

## Dilute-solution behavior of aeromonas gum, a heteropolysaccharide

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### Summary

Fifteen fractions of aeromonas gum, a heteropolysaccharide produced by the strain *Aeromonas nichidenii*, have been studied by static light scattering and viscometry with dimethylsulfoxide containing 0.2 M LiCl at 25°C as the solvent. Data for the z-average radius of gyration and the intrinsic viscosity covering a molecular weight range from  $4.5 \times 10^5$  to  $2 \times 10^6$  show the polymer to behave like a semiflexible chain in this solvent, and are analyzed on the basis of the wormlike chain by coarse-graining the heteropolysaccharide molecule. It is shown that these data and those for the particle scattering function are consistently explained by this model with a (mean) persistence length of 10 ( $\pm 1$ ) nm and a (mean) linear mass density of 1450 ( $\pm 100$ ) nm<sup>-1</sup>, and that the heteropolysaccharide chain is as stiff as cellulose derivatives.

### Introduction

In the past one or two decades, solutions of associating polymers, synthetic or naturally occurring, have received much attention of polymer chemists and physicists because of their wide applicability to food additives, cosmetics, paints, and so on [1]. Not a few associating polymers tend to form aggregates even in dilute solutions, so that experimental determination of their molecular characteristics, basic to the understanding of the formation of aggregates, is often a quite difficult task. The situation is more knotty when copolymer samples heterogeneous in composition and molecular weight are to be investigated. Aeromonas (A) gum, produced extracellularly by the strain *Aeromonas nichidenii*, is such an industrially useful heteropolysaccharide [2] forming aggregates in aqueous salts even at extremely low polymer concentrations [3]. Its major constituents are mannose (Man), glucose (Glu), xylose (Xyl), galactose (Gal), and mannuronic acid (ManA) [3]. Very recently, we found that A gum molecularly disperses in dimethylsulfoxide containing 0.2 M lithium chloride (0.2 M LiCl/DMSO) and is stable in it. The present study was thus undertaken to deduce the global conformation of the polysaccharide molecule in this solvent from static light scattering and viscosity measurements.

Our previous analysis [3] showed that an unfractionated (original) sample and five

middle fractions of A gum have molar ratios (Man : Glu : Xyl : Gal : ManA) of 1.0 : 0.55 ( $\pm 0.12$ ) : 0.46 ( $\pm 0.20$ ) : 0.17 ( $\pm 0.10$ ) : 0.46 ( $\pm 0.13$ ) and that the relatively small variation in the ratio with molecular weight is not systematic [4]. Thus, A gum fractions of different molecular weights may be regarded in a first approximation as a series of homologous samples if their composition distribution is assumed to be almost independent of molecular weight or random. Furthermore, copolymer effects on the light scattering determination of the weight-average molecular weight  $M_w$ , the second virial coefficient  $A_2$ , and the z-average radius of gyration  $\langle S^2 \rangle_z^{1/2}$  may be neglected in a good approximation, because the constituent sugars must have similar refractive indices. Accepting these plausible assumptions, we can circumvent serious impediments to the solution characterization of the heteropolysaccharide. In the work reported below, we analyze data of  $\langle S^2 \rangle_z$ ,  $P(\theta)$  (the particle scattering function), and  $[\eta]$  (the intrinsic viscosity) for A gum fractions in 0.2 M LiCl/DMSO at 25°C by applying relevant theories for homopolymer solutions.

## Experimental

### *Samples*

A sample (3.7 g) of A gum from *Aeromonas nichidenii* 5797 (Asahi Chemical Industry Co., Japan) was divided into 34 parts by repeating fractional precipitation four times with 0.2 M LiCl/DMSO as the solvent and acetone as the precipitant. The initial polymer concentration was adjusted to about 0.46%. Each product was reprecipitated from a DMSO solution (containing no LiCl) into acetone, washed with acetone four times (to remove LiCl), and finally dried in vacuum over a week. From the fractions thus prepared, 15 middle ones were chosen and designated below as AF-1, AF-2, ..., AF-15 in the order of decreasing molecular weight. A few of these fractions were investigated by  $^{13}\text{C}$  and  $^1\text{H}$  NMR to obtain some structural information, but our attempt ended up with observing too complicated, useless spectra.

