β-(1,4)-Polyglucuronic Acids – An Overview

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Abstract: Polyuronides are an acidic class of polysaccharides with interesting rheological and biological properties. However, except pectin and alginate, the structural variability of this class of polysaccharides is poor and low described in literature. In this context, a new generation of polyuronides has been isolated from two sources in the middle of the 90's. Firstly, a bacterial β-(1,4) polyglucuronic acid called glucuronan was identified as the sole exopolysaccharide produced by a bacteria belonging to the *Rhizobiaceae* family. Secondly, the development of the TEMPO chemistry led to the production at large scale of oxidized cellulose called cellouronate. Both new polyuronides were largely patented and found applications in several industrial areas. Moreover, the biodegradation study of these polysaccharides has led to the identification of a new family of polysaccharide lyases very specific for these substrates. This review focuses on the actual knowledge of this class of acidic polysaccharides and on the enzymes acting about them.

INTRODUCTION

A bibliographic research using SciFinder Scholar with cellouronate, glucuronan and polyglucuronic acid shows the present interest of the scientific community for β-D-(1,4)-polyglucuronic acids. Effectively, most of scientific productions (full papers, patents, reviews, book chapters and others) have been published after 2000. This recent consideration for this family of polyuronides originates firstly in the necessity to upgrade cellulose from vegetable by-products and secondly in the identification of natural forms of β-D-(1,4)-polyglucuronic acids. The most described one was the exopolysaccharide produced by the strain *Sinorhizobium meliloti* M5N1CS [1-2]. This anionic homopolymer called glucuronan is a (1,4)-β-D-polyglucuropyranosyluronic acid variably *O*-acetylated at C3 and/or C2 position depending to the Mg^{2+} concentration in the culture medium. It has been largely published and patented for various applications [1]. Since its identification in 1992 [2] it was the sole source of (1,4)-β-D-polyglucuropyranosyluronic acid even if this anionic polysaccharide has been described in cell walls of *Mucor rouxii* [3] or algae [4,5]. However these fungal and algal glucuronans are always mixed with other polymers and their extractions and separations are tedious. The second interesting source of (1,4)-β-D-polyglucuropyranosyluronic acid appeared between 1995 and the beginning of the 2000 years with the development of methods allowing regioselective oxidation of cellulose. In this way, the use of 2,2,6,6-tetramethylpyperidine-1-oxy(TEMPO)-NaBr-NaOCl system opens the way to regioselective modifications of this polysaccharide with good yields [6]. In the case of the TEMPO-mediated oxidation of cellulose, different products were obtained depending on cellulose forms (cellulose I crystal, regenerated and mercerized celluloses) employed as starting materials [7-9]. The most interesting was the cellouronic acid whose structure was comparable to an unacetylated bacterial glucuronan. In regards to the numerous applications of polyglucuronic acids, their biodegradability was investigated [10-13]. Studies led to the identification of a new family of polysaccharide lyases (EC 4.2.2.14), called glucuronan lyase and expressed by bacteria or fungi. These enzymes have been successfully employed for oligosaccharides production and have opened a reflection on their physiological roles. Effectively the ability of micro-organisms to grow with a β-D-(1,4)-polyglucuronic acid as single carbon source implies abundance of this polysaccharide in nature.

This review focuses on the actual knowledge of β-(1,4)-D-polyglucuronic acids from various origins and on enzymes degrading them.

MICROBIAL GLUCURONANS

The species *Sinorhizobium meliloti* belongs to *Rhizobiaceae*, a group of soil bacteria (gram-negative, motile, non-sporulating rods) that fix nitrogen after becoming established inside root nodules of plants from the *Leguminoseae* family. This symbiosis can be possible thanks to nodulating genes (Nod factors) and excretion of polysaccharides [14]. These exopolysaccharides (EPS) have been fully explored because of their original structures, their chemical and rheological properties in solution which confers them good industrial applications, in cosmetic, food-processing or pharmaceutical industry notably.

The strain *Sinorhizobium meliloti* M5N1 is a bacteria isolated from a root nodule of alfalfa (*Medicago sativa*) and able to excrete in its culture medium a well known heteropolysaccharide : the succinoglycan [15]. Chemical mutagenesis of this strain has been investigated using N-methyl-N'-nitro-N-nitrosogua-nidine [16]. A mutant revealed to pro-

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duce a new extracellular polysaccharide. This new strain, labelled *Sinorhizobium meliloti* M5N1CS (NCIMB 40472), was still able to induce nodule formation on alfalfa roots, but less bacteria than the wild-type strain were detected in the infected alfalfa cells [17].

The native polysaccharide excreted by the M5N1CS strain was characterized. It was not soluble in 1 M H_2SO_4 and was resistant to acid hydrolysis even under drastic conditions (H_2SO_4 70 %) [2]. After reduction of all uronic acids, the polysaccharide could be hydrolysed and only glucose has been detected [16]. The reduced EPS has then been methylated and hydrolyzed with trifluoroacetic acid. After acetylation, GC analysis has revealed the presence of a single peak corresponding to a 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-glucitol, which proved that the monomers were 1→4 linked. ^{1}H NMR analyses were monitored on polysaccharide. Signals in the 2 ppm region, characteristic of *O*-acetyl groups were detected. The integration value ratio between the acetyl region (from 1.9 to 2.2 ppm) and the upfield and downfield regions (4.3 ppm to 5.1 ppm and 3.1 ppm to 4 ppm, respectively) has enabled to determine the acetylation degree (generally about 0.5 acetate per glucuronic acid). It has been deduced that the M5N1CS strain produced a homopolymer composed of β-D-(1,4)-glucopyranosyluronic residues variably acetylated at C3 and/or C2 position (Fig. **1**). Conformational and configurational features of deacetylated or acetylated glucuronan have been investigated through glucuronan fibre X-ray diffraction analyses and molecular modeling [18, 19].

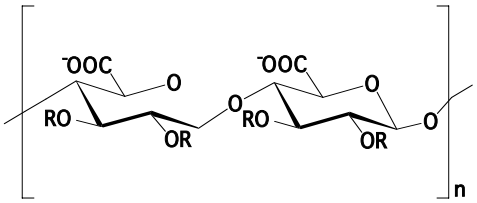

Fig. (1). Glucuronan structure.

The study of diglucuronic acid favourable conformations has allowed to distinguish four lowest energy regions A, B, C, D (Fig. **2**), which are typical of a (1→4) diequatorally branched disaccharide [20].

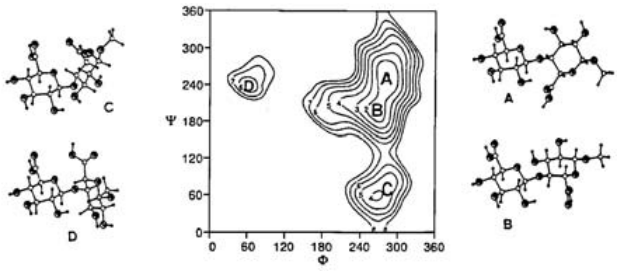

Fig. (2). Adiabatic map of a diglucuronic acid with the four minima structures of the wells labelled A, B, C, D Biopolymers, Vol. 45, 1998, p 170 [19]. Copyright 1998 John Wiley & Sons, Inc. Reprinted with permission of John Wiley & Sons, Inc

A and B wells corresponded to extended conformations whereas C and D ones corresponded to folded conformations. The A well was the most favourable because it allowed the formation of a hydrogen bond between O5' and O3. Introduction of acetate in position 3 caused the loss of this hydrogen bond. To the contrary, the addition of an acetate in position 2 had no effect on hydrogen bond. However, the presence of acetylation resulted in steric conflicts. Thus, bended conformations (corresponding to wells C and D) disappeared.

