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Synthesis of new glycosaminoglycans-like families by regioselective oxidation followed by sulphation of glucoglucuronan from *Rhizobium* sp. T1

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ABSTRACT

Glycosaminoglycan-like polysaccharides were prepared from *Rhizobium* sp. T1 polysaccharide using the TEMPO (nitroxyl radical 2,2,6,6-tetramethylpiperidine-1-oxyl radical) mediated oxidation. The structure of this new polyglucuronic acid sodium salt was analyzed by ^{13}C NMR spectra and HPAEC–PAD chromatography. Therefore, new polysaccharide containing only glucuronic acid monomers in both β-(1,3) and β-(1,4) linkage was obtained by the complete TEMPO-mediated oxidation of C6 primary hydroxyl groups of glucose of glucoglucuronan from *Rhizobium* sp. T1. Sulphation of this β-(1,3),β-(1,4)-polyglucuronic acid sodium salt was carried out using SO_3/DMF reagent. These results suggested a new synthetic route using both TEMPO-mediated oxidation and sulphation of polysaccharides from *Rhizobium* sp. in developing glycosaminoglycans mimic to enhance the profitability of its low-cost production and processing industries. This novel carbohydrate derivative might find use as cheaper surrogates of glycosaminoglycans in the cosmetics and pharmaceutical fields.

1. Introduction

Glycosaminoglycans (GAGs) are commonly described as a general family of highly functionalized, linear polysaccharides structure containing carboxyl and sulphate groups in their main repeating unit (Petit, Delattre, Papy-Garcia, & Michaud, 2006; Yeung, Chong, & Petillo, 2001). According to their structures and more exactly to their repeating unit identity, the GAG family can be separated into dermatan, chondroitin/chondroitin sulphate, hyaluronic acid and heparin/heparin sulphate (Petit et al., 2006). For a long time, GAGs have demonstrated key roles in many important biological processes such as regulation of cellular differentiation and proliferation, lymphocyte trafficking, inflammatory response and tumor metastasis (Carroll & Koch, 2003; Gama & Hsieh-Wilson, 2005; Petit et al., 2006). It was clearly established that glycosaminoglycans can easily bind growth factors and cytokines (Capila & Linhardt, 2002). The medical applications of glycosaminoglycans in anti-angiogenesis, antiviral and anticoagulation field were highlighted by the industrial development of the treatment of deep vein thrombosis and also by the production of drugs such as synthetic heparin pentasaccharide (Petitou & Van Boeckel, 2004). Therefore, these bio-applications revealed that chemical groups of GAGs can influence and modulate biological activities depending on various molecular compositions (Rabenstein, 2002). Consequently, chemical synthesis of Glycosaminoglycans was an extensive challenge following the recognition of their biological impact (Palmacci & Seeberger, 2004; Petit et al., 2006; Seeberger, 2001, 2003).

Various glycosaminoglycan-analogs have been synthesized by oxidation and/or sulphation of others natural polysaccharides in order to produce a very large range of bioactive glycosaminoglycan-like polysaccharides (Delattre, Michaud, Courtois, & Courtois, 2006; Delattre et al., 2009; Elboutachfaiti et al., 2010; Zhang et al., 2010).

Several publications have shown the interest in producing in large scale up polyglucuronic acid by using chemical oxidation of renewable, natural and abundant glucans such as: starch, cellulose, chitin and curdlan (Elboutachfaiti, Delattre, Petit, & Michaud, 2011; Tavernier, Delattre, Petit, & Michaud, 2007). As largely described in literature (Delattre et al., 2006, 2009; Elboutachfaiti et al., 2010; Muzzarelli, Muzzarelli, Cosani, & Terbojevich, 1999; Tavernier et al., 2007), the best method to produce neo-polyglucuronic acid sodium salt in large quantities was to use the nitroxyl radical 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO®) in the presence of sodium hypochlorite and sodium bromide. Therefore, by this oxidation method, several glycosaminoglycan structures analogs could be synthesized in high yields and purity (Delattre, Chaisemartin, Favre,

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Berthon, & Rios, 2012; Delattre, Michaud, Chaisemartin, Berthon, & Rios, 2012). Recently, Zhang et al. (2010) have proposed synthesis of sulphated polyglucuronic acid sodium salt from cellulose model. Nevertheless, very few studies have been proposed on both carboxylation and sulphation of other polysaccharides than cellulose.

