Molecular Size and Aggregation Behavior of Erwinia Gum in Aqueous Solution

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ABSTRACT: Erwinia (E) gum, a stabilizer and thickening agent of food, is composed of glucose, fucose, galactose, and glucuronic acid (1 : 0.1 : 0.05 : 0.3 by molar ratio). The apparent weight-average molecular weight M_w and intrinsic viscosity $[\eta]$ in 0.2 M NaCl aqueous solution were measured to be 7.83×10^5 and 268 mL g^{-1}, respectively, by light scattering and viscometry. The aggregation behavior of E gum in aqueous solution was investigated by gel permeation chromatography (GPC) and dynamic light scattering. The results showed that 7.5% E gum exists as an aggregate, whose diameter is 12 times greater than single-stranded chain, in aqueous solution at 25°C, and the aggregates' content decreased with increasing temperature or decreasing polymer concentration. The aggregates at higher temperature were more readily broken than in exceeding dilute solution. GPC analysis proved that a significant shoulder, corresponding to a fraction of higher molecular weight due to chain aggregation, appeared in the chromatogram of E gum in 0.05 M KH$_2$PO$_4$/5.7×10^{-3} M NaOH aqueous solution (pH 6.0) at 35°C, and decreased with increasing temperature, finally disappeared at 90°C. The disaggregation process of E gum in aqueous solution can be described as follows: with increasing temperature, large aggregates first were changed into the middle, then disrupted step by step into single-stranded chains. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 75: 1083–1088, 2000

Key words: Erwinia gum; molecular weight; aggregation; GPC; light scattering

INTRODUCTION

Erwinia (E) gum, an extracellular polysaccharide, produced by strains of *Erwinia mituyensis* 5796,

has been used as an additive for food such as a stabilizer and thickening agent.[1] Our recent work[2] indicated that the E gum is an ideal thickening agent, and its apparent viscosity η_a in water was more than that of xanthan, a well-known thickening agent. However, molecular weight and solution properties of E gum have been scarcely published. The thickening power of polysaccharides in aqueous solution depends upon molecular size and shape, i.e., molecular weight, conformation and viscosity. In general, large enhancement in viscosity of the polymer solution is attributable to chain stiffness,[3,4] large hydrodynamic volume of polyelectrolyte caused by electrostatic force[5]

and the aggregation resulted from inter-chain interaction.[6,7] Recently, much attention has been paid to the polymer aggregation, which play an important role in the thickening, micellization, gelation and microencapsulation etc.. The aggregation process in dilute solution,[8] aggregation mechanism,[9] aggregation behavior,[10] and the effects of temperature and polymer concentration on the aggregation of polymers[5,11,12] have been studied by static and dynamic light scattering, combining gel permeation chromatography (GPC) with infrared (IR) spectroscopy.

Aggregation behavior of some polysaccharides was investigated by using static and dynamic light scattering and GPC in our laboratory. It was proved that β-D-glucan PC3 from Poria cocos sclertium forms aggregates in aqueous solution and disassociates into single chains in cadoxen or dimethyl sulfoxide (Me$_2$SO).[13–15] Aeromonas gum, a stabilizer and thickening agent of food, exists as an aggregate in 0.5 M NaCl aqueous solution and as a single chain in cadoxen.[7] These results indicated that polysaccharides have generally a tendency to aggregate in aqueous solution because of the abundance of inter-chain hydrogen bonds. In this work, components, apparent weight-average molecular weight M_w, mean molecular size, aggregation behavior of E gum in aqueous solution were determined by the IR, high-performance liquid chromatography (HPLC), static and dynamic light scattering, and GPC. The effects of temperature and polymer concentration on the aggregation and the disaggregation process were studied and discussed.

