A Novel Technology for Removal/Recovery of Toxic and Precious Heavy Metal

R.A. Acey, G.R. Jordaan, P.H. Nugyen, and H. Nugyen

Department of Chemistry and Biochemistry
California State University, Long Beach, CA, USA, Roger.Acey@csulb.edu

ABSTRACT

We have developed a novel technology for the removal/recovery of heavy metal from aqueous and organic solvents. The patented technology is based on a low molecular weight metal binding protein known as metallothionein (MT). The protein has the unique ability to selectively bind a number of toxic and precious metals including, but not limited to, lead, mercury, arsenic, copper, gold, platinum, and uranium. The protein does not bind biologically essential metals such as sodium, calcium, or magnesium. The protein is heat stable and active at a wide range of pH. We have cloned the gene for MT gene and expressed the protein in bacteria. A chimeric SUMO-MT with a HisTag was cloned to enhance expression and simplify purification of the protein. The chimeric protein binds metal as efficiently as MT. The purified chimeric protein was linked to an agarose based resin and functions as a "Metal Sponge". As a solution contaminated with copper passes through the resin, the MT binds the metal. Water exiting the "sponge" is now metal free. If one were interested in recovering precious metal from the resin, rinsing with weak acid releases the metal. The material is reusable. The technology is also capable of extracting metal from organic solvents, e.g. extracting palladium (Pd) particles from chloroform.

Keywords: metal binding protein, toxic metal, water remediation, precious metal recovery.

1 INTRODUCTION

A major concern in today's society is exposure to toxic heavy metals found in surface, ground, and well water. The Agency for Toxic Substances & Disease Registry (ATSDR) is required by law to publish a list of hazardous substances proposed to be the most significant threat to human health, the CERCLA Priority List of Hazardous Substances. The top three members on the list are arsenic, lead, and mercury. Cadmium is number seven and hexavalent chromium number eighteen on the list. Studies funded by the Environmental Protection Agency (EPA) and the National Institutes of Health (NIH) have clearly shown that these metals have a significant impact on human health and the fiscal well-being of the health care system. Lead and mercury are associated with neurological problems. An alarming statistic is the EPA estimate that 630,000 children are born in the US each year with learning deficiencies due to *in utero* exposure to mercury [1]. Cadmium, arsenic, and hexavalent chromium [Cr (VI)] are well-documented carcinogens [2]. Government response has been a series of statutes to lower the acceptable limit of toxic heavy metal in potable water, e.g. the Copper and Lead Rule [3] and the Safe Drinking Water Act [4]. The EPA's Arsenic Rule recently lowered the acceptable limit of arsenic from 50 ppb to 10 ppb [5]. The California EPA set the target for Cr (VI) at 0.02 ppb. This is a 100 fold lower that what was discovered in some of the wells next to the PG & E facility in Hinkley, CA. In most cases, e.g., the new target for arsenic, the current technology is unable to meet these standards cost effectively, especially for small Public Works Systems (PWS). The EPA estimates that a major capital investment would be required to upgrade the infrastructure, an estimated $150 billion dollars over the next twenty years. We describe here a cost effective water remediation technology capable of meeting EPA guidelines.

2 THE TECHNOLOGY

The technology is based on a novel metal binding protein (metallothionein) isolated from the brine shrimp *Artemia*. The protein has the unique ability to ***selectively*** bind toxic (or precious) heavy metals ***instantaneously*** on contact. The metal binding activity is efficient at a wide range of temperature (4^{o}C-100^{o}C) and pH (4 -10). A device filled with MT fixed to a solid support will function as a "Metal Sponge". As water flows through the device, the MT will remove metal from the solution (see Fig. 1). Proof-of-principle data is shown in Table 1 [6].

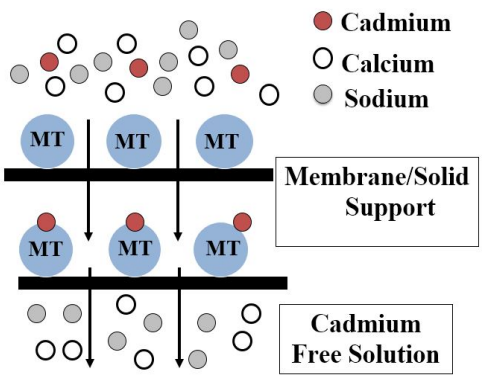

Figure 1: Heavy metal sponge

	Membrane Only	Membrane plus MT
Biodyne A	3768	152,876
Biodyne B	1774	158,762

Table: 1 Proof-of-principle data. MT was adsorbed to the surface of a Biodyne nylon membranes. A solution of ^{109}Cd in phosphate buffered saline (PBS) was passed through a membrane with or without MT. The membranes were washed with PBS and analyzed for radioactivity. Results are expressed as counts per minute (CPM) per 10 mm diameter membrane.

