Glycopeptide Analysis with the Finnigan™ LTQ™ with vMALDI™ Source and Pulsed Q Collision Induced Dissociation (PQD)

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Overview

Purpose: To implement pulsed Q collision induced dissociation (PQD) technique for the unequivocal identification of glycosylated protein sites.

Methods: An intermediate pressure Matrix-Assisted Laser Desorption/Ionization (vMALDI) source was coupled with a Finnigan™ LTQ™ linear ion trap, and a new technique, pulsed Q collision induced dissociation was used for Data Dependent™ MS/MS activation to analyze an N-Glycan acceptor substrate standard mix.

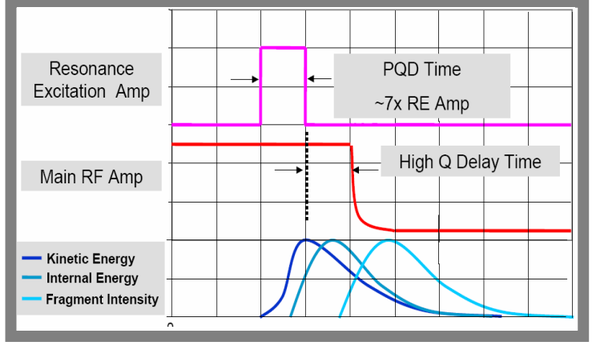
Results: PQD, a new ion activation technique for Finnigan linear ion traps, enabled detection of the *m/z* 204 and 366 product ions representing the oxonium ions HexNAc and Hex-HexNAc, respectively. Peptide fragments and diagnostic saccharides appeared in the fragmentation spectra only when the precursor ion was glycosylated.

Introduction

Post-translational modifications (PTMs), such as phosphorylation, glycosylation, and acetylation, increase the functional complexity of proteins inside cells. As a result, their chemical properties introduce inherent difficulties for qualitative analysis using mass spectrometry. For example, *N*- and *O*-linked glycosylation is usually heterogeneous, and without consistent mass shift or neutral loss following gas phase activation. Traditional mass spectrometric approaches to glycosylation analysis often employed the use of MALDI-ToF instruments because MALDI ionization generates predominantly singly-charged ions, the spectra are simple compared to ESI, and the sensitivity may be enhanced since ion current is not split among multiply-charged ions. MALDI is also used for the structural characterization of attached carbohydrates upon enzymatic release by PNGase F[1] or, in the case of O-glycosylation, through chemical cleavage that is more specific than existing O-glycanases[2].

In this experiment, we used a new ion activation technique to detect the presence of oxonium ions directly from the MS/MS of glycosylated precursors in MALDI. No LC-MS was required, no source decay was implemented, and no enzymatic deglycosylation was required with this approach. The new activation technique, PQD[3], dramatically lowers the low-mass detection limit for ion traps, allowing detection of diagnostic oxonium ions that indicate the presence of glycosylation. Using such a method, both unmodified peptides and *N*- and *O*-linked glycopeptides could in theory be identified and sequenced in one experimental analysis. Here, we demonstrate the technique using an N-linked glycopeptide standard.

FIGURE 1. Applied excitation amplitudes, delay times, and energy interconversion for the ions in the LTQ when using the PQD technique.



Methods

A vMALDI ionization source was coupled to a Finnigan LTQ for Data Dependent MS^n and subsequent high-throughput protein identification. We implemented the ion activation technique of PQD for MALDI ionization and optimized critical parameters to enhance the fragmentation at the low mass region.

A standard glycopeptide mixture, N-Glycan acceptor substrate (Calbiochem, San Diego, CA), was used to test and optimize the PQD technique in the LTQ with vMALDI source. This standard contains short peptides of amino acid sequences GENR or NK, with different glycosylated biantennary oligosaccharides attached through the asparagine (N) amino acid. Sample preparation consisted of spotting 0.5 μl on the MALDI plate, followed by the same volume of matrix after drying the analyte. 2,5-DHB (50 mg/ml) in 50/50 ACN/0.1% TFA was the matrix used. Analyte concentrations were in the 2 pmol/μl range. BSA digest (Waters, Milford, MA) 200 fmol/μl was mixed with 2 pmol/μl N-Glycan substrate standard to compare PQD fragmentation in glycosylated vs. non-glycosylated peptides in the same sample.

