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A practical perspective on ulvan extracted from green algae

Anabela Alves · Rui A. Sousa · Rui L. Reis

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Abstract Researchers have many times turned their attention to nature and biological processes to develop novel technologies and materials. In a medical perspective, nature-based products are believed to be a strategic alternative approach to the use of fully synthetic materials, particularly in the design of medical devices. In the past decades, marine organisms have become the focus of considerable attention as potential sources of valuable materials. The sustainable exploitation and valorisation of natural marine resources constitutes a highly attractive and strategic platform for the development of novel biomaterials, with both economic and environmental benefits. In this context, algae are known to synthesise large quantities of polysaccharides and are well established sources of these particularly interesting molecules, many of which are known for their applicability in the design of biomaterials. Agar, carrageenan and alginates are some of the most known examples, and their uses can range from food to biomedical applications. However, few of the world's available seaweed species are used commercially. Among the three main divisions of macroalgae (Chlorophyta, Phaeophyta and Rhodophyta), the green algae remain largely unexploited in this biomedical arena. While the demand for novel materials and technologies increases, so does the research of unexploited marine green algae including its unique polysaccharide ulvan.

Keywords Green algae · Ulvan · Polysaccharide · Biopolymer · Biomaterial

R. A. Sousa · R. L. Reis
3B's Research Group–Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho,
AvePark, Zona Industrial da Gandra, S. Cláudio do Barco, 4806-909 Caldas das Taipas,
Guimarães, Portugal

A. Alves · R. A. Sousa · R. L. Reis
ICVS/3B's–PT Associated Laboratory,
Braga/Guimarães, Portugal

Present Address:
A. Alves (✉)
3B's Research Group–Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho,
AvePark, Zona Industrial da Gandra, S. Cláudio do Barco, 4806-909 Caldas das Taipas,
Guimarães, Portugal
e-mail: anabela.pinto@dep.uminho.pt

Introduction

The world's oceans are a rich environment containing over 300,000 invertebrates and algal species (as cited in Pomponi 1999). These species survive and live within complex communities and in close association with other organisms. This diversity of living systems and habitats defines the basis of the wide variety of chemical classes typical of marine-derived molecules. Some organisms withdraw their rich chemistry from dietary sources, whilst others synthesise these compounds de novo (Cannell et al. 1998). Molecular diversity represents a vast and valuable chemical library, including saccharides, pigments, phenols or peptides, among others, which, together, are estimated to possess a potential market value of several billion dollars (Pomponi 1999).

In this scenario, marine algae, rich in different compounds of interest, are used in several industrial application contexts totalizing a consumption of 3.5 million tonnes of algae per year worldwide (Jensen 1993). The importance of these organisms and their constituents to humanity has justified intense research work; however, the full potential of algae molecules is yet to be unveiled.

Marine algae can be divided in three main groups—red (Rhodophyceae), brown (Phaeophyceae) and green

(Chlorophyceae)—classified on the basis of their photosynthetic pigments (Barsanti and Gualtieri 2006). These algae also differ on the type of storage material and cell-wall polysaccharides (Barsanti and Gualtieri 2006; Bocanegra et al. 2009). A large fraction of the world commercially exploited polysaccharides are of marine origin, namely alginate, agar and carrageenan (Jensen 1993; Lahaye and Axelos 1993; Michel and Macfarlane 1996). Within this context, green algae are still rather unexploited. Although they have been used in food for many centuries, it was the discovery of an important constituent of green algae, ulvan, which dramatically increased interest in these algae. Focused research on ulvan's characteristics and applicability, therefore, is required in order to grasp the full range of its capabilities and boost the industrial interest in green algae.

Historical outline

Classification of alga has not always been an easy task. Complexity is enhanced when recent genetic studies reveal that *Chloropelta*, *Enteromorpha* and *Ulva* in fact belong to the same genus (Hayden et al. 2003).

As the work with brown and red macroalga evolved to a well-established field of knowledge, the interest in green algae and especially its polysaccharides had a late and bumpy start. An interesting and complex polysaccharide composed of sulphate ester, uronic acids and xylose, rhamnose and glucose residues was identified in the early 1940–1950s. This pioneer work was, however, interrupted for several years due to the Second World War (Brading et al. 1954). In these early studies, Brading and co-workers (1954) already identified a possible structure present in this sulphated polysaccharide as -CH(OH)-CH(O-SO_3Na)-CH(OH)-. However, no evidence was found on what concerns the true position of the sulphate groups on the polysaccharide structure, although it seemed possible that they should be linked to glucose or rhamnose (Brading et al. 1954). This was also one of the first times rhamnose was identified as being part of an algal polysaccharide. In 1962, McKinnell and co-workers (McKinnell and Percival 1962b) found evidence that the sulphate groups were in fact linked to rhamnose. Ten years later, Abdel-Fattah and Edrees (1972) concluded on the heteropolysaccharidic nature of this polysaccharide, composed of particular entities of oligosaccharides of rhamnose and uronic acids composing its backbone, intercalated with sequences involving glucose, arabinose and xylose. The particularly acidic nature of the polysaccharide was assigned to the presence of glucuronic acids (Abdel-Fattah and Edrees 1972). In these early years, researchers were struggling with the identification and characterization of this unusual and complex polysaccharide. Extracts were obtained from different green algae genus and species and were characterised and compared between each other. Despite the fact that different researchers were extracting a polysaccharide from different genus and species of green algae, through diverse extraction methodologies, they all came to similar conclusions and agreed that the water-soluble polysaccharide from green algae was mainly composed of uronic acid and sulphated rhamnose with residues of diverse sugars, like xylose and glucose (Abdel-Fattah and Edrees 1972; Brading et al. 1954; Gosselin et al. 1964; Lahaye and Axelos 1993; McKinnell and Percival 1962a, b; Percival and Wold 1963; Ray and Lahaye 1995b). It was only in 1993 that the designation "ulvan" was proposed (Lahaye and Axelos 1993). It is now generally accepted that ulvan designates a class of sulphated polysaccharides mainly composed of glucuronic acid and sulphated rhamnose (Ray and Lahaye 1995b). The important discovery that iduronic acid was a constituent of the ulvan backbone was only made in 1997 (Quemener et al. 1997).

There is still a long way to go until the true nature of this polysaccharide is unravelled. However, in the last decade, research on ulvan has evolved as the knowledge on its occurrence and physiological function, chemical composition, polysaccharide conformation and properties has improved. This knowledge is now driving research a step forward towards the application development of this polysaccharide.

Physiological function

Marine algae are simpler organisms compared to land plants; however, they produce larger quantities of polysaccharides (Wood 1974). These polysaccharides are strikingly different with those found in higher plants, especially on what concerns the presence of sulphate groups and unusual sugar residues, high content of ionic groups, high water solubility and unique rheological properties (Jensen 1993; Michel and Macfarlane 1996; Popper et al. 2011; Wood 1974). This results in a range of distinctive characteristics and is the basis of their widespread industrial applicability.

In general, green algae are composed of ~11 % protein, ~36 % carbohydrate, ~53 % ashe and are rich in minerals like calcium, iron, phosphorous and chloride (Castro-González et al. 1996). Carbohydrates include cell-wall water-soluble sulphated ulvan, alkali-soluble hemicellulosic β(1,4)-D-glucuronan and β(1,4)-D-glucoxylan and amorphous α-cellulose with xylose residues (Bobin-Dubigeon et al. 1997b; Lahaye et al. 1997; Lahaye and Ray 1996; Ray and Lahaye 1995a, b).

Ulva spp. possess a characteristic blade-shaped frond, two-cell thick, with no tissue differentiation (Bobin-Dubigeon et al. 1997b). However, their cell walls are well organised in layers and rich in polysaccharides (Bobin-Dubigeon et al.

1997b; Popper et al. 2011). Ulvan is mostly homogeneously distributed throughout the frond being more predominant within the intercellular space and in the fibrillar wall (Bobin-Dubigeon et al. 1997b). Within this cell wall moiety, it is suggested that ulvan may be arranged in a bead-like structure, stabilised by cell wall proteins or strong physical interactions (Robic et al. 2009b). It is well known that boron is accumulated in algae, in the form of boric acid in green algae, and it was suggested that complexes with carbohydrates (Chuda et al. 1997). Given that ulvan is able to gel in the presence of boric acid, in a reaction mechanism mediated by calcium ions (Lahaye and Axelos 1993), it is easy to hypothesise that this polysaccharide may be present in green algae cell wall in the form of a gel, cross-linked by boron ions.

