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Reviews

Structure and Functional Properties of Ulvan, a Polysaccharide from Green Seaweeds

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With today's interest in novel renewable chemicals and polymers, the underexploited marine green algae belonging to species of *Ulva* and *Entermorpha* stimulated interest as sources of polysaccharides with innovative structure and functional properties. These algae are common on all seashores and can produce in time an important amount of biomass in nutrient-enriched waters. The major water-soluble polysaccharide, ulvan, extracted from the cell wall represents about 8−29% of the algae dry weight. The original physicochemical, rheological, and biological properties recently unraveled for this complex sulfated aldobiouronan open the way for novel potential applications.

Introduction

Ulvales (Chlorophyta) are very common seaweeds distributed worldwide. The two main genera *Ulva* and *Enteromorpha* are particularly known because members are grown or collected for food consumption[1] and others are associated with proliferations in eutrophicated coastal waters[2] or with contamination of algal closed cultures.[3,4] The opportunistic growth ability of these seaweeds makes them good candidates for water recycling in integrated invertebrates or fishes aquaculture systems[5,6] and of urban waters,[7] but most of the generated biomass is today of little value. The collected algae are most often incorporated into compost[8] but generally dumped although conversion to biogas is feasible.[9] With today's interest in new renewable sources of chemicals and polymers, this underexploited biomass represents a potential source to be explored. Among the polymers synthesized by these algae, cell wall polysaccharides represent around 38−54% of the dry algal matter.[10] These include four polysaccharide families in *Ulva* sp.: two major ones, the water-soluble ulvan and insoluble cellulose, and two minor ones, a peculiar alkali-soluble linear xyloglucan and a glucuronan. Their distribution and associations in *Ulva* cell wall have been summarized in a model which takes into account recent cytochemical and physicochemical data[11] (Figure 1). Most of the recent work on Ulvales cell wall polysaccharides focused on ulvan as it displays several physicochemical and biological features of potential interest for food, pharmaceutical, agricultural, and chemical applications. The objective of this paper is to review the chemistry and physicochemistry in some properties of ulvan.

Discussion

Chemistry of Ulvan. The name ulvan is derived from the original terms ulvin and ulvacin introduced by Kylin[12] in reference to different fractions of *Ulva lactuca* water-soluble sulfated polysaccharides. It is now being used to refer to polysaccharides from members of the Ulvales, mainly, *Ulva*, *Enteromorpha* sp. Extraction is generally achieved by water solutions at around 80−90 °C containing a divalent cation chelator such as ammonium oxalate.[13,14] The yield ranges from 8% to 29% of the algal dry weight, depending on the extraction and purification procedures.[13−21] Recovery of ulvan is generally done by precipitation by adding an alcohol or a quaternary ammonium salt.

Composition. The pioneering work of Brading et al.[22] and McKinnel and Percival[23] established that sulfate, rhamnose, xylose, and glucuronic acid are the main constituents of ulvan. They also identified that glucuronic acid and rhamnose occur mainly in the form of the aldobiouronic acid, 4-*O*-β-D-glucuronosyl-L-rhamnose (Figure 2). Rhamnose (16.8−45.0% dw), xylose (2.1−12.0%), glucose (0.5−6.4%), uronic acid (6.5−19.0%), and sulfate (16.0−23.2%) have since then been reported in ulvan from several Ulvales species,[14,16−19,24−30] but

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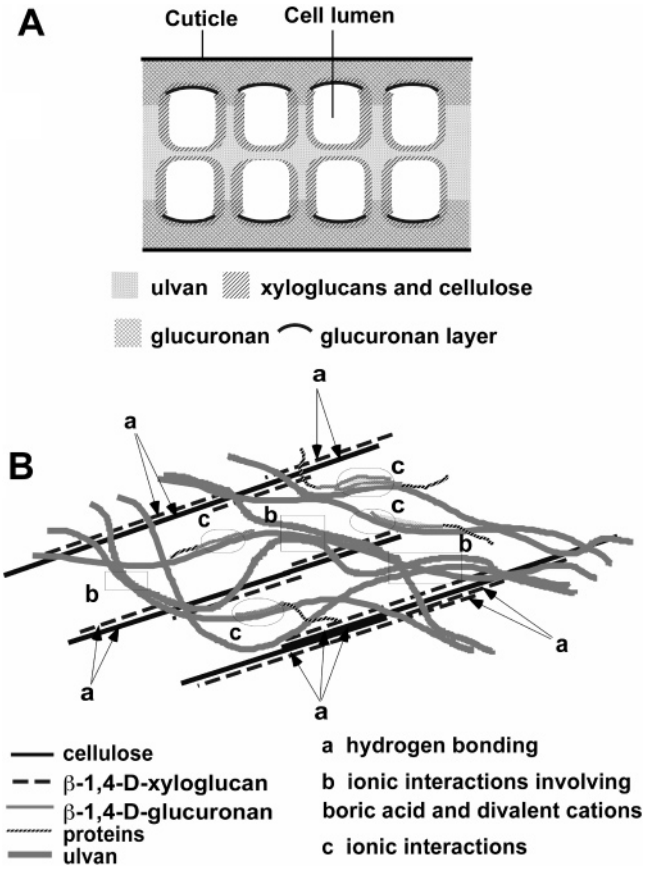

Figure 1. Distribution of the different *Ulva* sp. cell wall polysaccharides in a schematic cross section of a thallus (A) and proposed associations between the different cell wall polysaccharides (B).

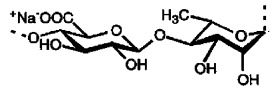

Figure 2. Structure of the main repeating disaccharide in *Ulva* ulvan: ulvanobiouronic acid.

it is only after the work of Quemener et al.[29] that iduronic acid (1.1−9.1%) was recognized as a constituent carbohydrate unit in ulvan. Variable amounts of mannose and galactose have been reported, but their belonging to ulvan has been questioned since they form a distinct neutral fraction in *U. mutabilis*.[24] Arabinose was reported to be present in *U. lactuca* ulvan collected in Egypt[13] and 3-*O*-methyl L-rhamnose in *E. compressa* and *Enteromorpha* sp.[23,31]

The variation in sugar composition can have methodological, taxonomic, and/or ecophysiological origins. To date, it is difficult to accurately determine the sugar composition of ulvan because the aldobiouronic linkage is refractory to acid hydrolysis[23,32] and iduronic acid is partially destroyed during acid hydrolysis.[33,34] To overcome these problems, a chemo−enzymatic degradation method was proposed to optimize the recovery of all the constituent sugars.[29] It involves a mild acid hydrolysis to depolymerize ulvan to aldobiouronic acid. The disaccharide is then cleaved by a β-D-glucuronidase into glucuronic acid and rhamnose. The acid treatment also releases all the sulfate groups from ulvan. The resulting neutral sugars and uronic acids can then be identified and quantified by HPLC. Even in the absence of a readily available iduronic acid reference sample, the contents of the two uronic acids in ulvan determined by HPLC compare well with those calculated from circular dichroism spectra.[35]

Confusions during species identification can also contribute to the compositional variability. Precise identification of Ulvales is difficult and cannot only rely on morphological characteristics.[36,37] Indeed, species exist in nature that have intermediate forms, such as *E. linza* with an *Enteromorpha*-like tubular base and *Ulva*-like distromatic blade distally, and several culture studies have revealed flexibility between tubular and blade morphologies. In fact, several evidences from molecular analyses[38−40] and culture studies[41−45] show that *Ulva* and *Enteromorpha* are not distinct evolutionary entities and belong to the same genera. In view of this complexity, it is likely that algae collected in different regions of the world and named *U. lactuca*, *U. rigida*, or *U. rotundata*, for example, may represent different species.

