Biogeochemical and Molecular Mechanisms of Mercury (Hg)

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Environmental Remediation Sciences

Oak Ridge National Laboratory – Science Focus Area

4. Molecular Structure, Dynamics and Simulation

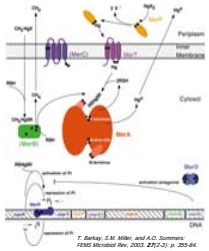
Background

Bacteria participate significantly in mercury transformation in natural and industrial environments. Bacterial mercury resistance is mediated by the *mer* operon, typically located on transposons or plasmids. It encodes specific genes that facilitate uptake of mercury species, cleavage of organomercurials, and reduction of Hg(II) to Hg(0). Expression of all structural genes is regulated by a highly specific Hg(II)-sensitive transcriptional regulator MerR.

Mer operons can be identified in many different classes of bacteria. Proteobacteria are prominent at East Fork Poplar Creek and have a major role in reduction and demethylation by the mer system. Summary of a phylogenetic analysis (right) performed in SFA Task 3 (see poster by Palumbo et al.)

phylum	%	*class*	%
Cyanobacteria	28.8		
Proteobacteria	26.0	β-proteobacteria	45.7
		γ-proteobacteria	31.6
		α-proteobacteria	11.5
		Unclassified proteobacteria	7.9
		δ-proteobacteria	3.3
		ε-proteobacteria	<0.1
Verrucomicrobia	15.1		
Unclassified bacteria	18.9		
Other bacteria	11.2		

Dissimilatory metal reduction also contributes to Hg(II) reduction in the environment. We investigate the role of outer membrane cytochromes in this process.



T. Barkay, S.M. Miller, and A.O. Summers: FEMS Microbiol Rev. 2003. 27(2-3): p. 355-84.

Objective, Hypotheses and Deliverables

The goal of Task 4 is to obtain fundamental understanding of cellular and subcellular processes that profoundly influence chemical speciation of Hg and to determine mechanisms at atomic detail concerning Hg transformation and transport processes in cells using integrated neutron studies with high-performance leadership-class computer simulation.

- Specific binding of Hg(II) to MerR induces allosteric conformational changes in MerR and can affect the operator DNA orientation with respect to the σ^{70} subunit of RNA polymerase.
- Hg-C protonolysis of organomercurials by MerB is the key step to bacterial demethylation. The roles of catalytic residues in the cleavage of the Hg-C bond will be revealed by quantum chemical calculations.
- Multiheme cytochromes in dissimilatory metal-reducing bacteria contribute to the reduction of Hg(II) in the environment.

Deliverables

- Overexpress and purify MerR, characterize by small angle x-ray scattering (SAXS), optimize DNA constructs for MerR/MerOP complexes using neutron scattering and computer simulations. Optimize conditions for MerR/MerOP/RNAP/σ^{70} complexes and deuteration of subunits for characterization by small angle neutron scattering (SANS).
- Construct active site models of MerB and investigate the detailed catalytic mechanism for the Hg-C bond cleavage reaction (Parks et al., *manuscript submitted to JACS*).
- Overexpress and purify the multiheme cytochrome OmcA, SAXS envelope shape, crystallization and diffraction data collection. Solve high resolution crystal structure of OmcA.

Allosteric Conformational Change of MerR

Dimeric MerR binds to a region of dyad symmetry between the -35 and -10 recognition sites of the DNA operator (MerOP). Binding results in the formation of an inactive pre-initiation complex with RNA polymerase (RNAP). Allosteric conformational changes in MerR/MerOP and the ternary MerR/MerOP/RNAP complex including the σ^{70} subunit are induced by Hg(II) and are the cause for initiation of transcription of *mer* genes.

