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Carbohydrate Polymers 68 (2007) 701–707

Carbohydrate Polymers

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AFM studies on gelation mechanism of xanthan gum hydrogels

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Received 19 July 2006; received in revised form 3 August 2006; accepted 3 August 2006

Abstract

The effect of annealing on xanthan gum molecules was investigated using atomic force microscopy (AFM). The values of height and width of xanthan gum molecules in AFM images are ca. 1 nm, which strongly indicates that xanthan gum molecules extended on the mica surface are in mono- or double layers. When xanthan gum aqueous solution was annealed, a network structure was observed. In contrast, a network structure was not observed for non-annealed solution. AFM images provide direct information concerning oscillational change of the network structure. It is concluded that xanthan gum molecular chains in aqueous solution aggregate and dissociate in an oscillational manner with increasing annealing time and that a homogeneous network structure was formed by annealing at 40 °C for 24 h.

Keywords: Xanthan gum; AFM; Hydrogel; DSC

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1. Introduction

Xanthan gum is an extracellular polysaccharide from *Xanthomonas campestris* (Jansson, Kenne, & Lindberg, 1975). The primary structure according to Morris's paper (1995) is shown in Fig. 1. The secondary structure of xanthan gum has been investigated by the use of X-ray diffraction studies of oriented fibre and molecular modelling method (Moorhouse, Walkinshaw, & Arnott, 1977; Okuyama et al., 1980).

It is known that xanthan gum does not readily form hydrogels by the usual gelation method (Fujiwara, Iwanami, Takahashi, Tanaka, Hatakeyama, & Hatakeyama, 2000; Quinn, Hatakeyama, Takahashi, & Hatakeyama, 1994; Yoshida, Takahashi, Hatakeyama, & Hatakeyama, 1998). However, it was found that xanthan gum forms hydrogels when aqueous solutions are annealed at a temperature higher than the characteristic temperature and subsequently cooled (annealing and subsequent cooling induced gelation) (Takahashi, Hatakeyama, & Hatakeyama, 1998). When xanthan gum aqueous solutions were annealed at 40 °C, gel–sol transition temperature ($T_{g\text{–}s}$) was clearly observed by falling ball method (FBM) rheological measurement (Yoshida et al., 1998). Similar results were obtained for hyaluronan aqueous solution (Fujiwara, Takahashi, Hatakeyama, & Hatakeyama, 2000). Furthermore, gelation of gellan gum, which is a representative gel forming polysaccharide electrolyte, is accelerated when the gellan aqueous solutions are annealed in the sol state (Quinn, Hatakeyama, Yoshida, & Hatakeyama, 1993).

The above phenomena are found to correlate with the structural change of water restrained by polysaccharide molecules. When xanthan gum aqueous solutions were measured by differential scanning calorimetry (DSC), it

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doi:10.1016/j.carbpol.2006.08.004

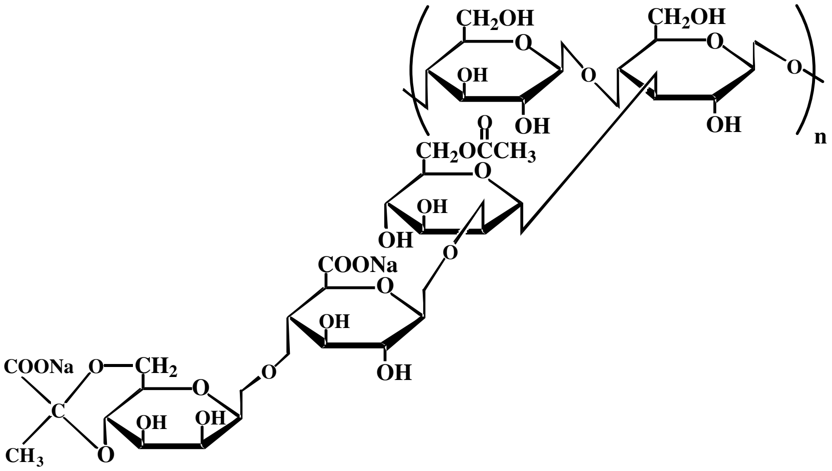

Fig. 1. Chemical structure of xanthan gum.

