CHAPTER 26

# Seaweed as a Source of Novel Nutraceuticals: Sulfated Polysaccharides and Peptides

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Contents




Abstract

Seaweeds and seaweed-derived products are underexploited marine bioresources and a source of natural ingredients for functional foods. Nutritional studies on seaweeds indicate that brown and red


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seaweeds possess a good nutritional quality and could be used as an alternative source of dietary fiber, protein, and minerals. Moreover, bioactive sulfated polysaccharides are the main components of soluble fiber in seaweeds and also bioactive peptides can be prepared from seaweed protein. This chapter gives an overview of the main biological properties of sulfated polysaccharides and peptides from brown and red seaweeds. Recent studies have provided evidence that sulfated polysaccharides from seaweeds can play a vital role in human health and nutrition. Besides, peptides derived from algal protein are most promising as antihypertensive agents. Further research work, especially *in vivo* studies, are needed in order to gain a better knowledge of the relation structure–function by which bioactive compounds from seaweeds exert their bioactivity.

## I. INTRODUCTION

### A. Seaweeds as an underexploited bioresource

Seaweeds have been used as a food in Asian countries, especially in China, Japan, and Korea, since ancient times (Chapman and Chapman, 1980; Indegaard and Minsaas, 1991; Nisizawa *et al.*, 1987). In Western European countries, seaweeds are mainly used in the pharmaceutical, food, and cosmetics industry as a source of hydrocolloids (Indegaard and Ostgaard, 1991; Juanes and Borja, 1991). Around 16 million tons of seaweeds (fresh weight basis) and other marine plants are annually produced or collected with an estimated value of 5575 million euros (FAO, 2007) worldwide; at the same time, seaweeds are currently considered as an underexploited natural resource (Cardozo *et al.*, 2007; Khan *et al.*, 2009). Moreover, seaweeds are a potential source of new biologically active substances and essential nutrients for human nutrition (MacArtain *et al.*, 2007; Smit, 2004). Therefore, systematic studies on nutrition and health protection of specific marine algae consumed in Europe (Denis *et al.*, 2010) and other countries are currently developed to provide the consumer with nutritional recommendations on a scientific base. These studies will also contribute to the economic exploitation of seaweeds.

### B. Nutritional assessment of seaweeds

Brown and red seaweeds possess a good nutritional value and can be an alternative source of proteins, minerals, and vitamins ( Jiménez-Escrig and Cambrodón, 1999; Plaza *et al.*, 2008; Rupérez and Saura-Calixto, 2001). Oil content is generally low but contains a great amount of essential fatty acids (Gómez-Ordóñez *et al.*, 2010; Rupérez and Saura-Calixto, 2001; Sánchez-Machado *et al.*, 2004).

Biochemical and nutritional aspects of seaweed proteins have been reported. Enzymatic degradation of algal fibers could be attempted to improve protein digestibility (Fleurence, 1999) and also to prepare bioactive peptides. A great deal of interest has been developed nowadays to isolate antihypertensive bioactive peptides, which act as angiotensin-converting enzyme (ACE) inhibitors because of their numerous health beneficial effects (Wijesekara and Kim, 2010).

Minerals are attributed to different ions associated with the charged polysaccharides of seaweeds. Seaweeds contain sulfate, representing different percentages of the ashes (Gómez-Ordóñez *et al.*, 2010; Rupérez and Saura-Calixto, 2001). Sulfate anion is derived from homo- or heteropolysaccharides in brown algae or from galactans in red ones. Sulfate seems to be a typical component of marine algal polysaccharides, related to high salt concentration in the environment and with specific functions in ionic regulation. Such sulfated mucilages are not found in land plants. Mineral bioavailability depends on the linkage type between polysaccharide and mineral and also on polysaccharide digestibility (Gómez-Ordóñez *et al.*, 2010). Typically, there is a strong positive correlation between sulfate content and biological activity of polysaccharides from seaweeds ( Jiao *et al.*, 2011).