Deacetylated glucuronan helical structure modeling (POLYs software) led to two possible conformations with similar energies: a 2-fold helix and a left-handed 3-fold helix (Fig. **3**). Compared to the X-ray diffraction of deacetylated glucuronan fiber, the 2-fold helix seemed to be favoured in solid state. Experimental and theorical values of periodicity along the fiber have been compared (10.3 Å and 10.32 Å respectively). A good fitting has confirmed the modeling method. Acetylated glucuronan modeling has led to a different helix stabilisation than for the acetyl-free glucuronan (Fig. **4**). X-ray fibre diffraction diagrams have confirmed a change in the polysaccharide conformation. Acetates encountering steric conflicts and formation or losses of hydrogen bonds, the helix stabilisation varied in function of acetate positions. An acetylation in position 2 promoted a stabilisation of the 2_1 conformation, whereas an acetylation in position 3 led to a better stabilisation of the 3_2 helix. So it was concluded that glucuronan adopted principally a 2-fold conformation with local defects corresponding to the presence of several monomers acetylated in position 2 and/or 3.

Glucuronan was obtained by culture of the *Sinorhizobium meliloti* M5N1CS strain in bioreactor with the Rhizobium complete (RC) medium supplemented with 1 % of fructose or sucrose [21]. The pH and temperature of the culture were respectively at 7.2 and 30°C. Agitation was maintained below 400 rpm to limit shear forces that led to a decrease in polysaccharide molecular weight. During fermentation, glucuronan excretion reached a peak in the growth stationary phase. Addition of magnesium salts (up to 3.25 mM per day) in the culture medium led to an increase in glucuronan acetylation degree [22].

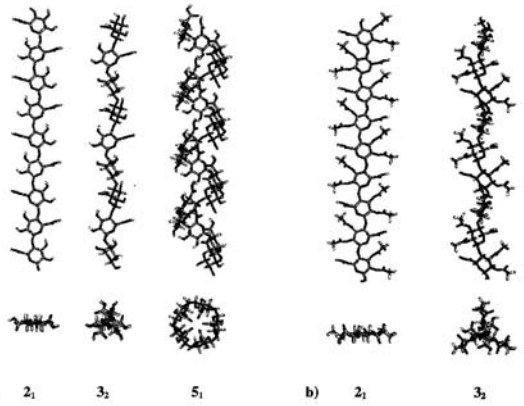

Fig. (3). Regular helical conformations of deacetylated (a) and fully acetylated (b) glucuronans Biopolymers, Vol. 45, 1998, p 170 [19]. Copyright 1998 John Wiley & Sons, Inc. Reprinted with permission of John Wiley & Sons, Inc.

Thus, a glucuronan obtained on medium without magnesium salts supplementation had an average acetylation de-

gree of 50 % against 75 % for one coming from a culture supplemented with $MgSO_4$. However, a decrease in average molecular weight of glucuronan combined with a loss of polysaccharide productivity has been observed for old cultures supplemented with $MgSO_4$. This was due to an enzymatic degradation of this bacterial polyuronide by a polysaccharide lyase specific of this substrate [10, 11].

All the forms of glucuronan (native, highly acetylated and deacetylated) have been characterized for their physicochemical and biological properties. First of all, glucuronan molecular weight has been measured between 6.10^4 and 4.10^5 using size-exclusion chromatography coupled to a refractometer and multi angle laser light scattering [2]. The intrinsic pK value (called pK_o associated with the removal of a proton from the uncharged chain) has been evaluated to 3. It is in accordance with pK_o of other well-known anionic polysaccharides such as alginates and pectins [23]. Furthermore, when glucuronan is acidified to a pH lower than 3, a precipitation is observed. This phenomenon is common to all polyuronic acids like for example polygalacturonic acid [2].

Polysaccharide aggregation capacity has been investigated using viscosity measurement at low glucuronan concentration and with increasing NaCl concentrations. It has been observed that for a salt concentration above 0.1 M, the Huggins constant k' increased greatly. This suggested that intermolecular interactions with aggregate formation occured. Moreover, the polysaccharide solubility was influenced by its acetylation degree. The less acetylated the more soluble. In fact, increase of *O*-acetyl groups promoted hydrophobic interchain associations. Consequently, polysaccharide aggregation was observed. These compulsions have been confirmed using configurational modeling of a glucuronan variably acetylated. The chain stiffness has been measured using persistence length (L_p) evaluation. A L_p of 105 Å has been evaluated for a glucuronan with an acetylation degree of 10 %, corresponding to a semi-rigid polymer in solution. This theorical value is in accordance with the experimental one (114 Å). An increase in the acetylation degree led to an increase in the chain stiffness, but this effect was slight. However, acetylated regions promoted intermolecular associations that conducted to local precipitations [24]. Furthermore, a relationship between intrinsic viscosity ($[\eta]$ in $mL.g^{-1}$) and molecular weight (Mw) was obtained: $[\eta]= 2.10^{-2} \times Mw^{0.9}$ [2]. A comparable relationship has been obtained for alginates [25].

Another property of glucuronan was its ability to form a gel in the presence of monovalent, divalent or trivalent cations, depending on polymer concentration and ionic strength [26]. In the presence of monovalent ions like Na^+, glucuronan formed a thermoreversible gel whose stability increased with ionic strength. In the presence of divalent cations, thermally stable gels were formed for cation concentrations higher than 10^{-2} M, whatever the divalent cation being used (Ca^{2+}, Cu^{2+}, Ba^{2+}). When the acetylation degree of polymer increased, the Young's modulus of glucuronan decreased. *O*-acetyl groups were responsible of the decrease of gel resistance because they inhibited the stability of ionic linkages. Even if the Young's modulus is comparable to the one obtained for alginates, the gel formation process seemed to be different. Analyses of the retention of calcium ions in glucuronan in the presence of sodium ions have been investigated. They have revealed that more than 50 % of calcium ions were removed when the gel was submerged in a high ionic strength solution of sodium without a decrease in the gel cohesion. This indicated that the gel formation process depended mainly upon the ionic strength. To the contrary pectin or alginate gels were very stable and calcium ions were very difficult to displace. These observations were confirmed thanks to a molecular modeling investigation of the interactions between β-(1→4)-D-polyglucuronic acid and calcium ions [24]. Favourable binding sites for calcium ions along both 2-fold and 3-fold helical structure of polyglucuronic acid were calculated (GRID program, version 15). It has revealed that glucuronan binded calcium essentially through electrostatic interactions, the only necessary condition being the closeness of a negatively charged carboxylic oxygen atom. A "cloud" of favourable positions for calcium ions around the chain was observed (Fig. **4**). A similar modeling has been obtained for β-(1,4)-D-polymannuronic acid. To the contrary, molecular modelling of α-(1,4)-D-polyguluronic acid and α-(1,4)-D-polygalacturonic acid showed tetradentate chelation sites that provided a strong calcium binding.

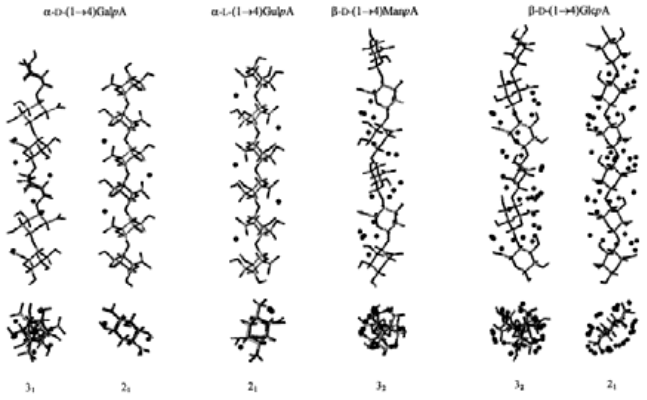

Fig. (4). Favorable positions of calcium ions along the stable ordered conformations of four polysaccharides (α-D-(1,4)-galacturonan, α-L-(1,4)-guluronan, β-D-(1,4)-mannuronan and β-D-(1,4)-glucuronan) over a 15 kcal/mol window above the lowest interaction energy Carbohydrate Research, Vol. 317, 1999, p 126. Copyright 1999 Elsevier Science Ltd. Reprinted with permission of Elsevier Science Ltd [24].

