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ORIGINAL ARTICLE

Purification and characterization of a soluble β-1,4-glucan from bean (*Phaseolus vulgaris* L.)-cultured cells dehabituated to dichlobenil

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Abstract Bean cells habituated to grow in the presence of dichlobenil exhibited reduced cellulose and hemicellulose content and an increase in pectic polysaccharides. Furthermore, following the extraction of pectins and hemicelluloses, a large amount of neutral sugars was released. These sugars were found to be part of a soluble β-1,4-glucan in a preliminary characterization, as reported by Encina et al. (Physiol Plant 114:182–191, 2002). When habituated cells were subcultured in the absence of the herbicide (dehabituated cells), the release of neutral sugars after the extraction of pectins and hemicelluloses was maintained. In this study, we have isolated a soluble β-1,4-glucan from dehabituated cells by sonication of the wall residue (cellulose fraction) remaining after fractionation. Gel filtration chromatography revealed that its average molecular size was 14 kDa. Digestion of the sample with endocellulase revealed the presence of cellobiose, cellotriose, and cellotetraose. Methylation analysis showed that 4-linked glucose was the most abundant sugar residue, but 4,6-linked glucose, terminal arabinose and 4-linked galactose for xyloglucan, and arabinogalactan were also identified. NMR analysis showed that this 1,4-glucan may be composed of various kinds of substitutions along the glucan backbone together with acetyl groups linked to the OH group of sugar residues. Thus, despite its relatively high molecular mass, the β-glucan remains soluble because of its unique configuration. This is the first time that a glucan with such characteristics has been isolated and described. The discovery of new molecules, as this β-glucan with unique features, may help understand the composition and arrangement of the polymers within plant cell walls, contributing to a better understanding of this complex structure.

Keywords Cellulose · Dehabituated cells · Dichlobenil · Soluble glucan · Xyloglucan

Abbreviations

DCB	Dichlobenil
XyG	Xyloglucan
snCR	Supernatant of cellulose residue

Introduction

Cellulose is the most abundant organic compound on Earth because of its high molecular stability and the fact that it is the main constituent of plant cell walls (Hon 1994; Álvarez et al. 2012, and references therein). It is also present in most algae, a large number of bacteria and, in the animal kingdom, in tunicates (De Leo et al. 1977). This polysaccharide is composed of linear chains made of anhydroglucopyranose units joined together by β-1,4-glycosidic bonds. Anhydrocellobiose is the repeating unit of cellulose,

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as adjacent anhydroglucose molecules are rotated 180° with respect to their neighbors. This rotation causes cellulose to be highly symmetrical, because each side of the chain has an equal number of hydroxyl groups. The coupling of adjacent cellulose molecules by van der Waal's forces and interchain (2 per glucose) and intrachain (2–3 per glucose) hydrogen bonds produces straight, stable supramolecular fibers of great tensile strength (Zhang and Lynd 2004). The crystalline state of cellulose is determined by the arrangement of the glucan chains with respect to each other. In nature, most cellulose is produced as crystalline cellulose and is defined as cellulose I (Saxena and Brown 2005), in which glucan chains are parallel to each other. The polymerization degree of cellulose (number of glucose molecules per glucan chain) is between 2,000 and 6,000 in the case of the primary cell wall of cotton fibers (Arioli et al. 2000). Additionally, cellulose may exist as a non-crystalline molecule, which is also present in cellulose microfibrils (Zhao et al. 2007).

