Intracellular Inducer Hg$^{2+}$ Concentration Is Rate Determining for the Expression of the Mercury-Resistance Operon in Cells

HONGRI YU,† LIEN CHU,† AND TAPAN K. MISRA*

Department of Microbiology and Immunology, University of Illinois College of Medicine, Chicago, Illinois

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Experiments involving mercury resistance mer operon-lacZ fusions, point mutations in the mercuric ion reductase merA gene, and transcomplementation have revealed that in Hg$^{2+}$-resistant cells, the inducer Hg$^{2+}$ concentration is rate determining for activation of transcription. mer operon expression is activated by the presence of nanomolar concentrations of Hg$^{2+}$ in liquid media only when the mercuric ion reductase function is artificially inactivated in cells, whereas cells with active mercuric ion reductase require micromolar concentrations of Hg$^{2+}$ for effective induction of the operon.

Several mercury resistance (mer) operons isolated from different gram-negative bacteria have been cloned into Escherichia coli, and the regulation of expression of these operons has been studied (recently reviewed in references 5, 10, 16, 18, and 19). The mer operons encoded by transposons Tn501 and Tn501 and the plasmid pDU1358 have genes with common functions. The major regulatory gene merR is transcribed divergently from the other genes (Fig. 1). The MerR protein represses its own expression as well as the expression of other mer genes from a different promoter, P$_T$. Genes encoding specific Hg$^{2+}$ transport proteins, mercuric ion reductase, organomercurial lyase (present in pDU1358), and an ancillary regulator protein, MerD, are expressed from the promoter P$_T$. In the presence of Hg$^{2+}$, MerR activates transcription from P$_T$ (3, 6, 8, 13, 15, 20). Tn501-derived mer operon expression is activated at nanomolar concentrations of Hg$^{2+}$ in vitro (17), and this phenomenon was correlated with in vivo Tn501-derived mer operon expression by using mer operon-reporter gene fusions (1, 7). Induction of mer operon expression in the presence of unusually low concentrations of the inducer Hg$^{2+}$ is unique among all the known metal ion resistance operons in bacteria. In the present study, we found that the induction of the mer operon by nanomolar concentrations of Hg$^{2+}$ is not biologically relevant. mer operon expression is activated by the presence of nanomolar concentrations of Hg$^{2+}$ in liquid media only when the mercuric ion reductase function is artificially inactivated in cells, whereas cells with active mercuric ion reductase require micromolar concentrations of Hg$^{2+}$ for effective induction of the operon. Most importantly, the concentration of the inducer Hg$^{2+}$ determines the rate of transcription in resistant cells and the inducer concentration never reaches a saturation level in such cells.

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Bacterial strain, medium, and plasmids. The bacterial strain used in this study is E. coli MC1061 (hsdR mcrB araD139Δ (araABC-leu)ΔlacX74 galU galK rps-l thi). Luria-Bertani (LB) medium supplemented with 100 μg of carbenicillin or 10 μg of tetracycline per ml was used to grow E. coli cells harboring different plasmids at 37°C. The plasmids used are listed in Table 1.

Construction of merA-lacZ transcriptional fusion. In plasmid pDH1, sequences from the 79th codon of merB to the 3′ end of the pDU1358-encoded broad-spectrum mer operon are deleted (11). A fragment from the EcoRV site (upstream of merR) to the EcoRI site (within merA) of pDH1 was blunt ended and cloned into the BamHI site (also blunted after BamHI digestion) of the promoterless lacZ transcriptional fusion vector pQF50 (2). Colonies expressing β-galactosidase activity were identified on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) plates containing 100 μg of carbenicillin per ml and 0.1 μM HgCl$_2$ as described elsewhere (20). Plasmid DNA from one of the clones expressing inducible β-galactosidase activity in the presence of Hg$^{2+}$ was named pHY1. In pHY1, the mercuric ion reductase activity was destroyed by deletion mutation at the 3′ end of the trpA gene. In the pQF50 vector, two copies of a synthetic trpA terminator were inserted upstream of its multiple cloning sites. This arrangement successfully eliminates read-through transcription (2).

Isolation of merA null mutant by chemical mutagenesis of pBmerlacZ. Plasmid pBmerlacZ contains the pDU1358 broad-spectrum mer operon with a lacZ reporter gene fused into its merB gene (20). pBmerlacZ DNA was mutagenized by hydroxyamine treatment to isolate merA mutants. Approximately 10 μg of the plasmid DNA was treated with 0.5 ml of a solution containing 0.4 M hydroxylamine, 0.5 M potassium phosphate buffer (pH 6.0), and 5 mM EDTA for 30 h at 37°C. Mutagenesis was terminated by overnight dialysis at 4°C against 1,000 volumes of water. E. coli MC1061 competent cells were transformed by the mutagenized plasmid DNA and spread on LB-carbenicillin plates. Colonies appearing on the LB-carbenicillin plates were replica plated on LB-carbenicillin supplemented with 5 μM HgCl$_2$. A few representative colonies that failed to grow in the presence of HgCl$_2$ but grew in the absence of HgCl$_2$ were further tested for Hg$^{2+}$ hypersensitivity by growth in liquid medium.

