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Review

Polyglucuronic acids: Structures, functions and degrading enzymes

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ABSTRACT

Totally unknown at the beginning of the nineties, polyglucuronic acids have aroused the interest of scientific community working with polysaccharides. These polyuronides, initially isolated from microorganisms, have been later identified in some complex cell walls such as those of green algae. The recent progresses in the regioselective oxidation of neutral glucans permits them to be obtained on a large scale under relatively mild conditions. At the same time, new enzymes able to degrade these polysaccharides have been identified leading notably to the description of a new polysaccharide lyase family. The different forms of polyglucuronic acids such as those of low molecular weight obtained after enzymatic degradation of β-(1,4)-polyglucuronic acids have been tested for biological activities with success. So, several industrial applications associated with these compounds have been patented. This review focuses on this class of polyuronides and the enzymes capable of cleaving them.

Contents

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1. Introduction

Polysaccharides are macromolecules possessing structural and functional diversity. Focusing on polyglucuronic acid (i.e. homopolymer of glucuronic acids), the glycosidic linkage, the stereochemistry of the anomeric carbon and the pattern of substitution may result in different structures. Nonetheless only few structures of this type were described. They can be separated into two families, those of natural polyglucuronic acid and this of synthetic one generally obtained after regioselective oxidation of natural glucans such as cellulose. Cellulose is the major polymeric component of plant matter. This β-(1,4) linked glucan is not only the most abundant organic compound but also the most intensively used as raw material for the production of paper, panel products, chemicals and other industrial compounds such as various esters and ethers. Cellulose has received much attention around all over the world (Chuan-Fu & Run-Cang, 2010). It is basically insoluble in most solvents and only substitution reactions of hydroxyl groups with carboxymethyl ether or sulfate ester groups give soluble compounds. Oxidation of primary hydroxyls groups of polysaccharides to carboxylate groups can also increase water solubility. Recently, regioselective oxidation of C6 primary hydroxyls using 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) was developed, and a water soluble β-(1,4)-D-polyglucuronic acid, named cellouronic acid, has been obtained from regenerated cellulose (Isogai & Kato, 1998). During the same period several natural sources of water-soluble β-(1,4)-D-polyglucuronic acids have been identified. Among them the exopolysaccharide excreted by the *Sinorhizobium meliloti* M5N1CS strain was described in 1993 as a linear homopolymer of D-glucuronic acids β-(1,4) linked and called glucuronan (Heyraud, Courtois, Dantas, Colin-Morel, & Courtois, 1993). It was partially *O*-acetylated at C3 and/or C2 positions and can be used as a substitute of pectin and alginate in food or cosmetic industries because of its remarkable gelling and thickening properties. Another β-(1,4)-D-polyglucuronic acid was also described in cell walls of *Mucor rouxii* (Ruiter, Josso, Colquhoun, Voragen, & Rombouts, 1992) or algae (Ray, 2006; Ray & Lahaye, 1995a, 1995b). Unlike a bacterial glucuronan, extraction from these resources required additional works to eliminate other polymers present. This review deals with of all these forms of polygucuronic acids and with enzymes acting about them. These polyglucuronic acid degrading enzymes led recently to the identification of a new family of polysaccharide lyases, those of glucuronan lyases (EC 4.2.2.14), but also to hydrolases acting about α-(1,4)-D-polyglucuronic acid and called α-(1,4)-glucuronidase. These enzymes are expressed by different bacteria and fungi (Da Costa, Michaud, Petit, Courtois, & Courtois, 2001; Delattre, Michaud, Lion, Courtois, & Courtois, 2005; Konno, Habu, Maeda, Azuma, & Isogai, 2006) or encoded by virus (Sugimoto, Onimatsu, Fujie, Usami, & Yamada, 2004). They were used with varying successes to obtain bioactive oligoglucuronans.

2. Overview of natural glucuronan polysaccharides from bacteria, fungi and algae

2.1. *β-(1,4)-D-Polyglucuronic acid from* Sinorhizobium meliloti *M5N1CS bacterial strain*

The bacterial strain *Sinorhizobium meliloti* M5N1CS (NCIMB 40472) was obtained by chemical mutagenesis of *Sinorhizobium meliloti* M5N1 using N-methyl-N′-nitro-N-nitrosoguanidine (Heyraud et al., 1993). This new strain produced an extracellular polysaccharide with interesting viscosimetric and gelling properties. The structural investigations revealed that the polysaccharide excreted was constituted only by glucuronic acids. An analytic sequence of reduction, permethylation, trifluoroacetic acid hydrolysis and gas chromatography (GC) analysis revealed the presence

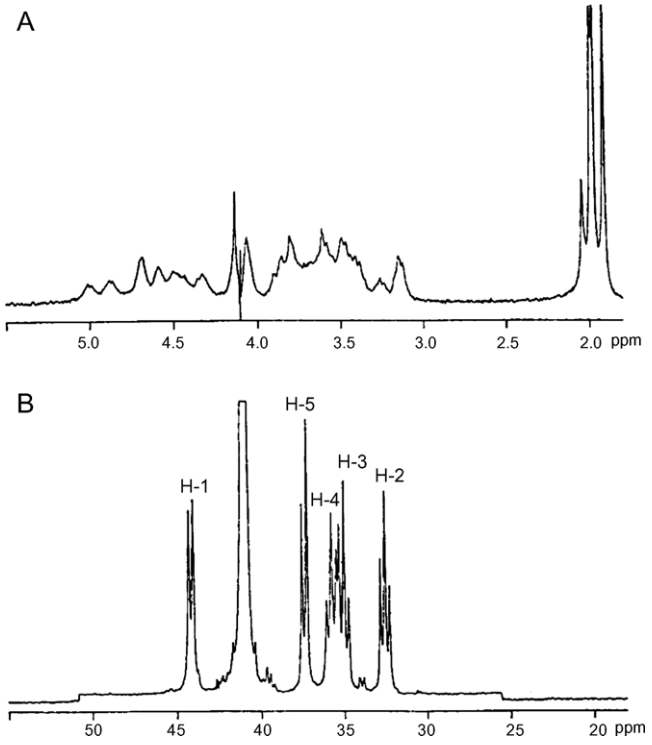


Fig. 1. ^{1}H NMR spectra (300 MHz) of native (A) and deacetylated (B) glucuronan (Heyraud et al., 1993).

of a single peak which corresponded to (1,4) linked glucuronic acid. ^{1}H NMR analyses characterized signals in the 2 ppm region as *O*-acetyl groups (Fig. 1A). The integration value ratio between the acetyl region (from 1.9 to 2.2 ppm) and the upfield and downfield regions (4.3–5.1 ppm and 3.1–4 ppm, respectively) has enabled the degree of acetylation of the macromolecule to be determined. A comparison between deacetylated polysaccharide (Fig. 1B) and β-glucuronic acid confirmed that the polymer was an homopolymer composed of β-D-(1,4)-glucopyranosyluronic residues variably acetylated at C2 and/or C3 position (Table 1) (Heyraud et al., 1993). Some authors have reported that the carbon source used (glucose, fructose, or sucrose) has no effect on polysaccharide production (Courtois et al., 1994) whereas other observed that increase of magnesium salts in the culture medium conducted to the production of highly acetylated glucuronan (Michaud et al., 1995). Thus, a glucuronan obtained without Mg^{2+} ions supplementation in the culture medium (the *Rhizobium* complete medium) has an average acetylation degree of 50% whereas another one from a culture supplemented with 3.25 mM $MgSO_4$ per day reached an acetylation degree of 74%. However, a decrease in average molecular weight combined with a loss of polysaccharide productivity has been observed and explained by an enzymatic degradation of the polysaccharide activated by Mg^{2+} ions (Michaud et al., 1994). In these different media, average molecular weights of glucuronans were measured between 6×10^4 and 4×10^5 Da using size-exclusion chromatography coupled to multi angle laser light scattering.

