

Effects of Hydrolysis Time on the Molecular Weight Distribution of Alginates Prepared from Sea Tangle *Laminaria japonicus*

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To prepare oligouronic acids from high-molecular-weight alginates, sea tangle *Laminaria japonicus* alginates were hydrolyzed at 80°C for various hydrolysis times (HT). The effects of hydrolysis time on the distribution ratios (DR) of the molecular weights (MW) of the hydrolyzed alginates were investigated. As HT increased, the DR of the alginates with MW>500 kDa decreased exponentially, while those with MW=50-100 kDa or MW<50 kDa increased exponentially. For MW=300-500 kDa, DR increased exponentially until HT reached 60 min, and then decreased exponentially. Similarly, for MW=100-300 kDa, DR increased exponentially until HT reached 90 min, and then decreased.

Key words: Alginates, Molecular weight, Distribution ratio, *Laminaria japonicus*, Hydrolysis time

Introduction

Alginates are binary copolymers of D-mannuronic acid and L-guluronic acid residues arranged in a block-wise pattern (Haug et al., 1967). This material is a linear polymer composed of (1→4)-linked β-D-mannuronopyranosyl and α-L-guluronopyranosyl units arranged in both homo- and heteropolymeric sequences (Haug et al., 1966, 1967; Penman and Sanderson, 1972; Sime, 1990). Recently, many studies of alginates have examined their physiological functions (Hidaka et al., 1986; Fujihara and Nagumo, 1993; Hideki et al., 1993; Suzuki et al., 1993; Fujiki et al., 1994; Hajime et al., 1994; Kobayashi et al., 1997), which are affected by their molecular weights and uronic acid composition (Kobayashi et al., 1997; You et al., 1997). Numerous studies have been devoted to the analysis of their uronic acid composition. In contrast, there are few studies of the effects of the distribution ratio (DR) of alginate molecular weight (MW) on the physicochemical properties of alginates. Both the molecular weight and the uronic acid composition vary with algal species, and the type and age of the tissues used to prepare the alginates (You and Lim, 2003; You et al., 2004). In addition, the molecular weights depend on the extraction conditions used for their preparation.

Therefore, control of the molecular weight of alginates is important in the alginates industry.

There are many methods of measuring the molecular weights of alginates, including light scattering (Turquois and Gloria, 2000), high-performance size-exclusion chromatography (Nagasawa et al., 2000), gel permeation chromatography (Hein et al., 2000), and calculating molecular weight by measuring the intrinsic viscosity of the alginate solution (Mancini et al., 1996; Chen et al., 1997; You and Lim, 2003; You et al., 2004). Frequently, these methods require expensive equipment beyond the means of small-to-medium-sized enterprises. Moreover, there are many difficulties in deducing the molecular weight of alginates from the intrinsic viscosity, because other components (e.g., ash, metals) contained in alginates can affect the viscosity of an alginate solution (You et al., 2004). Therefore, determining the degree of polymerization (DP) of alginates by measuring the reducing power of uronate residues is one way to evaluate the molecular weights. Very recently, we reported that determining the DP is very useful for evaluating the average molecular weights of alginates (You et al., 2004).

In this study, to prepare oligouronic acids from high-molecular-weight alginates, sea tangle alginates were hydrolyzed for various times and the distributions of the molecular weights of the resulting alginates were measured.

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Materials and Methods

Extraction and purification of alginates

The procedure used to extract alginates from sea tangle was based on the method described by You and Lim (2003). Dried powdered (60 mesh) sea tangle (*Laminaria japonica*) was suspended in 50x 0.5% Na₂CO₃ solution (w/v). The suspension was shaken in a water bath at 60°C for 6 hours, after which the mixture was filtered through a cloth. To precipitate alginates, the same volume of methanol was added to the filtrate. The precipitated alginates were washed three times with methanol and lyophilized. The dried alginates were ground and stored at 5°C.

Hydrolysis of alginates

The 1% alginates solution made from the prepared alginates was adjusted to pH 5.0 with citric acid. Then, it was hydrolyzed in a shaking water bath at 80°C for hydrolyzing times (HT) of 10, 20, 30, 40, 60, 90, or 120 minutes.

Fractionating the alginates by molecular weight (MW)

The hydrolyzed alginates were fractionated using ultrafiltration membranes (Millipore Corp., Billerica, MA, USA) that established five MW cutoffs: below 50, 50-100, 100-300, 300-500, and over 500 kilodaltons.

