Identification and Relative Quantitation of Peptides in Complex Un-fractionated Serum Matrices Using MALDI LTQ Orbitrap XL

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Overview

Purpose: Demonstrate the potential of high dynamic range and resolution of the MALDI LTQ Orbitrap XL to identify and quantify native or spiked peptides in complex biological matrices.

Methods: Mouse serum digest spiked with a mixture of peptides: SAVQGPPER and DGFFGNPR or human serum digests from different sources were analyzed using a selected ion or reaction monitoring acquisition modes on the MALDI LTQ Orbitrap XL and quantified with a custom-designed quantitation tool.

Results: The MALDI linear ion trap-Orbitrap™ mass spectrometry, which provides high mass measurement accuracy and resolving power, allows a rapid and sensitive identification and relative quantification of peptides in serum. We were able to achieve relative quantification of IgG peptide SAVQGPPER spiked into mouse serum over the linear range from 1 to 1000 fmol using FT SIM scan mode and peptide DGFFGNPR as internal standard. Moreover, MALDI screening of 18 peptides of interest in un-fractionated human serum digests identified 16 peptides using combination of Orbitrap full MS spectra and specific IT SRM MS2 scans.

Introduction

Quantitative serum proteomics is rapidly evolving requiring higher sensitivity instrumentation along with increased dynamic range. Triple quadrupoles remain the instrument of choice due to multiple mass analyzers allowing for specificity and selectivity of SRM transitions[1]. However, peptides must be fractionated via liquid chromatography limiting high throughput analysis. MALDI mass spectrometry has mainly been used in qualitative proteomics research due to matrix and ion suppression effects. However, recently, MALDI has started to attract interest for peptide characterization and quantitation because of its potential for high-throughput analysis[2]. Multiple myeloma is a cancer of plasma cells, which secrete antibodies. The amount and type of the antibody released by the tumor cells into blood and urine are used for diagnosis, staging, and evaluation of response to therapy. Current clinical measurements use gel or capillary electrophoresis to quantify and identify the type of antibody. After development of an LC-MRM based assay, we were interested to increase sample throughput with a MALDI approach. In this work, we probe instrumentation limits of the MALDI LTQ Orbitrap XL to identify and quantify peptides in serum digests.

Methods

Reduced and alkylated, diluted x10 or x20 human serum digests from different sources or mouse serum digest spiked with a mixture of peptides: SAVQGPPER and DGFFGNPR was loaded on a MALDI plate (1 µL/spot) and analyzed using CHCA matrix(Fig.1) and a Thermo Scientific MALDI LTQ Orbitrap XL hybrid mass spectrometer. The mass spectrometer was operated in positive ion mode with FT full mass survey scan at 30-100K RP at *m/z* 400, or ion trap using selected ion monitoring (SIM) of the targeted peptides or selected reaction monitoring (SRM) acquisition. For each sample, 20 microscans were collected in every acquisition mode (Figure 2). For each peptide, four b- or y-ions were monitored. Each selected SRM transition was unique for the peptide of interest. Acquired raw files were processed in batch mode to calculate the ratio between the intensities of analyte peak and internal standard peak with a special quant tool[3]. Thermo Scientific Xtract software was used to obtain monoisotopic masses. Thermo Scientific Proteome Discoverer 1.0 software the MASCOT™ v 2.1 search engine (Matrices Sciences, London, UK) was used for peptide identification and SRM selection.

FIGURE 1. Sample spot image with CHCA matrix (3.5 mg/mL in 0.003% TFA, 13% EtOH, 84% AcN)

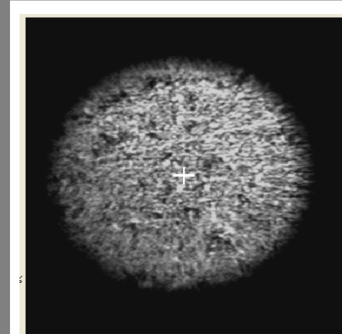
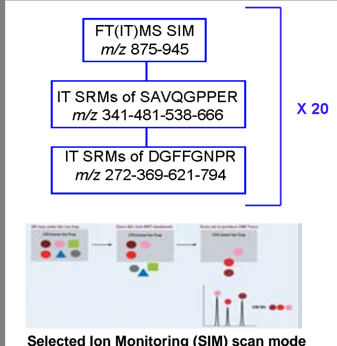


FIGURE 2. Instrument method set up for relative peptide quantification by MALDI LTQ Orbitrap XL



Selected Ion Monitoring (SIM) scan mode

Results

First we probed ability of MALDI LTQ Orbitrap XL™ to detect and quantify peptides spiked into mouse serum digest. Synthetic peptides were chosen for their biological relevance. Peptide 1 (SAVQGPPER) is from human IgG2 and peptide 2 (DGFFGNPR) is from human IgM. Figure 3 shows SIM scans of peptide mixture(100 fmol of each per spot) obtained either in the Orbitrap (A,B) or ion trap (C).

