NOTES

Catabolite Repression of the Bacillus subtilis gnt Operon Mediated by the CcpA Protein

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Inducer exclusion was not important in catabolite repression of the Bacillus subtilis gnt operon. The CcpA protein (also known as AlsA) was found to be necessary for catabolite repression of the gnt operon, and a mutation (crsA47, which is an allele of the sigA gene) partially affected this catabolite repression.

In the genus Bacillus, catabolite repression is observed not only in adaptive enzyme synthesis but also at the onset of sporulation (4). Catabolite repression of carbon utilization enzymes might be explained in part by inducer exclusion, because many of these enzymes are induced by their substrates. Recently, the cis-acting sequences responsible for catabolite repression of the Bacillus subtilis amyE (22), gnt (16, 17), xyl (12), hut (19), and bgI5 (14) operons were determined; these sequences are similar to the B. subtilis catabolite repression consensus sequence that was deduced from the results of site-directed mutagenesis of a catabolite repression operator for amyE (22). Moreover, a trans-acting gene product (CcpA [also known as AlsA]) which is involved in catabolite repression of the amyE gene was identified (11). A mutation, crsA47, which causes sporulation to be resistant to catabolite repression was isolated (21); this mutation is an allele of the sigA gene (also known as rpoD) encoding the σ^A factor of B. subtilis RNA polymerase (13). These facts imply that this organism might possess a common regulatory mechanism underlying catabolite repression. This mechanism is probably not one involving a cyclic AMP (cAMP) receptor protein-cAMP complex, such as which has been well demonstrated to exist in enteric bacteria (2), because vegetative cells of Bacillus species contain neither detectable cAMP nor adenylate cyclase under unstressed conditions (1, 20).

The B. subtilis gnt operon is responsible for gluconate catabolism and is subject to catabolite repression mediated by glycolytic intermediate(s), e.g., fructose-1,6-bisphosphate (18). Miwa and Fujita (17) proposed that catabolite repression of the gnt operon, which involves a sequence located within the first gene of this operon (gntR), might be partially explained by a transcription blockage mechanism, such as the transcription roadblock mediated by the PurR protein (10, 17); however, this remains to be proven. In this communication, we demonstrated that the gluconate-inducible system of the gnt operon did not participate in the catabolite repression of this operon but that the CcpA protein and the σ^A factor of RNA polymerase were involved in this catabolite repression.

The bacterial strains used are listed in Table 1. Strain YF223 (gntR43L) was constructed as follows. To introduce the gntR43L mutation into chromosomal DNA together with transformation of the gntOi mutation (Table 1) to the wild type, strain YF179 (gntOt) was transformed with plasmid pYF171(43L) carrying the gntR43L mutation (23) after linearization with BamHI. Because gntR43L transformants exhibited faster growth than strain YF179, they were enriched by overnight cultivation in S6 medium (5) plus tryptophan, methionine (50 μg/ml each), and 0.05% Casamino Acids (Difco) containing 25 mM gluconate and then plated on N medium (6) plus tryptophan, methionine, and 0.002% yeast extract containing 25 mM gluconate. Several larger colonies were assayed for constitutive synthesis of gluconate kinase, and then the DNAs of positive clones were isolated. The region surrounding the gnt operon of these DNAs was subjected to amplification by PCR, and then clones whose amplified fragments were of the same size as that of the wild-type gnt operon on 2% agarose gel electrophoresis were selected; the fragment carrying the gntOt mutation is 8 bp longer than that carrying the wild-type gnt operon. For the gluconate kinase assay, the cells were grown at 37°C to an A_600 of 1 in S6 medium containing Casamino Acids and the required amino acids, with or without 10 mM gluconate or with 10 mM each of gluconate and glucose (18). The preparation of a cell extract with an A_600 of 9 and spectrophotometric assays of gluconate kinase were performed as described previously (18).

The B. subtilis gntRKPZ operon is negatively regulated by the gnt repressor (GntR), which is antagonized by gluconate (9, 15). To examine whether inducer exclusion is involved in catabolite repression of the gnt operon, we used two gntR mutations, the mutated GntR proteins of which have completely lost their DNA-binding ability. The gntRI mutation is a frame-shift mutation consisting of a GTAC insertion between nucleotides 153 and 154, where the cis sequence for catabolite repression of the gnt operon is localized (8, 17). The other mutation used is the gntR43L mutation, which causes the amino acid substitution of Ser-43 → Leu in the GntR protein (23). The gntRI and gntR43L mutations were introduced into the B. subtilis chromosome by transformation to yield strains YF176 and YF223, respectively; construction of strain YF176 has been previously reported (8). Table 2 shows that gluconate kinase, which is the product of the second gnt gene (gntK), was largely induced by gluconate in our standard strain 60015, and this induction was repressed more than 27-fold by the addition of glucose. Even if the gntR43L mutation caused constitutive synthesis of gluconate kinase in strain YF223, this synthesis

