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Ultrasensitivity and heavy-metal selectivity of the allosterically modulated MerR transcription complex

(metalloregulatory protein/transcriptional activation/mercuric thiolate chemistry/mercury resistance)

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ABSTRACT **The MerR metalloregulatory protein is a heavy-metal receptor that functions as the repressor and Hg(II)-responsive transcription activator of the prokaryotic mercury-resistance (*mer*) genes. We demonstrate that this allosterically modulated regulatory protein is sensitive to $HgCl_2$ concentrations of $1.0 \pm 0.3 \times 10^{-8}$ M in the presence of 1.0×10^{-3} M dithiothreitol for half-maximal induction of transcription of the *mer* promoter by *Escherichia coli* RNA polymerase *in vitro*. Transcription mediated by MerR increases from 10% to 90% of maximum in response to a 7-fold change in concentration of $HgCl_2$, consistent with a threshold phenomenon known as ultrasensitivity. In addition, MerR exhibits a high degree of selectivity. Cd(II), Zn(II), Ag(I), Au(I), and Au(III) have been found to partially stimulate transcription in the presence of MerR, but concentrations at least two to three orders of magnitude greater than for Hg(II) are required. The molecular basis of the ultrasensitivity and selectivity phenomena are postulated to arise from the unusual topology of the transcription complex and a rare trigonal mercuric ion coordination environment, respectively. This mercuric ion-induced switch is to our knowledge the only known example of ultrasensitivity in a signal-responsive transcription mechanism.**

Ligand-responsive transcription factors must not only recognize their signal with specificity and sensitivity but must also efficiently transduce that signal into a change in transcription of a target promoter. The MerR metalloregulatory protein is a Hg(II)-responsive transcriptional regulator of the prokaryotic mercury-resistance (*mer*) operon that encodes proteins for Hg(II) sequestration and detoxification (1–3). MerR represses basal-level transcription of the *mer* resistance promoter (P_T) *in vivo* in the absence of Hg(II) and activates transcription in the presence of Hg(II) (4–10). Purified MerR has been shown to be necessary and sufficient for repression and metal-dependent activation of transcription *in vitro* (11). In contrast to other ligand-responsive transcription systems, MerR mediates both repression and mercuric ion-induced activation when bound to a single DNA site centered within the promoter (11). It is curious, in light of the deleterious effects of Hg(II) on both interior and exterior cellular components, that mercuric ion signal transduction utilizes an intracellular receptor such as MerR rather than a membrane receptor as commonly observed in other prokaryotic signal transduction systems (12). As we show below, a DNA-bound receptor allows establishment of an ultrasensitive switching mechanism that provides a sharp threshold response to small increases in mercuric ion concentration.

In the present study, we have investigated the dynamics of transcriptional regulation by MerR in response to metal ions other than Hg(II) to further elucidate the mechanism of MerR metal ion recognition and specificity. Nanomolar concentrations of Hg(II) are sufficient to stimulate high levels of transcription by MerR, and other metal ions may partially activate MerR when concentrations exceed the micromolar range. The response of the MerR transcription complex to concentrations of all activating metal ions demonstrates greater sensitivity (ultrasensitivity) than for systems obeying Michaelis–Menten kinetics (13).

MATERIALS AND METHODS

Proteins and Transcription Templates. MerR was purified and quantitated as described (11). Concentrations reported here correspond to MerR dimer. DNA templates used for transcription reactions were derived from plasmid pGMer (11). A 1-kilobase *Hin*dIII–*Sph* I P_T fragment and a 1.3-kilobase *Ava* I–*Pvu* I β-lactamase promoter fragment (βla) were gel-purified and recovered using Elutip D (Schleicher & Schuell). Template concentrations, expressed as units of molar fragment, were determined by optical density at 260 nm by using a molar extinction coefficient of 1.3×10^4 per nucleotide pair (14).

