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Quantitative analysis of low-abundance serological proteins with peptide affinity-based enrichment and pseudo-multiple reaction monitoring by hybrid quadrupole time-of-flight mass spectrometry

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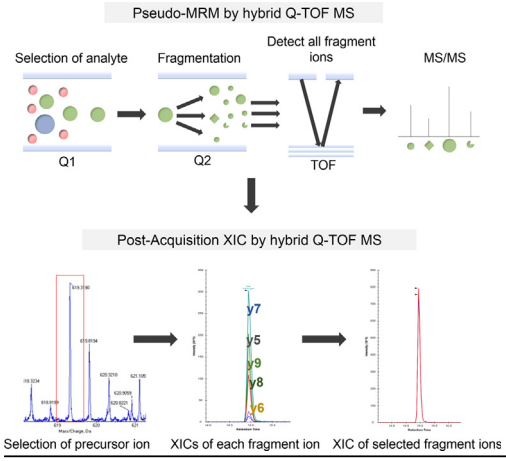
HIGHLIGHTS

- Development of a workflow for the quantitation of low-abundance serological proteins.
- Peptide affinity-based enrichment is effective for the analysis of low-abundance proteins.
- Optimization of hybrid Q-TOF MS for pseudo-MRM analysis.
- Pseudo-MRM provides an improved signal-to-noise ratio and reproducibility of measurement.

GRAPHICAL ABSTRACT



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ABSTRACT

Multiple reaction monitoring (MRM) is commonly used for the quantitative analysis of proteins during mass pectrometry (MS), and has excellent specificity and sensitivity for an analyte in a complex sample. In this study, a pseudo-MRM method for the quantitative analysis of low-abundance serological proteins was developed using hybrid quadrupole time-of-flight (hybrid Q-TOF) MS and peptide affinity-based enrichment. First, a pseudo-MRM-based analysis using hybrid Q-TOF MS was performed for synthetic peptides selected as targets and spiked into tryptic digests of human serum. By integrating multiple transition signals corresponding to fragment ions in the full scan MS/MS spectrum of a precursor ion of the target peptide, a pseudo-MRM MS analysis of the target peptide showed an increased signal-to-noise (S/N) ratio and sensitivity, as well as an improved reproducibility. The pseudo-MRM method was then

Abbreviations: MRM, multiple reaction monitoring; TIMP1, tissue inhibitor of metalloproteinases 1; PTPκ, tyrosine phosphatase κ; QqQ, triple quadrupole; Q-TOF, quadrupole-time of flight; CRC, colorectal cancer.

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Hybrid quadrupole time-of-flight mass spectrometer

used for the quantitative analysis of the tryptic peptides of two low-abundance serological proteins, tissue inhibitor of metalloproteinase 1 (TIMP1) and tissue-type protein tyrosine phosphatase kappa (PTPκ), which were prepared with peptide affinity-based enrichment from human serum. Finally, this method was used to detect femtomolar amounts of target peptides derived from TIMP1 and PTPκ, with good coefficients of variation (CV 2.7% and 9.8%, respectively), using a few microliters of human serum from colorectal cancer patients. The results suggest that pseudo-MRM using hybrid Q-TOF MS, combined with peptide affinity-based enrichment, could become a promising alternative for the quantitative analysis of low-abundance target proteins of interest in complex serum samples that avoids protein depletion.

1. Introduction

The quantitative analysis of proteins using mass spectrometry (MS) is an attractive strategy in proteomics research. Recently, MS-based quantitative analysis has been used for the discovery and verification of protein biomarkers due to its high selectivity and sensitivity for quantitation. Multiple reaction monitoring (MRM) with stable isotope-labeled internal standard peptides is the most widely used method for the absolute quantitative analysis of target proteins in the field of proteomics [1–6]. MRM is also suitable for multiple target quantitative analysis and high-throughput analysis in biomarker discovery and verification [4,7–9]. Typically, MRM is performed using triple quadrupole mass spectrometry (QqQ-MS) combined with a liquid chromatography (LC) system, in which the high selectivity of quadrupole mass filters is used to target specific ions from all fragment ions in the collision-induced dissociation (CID) tandem spectra [3]. MRM analysis requires the selection of precursor ions and product ion pairs (called transition ions). The first quadrupole (Q1) is set to allow only a specific precursor ion into the second quadrupole (Q2), which yields fragment ions based on collision energies. The fragment ions are then allowed into the third quadrupole (Q3) and subsequently detected. MRM has been successfully used in a variety of biological applications. QqQ-MS based-MRM analysis can quantify proteins as well as any other small molecule analytes, including pharmaceutical metabolites [10–13].