BIOLOGICAL ACTIVITIES AND PATENTS RELATIVE TO BACTERIAL GLUCURONANS AND OLIGOGLUCURONANS

A few bacterial polysaccharides have been developed in industry until now. Xanthan is obtained from the fermentation of *Xanthomonas campestris* and gellan is produced by *Sphingomonas elodea*. Despite relatively high production costs but thanks to their rheological properties, those polysaccharides have many industrial applications as food additives, thickeners, emulsifiers or stabilisers.

Concerning glucuronan, three patents have been registered until now [27-29]. Patent WO 9318174 registers the strain *S. meliloti* M5N1CS, the glucuronan as a polymer of glucuronic acids from 300 to 2500 residues with β-(1-4) links, and its derivatives (corresponding esters, ethers, and

mixes of all derivatives). This patent also protects glucuronan for its use in food products, pharmaceutics, cosmetics or water purification, particularly as a gelifying, thickening, hydrating, stabilizing, chelating or floculating agent. A second patent (FR2781673) registers the immunostimulating properties of glucuronan and oligoglucuronans. Actually, glucuronans have shown immunostimulating activities on human blood monocytes. In comparison with alginate, glucuronan induced a better production of Il-6 and TNF-α cytokines and an equivalent production of Il-1 cytokine. The third patent (WO 9913855) reports a composition for cosmetics and dermopharmaceutical use containing glucuronan combined with an algae extract from *Haematococcus pluvialis* [29]. The product obtained has showed action of skin nourishment, care and regeneration.

The activity of partially *O*-sulfated and *O*-acetylated glucuronans was assayed in tissue regeneration tests. Muscle regeneration is a complex phenomenon where specific agents of the extracellular matrix such as growth factors play a key role. *In vivo*, growth factor efficiency is countered by proteolytic activities coming from the multiple proteases which are released at the site of the injury. By interaction with growth factors, heparan sulfate may enhance the bioavailability of these factors. Thus, sulfation procedures were applied on glucuronan (dac = 0.7) and partially deacetylated glucuronan (dac = 0.2) in order to obtain heparan sulfate like. Sulfation of glucuronans by the SO_3-pyridin complex was performed on tetrabutylammonium polymer salts which provided solubility in organic medium. The degree of sulfation (dsS) estimated by conductimetric analyses were 1.4 and 1.6 for native and partially deacetylated glucuronan, respectively. Unfortunately, macroscopic and histological analyses of injured muscles treated by the sulfated polysaccharides showed global muscle degeneration because samples still contained pyridin. So, another sulfation reagent was applied on the polymer samples in order to obtain pure sulfated glucuronan. The SO_3-dimethylformamide complex led to a higher dsS (1.7 and 2.6 for native and partially deacetylated glucuronan, respectively). Muscular lesions were carried out on male rats Wistar. Under transitory anaesthesia, the *Extensor Digitorum Longus* muscles of the legs back were mechanically injured. A weak regenerating activity was observed with native, sulfated, or partially deacetylated glucuronans. Authors postulated that the yield of acetate groups may modulate the specific activity. However the native and sulfated glucuronan showed a good regenerating activity [30].

Concerning oligoglucuronans, the earlier patent WO 9318174 described the potential efficiency of oligoglucuronans in various domains [31]. It claimed their use in farming particularly for *in vitro* applications or for eliciting of plant natural defence mechanisms or also as additive for the coating of seeds. However, no example of these purposes were demonstrated until the patent FR 2795289 [31] where an application of plant natural defence elicitation by oligoglucuronans has been described. The model used was composed of protoplasts from *Rubus fruticosus L.* treated by a pool of oligoglucurans with dp <10 at 400 µg/L. These oligomers amplified a marker of defense reaction (β(1,3)-D-glucanase activity) by a factor of 1.5. The same claims were found in the FR 2885911 patent which revendicated also the use of the glucuronan lyase from *Trichoderma* sp. GL2 as a phytosanitary agent [32].

Another application concerned the immunostimulating properties on animal cells. It was reported the cytokine production (TNF-α, Il-1 and Il-6) by human blood monocytes. The LMW glucuronans induced a bigger production of IL-6 and TNF-α in the same conditions than those obtained by stimulating the cells with lipopolysaccharides or alginate molecules. Similar results were acquired for LMW and alginate molecules for the IL-1 cytokine [28].

CHEMICAL OXIDATION OF CELLULOSE

These last two years, lots of studies have been investigated to find inexhaustible ways to produce efficiently synthetic polyglucuronic acid by chemical oxidation of natural and abundant glucans such as cellulose and chitin. Cellulose is the world's most abundant natural polysaccharide. This renewable resource, composed of *β*-(1,4)-linked glucopyranosyl units, has been widely studied for the purpose of developing new biotechnologies for food, chemistry, pharmaceutical and fuels. Nevertheless, due to its intra- and intermolecular hydrogen bonds, cellulose is basically insoluble in most solvents. Then it has appeared necessary to modify the crystalline structure of cellulose by chemical substitution of the hydroxyl groups, allowing cellulose to be less crystalline and more water-soluble. Water soluble cellulose derivatives as many of other water-soluble polysaccharides play significant roles in many food, technical, industrial and medical applications [33] such as colloidal stabilizer or emulsifier. These properties and the non-toxic nature of polysaccharides have allowed to include them within regulating systems in pharmaceutical formulations to control the release rates of active substances [33]. In this context, cellulose chemical modifications such as esterification, have resulted in the development of biodegradable plastics used as food product packaging [34]. As already reported by Yalpani [35], polysaccharide chemical modifications represent promising ways to produce new polysaccharide structures possessing original properties.

Oxidized cellulose presents the advantage of being produced from a renewable material and being less expensive than carboxymethylcellulose (CMC), even if both provide comparable properties. However, cellulose oxidation is disadvantaged by the non-solubility of cellulose, making it inadequately accessible by oxidizing agents. Many medical applications attributed to polyglucuronic structures have been described for anionic cellulose such as sutures, hemostats, adhesion prevention devices, promotion of bone regeneration, promotion of antibacterial activity, and use in periodontal therapy… [36]. Moreover, because polyglucuronic acids have abundant hydroxyl groups, they can form intra and intermolecular hydrogen bonds to constitute edible films with high gas-barrier properties [37]. Considering the number of putative applications of these oxidized carbohydrates and their low representation in natural media, the selective oxidation of polysaccharide primary alcohol groups has been studied for more than half a century.

In the 40's, the polyglucuronic acid production by the selective oxidation of cellulose using nitrogen oxides was reported [38, 39]. This reaction has been improved by performing the experimentation in phosphoric acid and sodium nitrite [40-42]. Unfortunately, a large depolymerization of cellulose could not be prevented due to uncontrolled reac-

tions such as oxidative scission of 1,2-diols or formation of dicarboxylic by-products. In 2006, a successful method using (NO_2/N_2O_4) in supercritic phase was proposed for the quantitative production of polyglucuronic acids [43]. However the main drawback limiting this method is its dangerous and high-technological process.