To design SRM experiments, both neat peptides were first analyzed by full MS/MS with Orbitrap detection to assure identity of the fragments and then unique b and y ions (4 per peptide) were selected (Figure 4). Peptide 1 was spiked into neat serum digest in concentration range from 100 amol/µL up to 1 pmol/µL. Using high resolving power and dynamic range of MALDI LTQ Orbitrap XL we were able to perform relative quantification of peptide 1 in the linear range down to 1 fmol/µL in FT SIM mode (Figure 6A, table) and confirm its identity with SRM approach (Figure 5). Due to very complex matrix and ion suppression we were not able to detect spiked peptides in IT SIM spectra even using zoom scan mode at any concentrations (Figure 6B). However, effect of matrix suppression was observed even in case of FT SIM scans as linear range for detection of the same peptides in solution was down to 10 amol (data not shown). The average mass accuracy was ~3 ppm at 30K nominal resolution and ~ 1 ppm at 100K resolution (Figure 3A & 3B), but for most experiments 30K resolution was used to reduce acquisition time/spot without compromising the results. Average time to analyze each sample was ~2.5 min using method set up as in Figure 2.

In the next series of experiments we tried to screen 18 peptides of interest in un-fractionated human serum digests from healthy individuals or patients with multiple myeloma using combination of Orbitrap full MS spectra and specific IT SRM MS2 scans. Peptides of interests were chosen based on LC-MS/MS data as possible biomarkers of human multiple myeloma[4]. Using MALDI Orbitrap screening we were able to detect 16 out of 18 peptides. Figure 7 shows examples that for identity conformation in such complex matrix both full MS and SRM data are required. Even ions in range of 3 ppm for *m/z* 1149.6150 in myeloma samples were observed but its identity was not fully confirmed by SRM (Figure 7B & 7D).

FIGURE 3 SIM MALDI MS spectra of standard peptides obtained with Orbitrap (A,B) and Ion trap (C, zoom scan mode).

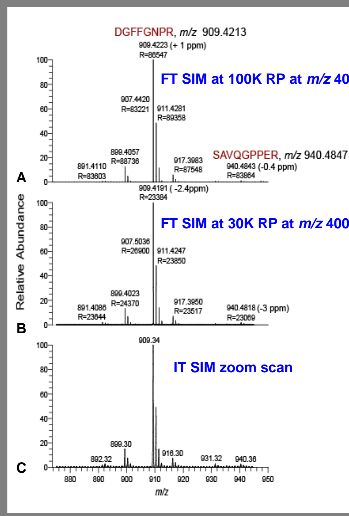


FIGURE 4. FT CID MS/MS spectra (7500 RP at *m/z* 400, Bottom panels) and selected IT SRM MS2 transitions (upper panels) of A) DGFFGNPR , and B) SAVQGPPER, 100 fmol each/spot.

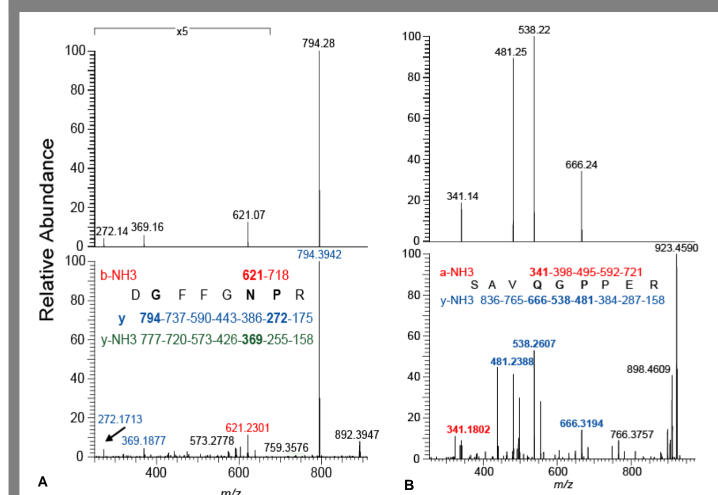


FIGURE 5. MALDI IT SRM MS2 transitions (20 scans/sample, Figure 4) acquired for SAVQGPPER (A) and DGFFGNPR (100 fmol/uL, internal standard, B) spiked into mouse serum digest

