Chain-Stiffness and Excluded-Volume Effects in Solutions of Sodium Hyaluronate at High Ionic Strength

Kanako Hayashi, Kikuko Tsutsumi, Fumio Nakajima, Takashi Norisuye,* and Akio Teramoto

Department of Macromolecular Science, Osaka University, Toyonaka, Osaka 560, Japan

Received December 1, 1994; Revised Manuscript Received March 7, 1995[⊗]

ABSTRACT: Twelve samples of sodium hyaluronate ranging in weight-average molecular weight from 3.8×10^3 to 3.5×10^5 have been studied by static light scattering, sedimentation equilibrium, and viscometry with 0.5 M aqueous sodium chloride at 25 °C as the solvent. Viscosity measurements have also been made at 0.2 M NaCl. Intrinsic viscosity ($[\eta]$) data confirm Cleland's early conclusion that the polysaccharide chain in 0.2 or 0.5 M aqueous NaCl has an unmistakable semiflexibility but undergoes appreciable excluded-volume effects unless the molecular weight is low. It is found that the molecular weight dependence of $[\eta]$ in the aqueous salts is described almost quantitatively by the combination of the Yamakawa−Fujii−Yoshizaki theory for $[\eta]$ of an unperturbed wormlike chain and the Yamakawa−Stockmayer−Shimada (YSS) theory for excluded-volume effects when the Barrett function is adopted for the viscosity expansion factor. The radii of gyration obtained for the four highest molecular weight samples in 0.5 M aqueous NaCl are also explained consistently by the YSS theory with the Domb−Barrett function for the radius expansion factor. The persistence lengths determined are 4.2 and 4.1 nm in 0.2 and 0.5 M aqueous NaCl, respectively, and the molar mass per unit contour length is 400−405 nm^{-1} in the aqueous salts.

Introduction

Hyaluronic acid is a linear polysaccharide consisting of *N*-acetyl-β-D-glucosamine and β-D-glucuronic acid alternately linked at the 1,3 and 1,4 positions, respectively. From a series of dilute-solution studies[1−4] Cleland concluded that the sodium salt of this polyacid in 0.2 or 0.5 M aqueous sodium chloride behaves like a wormlike chain[5] with a (total) persistence length q of 4−5 nm but undergoes intramolecular excluded-volume effects for molecular weights higher than 10^5. In the estimation of q, he corrected the measured intrinsic viscosities $[\eta]$, sedimentation coefficients, and z-average mean-square radii of gyration $\langle S^2\rangle_z$ (all at high molecular weights) for volume effects using a procedure based on the Orofino−Flory theory[6] for the second virial coefficient A_2 of long flexible chains. However, this theory is known to be a poor approximation to flexible polymers.

In contrast to Cleland's conclusion, Fouissac et al.[7] argued that the excluded-volume effect on $\langle S^2\rangle_z$ is negligibly small in 0.3 M aqueous NaCl and obtained a much larger q of 8 nm for the hyaluronate chain in the aqueous salt. The large discrepancy in q between the two groups appears to stem primarily from the different treatments of excluded-volume effect, and both chain-stiffness and excluded-volume effects in hyaluronate solutions at high salt concentrations ought to be studied by further experiment, preferably by making a more elaborate data analysis. Such work is a first step toward the settlement of a current problem[7,8] concerning the separate evaluation of q (or the electrostatic persistence length[9,10]) and excluded-volume effects at lower salt concentrations. The problem is not specific to sodium hyaluronate but common to any polyelectrolytes with intrinsically weak stiffness or high flexibility.

In the present work, 12 samples of sodium hyaluronate with molecular weights of 3.8×10^3−3.5×10^5 were prepared by extensive fractionation and investigated by viscometry in 0.2 and 0.5 M aqueous NaCl at 25 °C and also by sedimentation equilibrium or light scattering in the latter solvent. The radii of gyration were determined for the four highest molecular weight samples. As shown below, the polysaccharide in the aqueous salts is weakly (but unmistakably) stiff and undergoes pronounced excluded-volume effects, at least, for weight-average molecular weights M_w higher than 5×10^4. Thus, the molecular weight dependence of $[\eta]$ is analyzed by the Yamakawa−Fujii−Yoshizaki theory[11,12] for wormlike chains combined with the Yamakawa−Stockmayer−Shimada theory[13−15] for excluded-volume effects to estimate q, the molar mass per unit contour length, and the excluded-volume strength. With the model parameters thus determined, we finally examine whether the Yamakawa−Stockmayer−Shimada theory is capable of consistently explaining the measured $\langle S^2\rangle_z$.

Experimental Section

Samples and Preparation of Solutions. A hyaluronate sample with a purity higher than 99.9%[16] was supplied by Shiseido Co., Japan. This sample was produced by culture with the bacterium *Streptococcus zooepidemicus* and purified according to the method of Akasaka et al.[17] at Shiseido Co. Its viscosity-average molecular weight M_v was about 5×10^5 when estimated by use of Cleland and Wang's $[\eta]$ vs M_w relation[2] in 0.2 M aqueous NaCl.

The sample was degraded to different molecular weights by heating at 110 °C for 6−48 h, and some of the degraded samples were further hydrolyzed according to the method of Cleland[4] to obtain eight samples with M_v of 4×10^3−4×10^5. These products were divided each into several parts by fractional precipitation with 0.2 M aqueous NaCl as the solvent and acetone as the precipitant. Each fraction was reprecipitated from a 0.2 M aqueous NaCl solution into a large quantity of 95% aqueous acetone, washed with 95% and anhydrous acetone (each three times), and dried in vacuo. Middle fractions having nearly identical $[\eta]$ (in 0.2 M aqueous NaCl at 25 °C) were combined and again fractionated similarly. This procedure was repeated three to five times, and from 92 fractions thus obtained, 12 appropriate ones were chosen for the present study. They were designated HA1, HA2, ..., and HA12 in order of decreasing molecular weight.

Aqueous solutions of the chosen samples were passed through a mixed-bed ion exchanger (Amberlite IR-120 + IR-

[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1995.