The efficiency of the technology relative to ion-exchange is shown in Table 2. The calculations are based on the amount of material required to completely remove 100 parts per million (ppm) of cadmium from 1365 gal of a waste stream 500 ppm in TDS (Ca and Mg).

	Required Amount (lbs)	Disposal Volume (gal)
Marathon C	51	7.1
MT	7.7×10^{-3}	6.6×10^{-3}

Table 2: Efficiency of MT metal binding relative to Ion exchange. Marathon C is a standard ion exchange resin manufactured by Dow Chemical Corp.

3 EXPERIMENTAL

The isolation and purification of the protein and cloning of its gene are described elsewhere [7, 8]. The gene sequence is shown below (see Fig. 2). While the overall primary structure is distinct from other known MTs, the cysteine residues responsible for metal binding and their location are highly conserved.

M D C C K N G C T C A P N C K C A K D C K C C K
G C E C K S N P E C K C E K N C S C N S C G C H

Figure 2: The Primary Sequence of *Artemia* MT. The metal binding domains are underlined.

Molecular modeling (ICM, Molsoft) of *Artemia* MT based on its primary sequence supports a structure and metal binding stoichiometry consistent with other MTs i.e., seven divalent cations bound per MT molecule (see Fig. 3).

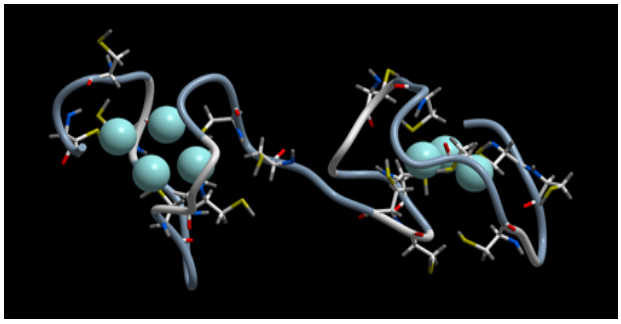

Figure 3: Structure of *Artemia* MT. Spheres represent zinc atoms.

The protein was initially expressed in bacteria. However, because of the metal binding activity of the protein, the level of expression was low. In order to enhance the expression levels and simplify its purification, a SUMO-MT chimeric protein (Small Ubiquitin-like Modifier MT) containing a His Tag was generated as described elsewhere [9]. The level of expression of the chimeric MT is dramatically enhanced over that of the MT construct. Typical yields are between 80 and 90 mg protein per liter of culture. Moreover, the metal binding activity is retained as the chimeric protein. Analysis by mass spectroscopy reveals a stoichiometry of 6 to 7 zinc ions per molecule of SUMO-MT. Moreover, with the His Tag, the protein can easily be purified from cells using Ni-NTA resin. The purity of a typical preparation is shown in Fig. 4.

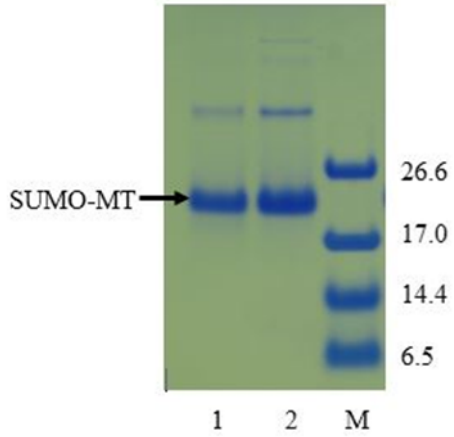

Figure 4: SUMO MT purified from bacteria. Cells were collected and sonicated in 0.05 M phosphate/300 mM NaCl, buffer, pH 8.0. Half the extract was placed in a boiling water bath for ten minutes. Both samples were centrifuged and the supernatants incubated with Ni NTA agarose. The chimeric protein was eluted with 400 mM histidine and analysed by SDS PAGE (12% Bis Tris gel). Lane 1: Boiled extract; Lane 2: Non Boiled extract; Lane M: markers with size indicated in kDa.

We have built a prototype "Metal Sponge" using SUMO-MT. Here the protein was covalently linked to an agarose-based resin. A 0.1 mM copper sulfate was then passed through the resin. The results are shown below (see Fig. 5). The metal binding is obvious. Exposure to acid regenerates the column. The column has been regenerated four times without loss of metal binding activity.

