

Searching for Biosignatures as Signs of Life using GALDI-FTMS

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ABSTRACT

Robust, unequivocal evidence of biosignatures in extraterrestrial and ancient terrestrial materials would have a profound impact on current understanding of the origins of life. In the search for signs of life, it is imperative to target minerals that are most likely to preserve and yield evidence of biological or organic compounds in order to design criteria for remotely operated instrumentation and optimize return sample missions. Laser desorption Fourier transform mass spectrometry (LD-FTMS) has been used to detect various biological and organic compounds associated with different minerals. For natural samples, LD-FTMS requires no sample preparation and offers high sensitivity to acquire spectra with a single laser shot for heterogeneously distributed biosignatures. Some organics compounds (i.e., polyaromatic hydrocarbons) self-ionize and are easily detected, but most biological and organic compounds require ionization assistance. Therefore, we are exploring how well different minerals assist in ionization, a process called geomatrix-assisted laser desorption/ionization (GALDI). The effectiveness of GALDI appears to depend not only on the composition of the biological

molecule and mineral moiety, but also on how they are associated. We report observations evaluating GALDI using LD-FTMS for laboratory-based and natural mineral samples. These observations have implications for which biological or organic compounds are detectable and persist in the rock record.

INTRODUCTION

Unequivocal evidence of microbial biosignatures in extraterrestrial and ancient terrestrial materials would have a profound impact on our current understanding of the origins of life. Laser desorption mass spectrometry (LDMS) has been touted as a preferred method to acquire chemical evidence for life without the need for sample preparation, especially since it was used so successfully to detect polycyclic aromatic hydrocarbons (PAHs) in meteorites [1]. We have previously demonstrated that detecting PAHs by LDMS is easy and independent of the associated mineral because PAHs can self-ionize [2]. Most bio/organic compounds, however, require ionization assistance; hence, the development of matrix-assisted laser desorption/ionization (MALDI) [3,4]. Therefore, we have been exploring a practical approach to search for signs of life with LDMS that relies on the geological matrix (i.e., minerals) to assist in the desorption/ionization of the bio/organic compounds, a process referred to as geomatrix-assisted laser desorption/ionization (GALDI) [5]. The ionization mechanisms involved in GALDI are mineral-dependent [5] and *can* differ from traditional MALDI mechanisms [6].

We have focused predominantly on the laser desorption Fourier transform mass spectrometry (LD-FTMS) because of its high mass resolution and accuracy; therefore, it easily allows simultaneous fingerprinting of the mineral and observation of bio/organic signatures. In addition, internal LD-FTMS can be quite sensitive for a single laser shot because only ~200 ions (~400 ions for our instrument) are necessary to produce a signal-to-noise (S/N) of 3 [2,5]. Other

mass analyzers, such as time-of-flight mass spectrometers, generally signal average data from 50–250 laser shots [7] because the geometry, ion optics, and/or characteristics of the detector may result in a detection efficiency of only ~5% of the ions generated from a single laser shot [8]. Single-shot sensitivity is necessary because the bio/organic compounds in biologically induced and natural mineral samples are likely distributed heterogeneously [9]. Hence, averaging multiple shots, as for a time-of-flight LDMS, results in a loss of the signal because the bio/organic signature becomes averaged into the noise [9]. The automated mapping capability of our LD-FTMS allows us to readily locate the bio/organic compounds in heterogeneous samples. In addition, the high mass accuracy coupled with the mass resolution of the LD-FTMS allows the chemical composition of peaks <1000 Da to be determined with high confidence [10,11].

We have analyzed an assortment of laboratory-based and natural mineral samples with LD-FTMS to determine what types of bio/organic compounds are detectable with different mineral matrices. The laboratory-based samples have focused on the potential for minerals to ionize bio/organic compounds by cationization. Here we focus on iron oxide laboratory samples. The natural samples have revealed various ionization mechanisms, from simple cationization to complex cluster ion formation.

EXPERIMENTAL

Sample Preparation. For the laboratory-based samples, two preparation methods [5] were adopted to simulate possible physical relationships that may occur between biomolecules and minerals in the natural environment: 1) aerospray [12] of a solution of bio/organic compound onto the mineral surface as a proxy for adsorption of biomolecules on mineral surfaces; and 2) homogenous mixture of analyte with ground geomatrix using a dry sample preparation technique [7] to mimic incorporation of biomolecules within geomaterials. Laboratory-based

and natural samples of loose mineral powders were pressed into pellets as described in the literature [6], while consolidated samples (i.e., rocks) were used as is as long as they fit on a 19 mm probe tip. The samples were attached to 316 SS FTMS probe tips using epoxy (Devcon 5 minute epoxy, Danvers, MA).