Ecophysiological growth conditions could also affect biosynthesis of ulvan and, thus, its chemistry. However, to date, no specific trends have yet been established between growth conditions and the chemical composition of the sulfated aldobiuronan.[21] Reports indicating variations in carbohydrate contents with seasons[20,27,46] may in fact reflect different proportions of starch or other cell wall polysaccharides such as glucuronan, xyloglucan, or some other glycoproteins coextracted with ulvan.[30,47,48]

Structure. Early sugar linkage analyses of *Enteromorpha* ulvan indicated terminal, 1,4-, 1,3-, 1,3,4-, and 1,2,3,4-linked rhamnose and 1,4- and 1,2,4-linked xylose.[31] Similar chemical studies on *U. lactuca* ulvan and its oligosaccharides after partial acid hydrolysis indicated a high proportion of 1,4-linked rhamnose substituted on C-3.[49] The latter report also showed some 1,4- and 1,3-linked xylose and 1,4- and 1,3-linked glucose originating from ulvan in which glucuronic acid residues had been previously reduced to glucose. The locations of sulfate esters were determined by chemical means and by infrared spectroscopy of native and chemically modified ulvan.[18,21,23] Small amounts of xylose 2-sulfate were deduced from the identification of 2-*O*-methyl-xylose in the reaction products of *U. lactuca ulvan* with sodium methoxide. IR spectra of ulvans from *U. lactuca* and *E. compressa* demonstrated a major band for sulfate esters in the region of 1250 cm^{-1} [18,21,23] and an absorbance at 850 cm^{-1} attributed to axial sulfate on C-2 of rhamnose by analogy to carrageenans.[18] However, though recent data confirmed the presence of xylose-2-sulfate, most of the sulfate in ulvan from *Ulva* spp. was to date found on C-3 of rhamnose.[27,50,51]

Determining the sugar sequence in ulvan represents, like in any other polysaccharide, a major challenge. Oligosaccharides and oxidation products released after Smith degradation or mild acid hydrolysis of native and chemically modified ulvan suggested the presence of other aldobiouronic acids and clearly demonstrated that rhamnose, xylose, glucuronic acid, or glucose were all present in the same chain.[49,52] The early sugar sequence studies were summarized in a seven-sugar model fragment[53] (1):

$$\text{GlcA}(1\rightarrow4)\text{Rha sulfate}(1\rightarrow4)\text{GlcA}(1\rightarrow3)\text{Xyl}(1\rightarrow4)\text{Rha sulfate}(1\rightarrow3)\text{Glc}(1\rightarrow4)\text{Xyl} \quad (1)$$

to which contiguous 3-/4-linked xylose and contiguous 3-/4-linked glucose residues are to be added.[52]

More recently, oligosaccharides recovered after mild acid hydrolysis of desulfated ulvan from *U. "rigida"*[54] indicated the existence of several new repeating sugar sequences (**2**−**5**) (Table 1). Among them, some (**4**, **5**) revealed that glucuronic acid can occur as branches on C-2 of rhamnose. Mild acid hydrolysis of native ulvan produced the sulfated aldobiouronic acid (**6**)

Table 1. Chemical Structure, Proposed Name, and Symbol for Oligosaccharides Isolated from Native and Chemically Modified Ulvan of Different Origins by Acid Hydrolysis or Enzymatic Degradation

	oligosaccharide/repeating structure	name	symbol	ulvan origin	ref
2	α-L-Rha (1→4) D-Xyl[a]			*Ulva* "*rigida*", desulfated ulvan	54
3	β-D-GlcA (1→2)-α-L-Rha (1→4) D-Xyl				
4	β-D-GlcA (1−4) [β-D-GlcA (1→2)] L-Rha				
5	β-D-GlcA (1−4) [β-D-GlcA (1→2)] α-L-Rha (1→4) D-Xyl				
6	β-D-GlcA (1→4)-L-Rha 3S→4) β-D-GlcA (1→4)-α-L-Rha 3S (1→	type A ulvanobiouronic 3-sulfate	**A_{3s}**	*Ulva* "*rigida*", native ulvan	
7	α-L-IdoA (1→4) α-L-Rha 3S→4) α-L-IdoA (1→4)-α-L-Rha 3S (1→	type B ulvanobiouronic 3-sulfate	**B_{3s}**	sea lettuce (*Ulva* sp.)	51
8	Δ (1→4) L-Rha 3S			sea lettuce (*Ulva* sp.)	55
9	Δ (1→4) α-L-Rha 3S (1→4) β-D-Xyl (1→4) L-Rha 3S				
10	Δ (1→4) α-L-Rha 3S (1→4) β-D-GlcA (1→4) L-Rha 3S				
11	Δ (1→4) α-L-Rha 3S (1→4) α-L-IdoA (1→4) L-Rha 3S				
12	Δ (1→4) α-L-Rha 3S (1→4) β-D-GlcA (1→4) β-D-GlcA (1→4) L-Rha 3S				
13	→4) β-D-Xyl (1→4) α-L-Rha 3S (1→	ulvanobiose 3-sulfate	**U_{3s}**		
14	Δ (1→4) α-L-Rha 3S (1→4) β-D-Xyl 2S (1→4) L-Rha 3S			*Ulva* "*rigida*", Canary Islands	57
15	Δ (1→4) α-L-Rha 3S (1→4) β-D-Xyl (1→4) α-L-Rha 3S (1→4) β-D-Xyl (1→4) L-Rha 3S				
16	Δ (1→4) α-L-Rha 3S (1→4) β-D-Xyl 2S (1→4) α-L-Rha 3S (1→4) β-D-Xyl (1→4) L-Rha 3S				
17[b]	Δ (1→4) α-L-Rha 3S (1→4) β-D-Xyl 2S (1→4) α-L-Rha 3S (1→4) β-D-Xyl (1→4) α-L-Rha 3S (1→4) β-D-Xyl (1→4) L-Rha 3S				
18	Δ (1→4) [β-D-GlcA (1→2)] α-L-Rha 3S (1→4) β-D-Xyl (1→4) L-Rha 3S (20)			*Ulva* "*rigida*", Brittany	
19	Δ (1→4) [β-D-GlcA (1→2))] α-L-Rha 3S (1→4) β-D-Xyl 2S (1→4) L-Rha 3S				
20	→4) β-D-Xyl 2S (1→4) α-L-Rha 3S (1→	ulvanobiose 2′,3-disulfate	**$U_{2's,3s}$**	*Ulva* "*rigida*", Canary Islands	
21	→4) β-D-GlcA (1→4) [β-D-GlcA (1→2)] α-L-Rha 3S (1→	type A ulvanobiouronic 2′-glucuronic acid, 3-sulfate	**$A_{2g,3s}$**	*Ulva "rigida*", Brittany	

