Research article

Impact of cadmium on early stages of flax fibre differentiation: Ultrastructural aspects and pectic features of cell walls

Olfa Douchiche [a,*], Azeddine Driouich [a], Claudine Morvan [b]

[a] Laboratory Glyco-MEV EA 4358, IFRMP 23, University of Rouen, 76821 Mont Saint Aignan Cedex, France
[b] Laboratory PBS-UMR 6270 CNRS, FR 3038, University of Rouen, 76821 Mont Saint Aignan Cedex, France

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ABSTRACT

The effect of 0.5 mM cadmium (Cd) was studied on the ultrastructural aspects and pectin features of the walls of flax cellulosic fibres when the thickening of secondary wall had just started in the hypocotyl of 10-day old seedlings. As seen by PATAg staining in controls, cell-wall formation displayed two distinct steps, secretion and remodelling, which did not occur simultaneously for all the neighbouring fibres. The inner part of the secondary wall, where the cellulose molecules had just been synthesized, appeared very reactive to PATAg. The outer part, where the cellulose fibrils associated in larger microfibril complexes, became non-reactive to PATAg. Under Cd treatment, we noticed some acceleration of fibre differentiation in terms of fibre number, wall thickness and yield. As revealed by PATAg staining, treated fibres exhibited a disturbed cell-wall texture, indicating a modified adhesion between the matrix polysaccharides and the cellulose microfibrils. The Cd impact on the distribution of highly methylesterified homogalacturonans (recognized by JIM7 antibody) was moderate in the cell junctions and low in the primary wall and outer part of secondary wall. The data meant that no early deesterification occurred in these domains, a behaviour related to the specificity of the CW-II metabolism. No large redistribution of low esterified homogalacturonans (recognized by JIM5 antibody) happened either. In parallel, the amount of uronic acid significantly increased in the so-called H_2SO_4 cell-wall extract, indicating a Cd impact on pectin structure not detected by JIM5 or JIM7 antibodies.

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1. Introduction

Flax hypocotyl is being considered as a laboratory model because i) hypocotyl and stem share many anatomical traits and have similarities in the composition of their cell-wall (CW) [1], and ii) common transcripts were identified associated with specific stages of hypocotyl development, in which phloem fibre cells were undergoing thickening of secondary wall [2]. As in stem, the cellulosic fibres of hypocotyl mature centripetally and their secondary wall contains mainly cellulose [3]. Both types of pectins, rhamnogalacturonan of type I (RG-I) and homogalacturonans (HGA) were also abundantly detected [1,4]. According to Alix et al. [5], these non-cellulosic polymers of the secondary wall contribute significantly to the physical properties of fibres. The differentiation of cellulosic fibres is a complex process that has been described in several stages, spatio-temporally separated in flax stem [6–8]. Some of these steps were also reported in the hypocotyl [1,4] including: i) synthesis of cellulose fibrils (3-6 nm) in the highly

hydrophilic matrix, that have been shown to be not reactive with cellobiohydrolase but highly reactive with uranyl acetate or PATAg (periodic acid thiocarbohydrazide silver proteinate), ii) remodelling of the matrix polymers and iii) association in the external part of CW-II of the fibrils in larger microfibril complexes, which have become reactive with cellobiohydrolase and non-reactive to PATAg.

Changes in the composition and synthesis of the wall can occur under environmental stresses, including heavy metals. For example, exposition to lead resulted in an increase of pectin level in *Funaria hygrometrica* protonemata [9]. Pectins were also observed to increase in *Salix viminalis* exposed to Cd [10]. According to Krzesłowska [11], high abundance of pectins and hemicelluloses increased the capacity of the CW to fix the metal, while the stimulated synthesis of callose and lipids limited the entrance of the metal into the cell. However, the most striking CW modifications that were reported concerned the abundance and distribution of methylesterified pectins. For example, in response to Al stress, it was found that the content of low and high methylesterified pectins in maize roots was correlated with the Al-sensitivity or tolerance of the genotype [12]. In *Funaria hygrometrica* protonemata tips, low

* Corresponding author.
E-mail address: olfa.douchiche@univ-rouen.fr (O. Douchiche).

methylesterified (JIM5 epitopes) and unesterified (PAM1 epitope) pectins were newly detected in the wall after exposition to Pb [9]. The impact of metals on cellulose appears to vary in different contexts. Some data showed an increase of cellulose in the thickened wall of Pb-treated protonemata tips [13] whereas Xiong et al. [14] reported a decrease of cellulose content in Cd-treated *Oryza sativa*. Under Cu-enriched condition, the cellulosic residue slightly increased in a metal-tolerant moss *Scopelophila cataracta* but the main remodelling dealt with moities rich in arabinose or galactose [15].

In the course of our studies on the effect of Cd on the CW of flax seedlings, we also observed specific responses of the CW related to the ultrastructure and pectin distribution [16,17]. Interestingly, flax hypocotyl offers the possibility to compare primary CW (CW-I) of cortical tissues and secondary CW (CW-II) from cellulosic fibre tissue. In both tissues of Cd treated seedlings, low-methylesterified pectins were compartmentalized in the outer part of the wall while the high methylesterified pectins (JIM7 epitopes) were immobilized in the inner part [16], probably to keep Cd away from the plasma membrane vicinity. In the primary tangential wall of the epidermis, marked changes in architecture, with polysaccharides deposited in an irregular fashion, appeared when sections had been stained with PATAg test [17]. Our study on fibres differentiated in 18-day old (d18) hypocotyls developed in the presence of Cd [16] indicated that the mechanisms which led to the compartimentalization of pectins were different from those reported in epidermis and cortical tissues. In fibres, only a moderate decrease of JIM7 labelling was found in the junctions; more importantly, there was a net increase of JIM7 labelling in the inner part of CW-II which was not observed in cortical tissues. Consequently, the questions raised in this paper deal with the impact of Cd on the fibre developmental process: how the architecture of CW-II is altered? How the homogalacturonan moities are affected?

