Pyrimidine, purine and nitrogen control of cytosine deaminase synthesis in *Escherichia coli* K12. Involvement of the *glnLG* and *purR* genes in the regulation of *codA* expression*

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Abstract. Cytosine deaminase, encoded by the *codA* gene in *Escherichia coli* catalyzes the deamination of cytosine to uracil and ammonia. Regulation of *codA* expression was studied by determining the level of cytosine deaminase in *E. coli* K12 grown in various defined media. Addition of either pyrimidine or purine nucleobases to the growth medium caused repressed enzyme levels, whereas growth on a poor nitrogen source such as proline resulted in derepression of cytosine deaminase synthesis. Derepression of *codA* expression was induced by starvation for either uracil or cytosine nucleotides. Nitrogen control was found to be mediated by the *glnLG* gene products, and purine repression required a functional *purR* gene product. Studies with strains harbouring multiple mutations affecting both pyrimidine, purine and nitrogen control revealed that the overall regulation of cytosine deaminase synthesis by the different metabolites is cumulative.

Key words: *Escherichia coli* - *codA* - *glnLG* - *purR* - Gene regulation - Cytosine deaminase

Cytosine deaminase catalyzes the hydrolytic deamination of cytosine to uracil and ammonia. Wildtype strains of *Escherichia coli* and *Salmonella typhimurium* can utilize cytosine as the sole pyrimidine and nitrogen source (Beck et al. 1972b; Gutnick et al. 1969) and they are sensitive to the cytosine analogue 5-fluorocytosine. Mutants defective in cytosine deaminase activity, *codA* mutants, cannot metabolize cytosine and they are resistant to 5-fluorocytosine (Neuhard and Ingraham 1968).

The *codA* gene in *E. coli* is located at 7 min on the chromosome, closely linked to the *lac* operon (de Haan et al. 1972). In *S. typhimurium* this region is absent from the chromosome and the *codA* gene in this bacterium is located at approximately 70 min (Beck et al. 1972a).

The utilization of cytosine for UMP synthesis, and thereby for the synthesis of all other pyrimidine nucleotides, requires the consecutive action of cytosine deaminase and uracil phosphoribosyltransferase. The synthesis of cytosine deaminase in *S. typhimurium* (West and O'Donovan 1982) and uracil phosphoribosyltransferase in *E. coli* (Rasmussen et al. 1986), have been reported to be negatively controlled by pyrimidine nucleotides; under conditions of low pyrimidine nucleotide pools these two pyrimidine salvage enzymes are found at increased levels.

In the course of our studies on cytosine metabolism we observed that the presence of the purine base hypoxanthine in the growth medium renders wildtype cells of *E. coli* resistant to 5-fluorocytosine. This led to the discovery that *codA* expression is repressed by purines, and that the repression involves a regulatory protein, encoded by the *purR* gene. The *purR* gene product controls the synthesis of purine biosynthetic enzymes (Kilstrup et al. 1989).

In the present study we have analyzed the regulation of *codA* expression in *E. coli* by measuring cytosine deaminase activity in wild-type and mutant cells grown in a variety of growth conditions. The results indicate that the regulation of cytosine deaminase synthesis is quite complex. The enzyme level is regulated in response to the intracellular pools of both pyrimidine and purine nucleotides and to nitrogen limitation.

Materials and methods

Bacterial strains and phages. The *Escherichia coli* K12 strains used are listed in Table 1. Generalized transduction by phage P1 *vir* (Miller 1972) was used in all strain constructions.

Media and growth conditions. The growth medium was AB medium (Clark and Maaløe 1967) containing thiamine (1 μg/ml) and glucose (0.2%) or glycerol (0.2%).

Enzyme assays. Exponentially growing cells were harvested at a cell density of about 5 x 10⁸/ml. Following wash in 0.9% NaCl cells were frozen at −20°C. Crude cellular extracts for enzyme assays were prepared by sonic disruption of cells.

