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Separation of low-molecular-mass acetylated glucuronans on L-histidine immobilized onto poly(ethylene–vinyl alcohol) hollow-fiber membranes

Anne-Sophie Pirlet[a], Olivier Pitiot[b], Linda Guentas[a], Alain Heyraud[c], Bernard Courtois[a], Josiane Courtois[a,*], M.A. Vijalayakshmi[b]

[a]*LBM, IUT/BA, Avenue des Facultés, Le Bailly, 80025 Amiens Cedex, France*
[b]*LIMTech.S, Centre de Recherches de Royallieu, BP 20 529, 60205 Compiégne Cedex, France*
[c]*CERMAV (CNRS) Université Joseph Fourier, BP 53X, 38041 Grenoble Cedex, France*

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Abstract

Low-molecular-mass polysaccharides produced by *Rhizobia* strains may be composed of a mixture of cyclic (1→2)-β-D-glucans substitued by *sn*-1-phosphoglycerol, succinic acid or methylmalonic acid or unsubstituted, and of oligosaccharides produced de novo or by degradation of the high-molecular-mass polysaccharides. The *Sinorhizobium meliloti* M5N1CS mutant strain (NCIMB 40472) produces unsubstituted cyclic glucans and these substituted by *sn*-1-phosphoglycerol, a wide variety of oligoglucuronans and high-molecular-mass glucuronans partially acetylated at the C2 and/or the C3 position. Until now, the purification of oligoglucuronans with a specific acetylation degree was not possible. In this work, we have studied the possibility of using L-histidine immobilized onto poly(ethylene–vinyl alcohol) hollow-fiber membranes for the separation of acetylated oligoglucuronans. Membranes with immobilized histidine known for purification of proteins based on dipole interaction were studied for the selective adsorption of glucuronans with discrimination of acetylation.

Keywords: Membranes; Poly(ethylene–vinyl alcohol) membranes; Histidine-poly(ethylene–vinyl alcohol) membranes; Glucuronans; Carbohydrates

1. Introduction

Purification of bacterial polysaccharides generally consisted of selective precipitation with organic solvents, or concentration by ultrafiltration on specific molecular mass cut-off membranes, or chromatography by gel permeation or on anion-exchange columns. Polymers obtained according to the different techniques are not generally pure, they may be composed of a mixture of osidic components presenting identical molecular masses as cyclic glucans (cyclosophoraoses) and oligosaccharides, or of osidic components presenting different substitution degrees. Acidic oligosaccharides from *Rhizobia* strains wich are generally necessary for the plant infection [1,2] are suspected to interact with specific plant proteins [3].

The *Sinorhizobium meliloti* M5N1CS mutant strain, obtained by *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (NTG) treatment on the wild type *Sino-*

*Corresponding author. Tel.: +33 22 534099, Fax: +33 22 956254.

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rhizobium meliloti M5N1 strain is able to induce nodule formation on alfalfa roots [4]. The mutant strain produces an homopolymer of glucuronic acid partially acetylated on the C2 and/or the C3 position [5,6] and cyclic β-(1→2)-glucans unsubstitued or substitued with *sn*-glycerol-1-phosphate residues at the C6 position (unpublished results). During fermentation, different oligoglucuronans partially acetylated (as the polymer) and presenting a 4-5 unsaturated residue at the non-reducing terminal unit are formed by degradation of the glucuronan with a glucuronan lyase [7,8]. We determined the degree of substitution (DS) of the low-molecular-mass (LMW) glucuronans ($5000<M_r<20\,000$) varied from 10% to 50% (unpublished results).

The first step in order to study the biological role of the different oliglucuronans, is to work out a method for obtaining the molecules in a pure form. In this study, we have tested L-histidine immobilized onto poly(ethylene–vinyl alcohol) (PEVA) hollow-fiber membranes to purify LMW polysaccharides produced by the *Sinorhizobium meliloti* M5N1CS mutant strain (NCIMB 40472) [4]; the molecules retained were characterized in terms of degree and position of acetylation.

2. Experimental

2.1. Fermentation

The *Sinorhizobium meliloti* M5N1CS strain (NCIMB 40472) was cultivated in a 2-l fermentor (from Setric France) containing 1.5 l of *Rhizobium* complete (RC) medium [9] supplemented with sucrose (1%, w/v) (RCS). The inoculum was 150 ml of a *S. meliloti* culture in RCS medium incubated 20 h on a rotary shaker (100 rpm) at 30°C, the cell density in the inoculum was $1.8\cdot10^9$ colonies forming units (CFU)/ml.

The pH in the fermentor was maintained at 7.2 by addition of KOH (2 *M*) and the pO_2 was stabilized at 80% (using a mass flow meter) during the exponential growth phase (20 h), and then at 50% for 71 h. The temperature was maintained at 30°C.

