Mapping Post Translational Modifications involved in Neisseria Meningitidis virulence by Top-Down Mass Spectrometry

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Abstract

Post translational modifications (PTMs) are increasingly being revealed as key intermediates in pathogenesis pathways[1-2]. Understanding their role on the molecular level not only greatly increases our comprehension of disease mechanisms but provides an essential basis onto which human intervention strategies can eventually be built. Detection and localisation of PTMs is not an easy task and mass spectrometry is often integrated into the process.
In recent work we identified an important PTM on the type IV pili of *Neisseria meningitidis*. Pili are extracellular, filamentous organelles highly involved in bacterial aggregation and host cell attachment. The pilus itself is a macro polymer built up of the repeating 17.5 kDa protein unit pilE, which is arranged helically to create long and flexible fibres.
The glycerophosphate modification, present on serine 93 of PilE, is induced *in vivo* after several hours of host cell contact. We hypothesise that subsequent alteration of the pilus surface, once modified by the phosphoester, ultimately leads to the dissemination of the bacterium; this step forcibly precedes invasive infection[3].
Because bottom-up methods are particularly inappropriate for the analysis of protein isoforms, we developed a top-down approach that employs FT-ICR mass spectrometry and multiple dissociation techniques for complete structural characterisation of pilE.
Here we present top-down results obtained using different mass spectrometers and various fragmentation techniques, for the localisation of all modifications present on the protein. Aspects of the techniques, applicability to other systems, including minor pilin isoforms from novel clinical strains, will be discussed along with the major implications of a “one shot” approach to understanding bacterial dissemination.

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