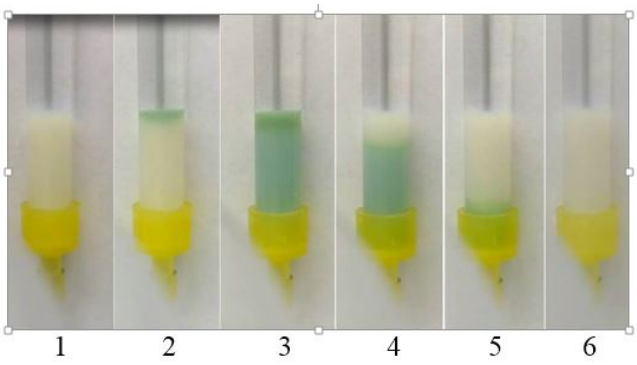

Figure 5: Prototype "Metal Sponge". A 0.1 mM solution of copper sulfate (colorless) is continuously passed through the column. Lane 1: MT bound to resin; Lane 2: After 15 minutes; Lane 3: After 60 minutes; Lane 4: After 30 sec acid wash; Lane 5: After 60 sec acid wash; Lane 6: Completely regenerated column.

Another application of the SUMO-MT resin is for recovery of precious (or rare) metals and their complexes from organic solvents. While most proteins are denatured by organic solvents and lose their biological activity, this is not the case with SUMO-MT. The material is capable of extracting Pd nanoparticles from chloroform (see Fig. 6).

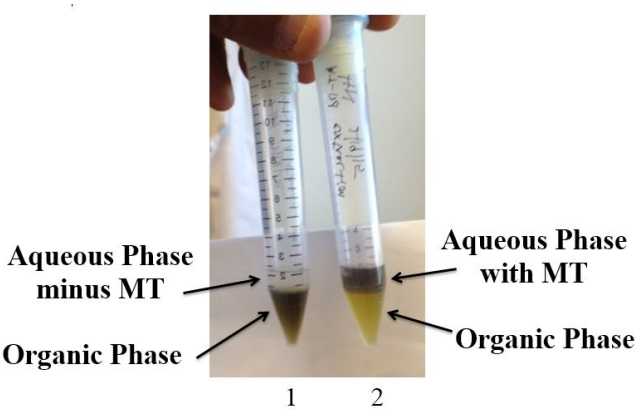

Figure 6: Recovery of Pd Nanoparticles from Chloroform. Tube 1: A solution of Pd nanoparticles in chloroform extracted with 50 mM Tris, pH 8.0; Tube 2: A solution of Pd nanoparticles in chloroform extracted with 50 mM Tris, pH 8.0 containing SUMO-MT.

4 CONCLUSION

What sets this "disruptive" technology apart from current methodologies are the following:

- MT is selective for a number toxic (or precious) heavy metals in the presence of a larger excess of biologically essential metals.
- Is active at a wide range of pH and temperature, making it adaptable to a number of environmental applications.
- The protein is reusable.
- Its selectivity, smaller carbon footprint, and lower downstream disposable costs make the technology cost effective.

REFERENCES

[1] C. Gundacker, M. Gencik, M. Hengstschläger, The Relavance of the Individual Genetic Background for the Toxicokinetics of Two Significant Neurodevelopmental Toxicants: Mercury and Lead, Mutation Research 705, 130-140, 2010.

[2] H. Shi, L.G. Hudson, K.J. Liu, Oxidative Stress and Apoptosis in Metal Ion-Induced Carcinogenesis, Free Radical Biology and Medicine 37, 582-593, 2004.
[3] Lead and Copper Rule-Code of Federal Regulations 40 CFR Part 141
[4] Safe Drinking Water Act – Code of Federal Regulations 42 U.S.C.§ 300f
[5] Arsenic Rule – Federal Register 66FR6976. Modified January 23, 2006.
[6] P.H.B Nguyen, H. Nguyen, G.R. Jordaan, and R.A. Acey. Use of a Novel Metal Binding Protein in Developing A "Toxic Metal Sponge" for Water Purification. ASBMB meeting, March 28-April 1, 2015, Boston, MA.
[7] B. Harpham, M.S. Thesis. Isolation of Metal Binding Proteins from Artemia, 1998.
[8] R.A. Acey, Compositions and Methods for Removing Heavy Metals from Contaminated Samples Using Membranes Provided with Purified Artemia Metallothionein (MT) Peptides, Patent Number 7,273,962, Issued 9/25/07
[9] R.A.Acey and G.R. Jordaan, Chimeric Metallothionein Proteins, Patent Pending, Application Number 62/301, 209. 2/29/16