Top Down Tandem Mass Spectrometric Analysis of a Chemically Modified Rough-type Lipopolysaccharide Vaccine Candidate

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Introduction

Recent advances in lipopolysaccharide (LPS) biology have led to its use in drug discovery pipelines, including vaccine and vaccine adjuvant discovery. Desirable characteristics for LPS vaccine candidates include both the ability to produce a specific antibody titer in patients and a minimal host inflammatory response directed by the innate immune system. However, in-depth chemical characterization of most LPS extracts has not been performed; hence, biological activities of these extracts are unpredictable. Additionally, the most widely adopted workflow for LPS structure elucidation includes nonspecific chemical decomposition steps before analyses, making structures inferred and not necessarily biologically relevant. Here we assign them directly

Experimental

In this work, several different mass spectrometry workflows that have not been previously explored were employed to show proof-of-principle for top down LPS primary structure elucidation, specifically for a rough-type mutant (J5) *E. coli*-derived LPS component of a vaccine candidate. First, ion mobility filtered precursor ions were subjected to collision induced dissociation (CID) to define differences in native J5 LPS v. chemically detoxified J5 LPS (dLPS). Next, ultra-high mass resolving power, accurate mass spectrometry was employed for unequivocal precursor and product ion empirical formulae generation. Finally, MS^3 analyses in an ion trap instrument showed that previous knowledge about dissociation of LPS components can be used to reconstruct and sequence LPS in a top down fashion.

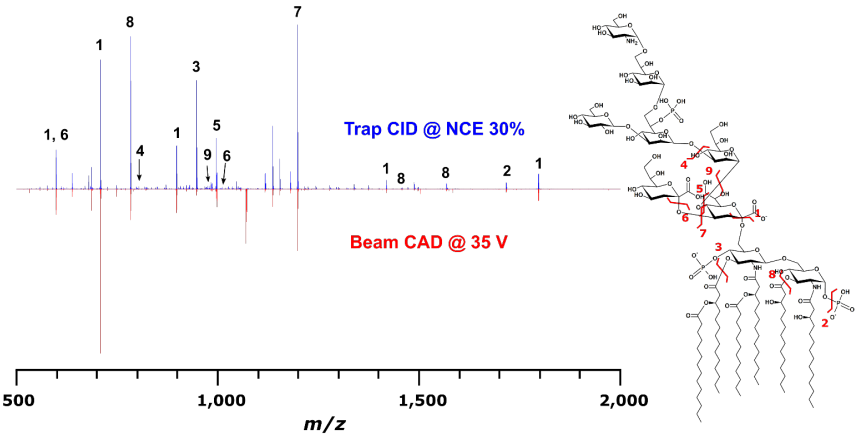

Fig. 1. Comparison of trap CID (blue) and beam CAD (red) for the same precursor ion at *m/z* 1071. Ninety-nine monoisotopic product ions common to both experiments were observed, fifteen of which are annotated in the CID mass spectrum with corresponding bond cleavages in the structure on the right. All product ion *m/z* were measured with less than 100 ppb error.

Results and Discussion

A structural rationale is explained for differential inflammatory dose-response curves, *in vitro*, when HEK-Blue hTLR4 cells were administered increasing concentrations of native J5 LPS v. dLPS, which will be useful in future drug discovery efforts.

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References

[1] Oyler, B.L*, et al.,* J Am Soc Mass Spectrom., **29**(6), 1221-1229 (2018).