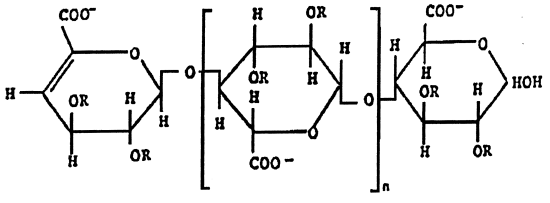

Fig. 1. Schematic representation of glucuronan containing a 4,5-unsaturated glucuronic unit partially acetylated. R=H or CO-CH_3.

2.2. Exopolysaccharide (EPS) isolation and purification

After 91 h of fermentation, the medium was centrifuged (34 000 *g*, 30 min) and the supernatant containing the EPSs was collected. Partial purifications were performed by successive ultrafiltrations of the previous EPS solution: the high-molecular-mass (HMW) and the medium-molecular-mass (MMW) EPS fractions were concentred in the retentate obtained by ultrafiltration on a 20 000 normal-molecular-mass cut-off (NMWCO) membrane (from Sartorius) of the crude EPS solution, the LMW EPSs contained in the previous filtrate were filtered through a 5000 NMWCO membrane, the LMW EPSs contained in the previous retentate were diluted with one volume of distilled water and purified by ultrafiltration with the same membrane as previously, this step was repeated six times, the LMW EPS fraction ($5000<M_r<20\,000$) obtained was dried by lyophilization.

2.3. Separation of oligoglucuronans and β-glucans from the LMW fraction

Five hundred μl of the LMW EPS fraction (5 g/l) in acetic acid/ammonium acetate ($AcOH/AcONH_4$) buffer ($5\cdot10^{-2}$ *M*, pH 4.8) were applied to a DEAE-Sepharose CL6B anion-exchange column (10.5×1 cm) equilibrated with $AcOH/AcONH_4$ ($5\cdot10^{-2}$ *M*, pH 4.8), the linear flow-rate was 0.5 ml/min. The eluent was first 12.5 ml of $AcOH/AcONH_4$ ($5\cdot10^{-2}$ *M*), then 75 ml of a linear gradient of $AcOH/AcONH_4$ from $5\cdot10^{-2}$ *M* to 1 *M*, finally, the elution was completed with 12.5 ml of the 2 *M* $AcOH/AcONH_4$ buffer. The column was connected to an ultraviolet detector (240 nm) and an evaporative light scattering detector (from Alltech). Oligoglucuronans (Fig. 1) were detected by the two analytical systems

while the cyclic glucans were detected only by the light scattering detector.

The same chromatographic conditions were applied to a larger amount of the LMW EPS fraction in order to purify cyclic glucans and pure oligoglucuronans. The chromatographed sample consisted of 1 ml of the LMW EPS fraction (13 g/l), in the $5 \cdot 10^{-2}$ *M* AcOH/$AcONH_4$ buffer. The eluted fractions absorbing at 240 nm containing oligoglucuronans were gathered. The eluted fractions characterized only by the light scattering detector were collected, this fraction contained only cyclic glucans. The two families of LMW EPSs: β-D-cyclic glucans unsubstituted or substituted with *sn*-glycerol-1-phosphate residue and the different species of oligoglucuronans were collected, pooled, desalted by chromatography on a Sephadex G-10 (M_r<700) column (50×1.6 cm) and dried by lyophilisation.

2.4. Preparation of L-histidine PEVA hollow-fiber membranes

PEVA hollow-fibers cartridges, Model Eval 3A, 0.4 m^2 surface area, 400 000 molecular mass cut-off, were from Kuraray, Osaka, Japan. 1,4-Butanedioldiglycidyl ether (bisoxirane) and L-histidine were from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

Activation and ligand coupling onto PEVA hollow-fibers were carried out as previously described [10]. Reactive oxirane groups of bisoxirane were introduced into matrix and subsequently opened and coupled with the α-NH_2 group of histidine.

2.5. Chromatography of the LMW polymer on L-His–PEVA hollow-fiber membranes

Membranes (70 cm^2 total surface) made of L-histidine immobilized onto PEVA hollow-fiber (His–PEVA module) were equilibrated with AcOH/$AcONH_4$ ($5 \cdot 10^{-2}$ *M*, pH 4.8) buffer, the flow-rate was 0.4 ml/min. The experiments were carried out using the system described in Fig. 2. LMW components (from 1 to 5 mg) in 5 ml of AcOH/$AcONH_4$ ($5 \cdot 10^{-2}$ *M*, pH 4.8) were applied on the small surface His–membrane (70 cm^2). The membrane was connected to a peristaltic pump and operated on a closed circuit during 1 h. Then the membranes were rinsed with 19 ml of the equilibration buffer; the elution was performed with 11 ml of 1 *M* AcOH/$AcONH_4$. Fractions (1 ml) were collected.