If sodium nitrite is replaced by nitrate [44], oxidation yield is increased and polysaccharide depolymerization is lower. Consequently "greener chemical reagents" have been looked for. The stable nitroxyl radical 2,2,6,6-tetramethylpyperidine-1-oxyl (TEMPO) in presence of NaOCl and NaBr has then been described as a specific catalyst for the regioselective oxidation of neutral polysaccharide primary hydroxyl groups [44, 45].

TEMPO/NaBr/NaOCl SYSTEM MEDIATED OXIDATION OF POLYSACCHARIDES

De Nooy *et al.* [44] first reported the efficiency of TEMPO/NaBr/NaOCl system for the yielded production of homogeneous polyuronic acid structures (Fig. **1**). Consequently, TEMPO reagent has become the exclusive catalyst for the regioselective oxidation of high molecular weight polysaccharides. The advantages of this chemical process are its high reaction level, its high production yield, and the suitable selectivity of primary alcohol groups (especially in carbohydrates). Until now, lots of studies and patents have described procedures for TEMPO mediated oxidations of water-soluble natural polysaccharides and their derivatives, as well as water-insoluble polysaccharides such as chitin [46-48] and cellulose [8, 45, 49-52]. The water-soluble cellulose and chitin derivatives synthesized by the way of this reaction present a great potential with respect to their numerous rheological properties, such as gelling or thickening.

As shown in Fig. (**5**), production of polyglucuronic acid by primary alcohol oxidations is successful because it needs less than 1 mol % of TEMPO, aqueous sodium hypochlorite as inexpensive co-oxidant and sodium hydroxyde as mild base. Additives such as NaBr or KBr can be used to increase the rate of oxidation reaction. In fact, the oxidation reaction is hampered by the high crystalline state of cellulosic and chitinic materials. It extremely decreases primary alcohol accessibility due to the hydrogen bond interactions. Even for a long oxidation experimentation (24 h) in alkaline medium (pH 10), the native polysaccharides keep being partially insoluble [8].

This way, it appeared essential to make hydro-soluble or hydro-dispersible cellulose and chitin to produce polyglucuronic acid in good conditions. Thus, a pseudo amorphous cellulose has been used for the preparation of polyglucuronic acid. It was obtained by the solubilization of a cellulose sample in 9% aqueous sodium hydroxide and was regenerated in anhydrous ethanol or triacetate cellulose (Table **1**). Consequently, a high level of oxidation (Table **2**) was obtained.

Another way to recover pseudo amorphous cellulose samples was a mercerization treatment of cellulose in 20 % of aqueous sodium hydroxide [45]. Pseudo amorphous celluloses were then oxidized using TEMPO–NaBr–NaOCl system allowing an increase of polyglucuronic acid production yield. Sun *et al.* [48] have recently produced chitouronic acid at large scale after the conversion of the chitin crystal structure. In fact, authors have shown that chemical and physical pretreatment of native chitin made it possible to change the crystal structure from α to β. Thus, the TEMPO mediated oxidation of pretreated chitin considerably increased the polyglucuronic acid yield from 36 % to 97%.

Chitouronic acids have been produced using TEMPO mediated oxidation of regenerated chitin after necessary pretreatments of crustacean chitins and fungal chitin–glucan [46]. Applying TEMPO/NaBr/NaOCl system to mercerized or regenerated cellulose, regenerated chitin or N-acetylated chitosan made it possible to recover β-(1,4)-polyglucuronic acids (cellouronic acids) and β-(1,4)-poly-*N*-acetylglucosaminuronic acids (chitouronic acids) (Fig. **6**) quantitatively contrary to the oxidation of native polysaccharide. In this way, few amounts of carboxyl residues were introduced because of the lower accessibility of TEMPO system to the native and insoluble polysaccharide [7, 37, 46, 48].

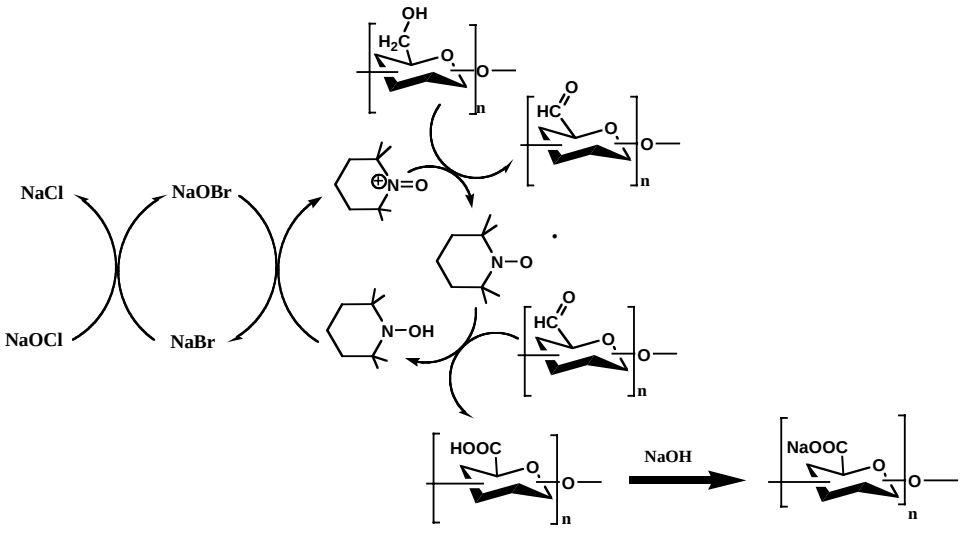

Fig. (5). General way to produce polyglucuronic acid by oxidation process using TEMPO/NaBr/NaOCl system.

Table 1. Production of Polyglucuronan by Oxidation of Natural *β*-(1,4) Glucan Using TEMPO/NaBr/NaOCl System

Polysaccharide Names	Pre Treatment	Yield (%)	References
Cellulose	- NaOH (20%)	8 >95	[53] [45]
TriAcetate cellulose	concentrated H_2SO_4 (72°C)	80	[51]
Chitin from Fungi	- -	25 45	[46] [54]
Chitin from crustacean	- soaked in H_20 (50°C, 4 h) cold concentrated H_2SO_4 DMAc-LiCl 5%	36 58 97 > 90	[48] [48] [48] [46]
Chitosan	Acetylated (Ds=0.6)	92	[48]

Table 2. Oxidation by TEMPO/NaBr/NaOCl System of Different Pseudo Amorphous Celluloses

Cellulose Sample	Solubility (%)	Polyglucuronic Acid Yield (%)	References
Bacterial	25	25	[8]
Cotton	26	31	[8]
Cotton Linter	35	39	[8]
Rayon	82	45	[8]
Microcrystalline	97	58	[8]
Triacetate cellulose (*DS=0.5)	100	80	[51]

*DS= Degree of substitution by acetate.

More recently the biodegradability of most of these synthetic polyuronic acids has been successfully tested [37]. This characteristic opens the way to applications in gas-barrier biomaterial and constitutes environmentally friendly biodegradable packaging films. Studies have led to the biodegradation of these synthetic polyuronides using new specific polysaccharide cleavage enzymes as polysaccharide lyases or hydrolases in order to consider large productions of potential bioactive anionic oligosaccharides [55, 56]. The use of these enzymatic activities could also generate new oligosaccharides with biological activities as described on lots of organisms such as bacteria, fungi, plant, *algae* and mammalian [57, 58].

Furthermore, other bio-applications can be contemplated for oxidized cellulose. Indeed, as previously mentioned, a bacterial *O*-acetylated polyglucuronic acid (glucuronan) and its oligoglucuronide derivates have been reported to be bioactive agents in animals [30] as well as elicitors of defense responses in plants [31].

