Aggregation of Aeromonas Gum in Aqueous Solution

Lina ZHANG,[†] Xiaojuan XU, Guowen JIANG,
Hideki IIJIMA,* and Hiromichi TSUCHIYA**

Department of Chemistry, Wuhan University, Wuhan 430072, China
** Chemicals Technology Department IV, Asahi Chemical Industry Co., Ltd.,
Miyazaki 882-0847, Japan*
*** Fourth Specialty Chemicals Sales Department, Asahi Chemical Industry Co., Ltd.,
Tokyo 100-8440, Japan*

ABSTRACT: Aeromonas (A) gum, a stabilizer, and thickening agent of food, is composed of xylose, mannose, galactose, glucose, and mannuronic acid. Weight-average molecular weights M_w and intrinsic viscosities $[\eta]$ of the unfractionated A gum and three fractions in water, 0.5 M NaCl aqueous solution and cadoxen were studied by light scattering and viscometry. Experimental results showed that A gum exists as an aggregate in 0.5 M NaCl aqueous solution and mainly as a single chain in cadoxen. The apparent aggregation number (N_{ap}) of A gum in 0.5 M NaCl aqueous solution was ca. 22, and decreased with increasing sample molecular weight. Change of aggregation and disaggregation of A gum in 0.5 M NaCl aqueous/cadoxen mixtures occurred from 0 to 0.4 of v_{cad} (volume fraction of cadoxen), and was reversible. The stable aggregation of A gum in 0.5 M NaCl aqueous/3% cadoxen mixture was achieved after 150 min.

KEY WORDS   Aggregation / Aeromonas Gum / Light Scattering / Viscometry / Polysaccharide Aqueous Solution /

Water-soluble polysaccharides, whose viscosity is very high, are of increasing interest as thickening agents in food and in enhanced oil recovery. Aeromonas (A) gum, an extracellular polysaccharide, produced by strains Aeromonas nichidenii, has been used as an additive for food such as a thickening agent and stabilizer.[1,2] A gum is composed of xylose, mannose, galactose, glucose, and mannuronic acid, and its molecular weight is ca. 10^5—10^7 by gel filter.[2] Understanding of the relation between solution behavior in different solvents and thickening is very important for application. In general, large enhancement in viscosity of the polymer is attributable to chain stiffness[3,4] and large hydrodynamic volume of polyelectrolyte caused by electrostatic force because of the charge groups in water.[5,6]

A water-soluble glucan A isolated from the fruit body of *Auricularia auricula-judae*, which dissolves in water as single-stranded helical chains, exhibits excessively high viscosity,[7] and intrinsic viscosity $[\eta]$ in water–Me$_2$SO mixtures decreased sharply in the w_{Me_2SO} (weight fraction of Me$_2$SO) range of 0.6—0.8.[8] Interestingly, β-D-glucan PC3 from *Poria cocos sclertium* forms aggregates in 20% cadoxen aqueous solution and disassociates into single chains in cadoxen, and the change of aggregation and disaggregation occurred in the v_{cad} (volume fraction of cadoxen) range from 0.2 to 0.3.[9] M_w and $[\eta]$ in 20% cadoxen aqueous solution are much larger than those in cadoxen. Many aggregation phenomena investigated by light scattering have been reported for synthesized copolymer,[5] sulfonated poly(phenylene terephthalamide),[6] trichosanthin,[10] block polyelectrolyte,[11] polystyrene/polyimide block copolymers,[12] and ω and α,ω-metal sulfonato polystyrene[13] in solution. In these cases, strong interchain interaction results in a large equilibrium constant for association in aqueous or other solutions, so that the polymer hardly exists in the single-chain state.[6] Cadoxen has been used as a solvent, which breaks the strong intermolecular interaction of polysaccharides. Xanthan dissolves in 0.1 M NaCl aqueous solution as double-stranded helix, which is sustained by intra- and intermolecular hydrogen bonds, but as single chain in cadoxen at 25°C.[3] In this work, the aggregation and solution behavior of A gum in 0.5 M NaCl aqueous solution and cadoxen were studied using light scattering and viscometry.