Abortive Transcription. Transcriptional activity was determined by the method of abortive initiation (15) in 20 μl containing 0.4 nM DNA template, 2.5–25 nM MerR dimer, and 50 nM *Escherichia coli* RNA polymerase (Pharmacia). Buffer was 100 mM potassium glutamate/10 mM Tris·HCl, pH 8/2 mM $MgCl_2$/5% (vol/vol) glycerol/bovine serum albumin (100 μg/ml)/1 mM dithiothreitol. Reaction mixtures containing DNA template, MerR, RNA polymerase, and metal ions were incubated for 45 min at 37°C before the addition of 450 μM ApU dinucleoside initiator and 0.2 μM [α-^{32}P]CTP. Transcription was allowed to proceed for 30 min, then terminated with formamide dye solution, heated to 90°C, and loaded directly to denaturing 15% polyacrylamide gels. Control reactions were carried out under similar conditions with the βla promoter fragment using the dinucleoside initiator GpA and [α-^{32}P]UTP. Transcription rates were found to be highly sensitive to reaction conditions, such as nucleotide and protein quality. Specific activities measured under directly comparable conditions are presented in the text. Despite variability in transcription maxima, the transcription sensitivity values (n_t) and half-maximal activation concentrations were consistent across individual determinations.

Heavy Metal Salts. Stock solutions at 10–100 mM of $NaO_2CCH_2CH(SAu)CO_2Na$ (Aldrich), $CdSO_4$ (Puratronic, Johnson-Matthey, Seabrook, NH), $HAuCl_4$, $CoCl_2$, $CuSO_4$, $Pb(NO_3)_2$, $MnSO_4$, $HgCl_2$ (99.9%, Aldrich), $NiSO_4$, K_2PtCl_4 (Johnson-Matthey), $AgNO_3$, $TlNO_3$ (Alfa), $ZnCl_2$ (99.9%, Aldrich), and $(CH_3CN)_4CuPF_6$ were made up on the day of assay. Stock solutions were analyzed by mercury atomic absorption spectroscopy and found to contain no detectable

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Abbreviations: P_T, promoter of *mer* resistance genes; βla, β-lactamase; n_t, transcription sensitivity coefficient.

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mercuric ion contamination. By using a detection limit of 0.2 ppm, we estimated that the Hg(II) concentration was <1 nM for all assays of other metal ions, including the highest concentrations where induction was observed (10^{-4} M). CH_3HgCl, *p*-($HOHgC_6H_4SO_3Na$), and $C_6H_5HgO_2CCH_3$ were assayed by run-off transcription (11).

Quantitation of Transcription. Transcription products were cut from gels and quantitated in a Beckman model LS600 scintillation counter. The cpm per gel slice were converted to mol of RNA product based on the specific activity of ^{32}P. Metal-dependent transcription profiles were fit by nonlinear regression to a logarithmic response function (Eq. 1) of the same form as the Hill equation (16, 17)

$$y = \frac{(M)^{n_t}}{(M)^{n_t} + (M_{0.5})^{n_t}}, \quad [1]$$

in which y represents the specific activity of *E. coli* RNA polymerase at the metal-responsive P_T in the presence of saturating concentrations of MerR and at a given metal ion concentration, M; $M_{0.5}$ represents the concentration of metal ion that results in the half-maximal response (18), and n_t represents the transcription sensitivity coefficient. The relationship between the function shown in Eq. 1 with the Hill equation is discussed below and n_t is comparable to the "apparent Hill coefficient," n_H, described in other studies of ultrasensitive phenomena (13).