0024-9297/95/2228-3824$09.00/0

400); several lower molecular weight samples lost significant amounts of the polymer probably because of polymer adsorption onto the ion-exchange resin. The solutions of the acid form polymer were neutralized with 0.1 N aqueous sodium hydroxide. Their pH's were monitored by a Beckman ϕ 70 pH meter. Dry sodium hyaluronate was obtained by lyophilizing the neutralized solutions over more than 4 days. Its solubility in aqueous NaCl did not detectably change before and after the lyophilization for any samples except the lowest molecular weight one (HA12), for which the solubility was less enhanced by this treatment. All the sodium salt samples were further dried in vacuo overnight just before the preparation of test solutions.

A weighed amount of each sample (except HA12) was dissolved directly in 0.2 or 0.5 M aqueous NaCl. The polymer mass concentration c was calculated from the gravimetrically determined polymer weight fraction with the solution density ϱ or the solvent density. Solutions of sample HA12 were prepared by dissolving the polymer in pure water followed by mixing of the solution with aqueous NaCl to the desired salt concentration of 0.2 or 0.5 M.

Light Scattering. Scattering intensities were measured for 0.5 M aqueous NaCl solutions of four sodium salt samples, HA1 through HA4, at 25 °C on a Fica 50 light scattering photometer in an angular range from 30 to 150°. Vertically polarized incident light of 436-nm wavelength was used. The experimental procedures including optical clarification were essentially the same as those described elsewhere.[18] Optical anisotropy effects on M_w, $\langle S^2\rangle_z$, and A_2 were negligible in the range of molecular weight studied by light scattering.

Excess refractive indices of 0.5 M NaCl solutions of sodium hyaluronate were measured at 25 °C for the wavelengths of 436 and 546 nm using a modified Schulz–Cantow type differential refractometer. Undialyzed solutions of two sodium salt samples with $M_v = 6 \times 10^3$ and 1.4×10^4 (see above for the preparation) yielded essentially the same values (0.147 cm^3 g^{-1} at 436 nm and 0.144 cm^3 g^{-1} at 546 nm) for the specific refractive index increment $(\partial n/\partial c)$, indicating that $\partial n/\partial c$ of the polymer is independent of molecular weight, at least above 6×10^3. This was also the case with the partial specific volume or the density increment $(\partial \varrho/\partial c)$ (see below for the measurement). On the basis of these findings, 0.5 M NaCl solutions of higher molecular weight sodium salt samples with $M_v = 1.7 \times 10^4$ and 5.3×10^4 were dialyzed against 0.5 M aqueous NaCl at 25 °C (see ref 19 for dialysis). The values of $(\partial n/\partial c)_\mu$ obtained for the dialyzed solutions were 0.141 cm^3 g^{-1} for 436 nm and 0.138 cm^3 g^{-1} for 546 nm at 25 °C (the subscript μ attached to $\partial n/\partial c$ signifies the condition that the chemical potentials of all diffusible components are held constant). These values agreed essentially with Cleland's results[1] (0.140 and 0.136 cm^3 g^{-1} for 436 and 546 nm, respectively) in the same solvent.

Sedimentation Equilibrium. Weight-average molecular weights for samples HA5 through HA12 were determined by sedimentation equilibrium in a Beckman Model E ultracentrifuge with 0.5 M aqueous NaCl at 25 °C as the solvent. A Kel-F 12-mm double-sector cell was used. The liquid column was adjusted to 1.2–3.0 mm. Except for sample HA12, the rotor speed was chosen so as to make the equilibrium polymer mass concentration (c_b) at the cell bottom about 3 times as high as that (c_a) at the meniscus; the rotor speed for HA12 was lowered by about 30% of the calculated value (just because of our machine condition), and a series of measurements on the sample was performed for high initial polymer concentrations c_0 to avoid the loss of accuracy in M_w.

The data were analyzed according to the equation[20]

$$M_{app}^{-1} = M_w^{-1} + 2A_2\bar{c} + \ldots \quad (1)$$

where the apparent molecular weight M_{app} and the mean polymer mass concentration $\bar{c}$ are defined by

$$M_{app} = (c_b - c_a)/\lambda c_0 \quad (2)$$

$$\bar{c} = (c_a + c_b)/2 \quad (3)$$

with

$$\lambda = (\partial \varrho/\partial c)_\mu (r_b^2 - r_a^2)\omega^2/2RT \quad (4)$$

In these equations, r_a and r_b are the radial distances from the center of rotation to the meniscus and the cell bottom, respectively, $(\partial \varrho/\partial c)_\mu$ is the density increment at constant μ (i.e., for dialyzed solutions), ω is the angular velocity of the rotor, R is the gas constant, and T is the absolute temperature.

The equilibrium concentration profiles were also analyzed to estimate the ratio of M_z (the z-average molecular weight) to M_w by use of the equation[21]

$$Q = (M_w/M_z)(1 + 2A_2M_w\bar{c} + \ldots) \quad (5)$$

where

$$Q = \frac{(c_b - c_a)^2}{c_0(r_b^2 - r_a^2)[(\partial c/\partial r^2)_{r=r_b} - (\partial c/\partial r^2)_{r=r_a}]} \quad (6)$$

For samples HA5 and HA12, however, Q (and hence M_z/M_w) could not be determined owing to remarkable nonideality for HA5 and the low rotor speed chosen for HA12.

Densities of 0.5 M aqueous NaCl solutions of sodium hyaluronate at 25 °C were determined by a bicapillary pycnometer with 30-cm^3 capacity. As in the measurement of $\partial n/\partial c$, samples with $M_v = 1.7 \times 10^4$ and 5.3×10^4 were used for dialyzed solutions and those with $M_v = 6 \times 10^3$ and 1.4×10^4 for undialyzed solutions. The value of $(\partial \varrho/\partial c)_\mu$ obtained was 0.380.