Besides, seaweeds are considered an excellent source of dietary fiber with a high proportion of soluble to total dietary fiber (Gómez-Ordóñez *et al.*, 2010; Jiménez-Escrig and Sánchez-Muniz, 2000; Rupérez and Saura-Calixto, 2001). Dietary fiber in seaweeds is mainly composed of indigestible sulfated polysaccharides (Gómez-Ordóñez *et al.*, 2010; Rupérez *et al.*, 2002), which are resistant to human digestive enzymes (Rupérez and Toledano, 2003). Several storage and structural polysaccharides commonly found in brown and red seaweeds are laminaran, alginate, fucan, carrageenan, and agar (Gómez-Ordóñez *et al.*, 2010; Rupérez *et al.*, 2002). Alginates from brown seaweeds are traditionally used as hydrocolloids, while fucans are most interesting because of their biological activity (Rioux *et al.*, 2007). Fucans from brown seaweeds are by-products in the preparation of alginates for the food and cosmetic industries (Boisson-Vidal *et al.*, 1995). Different biological activities and potential health benefits of sulfated polysaccharides derived from marine algae have been reviewed recently ( Jiao *et al.*, 2011; Wijesekara *et al.*, 2011).

Seaweeds have to survive in a highly competitive environment subjected to light fluctuation, oxygen exposure, dehydration process, etc.; therefore, they develop defense strategies in different metabolic pathways. Thus marine organisms are rich sources of structurally diverse bioactive minor compounds such as carotenoids, polyphenols, minerals, vitamins, and fatty acids (Cardozo *et al.*, 2007). Besides, they possess other major compounds such as complex carbohydrates and protein, from which bioactive sulfated polysaccharides and peptides can be isolated.

## II. SEAWEEDS AS A SOURCE OF BIOACTIVE SULFATED POLYSACCHARIDES

Sulfated polysaccharides play storage and structural roles in seaweeds and may exhibit many interesting biological properties. As mentioned above, seaweeds are the main source of sulfated polysaccharides in vegetables; thus different amounts of sulfated heteropolysaccharides can be found in green seaweeds (Chlorophyta), while other sulfated polysaccharides such as laminaran, alginate, and fucan are present in brown seaweeds (Phaeophyta) and sulfated galactans such as agar and carrageenan appear in red seaweeds (Rhodophyta) (Costa *et al.*, 2010).

Several studies have demonstrated that composition—sulfated polysaccharide and other nutrients—and biological properties of seaweed could depend on ripening stage or environmental factors such as geographical localization, seasonal variation, nutritional quality of sea water, and other postharvest factors such as seaweed drying or extraction procedures for phycocolloid preparation (Rioux *et al.*, 2007).

### A. Preparation of sulfated polysaccharides from seaweeds

They can be sequentially extracted based on their different solubility. For example, the extraction procedure in the brown seaweed *Fucus vesiculosus* includes water, acid, and alkali treatments (Rupérez *et al.*, 2002). Thus, laminarans are water soluble, but their solubility depends on branching level: the higher the branching degree, the higher the solubility. Fucans are extracted with diluted hydrochloric acid, while alginates are extracted with alkali. Alginates form insoluble precipitates of alginic acid at low pH, but they are stable in solution between pH 6 and 9. The acid- and alkali-insoluble material from *F. vesiculosus* contains residual polysaccharides plus cellulose.

For red seaweeds, the solubility of sulfated galactans is dependent on temperature. Thus, highly charged sulfated galactans are soluble in aqueous solution at 20 °C, while those less modified such as agar in Nori (Porphyra spp.) are soluble at 60–80 °C. A neutral galactan from agar, agarose, is soluble at acidic pH. Finally, in most red and brown edible seaweeds, cellulose is the main polysaccharide of the acid- and alkali-insoluble fraction (Rupérez and Toledano, 2003).