Ion Activation techniques in the LTQ ion trap

▪Normal collision-induced dissociation (CID) – in this case, the activation Q is kept constant during CID, fixing the low mass cutoff at 28% of the precursor *m/z*.

▪PQD, alternatively, lowers the low-mass cutoff in ion traps to 50 amu.

▪PQD of ions in the ion trap is described by three distinct steps (Figure 1):

➢1) Activation to a high Q value: This causes ions to oscillate at a very high frequency, achieving a high kinetic energy state, which is sustained for a certain time (PQD time in Fig. 1).

➢2) A delay time at high Q follows: Here, ions undergo energetic collisions with helium gas and kinetic energy is converted to internal energy of the ions. No significant fragmentation has occurred yet at this stage.

➢3) A pulse to low Q value and trapping of ion fragments: In this step, precursor ions with high internal energy now fragment. A low Q value allows trapping of low *m/z* fragments, from 50 amu and above.

FIGURE 2. Full MS of the N-Glycan acceptor substrate standard mix showing glycans and peptide structures

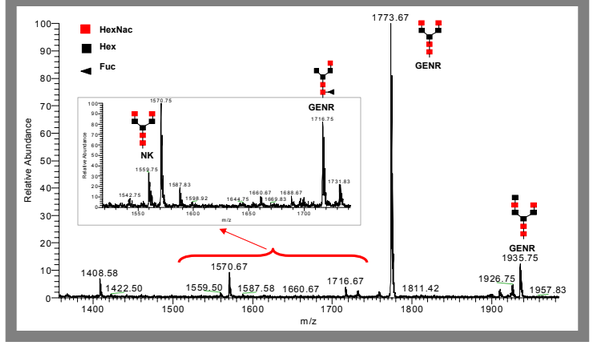
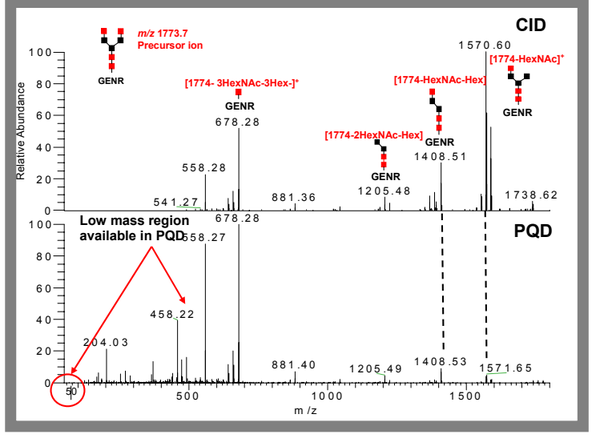


FIGURE 3. Fragmentation spectra with Collision Induced Dissociation (top panel) compared to Pulsed Q Dissociation (bottom panel). Notice fragmentation in the low mass region with PQD.



Results

Two parameters, High Q Delay Time, and Collision Energy were optimized for PQD in MALDI ionization and determined to be 0.3 ms and 45-47, respectively (data not shown), because the low mass region is optimally enhanced at these values.

Figure 2 shows the full MS spectrum of the N-Glycan standard mixture. Five glycosylated peptides were recorded as doubly-charged peaks in an electrospray ionization (ESI) Finnigan LTQ FT™ hybrid MS experiment (data not shown), while four singly-charged species were detected in the vMALDI spectrum. The vMALDI renders soft ionization comparable to ESI, which becomes important when analyzing potentially labile post-translational modifications in peptides.

Figure 3 shows a comparison between normal CID and the new PQD technique for the same glycopeptide mix. Notice the lack of fragments below *m/z* 540 in the CID spectrum (top panel). Sequential loss of oligosaccharides is shown in both fragmentation techniques. However, the diagnostic oxonium ions can only be found in the PQD spectrum, as seen in the expanded low mass region in Figure 4.