was found that the melting enthalpy (ΔH_m) of water changed oscillationally during the annealing process, and then approached a constant value (Takahashi, Hatakeyama, & Hatakeyama, 2000). In the initial stage of annealing, the assemblies of polysaccharide molecules dissociate by desorbing water molecules. Subsequently, the solution homogenizes by the diffusion of polysaccharide molecules, which are removed from the assemblies. After the homogenization of the system was attained, the xanthan gum molecules form a junction structure, which enables the system to form gels by adsorbing the non-freezing type bound water.

In this study, the effect of annealing on xanthan gum molecules is investigated using atomic force microscopy (AFM). By comparing morphological features and thermal data, the gelation process of xanthan gum hydrogels is clarified.

2. Experimental

2.1. Sample preparation

Xanthan gum in powder form was purchased from SIGMA, and xanthan gum was solved in pure water (Wako Chemical) and stirred at 25 °C. Xanthan gum aqueous solutions with concentration 10 or 20 g l^{-1} were prepared.

2.2. AFM measurement

Samples for AFM were prepared as follow. Ten grams per liter xanthan gum aqueous solution was annealed at 40 °C for 1 ~ 24 h (Fujiwara, Takahashi et al., 2000; Yoshida et al., 1998). One milliliter annealed or non-annealed 10 g l^{-1} (1%) xanthan gum aqueous solutions were diluted into 9 ml of pure water. This process was repeated. Ten nanograms per microliter ($10 \times 10^{-4}\%$) xanthan gum aqueous solutions were obtained. A drop of butanol was added to the xanthan gum diluted solutions. The 10 $\text{ng }\mu\text{l}^{-1}$ xanthan gum aqueous solution was spread on freshly cleaved mica using a syringe. Samples spread on mica were dried in air overnight.

An SII Nano Technology Inc., atomic force microscopy (AFM) SPA300HV equipped with a controller (SPI3800N, SII Nano Technology Inc.) was used. Tapping mode in air was employed in the present study. The size of the geometrical shape of the needle and the sample widths were calibrated using the method reported previously (Hatakeyama & Hatakeyama, 2004).

2.3. DSC measurement

Aqueous solution of xanthan gum with concentration 20 g l^{-1} was used. An SII Nano Technology Inc., differential scanning calorimeter (DSC) EXSTAR 6000 equipped with a cooling apparatus was used. Temperature and enthalpy calibrations were calibrated using water. Dry nitrogen was used as a purge gas and the flow rate was 30 ml min^{-1}. The scanning rate was 10 $°\text{C min}^{-1}$. The sample mass was ca. 4 mg and an aluminum sealed-type sample-pan was used. The sample-pan was hermetically sealed and the total mass was recorded. A Sartorius ultramicro-balance ($\pm 0.1 \times 10^{-6}$ g) was used for sample mass measurements. The samples were annealed at 40 °C for 0 ~ 24 h in a DSC sample holder. The sample was cooled from 40 °C to −80 °C and heated from −80 °C to 80 °C. Liquid nitrogen was used a coolant.

3. Results

As described in the experimental section, 10 g l^{-1} xanthan gum aqueous solution was diluted 10 times and the dilution repeated three times. AFM observation was carried out at each stage of dilution. When concentration reached 10 $\text{ng }\mu\text{l}^{-1}$, clear micrographs showing xanthan gum molecules extended on the mica surface were obtained. On this account, all AFM measurements were carried out at concentration of 10 $\text{ng }\mu\text{l}^{-1}$.