LD-FTMS Instrumentation and Parameters. Detailed description of the LD-FTMS has been published previously [13,14]. Experimental parameters for the FTMS analyses and the laser irradiance have also been reported [2,5,6].

RESULTS & DISCUSSION

Laboratory-based Bio/organic Compounds with Minerals. Direct laser desorption/ionization of biomolecules has never been highly successful [15]. It was not until 1988, when the MALDI process was developed, that detecting bio/organic compounds from solids became feasible [3,4]. The matrix is necessary to protect the analyte from the damaging effects of laser energy, help desorb the analyte from the surface into the gas phase, and facilitate ionization (e.g., protonation, proton abstraction, cationization, etc.). Because metals work as matrices for MALDI, we initially hypothesized that geomatrices (i.e., minerals) associated with bio/organic compounds could act as the matrices to desorb and ionize the organic molecules; hence, the term GALDI. We expected that the ionization process would form cation-attached molecular ions and that the process would be primarily facilitated by cations desorbed from the minerals that would attach to the bio/organic molecules in the laser desorption plume or in the gas phase. Indeed, we have observed sodium-attached molecular ions for amino acids (e.g., [His]Na⁺) and small proteins (e.g., [Gramicidin S]Na⁺) [5]. It is worth noting that this ionization process is effective for minerals such as halite that are simple salts known to suppress ion signals in traditional MALDI when present in “excess” (i.e., millimolar concentrations) [16]. Thus, our

ability to detect cation-attached (e.g., $[M+Na]^+$) peaks from pure mineral salts using LD-FTMS is actually surprising. Our success is probably from using a high sensitivity FTMS as the mass analyzer and using higher laser irradiation for the refractory minerals.

While cation attachment occurs for some bio/organic compounds with certain minerals, it does not work for all combinations [5]. When amino acids (e.g., Gly, Thr, Cys) are mixed with iron oxide minerals, such as hematite and goethite, no intact molecular ions were observed. Occasionally, fragment ions were observed for some amino acids (i.e., His with hematite). We have speculated that it was most likely the abundance of excited-state Fe ions in the gas-phase that were responsible for the fragmentation of the amino acids because Fe can insert between C-C bonds leading to fragmentation,

similar to other transition metals [17]. The small, cyclical protein gramicidin S (GramS) also failed to produce an unequivocal cationized molecular peak (Fig. 1a). However, we have observed intact biological compounds on iron-oxide minerals if PAHs are also present [2]

