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REVIEW

Progress in Mass Spectrometry Acquisition Approach for Quantitative Proteomics

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Abstract: As the major approach for quantitative proteomics, classic quantitative mass spectrometry (MS) faces new challenges, such as interferences from complex matrices and limits on analytical throughput. Recent progresses in MS technologies, including development of synchronous precursor selection (SPS), mass defect isobaric labeling, parallel reaction monitoring (PRM), multiplexing acquisition (MSX), and various novel data-independent acquisition (DIA) strategies, provide viable solutions for problems in relative and absolute quantification in proteomics. This review analyzed the current bottlenecks in the field of quantitative proteomics, summarized the most recent advances in quantitative MS acquisition, and highlighted the characteristics and the advantages of these new techniques in quantitative proteomics.

Key Words: Quantitative proteomics; Synchronous precursor selection; Parallel reaction monitoring; Data-independent acquisition; Review

1 Introduction

Currently, the focus of proteomics has shifted from qualitative to quantitative studies. Quantitative proteomics requires quantitation of the proteome of a cell, tissue, or even entire organism, and plays a significant role in investigation of mechanism of basic biological processes as well as biomarker identification and validation[1,2]. Proteome quantitation can be divided into relative and absolute quantification[3]. Relative quantitation is often applied in comparative studies, which involves large-scale, high-throughput quantitative MS analyses of samples collected under normal or pathological conditions aiming to identify the precise proteome differences. Two major techniques, stable isotope labeling or label-free strategies, can be employed in such studies[4,5]. Absolute quantitation seeks to determine absolute quantity of specific protein, which is typically achieved by monitoring unique peptides of target protein, and calculating concentrations based on peak area ratios to known amount of peptide standards (external method) or isotope-labeled "heavy" peptide standards (internal method). Selected/multiple reaction monitoring (SRM/MRM) of unique peptides is the most frequently employed MS approach[6].

Stable isotope labeling is the classic approach for relative quantitative proteomics. The labeled samples are mixed prior to MS analysis, so that relative quantitation can be achieved in one run, avoiding the instability in LC separation and minimizing error. Stable isotopes can be introduced metabolically (SILAC)[7], enzymatically (^{18}O labeling)[8], and chemically (dimethyl labeling)[9], whereas relative quantitation is usually based on the chromatogram peak area ratio of precursor ions. However, MS^1 based quantitation suffers from low labeling efficiency, narrow dynamic range, and poor sensitivity, and thus isobaric labeling based MS^2 quantitation is gaining more and more attention[10]. Using isobaric labeling, the same peptide originated from different samples displays identical m/z at MS^1 level, and thereby improves method sensitivity significantly. Meanwhile the reporter ions with different m/z are generated at MS^2 level, providing quantitation information as well as b/y ion identification. In addition, a wider dynamic range can be attained with this approach. iTRAQ[11] and TMT[12] are two major isobaric

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labeling reagents, with labeling capacity of 8-plex and 6-plex, respectively. Despite these improvements, isobaric labeling methods face interference issues caused by co-eluting peptides. Specifically, on the LC side, the matrices of proteomic samples are usually very complex, with a significant number of peptides co-eluted from the column; whereas on the MS side, an isolation window of 2 *m/z* is typically used when filtering precursor ions for MS^2 analysis. In combination, these technical pitfalls result in the lack of discrimination between target peptide and co-eluting near-*m/z* peptides, leading to erroneous incorporation of the reporter ions of co-eluting peptides, compromising quantitative accuracy[13,14]. Ting *et al*[15] has shown that the co-eluting peptides in complex samples can dramatically affect reporter ion intensity, distort peptide and protein ratios, leading to underestimation of proteome changes. Such problems have become the bottleneck in isobaric labeling method development.

