Comparison of quadrupole and ion trap collision induced dissociation for structure determination of *Francisella novicida* lipid A variants.

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Introduction (119/120)

Over 100 structural variants of *Francisella tularensis* subspecies *novicida* (Fn) lipid A have been characterized from two growth conditions using electrospray ionization with a linear ion trap (IT) Fourier transform ion cyclotron resonance (FT-ICR) hybrid mass spectrometer. These results were generated using hierarchical tandem mass spectrometry (HiTMS) by combination of manual and automated data analysis. Recently, we have investigated more efficient ways to characterize lipid A species using quadrupole-CID (q-CID) on a SYNAPT G2 Q-TOF-MS. Here we investigated use of q-CID for fragmentation of lipid A on a commercial Solarix XR 7T FT-ICR and by IT-CID on a bespoke 21T FT-ICR. We are also investigating our ability to interpret CID data from lipid A's much larger parent molecule, lipopolysaccharide (LPS).

Methods (113/120)

Gram-negative bacteria was grown at 37°C overnight. Lipid A was extracted using a rapid, hot ammonium isobutyrate micro-extraction protocol. Prior to mass spectrometric analysis, the extracted lipid A samples were dissolved in CHCl3/CH3OH (2:1 v/v) solution whereas the purified whole LPS was analyzed after being dissolved in water/methanol (50:50; 1 mg/mL). MALDI experiments were performed on a Bruker Solarix XR 7T FT-ICR mass spectrometer and ESI on a bespoke 21T FT-ICR constructed at Environmental and Molecular Sciences Laboratory at Pacific Northwest National Laboratory (Shaw JASMS 2016). Bacterial extracts were analyzed in negative ion mode using the matrix norharmane (Scott Pathog Dis. 2016) for MALDI or infused by ESI (Shaffer et al. JASMS 2009).

Preliminary Data (294/300 words)

LPS is the major component of the outer leaflet of the outer membrane of Gram-negative bacteria. The structure of LPS consists of three regions: O-antigen, core saccharide, and lipid A. As the hydrophobic anchor of LPS, lipid A resides in the outer membrane and is the biologically active component of LPS recognition by the innate immune system via TLR4. Lipid A is a disaccharide of glucosamine often with terminal phosphate at 1- and 4′-positions with primary fatty acid derivatives at 2-, 3-, 2′- and 3′-positions and secondary fatty acids also possible at 2′b- and 3′b-positions. Number and length of acyl chains varies between different bacteria. In our prior work we detected > 100 structural variants from Fn grown at 25°C and 37°C with about half coming from each condition. While mass spectra of either extract only contained a few obvious ions, use of high resolution FT-ICR MS allowed dozens of additional species to be detected (Shaffer JASMS 2009; Ting JASMS 2011).

Recently, we investigated q-CID as a surrogate for MSn (Yoon RCMS 2016) using a Waters SYNAPT G2S Q-TOF MS. Varying q-CID energies to dissociate lipid A prior to TOF analysis was an efficient and a faster method than MSn. Here, rather than use q-CID on a TOF instrument like the SYNAPT, we used q-CID in a commercial Bruker 7 T FT-ICR. We also compared these data to prior work on a Thermo 7T LTQ FT (an IT-FT-ICR) platform to new data on a 21T IT-FT-ICR platform. Varying collision energies from the nominal instrument values recorded as 30, 40 and 50 eV we were able to approximate prior data on a SYNAPT and the prior IT-FT-ICR MS work on the 7T LTQ FT platform. A comparison of the data from these two FT-ICR instruments will be presented.

Novel Aspect (16/20 words)

Comparison of q-CID on a MALDI 7T FT-ICR to IT CID on a ESI 21T FT-ICR.