Despite its abundance and relatively simple structure, cellulose biosynthesis process is not clearly understood nowadays (see Guerreiro et al. 2010; Carpita 2011 for recent reviews). The difficulty in synthesizing cellulose in vitro may be one of the most important challenges to definitively unravel the process. On the one hand, plant cellulose synthases have traditionally been difficult to study in vitro; on the other hand, the need of a careful analysis of synthesized products (because of the abundant synthesis of callose, β-1,3-glucan, together with cellulose), makes impossible to routinely assay cellulose synthesis in a high-throughput manner (Guerreiro et al. 2010). In addition, glucan chains composed of 7 or more glucosyl residues precipitate (Tonnesen and Ellefsen 1971), and the crystallinity of cellulose synthesized in vitro is higher than that of the microfibrils produced in vivo (Lai Kee Him et al. 2002). The discovery and analysis of several plant mutants with altered cellulose biosynthesis have produced a notable increase in the knowledge of this process. Nevertheless, several aspects remain unclear, including the crystallization of glucan chains. No protein that might be directly involved in such crystallization has been identified. It is known that the process does not occur immediately and spontaneously after the extrusion of glucan chains from the cellulose synthase, because treatment with agents that bind to glucan chains (e.g., calcofluor, Congo red, xylans, or carboxymethyl cellulose) prevents the correct crystallization of cellulose, provoking a higher polymerization degree of the molecule (Haigler 1991).

In some cases, the reduction in crystalline cellulose content has been accompanied by the accumulation of a soluble β-1,4-glucan. In the *Arabidopsis* temperature-sensitive mutant *rsw1*, this phenomenon has been associated with the disorganization of cellulose synthase rosettes. The authors suggested that the non-crystalline β-1,4-glucan comes from glucan chains, the assembly of which have been interrupted, hypothesizing that the correct conformation of terminal complexes is necessary for microfibril crystallization (Arioli et al. 1998). The accumulation of non-crystalline glucan also occurred in the *rsw2* mutants of *Arabidopsis* (allelic to the KORRIGAN gene) with an altered β-1,4-endoglucanase, suggesting that this enzyme is necessary for cellulose crystallization (Lane et al. 2001). On the other hand, treatment of cotton fibers with the herbicide CGA 325′615 provoked the inhibition of cellulose biosynthesis concomitantly with the accumulation of non-crystalline β-1,4-glucan (Peng et al. 2001). The authors found that the glucan was soluble because of its linkage to proteins, suggesting a bond with cellulose synthase subunits. These links may exist naturally or may have been created by the herbicide action. In this work, treatment with dichlobenil (2,6-dichlorobenzonitrile or DCB) did not promote the accumulation of soluble glucan (Peng et al. 2001). In contrast, bean cells habituated to grow in lethal levels of DCB, and presenting reduced amounts of cellulose and a concomitant increase in pectins (Encina et al. 2001, 2002; Alonso-Simón et al. 2004), released large amounts of glucose after the extraction of pectic and hemicellulosic polysaccharides (Encina et al. 2002). This glucose-rich fraction was stained by calcofluor white, a specific dye for β-linked fibrillar polymers (Hughes and McCully 1975), and the methylation analysis and other tests revealed that 95 % of the links were β-1,4- (Encina et al. 2002). When habituated cells were subcultured again without DCB (dehabituated cells), the cellulose content of cell walls increased up to levels similar to non-habituated (control) cells, although some features of habituated cells remained (Encina et al. 2002; García-Angulo et al. 2006, 2009). For example, the release of a glucose-rich fraction following the extraction of pectins and hemicelluloses from the cell wall (Encina et al. 2002) was observed, and some calcofluor-positive stained cell wall appositions were detected, suggesting the accumulation of an amorphous cellulose (García-Angulo et al. 2006).

It must be considered as well that β-1,4-glucan does not only appear in cellulose, but also in xyloglucan (XyG) backbone (Hayashi 1989b). This polysaccharide is the most abundant hemicellulose of most vascular dicot plants, and the XyG backbone has been proposed to work as a tether between cellulose microfibrils, contributing to cell wall rigidification (when it crosslinks adjacent microfibrils) or loosening (when it degrades) (Hayashi 1989b; Hayashi and Kaida 2011). Therefore, XyG plays a crucial role in plant cell wall architecture and plant cell enlargement in most plant cell wall models (Hayashi 1989b; Carpita and Gibeaut 1993; Cosgrove 2005). In addition to modifications regarding cellulose in DCB-habituated and -dehabituated

cells, hemicellulose content and structure also varied. In this way, XyG from habituated cells showed different fucosylation level, and when XyG was enzymatically digested, some cello-oligosaccharides characteristic of cellulose appeared (Alonso-Simón et al. 2010). These results would allow us to hypothesize that the β-1,4-glucan detected in previous work (Encina et al. 2002; García-Angulo et al. 2006) might be a low substituted xyloglucan rather than a non-crystalline cellulose.

