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Studies on the molecular chain morphology of konjac glucomannan

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Abstract

The chain geometry and parameters of konjac glucomannan were studied by using laser light scatter (LLS), gel permeation chromatography (GPC) and viscosimetry. The weight-average molecular weight (M_w), root-mean-square ratio of gyration ($\langle S^2\rangle^{1/2}$), second viral coefficient (A_2) and polydispersity index (M_w/M_n) were 1.036×10^6, 105 ± 0.9 nm, $(-1.587\pm0.283)\times10^{-3}$ \ mol ml g^{-2} and 1.015 ± 0.003 respectively. Mark-Houwink equation was established as $[\eta]=5.96\times10^{-2}M_w^{0.7317}$, and the molecular chain parameters were as follows: $M_L=982.82\ nm^{-1}$, $q=27.93$ nm, $d=0.74$ nm, $h=0.26$ nm, $L=1054.11$ nm. To confirm the above results, konjac glucomannan was observed by using atomic force microscopy (AFM) and transmission electron microscope (TEM). The physical image showed directly that the konjac glucomannan molecule was an extending semi-flexible linear chain without branches, and than the molecular dimension also conformed to the parameters above. Therefore the image of molecular chain geometry confirmed the deduction drawn by Mark-Houwink equation and molecular chain parameters magnificently.

Keywords: Konjac glucomannan; Single molecule; Chain geometry; Conformation; Laser light scattering; Atomic force microscope; Transmission electron microscope; Viscosimetry

1. Introduction

konjac glucomannan, a kind of neutral heteropolysaccharide, extracted from the tuber of *Amorphophallus konjac* C. Koch, consists of 1,4-linked β-D-mannose and D-glucose units in the ratio 1.6:1 (Kato, & Matsuda, 1969), with about 1 in 19 units being acetylated (Maekaji, 1978), the structural representation is shown as Fig. 1. konjac glucomannan has been regarded as no calorie food in China and Japan, and one of the primary benefits of traditional Chinese and Japanese foods made from konjac flour was that it was believed to provide indigestible high quality dietary fiber, the role of which had been demonstrated in weight reduction, modification of carbohydrate metabolism in diabetics and cholesterol reduction (Imeson, 1997). There was a long history of konjac tubers being used as a cure for certain diseases in China and Japan (Okimasu, 1993). The introduction of konjac glucomannan fiber in a diet might improve metabolic control in human beings (Vuksan et al., 1999; 2000) and low levels of konjac glucomannan exhibit lower plasma cholesterol in rats (Levrat-Verny, Behr, Mustad, Remesy, & Demigne, 2000). Additionally, konjac glucomannan could be extruded into films (Dave, Sheth, McCarthy, Ratto, & Kaplan, 1998) or form blend membranes (Cheng, Karim, Norziah, & Seow, 2002; Xiao, Gao, Wang, & Zhang, 2000; Yang et al., 1998) for coating and packaging applications in food Industry, and konjac glucomannan gels had promising applications to a controlled release matrix (Perols, Piffaut, Scher, Ramet, & Poncelet, 1997).

Maeda, Shimahara, and Sugiyama (1980) measured the weight-average molecular weight of konjac glucomannan as 1.12×10^6 or 2.619×10^5 by using light scattering, moreover viscosity-average molecular weight (M_η) of konjac glucomannan measured by Xu & Yang (1990) was 8.09×10^5. However the systematical research on molecular chain geometry of konjac glucomannan was still scarce as a whole, and whether or not the molecular had branches was still undecided.

