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Review

Proteomic approaches to study structure, functions and toxicity of legume seeds lectins. Perspectives for the assessment of food quality and safety

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ABSTRACT

Lectins are a structurally diverse class of (glyco)proteins which bind mono- and oligosaccharides with high specificity and in a reversible way. For many years, the unique sugar binding properties of plant lectins have been exploited for the development of biochemical tools for glycoprotein isolation and characterisation, and the use of lectins as a glycoprofiling tool has became much more sophisticated with the advent of lectin microarrays, in which a panel of lectins are immobilized on a single chip for glycomic analysis. Among the numerous lectins studied so far, those from legumes represent the largest family. They can be present at relatively high amounts depending on genetic as well as environmental factors, and are accumulated especially in the seeds. For this reason, some lectins as the phytohemagglutinin from the common bean *Phaseolus vulgaris* constitute a possible risk, since consumption of raw or incorrectly processed beans has been shown to cause outbreaks of gastroenteritis, nausea and diarrhoea. On the other hand, for these anti-nutritional properties, bean extracts enriched in lectins or in lectin-related amylase inhibitors are also finding a growing use as active ingredients of "weight-blockers" in dietetic preparations for obesity treatment. Current methods to determine the lectin levels in foods are based on immunoenzymatic or toxicity tests, which are largely aspecific. Very recently, the availability of proteomic methodologies has allowed to start development and validation of sensitive and specific assays for detecting trace amounts of harmful lectins in either raw or processed foods. In this review, the main aspects of current and perspective applications of mass spectrometry and proteomic technologies to the structural characterisation of legumes are presented, with focus on issues related to detection, identification, and quantification of phytohemagglutinins relevant for their biochemical, immunological and toxicological aspects.

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Contents

1. Plant lectins

The term "lectins" identifies proteins of non-immune origin which reversibly bind carbohydrates of glycoproteins, glycolipids, or polysaccharides with high affinity [1]. Because of their binding specificity, they play specific roles as mediators of cell recognition in a variety of biological processes, ranging from the symbiosis between nitrogen fixing bacteria and legumes to the adhesion of bacteria to host tissues and of leukocytes to endothelial cells. According to several studies, lectins have evolved through gene duplication and divergence, a process in which the carbohydrate-binding domains of lectins have become incorporated into families of proteins whose individual members play important roles in plant defense [2].

Lectins are generally named according to the plant they are extracted from. Of the many plant lectins that have been characterised extensively, most are secretory proteins, which means they enter the secretory system and subsequently accumulate either in vacuoles or in the cell wall and intercellular spaces. For example, the lectins concanavalin A, soybean agglutinin, phytohemagglutinin, pea lectin, and favin are all present at quite high levels and accumulate in vacuoles in the cotyledons (8–10% of total protein), and at lower levels in the embryonic axes of the seeds. These lectins are synthesized during seed development together with the more abundant seed storage proteins (see [3] for a discussion of plant lectins).

Despite their distinct sugar specificities, extensive homology in primary structure of lectins, also from unrelated taxonomic families, demonstrates a close evolutionary relationship. In addition, one plant species may contain structurally related lectin proteins with different biological properties. For example, the castor bean expresses two distinct but structurally related lectins with different biological properties: ricin and *Ricinus communis* agglutinin (RCA). Ricin is highly cytotoxic but is a weak agglutinin, whereas RCA is weakly cytotoxic but a strong agglutinin.

2. The use of lectins in proteomic research: from affinity chromatography to lectin microarrays and lectinomics

The different and high specificity of sugar-binding properties of plant lectins have made these molecules useful probes for glycan detection in carbohydrates and glycoproteins for many years, providing the basis for either biochemical analysis or for development of diagnostics tools in histology, blotting and biosensor applications [4,5]. Lectins are used as "glycan deciphers" to interpret the carbohydrate structure in living organs and cells [6,7]. Lectin affinity column chromatography, in which lectins are immobilized to agarose or other separation matrices, has been widely used as an efficient technique to fractionate not only N-glycosylated but also O-glycosylated peptides [8]. On this basis, the combination of mass spectrometry (MS) analysis with lectin chromatography has allowed the structural definition of highly complex glycoprotein systems, overcoming the limitations due to the glycan heterogeneity. In this integrated approach, the lectin-binding specificity provides the crucial, although partial, structural information to appropriately direct the fine structural MS analysis. The sequential use of different sets of available lectins may provide additional information to support glycopeptide MS analysis. Recently, the lectin affinity preparation of glycopeptides with *Sambucus nigra* agglutinin and concanavalin A provided the glycan structure outlines for the sialyl linkages and the core structure of N-glycans, soon confirmed by MS investigation [9]. On the other hand, the capability of the up-to-date mass spectrometric techniques in glycan profiling, especially when coupled with high-resolution separation techniques such as HPLC or capillary electrophoresis [10] allows for employing set of lectins for the capture of the entire glycoprotein panels and subsequent release of carbohydrates for analysis (Fig. 1). In this direction, multidimensional lectin affinity chromatography, based on a sequential approach [11], or multi-lectin affinity chromatography, in which a number of immobilized lectins are pooled in the stationary phase [12,13], has been developed for glycoproteome purposes. Glycoproteins selectively enriched by using the lectins can be separated by mono- or two-dimensional electrophoresis and identified with mass spectrometry based techniques. Because of the sensitivity of the MS techniques, the amount of glycoprotein sample required for multistage MS analysis is generally very low (about 1 nmol), also allowing to perform the glycoprotein enrichment at a microscale [14].

The electrophoresis-free proteomic approach usually addressed to the profiling of N-glycoproteins with the use of lectins is schematised in Fig. 1: the steps (Fig. 1, route 1) include lectin column-mediated affinity capture of glycoproteins, proteolytic digestion and PNGase F hydrolysis of glycans also mediating incorporation of ^{18}O as stable isotope label at