AXIMA-QIT™

Sequencing of Tryptic Peptides using a Novel CD Based Chemical Derivatization and a MALDI QIT TOF MS

- Versatility of target design for innovative sample application
- Decoupled ionization, fragmentation and mass analysis providing superior resolution and mass accuracy in all modes of operation
- Simplified MS/MS spectra for undemanding sequence interpretation of peptides

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Sequencing of Tryptic Peptides using a Novel CD Based Chemical Derivatization and a MALDI QIT TOF MS

Introduction

De novo sequencing may be required to unravel the complexities of protein sequence. This technique can be difficult and time-consuming, particularly if the operator has little experience of spectral interpretation.

In order to simplify MS sequencing experiments, a chemical derivatization[1] may be performed on the tryptic digest e.g. sulphonation of the N-terminus of the peptide resulting in a mass increase of 136 Da. This modification prompts the formation of predominantly y-ions in an MS/MS experiment providing a greatly simplified spectrum. The sequence may then be effectively "read" from the resultant fragment ions.

A novel CD based system has recently been introduced that is capable of performing the chemical derivatization steps and elution of the modified peptides onto an incorporated area specifically designed for MALDI acquisition. This may be carried out on multiple samples simultaneously, increasing the throughput of the technique. Advantages of this methodology include high reproducibility, increased sensitivity and reduced contamination.

We have applied this new technology to a novel MALDI based mass spectrometer[2], the AXIMA-QIT™ (Shimadzu Biotech) incorporating a MALDI ionization source, a quadrupole ion trap and a time-of-flight analyzer. This instrument is capable of MS and MS^n acquisition on low femtomole amounts of tryptic digests[3]. Identification of proteins was achieved using important MS/MS and MS^n data in conjunction with database searching. In addition, it has been demonstrated that the superior resolution and mass accuracy of the MALDI QIT TOF instrument provides a simple yet extremely effective and reliable method of *de novo* sequencing for CAF (Chemically Assisted Fragmentation) modified peptides of ambiguous sequence.

Methods

A 2D gel (rat endocrine pancreatic tissue) was stained firstly with Sypro® Ruby (Molecular Probes, Inc.), followed by a Coomassie blue stain. Protein spots were excised and digested with trypsin[3,4]. The samples were then lyophilized and reconstituted in 0.1% TFA. For underivatized samples, a 0.5 µl aliquot was spotted onto a stainless steel MALDI target and 0.5 µl of dihydroxybenzoic acid (DHB) added (12 mg/ml in 50:50 0.1% TFA/ACN). Spots were allowed to air dry. No further sample processing was undertaken.

For the CD based derivatized samples, a Gyrolab™ Workstation (Gyros AB) and a Gyrolab™ MALDI SP1 CD were used. The CD was conditioned with a solution of 50:50 0.1% TFA/ACN v/v, and the protein digests applied to individual channels on the CD containing C_{18} packing. The bound peptides were treated with the CAF reagent (Amersham Biosciences) and desalted (10% ethanol, 0.1% TFA). Derivatized peptides were subsequently eluted from the C_{18} bed using a matrix solution (20 mg/ml DHB in 50:50 0.1% TFA/ACN v/v) directly into a small incorporated MALDI target area. External calibration of the AXIMA-QIT™ was achieved using fullerite deposited directly onto the sample stage. Database searching was carried out using the Mascot® search engine (Matrix Science).

Mass Spectrometer - AXIMA-QIT™

High resolution mass spectra were acquired on an AXIMA-QIT™ MALDI quadrupole ion trap time-of-flight instrument. The design of the mass spectrometer is schematically depicted in Figure 1. The instrument consists of three main sections: the ion introduction region, the ion trap and the reflectron time-of-flight mass analyzer. These three sections are differentially pumped by turbo molecular pumps. The target, a 384 well mictrotitre format stainless steel MALDI plate, is positioned in the ion source using an xy sample stage. Matrix assisted laser desorption of peptides is produced by pulses of light (337 nm, 3 ns pulse width) generated by a nitrogen laser with a maximum pulse rate of 10 Hz. A small bias voltage is applied to the sample plate (2.5 to 30 V) depending on mass of the analyte under investigation allowing samples of an unconventional nature to be analyzed, for example, CDs or microscope slides.