Determining the average molecular weight (AMW) of the hydrolyzed alginates

The AMWs of the hydrolyzed alginates were determined using the method described by You and Lim (2003).

Measuring the ash, uronic acid, and water contents

The ash and water contents of the hydrolyzed alginates were measured using AOAC (1995) methods. The uronic acid content was evaluated using a previously reported method (You et al., 2004).

Results and Discussion

The physical properties of the alginates prepared from sea tangle are shown in Table 1. The ash content was 21.5%, the uronic acid content was 77.3%, and the AMW was 1,013 kDa. Table 2 shows the distribution ratios (DR) of the MW classes of the alginates before hydrolysis. Of the alginates, 67.4% had MW>500 kDa, with an AMW of 1,307 kDa, followed by 20.2% with MW=300-500 kDa and an AMW of 499 kDa.

The DRs of the MW classes of the alginates hy-

Table 1. The physical properties of alginates used in this study^{1,2)}

Ash (g/100 g)	Uronic acid (g/100 g)	AMW (kDa)
21.5±0.3	77.3±0.7	1,013±12

¹The alginates were extracted from sea tangle with 0.5% sodium carbonate solution for 6 hr at 60°C.

²All data were expressed as mean±SE of dry basis with 5 replications.

Table 2. The distribution ratio of MW cut-off sizes in alginates used in this study^{1,2)}

MW cut-off (kDa)	Distribution (%) ³⁾	AMW (kDa)
>500	67.4±3.7 ^a	1,307±13 ^a
300-500	20.0±2.2 ^b	499± 8 ^b
100-300	10.2±1.5 ^c	299± 5 ^c
50-100	1.8±0.5 ^d	99± 7 ^d
<50	0.6±0.2 ^e	49± 6 ^e

¹The alginates were extracted from sea tangle with 0.5% sodium carbonate solution for 6 hr at 60°C.

²All data (mean±SE with 5 replications) within a column with difference superscripts were significantly difference at p<0.05.

³The distribution ratios were expressed as the percentage of content (mg/100 g) of alginates having a certain MW cut-off size to total alginates content (mg/100 g).

drolyzed at 80°C are listed in Table 3. After the alginates were hydrolyzed for 10, 20, 30, or 40 min, the alginates with MW>500 kDa constituted 58.4, 49.4, 40.3, and 31.4%, respectively, with AMWs of 1,204, 1,101, 997, and 894 kDa. For HT of 10 to 40 min, as HT increased, the DRs of alginates with MW >500 kDa and their AMWs decreased gradually. For HT of 60 min, the alginates with MW=300-500 kDa had the highest DR (32.5%) and an AMW of 387 kDa. For HT of 90 min, the highest DR (30.8%) was for MW=100-300 kDa, with an AMW of 190 kDa. After hydrolysis for 120 min, the alginates with MW=50-100 kDa had the highest DR (34.8%), with an AMW of 80 kDa. For HT of over 60 min, as HT increased, the MW cutoff size of the alginates with the highest DR decreased.

The changes in the AMW of alginates hydrolyzed at 80°C for various times before or after fractionation by MW are shown in Fig. 1. Before fractionation, as HT increased, the AMW of the hydrolyzed alginates decreased exponentially. The change in the AMW of the alginates fit equation (1):

$$\text{AMW}=1,016.6 \exp(-0.019 \text{ HT}), r^2=0.996, \quad (1)$$

where r^2 is the correlation coefficient.

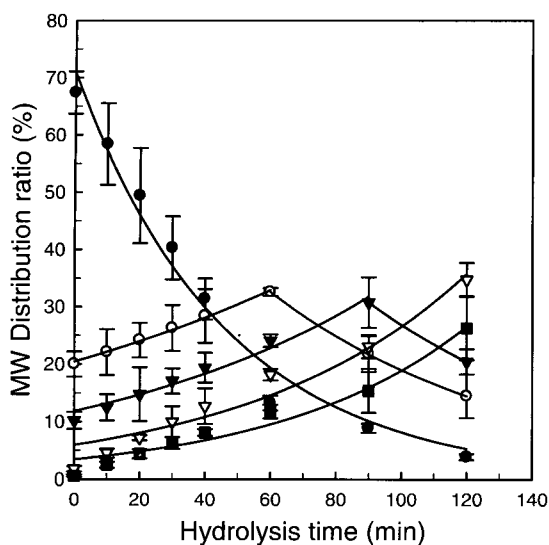


Fig. 2. The relationship between HT and MW distribution ratio in alginates hydrolyzed at 80°C and pH 5.0.