RESULTS

Metal Ion Specificity and Sensitivity of MerR. Activation of transcription by RNA polymerase at P_T requires the presence of MerR and submicromolar Hg(II) (11). In this study, we have determined the precise activation profile of MerR-mediated P_T transcription in response to mercuric ion (Fig. 1*B*). At very low concentrations of Hg(II) (10^{-10} to 10^{-9} M), low activity (9.7 mol of RNA per mol template per hr) was observed, corresponding to repressed transcription levels of P_T. Between 10^{-9} and 10^{-7} M Hg(II), the level of transcriptional activity shifted from 9.7 to 200.1 mol of RNA per mol of template per hr (100% activity). In fact, the range of 3.6×10^{-9} to 3.2×10^{-8} M Hg(II) resulted in a change from 10% to 90% of activation in this experiment. On average, an increase of a factor of 7 ± 2 in Hg(II) concentration was required for this change. The concentration of Hg(II) that resulted in half-maximal activation in Fig. 1*B* was 1.2×10^{-8} M. Between 10^{-7} and 10^{-4} M Hg(II), the maximal level of transcription was maintained.

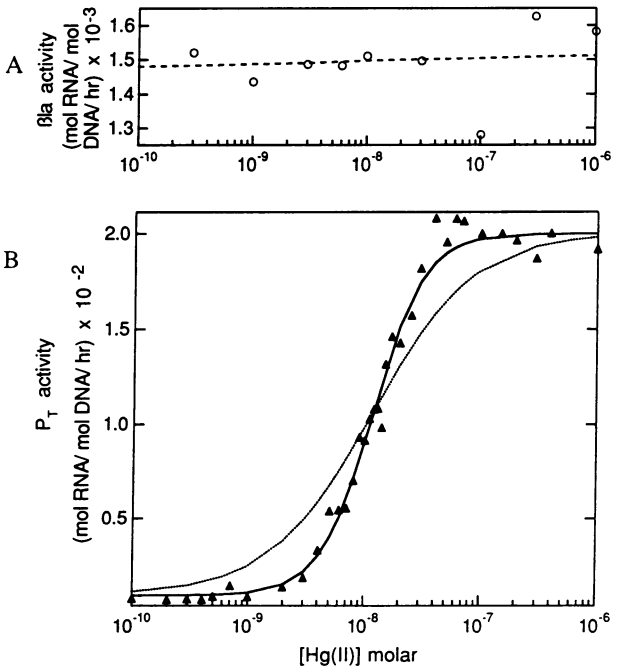

FIG. 1. Transcriptional activity of control and Hg(II)-responsive promoters in the presence of 2.5 nM MerR and a range of mercuric ion concentrations. (*A*) βla promoter. (*B*) P_T. The solid curve with an n_t of 2.0 is the least squares fit of the data to Eq. 1 (16, 17). The dotted curve was calculated using an n_t of 1.0, typical of Michaelis–Menten sensitivity.

To determine whether the effects of mercuric ion and other metal ions were specifically mediated by MerR or a result of the interaction of metal ions with RNA polymerase, we also investigated the effects of the same range of metal ion concentrations on the MerR-independent βla promoter. The βla promoter showed high levels of transcription (≈1400 mol of RNA per mol of template per hr) at all concentrations of Hg(II) in the presence (Fig. 1*A*) and absence of MerR. Between 10^{-6} and 10^{-4} M Hg(II), a slight (≈5%) increase in βla transcription was observed (data not shown).

The $CdSO_4$ activation profile shown in Fig. 2*A* was similar to that for Hg(II), with two important differences: the maximal level of transcription that occurred was 84% of that for Hg(II) and the half-maximal inducing concentration of the metal ion (2.0×10^{-6} M) was two orders of magnitude higher than required for Hg(II) stimulation. A threshold effect was also evident with cadmium between 1.0×10^{-6} and 4.0×10^{-6} M, where transcriptional activity shifted from repressed levels, at 8.5 mol of RNA per mol of template per hr, to the maximal response of 230 mol of RNA per mol of template per hr. In response to Zn(II) (Fig. 2*B*), the maximal activity was 94.4 mol of RNA per mol of template per hr [34% of the Hg(II) response] and the apparent half-maximal inducing concentration was 1.2×10^{-5} M Zn(II), a difference of three orders of magnitude from Hg(II).

