Enzyme Models

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Mercury–Alkyl Bond Cleavage Based on Organomercury Lyase

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cleavage reactions · coordination chemistry · enzyme models · mercury · S ligands

An enzyme with substrates that are organometallic species can justly be regarded as unusual. It is to be expected that the mechanism of action will have features that would be difficult to replicate in synthetic systems. One such enzyme is organomercury lyase (also known as MerB), for which functional models have recently been presented in the form of metal complexes.[1]

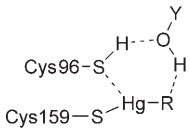
To classify the new work, it is helpful to first consider the global circulation of the element mercury, which is made up of numerous individual abiotic and biotic processes. The biomethylation of Hg^{2+} by sulfate-reducing bacteria in anaerobic marine sediments is an example of a biotic process, in which the methylmercury cation, abbreviated to "methylmercury", is formed. Methylmercury enters the food chain through plankton, and in this process it is concentrated by a factor of about 10^6. This extreme bioaccumulation leads to concentrations of up to 4 ppm being found at the end of the food chain, for example in sharks. Even humans can be directly affected by this, as methylmercury is highly neurotoxic. In unfavorable circumstances a health risk cannot be excluded when consuming saltwater fish.[2] There is also the "back reaction" in global mercury circulation, that is, the demethylation of methylmercury. Many bacteria possess the enzyme for this process, namely the above-mentioned MerB. Together with mercury(II) reductase (MerA) it endows these bacteria with so-called broadband resistance to mercury compounds: both methylmercury and Hg^{2+} ions are ultimately converted into metallic mercury, which diffuses from bacterial cells [Eq. (1)].[3]

$$MeHg^+ \xrightarrow{MerB} Hg^{2+} \xrightarrow{MerA} Hg^0 \qquad (1)$$

The bond cleavage catalyzed by MerB is a protonolysis reaction. The enzyme can transform numerous alkyl and aryl mercury compounds into Hg^{2+} ions and the respective hydrocarbon [Eq. (2)]. The proton donor HA is likely to be an amino acid side chain in the active center of the enzyme (see below). The three-dimensional structure of MerB has been determined in solution by NMR spectroscopy,[4] in which flexible regions in the active center and a neighboring, relatively large hydrophobic pocket which could accommodate the hydrocarbon moiety of the substrate were found. Both presumably contribute to the broad substrate specificity. The enzyme also cleaves certain organotin compounds to a lesser extent. However, the primary substrate is the naturally occurring methylmercury. The Hg–C bond of methylmercury is kinetically extraordinarily stable—almost no cleavage takes place even in concentrated strong Brønsted acids at room temperature. If methylmercury is to be destroyed on a laboratory scale, strong oxidizing agents such as aqua regia ($HNO_3 + 3HCl$) is used instead.[5] Considering this fact, the performance of MerB, namely a 10^6–10^7 fold acceleration compared to chemical protonolysis, is astonishing.

$$RHg^+ + HA \xrightarrow{MerB} Hg^{2+} + RH + A^- \qquad (2)$$

The mechanism that makes this bond cleavage possible is not known in detail, although it is known that the two cystein groups Cys96 and Cys159 (numbering as in the MerB of the plasmid R831b) are essential for the enzyme activity.[6] Proposal **1** is one suggestion for the key step of the protonation.[7] The group Y–O–H could be the tyrosine Tyr93. However, it is still unclear which amino acid actually functions as proton donor. There are also other candidates in addition to Tyr93, for example, Cys160.



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Hg–C bonds are activated by an increase in the coordination number of the mercury atom (see below). This knowledge is included in proposal **1** and was also the starting point for the work of Melnick and Parkin,[1] who used the potentially tridentate sulfur ligand tris(1-*tert*-butyl-2,3-dihydroimidazol-2-thion-1-yl)hydridoborate, $(Tm^{tBu})^-$, (Scheme 1)[8] and with it synthesized the alkyl mercury complexes $[(Tm^{tBu})HgMe]$, $[(Tm^{tBu})HgEt]$, and $[(Tm^{tBu})HgCH_2CN]$. Crystal structure analyses showed that the ligand has monodentate coordination in the methyl and ethyl complex (κ^1), but tridentate coordination in the cyanomethyl complex (κ^3). Thus, $[(Tm^{tBu})HgCH_2CN]$ is one of a handful of known pseudotetrahedral alkyl mercury complexes. Further examples are the macrocyclic thioether complex $[([9]aneS_3)HgMe]^+$[7] and the phosphane complex $[\{N(CH_2CH_2PPh_2)_3\}HgMe]^+$.[9] From ^1H NMR spectra it can

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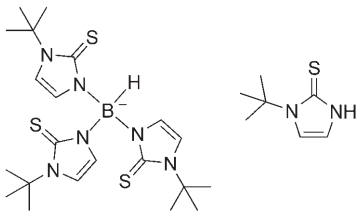
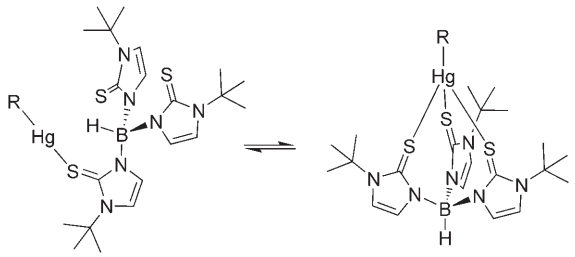


Scheme 1. Structures of the ligands $(Tm^{tBu})^-$ (left) and $Hmim^{tBu}$ (right).

be seen that in solution at −60 °C, the methyl and ethyl complexes of $(Tm^{tBu})^-$ also have pseudotetrahedral geometry. At higher temperatures the complexes fluctuate between the κ^1 and the κ^3 form (Scheme 2), whereby the κ^2 isomer is probably an intermediate.



