UMEÅ UNIVERSITET

Unraveling the importance of thiol compounds on mercury speciation, uptake and transformation by the iron-reducer *Geobacter sulfurreducens*

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Akademisk avhandling

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Abstract
The biogenic methylation of inorganic, divalent mercury (Hg(II)) by methylating microorganisms leads to formation and bioaccumulation of monomethyl mercury (MeHg) in the environment and can cause severe damage to ecosystems and human health. Diverse microorganisms carry the gene sequence *hgcAB* and are able to methylate Hg(II) intracellularly. The interplay of biological, chemical and physical parameters is driving mercury (Hg) transformation by microorganisms. The chemical speciation of Hg(II) with thiol compounds, both with dissolved low molecular mass (LMM) thiols and thiols present on microbial membrane surfaces, is one key factor for Hg availability and transformation. In this work the role of thiol compounds with respect to Hg speciation, uptake and transformation was studied by the iron-reducing model organisms *Geobacter sulfurreducens*. The turnover of dissolved thiols and the role of outer and inner membrane thiols was studied with novel experimental strategies.

In Paper I and II the formation of thiol compounds was studied under varying nutrient conditions. It was shown that the formation of LMM-thiol compounds was impacted by divalent iron, Fe(II). Furthermore, we showed the turnover of the small LMM-thiol cysteine to the branched LMM-thiol penicillamine, which was further amplified by the addition of exogenous cysteine or nutrients. This turnover of small to branched LMM-thiols impacted the Hg(II) speciation in methylation assays and the relative contribution between cysteine and penicillamine was important for Hg(II) availability, uptake and methylation. In addition, the partition of Hg(II) between the cell-adsorbed and dissolved phase was shifted towards the latter at higher LMM-thiol concentrations. Nutrient concentrations impacted cell physiology due to a shift to an active metabolism and a faster metabolization of LMM-thiols. We concluded that the interplay between thiol metabolism, Hg(II) speciation and cell physiology are key parameters for Hg(II) methylation by *G. sulfurreducens*. In Paper III The outer and inner membrane was characterized independently by two X-ray absorption spectroscopy techniques. The determination of the Hg speciation by both X-ray absorption spectroscopy techniques showed coherent results for both the outer and inner membrane of *G. sulfurreducens*. The concentration of thiol membrane groups was higher on the inner compared to the outer membrane. The differences between the outer and inner membrane suggested that thiol concentration and Hg coordination environment likely impact the Hg(II) internalization. The role of membrane thiols for Hg(II) uptake and transformation was further investigated in Paper IV by selectively blocking these functional groups. Partitioning and uptake of Hg was not affected by blocking the outer and inner membrane thiols of whole cell and spheroplast samples, respectively. However, the Hg(II) methylation was decreased by blocking thiols at the outer membrane, but no effect was observed by blocking thiols at the inner membrane. Blocking of membrane surface thiols changed the physiology in whole cells but not in spheroplasts. This result suggested weaknesses of the applied blocking approach. In addition, Hg(II) reduction was studied on the outer and inner membrane and showed the formation of liquid and gaseous elemental Hg, Hg(0), in Paper III and IV, respectively.

Overall, this work showed the central role of dissolved and cell-associated thiol compounds for Hg(II) uptake and the transformation reactions. Herby, concentration, compositions and distribution of thiols are crucial and impact the Hg(II) speciation, partitioning, uptake and availability for Hg(II) methylation and reduction. In addition, cell physiology is impacting the methylation potential and the turnover of LMM-thiol compounds. The role of membrane surface thiols for Hg(II) uptake was not fully identified, however such thiols were for the first time characterized selectively for the outer and inner membrane by X-ray absorption spectroscopy.

Keywords
mercury, low molecular mass thiols, speciation, uptake, methylation, reduction, Hg(0), Hg(II), MeHg, *Geobacter sulfurreducens*, ICP-MS, LC-MS/MS, X-ray absorption spectroscopy, cell physiology

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