Investigation of the Catalytic Properties of Cerium(IV) Oxide in Metal Oxide Laser Ionization-Mass Spectrometry Imaging

Background: Matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) is an emerging and powerful analytical technique, which allows the spatially resolved characterization of a wide range of analytes within biological specimens.[1] Metal oxide laser ionization (MOLI) is a recently described variation on MALDI in which a metal oxide, rather than an organic acid, is utilized as the matrix.[2] Unlike the other metal oxides, Cerium(IV) Oxide (CeO_2) demonstrates a unique property of laser induced fatty acyl catalysis when applied to phospholipids and energized by standard lasers found in MALDI-TOF MS instruments, as seen in Figure 1. This property of laser-induced catalysis by CeO_2 provides a considerable opportunity in various biological and clinical applications in which fatty acid profiling may be needed.[3,4] Beyond clinical applications, CeO_2-based materials also have a variety of applications as a catalytic system in fuel cells, thermochemical water-splitting, organic reactions, and photocatalysis.[5] Because of the involvement of CeO_2 in a variety of fields, and the potential it has to impact future technologies, a further investigation of the biological catalysis properties of this compound are warranted.

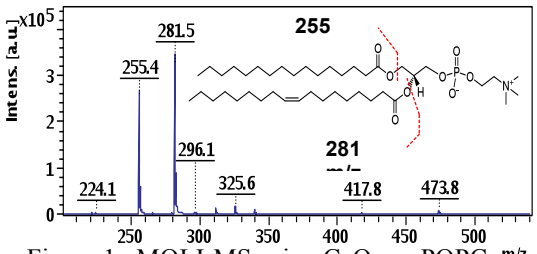

Figure 1. MOLI-MS using CeO_2 on POPC

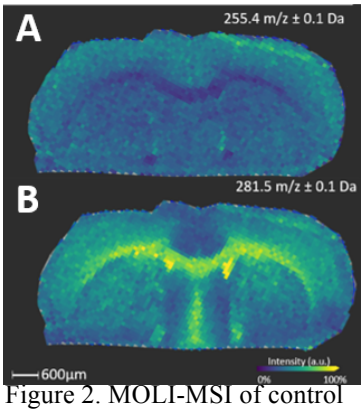

Figure 2. MOLI-MSI of control mouse brain: A) 255.4 m/z & B) 281.5 m/z

Preliminary Results: The Cox group in Colorado has headed the investigation for the use of MOLI techniques in the identification of bacterial species. This group discovered that CeO_2 could be utilized for identification with improved stability and reproducibility compared to other metal oxides.[6] Previously, MOLI has not been used in conjunction with MSI in order to induce fatty acyl catalysis directly from tissue for possible bacterial detection. While MSI is found to be a promising technique, there are only very few research groups that currently have the instrumentation available to conduct MSI studies. At Harvard Medical School, the Agar group has been working in the field of MSI for over a decade. During my time in the group this summer, I developed and optimized a technique for CeO_2 deposition on biological tissue, which is currently being prepared for submission. This technique describes the deposition of CeO_2 for MOLI-MSI, such as in Figure 2, as well as possible clinical applications.

Although MOLI using CeO_2 has shown considerable promise, the mechanism for which fatty acyl catalysis occurs when laser energy is applied to CeO_2 is still unknown. Most commonly, when MOLI-MS is used, analyte ionization of phospholipids typically occurs by protonation due to interactions with the Lewis acid/base sites on the metal oxide. However, only no protonation occurs with CeO_2-induced catalysis, indicating that the mechanism of cleavage is unique. It is postulated that much of the catalytic activity of CeO_2 arises from oxygen vacancy defects in the surface which occur at MALDI-like conditions (high temperature, low pressure).[6]

Proposed research: My preliminary results have developed a novel technique for the application of CeO_2 to clinical problems. However, it has left unanswered a critical question about the mechanism of fatty acyl cleavage that occurs when CeO_2 is used for MOLI-MS/MSI. Without understanding the mechanism of catalysis, it is difficult to fully interpret the mass

spectra generated by this method. My future research plans consist of three specific goals, detailed below. By achieving these goals, I plan to elucidate the mechanism of catalysis that is unique to CeO_2, when it is used with biological samples.

The first goal is to expand beyond phospholipids, and study compounds that also contain fatty acid chains, such as diacylglycerols, sphingolipids, triglycerides, acyl-carnitines, acyl-coenzyme A thioesters and other acyl-bound biomolecules. I am interested in determining if the cleavage of fatty acid chains is unique to phospholipids, or if CeO_2 can also induce this property on other compounds. This will serve to determine the depth and breadth of this application. Also, if certain compounds are unable to undergo catalysis by CeO_2, structural differences can be identified and studied. For these experiments, I plan to use commercially available lipid standards to evaluate catalysis in a simple, and direct way.

The second goal is to apply the knowledge gained in the first goal of this proposal to complex systems. Since isolated and purified compounds do not exist naturally, it is critical to see how effective this technique is when applied to a complex biological specimen. For this, I plan to correlate mass spectra obtained with a typical MALDI matrix and with CeO_2. I aim to correlate the known lipid composition with the fatty acid composition, to see if certain species are more prone to cleavage when present in a complex sample. This would be relevant in MOLI-MSI with heterogenous tissue samples, where lipid compositions can vary greatly throughout the specimen.

The third goal is to investigate the surface chemistry of CeO_2 using experimental and computational methods. I plan to perform studies where CeO_2 particles of varying sizes are probed by electron/neutron diffraction, since X-ray diffraction is not an ideal technique for this material, due to the low scattering power of oxygen. These studies aim to determine if a greater number of oxygen defects contributes to improved catalytic cleavage. Diffuse Reflectance Infrared Fourier Transform Spectroscopy will be performed to study the surface morphology of CeO_2 before and after it is subjected to MOLI-MS. Once the surface structure of CeO_2 is well understood, computational studies can be performed using density functional theory calculations.

Intellectual Merit & Broader Impact: Elucidating the mechanism of CeO_2-induced fatty acyl catalysis will allow scientists to use my developed MOLI-MSI technique and the MOLI-MS database for bacterial identification by the Cox group with increased confidence. Also, the information obtained from my first research goal has the potential to advance knowledge in fields that use fatty-acid containing molecules, such as cosmetics, nutrition, and metabolomics, in addition to biological applications. Furthermore, elucidation of the catalysis mechanism of varied metal oxides, and what induces this variance, can contribute fundamental knowledge to the field of catalysis chemistry, especially metal oxide catalysis. In regard to the broader impact in biological applications, fatty acid profiling of tissue specimens has been an extensive area of study, dating back nearly a century. Most current approaches use many time-consuming steps and, more concerningly, result in the loss of spatial relationships between these molecules. Preserving these spatial relationships is critical in the analysis of a wide variety of diseases, including many cancers, which demonstrate heterogenous tissue distribution. We have shown that MOLI-MSI using CeO_2 can provide in situ fatty acyl characterization of biological tissues while preserving regional distribution. By better understanding the chemistry of CeO_2 induced fatty acyl catalysis, a more informed interpretation of resulting MS spectra and therefore the tissue composition can be appreciated. This work will lay the groundwork for a potentially new clinical and translational diagnostic approaches.

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