Characterization of γ-irradiated Konjac Glucomannan by Light Scattering and Scanning Probe Microscope

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Konjac glucomannan (KM) is a water soluble glucomannan with high molar mass. KM aqueous solution shows extremely high viscosity. KM can be depolymerized by γ-rays irradiation. The molar mass of original KM was 1020 kDa. The molar mass of irradiated KM decreased significantly with increase of absorbed dose up to 2 kGy and then decreased gradually. The molar mass of 10 kGy irradiated KM was 236 kDa. The radius of gyration (Rg) of original KM was 98.0 nm. The Rg value decreased significantly with increase of the dose up to 2 kGy and then reached to 46.4 nm at 10 kGy of absorbed dose. KM molecules were solvated in the form of random coils in water. The flexibility of molecular chain increased with increasing temperature. SPM image of original KM molecular chain showed rod-like pattern. The chain length and thickness of original KM was 1150 nm and 1.04 nm, respectively. The chain length decreased significantly with decrease of molar mass. However, the chain height scarcely changed regardless of molar mass. The chain lengths of low molar mass KM with 507 kDa and 236 kDa were 440 nm and 250 nm, respectively.

Key words: Konjac glucomannan, low molar mass, radius of gyration, chain length, light scattering, SPM

1. INTRODUCTION

Konjac glucomannan (KM) is a neutral glucomannan derived from tubers of Amorphophallus Konjac C. Koch, having a β-1,4 linked β-D-glucose (G) and β-D-mannose (M) backbone approximately in the ratio of 1:1.6. KM has side chains and the branching positions are considered to be the C3 of G and M [1]. An acetyl group is attached to ca.13 sugar residues [1]. Deacetylation occurs in the presence of alkali and a non-thermoreversible elastic gel is formed [2]. KM aqueous solution shows extremely high viscosity. Polysaccharides undergo depolymerization by various methods: γ-irradiation [3, 4], ultrasonic irradiation [3], chemical [5] and enzymatic hydrolyses [6].

From our previous studies [7, 8], the γ-irradiation led to chain scission of KM, but introduced no significant new chemical groups into the structure, apart from a small increase in content of carbonyl groups. The intrinsic viscosity, molar mass and radius of gyration decreased rapidly with increasing dose up to 10 kGy and then at a slower rate. The Mark-Houwink-Sakurada equation for the KM gave [η] = 5.30 × 10^4 M^0.78.

Here, the molar mass and radius of gyration of irradiated KM were determined by a size exclusion chromatography equipped with a multiangle static light-scattering (SEC-MALS). The chain lengths of the KM samples were measured by a scanning probe microscope (SPM). The changes of molar mass and radius of gyration for KM by γ-irradiation were studied, and the chain length of low molar mass KM was also discussed.

2. MATERIAL AND METHODS

2.1. Material

Commercial konjac flour (Akaig Ohodama species) was supplied by Ogino Shoten Co. Ltd. The flour was purified by washing with aqueous ethanol solution several times before use.

2.2. γ-rays irradiation

The purified KM was irradiated with ⁶⁰Co γ-rays under reduced pressure (below 10⁻³ Torr) at room temperature. The dose rate was in the range of 0.5 to 3.33 kGy/h and the absorbed dose was varied from 1 to 10 kGy.

2.3. SEC-MALS measurement

Molar mass of KM samples was determined by a multangle static light-scattering (MALS) using a MALS detector from DAWN DSP(Wyatt Technology) with a vertically polarized He-Ne laser operated at wavelength of 632.8 nm. The photometer, which was calibrated using pure toluene, was connected to a size exclusion chromatography (SEC) column of GMPWXL (Tosoh) and a differential refractive index detector RI-71S (Shodex), which was used to determine KM concentration at each position of elution peak. The temperatures of the MALS flow cell and the column were controlled at 40 °C.

The KM sample solution was filtered through a 0.45 μm cellulose acetate membrane filter (Sartorius). Scattered light intensities at scattering angles between 14.4° and 163° were measured. 50 mM NaNO₃ solution was used as both solvent and eluent at 1 mL/min. A Berry plot was employed to obtain the weight-average molar mass (Mw) and z-average radius
of gyration ($R_g$) expressed as

$$[KC/R(0)]^{1/2} = M_w^{-1/2}[1 + (1/6)R_g^2q^2 + A_2M_wC]$$  \hspace{1cm} (1)

where, $K$, $C$, and $R(0)$ are the optical constant, the polymer mass concentration, and the reduced scattering intensity at scattering angle $\theta$, respectively. $q$ is the momentum transfer vector defined as $q = (4\pi/\lambda) \sin^2(\theta/2)$, with $n$ and $\lambda$ being the refractive index and wavelength [9, 10].

2.4. Scanning probe microscope measurement

The irradiated KM was dissolved in Barnstead water and filtered through a 0.45 µm cellulose acetate membrane filter (Sartorius). KM solution (5 x 10^{-3} mg/mL) was deposited on freshly cleaved mica and was dried under nitrogen gas stream for 3 h. KM molecular chains were observed by alternating current (AC) mode using a JSPM-5200 scanning probe microscope (JEOL) equipped with a silicon cantilever (NSC35/ALBS, μ-mash).

