Intact glycopeptide analysis by trapped ion mobility tandem mass spectrometry

HUPO2019

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Introduction

Recent introduction of parallel accumulation serial fragmentation (PASEF) on a trapped ion mobility quadrupole time-of-flight mass spectrometer (timsTOF Pro) provides unique possibilities for comprehensive glycopeptide profiling in complex samples such as blood plasma. Poor ionization efficiency during electrospray ionization was solved previously by providing acetonitrile enriched nitrogen gas into the CaptiveSpray source via the nanoBooster. Here, we show first results using nanoBooster dopants for glycopeptide analysis on the timsTOF Pro instrument.

Methods

Blood plasma from healthy individuals was subjected to tryptic digestion and glyco-peptides were enriched using Sepharose. Both the tryptic plasma digest and enriched glycopeptide fractions were measured by LC-IMS-MS/MS (Bruker Daltonics nanoElute and timsTOF Pro) using filtered air and dopant enriched nitrogen source gas using acetonitrile and ethanol as nanoBooster solvents. Data analysis was performed in DataAnalysis 5.0, ProteinScape 4.1, and in-house developed Perl scripts.

Summary

Use of acetonitrile and primary alcohols as nanoBooster dopant are compatible with TIMS and PASEF operation on the timsTOF Pro instrument. Ionization efficiency is significantly enhanced which enabled analysis of glycopeptides that were barely detectable under normal ESI source conditions (Fig1). PASEF using normal and elevated collision induced dissociation energies can be used to identify both the glycan- and peptide-moieties by GlycoQuest and MASCOT database searches as shown for selected IgG1 glycopeptides in Figure 2.