[a] Rha, GlcA, Xyl, IdoA refer to rhamnose, glucuronic acid, xylose, iduronic acid, respectively; Δ refers to the unsaturated uronic acid 4-deoxy-L-threo-hex-4-enopyranosiduronic acid at the nonreducing end. [b] Xylose-2-sulfate occurs equally in the central repeating disaccharide and at the nonreducing end.

together with rhamnose 3-sulfate monomer which clearly established sulfation on C-3 of rhamnose. The sulfated aldobiouronic acid **6** was found as one major disaccharide repeating structure in the ulvan from different *Ulva* samples together with another one in which iduronic acid is replacing glucuronic acid (**7**).[27,51] The two major aldobiouronic acids were named type A ulvanobiouronic acid 3-sulfate (**6**), and symbolized as **A_{3s}**, and type B ulvanobiouronic acid 3-sulfate for **7**, or **B_{3s}**.

To further characterize the fine structure of ulvan, ulvanolytic enzymes were searched and an extracellular ulvan-lyase was isolated from a marine Gram-negative bacterium.[55] This enzyme cleaved the (1→4) linkage between rhamnose 3-sulfate and glucuronic acid and produced oligosaccharides with an unsaturated uronic acid at the nonreducing end. It released several oligosaccharides from ulvan of edible *Ulva* sp. ("sea lettuce") (Figure 3A) that were identified as di-, tetra-, and pentasaccharides (**8**−**11**; Table 1). The authors demonstrated the presence of repeating **-A_{3s}-A_{3s}-**, **-A_{3s}-B_{3s}-**, **-A_{3s}-U_{3s}-**, **-A_{3s}-**GlcA-**A_{3s}-** sequences where **U_{3s}** refers to ulvanobiose 3-sulfate (**13**, Table 1). Isolation of oligosaccharides with GlcA flanked by **A_{3s}** structures showed that an extra 4-linked β-D-glucuronic acid residue can break the regularity of the disaccharide repetition. It provides a cleavage site of ulvan by glucuronan lyase.[56]

The activity of the ulvan-lyase preparation used in these studies was limited by iduronic acid in the **B_{3s}**-rich ulvan from *U. armoricana* from which mainly high molecular weight complex oligosaccharides were produced[55] (Figure 3B). Much less degradation was achieved on *E. compressa*[55] (13% degradation) and on *U. olivascens* ulvans (Lahaye, unpublished) indicating major structural differences between these polysaccharides. Thus, ulvan-lyase degradation can be helpful for distinguishing ulvans based on their fine structure and may be useful to differentiate Ulvales species. Applied to the ulvan from *U. rigida* collected in the Canary Islands (Spain) and in Brittany (France), the lyase preparation yielded two distinct degradation patterns[57] as shown by chromatography on Bio Gel P4 (Figure 3, parts C and D). From the Canary Islands sample, besides oligomers **8** and **9**, four new structures were identified (**14**−**17**, Table 1). From the Brittany sample, oligosaccharides **8**, **9**, **10**, **14** were produced together with two other sequences (**18**, **19**) reminiscent of the branched oligomer **5**. If the two ulvans shared **-A_{3s}-A_{3s}-**, **-A_{3s}-U_{3s}-**, **-A_{3s}-$U_{2's,3s}$-** sequences, they differed by **-A_{3s}-U_{3s}-U_{3s}-**, **-A_{3s}-$U_{2's,3s}$-U_{3s}-**, and **-A_{3s}-$U_{2's,3s}$-U_{3s}-U_{3s}-** sequences for the Canary Islands sample and by **-$A_{2g,3s}$-U_{3s}-** and **-A_{2g3s}-$U_{2's,3s}$-** sequences for the Brittany sample. In these, **$U_{2's,3s}$** refers to ulvanobiose 2′,3-disulfate (**20**, Table 1) and **$A_{2g,3s}$** to type A ulvanobiouronic acid 3-sulfate substituted on C-2 of the rhamnose 3-sulfate by a single glucuronic acid residue (type A ulvanobiouronic 2′-glucuronic acid, 3-sulfate, **21**, Table 1). These different ulvan oligosaccharides patterns indicated that either the polysaccharides originated from distinct species or that their biosynthesis was markedly affected by ecophysiological factors.

The relative high proportion of repeating sequences in ulvan makes these polysaccharides suitable for structural analysis by ^{13}C NMR spectroscopy. However, if the major **A_{3s}** and **B_{3s}** disaccharides are readily recognized[51] (Figure 4), identification of **U_{3s}**, **$U_{2's,3s}$**, and **$A_{2g,3s}$** structures is more difficult partly because of different distributions possible in the polysaccharide.[57] The ^{13}C NMR spectrum of *E. linza* is very complex, and besides signals for **A_{3s}** sequences, it shows additional resonances attesting for other unknown repeating structures that are also observed in other *Ulva* ulvan spectra such as that from *U. rotundata* from Brittany (Figure 4). Carbon signals for 1,4-linked β-D-glucuronan are often observed in the ^{13}C NMR spectra of ulvans[27] (Figure 4). Their origin can be due to the extra glucuronic acid in ulvan chains but can also reflect the presence of glucuronan occurring as a contaminant.

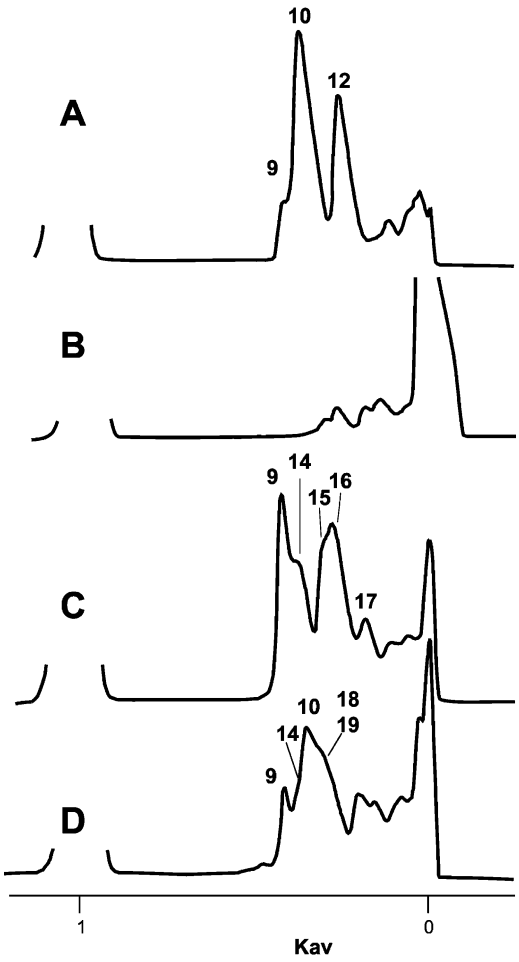

Figure 3. Bio Gel P4 profiles of ulvan-lyase degradation products of ulvan from "sea lettuce" (A), *U. armoricana* (B), and from *U. rigida* from the Canary Islands (Spain) (C) and Brittany (France) (D). Numbers on top of chromatographic peaks correspond to the oligosaccharide structures discussed in the text. The truncated peak eluting at a Kav of 1 is due to salts and corresponds to the total chromatographic volume of the column. The peak eluting at a Kav of 0 represents high molecular weight fragments of ulvan excluded from the column in the void volume.

