Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/euprot

The potential of fractional diagonal chromatography strategies for the enrichment of post-translational modifications

A. Saskia Venne, René P. Zahedi*

Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany

ARTICLE INFO

Article history:
Available online 24 July 2014

Keywords:
PTM
Enrichment
Signaling
PTM crosstalk

ABSTRACT

More than 450 post-translational modifications (PTMs) are known, however, currently only some of those can be enriched and analyzed from complex samples such as cell lysates. Therefore, we need additional methods and concepts to improve our understanding about the dynamic crosstalk of PTMs and the highly context-dependent regulation of protein function by so-called 'PTM codes'. The mere focus on affinity-based enrichment techniques may not be sufficient to achieve this ambitious goal. However, the complementary use of two-dimensional chromatography-based strategies such as COFRADIC and ChaFRADIC might open new avenues for enriching a variety of so far inaccessible PTMs for large-scale proteome studies.

© 2014 The Authors. Published by Elsevier B.V. on behalf of European Proteomics Association (EuPA). This is an open access article under the CC BY-NC-SA license (http://creativecommons.org/licenses/by-nc-sa/3.0/).

1. The relevance of post-translational modifications

Organisms have to adapt continuously their physiological processes in order to maintain homeostasis under a large range of environmental changes. Compared to gene expression and protein translation, post-translational modifications (PTMs) and protein degradation enable a faster regulation of cellular processes. Thus, PTMs allow the precise and dynamic response to internal and external stimuli, modulating for instance the subcellular localization, activity, stability and interaction of proteins. Consequently, understanding this sensitive and complex system is essential for cell biology, disease prevention and development of therapeutic approaches [1,2].

Currently, over 450 PTMs [3,4] are listed in the UniProt database including the most prominent members such as phosphorylation, glycosylation, ubiquitination and acetylation. The number of experimentally observed PTMs literally exploded in the past 10 years [5,6] mainly owing to the recent improvements in mass spectrometry (MS) and the availability of more sensitive and faster mass analyzers.

However, often more than 50% of MS/MS spectra acquired in an LC–MS/MS run cannot be identified by database searches. Such unmatched spectra can arise from *e.g.* (a) contaminations

Abbreviations: COFRADIC, combined fractional diagonal chromatography; ChaFRADIC, charge-based fractional diagonal chromatography; HPLC, high performance liquid chromatography; MOAC, metal oxide affinity chromatography; LC, liquid chromatography; MS, mass spectrometry; PTM, post-translational modification; SCX, strong cation exchange chromatography; TAILS, terminal amine isotopic labeling of substrates.

* *Corresponding author at*: Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Otto-Hahn-Str. 6b, 44227 Dortmund, Germany. Tel.: +49 0231 1392 4143.

E-mail address: rene.zahedi@isas.de (R.P. Zahedi).

http://dx.doi.org/10.1016/j.euprot.2014.07.001

2212-9685/© 2014 The Authors. Published by Elsevier B.V. on behalf of European Proteomics Association (EuPA). This is an open access article under the CC BY-NC-SA license (http://creativecommons.org/licenses/by-nc-sa/3.0/).

such as polymers or other biomolecules, (b) co-isolation of peptides (and such contaminations) so that MS/MS spectra represent fragment ions derived from a mixture of precursor ions, (c) point mutations or (d) sequences that are incorrectly annotated in databases, (e) degradation products, (f) enzymatic mis-cleavages and in-source decay [7,8], and (g) unknown or unanticipated PTMs (and their combinations, respectively). The possible extent of protein modification is well represented by histones, which are also among the best-characterized proteins. All core histones can be modified by up to eight different PTMs (acetylation, phosphorylation, mono-, di- and tri-methylation, butyrylation, crotonylation and propionylation) at the same time, while the individual sites can be modified by different PTMs with occupancies varying between <1% and 100% [9,10]. In histones these PTMs can act in concert so that the highly context-dependent and combinatorial pattern of different modifications referred to as 'PTM code', modulates and defines the final cellular output [11]. A similarly high degree of modification was shown for the tumor suppressor p53, with 10 different PTMs that act in concert and thus can induce different cellular responses. For both examples, different PTM codes could be mapped to specific molecular functions [11], however, this required a large number of elaborate experiments and studies.

