Involvement of *merB* in the Expression of the pMR26 *mer* Operon Induced by Organomercurials

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The inducibility by organomercury of the broad-spectrum *mer* operon on pMRA17 cloned from *Pseudomonas* K-62 plasmid pMR26 was assessed in the absence of a functional *merB* gene. The *mer* polypeptides encoded by the *mer* genes on pMRA17 were almost identified in maxicell induced not only by Hg^{2+} but also by $\text{C}_6\text{H}_5\text{Hg}^+$ or CH_3Hg^+ . Maxicell with pMRD103, a *merB*-deletion plasmid constructed from pMRA17, also produced the corresponding *mer* polypeptides when maxicell was induced by Hg^{2+} , whereas no *mer* polypeptides were detected in the maxicell induced by $\text{C}_6\text{H}_5\text{Hg}^+$ or CH_3Hg^+ . These results suggest that *merB* is needed for induction of the pMRA17 *mer* operon expression by organomercurials. Next, to test the inducibility of pMRA17 *mer* operon expression from its own promoter, a promoterless *lacZ* was fused with the *mer* operon, where *merB* was deleted in plasmid pB43*merlacZ*. Only Hg^{2+} , but not $\text{C}_6\text{H}_5\text{Hg}^+$ or CH_3Hg^+ , can activate β -galactosidase expression in bacteria with pB43*merlacZ*. These results not only imply that the pMRA17 MerR is a narrow-spectrum regulator that did not recognize organomercury as a direct inducer, but also confirms that *merB* is required for induction of the pMRA17 *mer* operon expression by organomercurials.

Key words — inducibility, organomercurials, MerR, *merB*, pMRD103, pB43*merlacZ*

INTRODUCTION

Bacterial resistance to organomercurials has been shown to be carried out by the coordinated action of mercury transport proteins, encoded by the *merT* and *merP* genes, and mercury detoxifying enzymes, encoded by the *merB* and *merA*

genes, acting in sequence.^{1–5)} In Gram-negative bacteria, the structural *mer* genes determining both transport and enzymatic functions are usually clustered in a single operon and regulated by the *merR* gene located at the beginning of the mercury resistance (*mer*) operon.^{3–7)} In general, *merR* protein (MerR) actively represses structural *mer* genes transcription in the absence of mercury, and activates expression of this transcript when mercury is present.^{8–10)} MerR from broad-spectrum *mer* operon, which determines bacterial resistance to both inorganic and organic mercurials, can activate the operon expression in the presence of Hg^{2+} and organomercurials, but the MerR from narrow-spectrum *mer* operon, which determines resistance only to inorganic mercurials, fails to recognize organomercury as an inducer.^{11–15)} Amino acid sequences for the two MerRs are quite similar, with the exception that the broad-spectrum MerR has 10 C-terminal amino acid residues that are completely different from those of the narrow-spectrum MerR.^{4,12,14)} Nucifora *et al.* reported that the 10 C-terminal amino acids of MerR from the broad-spectrum *mer* operon of pDU1358 is an organomercurial-sensing region required for induction of the operon by $\text{C}_6\text{H}_5\text{Hg}^+$, but not for induction with Hg^{2+} , since the 10 amino acids could be removed without abolishing the response to Hg^{2+} .^{4,12)}

In a previous paper, we reported that expression of the *mer* operon of pMRA17 cloned from *Pseudomonas* K-62 pMR26 could be induced not only by Hg^{2+} but also by $\text{C}_6\text{H}_5\text{Hg}^+$ or CH_3Hg^+ .^{2,7)} However, the 10 C-terminal amino acids of pMRA17's MerR are completely dissimilar to the sequence of pDU1358's MerR which can recognize organomercury as an inducer. One possible explanation of this phenomenon is that Hg^{2+} , produced by means of basal activity of the organomercurial lyase encoded by the *merB* gene, is actually an inducer. To clarify this matter, the

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activation of pMRA17 *mer* operon expression by organomercurials in the absence the *merB* was examined. Here, we report that *merB* is required for the pMRA17's *mer*-operon expression induced by organomercurials.

MATERIALS AND METHODS

Bacterial Strain, Plasmids and Growth Conditions

— Plasmid pMRA17 containing a typical broad-spectrum *mer* operon cloned from *Pseudomonas* K-62 plasmid pMR26 was used as the starting plasmid. *E. coli* XL1-Blue was grown at 37°C in Luria–Bertani medium.¹⁶⁾ When necessary, the medium was supplemented with 100 µg/ml ampicillin.

