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Effect of γ-Irradiation on Degradation of Alginate

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The aqueous solution of alginate was irradiated by ^{60}Co γ-rays in the dose range of 10−500 kGy. To assess the effect of irradiation on the degradation of alginate, the irradiation-induced changes in the viscosity, molecular weight, color, monomer composition, and sequence were measured. The molecular weight of raw alginate was reduced from 300000 to 25000 when irradiated at 100 kGy. The degradation rate decreased and the chain breaks per molecule increased with increasing irradiation dose. The viscosity of irradiated alginate solution reached a near minimum as low as at 10 kGy. No appreciable color changes were observed in the samples irradiated at up to 100 kGy, but intense browning occurred beyond 200 kGy. The ^{13}C NMR spectra showed that homopolymeric blocks, MM and GG, increased and the M/G ratio decreased with irradiation. Considering both the level of degradation and the color change of alginate, the optimum irradiation dose was found to be 100 kGy.

KEYWORDS: Alginate; oligosaccharides; irradiation; degradation

INTRODUCTION

Alginate is the polysaccharide obtained from brown seaweeds and is composed of (1−4)-linked β-D-mannuronic acid and α-L-guluronic acid (*1*). The monomers are arranged in a block structure, which may be homopolymeric block (M block, G block) or heteropolymeric block (MG block) (*2*). MG blocks form the most flexible chains. M blocks have been found to be strongly immunostimulating (*3*). G blocks form stiff chains and are cross-linked by divalent cations, resulting in gel formation (*3*). The relative amount of block-type varies with the source of alginate. The functional properties of alginate are found to be governed by the monomer composition (M/G ratio) and sequence (*4*).

Alginate has been widely used in the food and pharmaceutical industries because of its gel- and film-forming properties as well as its dietary fiber function (*5*). Alginate oligosaccharides have been prepared through depolymerization and reported to have bioactive functions, including stimulation of the growth of *Bifidobacteria* (*6*), growth promotion of plants (*7*, *8*), and prevention of hypertension (*9*). Alginate oligosaccharides are mainly utilized for their medical and agricultural applications.

Alginate has been previously depolymerized by acid (*1*) or enzymatic (*10*, *11*) hydrolysis. Acid hydrolysis is a common and fast method, but it has disadvantages, such as high cost, low yield, and spent acid (*12*). Enzymatic hydrolysis is preferred to acid hydrolysis because the hydrolysis can be controlled more easily and the chemical alteration of reaction products can be minimized. However, it is slow and expensive and thus unsuitable for commercial applications (*13*, *14*). Therefore, an irradiation depolymerization technique has been proposed as an alternative means for the mass production of alginate oligomers and monomers (*5*, *7*). The ionizing irradiation degrades polysaccharides through the free radical-induced scission of glycosidic bonds (*5*). Irradiation has been used for the degradation (*5*) and cross-linking (*15*) of polymers and the sterilization of food (*16*).

Alginate was irradiated at various doses to determine the effect of radiation on the alginate properties, such as molecular weight, viscosity, color, and monomer composition and sequence.

MATERIALS AND METHODS

Materials. Sodium alginate (low viscosity) derived from kelp (*Macrocystis pyrifera*) was purchased from Sigma (St. Louis, MO).

Irradiation. The aqueous solution (2% w/v) of alginate was irradiated by ^{60}Co γ-rays (Korea Atomic Energy Research Institute,

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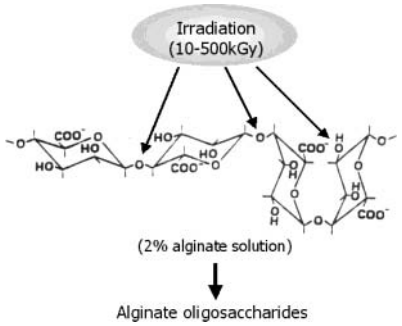

Figure 1. Scheme for the preparation of alginate oligosaccharides by irradiation.

Daejeon, Korea) in the dose range of 10−500 kGy at room temperature. **Figure 1** shows the scheme for the preparation of alginate oligosaccharides by irradiation.

