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Colocalization of low-methylesterified pectins and Pb deposits in the apoplast of aspen roots exposed to lead

Irena Rabęda [a], Henryk Bilski [b], Ewa J. Mellerowicz [c], Anna Napieralska [a], Szymon Suski [b], Adam Woźny [a], Magdalena Krzesłowska [a, *]

[a] Laboratory of General Botany, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland
[b] Laboratory of Electron Microscopy, Nencki Institute of Experimental Biology, Pasteur Street 3, 02-093 Warszawa, Poland
[c] Umea Plant Science Center, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, SE-90183 Umea, Sweden

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ABSTRACT

Low-methylesterified homogalacturonans have been suggested to play a role in the binding and immobilization of Pb in CW. Using root apices of hybrid aspen, a plant with a high phytoremediation potential, as a model, we demonstrated that the *in situ* distribution pattern of low-methylesterified homogalacturonan, pectin epitope (JIM5-P), reflects the pattern of Pb occurrence. The region which indicated high JIM5-P level corresponded with "Pb accumulation zone". Moreover, JIM5-P was especially abundant in cell junctions, CWs lining the intercellular spaces and the corners of intercellular spaces indicating the highest accumulation of Pb. Furthermore, JIM5-P and Pb commonly co-localized.

The observations indicate that low-methylesterified homogalacturonan is the CW polymer that determines the capacity of CW for Pb sequestration. Our results suggest a promising directions for CW modification for enhancing the efficiency of plant roots in Pb accumulation, an important aspect in the phytoremediation of soils contaminated with trace metals.

1. Introduction

Trace metal contamination of the environment is a widespread problem in the world. Pb contamination can arise from natural and anthropogenic sources, e.g. the mining technology, heavy automobile traffic in the past, smelting, manufacturing and deposition of agricultural waste in natural and agricultural areas (Ali et al., 2013). Lead is a persistent pollutant and is not degradable (Chapman et al., 2013; Werkenthin et al., 2014) and, in addition, it is one of the most hazardous trace metals affecting living organisms. According to some researchers there is no safe concentration of Pb. Medical studies found that any studied Pb concentration resulted in the impairment of biochemical processes in the brain causing, e.g. the Alzheimer's disease (for review see Bakulski et al., 2012). Therefore, Pb should be removed from contaminated areas, but the choice and application of suitable methods remains an unsolved problem (Sas-Nowosielska et al., 2004; Van Nevel et al., 2007; Ali et al., 2013). One cost-effective and environmentally friendly technology is phytoremediation, whereby plants are used for Pb extraction from the soil or for Pb stabilization in the soil (Salt et al., 1995; Bhargava et al., 2012). Fast growing trees, such as aspens and poplars, are considered to possess a high detoxification and phytoremediation potential (Pulford and Watson, 2003; Shim et al., 2013). However, at present the efficiency of these plants is too low for a practical use (Shim et al., 2013; Ali et al., 2013). For this reason the mechanisms involved in trace metal uptake, transport, accumulation and tolerance are central to the interests of researchers with the prospect of increasing the efficiency of these plants in phytoremediation (Bhargava et al., 2012; Ali et al., 2013).

Plant cell wall (CW) is the compartment which plays a key role in Pb binding and sequestration and is therefore important for the ability of plants to cope with Pb (for review see Krzesłowska, 2011; Ovečka and Takáč, 2014). Among the many compounds building the CW, pectins have been considered as the main polymers responsible for the binding and immobilization of Pb (Dronnet et al., 1996; Inoue et al., 2013). A large amount of Pb is often found in the pectin fraction of the CWs (Hu et al., 2012; Inoue et al., 2013; Wu et al., 2013). Moreover, root binding capacity for trace metal ions is usually higher in dicots than in monocots (Sattelmacher, 2001; Straczek et al., 2008) and the difference is attributed to a higher

* Corresponding author.
E-mail address: magdak@amu.edu.pl (M. Krzesłowska).

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pectin content in the dicot CWs (20–35% of the dry mass) than in monocot CWs (5% of the dry mass) (Pelloux et al., 2007; Vogel, 2008).

Pectins, however, are very complex molecules. Homogalacturonan (HGA) is considered as the main pectin domain responsible for binding trace metal ions (Dronnet et al., 1996; Maychik et al., 2014). This polymer is synthesized in a methylesterified form in the Golgi Apparatus (GA) (for review see Pelloux et al., 2007; Driouich et al., 2012) and exported via a secretion pathway into CWs (Kim and Brandizzi, 2014). In the CW methylesterified HGA is de-esterified *in muro* by cell-wall-bound pectin methylesterases (PMEs) resulting in the formation of free, negatively charged carboxyl groups (Pelloux et al., 2007). This leads to the formation of pectin gels through the Ca^{2+} cross-linking of neighbouring non-methylesterified pectin chains and CW stiffening (for review see Carpita and Gibeaut, 1993; Caffall and Mohnen, 2009). In isolated CWs *in vitro* it has been demonstrated that Pb^{2+}, which shows higher affinity to non-methylesterified pectin chains than Ca^{2+} (Ernst et al., 1992), may replace calcium in cross-linking (Dronnet et al., 1996). Therefore, for many years low-methylesterified homogalacturonans have been thought to be responsible for Pb binding and sequestration within CWs (for review see Krzesłowska, 2011; Ovečka and Takáč, 2014).