### B. Biological activity of sulfated polysaccharides from seaweeds

Bioactivity of sulfated polysaccharides seems to be due to a complex interaction of structural features including sulfation level, distribution of sulfate groups along the polysaccharide backbone, molecular weight,

sugar residue composition, and stereochemistry (Jiao *et al.*, 2011). Although research studies dealing with the chemical structure of seaweed polysaccharides have been reported (Deniaud *et al.*, 2003; Lahaye and Robic, 2007; Lahaye *et al.*, 2003; Lechat *et al.*, 2000), relationship between macromolecular structure and biological activity is not clearly established (Jiao *et al.*, 2011).

### 1. *In vitro* studies

Relevant pharmacological properties of algal sulfated polysaccharides, such as anticoagulant, antioxidant, antiviral, anticancer, and immunomodulating activities, have been reviewed recently (Jiao *et al.*, 2011; Wijesekara *et al.*, 2011). Besides, other less well known biological properties have been described for sulfated polysaccharide, namely, antimicrobial, antiproliferative, anti-inflammatory (Wijesekara *et al.*, 2011), liver protection (Charles and Huang, 2009), effect on glucose (Hoebler *et al.*, 2000; Vaugelade *et al.*, 2000) and lipid metabolism (Amano *et al.*, 2005; Bocanegra *et al.*, 2006; Hoebler *et al.*, 2000; Huang, 2010), and prebiotic effect (Devillé *et al.*, 2007).

*Anticoagulant*. The anticoagulant capacity of sulfated polysaccharides from seaweeds has been the most studied property in an attempt to find an algal substitute for heparin. For example, the anticoagulant activity of fucans was shown to depend on their sugar composition, molecular weight, extent of sulfation, and distribution of sulfate groups in the polysaccharide repeating units (Jiao *et al.*, 2011; Pereira *et al.*, 1999). Marine sulfated polysaccharides other than fucans have also been shown to possess anticoagulant and antithrombotic capacity. Thus, the sulfated galactofucan from a brown seaweed lacks significant anticoagulation activity, making it an ideal candidate as an antithrombotic agent (Rocha *et al.*, 2005). Results suggest that algal sulfated polysaccharides could be an alternative to heparin because they present a promising potential to be used as natural anticoagulant agents in the pharmaceutical industry (Wijesekara *et al.*, 2011). Moreover, the development of antithrombotic algal polysaccharides would avoid the potential for contamination with prions or viruses (Jiao *et al.*, 2011) of commercial heparins, currently obtained from pig and bovine intestine.

*Antioxidant*. Sulfated polysaccharides not only function as dietary fiber, but they also contribute to the antioxidant activity of seaweeds. It has been demonstrated that they exhibit potential antioxidant activity *in vitro* and several of them derived from brown seaweeds, such as fucoidan, laminaran, and alginic acid, have been shown as potent antioxidants (Rocha De Souza *et al.*, 2007; Rupérez *et al.*, 2002; Wang *et al.*, 2008, 2010).

The presence of sulfate groups seems to make feasible the interaction between polysaccharide and target centers of cationic proteins (Mulloy,

2005). Another factor which could specifically modulate the antioxidant activity of sulfated polysaccharide is molecular size (positively at lower size) and the presence of nonsulfated sugar units at polysaccharide terminals (negatively) (Silva *et al.*, 2005). This fact suggests a stereospecificity in anticoagulant activity and not just a quantitative presence of sulfate in the molecule (Costa *et al.*, 2010).

Likewise, the relationship between sulfated polysaccharides and antioxidant capacity *in vitro* has been shown for red seaweed extracts (Chandini *et al.*, 2008; Rocha De Souza *et al.*, 2007) from Indian seaweeds. Also, the biological activity of sulfated polysaccharides from tropical seaweeds collected in Brazil has been evidenced previously (Costa *et al.*, 2010).

### 2. *In vivo* studies in animals and cell model

The detoxifying effect of different seaweeds in a Wistar rat model indicates that the presence of sulfated polysaccharides is crucial in the liver protecting effect of macroalgae (Costa *et al.*, 2010). Other studies have evidenced the protective effect of seaweeds against liver toxicity induced by galactosamine in a rat model, concluding that this protecting effect is partly mediated by fucoidan, a sulfated polysaccharide from the brown seaweed *Laminaria* (Kawano *et al.*, 2007).