Viscometry. Viscosity measurements were made on all sodium salt samples in 0.2 and 0.5 M aqueous NaCl at 25 °C using a four-bulb low-shear capillary viscometer (apparent shear rates of 13–91 s^{-1}) for the highest molecular weight sample HA1 and conventional capillary viscometers of the Ubbelohde type (apparent shear rates of about 800 s^{-1}) for the other samples. The relative viscosity was evaluated by taking account of the difference between the solution and solvent densities for samples having $[\eta]$ smaller than 60 cm^3 g^{-1}. The Huggins plot, the Fuoss–Mead plot, and the Billmeyer plot were combined to determine $[\eta]$; the Huggins constant was normal for samples with $M_w > 1.8 \times 10^4$, being in the range 0.34–0.43, but it exceeded 0.5 for those with $M_w < 10^4$.

Results

Figures 1 and 2 show respectively the plots of M_{app}^{-1} vs $\bar{c}$ and Q vs $\bar{c}$ constructed from sedimentation equilibrium data for the indicated sodium salt samples in 0.5 M aqueous NaCl at 25 °C. The straight lines in the latter figure have been drawn with the aid of the A_2M_w values evaluated from the straight lines in the former figure. The data of M_w, A_2, and M_z/M_w obtained are summarized in Table 1. The M_z/M_w values are about 1.1, indicating that samples HA6 through HA11 are narrow in molecular weight distribution.

Figure 3 illustrates the Zimm plot in the square-root form for sodium salt sample HA4 (the lowest molecular weight studied by light scattering) in 0.5 M aqueous NaCl at 25 °C. Here, K is the optical constant, and R_θ, the reduced scattering intensity at scattering angle θ. The numerical data from light scattering measurements are presented in Table 1. The values of A_2 both from light scattering and sedimentation equilibrium are seen to be on the order of 10^{-3} mol cm^3 g^{-2} and comparable to or even larger than those for nonionic, flexible polymers in good solvents.[22] Cleland[1,2] and Ghosh et al.[8] observed similarly large A_2 values for sodium hyaluronate samples at high NaCl concentrations. Though not graphically shown, $\langle S^2\rangle_z^{1/2}$ in the table increases in proportion to $M_w^{0.58}$. This exponent is distinctly larger than the value 0.5 estimated at a lower

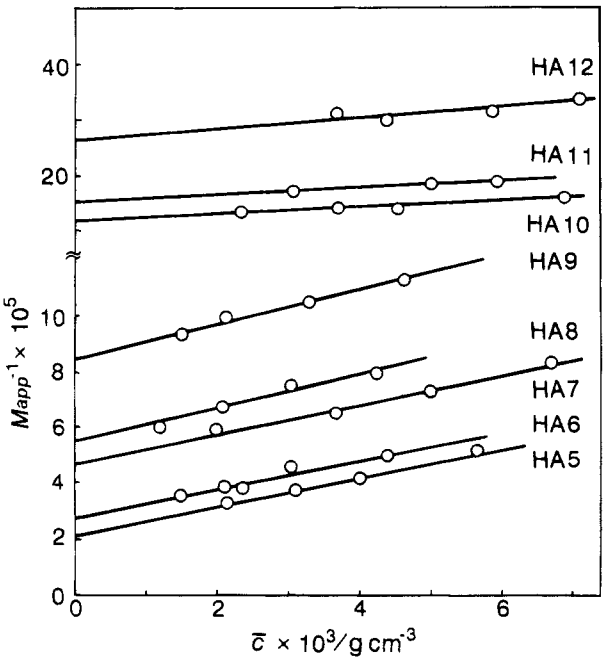

Figure 1. Plots of M_{app}^{-1} vs $\bar{c}$ for indicated sodium hyaluronate samples in 0.5 M aqueous NaCl at 25 °C.

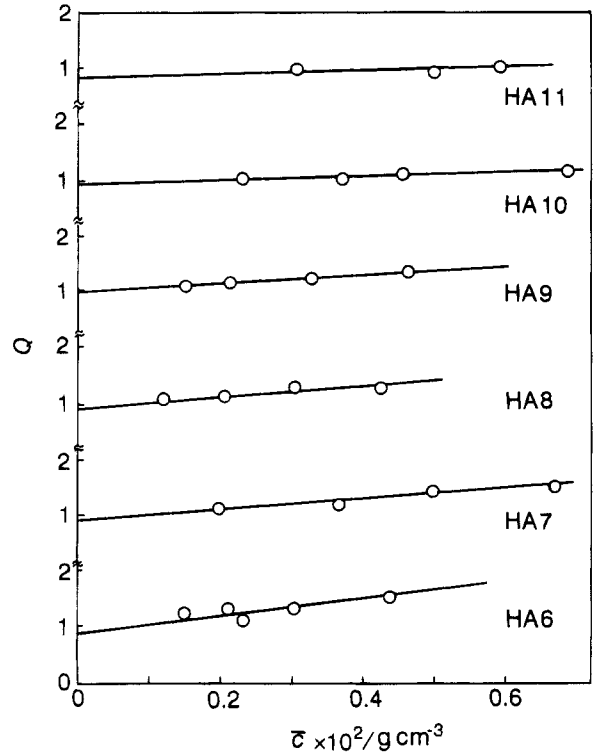

Figure 2. Plots of Q vs $\bar{c}$ for indicated sodium hyaluronate samples in 0.5 M aqueous NaCl at 25 °C. Q is defined by eq 6.

NaCl concentration of 0.3 M by Fouissac et al.,[7] though the $\langle S^2\rangle_z^{1/2}$ values of these authors are much larger than ours when compared at the same M_w. We also note that Cleland and Wang's $\langle S^2\rangle_z^{1/2}$ values[2] in 0.5 M aqueous NaCl are systematically larger than ours. This may be attributed at least partly to the broader-distribution samples they used.

