Biocompatible Hydrogel from a Green Tide-Forming Chlorophyta

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Abstract

The green-tide chlorophyta *Ulva* contains the functional acidic polysaccharide ulvan in its cell wall. Here, we focused on the development of a novel soft material that can be used as a biocompatible ion exchanger. Combining chitosan and ulvan solutions was found to yield a hydrogel with various functions. This ulvan-chitosan polyion complex gel was more stable than an alginic acid-chitosan gel under both acidic and basic conditions. However, an ulvan-chitosan gel-coated vessel showed only a mild effect of preventing blood clotting, whereas a heparin-chitosan gel-coated vessel prolonged clotting time. In terms of the ion-exchange behavior, the ratio of the $CuSO_4$-concentration in a $CuSO_4$ solution treated with the gel to that in a solution without the gel showed that increasing the initial $CuSO_4$ concentration increased $CuSO_4$ adsorption in the gel. These studies show that this novel hydrogel can be used as an ion exchanger as well as in other applications.

Keywords: Soft material, Ulvan, Chitosan, Polyion complex, Green-tide chlorophyta, Ion exchange

1. Introduction

A study on the economics of ecosystems and biodiversity has revealed the importance of biodiversity for economics (Bishop et al., 2010). Wise use of biodiversity is important for sustainable development because ecosystem services are provided by biodiversity. Scientific approaches to sustainable development have been reported in various fields such as nature conservation (Alvarez, 2010), chemical technologies for recycling plastics (Chen, 2006), materials science (Wu et al., 2009), and education for the wise use of biodiversity (Kanno et al., 2011). At the 10th conference of the parties of the convention on biological diversity (CBD-COP10) held in Nagoya, Japan, delegates from more than 100 countries agreed on the new strategic plan of the Convention on Biological Diversity (CBD), namely, the 'The Aichi Biodiversity Targets' (Strategic Plan for 2020 and the Aichi Targets, 2010). The Strategic Plan is comprised of a shared vision, a mission, strategic goals, and 20 ambitious yet achievable targets. A study on the utilization of species that are both problematic and a resource is one of the possible ways to achieve strategic goal B of the Aichi Targets-to reduce the direct pressures on biodiversity and promote sustainable use.

For species that are both problematic (e.g., invasive) and a resource, various means have been employed to increase their utility and thereby, in some cases, to reduce their environmental impact. New Zealand used the hair of the invasive possum by mixing it with lamb's wool and angora to produce wool. Another example is that of the crab. Although not an invasive alien species, a vast amount of crab shell waste is released by the aquatic food industry, creating an environmental problem. Chitin is a polysaccharide found in crab shells, shellfish, insects, and some fungi. A number of investigations on chitin and chitosan revealed that they possessed biological activities such as anti-bacterial effects. This research added value to crab shell waste to produce novel valuable materials.

Green algae blooms, which are often referred to as "green tides" occur throughout the world. Local self-governing bodies and NGOs (non-governmental organizations) engage in removing the algal mats from mudflat because they devastate the scenery and emit a foul odor. In Fukuoka, Japan, green tides and algal mats are removed by special ships, heavy machinery, and volunteer workers (Figure 1). Algal mats are incinerated or buried in landfills in Hakata bay. The Wajiro tideland is important because it is where the black-faced spoonbill (*Platalea minor*), an endangered species that numbers less than 1,000 worldwide, passes the winter. Although the seaweed *Ulva* is a natural resource, it is also an environmental challenge because it causes algal blooms.

Pollution and nutrient loading (e.g., of nitrogen and phosphorous) can result in an increase in seaweed growth and a resultant lack of oxygen because of rotting seaweed on the mudflats, and many animals living in the mud under the rotting *Ulva* suffocate (Donna et al., 1985). At the same time, *Ulva* is used in traditional (e.g., "seaweed soup") and frozen foods in Japan, and farmers also use it in compost. Mendo et al. reported that compost prepared with *Ulva* had lower total Kjeldahl nitrogen and produced maize plants with aerial biomass (Mendo et al., 2005). The chitin example described above has encouraged researchers engaged in studying *Ulva*. Many researchers have reported various biological activities for ulvan, a compound present in this seaweed, including anti-coagulant (Mao et al., 2009), anti-inflammatory (Leiro et al., 2010), anti-oxidant (Qi et al., 2006), immunostimulatory (Leiro et al., 2007), and anti-hyperlipidemic (Pengzhan et al., 2003) properties. A reduction in CO_2 emissions, which is a high political priority implemented by international policy, can be achieved by switching from petroleum to sustainable biological resources for producing materials. In this study, we report on the preparation and properties of a novel soft material consisting of an ulvan-chitosan polyion complex possessing biocompatibility.

