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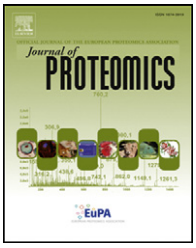

Review

An overview of proteomics approaches applied to biopharmaceuticals and cyclotides research☆

Diogo Ribeiro Demartini[a,*], *Giancarlo Pasquali*[a], *Célia Regina Carlini*[a,b]

[a]*Center of Biotechnology, Universidade Federal do Rio Grande do Sul., Av. Bento Gonçalves 9500, Prédio 43431, Sala 214, Porto Alegre, RS 91501-970, Brazil*

[b]*Graduate Program in Cellular and Molecular Biology Applied to Health, Universidade Luterana do Brasil, Canoas, RS 92425-900, Brazil*

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ABSTRACT

The evolution in proteomics approaches is notable, including quantitative proteomics and strategies for elucidation of post-translational modifications. Faster and more accurate mass spectrometers as well as cleverer bioinformatics tolls are making the difference in such advancement. Among the wide range of research in plant proteomics, biopharmaceutical production using plants as "biofactories" and the screening of new activities of new molecules, in this case, peptides, are quite important regarding translational proteomics. The present review is focused on "recombinant proteins and bioactive peptides", with biopharmaceuticals and cyclotides chosen as examples. Their application and challenges are focused on a "translational proteomics" point of view, in order to exemplify some new areas of research based on proteomics strategies.

This article is part of a Special Issue entitled: Translational Plant Proteomics.

Contents

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* *Corresponding author*. Tel.: +55 51 3308 6062.
E-mail addresses: diogord@terra.com.br, diogo.demartini@ufrgs.br (D.R. Demartini).

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1. Introduction

Considering the composition, the wide structural diversity and the consequent multitude of physical–chemical properties and functions of proteins, their study may be considered one of the most challenging tasks in analytical chemistry. Nevertheless notorious advances in proteomics approaches have been achieved [1]. The search for novel plant proteins or peptides associated to biochemical event(s) involved in disease or promoting resistance to conditions like stress or predators are examples of such studies [2–4]. The necessity to explore deeper and deeper in a particular proteome forced researchers to overcome the limits of pre-fractionation techniques, the robustness of mass spectrometers and the scope of bioinformatics softwares, as the complexity of the sample to be analyzed imposed different strategies for success [1,5]. To achieve a high number of identified proteins and eventually quantifying them depends on a range of equally important steps including protein preparation/extraction, sample pre-fractionation, protein digestion, data acquisition, and data analysis.

Protein extraction might involve previous organelle fractionation, membrane separation, or immunoprecipitation, among other approaches, in order to obtain the desired proteins sample, in sufficient amount for analysis [6,7]. Gel-based or multidimensional chromatography, at protein or peptide level, respectively, is mostly used for sample pre-fractionation [8].

Peptidases employed in protein digestion may influence the choice of fragmentation method. Trypsin, for instance, is not a good choice in electron-transfer dissociation (ETD), in which longer peptides, with higher charge states, are intended to be analyzed preserving post-translational modifications (PTMs) during the fragmentation step [9].

Quantitative proteomics represents one of the most studied approaches of proteomics research, encompassing tasks related to the identification of proteins involved in a given biological process and, eventually, the quantification of such proteins in one or more situations, uncovering differences in protein abundance among defined proteomes or conditions [10]. Several approaches for quantitative proteomics are nowadays available and were extensively reviewed, addressing their bottlenecks, challenges, advantages, and disadvantages [4,11–13]. In the case of label-free quantification, the abundance of one particular protein may be estimated based on the abundance of peptides derived from that particular protein, detected by any of the several liquid chromatography-tandem mass spectrometry analyses (LC-MS/MS). For spectral-counting, the signal intensities or peak areas at the MS^1 level, or the frequency of MS/MS scans attributed to peptides belonging to a particular protein, are considered [4,10,13].

Label-free approaches have been successfully used to compare biochemical events in different plants and other biological systems. In a study performed by Shen and collaborators [14], maize etiolated seedlings were analyzed using a label-free quantitative proteomics approach based on nano-UPLC-MS^E. About 400 proteins were identified and quantified yielding important data for further approaches on C4 plants like maize, sugarcane, millet, and sorghum, and providing a good example of the use of a nano-UPLC-MS^E label-free method in plant research [14]. In a more detailed and extended work performed by Ferro and collaborators, a total of 1323 non-redundant proteins from three plastid sub-compartments (envelope, thylakoids and stroma) were identified and quantified based on label-free spectral counting. The author's choice of sample pre-fractionation and combination of two high-resolution mass spectrometers to collect the data resulted in such high number of quantified proteins [15].

Protein post-translational modifications (PTMs) are another important field in proteomics. PTM analyses depend on the intended purpose of the study. The type of PTM to be addressed and the necessity of qualitative or quantitative data will define how the sample should be prepared, a critical step for a successful result [16]. Among the most studied PTMs are acetylation, acylation, glycosylation, methylation, oxidation, phosphorylation and ubiquitylation [17]. The amount of data already acquired in PTM analyses made available to researchers the use of prediction tools to identify possible modification sites and databases containing information about modified proteins [18].

2. Translational plant proteomics

In a recent publication, Agrawal and collaborators [19] proposed as a definition of translational plant proteomics "the application and outcomes of any discovery or technological development in plant proteomics to solve issues related but not limited to the recreational and economic values of plants, food security and safety, energy sustainability, and human health". Here we aimed to address the independent topics of "translational plant proteomics" where it relates to "recombinant proteins and bioactive peptides", focusing on biopharmaceuticals (for recombinant proteins) and cyclotides (for bioactive peptides), as examples of technological developments in plant proteomics translated into beneficial applications in several fields.

2.1. *Plant transformation for biopharmaceuticals*

The formidable advances of recombinant protein technologies brought new expression systems for proteins used as pharmaceutical products (named biopharmaceuticals from now on) [12,20]. In the meantime, the growing demand of new biopharmaceuticals for use in a large variety of diseases imposed to researchers and industries the need to develop new production strategies, aimed to minimize costs while improving yield and quality of final products. The challenge is to achieve a simple and inexpensive system able to produce high amounts of purified recombinant proteins [12,21]. In this scenario, plants have emerged as an efficient system to express biopharmaceuticals in high quantity and quality, reducing downstream process costs [20,22,23].

In general, plants are advantageous when compared to cultured cell systems or fermentation processes [20,24] because they are cost-effective and production-efficient systems [25]. The technologies for harvesting and processing in large scale are well known for a great number of plants [25,26] and, additionally, costs can be substantially reduced in the post harvesting phase in which the expressed protein could be accumulated and stored in seeds, maintaining sufficient