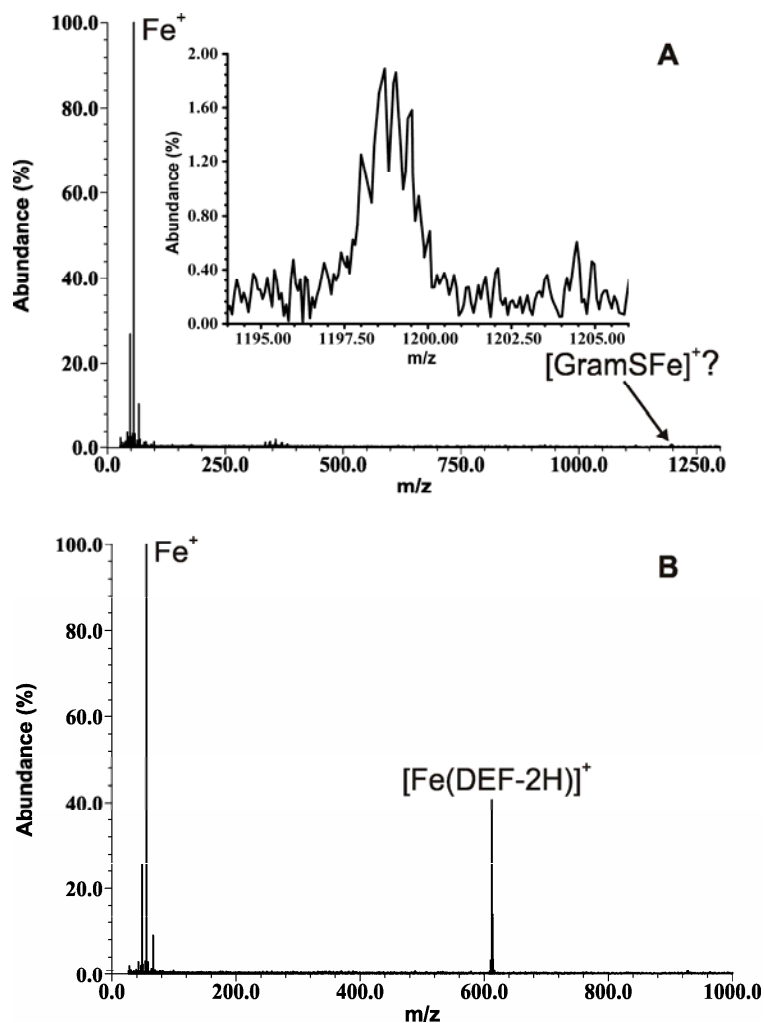


Fig. 1. LD-FTMS spectra of (A) GramS and (B) DEF areosprayed on hematite. Inset in (A) shows peaks with poor S/N near m/z 1198 that might be related to $[GramSFe]^+$.

because PAHs not only have the ability to self-ionize, but also ionize other organic compounds [18,19].

The only intact biological compound that we have observed on an iron-oxide mineral without the assistance of a PAH is the siderophore desferrioxamine B (DEF). When DEF was dissolved in a water-methanol solvent system and then aerosprayed onto a hematite surface, a distinct peak was observed at mass-to-charge (m/z) 614, which corresponds to $[\text{Fe}(\text{DEF}-2\text{H})]^+$

(Fig. 1b). This result is similar to that observed by Groenewold and co-workers for electrospray ionization mass spectrometry [20]. However, when DEF was physically mixed with hematite, no signal related to DEF was observed. One possible explanation is that there is a pH difference between the two sample preparations because it is known that the deprotonated version of DEF chelates iron efficiently [20]. It is possible that the DEF chelated with Fe on the surface forming $[\text{Fe}(\text{DEF}-2\text{H})]\text{X}$ (where X is a counterion), which

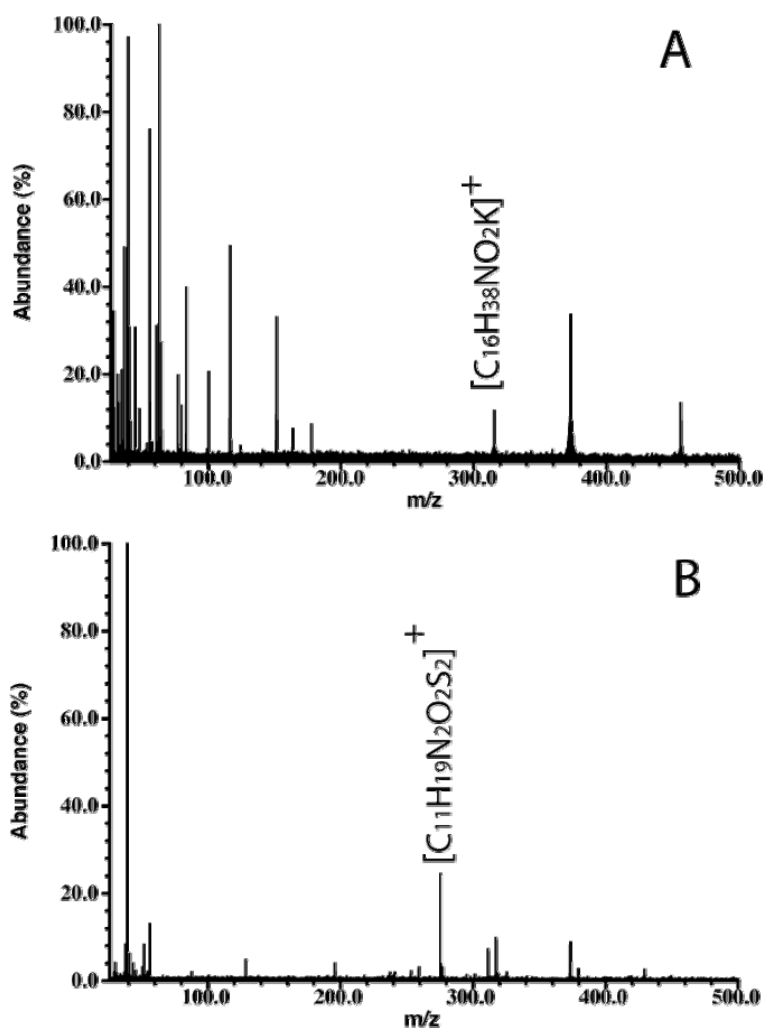


Fig. 2. LD-FTMS spectra of bio/organics associated with natural samples: A) Washburn site Yellowstone, WY and B) jarosite sample from New Zealand.

is then separated from its counterion during the desorption event.

