Mercury Operon Regulation by the merR Gene of the Organomercurial Resistance System of Plasmid pDU1358

GIUSEPPINA NUCIFORA,* LIEN CHU, SIMON SILVER, AND TAPAN K. MISRA
Department of Microbiology and Immunology, University of Illinois College of Medicine,
P.O. Box 6998, Chicago, Illinois 60680

Received 9 January 1989/Accepted 24 April 1989

The structural basis for induction of the mercury resistance operon with inorganic mercury and the organomercurial compound phenylmercuric acetate was addressed by DNA sequencing analysis and by lac fusion transcription experiments regulated by merR in trans from broad-spectrum-resistance plasmid pDU1358 (Hg\(^{2+}\) and phenylmercury responding). The lac fusion results were compared with those from a narrow-spectrum-resistance (Hg\(^{2+}\) responding but not phenylmercuric responding) operon and the pDU1358 merR deleted at the 3' end. The nucleotide sequence of the beginning region of the broad-spectrum mer operon of plasmid pDU1358 was determined, including that of the merR gene, the operator-promoter region, the merT and merP genes, and the first 60% of the merA gene. Comparison of this sequence with DNA sequences of narrow-spectrum mer operons from transposon Tn501 and plasmid R100 showed that a major difference occurred in the 3' 29 base pairs of the merR gene, resulting in unrelated C-terminal 10 amino acids. A hybrid mer operon consisting of the merR gene from plasmid pDU1358, a hybrid merT gene (determining mercuric reductase enzyme), and lacking the merB gene (determining phenylmercury lyase activity) was inducible by both phenylmercury and inorganic Hg\(^{2+}\). This shows that organomercurial lyase is not needed for induction by organomercurial compounds. A mutant form of pDU1358 merR missing the C-terminal 17 amino acids responded to inorganic Hg\(^{2+}\) but not to phenylmercury. Thus, the C-terminal region of the MerR protein of the pDU1358 mer operon is involved in the recognition of phenylmercury.

Bacterial cells can express mechanisms to counteract the toxic effects of mercury ions and organomercurial compounds. These resistance mechanisms are often encoded on plasmids or transposons and are highly specific for the Hg\(^{2+}\) ion or mercury covalently bonded to an organic component, as for example in phenylmercuric acetate (PMA) (4, 25, 26).

Three mercury resistance operons from gram-negative bacteria have been cloned and sequenced: the narrow-spectrum (Hg\(^{2+}\) resistance but not PMA resistance) resistance determinants from plasmid R100 (originally from a Shigella sp.) and from transposon Tn501 (originally from a Pseudomonas sp.) (2, 3, 17, 18) and the broad-spectrum (resistance to both Hg\(^{2+}\) and PMA) resistance determinant from pDU1358 (originally from a Serratia sp. [7; this work]). The operons contain several homologous genes: merT and merP, which code for proteins that transport the toxic ion into the cell, and merA, coding for mercuric reductase, which reduces Hg\(^{2+}\) ions to volatile and less toxic metallic Hg\(^0\). Plasmid pDU1358 confers resistance to organomercurial compounds such as PMA and has an additional gene, merB, which codes for the enzyme organomercurial lyase. The lyase cleaves the covalent C-Hg bond of PMA to give less toxic benzene and Hg\(^{2+}\), which is reduced by the reductase.

The expression of these genes is under the control of a trans-acting activator-repressor protein, encoded by the merR gene, which is transcribed divergently from the structural genes. The MerR proteins from different plasmids can cross-complement a merR mutant operon on plasmid R100 (4, 6). Expression of the structural genes can be induced by subtoxic levels of Hg\(^{2+}\) ions in narrow-spectrum mer operons or by either Hg\(^{2+}\) ions or organomercurial compounds such as phenylmercury, methylmercury, and ethylmercury in broad-spectrum mer operons.