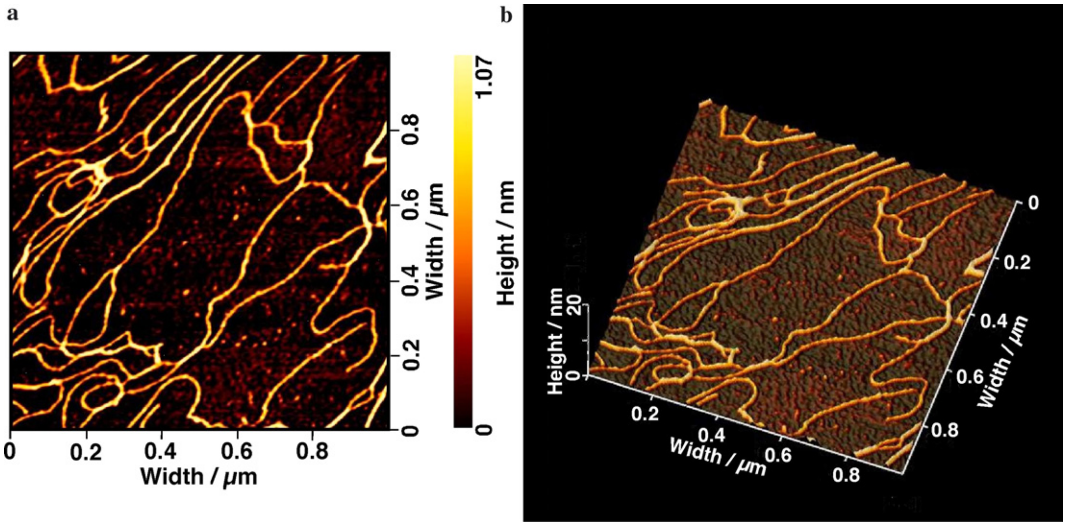

Fig. 2. AFM images of xanthan gum molecules (image size = 1 × 1 μm).

Fig. 2(a) shows AFM images of xanthan gum molecules. The size of the micrograph shown in figure is 1 × 1 μm. Fig. 2(b) shows a three dimensional AFM image. Eleven points of xanthan gum molecules shown in Fig. 2 were chosen, and the molecular height and width on AFM images were measured using AFM analysis software.

Table 1 shows the molecular size of xanthan gum molecules. Measured height and width in Table 1 are measured values, and calibrated width is calibrated value taken into consideration of the geometrical shape of needle (Hatakeyama & Hatakeyama, 2004). Average of measured height is 1.12 nm and standard deviation of measured height is ±0.20. Average of calibrated width is 1.22 nm and standard deviation of calibrated width is ±0.24. Values of both height and width were scarcely scattered.

Fig. 3 shows AFM images of annealed xanthan gum with various annealing times at 40 °C. The size of micrograph is 2 × 2 μm. Annealing time was 0 (non-annealing, Fig. 3(a)), 1 h (b), 3 h (c), 6 h (d), and 24 h (e), respectively. It is clearly seen that a network structure is observed when xanthan gum solution was annealed. In contrast, the network structure was not observed for non-annealed solution. When the sample was annealed for 1 h, xanthan molecules seem to assemble heterogeneously, however by annealing for 3 h, xanthan molecules spread homogeneously. By further annealing for 6 h, a heterogeneous molecular assembly was again observed. By 24 h annealing, xanthan solution became more homogeneous. As shown in Fig. 3(e).

Table 1
Molecular size of xanthan gum molecules by AFM

	Measured height (nm)	Measured width (nm)	Calibrated width (nm)
Data	0.80	12.19	0.93
	0.81	12.36	0.95
	0.90	12.88	1.04
	1.11	12.97	1.05
	1.15	13.13	1.08
	1.16	13.13	1.08
	1.19	14.48	1.31
	1.20	14.89	1.39
	1.22	15.28	1.46
	1.25	15.47	1.50
	1.53	16.24	1.65
Average	1.12	13.91	1.22
Standard deviation	0.20	1.33	0.24

The height and width of xanthan gum molecules at AFM images in Fig. 3 were measured at 11 points on the micrographs. Fig. 4 shows the relationship between measured height of xanthan molecules by AFM and annealing time at 40 °C. Height increased in the initial stage of annealing showing a maximum at 1 h and then levelled of at around 1.5 nm. The standard deviation of non-annealed sample was small. In contrast, the standard deviation of annealing sample is large.

