SEPARATION AND DETECTION OF PROTEIN POST-TRANSLATIONAL MODIFICATIONS BY LIQUID CHROMATOGRAPHY COUPLED WITH A NOVEL ION MOBILITY MASS SPECTROMETER

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OVERVIEW

A new class of mass spectrometer, Synapt™ High Definition Mass Spectrometry (HDMS™) system (Waters Corp.) has been developed which combines high efficiency Ion Mobility Separations (IMS) and quadrupole-time of flight mass spectrometry. This novel system helps to separate proteins based on their size, shape, and charge in the gas phase by size, shape, and charge, prior to mass spectrometric detection.

This poster presents the design and theory behind the Synapt HDMS system, and shows the different modes of analysis available for the analysis of post-translational modifications.

The instrument described here is equipped with both MALDI and atmospheric pressure ionization sources. Separation of differentially phosphorylated peptides, based upon ion mobility, has been demonstrated using the Synapt Trap and Transfer T-Wave devices providing additional flexibility.

RESULTS

The 2 phosphopeptides ions at m/z 706.3 corresponding to the di-phosphopeptide EQLS and the ion at m/z 766.3 corresponding to the mono-phosphopeptide species coelute at 11.5 minutes. However they have different drift time profiles when an orthogonal separation technique such as ion mobility is employed as shown in Figure 3 below.

INTRODUCTION

Post-translational modification (PTM) of proteins plays a fundamental role in cellular processes and their determination is one of the main goals of modern proteomics research. Among more than 200 known PTM’s, modifications such as glycosylation and acetylation are the best characterized. However, the variety, diversity and heterogeneity of these modifications requires novel analytical tools for qualitative and quantitative assessment of their structural and functional roles.

We have investigated the potential of a novel travelling wave ion mobility spectrometer for the separation, detection and mass determination of post-translational modified proteins.

METHODS

HDMS analysis mode

The Waters Synapt HDMS system schematic representation is shown in Figure 1. The V2 instrument combines time-of-flight (T-Wave) ion guide, an ion mobility drift tube, a quadrupole, and an orthogonal acceleration time of flight (oa-ToF) mass spectrometer. The quadrupole can be set to transmit a particular m/z or pass a selected mass range, then ions enter a novel three-stage Travay design [1]. The Travay device consists of three membranes: trapping, transfer (T-Wave) and oa-ToF (oa-ToF) mass spectrometer. The T-Wave is used to transport the separated ions into the oa-ToF for subsequent analysis. The oa-ToF mass spectrometer is used to transport the separated ions into the oa-ToF for subsequent analysis. The pressure in the oa-ToF mass spectrometer was 0.5 mbar of N2. The T-Wave pulse velocity and voltage were optimized to provide adequate ion mobility separation.

Segmented spray analysis

Three different types of ion mobility fragmentation experiments can be performed using the T-Wave device. These are illustrated in Figure 2.

Fragmentation modes

Three different types of ion mobility fragmentation experiments can be performed using the T-Wave device. These are illustrated in Figure 2.

MALDI MS experiments were performed on reconstituted glycan standards from HFD. The released glycans were initially separated by MALDI MS. Further analyses was performed on the individual glycans using TAP fragmentation. The glycans were released from the glycinan by treatment with P2-FA, the released glycans were then derivatized as a TAP, alkylated with Iodoacetate (Waters, Milford, MA), washed and finally eluted. Glycans were analyzed at an approximate level of ~10 nM target. The results were validated using a complementary orthogonal separation technique as shown in Figure 5 below.

CONCLUSION

The use of ion mobility combined with on-tof mass spectrometry for the analysis of phosphorylated ions has been presented. Separation of reconstituted and phosphorylated peptides using MALDI has shown dramatically improved sensitivity using their lower ion isotope ratio as the differentiating factor. The use of IMS-MS-MS abolishes fragmentation patterns from both the intact phosphopeptide and peptide-neutral ion pairs in parallel.

The HDMS system is currently used to generate secondary fragment ion information not available using conventional MALDI-TOF MS/MS.

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References


Figure 1. Diagram of the Synapt HDMS system showing the different fragment ions that can be generated.

Figure 2. Different T-Wave fragmentation experiments using the Travay device.

Figure 3. 3D view of an ion mobility spectrum showing the effect of drift time on the separation of multiply charged ions.

Figure 4. Mass spectra acquired at a retention time of 11.5 minutes, but at different IMS drift times of 55-60 and 67-73 scans respectively. Separate mass spectra showing the di-phosphorylated peptide and mono-phosphorylated peptide can easily be obtained.

Figure 5. Different T-Wave fragmentation experiments using the Travay device. The triglycerol matrix was selected as previously described [2]. For Electrospray, samples were solubilised and then 25mM EDTA, 50mM Amm Phosphate added prior to separation using Waters nanoACQUITY UPLC in HFD mode.

Figure 6. (A) A fragment ion obtained from MALDI mass spectrometry, (B) a fragment ion obtained from ESI mass spectrometry.

Figure 7. Comparison of MALDI and ESI fragment ion information from the same sample.

Figure 8. The 2 phosphopeptides ions at m/z 706.3 corresponding to the di-phosphopeptide EQLS and the ion at m/z 766.3 corresponding to the mono-phosphopeptide species coelute at 11.5 minutes. However they have different drift time profiles when an orthogonal separation technique such as ion mobility is employed as shown in Figure 3 below.

Figure 9. TAP fragmentation was performed on each of these glycans simultaneously. The results from the ion with m/z 1485.5 are shown here Figure 9. TAP fragmentation produces more analytical information than fragmentation conducted in either the Trap T-Wave or Transfer T-Wave regions as previously described [2]. For Electrospray, samples were solubilised and then 25mM EDTA, 50mM Amm Phosphate added prior to separation using Waters nanoACQUITY UPLC in HFD mode.

Figure 10. (A) A fragment ion obtained from MALDI mass spectrometry, (B) a fragment ion obtained from ESI mass spectrometry.

Figure 11. Selected second generation MS spectra showing different fragmentation patterns. Fragment ions align to a particular point in time and form a sequential mass range, then ions enter in a novel three stage Travay device [1]. The Travay device consists of three membranes: trapping, transfer (T-Wave) and oa-ToF (oa-ToF) mass spectrometer. The T-Wave is used to transport the separated ions into the oa-ToF for subsequent analysis. The oa-ToF mass spectrometer was used to transport the separated ions into the oa-ToF for subsequent analysis. The pressure in the oa-ToF mass spectrometer was 0.5 mbar of N2. The T-Wave pulse velocity and voltage were optimized to provide adequate ion mobility separation.

Figure 12. Four spectra showing different fragmentation patterns.