### *Light Scattering*

Intensities of light scattered from 0.2 M LiCl/DMSO solutions of A gum fractions were measured at 25°C on a Fica 50-light scattering photometer in an angular range from 30 to 150° using vertically polarized incident light of 436 nm in wavelength  $\lambda_0$ . Pure benzene at 25°C was used to calibrate the apparatus with its Rayleigh ratio taken as  $46.5 \times 10^{-6} \text{ cm}^{-1}$  at 436 nm [5]. The depolarization ratio of this liquid was determined to be 0.406 by the method of Rubingh and Yu [6]. Test solutions were made optically clean by filtration through 0.45  $\mu\text{m}$  Millipore filters, followed by 5-h centrifugation at  $2.5 \times 10^4 g$  in a Sorvall RC 5C centrifuge. The excess reduced scattering intensities  $R_\theta$  obtained as functions of scattering angle  $\theta$  and polymer mass concentration  $c$  were analyzed by use of the square-root plots of  $(Kc/R_\theta)^{1/2}$  vs.  $c$  and  $(Kc/R_\theta)^{1/2}$  vs.  $k^2$ . Here,  $K$  is the optical constant and  $k$  is the magnitude of the scattering vector defined by  $k = (4\pi n_0/\lambda_0) \sin(\theta/2)$ , with  $n_0$  being the solvent refractive index.

The specific refractive index increment  $(\partial n/\partial c)_\mu$  for dialyzed 0.2 M LiCl/DMSO solutions of A gum at 25°C and 436 nm was determined for fractions AF-1, AF-9, and

AF-12 using a modified Schulz-Cantow type differential refractometer. The subscript  $\mu$  attached to  $(\partial n/\partial c)$  signifies the condition that the chemical potentials of all diffusible components are kept constant. The  $(\partial n/\partial c)_\mu$  values obtained were  $0.0629 \text{ cm}^3 \text{ g}^{-1}$  regardless of the fraction.

### Viscometry

Conventional capillary viscometers of the Ubbelohde type were used to determine  $[\eta]$  and  $k'$  (the Huggins constant) for all A gum fractions in 0.2 M LiCl/DMSO at 25°C. Except for low molecular weight fractions AF-12, AF-13, and AF-15, the  $k'$  values were about 0.30, as may be expected for polymers molecularly dispersed in good solvents. Those for the three low molecular weight fractions were 0.12 - 0.23. Such small values are most likely due to the ionization of some acid groups in ManA, but we did not pursue the problem.

## Results and Discussion

### Light Scattering and Viscosity Data

Figures 1 and 2 illustrate the concentration dependence of  $(Kc/R_0)^{1/2}$ , i.e.,  $(Kc/R_\theta)^{1/2}$  at zero scattering angle, and the angular dependence of  $(Kc/R_\theta)_{c=0}^{1/2}$ , i.e.,  $(Kc/R_\theta)^{1/2}$  at infinite dilution, for the indicated A gum fractions in 0.2 M LiCl/DMSO at 25°C; in the latter, data at larger  $k$  are not shown (see Figure 5), since plotted points for high molecular weight fractions markedly deviate downward from the dashed lines representing the initial slopes. The apparent values of  $M_w$ ,  $A_2$ , and  $\langle S^2 \rangle_z^{1/2}$  evaluated were equated to the true values for the reason mentioned in the Introduction. The numerical results for all fractions are summarized in Table I, along with those for  $[\eta]$ . The values of  $A_2$  are on the order of  $10^{-4} \text{ mol g}^{-2} \text{ cm}^3$  for any fractions, so that intermolecular interactions between A gum chains in the solvent are predominantly repulsive.

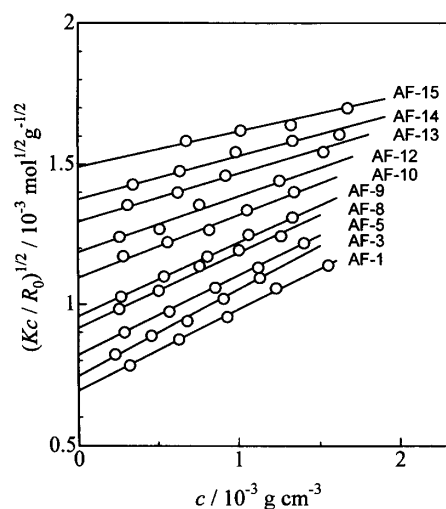


Figure 1. Concentration dependence of  $(Kc/R_0)^{1/2}$  for A gum fractions in 0.2 M LiCl/DMSO at 25°C.

