Coordination Chemistry of the Hg-MerR Metalloregulatory Protein: Evidence for a Novel Tridentate Hg-Cysteine Receptor Site

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The MerR metalloregulatory protein is a member of a class of metal responsive factors that trigger cellular responses at the genetic level.[1-3] A specific and ultrasensitive inorganic sensor, MerR switches on transcription of the bacterial mercuric ion resistance genes (*mer*) in the presence of nanomolar Hg(II) or micromolar Cd(II).[4,5] Mercuric ion binding in a stoichiometry of one metal ion per MerR dimer[6,7] converts MerR from a repressor to a strong activator of transcription.[5,6] Studies of site-specific mutations in each of the four cysteine residues per MerR monomer have led to the proposal that Hg(II) utilizes a linear bis-coordinate geometry in bridging Cys126 residues in the MerR dimer with possible ancillary ligation of the Cys82 residues.[8] However, low-energy LMCT transitions in the Hg-MerR UV difference spectra are characteristic of mercuric-thiolate complexes with a primary coordination number of 3 or 4.[9] We present evidence from extended X-ray absorption fine structure (EXAFS) spectroscopy and chemical modification experiments that Hg-MerR has a three-coordinate, $Hg(S\text{-}Cys)_3$ environment. This unusual tridentate heavy metal receptor site is consistent with the thermodynamic stability of $[Hg(SR)_3]^-$ complexes[10,11] and may account both for the high-affinity Hg(II) binding and for the selectivity for Hg(II) over other soft group IIB metal ions that prefer tetrahedral metal-thiolate coordination.[5]

Purified Tn501 MerR protein[6,12] was treated with excess mercuric ion in the presence of 1 mM dithiothreitol, purified by gel filtration, and precipitated with 2 M $(NH_4)_2SO_4$. The wet precipitate was studied directly or dissolved in 10 mM triethanolamine-bicarbonate buffer (pH = 7.5) and lyophilized. The Hg/protein ratio, determined for each sample by using graphite furnace atomic absorption and UV spectroscopy, was 0.94 ± 0.10 Hg/MerR dimer. XAS data collection and reduction followed standard methods.[13] Sample integrity was indicated by reconstitution of full transcriptional activity after X-ray measurements. Fourier transforms were calculated by using k^3-weighted data over the range 2-13.2 Å^{-1}. Empirical amplitude and phase functions were used for curve fitting.[14]

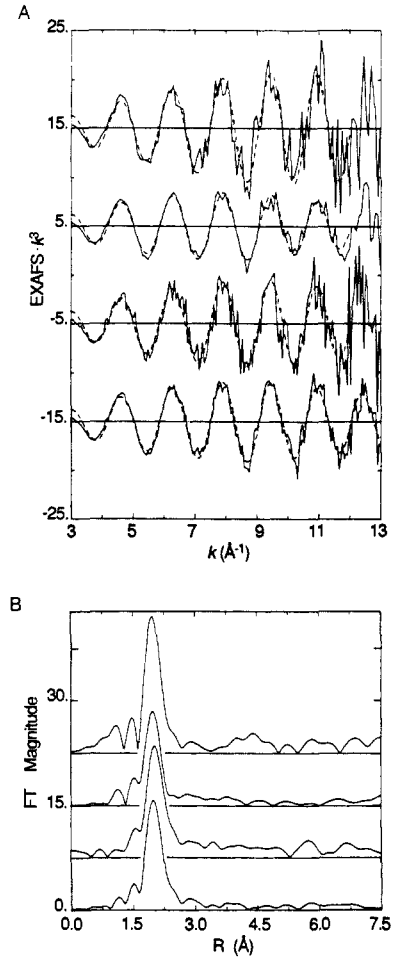

Figure 1. (A) MerR Hg EXAFS data. k^3-Weighted EXAFS spectra for MerR. Solid line = experimental data; dashed line = best fit using a single shell of sulfur. From top: sample 2, sample 3, sample 4, average of samples 2-4. Spectra offset vertically by +15, +5, -5, and -15, respectively, for clarity. (B) Fourier transforms. From top: sample 2, sample 3, sample 4, average of samples 2-4, showing lack of outer-shell peaks. Spectra offset vertically by 22.5, 15, 7.5, and 0, respectively, for clarity.

The EXAFS spectra (Figure 1A) and corresponding Fourier transforms (Figure 1B) for Hg-MerR show only one resolved shell of scatterers, regardless of sample preparation method. The best single-shell Hg-S fits are shown in Figure 1A and summarized in Table I. These suggest a three-coordinate Hg(II) site with an average Hg-S distance of 2.43 Å. As illustrated by the sulfur-only fits for the averaged data, the EXAFS goodness of fit alone cannot be used to determine uniquely the coordination