As far as the physiological function of ulvan within the cell wall goes, the literature is rich in hypotheses (e.g., Andrieux et al. 1998; Bobin-Dubigeon et al. 1997b; de Reviers and Leproux 1993; Lahaye et al. 1998; Paradossi et al. 1999; Popper et al. 2011; Quemener et al. 1997; Wood 1974). In general, the presence of sulphated polysaccharides can be associated with the organism's adaptation to ionic environments; this is true for marine plants and algae (seawater) as well as for vertebrates (physiological saline serum) (Popper et al. 2011). Therefore, ulvan, as part of the cell wall of green algae, would possess osmotic functions, including a role on ionic balance or on the prevention of algae desiccation due to its highly hygroscopic nature (Paradossi et al. 1999; Wood 1974). On the other hand, ulvan appears to inhibit the activity of cellulase, which indicates a protective role towards cell wall amorphous α-cellulose, protecting it from marine bacterial attack (Andrieux et al. 1998; Bobin-Dubigeon et al. 1997b; Lahaye et al. 1998). This protective function is also related with the fact that ulvan is associated with the low porosity of green algae (Bobin-Dubigeon et al. 1997b; Lahaye et al. 1998). Furthermore, ionic polysaccharides may be involved in mechanical regulation, spore release and adhesivity (de Reviers and Leproux 1993; Popper et al. 2011). The presence of acidic moieties in the ulvan polysaccharidic chain, particularly glucuronic and iduronic acid, may indicate that this polysaccharide is also involved in cell wall cohesion, in parallelism with mammalian glycosaminoglycans rich in uronic acids (Lahaye et al. 1998; Quemener et al. 1997).

Extraction of ulvan from green algae

This section is intended to provide guidance to those interested in extraction of natural products, especially from green algae. Success in working with nature-derived materials is very well summarised by Cannell and co-workers (Cannell et al. 1998): "One should always keep an open mind, expect the unexpected, use as many methods for purification as possible, and save all fractions."

The overall procedure to obtain polysaccharides from green algae can be divided in several different steps:

- Selection, collection, and identification of the raw material;
- Algae stabilisation and grinding;
- Extraction and purification;
- Precipitation; and
- Drying.

When working with nature-derived materials, one should always recognise and consider the inherent variability of extracted molecules from sources of natural origin (Jani et al. 2009); this can be due to varying extraction methodologies, seasonality or algae species and ecophysiology (Abdel-Fattah and Edrees 1972; Devaki et al. 2009; Hernández-Garibay et al. 2010; Lahaye and Axelos 1993; Robic et al. 2008, 2009b, c; Yamamoto 1980). One practical example is the variability of the obtained polysaccharide related with source as reported by Lahaye and Axelos (1993), when comparing the rheological behaviour of a polysaccharide obtained from *Ulva* spp. with one obtained from *Ulva lactuca*.

Selection, collection, and identification of the raw material

The first step in extraction of algae-derived materials involves the selection, collection and identification of the raw material. During these initial stages, one should try to answer some questions:

- What is the rationale for the extraction of a particular polysaccharide?
- What is the most accessible source of algae available suitable for the extraction of such polysaccharide?
- Can a compromise between yield and purity be obtained by appropriately selecting the algae species?
- Is it possible to have access to large quantities of the selected algae species?

The answer to these questions defines the basis of any future extraction procedure and allows the establishment of good criteria for potential industrial applications of ulvan.

In order to obtain the selected algal raw material, it is possible to collect it in loco, culture it or obtain it through specialised enterprises (Table 1). To address this issue, one should keep in mind that besides source dependence, cross-contamination by other organisms, habitat, geography and seasonality are relevant factors that influence and further complicate the work with marine natural products (Cannell et al. 1998; Lahaye and Robic 2007). Furthermore, among the biggest challenges when working with algae is their identification, which often can only be done correctly by

Table 1 Examples of companies specialised in the commercialization of algae

Company	Location	Algae	Culture system	Website
Sinaloa Seafields International Inc.	California, USA	*Ulva* sp.	Algae culture in non-arable land with sea water irrigation	http://www.sinaloa-seafields.com/home/
Atlantic Mariculture Ltd.	Grand Manan Island, Canada	*Ulva* sp.	Local harvest of native sea vegetables	http://organicdulse.com/
Setalg	Pleubian, France	*Ulva lactuca*	Harvest on algae fields	http://www.setalg.com/
Mariculture Technologies International, Inc.	Florida, USA	*Ulva* sp.	Local harvest (wild-collected)	http://www.mariculturetechnology.com/

an expert phycologist (Cannell et al. 1998; Lahaye and Robic 2007).

Algae can be obtained by harvesting natural seaweed beds or by algaculture (Michel and Macfarlane 1996). The availability of green algae allows a sustainable exploitation of this resource (Pomponi 1999). They are often involved in algal blooms with detrimental health and socio-economic impact (Charlier et al. 2007; Lahaye and Axelos 1993; Morand and Briand 1996). These algal blooms occur in nutrient-rich waters, stimulated by human activity in coastal regions (Charlier et al. 2007; Lahaye and Axelos 1993; Morand and Briand 1996). Partial or total utilisation of these algae and particularly of its polysaccharide ulvan would alleviate the effect of accumulation and elimination of these deleterious biomasses. Furthermore, it is easily cultured in (semi-) artificial conditions, both in-the-sea and land-based conditions, alone or in integrated systems (Barsanti and Gualtieri 2006; Bruhn et al. 2011; Matsuo 2004; Msuya and Neori 2008). These facts make green algae easily accessible to research work and application development.

Algae stabilisation and grinding

The first extractions of polysaccharides from green algae usually started with washing of algae prior to thermal drying (Bocanegra et al. 2009). Nowadays, relevant advances in seaweed and hydrocolloid industries reveal other alternatives to algae stabilisation treatments prior to polysaccharide extraction. Robic and co-workers (Robic et al. 2008) have studied the effect of different green algae pre-treatments, including freezing, drying methods, brining and dry salting. These different algae stabilisation treatments have a marked effect on the final yield of extraction and on ulvan's physicochemical traits, including molecular weight and viscosity. Higher yield is obtained for algae brined for 7 weeks; however, higher molecular weight and viscosity is obtained for frozen and freeze-dried algae (Robic et al. 2008). These results emphasise the need for a concise evaluation of the final application of the extracted polysaccharide and the expected cost and efficiency of the extraction procedure. Furthermore, it is important to note the effect of these pre-treatments on the chemical composition of the polysaccharide, in particular due to polysaccharide modification or even degradation (Robic et al. 2008).

Extraction and purification

Before and after extracting the polysaccharide per se, one must be aware of interfering substances that can be co-extracted with the molecule of interest and of other contaminants introduced during extraction, etc. These substances and contaminants can either affect purity of the extract or interfere with biological responses towards the polysaccharide (Cannell et al. 1998).

Removal of photosynthetic pigments (de-colouring) and lipids (de-fatting) may be considered as the first steps of purification. De-colouring and de-fatting can be achieved by different methods, including supercritical extraction, soxhlet extraction or simple immersion in organic solvent (Brading et al. 1954; Cannell et al. 1998; Gosselin et al. 1964; McKinnell and Percival 1962a,b; Percival and Wold 1963; Siddhanta et al. 2001). Being considered a green technology, the use of supercritical fluids avoids or reduces the use of organic solvents. However, this is an expensive technology, and the use of organic solvents is still more effective and widely used, both for de-colouring and de-fatting (Kitada et al. 2008). In this regard, soxhlet extraction is an old but effective technique (Luque de Castro and Garcia-Ayuso 1998). Different organic solvents may be used to remove pigments, including acetone (Brading et al. 1954; McKinnell and Percival 1962a; Percival and Wold 1963; Ray 2006), ethanol (Alves et al. 2010; McKinnell and Percival 1962b; Ray 2006; Xiong et al. 2010), methanol (Gosselin et al. 1964; Ray 2006), petroleum ether (Chattopadhyay et al. 2007; Jani et al. 2009) or chloroform (Jani et al. 2009). However, McKinnell and Percival (1962b) found that the best solvent to remove colouring matter from green algae is 85 % aqueous ethanol. Kitada and co-workers (2008) also found ethanol a more effective solvent to extract pigments, when compared to acetone; furthermore and considering safety issues, ethanol presents lower toxicity compared to acetone. On the other hand, treatment of algae with ethanol prior to polysaccharide extraction increases the total sugar content of the extract and decreases the presence of minor sugars and

glucose on the final polysaccharide extract (Ovodov 1975; Robic et al. 2009b).

