Carbohydrate Research 344 (2009) 2023–2027

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

# Photolytic depolymerization of alginate

Jankana Burana-osot [a], Saori Hosoyama [b], Yuzu Nagamoto [b], Sho Suzuki [b], Robert J. Linhardt [c], Toshihiko Toida [b,*]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Silpakorn University, Nakornpathom 73000, Thailand*
[b] *Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 260-8675, Japan*
[c] *Department of Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA*

ARTICLE INFO

*Article history:*
Received 8 May 2009
Received in revised form 6 June 2009
Accepted 21 June 2009
Available online 26 June 2009

*Keywords:*
Alginate
Photochemical depolymerization
NMR spectroscopy

ABSTRACT

A photochemical reaction has been developed for the partial de-polymerization of sodium alginate, a polysaccharide utilized in medicine, pharmacy, basic sciences and foods. An aqueous solution of sodium alginate was photochemically depolymerized to ∼40% of its average molecular weight using ultraviolet light in the presence of titanium dioxide catalyst at pH 7 over a period of 3 h. The products were separated giving four fractions all having an average molecular weight that was smaller than that of the starting material. Characterization of the guluronate (G) and mannuronate (M) contents, and determination of the M/G ratio of photochemically depolymerized alginate, were accomplished using $^1$H NMR spectroscopy. The resulting M/G ratio was compared to that obtained for alginate fractions produced by acid hydrolysis. The M and G content, of each alginate fraction, was also assigned with regards to their occurrence in G-rich, M-rich or M/G heteropolymeric domains. This new depolymerization method might also be applicable in the preparation of alginate oligosaccharides for use in the food and pharmaceutical industries.

© 2009 Elsevier Ltd. All rights reserved.

## 1. Introduction

Alginate is a polysaccharide produced by brown algae and bacteria. It is a linear binary copolymer consisting of (1→4) linked β-D-mannuronic acid (ManA) and α-L-guluronic acid (GulA), which are arranged in a block structure of homopolymeric (MM and GG) blocks and/or heteropolymeric (MG and GM) blocks as shown in Figure 1. Sodium alginate has been utilized in various fields, including the medicinal, pharmaceutical and food industries, for its gelling, stiffening and stabilizing properties. There are several reports of oligosaccharides prepared from alginate that exhibit a variety of biological activities, including anti-allergy properties, through the suppression of IgE,[1] anti-hypertensive activity,[2] an ability to enhance the growth of human endothelial cells and keratinocytes,[3,4] and an ability to induce cytokine production in a mouse macrophage cell line.[5] The proportion and sequential arrangement of the uronic residues such as ManA and GulA vary based on the algal species from which the alginate is prepared. This composition, ManA/GulA (M/G) ratio and alginate molecular weight are responsible for its biological and physico-chemical properties.[6–8]

Many methods have been used for preparation of alginate oligosaccharides, including acid hydrolysis,[9,10] enzymatic digestion,[11–14] γ-irradiation and ultraviolet photolysis,[15] γ-irradiation and oxidative–reductive reactions,[16] oxidative–reductive free radical depolymerization (ORD-reaction),[17] thermal degradation through autoclaving,[18] thermolysis under alkaline or acidic conditions[19] and breakdown using subcritical and supercritical water.[20] The disadvantages of the aforementioned methods are that strong conditions or harsh chemicals are used and decomposition of sugars can take place, reducing the yields and resulting in the formation of side products that can cause environmental pollution.

Photochemical reactions, generally involving ultraviolet (UV) light-initiated oxidation–reduction reactions are receiving increased attention with the advent of 'Green Chemistry'. Photochemical oxidation processes have been used for degradation of many different compounds.[21] Photocatalysis involves the acceleration of photochemical reactions through the use of catalysts. Such reactions are often applied for environmentally friendly treatments, including water purification, air cleaning, clean organic syntheses and disinfection.[22] The most widely used photocatalyst is titanium dioxide ($TiO_2$) because of its desirable properties, such as chemical stability, high catalytic activity under UV-light, low cost and most importantly its low biologically toxicity.[23–26] Photocatalysis using $TiO_2$ can be performed without special equipment or the need to control temperature. Moreover, the $TiO_2$ used as catalyst can be easily removed by filtration after the photoreaction takes place, resulting in highly pure products.

