J. Biol. Macromol., **10** (1), 3-11 (2010)

Regular Paper

Differences in Excluded Volume, Diffusion Coefficient, and Surface Charge of Alginates with Different Mannuronate to Guluronate Ratio

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Received September 9, 2009; Accepted October 19, 2009

Abstract

The difference in excluded volume of two types of alginates having high mannuronate (M) content (500M) and high guluronate (G) content (500G) were measured with gel filtration and viscosity together with dynamic and electrophoretic light scattering method. The molecular weight of both alginates was almost the same. The pattern of gel filtration of 500M eluted faster than 500G showing that the excluded volume of 500M is larger than 500G. The reduced viscosity of 500M was larger than 500G. The diffusion coefficient of 500M was smaller than 500G. Zeta-potential showing surface charge of molecular domain was about -60 mV for 500M and about -50 mV for 500G. These results may be attributed to the fact that the carboxyl groups of MM block of 500M exposed from the polymer chain are separated from each other and the 500M molecule takes the flexible structure as revealed by molecular mechanics program 3 (MM3). On the other hand, the carboxyl groups of one guluronic acid of GG block of 500G can form the hydrogen bond with the hydroxyl group on other guluronic acid and therefore the polymer chain of 500G takes more compact structure than the flexible structure. For these reasons, the excluded volume of 500G become smaller than 500M and the surface charge of 500G molecule indicates smaller negative value than 500M.

Key words: alginate, mannuronate to guluronate ratio, excluded volume, diffusion coefficient, zeta-potential

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Introduction

Alginate is an important commercial polysaccharide prepared from brown algae. It is a copolymer of 1,4-linked D-mannuronic

acid (M) and L-guluronic acid (G) consisting mainly of blocks of each and of alternating copolymer [1-4]. The exact composition depends on algal source, algal tissue and also season [1-4]. Alginate is used commercially for its ability to form very viscous solutions and gels with divalent and trivalent metal cations.

The uronic acid residues in alginate are arranged in the chains as homopolymeric blocks of M and G interspersed with sequences. The selectivity for cations and the gel forming properties of alginate are correlated with the content of G-blocks [4]. The enzymes mannuronan C-5 epimerases catalyse the in-chain epimerization of β-D-mannuronic acid to α-L-guluronic acid in the last step of alginate biosynthesis [5]. The technologically important physical properties of alginate in solution and/or gel are largely controlled by the relative amounts of the type of block present. Recently, Storz et el. [6]. analyzed about the ultra-high viscosity and highly biocompatible alginate in high salt buffer condition such as 150mM NaCl with fluorescence, NMR, viscometry and multi-angle light scattering (MALS) methods and showed that the monomer composition of alginate had no effect on coil expansion. However, the physical properties of alginate with different block compositions M and G have not yet been studied in details. In the present paper, the difference of the excluded volume measured with gel filtration and viscosity method of two types of alginates having high rich M content (500M) and high rich G content (500G) was discussed together with the data of diffusion coefficient measured with dynamic light scattering method, and surface charge measured with electrophoretic light scattering method.

Materials and Methods

Materials

The sodium alginates (bioreactor grade) were purchased from Kibun Food Chem. Ltd. (Tokyo, Japan) [7]. The ratio of mannuroic acid to guluronic acid residues (M/G) was determined using the method of Huang et al. [2]. The results are shown in Table 1. pH was measured with pH meter (Mettler-Toledo type Seven Easy) at 1 mg/ml alginate concentration in 10 mM NaCl.

Table 1 Analytical data of sodium alginate samples

Type	500M	500G
Viscosity(mPa·s)[1)]	568	550
pH	6.6	6.7
M/G ratio[2)]	1.05	0.41
$Mw \times 10^{-5}$	1.08	1.04

[1)]cited from catalog of Kibun Food Chem. Ltd.(alginate concentration is 1 g/100 ml at 20 ℃)

[2)]cited from catalog of Kibun Food Chem. Ltd.

The heavy metal was below 20 μg/g in each sample. All other reagents were of the purest grade available. After two types of alginate samples were dissolved in 0.01 M imidazole aqueous solution (pH 7.0), sodium

ions from alginates were removed with cation-exchange resin (Amberlite IR120B, Organo, Japan) [8]. The concentration of alginate was determined by Phenol-sulfuric method and prepared with 1 mg/ml. The carboxylate content of this solution was determined by titration with NaOH and the end point suggested one carboxylic acid per one monosaccharide.