Effective extraction of a polysaccharide of natural origin is mainly dependent on its solubility, stability and functional group considerations (Cannell et al. 1998). Being a water-soluble polysaccharide, ulvan can be effectively extracted with water (Alves et al. 2010; Ray and Lahaye 1995b). Some studies report that hot water extraction results in good extraction yields (McKinnell and Percival 1962b; Robic et al. 2009b; Yamamoto 1980). High temperatures, in the range of 80–90 °C, allow the extraction of higher molecular weight polysaccharides (Yamamoto 1980). However, above 100 °C, lower viscosity is detected, when compared with ulvan extracted at 80–90 °C (Yamamoto 1980), which may reflect some instability of the polysaccharide extracted in this range of temperature (Lahaye and Robic 2007). Ulvan can also be successfully extracted with sodium carbonate solution (Brading et al. 1954), calcium chelating agents, such as ammonium oxalate or ethylenediaminetetraacetic acid (Abdel-Fattah and Edrees 1972; Hernández-Garibay et al. 2010; Ray 2006; Robic et al. 2009b) or acidic solutions (Abdel-Fattah and Edrees 1972; Gosselin et al. 1964; Hernández-Garibay et al. 2010; Lahaye et al. 1998; Robic et al. 2009b). The use of calcium chelating agents facilitates ulvan extraction by means of sequestering calcium ions and disrupting chemical bonds formed by ulvan in the presence of calcium ions within the cell wall of green algae (Abdel-Fattah and Edrees 1972; Robic et al. 2009b). On the other hand, decrease of pH of the extraction media will de-stabilise aggregates of ulvan within the cell wall, increasing extraction yield (Robic et al. 2009b). The use of different solvents to extract ulvan will result in extracts with varying physicochemical and biological properties (Abdel-Fattah and Edrees 1972; Hernández-Garibay et al. 2010; Ray 2006; Robic et al. 2009b; Siddhanta et al. 2001). In this regard, it is important to define the objective of the extraction and the final properties expected from the resulting polysaccharide.

Being a cell wall material, common contaminants of this polysaccharide are amino acids and peptides (Brading et al. 1954; Lahaye et al. 1999; McKinnell and Percival 1962b). In fact, direct extraction with hot aqueous solutions will result in an extract mixture of polysaccharides, proteins, polyphenols and pigments (Béress et al. 1993). Methods to remove nitrogenous materials include enzymatic treatment with proteinase K (Alves et al. 2010) or precipitation with trichloroacetic acid (McKinnell and Percival 1962b). Besides nitrogenous material, McKinnell and Percival (1962b) found evidence of contamination by starch-like materials in hot water extracts. However, this starch can be effectively removed with salivary α-amylase (Costa et al. 2012; Love and Percival 1964; McKinnell and Percival 1962b; Percival and Wold 1963). Smaller molecular weight contaminants, including those responsible for odour and also colour can be removed with activated charcoal (Alves et al. 2010; Cannell et al. 1998; Gosselin et al. 1964); hydrogen peroxide can also be used as a de-colouring agent (Yang and Zhang 2009). Further purification may be achieved by other methods, including dialysis or even through final precipitation (Ovodov 1975).

Precipitation and drying

In general, water-soluble polysaccharides are removed from solution by precipitation with organic solvents. In the particular case of ulvan, it precipitates from aqueous solutions with ethanol or acetone, usually four volumes of the chosen organic solvent (Alves et al. 2010; Gosselin et al. 1964; Siddhanta et al. 2001). Ethanol precipitation allows the separation of the polysaccharide from low molecular ethanol-soluble compounds (Béress et al. 1993) and the removal of some pigments (Lahaye 1991); thus it is considered as a purifying step as well (Elboutachfaiti et al. 2011; Ovodov 1975; Tavernier et al. 2008).

Extracted polysaccharide can be dried by different methods. However, Jani and co-workers (2009) highlight the need to apply low temperature or vacuum on drying in order to avoid degradation of the polysaccharide.

Yield

Cell wall carbohydrate content of green algae ranges from 38 to 54 %, and ulvan content may vary between 18 and 29 % (Kaeffer et al. 1999; Lahaye and Robic 2007). Yield of extraction of ulvan varies between 1.2 to 27.5 % and the maximum extraction efficiency is in the range of 70 % (Abdel-Fattah and Edrees 1972; Alves et al. 2010; Chattopadhyay et al. 2007; El-Baky et al. 2009; Gosselin et al. 1964; Hernández-Garibay et al. 2010; McKinnell and Percival 1962b; Paradossi et al. 1999; Percival and Wold 1963; Ray 2006; Ray and Lahaye 1995b; Robic et al. 2008, 2009b, c; Siddhanta et al. 2001; Yamamoto 1980).

In general, a decrease in algae particle size will increase the yield of extraction (Robic et al. 2009b). This is also influenced by factors including algae pre-treatments, solvent used to extract the polysaccharide per se, number, duration and temperature of extractions, purification methodologies, algae species and seasonality (Abdel-Fattah and Edrees 1972; Hernández-Garibay et al. 2010; Lahaye and Robic 2007; Percival and Wold 1963; Robic et al. 2008, 2009b, c; Siddhanta et al. 2001; Yamamoto 1980).

Storage

As polysaccharides, and ulvan in particular, are hydrophilic and readily uptake water from the atmosphere, it is

recommended to store the extracted polysaccharide in a dry environment, ideally on desiccators, to avoid moisture uptake and degradation (Alves et al. 2010; Jani et al. 2009).

Chemical structure and conformation

Ulvan has been identified as being a sulphated single polydisperse heteropolysaccharide composed of variable amounts of uronic acids, including glucuronic and iduronic acids alternating with neutral sugar moieties, such as rhamnose, xylose and glucose residues, connected by α- and β-1→4 bonds (Brading et al. 1954; Lahaye 1998; Lahaye et al. 1997, 1998, 1999; Lahaye and Ray 1996; Percival and Wold 1963; Quemener et al. 1997). This polysaccharide accounts for 18–29 % of the carbohydrate fraction of green algae (Kaeffer et al. 1999). McKinnell and Percival (1962b) provided important insights on the structure of this polysaccharide by demonstrating that the sulphate groups were in fact linked to rhamnose, possibly in position 2; they suggested possible branching of the heteropolysaccharide, and uronic acid residues are pointed as possible end groups, being these attached to position 4 in rhamnose (McKinnell and Percival 1962b). Later on, it was proposed that sulphate groups may be linked to C-2 or C-3 in rhamnose and there is evidence of the presence of some sulphated xylose, with sulphate occurring in position 2 (Percival and Wold 1963; Ray and Lahaye 1995a, b). In 1997, an important study of Quemener and coworkers (1997) showed the presence of iduronic acid within the ulvan backbone. In general, green algae synthesise the same sulphated polysaccharide. However, the particular amount of each monosaccharide residue or the arrangement of the polysaccharide may vary with different factors, including method of extraction (Love and Percival 1964; Percival and Wold 1963; Siddhanta et al. 2001), geographical distribution or species (Lahaye 1998; Lahaye et al. 1999; Siddhanta et al. 2001), maturity, environmental condition and seasonality (Robic et al. 2009c; Wong and Cheung 2000), resulting in varying ulvan structures (Lahaye and Robic 2007). This variability influences the establishment of an accurate ulvan sugar composition, complicated by the difficulty of determining the presence of different characteristic sugars. This is due to the resistance of aldobiuronic acid to acid hydrolysis and due to the labile nature of iduronic acid, easily degraded by the strong acidic conditions needed to hydrolyse ulvan (Conrad 1980; Lahaye and Robic 2007; McKinnell and Percival 1962a; Ovodov 1975). In order to minimise these effects, innovative methods that combine mild acid hydrolysis with enzymatic degradation have been developed and allow an accurate insight into the ulvan sugar composition and the authentication of the presence of iduronic acid within its backbone (Costa et al. 2012; Quemener et al. 1997).