Sulfated polysaccharides from seaweeds are evidenced as protectors of the antioxidant status in a stressed induced rat model (Veena *et al.*, 2007). Besides, the protecting effect of aqueous and organic extracts from brown and green seaweeds against induced oxidation has been studied in cell models (Gunji *et al.*, 2007). Therefore, sulfated polysaccharides from edible seaweeds potentially could be used as natural antioxidants by the food industry (Rupérez *et al.*, 2002).

The influence of seaweed intake on glucose metabolism has been shown in a pig animal model (Amano *et al.*, 2005; Hoebler *et al.*, 2000; Vaugelade *et al.*, 2000). Other studies deal with the effect of edible seaweeds (Kombu (Laminaria spp.) and Nori) and fucoidan from *Laminaria japonica* on lipid metabolism in a hypercholesterolemic rat model (Amano *et al.*, 2005; Bocanegra *et al.*, 2006; Hoebler *et al.*, 2000) and prebiotic effect (Devillé *et al.*, 2007). Prebiotic effect of *Laminaria* polysaccharide has been shown in the gut metabolism through its effects on mucosal composition, intestinal pH, and short chain fatty acids production (Devillé *et al.*, 2007).

## III. EDIBLE SEAWEEDS AS POTENTIAL SOURCES OF BIOACTIVE PEPTIDES

Biologically active peptides are food-derived peptides that can exhibit diverse activities, including opiate-like, mineral binding, immunomodulatory, antimicrobial, antioxidant, antithrombotic, hypocholesterolemic,

and blood pressure-lowering actions (Erdmann *et al.*, 2008). Bioactive peptides have been detected in different animal and vegetable protein sources, milk peptides being by far the most commonly known source ( Jiménez-Escrig *et al.*, 2010; Pihlanto *et al.*, 2008).

Heart diseases, such as arteriosclerosis, coronary heart disease, stroke, peripheral arterial disease, and heart failure, may be caused by hypertension or blood pressure greater than 140 mmHg systolic and/or 90 mmHg diastolic pressures (Lo and Li-Chan, 2005). The ACE (dipeptidyl carboxypeptidase, EC 3.4.15.1) performs an important physiological function in the pathogenesis of cardiovascular and renal diseases through blood pressure regulation. In the renin–angiotensin system, ACE catalyzes the conversion of the inactive decapeptide angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) to the potent vasoconstrictor, the octapeptide angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), by hydrolytic removal of the histidyl leucine group from the C-terminal (Ondetti and Cushman, 1982). Further, ACE is implicated in cell oxidative stress, through the generation of reactive oxygen/nitrogen species ( Jung *et al.*, 2006).

Certain biologically active peptides may act as ACE-inhibitory peptides and thus may prevent hypertension and its pathological consequences. ACE-inhibitory peptides from foods are less active than synthetic drugs such as captopril; however, their significance lies in the fact that they meet the need for naturalness and safety (Wu and Ding, 2002).

Edible seaweeds have been considered over the past few decades as promising organisms for providing both novel biologically active substances and essential compounds for human nutrition (MacArtain *et al.*, 2007). However, to date, scarce work of the potential ACE-inhibitory compounds such as biopeptides (Sato *et al.*, 2002a,b; Suetsuna and Nakano, 2000) or phlorotannins ( Jung *et al.*, 2006) on seaweeds has been done.

