Mapping Silver-Binding Proteins in *Staphylococcus aureus* by Liquid Chromatography Combined with Gel Electrophoresis and Inductively Coupled Plasma Mass Spectrometry

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ABSTRACT: Metals play a crucial role in life processes and metallodrugs have been extensively used for therapeutic and diagnosis purposes.[1] To understand mode of action of metallodrugs, reduce toxicity, and overcome resistance as well as to design new drugs more rationally, exploring their protein targets is imperative. Silver has been used as an antimicrobial since antiquity, yet its targets and mechanism of action remain obscure.[1] LA-ICP-MS and SXRF are the main metallomic approaches utilized to determine metal-binding proteins, whereas the low sensitivity ascribed to the laser system and limitation to access the synchrotron facility prevent their routine use.[2]

Recently, by on on-line coupling of column-type gel electrophoresis with ICP-MS (GE-ICP-MS), we developed a robust and convenient approach for matching metals and proteins. We successfully separated and identified seven bismuth-binding proteins in *H. pylori*. The observed profile of Bi-binding proteins in *H. pylori* verified that Bi exhibits activity via multiple protein targets.[2] However, the relatively lower sensitivity of ICP-MS to metals with lower molecular weight and the compromised separating resolution of one dimensional gel column limits the fully exploration of silver-binding proteins.[3]

Herein, a robust two dimensional approach, namely liquid chromatography combined with gel electrophoresis and inductively coupled plasma mass spectrometry (LC-GE-ICP-MS) was developed and applied to dig out silver-binding proteins in *S. aureus*. The feasibility of the 2D method was validated with iodine-labelled standard proteins. MWs, pIs of proteins as well as intensity of metals contained in proteins can be mapped simultaneously by LC-GE-ICP-MS. Based on the 2D GE-ICP-MS, for the first time, we separated and identified more than 20 Ag-binding proteins in *S. aureus*. Both Gene Ontology biological process and function classification showed that the antimicrobial effect of silver is mediated mainly by disturbing intracellular oxidative stress homeostasis and energy metabolism. Further validation of these identified protein targets were conducted by comparing the alteration of their enzyme activities after treatment of silver.

KEY WORDS: metallomics, silver, antimicrobial, protein targets, GE-ICP-MS.

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