Transcriptional Switching by the MerR Protein: Activation and Repression Mutants Implicate Distinct DNA and Mercury(II) Binding Domains†

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ABSTRACT: Bacterial resistance to mercuric compounds is controlled by the MerR metalloregulatory protein. The MerR protein functions as both a transcriptional repressor and a mercuric ion dependent transcriptional activator. Chemical mutagenesis of the cloned *merR* structural gene has led to the identification of mutant proteins that are specifically deficient in transcriptional repression, activation, or both. Five mutant proteins have been overproduced, purified to homogeneity, and assayed for ability to dimerize, bind *mer* operator DNA, and bind mercuric ion. A mutation in the recognition helix of a proposed helix-turn-helix DNA binding motif (E22K) yields protein deficient in both activation and repression in vivo (a^-r^-) and deficient in operator binding in vitro. In contrast, mutations in three of the four MerR cysteine residues are repression competent but activation deficient (a^-r^+) in vivo. In vitro, the purified cysteine mutant proteins bind to the *mer* operator site with near wild-type affinity but are variably deficient in binding the in vivo inducer mercury(II) ion. A subset of the isolated proteins also appears compromised in their ability to form dimers at low protein concentrations. These data, taken with the results in the preceding paper (Shewchuk et al., 1989), support a model in which DNA-bound MerR dimer binds one mercuric ion and transmits this occupancy information to a protein region involved in transcriptional activation.

Bacterial resistance to the toxic effects of mercuric [Hg(II)] salts is mediated by the gene products of the *mer* operon [for reviews, see Foster (1987) and Summers (1986)]. The transcriptional activity of the *mer* operon is controlled by one of the operon gene products, the regulatory protein MerR, which displays both negative (repression) and positive (activation) control features in the absence and presence of its specific ligand Hg(II), respectively (Lund et al., 1986). In addition, MerR negatively regulates its own synthesis, from an overlapping but divergently oriented promoter, both in the presence and in the absence of Hg(II). Thus, MerR is a metal-regulated genetic switch.

In the preceding paper (Shewchuk et al., 1989), we have analyzed specific *mer* operator recognition by Tn*501* MerR protein and show that MerR has a K_d of 10^{-10}–10^{-11} M for DNA binding and does not dissociate from the DNA upon binding mercuric ion. We also determined specific ligation of one Hg(II) per MerR dimer. The effect of Hg(II) on the chemical reactivity of cysteine residues suggests that one or more cysteine residues may be important for Hg(II) binding, perhaps in a subunit bridging mode.

A complementary genetic analysis of the MerR protein from Tn*21* has been undertaken using chemical mutagenesis of the cloned *merR* structural gene. Three phenotypic classes of mutants have been identified by using β-galactosidase expression assays (W. Ross, unpublished data). Some alterations specifically impair repression (a^+r^-) and some impair activation (a^-r^+) while others impair both regulatory functions (a^-r^-). Sequencing of the mutations has indicated that DNA operator binding, transcriptional activation, and Hg(II) binding are mediated through distinct regions of the MerR protein. In this paper, we describe overproduction, purification, and functional characterization of wild-type and five mutationally altered Tn*21* MerR proteins. The mutant Tn*21* MerR proteins are variably affected in subunit dimerization, DNA binding, and Hg(II) ligation. These genetic and biochemical studies on the Tn*21* MerR protein are relevant to the Tn*501* protein since these two proteins are 94% homologous at the amino acid level (9 substitutions of 144, with conservation of all 4 cysteine residues). The Tn*21* MerR amino acid sequence is shown in Figure 1, with the five mutants examined in this work as noted.

Materials and Methods

Materials. Restriction endonucleases and DNA modification enzymes were from New England Biolabs. Deoxyadenosine [α-^{32}P]triphosphate and $^{203}HgCl_2$ were from New England Nuclear. Molecular weight standards and heparin–Sepharose CL-6B were purchased from Pharmacia. Phenylmethanesulfonyl fluoride (PMSF)[1] was from Boehringer Mannheim. All other chemicals and solvents were of the highest grade available.