It was well known that the conformation of biopolymers has great influence on their functionality or biological activity in food processing. In our previous work, we discussed the influence of conformation on the gel of konjac glucomannan (Li, & Xie, 2002a,b). Other reporters showed that the konjac

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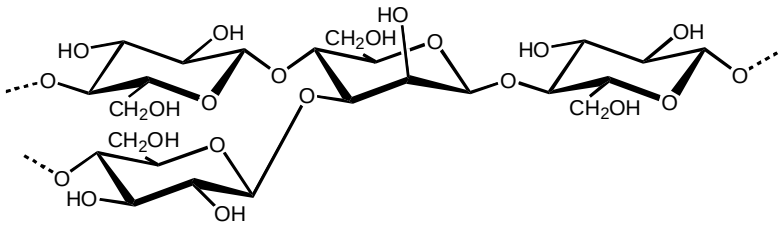

Fig. 1. The structural representation of konjac glucomannan.

glucomannan manifests its biological activity only if its molecular dimension was within a specified range (Okimasu, 1993). So it was of far reaching importance to study the secondary structure, especially the chain geometry and parameters, of konjac glucomannan. In this paper, both method relevant to determining of macromolecular solution behavior and methods of direct observation have been used to study the physical clarity of konjac glucomannan.

2. Materials and methods

2.1. Materials

ChuYe Konjac Food Co., Enshi County, Hubei province, P.R.C, provided the konjac tubers of *A. konjac* K. Koch. Amylase (40,000 U) and D-mannose were bought from Sigma Co. *Endo*-beta-1,4-D-mannanase (EC.3.2.1.78) was bought from Sigma Co. and its activity was assayed in 50 mmol L^{-1} sodium citrate buffer, pH 6.5, using a 0.5% solution of locust bean galactomannan as substrate. The release of reducing sugars in 5 min at 70 °C was measured as mannose equivalents by the dinitrosalicylic acid (DNS) method). One unit of enzyme activity is defined as the amount of enzyme producing 1 mmol of mannose per min under the given conditions. The other reagents were A.R. grade.

2.2. Separation and purification of konjac glucomannan

The extract and purify konjac glucomannan from tubers of *A. konjac,* tubers were sliced to about 8 mm in thickness, then air dried at 65 °C for about 6 h. The dried sheets were pulverized by a mill. The crude flour was immersed in 50% (v/v) aqueous methanol for 3 h, and then dried at 50 °C under reduced pressure. The raw konjac flour was extracted with benzene-absolute alcohol (4:1 v/v*)* and trichloromethane-*n*-butanol (Sevag method) for five times, respectively. This fat- and protein-extracted flour was dissolved in distilled water and heated at 40 °C for 2 h, and after environmental cooling to room temperature, the hydrosol was centrifuged at 16,000 rpm for 20 min (Himac Centrifuge, Hitachi). Then acetone was added to the supernatant and stirred. After filtration through 120 m sieve cloth, the white cotton-like precipitate was squashed and vacuum freeze-dried; its viscosity as a 1% (w/w) hydrosol was 15 Pa s.

2.3. Preparation of konjac glucomannan with different molecular weights

Konjac glucomannan (10.0 g) was dissolved completely in deionized water. A defined amount of mannanase (80 U) was added to obtain a series of konjac glucomannan with different molecular weights and hydrolysis allowed to proceed at room temperature for different times. The degraded product solutions were centrifuged, and the konjac mannans precipitated with aqueous methanol and vacuum freeze-dried. Thereby a series of powdered konjac glucomannans with different molecular weighs were obtained and were coded as KGM_1 KGM_2, KGM_3 and KGM_4.

2.4. Determination of molecular weight

Konjac glucomannan was dissolved in water (5 mg ml^{-1}) and determined by GPC on an HPLC instrument (Waters 600-410-system, USA) under the following conditions: Sephadex G-100 sugar column, injection volume 20 μL, mobile phase 0.2 mol L^{-1} NaCl aqueous solution, velocity of flow 1 μL min^{-1}, operation time 20 min.