Mutations in merA were confirmed by testing the resistance of cells to HgCl$_2$ following complementation with pCL324, which contains the merA gene under the control of the constitutive tet promoter in pACYC184. The isolated plasmid from one such merA mutant was named pHY2.
Intracellular inducer concentration is rate determining in mer operon expression by resistant cells. mer operon-lacZ fusions in merA and merB genes were used to quantitatively assay expression of the operon by measuring β-galactosidase activity. The maximum β-galactosidase activity in cells harboring pHY1 with the merA-lacZ fusion (merA function inactivated) was about twofold higher than that in cells containing the pBmer lacZ with a fusion within the merB gene (Fig. 2).

In growth studies, Nakahara et al. (12) first showed that cells with the functional mercury ion transport genes and an inactivated mercuric ion reductase gene were hypersensitive to Hg2+ because of hyperaccumulation of Hg2+ in the cell lacking the mercuric ion reductase. To address the question of whether Hg2+-hypersensitive cells respond to a significantly lower level of Hg2+ in the growth medium for transcriptional activation from P_T than the Hg2+-resistant cells, the MerA- mutant plasmid pHY2 was used in this study (Table 1). E. coli cells harboring different plasmids were induced by various concentrations of Hg2+ in the medium. Approximately a fourfold increase in Hg2+ concentration (50 to 200 nM) resulted in the activation of β-galactosidase activity from 10 to 90% in merA mutant cells (Fig. 2). This is in agreement with the data reported for the Tn21 mer system (with a mer-lux fusion) indicating that the “ultrasensitive threshold effect” observed in vitro can be correlated with that observed in vivo (1, 17). In our experiment, although the Hg2+ concentration that resulted in half-maximal activity (M_0.5) for merA mutant cells was 100 nM in the medium, the M_0.5 value for the resistant cells was 5,000 nM. The striking difference (50-fold) between the Hg2+ concentrations yielding M_0.5 can be explained by the fact that the intracellular concentration of Hg2+ never reaches the saturation level in resistant cells because of continuous reduction of Hg2+ by the mercuric ion reductase. Cells containing the intact mer operon maintain a certain level (steady state) of mer gene products including the mercuric ion reductase from the basal-level expression of the genes from the promoter P_T (4, 13).

### Table 1: Bacterial plasmids used in this study

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Description</th>
<th>Reference or source</th>
</tr>
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<tbody>
<tr>
<td>pBmerlacZ</td>
<td>Broad-spectrum mer-lacZ gene fusion in merB</td>
<td>20</td>
</tr>
<tr>
<td>pCL324</td>
<td>Broad-spectrum merA gene cloned for expression under the control of the tet promoter in pACYC184</td>
<td>This study</td>
</tr>
<tr>
<td>pDH1</td>
<td>pGN120 derivative with merD and part of merB deleted</td>
<td>11</td>
</tr>
<tr>
<td>pDU1358</td>
<td>Plasmid originally isolated from Serratia marcescens</td>
<td>4</td>
</tr>
<tr>
<td>pGN120</td>
<td>Broad-spectrum mer operon of pDU1358 cloned into pBR322</td>
<td>14</td>
</tr>
<tr>
<td>pOF50</td>
<td>Promoterless lacZ gene vector</td>
<td>2</td>
</tr>
<tr>
<td>pHY1</td>
<td>Broad-spectrum mer-lacZ gene fusion in merA</td>
<td>This study</td>
</tr>
<tr>
<td>pHY2</td>
<td>merA point null mutation in pBmerlacZ</td>
<td>This study</td>
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**FIG. 1.** pDU1358-encoded mer operon. The physical order of the genes, the functions of the gene products, and the operator-promoter region (OP) are shown. The 3’-most nucleotide of the merR gene is designated nucleotide 1. Nucleotide numbers above the operon define the boundaries of the different genes and the OP. The functions of the gene products are indicated below the gene designations. The orientations of the promoters are indicated by arrows.
tant cells with identical Hg$^{2+}$ concentrations in the medium. This explains why in merA mutant cells transcription can be activated by adding nanomolar concentrations of Hg$^{2+}$ to the medium (Fig. 2) (1) and why in resistant cells the effective inducer concentration is about 50-fold higher, in the micromolar range. Most importantly, the higher level of expression of β-galactosidase in merA mutant cells (cells with a large deletion or a point mutation in merA) containing plasmid pHY1 or pHY2 (Fig. 2) than in resistant cells containing the plasmid pBmerlacZ suggests that the intracellular concentration of the inducer Hg$^{2+}$ is rate determining for operon expression. Manipulations of the operon that may cause changes in the intracellular inducer concentration, directly or indirectly, could dramatically affect transcription activation from the promoter P$_r$.

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REFERENCES