Fig. 5 shows the relationship between calibrated width of xanthan molecules by AFM and annealing time at 40 °C. Similarly to height values, those for width showed a maximum at 1 h annealing and then decreased to 5 nm.

Phase transition of water in xanthan gum aqueous solution was measured by DSC. Melting temperature of water was observed at 5 °C (heating rate = 10 °C min^{-1}) and significant change was not observed by annealing xanthan gum aqueous solution (concentration = 20 g l^{-1}). Enthalpy of melting was calculated as a function of annealing time. Fig. 6 shows relationship between ΔH_m of xanthan gum aqueous solution (concentration = 20 g l^{-1}) by DSC and annealing time at 40 °C. ΔH_m of xanthan gum aqueous solution was smaller than ΔH_m of pure water 334 J g^{-1}. ΔH_m values increased in the initial stage, subsequently decreased, and then approached a constant value.

4. Discussion

The molecular size of xanthan gum has been previously investigated by X-ray analysis and electron microscopy.

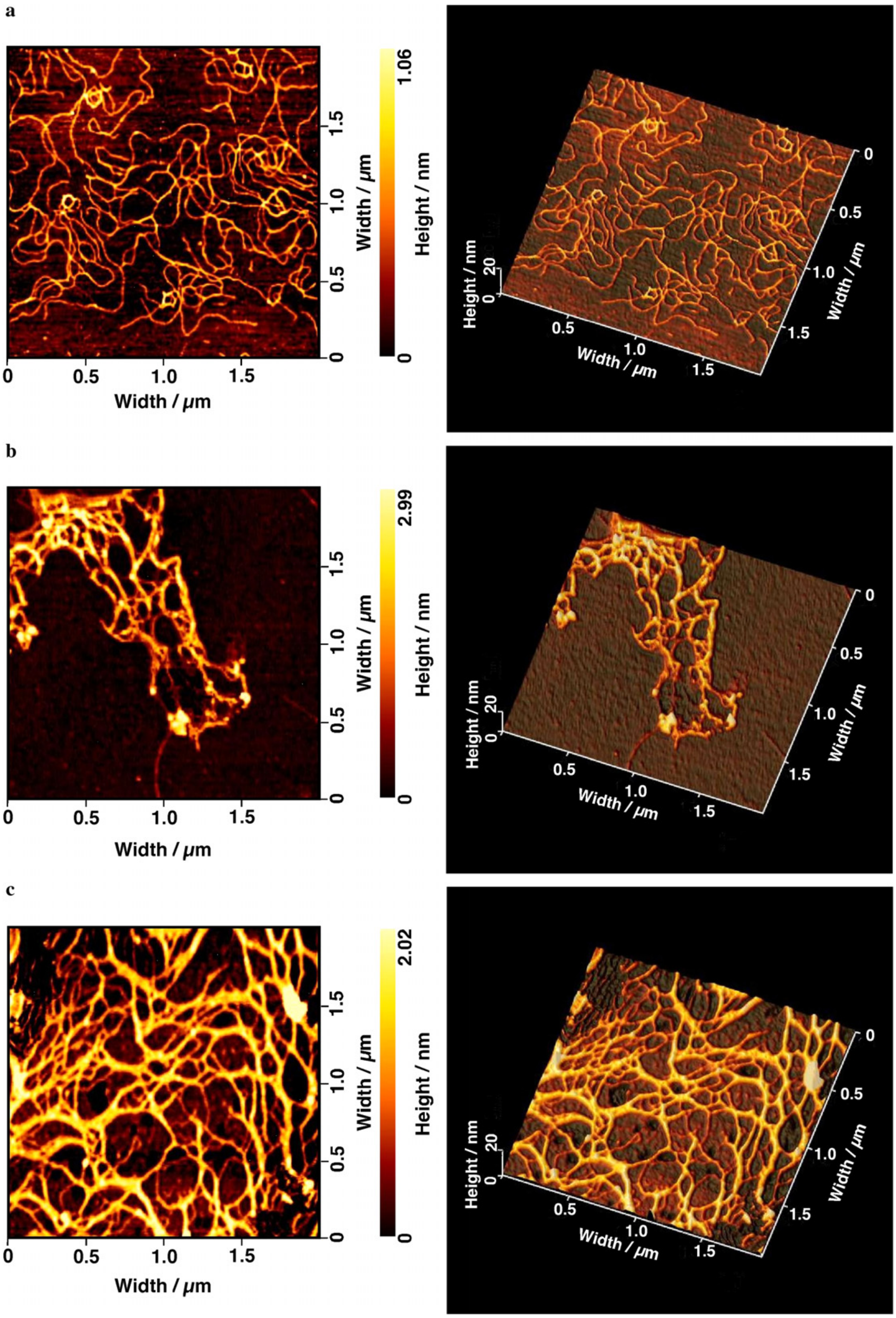

Fig. 3. AFM images of annealed xanthan gum with various annealing times at 40 °C (image size = 2 × 2 μm) (a) non-annealed, (b) 1 h, (c) 3 h, (d) 6 h, and (e) 24 h.

Morphological observation of xanthan gum has been carried out using electron microscopy since 1977 (Holzwarth & Prestridge, 1977). Holzwarth et al. showed evidence for multistranded molecules and concluded that xanthan is a stiff rigid rod-like molecule (Holzwarth & Prestridge, 1977). Stokke et al. analyzed electron micrographs obtained from different xanthan samples and determined the contour length distribution of the molecules and the