In the present paper, we perform a $TiO_2$-catalyzed photochemical reaction to depolymerize sodium alginate. Alginate is

* Corresponding author. Tel./fax: +81 43 226 2871.
*E-mail address:* toida@p.chiba-u.ac.jp (T. Toida).

0008-6215/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2009.06.027

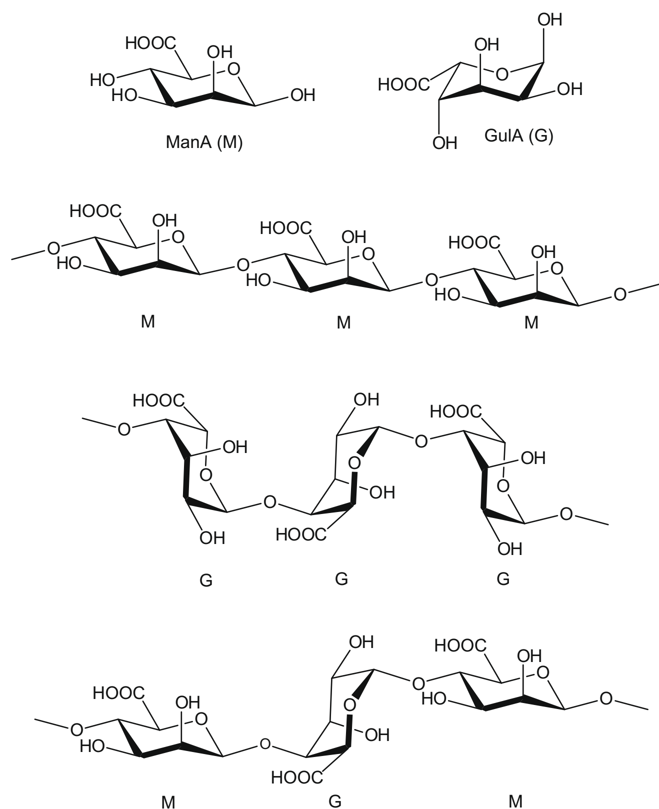


Figure 1. Chemical structure of alginate.

depolymerized under UV light and the resulting oligosaccharide products are fractionated.[9,10] The GulA and ManA contents and the M/G ratio of the depolymerized alginate fractions are then elucidated by $^1$H NMR spectroscopy.

## 2. Results and discussion

### 2.1. Degradation of alginate by photochemical UV/titanium dioxide process

The photochemical reaction was optimized, to obtain products having appropriate average molecular weight ($MW_{avg}$), by varying the pH of solvent and UV exposure time. The effect of pH was first investigated at pH 4 (water adjusted with 10 mM hydrochloric acid), pH 7 (water adjusted with 10 mM ammonium hydroxide) and pH 10 (water adjusted with 100 mM ammonium hydroxide). The dissolved alginate (1 g/L) samples were exposed to a low-pressure mercury UV light for 0, 1, 3 and 6 h. Polyacrylamide gel electrophoresis (PAGE) was used to qualitatively demonstrate that the alginate had been depolymerized by $TiO_2$-catalyzed photochemical reaction affording polysaccharides and oligosaccharides of lower molecular weight (Fig. 2).

The $MW_{avg}$ of each sample was determined by high performance size exclusion chromatography (HPSEC). The $MW_{avg}$ of alginate decreased to 148 kDa in 1 h after exposure to UV light in the presence of $TiO_2$. Alginate $MW_{avg}$ dramatically decreased after 3 h of photolysis and more slowly decreased after 6 h of photolysis. The $MW_{avg}$ of the alginate samples obtained at 3 h and 6 h of photolysis were 108 and 70 kDa, respectively, corresponding to a 45.5% and 64.6% decrease in the $MW_{avg}$ of the starting alginate polysaccharide. The $MW_{avg}$ of alginate samples obtained at pH 4, 7 and 10 for 3 h of the reaction time were not different (Table 1). Therefore, the optimal conditions for the photochemical reaction to produce fractionated sodium alginate were established as exposure to the UV light in the presence of $TiO_2$ for 3 h at pH 7. We also confirmed that these established conditions were general, obtaining the same results for another sodium alginate sample from a different biological source (data not shown).