It is now generally agreed (Lahaye 1998; Lahaye et al. 1999; Lahaye and Ray 1996; Michel and Macfarlane 1996) that the backbone of ulvan is mostly composed of α- and β-(1→4)-linked sugar residues, namely of α-1,4- and α-1,2,4-linked L-rhamnose 3-sulphate, with branching at O-2 of rhamnose, β-1,4- and terminally linked D-glucuronic acid and β-1,4-linked D-xylose, partially sulphated on O-2. The major structural units are the β-D-glucuronosyluronic acid-(1,4)-L-rhamnose 3-sulphate dimer (β-D-Glc*p*A-(1→4)-L-Rha*p* 3-sulphate) and α-L-Ido*p*A-(1→4)-α-L-Rha*p* 3-sulphate, also known as ulvanobiuronic acid A and B, respectively (Bobin-Dubigeon et al. 1997a; Lahaye 1998; Lahaye et al. 1997, 1998, 1999; Lahaye and Ray 1996; Quemener et al. 1997). The main difference between these aldobiuronic acids is the presence of glucuronic acid in A, which is replaced by iduronic acid in B (Lahaye et al. 1998). Variations in these main motifs can also be encountered (Lahaye 1998; Lahaye and Robic 2007).

One remarkable feature of ulvan is the presence of rare sugars within its backbone, namely sulphated rhamnose and iduronic acid. Rhamnose is an unusual sugar, usually found in bacteria, plants and being rare in animals, and branching of O-2 of 1,4-linked α-L-rhamnose residue was found only on an exopolysaccharide synthesised by the bacterium *Arthrobacter* sp (Fialho et al. 2008; Lahaye and Ray 1996; Oakes et al. 2010; Popper and Fry 2003; Yapo 2011). The presence of iduronic acid in the ulvan polysaccharidic chain represents another striking characteristic, as this sugar residue has never been identified in algal polysaccharides (Quemener et al. 1997).

Enzymatic degradation

Degradation of polysaccharides occurs through a sequence of abiotic and/or biological reactions. In order to get a sense on the biodegradation of ulvan, knowledge of its enzymatic cleavage becomes essential. In general, polysaccharide enzymatic degradation is carried by polysaccharide hydrolases or polysaccharide lyases (Tavernier et al. 2008). Polysaccharide lyases, in particular, degrade the polysaccharidic chain by β-elimination reactions and are usually isolated from bacteria, algae, gastropods and fungi (Linhardt et al. 1986; Tavernier et al. 2008). Despite this knowledge, only a few enzymes with ulvan lyase activity have been identified so far (Table 2). Generally, the discovery and isolation of novel polysaccharide enzymes is based on the need of enzymatic hydrolysis of polysaccharides as a tool for better understanding its structure (Ovodov 1975).

In this regard and, in order to try to elucidate the chemical structure of ulvan and understand the enzymatic degradation of this polysaccharide, an *endo*-ulvan lyase has been isolated from a marine gram-negative bacterium (Lahaye et al.

Table 2 Summary of identified enzymes with ulvanolytic activity

Enzyme	Origin	Activity	References
Endo-ulvan lyase	Marine gram-negative bacterium	Cleavage of (1→4) linkage between rhamnose 3-sulphate and glucuronic acid	Lahaye et al. (1997); Michaud et al. (2003)
β-Glucuronidase	Land snail	Cleavage of (1→4) linkage between rhamnose 3-sulphate and uronic acid	Quemener et al. (1997)
Ulvan lyase	Bacteria	Cleavage of the osidic bond between ulvanobiuronic acid A and ulvanobiuronic through β-elimination reaction	Elboutachfaiti et al. (2010)
Glucuronan lyase	Filamentous fungi	Acetylated and deacetylated glucuronans in general	Delattre et al. (2006)

1997). These bacteria were selected from slurry rich in decomposing *Ulva*, and their ulvanolytic activity requires the presence of calcium chloride and has optimum activity at pH 9 (Lahaye et al. 1997). De-polymerisation of ulvan is an endo-molecular process and ulvanobiouronic acid A motif (β-D-Glc*p*A-(1→4)-L-Rha*p* 3-sulphate) is cleaved resulting in different saccharides with 4-deoxy-L-*threo*-hex-4-enopyranosyluronic acid at the reducing end (Lahaye et al. 1997; Michaud et al. 2003). The isolation of this lyase permitted new insights into the chemical composition of ulvan.

The same objective was the basis of the work of Quemener and co-workers (1997) who were able to purify a β-glucuronidase from the snail *Helix pomatia* and used it to optimise studies on the different sugar composition of ulvans from various sources. In their study, a chemo-enzymatic method was developed to optimise the release of stable monosaccharides after ulvan degradation and β-glucuronidase was successively used to hydrolyse the resistant disaccharide aldobiuronic acid.

The work of Elboutachfaiti and co-workers (2010) resulted on the successful isolation of an ulvan lyase from a bacterium of the genus *Ochrobactrum*. This particular enzyme cleaves the osidic bond bridging ulvanobiuronic acid A and ulvanobiuronic B, through a β-elimination reaction (Elboutachfaiti et al. 2010).

Another enzyme with ulvan lyase activity was isolated from a fungus prevalent in soils, *Trichoderma* sp, and it was identified as a glucuronan lyase. It is dependent on the presence of some ions, including calcium and magnesium, and is sensitive to temperature. Ulvan degradation by this fungal glucuronan lyase originates low molecular weight polysaccharides (Delattre et al. 2006).

Due to the presence of uronic acids within the ulvan backbone, many authors (Lahaye et al. 1999; Leiro et al. 2007; Quemener et al. 1997) have compared it with mammalian glycosaminoglycans, such as chondroitin sulphate. In this sense, and in a biomedical application context, one can infer about the enzymatic susceptibility of ulvan towards naturally occurring mammalian enzymes that degrade this type of molecule into sulphate residues and monosaccharides, such as β-glucuronidase, hyaluronidase and aryl sulphate (Buermann et al. 1979). Given the evolution of the knowledge of ulvan so far, it should be expected that some of the research focus should now shift towards the understanding of enzymatic degradation of ulvan and the nature of the released products. These studies are especially important when applications such as in medicine, food or pharmaceutics are envisaged. In a particular example, within the context of tissue engineering and regenerative medicine, biodegradability (hydrolytic and/or enzymatic) in physiological conditions is one of the key and crucial properties of biomaterials (Mano et al. 2007).

Properties

The particular composition and conformation of a given polysaccharide constitute the basis of its physicochemical and biological properties, as well as its particular function on the organism (Jiao et al. 2011; Kreisman et al. 2007; Lazaridou et al. 2004). These unique properties, which distinguish each polysaccharide, define the boundaries of their applicability. Recently, Lahaye and Robic (2007) comprehensively reviewed the available literature on ulvan, with particular emphasis on its structure and properties. Therefore, for the sake of simplicity, this section will focus on some of the most studied properties of ulvan, with special emphasis on its molecular weight distribution, ion bonding, ability to gel and cross-link in the presence of ions, as well as an overview of its biological properties.

Molecular weight

The molecular weight of a polysaccharide is strongly influenced by various factors, and different molecular weights have been reported for ulvan polysaccharide, which may vary from 1.5×10^5 to 2×10^6 Da (Paradossi et al. 1999, 2002; Siddhanta et al. 2001; Yamamoto 1980). On the other hand, ulvan exhibits an aggregation tendency, which can affect molecular weight determinations (Paradossi et al. 2002). Presence of contaminants, different molecular weight distributions or the occurrence of varying ulvan species with

variable sugar content and distribution can also influence this property and may explain the polymolecular character of ulvan (Lahaye and Robic 2007).