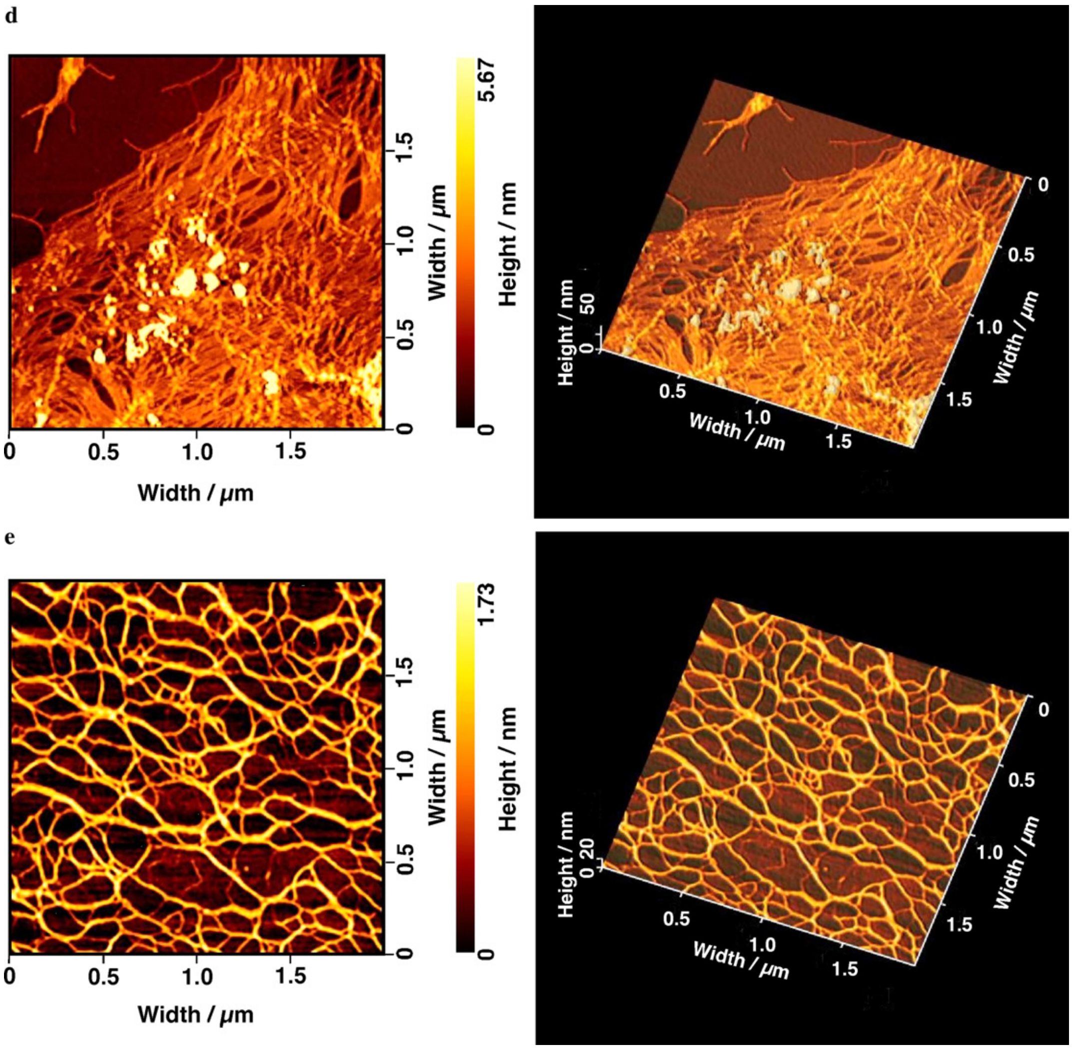

Fig 3. *(continued)*

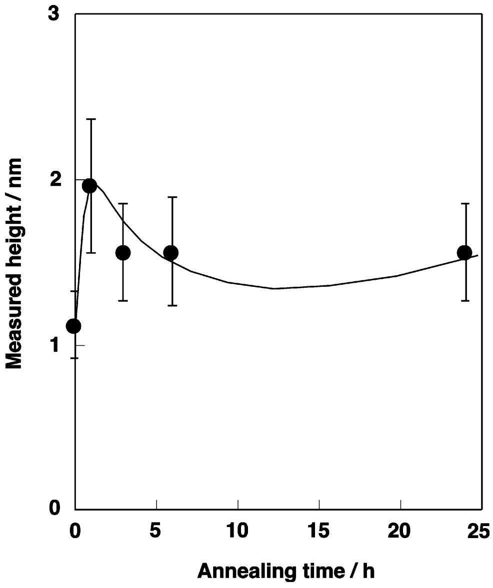

Fig. 4. Relationship between measured height of xanthan molecules by AFM and annealing time at 40 °C. Data is average value for 11 data.

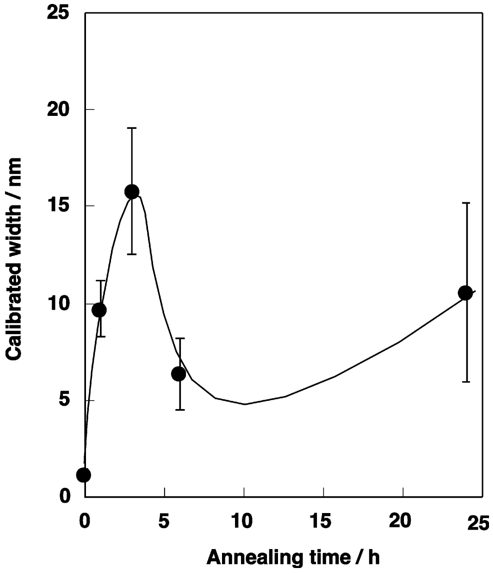

Fig. 5. Relationship between calibrated width of xanthan molecules by AFM and annealing time at 40 °C. Data is average value for 11 data.