Many microbial polysaccharides have chemical structures which could provide the basis for the synthesis of a new generations of glycosaminoglycan surrogates (Delattre et al., 2009). The *Rhizobium meliloti* wild type strains produce essentially succinoglycan and an galactoglucan under specific conditions, while *Rhizobium leguminosarum* strains have been described to produce EPS-containing uronic acid residues in their repeating unit (Delattre, Laroche, & Michaud, 2009). However, some uncommon *R. meliloti* strains have been studied for their ability to produce EPS with uronic acid residues as for example, the *R. meliloti* 201 strain, *meliloti* IFO 13336 strain and *R. meliloti* M5N1CS (Delattre, Laroche, & Michaud, 2009).

The last decades, a very interesting uronic-EPS has been produced by a *Rhizobium* sp. T1 strain able to infect clover and alfalfa (Guentas et al., 2000). In this work, the complete structure of this new polymer produced by a Rhizobia strain was elucidated and the effect of pH stabilization on the production was analyzed. Consequently, an heteropolysaccharide composed of glucuronic acid and glucose having the repetition units: ([,3)-β-D-GlcA-(1,4)-β-D-Glc-(1,]) and called glucoglucuronan was identified (Guentas et al., 2000; Seguin et al., 1996). Recently, it was shown that synthetic sulphated glucoglucuronan could modulate regenerating activities using an in vivo model of rat injured muscle regeneration (Petit et al., 2004). In fact a regenerating activity on injured *extensor digitorum longus* (EDL) muscles on rats was obtained with this sulphated anionic polymer. It was particularly mentioned that the degree of sulphation for glucoglucuronans (primary or secondary alcohol) could have influence on the biological activity.

In the present study, the regioselective oxidation of glucoglucuronan from *Rhizobium* sp. T1 following by sulphation was investigated in order to propose synthetic route to produce sulphated β-(1,3),β-(1,4)-polyglucuronic acid sodium salt which could constituted a new family of mimetic bioactive polysaccharides as glycosaminoglycan surrogates.

2. Material

2.1. Bacterial strain

The *Rhizobium* sp. T1 was obtained from atypical root nodules present on white clover plants inoculated by a *R. meliloti* M5N1 suspension (Heyraud, Rinaudo, & Courtois, 1986). Atypical nodules appeared on secondary plant roots forty days after the inoculation. Bacteria referred as *Rhizobium* sp. T1 have been extracted from these nodules in order to produce exopolysaccharides (Seguin et al., 1996).

2.2. Production of glucoglucuronan

Glucoglucuronan was produced as previously described by Guentas et al. (2000).

Briefly, the Rhizobia cells were grown at 30 °C in two, 20-L reactors (fermentors) containing 15 L of *Rhizobium* complete (RC) medium supplemented with sucrose (1%, w/v) (RCS). The inoculum was 1.5 L of *Rhizobium* sp. T1 culture in RCS medium, first grown at 30 °C in an Erlenmeyer flask on a rotary shaker, to a cell density corresponding to an optical density value at 600 nm (OD600) of 1. During growth, the oxygen partial pressure (pO_2) was stabilized at 80% (using a mass flow meter) for the stationary growth phase, and then at 50%. The pH was controlled and maintained at 7.2 with KOH 4 M The bacterial growth was followed by determination of the colony forming unit (CFU) on RC agar (1.5%, w/v) plates, and by measure of the OD600nm. The sugar consumption in the medium was studied with a sucrose: D-glucose enzymatic kit (from Boehringer). The polysaccharide production was determined from samples (100 mL) taken from the two reactors after 20, 28.5, 42, 50, 65 and 70 h of incubation.

First, the bacteria were removed from the medium by centrifugation (30 min at 14,000 × *g*), the supernatants were collected and the EPS were precipitated by 2 vol of isopropanol and freeze-dried. The polysaccharide structure was determined for the EPS collected at the end of the growth period from the bacterial free RCS media obtained as previously; the polymers were then purified by ultrafiltration on a 100,000 Da cut-off NMWCO membrane from Sartorius and freeze-dried under vacuum.