Aurothiomalate [$NaO_2CCH_2CH(SAu)CO_2Na$] was found to stimulate transcription with an apparent half-maximal inducing concentration of 1.2×10^{-6} M (Fig. 2*C*). Similar induction was observed with $HAuCl_4$ (data not shown); however, it is likely that this response results from Au(I) generated by reduction of Au(III) by buffer thiols (19). Transcriptional activation by MerR in response to $AgNO_3$ in a chloride-free buffer system [which allowed normal Hg(II)-responsiveness] gave varied results. When silver induction occurred, the apparent half-maximal effective concentration was $\approx 5 \times 10^{-5}$ M and the maximum induction was 98% of that in response to Hg(II).

In general, when metal ion concentrations approached 10^{-3} M in the abortive initiation assay, transcriptional activity at both P_T and βla promoter decreased abruptly to undetectable levels. The decline in transcriptional activity occurred when the metal concentration exceeded the buffer thiol capacity, resulting in the formation of inactive metal–biopolymer complexes. With aurothiomalate, P_T transcription decreased to background levels at concentrations greater than 1×10^{-5} M (Fig. 2*C*), whereas control reactions did not drop until 1×10^{-4} M, suggesting direct Au(I) inactivation of MerR.

Although the Tn*501* MerR protein mediates narrow spectrum resistance specifically to inorganic mercury salts, the organomercurial compounds CH_3HgCl, *p*-($HOHgC_6H_4SO_3Na$), and $C_6H_5HgO_2CCH_3$ were assayed by a run-off *in vitro* transcription assay (11) (data not shown), and no activation of transcription was observed in the latter two cases. CH_3HgCl, on the other hand, was effective at inducing P_T transcription, although it is likely that this response arises from inorganic Hg(II) that contaminates most sources of CH_3HgCl.