2. Experiment

2.1 Materials and Methods

All enzymes and polysaccharides were purchased from Wako Pure Chemical Industries, Ltd. *Ulva pertusa* was collected at the Wajiro mudflats in Fukuoka prefecture on October 2010. The samples were rinsed with seawater followed by pure water and subsequently dried *in vacuo*. Ulvan was extracted according to the hot water extraction method by using sodium chlorite (Robic et al., 2008). The extract was treated with glucoamylase and protease to remove contaminants. Further purification was accomplished by DEAE-cellulose column chromatography followed by dialysis and freeze-drying. Infrared spectra were recorded from powders in a KBr pellet on a Nicolet FTIR spectrometer. NMR spectra were recorded in D_2O on a Varian NMR 400 spectrometer. The APTT reagent kit and normal human plasma were purchased from EIDIA Co., Ltd.

2.2 Polyion Complex-Gel Formation

At 80°C, 5 ml of 1.5 wt% ulvan aqueous solution was added to 5 ml of 1.5 wt% chitosan solution in 5% acetic acid. The mixture was stored at 20°C for 20 h to form a hydrogel. The obtained hydrogel was stored for 3 h in 100 ml of water on 3 separate occasions to remove low molecular weight salts in the gel. The gel was then freeze-dried.

2.3 Swelling of the Gel

A hydrogel was created as described in section 2.2 but not freeze-dried. To study the effect of pH on gel swelling, the pH value was adjusted from 2 to 11 with 1 mM NaOH and 1 mM HCl. After storing a degassed gel in the solution at 20°C for 20 h, the volume of the gel was measured. As a control, the swelling of the alginic acid-chitosan polyion complex was examined at pH values of 2, 7, 8, and 11 the ratios of the gel volume at pH 7 to the gel volumes at other pH values are shown in Figure 2.

2.4 Activated Partial Thromboplastin Time (APTT) of Ulvan

Activated partial thromboplastin time (APTT) coagulation assays were performed as described earlier by using citrate-treated normal human plasma (Albuquerque et al., 2004). The APTT of the gel was examined by using a gel-coated test tube. Five milliliters of 5% chitosan solution in 5% acetic acid were applied to the inside wall of the test tube. Next, 5 ml of 5% ulvan aqueous solution were applied to the inside of the test tube and stored at 20°C for 20 h. Afterwards, the test tube was rinsed with water. The infrared spectrum of the powdered inside wall showed peaks assigned to ulvan.

2.5 Ion-Exchange of the Gel

Eighty milligrams of freeze-dried gel were swelled in 5 ml of $CuSO_4$ solution at 20°C for 20 h. The adsorption spectra of the supernatants were measured at 800 nm. The concentrations of $CuSO_4$ in the supernatants were determined by the calibration curve for $CuSO_4$. The blank spectra were measured with pure water and the supernatant of the water-containing gel for $CuSO_4$ solutions without and with the gel, respectively.

3. Results and Discussion

3.1 Ulvan

Ulvan was extracted according to the hot water extraction method by using sodium chlorite (Robic et al., 2008). Further purification was accomplished by DEAE-cellulose column chromatography. FT-IR spectra and NMR spectra suggested that the extract was ulvan. The characteristic absorptions of the sulfate group and uronic acids were identified in the FT-IR spectrum. The absorptions at 847, 1259, 1655, and 3500 cm^{-1} were attributed to the

C–O–S bending vibration of the sulfate group in the axial position, S=O stretching vibration of the sulfate group, C=O of uronic acids, and OH group, respectively.