2.3. Regio-selective oxidation of glucoglucuronan

β-(1,3),β-(1,4)-polyglucuronic acid sodium salt was prepared according to the oxidation procedure described by Delattre et al. (2009). Briefly, glucoglucuronan (10 g) was dissolved at room temperature in distilled water (1 L) during 60 min at pH 10 by adding NaOH (4 M). The solution was kept below 4 °C in an ice bath during oxidation step. TEMPO (90 mg), NaBr (2 g) and NaOCl (100 mL at 9.6%) were added to start oxidation. The pH was kept at 10 by addition of NaOH (1 M). The reaction was quenched by adding ethanol (50 mL) after 1 h and neutralized with HCl (5 M). After concentration (1/3) by evaporation, β-(1,3),β-(1,4)-polyglucuronic acid sodium salt was precipitated with cold isopropanol (3 vol). The precipitate was washed with isopropanol, dissolved in distilled water, dialyzed against water during 24 h at 10,000 Da cut off dialysis membranes and freeze-dried.

2.4. Sulphation of oxidized glucoglucuronan

The sulphation of β-(1,3),β-(1,4)-polyglucuronic acid sodium salt was realized according to Yuan et al. (2005). Briefly, the sulphation reagent (SO_3-DMF) was prepared by dropping very slowly 50 mL of chlorosulphonic acid ($HClSO_3$) into 250 mL of DMF in an ice-water bath.

Dry oxidized glucoglucuronan (2 g) was added to 80 mL DMF, and the mixture was vigorously stirred at 50 °C for 60 min to disperse oxidized glucoglucuronan. Then 20 mL of SO_3-DMF reagent was slowly added. After 3 h at 50 °C, the mixture was cooled at room temperature and filtered to remove insoluble material. The filtrate was then concentrated (1/10) under reduce pressure and precipitated with isopropanol (3 vol) at 4 °C overnight. The precipitate was filtered off, dissolved in distilled water at the concentration of 20 g/L and neutralized with NaOH (5 M). The solution was precipitated with isopropanol (7 vol) at 4 °C and filtered off. Finally, the sulphated oxidized glucoglucuronan was dissolved in distilled water (50 mL), dialyzed against water during 24 h at 10,000 Da cut off dialysis membranes and freeze-dried.

2.5. Sugar assay

Glucose and glucuronic acid contents of glucoglucuronan and oxidized form were assayed with *meta*-hydroxyldiphenyl (Van Den Hoogen et al., 1998) and resorcinol (Monsigny, Petit, & Roche, 1988) in order to quantify the oxidation level, D-glucose (Sigma) and D-glucuronic acid (Sigma) were used as standards. Quantification of neutral sugars was done according to the corrective formula described by Spick and Montreuil (1964). The glucuronic acid ratio (GA%) of polysaccharides obtained was expressed as the amount of

glucuronic acid compared with total sugars (glucose and glucuronic acid).

2.6. Analysis of glucuronic acid/glucose composition

The native glucoglucuronan and its fully oxidized form (1 mg) dissolved in 4 M TFA (1 mL) were heated at 100 °C for 8 h. The acid was removed by flushing the sample with air and the hydrolyzates were freeze-dried. The hydrolyzates (1 mg) were dissolved in pure water (1 mg/mL). Twenty-five microliters of these solutions was used for the ionic chromatography analysis by high performance anion-exchange chromatography (HPAEC) of Dionex ICS-2500 System. Analysis was performed on a Carbopac PA-10 column (4.5 mm × 250 mm) as described previously (Talaga, Vialle, & Moreau, 2002). Detection was performed using a pulsed amperometric detector (Dionex).

2.7. Sulphate content

For sulphate analysis, the sulphated polyglucuronic acids sodium salt was quantitatively assayed by turbidity measurement after hydrolysis with HCl (4 M) and addition of gelatin–barium chloride ($BaCl_2$) (Dodgson & Price, 1962).

2.8. NMR

NMR analyses were performed at 80 °C with a Bruker Advance 400 spectrometer of 400 MHz equipped with $^{13}C/^{1}H$ dual probe. The NMR experiments were recorded with a spectral width of 3000 Hz, an acquisition time of 1.36 s, a pulse width of 7 μs, a relaxation time of 1 s and a number of 1500 scans. Polysaccharides were dissolved in D_2O at a 50 g/L concentration.

