

Bacterial organomercury lyase: a protonolytic detoxification catalyst

Christopher Walsh, Tadhg Begley, Alan Walts

Departments of Chemistry and Biology
Massachusetts Institute of Technology
Cambridge, MA 02139

Abstract Bacteria became resistant to organomercurials by enzyme-catalyzed protonolytic fragmentation of the C-Hg bond by the enzyme organomercury lyase to hydrocarbon and mercuric ion products. The gene encoding the enzyme has been subcloned, expressed, and the lyase purified to homogeneity and analyzed for specificity and catalytic mechanism. The retention of stereochemistry as endonorbornyl mercurial is converted enzymically in D₂O to endo-D₁-norbornane and lack of other detectable radial pathways support an S_E2 pathway where C-H bond formation and C-Hg cleavage occur in a single transition state.

INTRODUCTION

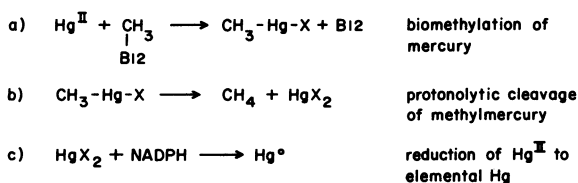
Among natural constituents in the environment are metals, usually in ionic form, some of which are toxic to cells and organisms, while others are essential components in biochemical processes. Williams and Silver (1984), have noted that volcanic soils and deep sea vents for example, have high concentrations of toxic heavy metals and have evolved specific and often sophisticated systems for resistance to metals. Bacterial resistance to such species as cadmium(II) and silver(I) ions are usually at the level of exclusion in uptake or active export of the metal ions back out of the cell. In the case of mercury toxicity, the metal is transformed by oxidation-reduction enzymology and by reversible conversion to organometallic species. The biochemical mechanism for metabolism of and resistance to organomercurials and inorganic mercury salts is the most well understood and is the focus of this paper.

The most common ore of mercury is red cinnabar (HgS), and this is the main anthropogenic source of metallic mercury by aerobic heating. Cinnabar from the Almaden mines in Spain has up to 7% mercury in the ore. Some 10¹⁰ tons of rock (av 80 µg Hg/Kg) are weathered globally per year, releasing 800 tons of mercury into the environment and the total oceanic reservoir of mercury is estimated at 200 million tons. The reservoir of volatile forms of mercury (Hg⁰ and CH₃HgX) in the atmosphere is about 4 million tons with an annual flux between these reservoirs of ~15,000 tons. The consequent dissolved Hg concentration in water is ca 0.03-2.0 µg/l (i.e. up to 10⁻⁸M).

Mercuric salts are biomethylated with methyl coenzyme B₁₂ to methyl mercuric salts and this organomercurial is a potent human neurotoxin, evidenced by the Minamata tragedy in Japan where Hg^{II} dumped from a oxymercuration plant led to accumulation of toxic levels of methylmercury in shellfish ingested by the townspeople. Bacteria have developed a two stage two enzyme detoxification strategy for recycling organomercurials back to nontoxic, volatile, elemental Hg⁰. As noted in Scheme 1, one enzyme, organomercury lyase, catalyzes a protonolytic cleavage of a carbon-mercury bond, e.g. to yield methane and Hg^{II} from a methylmercuric salt. The second enzyme, mercuric ion reductase, reduces Hg^{II} to Hg⁰ at the expense of stoichiometric oxidation of the NADPH coenzyme. While both RHgX and Hg^{II} salts are toxic by virtue of coordination to protein sulfhydryl groups, Hg⁰ is inert and nontoxic and volatilizes out of the bacterial cell. The genes that encode these enzymes and so confer resistance to mercurials are in general plasmid encoded and often on