Additionally, some studies with Arabidopsis mutants showed plants that, despite having cell walls lacking detectable xyloglucan, did not show severe phenotype, just aberrant root hairs (Cavalier et al. 2008). In this same work, a XyG with lower Xyl:Glc ratio, or a β-1,4-glucan backbone (synthesized by XyG glucan synthase) were proposed as substitutes of XyG in mutant cell wall to preserve its features. Both possibilities match the likely features of the glucan that have been repeatedly detected during habituation and dehabituation of bean cells to DCB.

The aim of this work was to isolate and characterize the soluble β-1,4-glucan from dehabituated cells, which showed higher growth rates than their DCB-habituated counterparts. The knowledge of this molecule may broaden our understanding of plant cell wall arrangement and structural plasticity.

Materials and methods

Plant material

Bean (*Phaseolus vulgaris* L. var. cannellini, from a local grocer in Santa Maria del Paramo, Leon, Spain) cells habituated to grow in 12 μM DCB for 8 subcultures were repeatedly subcultured in the absence of herbicide, at 27 °C on Murashige and Skoog medium (Murashige and Skoog 1962) solidified with 8 g l^{-1} agar, containing 30 g l^{-1} sucrose and 10 μM 2,4-dichlorophenoxyacetic acid (2,4-D). Cells were routinely subcultured for 30-day periods. These cells were designated the dehabituated cells (Encina et al. 2002). Cells were harvested at the end of the exponential growth phase and kept at −20 °C.

Cell wall fractionation

Dehabituated frozen cells (1,900 g FW, approximately), were homogenized with liquid nitrogen in a mortar. Homogenized cells were washed with 100 mM sodium phosphate buffer, pH 7.0, for 15 min (2×) at room temperature; 100 mM ethylenediamine tetraacetic acid (EDTA), pH 7.0 for 3 h at 85 °C (4×); and 24 % KOH (w/w) containing 0.1 % $NaBH_4$ for 3 h at 40 °C (4×). Pellets were always obtained by centrifuging at 15,000*g* for 10 min. After KOH incubation, the pellet was resuspended in distilled water and its pH adjusted to 5.0 with concentrated acetic acid; it was centrifuged and the supernatant was considered the snCR (SuperNatant of Cellulose Residue) fraction. The pellet was again resuspended in distilled water and sonicated for 2 min, 50 % intensity (8×) using a Branson 250 sonifier. This supernatant was designated the snCR-2 fraction and the final pellet was considered to be α-cellulose.

The snCR-2 fraction was dialyzed against distilled water for at least 3 days at 4 °C, changing the water every 3 h. The pore size of the dialysis membrane was 2 kDa. Subsequently, the fractions were concentrated in a rotary evaporator and treated with salivary α-amylase and proteinase K for 12 h at 39 °C and again dialyzed against distilled water for 3 days under the same conditions as described above. The sample was concentrated to a volume of 50 ml or less, frozen and freeze-dried.