Nevertheless, it is agreed that ulvan is composed of two major macromolecular populations, identified as a high molecular weight fraction (5×10^5 to 8×10^5 Da) and a medium molecular weight fraction (1.5×10^5 to 2×10^5 Da), being the high molecular weight fraction the most abundant and the one with higher viscosity (Costa et al. 2012; Robic et al. 2008, 2009a).

Selective ion binding

Green algae are well known for their ability to bind heavy metals, through covalent, electrostatic or redox reactions, removing them from contaminated waters (Bocanegra et al. 2009; Schijf and Ebling 2010; Webster and Gadd 1996). This ability is reported to be associated with the anionic polysaccharide ulvan, present in the cell walls of green algae, which are rich in functional groups with oxygen, including sulphate or hydroxyl groups, as potential metal binding sites (Andrade et al. 2004; Bocanegra et al. 2009; Schijf and Ebling 2010; Webster and Gadd 1996; Webster et al. 1997). In this regard, ulvan demonstrates different affinities towards various ions, such as Al>Cu>Pb>Zn>Cd=Mn>Sr>Mg=Ca (Lahaye and Robic 2007). In the particular case of copper (II) ion, both the uronic acids and sulphate groups of ulvan participate in the fixation of this ion (Paradossi et al. 1999, 2002).

Solution properties and ulvan gelation

Ulvan is soluble in water and its solubility may be enhanced with temperature (Warrand 2006). However, it cannot markedly thicken aqueous solutions and forms weak gels in deionized water, susceptible to pH and ions, with low intrinsic viscosity (Lahaye and Axelos 1993; Lahaye and Jegou 1993; Robic et al. 2009a).

When in aqueous solution, ulvan tends to arrange in a bead-like structure, partially linked by filaments (Robic et al. 2009a). This peculiar behaviour is explained by a localised hydrophobic character present within this charged polysaccharide, mostly related with the presence of hydrophobic methyl groups of rhamnose (Robic et al. 2009a). As water can be considered as a poor solvent for this polysaccharide, this may explain the low viscosities observed for aqueous ulvan solutions (Robic et al. 2009a, b). Decrease of pH towards acidic moieties forces ulvan beads to disperse into an isolated form (Robic et al. 2009a). Increasing solution pH promotes ionic interactions between carbonyl and sulphate groups, resulting in aggregation of ulvan beads (Robic et al. 2009a). The same behaviour is observed in saline ulvan solutions or in the presence of boron or copper, where polysaccharide self-associations are enhanced (Lahaye 2001; Paradossi et al. 1999, 2002; Robic et al. 2009a).

In general, ulvan gels are formed in the presence of boric acid and divalent cations, or copper, at slightly alkaline moieties (Lahaye 2001; Lahaye and Axelos 1993; Lahaye et al. 1998; Lahaye and Robic 2007; Paradossi et al. 1999; Robic et al. 2009a; Toskas et al. 2011). The important study of Lahaye and Axelos (1993) determined the gelation kinetics of ulvan in the presence of both boron and calcium ions, which led to the conclusion that gelation of this polysaccharide is time- and pH-dependent. In an appropriate ionic milieu, this polysaccharide forms a gel and the reaction mechanism is thought to involve the cross-linking between ulvan and boron, mediated by calcium ions (Lahaye and Axelos 1993; Lahaye et al. 1998; Toskas et al. 2011). A mechanism of gelation of ulvan in the presence of boron and calcium was proposed to require the presence of free *cis*-hydroxyls, involving rhamnose or uronic acids (Lahaye and Axelos 1993). However, further research on this matter has revealed that this scenario does not truly represent the gelation reaction mechanism. The high sulphation degree of ulvan, mostly localised in rhamnose, may make cross-linking difficult, which suggests that this particular sugar residue may not be involved in the gel formation through boron interactions (Lahaye et al. 1998). On the other hand, no borate complex was detected, neither involving glucuronic acid nor iduronic acid (Lahaye et al. 1998). Therefore, the particular gelation mechanism of ulvan in the presence of boron is yet to be determined and may involve minor sugars also present within the ulvan backbone (Lahaye et al. 1998).

In agreement with the involvement of ulvan in the ability of green algae to bind metal ions, Paradossi and co-workers (1999) found an affinity of this polysaccharide towards copper (II), and Lahaye and co-workers (as cited in Lahaye and Robic 2007) were able to induce gelation of ulvan in the presence of copper, zinc, magnesium and calcium.

The peculiar gelation behaviour of ulvan may result from the aggregation of ulvan bead-like structures through hydrophilic moieties present within this polysaccharide (Robic et al. 2009a). Nevertheless, the viscosity of polysaccharide solutions and gel-forming ability are highly dependent on the chemical composition of the polysaccharide itself (Robic et al. 2009c). In the particular case of ulvan, the high uronic acid content seems to affect the viscosity of ulvan solutions and the ability to form gel (Siddhanta et al. 2001). In fact, gel formation depends on intra- and inter-molecular cross-links, which are hampered by highly negative groups, including carboxylic acids (like uronic acids), sulphate groups and/or methyl groups (in rhamnose) (Lahaye 2001). An example of the striking effect of sulphation degree on the gelation ability of a polysaccharide is carrageenan. The three main types of carrageenan are kappa, iota and lambda and

the main difference between these polysaccharides is their increasing sulphate content (Williams 2009). Both kappa and iota carrageenan adopt an ordered double helical structure and form thermoreversible gels through coil-helix conformational transition (Williams 2009). However, the higher sulphation degree of lambda carrageenan impedes helix formation and consequently gel formation, possibly through steric hindrance or electrostatic repulsion (Williams 2009). Despite the results and hypotheses gathered concerning the gelation of ulvan, this is still an open field of research and the mechanisms of gelation are yet to be fully understood.

Biological properties

Marine algae constitute a rich and largely available source of sulphated polysaccharides with peculiar structures associated with different biological activities (Jiao et al. 2011; Wijesekara et al. 2011; Yang and Zhang 2009). These have been the focus of intense research and different structure-function studies reveal that these are many times correlated with the degree of sulphation of these polysaccharides (Jiao et al. 2011; Wijesekara et al. 2011).

In general, polysaccharides are regarded as non-cytotoxic polymers of natural origin (Jani et al. 2009). Ulvan, in particular, already has been studied and its toxicological effects revealed (Alves et al., unpublished). It was characterised in terms of its biological performance, evaluated by means of in vitro cytotoxicity assays, and it has been demonstrated that this polysaccharide is cytocompatible and is considered non-toxic in the range of concentrations studied (Alves et al., unpublished). Furthermore, its cellular effect was similar to hyaluronic acid, used as a control in their studies (Alves et al., unpublished). However, a change in the toxicity of this polysaccharide towards colonic epithelial cells was detected for modified ulvans, with low content or reduced uronic acids or de-sulphated ulvans (Kaeffer et al. 1999).