The values of $[\eta]$ for all samples in 0.2 and 0.5 M aqueous NaCl at 25 °C are given in the last two columns of Table 1 and plotted double-logarithmically against M_w in Figure 4. The curves fitting the plotted points

Table 1. Results from Light Scattering, Sedimentation Equilibrium, and Viscosity Measurements on Sodium Hyaluronate Samples in Aqueous NaCl at 25 °C

	in 0.5 M NaCl					in 0.2 M NaCl
sample	$10^{-4}M_w$	10^3A_2, mol cm^3 g^{-2}	M_z/M_w	$\langle S^2\rangle_z^{1/2}$, nm	$[\eta]$, cm^3 g^{-1}	$[\eta]$, cm^3 g^{-1}
HA1	35.0[a]	1.18[a]		45.3[a]	698	807
HA2	21.5[a]	1.25[a]		34.1[a]	454	494
HA3	10.4[a]	1.26[a]		22.8[a]	224	239
HA4	6.89[a]	1.57[a]		17.3[a]	162	176
HA5	4.81[b]	2.49[b]			112	119
HA6	3.64[b]	2.54[b]	1.1$_5$[b]		91.1	93.5
HA7	2.13[b]	2.67[b]	1.1[b]		57.0	57.9
HA8	1.83[b]	3.10[b]	1.1[b]		49.7	50.8
HA9	1.19[b]	3.13[b]	1.0$_5$[b]		34.4	34.5
HA10	0.838[b]	2.9[b]	1.1[b]		23.2	22.3
HA11	0.657[b]	3.3[b]	1.2[b]		17.5	17.5
HA12	0.377[b]	4.8[b]			10.5	10.7

[a] From light scattering. [b] From sedimentation equilibrium.

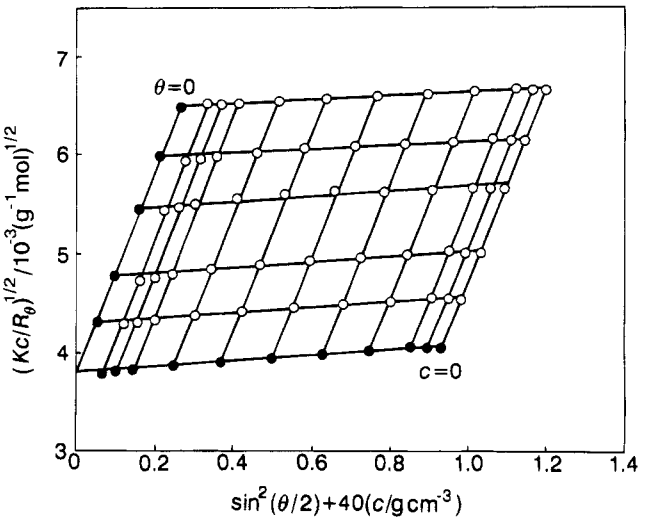

Figure 3. Zimm plot in the square-root form for sodium hyaluronate sample HA4 in 0.5 M aqueous NaCl at 25 °C.

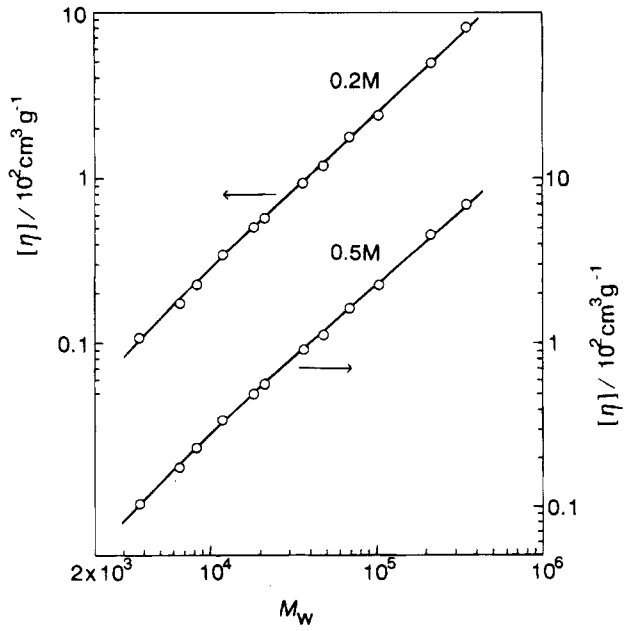

Figure 4. Molecular weight dependence of $[\eta]$ for sodium hyaluronate in 0.2 and 0.5 M aqueous NaCl at 25 °C.

for the respective salt concentrations C_s are slightly convex upward for M_w below 10^4 and almost linear for M_w above 10^4 with slope 0.92 for C_s = 0.2 M and 0.90 for C_s = 0.5 M. The downward curvature of each curve

at low M_w suggests a certain semiflexibility of the hyaluronate chain. Though not shown here, Cleland's data[2,4] in 0.2 and 0.5 M aqueous NaCl fall on the respective lines in the figure or their smooth extensions.

Discussion

Data Analysis. If the sodium hyaluronate molecule in a given aqueous NaCl is modeled by the wormlike chain, its $[\eta]$ in the unperturbed state is determined by L (the contour length), q, and d (the chain diameter).[11,12] The first parameter is related to the molecular weight M by

$$L = M/M_L \tag{7}$$

with M_L the molar mass per unit contour length of the chain. We first analyze the measured $[\eta]$ in 0.2 and 0.5 M aqueous NaCl by a conventional method applicable to typical stiff chains, putting weight on the data at low M_w where intramolecular excluded-volume effects should be negligible or small.

1. Conventional Method. According to Bushin et al.[23] and Bohdanecký,[24] the Yamakawa–Fujii–Yoshizaki theory[11,12] for the intrinsic viscosity $[\eta]_0$ of an unperturbed wormlike cylinder[25] can be expressed in a good approximation as

$$(M^2/[\eta]_0)^{1/3} = I + SM^{1/2} \tag{8}$$

where

$$I = 1.516 \times 10^{-8} I_0 M_L \ (\mathrm{g^{1/3}\ cm^{-1}}) \tag{9}$$

$$S = 1.516 \times 10^{-8} S_0 (M_L/2q)^{1/2} \ (\mathrm{g^{1/3}\ cm^{-1}}) \tag{10}$$

with I_0 and S_0 being known functions of $d/2q$. Equation 8 is applicable to a wide range of $L/2q$ from 1.6 to 2000 for weakly stiff chains ($d/2q \sim 0.1$) and from 0.4 to 300 for typical stiff chains ($d/2q \sim 0.01$). In such a range of chain length, the Bushin–Bohdanecký plot of $(M_w^2/[\eta])^{1/3}$ vs $M_w^{1/2}$ should give a straight line whose intercept and slope equal I and S, respectively. When excluded-volume effects are significant for high molecular weight samples, this plot bends down at large $M_w^{1/2}$.