2.9. Molecular Weight determination

Average molecular weights and molecular weight distributions were determined by high pressure size exclusion chromatography (HPSEC) with on line multi-angle laser light scattering (MALLS) and differential refractive index (DRI) detectors. The MALLS apparatus is the EOS from Wyatt Technology (CA, USA) filled with a K5 cell and a Ga–As laser (λ = 690 nm). The DRI detector is the ERC7515A from Erma Cr., Inc. (Japan). Columns [OHPAK SB-G guard column, OHPAK SB804 and 806 HQ columns (Shodex)] were eluted with $LiNO_3$ 0.1 M at 0.6 mL min^{-1}. Solvent was filtered through 0.1 μm filter unit (Millipore), degassed (ERC-413) and filtered through a 0.45 μm filter upstream column. The sample, filtered on 0.45 μm unit filter (Millipore) was injected through a 100 μL full loop. The collected data were analyzed using the Astra V-4-81-05 software package from Wyatt technology (CA, USA).

3. Results and discussion

3.1. Production of glucoglucuronan polysaccharide from Rhizobium sp. T1

As already mentioned in literature (Petit et al., 2004), we related that the exopolysaccharide (EPS) production was quite similar in the media with or without pH regulation. After 70 h of incubation time, the media was centrifuged and the EPS were precipitated with isopropanol and freeze-dried. The glucoglucuronan polysaccharide was then purified by ultrafiltration on a 100,000 Da cut-off NMWCO membrane from Sartorius and freeze-dried. Consequently, the EPS production by the *Rhizobium* sp. T1 strain in RCS medium was estimated at around 1.1 g/L with at least 3 repeats. ^{1}H NMR analysis was carried out to confirm the pure structure of glucoglucuronan (Fig. 1). In fact, ^{1}H NMR spectrum at 80 °C of exopolysaccharide produced by *Rhizobium* sp. T1, revealed two doublets at 4.4 ppm and 4.6 ppm (Fig. 1) presenting similar integration. According to the literature (Petit et al., 2004) they were assigned to the H-1 of β-glucuronic acid residue (H1-GlcA) and of β-glucose residue (H1-Glc), respectively. Proton assignment summarized in Table 1 was comparable to literature for *Rhizobium* sp. T1 (Petit et al., 2004). With this ^{1}H NMR analysis, we confirmed that this exopolysaccharide was only composed of [,3)-D-Glc*p*A (1,] and [,4)–D-Glc*p* (1,] residues in a 1:1 ratio after integration of anomeric proton H1-Glc and H1-GlcA. Moreover, the HPAEC analysis of the native glucoglucuronan (see Table 2) from *Rhizobium* sp. T1 was investigated in order to confirm the results described by Guentas et al. (2000) where it was mentioned the glucose/glucuronic acid ratio of 1/1. Finally, SEC-MALLS analysis of glucoglucuronan was investigated (see Table 2). Therefore, the weight average molecular weight was estimated at 1.5×10^{6} Da with polydispersity index of 1.2. Once again, this result compared to those obtained in the literature (Guentas et al., 2000; Petit et al., 2004) indicated that the production of glucoglucuronan from the *Rhizobium* sp. T1 is not affected by acidic pH conditions in contrast to the production of others *Rhizobium* sp. exopolysaccharides such as succinoglycan from the *R. meliloti* wild type strains (Delattre, Laroche, & Michaud, 2009).

3.2. Production of β-(1,3),β-(1,4)-polyglucuronic acid sodium salt by oxidation of glucoglucuronan mediated by TEMPO–NaBr–NaOCl system

Based on the work of Bragd, Van Bekkum, and Besemer (2004), new generations of β-(1,3),β-(1,4)-polyglucuronic acid sodium salt from glucoglucuronan have been easily produced using the TEMPO–NaBr–NaOCl system oxidation as shown in Fig. 2. This exopolysaccharide from *Rhizobium* sp. T1 composed of glucose and glucuronic acid having the repetition units: ([,3)-β-D-GlcA-(1,4)-β-D-Glc-(1,]) was oxidized using TEMPO/NaOCl/NaBr system in order to produce a pure β-(1,3),β-(1,4)-polyglucuronic acid sodium salt in a good yield (90%) after 1 h of oxidation time at pH 10 and 4 °C. Therefore, during oxidation step of glucoglucuronan, different samples were collected and analyzed by HPAEC in order to estimate the glcA/Glc ratio as related in Fig. 3. We have shown that the amount of NaOCl to complete oxidation of the C6 primary hydroxyl groups of 1 g native glucoglucuronan was estimated at around 7.5 mmol as already observed in common polysaccharides oxidation using TEMPO/NaOCl system (Delattre et al., 2009; Elboutachfaiti et al., 2010). Consequently, a new glucuronan family containing β-(1,3) glucuronic acid sodium salt residue and β-(1,4)-glucuronic acid sodium salt residue was obtained quantitatively (90%) by the complete TEMPO-mediated oxidation of glucoglucuronan using an amount of 7.5 mmol of NaOCl per gram of native exopolysaccharide.