A larger surface His–PEVA module (1000 cm^2) was used for scaled up operation in the same conditions as previously, the flow-rate was 5 ml/min. The tested sample was a LMW EPS fraction (M_r<20 000) obtained from 1 l of a *Sinorhizobium meliloti* M5N1CS RCS medium cultivated during 91 h. The pH in the retentate obtained by ultrafiltration on a M_r 20 000 cut-off membrane of the bacteria-free culture medium was stabilized at 4.8 with 1 *M* H_3PO_4. The adsorption of the saccharidic components on the 1000 cm^2 His–PEVA

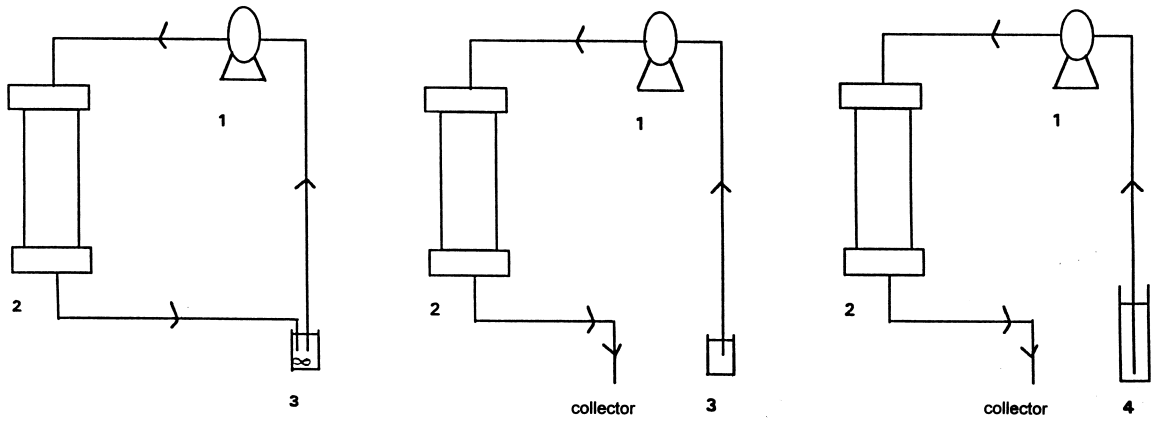

Fig. 2. Schematic representation of the chromatographic system composed of: 1, pump module; 2, column made of L-His–PEVA hollow fiber membranes; 3, sample injection; 4, elution by AcOH/$AcONH_4$, pH 4.8, $5 \cdot 10^{-2}$ *M*, then 1 *M*.

module was operated on the closed circuit during 3 h. The membranes were then rinsed with 1.5 l of the equilibration buffer and the elution was performed by 700 ml of 1 *M* $AcOH/AcONH_4$ buffer.

The remaining LMW EPS fraction (in the $5 \cdot 10^{-2}$ *M* $AcOH/AcONH_4$ buffer) collected after circulation in the L-His–PEVA module was ultrafiltred on a 5000 cut-off NMWCO membrane in order to eliminate the different medium components. The retentate was diluted with one volume of distilled water and purified by ultrafiltration with the same membrane as previously, this step was repeated six times, the LMW EPS fraction ($5000 < M_r < 20\,000$) was dried by lyophilisation. The same operations were applied on the fraction eluted by the 1 *M* buffer.

The total carbohydrate in each fractions and in the injected sample, was determined by the phenol–sulfuric acid method [11].

2.6. Nuclear magnetic resonance (NMR) studies

^1H NMR analyses were performed at 85°C with an AC-300 Bruker (Bruker Spectrospin, Wissembourg, France) Fourier transform spectrometer according to conditions described previously [12]. The degree of substitution (DS) with acetyl groups of glucuronans was determined by comparison of the total H1 resonances from oligoglucuronans (4.3 to 5.1 ppm) to protons resonances of the acetyl region (1.8–2.1 ppm) [13]. The degree of polymerization (DP) of glucuronans was determined by comparison of the integral of the H1 signal of the unsaturated unit to the integral of the H1 signals of the central units [12].

3. Results and discussion

The *Sinorhizobium meliloti* M5N1CS was cultivated during 91 h in a 2-l fermentor containing RCS medium, then the bacterial suspension was centrifuged. The supernatant was collected and successively ultrafiltered through 100 000, 20 000 and 5000 NMWCO membranes. The crude LMW EPS fraction ($5000 < M_r < 20\,000$) containing only a mixture of LMW glucuronans (Fig. 1) and cyclic (1→2)-β-D-glucans was collected and dried.