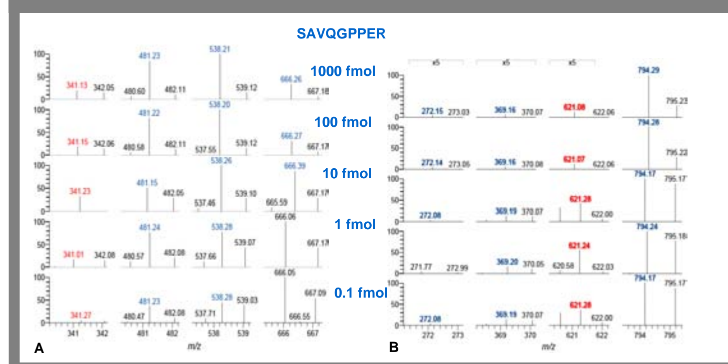
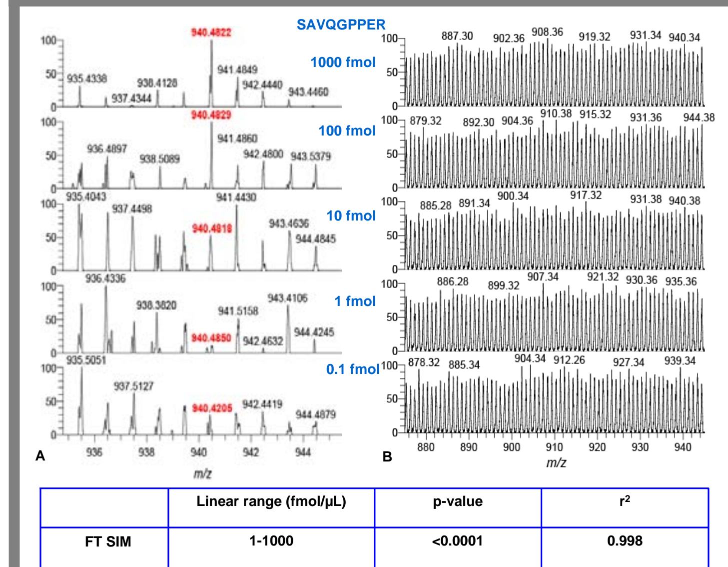
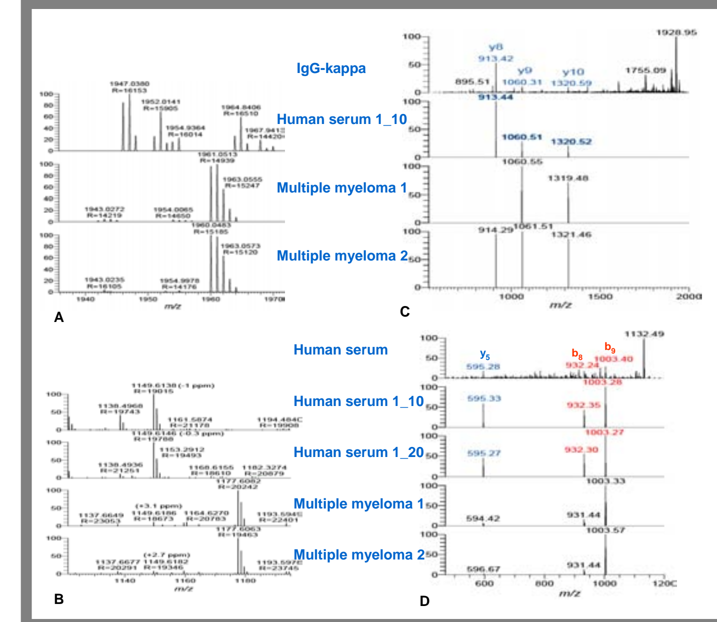


FIGURE 6. MALDI MS SIM scans (m/z 875-945) acquired for mouse serum digest spiked with a mixture of peptides: SAVQGPPER and DGFFGNPR (100 fmol/uL, internal standard) in the Orbitrap (30 K RP at *m/z* 400, A) or in Ion Trap (zoom scan, B). Table shows linear range of detection for SAVQGPPER on MALDI-Orbitrap using DGFFGNPR as internal standard.



	Linear range (fmol/µL)	p-value	r^2
FT SIM	1-1000	<0.0001	0.998

FIGURE 6. Monitoring of IgG-kappa TVAAPSVFIFPPSDEQLK (*m/z* 1946.0270 A,C) and human serum albumin LVNEVTFAK (*m/z* 1149.6150 B, D) in human serum or multiple myeloma samples using Orbitrap full MS spectra (A,B 30K RP at *m/z* 400) and its corresponding IT SRM MS2 transitions (C,D) selected based on IT full MS2 spectra (C,D top spectra).



Conclusions

- Data acquisition in FT SIM mode works best for relative peptide quantitation on the MALDI LTQ Orbitrap XL mass spectrometer
- A full 96-well target plate can be read in 4 hrs (20 FT SIM scans and 40 SRM scans/spot)
- Linear dynamic range from 1 to 1000 fmol/uL for peptide SAVQGPPER spiked into mouse serum digest is achievable on MALDI LTQ Orbitrap XL
- MALDI screening of 18 peptides of interest in un-fractionated human serum digest identified 16 peptides using combination of Orbitrap full MS spectra and specific IT SRM MS2 scans
- Total acquisition time required to screen 18 peptides (20 IT SRM scans/peptides) is 18 min in average.

References

1. Lange V, Picotti P, Domon B, Aebersold R. *Mol Syst Biol.* 2008;4:222. Epub 2008 Oct 14.
2. Schneider B, Hunter C, Champion M, Settineri, Covey TR. *Proceedings of the 55th ASMS conference* Indianapolis.
3. Viner R, Bui H, Miller K. *Proceedings of the 53th ASMS conference*, San Antonio.
4. Koomen JM, et al *Mol Cell Proteomics.* 2008 Oct;7(10):1780-94. Review.

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