Preliminary Communication

RpoS-Dependent Expression of the Second Lysine Decarboxylase Gene in Escherichia coli

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The second lysine decarboxylase gene (ldc) is at 4.7 min on the Escherichia coli chromosome [Kikuchi et al., J. Bacteriol. 179, 4486–4492 (1997)]. This report shows that the expression of ldc as well as cadA was induced at stationary phase in the wild type of E. coli. The ldc was not expressed in a rpoS deletion mutant of E. coli at any growing stage. In contrast, cadA was expressed in the rpoS mutant. Thus, we conclude that the expression of ldc but not cadA at stationary phase is regulated by a RpoS-dependent mechanism(s) in E. coli.

Key words: Escherichia coli; lysine decarboxylase gene; rpoS; stationary phase; expression

There are two types of amino acid decarboxylase in Escherichia coli, constitutive and inducible. The former includes decarboxylases speA (for arginine), speC (for ornithine), and speD (for S-adenosylmethionine). The latter includes adi (for arginine), speF (for ornithine), and cadA (for lysine). In E. coli, a previously characterized CadA of 78 kDa is encoded by cadA and participates in the synthesis of cadaverine from lysine. This enzyme is known to be inducible under anaerobic conditions at low pH (pH 5.5) and by adding lysine to the culture medium. While constructing the cadA-deletion mutant E. coli WC196C from strain WC196, which is a lysine analog (S-aminomethyl-L-cysteine)-resistant strain of W3110, we noticed that the strain WC196C could still degrade lysine to cadaverine. In our previous report, we demonstrated the existence of a second lysine decarboxylase gene (ldc) at 4.7 min on the E. coli chromosome besides cadA. We also found that the amino acid sequence of Ldc of 80 kDa deduced from the DNA sequencing data of ldc has 69.4% identity with that of CadA. However, no information on the expression mechanism(s) of ldc was available except it is expressed without lysine in the medium. Recently, Prüß et al. reported that a flagellar transcriptional activator gene, fliD, mediates the reduction of the rate of cell division of E. coli, and that the fliD gene product FliD is a positive regulator of the expression of cadA. They also mentioned that both a fliD- and a cadA-deletion mutant of E. coli show no CadA activity but still have lysine decarboxylase activity, which is caused by a potential second lysine decarboxylase gene. These findings and the presence of ldc in E. coli suggest that the ldc expression in E. coli is regulated by another gene(s) or its product(s). Therefore, we studied the expression mechanism(s) of ldc in E. coli. Here, we demonstrate that ldc is expressed during entry into stationary growth phase under aerobic conditions and its expression is regulated by a rpoS-dependent mechanism(s) in E. coli. We also confirm in this study that the expression of cadA induced at stationary phase is independent of RpoS.

We started to analyze the timing of the expression of ldc and cadA in the growing cells of E. coli by immunoblotting analysis using anti-Ldc antiserum in strains W3110 and WYK010 which are the wild type and its cadA deletion mutant, respectively. In this study, the anti-Ldc antiserum was found to cross-react with CadA as well as Ldc, although the intensity of the cross-reaction with CadA is approximately 1/3 of that to Ldc. The Ldc protein was detected in both strains during entry into the stationary phase, but not at exponential phase (Fig. 1, A, B, C, and D). In addition, CadA protein was also detected at stationary phase in W3110 strain (Fig. 1, A and C), which might be due to the expression of cadA mediated by fliD at the stationary phase, as suggested by Prüß et al.. From the data, we found that the expression of both ldc and cadA is induced at stationary phase in E. coli under normal growth conditions.

It is known that rpoS, which codes for a sigma factor of RNA polymerase is required for the expression of the genes that are induced at stationary phase in bacteria. Accordingly, we studied whether rpoS regulates the ldc expression by using the rpoS deletion mutant of E. coli harboring a plasmid that contains ldc::lacZ fusion gene. To construct a plasmid containing an ldc::lacZ fusion gene, we first obtained a whole structural gene of lacZ, which was excised as a 3083-bp [SmaI-PstI] fragment from the cloning vector pMC1871 (Pharmacia). The PstI site of the fragment was blunted with T4 DNA polymerase in the presence of dNTPs, and inserted into the EcoRV site of the plasmid pHLDC, which is at the 207th nucleotide from the translation start codon of ldc, to create a plasmid pHLZ1. In this