3. RESULTS AND DISCUSSION

3.1 Molar mass and radius of gyration for Konjac glucomannan

Fig.1 shows the SEC-MALS chromatograms of original KM determined by LS detectors. The angle dependency of scattering intensity for KM is applied to Zimm, Debye, and Berry formalisms. The result indicated that KM could fitted Berry’s equation.

Fig.2 shows Berry plot at peak top of the SEC-MALS chromatograms for original KM. The molar mass was determined by the intercept, and the radius of gyration was determined by the slope.

Fig.3 shows molar mass distribution of original KM. The molar mass linearly decreased with increase of eluent volume. The $M_w$, $R_g$, and polydispersity ($M_w/M_n$) were determined by the calculated line. The value of $M_w$, $R_g$, and $M_w/M_n$ were 1020 kDa, 98.0 nm and 1.23, respectively.

3.2 Molar mass and radius of gyration for irradiated Konjac glucomannan

SEC-MALS chromatograms of the irradiated KM are shown in Fig.4. The chromatogram shifted to lower molar mass and the peak width slightly expanded with increasing absorbed dose. It is inferred that the decrease of molar mass is caused by the main chain scission of KM induced by the irradiation.

The $M_w$ and $R_g$ were determined and plotted against the absorbed dose in Fig.5. The $M_w$ of original KM was 1020 kDa. The $M_w$ decreased significantly with increase of absorbed dose up to 2 kGy and then decreased gradually. The $M_w$ of 10 kGy irradiated KM was 236 kDa. The $R_g$ of original KM was 98 nm. The $R_g$ value decreased with increase of the dose up to 2 kGy and then reached to 46.4 nm at 10 kGy of absorbed dose.

Fig.6 shows relationship between $R_g$ and $M_w$ of irradiated KM measured at 40°C. The logarithmic value of $R_g$ increased linearly with increasing log $M_w$.

The relationship gave the following equation.

$$R_g = 6.1 \times 10^{-2} M_w^\nu$$  \hspace{1cm} (2)
where \( \nu \) is constant, with value 0.53. In general, the \( \alpha \) value of Mark-Houwink-Sakurada equation is related to the \( \nu \) as shown following equation.

\[
\alpha = 3\nu - 1 \tag{3}
\]

From Eq. (3), the \( \alpha \) value is 0.59, which is lower than the \( \alpha \) value of 0.78 determined at 25 °C [7]. The \( \alpha \) value is related to the shape of the molecular chain. The \( \alpha \) value from 0.5 to 0.8 indicates that KM molecules are solvated in the form of random coils in water. Since the flexibility of molecular chain increases with decreasing \( \alpha \) value, the flexibility of KM chain increased with increasing temperature.

3.3 SPM image of low molar mass Konjac glucomannan

To investigate the effect of molar mass on molecular shape of the irradiated KM, the molecular chain parameters were measured by SPM.

The topographical and the phase SPM images of original KM molecules are shown in Fig.7 and Fig.8, respectively. As seen in Fig.7, molecular chains of original KM showed rod-like pattern. The pattern was observed more clearly in the phase image.

Fig.4 SEC-MALS chromatograms of irradiated KM.

Fig.5 Effect of absorbed dose on Mw and \( R_G \) for irradiated KM.

Fig.6 Relationship between \( R_G \) and Mw of irradiated KM.

Fig.7 Topographical SPM image of KM deposited on mica.

Fig.8 Phase SPM image of KM deposited on mica.
Since the phase SPM image clearly observed, the phase SPM image of KM with 507 kDa and 236 kDa are shown in Fig. 9 (a) and (b). The chain length decreases significantly with decrease of molar mass.

The chain height and length of low molar mass KM were measured by topographical SPM images. The results are listed in Table 1 together with dose, Mw, and Rg. The chain height of original KM was ca. 1.04 nm. As chain diameter of KM molecule corresponding to the chain height was reported to be 0.7 to 1.0 nm, the observed chains are single molecular chains of KM [11, 12]. The chain height scarcely changed regardless of molar mass. The single molecular chain length of original KM was ca. 1150 nm. The value showed agreement with values 1050 nm reported by previous study [11, 12]. The chain lengths of low molar mass KM with 507 kDa and 236 kDa was 440 and 250 nm, respectively. The results showed good correlation with the result of Rg determined by light scattering.

4. CONCLUSIONS

KM can be depolymerized by high energy irradiation. The molar mass of original KM was 1020 kDa. The molar mass decreased significantly with increase of absorbed dose up to 2 kGy and then decreased gradually. The molar mass of 10 kGy irradiated KM was 236 kDa. The Rg of original KM was 98.0 nm. The Rg value decreased significantly with increase of the dose up to 2 kGy and then reached to 46.4 nm at 10 kGy of absorbed dose. KM molecules are solvated in the form of random coils in water. The flexibility of molecular chain increased with increasing temperature. SPM image of original KM molecular chain showed rod-like pattern. The chain length and height of original KM was 1150 nm and 1.04 nm, respectively. The chain length decreased significantly with decrease of molar mass. However, the chain height scarcely changed regardless of molar mass. The chain lengths of low molar mass KM with 507 kDa and 236 kDa were 440 and 250 nm, respectively.

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