Common methods for purification of polysaccharides were precipitation with polar alcohol or tangential ultrafiltration. Glucuronan structure permits another precipitation by acidifying the medium to a pH lower than 3. This phenomenon is common to all polyuronides such as polygalacturonic acid or alginate (Heyraud et al., 1993). Gelling properties was also reported in the presence of monovalent, divalent or trivalent cations depending on polymer concentration and ionic strength (Dantas et al., 1994). Glucuro-

Table 1
Structures and origins of polyglucuronic acids.

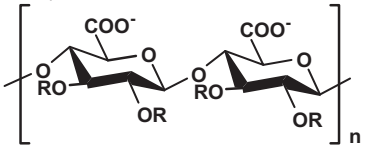
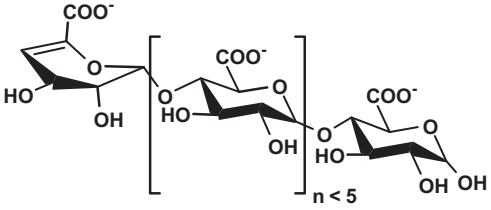
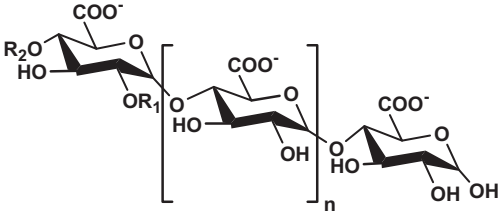
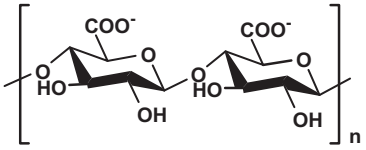
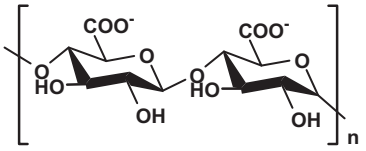
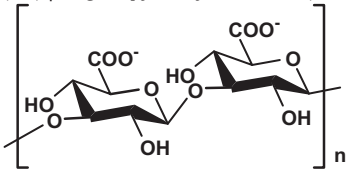
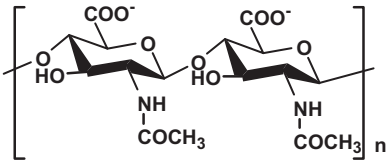
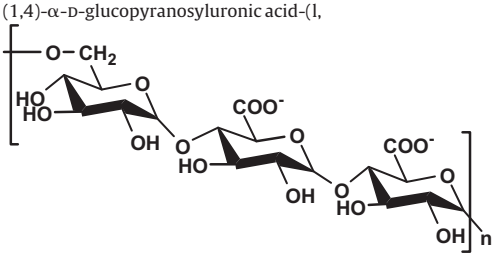
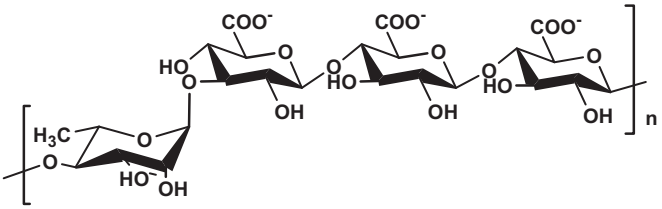
Name	Repeating unit		Source
Glucuronan	,4)-β-D-Glucopyranosyluronic acid-(1,4)-β-D-glucopyranosyluronic acid-(1,  R = H or CH_3-CO-		*Sinorhizobium meliloti* M5N1CS
Glucuronic acid oligomers Glucuronan oligosaccharides	,4)-α-D-Glucopyranosyluronic acid-(1,4)-α-D-glucopyranosyluronic acid-(1,  Non reducing end: 4-deoxy-l-*erythro*-hex-4-enopyranosyluronic acid		*Gluconacetobacter hansenii* PJK
Glucuronic acid oligomers Glucuronan oligosaccharides Amylouronic acid	,4)-α-D-Glucopyranosyluronic acid-(1,4)-α-D-glucopyranosyluronic acid-(1, 	Glucuronic acid oligomers: n <5, $R_1 = COCH_3$, $R_2 = CH_3$ Amyluronic acid: R_1 and R_2 = H	*Gluconacetobacter hansenii* PJK Regioselective oxidation of amylose
Mucoric acid Fungal β-(1, 4)-glucuronan Algal glucuronan	,4)-β-D-Glucopyranosyluronic acid-(1,4)-β-D-glucopyranosyluronic acid-(1, 		*Mucor rouxii* *Ulva* sp
Polyglucuronic acid Cellouronic acid Poly-α- and -β-(1,4)-glucuronic acid	,4)-α-D-Glucopyranosyluronic acid-(1,4)-β-D-glucopyranosyluronic acid-(1, 		Regioselective oxidation of cellulose *Bacillus* C-125
β-(1,3)-Polyglucuronic acid	,3)-β-D-Glucopyranosyluronic acid-(1,3)-β-D-glucopyranosyluronic acid-(1, 		Regioselective oxidation of curdlan

Table 1 (*Continued*)

Name	Repeating unit	Source
Chitouronic acid	,4)-*N*-Acetyl-β-D-glucoamінopyranosyluronic acid-(1,4)-*N*-Acetyl-β-D-glucoaminopyranosyluronic acid-(1, 	Regioselective oxidation of regenerated chitin or N-acetylated chitosan
Oxidized pullulans	,6)-α-D-Glucopyranosyluronic acid-(1,4)-α-D-glucopyranosyluronic acid-(1,4)-α-D-glucopyranosyluronic acid-(1, 	Regioselective oxidation of pullulan
Ulvan like	,4)-α-L-Rhamnopyranosyl-(1,3)-β-D-glucopyranosyluronic acid-(1,4)-β-D-glucopyranosyluronic acid-(1,4)-β-D-glucopyranosyluronic acid-(1, 	Regioselective oxidation of gellan

nan can form thermoreversible gels in the presence of monovalent cations as Na^+ at high ionic strength. Thermally stable gels were obtained with divalent cations (Ca^{2+}, Cu^{2+}, Ba^{2+}) and gel resistance was modulated by the acetylation degree of polymer which disturb the ionic interaction. Da Costa et al. (2001) first purified an enzyme belonging to glucuronan lyase family and opened the way to degradation of bacterial glucuronan in oligomers. This area has been well reviewed by Tavernier, Petit, et al. (2008). Patents describing the use of β-(1,4)-D-poly- and oligoglucuronic acids in different fields of application have been published. The first one claimed the production of glucuronan by the *S. meliloti* M5N1CS strain and its use in food products, farming, pharmaceutics, cosmetics or water purification, particularly as a gelling, thickening, hydrating, stabilizing, chelating or flocculating agent (Courtois-sambourg, Courtois, Heyraud, Colin-Morel, & Rinaudo-Duhem, 1993). In association with an algae extracted from *Haematococcus pluvialis*, a cosmetic and dermopharmaceutical application have been claimed (Lintner, 1999). Immunostimulating activities on human blood monocytes were also described in a patent as low molecular weight glucuronans induced the production of cytokines IL-1, IL-6 and TNF-α (Courtois & Courtois, 1998). Biological activities of these low molecular weight glucuronans on mammalian cells have been confirmed recently with a claim describing stimulation of elasticity of the dermis and epidermis by acetylated oligoglucuronans with degrees of polymerisation of 18–19 (Fournial, Grizaud, Le Moigne, & Mondon, 2008). On plant cell, other biological activities would be inclined to agronomic applications. Plant natural defence mechanisms (protoplasts from *Rubus frututicosus* L.) were elicited by oligoglucuronans of dp <10. They acted by amplification of the β-(1,3)-D-glucanase activity (Lienart, Heyraud, & Sevenou, 1999). An industrial preparation for these applications was mentioned in the patent FR2885911 by the use of glucuronan lyase from *Trichoderma* sp. GL2. It claimed also the phytosanitary status of the lyase (Delattre, Michaud, Courtois, & Courtois, 2005a). Finally, oligoglucuronans obtained by the action of the same polysaccharide lyase and the production of their derivatives was also claimed as they induced expression of genes implicated in plant natural defences (Rat et al., 2008).