The regulatory protein MerR of the narrow-spectrum mer operons from Tn501 and R100 binds to the operator-promoter region of the operon (9, 22, 23), possibly acting as a repressor by precluding access to RNA polymerase and repressing transcription. However, when Hg\(^{2+}\) ions are present, a complex is formed at the operator-promoter region of the DNA between MerR, RNA polymerase, and Hg\(^{2+}\). The complex activates transcription of the downstream structural genes (6, 9, 22). The mer operator of Tn501 has recently been analyzed with deletion mutants carrying mutations that result in constitutive synthesis of mer gene products (11). Recently published observations on the chromosomally encoded mer resistance operon of Bacillus sp. strain RC607 indicate that expression of the structural genes is regulated differently (8, 27), although the regulatory protein is significantly homologous to that of the gram-negative systems.

No specific information is available on the induction of the broad-spectrum mer operon with organomercurial compounds, and it is not clear whether activation depends on direct interaction between the regulatory protein (MerR) and organomercurial inducers, such as PMA. A low constitutive level of lyase could produce Hg\(^{2+}\), which actually acts as inducer.

In this report, we present the DNA sequence of the region of the broad-spectrum organomercurial resistance determinant of plasmid pDU1358, starting with the merR gene and continuing beyond the operator-promoter region through the merT and merP genes for 1,029 base pairs (bp) into the merA gene. (The sequence has been submitted to the GenBank data base under accession number M24940.) Together with the previously published sequence of the distal region of this mer operon (7), this completes the mer operon sequence of

---

* Corresponding author.
pDU1358 except for approximately 300 bp within merA. We also report complementation tests and in vitro recombination experiments which demonstrate that the ability of the plasmid pDU1358 MerR protein to respond to PMA resides in its carboxyl end, distal to the site for the Hg\(^{2+}\) response.

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** *Escherichia coli* strains MC1061 [F\(^{−}\) araD139 Δ ara leu)7696 lacY174 galU galK hsdR1 rpsL17491 thi rpoB Mu] harboring plasmid pDU1161 (encoding resistance to ampicillin, chloramphenicol, and kanamycin and supersensitivity to Hg\(^{2+}\)) and pW373 (phase M13-sensitive host [1]) were used.

Recombinants of plasmid pBR322 were selected on LB agar plates (13) for resistance to tetracycline (12 μg/ml), and derivatives of phase M13 were selected by screening single plaques on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) plates and agarose gel electrophoresis of DNA from infected-cell lysates (16). The cultures were grown either in LB broth (strains MC1061 and DU5111) or in 2XNY broth (strain W373). Growth was monitored by measuring the culture turbidity with a Klett-Summerson colorimeter.

The inducibility of the mercury operon was determined by addition of Hg\(^{2+}\) or PMA to exponentially growing cultures 60 min before harvesting the cells and assaying the β-galactosidase levels (15).

**Plasmids.** Plasmid pDU1003 containing the narrow-spectrum mercury resistance operon from plasmid R100 (21), plasmid pHG103 (7) containing the broad-spectrum mercury resistance operon from pDU1358, and plasmid pDU1161 (21), a derivative of R100, were used.

The construction of the plasmid containing the pDU1358-R100 hybrid mercury operon is outlined in Fig. 3. Plasmid pGN101 contained the proximal region of the mer operon of pDU1358 ligated at the conserved EcoRI site in the merA gene to the distal region of the mer operon of pDU1003. The correct recombinant was selected by agarose gel analysis after digestion with HindIII (which cuts twice in the pDU1358 mer, giving a 310-bp fragment, but has no recognition sequence in the R100 mer operon).

One of the M13 deletions generated for sequencing of pDU1358 mer by Bal31 exonuclease treatment (mGN1-267) was used to construct a recombinant operon containing a merR gene deleted at the 3′ end (see Fig. 3). Derivative mGN1-267 carries a merR gene lacking 54 nucleotides at the 3′ end and was digested with PvuI and HpaI and ligated to plasmid pGN101, also digested with PvuI and HpaI. The desired recombinant was selected by size and by the loss of the recognition sequence for SalI, lost in the deleted nucleotides.

**DNA sequencing.** A modification (16) of the dideoxy chain termination method (24) was used to sequence both strands of the 2.9-kilobase (kb) region of the mercury resistance operon from plasmid pHG103 (7). The 2.9-kb BglII-EcoRI fragment from plasmid pHG103 was cloned into M13 vector mTM010 (16), and overlapping ordered deletions were generated by using exonuclease Bal31.

