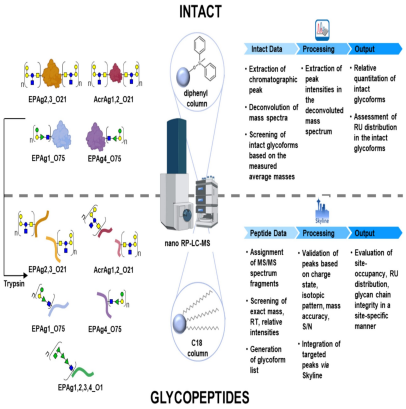
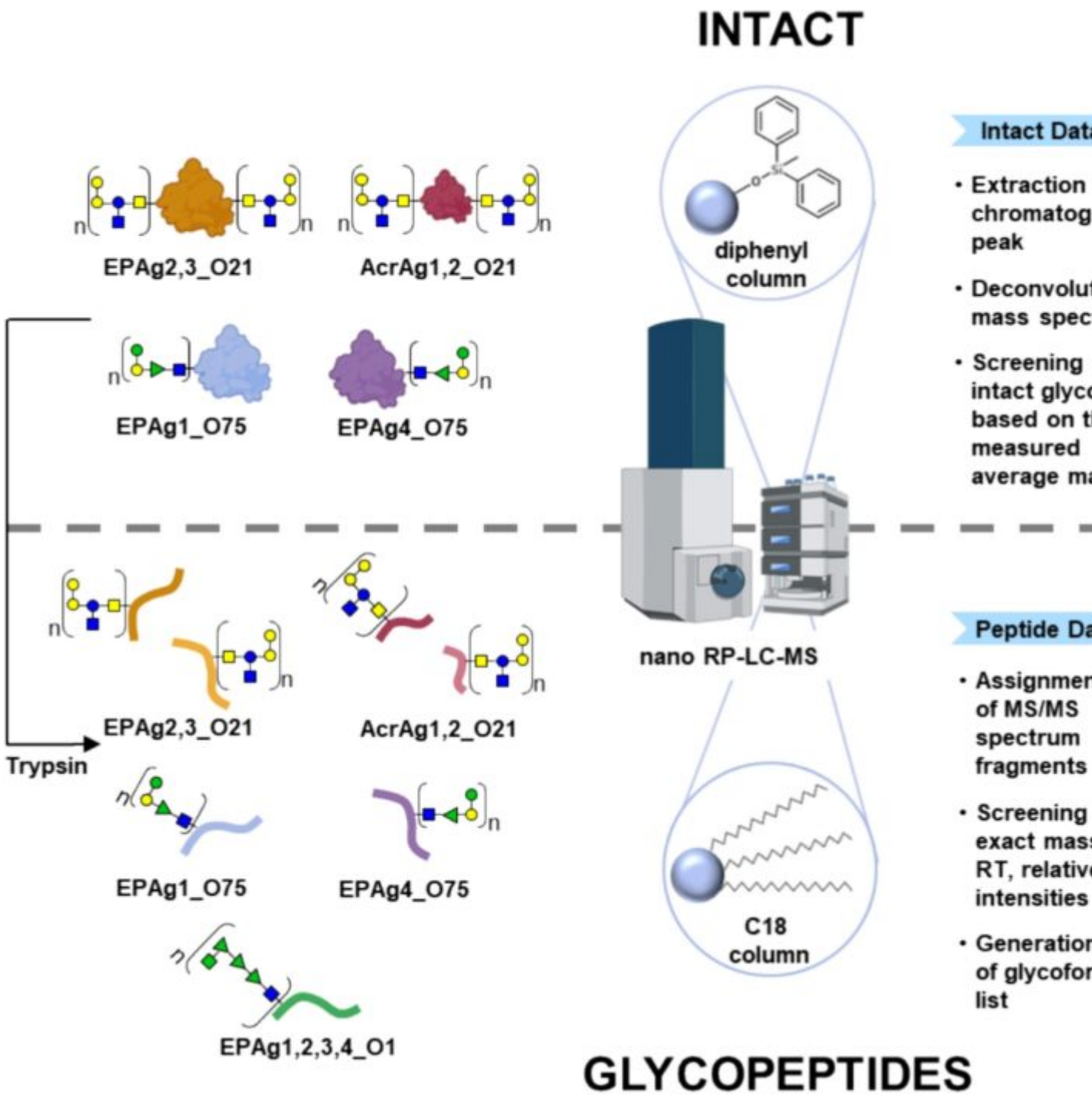


Comprehensive characterization of bacterial glycoconjugate vaccines by liquid chromatography – mass spectrometry

Description

Bacterial pathogens can cause a wide range of infections with adverse health effects. The development of vaccines is essential as multi-drug resistance in bacterial infections is an increasing concern. Recombinant O-antigen glycosylated proteins are promising glycoconjugate vaccine candidates for the prevention of bacterial infections. However, methods for their comprehensive structural characterisation are lacking.

The article presents a bottom-up approach for their site-specific characterization by detecting N-glycopeptides using nano reversed-phase liquid chromatography-mass spectrometry (RP-LC-MS). Glycopeptide analyses provided information on partial site occupancy and site-specific glycosylation heterogeneity and helped to confirm the polysaccharide structures and their modifications. The bottom-up analysis was complemented by intact glycoprotein analysis using nano-RP-LC-MS, which allowed rapid visualization of the polysaccharide distribution in the intact glycoconjugate. At the glycopeptide level, the model glycoconjugates analyzed showed different repeat unit (RU) distributions ranging from 1 to 21 RUs attached to each glycosylation site. Interestingly, the intact glycoprotein analysis showed an RU distribution ranging from 1 to 28 RUs, indicating the predominant species when the different glycopeptide distributions are combined in the intact glycoconjugate. The complete workflow based on LC-MS measurements allows a detailed and comprehensive analysis of the glycosylation state of glycoconjugate vaccines.



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Overview of the analytical workflows employed for the analysis of the five glycoconjugates EPAg2,3_O21, Acrag1,2_O21, EPAg1_O75, EPAg4_O75 and EPAg1,2,3,4_O1. RP-LC-MS: Reversed-phase liquid chromatography coupled to mass spectrometry.

Category

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