- MW 500 kDa over
10-120 min: $DR=70.9 \exp(-0.022 \text{ HT}), r^2=0.980$
- MW 300-500 kDa
10-60 min: $DR=20.5 \exp(-0.008 \text{ HT}), r^2=0.994$
60-120 min: $DR=73.2 \exp(-0.014 \text{ HT}), r^2=0.999$
- ▼ MW 100-300 kDa
10-90 min: $DR=11.8 \exp(-0.011 \text{ HT}), r^2=0.976$
90-120 min: $DR=104.5 \exp(-0.014 \text{ HT}), r^2=0.999$
- ▽ MW 50-100 kDa
10-120 min: $DR=6.0 \exp(-0.015 \text{ HT}), r^2=0.950$
- MW 50 kDa below
10-120 min: $DR=3.4 \exp(-0.017 \text{ HT}), r^2=0.962$

The exponent for the decrease in the AMW of the hydrolyzed alginates before fractionation by MW was -0.0019. After fractionation, the rates were -0.009, -0.005, -0.004, -0.002, and -0.001 for decreasing MW, respectively. These data indicate that the smaller the MW of the alginates, the less the decrease in their AMW and the more difficult it was to hydrolyze them.

Figure 2 shows the variation in the DR of the fractionated alginates hydrolyzed at 80°C for various times. As HT increased, the DR of the alginates with MW > 500 kDa decreased exponentially, according to equation (2).

$$DR=70.9 \exp(-0.222 \text{ HT}), r^2=0.980, \quad (2)$$

where HT is the hydrolysis time (min) at 80°C. The DR for the MW fractions 50-100 kDa and below 50 kDa increased exponentially, following equations (3) and (4), respectively.

$$DR=6.0 \exp(0.015 \text{ HT}), r^2=0.950 \quad (3)$$

$$DR=3.4 \exp(0.017 \text{ HT}), r^2=0.962 \quad (4)$$

The decrease in the DRs of the high-MW alginates with increasing HT agrees with the results of Haug and Larsen (1962), who also reported a loss of uronic acids during hydrolysis due to the breakdown of free uronic acids, which led to the formation of products with reducing power that formed colors with phenol-sulfuric acid. Therefore, a considerable portion of the material in alginates with MW < 50 kDa was products formed by the breakdown of free uronic acids.

The DR for MW 300-500 kDa increased exponentially until HT reached 60 min, and then decreased exponentially for longer HTs. The increase and decrease fit equations (5) and (6), respectively:

$$DR=20.5 \exp(0.008 \text{ HT}), r^2=0.994 \quad (5)$$

$$DR=73.2 \exp(-0.014 \text{ HT}), r^2=0.999. \quad (6)$$

The DR for MW=100-300 kDa followed a trend similar to the case MW=300-500 kDa: the DR increased exponentially until HT reached 90 min, and then decreased for longer HTs. The increase and decrease fit equations (7) and (8), respectively:

$$DR=11.8 \exp(0.011 \text{ HT}), r^2=0.976 \quad (7)$$

$$DR=104.5 \exp(-0.014 \text{ HT}), r^2=0.999. \quad (8)$$

Overall, the lower the MW of the alginates was, the greater the increase in the DR of the MW.

For all equations, the correlation coefficient (r^2) exceeded 0.95. The equations derived from the experimental data are very important for the production of alginates of a certain MW, because the manufacturer can use them to determine the appropriate hydrolysis conditions and the HT.

The MW cutoffs of alginates with the maximum DR for hydrolysis at 80°C are listed in Table 4. For HTs from 0 to 40 min, the MW cutoff of alginates with the greatest DR was over 500 kDa. For HT=60, 90, and 120 min it was 300-500, 100-300, and 50-100 kDa, respectively.

Table 4. The changes of maximum MW distribution in alginates hydrolyzed at 80°C and pH 5.0

Hydrolysis time (min)	MW cut-off (kDa)	Distribution ratio ¹ (%)	AMW (kDa)
before	>500	67.4±3.7	1,307±13
10	>500	58.4±7.1	1,204±11
20	>500	49.4±8.3	1,101±15
30	>500	40.3±5.5	997±17
40	>500	31.4±3.6	894±15
60	300-500	32.6±0.7	387±19
90	100-300	30.8±4.4	190±12
120	50-100	34.8±2.9	80±10

¹Refer to Table 2.

Acknowledgments

This research was supported by a grant from East Coastal Marine Bioresources Research Center in 2005.

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(Received June 2005, Accepted September 2005)