Plant Science 275 (2018) 36–48

Contents lists available at ScienceDirect

Plant Science

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

Structural network of arabinogalactan proteins (AGPs) and pectins in apple fruit during ripening and senescence processes

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ARTICLE INFO

Keywords:
Arabinogalactan protein
Immunofluorescence
Immunogold labelling
Malus x *domestica* fruit
Pectin
Quantitative analysis

ABSTRACT

The cell wall is an essential framework determining the overall form of the plant cell. Our study was focused on the distribution of arabinogalactan proteins (AGPs), arabinan, and homogalacturonan in fruit cells during ripening and storage with emphasis on quantitative analysis of their presence in particular regions of the cell wall - plasma membrane. The localization of the examined compounds was determined with immunohistochemistry techniques and immunogold labelling. Spatio-temporal colocalization between AGPs epitopes - [βGlcA(1→3)-αGalA(1→2)Rha] recognized by JIM13 and MAC207 antibodies, and arabinan labelled by the LM16 antibody was detected in the inner cell wall layer, in association with the plasma membrane. The specific arrangement of AGP and arabinan epitopes differentiated them from homogalacturonan epitopes, consisting of GalA residues recognized by LM19 and LM20 antibodies in all the examined fruit maturity stages. The disruption of cell wall - plasma membrane continuum, observed during ripening-associated softening process, was associated with both the substantial decrease of AGPs, pectins content and with remodeling of their arrangement. The results indicate that the textural properties of fruit during growth and postharvest storage, an attribute of fruit quality becoming selection criteria for consumers, depend on the existence of dynamic network organizing polysaccharides and glycoproteins in the extracellular matrix.

1. Introduction

The cell wall constitutes a protective barrier against biotic and abiotic factors. Dynamic changes in its structural integrity in response to environmental stress have an influence on the quality of the cell, tissue, and finally the whole plant. The quality of fruit is synonymous with tissue firmness, which depends on a series of biochemical events, i.e. cell wall constituents remodeling and metabolism [1,2]. The mechanical properties of the cell wall are mainly determined by polysaccharides with structural protein support [3]. The primary cell walls of dicots comprise 30–50% pectins, including mainly homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). From the cell expansion phase, accumulation of HG and RG-I in the cell wall contributes to the maintenance of tissue integrity under turgor pressure driving cell development [4,5].

Besides the major components, minor amounts of structural proteins are present in the plant cell wall. One of the most variable groups of cell wall proteins are hydroxyproline (Hyp)-rich-glycoproteins and among them – arabinogalactan proteins (AGPs). AGPs are characterized by a high proportion of sugar moieties up to 90% of the total mass, heterogeneity of their protein backbone and carbohydrate chains, and presence of the C-terminal glycosylphosphatidylinositol (GPI) sequence that anchors the molecule to the outer leaflet of the plasma membrane [6]. In general, AGPs have been implicated in a wide-range of physiological processes, including germination, cell extension, modulation of cell wall mechanics, and signaling between the cell wall and other cell compartments [7,8]. All these functions are related to associations between AGPs and various cell wall polysaccharides. In addition, AGPs serve as cross-linkers in cell walls and are involved in mediating pectin assembly as pectic plasticizers [9,10].

Tan and coworkers [11] explained the molecular basis of the biological activities of AGPs by identification of covalent linkages between

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https://doi.org/10.1016/j.plantsci.2018.07.019
Received 17 May 2018; Received in revised form 20 July 2018; Accepted 31 July 2018
Available online 02 August 2018
0168-9452/

Table 1
List of the primary antibodies used for immunolocalization.

mAb Name	Specificity	Epitope structure	References	Provider
JIM13	arabinogalactan protein	βGlcA(1→3)-αGalA(1→2)Rha	[18]	CCRC, University of Georgia, Athens, USA
JIM15	arabinogalactan protein	unknown	[18]	CCRC, University of Georgia, Athens, USA
MAC207	arabinogalactan protein	βGlcA(1→3)-αGalA(1→2)Rha	[19]	CCRC, University of Georgia, Athens, USA
LM16	arabinan/RG-I	(1→5)-α-L-arabinan	[20]	PlantProbes, Leeds, UK
LM19	de-esterified homogalacturonan	α-GalA(1→4)α-GalA(1→4)α-GalA(1→4)-αGalA	[20]	PlantProbes, Leeds, UK
LM20	methyl-esterified homogalacturonan	α-MeGalA(1→4)α-MeGalA(1→4) α-MeGalA(1→4)α-MeGalA	[20]	PlantProbes, Leeds, UK

cell wall polymers. It is postulated that there is a proteoglycan complex named ARABINOXYLAN PECTIN ARABINOGALACTAN PREOTEIN1 (APAP1) in cell walls, culture media, and extracellular exudates, which provides the possibility of forming a 'continuous network between wall polysaccharides and wall structural proteins'. The full proteoglycan structure of APAP1 is composed of an AGP core with covalently attached arabinoxylan, RG-I, and HG, which are linked through the rhamnosyl residue in the AG domain. The appearance of APAP1 has consequences in the cell wall arrangement and functions, including structural support, signaling, and cell-cell adhesion [11].