Figure 2. PAGE of sodium alginate under different photochemical reactions. *Lanes*: (1) dextran sulfate (500 kDa); (2) hyaluronate (100 kDa); (3) dextran sulfate (100 kDa); (4) intact sodium alginate; (5) sodium alginate reacted for 6 h without titanium dioxide; (6) sodium alginate reacted for 1 h at pH 7; (7) sodium alginate reacted for 3 h at pH 7; (8) sodium alginate reacted for 6 h at pH 7; (9) sodium alginate reacted for 3 h at pH 4; (10) sodium alginate reacted for 3 h at pH 7 (as same as *lane* 7); (11) sodium alginate reacted for 3 h at pH 10.

### 2.2. Characterization of alginate and its degraded products by $^1$H NMR

The $^1$H NMR spectra of sodium alginate sample at different temperatures are shown in Figure 3. The peaks of the components in an intact alginate sample were observed and are assigned in Figure 3 legend based on previously reported values.[27–30] $^1$H NMR spectra of the degraded alginate samples, obtained from photochemical reaction at pH 4, 7 and 10, are shown in Figure 4. Each signal was slightly changed by an altered distribution of alginate chain lengths. The signals for degraded alginate samples could be observed at the same chemical shifts of the intact alginate sample, indicating that the chemical structures were indistinguishable and suggesting that photochemical degradation occurs through the random breakage of the glycosidic bonds in the alginate polysaccharide.

Photochemical reaction of alginate gave four fractions (A-I, -II, -III and B) with a 90% recovery yield. Fraction A-I, which was insoluble at pH 3, consists of GluA-rich alginate (88% GluA). Fractions A-III and B,

**Table 1**
The average molecular weight (kDa) of the degraded sodium alginate in different pHs and at different light exposure times

| Exposure time (h) | Solvent | | |
|---|---|---|---|
| | pH 4 | pH 7 | pH 10 |
| 0 | 198 | 198 | 198 |
| 1 | 148 | 139 | 140 |
| 3 | 108 | 96 | 92 |
| 6 | 70 | 68 | 65 |

All data shown were tentatively estimated by using dextran and dextran sulfate as molecular weight standards.

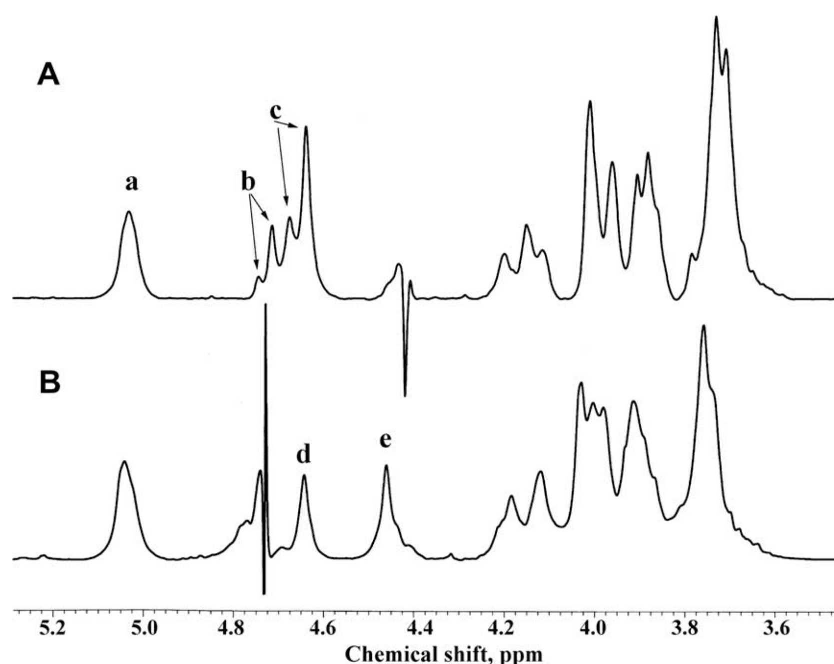


**Figure 3.** ^{1}H NMR spectra of sodium alginate collected at 60 (A) and 30 °C (B). *Signals*: (a) H-1 of G; (b) H-5 of G in GM-block; (c) H-1 of M; (d) H-1 of M; (e) H-5 of G in GG-block.