Analytical Procedure. The molecular weights of alginate samples were determined by HPLC (Waters Corp., Milford, MA) using a gel permeation column (Ultrahydrogel Liner, Waters Corp.) and 0.1 M $NaNO_3$ aqueous solution as eluent. The elution flow rate and temperature were 0.7 mL/min and 40 °C, respectively. Molecular weights were calculated from a calibration curve using the standard poly(ethylene glycol) (PEG) and poly(ethylene oxide) (PEO). The viscosity of alginate solutions was measured at the shear rate of 66.68 s^{-1} by a rheometer (RS-150, Haake Inc., Karlsruhe, Germany) at 25 °C using a coaxial cylinder. To determine the degree of color change, L values (whiteness index) were measured by a colorimeter (JP 7200F, Color Techno System Co.). ΔL was calculated as the difference of L values between the control and corresponding irradiated sample. ΔL has been used as a means for measurement of browning of food.

^{13}C NMR measurements were carried out to determine the monomer composition and sequence of the alginate irradiated at 0, 10, and 200 kGy. Alginate samples were dissolved in D_2O at pD 7. The ^{13}C NMR spectra were recorded with a Bruker Avance DRX 500 spectrometer (Bruker, Karlsruhe, Germany) at a resonance frequency of 75 MHz and a spectral width of 18. The measurement temperature was 60 °C. The inverse-gated decoupling method was selected as irradiation mode to eliminate the nuclear Overhauser effect for the quantitative measurement of spectra peak areas.

Calculation of Chain Breaks per Molecule and Breakdown Rate Constant. The number of chain breaks per molecule was calculated according to the following equation: $N = (M_0/M) - 1$, where M_0 is the initial molecular weight of alginate and M is the molecular weight of irradiated alginate (*17*).

To determine the degree of alginate degradation by irradiation and the destructive irradiation dose level, the degradation was kinetically studied. The correlation coefficient (r^2) was calculated from the plot of the logarithm of the molecular weight versus time. The breakdown rate constant (K, min^{-1}) was calculated using the following equation: $K = -\ln(M/M_0)/t$, where t = irradiation time at 83.3 Gy/min (*18*).

Statistical Analysis. Changes of molecular weight, viscosity, and ΔL value were measured in triplicate with irradiated samples from the same starting molecular weight of alginate samples. The error bars in **Figures 2**, **3**, **5**, and **7** represent the standard deviations for the plotted means.

RESULTS AND DISCUSSION

Changes in Molecular Weight of Alginate by Irradiation. As shown in **Figure 2**, an exponential increase in the degradation of alginate occurred with an increase in irradiation dose until the dose level reached 200 kGy, from which no further degradation was observed. The molecular weight of raw alginate was reduced by irradiation from 300000 to 15000. From the point of practical utilization of irradiation to produce depolymerized alginate, the maximum depolymerization can be achieved at 200 kGy. The depolymerization of alginate by irradiation results from the breakage of glycosidic bonds (*5*, *19*).

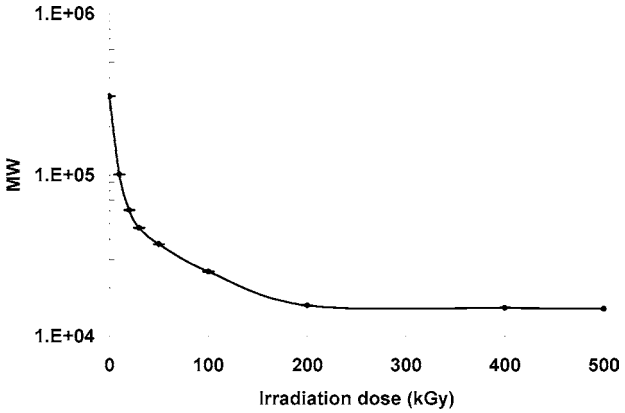

Figure 2. Changes in molecular weight of alginate with irradiation doses.

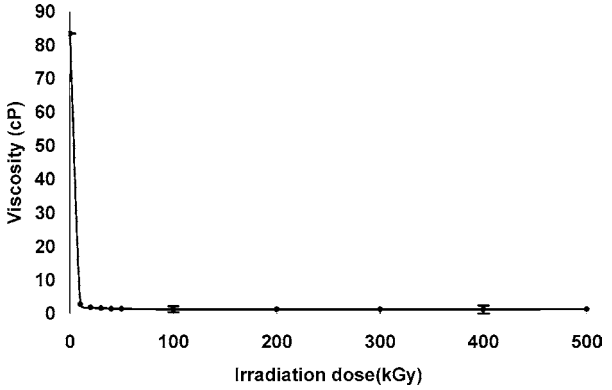

Figure 3. Changes in viscosity of alginate with irradiation doses.