Plasmid Constructions. The *merR* structural gene from transposon Tn*21* is carried on plasmid pWR2 and controls the transcription of β-galactosidase from the *merTPCAD* operon

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[1] Abbreviations: cAMP, cyclic adenosine monophosphate; crp, cAMP receptor protein; dNTPs, deoxynucleotide triphosphates; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); HPLC, high-pressure liquid chromatography; HTH, helix-turn-helix; IPTG, isopropyl β-D-thiogalactopyranoside; NMR, nuclear magnetic resonance; PMSF, phenylmethanesulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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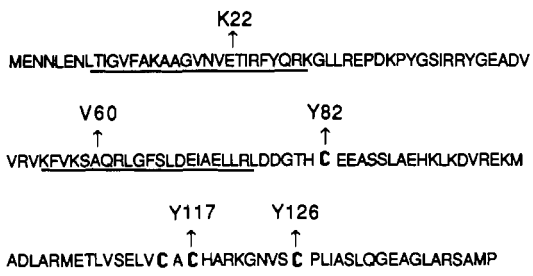

FIGURE 1: Amino acid sequence of the Tn*21* MerR protein. The four cysteine residues are shown in boldface, and the two regions proposed to form helix–turn–helix structures are underlined. Mutations studied in this paper are denoted with (↑).

Table I: Summary of in Vitro Properties of Purified Tn*21* MerR Proteins

mutation	phenotype[a]	dimerization[b]	Hg(II) binding	DNA binding [$\sim K_d$ (M)]
wild type	a^+r^+	++	++	$\sim 1 \times 10^{-10}$
E22K	a^-r^-	±	++	$\sim 1 \times 10^{-8}$
A60V	a^-r^+	+	++	$\sim 1 \times 10^{-9}$
C82Y	a^-r^+	+	+	$\sim 7 \times 10^{-10}$
C117Y	a^-r^+	+	+	$\sim 1 \times 10^{-10}$
C126Y	a^-r^+	++	±	$\sim 1 \times 10^{-10}$

[a] Phenotype determined from β-galactosidase expression assays, as will be described elsewhere (W. Ross, unpublished data). [b] Dimerization and Hg(II) binding affinity were determined as described under Materials and Methods. (++) infers near-wild-type activity, (+) infers less than wild-type activity, and (±) infers almost no activity.

promoter as will be described elsewhere (W. Ross, unpublished data). Mutagenesis of plasmid pWR2 gave rise to a series of plasmids carrying mutationally altered *merR* genes. The sequence changes of the various altered *merR* genes and their resulting phenotypes will be described elsewhere (W. Ross, unpublished data), but the data for the relevant mutations are summarized in Table I. The *merR* structural genes from pWR2 and the appropriate mutationally altered plasmids were used to construct the series of MerR-overproducing plasmids below.

The Tn*501 merR* gene has been previously engineered to give high-level overexpression from the P_{tac} promoter in plasmid pTO90-16 (O'Halloran & Walsh, 1987). For overexpression of the Tn*21*-derived proteins, pTO90-16 was first modified by digestion with *Nru*I and brief treatment with Bal31 exonuclease to remove the adjacent *Eag*I restriction site. The resulting plasmid, pJH501, still overproduces the Tn*501* MerR protein but now has a unique *Eag*I site at codons 16–18 of the *merR* gene.

To overproduce the Tn*21* MerR protein with a mutation of cysteine to tyrosine at position 117 (C117Y), the *merR* gene was excised from the appropriate pWR2 derivative (pWR135). Plasmid pWR135 was first digested with *Eco*RI, treated with Klenow fragment and deoxyribonucleotide triphosphates (dNTPs) to render the *Eco*RI site blunt, and subsequently digested with *Eag*I (at codons 16–18). The excised *Eag*I to *Eco*RI(blunt) fragment [carrying *merR* codons 18–145 (termination)] was gel purified and ligated to pJH501 that had been digested with *Sma*I(blunt) and *Eag*I to regenerate a hybrid *merR* gene (plasmid pJH135). The first 16 codons of the hybrid *merR* gene are derived from Tn*501* DNA, and the remainder of the *merR* gene is from Tn*21*. Although there are 5 nucleotide changes in these first 16 codons, the encoded proteins are identical in sequence in this region. Therefore, the MerR protein produced from plasmid pJH135 is identical in sequence with that produced from plasmid pWR135.