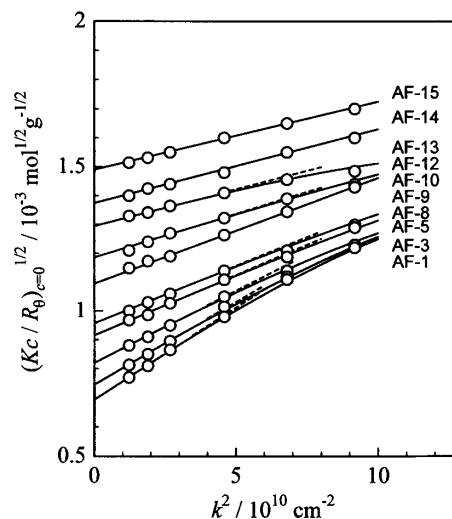


Figure 2. Angular dependence of  $(Kc/R_\theta)_{c=0}^{1/2}$  for A gum fractions in 0.2 M LiCl/DMSO at 25°C. The dashed lines indicate the initial slopes.

The Flory viscosity factor  $\Phi = [\eta]M_w / (6\langle S^2 \rangle_z)^{3/2}$ , included in Table I, is essentially independent of molecular weight and its average  $(2.0 \pm 0.3) \times 10^{23} \text{ mol}^{-1}$  is the one predictable for long flexible homopolymers in good solvents [7] or stiff chains in a limited molecular weight range [8]. This behavior of  $\Phi$  lends support to our expectation (mentioned in the Introduction) that A gum fractions of different molecular weights may be treated as a series of homologous samples. In other words, the present  $\Phi$  data and the previous composition data [3] allow us to examine how  $\langle S^2 \rangle_z^{1/2}$  and  $[\eta]$  vary with  $M_w$ .

Table I. Results from light scattering and viscosity measurements on A gum fractions in 0.2 M LiCl/DMSO at 25°C

Fraction	$M_w \times 10^{-6}$	$A_2 \times 10^4$ / mol g <sup>-2</sup> cm <sup>-3</sup>	$\langle S^2 \rangle_z^{1/2}$ / nm	$[\eta]$ / cm <sup>3</sup> g <sup>-1</sup>	$\Phi \times 10^{-23}$ / mol <sup>-1</sup>
AF-1	2.08	2.00	74.3	536	1.8
AF-2	1.84	2.32	68.2	505	2.0
AF-3	1.80	2.31	67.9	483	1.9
AF-4	1.52	2.61	59.0	451	2.3
AF-5	1.49	2.34	60.5	444	2.0
AF-6	1.43	2.51	60.0	422	1.9
AF-7	1.36	2.40	58.5	429	2.0
AF-8	1.19	2.47	52.5	398	2.2
AF-9	1.09	2.54	50.1	354	2.1
AF-10	0.834	2.46	44.8	296	1.9
AF-11	0.826	2.40	43.1	294	2.1
AF-12	0.711	2.38	39.2	247	2.0
AF-13	0.596	2.24	34.4	214	2.1
AF-14	0.529	2.13	33.4	191	1.8
AF-15	0.450	1.90	30.8	158	1.7

The molecular weight dependence of  $\langle S^2 \rangle_z^{1/2}$  and that of  $[\eta]$  are shown in Figures 3 and 4, respectively. The former relation is linear with a slope of 0.57, indicating that A gum in the  $M_w$  range examined behaves like a linear flexible chain expanded by large excluded-volume effect or like a stiff chain with or without excluded volume [8]. On the other hand, the latter relation slightly bends down and has a slope of about 0.75 for  $M_w > 8 \times 10^5$ . Although this viscosity exponent appears to be consistent with the expanded coil conformation, the downward curvature of the plot reveals an unmistakable stiffness of the polysaccharide. Hence, a semiflexible chain must be a reasonable model for A gum and excluded-volume effects on  $[\eta]$  and  $\langle S^2 \rangle_z$  may not be large, if any. The solid and dashed curves in the two figures are explained in the next subsection.