Scheme 1

Bacteria: Synthesis of Methylmercury and its Recycling to Hg⁰

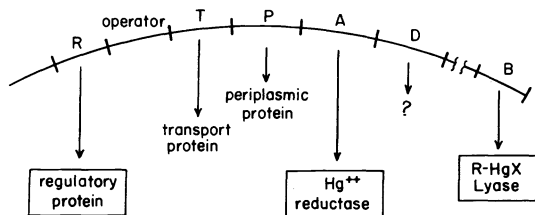


transposable elements within the plasmids, are inducible with low levels of mercurials, and are widespread, both in bacteria in soil and in aquatic environments (up to 1 in 10 bacteria) and in clinical isolates of drug resistant bacteria. Other plasmid determined resistances to toxic metals in bacteria include nickel, arsenic, antimony, cobalt, chromium, silver, cadmium, and copper (Williams and Silver, 1984).

The genes for mercury resistance are clustered together in a functional operon and a typical organization is illustrated in scheme II. The merR gene encodes a regulatory protein which, by binding to the operator region of the DNA in response to intracellular Hg^{II} concentrations, controls whether the structural genes of the mer operon are on or off. MerT and mer P encode membrane and periplasmic proteins required to transport Hg^{II} into the cell where the mer A gene product, the reductase, detoxifies it by reduction. In some, but not all Hg^{II} -resistant organisms, the mer B gene is present, encodes the lyase and enables resistance to organomercurials as well. We have constructed overproducers for the mer B, mer A, proteins, purified them to homogeneity, and begun to characterize them for structure and function (Begley, Walts, Walsh, 1982). We summarize studies below on the unusual organometallic cleaving enzyme encoded by the mer B gene.

Scheme II

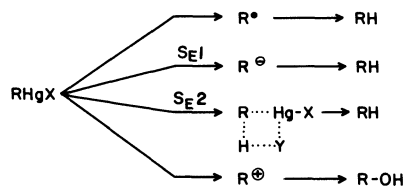
Mer Operon - Genes for Resistance to Mercurials



- Under study : mer R → regulatory protein controlling mer DNA activity
mer A → enzyme detoxifying Hg^{++} by reduction to Hg^0
mer B → enzyme cleaving organomercurials to RH and Hg^{++}

Scheme III

Mechanistic Pathways Available for R-HgX Cleavage



Mechanistic Route Dependant on Substrate Structure and C-Hg Cleavage Conditions

MER B GENE PRODUCT: ORGANOMERCURY LYASE

Carbon metal bonds are quite rare in biological systems, where the best studied example is the carbon-cobalt bond in coenzyme B_{12} (Halpern, 1985). Carbon-nickel bonds may be generated during carbonyl insertion processes to make acetyl units by the enzyme carbon monoxide dehydrogenase (Ragsdale and Wood, 1985). Organomercurial species, unlike many other carbon-metal compounds are very stable to protonolysis, with less than 1% protonolysis of methyl mercuric chloride by concentrated HCL in 100 minutes. Thus the job of organo mercury lyase is not a trivial one. While protonolysis of diorganomercurials has been studied (Jensen and Rickborn, 1968), less was known about the slower protonolysis of organomercurial salts and proposals involving carbanion, radical, and carbocation species have been proposed (Scheme III) with various substrates.

We have manipulated the mer B gene from plasmid R-831 to place it behind the bacteriophage T7 promoter and allow expression of the gene for overproduction of the encoded organomercurial lyase to 3% of the soluble cell protein. This has permitted purification of enzyme to homogeneity in 25mg quantities. It is a catalyst of 22,400 molecular weight (about 210-220 amino acid residues) and contains no metal ions or other cofactors for the organomercury protonolysis. Broad substrate specificity, for primary, secondary and tertiary (t-butyl) alkyl mercurials, vinyl allyl, and aryl mercurials is observed. The enzyme is relatively sluggish in turnover, with values of 1 cleavage per minute for methyl mercuric chloride and t-butyl mercuric chloride, 15 min^{-1} for n-butyl mercuric chloride to butane, for vinyl mercuric chloride to ethylene, for phenyl mercuric acetate to benzene. There is no detectable methane formation from dimethyl mercury. The enzyme require thiols in the buffer for activity, probably both for presentation of substrate as $RHgSR'$ salt and to assist removal of product Hg^{II} from the enzyme as the $Hg^{II}SR'$ bithiol complex (Begley, Walts, Walsh, 1986a).

