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Experimental investigation of amino acid binding as a mechanism for fractionating metal stable isotopes

CORDAY SELDEN[1], KATHRIN SCHILLING[2], ANIRBAN BASU[3] AND NATHAN YEE[1]

[1]Rutgers University
[2]Columbia University
[3]Royal Holloway University of London

Presenting Author: crselden@marine.rutgers.edu

Background: Cellular metabolism depends on transition metals as protein co-factors. As cellular activity partitions metal isotopes, their ratios can be used to trace biological function. *Ab initio* calculations based on density functional theory suggest that the incorporation of transition metals into proteins can impart an isotopic fractionation dependent on the metal binding site and, particularly, on the identity of the coordinating amino acid ligands. To experimentally test this hypothesis, we investigated the isotopic fractionation of zinc and copper due to coordination by amino acids that commonly act as ligands at protein metal-binding sites (e.g., cysteine, histidine, glutamic acid, aspartic acid). The objective of this study was to constrain equilibrium isotope fractionation values for amino acid-bound metals.

Materials and Methods: Free and bound metal ions were separated via equilibrium Donnan dialysis using a cation-permeable membrane (Nafion^{TM}). Experimental solutions were prepared at least one day in advance of dialysis to allow for isotopic equilibration of bound and unbound metals. Initial metal donor solutions for dialysis contained metal ions and amino acids at a ratio of 100 μM:200 μM. All experimental solutions were prepared at pH = 6.0 using MES hydrate as a buffer (5 mM) and strontium nitrate (10 mM) as a background electrolyte. Donor solutions were dialyzed for one day against metal- and amino acid-free acceptor solutions. Isotope ratios of pre- and post-dialysis solutions were measured via MC-ICP-MS following column purification under clean laboratory conditions.

Results and Discussion: In the copper experiments, oxygen ligands (glutamic and aspartic acid) yielded small, negative $\delta^{65}Cu_{complexed\text{-}free}$ values (~-0.1‰) while sulfur ligands (cysteine) yielded larger negative values (~-1.4‰), suggesting that both preferentially bind the light isotope relative to water. Experimentally determined isotope separation values ($\delta^{x}M_{complexed\text{-}free}$) are consistent with published *ab initio* predictions. This work adds to the growing body of evidence that metalloprotein biosynthesis can affect the distribution of transition metal isotopes in biological systems.