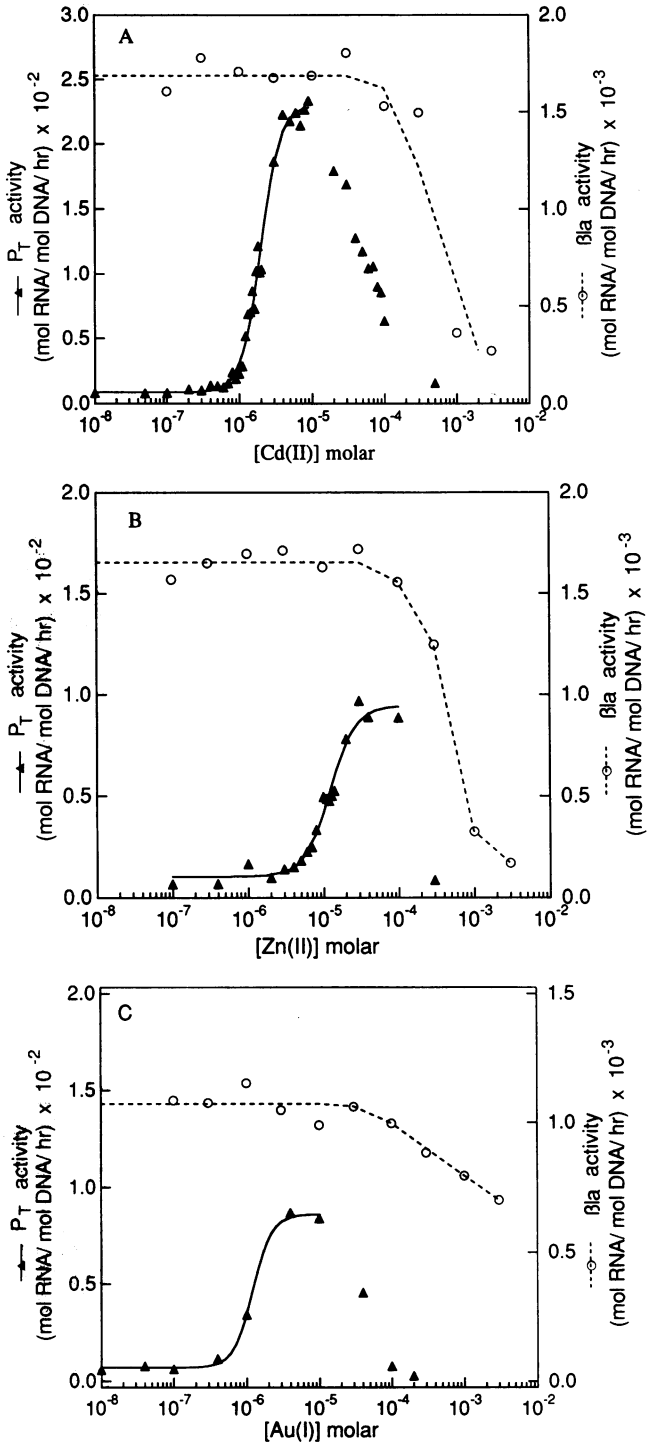

FIG. 2. Transcriptional activity of the P_T (▲) and βla (○) promoters in response to heavy metal ions. Transcriptional activity in response to the following compounds is shown. (*A*) $CdSO_4$. (*B*) $ZnCl_2$. (*C*) $NaO_2CCH_2CH(SAu)CO_2Na$. Solid curves shown for P_T are least squares fit of data to Eq. 1 (16, 17); dashed lines have been drawn to emphasize βla activity.

DISCUSSION

Two principal conclusions emerge from these studies of the effect of signal molecule concentration on the activity of an allosterically modulated transcriptional activator protein. Measurement of signal-response curves establishes key properties of the MerR receptor site and provides insights into the molecular basis of heavy metal recognition. The steepness of the signal-response curves is analogous to threshold phenomena observed in a variety of regulated enzymatic systems, and a possible mechanism involving RNA polymerase is elaborated below.

Heavy Metal Recognition: Receptor Sensitivity and Selectivity. We have established the sensitivity of MerR as a mercuric ion receptor to be $1.0 \pm 0.3 \times 10^{-8}$ M for half-maximal transcriptional activation under defined buffer conditions (Table 1). This high degree of sensitivity is striking due to the fact that nanomolar levels of MerR are able to detect trace amounts of Hg(II) in the presence of excess (1×10^{-3} M) competing Hg(II) ligands, such as 2-mercaptoethanol or dithiothreitol. Both of these thiols are stringent competitors for free Hg(II) and have binding constants on the order of 10^{45} for the formation of bismercuric thiolate complexes (20). The competition between thiols and MerR is best represented in Eq. 2.

$$Hg(SR)_2 + MerR \rightleftharpoons Hg\text{–}MerR + 2RSH \quad [2]$$

The receptor site in MerR must provide thermodynamic stabilization for Hg(II) binding well beyond that provided by buffer thiols. As elaborated below, it is likely that this additional stability arises from a combination of the chelate effect, competing-ligand exclusion, and a favorable enthalpic contribution from a third metal-thiolate bond not present in mercuric ion complexes with buffer thiols.

While the sensitivity of the metal ion receptor site is determined by Hg(II) titration in the presence of competitors, the characterization of different metal ion activation profiles establishes the selectivity of the receptor for Hg(II) relative to other metal ions. We have reported (21) that Hg(II) or Cd(II) can activate P_T transcription by way of Tn*501* MerR. In the present study, we delineate the relative K_m ($M_{0.5}$) values for Cd(II), Zn(II), Au(I), and Au(III) (Table 1). Relative to Hg(II), much higher concentrations of these other metals are required to induce transcription. The transcriptional response to all metal ions tested is summarized in Fig. 3. MerR from *Bacillus* responds to Hg(II) and Cd(II) but not

Table 1. $M_{0.5}$ and n_t data for relevant metal ions in *in vitro* MerR–RNA polymerase assays