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Abbreviations: Ldc, second lysine decarboxylase; CadA, inducible lysine decarboxylase; RpoS, sigma-38 subunit of RNA polymerase; LB, Luria-Bertani; bp, base pair(s).
plasmid, lacZ was confirmed to be fused to ldc in frame with lacZ at the position corresponding to between the 69th and 70th N-terminal amino acid residues of ldc by DNA sequencing. Next, the [EcoRI-HindIII] fragment of pHLZ1 containing the ldc::lacZ fusion gene and promoter region of ldc was cloned into the the EcoRI-HindIII site of a low-copy number plasmid vector pMW119 (Wako Pure Chemical Industries, Osaka, Japan) to construct a plasmid, pMLZ1. Finally, the strains E. coli MC4100 [F−, D (arg-lac)U169 araD139 rpsL150 ptsF25 fbiB5301 rbsR deoC relA1] and MC8301,10 which is an isogenic strain of MC4100 except for rpoSI3::Tn10, were transformed with pMLZ1. In this study, we confirmed that the strain MC8301 has no catalase activity. The cells of E. coli MC4100 (pMLZ1) and MC8301(pMLZ1) were grown with shaking at 37°C in LB medium containing ampicillin (100 µg/ml). At the time indicated in Fig. 2, cells were harvested and the β-galactosidase activity in the cells was assayed. As shown in Fig. 2, the strain MC4100 (pMLZ1) expressed lacZ at the basal level at exponential phase, but the expression level of lacZ markedly increased during entry into the stationary phase. In contrast, E. coli MC8301(pMLZ1) showed no significant amount of the β-galactosidase activity at either exponential or stationary phase (Fig. 2), indicating that the expression of ldc is dependent on rpoS. We confirmed this by assaying the lysine decarboxylase activity in E. coli strains WYK010 and its rpoS mutant (rpoSI3::Tn10) strain WYK12 which was constructed by Pl1vir phase-mediated transduction. As shown in the Table, a significant amount of lysine decarboxylase activity was detected at stationary phase in WYK010 (Table, lane 1). However, no activity of the enzyme was detectable in WYK12 (Table, lane 2). In the rpoS mutant MC8301 strain of E. coli, which has both cadA and ldc, the CadA protein but not Ldc was detected by immunoblotting analysis (Fig. 3). Thus, we conclude that the expression of ldc induced at stationary
Expression of ldc in E. coli

Table. Lysine Decarboxylase Activities at Stationary Phase in W3110 Derivatives

<table>
<thead>
<tr>
<th>Lane</th>
<th>Strain</th>
<th>Reference</th>
<th>Lysine decarboxylase activity (nmol/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WYK010 (W3110 cadA)</td>
<td>5</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>WYK012 (WYK010 rpoS)</td>
<td>this study</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

a. Each cell was grown at 37°C for 16 h under aerobic conditions in LB medium.
b. The crude extract of the cell was prepared as described previously. Lysine decarboxylase was assayed as described previously.
c. not detected.

Fig. 2. Course of Activity of β-Galactosidase in rpoS + and rpoS - E. coli Strains Harbouring the Plasmid pMLZ1 Which Contains ldc::lacZ.

The E. coli strains MC4100(pMLZ1) (open symbols) and MC8301(pMLZ1) (closed symbols) were grown with aeration at 37°C in LB medium. At the time indicated in the figure, the cell growth (circles) and the β-galactosidase activity (squares) were measured. The cell growth was monitored at 600 nm and the β-galactosidase activity was assayed by the method described by Miller. One unit of the enzyme activity was defined as the amount forming 1 nmol of nitrophenol released from o-nitrophenyl-β-d-galactopyranoside at 28°C in 1 min. The specific activity of the enzyme is indicated as units per mg of the total cellular protein.

Fig. 3. Western Immunoblotting Analysis of Ldc and CadA in rpoS Mutant of E. coli, Using Antisera Against Ldc.

The E. coli strains MC4100 (rpoS +) and MC8301 (rpoS -) were grown aerobically in LB broth at 37°C for 16 h. The cells were harvested and sonicated as described in the legend of Fig. 1. The sonicated extracts were then run on SDS-PAGE. After blotting on the nylon membrane, Ldc and CadA were detected by antisera against Ldc as mentioned in the legend of Fig. 1. Lanes 1 and 2 are from MC4100 and MC8301, respectively.

phase is regulated by rpoS.

It is known that in bacteria at least 30 genes are under the control of the sigma factor, σ70, encoded by rpoS. The σ70-dependent genes include katE (encoding catalase PHI), xth (exonuclease III), osmoregulatory pathway genes otsBA, fic (flammentation induced by cAMP), and so on. It is known that σ7 is structurally similar to the major sigma factor σ70 and that the promoters recognized by the σ7 subunit (E7) has some sequence similarity to the promoters recognized by the σ70 subunit (E70), such as TTGACA (the consensus −35 sequence for σ70) and TATAAT (the consensus −10 sequence for σ70). Recently, Hiratsu et al. reported that the −10 hexamer TATACT, but not the −35 sequence of fic, is required for the recognition of E70. Tanaka et al. also showed that the TATACT and TATAAT sequences of the −10 region are very important for promoter recognition by E70. In the promoter region of our ldc, −10 sequence, TATGAT is also conserved, although A of TATAAT is replaced by G.

Although the physiological role of Ldc is not clear yet, it is interesting to note that the amino acid sequence of Ldc showed 69.4% identity of that of CadA and that the expression of ldc is specific at stationary phase as well as that of cadA. The findings and our data presented here suggest that the level of expression of ldc as well as cadA correlates with the rate of cell division of E. coli.

Acknowledgments

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References


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