EXPERIMENTAL

Preparation and Analysis of Sample

Erwinia (E) gum of the first batch was kindly supplied by Fourth Specialty Chemicals Sales Department, Asahi Chemical Industry Co., Ltd. in Japan. The E gum aqueous solution (0.4 wt %) was centrifuged at 7000 rpm for 12 h to remove the water-insoluble gum, then concentrated by rotary evaporation at reduced pressure below 45°C, and finally added acetone as a precipitant to precipitate. The E gum precipitate was washed for 8 times by acetone, then dried in vacuum for 7 days to obtain E gum white powder.

The IR spectrum of the sample was recorded with a Nicolet FTIR spectrometer. It showed IR absorption at 890 cm^{-1} indicative of β-glucoside

and at 1610 cm^{-1} (CO$_2^-$). HPLC spectrum of sugars, obtained by hydrolyzing the sample with trifluoroacetic acid in sealed tube at 100°C, was performed on HPLC (LC-6A, Shimadzu) equipped with μ-Bondapak NH$_2$ column (7.8 mm × 300 mm) using refractometer detector. A mixture of CH$_3$CN/H$_2$O/CH$_3$OH (85 : 10 : 5 by volume) at 25°C was used as the mobile phase. The colorimetric method of Dishe's carbozole reaction for uronic acid in the presence of borate[16] was used for analysis of glucuronic acid. An absorbance of the reacted solution was monitored at 530 nm by UV spectroscopy (UV-160A, Shimadzu). The components of E gum were obtained to be glucose, fucose, galactose and glucuronic acid (1 : 0.1 : 0.05 : 0.3 by molar ratio).

Light Scattering

In static light scattering, the scattering light intensities were observed on a dynamic light scattering spectrophotometer (DLS-700, Otsuka Electronics Co.) with $\lambda = 633$ nm in an angular range from 30° to 150° at 15° intervals at 25°C. E gum solution was prepared with solvent of 0.2 M NaCl aqueous solution. The optical clarification of the solution was achieved by using a sand filter, with subsequent filtration through a 0.2-μm pore size filter (M-HJV) into the scattering cell. The refractive index increment (dn/dc) was 0.128 mL g^{-1} for the dialyzed solution measured with a double-beam differential refractometer (DRM-1020, Otsuka Electronics Co.) at 633 nm and 25°C.

Dynamic light scattering measurement was performed on BI-200SM laser light scattering (Brookhaven Co.) equipped with BI-9000 AT correlator and Innova 304 argon ion laser ($\lambda = 515$ nm) at $\theta = 60°$. The E gum solution purified was measured at 25, 40, 45, 50, and 60°C to obtain an intensity-intensity time correlation function $G^2(t, q)$ in the self-beating mode, which can be expressed by the normalized first-order electric field time correlation function $g^{(1)}(t, q)$[17]:

$$G^2(t, q) = A[1 + \beta|g^{(1)}(t, q)|^2] \qquad (1)$$

where A is a measured baseline; β, a parameter depending on the coherence of the detection; t, the delay time. $g^{(1)}(t, q)$ is related to the line-width distribution $G(\Gamma)$ by[18]

$$g^{(1)}(t, q) = \int_0^\infty G(\Gamma)e^{\Gamma t} \, d\Gamma \qquad (2)$$

$G(\Gamma)$ can be reduced to a translational diffusion coefficient distribution $G(D)$. Γ and D are systemertic averaged, so the average line width $\langle\Gamma\rangle$ and average translational diffusion coefficient $\langle D\rangle$ can be expressed by

$$\langle\Gamma\rangle = \int_0^\infty G(\Gamma)\Gamma\ d\Gamma \tag{3}$$

$$\langle D\rangle = \langle\Gamma\rangle/q^2 \tag{4}$$

The average diameter $\langle d\rangle$ of the polymer particle can be calculated by Stokes-Einstein equation:

$$\langle d\rangle = k_B T/(3\pi\eta_s\langle D\rangle) \tag{5}$$

where k_B is Boltzmann's constant; T, the temperature (K) and η_s, the solvent viscosity.