Figure 5 shows the Bushin–Bohdanecký plot constructed from the present data (the circles) for $M_w < 2 \times 10^5$ in 0.2 M aqueous NaCl and Cleland's data[2,4] (the triangles) in the same solvent. The plotted points follow a straight line in the range of $M_w^{1/2}$ between 80 and 220, yielding two relations, $I = 59\ \mathrm{g^{1/3}\ cm^{-1}}$ and $S = 0.969\ \mathrm{g^{1/3}\ cm^{-1}}$. Their downward deviation from the line for $M_w^{1/2}$ above 250 reveals pronounced excluded-volume effects on $[\eta]$. On the other hand, the deviation for $M_w^{1/2}$ below 70 can be ascribed to the inapplicability of eq 8 to short chains, but it may be utilized for finding one more relation and hence for uniquely determining the three parameters, q, M_L, and d. The procedure is as follows.[27]

First, different sets of q, M_L, and d are evaluated from the above I and S values and appropriately chosen d values. Then the $[\eta]_0$ values computed from the Yamakawa–Fujii–Yoshizaki theory with those parameter sets are compared with the experimental data for M_w below 5×10^4. The solid curve in the inset of Figure 5 represents the theoretical values for $d = 1.0$ nm ($q = 4.9$ nm and $M_L = 405\ \mathrm{nm^{-1}}$) in 0.2 M aqueous NaCl, while the dashed lines represent those for $d = 0.5$ nm

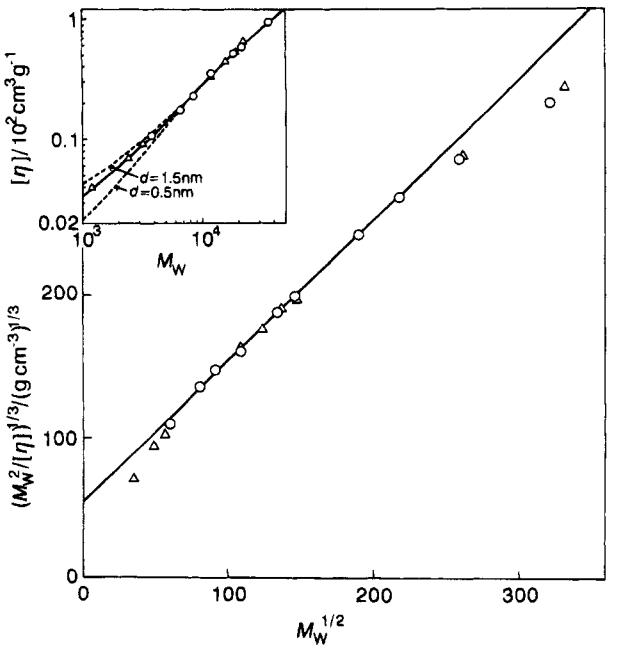

Figure 5. Plot of $(M_w^2/[\eta])^{1/3}$ vs $M_w^{1/2}$ constructed from the present data (circles) and Cleland's data[2,4] (triangles) for sodium hyaluronate ($M_w < 2 \times 10^5$) in 0.2 M aqueous NaCl at 25 °C. The solid line in the inset represents the theoretical values ($M < 5 \times 10^4$) calculated from the Yamakawa–Fujii–Yoshizaki theory[11,12] for the unperturbed wormlike chain with $q = 4.9$ nm, $M_L = 405\ \mathrm{nm^{-1}}$, and $d = 1.0$ nm, and the two dashed lines represent those for $d = 0.5$ ($q = 4.5$ nm and $M_L = 350\ \mathrm{nm^{-1}}$) and $d = 1.5$ nm ($q = 5.8$ nm and $M_L = 470\ \mathrm{nm^{-1}}$).

($q = 4.5$ nm and $M_L = 350\ \mathrm{nm^{-1}}$) and $d = 1.5$ nm ($q = 5.8$ nm and $M_L = 470\ \mathrm{nm^{-1}}$); note that, since I and S are fixed, q and M_L vary depending on the chosen d. It can be seen that the d value of 1.0 nm (with $q = 4.9$ nm and $M_L = 405\ \mathrm{nm^{-1}}$) gives the best fit to the data points for M_w below 5×10^4.

The M_L value of $405\ \mathrm{nm^{-1}}$ estimated above is consistent with the chemical structure of sodium hyaluronate, confirming that the wormlike chain is a good model for the polysaccharide in the unperturbed state. The d value of 1.0 nm is also reasonable if possible solvation of water molecules onto the hyaluronate chain is considered.[4]

The $[\eta]$ data in 0.5 M aqueous NaCl were similarly analyzed, but d at this C_s was assumed to be the same as that at 0.2 M because of the unavailability of $[\eta]$ data for oligomers. Excluded-volume effects were again significant for M_w above 5×10^4. The analysis yielded $q = 4.6$ nm and $M_L = 400\ \mathrm{nm^{-1}}$. As expected, the latter agrees with the above M_L value of $405\ \mathrm{nm^{-1}}$ in 0.2 M aqueous NaCl. We note that these experimental M_L values are quite close to the value $400\ \mathrm{nm^{-1}}$ invoked by previous workers[3,4,7] in evaluating q of the polysaccharide.