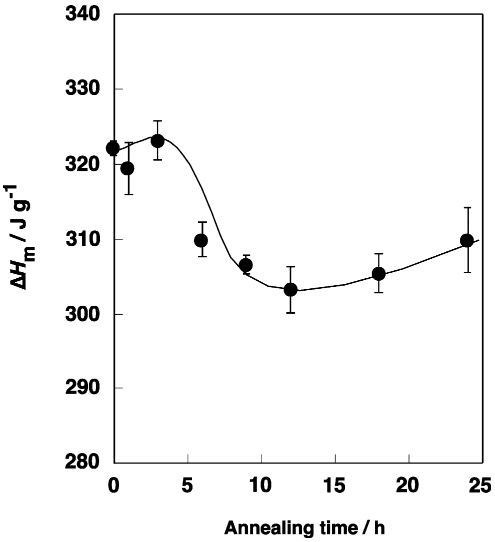

Fig. 6. Relationship between ΔH_m by DSC and annealing time at 40 °C (concentration = 20 g l^{-1}). Data is average value for three data.

persistence length (Stokke, Elgsaeter, Skjåk-Bræk, & Smidsrød, 1987). The molecules have molecular diameters of about 4 nm for double stranded and 2 nm for single stranded and persistence length of 150 nm for double stranded and 60 nm for single stranded structures (Stokke et al., 1987). Milas et al. reported xanthan exists only as single stranded wormlike molecules with an apparent thickness of 3–4 nm (Milas, Rinaudo, Tinland, & Murcia, 1988). Miles et al. reported that the width of the molecules in the STM images is about 1.5 nm, and the height is 1–1.5 nm (Miles, Lee, & Atkins, 1991). As shown in Table 1, values of height and width of xanthan gum molecules are ca. 1 nm, which strongly indicates that xanthan gum molecules extended on mica surface are in mono- or double layers. Widths are larger than heights by ca. 8%. It is considered that xanthan gum molecules extended on mica surface and placement of molecular chains is not uniform.

We reported that conformation of molecular chains of xanthan gum changes during annealing in sol state by rheology, falling ball method, SAXS and DSC (Fujiwara, Iwanami et al., 2000; Quinn et al., 1994; Yoshida et al., 1998). Based on experimental results, we proposed the following explanation concerning structural change of xanthan gum in aqueous media. When xanthan gum is dissolved in water at room temperature, large-scale molecular assemblies are formed and act as colloidal dispersion. On this account, the heterogeneous solutions do not form real gels. During annealing, the initial molecular assemblies decompose, and molecular chains start to rearrange with elapse of time. When xanthan molecules are liberated from the assemblies at a moderate temperature for a long time, the molecular chains move freely, and the system attains homogeneity. From the homogeneous solution thus obtained, firm gels are formed by subsequent cooling.

It is also noteworthy that the structural change of aqueous solution of xanthan gum does not proceed smoothly as a function of time, but equilibrates in a form of oscillatory decay. In order to explain the above oscillation, phenomenological theory on the change of bound water attaching to xanthan gum molecules was proposed (Takahashi et al., 2000).

In order to explain the behaviour of bound water (non-freezing water) in xanthan gum–water systems, the following model was proposed (Takahashi et al., 2000). Non-freezing water is thought to be water strongly bound to polysaccharide chains and forms a structure composed of polysaccharide chains and water molecules. Therefore, non-freezing water is considered to exist in the area where the density of polysaccharide chains is high. On this account, the total number of repeating units of polysaccharide molecules (N) is assumed to be composed of N_0, N_u and N_s as

$$N = N_0 + N_u + N_s \tag{1}$$

where N_0 is the number of repeating units which adsorb water molecules, N_u those not adsorbing water molecules and N_s those being incapable of adsorbing water molecules. N_0- and N_u- repeating units are considered to exist in the area where the structure described above is formed. While, N_s- repeating units exist in the area where the density of polysaccharide chains is low, and therefore do not participate in the structure formation by polysaccharide chains and water molecules. If s-monomers are needed to adsorb one water molecule, Eq. (2) is obtained

$$N_0 = sn, \quad N_u = sn_u \tag{2}$$

where n is the number of water molecules adsorbed on the polysaccharide molecular assemblies and n_u is the number of adsorption sites unoccupied by water molecules. It is thought that n increases when n_u is sufficiently large. The time evolution of n is expected to obey.

