Hydrocarbon Dissociation Pathways in MerB Catalysis: A Molecular Dynamics Study

Hao-Bo Guo[1], Jerry M. Parks[1], Hong Guo[1,2], Susan M. Miller[3], Anne O. Summers[4], Liyuan Liang[5], Jeremy C. Smith[1,2]

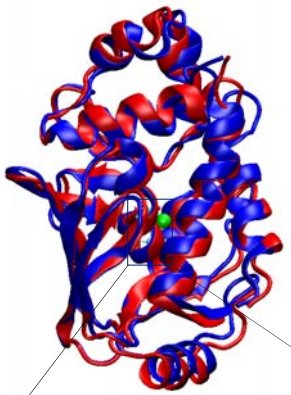
1. ORNL, Center for Molecular Biophysics; 2. University of Tennessee, Knoxville; 3.University of California, San Francisco; 4.University of Georgia, Athens; 5. ORNL, Environmental Science Division.

Oak Ridge National Laboratory – ERSP Science Focus Area

Introduction

The bacterial organomercurial lyase, MerB, catalyzes the protonolysis of organomercurial compounds. MerB cleaves Hg-C bonds of various substrates ranging from the methylmercury cation (MeHg) to merbromin. Upon Hg-C bond cleavage, Hg^{2+} and an organic molecule are produced. For example, methane is the product resulting from the protonolysis of MeHg. The release pathway and mechanism of the organic product are unclear. Here, we have applied molecular dynamics and free energy simulations to study the dissociation of a series of organic molecules. The x-ray crystallographic structure of MerB with a bound Hg^{2+} cation was used as the starting model, and the organic products were manually placed in the active site. The umbrella sampling method was used to obtain free energy profiles for the dissociation pathways. Several hydrophobic sidechains of MerB were found to interact with the organic molecules and may have important roles in the dissociation process. The relatively low free energy barriers of dissociation suggest that organic product dissociation is not rate limiting.

MerB Structure



$$R\text{-}Hg^{+} \xrightarrow{MerB} RH + Hg^{2+}$$

R= alkyl, aryl, …

X-ray crystallographic structures of MerB

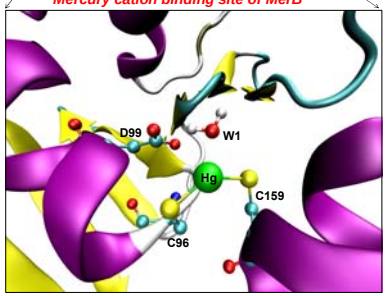
Blue: Unpublished structure, resolution 1.88 Å

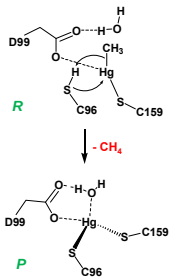
Red: Lafrance-Vanasse *et al*. J. Biol. Chem. in press. pdb id 3F0P, resolution 1.64 Å

Green: Mercury cation

Backbones of the two structures were superimposed with an RMSD of 1.3 Å.

Mercury cation binding site of MerB





R: reactant state; ***P***: product state

The X-ray crystal structure represents the Hg^{2+} bound product state of MerB after the protonolysis reaction and dissociation of the organic product (methane for methyl mercury, see bottom right).

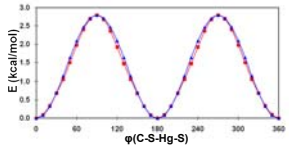
The rate-limiting step of MerB catalysis is the mercury cation release process driven either by *N*-terminal domain of MerA (NMerA) or by compounds with thiol groups (e.g., cysteine).

Starting from the product state (P) of MerB with a bound Hg^{2+} and product (methane) molecule that was manually placed into the mercury cation binding site, free energy simulations were used to study the product (methane) dissociation. A channel formed by hydrophobic sidechains may be important to this process.