Chromatography

Thin layer chromatography (TLC) was performed on aluminium sheets coated with silica gel F_{654} (Merck). The solvents used were 1-propanol:ethyl acetate:water (6:1:3, by vol.) and ethyl acetate:pyridine:water (10:4:3, by vol.), drying the TLC plate between both developments; and butanol:acetone:water (4:5:1, by vol.) (2×). Before TLC, aliquots of each fraction were dissolved in 1 mM sodium acetate buffer, pH 5.85, and centrifuged to separate soluble and insoluble parts. Later, the fractions were incubated with endocellulase from *Streptomyces* B514 (50 U) or XyG-specific endoglucanase (XEG) at 37 °C for 8 h, adding 1 μl of toluene to avoid contamination. The sample was then treated with DOWEX resin, H^+ and OH^- forms, to eliminate ions that could interfere in the separation of sugars, and filtered to eliminate the resin. The sample was frozen, freeze-dried, resuspended in a minimum volume of 10 μl of distilled water, and applied to TLC plates. Solutions of glucose, xylose, cellobiose, cellotriose, cellotetraose, and cellopentaose 1 % (w/v) in water:ethanol 1:1 (v/v) were used as markers. *Tamarindus* XyG digested by XEG was used as marker for oligosaccharides derived from XyG.

For gel filtration chromatography, Sepharose CL-6B columns (Pharmacia) (100 cm × 2.5 cm) with 0.1 M KOH as eluent were used. The Sepharose columns were calibrated using blue dextran and dextrans of 267, 72.6, 8.8 kDa, and glucose. The molecular size (Ms) of XyG was calculated as follows:

$$Ms = \frac{\sum (M_i \times X_i)}{\sum X_i}$$

where M_i is the molecular size corresponding to each fraction and X_i is the amount of XyG eluted in each fraction.

A DEAE A-25 Sepharose (Pharmacia) column (25 cm × 2.5 cm) was used for ion exchange chromatography. The eluent used was 10 mM sodium phosphate buffer, pH 7.0. Neutral sugars (not bound to the gel) were detected by the phenol–sulphuric acid method (DuBois et al. 1956). Samples of snCR-2 fraction containing 400–500 μg of neutral sugars were hydrolyzed in 2 N TFA for 1 h at 120 °C. The released neutral sugars were derivatized to alditol acetates and analyzed by gas chromatography.

Zone electrophoresis

Electrophoresis was performed on Toyo GA-100 glass filter paper at 1,350 V for 70 min with 0.1 M sodium tetraborate (pH 9.3). After electrophoresis, the filter paper was cut into strips that were then eluted with water. Carbohydrates in the eluted solution were determined by the phenol–sulphuric acid method (DuBois et al. 1956).

Linkage analysis

Samples of the snCR-2 fraction containing 500 μg of neutral sugars were methylated following the procedure initially described by Hakomori (1964) and modified by Sandford and Conrad (1966). The methylated sugars were derivatized to alditol acetates (Hayashi 1989a) and analyzed in a gas chromatograph connected to a mass spectrometer.

Nuclear magnetic resonance

The sample was dissolved in D_2O solution at a concentration of 0.1 %. Spectra were recorded using a 700-MHz NMR spectrometer (JNM-ECA700, Jeol). For ^{1}H NMR, a 45-degree observe pulse was used to record 256 transients of 16,384 data points with a relaxation delay of 5 s. The recorded spectrum was modified with the Dante presaturation method to remove signals resulting from water. ^{13}C NMR spectra were recorded with a single pulse decoupled for D_2O (sample) with an acquisition time (AQ) of 0.59 s and relaxation delay of 2 s.

Results

DCB-dehabituated bean cells were homogenized in liquid nitrogen and their cell walls were fractionated, thereby extracting pectins and hemicelluloses. After hemicellulose extraction with NaOH, the remaining pellet was resuspended in distilled water and adjusted to pH 5.0 with glacial acetic acid. After centrifugation, the supernatant was considered the snCR (supernatant of the cellulose residue) fraction. A soluble glucan was identified and isolated from this fraction, but in an insufficient amount for correct characterization (data not shown). The final insoluble residue (cellulose fraction) was again resuspended in distilled water and subjected to sonication, with the aim of inducing the release of a larger amount of soluble β-1,4-glucan, since ultrasound enhances polysaccharide extraction (Ebringerova and Hromádková 2010). The obtained supernatant was treated with amylase and protease and then dialyzed, and was designated the snCR-2 fraction. This fraction was subjected to anion exchange chromatography on DEAE-Sephadex to remove the minor uronic acids detected in the fraction (data not shown). The fraction eluted from DEAE-Sephadex was dialyzed and freeze-dried, and used for further analysis.