3.1. Chromatography of LMW EPS fraction on the L-His–PEVA hollow-fiber membrane

3.1.1. Determination of the chromatographic conditions

In order to purify specific saccharide components from the LMW EPS fraction ($5000 < M_r < 20\,000$), solutions (1 g/l) in $5 \cdot 10^{-2}$ *M* $AcOH/AcONH_4$ buffer were chromatographed on a closed circuit during 1 h on the L-His–PEVA prepared cartridge (70 cm^2). The module was rinsed with the same buffer (19 ml), then the elution was performed with 1 *M* $AcOH/AcONH_4$ (11 ml) (Fig. 2); fractions of 1 ml each were collected and the polysaccharide content was determined (Fig. 3). From 1.7 mg of LMW EPSs injected, about 0.05 mg were found to be retained on the His–PEVA module. In order to determine the nature of the specific saccharidic component (oligoglucuronan or cyclic glucan) retained selectively on the L-His–PEVA cartridge, 0.4 mg of cyclic β-(1→2)-D-glucan (unsubstitued and substitued by *sn*-1-phosphoglycerol) and 0.84 mg of LMW glucuronans in the same buffer as previously were run separately on the L-His–PEVA module during 1 h on a closed circuit (Fig. 2). Only LMW glucuronans were retained on the membranes (data not shown). We determined 0.09 mg of LMW glucuronans were retained on the membranes while 0.84 mg were injected, that is to say about 10% of the saccharidic molecules applied on the cartridge were retained. This raises the question whether the chromatographic conditions were specific to retain only one species of acetylated glucuronan or the retention rate was low due to a weak affinity between the saccharidic components and the L-His–PEVA hollow-fiber membrane.

In order to find elution conditions which may allow discrimination between the different classes of the LMW glucuronans, the elution conditions were modified. A linear gradient (60 ml) with $AcOH/AcONH_4$ (pH 4.8) from $5 \cdot 10^{-2}$ *M* to 1 *M* was used for elution instead of direct elution with the 1 *M* $AcOH/AcONH_4$ buffer. Nevertheless, the elution profile was comparable to that obtained with the 1 *M* buffer without gradient elution (data not shown), only one species of acetylated glucuronan was detected. This probably indicates that the membrane was not able to separate different oligoglucuronan species.

The concentration of molecules in the fraction eluted

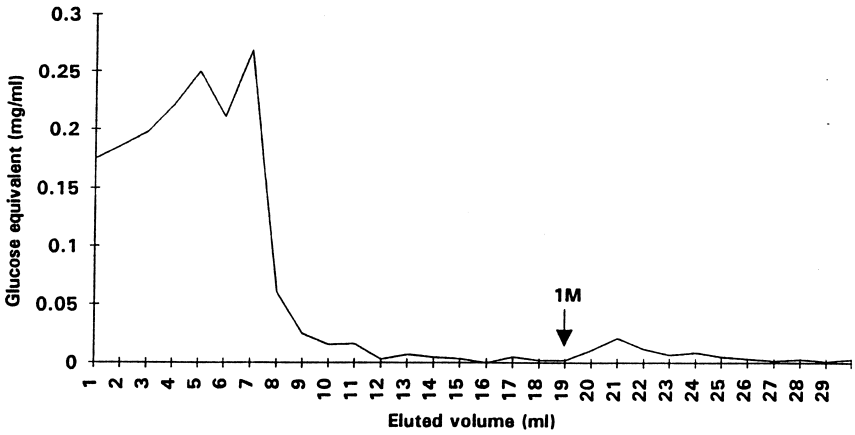

Fig. 3. Elution profile of LMW EPSs (cyclic β-glucans unsubstituted and substituted and oligoglucuronans) from the *S. meliloti* M5N1CS strain, on L-His–PEVA membranes (70 cm^2). Samples in 5 ml of $5 \cdot 10^{-2}$ $AcOH/AcONH_4$ *M* were applied 1 h on L-His–PEVA membranes, the flow-rate was 0.4 ml/min. The eluent consisted of first $5 \cdot 10^{-2}$ *M* $AcOH/AcONH_4$ buffer (19 ml) then 1 *M* (11 ml). Fractions (1.0 ml) were collected, total carbohydrate in each fraction was estimated by the phenol sulfuric method.

from the small surface module was too weak to allow further analyses, so larger surfaces membranes were studied.

3.1.2. Scale-up operation

For scale-up operation, a 1000 cm^2 membrane cartridge was used under the same adsorption and elution conditions as above. In order to study the feasibility of reducing the number of steps (avoid preparing a dry sample), a 1 l culture medium containing all the saccharidic molecules produced by the *S. meliloti* M5N1CS strain during 91 h, was filtered through a 20 000 NMWCO membrane, adjusted to pH 4.8, and was applied to the column on a closed circuit mode during 3 h. Both the non-retained fraction (noted F_1) and the retained fraction eluted by the 1 *M* buffer (noted F_2) were concentrated on a 5000 NMWCO membrane and dried for further studies. By carbohydrate analysis, we determined the eluted fraction (F_2) represented 8 mg of LMW ($5000 < M_r < 20\,000$) glucuronans. The non-retained fraction (F_1) represented 171 mg of LMW EPSs consisting of cyclic glucans and glucuronans ($5000 < M_r < 20\,000$). The ratio cyclic glucans/oligoglucuronans (w/w) in the LMW EPS fraction was about 1:1; thus, the retained fraction represent about 10% of oligoglucuronans present in the sample. This is comparable in terms of specific retention to the results obtained with the smaller cartridge, despite the fact that the injected sample contained not only cyclic glucans and oligoglucuronans, but also components from the culture medium.