The remainder of the Tn*21* MerR overproducers were derived from the appropriate pWR2 derivative and plasmid pJH135. Since the Tn*21 merR* gene (but not the Tn*501* gene) has a unique *Nco*I site approximately 70 bp following the *merR* termination codon, digestion of plasmid pJH135 with *Eag*I and *Nco*I allowed the insertion of *Nco*I to *Eag*I *merR* cassettes from the mutationally altered pWR2 derivatives. In this way, a series of plasmids has been constructed that overproduces both the wild-type and mutationally altered Tn*21* MerR proteins. The presence of the predicted DNA sequence changes in the resulting recombinants has been directly verified by DNA sequencing of all five mutationally altered Tn*21* *merR* genes.

MerR Overproduction and Protein Purification. Mutationally altered and wild-type Tn*21* MerRs were overproduced in *Escherichia coli* JM105 containing the appropriate overexpression vector and purified by using a modification of the previously published procedure (O'Halloran & Walsh, 1987). The high-salt extract of the crude cell lysate was precipitated with 2 M ammonium sulfate, resuspended in column buffer (CB) [10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 2 mM 2-mercaptoethanol, and 5% glycerol], and applied to a heparin–Sepharose column, equilibrated in the same buffer. The protein was eluted with a linear gradient from 100 to 1000 mM NaCl in column buffer. Fractions containing MerR, eluting at ca. 300 mM NaCl, were pooled, precipitated with 2 M ammonium sulfate, and stored as an ammonium sulfate pellet at 4 °C until required. PMSF (0.5 mg/mL) was included in all buffers up to the chromatography step.

The E22K mutant protein was overproduced in *E. coli* LC137 ($htpR^{165}$, lon^{R9}, Lac_{am}, trp_{am}, pho_{am}, rpsL, $supC^{ts}$, mal_{am}, tsx::Tn10). Cells were grown at 30 °C and lysed in the presence of 1 M NaCl. Following centrifugation to remove the cell debris, the supernatant was precipitated with 2 M ammonium sulfate. The protein pellet was resuspended in column buffer (containing no NaCl) and applied to a heparin–Sepharose column equilibrated in the same buffer. The E22K protein was eluted with a linear gradient from 0 to 500 mM NaCl column buffer.

Native Molecular Weight Determinations. Native molecular weights were determined on a BioSil TSK-125 HPLC gel filtration column (7.5 mm × 300 mm) calibrated with ribonuclease A (13 700), chymotrypsinogen (25 000), ovalbumin (43 000), and bovine serum albumin (67 000). One hundred microliter samples were applied to the column, equilibrated in 10 mM Tris-HCl (pH 7.5), 250 mM NaCl, 1 mM 2-mercaptoethanol, and 5% glycerol, and eluted at 0.5 mL/min. The elution profile was monitored at 280 nm.

Gel Binding Assay. Relative DNA binding affinities of the mutant proteins were determined by using a gel shift assay, as described previously for the wild-type protein (Shewchuk et al., 1989). A ^{32}P-labeled, 305 bp fragment from pMerOP (J. Helmann, unpublished data) was used as the source of *mer* operator/promoter DNA. The relative amounts of bound and free DNA were determined by densitometry using an LKB laser densitometer.

Gel Filtration Assay with $^{203}Hg(II)$. Relative binding affinities for $^{203}Hg(II)$ were determined on a BioSil TSK-125 HPLC gel filtration column essentially as described for the wild-type protein (Shewchuk et al., 1989). The stoichiometry of $^{203}Hg(II)$ binding was calculated from the ratio of bound to free $^{203}Hg(II)$, as determined by scintillation counting on a Beckman LS100 scintillation counter.

RESULTS

Chemical mutagenesis of the Tn*21* MerR regulatory protein by hydroxylamine (to be presented in detail elsewhere) gen-

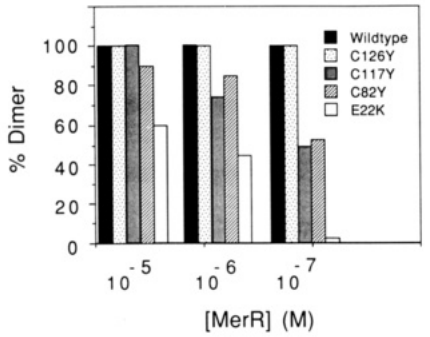

FIGURE 2: Concentration dependence of dimer formation of wild-type and mutant MerR proteins. Extent of dimerization was determined as described under Materials and Methods.