**RESULTS**

**DNA sequence.** Figure 1 shows the 2,924 bp of the broad-spectrum mercury resistance operon of plasmid pDU1358 from approximately 160 bp 3′ to the terminal BglII site in pHG103 (7) to the EcoRI site in merA. The first 676 bp shown are apparently not part of the mer system, and the function of this segment of DNA has not been studied. The pDU1358 mer operon starts with the termination codon of merR and continues to the EcoRI site that is conserved in the merA sequences of R100, Tn501, and pDU1358. Following a gap of approximately 300 bp that have not been sequenced, the pDU1358 merA gene continues in the sequence of Griffin et al. (7). The major reading frames are indicated with the direction of transcription and the predicted amino acid sequences of the translated gene products. The operator-promoter region and the coding regions of merR, merT, merP, and the proximal part of merA were identified by comparison with the sequences of the mer operons of R100 and Tn501 (5, 12, 17, 18).

The sequence in Fig. 1 from the end of the merR gene (position bp 677) through the end of the sequence shown (EcoRI site in merA) was compared with that of the previously sequenced mer operons from Tn501 and R100 (2, 17, 18). The comparisons of the sequences were made with the nucleotide alignment program of Wilbur and Lipman (28) with parameters K-tuple = 4, window = 20, and gap penalty = 10. All three sequences are very closely homologous. The plasmid R100 sequence showed 1,967 of 2,244 identical base pairs when matched with Tn501 (87.7% identity) and somewhat fewer (1,790 of 2,247) identical base pairs when matched with the R100 sequence (79.7% identity). For comparison, the Tn501 and R100 sequences are 81.5% identical (1,829 of 2,244 bp) over this region. Thus, the pDU1358 mer sequence shown is more similar to that of Tn501 than to that of R100. The only region where the pDU1358 DNA sequence is more dissimilar from that of Tn501 than are the Tn501 and R100 sequences is the 3′-most 29 bp of the merR gene, where there are only 6 differences between Tn501 and R100. 20 differences between pDU1358 and R100, and 21 differences between pDU1358 and Tn501.

This is the region where the amino acid sequence of the organomercurial compound-responding merR product of pDU1358 differs from that of the organomercurial compound-nonresponding merR product of R100 (see below).

The pDU1358 mer operon (like that of Tn501) lacks a merC gene. The termination codon of merP of pDU1358 is followed after 55 bp by the ribosome-binding site and ATG initiation codon of merA. The R100 sequence has an additional 438 bp representing the merC gene (17, 25) that is missing in Tn501 and pDU1358.

Comparison of the predicted amino acid sequences of MerR from pDU1358 with those from R100 and Tn501 (Fig. 2) shows strong homology, with the exception of the carboxyl-terminal nine amino acids, suggesting that this region is not essential for induction by inorganic mercury. A cysteine at position 115 is missing in pDU1358 MerR. The three cysteines at positions 82, 117, and 126 are conserved in the three MerR sequences. These three cysteines might be involved in Hg\(^{2+}\) binding.

**Inducibility of the mer operon: trans activation of the mercury operon with merR from R100 and pDU1358.** The ability of the pDU1358 merR product to respond to Hg\(^{2+}\) and PMA as inducers was studied in complementation tests between pDU1161, a derivative of plasmid R100 with an inactivated merR gene, and with a transcriptional merRlacZ fusion (7, 21).

In addition, in order to test the activation of the pDU1358 broad-spectrum merR in the absence of the merB lyase gene, a hybrid plasmid, pGN101, was constructed to complement pDU1161. Plasmid pGN101 (Fig. 3) contains the pDU1358 broad-spectrum merR gene, the transport
FIG. 1. DNA sequence of the 2,924 bp of the plasmid pD1358 broad-spectrum mer operon. Only one strand is shown. Putative transcription initiation signals, some restriction nuclease sites (used in these studies), and predicted amino acid sequences are shown. Starting codons are boxed, and stop codons are indicated by asterisks. Following a 300-bp gap after bp 2924, the merA sequence continues in Griffin et al. (7).
Restriction enzyme was recombinant (see Fig. 1) that is lost in the merR-deleted recombinant. mer genes are shown as R, T, P, A, B, D, and R' (3'-deleted version of merR). Restriction enzyme sites: E, EcoRI; Hd, HindIII; Hp, Hpal; Ps, PstI; Pv, PvuI; S, Sall. (Not drawn to scale.)

