novel lipopeptides with different chain length may be produced for clinical applications.

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Expanding the Biological Periodic Table

Javier Seravalli[1] and Stephen W. Ragsdale[2,*]
[1]Department of Biochemistry, University of Nebraska-Lincoln, 1901 Vine Street, Lincoln, NE 68588, USA
[2]Department of Biological Chemistry, University of Michigan Medical School, 1150 W. Medical Center Drive, 5301 MSRB III, Ann Arbor, MI 48109-0606, USA
*Correspondence: sragsdal@umich.edu
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Metal ions play an indispensable role in biology, enabling enzymes to perform their functions and lending support to the structures of numerous macromolecules. Despite their prevalence and importance, the metalloproteome is still relatively unexplored. Cvetkovic et al. (2010) now describe an approach to identify metalloproteins on a genome-wide scale.

Selected blocks from the iconic Periodic Table that looms over our chemistry lecture halls and classrooms are often highlighted by metallobiochemists and represented as the Biological Periodic Table, which includes the metal ions that play essential roles in the structure and function of macromolecules. We generally consider that about 40% of all enzymes depend on metals for activity because proteins have been isolated whose function depends on the presence of one of the first- (V, Mn, Fe, Co, Ni, Cu, Zn), second- (Mo), or third-row transition metal (W) ions. Yet one wonders how many elements in the Periodic Table might be required for life. As Joni Mitchell sang, "we are stardust," it seems likely that other elements found in the earth's crust and oceans might also be incorporated into proteins.

Genomic and metagenomic sequences are good starting points to predict whether a given organism or a selected ecosystem will utilize a particular metal ion (Andreini et al., 2009). More than 850 genome sequences have been completed. Yet, our current picture of the metalloproteome is quite incomplete; for example, user proteins may be present with no recognizable transporter, and vice versa (Zhang and Gladyshev, 2009). For metalloproteins, complete gene annotation is not possible at present because an experimental method for identifying all metal-binding proteins is been lacking. The study by Cvetkovic et al. (2010) is an important step forward in filling that gap.

While most individual metalloproteins have been identified after purification, characterization of their function, and their metal content, Mike Adams and his colleagues at the University of Georgia used a metal-based approach (Cvetkovic et al., 2010). They cultured the exemplary microbe *Pyrococcus furiosus* in a medium containing 44 metal ions and used a comprehensive method of metal analysis (inductively coupled plasma mass spectrometry [ICP-MS]) coupled with high-throughput mass spectrometry (HT-MS/MS) to determine all of the metals that this organism assimilates and to identify its metalloproteins on a genome-wide scale. Requiring only ~0.2 ml of sample, ICP-MS takes 0.5 s (or a total of ~3 min per sample) to quantify essentially all masses ranging from 6 (Li) to 238 (U) over a linear range of concentration spanning over six orders of magnitude for most elements. Surprisingly, 21 of the 44 elements present in the medium were found to accumulate in the cytoplasm and 343 distinct metal-containing peaks were identified after a single chromatographic separation step. Similar results were found for two other microbes (*Escherichia coli* and *Sulfolobus solfataricus*).

The value of the metal-based experimental approach chosen by Cvetkovic et al. (2010) relative to an in silico prediction of metal content based on genomic analyses is apparent from the finding that nearly half (158) of the 343 peaks did not align with any of the known metalloprotein conserved sequences. The type of thorough whole organism metal-based analysis described by Cvetkovic et al. (2010) promises to enrich the database (such as the InterPro-Metal database) with a wider array of metal coordination sites, eventually enabling researchers to better identify these essentia, yet diverse and poorly recognized components of protein structure. The metal-based

method described by Cvetkovic et al. (2010) also promises to shed light on metal-dependent regulation and toxicity because this type of analysis can be applied to environmental or laboratory samples gathered at varying metal concentrations. Metal accumulation by unicellular organisms has a strong dependence on the media composition; furthermore, studies in plants (Baxter et al., 2008) have revealed correlations between levels of multiple elements (ionomics) and the physiological state of the organism. For example, it is becoming increasingly recognized that iron-containing proteins from strict anaerobes, when overexpressed in *E. coli*, often contain zinc (Macauley et al., 2009). The methods described in this paper are ideal for evaluating how extensively and how generally zinc ions intrude into naturally occurring iron sites of proteins (as well as other metal substitutions as a function of media composition, redox conditions, etc.).

The authors purified eight proteins from the 158 unexpected metal peaks using the elemental elution profiles as the selection method. After an average of 5 to 6 columns per purification, two novel proteins for each nickel, molybdenum, lead, and uranium were isolated from *P. furiosus*. Discovery of these two nickel-containing proteins, an alanyl-tRNA editing hydrolase and a protein in the cupin family, raises the number of known Ni-containing proteins from 8 to 10. The alanine-tRNA hydrolase was also shown to be active only when Ni was bound. One of the Mo proteins appears to contain a $[Fe_4S_4]$ cluster and to be involved in reductive activation of ribonucleotide reductase, an exciting discovery because, prior to this study, molybdenum was not known to be a cofactor in any *P. furiosus* protein. Researchers need to be cautious in use of these high throughput methods because while the Ni and Mo contents of the purified enzymes approached 1 per monomer and Ni was demonstrated to be required for activity the stepwise purification of two uranium peaks, using cells grown in media with higher concentrations of lead (Pb) and uranium (U), yielded a Mg-dependent enolase and the iron chelating protein ferritin, both with less than stoichiometric amounts of U. More importantly, none of these proteins were predicted to exhibit binding to either Pb or U, but are perhaps involved in metal detoxification for those elements (as identified by the dbTEU database [http://gladyshevlab.bwh.harvard.edu/trace_element/]).

Other surprises were that *E. coli* cytoplasmic proteins showed accumulation of arsenic (As) and cadmium (Cd), while tin (Sn), Tallium (Tl), and antimony (Sb) accumulated in *S. solfataricus* proteins. Also intriguing is that while *E. coli* and *S.solfataricus* accumulated copper, no Cu accumulation was observed in *P. furiosus*, in spite of the fact that CopA was identified in this organism.

The novel methods described by Cvetkovic et al. (2010) will be extremely useful in evaluating the mechanisms of metal uptake, storage, excretion, metal remediation, and assembly of metal clusters in proteins. Furthermore, similar methodologies can be envisioned for identifying novel metal-binding RNA molecules and small cofactors.

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