Molecular Weight and Intrinsic Viscosity of Konjac Gluco-mannan

Noriko KISHIDA, Satoshi OKIMASU and Toshio KAMATA*

Laboratory of Food and Nutrition, Hiroshima Women's University, Hiroshima 734, Japan
*National Chemical Laboratory for Industry, Tokyo 151, Japan

Received December 6, 1977

For the purpose of making clear the macromolecular chemical properties of konjac gluco-mannan, the light scattering and viscosity measurements were carried out for the aqueous solutions of the partially methylated derivatives, and following results were obtained: 1) The weight-average molecular weight, \( M_w \), and the root mean square of radius of gyration, \( <S^2>^{1/2} \), were \( 100 \times 10^4 \sim 120 \times 10^4 \) and \( 1100 \sim 1300 \) Å, respectively. 2) The \( <S^2> \) versus \( M_w \) relationship was represented by the equation of \( <S^2> = 4.20 \times 10^{-1} \cdot M_w^{1.08} \), and this fact suggested a random coil as a molecular form in the solution. 3) The intrinsic viscosity, \([\eta] \), versus \( M_w \) relationship was represented by the equation of \( [\eta] = 6.37 \times 10^{-4} \cdot M_w^{0.74} \), which was considered to be useful for the purpose of estimating the molecular weight of konjac gluco-mannan by viscometric method.

Konjac gluco-mannan (KGM) is a main component of the konjac flour produced from the tubers of *Amorphophallus Konjac* C. Koch, and has the rheological and biochemical characteristics inherent in this polysaccharide. These characteristics vary considerably according to the methods of preparation and the strains or producing areas of tubers. Accordingly, it is of great interest to study such macromolecular chemical properties as molecular weight, molecular form, intrinsic viscosity and their mutual relationships etc. of KGM, but only a few reports have been made.

Torigata et al.\(^1\) have reported the results of the light scattering and viscosity measurements for an isoamyl acetate solution of nitro-KGM, but it is probable that KGM molecules may have suffered some degradation during nitration procedure. On the other hand, Sugiyama et al. have reported the results of the light scattering measurements\(^2\) for the aqueous solutions of native KGM, which was isolated by a method in which konjac flour washed with 50\% ethanol was dissolved in water, centrifuged, dialyzed, and then lyophilized.\(^3\) In their report, however, the viscosity measurement has not been made, perhaps owing to the fact that the stability of the solution was insufficient judging from the result of our close reexamination of their experiment.

In a previous paper,\(^4\) we have reported the method for preparing a water-soluble methyl-KGM by a moderate treatment which may not develop the depolymerization of KGM molecules. Thus, the present paper deals with the results of the light scattering and viscosity measurements for various methyl-KGM solutions.

**MATERIALS AND METHODS**

**KGM materials for methylation.** Four strains of konjac flours and 7 kinds of the partial hydrolysates shown in Table I were prepared as the KGM materials for methylation.

The flours were prepared specially for the present study from the tubers cultivated in 1974 at the agricultural...
tural experimental stations of Gunma and Hiroshima prefectures. Namely, the raw tubers were sliced ca. 5 mm in thickness, and dried in a hot-air dryer at 100°C until the moisture decreased to ca. 10%. The dried materials were pulverized for 8 hr by a stamp mill, and then elutriated and sieved from the pulverized materials. The flours were further purified by the following method reported previously by Sugiyama et al.3) : About 50 g of flour was stirred with 250 ml of 50% ethanol for 3 hr at room temperature, and filtered through double sheets of cheesecloth. This treatment was repeated 4 times. The final residue was successively washed with 80% and absolute ethanol, and dried in vacuo at 70°C.

It was confirmed by the following experiments that the main component of the purified flours thus obtained was KGM: The flour was dissolved in water at ca. 0.5% with stirring for 3 hr at room temperature, and the solution was centrifuged for 30 min at 10,000 rpm to remove a small amount of insoluble substance. The supernatant fluid was dialyzed for 3 days at 10°C through cellulose membrane against running distilled water. This solution did not show at all any reactions of starch-iodine, ninhydrin, and reducing sugars. In addition, a mixture of 40 ml of this solution and 10 ml of 0.5 N H₂SO₄ was heated in a boiling water bath for 24 hr under reflux. The hydrolyzate gave only two spots, corresponding to D-mannose and D-glucose, on usual paper chromatography. The molar ratio of D-mannose to D-glucose was found to be ca. 1.6: 1.0 by measuring the spots with a densitometer.