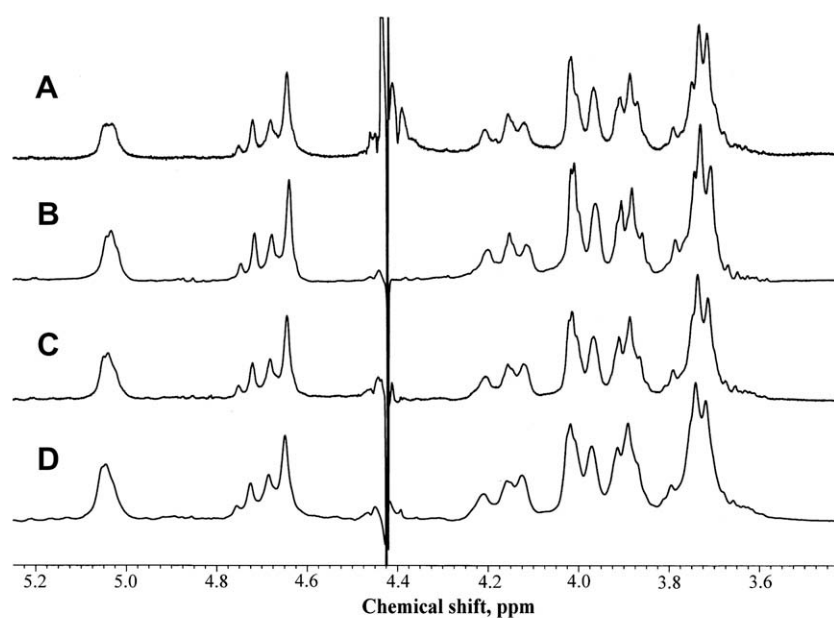


**Figure 4.** ^{1}H NMR spectra of sodium alginate samples. (A) intact alginate; (B) product after 1 h reaction at pH 7; (C) product after 3 h reaction at pH 7; (D) product after 6 h reaction at pH 7.

soluble at pH 0.85, were ManA-rich alginate (67% ManA). Fraction A-II had an M/G ratio of 1 (Table 2). In contrast, the total recovery of alginate and GluA residues, prepared by acid hydrolysis, was significantly lower (~20%) than those prepared by photolysis. These results suggest that $TiO_2$-catalyzed photolysis might be more suitable for preparation of alginate oligosaccharides than the conventional acid hydrolysis methods.

The characterization of four fractions of sodium alginate degraded by photolysis was accomplished by $^{1}H$ NMR spectroscopy at 30 °C and 60 °C (Fig. 5). The signals of all products were verified as alginate. However, the relative area of each signal was different (Fig. 5). The two predominant peaks at H-1 of GluA at 5.17 ppm and at H-5 of GluA at 4.40 ppm in the spectrum of fraction A-I, indicating that it is a GluA-rich alginate fraction. The small peak at 4.7 ppm showed ManA-residues present in the G-rich domain. Fraction A-II contained an M/G ratio of around 1, showing it to be a hetero-polymeric fraction. The signals assigned as H-1 of ManA at 4.65 and 4.67 ppm were the predominant peaks in the spectra of fractions A-III and B, indicating that these are ManA-rich alginate fractions. The peak at 5.03 ppm shows GluA-residues in the M-rich domain of both fractions A-III and B.