2.2. *α-(1,4)-D-Polyglucuronic acid from* Gluconacetobacter hansenii *bacterial strain*

Significant progresses have been made in discovering and developing of new bacterial polysaccharides that possess novel and highly functional properties (Laws, Gu, & Marshall, 2001; Sutherland, 1999). These microbial polysaccharides qualified as no toxic and highly biodegradable can be prepared in reliable quantities using conventional biotechnological processes (Laws et al., 2001). Moreover, these polysaccharides have special rheological properties and, thus, are useful in industrial applications as viscosifiers, stabilizers, or gelling agents (Kornmann, Duboc, Marison, & Stockar, 2003). So the screening of new microbial polysaccharides with new properties are of interest for industrial applications (Van Kranenburg, Boels, Kleerebezem & de Vos, 1999). In this context, research groups have isolated a cellulose-producing strain from rotten apples. It was identified as *Gluconacetobacter hansenii* based on its physiological characteristics and sequence of gene encoding for16S rDNA. Specifically named *G. hansenii* PJK (Park, Park, &

Jung, 2003a, 1993b), it was used to produce water-soluble polysaccharides composed of glucuronic acid (Jung, Park, & Chang, 2005; Jung, Park, & Park, 2003; Park, Hyun, & Jung, 2004; Shah, Ha, & Park, 2010). The strain was grown on a basal medium containing glucose (10 g/L), yeast extract (10 g/L), peptone (7 g/L), acetic acid (0.15%, v/v), and succinate (0.2 g/L). The water-soluble polysaccharides produced were isolated from the supernatant of culture broths by ethanolic precipitation followed by centrifugation (Khan, Hyun, & Park, 2007; Park, Khan, & Jung, 2006). The dried water-soluble polysaccharides were subjected to acid hydrolysis and the hydrolysates were analyzed using HPLC. Glucuronic acid was the sole component detected (Khan et al., 2007; Park et al., 2006). The polysaccharide structure was also investigated using NMR spectroscopy. The lack of signals around the 2 ppm region confirmed the absence of any *O*-acetyl groups (Khan et al., 2007; Park et al., 2006). The peak for the anomeric proton appeared as a doublet with a small coupling constant which indicated clearly the α-configuration (Khan et al., 2007; Park et al., 2006). It can be assumed that the polymer may be composed of α-glucuronic acid rather than β as reported previously (Khan et al., 2007; Park et al., 2006). The authors have emphasized that the ^{1}H NMR spectra exhibited a prominant peak at 5.8 ppm, characteristic of H-4 of an unsaturated glucuronic acid corresponding to the non-reducing terminal unit. This suggests that a part of the polysaccharide mixture was composed of unsaturated α-(1,4)-oligoglucuronic acids (Table 1). These results were also confirmed by ^{13}C NMR spectrum and molecular weights evaluation (Khan et al., 2007; Park et al., 2006).

These oligosaccharides possess good emulsifying properties and have sufficient thermal stability to be developed commercially (Khan et al., 2007). Their production from a chemically defined medium was expensive but the strain produced large quantities (112.65 g/L) of these oligomers on waste from beer fermentation broth (Khan et al., 2007; Khan & Park, 2008). In these conditions, the oligosaccharides have few structural differences compared with those obtained in defined medium including the presence of *O*-acetyl and *O*-methyl groups and a lack of unsaturation on the non reducing terminal unit (Table 1) (Jung et al., 2005; Khan et al., 2007; Khan & Park, 2008). However, their thermal and emulsifying characteristics were not modified.

2.3. *β- and α-(1,4)-glucuronans from the cell wall of the alkalophilic* Bacillus *strain C-125*

Two acidic polymers were identified in the cell walls of alkalophilic *Bacillus* C-125, a teichuronic acid composed of galacturonic acid, glucuronic acid and N-acetylfucosamine and a teichuronopeptide made up of polyglucuronic acid and poly-γ-L-glutamic acid.

Teichuronopeptides were extracted from the cell walls with 5% (w/v) trichloroacetic acid and separated by DEAE-cellulose column chromatography. Polyglucuronic acid was isolated by hydrazinolysis. The structural analysis of the polyglucuronic acid moiety from teichuronopeptide was performed (Aono, 1990). Smith-degradation indicated that hydroxy groups 2 and 3 of the glucuronic acid residue were free, and that hydroxy groups 1, 4 and 5 were blocked. Therefore the polyglucuronic acid is suggested to be composed of (1,4)-linked glucopyranosyluronic acids.

The ^{13}C NMR spectrum of the polyglucuronic acid showed several signals (102.2 and 99.3 ppm) suggesting that the glucuronic acid residue was not bound through a sole linkage (Fig. 2). The ^{1}H NMR analysis of anomeric carbon showed two signals for H-1 at 4.58 and 5.40 ppm, corresponding to β- and α-H-l, respectively. The integration of the H-1 signals suggested a molar ratio of 1:1.07 for β and α anomeric forms. Therefore the polyglucuronic acid was an equimolar mixture of α and β glucopyranosyluronic acids with (1,4) linkage.

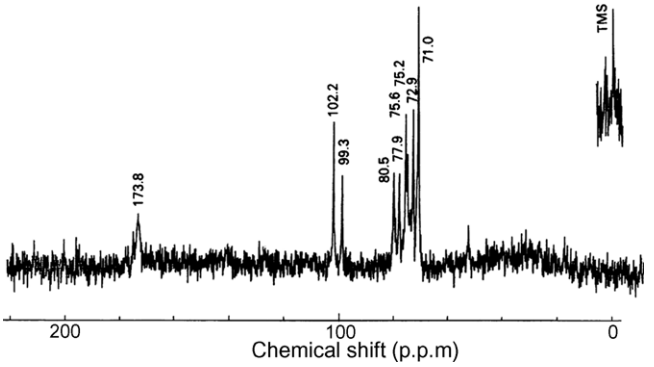


Fig. 2. ^{13}C NMR spectrum of the polyglucuronic acid extracted from the cell walls of alkalophilic *Bacillus* C-125 (Aono, 1990).

A final conclusion about structure of the polyglucuronic acid was obtained by enzymatic analysis of reduced oligosaccharides. The oligosaccharides were generated by chemical reduction and an acid hydrolysis step. A separation by a chromatography on Sephadex G-25 column was carried out. Monosaccharides, disaccharides, trisaccharides and tetrasaccharides fractions were obtained. The disaccharides were identified as maltose [α-D-glucopyranosyl-(1,4)-D-glucopyranosy] and cellobiose [β-D-glucopyranosyl-(1,4)-D-glucopyranosyl]. Maltotriose and cellotriose were not obtained. The tri- or tetrasaccharides were composed of D-glucose binding alternatively through α and β-(1,4)-linkages. In conclusion, the polyglucuronic acid obtained from the teichuronopeptide was composed by the following repeating unit: 4)-α-D-glucopyranosyluronic acid-(1,4)-β-D-glucopyranosyluronic acid-(l (Table 1).

2.4. *β-(1,4)-*D*-Polyglucuronic acid from* Mucor rouxii *fungal strain*

Mucor rouxii is a dimorphic phycomycete which can develop two different forms, depending on growth conditions. In an anaerobic environment with a carbon source (hexose), the development was yeast-like cells whereas it was mycelial in aerobic conditions (Bartnicki-Garcia & Nickerson, 1962a). These morphological differences were associated with two different patterns of wall growth: spherically symmetrical growth in the yeast form and apical in the mycelial one (Bartnicki-Garcia & Lippman, 1969). In *Mucor* species, the fibrillar structure of the wall is composed of chitin and chitosan (Bartnicki-Garcia & Nickerson, 1962b), which embedded in matrix materials mostly composed of polymers, containing large amounts of glucuronic acid (Bartnicki-Garcia & Reyes, 1968). *M. rouxii* was grown at 28 °C in yeast extract-peptone-glucose medium (Bartnicki-Garcia & Nickerson, 1962a) in a bioreactor gassed with air to obtain the mycelial phase and with a mixture of nitrogen and carbon dioxide (70:30, v/v) to obtain the yeast phase (Fig. 3). After 24 h, the cells were harvested by filtration and washed with distilled water. The polyuronides were extracted from yeast and mycelial walls by sequential treatments with lithium chloride and potassium hydroxide. Cell walls were prepared and suspended in 5.6 M LiCl (4 g/L, 18 h, 25 °C) with constant shaking (Fig. 3). Subsequent extraction of the residue was carried out for 30 min at 25 °C with 1 M KOH (20 g/L) (Dow, Darnall, & Villa, 1983). Polysaccharides were separated by chromatography on a DEAE-Sephadex column (Fig. 4). Two polymers (I and II) of different acidity were found in both wall types. The polymer I was a heteropolymer composed of D-glucuronic acid, D-mannose, D-galactose and L-fucose. It was designed as mucoran. Polymer II from both cell types contained large amounts of D-glucuronic acid and was designed as mucoric acid or fungal β-(1,4)-glucuronan (Table 1) (Dow et al., 1983). Mucorans from both sources were readily soluble in water

