Nano-Speciation on Metallomics

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Speciation of a bio-trace element is to determine its distribution of an element among defined chemical species in biological samples. The speciation analyses of such biological samples as blood plasma, tissue extract and urine using conventional HPLC hyphenated with inductively coupled plasma mass spectrometry (ICP-MS) require relatively large volumes of sample at the μL level due to the detection limit of ICP-MS. However, the volumes of samples including extracts of gene-modified cells, digested spots from two-dimensional electrophoresis and tissue biopsy extracts are limited in recent metallomics research. Thus, an analytical technique for samples having ultra small volumes, *i.e.*, a micro/capillary HPLC-ICP-MS, is needed in place of conventional HPLC. We constructed a two dimensional micro HPLC-ICP-MS system that consists of a gel filtration column and an anion-exchange column to separate metal-binding proteins in gene-modified cells, *i.e.*, specifically knocked down with the siRNA. To enhance the sensitivity of ICP-MS for the detection of the metal bound to the proteins, a low-volume spray chamber with sheath flow was newly designed and an enriched stable isotope was used. Indeed, a 100-nL portion of cell supernatant was sufficient for injection into the column, suggesting that the minimum cell number required for our micro HPLC-ICP-MS system was 2.0×10^3. The combination of nano-speciation with molecular biological techniques such as the RNAi technique may open new doors in the study of metallomics.