erated a series of mutant proteins with defects in positive regulatory (activation) and/or negative regulatory (repression) function. A subset of the mutations which have been sequenced is displayed in Table I. Two mutations, E22K and A60V, are within regions we previously identified as having helix–turn–helix potential, residues 9–29 (HTH1) and 55–75 (HTH2) (O'Halloran & Walsh, 1987), and thus were candidates for proteins with altered DNA binding motifs. A second phenotype, a^-r^+, is represented by the three cysteine mutants (C82Y, C117Y, and C126Y) which were immediate candidates for characterization in view of our studies in the preceding paper on cysteine residues as likely residues for Hg(II) ligation (Shewchuk et al., 1989). Mutations in the fourth and final cysteine, C115, have not been identified by this screen to date. Another representative of the a^-r^+ class is R120UGA (data not shown), a shortened polypeptide truncated by mutation to a stop codon. While this mutant MerR protein has not yet been purified, the in vivo phenotype does corroborate the in vitro data obtained with the 1–120 or 121 tryptic fragment of wild-type Tn*501* MerR (Shewchuk et al., 1989). Mutant proteins with the distinct phenotype, a^+r^-, will not be studied in this paper, though it is worthy to note that these proteins function as transcriptional activators in the absence of mercuric ion, although the extent of stimulation is increased further by the presence of mercuric ion.

The Tn*21* wild-type MerR protein and five mutationally altered derivatives (E22K, A60V, C82Y, C117Y, and C126Y) have been overexpressed and purified. The major purification step is heparin–Sepharose chromatography. Four of the mutant Tn*21* MerR proteins of Table I could be overproduced and purified by using the procedure developed for Tn*501* MerR. The E22K (a^-r^-) protein, however, was unstable in vivo, requiring it to be overproduced in a protease-deficient strain of *E. coli*. Mutant and wild-type Tn*21* MerR proteins used in these studies were essentially homogeneous by SDS–PAGE analysis (data not shown) and could be obtained in milligram quantities.

Effects of Mutations on Subunit Dimerization. Native Tn*501* MerR, like many prokaryotic repressor and activator proteins, is a homodimer and binds to an operator element containing dyad symmetry. Gel filtration studies on wild-type Tn*21* MerR now confirm its anticipated dimeric structure as well. A mutation affecting the equilibrium between the active dimer and the monomeric forms could influence both the activation and/or repression phenotype. Thus, we tested the five mutationally altered MerR proteins for functional defects in dimerization by examining their native molecular weights at various protein concentrations on a HPLC gel filtration column calibrated with molecular weight standards and with wild-type Tn*21* MerR dimer. Over a concentration range of

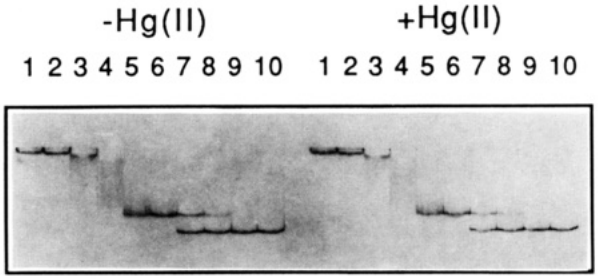

FIGURE 3: Concentration dependence of C117Y–operator DNA binding in the presence and absence of Hg(II). An autoradiogram of a typical gel binding assay in the presence or absence of Hg(II), performed as described under Materials and Methods, is shown for the C117Y protein. The lower band corresponds to free DNA, the middle band to a stoichiometric MerR–DNA complex, and the uppermost band to a MerR–DNA complex in which greater than one MerR dimer is bound, presumably nonspecifically. The protein concentration in each lane is as follows: (1) 10^{-7} M, (2) 5×10^{-8} M, (3) 10^{-8} M, (4) 5×10^{-9} M, (5) 10^{-9} M, (6) 5×10^{-10} M, (7) 10^{-10} M, (8) 5×10^{-11}, (9) 10^{-11} M, (10) no protein. The K_d determined from this experiment is 10^{-10} M both in the presence and in the absence of Hg(II).

10^{-5}–10^{-8} M, wild-type Tn*21* MerR exists exclusively as a dimer, as does the C126Y (a^-r^+) mutant. The other four mutant proteins all show a concentration-dependent dissociation to the monomeric form as the protein concentration is lowered to 10^{-8} M (Figure 2).