The review literature shown earlier indicated mutual dependences between components of the extracellular matrix in many kinds of plant cell walls. However, the subject of AGPs in the context of functions in physiological processes in fruits is still unknown. Therefore, the aim of the current study was to describe the distribution of AGP epitopes and their correlation with polysaccharides in the parenchyma tissue in fruits from the ripening process to senescence in postharvest storage. Our previous paper showed that the presence of AGP and arrangement of its epitopes depend on the kind of fruit tissue, fruit quality and storage limitations [12]. In present study, we focused on finding the association between the distribution of AGPs, arabinan, and HG as a crucial component affecting the fruit physiology. The study included a qualitative description of the occurrence of AGPs and pectin epitopes using several monoclonal antibodies (mAbs), determining cellular localization by immunofluorescence labelling (confocal laser scanning microscopy, CLSM) and by immunogold labelling (transmission electron microscopy, TEM). Also, detailed analysis of the quantification of immunogold labelling indicating the amount of AGPs, arabinan and HG epitopes in parenchyma cells were conducted.

2. Material and methods

2.1. Material

Malus x *domestica* (cv. 'Reneta') fruits were purchased from a local producer (Lublin, Poland) approximately a month before the optimal harvest date (green apple fruit, 10th Sept. 2017) and at the optimum maturity for this cultivar (red apple fruit, 10th Oct. 2017). The red apple fruit were stored in cold room at 2 °C in a normal atmosphere for 1 month (6th Nov. 2017) and 3 months (8th Jan. 2018). Apples were selected randomly, and the material was sampled from 3 apple fruits of each examined stage. From every examined fruit, 10 cube-shaped samples of the parenchyma tissue were taken from the depth of ca. 2 cm under the skin.

2.2. Tissue sectioning

The samples of the apple fruits were fixed in a solution of 4% paraformaldehyde (PFA, Sigma Aldrich, St. Louis, MO, USA) and 0.5% glutaraldehyde (Sigma Aldrich) in 0.15 M phosphate buffered saline (PBS, Sigma Aldrich), placed under vacuum for 3 h, and kept overnight. After fixation, the samples were dehydrated in a graded ethanol series from 30% to 99.8% ethanol and embedded in acryl resin LR White (Sigma Aldrich) according to the procedures described previously by Wilson and Bacic [13]. The final embedding in resin LR White was completed by polymerization in gelatin capsules at 55 °C for 48 h. The prepared material was sectioned using a Reichert Ultracut S ultramicrotome (Leica, Wetzlar, Germany) with a glass or diamond knife.

2.3. Immunolabelling for confocal laser scanning microscopy (CLSM)

The immunofluorescence labelling was performed as in our previous work [14]. Semi-thin Sections (1 μm) were placed on poly-L-lysine coated slides (Sigma Aldrich) and circled with a liquid blocker PAP Pen (Daido Sangyo, Tokyo, Japan). The sections were washed in PBS and pre-incubated with 1% bovine serum albumin (BSA, Sigma Aldrich) in PBS for 30 min to avoid nonspecific binding of antibodies. Then, the sections were incubated with the primary antibody adjusted to a 1:50 dilution in 0.1% BSA at 4 °C for 24 h and washed four times with PBS. The monoclonal antibodies recognizing AGP (JIM13, JIM15, MAC207), arabinan (LM16), and HG (LM19, LM20) epitopes selected in this work are generally used in plant biology research. Their characteristics are presented in Table 1. After incubation, the sections were washed four times in PBS and labelled with the secondary antibody. The slides were incubated with the secondary antibody: anti-rat IgG (whole molecule)–FITC antibody produced in rabbit (cat. no. F1763, Sigma Aldrich), diluted 1:50 in a blocking solution at 4 °C in darkness. After 24 h, the sections were washed in PBS twice and finally in deionized water. The observation was carried out using an Olympus BX51 CLSM equipped with corresponding software FluoView v. 5.0. (Olympus Corporation, Tokyo, Japan).

Immunohistochemical reactions were carried out on at least several serial sections from each sample from the apple fruits at different stages of ripening and after storage. All parameters (i.e. laser intensity, gain) were kept constant for all experiments. The excitation wavelength for the FITC was 475 nm, and the emission wavelength was 530 nm. The sections were analyzed against autofluorescence as well.

2.4. Immunogold labelling for transmission electron microscopy (TEM)

Immunogold labelling was performed using a method described previously by Segado et al. [15], Olmos et al. [16], and Sacharz et al. [17] with a few modifications. Ultrathin sections (65 nm) were collected onto formvar-coated nickel square mesh grids (300 MESH), washed in deionized water, and blocked by incubation on a drop of 1% BSA in PBS. The grids were incubated at 37 °C for 3 h with the primary monoclonal antibodies diluted (1:30) in PBS containing 0.1% BSA. After reaction with mAb, the sections were washed in 1% BSA in PBS and re-probed with a secondary anti-rat IgG (whole molecule)–gold antibody conjugated with 10 nm colloidal gold nanoparticles (cat. no. G7035, Sigma Aldrich) diluted (1:50) in PBS with addition of 0.1% BSA at 37 °C for 2 h. The grids were post-stained with 1% uranyl acetate for 5 min and Reynold's reagent for 1 min. The counterstain was followed by three washes in PBS and five washes in deionized water. The samples were examined using a TEM Zeiss EM900 operating at 80 kV acceleration voltage (Carl Zeiss AG, Oberkochen, Germany) and equipped with a digital camera with corresponding software ImageSP v. 1.1.2.5.

Control reactions in both labelling methods were carried out by omitting the primary antibody and keeping the rest of the protocol unchanged. The figures were edited using the CorelDrawX6 graphics