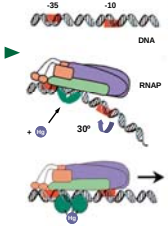
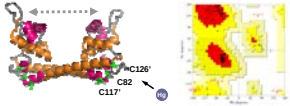
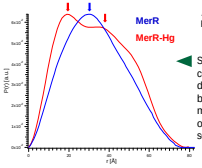


Figure adapted from: Brown, N. L, et al. FEMS Microbio. Rev. 27:145-163 (2003)

A sequence alignment of Tn*21* MerR to known homologous structures available in the PDB was used to find suitable templates for homology modeling.

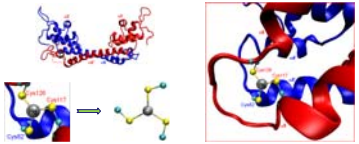


The program MODELLER was used to build a model of homodimeric MerR based on the Ag(I) form of CueR as a template. The Ramachandran plot of the refined model places more than 96% of the residues in the most favored regions of the plot.



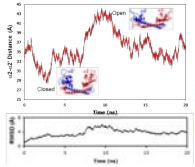
SAXS data reveals a distinct conformational change in the metal-dependent regulator MerR upon binding of Hg(II). Dimeric MerR makes a transition from a compact overall shape to a structure with two separate domains.

Molecular Dynamics Simulation of MerR



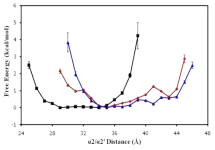
MerR contains two binding sites, but binds only one Hg(II) at a time via trigonal planar coordination with Cys117 and Cys126 of one monomer and Cys82' of the other monomer.

A simplified model, $[Hg(S\text{-}CH_3)_3]^-$, was used to develop CHARMM molecular mechanics parameters for trigonally coordinated Hg(II) in MerR.



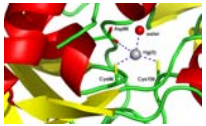
Molecular dynamics (MD) simulations revealed a closed-to-open conformational change in both Hg(II)-free and Hg(II)-bound MerR. The spacing between the DNA-recognition helices α2 and α2' increases ~6 Å upon Hg(II) binding.

Principal component analysis (PCA) also revealed that the principal movement of the lowest frequency mode is the closed-to-open conformational change.



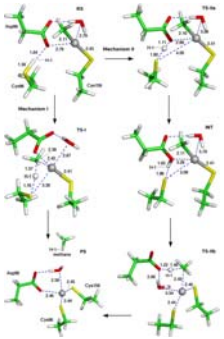
The figure to the right shows that the α2-α2' distance shifts from a closed state in Hg(II)-free MerR to an open state in Hg(II)-bound MerR. Free energy profiles were obtained from 20-ns MD trajectories for Hg(II)-free MerR (black squares), Hg(II)-bound to one binding site of MerR (red diamonds), and Hg(II)-bound MerR with Hg(II) bound at the other binding site (blue triangles).

Mechanism of Hg-C Protonolysis in MerB



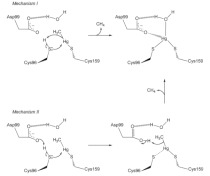
Lafrance-Vanasse, et al. *J. Biol. Chem.*, **2009**, 284, 938-944; Momany et al., *unpublished data.*

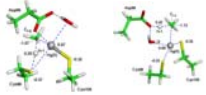
Demethylation is a key reaction in mercury resistance. The bacterial organomercurial lyase, MerB, catalyzes the demethylation of a wide range of organomercurials (including methylmercury) via Hg-C protonolysis. We have intestigated two proposed reaction mechanisms using a quantum chemical density functional theory (DFT) active site model derived from recent X-ray crystal structures of MerB (left).



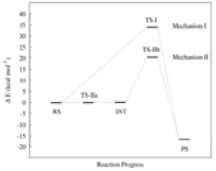
Substrate	Turnover no. (min^{-1})	$\Delta G^{\ddagger}_{exp}$	$\Delta E^{\ddagger}_{calc}$
$[CH_3Hg(II)]^+$	0.7	20.1	20.4
$[CH_2{=}CH\text{-}Hg(II)]^+$	12	18.4	18.3
$[cis\text{-}2\text{-butenyl-}2\text{-}Hg(II)]^+$	240	16.6	15.6

Experimental data from Begley, et al, *Biochem.*, 1986, 25, 7192.





