

# Mechanism of Hg-C Protonolysis in the Bacterial Organomercurial Lyase MerB

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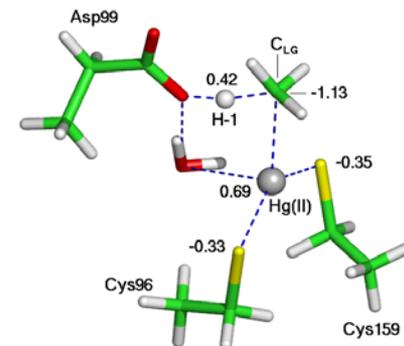
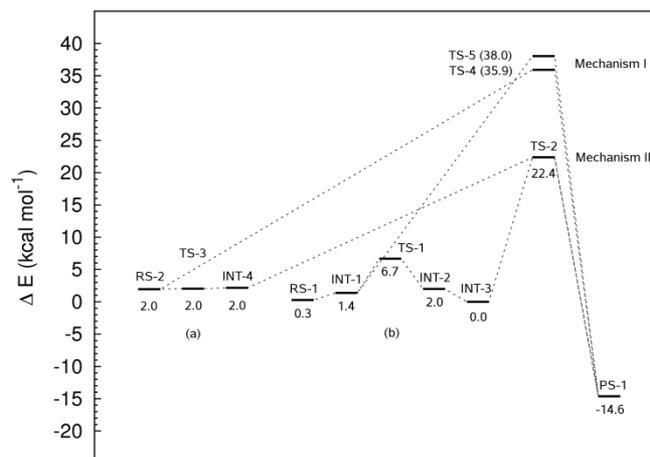
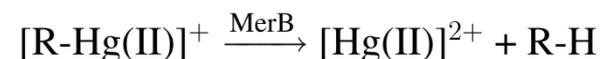
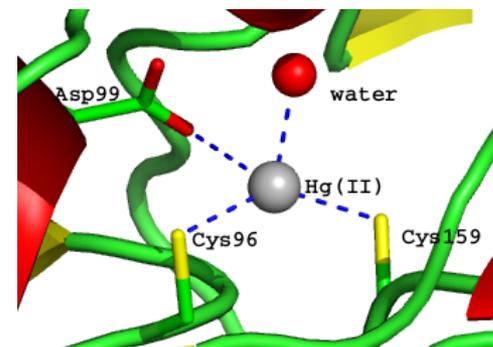
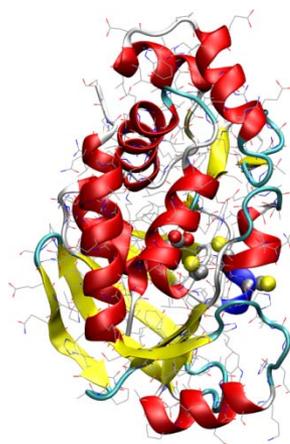
DOE/Office of Science/Biological & Environmental Research

↪ The chemical mechanism of Hg-C bond cleavage in organomercurials by the enzyme MerB was investigated using a quantum chemical cluster model of the active site.

↪ Verified the mechanism recently proposed by Lafrance-Vanasse et al., 2009

↪ Showed quantitatively how methylmercury coordination by two cysteines enables Hg-C bond cleavage

↪ Showed how an aspartic acid residue acts as a base and then an acid to deliver a proton to the hydrocarbon leaving group



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The article describes calculations revealing the mechanism of action of a key enzyme in bacteria that have the capability of detoxifying (transforming) methylmercury, a key contaminant that bioaccumulates in organisms. Using the recently-determined three-dimensional structure of the enzyme, named MerB, the calculations show how the protein stabilizes the transition state of the demethylation reaction that cleaves the methylmercury carbon-mercury bond, by priming the attacking hydrogen atom in the active site. Thus, MerB performs a crucial step in the detoxification process. The mechanistic principles revealed by these quantum chemical calculations may be useful in the future design of synthetic catalysts capable of detoxifying mercury-polluted streams and rivers.

**ABSTRACT:** Demethylation is a key reaction in global mercury cycling. The bacterial organomercurial lyase, MerB, catalyzes the demethylation of a wide range of organomercurials via Hg-C protonolysis. Two strictly conserved cysteine residues in the active site are required for catalysis, but the source of the catalytic proton and the detailed reaction mechanism have not been determined. Here, the two major proposed reaction mechanisms of MerB are investigated and compared using hybrid density functional theory calculations. A model of the active site was constructed from an X-ray crystal structure of the Hg(II)-bound MerB product complex. Stationary point structures and energies characterized for the Hg-C protonolysis of methylmercury rule out the direct protonation mechanism in which a cysteine residue delivers the catalytic proton directly to the organic leaving group. Instead, the calculations support a two-step mechanism in which Cys96 or Cys159 first donates a proton to Asp99, enabling coordination of two thiolates with R-Hg(II). At the rate-limiting transition state, Asp99 protonates the nascent carbanion in a trigonal planar, bis thiol ligated R-Hg(II) species to cleave the Hg-C bond and release the hydrocarbon product. Reactions with two other substrates, vinylmercury and *cis-2-butenyl-2-mercury*, were also modeled, and the computed activation barriers for all three organomercurial substrates reproduce the trend in the experimentally observed enzymatic reaction rates. Analysis of atomic charges in the rate-limiting transition state structure using Natural Population Analysis shows that MerB lowers the activation free energy in the Hg-C protonolysis reaction by redistributing electron density into the leaving group and away from the catalytic proton.

Parks, J.M., Guo, H., Momany, C., Liang, L., Miller, S.M., Summers, A.O., Smith, J.C. 2009. *Journal of the American Chemical Society*, 131:13278-13285.