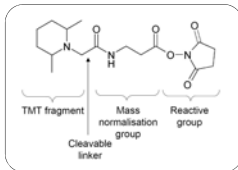


Tandem mass tags for selection and absolute quantification of brain damage markers in cerebrospinal fluids

Overview

1

Tandem Mass Tags (TMT[®], Scheme 1) can serve for relative or absolute quantitation. The technology was used previously for the discovery of protein markers of brain-damage related disorders¹. In order to verify these markers, mass spectrometric technologies are needed to circumvent the complex development of ELISA assays.



Scheme 1. Structure of the Tandem Mass Tag[®] (TMT[®]). The reporter ion provides the abundance of a peptide upon tandem mass spectrometry (MS/MS). The mass normalization group balances mass differences from individual ion reporter to ensure the same overall mass of the label reagents. The reactive group labels amino groups.

In this context, a strategy using TMT[®]-labeled reference peptides as calibrators (Figure 1) was developed.

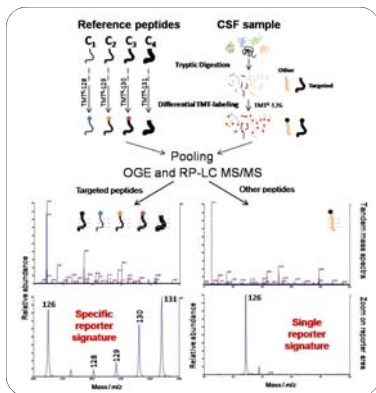


Figure 1. Description of the "TMT-reporter internal calibration curve" strategy for selective quantitation.

Introduction

2

High throughput absolute quantitation is essential for the verification and validation of potential biomarkers in biological fluids. In the past few years, mass spectrometry (MS) has emerged as a practical tool for the multiplexed measurement of these markers by isotope dilution. Despite the growing use of isobaric tagging to perform relative quantitation in biomarker discovery, no real application has been presented so far using these tags for the absolute protein measurement to assess them as biomarkers². In this study, 6-plex isobaric mass tagging technology (so-called Tandem Mass Tag[®] or TMT[®]) was used for monitoring and for absolute quantitation of an 8 potential biomarker panel in cerebrospinal fluids (CSF) by MS.

Methods

3

TMT[®] 6-plex reagents were used to label CSF (label releasing reporter at $m/z = 126$) and 4 identical mixtures of 41 synthetic proteotypic peptides (representative of 8 proteins), which were prepared at 4 defined concentrations (labels releasing reporters at $m/z = 128, 129, 130$ and 131). Labeled samples were mixed and the pooled sample was subjected to OFFGEL electrophoresis (OGE) as first dimension separation. The fractions were analyzed by LC-MS/MS on both ESI Orbitrap and MALDI TOF/TOF instruments. Quantitative data were extracted from the raw tandem mass spectra to build calibration curves from the reporter ions released by the reference synthetic peptides (Figure 2).

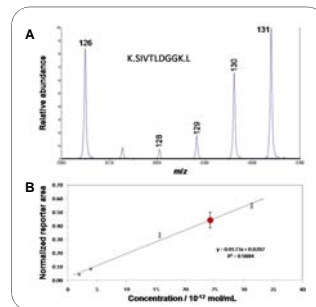


Figure 2. Zoomed MALDI tandem mass spectrum of the TMT[®] reporter region for matched SIVTLGGK peptide from fatty acid-binding protein, heart (FAPBH) (A). TMT[®] reporter internal calibration curve obtained for measurement of SIVTLGGK in CSF (B).

Results

4

After collision-induced dissociation, each TMT[®] label releases a specific reporter ion. The ions at $m/z = 128, 129, 130$ and 131 reflect the amount of the 41 synthetic peptides and the ion at $m/z = 126$ the peptides from the CSF. When reporting TMT[®] reporter peaks along chromatograms (Figure 3), it clearly appears that simplified patterns are consequently observed for the reporters at $m/z = 128, 129, 130$ or 131 with respect to reporter at $m/z = 126$ which reveals the complexity of the CSF sample. The reporter ions generated by the TMT[®]-labeled reference synthetic peptides can therefore be used as a constrained tool to selectively identify and quantify these peptides in CSF. Only the targeted peptides show specific fragmentation pattern in the reporter zone of the tandem mass spectra. When calculating ratio of the reporter 126 over the sum of reporter 128 to 131, it was possible to discriminate targeted peptides from other peptides with 95% specificity and 93% sensitivity at a ratio cut-off of 3.065. The area under the ROC curve was 0.9692.

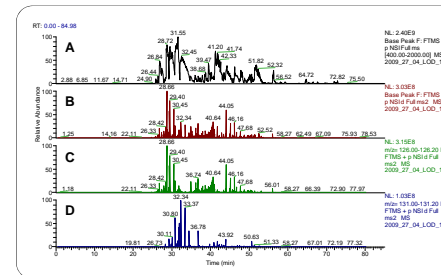


Figure 3. Reversed-phase LC ESI Orbitrap MS analysis (the sample was first fractionated by OGE; the displayed analysis corresponds to fraction number 3). MS base peak chromatogram (A). MS/MS base peak chromatogram at $m/z = 126.1$ (C) and 131.1 (D), respectively corresponding to the CSF sample and the spiked peptides at the highest concentration.

In addition, the peak areas of TMT[®] reporter ions at $m/z = 128, 129, 130$ or 131 were used to build a calibration curve for the 8 proteins represented by the 41 spiked synthetic proteotypic peptides in 4 known amounts (Figure 2). The quantitation requires that sufficient pre-fractionation and chromatographic separation of the peptides are achieved to prevent co-fragmentation of peptides with similar precursor-ion masses. OGE fractionation showed very good reproducibility behaviors (data not shown) and was used as an additional filter to rule out falsely identified peptides.

Conclusions

4

Data obtained by MS/MS provided at this stage semi-absolute quantitative results for the 8 probed proteins in CSF (Figure 4). Prostaglandin-H2 D-isomerase (PTGDS) which is one of the most abundant protein in CSF³ was found effectively as the most concentrated protein in the analyzed sample.

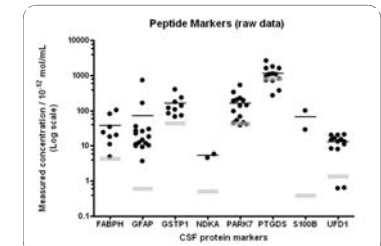


Figure 4. Protein concentration determined by isobaric tagging and MS/MS. These proteins pertain to a potential panel of markers related to brain damages. Grey bars indicate reference concentrations from ELISA^{1,4,5}.

Conclusions

5

This study is a preliminary step for the development of assays for the verification of potential markers by MS. Reference TMT[®]-labeled synthetic peptides were used to fish out specific peptides in complex samples and obtained semi-absolute quantitative data by MS.

Further works include the selection of the peptides which present the best behaviors for absolute quantitative purposes (background evaluation, specificity, sensitivity...), and the measurements of these peptides in several samples to fully validate the quantitative assay.

Acknowledgments and references

The authors thank Proteome Sciences plc for financial support and providing the TMT[®] reagents.

^[1] Anal Chem. 2008, 80, 2921-2931.

^[2] Anal. Biochem. 2008, 379, 26-31.

^[3] Electrophoresis 2005, 26, 4563-4570.

^[4] J. Proteome Res. 2006, 5, 1674-1681.

^[5] unpublished data obtained for *post-mortem* CSF.