An aliquot from the sample was dissolved in distilled water. A small part of the sample did not dissolve, and it was separated by centrifugation. Both (soluble and insoluble parts) were digested with endocellulase or XEG. The products of the digestions, as well as aliquots of non-digested samples, were subjected to TLC, to detect cello-oligosaccharides which would indicate the presence of a soluble glucan. Chromatography revealed spots identified as glucose, cellobiose, cellotriose and even cellotetraose corresponding to endocellulose-digested soluble aliquot (Fig. 1), but no signs of XyG oligosaccharides were detected. In addition, no cello-oligosaccharide was detected from insoluble or non-digested samples, supporting that the detected cello-oligosaccharides were released from a glucan that remains soluble.

The molecular size distribution of the sugars in the snCR-2 fraction was studied by gel filtration chromatography in Sepharose CL-6B gel (Fig. 2). The elution profile showed a single peak, with an average molecular size of 14 kDa and a small shoulder on each side of the peak. The fractions of the main peak were collected and chromatography was performed again to remove sugars of both shoulders. The fractions of the main peak (tube nos. 78–94) were collected and subjected to zone electrophoresis. The purified fraction exhibited a single symmetrical peak (Fig. 3), indicating that the polysaccharide may be homogenous in gel filtration and electrophoresis.

Methylation analysis revealed that 1,4-linked glucose was the most abundant sugar residue of the sample, comprising 61.3 % of total sugar. 4,6-Linked glucose, the typical sugar residue for XyG, comprised 9.5 % of the total and sugars characteristic of arabinogalactan, 21.1 % (Table 1). A sugar composition analysis carried out by gas chromatography revealed that glucose constituted 85 % of the fraction, together with other neutral sugars (mainly xylose, arabinose and galactose) that never exceeded 5 % of the sample (data not shown). However, it may be difficult to achieve perfect hydrolysis of the sample, because