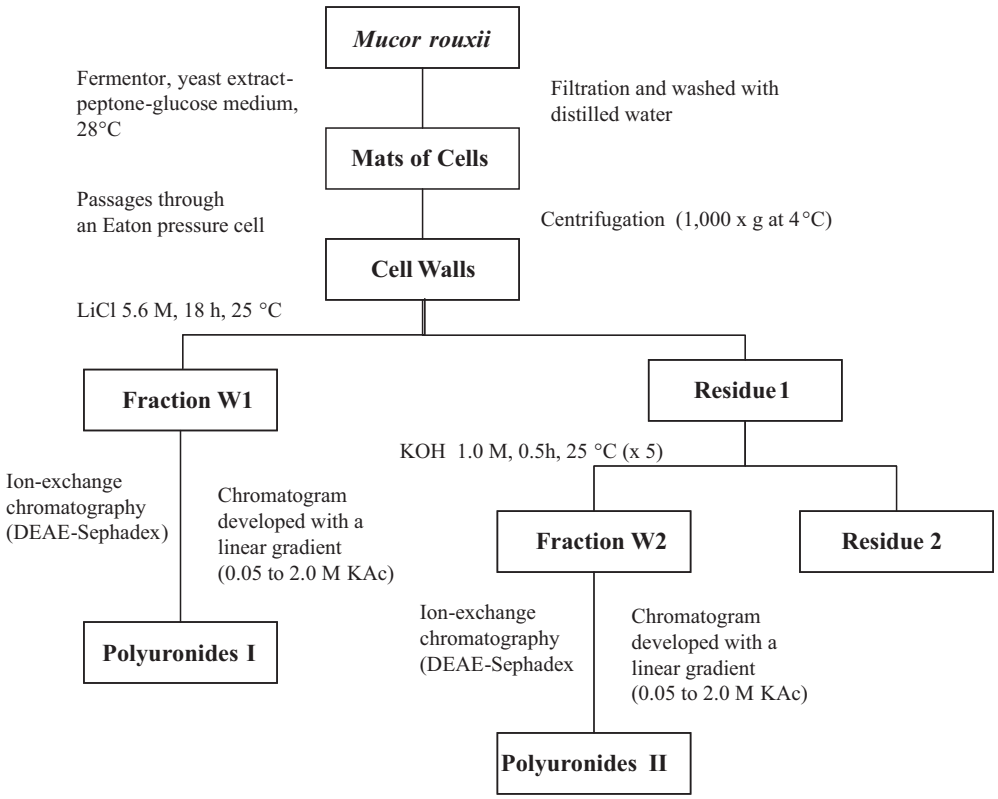


Fig. 3. Extraction and purification mucoran (polyuronides I) and mucoric acid (polyuronides II) from *Mucor rouxii* (Maxwell Dow et al., 1983).

and were not precipitated by acids or after chelating with $CaCl_2$. In contrast, mucoric acids from both sources were only soluble at pH above 5. Polymers could be precipitated from neutral aqueous solution by the addition of $CaCl_2$ and were insoluble in 5.6 M LiCl. The mucoric acid was quantitatively precipitated by 1 M acetic acid or 10 mM HCl (Dow et al., 1983). The first studies led by Bartnicki-Garcia and Reyes (1968) have suggested that the mucoric acid and mucoran may be derived from a single heteropolymer rather than from distinct polymers. A few years later, researches showed that cell walls from the yeast and mycelial phases of *M. rouxii* contained two distinct polyuronides. They were not part of a larger heteropolymer since they could be extracted with lithium chloride (Dow et al., 1983).

2.5. β-(1,4)-Glucuronan from green algae

It is true that bacterial glucuronans appear to have some advantages over the synthetic glucuronans (oxidized celluloses). They are non-toxic, less expensive, and readily available. Moreover, appropriate strains can be genetically modified to acquire a product with desired properties. For this reason, new sources of natural glucuronans have been explored. Previous studies have revealed the presence of β-(1,4)-D-polyglucuronic acids (algal glucuronan) in the cell walls of a number of green seaweeds (Ray, 2006). It was reported that the algal glucuronan was co-extracted together with ulvans, major sulfated water-soluble polysaccharides usually extracted from the cell wall of *Ulva* sp. using hot water often containing a calcium chelating agent such as sodium oxalate (Ray & Lahaye, 1995a). The presence of the algal glucuronan poses several problems for the fine chemical structure analysis of ulvans. Thus, in previous studies some authors have successfully used ion-exchange chromatography to eliminate the algal glucuronan (Ray & Lahaye, 1995a, 1995b). Nevertheless, these chromatographic techniques, which are labor, intensive and time consuming, limit the industrial scale production of this algal glucuronan.

A new procedure was developed to efficiently and quantitatively extract the glucuronan from *Ulva lactuca* (Elboutachfaiti et al., 2009). By combining oxalate buffer extraction with acid precipitation, authors were able to isolate, with a high yield, pure glucuronan using only a few reaction steps. The method was carried out according to the scheme shown in Fig. 5. Fresh seaweeds were pretreated with EtOH, acetone, and chloroform. Samples of defatted algae (100 g) were extracted with hot oxalate solution (3 L, 0.05 M, pH 6, 90 °C, 3 h). The supernatants were subjected to acid precipitation (pH 2) and the precipitated glucuronan was collected. After sugar and sulfate analysis, the separation of glucuronan and ulvan was confirmed. In fact, the high degree of purity, estimated at 94% by measure of uronic acid contents, associated with the absence of sulfate groups confirmed the presence of pure glucuronan (Elboutachfaiti et al., 2009). The algal glucuronan was obtained with a yield of 2.5%. It has to be mentioned that contrary to other extraction processes of glucuronan from *Ulva* sp. the present method does not need any chromatographic purification steps. To confirm the efficiency of the separation, glucuronan fraction was analyzed by ^1H NMR. Five resonance peaks, characteristics of glucuronan were observed according to previous works obtained from bacterial and synthetic glucuronans (Dantas et al., 1994; Fraschini & Vignon, 2000).