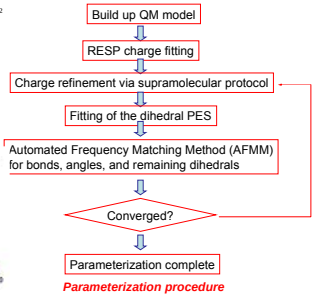
Parameterization of Mercury Cation in MerB

$$U(\vec{R}) = \sum_{bonds} K_b (b-b_0)^2 + \sum_{angles} K_\theta (\theta-\theta_0)^2 + \sum_{UB} K_{UB}(s-s_0)^2 + \sum_{dihe} K_\varphi [1+\cos(n\varphi-\varphi_0)] + \sum_{impr} K_\psi (\psi-\psi_0)^2 + \sum_{ab} \left\{ \varepsilon_{ij} \left[\left(\frac{R_{\min,i,j}}{r_{ij}}\right)^{12} - \left(\frac{R_{\min,i,j}}{r_{ij}}\right)^{6} \right] + \frac{q_i q_j}{4\pi D r_{ij}} \right\},$$

A typical force field that describes the atomic interactions in a bio-macromolecule complex



e.g. Dihedral C-S-Hg-S, K_φ = 1.395 kcal/mol, n = 2, φ_0=90°
Blue: QM PES, Red: MM PES after fitting to the QM PES.



Parameterization procedure

Force field parameters for mercury (in CHARMM format)

Bonds:

		K_b	b_0
Hg	S	324.672	2.486
S	C	205.0	1.833

Angles:

			K_θ	θ_0
Hg	S	C	391.323	102.072
S	Hg	S	15.685	120.000
S	C	H	339.904	110.530

Dihedrals:

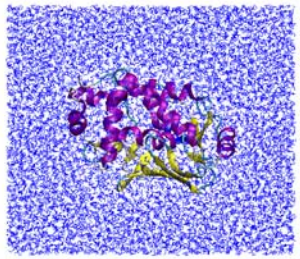
				K_φ	n	φ_0
H	C	S	Hg	0.346	3	0.0
C	S	Hg	S	1.395	2	90.0

vdW:

	ε	$R_{min}/2$
Hg 0.0	-0.44228	1.7745

Charge:
RESP fitting (+0.78 for Hg)

Molecular Dynamics of MerB



MerB was placed in an 81×70×67 Å³ water box, with approximately 38,000 atoms

NAMD software was used for the molecular dynamics (MD) and free energy simulations.

The CHARMM force field and TIP3P water model were used.

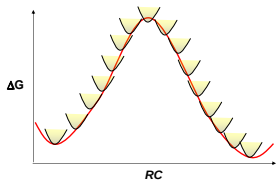
The Particle Mesh Ewald (PME) method was applied for the electrostatic interactions, and a 12 Å cutoff was used for van der Waals interactions.

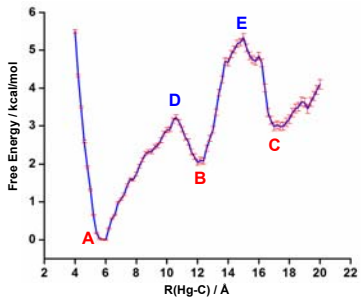
The simulations were carried out in a constant volume and constant pressure (1.0 atm) ensemble.

Product Dissociation -- Free Energy profile

In umbrella sampling, harmonic potentials along the reaction coordinate (RC) to enhance the sampling in high free energy (ΔG) or rare event region, are added. So that we can obtain dynamics and free energy information for the entire process.

In the current study, RC was selected as the distance between the bound Hg^{2+} and carbon atom of the leaving methane molecule. A harmonic force of 10 kcal/mol/Å² was added to RC. Sixteen windows, each of which underwent 200 ps equilibration and 200 ps productive run were sampled for the methane dissociation.