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(14) $[NEt_4]_2[Hg(SPhCl)_4]$,[15] $Hg(Cys)_2Cl\cdot H_2O$,[16] and $Hg(SCN)_2$[17] for Hg-S; $Hg(pyridine)_2^{2+}$[18] for Hg-N. Identical structural results were obtained with the different models. $Hg(SEt)_2$ was also examined; however, the EXAFS Hg-S distance (2.36 Å) differs from the crystallographic distance (2.45 Å), suggesting an error in the crystal structure. See ref 19.
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Table I. Best Fits to Hg-MerR EXAFS[a]

sample	condition	fit[b]	R, Å	N_S	$\Delta\sigma^2 \times 10^3$, Å^2	R, Å	N_N	$\Delta\sigma^2 \times 10^3$, Å^2	F[c]
1	ppt	S	2.42	*d*					
2	ppt	S	2.43	3	-2.3				4.5
		S + N	2.42	3	0.5	2.36	1	-7.5	2.7
3	lyoph	S	2.43	3	0.9				4.4
		S + N	2.43	2	-1.0	2.27	2	8.6	4.0
4	ppt	S	2.43	3	-0.8				4.5
		S + N	2.44	2	-3.1	2.21	2	5.5	2.4
average	-	S	2.43	2	-2.7				3.8
		S	2.43	3	-0.6				2.5
		S	2.43	4	+1.2				4.2
		S + N	2.44	2	-2.6	2.25	2	10.2	2.2

[a] Fits used a range of fixed integer coordination numbers (N) with bond length (R) and Debye-Walter factor ($\Delta\sigma^2$) as freely variable parameters. [b] Fits using sulfur only (S) and using sulfur + nitrogen (S + N) are reported. For samples 2-4, tabulated fits are for values of N giving the best fit for a given fit type (S or S + N). [c] Goodness of fit $F = [(\chi_{calcd}k^3 - \chi_{exptl}k^3)^2/(\mathrm{NPTS} - 1)]^{1/2}[(\chi k^3)_{max} - (\chi k^3)_{min})](100\%)$, where χ_{exptl} and χ_{calcd} refer to measured and simulated EXAFS and max and min refer to the maximum and minimum in the weighted, experimental data. [d] Data were extremely noisy and could be fit with one or two shells of sulfur. Subsequent data gave the same average Hg-S distance but did not confirm the two-shell HgS_2S_2' model.

number. However, the strong dependence of Hg-S bond length on coordination number clearly excludes simple HgS_2 or HgS_4 structures. Hg-S bond distances in mononuclear $Hg(SR)_2$ complexes are found from 2.32 to 2.36 Å (R_{av} = 2.34 Å) while mononuclear $Hg(SR)_4$ complexes exhibit Hg-S distances from 2.50 to 2.61 Å (R_{av} = 2.54 Å).[19] Although there are few crystallographically characterized mononuclear $Hg(SR)_3$ complexes, the examples that are known have Hg-S distances from 2.40 to 2.51 Å (R_{av} = 2.44 Å), consistent with the distance found in MerR.[9,19,20] A Fourier transform of the averaged Hg-MerR data from samples 2-4 is shown in Figure 1B. No detectable contribution from scatterers at >2.5 Å is observed for Hg-MerR; thus there is no EXAFS evidence for secondary bonding interactions.[21]

We see no evidence for two unresolved shells of scatterers in MerR. Although it is difficult to rigorously exclude contributions from a weak scatterer (Hg-N or Hg-O) in the presence of strong Hg-S EXAFS, two-shell fits (Hg-S + Hg-N) give only modest improvement over one-shell Hg-S fits. Improvement is only seen for the noisiest data, and the refined Hg-N distances vary from sample to sample. This argues against Hg-N ligation, although additional structural data on mercuric complexes having mixed sulfur/nitrogen ligation are necessary. No improvement is observed for two-shell (Hg-S + Hg-S') fits. Chloride or exogenous buffer thiol ligation are unlikely on the basis of spectrophotometric titrations and gel filtration studies using radiolabeled thiols;[9] thus the EXAFS results suggest coordination by three endogenous sulfur ligands.

Chemical modification experiments corroborate this model. DTNB titrations[22] repeated in triplicate on the apoprotein reveal that 6.3 (SD = 0.3) of 8 cysteines per dimer are accessible, consistent with results of Schewchuk et al.[7] In contrast to that report, titration of the Hg-MerR samples prepared as described for EXAFS reveal that 3.2 (SD = 0.4) cysteines per dimer are available for reaction with DTNB, yielding a net protection of three cysteines per dimer in the Hg-protein.

One of the striking attributes of MerR is its avidity for mercuric ion; the binding is stoichiometric for nanomolar protein and Hg(II) concentrations, even in the presence of 10^5-fold excess thiol. The tridentate model for Hg-MerR coordination suggests a structural and thermodynamic rationale for the ability of this receptor to discriminate between Zn(II), Cd(II), and Hg(II) while maintaining a nanomolar sensitivity to the latter.[5] Work aimed at further characterizing MerR metal binding is in progress.

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Recognition of Mixed-Sequence Duplex DNA by Alternate-Strand Triple-Helix Formation

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Oligodeoxyribonucleotide-directed triple-helix formation offers a chemical approach for the sequence-specific binding of double-helical DNA that is 10^6 times more specific than restriction enzymes.[1,2] Because triple-helix formation by pyrimidine oligonucleotides is limited to purine tracts, it is desirable to find a general solution whereby oligonucleotides could be used to bind all four base pairs of intact duplex DNA (37 °C, pH 7.0). Approaches toward such a goal include the following: the search for other natural triplet specificities, such as G·TA triplets;[1f] the design of nonnatural bases for completion of the triplet code; the incorporation of abasic residues for nonreading of certain base

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