## A. *In vitro* and *in vivo* evaluation of antihypertensive activities: different approaches

The isolation of protein from seaweeds is a difficult task due to the link between polysaccharides and protein within the seaweed matrix. It is described that the extraction of proteins from the tissues of laminarialean algae (Nagai *et al.*, 2008) or *Saccharina japonica* (Kim *et al.*, 2011) is difficult due to high levels of nonprotein interfering compounds, mainly viscous polysaccharides. As a consequence, isoelectric point (Ma *et al.*, 1996) or ammonium sulfate saturation (Hernández-Mireles and Rito-Palomares, 2006) or trichloro acetic acid (Barbarino and Lourenço, 2005) approaches, which are commonly used for protein precipitation, are not completely useful for seaweeds. Thus, to solve this task, different approaches are proposed such as proteolytic treatment of the whole seaweeds followed by filtration and dialysis (Suetsuna and Nakano, 2000) or treatment

of seaweed matrix with alginate lyase S to obtain an enriched-protein precipitate which is recovered by centrifugation (Sato *et al.*, 2002a,b).

It is described the identification of ACE-inhibitory peptides derived from *Undaria pinnatifida* (Wakame), and hypotensive action of orally administered peptides on spontaneously hypertensive rats (SHRs). These studies are based on the previous evidence that dietary ingestion of whole Wakame, one of the most widely eaten brown seaweeds in Japan, has been shown to decrease blood pressure in humans. Specifically, the systolic blood pressure (SBP) of patients decreased significantly after daily oral administration of 3.3 g of dried Wakame after 4 weeks (Nakano *et al.*, 1999). In the work of Suetsuna and Nakano (2000), Wakame powder was digested using pepsin. Then, the filtrate of enzymatic digestion is dialyzed, the outer solution is applied sequentially to a Dowex 50W column $H^+$ form, and peptides were eluted with ammonium solution. After concentration under vacuum, the residue was fractionated on a SP-Sephadex C-25 column and a peptide power was obtained. The fractions having a molecular weight of 300–1000 kDa were collected and concentrated to dryness. The total yield of the peptide powder from 23.6 g of seaweed powder was 3.7 g. The peptides on the most ACE-inhibitory potent fraction were purified further by HPLC with an ODS-5 column. Although approximately 100 peaks were detected by this chromatography, potent inhibitory peptides were obtained in four peaks. Afterward, using protein sequencing, primary structures of the individual peptides were identified. The amino acid sequences of the peptides were Ala-Ile-Tyr-Lys, Tyr-Lys-Tyr-Tyr, Lys-Phe-Tyr-Gly, and Tyr-Asn-Lys-Leu. All of the active peptides had a tyrosine and lysine residue in the structure. Apart from this research, some peptides with potent ACE-inhibitory activity *in vitro* or intravenously are inactive in oral administration. Thus, hypotensive activity of each tetrapeptide are evaluated by measuring the SBP on SHR after oral administration of chemically synthesized tetrapeptides [50 mg/kg of body weight (BW)] (Suetsuna and Nakano, 2000). SBP did not change in control rats during the study period (6 h). Captopril (10 mg/kg BW) lowered SBP significantly. A single dose (50 mg/kg BW) of the tetrapeptides significantly reduced SBP in SHR. This work firstly isolated the bioactive peptide and then evaluated the activity of each synthesized peptide in a rat model.

The ACE-inhibitory and antihypertensive activities of Wakame hydrolysates have been investigated in another study, with a different research design (Sato *et al.*, 2002a,b). To obtain an isolated protein residue, Wakame was treated with alginate lyase S at 45 °C for 18 h and an enriched protein precipitate (46.3% dry matter) was recovered by centrifugation. Then Wakame was hydrolyzed using 17 kinds of proteases at different pH and temperature conditions, and ultrafiltered hydrolysates were tested for the inhibitory activity of the ACE. Among the proteases used in this study,