FIG. 2. Alignment of the predicted amino acid sequences of the MerR regulatory protein from pDU1358 with those of MerR from R100 and Ts501. Asterisks indicate amino acids identical to those of pDU1358.

FIG. 3. Construction of plasmids pGN101 and pGN102. For the construction of these plasmids, M13 derivatives were used as donors of the mer operon containing the broad-spectrum merR. Phage mGN3 replicative form (RF) DNA containing the intact mer operon of plasmid pDU1358 was digested with PstI and EcoRI and ligated to plasmid pDU1003, digested with PstI and partially digested with EcoRI. The two restriction enzymes cut in both molecules outside the mer operon in the vector (PstI) and at corresponding sites in merA (EcoRI). The correct recombinant, containing the PstI-EcoRI fragment of the broad-spectrum mer operon replacing the comparable fragment of the R100 mer operon, was selected by screening the size of the recombinants and by digestion of the recombinants with HindIII, which cuts twice in the pDU1358 merR, giving a 310-bp fragment; HindIII has no recognition sequence in the R100 mer operon. For construction of plasmid pGN102, M13 derivative mGN1-267 (created by Bal31 digestion for sequencing) was used. In this construct, the pDU1358 merR gene lacks 54 bp at the 3' end. mGN1-267 was digested with PvuI and Hpal and ligated to pGN101 digested with the same two restriction enzymes. The correct recombinant was selected by size and by the inability to be digested by Sall, which has a recognition sequence 383 bp 3' of merR of pDU1358 (see Fig. 1) that is lost in the merR-deleted recombinant. mer genes are shown as R, T, P, A, B, D, and R' (3'-deleted version of merR). Restriction enzyme sites: E, EcoRI; Hd, HindIII; Hp, Hpal; Ps, PstI; Pv, PvuI; S, Sall. (Not drawn to scale.)
These results suggest interaction between PMA and merR in activation of the broad-spectrum mer operon.

Recognition of different inducers by the pDU1358 broad-spectrum MerR protein. Comparison of the predicted amino acid sequence of the MerR protein from pDU1358 with those of the MerR proteins from R100 and Tn501 (Fig. 3) showed a lack of homology at the carboxyl terminus. In contrast, the rest of the sequence had an overall conservation of more than 90%.

We investigated the possibility that this C-terminal region plays a role in induction with PMA by replacing the wild-type pDU1358 merR in plasmid pGN101 with a deletion mutant lacking 54 bp at the 3′ end in plasmid pGN102 (Fig. 3). In plasmid pGN102, the C-terminal 17 amino acids (starting with Leu-Ile-Ala) (Fig. 2) are missing and are replaced with 7 amino acids (Ile-Ser-Arg-Ser-Leu-Trp-Asn). These seven amino acids are encoded by nucleotides from the M13 phage in which the Bal31 deletion was generated. Despite the deletion of the 17 carboxyl-end residues, the MerR protein was capable of regulating expression of the R100 operon in pDU1161. Plasmid pGN102 expressed the same level of resistance to mercury as did plasmid pGN101 (Fig. 4A). However, the level of induction was affected by the altered carboxyl end of the protein. With Hg²⁺ as the inducer, the production of β-galactosidase was reduced in the complementation experiment with plasmid pGN102, and the activity of PMA as an inducer was completely lost (Fig. 5).