During the oxidation of glucoglucuronan mediated by TEMPO/NaOCl system, we observed the decrease of gelling power once the amount of NaClO added was increased in the oxidation step. Thus, active liquefaction phenomenon of viscous glucoglucuronan solution is started once the amount of NaClO reaches a value of surrounding 5.0 mmol/g. As against, when the NaClO level reach around 2.0 mmol, the oxidized products still allows the ability to form a weak gel. As largely described (Elboutachfaiti et al., 2010), the changes in macromolecular conformation after oxidation of polysaccharides allowed polyelectrolyte properties which increase the solubility of polyuronides according to the global anionic charge. Thus, this new polyelectrolyte polysaccharide could constitute a good model for gelling ingredient in the cosmetic or therapeutic field.

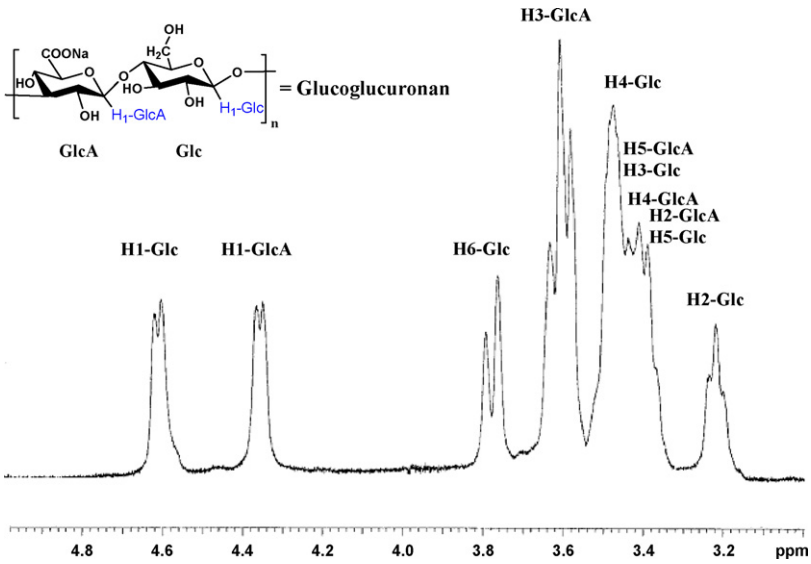

Fig. 1. ^{1}H NMR analysis of glucoglucuronan synthesized by *Rhizobium* sp. T1 strain.

Table 1
^{1}H NMR chemical shifts (at 80 °C) (expressed in ppm) and of glucoglucuronan excreted by *Rhizobium* sp. T1.

	Chemical shifts (δ ppm)					
	H-1	H-2	H-3	H-4	H-5	H-6
[→3)-β-D-GlcA-(1→]	4.38	3.41	3.58	3.43	3.49	–
[→4)-β-D-Glc-(1→]	4.62	3.23	3.49	3.50	3.41	3.75

3.3. NMR analysis of β-(1,3),β-(1,4)-polyglucuronic acid sodium salt

This new β-(1,3),β-(1,4)-polyglucuronic acid sodium salt produced by oxidation of *Rhizobium* sp. T1 exopolysaccharide was analysis by ^{13}C NMR as shown in Fig. 4.

As mentioned in Table 3, the ^{13}C NMR analysis shown 12 specifics resonance peaks for glucoglucuronan from *Rhizobium* sp. T1 (Petit et al., 2004) and oxidized glucoglucuronan corresponding to [→3)-β-D-GlcA-(1→)] and [→4)-β-D-Glc-(1→] residues and, [→3)-β-D-GlcA-(1→)] and [→4)-β-D-GlcA-(1→] residues respectively. This ^{13}C NMR analysis confirmed, based on the comparison with other chemical modification of polysaccharides described in literature (Delattre et al., 2006, 2009; Delattre, Chaisemartin, et al., 2012; Delattre, Michaud, et al., 2012; Elboutachfaiti et al., 2010), the absence of the C-6 resonance at 61.5 ppm after 60 min of oxidation, indicating the total oxidation of the primary hydroxyl group C-6 of [→4)-β-D-Glc-(1→] residues of glucoglucuronan from *Rhizobium* sp. T1 (Fig. 4B). The spectrum of oxidized glucoglucuronan shows a new signal due to the carboxyl group around 175.7 ppm as evidence of oxidation of C6 from [→4)-β-D-Glc-(1→] residues of glucoglucuronan from *Rhizobium* sp. T1. The presence of specific signals from oxidized [→4)-β-D-Glc-(1→] residues at 103.1 ppm (C-1), 82.1 ppm (C-4), 76.8 ppm (C-5), 76.6 ppm (C-3), 74.3 ppm (C-2) and more especially 175.7 ppm (C-6) validated the fact that we obtained a pure form of this fully oxidized glucoglucuronan from *Rhizobium* sp. T1 after 1 h of the process.