The alginate solution was irradiated in air in this study because oxygen and water are important factors in the degradation of alginate. Nagasawa et al. (*5*) reported that the irradiation of an aqueous solution was more effective than that of a solid in reducing molecular weight. It is believed that the high mobility of alginate chains in aqueous solution may have prevented recombination of main chains. Free radicals generated by irradiation promoted the chain scission of alginate molecule (*5*). Zegota (*17*) reported that oxygen plays an important role in the degradation of polysaccharides. Peroxyl radicals formed by the addition of O_2 to primary carbohydrate radicals initiated the oxidative degradation and resulted in chain scission.

Changes in Viscosity of Irradiated Alginate. Upon irradiation, the viscosity of raw alginate solution sharply reduced from 83 to 2.75 and 1.3 cP at 10 and 200 kGy, respectively, with no further changes through 500 kGy (**Figure 3**). Similar results were obtained from other irradiated polysaccharide solutions, such as agar, chitosan, and carrageenan solutions (*17*). The reduction of viscosity by irradiation was a result of the breakage of marcromolecules (*5*). Irradiation-induced scissions of 1−4-glycosidic bonds of alginate reduced the molecular weight of the polymer, resulting in a sharp decrease in the viscosity of the alginate solution.

Color Change of Irradiated Alginate. Figure 4 shows the color changes of alginate solution by irradiation. The color of the alginate solution turned brown with increasing irradiation dose. The color change of irradiated alginate was not as severe as that of irradiated chitosan (*14*). The color did not change appreciably in the dose range of 0−100 kGy but changed markedly beyond 200 kGy. Low ΔL values without appreciable color change were obtained up to 100 kGy (**Figure 5**).

Figure 4. Color changes of alginate solution by irradiation.

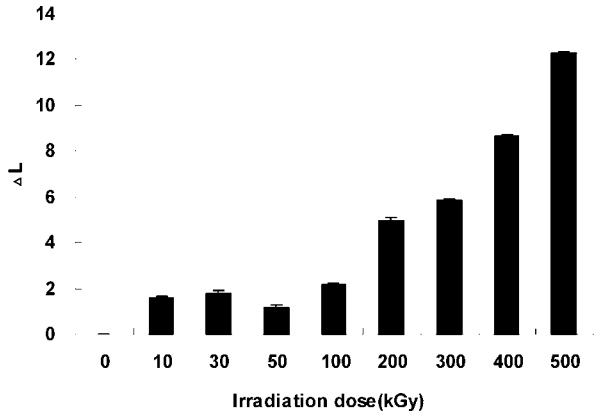

Figure 5. Changes in ΔL values of alginate solution by irradiation.

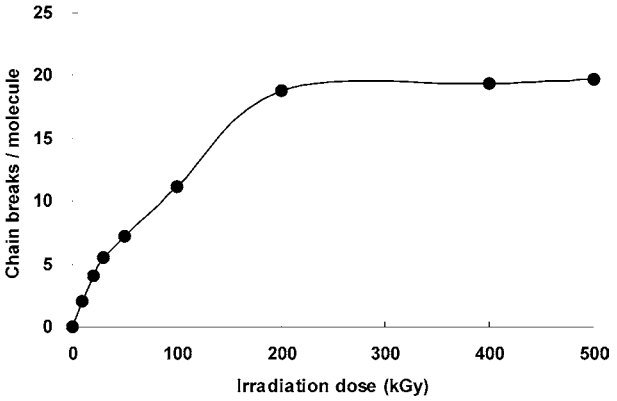

Figure 6. Changes in the number of chain breaks per molecule with irradiation doses.

Therefore, the maximum irradiation dose that did not cause an appreciable browning of alginate solution was 100 kGy. Nagasawa et al. (*5*) reported that the browning of irradiated alginate was due to the formation of double bonds after scission of the main chains.

Number of Chain Breaks and Breakdown Rate Constant of Irradiated Alginate. Figure 6 shows the increase in the calculated number of chain breaks per alginate molecule with irradiation dose. The number of chain breaks markedly increased with doses up to 200 kGy. Therefore, the minimum irradiation dose for the maximum chain scission of alginate was 200 kGy. The scission of glycosidic bonds resulted in chain breaking.

