In-depth Characterization of Neutral and Acidic Glycopeptides by ZIC-HILIC Enrichment and Mass Spectrometry

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Introduction

- Analysis of glycopeptides is challenging due to high glycan heterogeneity.
- Ion suppression effects require additional selective methods for glycopeptide enrichment.
- In this study, we used ZIC-HILIC glyco capture resins for glycopeptide enrichment.
- We analyzed a tryptic digest of bovine α1-acid-protein (AGP), a protein with complex di-, tri- and tetra-antennary as well as highly sialylated glycans N-linked to 5 sites containing both N-acetyl-neuraminic and N-glycolyl-neuraminic acid.
- LC-MALDI TOF/TOF-MS was used for in depth analysis of the peptide and the glycan moieties of each glycosylation site.

Methods

- Sample: tryptic digest of bovine AGP
- Glyco enrichment: ProteoExtract™ Glycopeptide Enrichment Kit (Art. No. 72103, EMD Chemicals Inc.).
- LC-MALDI Analysis: ultraflextreme in linear ion mode for detection of acidic glycopeptides and in LIFT mode for MS/MS analysis (all Bruker).
- Software: All LC-MS/MS data were organized and analyzed in the ProteinScape 3.0 bioinformatics platform (Bruker Daltonics), which was used for a detailed characterization of the glycosylation pattern and visualization of the relevant mass spectra. Glycans searches were searched in the database GlycomeDB using the search engine GlycoQuest. Peptide searches were performed in the SwissProt database using Mascot (Matrix Science Ltd.).

Results

LC-MALDI analysis of glycopeptides
- RP-HPLC separation according to peptide moieties
- Specific glycopeptide fragmentation pattern in MALDI MS/MS spectra allows automatic determination of peptide mass.
- MS/MS spectra were used for protein and for glycan identification via database searches in ProteinScape 3.0 (Fig. 1)
- The workflow is outlined for the analysis of glycans at Asn 136 (Fig. 2).

Summary

- Glycopeptide enrichment facilitates the analysis of glycosylation sites.
- LC-MALDI of glycopeptides allowed to identify peptide sequences and glycan structures from a single MS/MS spectrum, simultaneously.
- LC-MALDI allowed the in depth analysis of all glycosylation sites of a protein in one experiment.
- Dedicated software (ProteinScape 3.0) was used for glycan and peptide identification and for result validation.

Conclusions

Straightforward glycopeptide analysis using:
- Glycopeptide enrichment
- LC-MALDI
- Software platform ProteinScape 3.0 for glycan database searches