EXPERIMENTAL

Preparation and Analysis of Samples

A gum was provided by Fourth Specialty Chemicals Sales Department, Asahi Chemical Industry Co., Ltd. in Japan. A gum aqueous solution (5500 mL 0.56 wt%) was prepared, and the water-insoluble gum was removed by centrifugation. Half of the clear solution obtained was diluted to 0.1 wt%, and according to the nonsolvent addition method fractionated by the addition of isopropanol as a precipitant at room temperature. Isopropanol was added until the solution turned slightly milk-white. After standing for 12 h, the precipitate was removed by centrifugation to obtain the first fraction. The liquid was subjected to next fractionation step. Eight fractions obtained were dialyzed to purity, and rotary evaporated at reduced pressure below 45°C to dry. The unfractionated sample A and three fractions A-5, A-6, and A-7 were used in this work.

The IR spectra of the samples were recorded with a Nicolet FT-IR spectrometer. Each sample showed IR absorption at 890 cm^{-1} indicative of β-glucoside as well as at 1610 cm^{-1} (CO$_2^-$) and 800 cm^{-1} (mannoside), similar to acidic heteropolysaccharide.[14] High performance liquid chromatography (HPLC) of sugars, obtained by hydrolyzing the samples with trifluoroacetic acid in sealed tubes at 100°C, was performed on HPLC (LC-6A, Shimadzu) equipped with u-Bondapak NH$_2$

[†] To whom correspondence should be addressed.

(7.8 × 300 mm) using refractometer detector. A mixture of $CH_3CN/H_2O/CH_3OH$ (85 : 10 : 5 by volume) at 25°C was used as the mobile phase. The colorimetric method of Dische's carbozole reaction for uronic acid in the presence of borate[15] was used for analysis of mannuronic acid. An absorbance of the reacted solution was monitored at 530 nm by UV spectroscopy (UV-160A, Shimadzu). The determined components of the samples A, A-5, A-6, and A-7 are summarized in Table I.

Light Scattering

Scattering light intensity was 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. Solutions were prepared with solvents of 0.5 M NaCl aqueous solution and cadoxen, respectively. Measurements were performed at 1×10^{-4}—8×10^{-4} g mL^{-1}. Optical clarification of the solutions was achieved using a sand filter, with subsequent filtration through a 0.2-μm pore-size filter (M-HJV) for NaCl aqueous solution or a 0.45-μm pore-size filter (Nylon Acrodisc, Gelman Sci. Inc.) for cadoxen into the scattering cell. The refractive index increments (dn/dc) were measured with a double-beam differential refractometer (DRM-1020, Otsuka Electronics Co.) at 633 nm and 25°C. dn/dc was measured to be 0.122 mL g^{-1} for the sample A and fractions in 0.5 M NaCl aqueous solution, dialyzed by using a regenerated cellulose tube for three days before use. dn/dc of 0.161 mL g^{-1} for glucan in cadoxen under the same experimental conditions[9] was used as A gum and its fractions in cadoxen.

Viscometry

Viscosity of the solutions was measured using a Ubbelodhe capillary viscometer and low-shear three-bulb capillary viscometer at 25±0.1°C. Water, 0.5 M NaCl aqueous solution, cadoxen, and 0.5 M NaCl aqueous/cadoxen mixtures were used as solvents. The intrinsic viscosity [η] of A gum in aqueous solution depended on the shear rate $\dot{\gamma}$. Huggins and Kraemer plots were used to estimate [η] of the samples in 0.5 M NaCl aqueous solution, and zero shear-rate viscosity [η]$_0$ was obtained from plots of [η] against $\dot{\gamma}$. [η] of the samples in cadoxen, variant NaCl aqueous solution and 0.5 M NaCl aqueous/cadoxen mixtures were measured using a Ubbelodhe viscometer and calculated by Huggins and Kraemer plots. [η] of the samples in water were measured using the Ubbelodhe viscometer and estimated by Fuoss experimental equation suitable for poly- electrolyte solution as following:

$$(\eta_{sp}/c)^{-1} = ([\eta])^{-1} + B([\eta])^{-1}c^{1/2} \qquad (1)$$

where c is concentration, g mL^{-1}.