Light scattering measurements

Measurements of the intensity of the light scattering were done with a modified Ellipsometer, an automatic light scattering analyzer AEP-100 (Shimadzu Co., Ltd., Japan), at 20°C [9-12]. The linearly polarized monochromatic incident light passed through the cylindrical scattering cell, and the light scattered at any scattering angle was detected through the linear analyzer by a photo-multiplier in a telescope arm that can rotate from 45° to 135° by a stepping motor.

The sample was dissolved in 0.2 M NaCl aqueous solution and diluted using same buffer solution from stock solution to desired concentration of alginate and was made optically clean by filtrating with a Millipore filter (pore size, 0.45 μm). The apparatus constant was obtained by using a solution of bovine serum albumin in 0.1 M phosphate buffer (pH 7.2), whose molecular weight was taken as 65,800 optically clarified through a Gelman (Germany) filter (pore size, 0.2 μm).

Gel filtration

Gel filtration was carried out on a Sepharose 6B column (Amersham Pharmacia Co. Ltd.) in 5 mM NaCl solution. Alginate concentration in each elution tubes was detected by optical density at 480 nm with Phenol-sulfuric method [13].

Viscosity

Viscosity measurements were carried out with an Ostwald-type viscometer at 25 °C. Flow time was about 120 s for pure water [14]. The viscometer was at first rinsed with concentrated nitric acid and then washed exhaustively with pure water. Each alginate solution of several concentrations was made by diluting the stock solution with 10 mM NaCl solution.

Dynamic light scattering measurement

The diffusion coefficients of alginate solutions were measured with the dynamic light scattering instrument of a light scattering analyzer ELS-800 (Ohtsuka Co., Ltd., Japan) at 20 °C [11]. He-Ne laser of 632.8 nm, and the scattering angle was 90°. The autocorrelation function of the photo-multiplier photocurrent was automatically analyzed by standard computer fitting techniques to obtain the translational diffusion coefficient of each solution of alginate [14]. The sample was made optically clean by filtering with a Millipore (Germany) filter (pore size, 0.45 μm).

Zeta-potential measurement

The zeta-potential of optically clean alginate by filtering with Millipore filter was measured with laser electrophoretic zeta-potential analyzer ELS-80 (Ohtsuka Co., Ltd., Japan) at 25 °C [15]. The photomultiplier was mounted on a computer-controlled goniometer by which the scattering angle could be varied over a range of 5-30°. An electrophoretic cell with a square cross section was used. The dimensions cell were 17 mm in length (in the direction of applied electric field), 10 mm in width (in that of the incident beam), and 2 mm in thickness (in that vertical to both of them). The mounting of the electrophoretic cell was designed so that it can be moved in the vertical direction and the scattering volume in the cell can be set at a mechanical precision of 0.01 mm. The temperature of the cell was controlled by the circulation of thermostated water through the mounting block of the cell. The cell holder is provided with a stage, the height of which is adjustable with an automated micrometer so that the laser beam path can be moved from the top to the bottom of the cell. Electroosmosis leading to flow with a parabolic velocity profile cannot be avoided in the case of a closed electrophoresis cell. The true mobility can be calculated by determining the apparent mobilities at various positions and the data points were fitted by the least-square method to obtain the velocities at the stationary layer.

Results and Discussion

Molecular weight of alginate samples

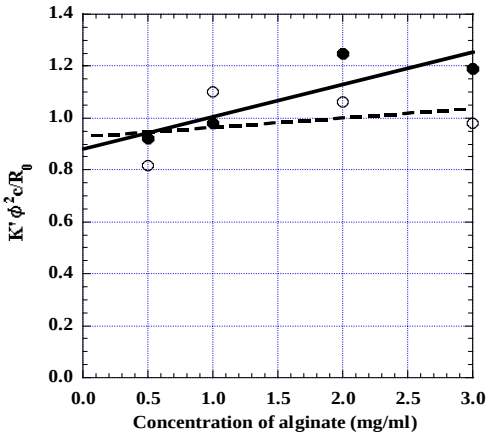

Fig. 1 Zimm plots of 500G (open circles) and 500M (closed circles) in 0.2M NaCl by laser light scattering method