FIGURE 4. PQD of precursor at *m/z* 1774 showing the low mass region. Peptide sequencing information is displayed in addition to diagnostic oxonium ions.

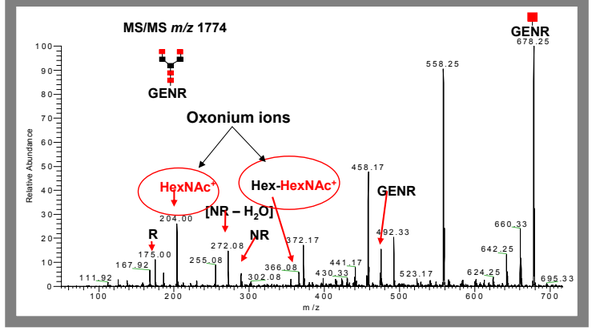
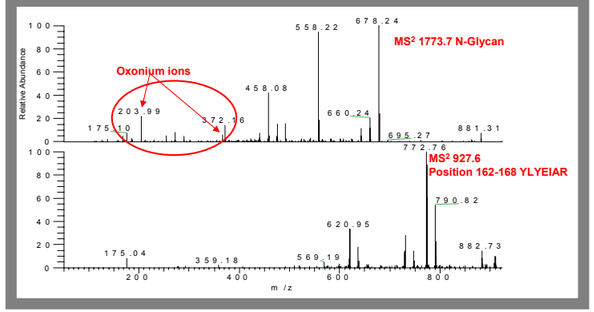


FIGURE 5. Data Dependent experiment using PQD ion activation. Sample is a mixture of BSA digest and the N-Glycan standard. Top trace shows fragmentation of a glycosylated-peptide. Bottom trace shows PQD of a non-glycosylated peptide.



PQD enabled detection of the *m/z* 204 and 366 product ions representing the oxonium ions HexNAc+ and Hex-HexNAc+, respectively, despite the precursor ions having intact mass-to-charge values of 1700-1900 Da (Figure 4). The oxonium ions are diagnostic of the presence of glycosylation in that particular precursor ion.

Figure 5 shows two spectra from a Data Dependent experiment consisting of one full MS scan followed by 22 MS/MS scans using PQD activation. The sample used was a 1:1 mix of BSA digest (non-glycosylated) and N-Glycan substrate standard. The fragmentation spectrum from a glycosylated peptide (*m/z* 1773.7, scan #2, top trace) contains both diagnostic oxonium and peptide ions corresponding to the glycopeptide (peptide ions: *m/z* 175, 272, 289, 301). The bottom trace, which corresponds to one of the BSA digest peptides, shows no oxonium ions at all. This demonstrates the utility of the PQD technique to quickly scan a series of MS/MS spectra and determine whether the peptide is glycosylated.

Conclusions

- PQD was successfully implemented in an ion trap with intermediate pressure MALDI ionization.
- Diagnostic low marker oxonium ions are reported for the first time from singly-charged ions in a MALDI ion trap.
- Unequivocal identification of a glycosylated peptide was accomplished by the appearance of both diagnostic oxonium ions alongside peptide sequence information.
- PQD allows rapid identification of glycosylated and non-glycosylated peptides in one Data Dependent automated experiment.
- Future work includes the application of this technique to protein digests and testing on O-glycosylated peptides.

References

1. H.J. An, T.R. Peavy, J.L. Hedrick, and C.B. Lebrilla (2003). Determination of N-glycosylation sites and site heterogeneity in glycoproteins. *Anal. Chem.* 75, 5628-5637.
2. Y. Huang, T. Konse, Y. Mechref, and M.V. Novotny (2002). MALDI MS compatible b-elimination of O-linked oligosaccharides. *Rapid Commun. Mass Spectrom.* 16, 1199-1204.
3. J.C. Schwartz, J.E.P. Syka, and S.T.Quarmby (2005). Improving the fundamentals of MS^n on 2D linear ion traps: new ion activation and isolation techniques. Proceedings Amer. Soc. MS, San Antonio, Texas.

Acknowledgements

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