The most dramatically compromised protein is that altered by the E22K mutation in the recognition helix of the proposed helix–turn–helix motif. Not only is this protein predominantly monomer at concentrations less than 10^{-6} M, but it is unstable in vivo and could only be purified from a protease-deficient strain of *E. coli*. Given that the MerR protein appears to be present at very low concentrations in vivo, the decreased proportion of dimer could contribute to the observed phenotypic defect in the E22K mutant. In contrast, the somewhat compromised ability of the C82Y and C117Y proteins to form dimers is probably not significant in vivo, since these proteins repress transcription at least as efficiently as the wild-type protein.

Most of these Tn*21* MerR proteins were also susceptible to the facile oxidation to disulfide-linked dimers, as was observed for wild-type Tn*501* MerR (Shewchuk et al., 1989). In the absence of exogenous buffer thiols, the wild-type and four of the mutant proteins were rapidly converted to dimers, detectable on SDS gels under nonreducing conditions. The exception was the C82Y MerR. Taken together with our results in the preceding paper (Shewchuk et al., 1989), that C82 is not susceptible to alkylation in thiol-free buffers, it is likely that C82 contains the thiol involved in the facile oxidation reaction to yield less active disulfide-linked MerR dimer. Consequently, we conclude that C82 is at or near the subunit interface.

DNA Binding Affinity. The absolute and relative binding affinity of the five mutant MerR proteins to *mer* operator DNA was examined by using the gel mobility shift assay on ^{32}P end-labeled restriction fragments. A typical assay is shown in Figure 3: the lower band corresponds to free DNA, the middle band to a stoichiometric MerR–DNA complex, and the uppermost band to a MerR–DNA complex in which greater than one MerR dimer is bound, presumably nonspecifically. Scanning laser densitometry measurements were used to quantitate the relative amounts of bound and free DNA. The concentration of MerR protein required for half-maximal binding to its operator is reported as the apparent K_d value in Table I.

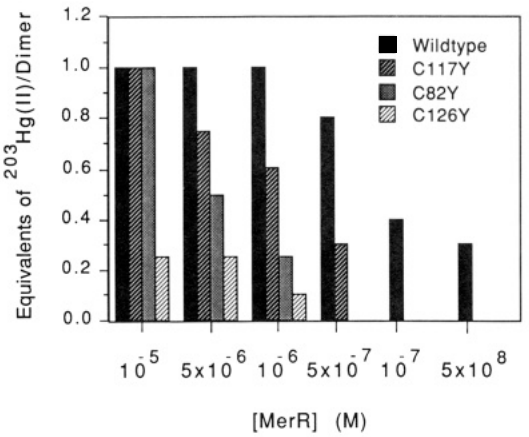

FIGURE 4: Relative Hg(II) binding affinity of wild-type and mutant MerR proteins. Equivalents of Hg(II) per dimer were determined for protein concentrations between 10^{-5} and 5×10^{-8} M, as described under Materials and Methods.

Three items emerged from these studies. First, the E22K mutant, with an a^-r^- phenotype, is dramatically affected in its ability to recognize and bind to *mer* operator DNA. No specific binding (over the nonspecific background) could be detected, indicating at least a 100-fold decrease in specific DNA recognition. Second, the alanine to valine mutation at position 60 (A60V), in the second potential helix–turn–helix structure, and the C82Y mutation result in proteins with slightly reduced affinity (ca. 10-fold) for the operator sequence. Third, C117Y and C126Y are unaffected in operator DNA binding.

For all five mutant MerR proteins examined, addition of Hg(II) does not effect a detectable change (increase or decrease) in DNA binding affinity. In contrast, up to a 10-fold increase in DNA binding by the wild-type Tn*501* protein in the presence of mercuric ion is observed (Shewchuk et al., 1989).

Hg(II) Binding Affinity. To test the effects of specific mutations on the affinity for the inducer ion, Hg(II), gel filtration assays with radioactive ^{203}Hg(II) were conducted. Each MerR protein was incubated with a 10-fold molar excess of ^{203}Hg(II) (in a dilution series from 10^{-5} to 10^{-8} M protein), followed by gel filtration to separate protein-bound ^{203}Hg(II) from free ^{203}Hg(II). The mole fraction of bound mercuric ion at various concentrations is summarized in Figure 4. It should be noted that both MerR protein and the mercuric ion ligand are being diluted, while the concentration of buffer thiol remains constant.

