Imaging Analysis of Metals, Lipids, and Proteins in Biological Tissues via Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

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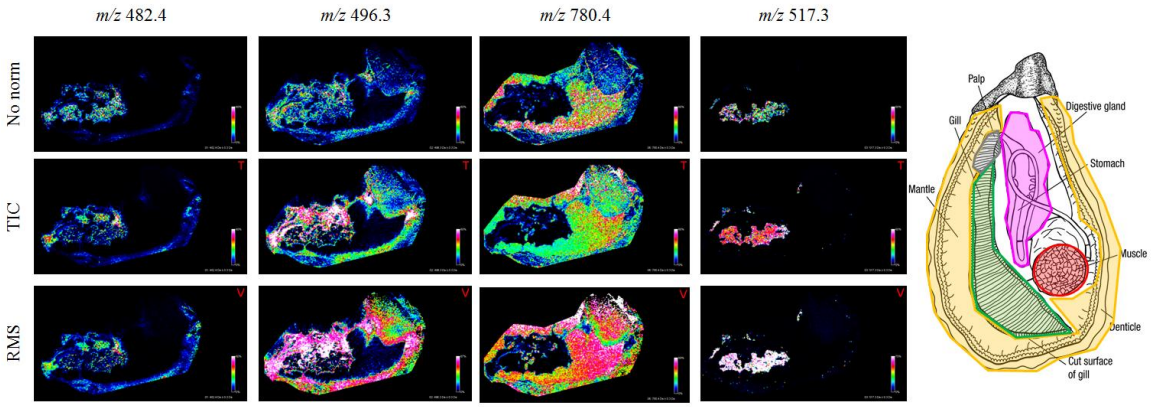
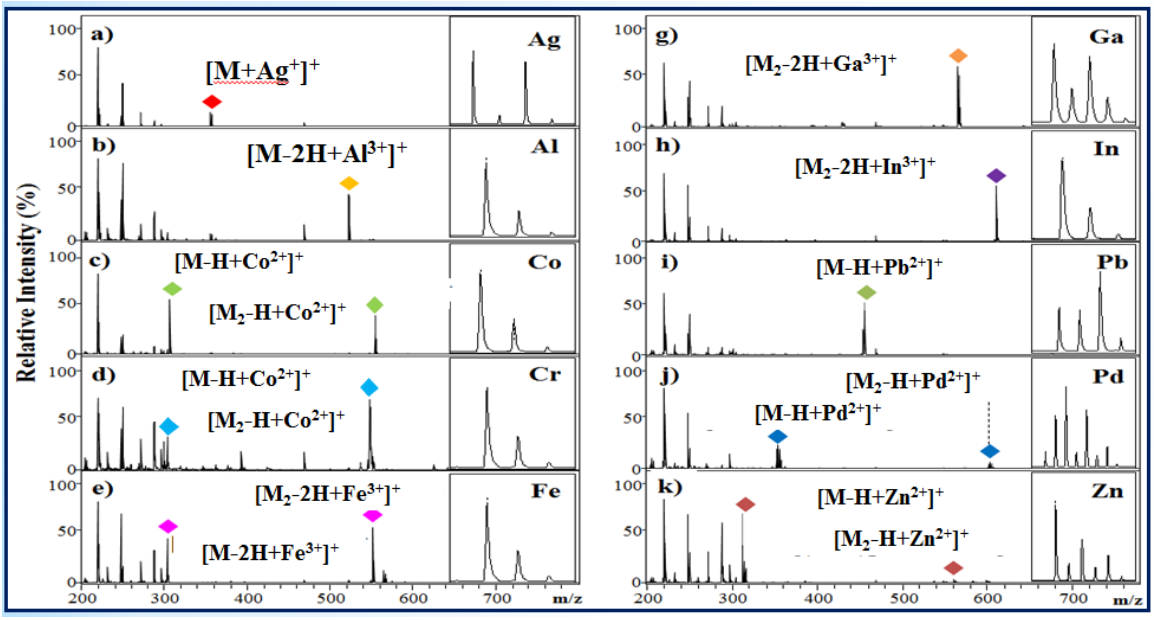
MALDI-TOF/MS is an established technique for the analysis of proteins, lipids, and other molecules, but there are few studies on its use for the detection of metals. An analytical technique that is able to provide metal, lipid, and protein profiles can prevent data variations due to differences in instrumentation. More importantly, analysis of different analyte distributions may reveal correlations that can help understand the relationships between metals, proteins, and lipids in biological tissues. Such results have promising applications in clinical diagnosis. In this study, metal, lipid, and protein distributions in biological tissues were determined using MALDI-TOF imaging mass spectrometry.

In order to perform metal imaging analyses using MALDI-TOF, an azo dye was selected that served as both a matrix and chelating agent. DHB and CHCA were used as matrices for lipid and protein imaging, respectively. Aqueous metal standards (Ag, Al, Cd, Co, Cr, Cu, Fe, Ga, In, Mn, Ni, Pb, Pd, and Zn) were tested prior to real sample analysis, during which azo dye chelation complexes were detected. MS/MS analyses were also performed to obtain fragmentation spectra and confirm analyte signals. Metal, lipid, and protein analyses were performed on homogenized oyster extracts prior to imaging analyses to check the presence of analytes. Results from metal imaging analysis indicate the distribution of Zn in the digestive system of oysters and the presence of unknown signals localized to the adductor muscle. Different lipid distributions exclusive to the mantle, digestive system, or gills were observed. Protein distributions for oysters will be acquired in future studies, but homogenized extracts show the presence of peptides and proteins smaller than 19 kDa. On the other hand, data from imaging analyses of human breast and rat liver and kidney tissues indicate potentially meaningful distributions of Na and K that require further confirmation and comparison with lipid and protein profiles. Histological