Plasmid Construction — Plasmid pMRD103 containing *merRTPAG* was constructed by eliminating the gene encoding the organomercurial lyase from plasmid pMRA17.⁷⁾ A *mer-lacZ* fusion plasmid, pB43*merlacZ* was constructed by cloning the 4.2-kb *Bsa*AI fragment that contains *merRTPAG* genes of pMRA17, into the *Sma*I site of the *lacZ* transcriptional fusion vector pQF52.¹⁷⁾

Mercury Transforming Activity — HgCl₂-induced bacterial cells were harvested, and washed twice with an equal volume of L-broth. The washed cells were then resuspended in the original volume of fresh L-broth containing 100 µM EDTA, 100 µg/ml ampicillin and 5 µM ²⁰³Hg²⁺ (136 mCi/mmol), 50 µM ¹⁴C₆H₅Hg⁺ (30 Ci/mmol) or 5 µM ¹⁴CH₃Hg⁺ (57 mCi/mmol). After incubation at 37°C, aliquots (500 µl) were taken out periodically and counted in a Beckman gamma scintillation counter (GAMMA-5500) or liquid scintillation spectrometer (Aloka LSC-3500). The assays were carried out in triplicate and had an S.E.M. of less than 5%.

Maxicells Analysis — The transformants of *E. coli* CSR603 carrying the plasmids of interest were incubated aerobically at 37°C in K medium (M9 medium supplemented with 1% Casamino acids and 0.1 µg/ml thiamine). After 50 s UV irradiation (50 J/m²), the cells were treated with D-cycloserine (150 µg/ml), and the plasmid-encoded proteins were labeled with [³⁵S] methionine (1000 Ci/mmol) according to the original protocol.¹⁸⁾ For induction, 1 µM mercurial was added to the cells during the 1 h period of labeling with [³⁵S] methionine. Gel electrophoresis was performed by the method of Laemmli¹⁹⁾ and sodium salicylate was used to detect ³⁵S-labeled polypeptide.²⁰⁾

β-Galactosidase Activity — The activity was assayed as described by Miller.²¹⁾ Cells were grown

overnight in supplemented M9 medium. The cultures were then diluted 1 : 50 into fresh medium and grown at 37°C until the OD₆₀₀ was between 0.4 and 0.8. Cells were then induced with 10 µM Hg²⁺, 2 µM C₆H₅Hg⁺ or 2 µM CH₃Hg⁺ for 1 h, and the activity was measured.

RESULTS AND DISCUSSION

MerR is the transcriptional regulator of *mer* operon, acting at the *mer* promoter as both an activator in the presence of mercurials and a repressor in their absence.^{8–10)} In the Gram-negative MerRs, the C-terminal region of the protein specifies whether there is a broad- or narrow-spectrum response.^{4,12)} Despite lacking the reported organomercury-sensing sequence^{4,12)} in the MerR molecule, the *mer* operon of pMRA17 is capable of inducing by C₆H₅Hg⁺ or CH₃Hg⁺.^{2,7)} To establish whether or not the organomercurials functioned directly as an inducer, it is necessary to inactivate the *merB* gene on pMRA17. Because a low constitutive level of organomercurial lyase encoded by *merB* could produce an inducing concentration of Hg²⁺ from the organomercurials used as inducers, we first attempted to construct a *merB*-deleted mutant from pMRA17 and examined its inducibility by organomercurials.

Since the complete sequence of the *mer* operon on pMRA17 is known,⁷⁾ we were able to create our deletion mutation *in vitro* by manipulating strategically located restriction sites. As shown in Fig. 1, pMRD103 failed to confer activity to degrade C₆H₅Hg⁺ and CH₃Hg⁺, but still had activity to volatilize Hg²⁺. Bacteria with pMRD103 expressed CH₃Hg⁺-sensitive and C₆H₅Hg⁺-hypersensitive phenotypes but still retained their full Hg²⁺ resistance (data not shown). These results clearly demonstrate that the *merB* encoding the enzyme organomercurial lyase is certainly deleted. The hypersensitivity to C₆H₅Hg⁺ shown by the bacterium with pMRD103 is thought to result from expression of the mercury transport proteins encoded by *merT* and *merP*, in the absence of a functional *merB* protein. The transport proteins encoded by *merT* and *merP* are known to be involved in the transport of C₆H₅Hg⁺ but do not participate in the transport of CH₃Hg⁺ into the cells.^{22,23)} Next, we tested whether or not the organomercury functions directly as an inducer of the *mer* operon in