The molecular weight of both alginate samples were determined by Zimm plot method of laser light scattering [16]. A grid-like Zimm plot method is normally used with both the angular dependence correlated to the radius of gyration and the concentration dependence associated with both the molecular weight and the second virial coefficient. The characterization of alginate samples such as in this paper is especially a necessity in the case of the latter case. The extrapolation of zero angle was done by the computer simulation method. As is shown in Fig.1, $K'Ø^2c/R_0$ was plotted against the weight concentration of alginate c, where K' is the optical constant, Ø is the refractive index increment and R_0 is the reduced scattering intensity at scattering angle equal to zero. The Ø was used as 0.154 ml/g for both alginate samples [17]. Fig. 1 gives the weight-average molecular weight

from the intercept of the ordinate and the second virial coefficient showing polymer-polymer and polymer-solvent interactions from the inclination of the straight lines. The data was little scattered. However, the concentration dependence of 500M was appreciably little higher than 500G. The linear extrapolation of zero concentration was done with the computer simulation method.

The molecular weight of both alginate samples was estimated as almost the same and approximate 1.0×10^5, as is shown in Table 1. The charged polyelectrolyte such as alginate is normally good soluble in water. Both alginate samples have a high negative charge and are highly soluble in water as a monomolecular molecule at the diluted concentration range. In this case, the second virial coefficient shows a positive value due to mainly the polymer-polymer repulsive interaction. At the much higher salt buffer condition, the alginate decreases the negative surface charge due to the charge shielding effect with salt. In this condition, the second virial coefficient shows almost zero. If the alginate forms intermolecularly the association, the second virial coefficient shows normally a negative value [18]. In the present paper, the second virial coefficient took a positive value in both cases and was attributed to mainly the negative charged polymer-polymer interaction. The positive second virial coefficient of 500M was larger than 500G, because the negative surface charge of 500M molecule has much higher than 500G molecule as is discussed later.

Gel filtration of alginate samples

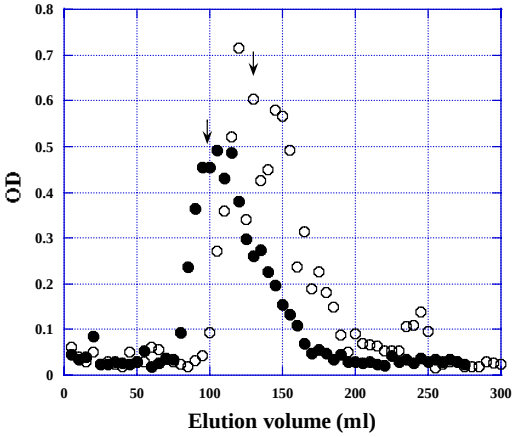

Fig. 2 Elution pattern of gel filtration of 500G (open circles) and 500M(closed circles)

Fig. 2 shows the elution pattern of gel filtration of both samples, where the horizontal axis shows the elution volume and the vertical axis indicates the optical density of each fraction by phenol sulfuric acid method. The 500M eluted faster than 500G. This means that the excluded volume of 500M is larger than 500G and the tertiary molecular domain structure of 500G takes more compact structure than 500M, although the molecular weight of both samples is almost same.

Viscosity of alginate samples

The reduced viscosity, η_{sp}/c, was calculated from the average values of three experimental times. Fig. 3 showed the concentration dependence of the reduced viscosity of both alginate samples. The reduced viscosity of 500G decreased almost straightly with the decrease of alginate concentration.

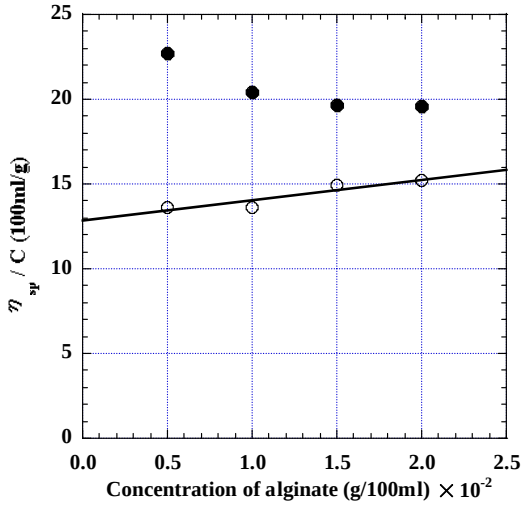

Fig. 3 Concentration dependence of reduced viscosity of 500G (open circles) and 500M (closed circles)

The reduced viscosity of 500M, however, increased monotonously with decreasing the concentration of alginate because the buffer was low ionic strength and 500M sample has higher negative surface charge than 500G, as is discussed later. The reduced viscosity of 500M was always larger than 500G. This result is good consistent with gel filtration chromatography, and the size of excluded volume for 500M is larger than 500G.