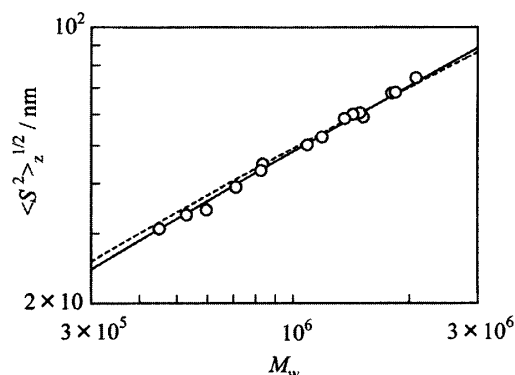


Figure 3. Radii of gyration for A gum fractions in 0.2 M LiCl/DMSO, compared with the theoretical curves for the unperturbed wormlike chain (the dashed line) with  $q = 11$  nm and  $M_L = 1450$  nm<sup>-1</sup> and for the perturbed wormlike chain (the solid line) with  $q = 9.3$  nm,  $M_L = 1450$  nm<sup>-1</sup>, and  $B = 1.6$  nm.

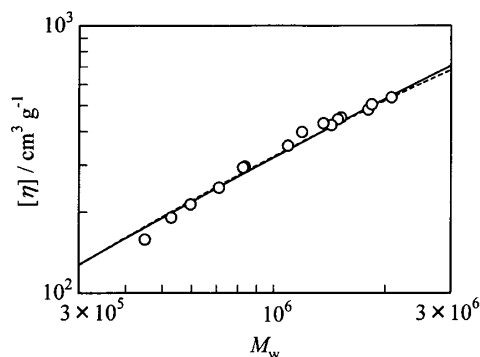


Figure 4. Intrinsic viscosities for A gum fractions in 0.2 M LiCl/DMSO, compared with the theoretical curves for the unperturbed wormlike chain (the dashed line) with  $q = 11$  nm,  $M_L = 1450$  nm<sup>-1</sup>, and  $d = 2.0$  nm and for the perturbed wormlike chain (the solid line) with  $q = 9.3$  nm,  $M_L = 1450$  nm<sup>-1</sup>,  $B = 1.6$  nm, and  $d = 2.7$  nm.

We confirmed the irrelevance of the random coil conformation by observing that the linear plot of  $[\eta]/M_w^{1/2}$  against  $M_w^{1/3}$  [9] expected for expanded random coils is markedly convex upward when applied to the present data; we note that excluded-volume interactions (in a flexible copolymer chain) can be treated in the conventional scheme with the mean binary cluster integral (a linear combination of the cluster integrals for all possible pairs of like or unlike units) provided the composition distribution is random [9]. Thus, it seems reasonable to analyze the  $\langle S^2 \rangle_z$  and  $[\eta]$  data for A gum on the basis of the Kratky-Porod wormlike chain [10] (a typical model for stiff polymers) by coarse-graining the heteropolysaccharide molecule, i.e., by replacing it with a continuously bending chain.

#### Data Analysis as a Wormlike Chain

The unperturbed mean-square radius of gyration  $\langle S^2 \rangle_0$  of the wormlike chain is expressed by [11]

$$\langle S^2 \rangle_0 = \frac{qL}{3} - q^2 + \frac{2q^3}{L} - \frac{2q^4}{L^2} [1 - \exp(-L/q)] \quad (1)$$

where  $L$  and  $q$  denote the contour length and persistence length of the chain, respectively. Since  $L$  equals the ratio of  $M$  (the molecular weight) to  $M_L$  (the molar mass per unit contour length), the molecular weight dependence of  $\langle S^2 \rangle_0$  is determined by  $q$  and  $M_L$ . We note that for A gum these two parameters are composition-averages [9]. The theory [12] for  $[\eta]_0$  (the unperturbed intrinsic viscosity) of the wormlike chain contains one additional parameter, the chain diameter  $d$ . Although the diameter depends on the kind of sugar in A gum, we assume a single (coarse-grained)  $d$  value.