RESULTS AND DISCUSSION

Molecular Weight and Aggregation Number

Static light scattering is convenient for measuring weight-average molecular weight M_w and mean-square radii of gyration $\langle S^2 \rangle$ of polymers. However, light scattering does not give true values of M_w and $\langle S^2 \rangle$ for heterogeneous copolymers. Fortunately, the A gum

Table I. Components and content of samples

Sample	Components sugar[a] (molar ratio)				
	Glc	Man	Xyl	Gal	ManA
A	1.0	2.0	1.0	0.3	0.8
A-5	1.1	2.5	1.0	0.3	1.0
A-6	1.5	2.9	1.0	0.2	1.7
A-7	2.2	3.9	1.0	0.3	1.5

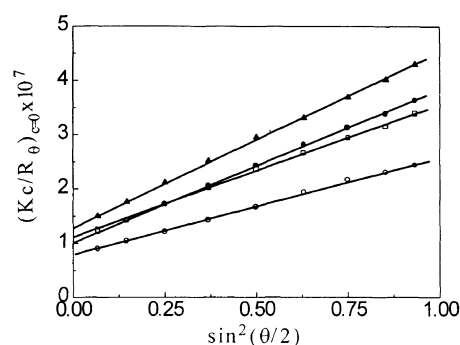[a] Glc, glucose; Man, mannose; Xyl, xylose; Gal, galactose; ManA, mannuronic acid.

Figure 1. Angular dependence of $(Kc/R_\theta)_{c=0}$ of A (▲), A-5 (○), A-6 (●), and A-7 (□) in 0.5 M NaCl aqueous solution at 25°C.

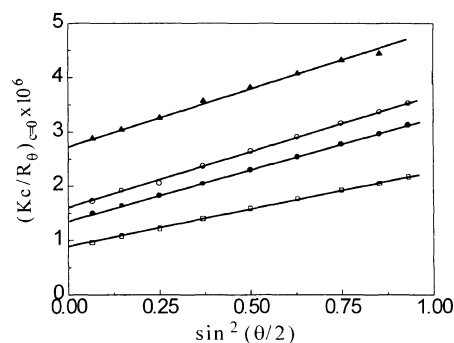**Figure 2.** Angular dependence of $(Kc/R_\theta)_{c=0}$ of A (▲), A-5 (○), A-6 (●), and A-7 (□) in cadoxen at 25°C.

components given in Table I can be expected to have similar refractive indices, so that apparent weight-average molecular weight and apparent radii of gyration directly derived may be equated to the true values without significant error.

Figures 1 and 2 illustrate the angular dependence of $(Kc/R_\theta)_{c=0}$ of four samples in 0.5 M NaCl aqueous solution and cadoxen at 25°C, respectively. K is the light scattering constant, and R_θ is the reduced Rayleigh ratio at angle θ. M_w and $\langle S^2 \rangle^{1/2}$ of the samples are summarized in Table II. M_w of the samples A, A-5, A-6, and A-7 in 0.5 M NaCl aqueous solution are significantly higher than those in cadoxen. The large ratios of the M_w in 0.5 M NaCl aqueous solution to those in cadoxen suggest aggregation of A gum in 0.5 M NaCl aqueous solution. The apparent aggregation number (N_{ap}) can be deduced from the mass of aggregates, namely $N_{ap} = M_w$ (in NaCl aq.)/M_w (in cadoxen). N_{ap} are given in Table II. The dependence of N_{ap} on M_w in cadoxen is shown in Figure 3. Clearly, the aggregation numbers of A gum and its fractions increased with decreasing molecular weight, as has been reported.[13]

Table II. Experimental M_w, $\langle S^2 \rangle^{1/2}$, $[\eta]_0$, $[\eta]$, and N_{ap} of samples in various solvents at 25°C

Sample	Sovelnt	$M_w \times 10^{-5}$	$\dfrac{\langle S^2 \rangle^{1/2}}{\text{nm}}$	$\dfrac{[\eta]}{\text{mL g}^{-1}}$	$\dfrac{[\eta]_0}{\text{mL g}^{-1}}$	$\dfrac{[\eta]_{0\,\text{NaCl}}}{[\eta]_{\text{cad}}}$	N_{ap}
A	0.5 M NaCl	79.0	105	5280[a]	944	5.0	22
A-5	0.5 M NaCl	128	99	6070[a]	995	3.9	21
A-6	0.5 M NaCl	98.6	112	5490[a]	1090	3.2	14
A-7	0.5 M NaCl	90.1	97	5100[a]	1050	3.1	8
A	Cadoxen	3.67	57	188			
A-5	Cadoxen	6.18	74	254			
A-6	Cadoxen	7.26	77	337			
A-7	Cadoxen	11.4	83	339			