3.2. Identification of the LMW glucuronans retained on the L-His–PEVA membranes

3.2.1. Chromatography on a DEAE–Sepharose column

In order to characterize the non-retained fraction (F_1) and the fraction retained (F_2) after chromatography of the complete LMW EPS fraction on the 1000 cm^2 His–PEVA cartridge, each fraction (5 mg) was solubilized in 500 μl of the $5 \cdot 10^{-2}$ *M* $AcOH/AcONH_4$ buffer and applied to a DEAE-Sepharose column (10.5×1 cm).

The elution profile of the LMW polymers present in the fraction F_1 reported on Fig. 4 was similar to that obtained with the complete $5000 < M_r < 20\,000$ fraction (data not shown). This result indicated the F_1 fraction contained LMW glucuronans and cyclic glucans (substituted or unsubstituted).

The elution profile of the F_2 fraction after chromatography on the DEAE column under the same conditions as previously revealed the presence of only one fraction (data not shown), this fraction was eluted in the

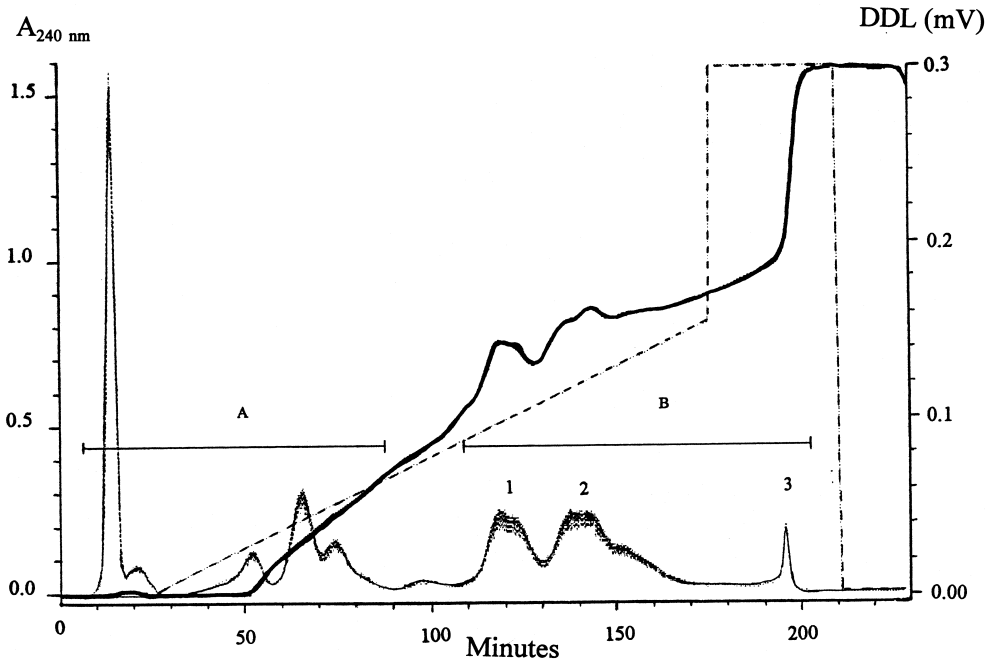

Fig. 4. Elution profile on a DEAE-Sepharose column (10.5×1 cm) of the F_1 fraction corresponding to the remaining LMW EPS crude fraction after chromatography on the L-His–PEVA membranes. The flow-rate was 0.5 ml/min. The eluent was AcOH/$AcONH_4$ from $5 \cdot 10^{-2}$ to 2 *M* (- - -). UV detection at 240 nm (—) and detection by a light scattering detector (—). (A) Cyclic glucan fraction; B1-2-3: LMW glucuronan fraction).

same conditions as the LMW glucuronan species noted B_3 purified by DEAE chromatography of the crude LMW EPS fraction or of the F_1 fraction. This result confirmed that only LMW glucuronans present some affinity with L-His–PEVA membranes.

3.2.2. ^{1}H NMR study

3.2.2.1. Characterization of the DP and the DS

In order to characterize the DP and the DS of the glucuronans present in the fraction retained on the membrane, ^{1}H NMR studies according to conditions described previously [13] were performed on the F_1 and F_2 fractions. The spectrum of the glucuronans present in the F_2 fraction (Fig. 5A) is similar to spectra obtained with partially acetylated glucuronans [7]. The ^{1}H NMR spectrum of the F_1 fraction (Fig. 5B) was similar to that obtained with the crude LMW EPS fraction ($5000 < M_r < 20\,000$) [6], the ratio of the signals between 4.7 and 4.85 ppm characteristic of H1 in the cyclic glucans to the signals from 4.3 to 5.2 ppm corresponding to H1 in the glucuronans was calculated. We determined the fraction not retained on the 1000 cm^2 His–PEVA cartridge is comparable to the LMW EPS crude fraction. This result allowed us to conclude that the non-retained fraction was composed of about 50% of glucuronans and the same proportion of cyclic glucans.