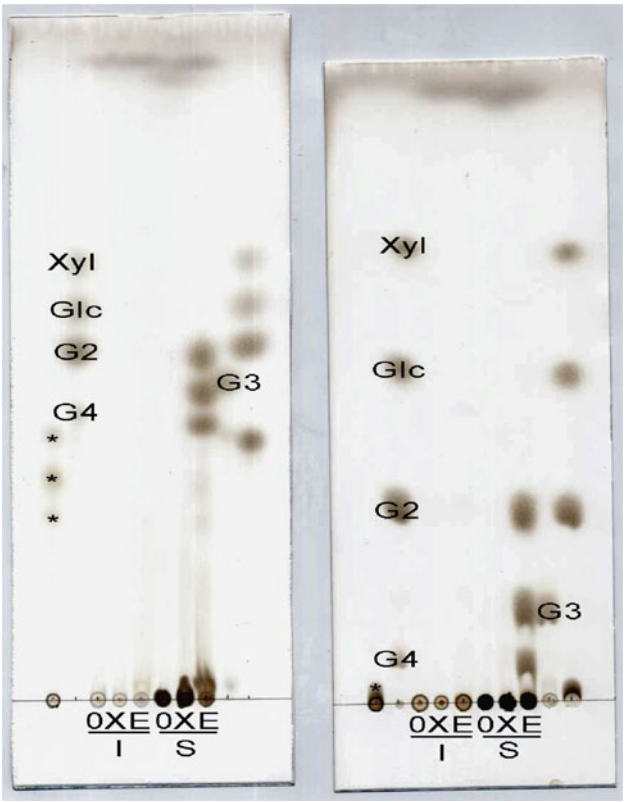

Fig. 1 TLC of the snCR-2 fraction in two different solvents: 1-propanol:ethyl acetate:water (6:1:3, by vol.) followed by ethyl acetate:pyridine:water (10:4:3, by vol.) (*left*), and butanol:acetone:water (4:5:1, by vol.) (*right*). *0* non-digested snCR-2 fraction, *X* same fraction digested by XEG, *E* digested by endocellulase, *I* insoluble part of snCR-2 fraction, and *S* soluble part of snCR-2 fraction. Markers: xylose (*Xyl*), glucose (*Glc*), cellobiose (*G2*), cellotriose (*G3*), cellotetraose (*G4*), and XyG digested with XEG (*)

its main component is 1,4-β-glucan, which is very resistant to chemical hydrolysis.

Nuclear magnetic resonance (NMR) measurements were performed directly in D_2O for the water-soluble glucan fraction. The proton ^{1}H-NMR spectrum of the glucan revealed protons due to the acetyl group at 1.3 ppm, which correspond to the chemical shifts of carbonyl and methyl carbons at 182 and 20 ppm in the carbon-13 ^{13}C-NMR spectrum (Fig. 4). The ^{13}C-NMR spectrum of the glucan illustrates shifts that are different from those of pure cellulose, but are similar to those of XyG (Jarvis and Apperley 1990). The individual chemical shifts due to C1, C4, and C6 can be assigned to the signals at δ 99.8, 99.6, and 99.4 (Fig. 4b), indicating the presence of a variety of β-1,4-linked glucans. The data are in agreement with previous findings regarding XyG on NMR spectra, in which the 1,4-β-linked glucan backbone may not have intra- and intermolecular hydrogen bonding. However, the glucan may be composed of various kinds of substitutions along the glucan backbone together with acetyl residues linked to the OH group of sugar residues. In this sense, chemical shifts

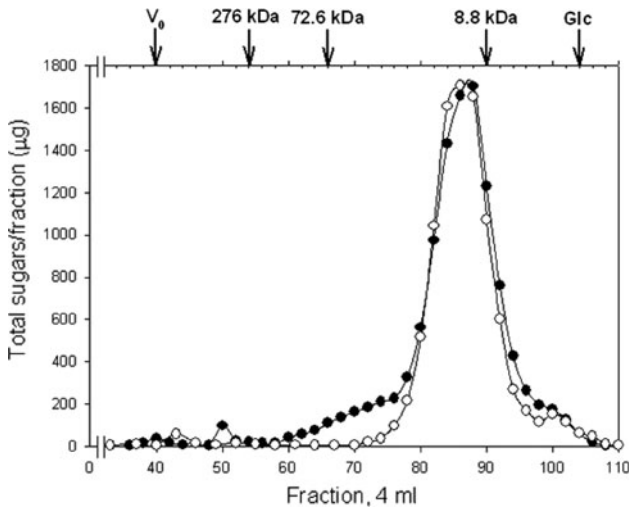

Fig. 2 Gel filtration chromatography in Sepharose CL-6B (100 cm × 2.5 cm) of snCR-2 fraction eluted from the DEAE-Sepharose column. *Black circles* elution profile of the first gel filtration chromatography; *white circles* elution profile of fractions 78–94 of the first gel filtration chromatography. The column was calibrated with blue dextran (V_0) and dextrans of *276*, *72.6*, and *8.8 kDa* and glucose (*Glc*)

