Identifying Components of Toxic Metal Stress and Hg Methylation with Global Proteomics

Anne Summers (summers@uga.edu) - University of Georgia (PI); Susan M. Miller, University of California-San Francisco; Mary S. Lipton, EMSL, Pacific Northwest National Laboratory; Judy Wall, University of Missouri-Columbia (Co-PIs).

We study in vivo and in vitro modifications of proteins by organic and inorganic mercury compounds, specifically:

INORGANIC Hg(II) EXPOSOME OF A NON-METHYLATING GAMMA PROTEOBACTERIUM: The inorganic-Hg(II) exposome of the model bacterium E. coli, reveals a large decrease in modifiable protein cysteines as measured by iodoacetamide (IAM)-induced carboxyamidomethyl (CAM)-modifications (also seen previously in organo-Hg exposure). Surprisingly, few proteins had detectable Hg(II)-adducts and all that did had at least two cysteines in the same peptide, affording strong chelation of Hg(II). Hg(II) exposure produced more unmodified cysteines than PMA exposure, suggesting biologically-formed Hg(II)-adducts may have been lost during MS analysis. Regardless of whether this differential observation of organic- and inorganic-Hg adducts occurs biologically or during analysis, it reflects a chemical difference in these ligands with potential biological implications. We're pursuing this question in well defined pure proteins and peptides (below).

INORGANIC Hg(II) EXPOSOME OF A METHYLATING SULFATE-REDUCING BACTERIUM: Desulfovibrio ND132 growing facultatively with or without Hg(II) exhibited 54% of 3455 encoded proteins. Unlike the E. coli observations, we saw no change in (CAM)-modifications or unmodified protein cysteines with or without Hg(II). Remarkably, we observed no Hg(II)-modified proteins with or without IAM, perhaps because some of the 1 mM cysteine in the medium was metabolized to H2S, thus sequestering Hg(II) as insoluble HgS. Since ND132 methylates Hg(II) under these conditions, HgS may be the direct substrate for HgcAB or unidentified proteins may transfer Hg(II) from HgS to HgcAB. Possible candidate metallochaperones are being screened genetically.

LC-MS/MS OBSERVATION OF RHg- VERSUS Hg(II)-MODIFICATIONS: Differences in proteomic detection of Hg- and RHg-adducts could arise from differences in formation during cell exposure or in stability to LC-MS/MS processing. Such differences could underlie documented differential toxicity and bioaccumulation of these compounds, impacting remediation and stewardship. We found a pure synthetic peptide PMA-adduct stable over a range of MS inlet temperatures and voltages but its signal was 10-fold less than the unmodified peptide. With two well defined cysteine-rich proteins [the secreted enzyme lysozyme, (8 cysteines as disulfides) and the metalloregulator MerR, (8 cysteines as thiols)], we found that: (a) the most readily reduced lysozyme disulfides are highly modified by PMA or Hg(II); (b) either modification occurs less than CAM-adducts in the same peptide; and (c) PMA yields more adducts than Hg(II). These simple systems mimic proteomic results and will aid in defining the differential detectability/stability of organic and inorganic Hg-protein adducts.