The samples were determined by LLS using a DAWN-DSP multi-angle laser photometer equipped with a He–Ne laser ($\lambda =$ 663 nm, $T = 25$ °C, Wyatt Technology Co., USA), the photometer was also combined with a pump P100 and thermo separation equipment. The analysis was conducted by GPC using a TSK-GEL G7000 HHR column (7.8 mm × 300 mm), detector: differential refractive index detector (RI-150), and Astra software (also supplied by Wyatt Technology Co., USA) for the data acquisition and analysis.

The konjac glucomannan solution was prepared for LLS analysis as follows: the konjac glucomannan was dissolved completely in 0.2 mol/L NaCl then the solution was filtered through a bacterium funnel with a micropore diameter of 1–5 μm and through a 0.2 μm microstraining film filter (M-HJV, Millipore Co.) in turn to remove the microparticle impurities in the solution. After that the refined solution was just allowed to stand for about 48 h before LLS analysis. The refractive index increment (d*n*/d*c*) of konjac glucomannan in 0.2 mol/L NaCl aqueous solution measured at 25 °C was 0.140.

The intrinsic viscosity in 0.2 mol L^{-1} NaCl aqueous solution was measured by Ubbelohde viscometer (0.58 mm) at 25 °C (ultra thermostat, ± 0.05 °C), and adsorption adjustment was carried in the experiment.

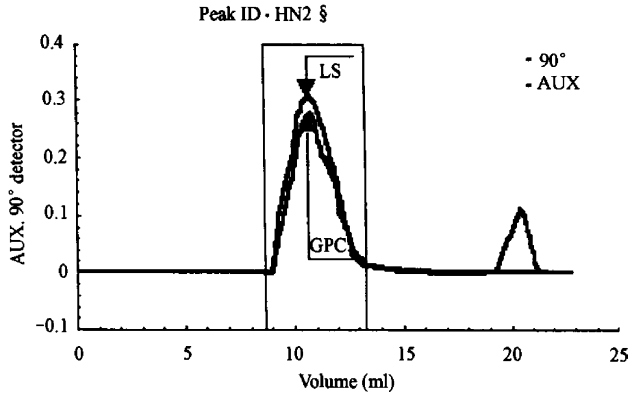

Fig. 2. GPC–LLS spectrum of konjac glucomannan in 0.2 mol L^{-1} NaCl.

2.5. Observation of molecular chain geometry of konjac glucomannan

2.5.1. TEM

Konjac glucomannan was dissolved in distilled water and its accurate concentration determined by the 2,4-di-nitro-salicylic acid (DNS) colorimetric method to be 1.5×10^{-4} mg mL^{-1} (Hu & Li, 1999). The copper grid pre-painted with carbon film was used to catch/absorb the konjac glucomannan molecular chain from this solution, then the palladium–iridium alloy was sprayed on the copper grid at 7° angle after the solution had volatilized completely. So by this technique the konjac glucomannan sample for TEM observation was obtained. The transmission electron microscope (PHILIPS TEM400ST, Holland) gave an enlargement of 40,000 times.

2.5.2. AFM

Konjac glucomannan solution (1.5×10^{-3} mg mL^{-1}) was observed using a AJ-III scan probe microscope (Shanghai AiJian Co. P.R.C.), sample size $\leq$10 mm, scan range 6 μm× 6–20 μm×20 μm, distinguishable (based on crystal lattice of mica surface): *X*-axis and *Y*-axis 0.4 nm, *Z*-axis <0.1 nm, needlepoint approaching range $\geq$20 mm, precision $\leq$0.1 μm. The images were obtained under tapping mode, touch force was controlled in the range of 3–4 nN, and the experiment was carried out under an air atmosphere directly at room temperature and 30% relative humidity.