GLUCURONAN/CELLOURONATE ACTING ENZYMES

Seeing that polyglucuronic acid structures have been recently described, enzymes that are able to degrade them have been investigated only for one decade. All available articles, book chapters and reviews focus on enzymes degrading the β-(1,4) glycosidic bond between two glucuronic acids. No studies have been published on enzymatic synthesis of β-(1,4) polyglucuronic acids until now.

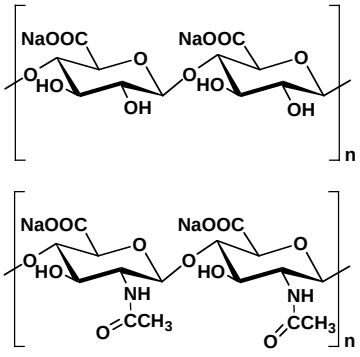

Fig. (6). Chemical structures of (a) cellouronic acid and (b) chitouronic acid obtained by TEMPO/NaBr/NaOCl mediated oxidation of regenerated cellulose and chitin or chitosan respectively.

The goal of these studies is double. First, in regards to the numerous putative applications of cellouronate and bacterial glucuronan in agronomy, cosmetic and therapeutic, authors have to validate the biodegradability of these polysaccharides. Secondly other investigations to produce at large scale

oligomers derivating from cellouronate or glucuronan lead to use them as bioactive compounds knowing that oligosaccharides are often implicated in biological activities [57].

Polysaccharide enzymatic cleavages may be catalyzed by two families of carbohydrate enzymes: polysaccharide hydrolases (EC 3.2.1.-) and polysaccharide lyases (EC 4.2.2.-) (Fig. **7**). Polysaccharide lyases (PL) are a group of enzymes that act on polysaccharides by β-elimination. This mechanism leads to Δ-(4,5)-unsaturated oligouronic acid production. This reaction consists in the general base-catalyzed elimination of the proton at C-5 of an uronic acid. An electron is transferred from the carboxyl group to form a double bond between C-4 and C-5. It results in the elimination of the 4-*O*-glycosidic bond and in the formation of 4-deoxy-l-*erythro*-hex-4-enopyranosyluronic acid. This reaction conducts to the formation of an unsaturated uronate at the new non-reducing end [59, 60]. The PL substrates consist in (α and β)-(1,4) linked polyuronic acids such as alginates, or polymers including this kind of linkage in their global structure such as pectins, gellan, glycosaminoglycans and xanthan [59, 60]. Nevertheless we noticed in literature the existence of α-(1,4) glucan lyases [61]. Unsaturated oligosaccharides with a deoxy-L-erythro-hex-4-enopyranosyluronate terminal unit and polymerization degrees between 2 and 5 are the main degradation products of the PL activities. However, in some cases, unsaturated monosaccharides can be formed [62].

Polysaccharide lyases are widely distributed amongst Gram (–) bacteria. Nevertheless, some of them have been purified and characterized from Gram (+) bacteria, *algae*, gastropods and fungi.With regards to β-(1,4) polyglucuronic acid depolymerization, most of the clearly identified activities are glucuronan lyases from bacteria and fungi (Table **3**) even if some glucuronan hydrolases have been described [13, 63].

However, if the obvious polyglucuronic acid degradations using polysaccharide cleavage enzymes were effective in all these studies, enzymes employed were often crude extract from filamentous fungi including cellulases, hemicellulases, pectinases and others. So the unsaturated products generated by these putative polysaccharide hydrolases are probably polysaccharide lyases and more especially glucuronan lyases. From now, glucuronan and cellouronate have actually never been identified as substrate for cellulases. In the same way, Dow *et al.* have previously detected oligoglucuronides in extracellular media of *Mucor rouxii* cultures [64]. These authors have related an action of lytic enzymes which could release soluble hydrolysis products from acidic polymers included in the cell wall (the mucoran). Moreover, some extracts from *Mucor rouxii* contained an enzyme activity which could degrade the homopolymeric β-(1,4)-D-glucuronan (mucoric acid) of the fungal wall. It could also degrade the homopolymeric glucuronan domains of mucoran. This enzyme was active at neutral pH and had an estimated M_w of 27500 Da. The major digestion product was a disaccharide, suggesting an endolytic mechanism [64]. However, the lack of investigations relative to the structure of these oligomers did not allow to conclude on the cleavage mechanism. Nevertheless, the presence of polyglucuronic blocks in the mucoran [64], the biodegradation of cellouronate [13] and the identification of deacetylated glucuronan cleavage by crude enzyme extracts from fungi [63] led authors to the conclusion of a relative abundance of polyglucuronic structures in nature. This abundance was confirmed by the detection of polyglucuronic structures in ulvan, a sulfated glucuronorhamnoxyloglycan extracted from the green seaweed *Ulva lactuca* [4, 5]. We noticed in this context that a marine bacterium was able to cleave ulvan to generate oligosaccharides with 4-deoxy-L-threo-hex-4-enopyranosiduronic acids at the non-reducing end [5, 65]. This enzyme could be a glucuronan lyase but its cleavage site in the ulvan chain has not been clearly identified.

Glucuronan lyase (EC 4.2.2.14) characterization has begun with the detection of unsaturated and deacetylated oligoglucuronans in culture broths of *S. meliloti* M5N1CS strain [11]. Associated to glucuronan synthesis, this glucuronan lyase was purified and characterized [60, 66]. It degraded various acetylated (apart from 2,3-di-O-acetylated) and non-acetylated glucuronans with an endolytic mode (Fig. **8**).

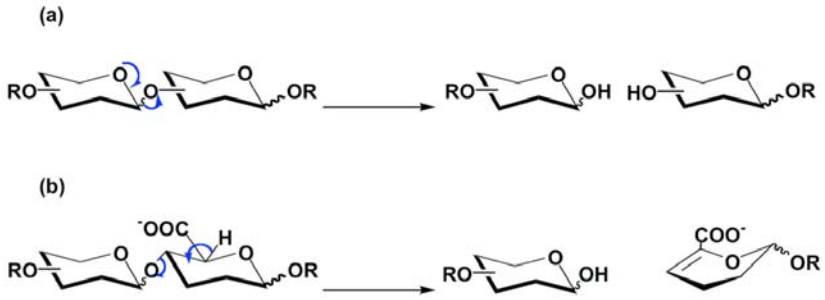

Fig. (7). Enzymatic cleavage of polysaccharides by polysaccharide hydrolases (a) and polysaccharide lyases (b).

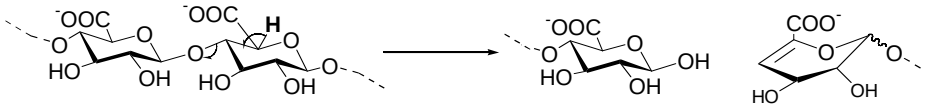

Fig. (8). Cleavage of deacetylated glucuronan by glucuronan lyase from *S. meliloti* M5N1CS.

Authors tried with poor success to employ this endopolyglucuronic acid lyase in order to produce unsaturated oligoglucuronans with putative biological activities [66]. In fact, this activity was highly specific of deacetylated glucuronan. No significant activity was observed when highly acetylated glucuronan was used as substrate. 2,3-di-*O*-acetyled residues had the most inhibitory effect.

These results agreed with those obtained with alginate lyases inhibited by *O*-acetylated mannurosyl residues [67, 68]. This high level of inhibition by acetate, associated to a low specific activity expressed by the bacterial strain synthesizing the polysaccharidic substrate suggested that this lyase was used by the micro-organism to control the molecular weight of its exopolysaccharide. This hypothesis was corroborated by literature data since polysaccharide lyase biosynthesis is often associated to polysaccharide substrate production. Indeed, molecular biological studies have shown a genetic association between polysaccharide biosynthesis encoding genes and PL encoding genes. This association could be implicated in the regulation of medium viscosity for metabolite diffusions or for polysaccharide release [60, 69, 70].