Matsumoto and Mashiko [19] showed that the values of the dynamic viscoelastic moduli, the storage modulus and loss modulus, are almost independent of M/G ratio of alginate and suggested that the viscoelastic properties of alginate aqueous solution are strongly influenced by the molecular weight and not by M/G ratio of alginate samples. This may be caused by the fact that the viscoelastic properties are measured at much higher concentration of the samples than the diluted measurements of gel filtration chromatography and/or the reduced viscosity in this paper.

Diffusion coefficient of alginate samples

The diffusion coefficient, D, of alginates was monitored with the quasi-elastic light scattering method [11,14]. The experiments were accumulated over 100 times for the same samples and repeated 3 times. The data were very reproducible.

The autocorrelater was connected to the light scattering analyzer and the autocorrelation function of the photo-multiplier photocurrent was automatically analyzed by standard computer fitting techniques to obtain the translational diffusion coefficient of alginates. The observed single-clipped photocount correlation function could be described by a single exponential curve. The translational diffusion coefficient was obtained by the first cumulant of the electric correlation function.

The diffusion coefficient was determined by the initial slope of autocorrelation function. The diffusion coefficient obtained was 2.51×10^{-10} cm^2/sec for 500G and 1.76×10^{-10} cm^2/sec for 500M, respectively. The diffusion coefficient of 500G is larger than 500M, indicating that the size of excluded volume of 500G is smaller than 500M. This result is very good coincident with the results of gel filtration and viscosity.

Surface charge of alginate samples

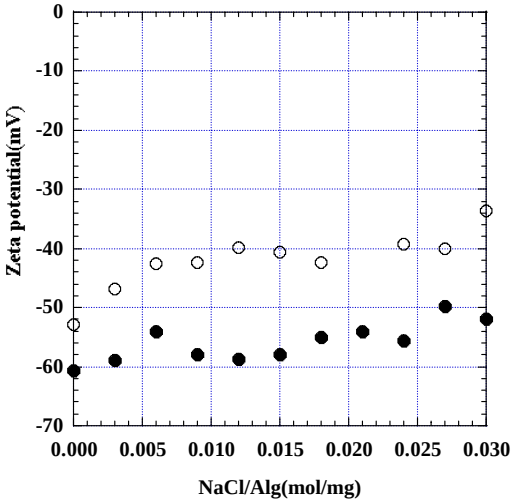

Fig. 4 Zeta-potential of 500G (open circles) and 500M (closed circles) by electrophoretic light scattering method.

The study of electrophoretic behavior of alginates affords the important and unique information about surface properties of alginate molecule. Zeta-potential was measured by electrophoretic light scattering method. As is shown in Fig. 4, zeta-potential was about -60 mV for 500M and about -50 mV for 500G, respectively. The negative surface charge of 500M was much higher than 500G. This result is coincident with the data of the higher second virial coefficient and also the stronger concentration dependence of the reduced viscosity of 500M than 500G.

Alginic acid is composed of GG block (guluronic acid-guluronic acid), GM block (guluronic acid-mannuronic acid), MG block (mannuronic acid-guluronic acid), MM block (mannuronic acid-mannuronic acid). The 500G alginate consists of many GG block because of having much G content. On the other hand, the 500M alginate consists of many MM block because of having much M content. As is shown in Fig. 5, the carboxyl groups in MM block is exposed from the polymer chain of alginate molecule and the carboxyl groups exposed are separated from each other because of the electrostatic repulsive force, where the polymer chain of alginate molecule takes the flexible structure [20]. On the other hand, the carboxyl group on one guluronic acid in GG block can form the hydrogen bond to the hydroxyl group on other guluronic acid, where the polymer chain of alginate takes more compact structure than the flexible structure and also the negative surface charge of alginate molecule in GG block becomes smaller than MM block.

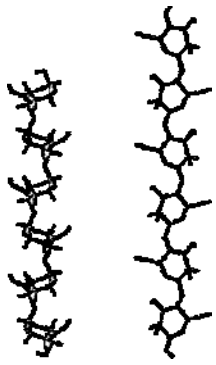

Fig. 5 Schematic illustration of alginic acid by molecular mechanics program 3 (MM3). Left chain shows GG block and right chain indicates MM block.

For these reasons, it is concluded that the excluded volume of 500G becomes smaller than 500M and the surface charge of 500G molecule indicates smaller negative value than 500M.

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Communicated by Yoko Nitta