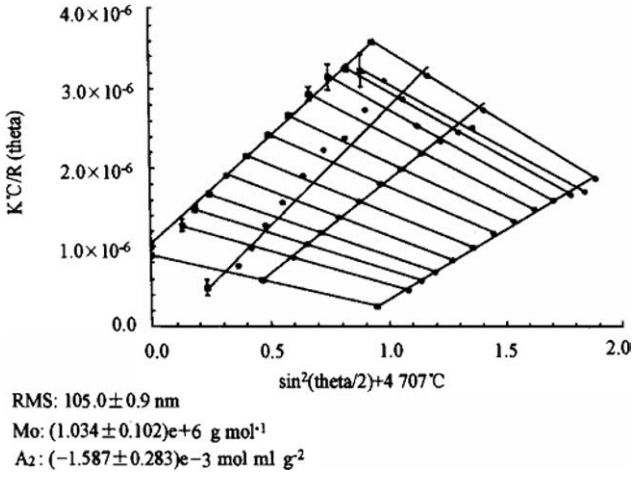

Fig. 3. Zimm-plot of konjac glucomannan in 0.2 mol L^{-1} NaCl.

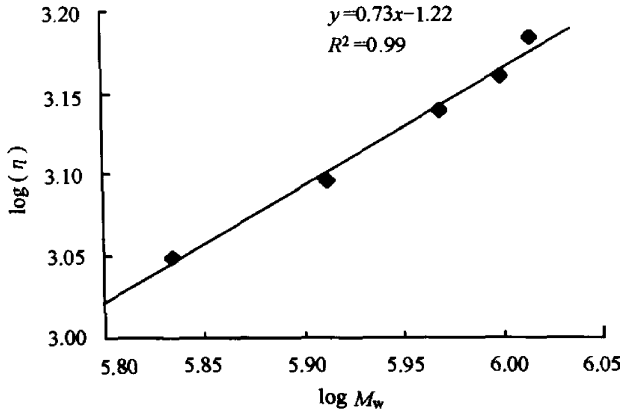

Fig. 4. Relationship between the molecular weight and the intrinsic viscosity for the Mark-Houwink equation.

3. Results and discussion

3.1. Molecular weight of konjac glucomannan

The distribution of konjac glucomannan molecular weight was a single peak as shown by gel permeation chromatography, and the M_n (number average molecular weight), M_p (peak position molecular weight) and M_w (weight average molecular weight) values were 1.044×10^6, 1.033×10^6, and 1.088×10^6 respectively, but $M_w/M_n=1.04$. Such a result indicated that the konjac glucomannan has a largely undispersed molecular weight distribution.

LLS combined with GPC was used to determine molecular weight of konjac glucomannan. (Fig. 2), The outflow curve as determined by differential refractive index detector synchronized with the curve determined by laser light scatter intensity detector very well. The polydispersity index $M_w/M_n=1.015$, M_n, M_w, M_z (Z average molecular weight) and $\langle S^2\rangle^{1/2}$ were 1.034×10^6, 1.036×10^6, 1.038×10^6 and 105 nm respectively.

The molecular weight values for konjac glucomannan were therefore very similar for the relative value determined by GPC and the absolute value determined by GPC–LLS, indicating

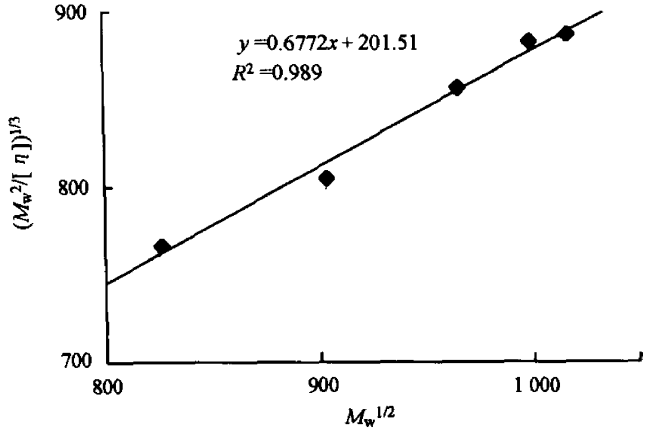

Fig. 5. Relationship Between $(M_w^2/[\eta])^{1/3}$ and $M_w^{1/2}$.

that the molecular weight values of konjac glucomannan as determined were authentic.