3. Synthetic polyglucuronic acids

3.1. Some generalities

Over many years, several patents and publications have shown that there is interest in producing in large quantities of polyglucuronic acids using chemical processes. Consequently, over the last two decades, numerous studies have investigated ways to generate

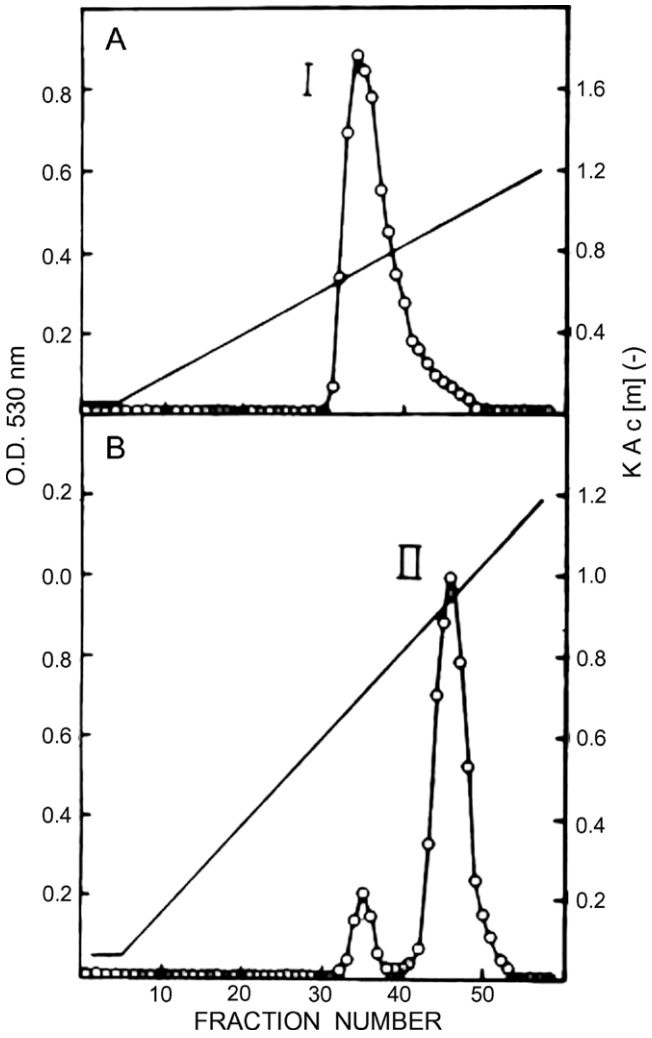


Fig. 4. Ion-exchange chromatography on DEAE-Sephadex of polyuronides extracted from *Mucor rouxii* by sequential treatments with lithium chloride (A) and potassium hydroxide (B). Chromatograms were developed with an acetate gradient (Maxwell Dow et al., 1983).

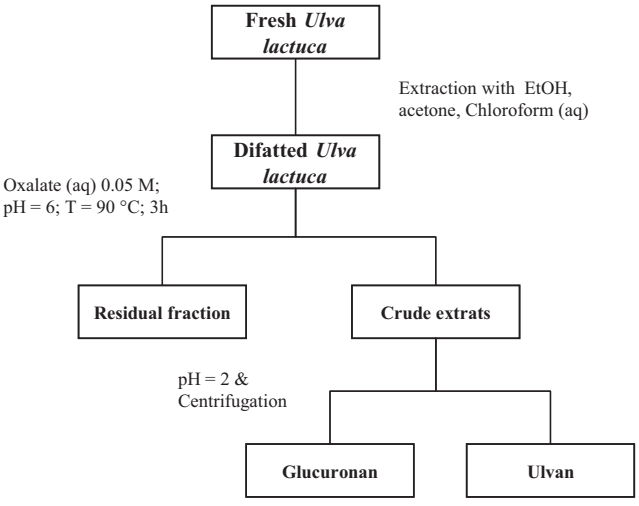


Fig. 5. Extraction procedure of glucuronan from *Ulva lactuca*.

synthetic polyglucuronic acids by chemical oxidation of renewable, natural and abundant glucans such as for examples: starch, cellulose and chitin. Utilization of oxoammonium salt is actually the best method to produce neo-polyglucuronic acids in large scale. This technique uses nitroxyl radical 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO®) as catalyser in the presence of sodium hypochlorite and sodium bromide (De Nooy, Besemer, & van Bekkum, 1995; Gomez-Bujedo, Fleury, & Vignon, 2004; Isogai & Kato, 1998). Specific modifications generated by this regioselective oxidation have been widely studied as a mean to develop and propose new polymers for food, chemistry, pharmaceutical and fuels.

3.2. *Synthetic polyglucuronic acid: «A long Chemical Story»*

Since 1940s, Yackel and Kenyon (1942) as well as Maurer and Reiff (1943) have firstly described a process using nitrogen oxides to produce polyglucuronic acids derivatives by selective oxidation of cellulose. However, even if this reaction has been improved later by Painter (1977) and Painter, Cesaro, Delben, and Paoletti (1985) performing the experiments in phosphoric acid and sodium nitrite, the main drawback was the uncontrolled reactions such as oxidative scission and production of dicarboxylic by-products resulting in a large depolymerization of cellulose. This oxidation of cellulose using nitrogen oxide and derivatives was developed until now as for example by Vignon et al. who proposed a successful method using supercritical NO_2/N_2O_4 to produce polyglucuronic acids (Vignon, Montanari, Samain, & Condoret, 2006). Although this method seems successful, its main disadvantage limiting its utilization is its dangerous and technical processing. From De Nooy et al. (1995) we know that if we replace sodium nitrite by nitrate, the oxidation yield is higher and the depolymerization of polysaccharides was considerably reduced during the oxidation process. Consequently a more "green" chemical reagent has been sought and the stable nitroxyl radical 2,2,6,6-tetramethylpyperidine-1-oxyl (TEMPO) in presence of NaOCl and NaBr was then described as specific catalyst for the regioselective oxidation of primary hydroxyl group of natural polysaccharides (Nooy, Besemer, & van Bekkum, 1995).

Historically, one of the earlier publication which described the oxidation of compounds by using oxammonium salts as reagent was written by Anelli et al. in Journal of Organic Chemistry in 1989 (Anelli, Banfi, Montanari, & Quici, 1989). In this work, the oxidation of 1,4-butanediol and 1,5-pentanediol was proposed using a system of 2,2,6,6-tetramethyl-piperidinyloxy free radical (TEMPO) with sodium hypochlorite and sodium bromide in a two phase system. In 1990, Andersson et al. have proposed the oxidation of cellulose with sodium nitrite in orthophosphoric acid and described the reaction products after NMR characterisation (Andersson, Hoffman, Nahar, & Scholander, 1990). Later, patents have proposed the specific oxidation of polymeric substances and more especially the regioselective oxidation of primary hydroxyl groups from alkyl polyglucosides by the use of sterically hindered N-oxides (Casciani, Likibi Parfait, & Mccraw, 1992). In this work TEMPO was suggested as preferred oxidant of polyglucosides although many others related nitroxides. Moreover, the use and reaction mechanism of TEMPO reagent with sodium hypochlorite (NaOCl) was described by Davis and Flitsch (Davis & Flitsch, 1993) in order to achieve the regioselective oxidation of primary hydroxyl groups of monosaccharides. These studies have marked the beginning of the "revolution" in polyuronide chemistry synthesis of 90s with the development of new and original syntheses of several polyglucuronic acids (polyuronans). In fact, following the Davis et al. paper, this route to carboxylation began to be very actively explored, particularly in the Netherlands and later in the United States. Therefore, De Nooy, Besemer, and van Bekkum (1994) have reported the selective oxidation of primary alcohol groups of inulin

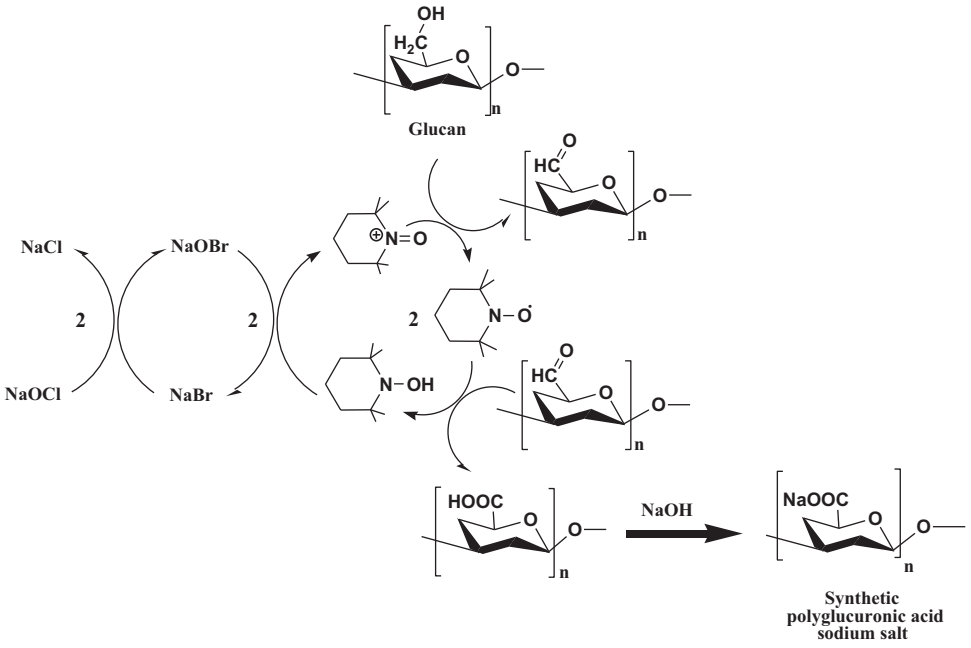


Fig. 6. General procedure to produce synthetic polyglucuronic acid from oxidation of glucans mediated by TEMPO/NaOCl/NaBr.