2. Consideration of Excluded-Volume Effects. The above data analysis shows that the sodium hyaluronate chain in 0.2 or 0.5 M aqueous NaCl is weakly stiff ($d/2q \sim 0.1$) and perturbed by excluded-volume effects, at least for M_w above 5×10^4. If there is a certain, narrow M_w region in which stiffness and excluded-volume effects lead to similar dependences of $[\eta]$ on chain length, the q values estimated above may be somewhat overestimated. This is likely since A_2 for sodium hyaluronate is quite large even at $C_s = 0.5$ M (see Table 1). We therefore reanalyze the $[\eta]$ data covering the entire M_w range by considering excluded-

volume effects in the framework of the Yamakawa–Stockmayer–Shimada (YSS) theory[13–15] (i.e., the quasi-two-parameters theory) for helical wormlike[28,29] or wormlike chains. In so doing, we fix M_L and d to the values estimated above by the conventional method.

In the YSS theoretical framework, the viscosity expansion factor α_η [$\equiv([\eta]/[\eta]_0)^{1/3}$] is a universal function of the scaled excluded-volume parameter $\tilde{z}$ defined by

$$\tilde{z} = (3/4)K(\lambda L)z \tag{11}$$

with

$$z = (3/2\pi)^{3/2}(\lambda B)(\lambda L)^{1/2} \tag{12}$$

Here, $K(\lambda L)$ is a known function of λL,[15] λ^{-1} is the stiffness parameter, z is the conventional excluded-volume parameter, and B is the excluded-volume strength representing the interaction between a pair of beads; $K(\lambda L)$ is essentially zero for λL below unity owing to the stiffness effect and approaches the coil-limiting value of $^4/_3$ at infinite λL. In the wormlike chain limit (of the helical wormlike chain), with which we are concerned here, we have

$$\lambda^{-1} = 2q \tag{13}$$

$$B = \beta/a^2 \tag{14}$$

with β and a being the binary cluster integral and the bead spacing, respectively. For α_η^3, we adopt the Barrett function[30] with $\tilde{z}$ in place of z:[31]

$$\alpha_\eta^{\,3} = (1 + 3.8\tilde{z} + 1.9\tilde{z}^2)^{0.3} \tag{15}$$

This equation fairly accurately describes α_η^3 data for nonionic, flexible polymers (with λ^{-1} of 1.3–5.8 nm) over a very broad range of molecular weight.[32,33]

The Yamakawa–Fujii–Yoshizaki theory combined with the YSS theory (with eq 15) contains four parameters, q, M_L, d, and B. A curve-fitting procedure was employed to estimate a set of q and B for the known values of M_L and d in 0.2 or 0.5 M aqueous NaCl. Figures 6 and 7 show that the theoretical solid curves for the two C_s closely fit the data points throughout the entire range of molecular weight examined; the dashed lines here refer to the unperturbed state (i.e., $B = 0$). The parameter values used for the calculation are summarized in Table 2, in which the q values from the conventional method are included with parentheses for comparison. It can be seen that explicit consideration of excluded-volume effects lowers q by 11–14%. The q values at the two salt concentrations (4.2 nm at 0.2 M and 4.1 nm at 0.5 M) are essentially the same, suggesting that for C_s above 0.2 M electrostatic interactions between neighboring charged groups are not strong enough to appreciably contribute to the total persistence length. These q values do not differ much from Cleland's estimate (4–5 nm)[3,4] mentioned in the Introduction, but they are only about half that of Fouissac et al. (8 nm at C_s = 0.3 M)[7] from $\langle S^2\rangle_z$.

Excluded-Volume Effects on Chain Dimensions. In the YSS theoretical framework, the radius expansion factor α_s [$\equiv(\langle S^2\rangle/\langle S^2\rangle_0)^{1/2}$] is also a universal function of $\tilde{z}$, where $\langle S^2\rangle_0$ denotes the unperturbed mean-square radius of gyration and is given by[34]

$$\langle S^2\rangle_0 = (qL/3) - q^2 + (2q^3/L) - (2q^4/L^2)[1 - \exp(-L/q)] \tag{16}$$

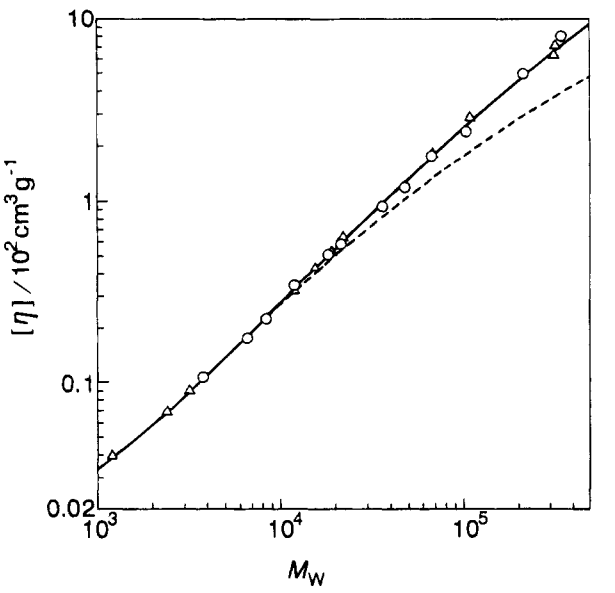

Figure 6. Comparison between the measured $[\eta]$ in 0.2 M aqueous NaCl and the YSS[13–15] theoretical values (the Yamakawa–Fujii–Yoshizaki theory[11,12] + the Barrett function[30] with eq 11) for the perturbed wormlike chain with q = 4.2 nm, M_L = 405 nm^{-1}, d = 1.0 nm, and $B/2q$ = 0.41. The dashed line refers to the unperturbed state (B = 0). The symbols are the same as those used in Figure 5.