Gel Permeation Chromatography (GPC)

An HPLC instrument (Waters Co.) equipped with TSK G 5000 PW column (7.5 mm $\times$ 300 mm), 600 pump, 410 differential refractometer and 2010 Millennium Workstation was used for GPC analysis. The pullulan standards (P-20, P-50, P-200, P-400, and P-800), and the E gum solution were measured at 35, 60, 70, 80, and 90°C. The eluant was 0.05 M KH$_2$PO$_4$/5.7 $\times$ 10^{-3} M NaOH (pH 6.0) aqueous solution and the flow-rate was 1.0 mL min^{-1}. E gum was dissolved in 0.05 M KH$_2$PO$_4$/5.7 $\times$ 10^{-3} M NaOH to prepare about 3 $\times$ 10^{-3} g mL^{-1} concentration, and the injected volume was 10 μL.

Viscometry

Viscosity of E gum in 0.2 M NaCl aqueous solution was measured by using Ubbelodhe capillary viscometer at 25 $\pm$ 0.1°C. The kinetic energy correction was always negligible. Huggins and Kraemer plots were used to estimate the intrinsic viscosity [η].

RESULTS AND DISCUSSION

Apparent Molecular Size

Figure 1 illustrates the Zimm plots for E gum in 0.2 M NaCl aqueous solution. Here K is the light-scattering constant, R_θ is the reduced Rayleigh ratio at angle $\theta°$, and c is polysaccharide concen-
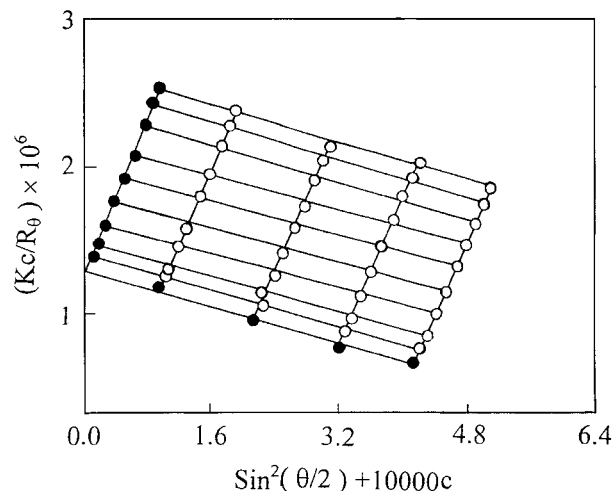

Figure 1   Zimm plot of E gum in 0.2 M NaCl aqueous solution at 25°C.

tration. The apparent weight-average molecular weight M_w, mean radius of gyration $\langle S\rangle$ and second virial coefficient A$_2$ were calculated to be 7.83 $\times$ 10^5, 65 nm, and -6.8 $\times$ 10^{-4} mL mol g^{-2}, respectively. From $\langle S\rangle$ value, the apparent mean molecular size can also be described with 2$\langle S\rangle$ = 130 nm. [η] of E gum in 0.2 M NaCl aqueous solution was determined to be 268 mL g^{-1}, which is much more than 172 mL g^{-1} of pullulan, a flexible glucan, with same M_w in 0.02 wt % sodium azide aqueous solution,[19] indicating a larger thickening power in the dilute solution.

