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SYNAPT HDMS: COMPARING THE GAS- AND SOLUTION-PHASE STRUCTURE OF A PHOSPHOPEPTIDE USING ION MOBILITY AND NMR

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In this technical note, we demonstrate how two different analytical structural determination techniques can be used to elucidate the structure of a phosphopeptide.

We have derived a SYNAPT™ HDMS™ gas-phase collision cross-section (Ω) of a phosphopeptide. We also demonstrate how the Nuclear Magnetic Resonance (NMR) solution-phase structure of a phosphopeptide can be used to determine the theoretical Ω value, through a new and improved algorithm developed by Waters based on the Projection Approximation method.[1,2]

INTRODUCTION

The mechanism of peptide and protein folding can have profound effects on its biological activity; it is imperative to understand the three-dimensional (3D) structure of a biological molecule. For example, the mis-folding of an amyloid protein results in Alzheimer's disease. Phosphorylation is an important regulatory event involved in cell-signalling pathways. It has been demonstrated that phosphorylation can also change the shape of protein kinases involved in cellular signalling. Therefore, it is beneficial to use more than one analytical technique to determine, predict, and infer structure or structural changes that are the result of phosphorylation.

EXPERIMENTAL

Travelling Wave (T-Wave™) ion mobility calibration was performed using singly-charged peptides whose Ω value has previously been determined utilizing a drift tube instrument.[3] The gas SF_6 was used in the Trap and Transfer T-Wave regions at a pressure of 2.0 e^{-2}mbar. Helium was used in the ion mobility T-Wave region at a pressure of 3.0 mbar, and travelling-wave of 250 m/sec with a pulse height of 5 V. The synthetic phosphopeptide, HLADL(pS)K, was infused as an aqueous solution into the SYNAPT HDMS System at a concentration of 1 µM.

For NMR spectroscopy, the sample was diluted in 90:10 H_2O/D_2O for series of 2D NOESY (Nuclear Overhauser Effect), TOCSY (Total Correlation) and COSY (Homonuclear Correlation) NMR spectra. Spectra were acquired at 298 K using a 700 MHz spectrometer with TCI CryoProbe equipped with z-gradient coil. Structure refinement was carried out manually using XPLOR-NIH v. 2.14.[4]

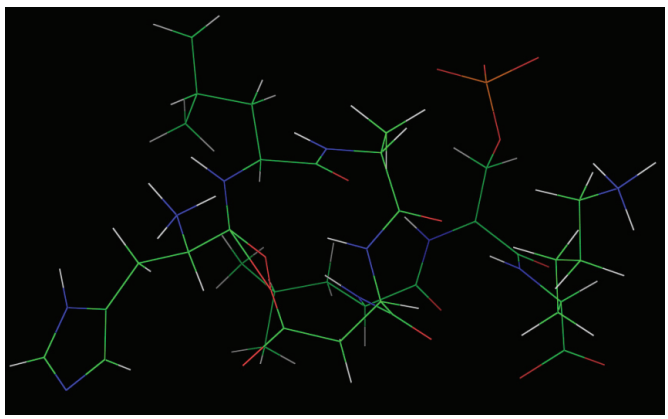

Figure 1. PYMOL displayed solution-phase NMR structure of the phosphopeptide HLADL(pS)K.

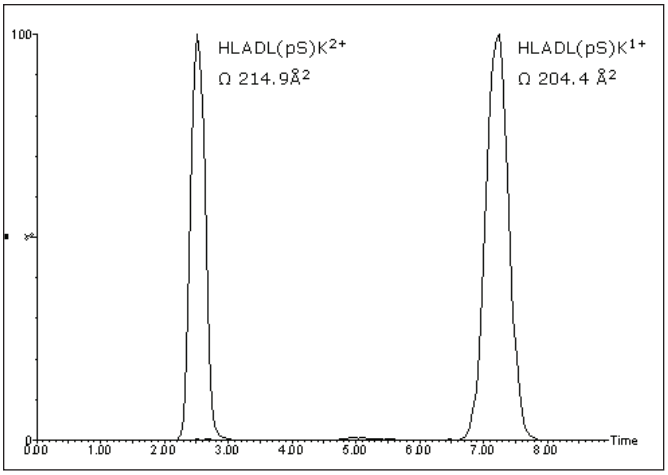

Figure 2. SYNAPT HDMS T-Wave derived drift-times (msec) and annotated Ω values for the singly and doubly charged ions of the phosphopeptide HLADL(pS)K.

The solution-phase structure of the phosphopeptide was determined by NMR, and is displayed in Figure 1 using the molecular modelling viewing program PYMOL[5]. The gas phase Ω values can also be determined for all observable charge states of the phosphopeptide using the SYNAPT HDMS System. The calculated Ω values for the $[M+2H]^{+2}$ and $[M+H]^{+1}$ were 214.9 $Å^2$ and 204.4 $Å^2$ respectively (Figure 2).

We also compared the SYNAPT HDMS T-Wave-derived Ω values to the theoretically calculated Ω values (based on the Projection Approximation method) using the open source code MOBCAL[1,2] and the newly developed Waters collision cross-section algorithm.

Briefly, MOBCAL and the Waters algorithm allows one to input a 3D coordinate file (PDB File), in this case generated from the NMR analysis of the phosphopeptide structure, and generate a theoretical Ω value. The theoretical Ω value can be compared to the T-Wave-derived Ω value.

HLADL(pS)K $[M+H]^{+1}$	HLADL(pS)K $[M+2H]^{+2}$	Waters Ω algorithm	MOBCAL PA
204.4$Å^2$	214.9 $Å^2$	204.8 $Å^2$	205.6 $Å^2$

Table 1. A comparison of T-Wave-derived Ω values of the $[M+H]^{+1}$ and $[M+2H]^{+2}$ phosphopeptide charge states with the Waters and MOBCAL theoretical Ω values.

The singly charged T-Wave-derived gas-phase structure closely matches that of the theoretical Ω value obtained by processing of the solution-phase NMR structure, as shown in Table 1. This observation is consistent with previous studies that have shown the lowest charge state observed with an electrospray spectra is most representative of the native solution-phase structure.[6]

CONCLUSION

The solution-phase structure for the phosphopeptide HLADL(pS)K was determined by NMR and was demonstrated to be consistent to the gas-phase Ω value of the singly-charged phosphopeptide as determined using the SYNAPT HDMS System.

Within the SYNAPT HDMS System the native, biologically active shape of the molecule is retained, which allows comparison to the solution-phase structure. This also demonstrates that the gas-phase structure is consistent with that of the solution-phase structure. More importantly, the solution-native structure can be retained within the vacuum of the mass spectrometer.

The calculation of a Ω value, whether T-Wave or theoretically derived, is a further, orthogonal physiochemical property that could be used for structural identification or confirmation.

Utilizing different but complimentary analytical techniques, such as Nuclear Magnetic Resonance and Ion Mobility coupled to mass spectrometry, represents a powerful addition to biomolecule structural elucidation.

References

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July 2009 720003172EN AG-PDF

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