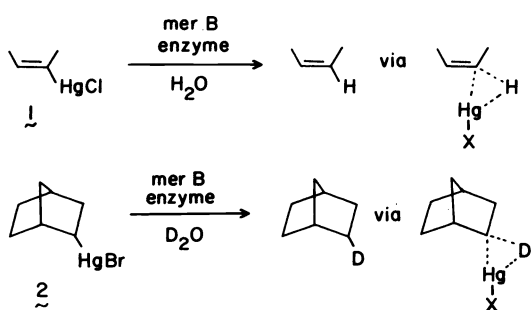
To assess the possible mechanistic routes of scheme III, we determined that CH_3HgCl yields CH_4 and Hg^{II} at equal rates, ruling out the solvolytic S_N1 carbocation, Hg^0 route ab initio. The S_E1 route via carbanion intermediate is also highly unlikely, proceeding in the nonenzymic cases only when the resulting carbanion is particularly stabilized (Dodd

& Johnson, 1969, Coad and Johnson, 1967; Hughes et al, 1964), not obviously the case in the above enzymic substrates. The homolytic radical cleavage pathway, S_H2 , has been shown nonenzymically for protonolysis of diorganomercurials but not conclusively for organomercurial salts (Ingold and Roberts, 1971). The last alternative, a concerted S_E2 cleavage with simultaneous C-Hg cleavage and C-H formation, has been suggested with vinyl mercurials, diorganomercurials, and alkylmercurials (Jensen and Rickborn, 1968; Kreevoy, 1957; Kreevoy and Hansen, 1961). We have used probes for stereochemistry and radical-based rearrangements to attempt to distinguish between S_H2 and S_E2 mechanisms used by the enzyme (Begley, Walts, Walsh, 1986b).

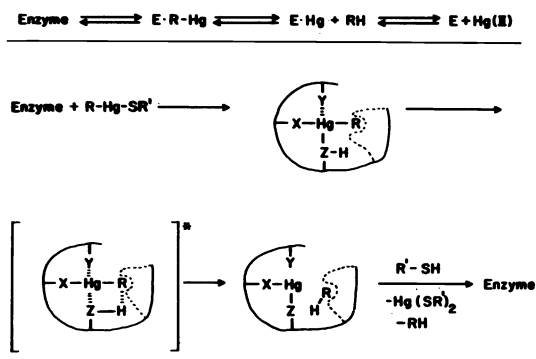
STEREOCHEMICAL AND RADICAL PROBES OF ORGANOMERCURY LYASE

Thus *cis*-butenyl mercuric chloride **1** is processed, with the highest turnover of any substrate detected (240 min^{-1} , to *cis*-butene without detectable *trans*-butene (1% detection limit by GC analysis). Also the endo norbornyl mercurial **2**, is cleaved enzymically in D_2O to the 2-D-norbornane with deuterium only at the endo position as monitored by FTIR which can distinguish the C-D stretches for endo and *exo*-2- D_1 -product. The high degree of configurational retention (Scheme IV) is consistent with an enzymic S_E2 process rather than a radical route which would have been expected to yield some *trans* butene (35% on full equilibration) and *exo*- D_1 -norbornane respectively.