Fig. 1. Volatilization of ^{203}Hg from $^{203}\text{Hg}^{2+}$ (A) and Degradation of the C–Hg Bond from $^{14}\text{C}_6\text{H}_5\text{Hg}^+$ (B) and $^{14}\text{CH}_3\text{Hg}^+$ (C) by *E. coli* XL1-Blue Carrying pBluescriptII (Δ), pMRA17 ($\blacksquare$) or pMRD103 ($\blacktriangledown$)

All values are the means of triplicate experiments.

Fig. 2. Inducible Synthesis of *mer* Polypeptides in Maxicells by Mercurials

Lane 1, pBluescriptII uninduced; lane 2, pMRA17 induced with $1 \mu\text{M}$ Hg^{2+} ; lane 3, pMRD103 induced with $1 \mu\text{M}$ Hg^{2+} ; lane 4, pMRA17 induced with $1 \mu\text{M}$ $\text{C}_6\text{H}_5\text{Hg}^+$; lane 5, pMRD103 induced with $1 \mu\text{M}$ $\text{C}_6\text{H}_5\text{Hg}^+$; lane 6, pMRA17 induced with $1 \mu\text{M}$ CH_3Hg^+ ; lane 7, pMRD103 induced with $1 \mu\text{M}$ CH_3Hg^+ . Molecular mass markers (in kilodaltons) are indicated at the left.

the absence of a functional *merB* gene. The inducibility was directly assessed by examining the expression of *mer* polypeptides in maxicell. Consistent with our previous results,⁷⁾ the *mer* polypeptides encoded by pMRA17 which contains the *merR*, *T*, *P*, *A*, *G* and *B* genes, were almost identified in the maxicell induced by Hg^{2+} , $\text{C}_6\text{H}_5\text{Hg}^+$ or CH_3Hg^+ , but not in the cells without mercury induction as shown in Fig. 2. These results reveal that expression of the *mer* genes is under regulation by MerR in the presence of not only Hg^{2+} but also $\text{C}_6\text{H}_5\text{Hg}^+$ or CH_3Hg^+ . Maxicell harboring pMRD103 in which *merB* was

Fig. 3. β -Galactosidase Activity in Cells with pB43–*merlacZ* Induced by Mercurials

All values are the means of triplicate experiments.

deleted, also produced the corresponding *mer* polypeptides when the cells were induced by Hg^{2+} , but not in the cells induced by $\text{C}_6\text{H}_5\text{Hg}^+$ or CH_3Hg^+ (Fig. 2). These results clearly demonstrate that *merB* is needed for induction of the pMRA17 *mer* operon expression by organomercurials.

Finally, to study the inducibility of pMRA17 *mer* operon expression from its own promoter in the presence of mercurials, a promoterless *lacZ* gene was fused with the pMRA17 *mer* operon, where *merB* was deleted in plasmid pB43*merlacZ*. The ability of the pMRA17 *merR* to activate the *mer* operon in pB43*merlacZ* was tested

by monitoring the transcriptional activation of the *mer* gene-*lacZ* fusion in pB43*merlacZ*. As shown in Fig. 3, only Hg^{2+} but not $\text{C}_6\text{H}_5\text{Hg}^+$ or CH_3Hg^+ is able to induce β -galactosidase expression in the bacterium with pB43*merlacZ*. These results not only implied that pMRA17 MerR is a narrow-spectrum regulator that did not recognize the organomercury as direct inducer, but also confirmed that the *merB* gene encoding organomercurial lyase is required for induction of the pMRA17 *mer* operon expression by organomercurials.

In most cases, the MerR of the broad-spectrum *mer* operon can recognize both inorganic and organic mercury as inducers.^{12–15} Although the *mer* operon of pMRA17 is a typical broad-spectrum *mer* operon, the pMRA17 MerR fails to recognize organomercury as a direct inducer. Therefore, the expression of pMRA17 *mer* operon in the presence of organomercury seems to be due to expression of a low constitutive level of organomercurial lyase that produce sufficient quantities of Hg^{2+} for induction of the *mer* operon expression by cleaving the C–Hg bond of organomercury.

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