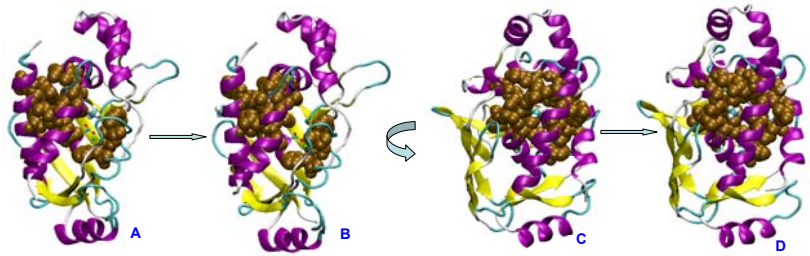




Free energy profile for the hydrocarbon dissociation was computed using the umbrella sampling method. Three minima A, B, and C, and two maxima D and E, were found. The dissociation process shows a free energy barrier of ~5 kcal/mol, indicating that it is not rate-limiting in MerB catalysis.

Hydrophobic sidechains of MerB were found to form a hydrophobic channel. The methane molecule was found to leave the reactive center through a hydrophobic channel formed by hydrophobic sidechains of MerB (see below).

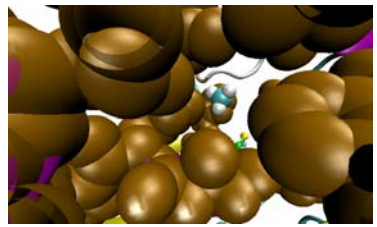
Hydrophobic Interactions Guide the Release Pathway



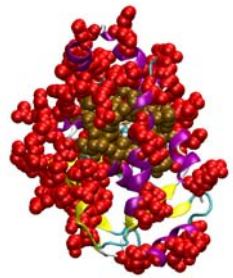
Gold: Channel formed by hydrophobic sidechains. The methane molecule shown in spheres.

The methane product dissociates through the hydrophobic channel. Walls formed by hydrophilic sidechains surrounding the hydrophobic channel prevent the product from escaping in other directions. At the end of the channel, L14, W95, A154, and F158 form a hydrophobic pocket that may function to hold the substrate such as methylmercury.

Methylmercury may also enter the MerB active site through the hydrophobic channel, though the substrate binding and product dissociation processes are not necessarily the same. It should be pointed out that the organic products of other mercurial compounds (e.g. benzene molecule from the benzylmercury) may dissociate by different pathways.



Close-up view of the hydrophobic channel (gold). The methane molecule is shown in spheres.



Hydrophilic sidechains (red) surround the hydrophobic channel (gold)

Conclusions

Charmm force field was parameterized for mercury cation in MerB, the organomecurial lyase, for molecular dynamics simulations. Umbrella sampling was used to study the product dissociation after the cleavage of the mercury-carbon bond that yields the hydrocarbon molecule. The results of the free energy calculations showed that the product dissociation is not rate-limiting in MerB catalysis. It was found that the dissociation pathway may be driven by a channel formed by the hydrophobic sidechains of MerB.

Acknowledgements

This research is sponsored by the U.S. Department of Energy's Office of Science Biological and Environmental Research, Environmental Remediation Sciences Program (ERSP). Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract DE-AC05-00OR22725.

References

1. Di Lello, P.; Benison, G.C.; Valafar, H.; Pitts, K.E.; Summers, A.O.; Legault, P.; Omichinski, J.G. *Biochemistry* **2004**, *43*, 8322-8332.
2. Lafrance-Vanasse, J.; Lefebvre, M.; Di Lello, P.; Sygusch, J.; Omichinski, J.G. *J. Biol. Chem*. In press.
3. Pitts, K.E.; Summers, A.O. *Biochemistry* **2002**, *41*, 10287-10296.
4. Melnick, J.G.; Parkin, G. *Science* **2007**, *317*, 225-227.
5. Vaiana, A.C.; Cournia, Z.; Costescu, I.B.; Smith, J.C. *Comp. Phys. Commun*. **2005**, *167*, 34-42.
6. Petridis, L.; Smith, J.C. *J. Comput. Chem*. In press.
7. Fuchs, J.-F.; Nedev, H.; Poger, D.; Ferrand, M.; Brenner, V.; Dognon, J.-P.; Crouzy, S. *J. Comput. Chem*. **2006**, *27*, 837-856.