Identification of Metal Reductases and Determination of their Relative Abundance in Subsurface Sedimentary Systems using Proteomic Analysis

Dwayne A. Elias, Heather M. Mottaz, Carrie D. Goddard, Alexander S. Beliaev, and Mary S. Lipton

Abstract
Heavy metal and radioactive contamination is a serious problem throughout the Department of Energy’s (DOE’s) sites. The DOE sponsors research on bioremediation and biomineralization approaches to decrease the toxicity of radionuclides and heavy metals. We describe here a multistep approach to identify specific reductases involved in metal and radionuclide reduction in the Fricke dosimeter, a sedimentary anaerobic community of G. metallireducens. We describe how proteins are isolated from the community, identified by mass spectrometry, and quantified to determine their relative abundance.

Results and Discussion
We have used cell fractionation techniques to resolve sub-cellular protein fractions and quantify the purity of proteins within each enriched fraction. We have used classical biochemical separations of fractions and NTA column enrichment to enrich for specific groups of proteins. Anion, Cation, Ni-NTA, and Cu(II)-NTA columns were used to enrich different groups of proteins. We have used MS/MS fragmentation to identify the proteins and functional categorization to understand the metabolic pathways involved. The MS/MS fragmentation data was used to identify metal-reductase candidates in the different fractions.

Accurate Mass and Time tag (AMT) Approach

Concept of Experiments

Accurate Mass and Time tag (AMT) Approach

Discussion and Future Directions

- Subcellular fractions of S. oneidensis MR-1, Dsr. desulfuricans G20, G. metalreducens and G. sulfurreducens have been enriched by multiple matrices and the proteins from fractions displaying Facill reduction activity identified as potentially being involved in metal-reduction in each of these organisms.
- The combination of reductase enrichment with high-throughput, comprehensive MS analysis yields more information without lengthy purification of each protein and the possible loss of activity during purification.
- These amino acid sequences will be used to reconstitute enzymes in these organisms against newer sequencing efforts, thus improving their functional characterization.
- These sequences will also be used together with our home-containing peptide detection methods to identify organisms from sediment extracts of DOE sites including FRC, PX4 and Hanford.

Acknowledgements

References

Table 1: Proteins in Different Fractions

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<thead>
<tr>
<th>Matrix</th>
<th>Number of Proteins</th>
<th>Percent of Total</th>
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<tbody>
<tr>
<td>Cell Extract</td>
<td>272</td>
<td>100</td>
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<tr>
<td>Anion Exchange</td>
<td>108</td>
<td>39</td>
</tr>
<tr>
<td>Cation Exchange</td>
<td>132</td>
<td>48</td>
</tr>
<tr>
<td>Ni(II)-NTA</td>
<td>90</td>
<td>33</td>
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<tr>
<td>Hydrophobic</td>
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<td>9</td>
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<tr>
<td>Cofraction</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Common to all Matrices</td>
<td>70</td>
<td>25</td>
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</tbody>
</table>

References


Graphs and Figures

Graph A: MS analysis of fractions

Graph B: Corroborating Lines of Evidence

Graph C: Discussion and Future Directions