Thus in the present work, the solutions of purified flours were used as the KGM materials for the preparation of hydrolyzates or methyl-derivatives after the centrifugation and/or the dialysis were performed.

The partial hydrolyzates were prepared from the purified flour of Hiroshima prefecture. About 0.5% solution of the flour was prepared and centrifuged in the same manner as described above. The hydrolysis was carried out by heating 400 ml of the supernatant with 100 ml of 0.5 N H₂SO₄ for a limited time at the fixed temperature. The solution was then neutralized with 1 N NaOH, and dialyzed for 2 days. The product was separated into 3 fractions by the fractional precipitation with methanol. The middle fraction was taken, dehydrated with acetone, and then dried in vacuo at 50°C. Each reaction conditions of hydrolysis is shown in Table I.

Preparation of methyl-KGM. The KGM materials mentioned above were dissolved in water at 0.5% with stirring for 3 hr at room temperature. In case of flour, however, the solution was centrifuged and dialyzed in the same manner as described above. The exact concentration of KGM in the dialyzed solution was estimated by evaporating 30 g of the solution to dryness at 105°C and weighing.

These solutions were submitted to methylation by our method reported previously on purpose to prepare the water-soluble methyl-KGM having a degree of substitution (DS) of ca. 0.45, where a DS of 1 was defined as one methoxyl group per hexose residue of KGM.

To an appropriate amount of each solution was added 60% NaOH to a final concentration of 1%, and half the specified amount of dimethyl sulfate was added to this little by little, followed by standing for 1 hr at 30°C. After the concentration of NaOH in the solution was adjusted again to 1% with 60% NaOH, the other half of dimethyl sulfate was added in the same manner, and the solution was allowed to stand for 3 hr at 30°C with occasional shaking. The resulting solution was successively dialyzed for 3 and 2 days through cellulose membrane against running tap and running distilled water, respectively. The product was precipitated and dehydrated with acetone, and dried in vacuo at 30°C. Each methyl-KGM thus obtained was entirely free from ash and nitrogen, and very soluble in water. The DS was estimated by the previous method as shown in Table II.

Light scattering measurements. When unpolarized light is used as a light source at the light scattering measurement of a dilute polymer solution, the reduced intensity, R₀, of scattered light is given by the following equation.5)

\[
\frac{KC}{R_0} = 1/M_w \cdot P(\theta) + 2A_2c
\]  
(1)

\[
K = 2\pi^2n_0^2(dn/dc)^2/Na^2 \lambda_0^4
\]  
(2)

Where M_w is the weight-average molecular weight of polymer, c concentration (g/cc), A₂ second virial coefficient, n₀ refractive index of solvent, dn/dc the specific refractive index increment, \(\lambda_0\) wavelength of incident light in vacuum, and Na Avogadro number. \(P(\theta)\) is called the particle scattering function, and the relationship between \(P(\theta)\) and the mean square of radius of gyration, \(\langle S^2\rangle\), is represented by the following equation.

\[
P(\theta) = 1 - (1/3)(4\pi/\lambda_0^2)\langle S^2\rangle \sin^2(\theta/2) + \cdots
\]  
(3)

On the above equations, \(K\) is the optical constant decided according to the kinds of polymer and solvent. Therefore, \(R_0\) of various concentrations of polymer solution is measured at various angles, and then M_w, A₂ and \(\langle S^2\rangle\) are generally found by the well-known Zimm-plot method (See Fig. 1). However, the aqueous solution of methyl-KGM exhibits very high viscosity in ordinary concentration for light scattering measurements, and the optical clarification is difficult. Furthermore, such a Zimm-plot is usually time-consuming. Thus in the present work, all the light scattering measurements were carried out for extremely dilute solution, and the calculation of these molecular constants was treated with one concentration method in light scattering.
Molecular Weight and Intrinsic Viscosity of Konjac Gluco-mannan