The DS average of glucuronan unit by acetyl residue, determined by the ratio of the total H1 resonances from glucuronan (signals from 4.3 to 5.2 ppm) to protons of the acetyl region (1.8–2.1 ppm), was 16%.

The DS and the molar ratios of the different species of glucuronic residues (2-*O*-acetyl; 3-*O*-acetyl; 2,3-di-*O*-acetyl; and unacetyled) present in the LMW crude fraction and LMW fraction retained on His membranes are reported in Table 1. We detected the DS average by acetyl residues of the LMW glucuronans retained on the His–PEVA cartridge was lower than the DS average of the whole LMW EPS fraction (15.5% instead of 37%). No 2,3-di-*O*-acetyl residue was detected in the

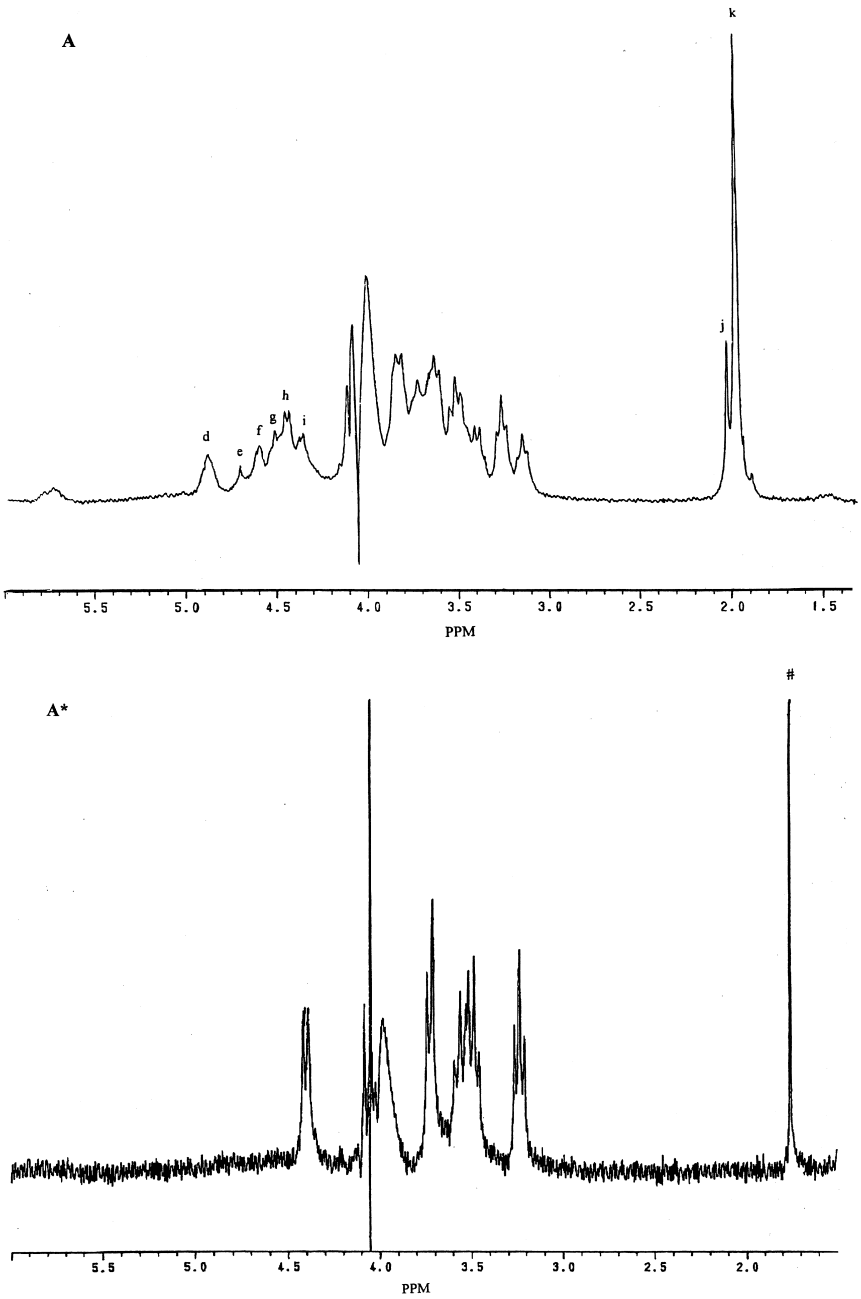

Fig. 5. ^{1}H NMR spectrum (at 300 MHz; *T*, 85°C) of oligoglucuronans in the F_2 fraction (in $^{2}H_2O$) retained on the L-His–PEVA module (1000 cm^2) (A); of the F_2 fraction (in $^{2}H_2O$) after deacetylation with $NaO^{2}H$ (A*); of oligoglucuronans in the F_1 fraction (in $^{2}H_2O$) (B); of the complete LMW EPS fraction ($5000 < M_r < 20\,000$). [(a) H4 of the unsaturated non-reducing terminus; (b) H-1α