Biochimica et Biophysica Acta 1864 (2016) 983–990

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Post-translational modifications of plant cell wall proteins and peptides: A survey from a proteomics point of view☆

Hervé Canut, Cécile Albenne, Elisabeth Jamet *

Université de Toulouse, CNRS, UPS, 24 chemin de Borde Rouge, Auzeville, BP42617, 31326 Castanet Tolosan, France

ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 12 February 2016
Accepted 24 February 2016
Available online 2 March 2016

Keywords:
Cell wall
N-glycosylation
O-glycosylation
GPI anchor
Plant
Protein processing
Proteomics
Peptidomics

ABSTRACT

Plant cell wall proteins (CWPs) and peptides are important players in cell walls contributing to their assembly and their remodeling during development and in response to environmental constraints. Since the rise of proteomics technologies at the beginning of the 2000's, the knowledge of CWPs has greatly increased leading to the discovery of new CWP families and to the description of the cell wall proteomes of different organs of many plants. Conversely, cell wall peptidomics data are still lacking. In addition to the identification of CWPs and peptides by mass spectrometry (MS) and bioinformatics, proteomics has allowed to describe their post-translational modifications (PTMs). At present, the best known PTMs consist in proteolytic cleavage, *N*-glycosylation, hydroxylation of P residues into hydroxyproline residues (O), *O*-glycosylation and glypiation. In this review, the methods allowing the capture of the modified proteins based on the specific properties of their PTMs as well as the MS technologies used for their characterization are briefly described. A focus is done on proteolytic cleavage leading to protein maturation or release of signaling peptides and on *O*-glycosylation. Some new technologies, like top–down proteomics and terminomics, are described. They aim at a finer description of proteoforms resulting from PTMs or degradation mechanisms. This article is part of a Special Issue entitled: Plant Proteomics— a bridge between fundamental processes and crop production, edited by Dr. Hans-Peter Mock.

1. Introduction

Plant cells are surrounded by cell walls mainly composed of polysaccharides and proteins which are organized in intricate networks interacting which each other [1,2]. Cell wall proteins (CWPs) and peptides are important players in cell walls contributing to their assembly and to their remodeling during development and in response to environmental constraints, but also to cell-to-cell signaling processes [3–6]. Since the rise of proteomics technologies at the beginning of the 2000's, the knowledge of CWPs has greatly increased leading to the discovery of new CWP families and to the description of the cell wall proteomes of different organs of many plants [7,8]. The most described cell wall proteomes are those of *Arabidopsis thaliana* and of *Brachypodium distachyon* which are respectively dicot and monocot model plants: 715 and 500 different CWPs are respectively listed in the *WallProtDB* database dedicated to plant cell wall proteomics (http://www.polebio.lrsv.ups-tlse.fr/WallProtDB/) [9]. Conversely, plant peptidomics is still in its infancy and cell wall peptidomics data are lacking [10]. Peptidomics has been only recently developed in plants based on work done in neuroscience and on the study of the degradomes generated by protease activities [11].

In addition to the identification of CWPs and peptides by mass spectrometry (MS) and bioinformatics, proteomics has allowed to describe their post-translational modifications (PTMs). CWPs can undergo several types of PTMs among which proteolytic cleavage leading to protein maturation or release of signaling peptides, *N*-glycosylation, hydroxylation of P residues into hydroxyproline residues (O), *O*-glycosylation and glypiation [12,13]. These PTMs are performed in different cell compartments. The proteolytic cleavages may occur in the Golgi apparatus or in secretory vesicles before the secretion of proteins [14] or in cell walls for the maturation of proteins or to the release of biologically active peptides [15,16]. *N*-glycosylation has been the most studied PTM (for recent reviews see [17,18]). It occurs in the endoplasmic reticulum and most CWPs are expected to be *N*-glycosylated since they pass through the secretion pathway to reach the extracellular space [12]. The *N*-glycosylation rule on N residues is a simple one and it has been well-described for a long time [19]. The consensus *N*-glycosylation amino acid motif (NXS/TX, with X = any amino acid except P) has been listed in the Prosite database under the accession PS00001 [20]. Due to its short size, this pattern has a high probability of occurrence which means that its presence is not sufficient to conclude that the N residue is *N*-glycosylated. Only a

☆ This article is part of a Special Issue entitled: Plant Proteomics— a bridge between fundamental processes and crop production, edited by Dr. Hans-Peter Mock.

* Corresponding author: Laboratoire de Recherche en Sciences Végétales (UMR 5546 UPS/CNRS), 24 chemin de Borderouge, Auzeville, BP42617, 31326 Castanet-Tolosan Cedex, France.
E-mail address: jamet@lrsv.ups-tlse.fr (E. Jamet).

http://dx.doi.org/10.1016/j.bbapap.2016.02.022
1570-9639/

few CWP families have been shown to contain no *N*-glycosylation site, such as expansins [21]. Hydroxylation of P into hydroxyproline (O) residues occurs in the endoplasmic reticulum and can be followed by *O*-glycosylation in the Golgi apparatus [17,22]. This PTM is mostly found in the so-called Hyp-Rich Glycoproteins (HRGPs) like extensins, arabinogalactan proteins (AGPs) and Hyp/Pro-rich proteins (H/PRPs), but it is also found in signaling peptides [23,24]. In extensins, S residues adjacent to O residues can also be *O*-glycosylated. Glypiation consists in the attachment in the endoplasmic reticulum of a glycosylphosphatidylinositol (GPI) anchor at the C-terminus of the protein [25]. The GPI anchor provides stable attachment of the protein to the plasma membrane on its outer surface. The phosphatidylinositol moiety of the anchor can be cleaved by phosphatidylinositol-specific phospholipases C (PI-PLCs) [26]. Thus, GPI-anchored proteins may exist both at the plasma-membrane outer surface after cleavage and in a soluble form inside cell walls. A proteomic study combined to a genomic analysis has confirmed the presence of proteins predicted to be GPI-anchored in Triton-X114 resistant membrane domains purified from *A. thaliana* plasma membranes [27,28].

