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Pb induces plant cell wall modifications - in particular - the increase of pectins able to bind metal ions level

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Abstract. Low - methylesterified pectin fraction, able to bind metal ions, is the cell wall compound which participates in land and water plant cell response to toxic metals. Protonemata of Funaria hygrometrica (Hedw.), root tips of Populus tremula x P.tremuloides and Lemna trisulca fronds, were used for studying the effects of Pb on plants cell walls (CW). The study were focused on the low- methylesterified pectins level and distribution. It was carried out by immunocytochemical methods, using JIM5 antibody which recognized low-methylesterified pectins fraction - up 40%. Pb exposure resulted in the cell wall modifications in all investigated objects. The most striking result was the marked increase of the low-methylesterified pectins level. Moreover, cell walls thickenings were formed both in the moss protonemata and the poplar roots. The cell wall thickenings in both objects contained especially high level of low-methylesterified pectins. Simultaneously, cell wall thickenings accumulated extremely large and numerous Pb deposits. In many regions of the cell wall and cell wall thickenings the colocalization of low- methylesterified pectins and Pb deposits occurred. Low - methylesterified pectins level increased also in the cell walls of Lemna trisulca fronds and some of Pb deposits were colocalized with this pectin fraction in the CW. In fronds several Pb deposits occurred between plasma membrane and cell wall and only occasionally they were colocalized with low-methylesterified pectins. However, in L. trisulca - cell wall was generally thicker in response to Pb. We did not observed almost any local cell wall thickenings as in Funaria and Populus. Taken these facts together we can conclude that plant cell walls were actively and intensively modified in response to Pb. In particular, the amount of low - methylesterified pectins, able to bind toxic Pb ions, markedly increased. Simultaneously, both cell wall and cell wall thickenings were the compartments which accumulate large amount of Pb. Hence, modified cell walls appear to be a very important repository for Pb^{2+} in different types of plant cells and different species. Detection of such a reaction in three different plant species and three different types of plant cells indicates that it may be more common plant tolerance strategy to Pb.

Key words: heavy metal, cell wall modification, tolerance

Introduction

Lead pollution has become an increasingly serious environmental problem over recent decades due to its toxicity and the susceptibility of the environment. It leads to many detrimental effects in living organisms including plants. Plants are sessile and, unlike animals, are unable to escape contaminated areas. Therefore they have developed many defense reactions to survive (Patra et al., 2004). One of the most common is the deposition of toxic trace metals within the CW. The essential capacity of the CW for binding metal cations depends mainly on the amount of pectins abundant in carboxyl groups Among many pectin fractions probably only the low-methylesterified pectins, up to 40%, (JIM5 pectin epitope) are able to bind trace metals. It has been shown that JIM5 pectin epitope can interact through calcium bridging between two free carboxyl groups (Grant et al., 1973).

Many trace metals, especially Cu and Pb, show higher affinity to the pectin fraction than Ca. When plants grow in contaminated areas then trace metals can replace Ca in the "egg-box" structure and are bound by pectins within the CW (Dronnet et al., 1996). Therefore, Pb is preferentially accumulated in the CW where it often forms large and numerous deposits (Kopittke et al., 2007, 2008; Meyers et al., 2009).

Recent studies concerning different plant species have shown that plant CW can be modified in response to trace metals (for review Krzesłowska 2011). Modifications of the CW mainly concern an increase in the amount of polysaccharides (in particular pectins). The modifications of the CW and CWts formation were detected in our previous studies which dealt with *Funaria hygrometrica* protonemata response to Pb (Krzesłowska et al., 2009, 2010). The CWts were different in structure and composition than control CW surrounding the tip of apical cells. One of the most striking difference was the extremely large amount of JIM5 pectin epitope able to bind trace metals - absent from control apical CW. In CWts JIM5 pectin epitope co-occurred with numerous and large Pb deposits and occupied the largest area of the CWts (Krzesłowska et al., 2009, 2010). Thus, CWts formation increased CW capacity for Pb accumulation.

Taking all the above facts into consideration we put forward the hypothesis that the ability of CWts formation to increase the capacity for Pb^{2+} accumulation could be a defense strategy not limited to one plant species only but is universal.

Material and Methods

Land and water plants were use to verify the hypothesis: protonemata of *Funaria hygrometrica* (Hedw.), root tips of *Populus tremula* x *P.tremuloides* and *Lemna trisulca* fronds. *F. hygrometrica* and *Populu*s both were treated with 1000µMPb (supplied as $PbCl_2$) for 4h, while *L. trisulca* was treated with 15µMPb (supplied as $Pb(NO_3)_2$) for 12h. The concentrations for metal exposure were based on a preliminary tests (Krzesłowska et al., 2010; Samardakiewicz et al., 2012).

