Complete Post-Translational Modification Mapping of Pilins from Clinical Strains of Pathogenic Neisseria meningitidis Requires Top-Down Mass Spectrometry

View Presentation Detail

Authors
Joseph Gault1; Christian Malosse1, 2; Marie-Cécile Ploy6; Catherine E. Costello3; Guillaume Dumenil4, 5; Julia Chamot-Rooke1, 2

Institutes
1 Institut Pasteur, Paris, FRANCE; 2 CNRS UMR3528, Paris, France; 3 Boston University School of Medicine, Boston, MA; 4 INSERM U970, Paris, France; 5 Université Paris Descartes, Paris, France; 6 INSERM UMR1092, Limoges University Hospital, Limoges, France

Introduction
In pathogenic bacteria post-translationally modified proteins have been found to promote bacterial survival and evasion from the host immune system. For the human pathogen Neisseria meningitidis, pathogenesis is mediated by pili, extracellular filamentous organelles primarily built of a single protein subunit or major pilin, PilE. Previous reports have shown that PilE can be expressed as different proteoforms, each harboring its own set of post-translational modifications (PTMs) and that specific proteoforms are key in promoting virulence [1]. Efficient tools that allow complete PTM mapping of proteins involved in bacterial infection are therefore strongly needed. We show here, using novel clinical strains, that top-down mass spectrometry is required to achieve this goal when more than two proteoforms are present simultaneously [2,3].

Methods
Clinical isolates of N. meningitidis were obtained from patients that had been hospitalised with evidence of meningitis.

Top-down experiments were performed on a 12T solariX FT-ICR equipped with a hollow dispenser cathode. Purified pilins were introduced into the mass spectrometer using a Triversa Nanomate (Advion). MS spectra were acquired in broadband mode and deconvoluted using the maximum entropy option. For MS/MS experiments, ions of interest were submitted to Electron Capture Dissociation (ECD). Peak picking was performed using the SNAP 2.0 algorithm and a home-built package was used for ion assignment.

For bottom-up experiments, samples were trypsin digested and analysed in infusion mode on an LTQ-Velos Orbitrap mass spectrometer using Higher energy Collision Dissociation (HCD) and Electron Transfer Dissociation (ETD).

Preliminary Results/Abstract
FT-ICR MS profiling of PilE purified from the N. meningitidis 278534 isolate revealed the presence of four major proteoforms in a 1:1:1:1 ratio. Comparison of the theoretical mass and the lowest mass major peak observed in the MS profile indicated a difference of over 620 Da, showing that PilE could be highly post-translationally modified. PilE was trypsin digested and analyzed by nanoESI-FTMS on an Orbitrap mass spectrometer. Comparison of the measured tryptic peptide masses with theoretical ones revealed the presence of non-modified peptides leading to sequence coverage of 80 %. HCD/ETD experiments further allowed the characterization of several novel peptides modified with DATDH (diacetamido trideoxy hexose) and PG (phosphoglyceryl) groups, extending the sequence coverage to 96 %.

Armed with these results, we tried to assign peptide combinations, and thus PTMs, to specific proteoforms. Only the lowest and highest mass proteoforms could be fully characterized, highlighting the severe limitations of bottom-up for complete proteoform mapping.

A top-down ECD MS/MS approach allowing each proteoform to be investigated separately was therefore developed. The results obtained led to a complete characterization of all four proteoforms. Interestingly, an unexpected high content of glycosylation and multiple sites of modification were found.

Using the PTM localisation data provided by top-down mass spectrometry, molecular modeling of the pilus fibre was performed and indicated that the surface is completely covered by glycan subunits, precluding any interaction with an
antibody. This high level of glycosylation is an efficient way for the bacteria to generate antigenic variants and escape host immune system.

This study highlights the interest of using top-down mass spectrometry to fully characterize virulence proteins and probe new mechanisms of immune evasion.

3. Gault et al., Proteomics DOI: 10.1002/pmic.201300394

**Novel Aspect**
First example showing how top-down MS can be used on clinical samples to characterize virulence proteins and probe immune evasion.