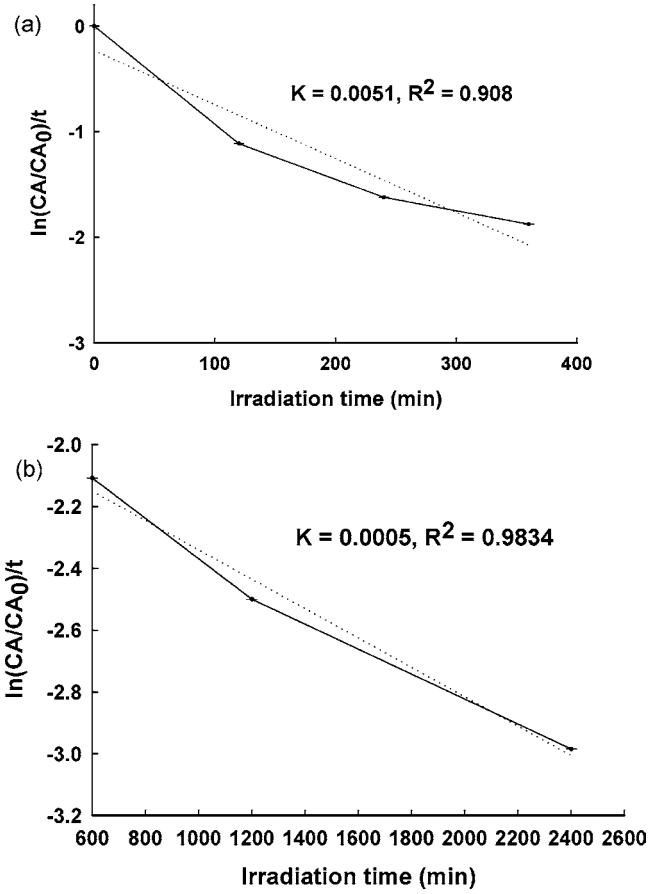

Figure 7. First-order plot for the breakdown of alginate by irradiation: (a) 0–360 min; (b) 600–2400 min.

By applying the molecular weight data to kinetic model, the breakdown rate was predicted (*18*). **Figure 7** shows the first-order breakdown of alginate by irradiation. The breakdown rate constants (K) were calculated to determine the most effective irradiation dose interval for the degradation of alginate. To compare the low irradiation doses with high irradiation doses, they were divided into two separate intervals. Two plots fit the first-order model because of linear correlation ($r^2 > 0.9$). The breakdown rate constants (K) of the first interval ranging from 0 to 360 min (0−30 kGy) and the second interval ranging from 600 to 2400 min (50−200 kGy) were 0.0051 and 0.0005 min^{-1}, respectively. The breakdown rate of the first interval was considerably high, compared with that of second interval. This suggests that alginate can be effectively degraded by low-dose irradiation. The degradation rate decreased as the irradiation dose increased.

Structural Analysis of Irradiated Alginate by ^{13}C NMR Spectra. The peaks in the spectra are interpreted according to references *1* and *20*. The spectra in the region of anomeric carbon and C2−C5 were investigated to determine their sequential structure and monomer composition.

Figure 8 shows the ^{13}C NMR spectra of the anomeric region. Peaks in the range of 100−102 ppm indicate mannuronic and guluronic acid sequences of the C1 carbon. Four peaks in the anomeric region are assigned to MG, GG, MM, and GM in the order from low magnetic field side. ^{13}C NMR spectra were measured with the quantitative method to determine the intensities of peaks. According to the NMR spectra, raw alginate was found to have mannuronic acid rich heteropolymeric blocks. Alginate irradiated at 10 kGy was rich in MM block, whereas that irradiated at 200 kGy was rich in GG blocks. Raw alginate