3.2 Polyion Complex-Gel Formation

The sequence of repeating units in ulvan is composed of the disaccharides ulvanobiuronic acid 3-sulfate[→4)-β-D-GlcA-(1→4)-α-L-Rha 3-sulfate-(1→], xylosylrhamnose sulfate [→4)-β-D-Xyl-(1→4)-α-L-Rha 3-sulfate-(1→] and [→4)-β-D-Xyl 2-sulfate-(1→4)-α-L-Rha 3-sulfate(1→] (Lahaye, 1998). The cross-links of the gel consist of reversible ionic bonds, so that ion exchange can result in the loss of cross-links and thereby make the gel swell. As shown in Figure 2, the alginic acid-chitosan gel swelled under both acidic and basic conditions and was partially dissolved at pH 11. In contrast, the ulvan-chitosan gel did not swell at any of the tested pH values, showing that this gel is more stable than the alginic acid-chitosan gel under both acidic and basic conditions. This resistance of the ulvan-chitosan gel to swelling under various pH conditions may be caused by the integrity of the ulvan chain. The carbonic acid and sulfate groups of ulvan form ionic cross-links with the amino groups of chitosan. Ulvan also forms a hydrogel with boron; however, the boron complex forms only at pH values over 9 (Lahaye et al., 1998). The ulvan-chitosan polyion complex-type gel was stable over a wide range of pH values. The swelling of the ulvan-chitosan and alginic acid-chitosan gels was also examined at various temperatures. The ratios of the gel volumes at various temperatures to the control volume at 20°C are shown in Figure 3. The ulvan-chitosan gel swelled only 1.09 times compared to the control. In addition, both the ulvan-chitosan and alginic acid-chitosan gels were stable within the temperature range of 0 to 80°C.

3.3 Activated Partial Thromboplastin Time (APTT) of Ulvan

Blood coagulation is part of an important host defense mechanism termed hemostasis, and ulvan shows anticoagulant activity for all conditions tested thus far in the literature. Moreover, different types of surfaces are known to affect the clotting time of blood because interfacial adsorption is a factor in the clotting of blood plasma. Here, we focused on the biocompatibility of the ulvan-chitosan gel as a novel soft material. To examine the anticoagulant activity of the polyion complex, we prepared a polyion complex-coated vessel. For various sulfated polysaccharides, Figure 4 shows the ratio of the clotting time for the plasma with sulfated polysaccharide to that for the control plasma without sulfated polysaccharide. Three kinds of sulfated polysaccharide (heparin, fucoidan, and ulvan) shown to possess anticoagulant activity and prolong clotting time are displayed for comparison. Both the heparin-chitosan and fucoidan-chitosan gel-coated vessels prolonged clotting time by more than 18-fold compared to the control. In contrast, the ulvan-chitosan gel-coated vessel only weakly prevented clotting. Although anti-coagulant activity is just one test for examining biocompatibility, these results suggest that the ulvan-chitosan gel may be a biocompatible soft material because it did not strongly affect the clotting time of blood.

3.4 Ion-Exchange of the Gel

An ion-exchanger is an important biomaterial. For instance, Christoforou et al. (1998) reported on ion-exchange beads, which have been shown to promote a variety of wound-healing responses in several model systems. We examined the ion-exchange behavior of the ulvan-chitosan gel (Figure 5). Figure 5 shows the ratio of the $CuSO_4$ concentration for a $CuSO_4$ solution treated with the gel to the initial $CuSO_4$ concentration without gel treatment. Increasing the initial concentration of $CuSO_4$ increased the adsorption of $CuSO_4$ in the gel.

Given that ulvan contains about one carboxylic acid per repeating unit, the mol percent of the carboxylic acid groups in ulvan involving the formation of an ionic bond with the Cu ion is about 1.4% for 0.0140 M $CuSO_4$, 4.6% for 0.00562 M $CuSO_4$, and 12% for 0.00140 M $CuSO_4$.

