Structural characterization of lipid biomarkers from *Staphylococcus aureus* following microextraction for mass spectrometric phenotyping

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Abstract

Bacteria contain essential complex lipoglycans within their membranes. These are easily extractable for analysis by mass spectrometry (MS), and glycolipid mass spectra are distinguishable between different bacteria. Therefore, we propose this class of lipids as a novel biomarker to permit identification of bacteria by MS phenotyping. Here we elucidate the structure of glycolipids isolated from Gram-positive *Staphylococcus aureus* during rapid microextraction on our diagnostic platform. Tandem MS demonstrate that the primary glycolipid species detected in these extracts is a partially truncated lipoteichoic acid (LTA) molecule. LTA are abundant in Gram-positive membranes and unique between species. These findings suggest the potential of this novel diagnostic platform for rapid identification of pathogens during infection.

Introduction

Bacterial membranes are composed of lipids of diverse structure and fluid composition. Similar to eukaryotic cell membranes, microbial membrane structure is a bilayer of amphiphilic glycerophospholipids. In Gram-negative bacteria, there are two distinct membranes separated by a periplasm and in Gram-positive bacteria, the membrane is enclosed by a thick peptidoglycan cell wall. Extensive preliminary data and published literature indicates that lipid A (LA) derived from lipopolysaccharide (LPS) of Gram-negative bacteria, which comprises the majority of the outer membrane, is unique and could be used as a molecular signature for pathogen identification. (**U.S. Patent # US20120197535 A1**) However, little research has been conducted to determine the diagnostic potential for glycolipids in Gram-positive bacteria. They have numerous unique cell wall glycans including lipoteichoic acid (LTA) which is composed of a diacylglycerol lipid that anchors in the membrane and a complex oligosaccharide that penetrates the cell wall. These provide species-specific variability in the arrangement of fatty acid side chains and in response to changing growth conditions. We hypothesize that these signature glycolipids represent a novel biomarker that would enable identification of a pathogen.

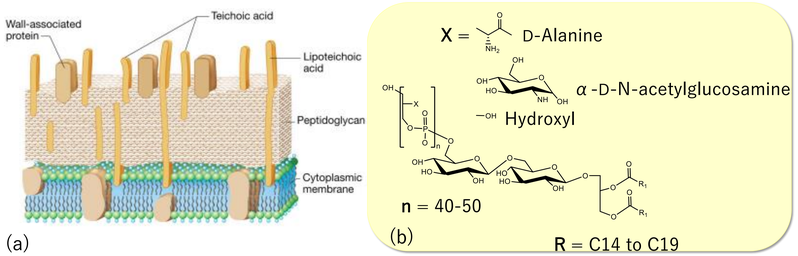


Figure 1. (a) Schematic representation of Gram-positive bacterial membrane and (b) general structure of lipoteichoic acid from *Staphylococcus aureus*

Methods

We optimized a micro-scale hot ammonium isobutyrate lipid isolation protocol described by El Hamidi *et al.* (2005) that utilizes mild acid hydrolysis to disrupt the membrane and liberate the lipid molecules from their complex carbohydrate components. Overnight growth cultures (1-5 mL) of methicillin-resistant *S. aureus* (MRSA) M2 strain were harvested and reacted in a 5:3 isobutyric acid/ammonium hydroxide mixture at 100° C for 30-45 minutes. Reactions were lyophilized overnight to generate dry pellets which were solubilized in 100-200 µL 2:1:0.2 chloroform/methanol/water solvent mixture. Extracts were diluted 10:1 and samples were analyzed by ESI-TOF in negative ion mode using a Waters Synapt G2 HDMS. Samples were injected at a rate of 5 µL/min, and MS/MS fragmentation was performed by energizing to 60 eV.

Preliminary Data

A truncated LTA molecule was proposed based on the base peak at *m/z* 1352 of the MS^1 spectrum (**Figure 2**). This results from the cleavage of the majority of the teichoic acid during the extraction process and loss of the N-acetyl glucosamine substituent from the remaining glycerophosphate repeating units. Neighboring mass spectral peaks are separated from one another by 14 mass units which suggest differences of a single methylene group and likely results from variations in the fatty acid chain lengths of the diacylglycerol.