Aggregation Behavior

Figure 2 shows GPC chromatograms of E gum with 0.05 M KH$_2$PO$_4$/5.7 $\times$ 10^{-3} M NaOH aqueous solution as mobile phase at 35, 60, 70, 80, and 90°C. The left in the GPC patterns was almost vertical line, because the exclusion limit of the column was lower than the highest apparent molecular weight of the sample used here. A significant shoulder, corresponding to a fraction of higher molecular weight, appeared in the chromatogram at 35°C. The shoulder of the high molecular weight fraction is due to chain aggregation, similar to the result from β-D-glucan aggregates in 0.25 M LiCl/Me$_2$SO at 40°C.[14] Obviously, the shoulder peak decreased with increasing temperature, and disappeared at 90°C, showing a sharp peak of single-chain stranded. It indicated that higher temperature could disrupt the aggregates of E gum in aqueous solution. Interestingly, the aggregates were dissociated as a single-
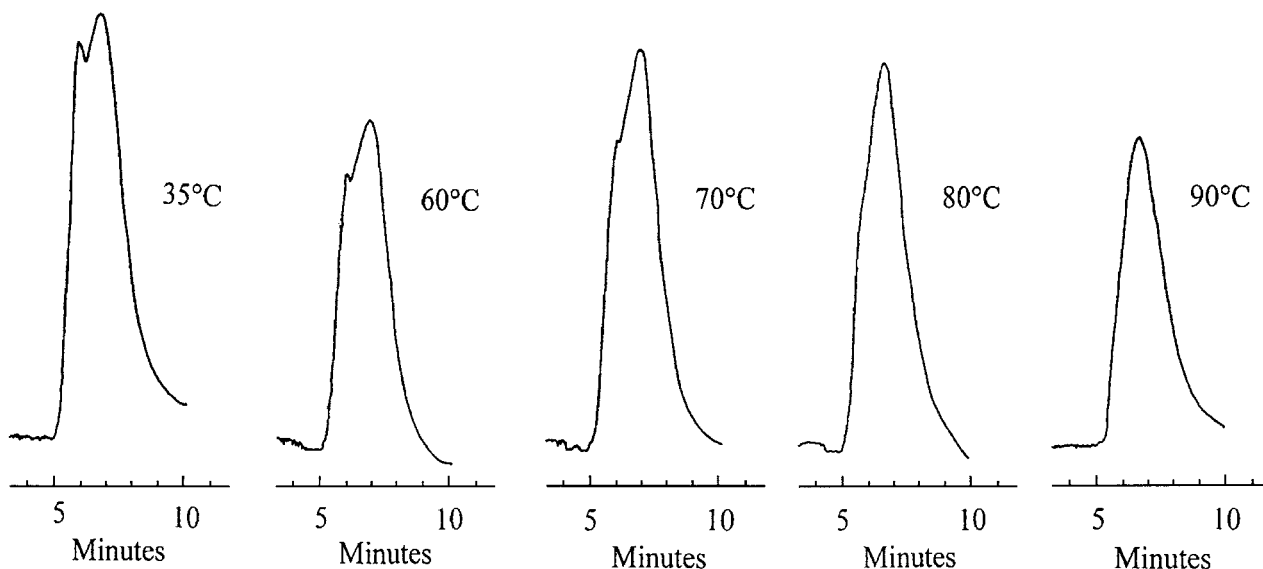
Figure 2 GPC chromatograms of E gum in 0.05 M KH$_2$PO$_4$/5.7 × 10^{-3} M NaOH aqueous solution at 35, 60, 70, 80, and 90°C.

stranded chain at 90°C, which is in agreement with the GPC result of β-D-glucan from poria cocos sclerotium at 80°C.[14] This gives the further evidence that the aggregation can be avoided by carrying out GPC measurement at high temperature.

The average diameters $\langle d \rangle$ of the E gum particles in 0.2 M NaCl aqueous solution at 25, 40, 45, 50, and 60°C were obtained to be 124, 101, 99, 98, and 91 nm, respectively, and the diameter distribution is shown in Figure 3. The $\langle d \rangle$ value at 25°C is close to apparent mean molecular size (130 nm) of E gum from static light scattering at 25°C. These measurements were performed in 3–4 h by increasing temperature step by step. Obviously, there are aggregates in the E gum solution at room temperature, supporting the conclusion from GPC. At 25°C, mainly two kinds of particles exist in the solution, namely, single-stranded chain with diameter of about 50 nm, and aggregate with diameter of about 600 nm. The content of aggregates at 25°C was calculated to be 7.5%. With increasing temperature, middle particles appeared and changed. The contents of middle particles are 0 at 25°C, 9% at 40°C, 19% at 50°C and 6.2% at 60°C, respectively. The contents of large particles decreased with increasing temperature to be 7.5% at 25°C, 5.9% at 40°C, 5.0% at 50°C, and 4.0% at 60°C. It is worth noting that the diameter of single-chain is still about 50 nm, while the diameter and number of large aggre-