Proton NMR spectroscopy has also been used to study the chemistry of these polysaccharides. The high number of possible disaccharide sequences and distributions in addition to other structural irregularities complicates the analysis of the signals that distribute within a narrow chemical shift range in the spectra[51] (Figure 5). Information can nevertheless be obtained on the presence of iduronic acid, but further work is required before this method of analysis can be used to quantify specific structures.

Ulvan samples from several Ulvales species are composed of variable proportions of different repeating sequences mostly based on disaccharide domains made of rhamnose, glucuronic acid, iduronic acid, xylose, and sulfate. The different enzymatic degradation susceptibility by the ulvan-lyase preparation used in these studies and the variations in the ^{13}C NMR spectra of ulvans clearly demonstrate that other linkages, sugar distributions, branching, and/or sulfation patterns exist. Whether the nature and the proportion of these sequences are species specific and can be used as chemotaxonomic markers remains to be clearly established. Interestingly, water-soluble cell wall polysaccharides of members of the Ulotrichales, another order of the Ulvophyceae besides Ulvales,[58] share several compositional and structural similarities. They are made of rhamnose, uronic acid and xylose as main sugars and of type A ulvanobiouronic acid.[59−63] However, the polysaccharides of *Spongomorpha indica*[64] also belonging to this order resemble Cladophorales sulfated arabinogalactans.

Biosynthesis of Ulvan. The mechanisms by which the ulvan building units are synthesized, assembled, exported, and integrated in the algal cell wall will influence extraction and properties of the polysaccharides. Although no study dealt with the biosynthesis of ulvan, a general scheme can be sketched taking into account recent advances made in the biosynthesis of matrix polysaccharides in different organisms. It involves building of the sugar nucleotide precursors in the cytoplasm, polymerization of precursors in the cellular endomembrane systems, export and assembly in the cell wall.[65,66]

Monosaccharides in polysaccharide synthesis are generally incorporated from their corresponding sugar nucleotide donors.[65,66] These donors mostly arise from the interconversion of UDP-Glc. The synthesis of glucuronic acid, rhamnose, and xylose precursors in ulvan is expected to follow the pathways depicted in Figure 6. UDP-GlcA arises from UDP-Glc or from inositol. UDP-Xyl is then formed by interconversion of UDP-GlcA. The nucleotide precursor of rhamnose may be TDP-L-Rha as in bacteria or UDP-L-Rha as suggested in plants.[67,68] In contrast, iduronic acid in ulvan may be formed at the polymer level as for animal glycosaminoglycans biosynthesis[69,70] by action of an epimerase converting glucuronic acid to iduronic acid. A similar conversion is known to occur in the cell wall of brown seaweeds, i.e., the epimerization of mannuronic acid to guluronic acid in alginate.[71] The nucleotide sugar precursors are transported in the Golgi apparatus by specific transporters or by glycosyltransferases. They serve as substrates to bound glycosyltransferases and synthases complexes in the biosynthesis of the polysaccharide.[65,66] Branches and sulfation may occur concomitantly or independently and sequentially to chain polymerization and in a concerted manner to yield the different structural repeating domains of ulvan. Although sulfation of ulvan by specific sulfotransferases may occur in the Golgi as for animals and brown seaweeds sulfated polysaccharides,[72,73] direct sulfation in the cell wall as proposed in red seaweeds cannot be excluded.[74] Then, the vesicles containing the newly formed polysaccharides are discharged in the apoplast. The mechanisms of higher plant cell wall polysaccharides assembly and organization remain unclear but involve further processing of the polysaccharides by wall enzymes in response to various biological and environmental factors. In the case of ulvan, as mentioned above, epimerization of glucuronic acid to iduronic acid, sulfation and desulfation may also occur as reported for glycosaminoglycans in the animal cell matrix and for algal galactans, particularly in response to growth and environmental factors.[75,76]

Physicochemistry of Ulvan. *Molecular Heterogeneity.* Ulvan from *U. lactuca* was described as a family of chemically related branched molecules of broad distribution in term of charge density and molecular weight.[53] The polymolecular character of ulvan may have different origins: presence of contaminants, different molecular weight distributions, numerous populations of ulvan varying in the content and distribution of the repeating structures. Ulvans from *U. pertusa*, *U. conglobata*, and *E. prolifera* show a multimodal distribution by gel permeation chromatography.[14] On anion-exchange chromatography of *U. pertusa* and *U. conglobata* ulvan, the major fraction eluted with 1 M NaCl and distributed as a single polydispersed population on gel permeation chromatography. For *U.* "*rigida*" ulvan, the polymolecularity was due to contaminating linear xyloglucan