With the same aim to generate anionic oligosaccharides at large scale, Delattre *et al.* [71] have investigated other sources of glucuronan lyases. Knowing the potential of filamentous *fungi* to produce polysaccharide cleavage enzyme and the previous characterisation of glucuronan pattern in *fungi* cell wall [3], a strain of *Trichoderma* sp. called *Trichoderma* sp. GL2 was isolated from compost using bacterial glucuronan as single carbon source. This new fungal glucuronan lyase degraded all glucuronans (acetylated or not), ulvan extracted from the green seaweed *Ulva lactuca* and also cellouronate. [71, 55]. This endolytic enzyme (GL2) with a molecular weight of 27 kDa had a higher specific activity than the one from the *S. meliloti* M5N1CS strain.

The GL2 activity led to the formation of unsaturated oligomers with various degrees of polymerization depending of the substrate acetylation degree. The smallest substrate cleaved by the enzyme was an unsaturated trisaccharide. When deacetylated glucuronan was used, reaction products were a pool of oligomers from dp 1 to dp 3. The spontaneous transformation of the unsaturated monomer in α-keto-glucuronic acid has been considered thanks to literature data [72]. When the glucuronan lyase from *Trichoderma* sp GL2 was applied to nascent glucuronan, product was an unsaturated oligomer with a dp 3 and not a mix of acetylated oligomers as noticed with the purified polymer [58]. The biological function of this glucuronan lyase is unknown from now on. Nonetheless, a number of *Trichoderma* strains are well-known for their mycoparasitic action against phytopathogenic fungi. They can produce fungal cell wall cleavage enzymes when they are cultivated on specific substrates (like chitin or laminarin) or directly on fungal cell wall as carbon sources [73]. Moreover, glucuronan patterns or uronic acids are included in the structure of some fungal cell wall [3, 74]. These two knowledges suggested that this new glucuronan lyase could be implicated in the lysis of fungal cell wall in association with other polysaccharide cleavage enzymes [75]. At the same time of these investigations, other authors have screened from a sample of soil micro-organisms which were able to produce cellouronate lyase [56]. Surprisingly, even if cellouronate is not recognized as an abundant natural polymer, four different strains grew on media with cellouronate as sole carbone source (Table **3**). Production of cellouronate lyase activity was confirmed for all the strains. The one expressed by the strain *Brevundimonas* sp. SH203 has been purified, characterized and labelled CUL-I. Once more, this enzyme has revealed to be an endolyase with a high specificity to cellouronate. Residual activities were noticed with other polyuronates like alginate and amylouronate. After cellouronate degradation with CUL-I, the main products

Table 3. Biochemical and Physicochemical Properties of β-(1,4) Glucuronan Lyases

Source	Nature	Molecular Weight (kDa)	Substrate Specificity	pI	Specific Activity ($U.mg^{-1}$)	*Km* (mg/mL)	Optimum Temperature (°C)	Temperature Stability (°C)	Optimum pH	pH Stability	Ref.
Sinorhizobium meliloti	endo	20	Glucuronan	4.9	15.76*	-	50	<50	6.5	-	[10]
Brevundimonas sp.SH203	endo	39	Cellouronate Alginate Amylouronate	-	13700**	0.43	-	10-40	7.5	5-9	[56] [76] [55]
Brevundimonas sp.SH203	exo	62	Cellouronate	-	3800**	3.4	-	10-60	7.5	5-9	[76]
Brevundimonas sp.KH403Y	-	-	Cellouronate	-	-	-	-	-	-	-	[56]
Sphingomonas sp.	-	-	Cellouronate	-	-	-	-	-	-	-	[56]
Sphingopyxis sp.	-	-	Cellouronate	-	-	-	-	-	-	-	[56]
Trichoderma sp.	endo	27	Glucuronan Cellouronate Ulvan	6.95	162***	-	55	<35	5.5	4-8	[55] [55]
Trichoderma reesei	-	-	Glucuronan	-	-	-	-	-	-		[63]

*1U: amount of enzyme necessary to increase 1 U in the absorbance at 235 nm per min.
** 1U: amount of enzyme necessary to increase 0.01 U in the absorbance at 235 nm per min.
*** 1U: amount of enzyme necessary to release 1 μmole of product per min.

were similar to those obtained after a degradation of deacetylated glucuronan with the glucuronan lyase from *Trichoderma* sp. GL2.

To resume, glucuronan and/or cellouronate lyases are until now the only enzymes working on β-(1,4)-D-polyglucuronic acids. All the identified enzymes are endolyases. As these enzymes were discovered in various micro-organisms, we can deduce their abundance and diversity in nature. The isolation of an exolyase working specifically on oligoglucuronans and/or oligocellouronates could improve the comprehension of polyglucuronic acid assimilation. Indeed, Konno *et al.* have recently identified an exocellouronate lyase (CUL-II) from the CUL-I producing strain (*Brevundimonas* sp. SH203). Until now, this is the first exolytic enzyme described for the degradation of β-D-(1,4)-polyglucuronic acid. CUL-II can degrade saturated or unsaturated dimers in monomers more rapidly than the cellouronate polymer. Thus depolymerization of cellouronate is more efficient when CUL-I and CUL-II are both present in the mixture [76].

In future, to increase the number of proteins in this enzyme family (EC 4.2.2.14), the clonage of glucuronan/cellouronate lyase genes seems to be unavoidable.

PURIFICATION OF POLY- AND OLIGOGLUCURONANS

Oligoglucuronans are negatively charged oligosaccharides composed of glucuronic acid residues, which can be partially acetylated on C2 and/or C3 position. They are obtained from enzymatic β-elimination of glucuronan or cellouronate with glucuronan/cellouronate lyases as described above. The oligoglucuronan structure can be analyzed in term of acetylation degree (dac) and polymerization degree after purification.

The most common treatment for the purification of β-(1,4)-polyglucuronic acids is the precipitation (with addition of 3 volumes of alcohol or in acidic media) since the polysaccharide has a high molecular weight [1, 27, 77, 78]. Another useful and common method for purification is also the tangential ultrafiltration (UFT) on membranes with a molecular weight cut off of 100 kDa [1, 10, 30, 58, 66, 78].

These techniques can be easily adapted to oligomer purification. Alcohol precipitation with addition up to 7 volumes of alcohol allows to isolate a mixture of oligomers. In our team, alcohol precipitation is the common protocol to recover oligosaccharides obtained from enzymatic hydrolysis of glucuronan with *Trichoderma* sp. GL2 crude enzyme extract. Another way is UFT because this process can be easily applied to all types of synthetized oligoglucuronans with the available membranes cut-off [79-81]. Delattre *et al.* [12, 57, 71, 78] investigated the production of oligoglucuronan by cleavage of highly (dac = 0.96) and normal *O*-acetylated glucuronan (dac = 0.7) during a fermentation process. A common *S. meliloti* M5N1CS culture was implemented in bioreactors. Fungal glucuronan lyase crude extract was added to broths before or after glucuronan synthesis. The fermentation media were centrifuged (34 000×*g* for 30 min) to remove bacteria. The supernatants containing poly- and oligoglucuronic fractions were treated by UFT through 100, 30, 10 and 5 kDa membranes successively. In these conditions, depolymerization of nascent mainly 3-*O*-acetylated glucuronan produced a large amount of a deacetylated oligoglucuronan of dp 3. With 2,3-di-*O*-acetylated glucuronan, the fungal glucuronan lyase crude extract made it possible to generate acetylated glucuronan with molecular weight below 100 kDa but did not allow to recover oligosaccharides. In 1999, Harscoat *et al.* [82] described the treatment of fermentation broths using dynamic cross-flow microfiltration (with a 0.2 μm nylon membrane on a 16 cm rotating disc holder) to obtain a bacteria free medium. Improvements were noticed thanks to addition of valves on the holder which produced an increase of the permeate flux. Moreover, the use of a rotating disc made it possible to recover the polysaccharide by UFT through a 50 kDa membrane [83]. The same technique was employed using other cut-off corresponding to ultra- or nanofiltrations to separate various families of glucuronans and oligoglucuronans. The filtration module was composed of a 15 cm diameter aluminium disc equipped with 8 radial valves 6 mm high, rotating at adjustable speeds. The 190 cm^2 membrane was fastened on the holder. The best result was obtained with a cascade of 3 ultrafiltrations at 50, 20 and 10 kDa followed by 2 nanofiltrations at 1 kDa and 700 Da. The first permeate at 50 kDa contained oligomers of dp < 25. The 20 kDa permeate contained oligomers of dp < 10. In the 10 kDa permeate oligomers of dp < 5 were recovered and finally oligoglucuronans with dp between 3 and 5 were collected in the 700 Da retentate [84].