This review comprises several parts: a brief overview of the strategies used to isolate sub-proteomes based on the properties of CWPs and to characterize glycoproteins, *i.e.* both their glycan and their polypeptide moieties; a description of proteolytic cleavages of CWPs including maturation and release of biologically active peptides; a focus on P hydroxylation and *O*-glycosylation of CWPs; and technological advances to further characterize proteoforms resulting from PTMs or degradation mechanisms.

2. Some strategies to capture and study CWPs carrying PTMs

The various strategies used to study cell wall proteomes have been previously reviewed and their advantages and limitations have been discussed [7,8,29,30]. They mainly consist in three approaches: (i) non-destructive methods aiming at the elution of CWPs from cell walls using salt solutions without disrupting the plasma membrane; (ii) destructive methods starting with the grinding of the plant material followed by a purification of cell walls and an extraction of CWPs with salt solutions; (iii) purification of CWPs sharing common biochemical properties such as GPI-anchored proteins, AGPs or glycoproteins. This review will focus on the last type of strategy because the characterization of CWP PTMs requires enrichment in the proteins of interest due to the level of sensitivity of the analytical methods (Fig. 1).

2.1. Isolation of GPI-anchored proteins

As mentioned above, proteins attached to the external side of the plasma membrane by GPI-anchors can be considered as CWPs. Actually, AGPs devoid of their GPI-anchors have been found in culture medium of cell suspension cultures or have been released from purified plasma membranes after PI-PLC treatment [27,31]. Usually, it is possible to isolate GPI-anchored proteins after fractionation of microsomal membranes by Triton X-114 detergent phase partitioning followed by PI-PLC treatment (Fig. 1) [27,32]. Thirty GPI-anchored proteins have been identified among which six β-1,3 glucanases, five phytocyanins, four fasciclin-like arabinogalactan proteins (FLAs) and two proteins of the SKU family (SKU5 and SKS1) [27].

2.2. Isolation of N- and O-glycoproteins

With regard to other PTMs, the enrichment in proteins sharing common features can be done in several ways, starting either from a total protein extract, from the elution of intercellular fluids using a non-destructive method, or from the purification of cell walls using a destructive method followed by the extraction of proteins (Fig. 1). Up to now, three types of CWPs have been specifically targeted: *N*- or *O*-glycoproteins and AGPs. Affinity chromatography has been a method of choice to capture glycoproteins. The most used lectin for *N*-glycoprotein enrichment has been Concanavalin A (ConA) [21,33]. However, the *Galanthus nivalis* agglutinin (GNA) and the *Lens culinaris* hemagglutinin (LCH) have also been used and shown to enlarge the *N*-glycoproteome of the tomato fruit pericarp [34]. With regard to *O*-glycoproteins, the tools are scarcer. Classical AGPs have been precipitated using the β-glucosyl or β-galactosyl Yariv phenylglycosides which specifically interact with type II arabinogalactans (AGs) of AGPs [35–37]. AtAGP31 has been purified by affinity chromatography on peanut agglutinin (PNA) which seems to recognize the Ara-Gal rich *O*-glycans of its central H/PRP domain [38]. Besides, boronic acid chromatography has been used, but did not prove to be very efficient [21]. The purification of *O*-glycoproteins remains a challenge and tools are lacking. A major bottleneck for the analysis of *O*-glycoproteins is the fact that many of them are covalently linked together or with other cell wall components [39–41]. Recently, a new strategy involving direct release of peptides from cell walls by tryptic digestion has allowed to identify several structural proteins among which extensins, H/PRPs and Leucine-Rich repeat extensins (LRXs) [42].

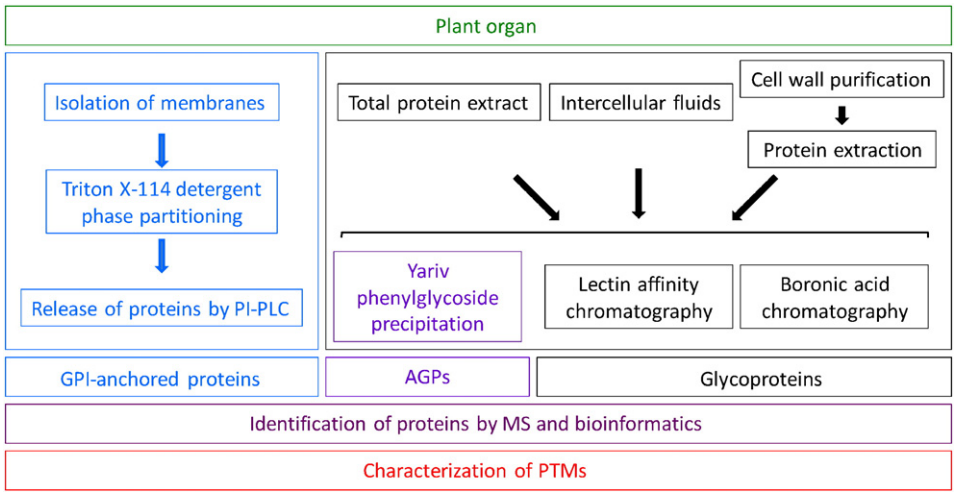

Fig. 1. Main strategies used to isolate and characterize CWPs carrying PTMs like GPI-anchors or glycosylations.