The molecular weight of konjac glucomannan was also determined only by LLS (uncombined with GPC), and its Zimm plot is shown in Fig. 3. The M_w, $\langle S^2\rangle^{1/2}$, A_2 and M_w/M_n values were 1.036×10^6, 105 ± 0.9 nm, $(-1.587\pm0.283)\times10^{-3}$ mol ml g^{-2} and 1.015 ± 0.003, respectively. The reason why A_2 is negative is undefined and needs further studies. Maybe there is a special relationship between the complicated chemical structure and solution behavior of the konjac. The value of $\langle S^2\rangle^{1/2}$ of konjac glucomannan was analogous to those of black fungus glucosan (single helix), schizophillan (triple helix), xanthan and mushroom polysaccharides L-F_V(double helices)(Diao, Liang, Liang, 2001; Zhang, & Yang, 1995; Zhang, Zhang, & Li, 1998) in aqueous solution with corresponding molecular weights, indicating that the konjac glucomannan molecular chain was relatively extended in aqueous solution.

3.2. Judgment of molecular morphology

Based on the respective M_w and intrinsic viscosity [η] values of konjac glucomannan, KGM_1 KGM $_2$, KGM $_3$ and KGM_4, a plot of lo g (η) against lo g M_w, yielded a straight line (Fig. 4). According to the slope and intercept of the line, $K=5.96\times10^{-2}$ and $\alpha=0.7317$, so the Mark-Houwink equation could be established as:

$$[\eta] = 5.96\times10^{-2}M_w^{0.7317}$$

The character constant α, has an intimate relationship with the rigidity degree and solvation ability of a polymer molecular chain, and its value depends on the polymer solution system property and polymer chain structure. That α value of konjac glucomannan was 0.7317 indicating that the konjac glucomannan molecule is a kind of semi-flexible chain in aqueous solution (Zhu, 1996).

3.3. Parameters of molecular chain

Having established that konjac glucomannan belongs to a semi-flexible chain type, using formulas (1)–(3) (Zhu, 1996) was dependable.

$$\left(\frac{M_w^2}{[\eta]}\right)^{1/3} = I + SM_w^{1/2} \tag{1}$$

$$I = 1.5416\times10^{-8}A_0M_L(g^{1/3}\mathrm{cm}^{-1}) \tag{2}$$

$$S = 1.5416\times10^{-8}B_0\left(\frac{2q}{M_L}\right)^{-12}(g^{1/3}\mathrm{cm}^{-1}) \tag{3}$$

Where M_L is the molar mass per unit length (nm^{-1}); q the persistent length (nm); and B_0 a constant, its value being in the range 1.05–1.08, $B_0=1.065$, in this paper.

The value of M_w and [η] of konjac glucomannan, KGM_1 KGM $_2$, KGM $_3$ and KGM_4 respectively were substituted into formula (1), and $(M_w^2/[\eta])^{1/3}$ was plotted against $M_w^{1/2}$ (Fig. 5).

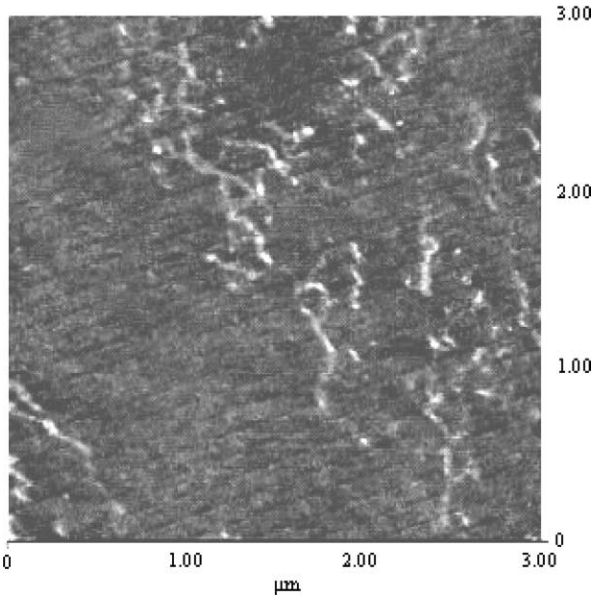

Fig. 6. AFM image of konjac glucomannan deposited onto mica.