of the reducing end unit; (c) H1 of the unsaturated non-reducing teminus; (d) H3 of *O*-acetylated residues; (e) H1+H2 of 2,3-di-*O*-acetylated residues; (f) H1+H2 of 2-*O*-acetylated residues; (g) H1β of the reducing terminus; (h) H1 of the unacetylated residues; (i) H1 of unacetylated residues before or after acetylated residues, (j) protons of the acetyl group at the C2 position in the 2-*O*-acetylated residues; (k) protons of the acetyl group at the C3 position in the 3-*O*-acetylated residue and at C2 in the 2,3-di-*O*-acetylated residues; (l) protons of the acetyl group at the C3 position in the 2,3-di-*O*-acetylated residues from glucuronan units]. [(e′) H1 of glucose from cyclic glucans). (≠ protons of free acetate).

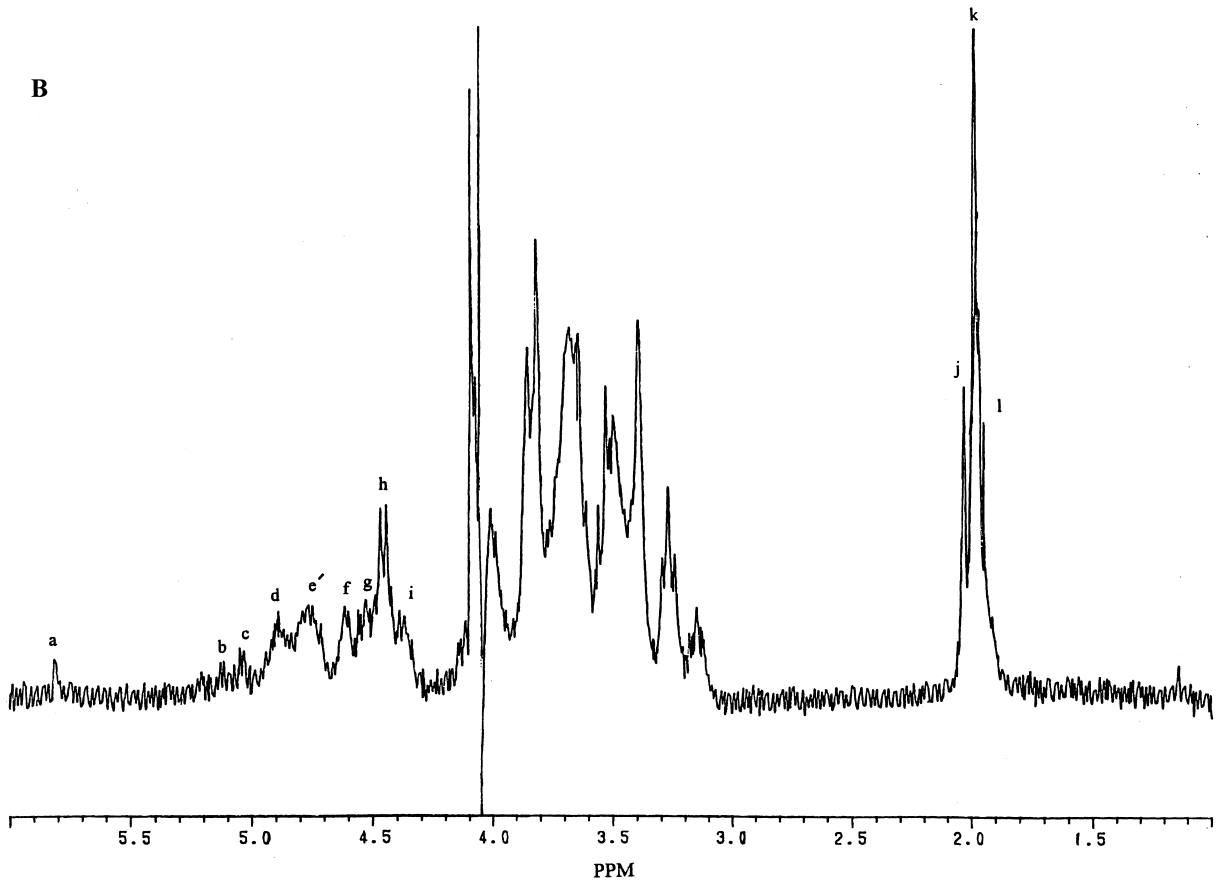

Fig. 5. (*continued*)

retained fraction while this species of acetylated glucuronan correspond to 6.5% of the glucuronic residues present in the injected sample. The molar proportion of 2-*O*-acetyl and 3-*O*-acetyl glucuronic residues was lower in the F_2 fraction than in the initial fraction, moreover, the ratio of the of 2-*O*-acetyl glucuronic