3.5 Ulvan-Chitosan Gel as a Novel Soft Material

Haug reported gel formation of a sulfated polysaccharide extracted from *Ulva*. However, at that time, gel formation of the sulfated polysaccharide required both borate and calcium ions (Haug, 1976). After some time, Lahaye et al. reported the formation of a hydrogel of ulvan with boron, although the boron complex formed only at pH values over 9 (Lahaye et al., 1998). Interestingly, the ulvan-chitosan polyion complex type gel was stable over a wide range of pH values. The reason for this may be that the polyion complex has many cross-linking groups (e.g., carboxyl groups and amino groups) in a single molecule. Although the mol percent of the carboxylic acid groups in ulvan involving the formation of an ionic bond with the Cu ion is low, this novel soft material possess a number of advantages and possibilities. First, this gel is prepared with only a few chemical reagents. This is in contrast to gels synthesized from petroleum, which require many kinds of pollutive reagents such as polymerization initiators and organic solvents. Second, although non-cross-linked ulvan possesses anti-coagulant activity, the ulvan-chitosan gel does not prevent blood clotting. This suggests that the gel can be used for biocompatible materials. Third, the gel is prepared from problematic algal mats. Fourth, it is a renewable organic material.

As reported by Sari (2008), *Ulva* shows good biosorption of Pb(II) and Cd(II) from aqueous solutions. Our research focused on the development of a novel soft material, in particular for a biocompatible ion-exchanger, because investigation of high value-added products accelerates the recycling of waste or utilization of problematic (but resourceful) species. As an example, an enormous amount of research on chitin and chitosan and their biological activities accelerated the utilization of crab shell waste to produce valuable materials. Although research on the utilization of *Ulva* is still in progress, the example of green tide waste may yield valuable materials.

4. Conclusion

In this study, we reported on the preparation and properties of a novel soft material consisting of an ulvan-chitosan polyion complex. Ulvan was obtained from the green-tide chlorophyta *Ulva*. Combining chitosan and ulvan solutions were found to yield a hydrogel with various functions. This ulvan-chitosan polyion complex gel was more stable than an alginic acid-chitosan gel under both acidic and basic conditions. An ulvan-chitosan gel-coated vessel showed only a mild effect of preventing blood clotting, whereas a heparin-chitosan gel-coated vessel prolonged clotting time. This suggests that the ulvan-chitosan gel does not significantly affect blood coagulation. The ratio of the $CuSO_4$ concentration in a $CuSO_4$ solution treated with the gel to that in the $CuSO_4$ solution without the gel showed that increasing the initial $CuSO_4$ concentration increased the adsorption of $CuSO_4$ in the gel.

A reduction in CO_2 emissions can be achieved by switching from petroleum to biological resources to produce materials. An enormous number of polymer materials including plastics, elastomers, fivers, and paints are synthesized from petroleum (e.g., a polystyrene sulfonic acid derivative as an ion-exchanger, polyacrylic acid as a superabsorbent polymer). Our studies suggest that the novel hydrogel can be used as a biocompatible ion exchanger as well as in other applications. *Ulva*, which is a species that is both problematic and a resource yielded the ‘ulvan-chitosan gel,’-an alternative to polymer materials derived from petroleum. A study on the utilization of species that are both problematic and a resource is one possible way to achieve strategic goal B of ‘The Aichi Biodiversity Targets’: Reduce the direct pressures on biodiversity and promote sustainable use.

The International Union for Conservation of Nature (IUCN) has produced a list of ‘100 of the world’s worst invasive alien species’ (Lowe, S. et al., 2000). Invasive alien species that are introduced into areas outside of their natural habitats cause biodiversity loss by eliminating native species through competition, predation, or transmission of pathogens. Wakame seaweed (*Undaria pinnatifida*) and Kudzu (*Pueraria montana var. lobata*) are two of ‘100 of the world’s worst invasive alien species’. However, they contain useful polysaccharide molecules and have been traditional foods in Japan. Hence, despite their insufficient utilization and current status as waste materials, some invasive alien species may not only problematic but also a resource. Identifying the biological and chemical functions of these apparent waste materials and reducing petroleum-derived material is one possible way to achieve strategic goal B of ‘The Aichi Biodiversity Targets’: Reduce the direct pressures on biodiversity and promote sustainable use.