Therefore the values of I and S could be obtained according to the slope and intercept ($I=201.51$, $S=0.6772$). Subsequently by using formulas (4) and (5).

$$\frac{d_r^2}{A_0} = \left(\frac{4\phi_0}{1.215\pi N_A}\right)\left(\frac{\nu}{I}\right)S^4 \tag{4}$$

$$Ig\left(\frac{d_r^2}{A_0}\right) = 0.173 + 2.158\,Igd_r \tag{5}$$

where ϕ_0 is the Flory constant, $\phi_0=2.86\times10^{23}$ ($d_r\leq0.1$); N_A the Avogadro constant; and ν the partial differential specific

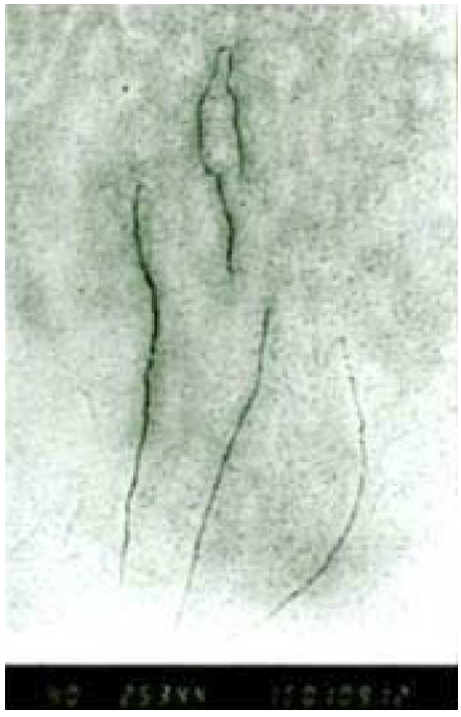

Fig. 7. TEM image of konjac glucomannan.

volume.

$$d_r^2/A_0 = 1.23 \times 10^{-4}$$

(deduced from formula (4)) was substituted into (5) and yielding $d_r = 0.01315$, and $A_0 = 1.33$. Finally by using formulae (6)–(9)

$$d = \mathrm{d}r^* 2q \tag{6}$$

$$h = \left(\frac{(M_0/x)}{M_L}\right) \tag{7}$$

$$L = \frac{M_w}{M_L} \tag{8}$$

$$X = \frac{L}{d} \tag{9}$$

where d is the chain diameter (nm); H the girth per glucose residue of the chain (nm); M_0 the molecular weight of repeating unit; x the number of glucose residue per repeating unit; L the outline length (nm);and X the axis ratio.

The parameters of the molecular chain were therefore figured out as follows:

$$M_L = 982.82\ \mathrm{nm}^{-1}, \quad q = 27.93\ \mathrm{nm}, \quad d = 0.74\ \mathrm{nm},$$

$$h = 0.26\ \mathrm{nm}, \quad L = 1054.11\ \mathrm{nm}, \quad X = 4054.27$$

One of the important parameters for characterization of molecular chains, including conformation and rigidities M_L, and the greater the value is, the more rigid is the chain. The value of M_L for konjac glucomannan (982.82 nm^{-1}) was in the range of 500–2200 nm^{-1}, this value was less than that for single helix β-D-glucan (1030 nm^{-1}) and much smaller than that of xanthan (1940 nm^{-1}) and schizophillan (2170 nm^{-1}), but greater than that for flexible trinitrocellulose (520 nm^{-1}), Therefore it could be concluded that konjac glucomannan molecular chain morphology was a kind of semi-flexible chain with some degree of rigidity between that of a single helix and a flexible chain.