* This paper is dedicated to Professor John Ingraham, Department of Bacteriology, University of California, Davis, on the occasion of his retirement, in recognition of his many contributions in the field of bacterial growth and metabolism

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resuspended in 100 mM Tris-HCl buffer pH 7.8 containing 1 mM EDTA and 1 mM dithiothreitol.

Cytosine deaminase activity was determined by measuring the amount of uracil formed per minute in a reaction mixture containing 5.0 mM (14C)cytosine (0.45 Ci/mol), 100 mM Tris-HCl pH 7.8 and about 0.5 mg cellular protein/ml. At different times during incubation at 37 °C 5 gg mol), 100 mM Tris-HCl pH 7.8 and about 0.5 mg cellular reaction mixture containing 5.0 mM (14C)cytosine (0.45 Ci/

Results and discussion

Regulation of cytosine deaminase synthesis in Escherichia coli

The sole route by which cytosine can be metabolized in Escherichia coli and Salmonella typhimurium is through deamination to uracil and ammonia, catalyzed by cytosine deaminase (codA). Thus, cytosine deaminase enables cells to utilize cytosine as a source of purines (Beck et al. 1972b) and nitrogen (Gutnick et al. 1969). In order to investigate whether expression of the enzyme in E. coli is regulated by intermediates of the two pathways, the level of cytosine deaminase was determined in strain SO5075 grown exponentially in various defined media. The results obtained are summarized in Table 2. As shown by exp. 1, 2 and 3 addition of the pyrimidine bases uracil or cytosine reduces the level of the enzyme. This indicates that codA expression is repressed during growth on exogenous pyrimidines, and that cytosine deaminase synthesis is not induced by the substrate.

A significant repression is observed also in cells grown in the presence of the purine base hypoxanthine (exp. 4). This agrees with previous observations showing that exogenous purine compounds affect codA expression (Kilstrup et al. 1989).

Finally it can be seen (exp. 5 and 6, Table 2) that growth on a poor nitrogen source such as proline resulted in 3-fold derepression of cytosine deaminase synthesis. Thus codA expression in E. coli appears to be under both pyrimidine, purine and nitrogen control.

Cytosine deaminase activities in mutants with altered pyrimidine nucleotide pools

To gain further insight into the nature of the effector(s) involved in pyrimidine control of codA expression the level of cytosine deaminase was determined in E. coli mutants which contain altered intracellular pyrimidine nucleotide pools. Strain SO5072 carries a pyrH mutation which renders UMP kinase more thermolabile. Thus, the UMP kinase activity of SO5072 grown at 37°C is low, resulting in cells with low UTP and high UMP pools. As shown in Table 3, the level of cytosine deaminase in SO5072 grown at 37°C is 3-fold higher than in strain SO5075 which carries a normal UMP kinase.

Table 2. Regulation of cytosine deaminase synthesis*

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Carbon source</th>
<th>Nitrogen source</th>
<th>Supplements</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose</td>
<td>Ammonia</td>
<td>None</td>
<td>68</td>
</tr>
<tr>
<td>2</td>
<td>Glucose</td>
<td>Ammonia</td>
<td>Uracil</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>Glucose</td>
<td>Ammonia</td>
<td>Cytosine</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>Glucose</td>
<td>Ammonia</td>
<td>Hypoxanthine</td>
<td>23</td>
</tr>
<tr>
<td>5</td>
<td>Glucose</td>
<td>Ammonia</td>
<td>Proline</td>
<td>62</td>
</tr>
<tr>
<td>6</td>
<td>Glucose</td>
<td>Ammonia</td>
<td>Proline</td>
<td>185</td>
</tr>
</tbody>
</table>

* Strain SO5075 was grown at 37°C in minimal medium containing thiamine and methionine. The carbon source was either 0.2% glucose or 0.2% glycerol. When the nitrogen source was ammonia, AB medium was employed; when the nitrogen source was proline (10 mg/ml), the NH4Cl in the AB medium was replaced by equimolar amounts of KCl. Supplements were added in the following final concentrations: Uracil, 20 μg/ml; cytosine, 20 μg/ml; hypoxanthine, 50 μg/ml; proline, 10 mg/ml. Nanomol cytosine deaminated per min per mg protein

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