All techniques described above allowed to separate families of different molecular weights but were not sufficiently accurate to obtain pure oligosaccharides with define dp and dac. So other methods such as liquid chromatography had to be used in order to purify oligosaccharides.

Unsaturated oligomers have been separated with various LC modes such as size exclusion, anion-exchange and pseudobioaffinity chromatography (Table **4**). The most common detection method was UV absorption at around 230 nm attributed to Δ-(4,5)-unsaturated uronic acids at the oligoglucuronan non-reducing end, with routinely detectable amounts in the nanogram range. Other detection methods such as refractive index (RI) or evaporative light scattering detection (ELSD) could be easily used for sugar detection. The detection mode was largely dependent on the kind of chromatography used.

Dantas *et al.* [63], in the study of oligoglucuronate prepared by Celluclast 1.5L (Novo) hydrolysis, implemented a conventional size exclusion chromatography to separate easily deacetylated oligomers up to dp 9 on a Biogel P6 column (Fig. **9**). Many other authors [10, 12, 55, 58, 66, 78, 80] used this technique in order to purify oligoglucuronans. Nonetheless, this kind of separation was disturbed by the polyanionic character of oligomers. In order to suppress this effect, an ionic strength had to be added in the eluent. Consequently a desalting step was necessary to recover pure oligosaccharides, unless a volatile eluent such as ammonium formate was employed. Analytical SEC-HPLC, used for example on Shodex columns (SB-802.5 coupled with SB-802), offered identical separation and could be used to monitor enzymatic degradation. However, same limitations than previously described have been observed, because of the polyanionic character. Good resolution for deacetylated oligoglucuronans were obtained but this technique revealed to be not enough efficient to separate accurately a mixture composed of acetylated or deacetylated oligomers.

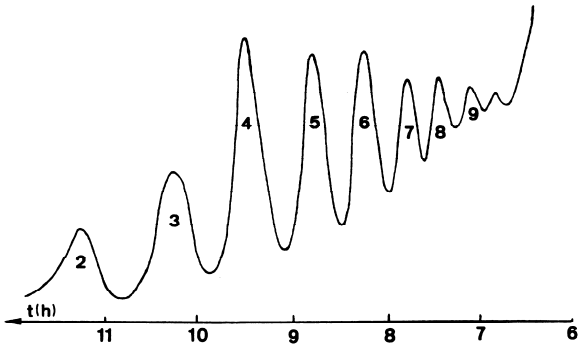

Fig. (9). Fractionation of oligoglucuronans obtained by enzymatic hydrolysis with Celluclast 1.5L on a Biogel P6 column (100 x 2.5 cm, 60 ml/h $NaNO_3$ 50mM, R.I. detector) The dp is indicated by the numbers under the peaks. Carbohydrate Research, Vol. 265, 1994, p 304. Copyright 1994 Elsevier Science B.V. Reprinted with permission of Elsevier Science Ltd.

The ionic character of oligoglucuronans made it possible to purify them on a weak anion–exchange column. The first example concerns the separation of neutral and acidic compounds obtained after *in situ* degradation of glucuronan during a fermentation process. After an initial treatment by UFT, the last permeate recovered from a 5 kDa cut-off membrane contained cyclic β-(1,2) glucans which were identified thanks to their ^{1}H NMR spectra in accordance to the literature [22]. In order to separate these neutral compounds from oligouronates, the 5 kDa permeate was fractionated on a DEAE Sepharose CL-6B column. The uronic fractions were also applied on a SEC column (Biogel P6) and an unique and deacetylated oligoglucuronan of dp 3 was identified thanks to ^{1}H NMR and mass spectrometry [58]. Another example consisted in the separation of deacetylated oligoglucuronans which were generated by enzymatic degradation of purified glucuronan using the *Trichoderma sp.* GL2 lyase with a similar DEAE Sepharose CL-6B column. Thus, a mix

Table 4. Chromatographic Conditions for Oligoglucuronans Separation

Ref.	Column (Length in cm x I.D. in mm)	Eluent	Detection
[55, 58, 78]	Biogel P6 fine (100 x 26)	50 mM ammonium formate-0.8 mL/min	UV 254 nm
[12]	Biogel P6 fine (100 x 26)	50 mM ammonium formate-0.8 mL/min	RI
[10, 66]	Biogel P6 fine (100 x 25) Desalting step by Toyopearl HW 40F/50F (50 x 25)	50 mM sodium nitrate-50 mL/h Distilled water	RI
[26]	Biogel P6 fine (100 x 25) Desalting step by Biogel P2-(210 x 15)	50 mM sodium nitrate-60 mL /h Distilled water	RI RI
[80]	Biogel P6 fine (100 x 25) Desalting step by Toyopearl HW 40F/50F (50 x 25)	50 mM sodium nitrate-94 mL /h Distilled water	RI RI
[78]	DEAE Sepharose CL-6B (20 x 26) Desalting step by Biogel P6 (100 x 26)	Linear gradient from 40 mM to 1 M ammonium acetate / acetic acid pH 4.8 - 1.5 mL/min Distilled water	UV 254 nm RI RI
[12]	DEAE Sepharose CL-6B-(20 x 16) Desalting step by Biogel P6 (100 x 26)	Linear gradient from 40 mM to 1 M ammonium acetate / acetic acid pH 4.8 1.5 mL/min Distilled water	UV 254 nm RI RI
[80]	DEAE Sepharose CL-6B - (10.5 x 10) Desalting step by Sephadex - G10 (50 x 16)	Linear gradient from 50 mM to 1 M ammonium acetate / acetic acid pH 4.8 and washing with 2 M eluent-0.5 ml/min Distilled water	UV 240 nm ELSD RI
	Carbopac PA1-(25 x 4)	Solvent A: 160 mM NaOH Solvent B: 200 mM NaOH 600mM sodium acetate 0-5 min 0%B, 5-35 min 0-100% B, 1 ml/min	Pulse amperometric detector
[77]	L-histidine - PEVA hollow-fiber membranes surface: 110 cm^2 or 0.1m^2	All: 50 mM pH 4.8 - ammonium acetate / acetic acid - MES / ammonium acetate - Phosphate - Tris / succinate Linear gradient from 50 mM to 1 M and washing with 2 M eluent, 0.4 mL/min	Uronic oligosaccharide assay
[81]	L-histidine - PEVA hollow-fiber membranes surface: 70 cm^2 surface: 0.1m^2	50 mM ammonium acetate / acetic acid pH 4.8 and washing with 1 M 0.4 mL/min 5 mL/min	Phenol sulphuric assay
[80]	L-histidine - PEVA hollow-fiber membranes surface: 70 cm^2 surface: 0.1m^2	Linear gradient from 50 mM to 1 M ammonium acetate / acetic acid pH 4.8 and washing with 2 M eluent, 0.4 ml/min 50 mM ammonium acetate / acetic acid pH 4.8 and washing with 1 M, 5 mL/min	ELSD