3.4. SEC MALLS analysis

SEC MALLS analysis was performed by coupling on-line a size exclusion chromatography (SEC), a multi-angle laser light scattering (MALLS) and a differential refractive index detector (DRI) in order to evaluate the molecular weight of this new β-(1,3),β-(1,4)-polyglucuronic acid sodium salt. As observed in Table 2, the average molecular weight of β-(1,3),β-(1,4)-polyglucuronic acid sodium salt was estimated as 490 kDa which correspond to around 1/3 of that of glucoglucuronan from *Rhizobium* sp. T1. This result clearly indicated that the oxidation process of glucoglucuronan from *Rhizobium* sp. T1 reduced the MW. This observation is in accordance with to literature since in lot of publications of regioselective oxidation of polysaccharides by TEMPO/NaOCl system, a significant depolymerization of polysaccharides was observed (Elboutachfaiti et al., 2010). In fact, the mechanism of this depolymerization of polyuronides such as cellouronic acid, amylouronic acid,

Table 2
Characterization of glucoglucuronan and its derivatives.

Derivatives	Glc (%)	GlcA (%)	Mw (Da)	Ip	DS (%)
Glucoglucuronan	50	50	1.50×10^{6}	1.2	0
Oxidized glucoglucuronan	1	99	0.49×10^{6}	1.1	0
Sulphated β-(1,3),β-(1,4)-GlcA	1	99	0.24×10^{6}	1.3	41

GlcA: glucuronic acid estimated by HPAEC.
Glc: glucose estimated by HPAEC.
DS: degree of sulphation estimated by turbidimetry assay (Dodgson & Price, 1962).
MW: molecular weight estimated by SEC MALLS.
Ip: index of polydispersity.