and starch using TEMPO/hypobromite system. The following years, numerous studies have proposed the highly selective oxidation of primary alcohol groups of glucans using TEMPO and sodium hypochlorite/sodium bromide oxidant as summarized in Fig. 6 adapted from Delattre et al. (2009) (Barzyk, Page, & Ragauskas, 1997; Chang & Robyt, 1996; De Nooy, Besemer, & van Bekkum, 1995). Consequently, TEMPO reagent became the catalyst of choice for the regioselective oxidation of high molecular weight polysaccharides. This chemical process was currently described for its high reaction level, high production yield, and suitable selectivity of primary alcohol groups. As shown in Fig. 6, production of polyglucuronic acid by oxidation of primary alcohols was successful using less than 1 mol% of TEMPO and aqueous sodium hypochlorite as inexpensive co-oxidant with sodium hydroxide as a mild base. Additive, such as NaBr (or KBr), have to be used to accelerate the rate of oxidation reaction. This proposed chemical mechanism (adapted from Delattre et al., 2009) shows that the regioselective TEMPO oxidation of polysaccharides is a complex reaction. In fact, when TEMPO is added as catalyser; the oxidation process involves different reaction steps as mentioned in Fig. 6. More generally, oxidation of 1 mol of primary alcohol in carboxylate requires 2 mol of NaOCl and 2 mol of TEMPO radical.

In the same period, De Nooy and Besemer, and Chang and Robyt have described the oxidation of 10 different natural polysaccharides (Chang & Robyt, 1996; De Nooy & Besemer, 1996). In this work, polysaccharides such as pullulan, cellulose, chitin, amylose, etc. with widely different structures and water solubilities, have been yielded regioselectively oxidized into polyglucuronans using TEMPO at pH 9–11 and 0–4 °C. These water soluble polyglucuronans had their degree of solubility doubled or tripled. They represented new anionic polymers with unique structures that could have several applications in cosmetic and pharmaceuticals as gums, gels, and films.

From the beginning of polysaccharide oxidation, cellulose was most of time, used as model substrate. Nevertheless, a lot of work established the real difficulties occurred during the oxidation of native cellulose and also of native chitin due to their insolubility (Isogai & Kato, 1998; Sun et al., 2006). In fact, the oxidation reaction is disadvantaged by the high crystalline state of cellulosic and chitin materials which considerably reduces the accessibility of primary alcohols due to high number of hydrogen bonding interactions. Then, pre-treatments for making hydro-soluble or hydro-dispersible polysaccharides are primordial for the oxidation of non-soluble glucans into polyglucuronic acid with yields superior to 80%. Consequently, pseudo amorphous cellulose obtained by solubilization of cellulose in more than 10% aqueous sodium hydroxide and regenerated into anhydrous ethanol or triacetate cellulose have been used for the preparation of polyglucuronic acids (Gomez-Bujedo, Fleury, & Vignon, 2004; Isogai & Kato, 1998; Sun et al., 2006; Tahiri & Vignon, 2000). For example, oxidation of cellulose in good yield has been proposed by Isogai and Kato (1998). In order to produce large amount of soluble polyglucuronic acids (cellouronic acid), treatment of several native, mercerized, and regenerated celluloses with TEMPO/NaBr/NaOCl allowed to produce cellouronate with interesting yields. The Isogai team noted that the water soluble products had almost 100% carboxyl substitution at the C6 site. They further note that oxidation proceeded heterogeneously at the more accessible regions on solid cellulose. Using native celluloses, it was unable to obtain water soluble polyglyglucuronic acid material since it was achieved only very low amounts of carboxylate conversion. Nevertheless, it was noted the putative beneficial properties of these insoluble polyanionic materials in papermaking market as additives (Isogai & Kato, 1998; Kitaoka, Isogai, & Ouabe, 1999).

Recently, new «chemoenzymatic» approach was proposed in order to produce polyglucuronic acids. In fact, it was found that TEMPO combined with laccase can oxidise cellulose on a mild and environmental friendly condition (Mathew & Adlercreutz, 2009). The enzymatic approach with laccase and TEMPO was compared to the well known chemical approach using TEMPO and NaBr/NaOCl. It was found that both approaches yield similar distributions of functional groups (Fig. 7) indicating a similar reaction mechanism. As it was proposed by authors, the laccase generates the oxoammonium ion which forms the C6-aldehyde (Mathew & Adlercreutz, 2009). The drawback of the chemical variant of TEMPO oxidation is the degradation of cellulose during the course of reaction due to the beta-elimination in the alkaline reaction medium. Therefore, the advantage of the enzymatic approach should be the slightly

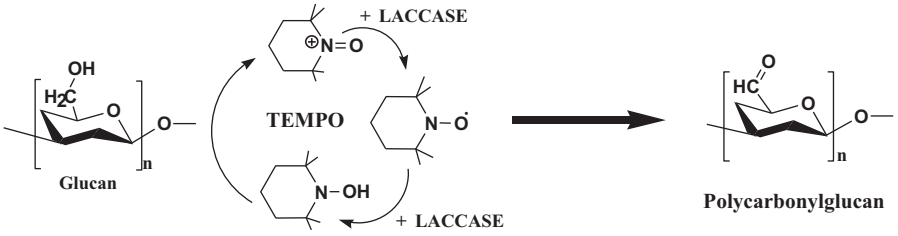


Fig. 7. Proposed mechanism of glucan oxidation by enzymatic process by using laccase.

acidic reaction medium, which should suppress degradation due to a beta-elimination process. Nevertheless, by this enzymatic approach using laccase, the reaction time is higher and a lot of carbonyl groups (aldehyde functions) are produced instead of carboxyl groups as observed with the conventional TEMPO/NaBr/NaOCl oxidation system.

In conclusion, TEMPO/NaBr/NaOCl is the preferable and suitable method used for carboxylation of many carbohydrate products such as simple sugars, relatively low molecular weight oligomers of sugars, starch, chitin, chitosan, cellulose and many others that have an accessible primary hydroxyl group. The lower cost of oxidation process for the preparation of interesting polyglucuronic acid is significant for industrial applications.

3.3. Toward new generation of bioactive polyglucuronic acids surrogates

In recent years, chemical analogues of pharmaceutical and cosmetic polysaccharides have been proposed as new surrogates of known bioactive polyuronic acids. The followed paragraph present the last examples of these polymers produced from oxidation of natural polysaccharides mediated by TEMPO/NaBr/NaOCl.

3.3.1. β-(1,4)-Polyglucuronic acid and derivatives as biological active molecules

Most of these synthetic polyuronic acids have been successfully tested for their biodegradability (Kato, Kaminaga, Matsuo, & Isogai, 2005) and this characteristic opens the way to applications in gas-barrier biomaterial and constituted environmentally friendly biodegradable packaging films. Studies about the biodegradation of these synthetic polyuronides using new specific polysaccharide cleavage enzymes as polysaccharide lyases or hydrolases could result in the large production of anionic oligosaccharides (Delattre, Michaud, Elboutachfaiti, Courtois & Courtois, 2006; Iihashi, Nagayama, Habu, Konno, & Isogai, 2009; Konno et al., 2006). The use of these enzymatic activities could also generate new oligosaccharides with biological activities as it has been largely described on numerous organisms such as bacteria, fungi, plant, algae and mammalian (Delattre, Michaud, Courtois & Courtois, 2005b). Furthermore, others bio-applications can be envisaged for oxidized cellulose. Effectively, as previously mentioned, a bacterial *O*-acetylated polyglucuronic acid (glucuronan) and its oligoglucuronides derivates have been reported to be bioactive agents in animals (Petit et al., 2004) as well as elicitors of defense responses in plants (Lienart et al., 1999).