**DISCUSSION**

A major question concerning gene regulation today is the topology of regulatory proteins and how the domains involved in inducer recognition and binding and DNA target recognition are arranged within the three-dimensional structure of the protein. For the merR gene product of pDU1358, five domains must be considered. (i) A helix-turn-helix motif is presumed to be involved in binding to the operator DNA. Its sequence has been deduced from homologies with other regulatory proteins (5, 23). Since MerR proteins from other mer operons can recognize the R100 mer operator sequence,
the DNA recognition amino acids are presumably conserved among many MerR proteins. (ii) Since MerR protein exists as a homodimer (22, 23), there must be residues at the interface between the subunits determining subunit-subunit interactions. There are no data concerning where these determinants lie. (iii) Inorganic Hg\(^{2+}\) acts as an inducer of R100, Tn501, and pDU1358 mer transcription, so the amino acid residues interacting with Hg\(^{2+}\) ions are probably conserved. (iv) This report shows that the response to the phenylmercury ions requires the carboxyl-terminal end of MerR, which can be removed without abolishing the response to Hg\(^{2+}\) (Fig. 6). The Cys-126 residue shown by our laboratory (K. P. Yoon, unpublished data) to be essential for response to Hg\(^{2+}\) is only 2 amino acids from the beginning of the deletion (of 17 amino acids) that removed the phenylmercury response. (v) A fifth function comes from the findings of O’Halloran and Walsh (22, 23) that the same DNA region is protected from DNase I attack by MerR protein in the presence or absence of Hg\(^{2+}\). In the absence of MerR function, the mer operon transcription is about 1% of its maximum induced rate (100% in the presence of Hg\(^{2+}\) and MerR). In the presence of MerR, the transcription is repressed to less than 0.1% (21). Activation by Hg\(^{2+}\) may not involve a shift in position of MerR protein on the DNA. The addition of Hg\(^{2+}\) activates mer transcription by allowing a specific interaction between MerR and another component, probably RNA polymerase (22).

The broad-spectrum mercury resistance operon of plasmid pDU1358 differs from those of R100 and Tn501 with the additional inducibility by PMA (7). Griffin et al. (7) reported Southern blot DNA-DNA hybridization experiments with pDU1358 DNA in which a probe containing the mer gene of R100 was used. In these experiments, hybridization was not detected. However, when these experiments were repeated with the same probe, hybridization was seen when either HindIII or EcoRI was used to cut the pDU1358 plasmid DNA (data not shown). The small 310-bp HindIII fragment within mer of pDU1358 (Fig. 1) was apparently lost in the experiments of Griffin et al. (7).

When the mer genes of Tn501 and pDU1358 were aligned by the Wilbur and Lipman (28) method with parameters that allowed gaps, a 21-nucleotide gap appeared followed by a perfect match of 19 bp, including the 3’-most 7 bp of merR (ends with TAG), followed by 12 bp, including 7 T residues (Fig. 7A), potentially a rho-independent transcription termination site. When the Wilbur and Lipman (28) gap penalty parameter was increased to suppress gaps, a different TAG chain terminator codon aligned with the Tn501 sequence (Fig. 7B), and this was preceded by 29 unrelated nucleotides.

Possibly the 29 unrelated bp of Tn501 were replaced in a single substitution event with the 50 bp of pDU1358 from bp 659 (in the merR stop codon) to 708 (Fig. 2). One can speculate that a phenylmercury-binding “cassette” might have been inserted toward the 3’ end of the merR gene, in a region which is not needed for the Hg\(^{2+}\) response. The DNA sequence alignment in Fig. 7 suggests this possibility.

The merR deletion mutant lacking the C-terminal 17 amino acids of MerR was fully resistant to inorganic Hg\(^{2+}\) but sensitive to phenylmercury (Fig. 4), whereas the results with the lac fusion in Fig. 6 show lesser response to Hg\(^{2+}\) as an inducer with the deletion mutant merR than with wild-type pDU1358 merR. It is possible that the deletion of the C-terminal 17 amino acids reduced the ability of MerR to respond to Hg\(^{2+}\) quantitatively (Fig. 6), but that merR function is not rate limiting for mercury ion resistance. Barrineau et al. (2) also found that a merR deletion mutant (in this case missing the C-terminal 15 amino acids of R100 MerR) conferred full Hg\(^{2+}\) resistance. It seems that a balance between adequate mercury transport activity and intracellular mercuric reductase determines the mercury resistance level (10, 19, 21).

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI24795 and GM36722 from the National Institutes of Health and by funds from the University of Illinois.

We appreciate the continuing collaboration of N. L. Brown and T. J. Foster (who originally provided the mer region of plasmid pDU1358 and the merA-lacZ fusion) in this effort.

LITERATURE CITED


VOL. 171, LUND, 12.


MERCURY OPERON REGULATION