$^{1}H$ NMR has been a routine method for the analysis of GluA content in alginate. Previous studies have shown that the signals and the relative area of anomeric protons could be used for the quantitative analysis of GluA (G%) and G-G sequence (GG%).[18,27,29,30] The

**Table 2**
The amounts of guluronic acid (G), mannuronic acid (M), G-G sequence (GG) and M/G ratio in fractionated alginate analyzed by $^1$H NMR

| Substance | % Yield | GG% | G% | M% | M/G |
|---|---|---|---|---|---|
| Intact | | 23.0 | 37.6 | 62.4 | 1.66 |
| After reaction | 90.0 | 23.9 | 37.4 | 62.6 | 1.67 |
| | 70.4 | 18.4 | 32.2 | 67.8 | 2.10 |
| A-I | 3.4 | 65.6 | 87.9 | 12.1 | 0.14 |
| | 2.1 | 59.2 | 80.2 | 19.8 | 0.24 |
| A-II | 37.6 | 43.4 | 50.5 | 49.5 | 0.98 |
| | 33.5 | 47.1 | 44.2 | 55.8 | 1.26 |
| A-III | 8.4 | 18.2 | 32.8 | 67.2 | 2.05 |
| | 6.2 | 15.4 | 28.3 | 71.7 | 2.53 |
| B | 31.0 | 10.5 | 33.0 | 67.0 | 2.03 |
| | 20.5 | 8.2 | 25.3 | 74.7 | 2.95 |

Upper and lower rows indicate data obtained by photolysis and hydrolysis, respectively.

relative areas of signals for H-1 of GluA, those of H-1 of ManA, H-5 of GluA in GM-block and H-5 of GluA in G-block were measured, and the G% and GG% were calculated based on previously proposed equations.[20,29]

The M% was derived by subtracting the G% from 100. The calculated data of sodium alginate and its degradation products are presented in Table 2. The value of M/G ratio and GG% of the degraded sodium alginate and intact alginate showed the same value. These results strongly suggest that the photochemical reaction leads to the formation of oligomers without compositional changes.

## 3. Conclusions

The degradation of sodium alginate was accomplished under a photochemical UV/$TiO_2$ reaction at pH 7 for 3 h. The results showed that the fragmentation of alginate occurred using this process, leading to the formation of alginate oligomers without a measurable change in their chemical structure. The GluA-rich and ManA-rich alginate and GM-alternating sequence blocks were obtained in 90% yield from this photochemical reaction. The $TiO_2$ used as a catalyst could be easily removed, resulting in the convenient production of purified degraded products. This process is simpler, higher yielding and more environmentally benign than conventional methods, such as acid hydrolysis. Furthermore, this procedure might represent a convenient and reliable method for a routine production of alginate oligosaccharides for the food and pharmaceutical industries.

## 4. Experimental

### 4.1. Chemicals

A sodium alginate sample prepared from *Lessonia nigrescens* having a $MW_{avg}$ of ~198 kDa was kindly provided by KIMICA Corporation, Japan. Titanium dioxide (anatase type, particle size aver-