Peterson et al. [14] proposed a novel targeted quantitative method using a quadrupole-equipped Orbitrap MS analyzer. Because high resolution and accurate masses are required for the parallel detection of all fragment ions instead of the third quadrupole analyzer, this approach can efficiently distinguish target analytes from interference in a complex sample matrix [14–16]. Recently, an improved hybrid quadrupole time-of-flight (hybrid Q-TOF) MS instrument was introduced that shows high resolution and acquisition speeds [17]. In this system, a time-of-flight (TOF) analyzer is not used for the ion-filtering process as a quadrupole analyzer is in QqQ-MS. However, like an Orbitrap analyzer, the hybrid Q-TOF MS can provide higher-resolution CID MS/MS spectra than a QqQ-MS. This suggests that hybrid Q-TOF MS may be used to perform quantitative analysis, similar to MRM MS. Because hybrid Q-TOF MS has higher resolving power (~~30,000) than QqQ-MS, with mass accuracy below 3 ppm and high speed scans (20 Hz) [17], it may reduce the number of peaks that show interference with the numerous peptide peaks detected. Moreover, the faster scan speed of hybrid Q-TOF MS is advantageous when analyzing a large number of proteins. The high scanning speed can provide more informative data in the analysis of complex biological samples, such as blood. Hybrid Q-TOF MS can also be used to identify and quantify target proteins. For quantitative analysis using hybrid Q-TOF MS, the full scan MS/MS spectra of selected precursor ions of the target peptide are obtained first, after which peptide fragments observed in the high-resolution MS/MS data can be selected to obtain a quantitative result. High-resolution extracted ion chromatograms (XICs) from each transition are combined to yield quantitative information on the target peptide (called pseudo-MRM MS).

In general, QqQ-MS provides quantification results from a single transition, although further transitions can be considered in MRM analysis to obtain subsidiary information, such as the co-eluting time confirmation of a target peptide. In contrast, using post-acquisition XICs from MS/MS data and considering more fragment ions in full-scan MS/MS spectra, the identification and quantification of target peptides can be achieved with enhanced sensitivity and an improved signal-to-noise (S/N) ratio using hybrid Q-TOF MS. These benefits of hybrid Q-TOF MS may be especially useful for verification studies of known biomarker candidates in clinical proteomics.

The quantification of low-abundance proteins in biological samples (such as serum or plasma) using MS is challenging due to sample complexity. In this study, to determine whether hybrid Q-TOF MS can be used for pseudo-MRM mass analysis, we selected two target glycoproteins, tissue inhibitor of metalloproteinase 1 (TIMP1) and tissue-type protein tyrosine phosphatase kappa (PTPκ), both of which are serological colorectal cancer (CRC) biomarker candidates. These target peptides are typically present at low concentration (a few dozen ng mL^{-1}) in human serum. TIMP1 normally inhibits matrix metalloproteinase 9 (MMP-9), which is known to increase in CRC [22]. Thus, overexpressed TIMP1 in plasma or serum can be used to diagnose CRC [23,24]. Also, during tumor growth and metastasis, TIMP1 is known to be aberrantly glycosylated by *N*-acetylglucosaminyltransferase-V (GnT-V), a glycosyltransferase associated with pathogenic processes in cells, which is upregulated in cancer cells [18–20]. Thus, upregulated GnT-V in cancer cells catalyzes the abnormal formation of a ß-1, 6-*N*-acetylglucosamine (ß-1, 6-GlcNAc) moiety on the *N*-linked core glycans of TIMP1. An increase in ß-1, 6-GlcNAc branching on *N*-linked glycans is known to be associated with the metastasis of colon cancer cells [20]. PTPκ is similarly aberrantly glycosylated by GnT-V and is a potential colon cancer biomarker [21]. Therefore, quantitative monitoring focusing on these cancer-related proteins could be used to investigate the relationship between target serum proteins and the progression of a cancer.

Mass spectrometric analysis of glycoproteins present at low concentrations is difficult because of the high complexity and wide range of protein abundance in serum samples. Therefore, enrichment of the target peptide is required for the effective detection of the targets by MS. Peptide affinity-based enrichment, an immunoaffinity method for the enrichment of a target peptide from complex proteome samples, can reduce sample complexity and significantly improve the sensitivity of the target peptide [25,26]. This peptide-based immuno-enrichment method, coupled with MRM MS, has been used for the quantitative analysis of the low-abundance TIMP1 and PTPκ present in the serum of cancer patients [6,27]. In this study, we evaluated the analytical performance of pseudo-MRM-based techniques using hybrid Q-TOF MS for the quantitative analysis of TIMP1 and PTPκ (CRC biomarker candidates) using peptide affinity-based enrichment (Fig. 1).

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