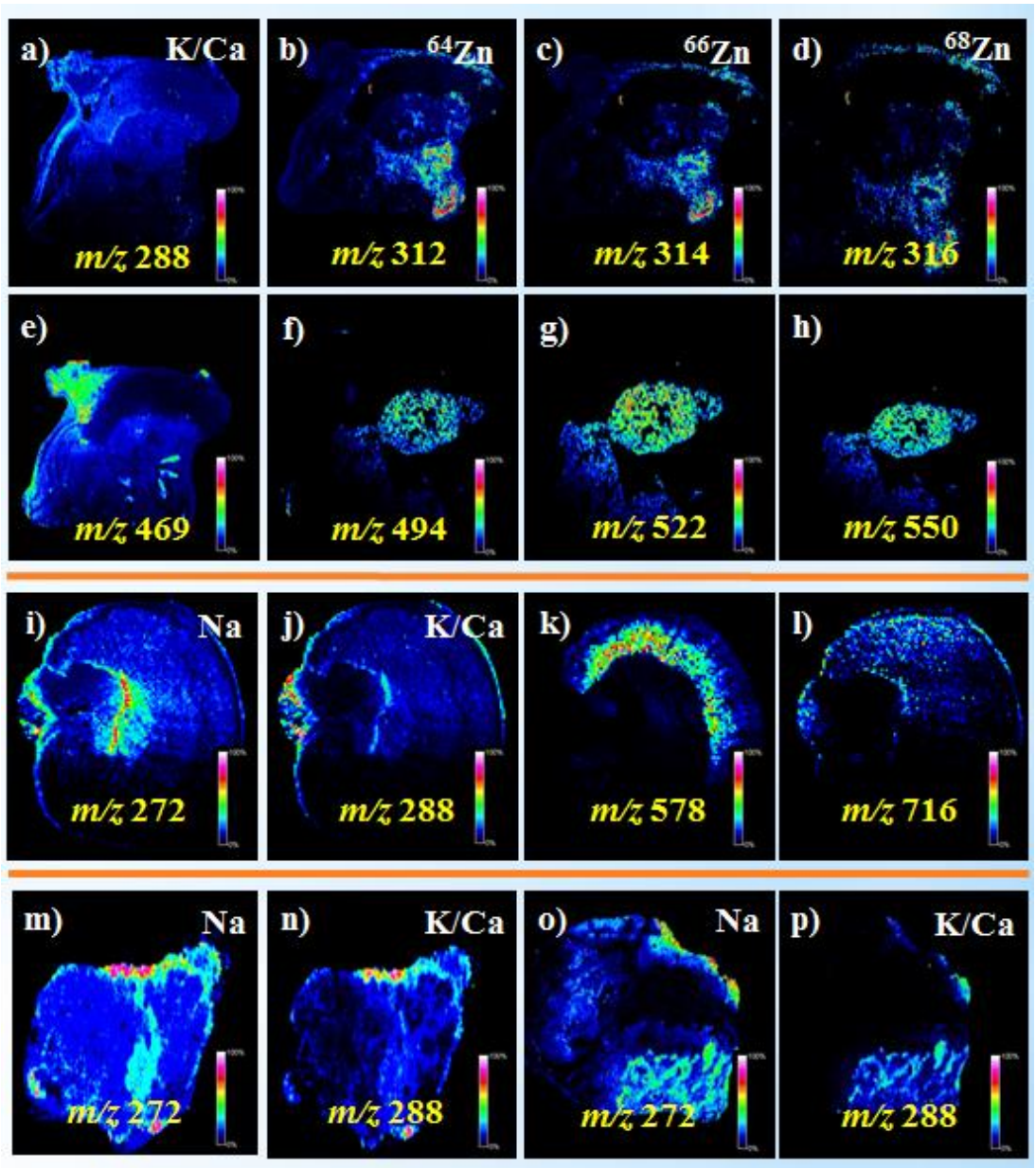
comparisons of images with H&E stains are necessary confirmation steps in future studies. The metal, lipid, and protein imaging of other biological tissues such as those of rat organs and biomedical samples will be performed for possible clinical applications in differentiating between healthy and cancerous tissues. In conclusion, the use of MALDI-TOF to perform analyses of both organic and inorganic analytes allows for a more holistic understanding of the protein, lipid, and metal profiles of samples for potential clinical applications.



MALDI-TOF imaging results of lipid distributions in oyster tissues. Note the signal localization in the mantle, palp, digestive system, gills, and adductor muscle (compare with oyster anatomical image). Non-normalized and RMS- and TIC- normalized spectra are shown.



Spectra of azo dye (M) chelation complexes with metals in aqueous metal standards. Isotope patterns of chelation complexes with the respective metals are shown in insets.



Images of azo dye chelation complexes with metals in a-h) oyster, i-l) rat kidney, and m-p) human breast tissues (m, n from one tissue; o, p from another). Images b-d show Zn chelation complexes in the oyster digestive system. Unknown signals were observed in the oyster adductor muscle (f-h) and rat kidney (k, l). Sodiated and potassiated/calciated azo dyes were observed at *m/z* 272 (i, m, o) and 288 (a, j, n, p), respectively. Color scales are based on relative intensity (pink highest).