[a] In water.
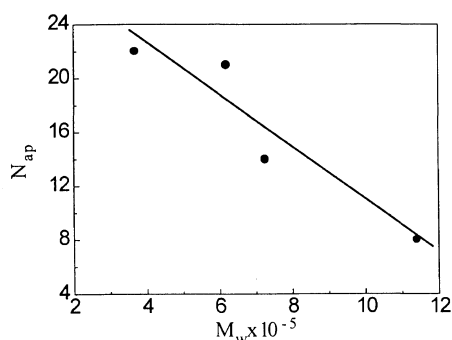
Figure 3. Dependence of aggregation number N_{ap} on molecular weight M_w for A gum in solution.

Figure 4. Salt concentration dependence of $[\eta]$ of A-6 at 25°C.

The fractions from A-1 to A-4 were not used in this work because of the presence of large gel particles in aqueous solution, resulting in failure of light scattering. This implies that fractions A-1 to A-4 may have much larger N_{ap} than those used here. It was not possible to prepare usable fractions by precipitating A gum aqueous solution with isopropanol, because the water-solubility of A gum depends on aggregating number, molecular weight and content of uronic acid.

Viscosity Behavior

The intrinsic viscosity $[\eta]$ and zero shear-rate viscosity $[\eta]_0$ of the samples in water, 0.5 M NaCl aqueous solution and cadoxen are summarized in Table II. $[\eta]_0$ of the samples in 0.5 M NaCl aqueous solution are higher than $[\eta]$ in cadoxen, but the ratios of $[\eta]_0$ in 0.5 M NaCl aqueous solution to $[\eta]$ in cadoxen are much lower than those of the corresponding M_w. Usually, $[\eta]$ of the flexible polymer in water are lower than in cadoxen, because of the solvation. $[\eta]$ of schizophyllan[4] and xanthan[3] in aqueous solution are much higher than in Me$_2$SO or cadoxen, and the ratios of $[\eta]$ in aqueous solution to those in Me$_2$SO or cadoxen are higher than those of M_w, which decrease in Me$_2$SO or cadoxen by factors of 3 and 2, respectively. The viscosity behavior of A gum differs substantially from that of the flexible single-chain and multiplex-stranded helix. Disruption of A gum aggregates in cadoxen may lead to extensive decrease in $[\eta]$, but the solvation in cadoxen causes increase in chain dimensions. For example, $[\eta]$ of amylose rises by a factor ~ 2 on passing from water to Me$_2$SO.[16]

$[\eta]_0$ in 0.5 M NaCl aqueous solution and $[\eta]$ in

cadoxen of A-6 and A-7 samples were significantly higher than those of A and A-5, respectively. Interestingly, charge in the contents of A-6 and A-7 samples was larger than for A and A-5 as shown in Table I. Electrostatic repulsion between uronic acid groups thus be important in the enhancement of viscosity of A gum in solution. As given in Table II, the samples exhibit excessive high $[\eta]$ in pure water, owing to extension of chains caused by electrostatic repulsion between chrged groups. Therefore, A gum is a good thickening agent in water.

Influence of Salt Concentration on $[\eta]$

Figure 4 shows salt concentration c_s dependence of $[\eta]$ for A-6 in desired NaCl concentration aqueous solution. $[\eta]$ decreased with salt concentration. Eisenberg et al. reported[11] two block polyelectrolyte samples as a function of NaCl concentration, and found that at low salt concentration ($c_s < 0.2$ M) aggregation numbers initially increased with salt concentration and then eventually leveled off. The aggregation number of A gum in NaCl aqueous solution may increase with c_s, but reduced dimensions caused by decrease of electrostatic force is likely to have stronger effect on $[\eta]$ than enhancement of N_{ap}.