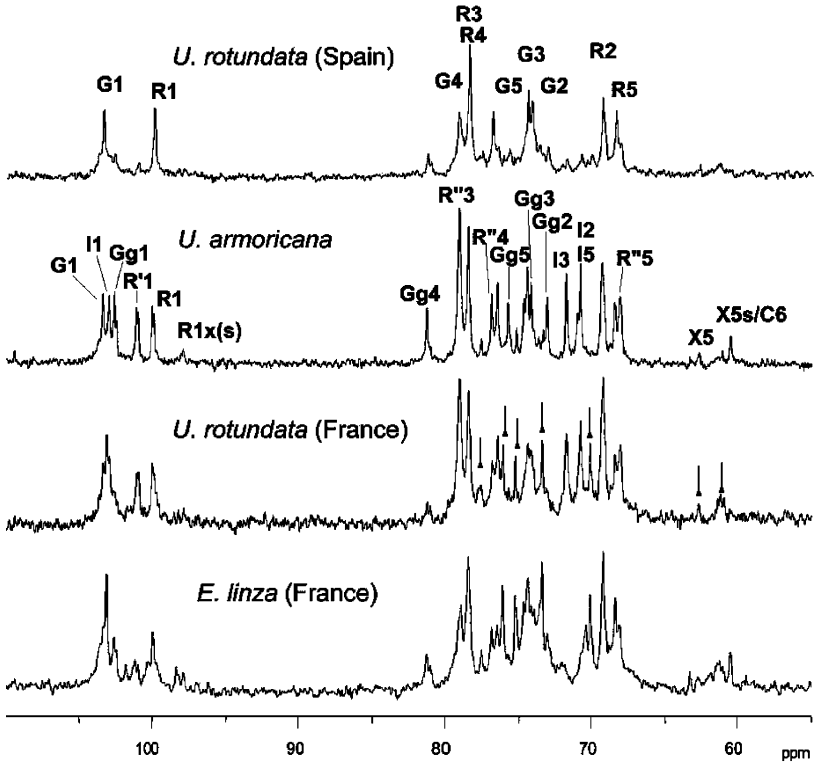

Figure 4. ^{13}C NMR spectra recorded at 70 °C of ulvan from *U. rotundata* collected in Spain and in Brittany (France), from *U. armoricana* ("green-tide" *Ulva* from Brittany, France) and *Enteromorpha linza* from Brittany (France). Letters G and R and numbers refer to carbons of glucuronic acid and rhamnose 3-sulfate, respectively, in $\mathbf{A_{3s}}$ structures; the letters Gg and numbers refer to carbon in the glucuronic acid residues of 1,4-linked β-D-glucuronan; R′ and R″ and numbers associated refer to carbons of rhamnose 3-sulfate linked to C-4 of iduronic acid and of rhamnose 3-sulfate to which iduronic acid is linked on C-4, respectively, and reflect $\mathbf{B_{3s}}$ sequences. R1x(s) refers to the anomeric carbon of rhamnose 3-sulfate linked to xylose or xylose-2-sulfate; X5 and X5s correspond to C-5 of 1,4-linked xylose and xylose 2-sulfate, respectively. These signals reflect the presence of $\mathbf{U_{3s}}$ and $\mathbf{U_{2's,3s}}$ sequences, respectively. C6 corresponds to the signal of a C-6 originating probably from glucose. Arrows on the spectrum of ulvan from *U. rotundata* collected in France correspond to carbons of unknown structures also found on the spectrum of the *E. linza* ulvan. Not shown on the spectra are the resonances for C-6 of rhamnose 3-sulfate at 17.5 ppm and glucuronic and iduronic acids at 175.0 and 174.6 ppm, respectively (DMSO reference signal attributed at 39.6 ppm).

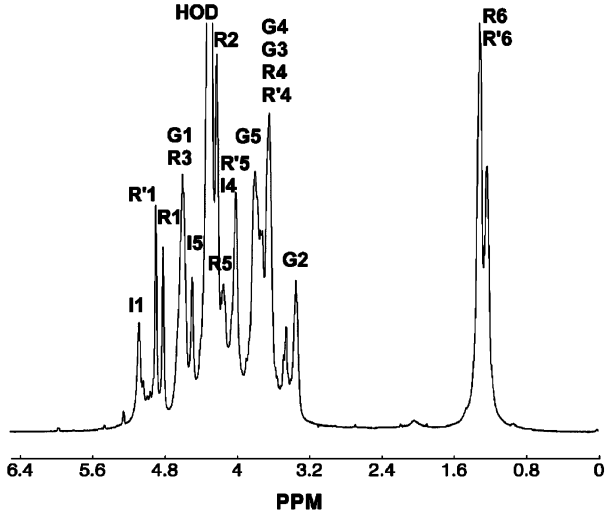

Figure 5. ^{1}H NMR spectrum recorded at 80 °C of ulvan from a "sea lettuce" sample rich in $\mathbf{A_{3s}}$ and $\mathbf{B_{3s}}$ repeating structures. Letters I, R, and G and numbers correspond to protons in iduronic acid, rhamnose 3-sulfate, and glucuronic acid, respectively. HOD refers to the signal of the residual water in the sample.

and glucuronan coeluting with proteins beside ulvan of narrow charge density distribution.[30] Several ulvan fractions varying in charge and sugar compositions and with different molecular weights and intrinsic viscosities were isolated from *E. intestinalis*.[25]

Different molecular weights and molecular weight distributions of ulvan have been reported. Sedimentation measurements gave molecular weights ranging from 5.3×10^5 to 3.6×10^6 g/mol for *U. pertusa*, *U. conglobata*, and *E. prolifera* ulvans[14] with large variations in case of *U. conglobata* ulvan in relation with the temperature at which the polysaccharides were extracted. The lowest and highest molecular weights, recorded for the extract obtained at 30−40 °C (5.3×10^5 g/mol) and 80−90 °C (3.6×10^6 g/mol), respectively, indicate that high temperature are required to extract high molecular weight ulvan. On more defined fractions from *U. pertusa* and *U. conglobata* ulvans, molecular weights of 9.1×10^5 and 8.2×10^6 g/mol were obtained by sedimentation, respectively, and 1.1×10^6 and 7.7×10^6 g/mol by gel permeation chromatography.[77] Narrow polydispersity indices of 1.5 and 1.3 were also calculated for these ulvans demonstrating narrow molecular weights distributions. Osmometry measurement of *U. "rigida"* ulvan gave molecular weight of 1.7×10^5 g/mol,[78] while that of *E. intestinalis* ulvan determined by light scattering varied between 1.9×10^5 to 5.0×10^5 g/mol depending on the temperature and the nature of the solvent used to extract it.[25] This time, the highest molecular weight was recorded for the polysaccharide fraction extracted at low temperature. It thus appears that molecular weight of ulvan varies according to its origin and to its mode of extraction. However, variations in molecular weights may also reflect different methods and conditions used for their measure. The tendency of ulvan to form microaggregates in dilute solutions in the presence of salts[35] (Lahaye et al.,