Wakame hydrolysates of pepsin, protease S and N Amano, and proleather FG-F were able to produce potent ACE inhibitors *in vitro*. The yield of the different enzymes used ranged from 115 to 239 mg as the weight of the solid contents obtained from 1 g of dried Wakame. In a second step, in order to evaluate the antihypertensive activity *in vivo* of hydrolysates produced by the four selected proteases, single oral administrations of hydrolysates were given to SHR ($n = 6$) at dosages of 100 and 1000 mg protein/kg BW. All the Wakame hydrolysates used in this test decreased the SBP in SHR, especially hydrolysates from protease S Amano or proleather FG-F. Digestion stability was evaluated by the change in $IC_{50}$ values of hydrolysates before and after treatment with gastrointestinal proteases (pepsin, trypsin, and chymotrypsin) to simulate *in vivo* resistance to digestion. In addition, a long-term feeding of hydrolysates was assayed on SHR. Seven-week-old SHR were fed a diet containing 0%, 0.01%, 0.1%, and 1.0% of the protease S Amano hydrolysate for 10 weeks. The SBP in the Wakame hydrolysate group tended to be lower than in the control group. Summarizing, there is no correlation between the *in vitro* and *in vivo* studies. These results indicated that *in vivo* experiments—single oral administration test and long-term feeding test—are important for the final evaluation of the antihypertensive effects of peptides. Among 17 proteolytic enzymes tested *in vitro*, it has been found that hypertension in SHR was suppressed by the Wakame protease S Amano hydrolysates.

Moreover, a study of isolation of potential antihypertensive agents (fucosterol and polyphenols) has been derived from seaweeds: Phaeophyta (*Ecklonia stolonifera*, *E. cava*, *Pelvetia siliquosa*, *Hizikia fusiforme*, and *U. pinnatifida*), Rhodophyta (*Gigartina tenella*, *Gelidium amansii*, *Chondria crassicaulis*, and *Porphyra tenera*), and Chlorophyta (*Capsosiphon fulvescens*) ( Jung *et al.*, 2006). The study includes the crude extracts of selected edible Korean seaweeds which were screened for ACE-inhibitory activity. Seaweed bioactive constituents are extracted with ethanol followed by partitioning with organic solvents: *n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol. Then the fractions extracted were chromatographed over a silica gel column yielding different subfractions and evaluated. In the case of the extract containing phloroglucinol, purification over an RP-18 column is used. Among the tested seaweeds, the ethanol extracts at a concentration of 163.93 μg/mL of *E. stolonifera* and *E. cava* appeared to be the most active, with inhibition of $64.86 \pm 0.58\%$ and $166.67 \pm 4.20\%$, respectively. With the notable exception of *H. fusiforme*, the other brown algae *P. siliquosa* and *U. pinnatifida* also exhibited favorable ACE-inhibitory activity, between 46% and 53%. Among the red algae tested, only *G. amansii* exhibited significant ACE-inhibitory effects, with an inhibition of $58.11 \pm 1.73\%$. Column chromatography of the *n*-hexane and ethyl acetate fractions led to the isolation of fucosterol and six phlorotannins, as phloroglucinol, and its oligomers eckstolonol, eckol, phlorofucofuroeckol A (a pentamer), dieckol (a hexamer), and triphlorethol A (a trimer) from the *Ecklonia* and

*Eisenia* species of brown algae. The ACE-inhibitory properties of phlorofucofuroeckol A, dieckol, and eckol ranked high, with $IC_{50}$ values of 12.74 ± 0.15, 34.25 ± 3.56, and 70.82 ± 0.25 μM, respectively. Summarizing, other bioactive compounds, besides peptides, may be responsible for the antihypertensive capacity of seaweeds.

## IV. CONCLUSION

The addition to traditional foods of edible seaweeds or seaweed-derived ingredients such as bioactive sulfated polysaccharides or peptides can be considered as a good strategy in order to increase the offer of the functional food market. To date, scarce work of the potential ACE-inhibitory compounds such as biopeptides has been done on seaweeds. The required isolation of protein is a difficult task due to the strong link between polysaccharides and protein in the seaweed matrix, and thus different extraction approaches have been proposed. Regarding antihypertensive effects of peptides, there is no correlation between *in vitro* and *in vivo* studies. Therefore, further research work on edible seaweeds through the systematic study of their sulfated polysaccharides, biopeptides, and related biological properties *in vitro* and especially *in vivo* will make possible a better knowledge of their potential benefit on human health and will contribute at the same time to their use as natural ingredients for the preparation of novel nutraceuticals.

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