Table 1
Substitution degree average [determined by comparison of the total H1 resonances from oligoglucuronan (from 4.3 to 5.1 ppm) to protons in the acetyl region (from 1.8 to 2.1 ppm) of a complete LMW oligoglucuronan fraction (a), and of glucuronan species retained on: DEAE–Sepharose column (B3 fraction) (b) and on L-His–PEVA membranes (c) after chromatography of the complete glucuronan fraction; and (d) molar ratio of the different species of glucuronic residues acetylated (2-*O*-Ac-GlcpA, 3-*O*-Ac-GlcpA, 2,3-*O*-Ac-GlcpA) and unacetylated contained in the a, b, and c LMW EPS samples (expressed as a % of the molar ratio: specific glucuronic residue/glucuronic residues entirety)

	LMW EPSs $5000<M_r<20\,000$ (a)	LMW EPSs B3 fraction (DEAE-Sepharose) (b)	LMW EPSs retained on L-HIS–PEVA membranes (1000 cm^2) (c)
Substitution degree	37	17	15.5
2-*O*-Acetyl Glc pA (d)	8	4.5	3
3-*O*-Acetyl Glc pA (d)	16	6.5	11
2,3-Di-*O*-acetyl Glc pA (d)	6.5	3	0
Unacetylated Glc pA (d)	69.5	86	86

residues in the retained fraction was 2.6-times lower than in the crude fraction while the ratio of the 3-*O*-acetyl residues was less reduced (1.45-times), in addition, we noted the molar proportion of unacetylated residues was higher in the F_2 fraction. We determined the F_2 fraction differ from the LMW B_3 fraction collected after chromatography on a DEAE-Sepharose column of the LMW EPS crude fraction, the glucuronans in the latter fraction were more acetylated than the glucuronans in the former fraction, more, 2,3-di-*O*-acetyl residues were detected in the LMW B_3 fraction.

In the F_2 fraction, the DP average of the LMW glucuronans, evaluated by ^{1}H NMR spectroscopy, was: DP>16.

These results lead us to conclude that L-His–PEVA hollow-fiber membranes are convenient to purify a specific species of LMW glucuronan acetylated at the C2 or at the C3 position, the oligoglucuronan wich interact with the membrane present a low acetylation degree and a high concentration of unacetylated residues.

3.2.2.2. Identification of the glucuronic residue at the glucuronan non-reducing end

A signal at 5.75 ppm on the ^{1}H NMR spectrum of the F_2 fraction present a weak intensity (Fig. 5A), therefore in previous works we detected, for LMW EPSs ($5000<M_r<20\,000$) obtained by a lyase degradation of the polymer, a higher signal in the same region [8]. The signal was previously attributed to H4 of 4-5 unsaturated terminal residues [8]. The weakness of the signal detected in this work was surprising, the glucuronans in the F_1 and the F_2 fractions presented the same DP, and the F_1 fraction chromatographed on the DEAE-Sepharose column contained oligoglucuronans presenting an unsaturated unit characterized by the absorbance at 240 nm. In order to explain the weakness of this small signal, the F_2 glucuronan fraction in ^2H_2O was deacetylated with NaO^2H.

No signal at 5.75 ppm was detected on the ^{1}H NMR spectrum obtained with the deacetylated F_2 glucuronan fraction (Fig. 5A*), while the whole LMW glucuronan fraction deacetylated under the same conditions present an important signal at 5.75 ppm (data not shown). The small signal was probably due to the presence of substituent residues on the glucuronans, as no 4-5 unsaturated residue was detected in the F_2 glucuronan fraction, we conclude that the molecules retained on the L-His–PEVA membrane present only saturated residues. Until now, such molecules were not detected in the LMW EPS fraction. The purification by the L-His–PEVA hollow-fiber membranes of this new species of oligoglucuronan indicate such oligosaccharide was not obtained by degradation of the polymer with a glucuronan lyase [8].

4. Conclusions

L-His–PEVA hollow-fiber membranes present a selective affinity for monoacetylated oligoglucuronans, and no di-*O*-acetyl residues were retained. The high selectivity between the glucuronan species and the membrane lead us to purify a new species of oligoglucuronan probably not formed by action of a glucuronan lyase on the polymer, this new species may be formed by action of a β-(1→4) glucuronan hydrolase (not detected until now) on the polymer or produced de novo by the Rhizobia strain. The retention of the specific glucuronan from a crude LMW EPS fraction containing LMW glucuronan, β-(1→2)-D-glucan cyclic and components of the culture medium indicates L-His–PEVA hollow-fiber membrane can be considered as a new chromatographic method useful to separate specific LMW glucuronans from a LMW EPS crude fraction.

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