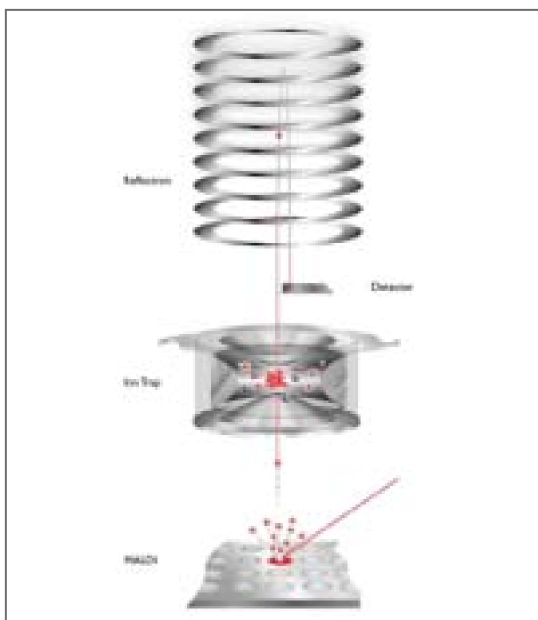


Figure 1. MALDI QIT TOF schematic

Following ionization, ions are immediately extracted from the ion source region and injected into the trap. During ion introduction, there is no RF potential applied to the ion trap. In order to trap ions, a retarding potential is applied to the end-cap and an RF with a frequency of 500 kHz is applied to the ring electrode (this is known as "rapid RF-startup"). The rapid RF-startup method provides trapping efficiency close to 100% . Once trapped, the ions are cooled using helium. The time-of-flight mass analyzer incorporating an ion reflectron is employed to acquire the data in both MS and MS/MS modes of operation.

Prior to MS/MS analysis, precursor ions can be isolated using the filtered noise field (FNF) method which ejects all unwanted ions from the trap. In order to promote fragmentation of the precursor ion, resonant excitation is induced. Following decomposition, the product ions are extracted into the TOF for mass analysis.

The mass spectrometer has three gas inlets - one for cooling gas and two for a collision gas which may be varied. In these experiments, helium was used as a cooling gas and argon as the collision gas both delivered to the ion trap via precisely controlled fast pulsed valves.

Results

Ten underivatized in-gel tryptic digests were analyzed in positive ion mode on an AXIMA-QIT™ and a peptide mass fingerprint generated for each. The results were database searched using Mascot and putative identifications made. These findings are collated in Table 1. Typical spectra are presented in Figures 2-4. These samples were crude tryptic digests and have undergone no extra clean-up procedures.

The CD based chemically modified samples were also analyzed on the AXIMA-QIT™ directly from the CD using a specifically designed target adaptor. The underivatized samples were also processed on the CD providing straightforward PMF capability. Underivatized sample 2 was found to contain peptide ion signals at m/z 1271 and m/z 1615 that when derivatized exhibit masses of m/z 1407 and m/z 1752 respectively, corresponding to the expected increase of 136 Da. These modified peptides were subjected to MS/MS and the results are displayed in Figures 5a and 5b. These fragmentation spectra display a dominant y-ion fragmentation series allowing either Mascot searches of the data or simplified *de novo* sequencing.

Sample No	Accession	Protein Mass (Da)	Mascot Score	Identification
1	DHE3 RAT	61731	116	Glutamate dehydrogenase, mitochondrial PR
2	AMYP RAT	57207	106	Alpha-amylase, pancreatic precursor
3	LIPP RAT	52149	180	Triacylglycerol lipase, pancreatic precursor
4	CTRB RAT	28458	72	Chymotrypsinogen B precursor
5	TRY3 RAT	26936	100	Trypsin III, cationic precursor
6	LIPI RAT	53086	218	Pancreatic lipase related protein I precursor
7	GATM RAT	48724	225	Glycine amidinotransferase, mitochondrial
8	PDI RAT	57315	137	Protein disulphide isomerase precursor
9	GAMT RAT	26544	111	Guanidinoacetate N-methyltransferase
10	PDA3 RAT	57044	190	Protein disulphide isomerase A3 precursor

Table 1. Putative protein identification

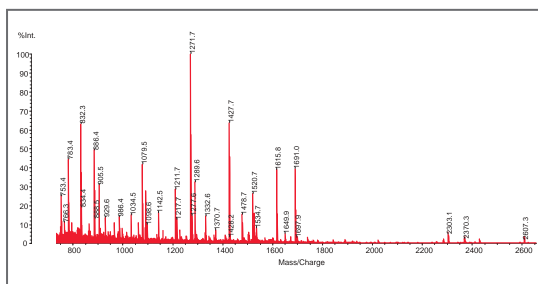


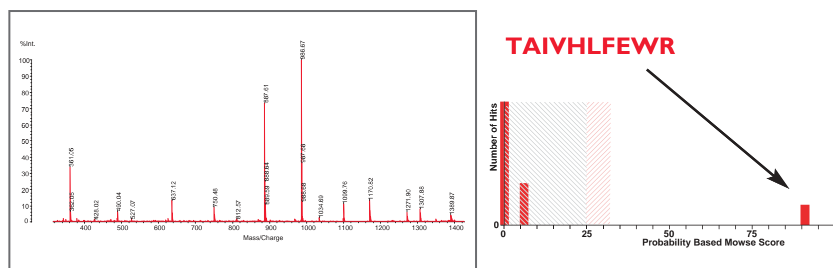
Figure 2. MALDI QIT TOF MS spectrum of unmodified sample 2