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Figure 1. Civic organization removing algal mats from Wajiro mudflats

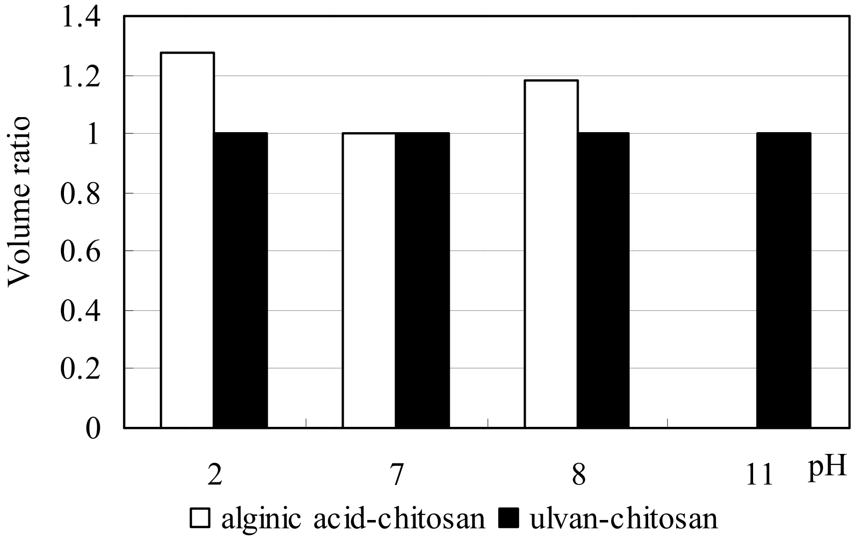


Figure 2. Swelling of ulvan-chitosan polyion complex at various pH values

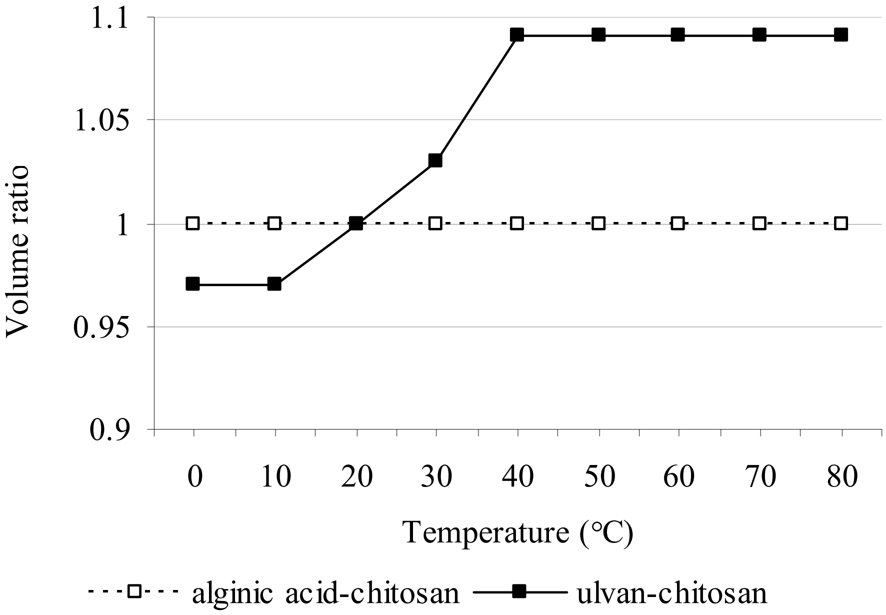


Figure 3. Swelling of ulvan-chitosan polyion complex at various temperatures