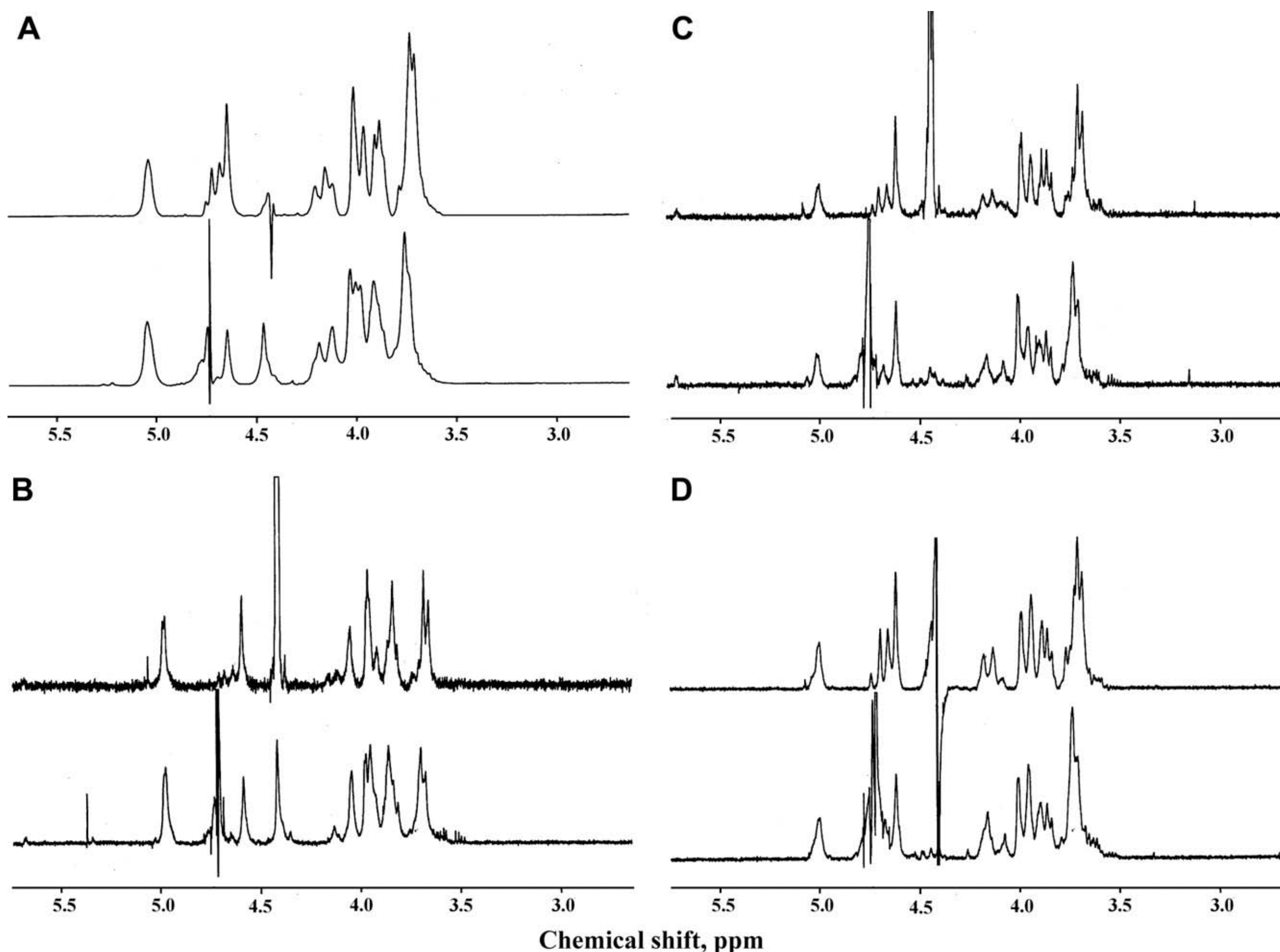


**Figure 5.** $^1$H NMR spectra of photochemically degraded product fractions of sodium alginate. (A) fraction A-I; (B) fraction A-II; (C) fraction A-III; (D) fraction B.

age, 50 μm) was purchased from Wako. Other chemicals used were of analytical grade.

### 4.2. Photochemical reaction apparatus

The photochemical reaction experiment device (Sen Lights Corporation, Osaka, Japan) consists of a VG1500 reaction tank with 5 inlets, a UV light source (low-pressure mercury lamp HL400B-8, 400 W), a power source (HB400P-1) and a lamp jacket-quartz glass JW-2Q. The apparatus is connected with a water circulating system to cool the lamp.

### 4.3. Degradation of sodium alginate by photochemical reaction

Sodium alginate (10 mg) was dissolved in 1 mL water with 1 mg of titanium dioxide ($TiO_2$) particles and closed loosely by a screw cap. The sample tube (borosilicate glass) was then placed in the photochemical reaction tank and was exposed to UV-light. The alginate was degraded at room temperature and the light exposure time was varied between 1 h and 6 h. A mechanical stirrer was used in addition to a magnetic stirrer to ensure the dissolution of air into the solution. After the reaction, the sample was centrifuged at 1500*g* for 5 min at 20 °C and the supernatant was filtered through 0.45 μm membrane filter to eliminate all of the $TiO_2$, and the product solution was dialyzed and lyophilized. The change in molecular weights of degraded alginate samples were monitored by PAGE.

The alginate sample solutions with different pH values were prepared to study the influence of pH on the degradation. An aqueous alginate solution, at a pH value of 4, was made by adjusting the pH of a 1% (w/w) solution with 10 mM hydrochloric acid using a mechanical stirrer. Alginate with pH value of 7 was made by adjusting the pH of 1% (w/w) sample solution with 10 mM ammonium hydroxide. Alginate with pH value of 10 was made by adjusting the pH of a 1% (w/w) sample solution with 100 mM ammonium hydroxide.