The support ability of the molecular chain (q) is a polymer parameter, which means that the chain becomes more rigid when the value of q increases. The q value for several kinds of natural polysaccharides derivatives is as follows: trinitrocellulose (17 nm) <konjac glucomannan (27.93 nm)< black fungus β-D-glucosan (90 nm)<xanthan (120 nm)<schizophillan (200 nm). The value of q for konjac glucomannan was much less than for the rigid chain such as black fungus glucan and larger than that of a flexible chain such as trinitrocellulose in some degree, which meant that the chain morphology of konjac glucomannan stands between a rigid chain and a flexible chain. Such a result could offers support to the deduction made above in Section 3.2 that the konjac glucomannan molecular chain morphology was a kind of semi-flexible chain.

3.4. Observation of molecular morphology

TEM, a standard and effective method of the directly observing and studying long chain polymer chain geometry gives two-dimensional information of the sample surface. AFM, a new method of exploring the microcosm, overcomes the microscope resolution limit caused by light and electronic wavelength and can give three-dimensional information. AFM by adopting tapping mode in atmosphere conditions observes the polysaccharide morphology directly and does not destroy the molecular chain (Rief, Oesterhelt, Heymann, & Gaub, 1997).

In the AFM image of konjac glucomannan (Fig. 6) the molecular chain took on an extending chain structure. The density of the chains depends on the original concentration. of konjac glucomannan and the amount deposited on the mica surface. The image quality depended on the operating force. The chain could be destroyed by the force being too strong, but a clear and stable image could not be obtained when the force was too little, so it was necessary to control the work force in range of 3–4 nN. The chain length was 950–1100 nm in the image and the average length was about 1020 nm, and the height was 0.7 1.0 nm. Comparing these data to the values calculated above, it was obvious that the chain length observed differed from the calculated value by only about 3.5%, and the chain diameter calculated met the chain height observed well.

However the width observed was about 35.0 nm, (in general, the width of polysaccharide chains is in the range of 0.1–1 nm), and is much greater than the theoretical width. The possible reason for the difference was a broadened domino effect caused by interaction between the tiny needlepoint and different section of molecular chain in the course of scanning. Analogous broadened domino effect has also been observed in the case of DNA (Bustamanter et al., 1992).

The TEM image of konjac glucomannan (Fig. 7). It showed that the molecule is an extending linear chain without branch points. This result differed from the molecular structure with branch points provided by Xu & Yang (1990), and differed again from the bi-fold helix provided by Ogawa, Yui and Mizuno (1991). This may be caused by species differences of source of the konjac glucomannan. The length of chain lay in the range of 950–1125 nm and the average length was 1050 nm. The chain in the TEM image was more extending than that shown in the AFM image (it was caused assumedly by the gold spray and vacuum operations); but the two results were similar. Moreover the molecular chain diameter confirmed by TEM was 0.6–1.0 nm, the average chain diameter was 0.7 nm, and these results also conformed to calculated values of 0.74 nm.

4. Conclusions

The molecular morphology of a konjac glucomannan has been discussed in terms of data obtained by using both macromolecular solution behavior and direct observation. The two chain lengths differ only by 3.5% and the two chain diameters were nearly equal. This demonstrated that the results of direct observation met the calculated value from solution behavior well, and could definitely validate the deductions from macromolecular solution behavior.

Konjac glucomannan, the material evaluated in this paper, is a kind of natural polysaccharide whose molecular weight distribution was fairly mono disperse, and the molecular chain was extending, semi-flexible, linear, a little rigid and without branching. The parameters of konjac glucomannan molecular chain were as follows: $M_L = 982.82\ nm^{-1}$, $q = 27.93$ nm, $d = 0.74$ nm, $h = 0.26$ nm, $L = 1054.11$ nm.

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