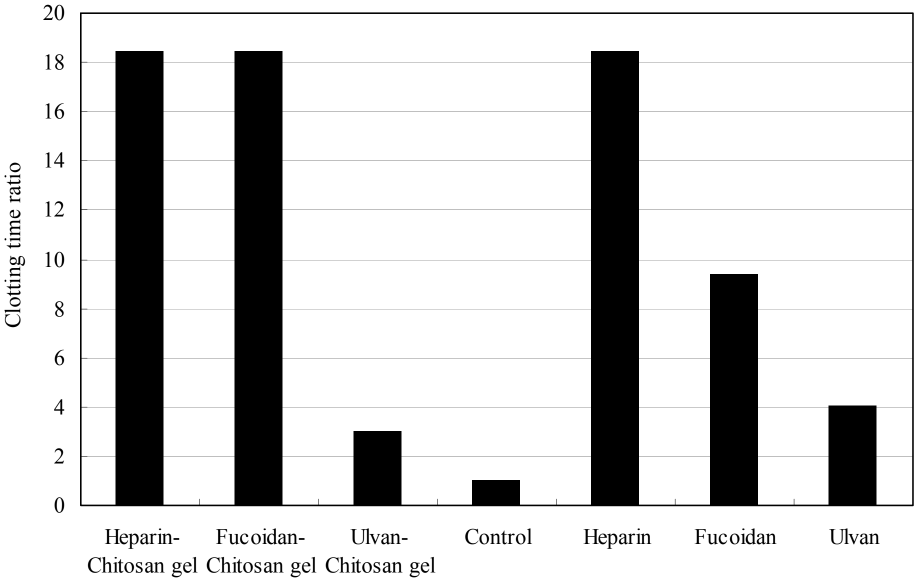


Figure 4. Anti-coagulant activity of ulvan-chitosan polyion complex

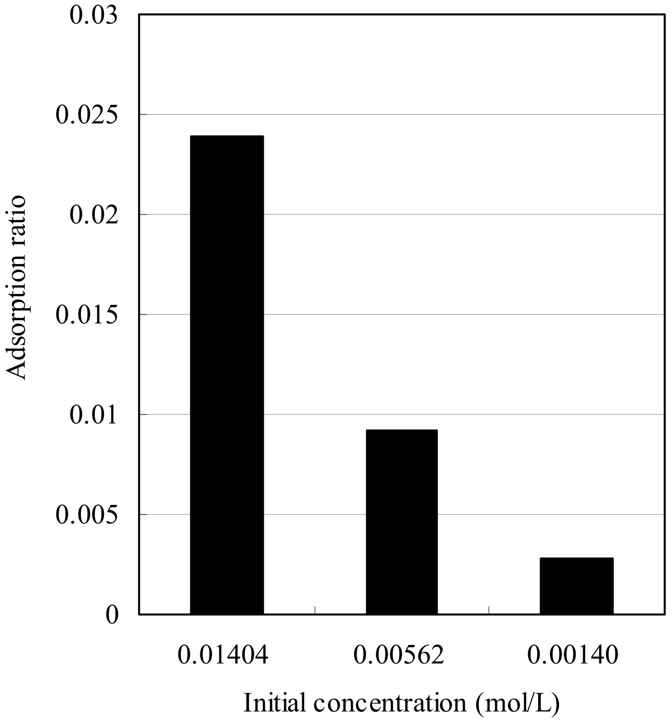
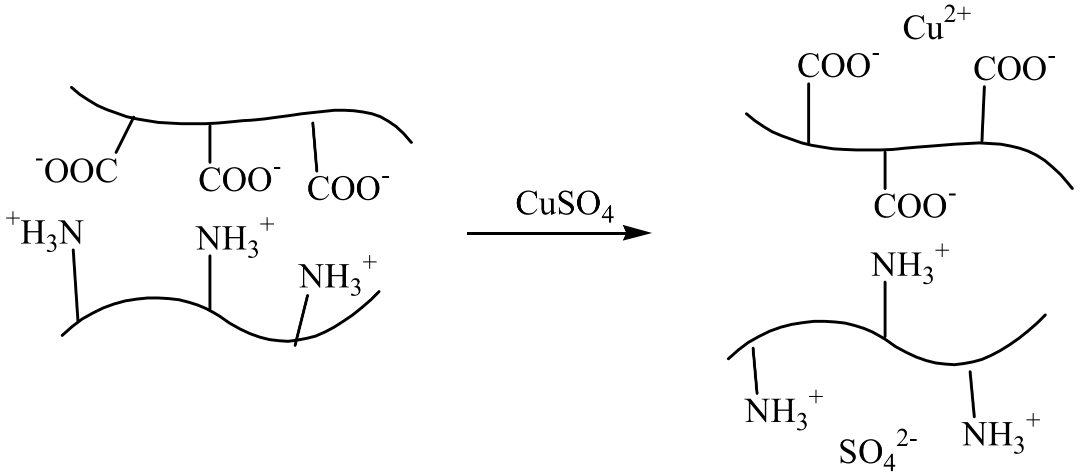


Figure 5. Adsorption of Cu ion in ulvan-chitosan polyion complex



Scheme 1. Adsorption of Cu ion in ulvan-chitosan polyion complex