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Note

Isolation and Characterizartion of Alginic Acid from Commercially Cultured *Nemacystus decipiens* (Itomozuku)

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An alginate was isolated from commercially cultured *Nemacystus decipiens* which had been harvested in Yonashiro Town (Okinawa, Japan). The yield of the alginate was 1.6% (w/w of wet alga), and the uronic acid, ash and moisture contents of the alginate were 86.0%, 12.0%, and 2.3% (w/w), respectively. The molecular mass of the alginate was estimated to be about 1.5×10^5. The infrared spectrum and optical rotation of the alginate were in agreement with those of the standard alginate. D-Mannuronic acid and L-guluronic acid were identified by ^{1}H- and ^{13}C-NMR spectroscopy, the molar ratio of both sugar residues being estimated to be 0.72:1.00.

Key words: alginic acid; Itomozuku; *Nemacystus decipiens*

Alginate is a major gelling carbohydrate polymer obtained from a number of brown seaweeds (Phaeophyceae) and is of considerable technological importance for both its solution properties and as a gelling agent.[1] Alginate is a linear, (1→4)-linked copolymer of α-L-guluronate and β-D-mannuronate, the relative proportions of which, and their sequence within the primary structure, show substantial variations between species, and between different tissues within the same plant.[2] We have proposed a gelation mechanism for alginate at the molecular level in an aqueous solution.[3]

In the previous study,[4] we isolated alginate which consisted of D-mannuronate and L-guluronate in a molar ratio of 0.27 to 1.00 from *Cladosiphon okamuranus* Tokida (Okinawamozuku) which had been commercially cultured on artificially seeded nets (1.5×20 m). The yield of alginate was estimated to be 0.1% (w/w) based on wet algae, while the acetyl fucoidan content was 2.3%. This suggested that fucoidan could be commercially produced on a simple industrial scale from artificially cultured Okinawamozuku. The annual production of this alga in Okinawa was reported to have been approximately 18,500 t in 1999.

Nemacystus decipiens Kuckuck (Itomozuku), one of the brown algae, is widespread on the shore between Akita and Okinawa Prefectures in Japan. In Okinawa Prefecture, the production of Itomozuku by artificially seeded culture nets has been carried out in the sea since 1979. The annual production of Itomozuku in Okinawa was reported to have been approximately 2,500 t in 1999. The seaweed is used together with Okinawamozuku in salad (Sunomono), so that their utilization by the food industry has been increasing. A fucoidan has recently been prepared on an industrial scale from Okinawamozuku and used as an additive to health foods and drinks in Japan.

We have previously isolated a fucoidan of about 0.5% in yield based on the wet seaweed from Itomozuku.[5] However, an alginate of the spontaneous and artificially cultured alga has not previously been studied. We therefore report here the isolation and identification of an alginate from artificially cultured Itomozuku.

Itomozuku, which was commercially cultured on nets (1.5×20 m) in the sea of sea off Yonashiro Town (Heanza Island, Okinawa) from November 1995 to May 1996, was used in this study. An alginate (45G, molar ratio of D-mannuronic acid to L-guluronic acid = 0.26:1.00; Kibun Food Chemipha Co., Japan) was used as the standard.

A wet seaweed sample (100 g) was homogenized with double its weight of 0.2 M HCl (200 g), stirred at room temperature for 2 h, centrifuged at 23,000 g for 20 min and filtered through Celite 545.[4,5] The residual precipitate was washed with distilled water and then suspended in a 3.0% Na_2CO_3 solution at room temperature for 3 h to extract the alginate. The extract was neutralized with 0.1 M HCl and then gelled by the addition of 2.0% $CaCl_2$. The gelatinous precipitate was separated by centrifugation at 23,000 g for 20 min. This precipitate was washed with distilled water, and then dissolved in a 1.0 M NaOH solution. The resulting solution was filtered through

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Celite 545 and then dialyzed overnight against distilled water. In the presence of NaCl (200 mg), ethanol (2 vols.) was added and the precipitate was dried *in vacuo*. The crude alginate (2.0 g) was dissolved in 300 ml of distilled water and passed through a column of Amberlite IR-120 (H^+) to deionize it, before being then neutralized with 0.5 M NaOH. The solution was dialysed against distilled water and then freeze-dried.

Uronic acid of the alginate was determined by the carbazol-sulfuric acid method,[6] using D-mannuronic acid (Sigma Co., U.S.A). High-performance liquid chromatography (SCL-6B; Shimadzu Co., Japan) with a column of TSKgel G3000PWXL (7.8×300 mm, Tosoh Co., Japan) was carried out to estimate the molecular mass. Pullulan P-20, P-100, and P-400 (Showa Denko Co., Japan) of Mw 2.28×10^4, 1.12×10^5 and 4.05×10^5, respectively, were used as molecular weight markers, detection being made with a refractive index detector. Infrared spectra (FT-IR) were recorded with an IR-8200 infrared spectrophotometer (Shimadzu Co., Kyoto, Japan) for a sample dispersed on KBr discs. Optical rotation was measured at 589 nm by a DIP-180 polarimeter (Jasco Co., Tokyo, Japan) for a 0.2% (w/v) solution.