Trial	$M_{0.5}$,* M	n_t*	MerR, nM
Hg(II)			
1	5.7×10^{-9}	2.4	25
2	1.0×10^{-8}	2.8	25
3	1.3×10^{-8}	2.0	2.5
4	1.2×10^{-8}	2.0	2.5
Average	1.0 (0.3) × 10^{-8}	2.3 (0.4)	
Cd(II)			
1	1.8×10^{-6}	3.6	25
2	8.3×10^{-7}	3.1	25
3	2.0×10^{-6}	3.2	2.5
Average	1.5 (0.6) × 10^{-6}	3.3 (0.3)	
Zn(II)			
1	1.8×10^{-5}	2.6	25
2	1.2×10^{-5}	2.5	2.5
Average	1.5×10^{-5}	2.55	
Au(I)			
1	1.2×10^{-6}	3.3	25
2	1.4×10^{-6}	3.1	38
3	1.3×10^{-6}	2.5	25
Average	1.3 (0.1) × 10^{-6}	3.0 (0.4)	
Au(III)			
1	1.1×10^{-6}	3.6	38
2	1.1×10^{-6}	2.1	25
Average	1.1×10^{-6}	2.8	

Numbers in parentheses represent the standard deviation of the data.
*Values were determined by computer fit of the data to Eq. 1.

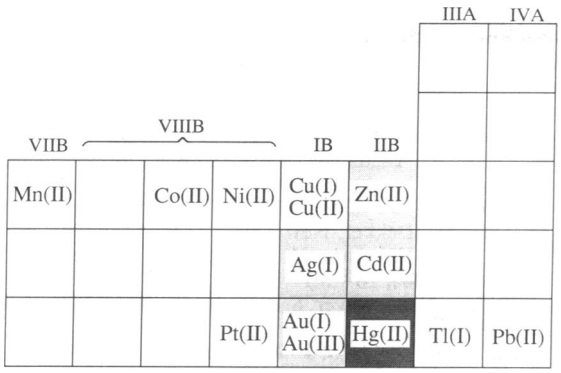

FIG. 3. Metal ions assayed in the *in vitro* MerR–RNA polymerase transcription system. Unshaded boxes indicate metal ions that exhibited no activation of P_T transcription under these assay conditions. Light shading indicates stimulatory metal ions that exhibit a $M_{0.5}$ (K_m) $\geq 10^{-6}$ M. Dark shading indicates stimulatory metal ions that exhibit a $K_m \leq 10^{-8}$ M. The effect of Co(II), Ni(II), Cu(I), Cu(II), Ag(I), Au(I), Au(III), Zn(II), Cd(II), and Hg(II) on the βla promoter was also examined. No effect on transcription was observed at the βla promoter at metal ion concentrations $<10^{-4}$ M.

to other divalent metal ions (J. Helmann and C. T. Walsh, personal communication).

Metal-dependent transcriptional response curves are not simple metal–receptor binding isotherms but rather arise from several consecutive equilibria involving mercuric ion (or its analogue), MerR, the DNA fragment bearing the P_T sequence, and RNA polymerase. Saturating conditions were established for each of the biopolymer components by varying the respective concentrations in the presence of excess Hg(II). Thus the steady-state rates of transcript formation at saturating metal ion concentrations are V_{max} values and the concentrations of metal ions giving rise to half-maximal transcription activity ($M_{0.5}$) are K_m values for the specific metal. It should be noted that $M_{0.5}$ values in response to Zn(II) and Au(I) are lower limits for K_m. The rapid fall-off in RNA polymerase activity in both the βla control and the MerR-mediated systems at metal concentrations greater than $\approx 1 \times 10^{-4}$ M prevents a more precise delineation of $M_{0.5}$ and n_t for Zn(II) and Au(I).