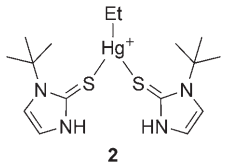
Scheme 2. Equilibrium between the κ^1 and the κ^3 form of $[(Tm^{tBu})HgR]$ (R = Me, Et).

One of the decisive experiments was the reaction of $[(Tm^{tBu})HgR]$ (R = Me, Et) with thiophenol in deuterated benzene. It was found that the Hg–C bond cleavage was almost quantitative after just one day at room temperature [Eq. (3)]. This finding is very unusual, because mercury–alkyl

$$[(Tm^{tBu})HgR] + PhSH \rightarrow [(Tm^{tBu})Hg(SPh)] + RH \quad (3)$$

bonds (unlike mercury–aryl bonds) are normally extremely difficult to cleave with thiols.[10] Reaction (3) outperforms the previous best model reactions for bacterial Hg–C cleavage. Thus, the reaction of $[\{N(CH_2CH_2PPh_2)_3\}HgMe]^+$ with thiophenol ($pK_a = 6.6$) is incomplete under comparable conditions,[9] and the very strong trifluoromethanesulfonic acid ($pK_a \approx -13$) is required for the cleavage of the Hg–C bond in $[([9]aneS_3)HgMe]^+$.[7]

The previous findings lead to the conclusion that activation of the Hg–C bond occurs when the mercury coordination number exceeds the value of two preferred by organomercury compounds. Quantum mechanical calculations also clearly point in this direction: the calculated activation barriers for protonolysis falls drastically with increasing coordination number.[7,11,12] Clearly a higher coordination number increases the bond polarity ($Hg^{\delta+}$–$C^{\delta-}$), and thus proton transfer to the alkyl group is facilitated. The theoretical results agree qualitatively with experiments, for example, on methylmercury–thioether complexes. Whereas for $[([9]aneS_3)HgMe]^+$ (CN 4) 25% conversion is observed after just one hour with trifluoromethanesulfonic acid, $[(Et_2S)HgMe]^+$ (CN 2) shows no change even after a day. Melnick and Parkin were able to confirm the influence of coordination number even more directly.[1] To this end they synthesized the ethylmercury complex of the ligand 1-*tert*-butyl-2,3-dihydroimidazol-2-thione, $Hmim^{tBu}$. This ligand represents the basic structural element that occurs three times in the larger ligand $(Tm^{tBu})^-$ (Scheme 1). The mercury atom is coordinated linearly in the complex $[(Hmim^{tBu})HgEt]^+$. As expected, the reaction with thiophenol is correspondingly slow. At room temperature no ethane can be detected even after two days; ten days at 60 °C are required for complete conversion. However, if along with thiophenol further $Hmim^{tBu}$ ligand is added, the formation of ethane is complete after just two days at room temperature. The cause of this is the formation of the complex $[(Hmim^{tBu})_2HgEt]^+$ (**2**), in which the metal center is triply coordinated. Evidence for the existence of **2** was obtained when the complexes $[(Hmim^{tBu})HgR]^+$ (R = Me, Et) were titrated against $Hmim^{tBu}$ and the titration was followed by ^1H NMR spectroscopy. During the course of the titration the signals for $Hmim^{tBu}$ and the alkyl group were shifted, while signals of noncoordinated $Hmim^{tBu}$ were absent. From this a rapid reversible coordination of the added $Hmim^{tBu}$ could be concluded.



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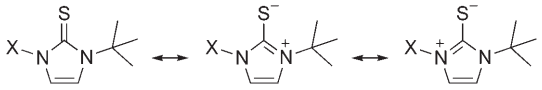
The work presented in reference [1] can be regarded as a breakthrough in the search for functional models of organomercury lyase. The complexes described can probably be looked upon to a certain extent also as structural models. In the enzyme, a key role is attributed to cystein groups, that is, to thiols as in **1**. Indeed, in addition to thioketone character, the sulfur atoms of the model ligands $(Tm^{tBu})^-$ und $Hmim^{tBu}$ also have thiolate character (Scheme 3). There can now be no doubt that the increase in the mercury coordination number is



Scheme 3. Resonance structures of $(Tm^{tBu})^-$ (X = BH(mim^{tBu})$_2^-$) and $Hmim^{tBu}$ (X = H).

a general principle for the activation of mercury–alkyl bonds for protonolysis. Why should organomercury lyase also not make use of this principle? Over and above the pure acquisition of knowledge, possibilities for practical applications are also imaginable for the synthetic models. Thus ligands based on $Hmim^{tBu}$ could be anchored to insoluble solid supports, such as cross-linked polymers, for use in the detoxification of effluent contaminated with methylmercury. The industrial use of mercury-resistant bacteria is already being practiced.[3] Transgenic plants have already been produced that express MerB (alone or together with MerA).[13,14] With such plants, methylmercury could be removed from contaminated soil. The findings of Melnick and Parkin could be helpful in improving the catalytic

properties of MerB specifically for such applications.[15] Finally there remains the interesting question as to whether $(Tm^{tBu})^-$ and similar ligands would also make possible the protonolysis of highly toxic alkyl tin compounds; the capabilities of the enzyme is in this aspect only modest.

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