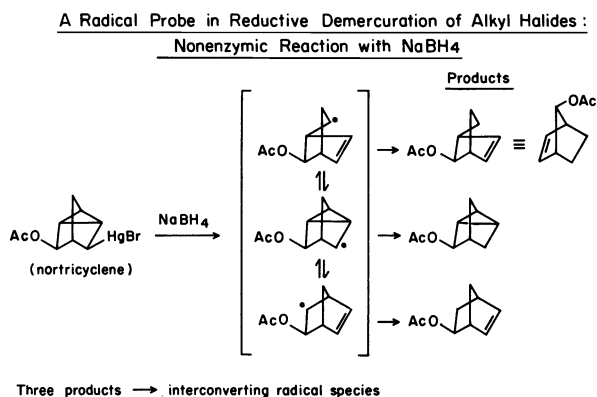
Scheme IV



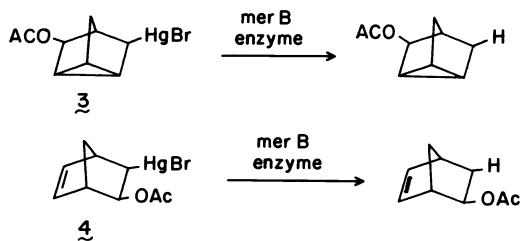
Scheme VI



Scheme V



G.M. Whitesides + J. San Filippo, Jr.
J. Am. Chem. Soc. **1970**, **92**, 6611



Whitesides and SanFilippo (1970) used a nortricyclic mercurial (*exo*-3-acetoxynortricyclyl-5-mercuric bromide), **3**, and the bicyclic mercurial (*cis*-*exo*-bicyclo[2.2.1]hept-5-ene-2-acetoxy-3-mercuric bromide), **4**, to show that borohydride reduction yielded a constant mixture of nortricyclylene and norbornene isomers, interpreted in favor of rapidly equilibrating radical intermediates. In contrast the organomercury lyase processes **3** and **4** only to the unrearranged products from Hg replacement by a proton (Scheme V). These results, coupled with no isomerization of *cis*-butenyl-mercurial **1** to *trans* butene and no isomerization of **2** to the *exo*-2- D_1 -norbornane (9/1 *exo*/*endo* ratio seen from radical intermediates nonenzymically) all argue strongly against radical S_H2 type C-Hg cleavage by the enzyme in substrates **1-4**.

MECHANISTIC PROPOSAL

We suggest the organomercury lyase catalyzes organomercury C-Hg bond cleavage by general acid catalysis and by participation of one or preferably two basic/nucleophilic groups (e.g. cysteine-SH groups) to activate the C-Hg bond for protonolysis (Scheme VI). X-ray structure of organomercurial complexes with amino acids suggests such secondary

coordination (Wong et al, 1973, 1974; Furmanova et al, 1980) and nucleophilic acceleration of electrophilic reactions of the carbon-mercury bond (for example, with I_2) are well-known (Beletskaya et al, 1965; Charman et al, 1961; Kreevoy et al, 1966; Sayre and Jensen, 1979). Cleavage via S_E2 transition state would yield product hydrocarbon and bis enzyme-coordinated Hg^{II} , to exchange with buffer thiol to yield free enzyme ready for another catalytic cycle. The rate acceleration seen with cis-butenyl mercury substrate suggests partial protonation to stabilize a carbenium ion contributor. The stabilization by metals in carbenium ion intermediates has been explained to propose the ready acid protonolyses of alkenyl metal species (Kreevoy and Kretchmer, 1964; Negishi, 1980). In accord with a rate-limiting S_E2 transition state is the solvent D_2O V_{max} isotope effect VH_2O/VD_2O of 2.1 for cis-butenyl- Hg \perp seen with the enzyme (Begley, Walts, Walsh, 1986B).

The turnover numbers ($1-240 \text{ min}^{-1}$) for organomercurial protonolyses by this bacterial detoxification enzyme are relatively modest in the context of absolute rates of enzymic catalysts but are still 10^6-10^7 -fold faster than the equivalent chemical protonolysis rates (Begley, Walts, Walsh, 1986b). There is no obvious correlation of rates of chemical protonolysis or of carbon-mercury bond strengths with V_{max} values of organomercury lyase. This is the first enzyme catalyzing organometallic cleavage to be isolated and characterized and may be a paradigm for such catalyst design. For example organotin compounds are widely dispersed in the environment from use as biocides in agriculture and in antifouling paints. Resistant organisms are known and may well protonolyse the tetravalent organostannanes. In this regard we have detected weak activity of organomercury lyase in converting tetravinyl tin to ethylene (Walts & Walsh, 1986).

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