Nowadays, large-scale characterization of PTMs is performed routinely [12–17] and improved methods enable PTM localization and quantification with high confidence [18–22]. Nevertheless, this vibrant research field is still emerging and accompanied by incomplete assignment of identified PTM sites to specific cellular functions [23]. Although extensive databases about PTMs exist, complete 'PTM catalogs' [23] summarizing all possible modifications (or sites) for protein entries are still far from reality. Such catalogs will represent valuable resources for both functional/structural studies and modeling.

This holds true for extensively analyzed PTMs such as phosphorylation or glycosylation, but even more for the huge proportion of so far known but largely uncharacterized PTMs [6]. Because many PTMs have a low stoichiometry and thus are almost inaccessible for the global proteome analysis of complex samples such as cell lysates, their detection requires specific enrichment prior to analysis. For some PTMs antibody- or affinity-based enrichment is routinely used, but most PTMs remain hidden from large-scale MS-based detection owing to the lack of dedicated enrichment methods. Hence, there is a strong need for alternative and versatile strategies for PTM enrichment that provide high sensitivity and flexibility, to allow for a more comprehensive analysis of PTMs and PTM codes in the future.

2. Strategies for MS-based analysis of PTMs

In general there are three strategies to analyze proteins *via* MS: (i) top-down, (ii) middle-down and (iii) bottom-up (see Fig. 1).

In top-down proteomics intact proteins are analyzed, potentially revealing complete protein PTM patterns such that the information about the number, type and the localization of PTMs is retained [24]. One substantial advantage is the capability to determine the relative abundance of different proteoforms and therefore their relative proportion in the sample. Relative site occupancies can be calculated for different PTMs to determine stoichiometries [24]. Furthermore, top-down allows characterizing structural changes induced upon PTM of proteins [25]. However, certain limitations still impede the dissemination of top-down as routinely used method for high-throughput PTM analysis [26]. So far efficient fragmentation is mainly achieved for small proteins (<30 kDa) and the need for elaborate pre-fractionation involves high amounts of starting material [26–28], while PTM enrichment is much more challenging on the protein compared to the peptide level.

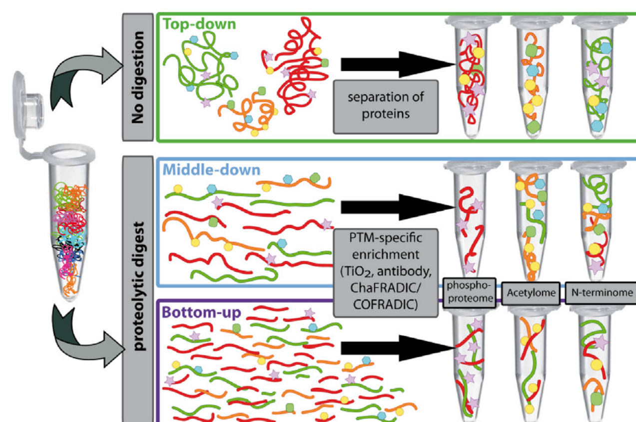

Fig. 1 – Strategies for MS-based analysis of PTMs. (i) In top-down experiments intact and purified proteins are analyzed. As prior digestion is not required, the comprehensive identification and mapping of PTMs is possible. (ii) Middle-down approaches produce relatively long peptide-stretches that can potentially contain multiple PTMs. Like in bottom-up experiments the modified peptides can be enriched to obtain specific sub-proteomes (*e.g.* phosphoproteome, acetylome, N-terminome). (iii) In bottom-up strategies shorter peptides are generated. This is usually accompanied by a loss of information about complex PTM patterns of different proteoforms.

In contrast, bottom-up strategies mostly generate shorter peptides [8,29] (6–30 amino acids [8,29]), which, compared to proteins, are less heterogeneous and thus can be separated and detected more efficiently. This allows detection down to the amol range, even for complex samples. The physicochemical properties of peptides can be exploited effectively to enrich for certain PTMs that can be mapped with high localization probabilities [30,31]. However, the improved enrichment and detection capabilities of bottom-up approaches are accompanied by an inherent loss of qualitative and quantitative information, considerably impeding the differentiation of proteoforms, PTM stoichiometry and consequently also PTM crosstalk [32]. Despite those inherent limitations, peptide-centric bottom-up proteomics is still the method-of-choice to screen for PTMs and their dynamics, providing important information about PTM localization and changes between different cellular states or time points.

More recently, a promising alternative, so-called middle-down, was established. Here, proteases that generate peptides in the range of 3–9 kDa are used, allowing the identification of

Download English Version:

https://daneshyari.com/en/article/1183624

Download Persian Version:

https://daneshyari.com/article/1183624

Daneshyari.com