Sample methyl-KGM (10−25 mg) was dissolved in ca. 40 ml of dust free water, and the resulting solution was centrifuged for 1 hr at 30,000 rpm (90,000 G) by Spinco Model L 4 Ultracentrifuge. After moderately diluting the supernatant with clean water, the solution was filtered into the light scattering cell through a Corning ultrafine sintered glass filter. The light scattering measurements were carried out by Shimadzu light scattering photometer (Type PG-21) at room temperature using the wavelength of 4358 Å, and the angular distributions of light scattering were observed. The dn/dc of methyl-KGM solution was measured by Shimadzu differential refractometer, and was found to be 0.1453 (cc/g) for 4358 Å.

All results of light scattering measurements were calculated with least-squares method by an electronic computer.

On one concentration method of light scattering, it is desirable to measure the reduced intensity of scattered light on the condition that the error brought about by ignoring the concentration dependent terms, A2, is less than 2%. Consequently, one concentration method must be carried out for the solution of such concentration as satisfies the following equation.6)

\[ R_0 \leq K \times 10^{-2}/A_2 \]  

As the value of K is already known in the present experiment, if the order of A2 is known it is possible to set such measuring conditions as satisfies Eq. (4). The value of A2 was found to be 3.5 × 10^{-4} from the Zimm-plot (see Fig. 1) for sample MH2. Thus the condition of the present experiment was set using this value, and the correction by A2 was applied to all data obtained.

**Viscosity measurements.** Sample methyl-KGM was dissolved in water at 0.04−0.12 g/dl, and the solution was centrifuged for 1 hr at 10,000 rpm. The viscosity of the supernatant was then measured in various concentrations at 30±0.02°C with Ubbelohde-type viscometer. The flow time for water was 93.0 sec. The intrinsic viscosity, [η], was found by plotting reduced viscosity against concentration and extrapolating to zero concentration.

**RESULTS AND DISCUSSION**

**Molecular weight and mean square of radius of gyration**

The Kc/Rg versus sin²(θ/2) plots obtained for 11 kinds of methyl-KGM are shown in Fig. 2, and the values of Mw and \( <S^2>^{1/2} \) found in this figure are summarized in Table II. As seen in Table II, the values of Mw and \( <S^2>^{1/2} \) for methyl-derivatives of native KGM are high as 100 × 10⁴−120 × 10⁴ and 1100−1300 Å, respectively, and no significant difference due to the strain of KGM is observed.
On the other hand, Sugiyama et al.\textsuperscript{2)} have carried out the same one concentration method of light scattering as our work for various sources of native KGM, and reported the values of $M_w=25.5 \times 10^4 \sim 190 \times 10^4$ and $\langle S^2 \rangle^{1/2}=630 \sim 2330$ Å. However, these values for KGM prepared from common flours are of almost the same order as our data, except that these values for Shina Shu (Chinese species) are extremely high ($M_w=190 \times 10^4$, $\langle S^2 \rangle^{1/2}=2330$ Å).

Furthermore, they have reported the values of $M_w=27.0 \times 10^4$ and $\langle S^2 \rangle^{1/2}=570$ Å (correspond to the root mean squares of end to end distance, $\langle r^2 \rangle^{1/2}$, of 1396 Å) for the deacetylated KGM, which was prepared by the deacetylation of acetyl-KGM with alkali. On the other hand, Torigata et al.\textsuperscript{1)} have reported the values of $M_w=27.1 \times 10^4$ and $\langle r^2 \rangle^{1/2}=1380$ Å (correspond to $\langle S^2 \rangle^{1/2}$ of 563 Å) for the isoamyl acetate solution of nitro-KGM. Sugiyama et al.\textsuperscript{2)} have explained these results by the assumption the KGM molecules are degraded to one-fourth through acetylation and deacetylation as well as in case of nitration. These values of $M_w$ and $\langle S^2 \rangle^{1/2}$ are nearly equal to those that we have obtained for a methyl-derivative (MH3 in Table II) of hydrolyzed KGM. Accordingly, it is considered that the degradation of KGM molecules surely occurs during the preparation.

