Fast Lipid Analysis of Lipid A Coupled with Tandem Mass Spectrometry

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Overview

- Rapid citric acid hydrolysis of lipid A from various lipopolysaccharides
- Tandem MS of *E. coli* bacteria colonies, four LPS bacteria strains, and alkali metal adducted bacteria colonies

Introduction

Gram-negative bacteria contain an embedded layer of endotoxic lipopolysaccharides (LPS) which contributes to antibiotic resistance owing to its ability to adapt and hinder antibiotics from entering and killing the bacteria.[1] Gastric adenocarcinoma is a stomach cancer largely initiated by inflammation from chronic *Helicobacter pylori* bacterial infections.[2] Bacterial modifications to lipid A, a structural component of lipopolysaccharides, may obstruct the entry of cationic antimicrobial peptides into the bacteria, diminishing their efficacy.[1] Here, we aim to characterize the structures of lipid A of *H. pylori* and other bacteria by implementing the fast lipid analysis technique (FLAT)[3] utilized with matrix-assisted laser desorption/ionization (MALDI) and collision-induced dissociation (CID) or 193 nm ultraviolet photodissociation (UVPD). FLAT streamlines lipid A extraction to under an hour, allowing rapid identification of free lipid A in bacteria colonies compared to traditional processes.[3] For characterization of lipid A, CID cleaves primarily labile bonds. Alternatively, UVPD is a higher energy activation method capable of generating cross-ring cleavages and fragment ions that allow localization of modifications and determination of acyl chain compositions of lipid A. UVPD has successfully been used to analyze lipopolysaccharides in combination with other activation methods.[4] Owing to the frequent prevalence of various cation sources in bacteria samples, lithium adduction to lipid A can be used to increase fragment ion intensity and identification by consolidating adducts and diminish distribution of ion signal.[5, 6]

Methods

Experiments were performed on a Bruker timsTOF Flex mass spectrometer equipped with a MALDI source. For MALDI experiments, a bacteria colony or LPS bacterial extract was deposited on a glass slide and 1 µL of an extraction buffer (0.2 M citric acid, 0.1 M trisodium citrate dihydrate, pH 3.5) was applied to the sample before incubation in a humidified chamber at 110°C for 30 minutes. Subsequently, the sample was rinsed with water, air-dried, and 1 µL of norharmane matrix (10 mg/mL in 12:6:1, v/v/v chloroform/methanol/water) was applied. In the case of lithium adduction experiments, samples were prepared with the addition of 1 µL of 100 µM lithium trifluoroacetate to 99 µL norharmane matrix, and 1 µL of the matrix mixture was applied to the bacteria colony sample. For spectral acquisition, 1000 laser shots were averaged with a laser energy of 70%. For ESI experiments, a 10 µM E. coli lipid A standard was prepared in 50:50 (v/v) isopropanol/water. ESI and UVPD experiments were performed on a Thermo Orbitrap Fusion Lumos Tribrid mass spectrometer equipped with a MassTech AP/MALDI (ng) UHR source and a 193 nm excimer laser. Mass spectra were collected in negative mode with a spray voltage of 2.8 kV, an ion transfer tube temperature of 300°C, and a resolution of 120,000 at m/z 200.

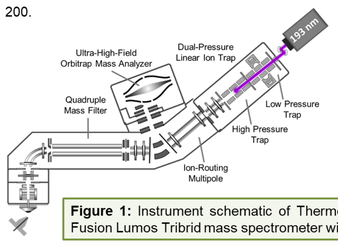
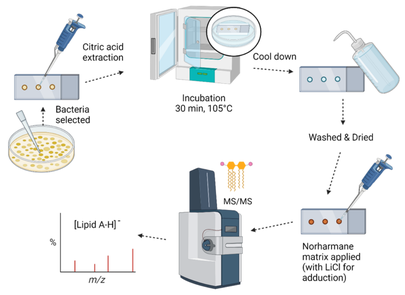


Figure 1: Instrument schematic of Thermo Scientific Orbitrap™ Fusion Lumos Tribrid mass spectrometer with a 193 nm laser.

Results & Discussion



Scheme 1. Workflow for FLAT lipid A extraction and lithium adduction prior to MALDI-MS analysis.

FLAT-MALDI: Previous work with FLAT has showcased rapid extraction of lipid A from bacterial colonies and liquid bacteria cultures followed by tandem MS analysis.[3] The workflow for FLAT is demonstrated in **Scheme 1** and was modified from using bacteria colonies to also using bacterial LPS extracts. **Figure 2** shows the difference between FLAT analysis of lipid A from bacteria colonies without and with use of the extraction buffer based on monitoring the lipid A ion of *m/z* 1796. The use of the extraction buffer allows successful identification of lipid A and sufficient signal intensity for further MS analysis.