All three cysteine to tyrosine mutants are dramatically affected in Hg(II) binding affinity relative to the wild-type MerR protein. In the preceding paper (Shewchuk et al., 1989), it was shown that the homologous wild-type Tn*501* MerR protein binds 1 equiv of Hg(II)/protein dimer. At high protein concentrations, e.g., 5×10^{-6} M, the C82Y and C117Y mutants can in fact achieve this stoichiometry of 1 Hg(II)/dimer. Upon MerR [and Hg(II)] dilution, however, mercuric ion binding is rapidly lost. The C126Y mutant is most disturbed in its binding properties, binding a maximum of 0.25 equiv of Hg(II)/dimer, even at the highest MerR concentration tested, 5×10^{-5} M. Given the decreased affinity for Hg(II) ligation and the a^-r^+ phenotype in vivo, it may be that the failure of these mutants to stimulate *mer* operon transcription stems from their inability to bind Hg(II) through one or more of these cysteines. In contrast, initial studies indicate that the A60V and E22K mutant proteins possess near-wild-type affinity for Hg(II) (data not shown).

Discussion

The studies presented here represent the initial biochemical characterization of mutant Tn*21* MerR proteins and demonstrate that the DNA binding and metal recognition regions are genetically separable. Mutation of any of three cysteine residues to tyrosine dramatically compromises the affinity of the protein for mercuric ion while having a negligible effect on operator recognition. This is apparent both from in vitro DNA binding studies and from the ability of these mutant proteins to efficiently repress transcription in vivo. Conversely, the E22K mutant protein lacks detectable operator binding yet binds mercuric ion with near-wild-type affinity (Table I).

The lack of detectable operator binding by the purified E22K protein supports a model in which amino acids 9–29 (HTH1) form a classical helix–turn–helix DNA binding motif (Figure 1). In contrast, the mutation of the critical alanine in HTH2 to valine (A60V) has only a small effect on operator binding in vitro and allows efficient repression in vivo. All known HTH structures have either a glycine or a alanine residue at the corresponding position (Dodd & Egan, 1987). Sequence comparisons of homologous MerR polypeptides reveal a high degree of amino acid conservation in the specificity determining positions of the proposed recognition helix (HTH1), and in vitro studies demonstrate that the encoded proteins recognize similar operator sequences (J. Helmann, unpublished data). In contrast, the second possible recognition region, HTH2, is not highly conserved between different MerR polypeptides. Together, these results provide a compelling argument for the assignment of HTH1 as the critical region for site-specific DNA binding.

A priori, we suspected that in a genetic switch protein regulated by Hg(II), cysteine thiols would be the high-affinity sites for metal ligation. Since the Tn*501* MerR protein binds a maximum of one mercuric ion per dimer (Shewchuk et al., 1989), it is simplest to assume that symmetrically disposed cysteines in each subunit are the biologically significant ligands in formation of an energetically favorable bis-, tri-, or tetracoordinate Hg(II)–thiolate complex. Indeed, sequencing of mutants within the a^-r^+ phenotypic class has turned up mutations in three of the four cysteines. All three mutant proteins (C82Y, C117Y, and C126Y) produce equally dramatic affects on activation in vivo, yet all are efficient transcriptional repressors. This suggests that up to three cysteine residues (C82, C117, and C126) in each subunit are important for transcriptional activation, either directly or by contributing to a Hg(II)-dependent conformation of the MerR dimer.

In an attempt to distinguish roles for these three cysteines, the relative Hg(II) binding affinity and dimer–monomer equilibria of the three cysteine mutant proteins were examined, and the results correlated with those obtained from DTNB titrations, iodoacetamide alkylations, and Hg(II) kinetic protection experiments on wild-type Tn*501* MerR (Shewchuk et al., 1989). In vitro, the protein most deficient in Hg(II) ligation is C126Y, where fully stoichiometric ratios of ^{203}Hg(II) binding could not be obtained (Figure 4). In addition, an essential role for C126 is suggested by studies on the 1–120 or 121 amino acid tryptic fragment of wild-type Tn*501* MerR (Shewchuk et al., 1989) which binds DNA specifically but not Hg(II). In contrast, the C82Y and C117Y mutant proteins can still bind one Hg(II) per dimer at high protein and Hg(II) concentrations (Figure 4), suggesting that neither C82 nor C117 is an essential ligand for specific complexation with Hg(II). However, chemical protection experiments with the Tn*501* protein suggest that either C115 or C117 is specifically protected against alkylating agents when