From the facts mentioned above, it is concluded that no degradation of KGM molecules occurs through the methylation and such values obtained for methyl-derivatives show those of native KGM. Moreover, the partial methylation of KGM brings about a remarkable merit of the stability of the solution.

In addition, it has been well-known\textsuperscript{8)} that the molecular form of polymer in the solution is elucidated from the $\langle S^2 \rangle$ versus $M_w$ relationship. Accordingly, this relationship of methyl-KGM was examined, and a good linear relationship was then observed between $\log \langle S^2 \rangle^{1/2}$ and $\log M_w$ as shown in Fig. 3. This is represented by the following equation, and $\langle S^2 \rangle$ may be said to be almost proportional to $M_w$.

$$\langle S^2 \rangle=4.20 \times 10^{-1} \cdot M_w^{1.08} \quad (5)$$

This fact is naturally understood to suggest...
Intrinsic viscosity versus molecular weight relationship. A good linear relationship was observed between log \([\eta]\) and log Mw as shown in Fig. 4. This means that the \([\eta]\) versus Mw relationship is in good conformity with the following Sakurada-Houwink's equation for linear polymer.

\[
[\eta] = KM^a
\]  \hspace{1cm} (6)

In this equation, the values of \(K\) and \(a\) were found to be \(6.37 \times 10^{-4}\) and 0.74, respectively. These values are compatible with the chemical structure and the \(<S^+>\) versus Mw relationship of methyl-KGM.

As for the intrinsic viscosity versus molecular weight relationship of KGM, Torigata et al.\(^1\) have reported the equation of \([\eta] = 11.6 \times 10^{-4} \cdot P^{0.95}\) (\(P\) is degree of polymerization.) for the isoamyl acetate solution of nitro-KGM. We have attempted to reexamine their results with great interest, but we could not obtain the nitro-derivative in spite of the repeated trial of preparation by the reported procedure or under various conditions. Therefore, the nitration itself seems to be questionable as the method for the macromolecular chemical study of KGM.

On the other hand, as for the relationship between the intrinsic viscosity for a linear polymer solution and the molecular weight, following Flory-Fox's equation \(^1^0\) reflected an excluded volume effect has been reported.

\[
[\eta] = KM^{1/2}a_s^3
\]  \hspace{1cm} (7)

Where \(a_s\) is the expansion coefficient, which is a parameter concerning the extension of polymer chain in the solution. The value of constant, \(K\), can be found from the \([\eta]/M^{1/2}\) versus \(M^{1/2}\) plots based on the Stockmayer-Fixman's equation.\(^1^1\)

From the above standpoint, the \([\eta]/M^{1/2}\) versus \(M^{1/2}\) plots for methyl-KGM solution were made as shown in Fig. 5, and the value of \(K\) was found to be 0.85 in this figure. The values of \(a_s\) calculated from Eq. (7) are summarized in Table II. The \(a_s\) of the native KGM derivatives shows almost the same value of ca. 1.30, but that of the hydrolyzed KGM derivatives becomes somewhat smaller than this with the lowering of molecular weight. On the other hand, it has been known that the \(a_s\) values of various polymers show the highest of ca. 1.7 for good solvent and the lowest of ca. 0.88 for poor solvent. Thus, it is concluded that the methyl-KGM molecules in aqueous solution are solvated considerably in the form of a stretched random coil, and this fact is supported also by the stability of the solution.

Thus in the present paper, such macromolecular chemical properties as molecular weight, molecular form, intrinsic viscosity and their mutual relationships etc. of KGM have been
clarified for methyl-KGM solution. The relationship shown in Eq. (6) is expected to be conveniently used for the purpose of estimating the molecular weight of KGM by viscometric method. However, as for the connection of these properties with the rheological and biochemical characteristics, further investigation will be necessary.

Acknowledgements. We are sincerely grateful to Dr. R. Senju and Prof. I. Sakata, Kyushu University, for their helpful advice and instruction.

REFERENCES