Detection of low-methylesterified pectins (JIM5 pectin epitope) in TEM was carried out by applying the primary monoclonal antibody (mAb) JIM5 and the secondary anti-rat IgG-gold conjugate 10nm, (BioCell) on the ultrathin sections.

Lead was detected in TEM as very characteristic deposits easy to recognise, because of its irregular shape and extremely high-electron density.

Ultrastructural observations were conducted in a transmission electron microscope (TEM) JEM – 1200 EXII JEOL, Tokyo, Japan.

Results and Discussion

Pb exposure resulted in the cell wall modifications in all investigated objects. The most striking result was the marked increase of the low-methylesterified pectins level. In protonemata the CW was the plant cell compartment which preferentially accumulated Pb In response to this metal, protonemata additionally modified their CW. The CWts were formed mainly at the tip of the cell. Moreover, the lateral CWs were locally thickened in numerous sites. The CW of the Pb exposed material differed from the CW of the control which generally did not indicate such modifications.

Thickened CW, both tip and lateral were especially abundant in the JIM5 pectin epitope, indicated by gold particles and preferentially accumulated Pb deposits. Moreover, the general level of this pectin epitope in *Funaria* protonemata cell walls increased.

In poplar root cells the CWs were modified in many sites. In several regions CWts occurred. The thickenings were characterized by different sizes and shapes but were generally the CW regions that indicated the highest level of Pb deposit concentration. In some of them Pb deposits as often as not occupied almost the entire CWts. Moreover, the CWTs contained high levels of JIM5 pectin epitope. Thus, the colocalization of Pb deposits and JIM5 pectin epitope occurred. The CWts were formed in various regions of the CWs. Mostly at the CW surrounding the IS, at CW connections of a few cells, at the cross CWs.

Low - methylesterified pectins level increased also in the cell walls of *Lemna trisulca* fronds and some of Pb deposits were colocalized with this pectin fraction in the CW. In fronds several Pb deposits occurred between plasma membrane and CW and only occasionally they were colocalized with low-methylesterified pectins. However, in *L. trisulca* - cell wall was generally thicker in response to Pb. We did not observed almost any local CWts as in *Funaria* and *Populus.*

The examined cells belonged to different plant species moss *Funaria hygrometrica*, flowering plant *Populus tremula x P. tremuloides* and water flowering plant *Lemna trisulca*. Therefore, the detection of a highly similar response in such different types of plant cells to Pb indicated that it could be a universal strategy in plant cell defense response to this metal.

Such a conclusion would also support the results of the recent studies concerning plant cell defense responses to trace metals including CW modifications, especially pectins and hemicelulose increase in different plant species, and also CWts formation (for review Krzeslowska 2011).

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References

Dronnet VM, Renard CMGC, Axelos MAV, Thibault JF. Heavy metals binding by pectins" selectivity, quantification and characterization. Carbohydr Polym 1996;30:253–263.

Grant GT, Morris ER, Rees DA, Smith PJC, Thom D. Biological interactions between polysaccharides and divalent cations: the “egg-box” model FEBS Lett 1973;32:195–198

Kopittke PM, Asher CJ, Kopittke RA, Menzies NW. Toxic effects of Pb^{2+} on growth of cowpea (*Vigna unguiculata*). Environ Pollut 2007;150: 280-287.

Kopittke PM, Asher CJ, Blamey FP, Auchterlonie GJ, Guo YN, Menzies NW. Localization and chemical speciation of Pb in roots of signal grass (*Brachiaria decumbens*) and Rhodes grass (*Chloris gayana*). Environ Sci Technol 2008;42:4595-4599.

Meyers DE, Kopittke PM, Auchterlonie GJ, Webb RI. Characterization of lead precipitate following uptake by roots of *Brassica juncea*. Environ Toxicol Chem 2009;28:250-255.

Krzesłowska M., Lenartowska M., Samardakiewicz S., Bilski H., Woźny A. 2010. Lead deposited in the cell wall of *Funaria hygrometrica* protonemata is not stable – a remobilization can occur. Environmental Pollution 158: 325–338.

Krzesłowska M, Lenartowska M, Mellerowicz EJ, Samardakiewicz S, Woźny A. Pectinous cell wall thickenings formation – a response of moss protonemata cells to Pb. Environ Exp Bot 2009;65:119–131.

Krzesłowska M. The cell wall in plant cell response to trace metals: polysaccharide remodeling and its role in defense strategy. Acta Physiol Plant 2011;33:35–51.

Patra M, Bhowmik N, Bandopadhyay B, Sharma A. Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environ Exp Botany 2004;52:199–223.

Samardakiewicz S., Krzesłowska M., H. Bilski., Bartosiewicz R., Woźny A. 2012. Is callose a barrier for lead ions entering *Lemna minor* L. root cells? Protoplasma 249: 347-351.