than one mechanism may permit discrimination between different *cre* elements and hence would form a plausible explanation for data suggesting that HPr(Ser-P) is necessary for catabolite repression of some genes and not required for others. For example, CcpA could interact with HPr to regulate one set of genes while interacting with another effector to regulate a second set of genes that includes the α-amylase gene. Alternatively, the regulation by CcpA of a set of genes may require HPr(Ser-P) plus a second factor, possibly an effector molecule, while other genes only require the second factor. If one of these models is correct, one should be able to find predictable differences in the CcpA target sites of genes that require CcpA-HPr(Ser-P) complexes and those that interact with other CcpA-effector complexes. Several mechanisms can be proposed for the activation of CcpA; however, it seems likely that other factors besides HPr(Ser-P) can activate CcpA in *B. subtilis* catabolite repression.

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