Aggregation Process

To study aggregation, sample A-6 was dissolved in 50% cadoxen, which disassociates aggregates to single chains, and then diluted by 0.5 M NaCl aqueous solution at once to a final solvent of 0.5 M NaCl/3% cadoxen. Viscosity measurements were carried out as a function of time, and the time dependence of η_{sp}/c obtained from A-6 in 3% cadoxen aqueous solution diluted with 0.5 M NaCl aqueous solution is shown in Figure 5. η_{sp}/c
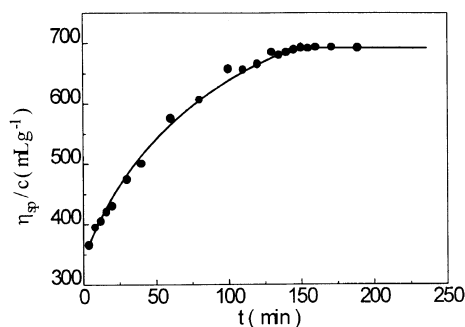
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Figure 5. Aggregation-time dependence of η_{sp}/c of A-6 in 0.5 M NaCl/3% cadoxen at 25°C.
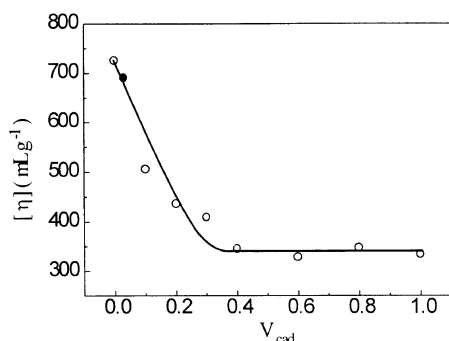

Figure 6. Plots of $[\eta]$ against v_{cad} for A-6 in 0.5 M NaCl/cadoxen mixtures at 25°C. See the text for solid circle explanation.

increased with time from 0 to 150 min, then kept constant η_{sp}/c for over one week, indicating stable aggregation. The aggregation of A gum forms more easily than schizophyllan, which formed associates ($c = 0.194 \times 10^{-2}$ g mL^{-1}) after 8 h.[4]

Figure 6 shows that the dependence of $[\eta]$ on the volume fraction of cadoxen in the mixtures (v_{cad}) for A-6 in 0.5 M NaCl aqueous/cadoxen mixtures. As v_{cad} increased from 0 to 0.4, the $[\eta]$ values decreased sharply, and then almost kept the constant with continuous increase of v_{cad}. This suggests that the aggregates were broken up to single chains in a range of v_{cad} from 0 to 0.4. The solid circle in Figure 6 refers to the value of $[\eta]$ obtained from A-6 dissolved in 50% cadoxen and then diluted to 0.5 M NaCl/3% cadoxen with 0.5 M NaCl aqueous solution. The results mentioned above are similar to those of glucan PC3 from Poria cocos sclerotium in water/cadoxen mixtures, in which the change of aggregation and disaggregation occurred in the range of v_{cad} from 0.2 to 0.3, and was reversible.[9] These results proved that the aggregates of the polysaccharides were diassociated to single chains in cadoxen more readily than triple helix of schizophyllan in Me$_2$SO[4] and double-stranded helix of xanthan in cadoxen.[3] When the weight fractions of Me$_2$SO or cadoxen in the mixtures reached 0.87 and 0.7,[3,4] the triple helix or double helix melted to single chains.

CONCLUSIONS

The M_w of Aeromonas gum was measured to be 7.90×10^6 in 0.5 M NaCl aqueous solution and 3.67×10^5 in cadoxen by light scattering, respectively. It indicated that A gum exists as aggregates in 0.5 M NaCl aqueous solution and mainly as single chains in cadoxen, and the apparent aggregation number was given to be *ca.* 22. The aggregation number decreased with increase of the molecular weight of the sample. The change of aggregation and disaggregation of A gum in 0.5 M NaCl aqueous/cadoxen mixtures occurred from 0 to 0.4 of v_{cad}, and were reversible. Stable aggregation of A gum in 0.5 M NaCl aqueous/3% cadoxen was achieved after 150 min. The aggregation of A gum in aqueous solution took place more easily and was broken in cadoxen than multiple helix chain. $[\eta]$ and $[\eta]_0$ of A gum in water and 0.5 M NaCl aqueous solution were much higher than in cadoxen, and electrostatic repulsion between uronic acid groups was essential to the enhancement of viscosity of A gum in water.

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