### 4.4. Gel electrophoresis

PAGE was carried out in 15% polyacrylamide gels at 200 V for 1 h using Mini Protean® 2 system (Bio-Rad Laboratories, Inc., USA). Sodium alginate samples were dissolved in 50% (w/v) sucrose mixed with BPB 0.01% (w/v) (1:1 by volume). The resulting gels were visualized by staining with Alcian blue. Molecular mass markers consisted of dextran sulfate (500 kDa and 10 kDa) and hyaluronan (100 kDa).

### 4.5. Estimation of the average molecular weight

The average molecular weights of sodium alginate samples were estimated by HPSEC system consisted of a Hitachi L-600 pump (Hitachi Seisakucho Co., Japan), Rheodyne 7725i loop injector (USA) and an YRD-880 refractive index detector (Shimamura Instruments Co., Japan). The column used was an Asahipak 510 HQ column (7.6 mm, i.d. × 300 mm) (Showdex Co. Ltd, Tokyo) and eluted with 10 mM ammonium bicarbonate at a flow rate of 0.3 mL/min. The calibration curve for molecular weight estimation was performed using T-series dextran standards.

### 4.6. Fractionation of sodium alginate

The fractionation, of alginate decomposed by hydrolysis and by photolysis, was performed according to Haug et al., with slight modifications.[28] Hydrolysis of sodium alginate was carried out in 1.0 M oxalic acid at 100 °C for 10 h.[9,10] Each decomposed alginate sample obtained by acid hydrolysis and photolysis was dissolved in water and acidified to pH 0.85 with 1 M hydrochloric acid. The precipitate was collected to obtain fraction A and the supernatant was recovered to obtain fraction B. Fraction A was further separated after re-dissolving the precipitate by neutralizing with 0.1 M NaOH, and the solution was acidified to pH 3, by the addition of 1 M hydrochloric acid, to form a suspension. The suspension was then separated by centrifugation at 3000*g* for 5 min at 4 °C and the precipitated fraction (A-I) and the supernatant were obtained. The supernatant was adjusted to pH 0.85 and centrifuged at 3000*g* for 5 min at 4 °C to obtain A-II (precipitate) and A-III (the supernatant), which was collected and freeze dried. All products were neutralized before dialysis (MWCO 500) and then freeze-dried.

### 4.7. Structural analysis by $^1$H NMR

The sample was kept in a desiccator over phosphorus pentoxide in vacuo overnight at room temperature. The thoroughly dried sample was then dissolved in 0.5 mL of $D_2O$ (99.96%), centrifuged at 2000*g* for 15 min and transferred to an NMR tube (5.0 mm o.d. × 25 cm; Wilmad Glass Co. (Buena, NJ)). NMR experiments were performed on a JNM-400A spectrometer equipped with a 5-mm field-gradient tunable probe with standard JEOL software at 30 °C and 60 °C for all experiments on 500 μL samples. The HOD signal was suppressed by pre-saturation for 3 (30 °C) or 1.5 s (60 °C).


## Acknowledgements

The authors are grateful to KIMIKA Corporation for a gift of sodium alginate samples. This work was supported in part by Grants-in-Aid from the Ministry of Culture, Sports and Education of Japan (20590032 (T.T.)).