Results

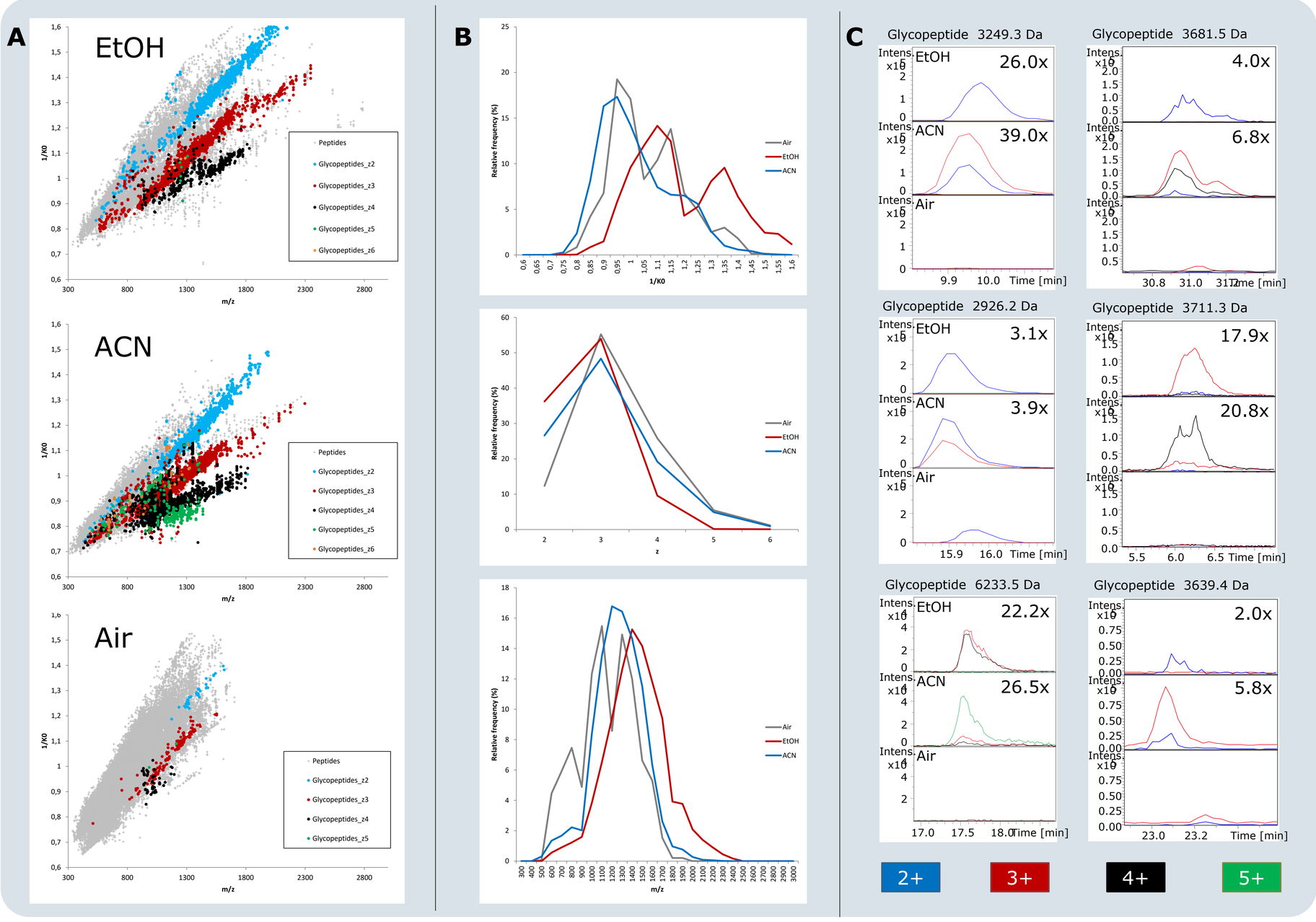

***Fig. 1: Effects of nanoBooster solvents compared to air for peptides and glycopeptides from human blood plasma. (A)**: m/z versus 1/K0 precursor maps for peptides and glycopeptides with charge z=2⁺ up to z=6⁺ using air or ethanol and acetonitrile dopants. **(B)**: Relative distributions of m/z, z, and 1/K0 values for all precursor ions for each nanoBooster condition. **(C)**: Representative extracted ion currents (EIC) for each charge state of six randomly selected glycopeptide precursor ions. The fold change increase in signal intensity relative to air was calculated using the dominant charge state of each condition. Colors of the EIC traces correspond with charge states : blue: z=2⁺, red: z=3⁺, black: z=4⁺, green: z=5⁺*

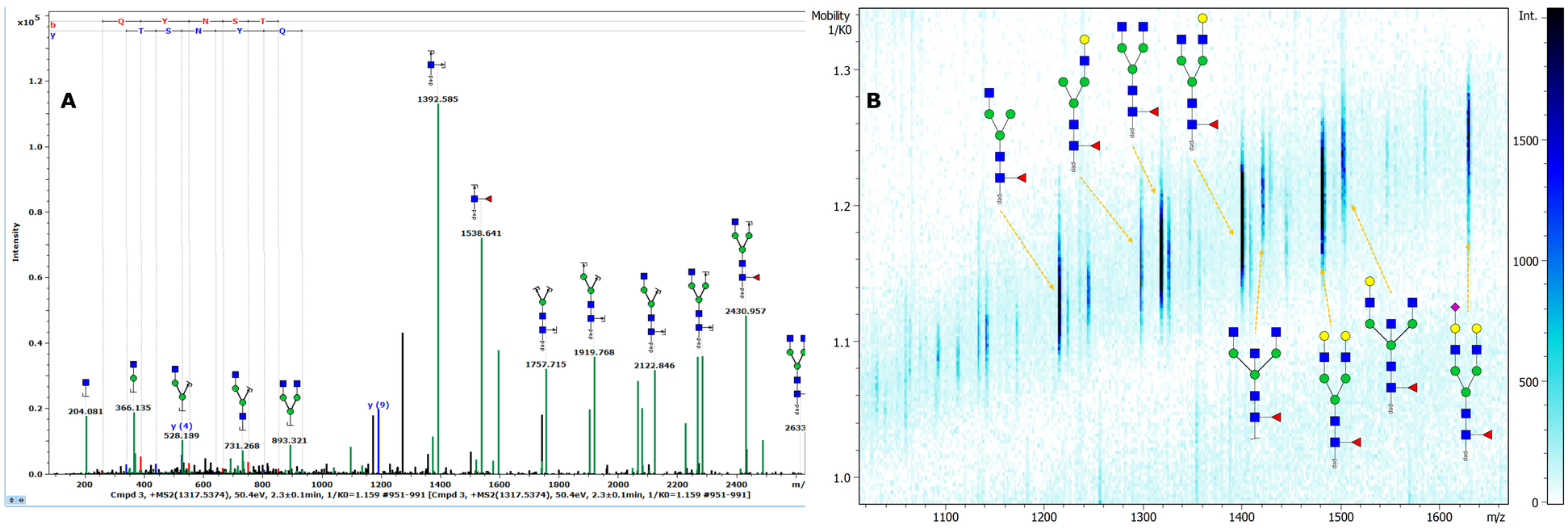

***Fig. 2: IgG glycopeptide identifications example.(A)**: Combined GlycoQuest and MASCOT search result of G0F with glycan and peptide moiety fragmentation. **(B)**: Selection of the most abundant identified IgG1 glycopeptides is highlighted in the m/z versus 1/K0 precursor map.*

Conclusions

- nanoBooster dopants are compatible with TIMS operation
- Acetonitrile and primary alcohols enhance glycopeptide ionization efficiency
- Acetonitrile dopant supercharges glycopeptide precursor ions and increases the number of charge states per precursor ion
- Primary alcohols subcharge glycopeptide precursor ions and reduce charge state heterogeneity
- PASEF enables comprehensive structural elucidation of glycopeptides

This work was supported, in part, by the NWO ZonMw medium investment programme under project number 9118025.

timsTOF PASEF