Lipid A from four different LPS bacterial samples were analyzed using MALDI-TOF (**Figure 3**). The dominant lipid A molecules included *H. pylori* 26695 (*m/z* 1547.80), *Salmonella enterica* RD1 (*m/z* 1716.25), *Acinetobacter baumannii* WT 17978 (*m/z* 1892.58), and *Escherichia coli* R1 (*m/z* 1279.85).

The corresponding CID spectra obtained for the most abundant lipid A for each bacteria are shown in **Figure 4A**. Fragmentation maps are shown in **Figure 4B**. Key cleavage sites are numbered (1 – 8), and the resulting fragment ions are listed next to the cleavage site numbers. Fragment ions that require multiple cleavages are listed next to each cleavage sites. The dominant fragment ions generated by CID result from acyl chain cleavages. No cross-ring cleavages that could help confirm the overall lipid A structure occurred.

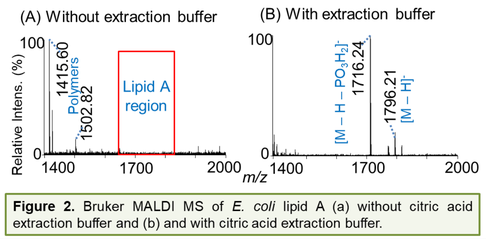


Figure 2. Bruker MALDI MS of *E. coli* lipid A (a) without citric acid extraction buffer and (b) and with citric acid extraction buffer.

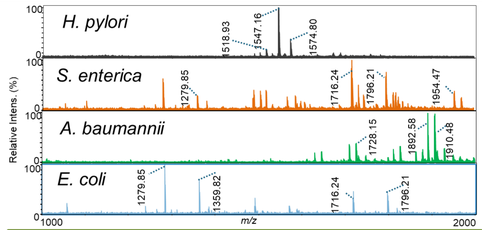


Figure 3. MALDI MS1 spectra of lipid A molecules from four bacteria strains.

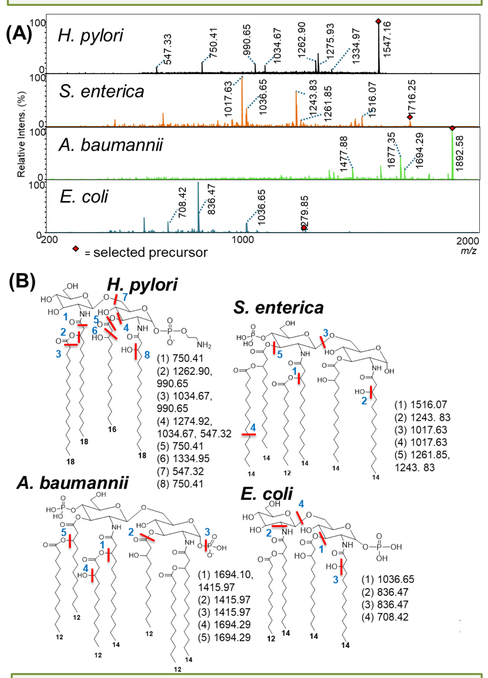
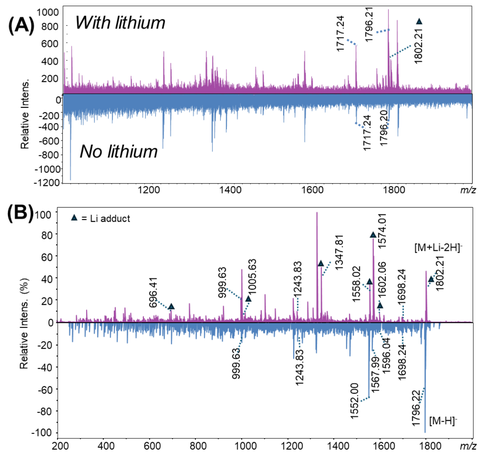


Figure 4. (A) CID (100 eV collision energy) of deprotonated lipid A molecules from four bacteria strains. (B) Corresponding fragmentation maps. The number listed beneath each acyl chain indicates the number of carbon atoms.

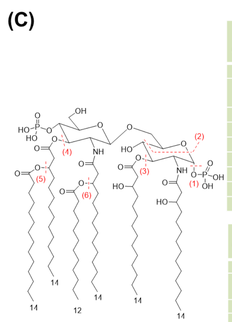
Lithium Adduction: To improve signal intensity, lithium adduction with FLAT-MALDI was optimized using an *E. coli* bacterial colony, as adapted from a prior report.[6] Addition of lithium salt resulted in a 2X increase in signal abundance (**Figure 5A**). Consolidating all adducts into the lithium complex overcame dilution of the ion signal into many competing metal adducts.