Bio/organic Compounds in Natural Mineral Samples. The best illustration of the variety and unexpected complexity of the bio/organic compound–mineral analyses is our work with bio/organic compounds associated with jarosite minerals that spans synthetic, biologically induced, and natural mineral samples. Jarosite has the general formula $XFe(SO_4)(OH)_6$ (where $X = Na, K, NH_4, \text{etc.}$). For example, a peak at m/z 315 was detected (Fig. 2a) in a yellow powder from Washburn hot springs, Yellowstone National Park, WY that was confirmed to be jarosite by X-ray powder diffraction (XRPD). With only the mass accuracy of a time-of-flight mass spectrometer, the peak at m/z 315 would have been assigned to a fatty acid of biogenic origin. However, the mass accuracy of the LD-FTMS shows that the peak is at m/z 315.253 and, therefore, the chemical formula must contain one nitrogen atom to produce the correct mass defect (i.e., the numbers after the decimal point). The process of assigning chemical compositions to accurate m/z values has been explained in detail in the literature [6,10,11,21,22]. The peak at m/z 315 most likely corresponds to a chemical composition of $C_{16}H_{38}NO_2K^+$; thus, it appears to be a straightforward case of ionization by cation attachment. However, it is likely to be a cluster ion with NH_3 and H_2O noncovalently attached given the high ammonia concentration at the Washburn site. Further experiments to elucidate the structure of the ion are necessary to fully identify this peak as a type of lipid.

In several other jarosite mineral samples from different locations around the world, a peak at m/z 275.087 was observed repeatedly and assigned a chemical composition of $C_{11}H_{19}N_2O_2S_2$ based on the mass and isotopic distribution (Fig. 2b) [6]. This peak was also observed in laboratory biologically induced minerals created by *Acidithiobacillus ferrooxidans*, and though it appears to be due to a large biomolecule, it is not. Laboratory-based samples of various

synthetic jarosites (free of any organic contaminants) mixed with glycine (75 Da) revealed the same peak at m/z 275. Other lab experiments with K_2SO_4 and Na_2SO_4 confirmed that this is a cluster ion formed in conjunction with the sulfate anion [6]. If the amino acid alanine (89 Da) is substituted for glycine, a different peak at m/z 266 is observed; the ionization mechanisms for the peaks at m/z 275 and 266 are complex because these peaks would not be predicted based on the masses of the amino acids. These types of reactions have not been reported before; however, there is precedence in the mass spectrometry literature for gas-phase reactions of oxyanions with water and/or small organic molecules [23-30]. It is plausible that a recombination reaction with the glycine and sulfate occurs in the laser plume [31]. Recently, we have performed similar experiments with amino acids mixed with other oxyanion minerals (e.g., NO_3^- , SiO_4^{2-} , CO_3^{2-} , etc.) and observed formation of complex cluster ions. Further investigation of these cluster ion formation mechanisms is required to understand which bio/organic compounds are susceptible to this type of ionization in the presence of oxyanion minerals.

LD-FTMS Detection Limits. The type or nature of bio/organic compound–mineral association affects our ability to detect bio/organic signatures using LD-FTMS. If the ratio of bio/organic compound to mineral is high, as for surface applied bio/organic compounds or inhomogenous physical mixtures, the signal intensities are poor, which is not surprising given that LDMS of biomolecules alone has never worked well [15]. We speculate that as the bio/organic compound is incorporated into the mineral, the signal intensity increases. Indeed, we have observed that as the relative organic concentration decreases, the S/N increases; presumably because the ionization efficiency is greater as the organic molecules are surrounded by the mineral matrix. Based on our analyses of laboratory and natural samples, these lower concentrations of bio/organic compounds are more representative of real-world samples. Our

bio/organic compound detection range is typically picomoles (10^{-12}) to attomoles (10^{-18}) [5], equivalent to ppm and ppb, respectively. We have not yet pressed for lower detection limits because of heterogeneity issues.

CONCLUSIONS

Though we have observed that organic signatures can be detected in laboratory-based, biologically induced, and natural minerals; bio/organic signatures cannot be detected in all combinations of bio/organic compounds and mineral moieties. Detection of bio/organic compounds with iron-oxide minerals is particularly difficult. Our results also indicate that bio/organic signatures are the same, regardless of how the organic matter and the mineral are associated (e.g., the same signatures are observed from physical mixtures of organic materials and minerals, surface coatings of organic materials on minerals, and bio/organic materials procedurally included during synthesis of minerals). While the GALDI fingerprint of the bio/organic signature from each of these types of samples is the same, we have observed a difference in the detectability of the bio/organic signature that depends upon the type of mineral association because the ionization efficiency appears to increase as the contact between the bio/organic compound and mineral becomes more intimate.

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