We searched for a set of  $q$ ,  $M_L$ , and  $d$  allowing eq 1 and the  $[\eta]_0$  theory (the cylinder model) to consistently explain the experimental data in Figures 3 and 4; the  $\langle S^2 \rangle_z$  data allow unique determination of  $q$  and  $M_L$ , while the  $[\eta]$  data give two of the three parameters. The dashed curves in Figures 3 and 4 represent the theoretical values computed with the resulting parameters,  $q = 11$  nm,  $M_L = 1450$  nm<sup>-1</sup>, and  $d = 2.0$  nm. They fit the data points fairly well, though the agreement at low  $M$  is not very good in either graph.

According to well documented studies [13, 14], excluded-volume effects on  $\langle S^2 \rangle_z$  and  $[\eta]$  of typical stiff chains begin to be appreciable when the Kuhn segment number ( $= M/2qM_L$ ) exceeds about 50. For A gum, this number corresponds to a molecular weight of  $1.6 \times 10^6$ , which is not very different from the highest molecular weight studied. Thus, the estimated  $q$  and  $M_L$  values appear to be consistent with the unperturbed wormlike chain behavior of the polysaccharide. To check this, we take account of excluded-volume effects on  $\langle S^2 \rangle_z$  and  $[\eta]$  in the quasi-two-parameter (QTP) scheme [15], in which the expansion factors  $\alpha_s^2 (= \langle S^2 \rangle / \langle S^2 \rangle_0)$  and  $\alpha_\eta^3 (= [\eta] / [\eta]_0)$  are universal functions of the scaled excluded-volume parameter  $\tilde{z}$  defined (for the wormlike chain) by

$$\tilde{z} = \frac{3}{4} K(L/2q) z \quad (2)$$

and are expressed by

$$\alpha_s^2 = \left[ 1 + 10\tilde{z} + \left( \frac{70\pi}{9} + \frac{10}{3} \right) \tilde{z}^2 + 8\pi^{3/2} \tilde{z}^3 \right]^{2/15} \times [0.933 + 0.067 \exp(-0.85\tilde{z} - 1.39\tilde{z}^2)] \quad (3)$$

$$\alpha_\eta^3 = (1 + 3.8\tilde{z} + 1.9\tilde{z}^2)^{0.3} \quad (4)$$

if the Domb-Barrett [16] and Barrett [17] equations are adopted. Here,  $K(L/2q)$  is a known function of  $L/2q$  and  $z$  is the conventional excluded-volume parameter defined by  $z = (3/2\pi)^{3/2} (B/2q)(L/2q)^{1/2}$ , with  $B$  being the excluded-volume strength ( $=$  the binary cluster integral divided by the square of the bead spacing);  $B$  is also a composition-average for A gum. Using the expression  $A_2 = N_A B / 2M_L^2$  in the single-contact approximation [15] ( $N_A$  is the Avogadro constant) with  $A_2 = 2.3 \times 10^{-4}$  mol g<sup>-2</sup> cm<sup>3</sup> (the mean of the values in Table I), we have a relation between  $B$  and  $M_L$ , so that a set of parameters ( $q$ ,  $M_L$ ,  $d$ , and  $B$ ) may be estimated from the  $\langle S^2 \rangle_z$  and  $[\eta]$  data as was done above for the case of the unperturbed wormlike chain.

The solid lines in Figures 3 and 4 show the theoretical values calculated as a perturbed wormlike chain with  $q = 9.3$  nm,  $M_L = 1450$  nm<sup>-1</sup>,  $B = 1.6$  nm, and  $d = 2.7$  nm. The fit to the  $\langle S^2 \rangle_z$  data is improved, whereas that to the  $[\eta]$  data is almost unchanged. Equally good fits were obtained for different parameter sets in the ranges of  $q$ ,  $M_L$ , and  $B$  from 9.0 - 10 nm, from 1350 - 1550 nm<sup>-1</sup>, and from 1.5 - 1.8 nm, respectively, for the fixed  $d$  of 2.7 nm. The uncertainties arose from the fact that  $q$  and  $B$  contribute toward similarly increasing both  $\langle S^2 \rangle_z$  and  $[\eta]$ . In any case, introduction of  $B$  only leads to a slight decrease in  $q$  with  $M_L$  kept essentially unchanged, convincing us that the wormlike-chain parameters for A gum are little affected by the presence or

absence of excluded volume effects in the molecular weight range studied. Hence, we conclude that the coarse-grained heteropolysaccharide molecule in 0.2 M LiCl/DMSO at 25°C is characterized by  $q = 10 (\pm 1)$  nm and  $M_L = 1450 (\pm 100)$  nm<sup>-1</sup>. The chain diameter should be 2 to 3 nm, its precision being only moderate.