Although MerR selectivity decreases in an order [Hg(II) >> Cd(II) > Zn(II)] that parallels the affinity of the metal ions for thiol in general, this selectivity is exhibited in the presence of 100,000-fold excess of buffer thiol over MerR. Under these conditions the free metal ion concentration in the thiol buffer is opposite: [Zn(II)] > [Cd(II)] >> [Hg(II)]. Uncomplexed Zn(II) is more available for receptor binding yet the receptor discriminates against Zn(II) by a factor of at least 10^3. The high degree of selectivity and sensitivity of MerR for nanomolar Hg(II) levels can be explained in part by recent physical studies of Hg–MerR that indicate unusual Hg(II) coordination in the receptor site. Although Hg(II) is commonly found in linear, two-coordinate complexes, data from extended x-ray absorption fine-structure spectroscopy (EXAFS) (22), thiol titrations (22) and UV difference spectroscopy (23) support Hg(II) coordination to three cysteinyl residues in the metal ion receptor site of the Tn*501* MerR protein. While a model of linear biscoordination of Hg(II) to Cys-126 with ancillary coordination by Cys-82 has been proposed based on site-directed mutagenesis (24), mutant heterodimer complimentation for the *Bacillus* sp. RC607 MerR protein is consistent with models for a tricoordinate binding environment (25). Three-coordinate Hg(II)–thiolate complexes, although less commonly encountered in structural studies of Hg–SR compounds, have association constants on the order of 10^2 (from the respective two-coordinate complex) and are therefore present in trace amounts in the buffer at Hg(II) and thiol concentrations employed in these studies (20, 26). Assuming a tridentate mercuric–cysteinate environment in Hg–MerR, an enthalpic stabilization corresponding to the third mercuric thiolate bond is realized upon metal–protein complexation, as shown in Eq. **2**. In addition, a favorable entropic term arising from the chelate effect is expected to further stabilize the metal–protein complex.

The two to three orders of magnitude differences between the apparent K_m ($M_{0.5}$) values of Hg(II) and the gratuitous inducers are indicative of signal discrimination by MerR and are consistent with known coordination chemistry. Two of the gratuitous inducers, Zn(II) and Cd(II), can form mononuclear trigonal, metal–thiolate complexes (27, 28) analogous to the known $Hg(SR)_3$ complexes (20, 23, 28). The Cd–MerR complex, based on analogy with the model complexes, may be similar to the Hg–MerR complex. Although this possibility is supported by the correspondence of the metal protein stoichiometry in these two cases (S. L. Johnson and T.V.O., unpublished results), conclusive delineation of metal recognition awaits physical and chemical characterization of the Cd–MerR coordination environment. CdL_n formation constants slowly decrease from n = 1–4, whereas, for the HgL_n series, the respective constants for n = 1 or 2 are large but for n = 3 or 4, they are much smaller (26, 29). In fact the fourth formation constant for an Hg(II) thiolate has never been reported in spite of direct attempts to measure it (30). Accordingly, a three-coordinate receptor site would enthalpically favor Hg(II) and also disfavor Cd(II) when competing thiol concentrations are high.

Metal ions that activate transcription in this system have also been shown to alter the interactions of the MerR protein with DNA in a manner that parallels the effect of Hg(II). Both Hg(II) and Au(I) have been found to cause a slight (3-fold) increase in the K_d of MerR for its operator sequence (ref. 11; M. K. Shin and T.V.O., unpublished data). In addition, both Cd(II) and Hg(II) induce a similar MerR-mediated hypersensitivity to intercalating chemical nucleases, such as methidiumpropyl-EDTA-Fe(II), and this has been interpreted as a distortion in the center of the palindromic operator sequence (31). The operator sequence spans the −10 and −35 promoter spacer region, thus the distortion also involves a change in promoter structure in response to metal ions. Current data support a model in which a metal-induced change in MerR conformation drives a local underwinding of promoter DNA that facilitates transcription initiation by *E. coli* RNA polymerase (11, 31).