However, until now *in situ* evidence that low-methylesterified pectins are really essential in Pb^{2+} binding and sequestration in plant tissues has been lacking. Moreover, it has been recently demonstrated that other CW compounds may also participate in Pb binding. For example, Pb was bound to the ligno-cellulosic fraction in roots of *Juglans regia L.* (Marmiroli et al., 2005). In *Brachiaria decumbens* Stapf., Pb was deposited in the CW as chloropyromorphite crystals (Kopittke et al., 2008), whereas in *Thypha latifolia* L. root tissues (mainly rhizodermis and cortex cells) Pb was colocalized with phosphorus and sulphur suggesting that Pb may form complexes with these elements (Lyubenova et al., 2012). Furthermore, recent results have indicated that the ability of isolated CWs to bind trace metals *in vitro* is much lower than *in vivo* (Guigues et al., 2014). CWs form a continuum with the plasma membrane (PM) and their marked capacity for binding trace metals depends on phosphate groups of phospholipids and carboxyl groups of proteins, especially arabinogalactan proteins, which link CWs and PM (Guigues et al., 2014). It has been shown that a marked accumulation of Zn^{2+} in the hyperaccumulating plant *Solanum nigrum* L., occurs within the PM–CW complex (Samardjieva et al., 2015).

The present study was inspired by observations of uneven distribution of low-methylesterified homogalacturonan epitope recognized by the monoclonal antibody JIM5 (JIM5-P) reported earlier in developing tissues of the root apex in several species, e.g., *Daucus carota* L., *Avena sativa* L. (Knox et al., 1990) and *Beta vulgaris* L. (Guillemin et al., 2005). This distribution was principally limited to some CW domains such as cell junctions (CJ), CW lining the intercellular spaces (ISs) and the corners of ISs. Moreover, in these regions, a high level of Ca^{+2} was detected (Guglielmino et al., 1997). Roots of hybrid aspen, a plant which shows a high detoxification and phytoremediation potential due to its rapid growth, and which serves as a tree model species (Jansson and Douglas, 2007), were used as the experimental system. The detection of a characteristic distribution pattern of JIM5-P in aspen roots provides an excellent model system to examine the relationship between the occurrence of JIM5-P and Pb *in situ*. It is worth noting that plant roots, due to their simple and predictable structural organization and developmental zonation, are considered as an ideal model systems for studying plant adaptations to abiotic stresses (Baluška et al., 1996; Verbelen et al., 2006). Moreover, they are the organs that are directly exposed to the trace metal content of contaminated soils (Ovečka and Takáč, 2014).

Based in these reports we used *in situ* detection techniques to test the hypothesis that low - methylesterified homogalacturonan (pectin epitope JIM5) is one of the main plant CW compounds binding Pb *in vivo*. We examined (1) whether the distribution pattern of JIM5-P in root apex tissues and cells is similar to the patterns described above for other plant species and shows characteristic regions of abundance such as cell junctions or CW lining the intercellular spaces; (2) whether the distribution of JIM5-P corresponds to Pb accumulation pattern, in particular, if the regions which indicate the highest JIM5-P level are simultaneously the sites of highest Pb accumulation and (3) whether JIM5-P and Pb show a tendency for co-localization.

2. Material and methods

2.1. Growth conditions and lead exposure

Hybrid aspen (*Populus tremula* L. *x P. tremuloides* Michx.) plants, clone T89, were propagated vegetatively *in vitro* by shoot cuttings. The cuttings were cultured for two weeks on Murashige and Skoog's (1962) medium before treating with $PbCl_2$, applied as an aqueous solution, up to the final concentration of 1 mM for 4 h. Control material was treated with distilled water. Further analyses were focused on the 3–4 mm apical segments of the roots, because in preliminary experiments these regions showed the highest Pb levels. All experiments were conducted in three replicates.

2.2. Fixation

Root tips were fixed after 4 h of Pb treatment and dehydrated using an ethanol series as previously described (Krzesłowska et al., 2009). Dehydratated material was embedded in LR Gold (Sigma) or LR White (Polysciences). Tissue specimens were sectioned with a diamond knife on the ultramicrotome (Leica Ultracut EM UC6, Vienna, Austria) into semi-thin and ultra-thin sections. Semi-thin sections were transferred onto Poly-L-lysine (Sigma Aldrich) covered microscope slides and used for pectin and Pb localization in light and confocal microscopy. Ultrathin sections were collected on nickel grids (Polysciences) coated with 0.3% Formvar (Sigma) for pectin and Pb localization in transmission electron microscopy (TEM).

2.3. Immunolabelling of low-methylesterified pectin epitope

Detection of low-methylesterified pectin epitope (JIM5-P) was carried out using primary monoclonal antibody (mAb) JIM5 (Plant Probes). The immunolabelling procedure was carried out according to Lenartowska et al. (2001). For detection of JIM5-P in fluorescent and confocal microscopy secondary anti-rat IgG Cy™3 conjugated antibody (Jackson ImmunoResearch Laboratories, Inc.) was used. After the immunolabelling procedure the material was mounted in Citifluor anti-fade mounting medium (Citifluor Ltd.). Negative controls were performed with the omission of incubation with primary antibodies.

Identification of JIM5-P in TEM was done by primary mAb JIM5 and the secondary anti-rat IgG-gold conjugate (10 nm particles; BioCell). Control of the reaction was performed with the omission of incubation with the primary antibody.

2.4. Identification of Pb by light microscopy and TEM

In light microscopy, Pb was detected using sodium rhodizonate ($C_6O_6Na_2$) which forms a scarlet coloured precipitate with lead (Glater and Hernandez, 1972). For Pb detection at the tissue level,