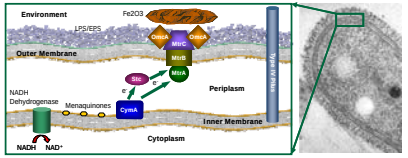
Transition state structures for Mechanism I (left) and Mechanism II (right). Natural Population Analysis (NPA) partial charges are shown for selected atoms.



The calculations favor Mechanism II, in which Cys96 donates a proton to Asp99, and subsequently activates the Hg-C bond by coordinating with Hg at the transition state. Asp99 then protonates the hydrocarbon leaving group. Coordination by two Cys thiolates is necessary and sufficient to activate the Hg-C bond toward protonolysis, and Asp99 serves as a proton mediator in the reaction.

Computed activations barriers reproduce the experimental reaction rates for three organomercurial substrates, methylmercury, vinylmercury, and *cis*-2-butenyl-2-mercury to within ~1.0 kcal/mol.

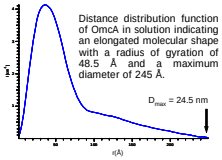
Role of the outer membrane cytochrome OmcA



L. Liang, R. Rinaldi, H. Schober(Eds.): Neutron Applications in Earth, Energy and Environmental Sciences, 16, 461-486, Springer, 2009.

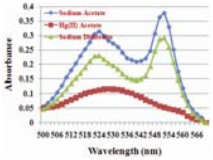
Gram-negative bacterial cell

The genome of *Shewanella oneidensis* MR-1 contains 42 possible *c*-type cytochrome coding sequences. The decaheme outer membrane cytochrome OmcA is the terminal electron donor that transfers electrons to mineral surfaces and may also be responsible for the reduction of Hg(II) at concentrations too low to induce *mer* operon functions.

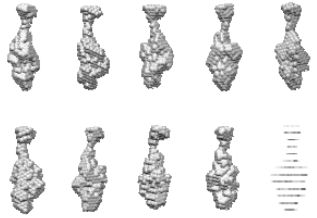


Distance distribution function of OmcA in solution indicating an elongated molecular shape with a radius of gyration of 48.5 Å and a maximum diameter of 245 Å.

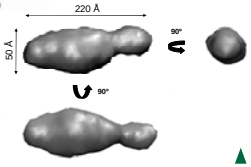
D_{max} = 24.5 nm



Hg(II) acetate oxidizes chemically reduced OmcA as evidenced by disappearance of the peak at 552 nm (red). This demonstrates the direct electron transfer from OmcA to Hg(II). Addition of sodium dithionite re-reduces the oxidized OmcA, showing that OmcA is still redox active after exposure to Hg(II) (green).



Ten single independent models restored by *ab initio* shape reconstruction using the program DAMMIN. Bead size: 10Å



Averaged shape of the molecular envelope of OmcA derived from 20 independent *ab initio* models (left) using the programs SUBCOMB and DAMAVER.

To reveal the details of protein function, atomic resolution structure information is required. OmcA was crystallized in our lab and preliminary X-ray diffraction data was collected.

Summary/Conclusions

- **Initiation of transcription is triggered by a specific Hg(II)-induced change in the conformation of homodimeric MerR. SAXS reveals a conformation shift between the Hg(II)-bound and unbound states.**
- **Developed CHARMM parameters for molecular dynamics simulation of trigonally coordinated Hg(II) in MerR.**
- **Obtained homology model of MerR and observed a closed-to-open conformational change of MerR by MD simulations and principal component analysis.**
- **In MerB, coordination by two cysteines activates the Hg-C bond in organomercurials. Asp99 is the catalytic acid residue in the protonolysis reaction.**
- **Provided evidence that the decaheme outer membrane cytochrome OmcA can directly transfer electrons to Hg(II). Structural data was obtained for this key player in dissimilatory metal reduction.**

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