gates decreased step by step with increasing temperature. Figure 4 shows temperature dependence of the average diameter $\langle d \rangle$ of E gum in 0.2 M NaCl aqueous solution. It indicated that the $\langle d \rangle$ decreased with increasing temperature, suggesting that the interchain interaction of E gum was disrupted. Unfortunately, data at 70–90°C were not obtained because of the temperature limit (<65°C) of the light scattering instrument. Based on the results mentioned above, the disaggregation process of the E gum solution can be described as follows: with increasing temperature, the large aggregates first were changed into middle, then disrupted into single-stranded chains.

Figure 5 shows the relationship between the average diameter $\langle d \rangle$ and concentration c of E gum in 0.2 M NaCl aqueous solution at 25°C. The concentration used in the present work changed from 7.172 × 10^{-4} to 1.544 × 10^{-3} g mL^{-1}. Obviously, $\langle d \rangle$ values increased sharply with increase of the concentration. It can be interpreted that the lower polymer concentration in solution, is more effective at interacting the solvent with the polymer. With increasing the polymer concentration, the solvent-polymer interaction will decrease, while the polymer-polymer interaction increases, resulting in a great increase of diameter due to aggregation.[12] Raspaud et al.[8] have reported that triblock copolymers in a selective solvent formed aggregates above the critical aggre-
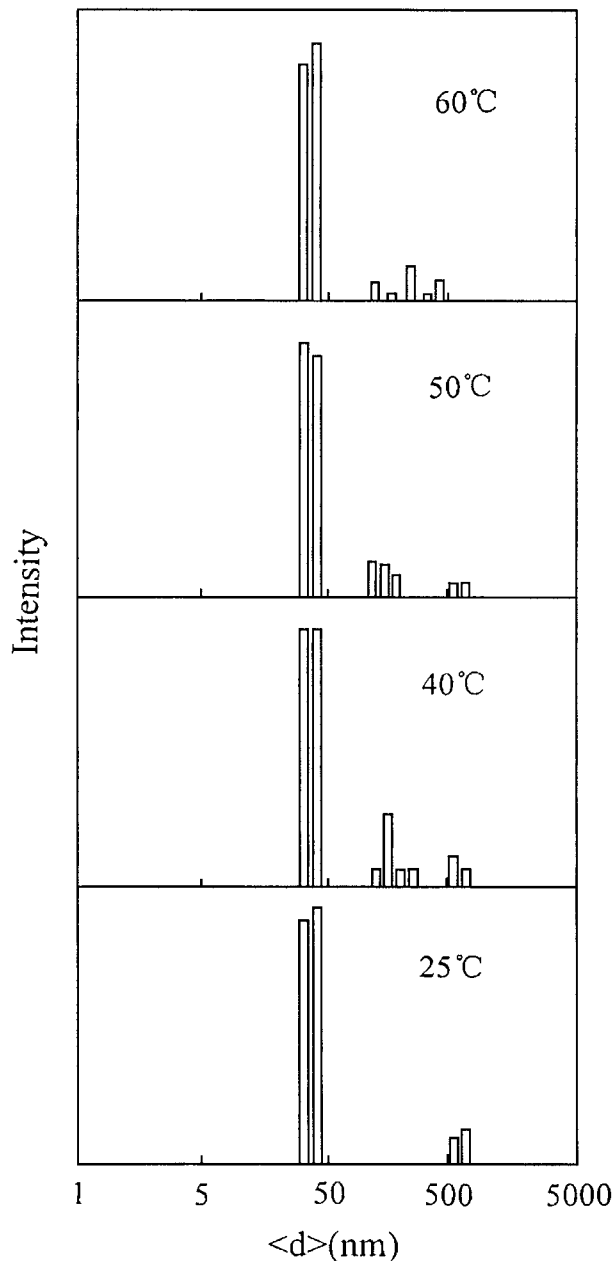
Figure 3 Diameter distribution of E gum in 0.2 M NaCl aqueous solution at 25, 40, 50, and 60°C.