MerR is complexed with mercuric ions, in the presence of low concentrations of buffer thiols (Shewchuk et al., 1989). Of these two cysteines, the significant ligand is presumably C117 since this residue is evolutionarily conserved and C115 is not (T. Misra and I. Mahler, personal communications). Thus, C117 and C126 are still both candidates for specific Hg(II) ligands.

Taken together, these observations suggest that specific, high-affinity mercuric ion binding by MerR is complex and at least three of the four cysteine residues present in each monomer contribute to transcriptional activation (an a^+ phenotype). The requirement for multiple cysteine residues may reflect sequential interaction with Hg(II) or may be due to adoption of a tri- or tetracoordinate geometry. Site-directed mutagenesis experiments using cysteine → alanine changes may clarify these possibilities (in progress). The availability of cysteine to alanine mutants will rule out specific perturbation of structure due to cysteine to tyrosine changes and may allow specific assignment of [β-^{13}C]cysteine resonances for [^{13}C]cysteine-enriched NMR contact shift analyses.

Genetic and biochemical analysis of the MerR protein suggests that mercuric ion binding is mediated by the cysteine residues clustered near the C-terminus and DNA binding is mediated by a helix–turn–helix motif near the N-terminus. These two activities are genetically separable, but we have been unable to demonstrate a two-domain structure using classical proteolysis techniques [see Shewchuk et al. (1989)]. This suggests that the activation of transcription by MerR may require only subtle changes in gross protein structure. The mode of action of this metal-responsive genetic switch appears distinct from previously studied regulatory proteins that use ligand binding to directly alter DNA recognition (Garges & Adhya, 1988; Zhang et al., 1987).

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Determination of Amino- and Carboxyl-Terminal Sequences of Guinea Pig Liver Transglutaminase: Evidence for Amino-Terminal Processing†

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ABSTRACT: Transglutaminases (EC 2.3.2.13) catalyze the formation of ϵ-(γ-glutamyl)lysine cross-links and the substitution of a variety of primary amines for the γ-carboxamide groups of protein-bound glutaminyl residues. These enzymes are involved in many biological phenomena. In this study, the amino- and carboxyl-terminal sequences of guinea pig liver transglutaminase were identified by sequence analysis to determine whether this enzyme is processed posttranslationally at its terminal regions. Two peptides, believed to contain the amino-terminal sequences of transglutaminase, were isolated from the Pronase digest of the enzyme protein with SP-Sephadex C-25 column chromatography and reverse-phase HPLC. Analyses (amino acid analysis, sequencing after the treatment with an acylamino-acid-releasing enzyme, and fast atom bombardment mass spectrometry) of these peptides indicated that the amino-terminal structure of this enzyme is acetylAla-Glu-Asp-Leu-Ile-Leu-Glu. The candidate for the carboxyl-terminal peptide in the trypsin digest of enzyme was isolated from the unadsorbed fraction of affinity chromatography with anhydrotrypsin agarose gel. The peptide was found to be Asn-Val-Ile-Ile-Gly-Pro-Ala. Both the terminal sequences were completely consistent with those predicted from the cDNA sequence [Ikura, K., Nasu, T., Yokota, H., Tsuchiya, Y., Sasaki, R., & Chiba, H. (1988) *Biochemistry 27*, 2898–2905]. These results indicated that the amino-terminal processing occurred after or in the course of translation of this enzyme, namely, removal of the initiator methionine and a subsequent acetylation of the alanine residue adjacent to the methionine. Our results did not indicate carboxyl-terminal processing of guinea pig liver transglutaminase.

Transglutaminases (protein-glutamine:amine γ-glutamyltransferase, EC 2.3.2.13) are calcium-dependent acyltransferases that catalyze the formation of an amide bond between the γ-carboxamide groups of peptide-bound glutamine residues and the primary amino groups in a variety of compounds, including the ϵ-amino group of lysine in certain proteins. These enzymes are widely distributed in most tissues and body fluids, and several are involved in diverse biological

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