Figure 5 compares experimental  $P(\theta)^{-1} [= M_w(Kc/R\theta)_{c=0}]$  for several A gum fractions (over the entire angular range studied) with theoretical values for the unperturbed wormlike chain [18]; it is well known that  $P(\theta)$  is insensitive to excluded-volume effect. We have used  $q = 11$  nm for AF-1, AF-3, and AF-10 and 10 nm for the rest, with  $M_L$  fixed to 1450 nm<sup>-1</sup>. The generally good agreement between theory and experiment confirms the wormlike-chain parameters estimated from  $\langle S^2 \rangle_z$  and  $[\eta]$ . Since  $P(\theta)$  is known to be relatively sensitive to the heterogeneity of the sample, the agreement suggests that our fractions are reasonably sharp in molecular weight distribution.

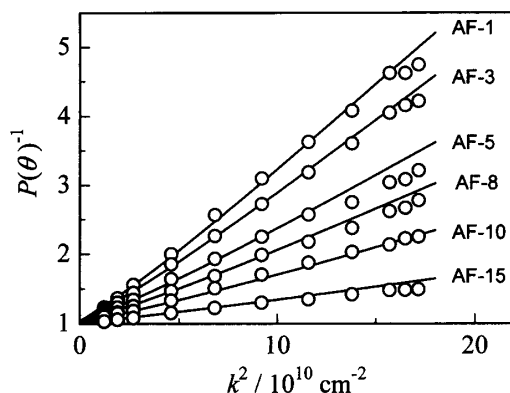


Figure 5. Comparison of experimental scattering functions for A gum fractions in 0.2 M LiCl/DMSO (circles) with theoretical curves for the unperturbed wormlike chain with  $M_L = 1450$  nm<sup>-1</sup> and  $q = 11$  nm (for AF-1, AF-3, and AF-10) or  $q = 10$  nm (for AF-5, AF-8, and AF-15).

### Conformational Characteristics

We have found that A gum behaves like a stiff chain with a (mean or composition-averaged) persistence length of  $10 (\pm 1)$  nm and a mean linear mass density of  $1450 (\pm 100)$  nm<sup>-1</sup> in 0.2 M LiCl/DMSO at 25°C. These parameters yield  $2.3 \times 10^{-3}$  nm<sup>2</sup> for the unperturbed dimension  $(\langle S^2 \rangle_0/M)_\infty$  of the polymer at infinite molecular weight. The persistence length of 10 nm is comparable to those reported for cellulose derivatives [19], indicating that the heteropolysaccharide chain is as stiff as cellulose derivatives.

The chain stiffness of A gum or conversely its considerable flexibility may rule out the possibility of a multi-helical conformation in 0.2 M LiCl/DMSO. The molecular weight dependence of  $\langle S^2 \rangle_z^{1/2}$  and  $[\eta]$  in Figures 3 and 4 also precludes the presence of long branches. The estimated  $M_L$  value ( $1450 \pm 100$  nm<sup>-1</sup>) then suggests that the polysaccharide should have side chains or short branches composed of 2 - 3 sugars (on the average) per main-chain residue, because without side chain,  $M_L$  should be 400 - 500 nm<sup>-1</sup>. In this case, a chain diameter of 2 - 3 nm may be expected, which is indeed consistent with the present estimate from  $[\eta]$ . These pieces of structural information may be ascertained if the primary structure of A gum is established by a certain elaborate chemical analysis. Since most heteropolysaccharides are left unexplored in

the field of polymer solutions despite their growing importance, it is highly desirable to extract conformational or molecular characteristics of those polymers from solution data by utilizing the current knowledge of dilute polymer solutions, as was done in this work. The relatively high stiffness of the A gum backbone revealed by the present analysis and the tendency of aggregate formation in aqueous salts mentioned in the Introduction are major physical factors that are responsible for viscosity enhancement and hence for potential use of the heteropolysaccharide as food additives [2].

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