^{1}H- and ^{13}C-NMR spectra were recorded by an FT-NMR spectrometer (JNM-α500, JEOL Co., Tokyo, Japan) at 500.00 and 125.65 MHz. Alginate (2.0%) isolated from Itomozuku was dissolved in 4 ml of D_2O, and data were recorded at 80°C. The ^{1}H- and ^{13}C-NMR chemical shifts are expressed in parts per million (ppm) relative to the internal sodium 3-(trimethylsilyl)propionic-2,2,3,3-d4 (TSP, 0.00 ppm).

The Itomozuku sample reached 20–30 cm long having a cylindrical axil, the thick part of which was about 1.5 mm in diameters with a sharp tip. The alginate was prepared and purified as already described. This alginate obtained from Itomozuku was a colorless fibrous powder in a yield of 1.6% (w/w; based on the wet alga). The uronic acid, ash, and moisture contents were 86.0%, 12.0%, and 2.3%, respectively (Table 1).

Liquid chromatography, using standard pullulan, gave a value of approximately 1.5×10^5 for the molecular mass (Fig. 1). The infrared spectrum of the alginate isolated from Itomozuku was measured (not shown in Figure), the spectrum being in agreement with that of the standard alginate (45G). The optical rotation of the alginate showed a value of $-0.103°$ at a temperature of 50°C, then it increased a little with decreasing temperature, before showing a value of $-0.098°$ at 20°C. These values are in agreement with those of the standard alginate.

The ^{1}H nuclear magnetic resonance (NMR) spectrum at 500 MHz of the alginate isolated from Itomozuku is shown in Fig. 2. Signal assignment of the alginate was achieved by the comparing with previously reported chemical shifts.[7-9] The spectrum

Table 1. Chemical Components of the Alginate Isolated from *Nemacystus decipiens*

% (w/w)

	Uronic acid	Ash	Moisture
Alginate	86.0	12.0	2.3

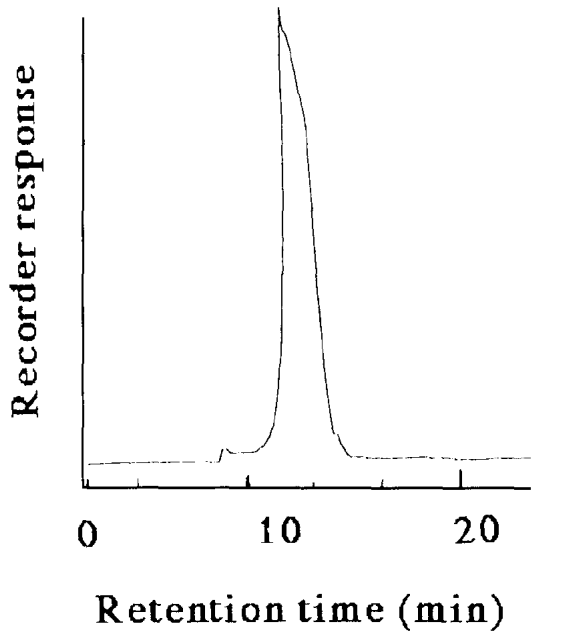

Fig. 1. Gel Filtration Chromatogram of Alginic Acid from Itomozuku.

Column, TSKgel G4000PWL (7.8×300 mm), eluent, 150 mM sodium chloride in a 50 mM phosphate buffer (pH 7.0); eluent flow, 0.5 ml/cm.

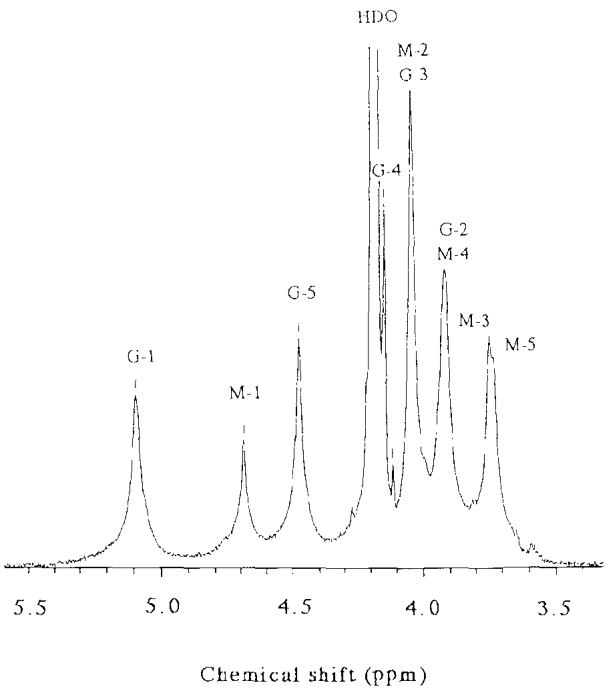

Fig. 2. ^{1}H-NMR Spectrum of Alginic Acid from Itomozuku in D_2O at 80°C.