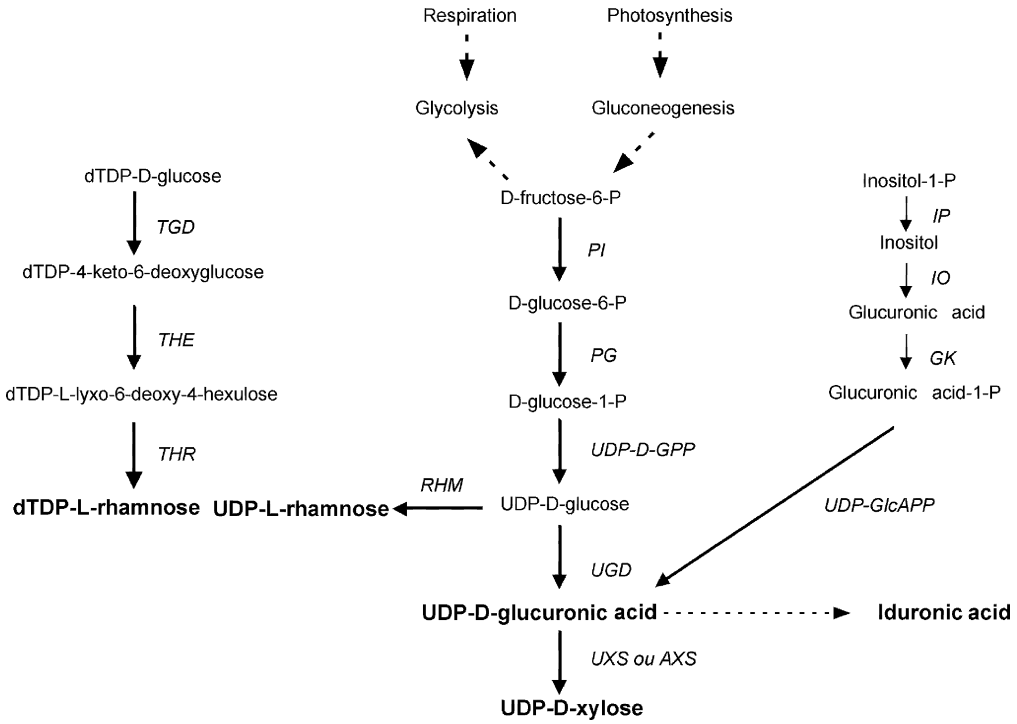

Figure 6. Proposed pathways for the biosynthesis of the main nucleotide sugar precursors of ulvan. Iduronic acid is proposed to be formed at the polymer level by action of parietal epimerase converting glucuronic acid to iduronic acid. Abbreviations: AXS, UDP-D-apiose/UDP-D-xylose synthase; GK, glucuronokinase; IO, inositol oxygenase; IP, inositol-1-phosphatase; P, phosphate; PG, phosphoglucomutase; PI, phosphoglucose isomerase; Rha, rhamnose; RHM, rhamnose synthase composed by a 4,6-dehydratase, a 3,5-epimerase, and a 4-reductase; TDP, thymidin 5′-diphosphate; TGD, dTDP-D-glucose 4,6-dehydratase; THE, dTDP-6-deoxy-D-xylo-4-hexulose 3,5-epimerase; THR, dTDP-6-deoxy-L-lyxo-4-hexulose reductase; UDP, uridine 5′-diphosphate; UDP-D-GPP, UDP-D-glucose pyrophosphorylase; UDP-GlcAPP, UDP-glucuronic acid pyrophosphorylase; UGD, UDP-D-glucose dehydrogenase; UXS, UDP-D-xylose synthase.

unpublished) may largely contribute to the wide disparity in the reported values.

Solution Properties. Ulvan solutions develop low viscosities. The intrinsic viscosity in saline solutions is in the order of 95−285 mL/g for *Ulva* extracts[14,16,21,77] and is lower for *Enteromorpha* extracts, between 24 and 61 mL/g.[14,16,25] A lower viscosity was recorded for ulvan extracted from *U. pertusa* at 120 °C compared to that extracted at 20−90 °C[14] likely due to instability of the polysaccharide at high temperature. Such low viscosity may reflect the polymolecularity of ulvans with high proportions of short-chain polysaccharides and/or highly branched structure as proposed.[53] However, the structure and the branching pattern of the ulvan-lyase fragments characterized so far are not in support of a highly branched polysaccharide.

Ion Binding Properties. Ulvales cell walls bind heavy metal ions[79] and besides glucuronan and probably proteins, ulvan with between 2.8 and 3.77 mequiv charge/g is the main contributor. Cadmium is able to displace calcium in the cell wall of *U. lactuca*,[80] and this is in harmony with the higher affinity of ulvan for this cation than for calcium. pHmetric titration of ulvan from *U. armoricana* showed an affinity for ions in the following order: Al > Cu > Pb > Zn > Cd = Mn > Sr > Mg = Ca (Lahaye et al., unpublished). Fixation of copper (Cu(II)) by *U. rigida* ulvan is proportional to the content of uronic acid, and sulfates participate to the binding at high copper concentrations.[78] The extent of fixation is controlled by the content in iduronic acid[35] likely in relation with its ring flexibility.[81]

Gelling Properties of Ulvan. One particular interesting feature of ulvan is its ability to form gels. McKinnel and Percival[23] were the first to mention the formation of a stiff gel on concentrating an *U. lactuca* ulvan extract. Later on, Haug[82] discovered that boric acid, calcium ions, and a pH between 7.5−8.0 were required to form a weak gel with the ulvan from *U. lactuca*. Such gel was also obtained with ulvan from proliferating *Ulva* spp.[15] (*U. armoricana*) as well as several from other ulvan samples ("sea lettuce", *U. rigida*, *U. rotondata*, and *Enteromorpha* sp., Lahaye, unpublished). Optimal concentrations of boric acid and calcium of 15−33 and ∼7 mM, respectively, and pH 7.5 produced a gel with *U. armoricana* ulvan which, at 1.6% (w/v) concentration, had a storage modulus of about 250 Pa. Higher ion concentrations, higher and lower pH, or Tris and phosphate buffering ions were detrimental to the gel. Ulvan in the sodium form does not form a gel on addition of boric acid at pH 7.5 confirming that calcium or a divalent cation is required for gelation.[48] Gels of increasing elastic modulus were obtained with Cu > Zn > Mn > Ca for *U. armoricana* ulvan in agreement with ulvan affinity for these ions, but no gel was obtained with Mg (Lahaye et al., unpublished).

The mechanism of gel formation is not understood. Haug[82] proposed that very few borate esters are formed with the cis-diol functions of unsulfated rhamnose residues to cross-link ulvan chains (Figure 7). Calcium ions would bridge complexes and/or stabilize the borate esters. Sulfate and carboxylic acid groups were later on proposed to coordinate to Ca(II) and participate to the gel formation. Since the gel is thermoreversible and does not show thermal hysteresis, the interchain "junction zones" in ulvan gel are reversible and involve weak linkages. Thus, direct ulvan cross-links by borate esters are unlikely (Figure 7 **II**).[15,48] Haug[82] indicated that boric acid had no effect on the rotary power of ulvan from *U. lactuca* and concluded that the amount of modifications brought by borate ester complexes were below the detection limit of the technique. Borate complexes were not observed by ^{11}B NMR spectroscopy,[51] a highly sensitive technique to measure the different forms of borate ester complexes.[83,84] Several explanations were