Figure 3. MALDI QIT TOF MS spectrum of unmodified sample 3

Figure 4. MALDI QIT TOF MS spectrum of unmodified sample 10

This CD-based chemistry is also useful for larger peptides, for example, in sample 6, a modified peptide of m/z 2005 is observed. MS/MS of this peptide again clearly demonstrates a y-series of ions from which the sequence may be determined, as shown in Figure 6. A zoom view of this spectrum reveals the baseline isotopic resolution of the MS/MS ions which is calculated to be 10,000 or greater. This high resolution and mass accuracy in all modes of operation can be vital when performing sequencing experiments of any kind enabling the differentiation of residues of similar nominal mass.

The MS/MS spectrum in Figure 6 provides a y-series of ions identifying a sequence tag of 13 amino acids - **HLIGHSLGAHVAG**. These data were searched and provided a highly confident MS/MS Mascot score of 137 relating to a peptide the full sequence of which is **VHLIGHSLGAHVAGEAGSR**. This series of amino acids was also subjected to a BLAST search to provide sequence homology (as could be carried out in the case of total unknowns). The results indicated pancreatic lipase related protein 1 derived from either *Mus musculus* or *Rattus norvegicus*, both with a score of 60 (E value of 5e-09).



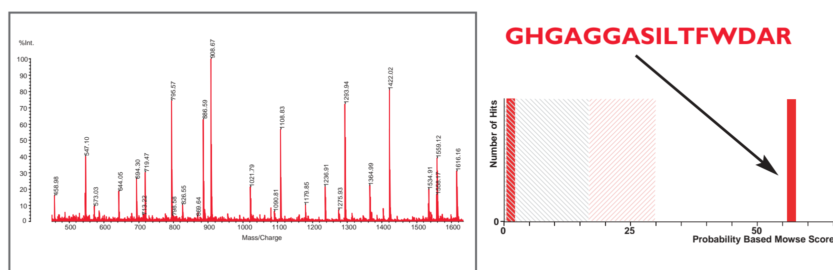


Figure 5. (a) & (b) MALDI QIT TOF MS/MS spectrum of modified sample 2 and y-ion identification

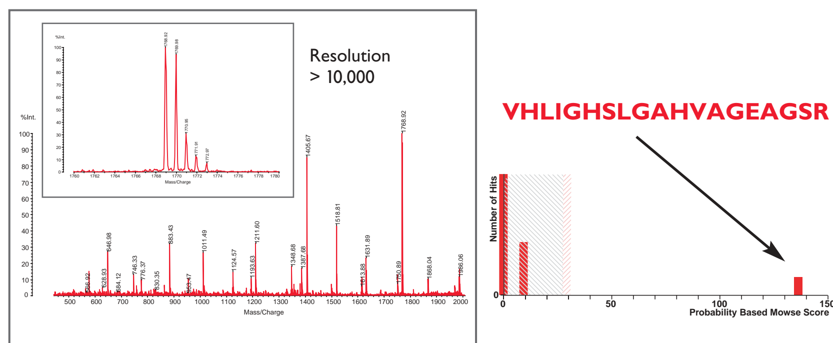


Figure 6. MALDI QIT TOF MS/MS spectrum of modified sample 6 and zoom view

Conclusions

- PMF experiments carried out on the AXIMA-QIT™ have demonstrated the ability to identify protein digests simply and rapidly
- often sequence information is required via MS/MS experiments. This can be daunting for the inexperienced mass spectroscopist as in many cases complicated spectra are produced requiring detailed interpretation
- the combination of the Gyrolab™ CD based chemical derivatization technology and the AXIMA-QIT™ has been shown to be of significant value when performing protein sequencing experiments
- economical use of reagents through microfluidic based processing
- analyzing peptides directly from the CD allows a seamless transition from sample processing directly through to MS acquisition and MS/MS experiments providing either protein identification or novel sequence information
- the simple y-series of ions produced when MS/MS is performed on the modified peptides allows undemanding interpretation of the spectrum
- this series of acquisitions can be automated - the MS spectrum being acquired initially and a series of MS/MS spectra subsequently generated

REFERENCES

1. *Amersham Biosciences CAF MALDI sequencing kit Part No 17-6002-97*
2. *R.L. Martin and F.L. Brancia, Rapid Commun. Mass Spectrom., 17, (2003), 1358-1365*
3. *C. Koy et al, Proteomics, 2003, volume 3*
4. *Shevchenko et al, Anal. Chem., 68, (1996), 850-858*

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