Ulvan has been described as a heparinoid agent, meaning that it possesses biological activity similar to that of heparin as a potent anti-coagulant (El-Baky et al. 2009; Harada and Maeda 1998; Mao et al. 2006). It also has been found that this polysaccharide is a potent anti-viral agent, particularly against influenza virus, both human and avian, and Herpes Simplex Virus 1 (El-Baky et al. 2009; Kaeffer et al. 1999). Furthermore, it has anti-hyperlipidemic properties, and both ulvan and its low molecular weight oligosaccharides demonstrate an effect on lipid metabolism, limiting hyperlipidemy (Bocanegra et al. 2009; Pengzhan et al. 2003a, b; Sathivel et al. 2008). Its anti-peroxidative and anti-hyperlipidemic properties have shown to exert a protective effect over the liver of rats exposed to a hepatitis-inducing toxin (Devaki et al. 2009; Sathivel et al. 2008). Anti-oxidant activity was additionally recognised in ulvan, namely scavenging activity towards superoxide and hydroxyl radicals, metal chelating activity and reducing power (Costa et al. 2010; El-Baky et al. 2009; Kuda and Ikemori 2009; Qi et al. 2005a, b, 2010). This trait is influenced by the molecular weight of the polysaccharide and its oligosaccharides, as well as by the sulphate content of ulvan and its derivatives (Qi et al. 2005a, b, 2010). Chemical modifications of the natural polysaccharide, such as acetylation and benzoylation described by Qi et al (2006, 2010) may enhance this ability. Another important feature of ulvan is its immunostimulating ability, comparable to other algal polysaccharides (Castro et al. 2004, 2006; Leiro et al. 2007). It has been reported to induce respiratory burst in flatfish and mammalian phagocytes (Castro et al. 2004, 2006; Leiro et al. 2007; van Rooijen and Sanders 1997). Phagocyte activation is a key process in the host defence against microorganisms, and it is mostly related with the anti-oxidant properties already recognised in this polysaccharide (Castro et al. 2004, 2006; De la Fuente et al. 2011; Leiro et al. 2007). This activity is strongly influenced by the presence of sulphate groups as well as by exposure time and concentration and may occur through interaction of the polysaccharide with cell surface receptors (Castro et al. 2004, 2006; De la Fuente et al. 2011; Leiro et al. 2007). Ulvan can also mediate intestinal epithelial growth and take part on the repair of wounds by stabilising and promoting binding of relevant growth factors to intestinal cells (Warrand 2006). Ulvan has demonstrated anti-proliferative activities against human cancer cells, in a dose-dependent manner, particularly for breast adenocarcinoma cells (El-Baky et al. 2009). This may be correlated with its sulphate content and richness in uronic acids (El-Baky et al. 2009).

As mentioned before, many of the biological properties of ulvan are strongly influenced by its degree of sulphation. However, they can also be related to the ubiquitous presence of rhamnose within the backbone of this algal polysaccharide (Andrès et al. 2006). In general, rhamnose-rich polysaccharides possess anti-inflammatory properties, reduce bacterial adhesion to the skin, protect skin from UV-induced and age-related damage and stimulate cell proliferation and collagen biosynthesis (Andrès et al. 2006; Faury et al. 2011). In fact, skin keratinocytes and fibroblasts possess lectins that recognise rhamnose moieties present within a polysaccharide (Andrès et al. 2006; Faury et al. 2011). These peculiarities of this type of polysaccharides have generated a unique interest for the treatment of skin pathologies, particularly the ones related with age and its effects (Andrès et al. 2006; Faury et al. 2011).

Polysaccharides of marine origin are often associated with important biological activities, which are affected by different factors, including molecular weight, chemical composition and chain conformation (Yang and Zhang 2009). Ulvan is no exception and, as it happens with many

other polysaccharides, these properties justify the applicational interest on this green algal polysaccharide.

Applications

The interest in marine algae, as sources of unique polysaccharides with novel structures and interesting biological activities for innovative potential applications, is increasing. These include food, pharmaceutical and medical industries as well as microbiologican and biotechnological applications (Bocanegra et al. 2009). However, few of the world's available algae species are used commercially. Among the three main divisions of macroalgae (Chlorophyta, Phaeophyta and Rhodophyta), green algae remain largely unexploited in these areas. Gosselin and co-workers (1964) noticed, in the early 1960s, the discrepancy in knowledge of green algae polysaccharides when compared to brown and red algae. Almost half a century later, this is still a quite valid consideration. Although knowledge has evolved, green algae are still a rather underexploited biomass. Their polysaccharides remain in the field of possibilities, against, for instance alginate and carrageenan or agar. These are well established and worldwide-accepted polysaccharides for diverse applications, ranging from the food industry to biomedical field (Bocanegra et al. 2009; Ertesvag and Valla 1998; Jensen 1993; Renn 1984).

Ulvan from different sources is demonstrating ubiquitous potential to be successfully used in various applications. In this section, the applicability of ulvan as a polysaccharide will be discussed, although one should bear in mind that ulvan oligosaccharides also demonstrate interesting properties that may justify more research and development of future applications, particularly those related to its biological properties (Courtois 2009; Pengzhan et al. 2003a; Qi et al. 2005b; Zhang et al. 2008).

Active agent for pharmaceutical applications

A review of algae applicability focused on pharmaceutical and medical applications was written by Albertus Smit (2004), who notes the ecological significance and potential of algae: "Phycologists may be surprised to discover how frequently seaweed natural products are discussed in medicine".

In ancient times, green algae were used to treat different pathologies, including hyperlipidemia and urinary diseases (Pengzhan et al. 2003a). Nowadays the medicinal interest in green algae is centred in its polysaccharidic part, particularly ulvan, to be used as a therapeutic active agent.

The presence of glucuronic and iduronic acids makes ulvan a very special polysaccharide. This fact gains importance as we think of this polysaccharide for pharmaceutical and biomedical applications. Both these sugar residues are important constituents of mammalian glycosaminoglycans, including heparin and chondroitin sulphates (Lahaye et al. 1999; Leiro et al. 2007; Quemener et al. 1997). Another remarkable property of ulvan is its sulphation degree. Sulphate groups have long been associated with different biological activities (Jiao et al. 2011). In fact, sulphated polysaccharides are abundant within animal cells and participate in cell recognition, adhesion or regulation of receptor functions (Leiro et al. 2007; Pengzhan et al. 2003b).

The use of ulvan as a strategic alternative to various synthetic or animal bioactive agents would take advantage of its algal origin, together with high availability and low expected production costs, low cytotoxicity and broad spectrum of biological activities. It could be applied as an antiviral agent (El-Baky et al. 2009; Kaeffer et al. 1999), antioxidant (Costa et al. 2010; Kuda and Ikemori 2009; Qi et al. 2005a, b, 2006, 2010), as an anti-coagulant alternative to heparin (El-Baky et al. 2009; Harada and Maeda 1998; Mao et al. 2006), anti-hyperlipidemic (Bocanegra et al. 2009; Devaki et al. 2009; Pengzhan et al. 2003a, b; Sathivel et al. 2008) or of its anti-proliferative activity towards cancer cells (El-Baky et al. 2009), or for therapy for diseases where the immune system is impaired (Castro et al. 2004, 2006; Leiro et al. 2007). Furthermore, due to its similarity with mammalian glycosaminoglycans, it could be exploited as a pharmaceutical where the delivery of glycosaminoglycans is needed, such as for the treatment of musculoskeletal disorders (Ghisalberti 2010; Larraz et al. 2007). On the other hand, rhamnose moieties ubiquitous in the ulvan backbone, as mentioned above, may be the basis for its use for the treatment of skin pathologies, particularly the ones related with age and its effects (Andrès et al. 2006; Faury et al. 2011). Massarelli et al (2007) have studied the interaction of ulvan with hepatocyte lectins and found that the presence of xylose within the backbone of this polysaccharide mediates the interaction with these membrane receptors. As ulvan is readily recognised by hepatocyte membrane receptors, it could be used as a biomaterial for diagnostic or therapeutic purposes (Massarelli et al. 2007). Moreover, and taking advantage of the ability of ulvan to complex with metal ions (Lahaye and Robic 2007), it can find applications where the removal of these ions from the body is required or to warrant the presence of ions when they are needed. For example, it can be used in the therapy for metal poisoning or even be used as part of drugs relevant for targeted radioactive treatment of tumours, as already proposed for carrageenan (Khotimchenko et al. 2010). Ulvan's biological activities and possible applicative scenario are summarised in Table 3.