of oligomers was fractionated and deacetylated oligoglucuronans of dp 3 were obtained with a higher yield than previously [12]. A more exhaustive study about the separation by anion-exchange chromatography (DEAE, eluent ammonium acetate / acetic acid pH 4.8) was published by Pirlet *et al.* [80]. Oligoglucuronans were first isolated from the fermentation broth by UFT. Products having a molecular mass between 5 and 20 kDa were loaded on a DEAE column. Two major fractions of oligoglucuronans were isolated. The first one contained dp from 3 to 5 with a higher content of dp 5. The second was composed of molecules ranging from dp 4 to dp 7. Another fraction of higher dp (>10) was obtained by a 2M washing step (Fig. **10**). Molecules with a similar dp presented different retention times on DEAE column. The first fraction was more acetylated than the second one. Indeed, those different retention times could be explained by the dac of the molecules. In the case of deacetylated oligoglucuronans, the separation on DEAE column was only controled by the dp of oligomers (Fig. **11**).

High Pressure Anion Exchange Chromatography (HPAEC) with a PA-1 column has also been carried out to separate oligoglucuronans up to dp 15 (Fig. **12**). The detection was achieved with pulsed amperometric detector in alkaline conditions (Table **4**).

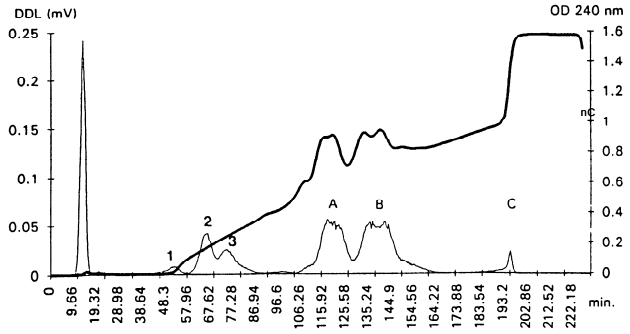

Fig. (10). Fractionation on DEAE column of oligoglucuronans from fermentation broth (fractions between 20 and 5 kDa) (-) UV detector at 240 nm (-) evaporative light scattering detector (10.5 x 1 cm, 0.5 ml/min, linear gradient from 50 mM to 1 M ammonium acetate / acetic acid pH 4.8 and washing with 2 M eluent). Peaks 1, 2, 3 correspond to cyclic β(1→2) glucans and peaks A, B, C to oligoglucuronans. Fractions A (3 < dp <5) were more acetylated than fractions B (4 < dp < 7) and high dp(> 16) fraction C. Carbohydrate Polymers, Vol. 38, 1999, p 156. Copyright 1999 Elsevier Science Ltd. Reprinted with permission of Elsevier Science Ltd.

An original method of separation was studied using L-histidine grafted onto poly(ethylene–vinyl alcohol) (PEVA) hollow-fiber membranes.

This support was originally designed for protein purification such as immunoglobulin G [85]. Oligoglucuronans from fermentation broths were applied on the L-histidine-PEVA module. Only one fraction was eluted with 1 M ammonium acetate / acetic acid pH 4.8. A ^{1}H NMR analysis revealed that the fraction was composed of acetylated oligoglucuronans [81].

The same authors completed their study by the injection of different species (with various acetylation degrees and 3 < dp < 5) and concluded that the affinity between the oligoglucuronan and the L-histidine - PEVA module was furthered by low dac. They observed also that successive purifications of fermentation broth on the L-histidine - PEVA module permitted to isolate different oligoglucuronans with various dp and dac [80]. In another study using unsaturated oligomers, it was observed that the adsorption was dependent on the composition of the eluent. Moreover it was identified that the binding process was an ionic interaction depending essentially on the dp of oligomers. Acetylation increased the fixation by other interactions such as hydrogen bonding and/or hydrophobic interactions [77].

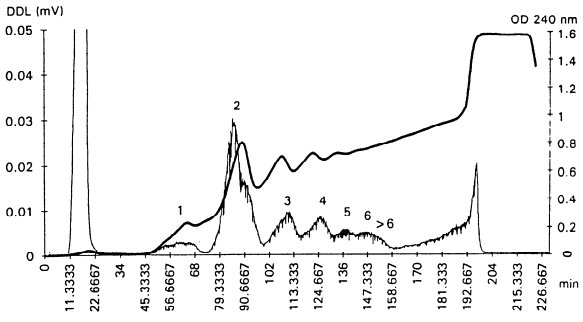

Fig. (11). Fractionation on DEAE column of deacetylated oligoglucuronans (-) UV detector at 240 nm (-) evaporative light scattering detector (10.5 x 1 cm, 0.5 ml/min, linear gradient from 50 mM to 1 M ammonium acetate / acetic acid pH 4.8 and washing with 2 M eluent). The number above each peak correspond to dp of the oligomers. Carbohydrate Polymers, Vol. 38, 1999, p 158. Copyright 1999 Elsevier Science Ltd. Reprinted with permission of Elsevier Science Ltd.

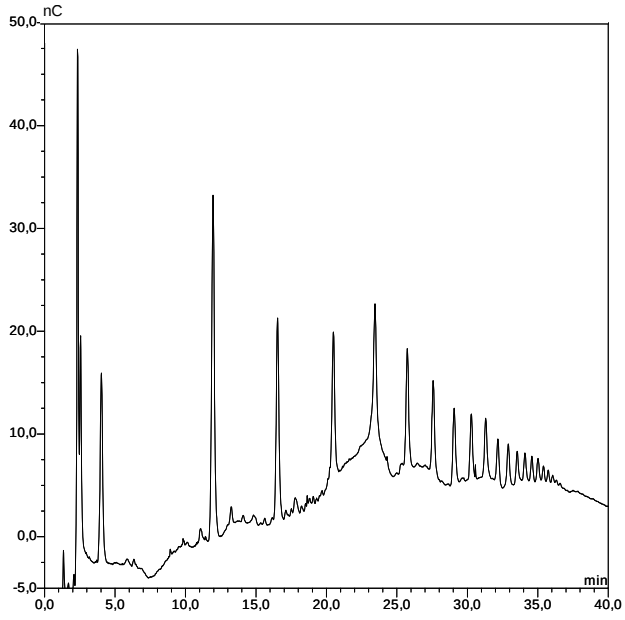

Fig. (12). Elution of deacetylated oligoglucuronans on Carbopac PA-1 column 250 x 4 mm.

CONCLUSION

Structural features and biodegradability of bacterial glucuronans and cellouronate have been well investigated during these last years. Studies highlighted lots of applications

in various industrial areas but patents were not always successfully associated with industrial developments. In this context the most promising way for valorisation could be the characterization of biological activities of oligomers. This implies to obtain them at large scale and as mixes well characterized (dp and dac). Thus degradation of macromolecules by glucuronan lyases associated to ultra and/or nanofiltration processes could open the way to new enzymatic reactors. Moreover, developments of immobilized glucuronan lyases would allow a better control of dp. However, significant improvements of polyglucuronic acids degradation seem to be dependent on molecular biology engineering. The avaibility of bacterial and fungal genes coding for these enzymes could make achievable the enzyme directed mutagenesis by PCR strategies. So the biodegradation of acetylated glucuronans in oligomers, often described as bioactivators in animals and vegetables could be achieved. To conclude, the role of the glucuronan or cellouronate lyases in the physiology of micro-organisms and their detection in other micro-organisms will be a challenge to identify other sources of natural glucuronans.

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