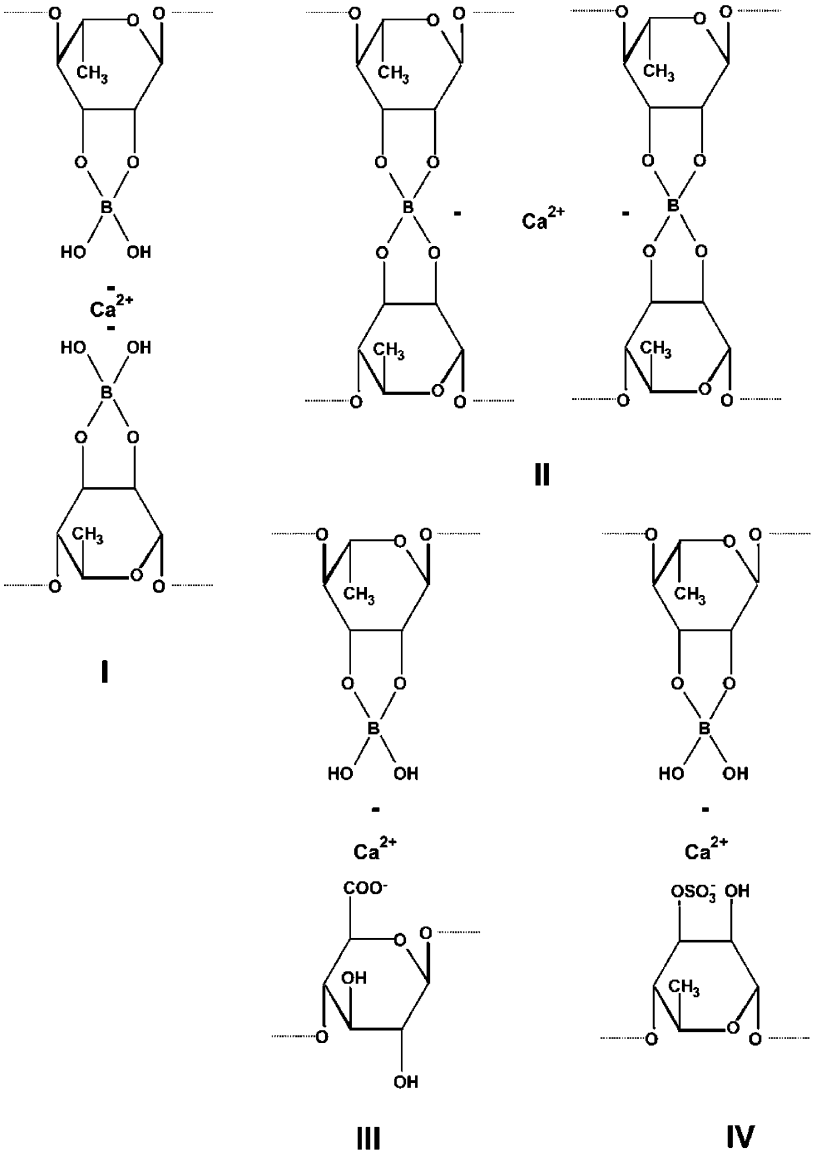

Figure 7. Proposed borate and calcium interactions in the establishment of junction zones leading to ulvan gel. Structures **I** and **II** were proposed by Haug (ref 82), and structures **III** and **IV** were added by Lahaye and Axelos (ref 15). Redrawn with permission from ref 15. Copyright 1993 Elsevier.

proposed: borate complexes are absent, the exchange rate in the equilibrium borate ester−ulvan/free boric acid is too fast at the time scale of NMR spectroscopy, and/or the complexes are formed with boric acid and cannot be distinguished from the free acid. Ulvan chains in the gel state yielded a ^{13}C NMR spectrum similar to that in solution in support of the implication of very few or short-lived transitory "junction zones" between ulvan chains.[51] The role of borate and cations in ulvan gelation is not clear. Haug[82] noted that ulvan dialyzed against a solution of borate at pH 8 then against buffer at pH 8 without borate failed to form a gel with calcium. Thus, ulvan−boric acid/borate complexes are highly unstable unlike others formed with polyols or other polysaccharides.[85−87] Calcium has been reported to help boric acid ester formation with carboxylates[88,89] and with the higher plant rhamnogalacturonan II−borate cross-link formation.[90] Borate, divalent cations, and pH may play important roles in promoting and/or stabilizing ulvan conformations in the formation of "junction zones". At any rate, the ulvan gelation mechanism appears unique among polysaccharide hydrogel and remains to be unraveled.

Conformation. Self-assembling, gel formation ability, physicochemical and biological properties of polysaccharides are bound to specific conformations resulting from their primary structures.[91,92] Although the overall ulvan chains do not appear to show a particular ordered conformation[78] due to the presence of different repeating sequences and different distributions, locally, the regularity could be sufficient to transiently lead to ordered conformations. These in turn, could be at the origin of "junction zones" leading to gel formations or of biological properties. The glycosidic linkage geometry is an important determinant of the overall conformation of regular sequences[92] and according to those encountered in $\mathbf{A_{3s}}$, $\mathbf{U_{3s}}$, $\mathbf{U_{2's,3s}}$, $\mathbf{A_{2g,3s}}$, and $\mathbf{B_{3s}}$, ordered helical conformations can be expected for homogeneous sequences of these repeating units. Molecular models of $\mathbf{A_{3s}}$ and $\mathbf{B_{3s}}$ sequences indicated that one hydrogen bound between the oxygen atoms of the rhamnose sulfate group and the hydroxyl group on C2 of the preceding uronic acid or with the carboxylic oxygen of the following uronic acid can stabilize helical conformations.[35] Extended models of such homogeneous repeating unit sequences predicted left-handed

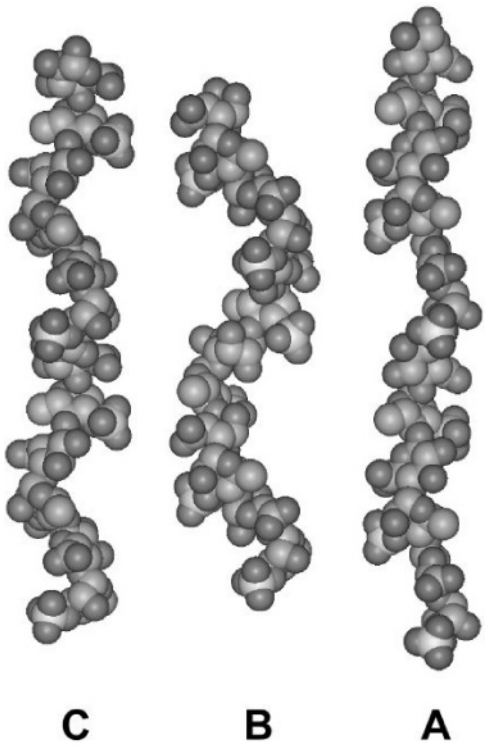

Figure 8. Structural models of ulvan chains composed of $\mathbf{A_{3S}}$ repeating sequences (A), $\mathbf{B_{3S}}$ sequences with iduronic acid in the $^1C_4$ conformation (B), and in the $^2S_0$ conformation (C). Redrawn with permission from ref 35.