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TABLE 1. Bacterial strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>60015</td>
<td>trpC2 metC7</td>
<td>Our standard strain</td>
</tr>
<tr>
<td>YF176</td>
<td>gntR1 trpC2 metC7</td>
<td>This study</td>
</tr>
<tr>
<td>YF223</td>
<td>gntR43L trpC2 metC7</td>
<td>This study</td>
</tr>
<tr>
<td>1A250</td>
<td>trpC2 alsL1 iibBΔ1</td>
<td>BGSC</td>
</tr>
<tr>
<td>1A147</td>
<td>alsA1 trpC2 alsL1 iibBΔ1</td>
<td>BGSC</td>
</tr>
<tr>
<td>WLN-29</td>
<td>araG932 trpC2</td>
<td>G. H. Chambliss</td>
</tr>
<tr>
<td>gnt-26::Tn917ac</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ccpA::Tn917)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YF168</td>
<td>trpC2 lys-1</td>
<td>I. Takahashi via R. Doi (21)</td>
</tr>
<tr>
<td>CS3</td>
<td>crsA47 trpC2 lys-1</td>
<td>I. Takahashi via R. Doi (21)</td>
</tr>
<tr>
<td>YF179</td>
<td>trpC2 metC7 gntOF</td>
<td>This study</td>
</tr>
</tbody>
</table>

* Bacillus Genetic Stock Center (Ohio State University).
* Sister strain of strain CS3 in PBS1-mediated transduction.
* The gntOF mutation carries an ATACAAAGT insertion between the two AccI sites located in the gnt operon region. Details of the isolation and characterization of this mutation will be presented elsewhere.

was repressed 44-fold by the addition of glucose (Table 2), indicating that catabolite repression almost normally occurs even in the absence of the gluconate-inducible system of the gnt operon. (The residual activity in strain YF223 grown with glucose was a little bit higher than that of strain 60015, but it was very difficult to judge that there was a significant difference in their repression ratios.) However, constitutive synthesis of gluconate kinase, caused by the gntR1 mutation, could be repressed only partially by the addition of glucose (repression ratio = 2.3) (Table 2), indicating that this mutation only partially affects catabolite repression of the gnt operon. This difference in the effect on this catabolite repression between the gntR43L and gntR1 mutations can be explained well by our previous idea that the gnt sequence (nucleotides 140 to 155) might be the cis sequence for catabolite repression of the gnt operon (17), because the gntR1 insertion mutation disrupts this cis sequence. Therefore, it was concluded that inducer exclusion does not affect catabolite repression of the gnt operon, or that it affects it only a little if it affects it at all.

Henkin et al. (11) reported that a trans-acting gene product, CcpA, is involved in catabolite repression of the B. subtilis α-amylase gene. The ccpA gene is allelic to the alsA gene (24), which is involved in the regulation of acetalactate synthase activity. Thus, we examined whether this gene product was involved in catabolite repression of the gnt operon. As shown in Table 2, catabolite repression of the gnt operon was completely abolished in strains WLN-29 (ccpA::Tn917) and 1A147 (alsA1), while it was normally observed in strain 1A250 (repression ratio > 27), which is isogenic to strain 1A147, indicating that catabolite repression of the gnt operon does not occur without the CcpA (or AlsA) protein.

Since a mutation that causes sporulation to be resistant to catabolite repression (crsA47) is an allele of the sigA (or rpoD) gene encoding the σ^B factor of the B. subtilis RNA polymerase (13), we examined whether it affects catabolite repression of the gnt operon. As shown in Table 2, catabolite repression of the gnt operon of strain CS3 (crsA47) was partially relieved (repression ratio = 2.7), in contrast to that of its isogenic strain, YF168 (repression ratio > 35), indicating that the crsA47 mutation partially affected catabolite repression of the gnt operon.

Almost normal catabolite repression of the chromosomal gnt operon was observed for the gntR43L mutation, which causes the gnt operon to completely lose its DNA-binding ability, implying that inducer exclusion is not involved in its catabolite repression (Table 2). In our most recent communication (17), we stated that inducer exclusion might be involved in catabolite repression of the gnt operon. This wrong conclusion was derived from the finding that the gntR1 insertion mutation, which also completely abolishes the gluconate induction system, affected catabolite repression of the chromosomal gnt operon (Table 2), which is now explained by an insertion into the cis sequence necessary for the catabolite repression of this operon.

The ccpA::Tn917 and alsA1 mutations completely overcame the catabolite repression of the gnt operon (Table 2), suggesting that the CcpA (or AlsA) protein probably plays a central role in catabolite repression of the gnt operon. Although B. subtilis catabolite repression involving the consensus cis sequence seems to be generally mediated by the negative regulator, CcpA (3, 11, 17a), this protein when synthesized in Escherichia coli could not bind specifically to the cis sequence for catabolite repression of the gnt operon (17a). The direct function of CcpA in B. subtilis catabolite repression remains to be revealed.

The crsA47 mutation significantly affected the catabolite repression of this operon (Table 2); this mutation is a mutation of the sigA gene encoding the B. subtilis major sigma factor (σ^B) which recognizes the promoter of the gnt operon (7, 15). However, it remains to be seen whether the crsA47 mutation affects gnt transcription directly or whether it affects transcription of the ccpA gene and/or the gene(s) for another protein factor(s).

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REFERENCES