$$\frac{dn}{dt} = k_1 n_u - k_2 n \tag{3}$$

where k_1 and k_2 are rate constants for adsorption and desorption of water molecules. If n deviates from the equilibrium value, n changes with time t according to the Eq. (3). Eqs. (1)–(3) give

$$\frac{dn}{dt} + (k_1 + k_2)n = \frac{k_1}{s}(N - N_s) \tag{4}$$

which describes the annealing time t dependence of n. Here, we assume the simple relation to describe the time evolution of $N - N_s$,

$$\frac{d(N - N_s)}{dt} = -\alpha[n - n_{eq}(T)] \tag{5}$$

Here, α is the phenomenological constant and $n_{eq}(T)$ is the equilibrium value of n at the annealing temperature T. From Eqs. (4) and (5), the differential equation is obtained.

$$\frac{d^2n}{dt^2} + (k_1 + k_2)\frac{dn}{dt} + \frac{k_1 \alpha n}{s} = \frac{k_1 \alpha n_{eq}}{s} \tag{6}$$

New parameters γ and ω and the new variable n are redefined as follows,

$$\gamma = \frac{(k_1 + k_2)}{2}, \quad \frac{k_1 \alpha}{s} = \omega_0^2, \quad n = n - n_{eq} \tag{7}$$

Here, γ represents the averaged rate constant for adsorption and desorption of water molecules and ω_0 the constant representing the effect of the change of the number of adsorption sites on the time evolution of n. Eq. (6) is transformed by the replacement of Eq. (7) and Eq. (8) is obtained.

$$\frac{d^2n}{dt^2} + 2\gamma \frac{dn}{dt} + \omega_0^2 n = 0 \tag{8}$$

In the case of $\gamma < \omega_0$, Eq. (8) has the oscillational solution

$$n = C \exp(-\gamma t) \cos(\omega t + \delta) \tag{9}$$

where $\omega = (\omega_0^2 - \gamma^2)^{\frac{1}{2}}$. From Eq. (9), the number of water molecules attaching to xanthan molecules varies in an oscillational manner and experimental results on the amount of bound water could fit with Eq. (9) assuming appropriate parameters.

AFM images provided us direct information concerning the oscillational change of network structure. In the AFM images shown in Figs. 2 and 3(a), a part of the non-annealed xanthan molecules assemble and another part of the molecules is entangled in relaxed form. The placement of the assembled portion is not uniform. By 1 h annealing, molecular bundles expand by swelling and a heterogeneously assembled structure is observed. By 3 h annealing, network changed into homogeneous structure. By further annealing, homogeneity of network structure increased. A homogeneous network structure appeared by 24 h annealing. Molecular sizes calculated from AFM images, both height and width, (Figs. 4 and 5) correspond with the above variation of AFM images. AFM results support the fact that homogeneous hydrogels are formed when xanthan gum solution is annealed for 24 h. Oscillational change of ΔH_m by DSC (Fig. 6) indirectly shows that polysaccharide molecular chains aggregated and dissociated. These AFM images accord well with variation of non-freezing water content measured by DSC.

From the above AFM images, it is concluded that xanthan gum molecular chains in aqueous solution aggregate and dissociate in an oscillational manner with increasing annealing time. The homogeneous network structure is formed by annealing at 40 °C for 24 h.

Acknowledgement

This work was supported by a Grant-in-Aid for Scientific Research from Nagasaki University, Japan.

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