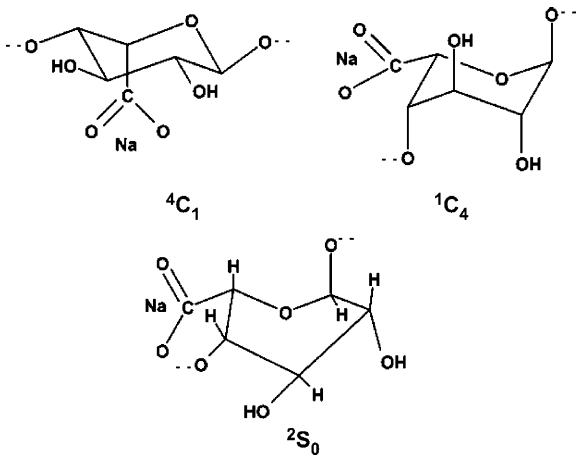

Figure 9. Different chair ($^4C_1$, $^1C_4$) and skewed ($^2S_0$) conformations of iduronic acid.

helices made of 2−5 $\mathbf{A_{3s}}$ dimers per helix period (2−4 helical turns per helix period) yielding a highly extended structure with up to 9.9 Å advance along the helix axis per dimer (Figure 8). For the $\mathbf{B_{3s}}$ segments, the helical conformation would depend on the sugar ring conformation of iduronic acid. Studies on heparin, a mammalian glycosaminoglycan mainly made of disaccharide sequences of alternating, α-1,4-linked 2-L-iduronic acid 2-*O*-sulfate and D-glucosamine *N*,6-disulfate, showed that iduronic acid, sulfated or not, can adopt the $^1C_4$, $^4C_1$ chairs as well as the $^2S_0$ skewed ring conformations (Figure 9) depending on its glycosidic environment, on pH, temperature, and ionic composition of the solvent.[93−98] In the $^1C_4$ conformation, molecular modeling of ulvan $\mathbf{B_{3s}}$ segments predicts two equiprobable 4-fold symmetry left-handed helical conformations with 7.4 or 8.4 Å advance along the helix axis per dimer. For the $^2S_0$ skewed ring conformations, only 1.5-fold symmetry left-handed helices are predicted with 2.5 $\mathbf{B_{3s}}$ unit per helical period and a rise of 8.4 Å per dimer[35] (Figure 8). Other sugars along the ulvan chains may show some ring flexibility according to their substitution and distribution. This may be the case for rhamnose 3-sulfate, which demonstrated different proton chemical shifts and coupling constants according to its distribution in $\mathbf{U_{3s}}$ and $\mathbf{U_{2s,3s}}$ sequences.[57] Such sugar ring flexibilities modulated by the nearby chemical structure and the physicochemical environment are likely to play important roles on cation and borate reactivities and on hydrogen bond formations impacting ulvan chain associations.

What Are Potential Uses for Ulvan? The unique chemical and physicochemical properties of ulvan make this family of polysaccharides attractive candidates for novel functional and biologically active polymers for the food/feed, pharmaceutical, chemical aquaculture, and agriculture domains.

On the basis of the peculiar chemical composition of ulvan, the biomass can be a source of rare sugar precursors for the synthesis of fine chemicals. The sulfated polyaldobiuronan is among the few polysaccharides found in nature built on high amounts of rhamnose. This rare sugar is used in the synthesis of aroma,[99] and its production has been patented from *Monostroma* (Codiolales) containing ulvan-like cell wall polysaccharides.[100] Other pharmaceutical applications based on rhamnosylated saccharides can be expected since L-rhamnose is a fundamental component of the surface antigens of many microorganisms and is specifically recognized by a number of mammalians lectins.[101] Ulvan is also a potential source of iduronic acid, another rare sugar found in mammalian glycosaminoglycans and required in the synthesis of heparin analogs with antithrombotic activities.[102] To date, iduronic acid is synthesized in several steps[103,104] that could be saved by this natural source.

Besides monomers, ulvan oligomers and polymers could find applications related with their biological properties. Different works demonstrate that ulvan and its oligosaccharides have antitumor and immune modulation activities,[105] strain-specific anti-influenza activities,[106] and anticoagulant activities.[107]

The use of rhamnan, rhamnose, and oligomers from desulfated ulvan-like polysaccharides of *Monostroma* was patented[108] for the treatment of gastric ulcer. This property is in line with the ability of ulvan to induce mucin secretion in rat colon and thus to increase the protection of the colonic mucosa.[109] Ulvan has also antioxidant activities and reduces hepatotoxicity against acetaminophen-induced hepatotoxicity in experimental albino rats.[110−112]

Several studies deal more specifically on the dietary contribution of ulvan in "sea lettuce". As member of the cell wall polysaccharides, ulvan is not degraded by human endogeneous enzymes and belongs to the dietary fibers of "sea lettuce".[10] It limits degradation by human colonic flora of the other dietary fiber components in the edible algae[113−115] and thus contributes to the water retention capacity of the fibers.[16,116] Such characteristics are typical of dietary fiber acting as bulking agents and helping in the prevention of pathologies related to intestinal transit dysfunctions.[117] Ulvan may as well modulate lipid metabolism. It limits hyperlipidemy in rats and mice. A decrease of serum high-density lipoprotein cholesterol (HDL-cholesterol) and an increase of low-density lipoprotein cholesterol (LDL-cholesterol) and triglyceride are considered to be significant risk factors in cardiovascular diseases.[118] Ulvan or derived oligosaccharides significantly lowered the level of serum total cholesterol, LDL-cholesterol, and reduced triglyceride, while they increased the levels of serum HDL-cholesterol. Thus, ulvans

can decrease significantly the atherogenic index.[119,120] Although all these studies were carried out in the context of human nutrition, the results apply to the design of functional foods and feed. Enzymatic transformation of Ulvales biomass with cellulase activities could increase both the algal protein availability and digestibility[121] and ulvan degradation to oligosaccharides could be beneficial for stimulating animal defenses against diseases. *U. rigida* ulvan as polymer can stimulate macrophage and contribute to disease resistance in fish.[122] A recent patent describing the particular capacity of ulvan to intercalate into clay was described with applications in animal feed detoxification.[123] This characteristic opens the way for the preparation of new nanocomposites of interest for applications in many different domains.

Interestingly, little work in the literature is related to the agricultural uses of ulvan. Beside compost, the elicitation of defense or growth of ulvan has rarely been addressed. An ulvan extract was shown to elicit defense mechanisms in *Medicago truncatula*, and previous treatment of the plant by ulvan protected it from infection by the pathogenic fungus *Colletrichum trifolii*.[124] Other recent patents concern nitrogen uptake improvement and disease resistance conferred by ulvan.[125,126]

The mechanisms by which ulvan interferes with the different biological systems are yet to be identified. They may be a mixture of specific cell receptors competition and physicochemical properties related to particular ion-exchange properties. The latter are at the basis of the choice of these seaweeds as bioindicators for monitoring coastal water heavy metal pollutions.[127] They could be further exploited to develop ion-exchangers from Ulvales cell walls with particular ion selectivity[128] for industrial effluents depollution or the enrichment of food, feed, or soils with specific trace mineral elements.

The unique gelling properties of ulvan offer potential applications where texture need to be precisely controlled by cations, pH, or temperature. It could be used to design gels able to release entrapped molecules/particles under specific physicochemical conditions.

Conclusion

The recent interest for ulvan arises from its unique physicochemical, rheological, and biological properties that are beginning to be described. Its unusual chemical composition and regular structure combining uronic acids, sulfate groups, and rare sugars, such as rhamnose and iduronic acid, are starting to be unraveled. Further work is required to explore its structural diversity in relation with its functional properties among members of Ulvales and, more generally, among Ulvophyceae. In particular the mechanisms controlling the atypical gelling mechanism remain to be discovered and will undoubtedly contribute to the development of many uses from the abundant renewable green algal biomass.

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