Medical devices

Side by side with its biological properties and potential pharmaceutical relevance, one can think of ulvan for

Table 3 Summary of relevant biological activities associated with ulvan and its potential strategic application in a pharmaceutical context, according to diverse studies reported in the literature

Biological activity	Strategic possible applications	References
Anti-viral	Treatment of viral infections, particularly influenza and HSV-1	El-Baky et al.(2009); Kaeffer et al. (1999); Muto et al. (1992)
Anti-oxidant	Prevention of oxidative stress and be used as a protective drug for several pathologies, including age-related or cancer	Costa et al. (2010); Kuda and Ikemori (2009); Qi et al. (2005a, b, 2006, 2010); Daniels (2004a, b)
Anti-coagulant	Surrogate of heparin	El-Baky et al. (2009); Harada and Maeda (1998); Mao et al. (2006); Maeda et al. (1992); Daniels (2004a, b)
Anti-hyperlipidemic	Regulation of lipid metabolism	Bocanegra et al. (2009); Devaki et al. (2009); Pengzhan et al. (2003a, b); Sathivel et al. (2008); Daniels (2004a, b)
Immunostimulating	Therapy for diseases where the immune system is impaired	Castro et al. (2004, 2006); Leiro et al. (2007); Daniels (2004a)
Anti-proliferative towards cancer cells	Agent for inhibition of cancer cells proliferation	El-Baky et al. (2009)

biomedical applications, in particular for the production of medical devices. In this regard, the technological development of ulvan is still in the field of possibilities and mainly focused on its applicability as a biomaterial for tissue engineering and regenerative medicine. Within the context of tissue engineering and regenerative medicine, the main objective is to guide cells into forming a functional living tissue (Mano et al. 2007). A common strategy involves the use of biodegradable scaffolds that provide structure and support cell adhesion, differentiation and proliferation (Mano et al. 2007). Depending on the target tissue, one is trying to repair, regenerate or substitute polysaccharide-based systems can assume different forms, with different functionalities, ranging from nano-particulate structures to complex 3-D scaffolds, passing through smart systems. Materials of natural origin present the additional advantage of possessing a variety of distinctive biochemical cues that may enhance and define their applicability in a biomedical context (Barbosa et al. 2005; Mano et al. 2007; Oliveira and Reis 2011). The promise that tissue engineering and regenerative medicine holds is becoming more realistic as significant milestones are achieved, particularly by the approval and commercialization of different developed systems based on natural polymers. Examples of commercial medical devices include Colla-Guide™ and Novocart 3D Autologous Chondrocyte Transplantation, based on collagen, which are used for guided tissue regeneration and cartilage engineering, respectively, and alginate and chitosan wound dressings (Tegagen™ Alginate Dressing, Sorbsan® and ChitoFlex®).

In this area, as it happens in a general applicative overview, when one thinks about polysaccharides of marine origin, both research and market are generally based on chitin and chitosan (Correlo et al. 2010; Cruz et al. 2008; Grenha et al. 2009; Santos et al. 2007; Silva et al. 2011), carrageenan (Grenha et al. 2009; Pereira et al. 2009; Popa et al. 2011; Santo et al. 2009) and alginate (Bernhardt et al. 2009; Popa et al. 2011) (Costa-Pinto et al. 2011; d' Ayala et al. 2008; Mano et al. 2007; Oliveira and Reis 2011).

The use of ulvan as a medical device, for applications such as tissue engineering and regenerative medicine, is at its early stage, particularly on what concerns polysaccharide modification and processing and biomaterial design. Nevertheless, diverse ulvan structures for different end applications have been so far developed and are already reported in the literature. These include nano-fibres (Toskas et al. 2011), membranes (Alves et al. 2012b), particles (Alves et al. 2012a), hydrogels (Morelli and Chiellini 2010) and 3D porous structures (Alves et al., unpublished). An overview of these structures is presented in Table 4. Proposed applications include drug delivery, wound dressing or bone tissue engineering (Alves et al. 2012a, b; Morelli and Chiellini 2010; Toskas et al. 2011).

Personal care products

The array of personal care products is quite broad and there is a tendency towards the use of products of natural origin, including algae extracts (Barsanti and Gualtieri 2006; Kim et al. 2008; Weisberg and Baumann 2009). The use of ulvan in this particular industry is poorly described, being mostly limited to patents that illustrate and claim the use of ulvan or a water extract from green algae, in this field (Blin 2007; Briand 1991; Demais et al. 2006). However, it is easy to envision the potential applicability of this polysaccharide in personal care products, especially if one considers its described biological properties. First of all, ulvan presents

Table 4 Structures based on ulvan developed for biomedical applications, including tissue engineering and regenerative medicine

Structure	Processing methodology	References
Nano-fibres	Electro-spinning of a blend solution based on ulvan and poly(vinyl alcohol)	Toskas et al. (2011)
Membranes	Modification of ulvan by chemical cross-linking with butanediol diglycidyl ether followed by solvent casting	Alves et al. (2012b)
Particles	Extrusion-dripping method to form ulvan particles by electrostatic interaction with a polycation (chitosan)	Alves et al. (2012a)
Hydrogels	Modification of ulvan with methacryloyl groups followed by photopolymerization	Morelli and Chiellini (2010)
3-D porous structures	Freeze-drying of a solution prepared with ulvan cross-linked with butanediol diglycidyl ether	Alves et al. (unpublished)

similarities with glycosaminoglycans, like chondroitin and dermatan sulphate, ubiquitous in skin tissue (Baumann and Saghari 2009; Lahaye et al. 1999; Leiro et al. 2007; Quemener et al. 1997). Furthermore, being a polysaccharide rich in rhamnose moieties, it may induce cell proliferation and collagen biosynthesis (Andrès et al. 2006; Faury et al. 2011). On the other hand, it has proven anti-oxidant properties and high hydration ability (Bocanegra et al. 2009; Costa et al. 2010; Kuda and Ikemori 2009; Qi et al. 2005a, b, 2006, 2010). In fact, the presence of glucuronic acid within the ulvan chain confers moisturising properties to this polysaccharide, important in protecting skin and preventing damage by exposure to dry environments (Kim et al. 2008). These particularities reinforce ulvan's value for cosmetic applications.

Food industry

Green algae have been present in the diet of humans since ancient times and are considered as food and an alternative and rich source of vegetables (Bocanegra et al. 2009; Castro-Gonzalez et al. 1996; Jensen 1993; Wong and Cheung 2000). They are traditionally consumed in Asia and are also approved for consumption in Europe, namely in France (Bocanegra et al. 2009; Castro-Gonzalez et al. 1996; Lahaye et al. 1994; Pengzhan et al. 2003a; Wong and Cheung 2000). In fact, the consumption of Ulva poses no threat to human health (Andrieux et al. 1998). These algae are rich in soluble and insoluble dietary fibres and important minerals, as well as vitamins, polysaccharides, chlorophyll and protein, and low lipid content (Bocanegra et al. 2009; Ortiz et al. 2006; Pengzhan et al. 2003a; Wong and Cheung 2000). In fact, nutritional interest in algae resides on their wealth on vitamins, oligoelements, minerals and dietary fibres (Lahaye and Jegou 1993; Ortiz et al. 2006). On the other hand, algae are considered as low-energy food due to their low lipid content and the fact that their carbohydrates are resistant to digestion and fermentation (Andrieux et al. 1998; Bobin-Dubigeon et al. 1997a; Bocanegra et al. 2009; Lahaye 1991; Michel and Macfarlane 1996). Besides consumption of algae, one can make use of their constituents for food-related applications. The majority of algal polysaccharides, including ulvan, are resistant to endogenous human digestive enzymes (Lahaye 1991; Michel and Macfarlane 1996; Wood 1974), and for this reason, they can be considered as good sources of dietary fibres (Bocanegra et al. 2009; Lahaye 1991). These fibres are present in algae in larger quantities compared to land plants and can be divided into water soluble and insoluble fibres (Andrieux et al. 1998; Lahaye 1991; Lahaye and Jegou 1993; Wong and Cheung 2000). Ulvan is recognised as a soluble dietary fibre (Andrieux et al. 1998; Bobin-Dubigeon et al. 1997a; Lahaye 1991), and it is resistant to hydrolysis by digestive enzymes. Its digestive fate has been proposed to be mostly related with de-polimerization and fermentation by the large intestinal bacteria (Michel and Macfarlane 1996). However, ulvan demonstrates low fermentability and is poorly degraded by faecal bacteria (Andrieux et al. 1998; Bobin-Dubigeon et al. 1997a). This trait is strongly influenced by its characteristic chemical structure, with little influence of sulphate groups on the resistance of this polysaccharide to bacterial degradation (Bobin-Dubigeon et al. 1997a). In fact, its resistance to degradation is maintained after the removal of sulphate groups or oxidation of uronic acid and can be associated with the absence of ulvan-specific de-polymerases in colonic microflora (Durand et al. 1997; Ray and Lahaye 1995a). This is in accordance with the fact that different bacteria present in human flora do not use ulvan as a source of carbohydrates (Rochet and Bernalier 1997). Even though ulvan is poorly fermented, it induces positive effects on the metabolism of colonic microflora, including regulation of the activity of the enzymes β-glucuronidase and β-glucosidase (Andrieux et al. 1998) and induction of intestinal mucin secretion (Barcelo et al. 2000). Interestingly, it appears that sulphate groups are not the main responsible for some of these effects (Barcelo et al. 2000; Bobin-Dubigeon et al. 1997a; Ray and Lahaye 1995a).