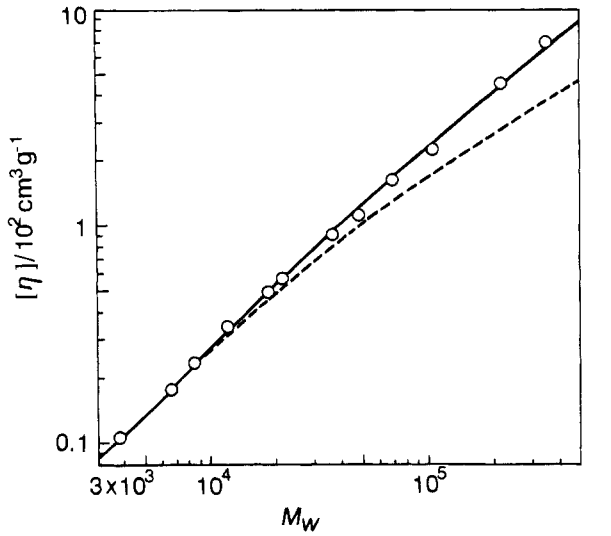

Figure 7. Comparison between the measured $[\eta]$ in 0.5 M aqueous NaCl and the YSS theoretical values for q = 4.1 nm, M_L = 400 nm^{-1}, d = 1.0 nm, and $B/2q$ = 0.34. The dashed line refers to the unperturbed state.

Table 2. Values for the Wormlike Chain Parameters and the Excluded-Volume Strength for Sodium Hyaluronate in 0.2 and 0.5 M Aqueous NaCl at 25 °C

C_s, M	q, nm	M_L, nm^{-1}	d, nm	$B/2q$
0.2	4.2 (4.9)[a]	405	1.0	0.41
0.5	4.1 (4.6)[a]	400	1.0[b]	0.34

[a] Estimated by a conventional method applicable to typical stiff chains with negligible excluded volume (see the text). [b] Assumed.

for the wormlike chain. Thus, $\langle S^2\rangle$ of the hyaluronate chain with a given M should be determined by q, M_L, and B. We examine whether the YSS theory with these parameters from $[\eta]$ in 0.5 M aqueous NaCl consistently explains the measured $\langle S^2\rangle_z$, using the Domb–Barrett function[35] for α_s. This function in the YSS theory reads[14]

$$\alpha_s^2 = \left[1 + 10\tilde{z} + \left(\frac{70\pi}{9} + \frac{10}{3}\right)\tilde{z}^2 + 8\pi^{3/2}\tilde{z}^3\right]^{2/15}[0.933 + 0.067\exp(-0.85\tilde{z} - 1.39\tilde{z}^2)] \quad (17)$$

which reduces to the original Domb–Barrett equation in the coil limit. We note again that eq 17 quite accurately describes α_s^2 data for nonionic, flexible polymers.[32,33,36,37]

For $q = 4.1$ nm, $M_L = 400$ nm^{-1}, and $M \geq 6.9 \times 10^4$, $\langle S^2\rangle_0$ in eq 16 is determined substantially by the leading term $qL/3$, the contribution from the second and higher terms being at most 7%; the M value of 6.9×10^4 corresponds to the lowest molecular weight studied by light scattering. Thus, the measured $\langle S^2\rangle_z$ in 0.5 M aqueous NaCl may be converted to the weight-average values $\langle S^2\rangle_w$ in a good approximation using the relation $\langle S^2\rangle_w = \langle S^2\rangle_z M_w/M_z$ with $M_z/M_w = 1.1$ (see Table 1). In Figure 8, the resulting $\langle S^2\rangle_w$ values (the circles) are compared with the theoretical values (the solid line) calculated from eqs 16 and 17 for the perturbed wormlike chain with $q = 4.1$ nm, $M_L = 400$ nm^{-1}, and $B/2q = 0.34$ (see Table 2). The dashed line refers to the unperturbed state. The close fit of the solid curve to the data points substantiates that the YSS theory is capable of consistently explaining the excluded-volume effects on both $[\eta]$ and $\langle S^2\rangle$ of sodium hyaluronate in 0.5 M aqueous NaCl.

In their analysis of $\langle S^2\rangle_z^{1/2}$ data at $C_S = 0.3$ M, Fouissac et al.[7] took α_s to be nearly unity over the molecular weight range ($1.3 \times 10^5 - 1.86 \times 10^6$) they studied, on the basis of the observation that $\langle S^2\rangle_z^{1/2}$ was roughly proportional to $M_w^{0.5}$ as expected for unperturbed long wormlike chains. Probably, they put much weight on the $\langle S^2\rangle_z^{1/2}$ values for their two lowest M_w samples to derive the $\langle S^2\rangle_z^{1/2}$ vs $M_w^{0.5}$ relation (see Figure 2 of ref 7). In fact, if the data for these two samples are omitted, $\langle S^2\rangle_z^{1/2}$ of the group varies as $M_w^{0.59}$ in the range of M_w between 2.6×10^5 and 1.86×10^6. This is in line with the present data not only of $\langle S^2\rangle_z^{1/2}$ but also of $[\eta]$, both of which consistently show that excluded-volume effects are significant at high molecular weights.

Conclusions

Sodium hyaluronate in aqueous NaCl with salt concentrations of 0.2 and 0.5 M undergoes intramolecular excluded-volume effects when the molecular weight exceeds 10^4. The $[\eta]$ data at either salt concentration are described almost quantitatively by the combination of the Yamakawa–Fujii–Yoshizaki theory[11,12] for unperturbed wormlike chains and the Yamakawa–Stockmayer–Shimada theory[13–15] for volume effects (with the Barrett function[34] for the viscosity expansion factor) throughout the entire range of molecular weight studied (i.e., from 3.8×10^3 to 3.5×10^5). The $\langle S^2\rangle_z$ data can also be explained in the same theoretical framework. In other words, the quasi-two-parameter theory (for α_s and α_η) is a good approximation to sodium hyaluronate in aqueous NaCl at high ionic strength, as is the case for uncharged flexible polymers. The persistence lengths of sodium hyaluronate in 0.2 and 0.5 M aqueous NaCl are 4.2 and 4.1 nm, respectively, essentially confirming Cleland's estimate (4–5 nm).

Acknowledgment. We thank Dr. T. Yanaki of Shiseido Co. for providing a hyaluronate sample. This work was supported in part by a Grant-in-Aid (06403027) for Scientific Research from the Ministry of Education, Science and Culture, Japan.