## References


1. Uno, T.; Hattori, M.; Yoshida, T. *Biosci., Biotechnol., Biochem.* **2006**, *70*, 3054–3057.
2. Tsuchida, T.; Chaki, T.; Hiura, N.; Satou, R. *Med. Cons. New-Remed.* **2001**, *38*, 555–560.
3. Kawada, A.; Hiura, N.; Tajima, S.; Takahara, H. *Arch. Dermatol. Res.* **1999**, *291*, 542–547.
4. Kawada, A.; Hiura, N.; Shiraiwa, M.; Tajima, S.; Hiruma, M.; Hara, K.; Ishibashi, A.; Takahara, H. *FEBS Lett.* **1997**, *408*, 43–46.
5. Iwamoto, M.; Kurachi, M.; Nakashima, T.; Kim, D.; Yamaguchi, K.; Oda, T.; Iwamoto, Y.; Muramatsu, T. *FEBS Lett.* **2005**, *579*, 4423–4429.
6. Haug, A.; Myklestad, S.; Larsen, B.; Smidsrød, O. *Acta Chem. Scand.* **1967**, *21*, 768–778.
7. Draget, K. I.; Bræk, G. S.; Smidsrød, O. *Carbohydr. Polym.* **1994**, *25*, 31–38.
8. Heyraud, A.; Gey, C.; Leonard, C.; Rochas, C.; Girond, S.; Kloareg, B. *Carbohydr. Res.* **1996**, *289*, 11–23.
9. Haug, A.; Larsen, B. *Acta Chem. Scand.* **1962**, *16*, 1906–1918.
10. Haug, A.; Larsen, B.; Smidsrød, O. *Acta Chem. Scand.* **1966**, *20*, 183–190.
11. Suzuki, H.; Suzuki, K.; Inoue, A.; Ojima, T. *Carbohydr. Res.* **2006**, *341*, 1809–1819.
12. Zhang, Z.; Yu, G.; Guan, H.; Zhao, X.; Du, Y.; Jiang, X. *Carbohydr. Res.* **2004**, *339*, 1475–1481.
13. Sawabe, T.; Ohtsuka, M.; Ezura, Y. *Carbohydr. Res.* **1997**, *304*, 69–76.
14. Wong, T. Y.; Preston, L. A.; Schiller, N. L. *Annu. Rev. Microbiol.* **2000**, *54*, 289–340.
15. Wasikiewicz, J. M.; Yoshii, F.; Nagasawa, N.; Wach, R. A.; Mitomo, H. *Radiat. Phys. Chem.* **2005**, *73*, 287–295.
16. Humphreys, E. R.; Howells, G. R. *Carbohydr. Res.* **1971**, *16*, 65–69.
17. Smidsrød, O.; Haug, A.; Larsen, B. *Acta Chem. Scand.* **1963**, *17*, 2628–2637.
18. Shinohara, M.; Kamono, H.; Aoyama, T.; Bando, H.; Nishizawa, M. *Fish. Sci.* **1999**, *65*, 816–820.
19. Holmea, H. K.; Lindmoa, K.; Kristiansena, A.; Smidsrød, O. *Carbohydr. Polym.* **2003**, *54*, 431–438.
20. Matsushima, K.; Minoshima, H.; Kawanami, H.; Ikushima, Y.; Nishizawa, M.; Kawamukai, A.; Hara, K. *Ind. Eng. Chem. Res.* **2005**, *44*, 9626–9630.
21. Chiron, S.; Fernandez-Alba, A.; Rodriguez, A.; Garcia-Calvo, E. *Water Res.* **2000**, *34*, 366–377.
22. Carp, O.; Huisman, C. L.; Reller, A. *Prog. Solid State Chem.* **2004**, *32*, 33–177.
23. Mills, A.; LeHunte, S. J. *Photochem. Photobiol., A* **1997**, *108*, 1–35.
24. Waki, K.; Zhao, J.; Horikoshi, S.; Watanabe, N.; Hidaka, H. *Chemosphere* **2000**, *41*, 337–343.
25. Fujishima, A.; Rao, T. N.; Tryk, D. A. J. *Photochem. Photobiol., C* **2000**, *1*, 1–21.
26. Agustina, T. E.; Ang, H. M.; Vareek, V. K. J. *Photochem. Photobiol., C* **2005**, *6*, 264–273.
27. Penman, A.; Sanderson, G. R. *Carbohydr. Res.* **1972**, *25*, 273–282.
28. Haug, A.; Larsen, B.; Smidsrød, O. *Carbohydr. Res.* **1974**, *32*, 217–225.
29. Haug, A.; Larsen, B.; Smidsrød, O. *Carbohydr. Res.* **1979**, *68*, 3–31.
30. Grasdalen, H. *Carbohydr. Res.* **1983**, *118*, 255–260.