Triple quadrupole-based SRM (or MRM), the gold standard for MS quantitation, is widely used for absolute quantitation of proteins[6]. SRM monitors the precursor and product ion mass of specific peptides. The first quadrupole (Q1) filters for the precursor ions, and Q3 selects for product ions after precursor fragmentation in Q2. By monitoring the signal of unique precursor-product transition, interfering ions can be mostly excluded. Peptide confirmation and quantitation can be achieved either with external standard using calibration curves, or by spiking with known amount of stable isotope-labeled peptide internal standards[6,16]. There is no doubt that SRM method offers high sensitivity and wide dynamic range, and is indispensible for target protein verification and quantitation. However, accompanying the progress of quantitative proteomics, SRM is challenged with more complex sample matrices, lower abundance of target proteins and ease of suppression by high abundance components. In addition, the low resolution SRM fails to effectively exclude interferences from complex matrices, and is prone to false positive results[17,18]. On the other hand, the demand on analysis throughput also increases rapidly, with the need to monitor tens of thousands of transitions in one analytical run, while SRM method can only manage a limited number of transitions simultaneously due to its speed and sensitivity limit[19]. Moreover, optimization of SRM transition and collision energy can be overwhelming for proteomic research, especially for those biomarker and systems biology studies requiring large sample sizes[20,21]. Therefore, absolute proteome quantitation also faces intimidating technical challenges.

Recently, technical improvement of high-resolution mass spectrometers (such as Orbitrap), and innovations in data acquisition strategies, including isobaric labeling-based synchronous precursor selection (SPS) and mass defect isobaric labeling, as well as high-resolution parallel reaction monitoring (PRM) and multiplexing acquisition (MSX) for SRM type analysis, and various novel data-independent acquisition (DIA) strategies, have brought new opportunities to solve these challenges in quantitative proteomics.

2 Progress in isobaric labeling technique: synchronous precursor selection (SPS) and mass defect isobaric labeling

As mentioned earlier, isobaric labeling techniques represented by iTRAQ and TMT face the challenges on two fronts: (1) massive co-eluting near-*m/z* peptides co-fragment with target peptide, which affect the measurement of reporter ion intensity, and distort quantitative results[13–15]; (2) isobaric tags have limited labeling capacity. Taking iTRAQ as an example, its reporter group (*N*-methylpiperazine) consists 6 C and 2 N, with limiting labeling capacity to 8-plex, thus unsuitable for higher sample throughput[22]. Although larger reporter group supports higher labeling capacity, it hurts sensitivity[23].

To solve the problem caused by co-eluting peptides, Ting *et al*[15] analyzed TMT-labeled samples using MS^3 scan in LTQ-Orbitrap (Fig.1). They first employed relatively low collision energy for MS^2 CID (35%) fragmentation to generate b/y ions for sequence identification without over-fragmenting the TMT tags; then within the 110%–160% *m/z* range of parent ion, selected the product ion with highest intensity to undergo thorough reporter group fragmentation by high energy HCD (60%) and to determine reporter ion ratios with MS^3 detection. They demonstrated that, through selection on both precursor ion and product ion level, elimination of co-eluting interference can be achieved, yielding a quantitative ratio matching the theoretical one. For example, in a model two-proteome peptide mixture sample from Lys-C protein digests of yeast (mimicking sample) and the human HeLa cell line (functioned as interference), when the theoretical ratio was 10:1, classic MS^2 analysis only got about 3, while MS^3 reported a ratio of 11.7, which was very close to the theoretical ratio.

However, signal intensity drops significantly in MS^3 results compared to MS^2, leading to a much lower sensitivity of MS^3 in quantitation despite its high quantitation accuracy, thus limiting its application in real sample analysis[24]. Although the recently developed TMTc[25] and QuantMode[26] methods are effective in dealing with co-eluting peptides, these methods involve tedious procedures without offering a quantitative accuracy comparable to that of MS^3.

Synchronous precursor selection (SPS) technique completely solved the signal intensity drawback of MS^3. SPS uses MultiNotch technique which allows for synchronous isolation of multiple ions (≤ 15, Fig.2A) in a linear trap[27]. With this technique, SPS method can select multiple b/y fragments from the target peptide in MS^2 for MS^3 experiment, resulting in accumulation of reporter ions and a substantial improvement