JOURNAL OF PROTEOMICS 92 (2013) 2–27

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot

Review

Advances in purification and separation of posttranslationally modified proteins☆

Martin Černý, Jan Skalák, Hana Cerna, Břetislav Brzobohatý*

Department of Molecular Biology and Radiobiology, Mendel University in Brno & CEITEC — Central European Institute of Technology, Mendel University in Brno, Zemědělská 1, CZ-613 00 Brno, Czech Republic

ARTICLE INFO

Available online 15 June 2013

Keywords:
Posttranslational modification
PTM
Protein enrichment
PTM detection
Proteome
Top-down proteomics

ABSTRACT

Posttranslational modifications (PTMs) of proteins represent fascinating extensions of the dynamic complexity of living cells' proteomes. The results of enzymatically catalyzed or spontaneous chemical reactions, PTMs form a fourth tier in the gene – transcript – protein cascade, and contribute not only to proteins' biological functions, but also to challenges in their analysis. There have been tremendous advances in proteomics during the last decade. Identification and mapping of PTMs in proteins have improved dramatically, mainly due to constant increases in the sensitivity, speed, accuracy and resolution of mass spectrometry (MS). However, it is also becoming increasingly evident that simple gel-free shotgun MS profiling is unlikely to suffice for comprehensive detection and characterization of proteins and/or protein modifications present in low amounts. Here, we review current approaches for enriching and separating posttranslationally modified proteins, and their MS-independent detection. First, we discuss general approaches for proteome separation, fractionation and enrichment. We then consider the commonest forms of PTMs (phosphorylation, glycosylation and glycation, lipidation, methylation, acetylation, deamidation, ubiquitination and various redox modifications), and the best available methods for detecting and purifying proteins carrying these PTMs. This article is part of a Special Issue entitled: Posttranslational Protein modifications in biology and Medicine.

Contents

1. Introduction . . . 3
   1.1. So few, and yet so many . . . 3
2. Methods applied in PTM analyses . . . 3
   2.1. Protein separation or "Thou shalt not use only bottom-up proteomic approaches" . . . 3
      2.1.1. Size does matter . . . 4
      2.1.2. Isoelectric focusing . . . 5
      2.1.3. Two-dimensional electrophoresis (2-DE) . . . 5
      2.1.4. Liquid chromatography (LC) . . . 5

☆ This article is part of a Special Issue entitled: Posttranslational Protein modifications in biology and Medicine.

* *Corresponding author at:* Department of Molecular Biology and Radiobiology, Mendel University in Brno & CEITEC MENDELU, Zemědělská 1, CZ-613 00 Brno, Czech Republic. Tel.: +420 545 133 374.
E-mail address: martincerny83@gmail.com (M. Černý).

1874-3919/$ – see front matter
http://dx.doi.org/10.1016/j.jprot.2013.05.040

2.1.5. Diagonal separation . . . 6
2.1.6. Affinity separation and enrichment . . . 7
2.2. Deeper mining to detect hidden proteins and PTMs . . . 8
2.2.1. Immunoaffinity depletion . . . 8
2.2.2. Proteome equalization . . . 8
3. Major protein PTMs found in proteomic studies . . . 8
3.1. The phosphoproteome . . . 9
3.1.1. Visualization of phosphorylated proteins . . . 11
3.1.2. Phosphoproteome enrichment by affinity chromatography . . . 11
3.1.3. Uses of antibodies and derivatization in phosphoproteome analyses . . . 12
3.1.4. The future of phosphoproteomics . . . 12
3.2. Redox proteomics . . . 12
3.2.1. Cysteine oxidative modifications . . . 13
3.2.2. Tyrosine and tryptophan nitration . . . 14
3.2.3. Methionine oxidation . . . 14
3.2.4. Protein carbonylation . . . 14
3.2.5. Troublesome path of redox proteomics . . . 15
3.3. Protein lipidation . . . 15
3.3.1. Analysis of protein lipidation . . . 15
3.3.2. Detection and enrichment of lipidated proteins . . . 15
3.4. Glycoproteomics . . . 16
3.4.1. Visualizing a glycoproteome . . . 16
3.4.2. Enrichment of glycosylated or glycated proteins . . . 17
3.5. Protein deamidation . . . 18
3.6. Protein acetylation . . . 18
3.7. Protein methylation . . . 18
3.7.1. Protein methylation detection and enrichment of protein methylomes . . . 19
3.8. Protein ubiquitination . . . 19
3.8.1. Protein ubiquitination — enrichment . . . 19
4. Summary . . . 19
Acknowledgements . . . 20
References . . . 21

1. Introduction

1.1. *So few, and yet so many*

Proteomes are expected to be two to three orders of magnitude more complex than would be predicted from numbers of protein-encoding genes present in the respective genomes. There are over 300 known naturally occurring amino acids, but only 64 codons in the genetic code and the actual number of proteinogenic amino acids is even lower. Altogether, 22 amino acids have been proven to be encoded by DNA. However, after protein translation these amino acids may undergo further modifications, which considerably increase the diversity of proteins present in living cells. These modifications can be either transient or permanent, and may result from either targeted, enzymatically catalyzed reactions or spontaneous chemical reactions in the cell. The Unimod Database (http://www.unimod.org; 10/2012) lists almost 1000 different protein modifications that have been detected in mass spectrometric analyses of proteins, but some of them could be artifactual, like methionine and cysteine oxidations during two-dimensional electrophoresis (2-DE), carbamylation by urea on any free amino group in the protein sample (at N-termini, or side chains of arginine or lysine), and modifications introduced by MS analysis (e.g. conversion of phosphoserine to dehydroalanine with neutral loss of phosphoric acid). The RESID database hosted by the European Bioinformatics Institute, EBI (http://www.ebi.ac.uk/RESID/; 10/2012), compiles information on protein posttranslational modifications (PTMs) found in nature and its release 70.01 (08/2012) lists 591 entries. Covalent protein modifications can be classified as a covalent addition of some chemical group, or a covalent cleavage of peptide backbones [1]. Side chain modifications are known for all of the proteinogenic amino acids except Ala and Pyr, and all can undergo crosslink reactions and/or N- or C-modification if they are at the N- or C-terminus, respectively, of their respective proteins (Fig. 1).

2. Methods applied in PTM analyses

2.1. *Protein separation or "Thou shalt not use only bottom-up proteomic approaches"*

Widely used MS-based approaches in so-called "bottom-up proteomics" (involving digesting samples by selected proteases, MS analysis of the resulting peptides and finally assembly of the