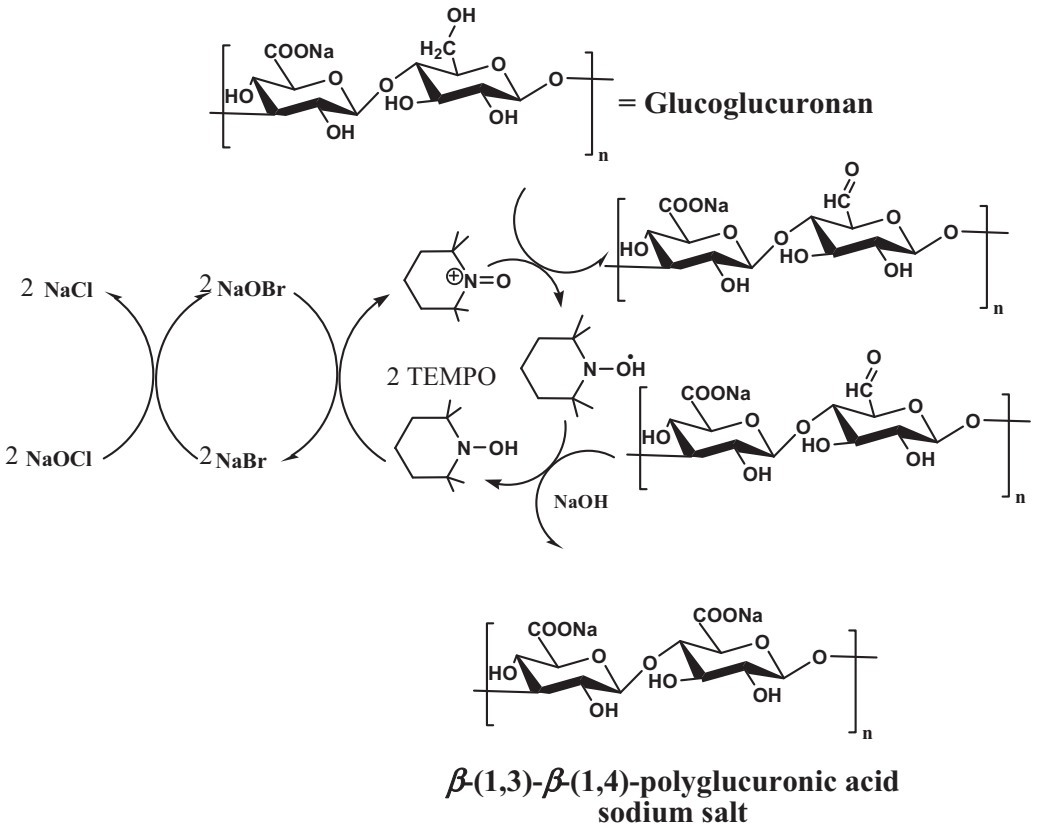

Fig. 2. Schematic representation of the oxidation process of glucoglucuronan using TEMPO/NaOCl/NaBr system.

chitouronic acid, etc. during TEMPO-mediated oxidation was studied. It was established that this phenomenon could be attributed to hydroxyl radicals formed such as super oxide anion radical and/or hydroperoxide radical (HOO—) from NaOBr and TEMPO at pH 10–11 leading to the depolymerisation of the polysaccharide by β-elimination (Elboutachfaiti et al., 2010; Ishizu, 1973; Isogai & Kato, 1998; Kitaoka, Isogai, & Onabe, 1999; Matsumoto, 2000; Miyazawa, Endo, & Okawara, 1985).

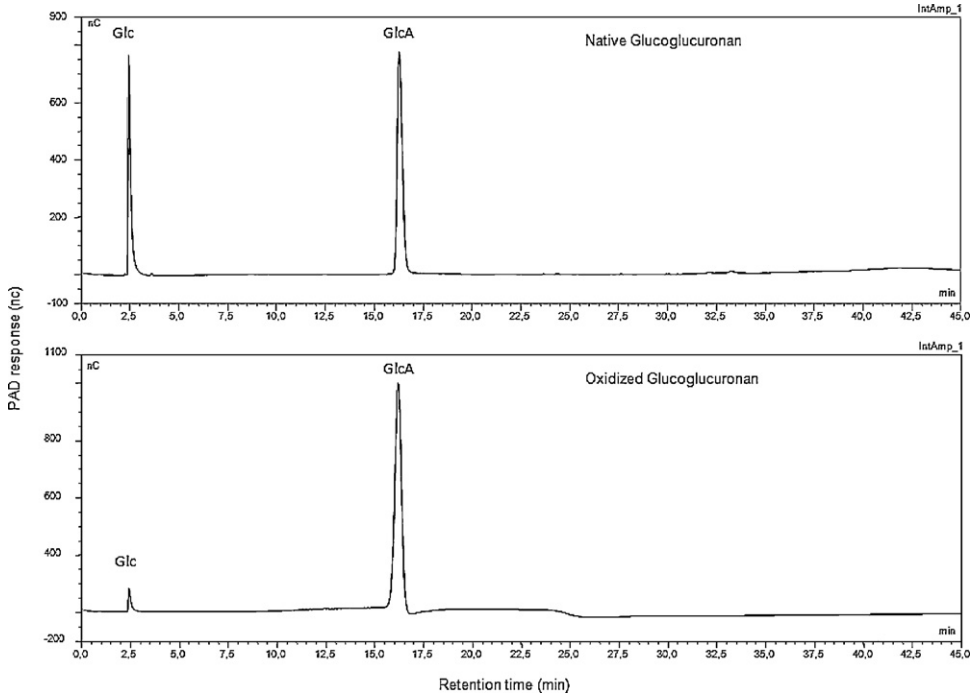

Fig. 3. HPAEC–PAD chromatograms of native glucoglucuronan from *Rhizobium* sp. T1 and TEMPO-mediated oxidation products hydrolyzates. Glc and GlcA referred to glucose and glucuronic acid respectively.