3.3.2. β-(1,3)-Polyglucuronic acid as glycosaminoglycans surrogate

Recently, the synthesis and characterization of a new β-(1,3)-polyglucuronic acid (Table 1) from curdlan modification has been described as a putative bioactive glycosaminoglycan-like for pharmaceutical and cosmetic field (Delattre et al., 2009; Rios, Delattre, Laroche, Michaud, & Berthon, 2008; Tamura, Hirota, Saito, & Isogai, 2010; Tamura, Wada, & Isogai, 2009). It has been shown that after regioselective oxidation of β-(1,3)-glucan by TEMPO/NaOCl/NaBr, this polyuronide could be used in cosmetic composition as slimming agent (Rios, Delattre, Patriarca, Favre & Berthon, 2010). It provoked a significant decrease in the hyperplasia and hypertrophy of adipose tissues via a direct and localized action at the level of the adipocyte itself. It locally induced an overexpression of the gene encoding for fasting induced adipose factor. As a result adipokine FIAF synthesis increased. Therefore, four new and unique actions were described: (i) inhibition of activity of lipoprotein lipase with a significant decrease in lipogenesis and hence in fat storage; (ii) activation of adipose triglyceride lipase, a second lipase non hormone sensible but with an important role in the lipolysis at level of lipidic vacuoles; (iii) increasing the transport of all these free fatty acids from the adipocyte towards peripheral tissues to be used as energy source and finally, (iv) inhibition of the of pre-adipocyte differentiation. Consequently, this new β-(1,3)-polyglucuronic acid can be used in the cosmetic field as slimming and body care products.

3.3.3. Oxo-chitin as hyaluronan like polysaccharide

Numerous studies and patents continue to propose improvements of chemical procedures for TEMPO mediated oxidation of water-insoluble polysaccharides such as chitin and chitosan (Kato, Kaminaga, Matsuo, & Isogai, 2004; Muzzarelli, Muzzarelli, Cosani, & Tjerbojevich, 1999; Sun et al., 2006). These soluble chitouronic acids (from chitin/chitosan oxidation) have a great potential with respect to their sequestering, gelling and thickening properties. Moreover, these anionic polysaccharides are soluble over an extended pH range could be proposed as surrogates of hyaluronic acid and as commodities for industrial use (Muzzarelli & Peter, 1997). The last two decades, chitouronic acids were produced by TEMPO mediated oxidation of regenerated chitin after pretreatment of crustacean fungal chitins and chitin–glucan (Muzzarelli et al., 1999). When TEMPO/NaBr/NaOCl system was applied on these polysaccharides, β-(1,4)-poly-N-acetylglucosaminuronic (chitouronic acids) and β-(1,3)-polyglucuronic acids (Table 1) were quantitatively obtained. In contrary, oxidation of native polysaccharide gave few amounts of carboxyl residues due to the lower accessibility of TEMPO system to the native and insoluble polysaccharide (Isogai & Kato, 1998; Kato et al., 2005; Muzzarelli et al., 1999; Sun et al., 2006).

Consequently, Muzzarelli et al. have defined the suitable procedure for the production of C6-carboxylated polysaccharides from industrial *A. niger*, *Trichoderma reesei* and *Saprolegnia* sp biomasses (Muzzarelli et al., 2000). By this method, biomass from *A. niger* was easily changed into polyuronans that included 20% of 6-oxychitin. These polyglucuronic acids, in the form of sodium salt, were an off white powder water soluble over the pH range 3–12. This study offered the opportunity of products based on industrial spent fungal biomasses making use of common chemicals, i.e. NaOCl, NaBr and TEMPO as catalyst. The final polysaccharide products were mixtures of polyuronans suitable for major industrial applications in agriculture, cosmetic and pharmaceutical fields.

Recently, Sun et al. (2006) have proposed the large scale production of pure chitouronic acid after conversion of crystal structure of chitin by alkaline treatment. The TEMPO mediated oxidation of

pretreated chitin considerably increased the polyglucuronic acid yield from 36% to 97%.

To summarise these studies showed the conditions under which animal and fungal chitins are regioselectively oxidized. Novel soluble biopolymers may result which might be easily proposed and defined as low cost surrogates of hyaluronans for pharmaceutical uses (Muzzarelli & Peter, 1997).

3.3.4. Rhamnoglucuronan polysaccharide from gellan carboxylation

As largely described in this present review, TEMPO oxidation is an attractive method for producing new anionic macromolecules. Therefore, Elboutachfaiti et al. (2010) have proposed a new ulvan-like polymer after regioselective oxidation of gellan using TEMPO reagent. Thus, an original water-soluble rhamnoglucuronan was obtained quantitatively with a homogeneous chemical structures. This polysaccharide might find use in pharmaceutical and medical areas as surrogates of ulvan, a sulfated polysaccharides extracted from green seaweeds (Lahaye, Brunel, & Bonnin, 1997). At present, work is in progress to produce large amount of oligo-rhamnoglucuronic acids from this new ulvan-like which could be used in cosmestic, pharmaceutic, neutraceutic and agriculture.

3.3.5. Cellulose oxidation and sulfatation to produce Glycosaminoglycan like

Glycosaminoglycans (GAG), such as for example heparan sulfate or heparin have for long time demonstrated important rules in regulation of cellular differentiation and proliferation (Carroll & Koch, 2003). Then, it is well established that glycosaminoglycans can easily bond a large variety of cytokines and growth factors (Capila & Linhardt, 2002). These GAGs generally contain carboxyl and sulfate groups in their main repeating units and these chemical groups can modulate biological activities depending on various molecular compositions (Rabenstein, 2002). To overcome this limitation, diverse glycosaminoglycan–analogues have been realized by sulfating other natural polysaccharides in order to produce a very large range of biological polysaccharides.

Up to now, few studies have been carried out on polysaccharide derivatives with both carboxyl and sulfate groups. In fact, cellulose derivatives bearing both functional groups may possess new properties and new potential applications. Recently Zhang et al. (2010) have published synthesis routes resulting in sulfated polyglucuronic acids containing both sulfate and carboxyl groups from cellulose. These new synthetic glycoaminoglycans were investigated for their mitogenic activity on 3T3 mouse fibroblasts. Consequently, it was shown that with higher contents of sulfate groups instead of carboxyl groups these glycosaminoglycans were able to promote significantly the proliferation of fibroblasts in the presence of FGF-2 (Zhang et al., 2010). In the same way, natural polyglucuronic acid from *S. meliloti* M5N1CS has been partially *O*-sulfated and tested in muscle regeneration. A regenerating activity on injured *extensor digitorum longus* muscles of rats was obtained with this sulfated polysaccharide. The location of sulfates appeared without effect on the biological activity contrary to the degree of sulfatation. The rate of glucuronan acetylation modulated the activity (Petit et al., 2004).