To answer these questions, it was necessary to investigate early effects of the metal, as soon as the first deposition of secondary wall happened in fibres. We previously reported [3] that, in flax cultivated in "hydroponic" conditions the deposition of secondary walls began when the seedlings had been grown for ~ 10 days (d10). We therefore chose to study the impact of Cd at d10. The alterations of polysaccharide deposition and the fine structure of CW-II were observed using PATAg test. The effects on homogalacturonan distribution were vizualised using JIM5 and JIM7 antibodies. Pectin composition was also studied. To discuss the data in term of developmental process of fibre, we compare the d10 results to those obtained at d18 [16].

2. Results

2.1. Anatomical organization of flax fibres and effect of cadmium

Transverse sections in the middle of d10 hypocotyl showed the organization of differentiating fibres located between a thin-walled parenchymatous tissue in one side and phloem cells in the other (Fig. 1A). Fibres consisted of thick-walled cells of various shapes, arranged in a single layer that followed the prominent arc of the nearby xylem tissue.

As a first impact of Cd, a second layer of fibres started to differentiate on the side of the existing bundle nearest to the xylem (Fig. 1B). These fibres appeared to have a compressed and flattened shape in contrast to the first-differentiating layer fibres, which appeared polygonal or round. The maximum number of fibres was the highest in the presence of Cd (Fig. 1C) although the mean number of thickened fibres did not significantly increase (51 ± 14). The number of fibres increased with the hypocotyl length, with no thickened fibre when hypocotyl length was <0.5 to 1 cm in Cd treated seedlings and controls, respectively. Due to the reduction of the hypocotyl length, the slope was much larger in the presence of Cd.

2.2. Ultrastructure of early-differentiated fibre-cells

Control fibres, observed in transmission electron microscopy, appeared as living cells with a well-developed multivesicular cytoplasm, indicating a high metabolic activity (Fig. 2A,B). Mitochondria with cristae and matrix as well as chloroplasts with numerous compartmentalized grana stacks and plastoglobules were often observed (Fig. 2A). Small non-contrasted vesicles as well as electron-dense vesicles were also present in the cytoplasm. Interestingly, newly secreted polymers appeared with a vesicular shape in the inner part of the wall (Fig. 2B). The average thickness of the compound middle lamella (CML, defined as the layer including the middle lamella and both adjacent primary walls) was estimated to be 9 ± 1 nm (Table 1). The CML appeared highly contrasted, with PATAg staining indicating high abundance of polysaccharides (mainly pectins) with free OH (Fig. 2C). In the core of tri-cellular junctions, the staining was less intense.

The cell-wall thickness of control fibres was in the range of 1 μm although some variability was observed (Fig. 2). The PATAg test allowed us to distinguish between two main types of CW-II ultrastructure with different reactivity, accounting for 41 and 52% of the observed fibres, respectively (Table 1). The first type was characterized by homogeneous reactivity and only moderate staining (called monolayered reactivity; see S2HR in Fig. 2A,D). ii) A second type of CW-II ultrastructure displayed two differently stained parts (designated as bi-layered reactivity; see fibre F2 in Fig. 2D). In the latter type, the inner layer was generally more electron opaque due to either a large amount of newly synthesized hydrosoluble matrix polysaccharides or to non-crystalline cellulose [6]. Discrete lamellations could be distinguished in this layer (Fig. 2C) which suggested that the synthesis of cellulose and matrix polysaccharides alternated. The outer layer appeared weakly contrasted as it was mainly constituted of crystalline cellulose (where the vic-glycol functions are engaged into hydrogen bonds [18]).
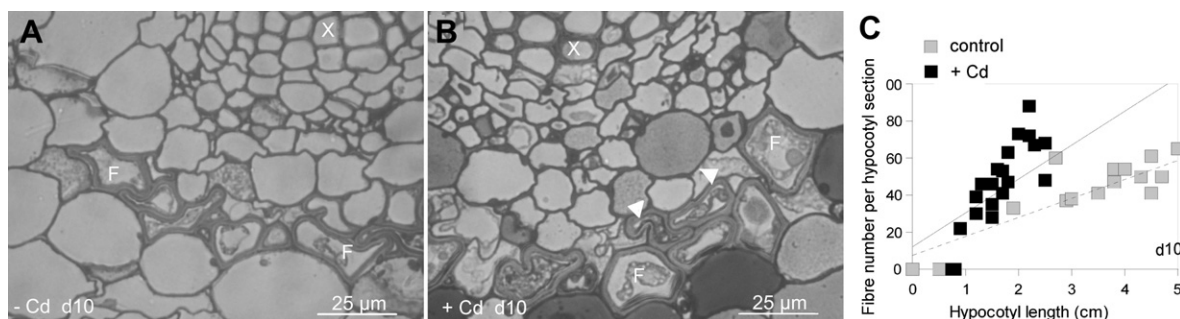

Fig. 1. Differentiation of fibres in d10 flax hypocotyl. (A-B) Micrographs of semi-thin sections in the absence (A) and presence (B) of cadmium (Cd). White arrows pointed out the second layer of fibres. F: fibre; X: xylem. (C) Variation of the number of fibres in control (grey symbol) and treated (black symbol) hypocotyls as a function of hypocotyl length (cm).