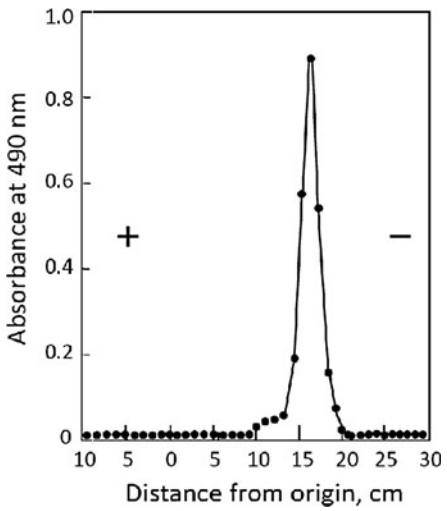

Fig. 3 Zone electrophoresis of the glucan obtained from snCR-2 fraction

Table 1 Methylation analysis of the snCR-2 fraction

Sugar linkage		%
Xylosyl	Terminal	2.7
Arabinosyl	5-Linked	13.6
Glucosyl	Terminal	4.1
	1,4-Linked	61.3
	4,6-Linked	9.5
Galactosyl	Terminal	1.3
	2,4-Linked	7.5

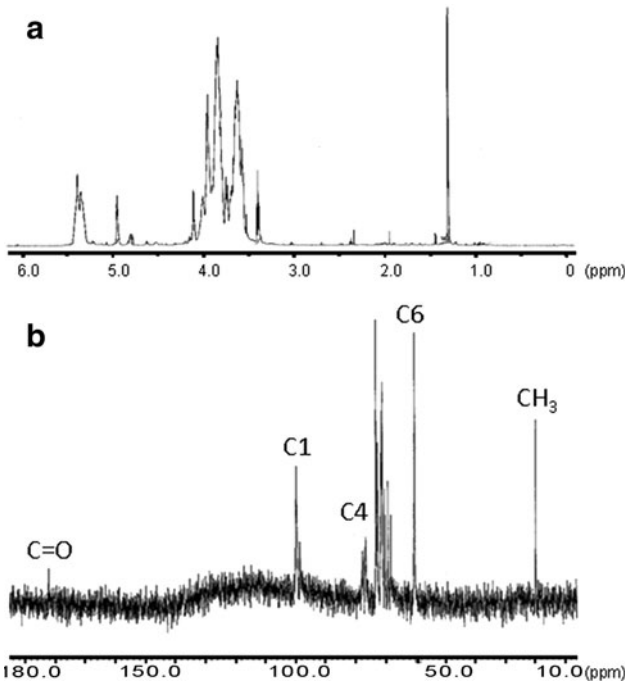

Fig. 4 NMR spectra of the glucan from the snCR-2 fraction. **a** ^{1}H-NMR spectrum. **b** ^{13}C-NMR spectrum. Characteristic chemical shifts for glucans are *highlighted*

at around δ 78.0, and the single signal at δ 60.3 can be assigned to C4 and C6 of galactan, respectively (Navarro et al. 2002).

Discussion

In this study, we purified and characterized a soluble β-1,4-glucan from the cell walls of bean cells dehabituated to dichlobenil. Two different populations were detected. The first, as Encina et al. (2002) has described, was easily released when the suspension of cell wall residue following pectin and hemicellulose extraction was neutralized. The second was obtained after sonication of the fraction, usually considered to be α-cellulose. This latter glucan population showed an average molecular size of 14 kDa, which corresponds to 86 glucosyl residues, approximately.

Glucan chains have been reported to precipitate when possessing more than 7–8 glucosyl residues (Tonnesen and Ellefsen 1971). Peng et al. (2001) reported the presence of a soluble β-1,4-glucan in cotton cells treated with the herbicide CGA 325′615, suggesting that the solubility was the result of a tight association of glucan chains to proteins, specifically to any cellulose synthase sub-unit. In our study, this possibility was discarded, because all fractions were treated with protease and the glucan remained soluble. In addition to CGA 325′615, Peng et al. (2001) also treated cotton cells with DCB, but did not find any soluble glucan in that case. This difference with our results may be due to the different extraction protocol for the soluble glucans. In the study by Peng et al. (2001), the snCR fraction (where we found the soluble glucan) was not obtained. Thus, if DCB-treated cells had synthesized a soluble glucan, it would not have been detected and would have remained in the final residue of cell wall fractionation. It is also likely that a short treatment with DCB would not provoke the accumulation of soluble β-1,4-glucan in the same way as the habituation procedure did. Arioli et al. (1998) also described the extraction of a soluble β-1,4-glucan from Arabidopsis *rsw1* mutant cell walls. In this case, the solubility was associated with a defective crystallization of cellulose as a result of the disorganization of cellulose synthase rosettes. In the case of dehabituated bean cells, the conformation of cellulose synthase may be correct, because previous research corroborates the finding that the amount of crystalline cellulose synthesized by these cells is very similar to that of non-habituated cells (Encina et al. 2002; García-Angulo et al. 2009).