gation concentration, which equals to $(1.6 \pm 0.2) \times 10^{-3}$ g mL^{-1}, and the diameter increased sharply with increasing concentration. Compared with the triblock copolymer, E gum is more easily aggregated, and the critical aggregation concentration may be much lower than that of the triblock copolymer. Clearly, E gum can form aggregates even in exceeding dilute solution, showing a larger thickening action.
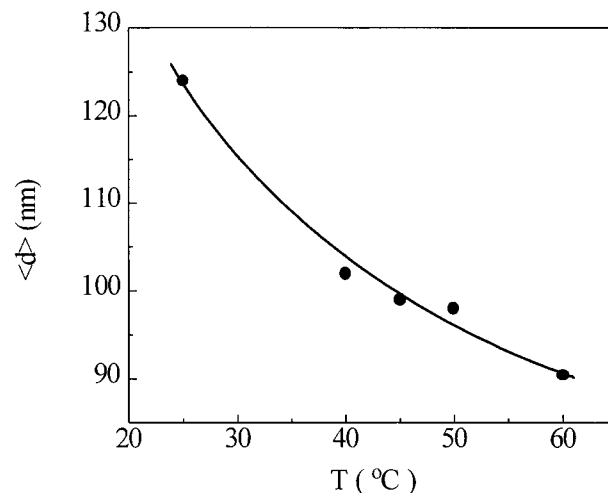

Figure 4 Temperature (T) dependence of the average diameter ($\langle d \rangle$) of E gum in 0.2 M NaCl aqueous solution with polymer concentration of 0.9894×10^{-3} g mL^{-1}.

CONCLUSIONS

The apparent weight-average molecular weight M_w, mean radius of gyration $\langle S \rangle$, intrinsic viscosity of $[\eta]$ Erwinia gum in 0.2 M NaCl aqueous solution at 25°C were 7.83×10^5, 65 nm, and 268 mL g^{-1}, respectively. 7.5% E gum exists as larger aggregate with average diameter of 124 nm in aqueous solution at 25°C. The numbers of aggregates of E gum in aqueous solution significantly decreased with increasing temperature or decreasing polymer concentration. The aggregates
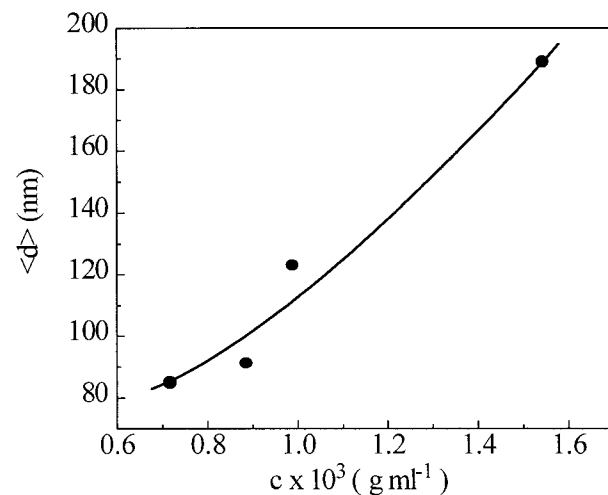

Figure 5 Concentration (c) dependence of the average diameter ($\langle d \rangle$) of E gum in 0.2 M NaCl aqueous solution at 25°C.

were disrupted completely into single-stranded chains at 90°C, but E gum can still form aggregates in very dilute aqueous solution at 25°C. The aggregates at higher temperature were more readily broken than in exceeding dilute solution, which is more effective at interacting the solvent with polymer chain.[12] The disaggregation process of E gum in aqueous solution can be described as follows: with increasing temperature, larger aggregates first were changed into the middle and then weredisrupted step by step into single-stranded chains.

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