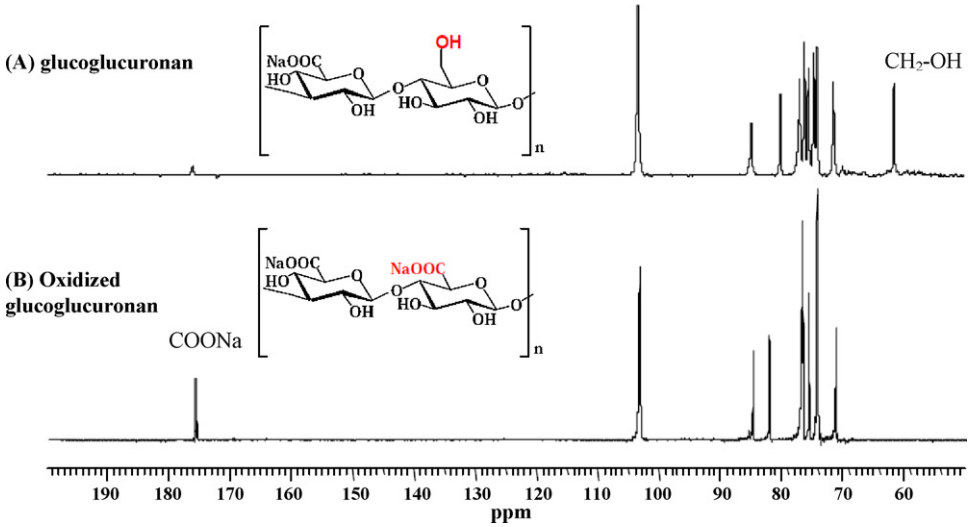

Fig. 4. ^{13}C NMR analysis of (A) glucoglucuronan and (B) oxidized glucoglucuronan.

Table 3
^{13}C NMR chemical shifts (at 80 °C) (expressed in ppm) of glucoglucuronan and oxidized glucoglucuronan.

	Chemical shifts (δ ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
Glucoglucuronan						
[→3)-β-D-GlcA-(1→...	103.3	74.1	84.7	71.3	75.6	175.9
[→4)-β-D-Glc-(1→...	103.5	75.6	76.1	80.0	76.9	61.5
Oxidized glucoglucuronan						
[→3)-β-D-GlcA-(1→...	103.3	74.1	84.7	71.3	75.6	175.9
[→4)-β-D-GlcA-(1→...	103.1	74.3	76.6	82.1	76.8	175.7

3.5. Production of sulphated β-(1,3),β-(1,4)-polyglucuronic acid sodium salt

Once produced, the pure β-(1,3),β-(1,4)-polyglucuronic acid sodium salt was sulphated in good yield (80–90%) as described in material and method part. Therefore, we produced a sulphated polyglucuronic acid sodium salt with degree of sulphation (DS) of 41% (see Table 2). Note to mention that when carbohydrates are subjected to sulphation, hydroxyl groups in the chains are replaced with sulphate groups. Therefore, the presence of sulphur could be confirmed in polysaccharides by FT-IR analysis (data not shown) with the observation of the specific characteristic vibrations for C—O—S (810–815 cm^{-1}) and S=O (1250–1260 cm^{-1}) (Yuan et al., 2005; Zhang et al., 2010).

Once again, by correlation with literature (Petit et al., 2004) during sulphation process, the β-(1,3),β-(1,4)-polyglucuronic acid sodium salt backbone was partially depolymerized and the sulphated form showed average molecular weight lower than the fully oxidized precursors. In fact, as mentioned in Table 2, the MW was reduced from 490 kDa for the fully oxidized glucoglucuronan up to 240 kDa for the sulphated β-(1,3),β-(1,4)-polyglucuronic acid sodium salt.

4. Conclusion

In modern biotechnologies, sulphated polysaccharides are biomolecules of great interest for medical and cosmetic field. Due to the worldwide boost in the industrial utilization of glycosaminoglycans in pharmacology, cosmetology and agronomy, the natural sources will not be enough for the biotechnological need in future. Consequently, scientists have to propose new advances in sulphated polysaccharides synthesis route to glycosaminoglycan surrogates. Here, we proposed an original and attractive synthesis route of glycosaminoglycan-like by regioselective oxidation and sulphation of glucoglucuronan from *Rhizobium* sp. T1 yielding to homogenous highly sulphated β-(1,3),β-(1,4)-polyglucuronic acid sodium salt. It is important to note that work is in progress to assay the biological activities of this highly sulphated β-(1,3),β-(1,4)-polyglucuronic acid sodium salt in order to find the carboxylate/sulphate ratio which could explain the structure/biological activity relationship notably as regenerating agent as already studied with the sulphated glucoglucuronan.

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