Dietary fibres possess many interesting properties which render them well-established additives for food industry applications, as thickeners, stabilisers, emulsifiers or as bulking or gelling agents (Bocanegra et al. 2009; Wood 1974). In general, algae dietary fibres, including ulvan, have remarkable hydration ability, form solutions with different viscosities, and are able to interact with relevant biological molecules, including cholesterol, and provide bulk to faeces (Bocanegra et al. 2009). Furthermore, ulvan can be used in this particular industrial niche for its anti-oxidant properties, preventing food deterioration (Wijesekara et al. 2011).

Other applications

Besides pharmaceutical, biomedical or food-related applications, ulvan has been studied and can be applied in many other areas, ranging from agriculture to more technical usages.

Plant pathogens pose an important threat to agricultural production. In this regard, elicitors of plant defence mechanisms represent a powerful alternative to pesticides (Mejía-Teniente et al. 2010). A good elicitor triggers defence responses and ensures protection to different diseases without disturbing the organism's primary metabolism (Cluzet et al. 2004).

Ulvan has been demonstrated to activate signalling pathways of intracellular plant defence and exerted a protective effect against plant diseases, proving its potential applicability as an environmental friendly plant pathogens' control (Araújo et al. 2008; Briand et al. 2005; Cluzet et al. 2004; Freitas and Stadnik 2012; Jaulneau et al. 2010; Montealegre et al. 2010; Paulert et al. 2009). In fact, pre-treatment of plants with ulvan induces plant resistance and reduces the impact and severity of fungal diseases; however and despite this effect on the plant itself, ulvan does not impede mycelia growth (Araújo et al. 2008; Montealegre et al. 2010; Paulert et al. 2009). This activity may be associated with its sulphate content as well as with the presence of rhamnose and uronic acid in its composition, acting through the jasmonic acid pathway (Jaulneau et al. 2010). In a more technical application of this polysaccharide, Castro et al. (2008) have used ulvan to purify a neutrophil fish mieloperoxidase (Castro et al. 2008). This particular application of ulvan is based on its anionic nature and on the fact that this polysaccharide resembles some glycosaminoglycans, enabling the binding of the enzyme via electrostatic interactions and its isolation and purification by affinity chromatography (Castro et al. 2008). Furthermore, being largely composed of rhamnose, ulvan can be considered a good and cheap raw material to obtain this valuable monosaccharide (Takemura et al. 1986).

Relevant patents

The increasing demand for novel polymers side by side with the sustainable exploitation of natural resources is forcing the focus of research towards polymers of natural origin, particularly from marine origin. This mining of the ocean's resources is the basis of different emergent industries based

Table 5 Summary of patents related to ulvan or its derivatives

Product based on/including	Application	Priority	Patent number
Water extract from green algae	Source of L-rhamnose	1986	US 4758283 (Takemura et al. 1986)
Water extract from green algae	Active agent for anti-viral drugs	1987	US 5089481 (Muto et al. 1992)
Water extract from green algae	General use for cosmetic, pharmaceutical, food or agricultural applications	1989	WO 91/07946 (Briand 1991)
Water extract from green algae	Agent with anti-coagulant activity	1990	EP 0475383 (Maeda et al. 1992)
Water extract from green algae	Therapeutic agent for treatment of cardiovascular pathologies	2004	US 2004/0170645 (Daniels 2004a)
Ulvan	Elicitor of plant defence mechanisms for agricultural applications	2004	WO 2005/094588 (Briand et al. 2005)
Ulvan	Use of ulvan as interspacing component to prepare an interspersed clay for cosmetic, pharmaceutical, food or packaging applications	2004	WO 2006/030075 (Demais et al. 2006)
Water extract from green algae	Pharmacological agent for therapy for neuropathies associated with diabetes and preservation of renal function and vasculopathies	2004	WO 2004/103280 (Daniels 2004b)
Ulvan	Modification of ulvan and use of the resulting product as a surfactant	2005	WO 2007/045795 (Ranson et al. 2007)
Ulvan	Cosmetic composition, making-up or caring for skin and lips	2005	WO 2007/007294 (Blin 2007)
Ulvan	Cleansing and detoxifying agent composed of alginate and ulvan	2007	US 2009/0060942 (O'Mara and O'Mara 2009)
Water extract from green algae	Agent for topical application for the treatment of hot flashes	2008	WO 2009/142745 (France 2009)

on the unique properties of molecules of marine origin. Examples of successful enterprises include Porifarma B. V. (The Netherlands), BioTechMarine (France) and Seanergy (Faroe Islands), among others. In the particular case of ulvan, the interest in this polysaccharide is being revealed by the increasing number of patents focused on the different possibilities of application development based on this polysaccharide, ranging from cosmetics, food, agricultural, pharmaceutical to more technical applications. A resume of this intellectual property is disclosed in Table 5.

Outlook and perspectives

The interest on molecules of marine origin is not recent and has always attracted attention of visionary entrepreneurs for innovative industrial applications. Within this context and given the evolution and advances of polysaccharides of marine origin of different sources, it is surprising to see the limited evolution of green algae polysaccharides. This is particularly striking if one considers that the study of green algae polysaccharides is being documented since the beginning of the twentieth century. A possible justification for this impaired development may reside on the peculiar and complex structure of ulvan, only completely unravelled in 1997 with the definite confirmation of the presence of iduronic acid within the ulvan backbone (Quemener et al. 1997). It was only in 2009 that Robic and co-workers shed some light on the abnormal behaviour of this polysaccharide in aqueous solutions (Robic et al. 2009a). This knowledge has strong impact on the future application development of this polysaccharide. Since the early 1990s, research focused on ulvan has been increasing, with a particular focus on its structure and composition, as well as on its properties. Interest on the application development is far more recent. However, the potential of this green algae polysaccharide is such that intellectual property is being reported since mid-1980s. When compared with other polysaccharides originated from red and brown algae, ulvan may be considered as a late bloomer. However, the limits of its potential applicability are being drawn, and the knowledge generated during the past few years will drive research a step further towards the application development of this green algae polysaccharide.

Final remarks

As the fundamental knowledge on ulvan increases, research on this polysaccharide tends to shift towards applied science. However, this evolution is naturally impaired by a lack of a standardised commercial form of this polysaccharide. In this regard, the design of a novel and effective extraction procedure, focused towards targeted applications, becomes crucial. This certainly will open the way to the possibility of scaling up. This is an important consideration, if continuous research on ulvan is intended.

The literature is rich on reports highlighting the peculiar nature and diverse properties associated to this polysaccharide. In fact, a large portion of research efforts are focused on the study of ulvan chemistry and properties. This knowledge constitutes the fundamental basis that supports applied studies in an attempt to position ulvan as a valid alternative to other polymers, in diverse areas of knowledge. In this sense, this polysaccharide can find niches of application in areas like the pharmaceutical industry or the biomedical arena, for instance as a bioactive compound or as a medical device, or find applicability in the personal care products' market, or in the food industry or even in agriculture. However, it is the authors' belief that a practical understanding on ulvan's applicability is still a rather open field of research. This is of course enhanced by the particular demands of each niche of application. For example, if one considers ulvan in a biomedical context to be used as a medical device for regenerative purposes, considerable research endeavour is still needed to accomplish clinical relevance based on ulvan. In this particular context, research strategies may be focused, for instance, on polysaccharide modification, processing and material design and/or material–cell interactivity in order to achieve successful development.

Although the task of translating fundamental research on ulvan into practical achievements appears substantial, this polysaccharide holds great potential and versatility. Taking advantage of the knowledge, gathered research can now be driven towards practical applications of ulvan in an attempt to decrease the distance between scientific understanding and industrial awareness. The promise of success is feasible and for now, one can only say "*alea jacta est*".

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