Ultrasensitivity: Threshold Response. Koshland *et al.* (18) have described biological switches that govern protein activity as being subsensitive, hyperbolically sensitive (Michaelis–Menten), or ultrasensitive in regard to the range of concentration of ligand required to result in a change from 10% to 90% activity. This range has been referred to as the response coefficient, R_s or R_v (13, 18), and has a value of 81 for Michaelis–Menten sensitivity. All metals that activate P_T transcription were found to have R_s values between 3 and 9, indicative of ultrasensitivity. Furthermore, when Hg(II), Cd(II), Zn(II), and Au(I) response curves are fit to Eq. **1**, they are found to have n_t values in the range of 2.3–3.3 (Table 1). n_t values >2 are also observed for Hg(II) response curves carried out with 0.1 or 10 mM dithiothreitol or 2 mM 2-mercaptoethanol (data not shown). The term n_t is analogous to the Hill coefficient n_H; however, we emphasize that the sigmoid mercuric ion response of P_T transcription is different from classical Hill behavior since MerR binds only one Hg(II) per dimer.

Goldbeter and Koshland (13) further suggest that enhanced sensitivity may arise as the result of (*i*) cooperative ultrasensitivity, such as that demonstrated by allosteric multiple-ligand binding proteins; (*ii*) multistep ultrasensitivity arising from a stimulus that affects more than one protein in a

pathway; or (*iii*) the zero-order ultrasensitivity of reversible covalent modification systems.

Based on data from *in vitro* (31) and *in vivo* (7) footprinting results indicating that RNA polymerase is trapped in a closed inactive complex with MerR at P_T in the absence of Hg(II), the zero-order ultrasensitivity mechanism best accounts for the abrupt transcriptional activation at P_T. The ultrasensitive response in the zero-order scheme (Eq. 3)

$$\text{Effector} \Downarrow$$

$$\text{Low activity protein} \underset{\text{Modifying protein'}}{\overset{\text{Modifying protein}}{\rightleftharpoons}} \text{High activity protein} \quad [3]$$

is dependent on the condition that the modifying protein be saturated with respect to a low-activity protein substrate; i.e., in the zero-order range of concentration. MerR bound at its operator sequence may function as the modifying protein where Hg(II) is the effector as in Eq. 4.

$$\text{Hg(II)} \Downarrow$$

$$[\text{RNAP}\text{–}P_T\text{–MerR}]_{\text{closed}} \rightleftharpoons [\text{RNAP}\text{–}P_T\text{–MerR}]_{\text{open}} \quad [4]$$

The "low-activity" protein in this instance is an RNA polymerase (RNAP) complex with its cosubstrate, P_T. This particular transcription complex exhibits low activity even in the absence of MerR because the promoter spacer region between the −10 and −35 sequences deviates from consensus by an additional two base pairs (6). Under the conditions of the transcription assay, the closed complex containing MerR apparently leads to the saturation of *E. coli* RNA polymerase at P_T. Furthermore, kinetic data indicate that Hg(II) binding by MerR effectively increases the RNA polymerase turnover rate by specifically stimulating the isomerization of RNA polymerase–promoter complexes from a closed inactive form to an open transcriptionally active form (D.M.R., M. T. Szatkowski, M. K. Shin, and T.V.O., unpublished data). This activity parallels the origin of zero-order ultrasensitivity in other systems, such as the phosphorylation of isocitrate dehydrogenase (32) and glycogen phosphorylase (33). In such systems, ultrasensitivity arises from the specific enhancement of one activity in a pair of opposing activities: the rate of kinase activity over the rate of dephosphorylation. In these *in vitro* MerR assays, the simple enhancement of the forward rate of open transcription complex formation over the repressing activity of MerR without Hg(II) may establish a similar set of opposing modifying activities. An additional criterion of the zero-order ultrasensitivity mechanism (13) is met by the fact that the effect of Hg(II) on MerR results in a change in steady-state transcriptional activity.

The single metal ion receptor site of MerR limits the interpretation of the acute transcriptional response to multistep or zero-order ultrasensitivity. A determination of the effect of Hg(II) on reverse rate constants will discriminate between mechanisms. Alternatively, it is possible that MerR forms a tetramer or other oligomer when bound to DNA in the transcription complex.

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