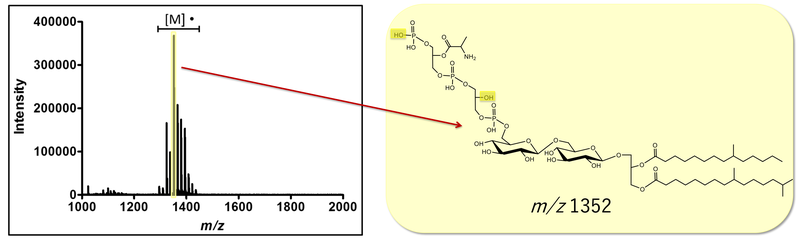


Figure 2. Predicted structure for truncated LTA molecule from *Staphylococcus aureus* following ammonium butyrate whole cell extraction of lipids.

Conclusions

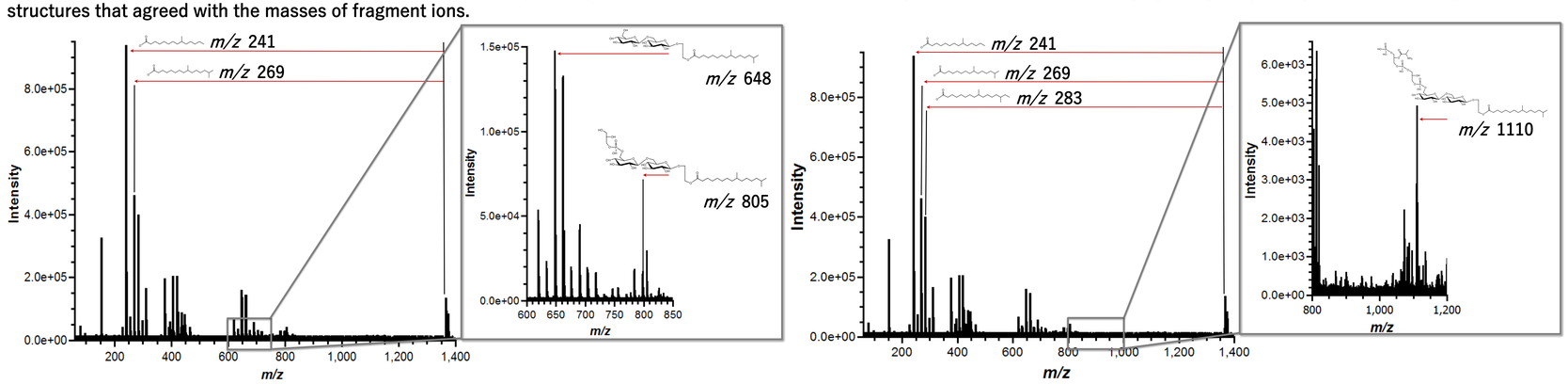
- Mass peaks at *m/z* 241, 269, and 283 as well as minor mass peaks at *m/z* 227, 255, 297 and 311 are attributed to 14:0, 15:0, 17:0, 18:0, and 19:0 chain fatty acids with branched chains at C-9 and C-14 positions.
- Mass peaks were also observed at *m/z* 1110 (representing a loss of one C14:0), *m/z* 805 (representing loss of two glycerophosphate units), and *m/z* 649 (representing loss of one C14:0 and all of the glycerophosphate repeating units from the dihexose sugar).
- A mass peak distribution was noted at these positions with mass peaks separated by 14 mass units suggesting a difference of a single methylene group.
- Fatty acid (FA) composition accounts for the multiple LTA molecules present in the M2 profile reflecting the overall diversity of the molecule.

Future directions

- Gas chromatography with flame ionization detector (GC-FID) will be used to confirm the heterogeneity of lipid isomers within each peak.
- Additional Gram-positive organisms, particularly with different LTA types, will be included for structural studies.

Results: Tandem MS on most abundant mass peaks from *S. aureus* LTA extracted by hot ammonium isobutyrate

Figure 3. Tandem mass spectrometry was performed on mass spectral peaks at *m/z* 1352 and 1366. Data analysis was conducted manually by using the primary structure to assign structures that agreed with the masses of fragment ions.



References

1. Fischer *et al.*, *Eur. J. Biochem.*, 133: 523-530 (1983)
2. Morath *et al.*, *J. Exp. Med.*, 193(3): 393-397 (2001)
3. Morath *et al., J. Endo. Res.*, 11(6): 348-356 (2005)
4. El Hamidi *et al.*, *J. Lipid Res.,* 46: 1773–1778 (2005)
5. Percy and Gründling, *Annu. Rev. Microbiol.*, 68: 81-100 (2014)

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