4. Polyglucuronic acids acting enzymes

4.1. Enzymatic degradation of polyglucuronic acids

As for the majority of enzymes acting on polyuronides, polysaccharide lyases are the main polysaccharide cleavage enzymes acting on polyglucuronic acids. They recognize uronic acid residues β or α-(1,4) linked and cleave their substrates by β-elimination leading to the formation of a 4-deoxy-L-*erythro*-hex-4-enopyranosyluronate in the newly formed nonreducing terminal unit (Michaud, Da Costa, Courtois, & Courtois, 2003). Polysaccharide lyases (EC 4.2.2.x) are classified into 22 families based on their amino acids sequences similarities in the carbohydrate-active enzymes database (CAZy, http://www.cazy.org, Cantarel et al., 2008). A previous review focused on glucuronan and cellouronate degrading enzymes (Tavernier, Delattre, Petit, & Michaud, 2008). All the enzymes identified are β-(1,4)-polyglucuronic acid lyases (EC 4.2.2.14) even if some of them have been previously described mistakenly as hydrolases. These enzymes are always endolyases except an exolyase purified from a bacterial strain belonging to the genus *Brevundimonas* (Konno, Habu, Iihashi, & Isogai, 2008). They degrade preferentially deacetylated glucuronan and cellouronate. However residual activities on acetylated glucuronan, alginate, amylouronate and glucuronan blocks of ulvan have been also described for some of them (Delattre et al., 2005b; Konno et al., 2006). These enzymes are robust and the degradation can be operated in water in large scales of pH and temperatures. Final unsaturated products had a dp between 1 and 5 and unsaturated monomer was described as unstable and spontaneously transformed in α-keto glucuronic acid (Preiss and Ashwellb, 1962). Note that when substrates were acetylated glucuronans, mixes of unsaturated oligomers generated had generally dp higher than those observed with not acetylated substrate. Enzymatic activity was easily quantified by measure of absorbance of the generated unsaturated terminal unit at 235 nm. One unit was expressed as the amount of enzyme necessary to increase 1 U in the absorbance at 235 nm/min or the amount of enzyme necessary to release 1 μmol/min of an unsaturated oligomer with a dp of 3. Biological degradations of β-(1,4)-polyglucuronic acid seems to be a common phenomenon in nature. In fact these enzymes could play an important role in the degradation of complex cell wall such as those of algae and fungi where polyglucuronic blocks or β-(1,4) linked glucuronic acids have been detected (Dow et al., 1983; Ray, 2006). More recently some of them have been identified as substrates of glucuronan lyase (Elboutachfaiti et al., 2009) or enzyme assimilated as this encoded by the chlorovirus CVK2 (Sugimoto et al., 2004). Glucuronan and cellouronate lyases are express by bacterial strains isolated from soil such as *Brevundimonas* sp., *Sinorhizobium meliloti*, *sphingomonas* sp and *Sphingopyxis* sp but also from *Trichoderma* (teleomorph *Hypocrea*) strains (Tavernier, Delattre, et al., 2008). *Trichoderma* spp are among the most frequently isolated soil fungi and are present in plant root ecosystems. These fungi are opportunistic, avirulent plant symbionts and function as parasites and antagonists of many phytopathogenic fungi, thus, they protect plants from diseases. These Ascomycetes express various enzymes for polysaccharide degradation often called cell wall degrading enzymes (Vinale et al., 2008). Delattre et al. isolated from compost a *Trichoderma* sp. that secreted a glucuronan lyase when it was cultured in the presence of glucuronan (Delattre et al., 2005b). The recent publication of the cellulolytic fungi *Trichoderma resei* genome led Konno et al. to clone a cDNA encoding for a glucuronan lyase from this strain and heterologously expressed it in the methylotrophic yeast *Pichia pastoris* (Konno, Ishida, et al., 2009; Konno, Igarashi, Habu, Samejima, & Isogai, 2009). No site for *N*- and *O*-glycosylation was found in the gene. The amino acid sequence of the mature protein (238 aa) showed no significant similarities with amino acid sequences of previously described functional proteins. This new enzyme was then classified in the family 20 of polysaccharide lyases. This family is actually made up of 11 proteins but 10 of them are hypothetical proteins mainly identified on eukaryotic genomes. No homologies of amino acid sequences were detected with proteins from the family 14, the sole other family where similar activities as this of the glucuronic acid specified polysaccharide lyase of *Chlorovirus* CVK2 were classified. The recombinant pro-

tein was able to degrade cellouronate with high specifically. The enzyme was most active with Ca^{2+} suggesting its calcium dependence. The crystal structure of the enzyme called TrGL has been determined at 1.8 Å resolution. The model obtained contains all the mature protein, a calcium ion, a citrate and water molecules. The enzyme showed a typical β-jelly roll fold. This structure has similarities with enzymes belonging to the family 16 of glycoside hydrolases and family 7 and 18 of polysaccharide lyases (Konno, Ishida, et al., 2009). Authors identified possible catalytic residues by alignment of the structures of the A1-II alginate lyase belonging to the family 7 of polysaccharide lyase with this of TrGL but concluded that mutational analysis and determination of complex structures with substrate or analogues will be required.

Besides these glucuronan and cellouronate lyases acting on β-(1,4)-polyglucuronic acids another enzyme have been described acting about α-(1,4)-linked glucuronan (also called amylouronate). This enzyme isolated and purified from *Paenibacillus* sp TH501b, a telluric bacterial strain (Iihashi et al., 2009), was designed as a α-(1,4)- glucuronan hydrolase or α-glucuronidase. It cleaved amylouronate by an exohydrolytic mode leading to glucuronic acid release as sole product. A second α-(1,4)-glucuronan hydrolase degrading amylouronate by an endolytic mode was suspected by authors.

4.2. Potential application of polyglucuronic acid degrading enzymes

In recent years the number of patents relating to oligoglucuronans has increased significantly. These compounds have been described as biological response modifiers on vegetable and mammalian cells (Courtois & Courtois, 1998; Fournial et al., 2008; Lienart et al., 1999; Michaud, Delattre, Courtois, & Courtois, 2005; Rat et al., 2008). Biological activities claimed and described focus on elicitation of plant defense response, stimulations of immune system and dermo-epidermal cohesion. However, the obtaining of these oligomers is actually the main drawback limiting their utilization. The use of a fungal glucuronan lyase from a *Trichoderma* strain during the last decade by Delattre et al. and the development of new strategies for purification and fractionation of anionic oligomers have resulted in significant progresses in obtaining significant amounts of pure and controlled mixtures of oligomers (for a review see Tavernier, Delattre, et al., 2008). However, these amounts are still not really competitive in terms of industrial manufacture and the processes need to be more efficient. In this context, recent publications have described continuous glucuronan depolymerization after immobilization of the *Trichoderma* sp GL2 glucuronan lyase on various matrices. These studies showed that it was possible to control the large depolymerization of deacetylated or acetylated glucuronan with a productivity ranging from 120 mg h^{-1} to 1.2 g h^{-1} with less than 1 mL of support. Depending to the flow rate and degree of glucuronan acetylation, oligomers had a degree of polymerization between 2 and 25. The enzyme was immobilized without apparent lack of activity and was stable for several weeks (Tavernier, Petit, et al., 2008; Tavernier, Michaud, Wadouachi, & Petit, 2009).

In the future two other ways for the large scale production of polyglucuronic acid degrading enzymes could be developed. The first will involve macerating of fungal and/or plant cell walls with complex structures including (1,4) linked glucuronic acid or β-(1,4)-polyglucuronic acid blocks resistant to traditional enzymatic complexes. The second approach could be glucuronic production after enzymatic degradation of amylouronate by the α-(1,4)-glucuronidase recently identified (Iihashi et al., 2009). Effectively, glucuronate and glucuronolactone (its intramolecular ester) are abundantly employed in industry, notably for medical applications. Iihashi et al. (2009) suggest that the α-(1,4)-glucuronidase from *Panebacillus* has the potential to be applied to a new process for the efficient and environment friendly production of glucuronate.

5. Conclusions

Since the nineties the production relative of polyglucuronic acid and related materials has increased significantly. An recent examination of Scopus, one of the largest databases of peer reviewed literature using the key words glucuronan; cellouronate and polyglucuronic acid gave 106 references including patents. The majority of the more recent patents focused on oligoglucuronans applications for their biological activities apparently limited by the strategies available to produce them in large quantities. The recent progresses in molecular biology of genes encoding for glucuronan lyases and the development of reactors with immobilized enzymes could counteract this drawback. However, the most exciting question about glucuronan lyases is their conservation in genomes of a large variety of organisms and virus. They are generally encoded by genes which are induced by their substrate. The actual organisms producers identified are telluric bacteria and fungi in strong interactions with plant and fungi. So polyglucuronic acid and notably those linked in β-(1,4) or simple β-(1,4) linked glucuronic acid are probably often present in the complex cell walls of some plant and fungi. Glucuronan lyases could be implicated in their degradation. The recent identification of these uronic structures in algae such a *Ulva lactuca* and microalgae such as *Chlorella* reinforces this idea.

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