Another explanation for the solubility of the glucan could be the association with XyG molecules. In this sense, when XyG from habituated and dehabituated cells was analyzed, some cello-oligosaccharides were detected, either covalently bound to XyG or because glucan was contaminating the isolated XyG (Alonso-Simón et al. 2010). However, XEG digestion of the fraction containing the glucan did not provoke either its precipitation or xylo-oligosaccharides detectable by TLC, thus a covalent link to XyG molecules can be discarded. Nevertheless, methylation analysis of the glucan revealed the presence of 4-linked glucose with substitution in carbon 6 (a residue distinctive of XyG) as 9.5 % of the molecule. This percentage renders approximately one branch per 8–9 glucose residues, considering the size of the molecule. Taking into account that the substitution of only one hydroxyl group in 10 glucose residues may solubilize cellulose in a neutral aqueous medium (Atalla 1998), this rate of branching would be sufficient to maintain the glucan soluble. Due to the low amount of xylose, some of the branches should be arabinans, galactans or arabinogalactans, which have also been detected by methylation analysis, NMR and gas chromatography. To date, no β-1,4-linked glucan presenting arabinans and galactans as branches have been described (Fry 2011), even though in certain cell walls, cellulose has been found in a close association with arabinans (Vignon et al. 2004). In contrast, XyG has been shown to be strongly linked to an arabinose/galactose-rich pectic domain, probably rhamnogalacturonan I (RG I) (Thompson and Fry 2000). In later research, this XyG–pectin complex was found to be synthesized intra-protoplasmically, and it has been suggested that some RG I sidechains were used as primers for the synthesis of

XyG (Popper and Fry 2008). Interestingly, the authors of this study found this XyG–pectin complex to be the answer to how XyG could be firmly integrated in DCB-treated cell walls (which therefore possessed a lower amount of cellulose microfibrils to bind to) (Edelmann and Fry 1992). XyG would integrate in cell walls by means of its pectic moiety, being covalently linked to RGI instead of hydrogen-bonded to cellulose. This linkage would also explain the detection of galactan and arabinan epitopes in cell wall fractions tightly bound to cellulose in DCB-habituated and -dehabituated cells (García-Angulo et al. 2006). In our case, arabinans and galactans seem to be branches of a XyG which presented quite a few xylose branches, according to methylation analysis.

Therefore, the glucan we have isolated is likely to be a XyG backbone with such a low proportion of xylose residues that they cannot be detected by TLC. A glucan with similar properties has been proposed to replace XyG in mutant plants (Cavalier et al. 2008), and it would fulfill the characteristics of the XyG–cellulose complex recently proposed by Park and Cosgrove (2012) to link cellulose microfibrils together in plant cell wall: being digested by enzymes with both xyloglucanase and cellulase activity and being located in a relatively inaccessible site, acting as an adhesive between two adjacent microfibrils (Park and Cosgrove 2012).

To summarize, we have isolated a soluble β-glucan of relatively high molecular mass that can remain soluble given its unique configuration, which includes acetyl groups, a few xylose branches, and the association to other cell wall components, such as arabinan and/or galactan chains. To our knowledge, this is the first time that a polysaccharide with such characteristics has been isolated and characterized. The discovery and description of such new polysaccharides will broaden the comprehension of plant cell wall composition and arrangement, contributing to the better understanding of this important plant cellular structure.

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