CID of deprotonated lipid A and the corresponding lithium complex are displayed in **Figure 5B**, and the key fragment ions are summarized in **Figure 5C**. There are a few fragment ions produced for the [M + Li – 2H] complex, such as *m/z* 999.63 and 1243.83 that are higher in intensity than observed in the bottom spectrum (**Figure 5B**).

Shotgun ESI-MS³. LPS were also introduced by ESI in a direct infusion shotgun manner, and one MS1 spectrum for LPS from an *S. enterica* RD1 extract is shown in **Figure 6A**. Specific LPS were selected for MS³ analysis. For example, CID was performed on the core lipooligosaccharide (*m/z* 1200.18^{2-}) to cleave it into lipid A (*m/z* 1796.20) and oligosaccharide (*m/z* 603.18) portions **(Figure 6B)**. UVPD was used to characterize the released lipid A (**Figure 6C**). Nine diagnostic fragment ions are labeled on the fragmentation map in **Figure 6D**. The fragment ions observed help to identify the acyl chain lengths, but no backbone cleavages were observed that could be used to localize possible modifications on the phosphate positions.



(C)



Li adduct *m/z* (theor.)	Li adduct *m/z* (expt.)	Cleavages	Error (ppm)
696.41	696.41	(2) + (4)	0.00
1005.64	1005.63	(1) + (3) + (4)	-9.94
1249.84	n/a	(1) + (4)	n/a
1347.82	1347.81	(4)	-7.42
1558.02	1558.02	(3)	0.00
1574.01	1574.01	(5)	0.00
1602.04	1602.06	(6)	12.48
1704.25	n/a	(1)	n/a
1802.22	1802.21	n/a	-5.55

m/z (theor.)	*m/z* (expt.)	Cleavages	Error (ppm)
690.40	n/a	n/a	n/a
999.63	999.63	(1) + (3) + (4)	0.00
1243.83	1243.83	(1) + (4)	0.00
1341.80	n/a	n/a	n/a
1552.01	1552.00	(3)	-6.44
1568.00	1567.99	(5)	-6.37
1596.03	1596.04	(6)	6.26
1698.23	1698.24	(1)	5.88
1796.21	1796.22	n/a	5.56

Figure 5. (A) MALDI-MS^1 spectra obtained for *E. coli* lipid A with and without lithium adduction. (B) CID (100 eV collision energy) of (M + Li – 2H)$^-$ *m/z* 1802 (top) versus (M – H)$^-$ *m/z* 1796 (bottom). (C) Corresponding fragmentation map and summary of key fragment ions.

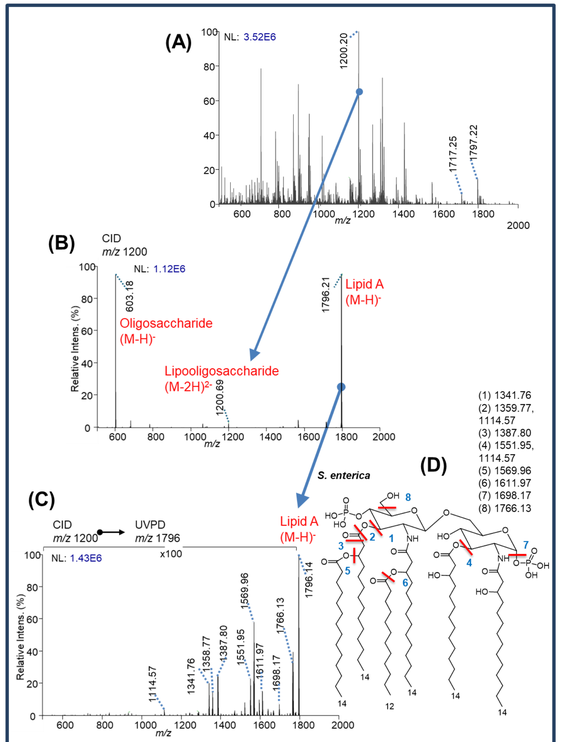


Figure 6. (A) ESI spectrum of *S. enterica* LPS extract. (B) CID (NCE 30) was used to cleave one selected LPS (*m/z* 1200.18, 2-) into oligosaccharide and lipid A portions. (C) UVPD (5 pulses, 3 mJ) was used to characterize the lipid A moiety (*m/z* 1796.20). (D) Fragmentation map of lipid A.

Conclusions

- FLAT-MALDI used on bacteria colonies and LPS extracts
- Signal intensity increased by 2X using lithium adduction with MALDI
- MS³ used to characterize lipid A from LPS, *S. enterica* RD1

References & Acknowledgements

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