SCIENTIFIC ANALYSIS INSTRUMENTS

Application Note

LaserToF LT3 Plus

Sequencing Phosphorylated Peptides

Introduction

The reversible phosphorylation of hydroxyl amino acids is an important method of switching the activity and behaviour of proteins which are key to cellular processes. For example, growth factors such as insulin trigger the phosphorylation of tyrosine.

Certain tumour viruses produce cancer by promoting the excessive phosphorylation of tyrosine residues that promote cell growth. The ability to detect phosphorylation sites is hence an important one. This note describes a method of detecting the site of phosphorylation in a synthetic peptide.

Materials and Methods

The lyophilised sample was reconstituted and diluted to approximately 10 µM in 0.1% TFA, without the need for further purification. A 10 µl aliquot was mixed 1:1 with the matrix liquor (5 mg/ml re-crystallised CHCA in 50% ACN, 0.1% TFA). Samples were spotted onto a slide and analysed on the LaserToF in positive ion reflectron mode.

A seamless PSD analysis (pPSD) was performed utilising the unique harmonic reflectron of the LaserToF LT3 Plus, which obviates the tiresome need to stitch spectra. The analysis of modified peptides is greatly simplified using SAI's PSD analysis tool. Specific amino acids can be custom-modified, in this case serine was phosphorylated, resulting in the correct interpretation of the modified PSD fragments.

Results

Figure 1 shows the seamless PSD mass spectrum of the phosphorylated LEYDpSTGFKA to m/z 1000, showing the neutral losses of phosphate and water from the peptide.

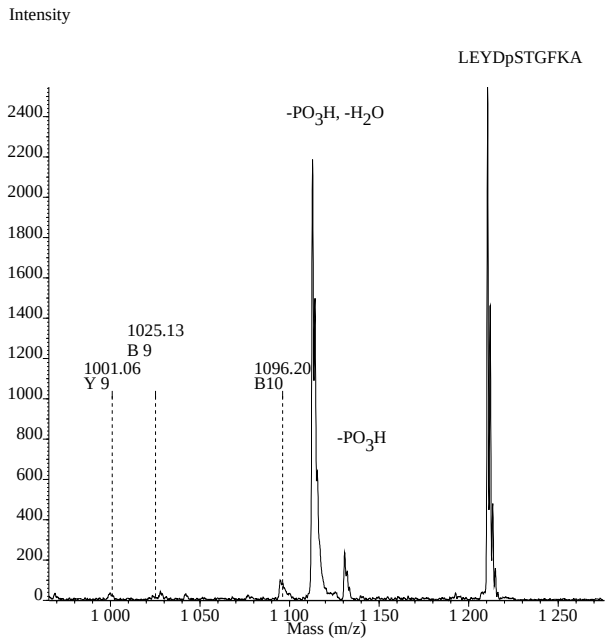

Figure 1: sPSD of LEYDpSTGFKA to m/z 1000

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Figure 2 shows the continuation of the fragment ion series.

Conclusions

1) PSD of phosphorylated peptides gives sequence information and correctly identifies the site of phosphorylation.

2) The harmonic mirror obviates tiresome stitching.

3) The mass accuracy of the system calibration was proven to be adequate for the confirmatory analysis of the synthetic peptide used. Improved mass accuracy would result from an external calibration.

4) The propriety proteomics software allows for the modification of residues so enabling a quick scan of the fragmentation pattern for derivatized peptides.

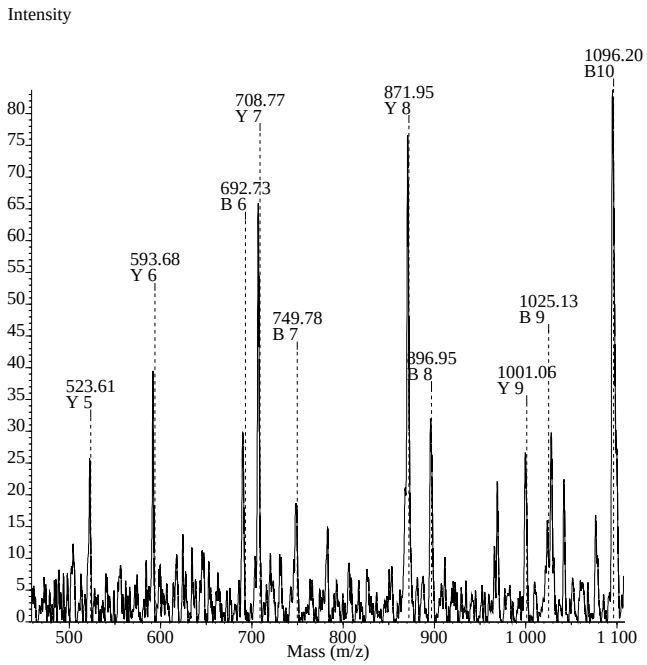

Figure 2: The continuation of the fragmention series.

Acknowledgement

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