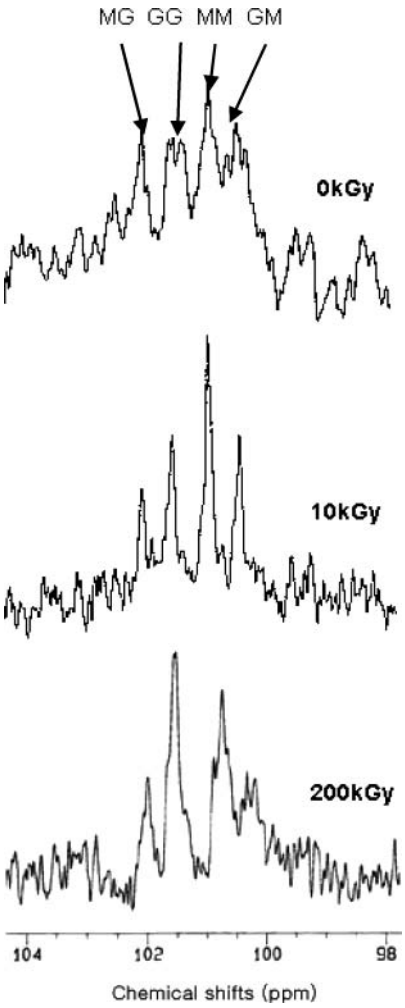

Figure 8. ^{13}C NMR spectra of the anomeric region.

Table 1. M/G Ratio of Raw and Irradiated Alginate

irradiation dose (kGy)	M/G ratio (C-4)
0	1.88
10	1.42
200	0.79

had heteropolymeric blocks, but homopolymeric blocks, such as MM and GG, became larger when irradiated. Fujihara et al. (*21*) reported that the antitumor activity of alginate depends on the composition of MM and GG blocks. Therefore, irradiated alginate that is rich in homopolymeric block may be desirable for antitumor activity.

Figure 9 shows spectra of C-2, -3, -4, and -5. The peaks of G-4 and M-4 appeared at 80.8 and 78.8 ppm, respectively. The M/G ratio at C-4 of raw and irradiated alginate is shown in **Table 1**. The M/G ratio was calculated from the integrated peak areas of G-4 and M-4. The M/G ratio of raw alginate was 1.88, whereas the M/G ratios of alginates irradiated at 10 and 200 kGy were 1.42 and 0.79, respectively. The M/G ratio decreased as the irradiation dose increased. A decrease in the M/G ratio with irradiation suggests that the mannuronic acid monomer was more easily broken and reduced in quantity than the guluronic acid monomer.

Conclusion. The molecular weight of raw alginate was reduced from 300000 to 25000 when irradiated with 100 kGy and to a minimum at 200 kGy with no further changes. The degradation followed the first-order kinetics in two irradiation

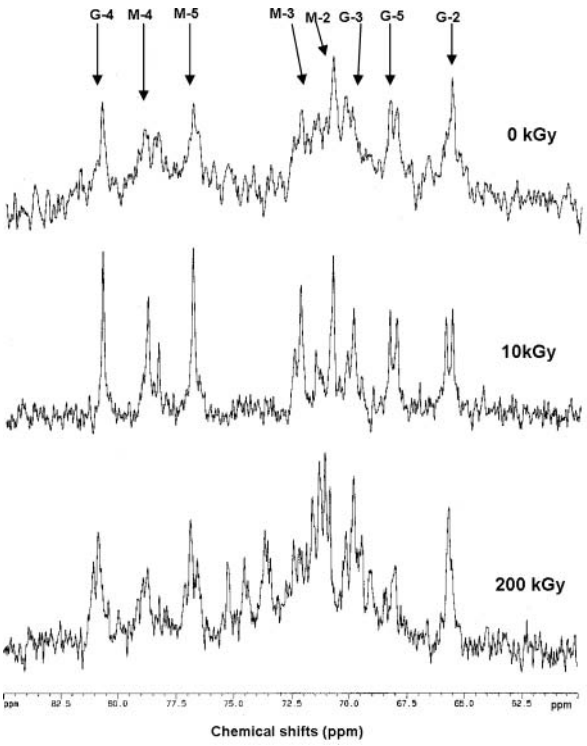

Figure 9. ^{13}C NMR spectra of C-2, -3, -4, and -5.

dose ranges of 0−30 and 50−200 kGy. The degradation rate decreased and the chain breaks per molecule increased with increasing irradiation dose. The viscosity of irradiated alginate solution sharply decreased to a near minimum with a dose as low as 10 kGy. No appreciable color change was observed in the samples irradiated up to 100 kGy, but intense browning occurred beyond 200 kGy. The ^{13}C NMR spectra showed that homopolymeric blocks, MM and GG, increased and the M/G ratio decreased with irradiation. Considering both the level of degradation and the color change, the optimum irradiation dose was found to be 100 kGy.

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