Abbreviations: M, D-mannuronic acid; G, L-guluronic acid.

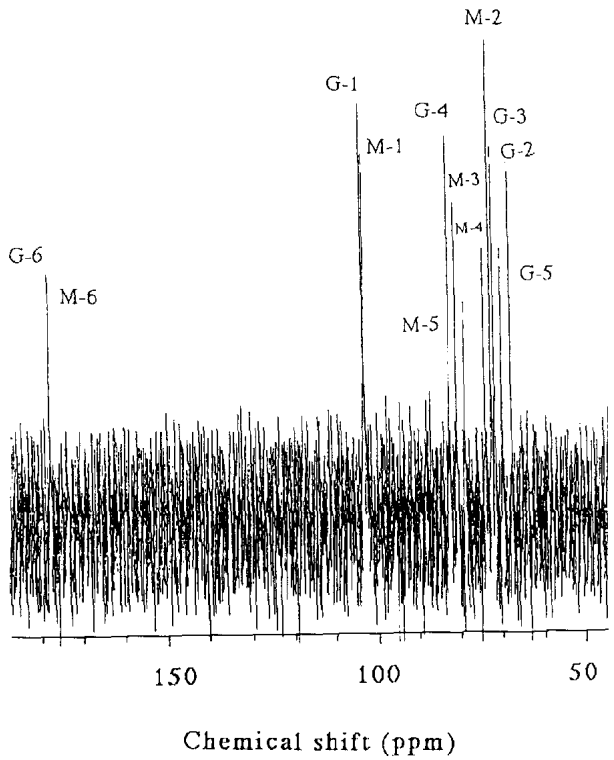

Fig. 3. ^{13}C-NMR Spectrum of Alginic Acid from Itomozuku in D_2O at 80°C.
Abbreviations: M, D-mannuronic acid; G, L-guluronic acid.

showed ten peaks (M1, 4.67; M2, 4.03; M3, 3.74; M4, 3.90; M5, 3.72; G1, 5.07; G2, 3.90; G3, 4.03; G4, 4.13; G5, 4.46 ppm). The chemical shifts are in agreement of those of the standard alginate (45G). The chemical shifts of the anomeric protons were assigned at 5.07 (L-guluronic acid) and 4.67 (D-mannuronic acid) ppm. The molar ratio of D-mannuronic acid to L-guluronic acid for the alginate was estimated from the signal area of both the anomeric protons to be 0.72:1.00.

As shown in Fig. 3, the ^{13}C-NMR spectrum of the alginate from Itomozuku indicated twelve peaks. The ^{13}C-NMR spectrum of the alginate had resolved chemical shifts in the carbonyl carbon region of 175.75 (G6) and 175.58 (M6) ppm,[10,11] in the anomeric region of 101.44 (G1) and 100.97 (M1) ppm, respectively, and of 66.04 (G2), 70.83 (M2), 70.01 (G3), 72.29 (M3), 60.71 (G4), 78.94 (M4), 68.18 (G5), 77.01 (M5) ppm. These data are consistent with those of the standard alginate (45G). The chemical shifts were assigned to the repeating unit of L-guluronic acid-rich alginate.[12,13] No any other chemical shift assigned to a carbon atom was evaluated.

Alhough Itomozuku is widespread between Akita and Okinawa Prefectures, the production of the alga has been decreased annually by an effect probably caused by sea pollution. However, the alga has been artificially cultured on a commercial scale off the Okinawa Islands since 1979. The optimum depth for growth of the alga has been estimated to be 2 meters below the low-tide level. The harvest from the one sheet of a culture-net (1.5 × 20.0 m) of the alga is about 100 kg in wet weight.

Itomozuku contains a fucoidan of about 0.5% yield based on the wet seaweed.[5] This fucoidan consisting of L-fucose, D-galactose and sulfuric acid in the molar ratio of 1.0:0.1:1.0. Fucoidans seem to have such biological activities as anti-coagulant,[14] anti-thronbin,[15] anti-tumor[16] and anti-HIV[17] infection, because of the high number of substituting sulfate groups. Thus, fucoidan and alginate can be produced on an industrial scale from *Itomozuku* artificially cultured in Okinawa, Japan.

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