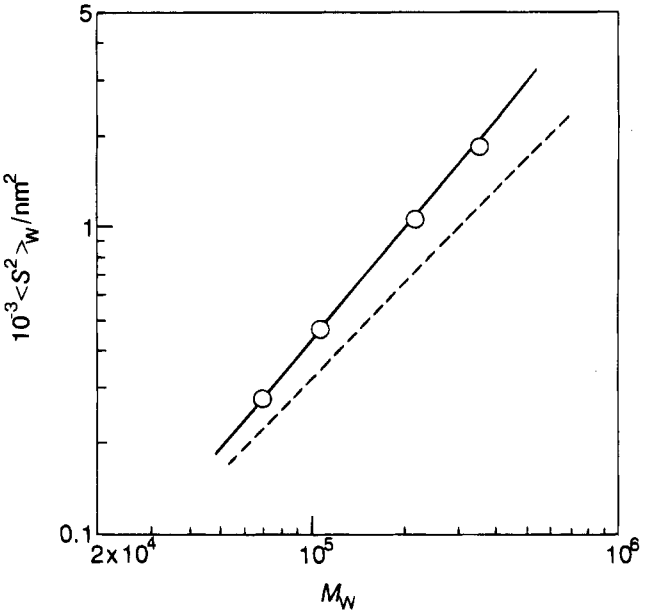

Figure 8. Comparison between the experimental $\langle S^2\rangle_w$ (corrected for polydispersity) in 0.5 M aqueous NaCl and the YSS theoretical values (eqs 16 and 17 with eq 11) for the perturbed wormlike chain with $q = 4.1$ nm, $M_L = 400$ nm^{-1}, and $B/2q = 0.34$. The dashed line refers to the unperturbed state.

References and Notes

(1) Cleland, R. L. *Biopolymers* **1968**, *6*, 1519.
(2) Cleland, R. L.; Wang, J. L. *Biopolymers* **1970**, *9*, 799.
(3) Cleland, R. L. *Arch. Biochem. Biophys.* **1977**, *180*, 57.
(4) Cleland, R. L. *Biopolymers* **1984**, *23*, 647.
(5) Kratky, O.; Porod, G. *Recl. Trav. Chim. Pays-Bas* **1949**, *68*, 1106.
(6) Orofino, T. A.; Flory, P. J. *J. Chem. Phys.* **1957**, *26*, 1067.
(7) Fouissac, E.; Milas, M.; Rinaudo, M.; Borsali, R. *Macromolecules* **1992**, *25*, 5613.
(8) Ghosh, S.; Li, X.; Reed, C. E.; Reed, W. F. *Biopolymers* **1990**, *30*, 1101.
(9) Odijk, T. *J. Polym. Sci., Polym. Phys. Ed.* **1977**, *15*, 477.
(10) Skolnick, J.; Fixman, M. *Macromolecules* **1977**, *10*, 944.
(11) Yamakawa, H.; Fujii, M. *Macromolecules* **1974**, *7*, 128.
(12) Yamakawa, H.; Yoshizaki, T. *Macromolecules* **1980**, *13*, 633.
(13) Yamakawa, H.; Stockmayer, W. H. *J. Chem. Phys.* **1972**, *57*, 2843.
(14) Yamakawa, H.; Shimada, J. *J. Chem. Phys.* **1985**, *83*, 2607.
(15) Shimada, J.; Yamakawa, H. *J. Chem. Phys.* **1986**, *85*, 591.
(16) Yanaki, T.; Yamaguchi, T. *Biopolymers* **1990**, *30*, 415.
(17) Akasaka, H.; Seto, S.; Yanagi, M.; Fukushima, S.; Mitsui, T. *J. Soc. Cosmet. Chem. Jpn.* **1988**, *22*, 35.
(18) Nakanishi, Y.; Norisuye, T.; Teramoto, A.; Kitamura, S. *Macromolecules* **1993**, *26*, 4220.
(19) Sato, T.; Norisuye, T.; Fujita, H. *Macromolecules* **1983**, *16*, 185.
(20) Fujita, H. *Foundations of Ultracentrifugal Analysis*; Wiley: New York, 1975.
(21) Norisuye, T.; Yanaki, T.; Fujita, H. *J. Polym. Sci., Polym. Phys. Ed.* **1980**, *18*, 547.
(22) Fujita, H. *Polymer Solutions*; Elsevier: Amsterdam, The Netherlands, 1990.
(23) Bushin, S. V.; Tsvetkov, V. N.; Lysenko, E. B.; Emel'yanov, V. N. *Vysokomol. Soedin.* **1981** *A23*, 2494.
(24) Bohdanecký, M. *Macromolecules* **1983**, *16*, 1483.
(25) When the theory for the wormlike touched-bead chain[26] is adopted instead of that for the wormlike cylinder, the chain (hydrodynamic) diameter alters. However, these theories are equivalent if the difference in hydrodynamic diameter is taken into account.
(26) Yoshizaki, T.; Nitta, I.; Yamakawa, H. *Macromolecules* **1988**, *21*, 165.
(27) Kuwata, M.; Murakami, H.; Norisuye, T.; Fujita, H. *Macromolecules* **1984**, *17*, 2731.

(28) Yamakawa, H. *Annu. Rev. Phys. Chem.* **1984**, *35*, 23.
(29) Yamakawa, H. In *Molecular Conformation and Dynamics of Macromolecules in Condensed Systems*; Nagasawa, M., Ed.; Elsevier: Amsterdam, The Netherlands, 1988; p 21.
(30) Barrett, A. J. *Macromolecules* **1984**, *17*, 1566.
(31) Abe, F.; Einaga, Y.; Yamakawa, H. *Macromolecules* **1993**, *26*, 1891.
(32) Abe, F.; Horita, K.; Einaga, Y.; Yamakawa, H. *Macromolecules* **1994**, *27*, 725.
(33) Kamijo, M.; Abe, F.; Einaga, Y.; Yamakawa, H. *Macromolecules* **1995**, *28*, 000.
(34) Benoit, H.; Doty, P. *J. Phys. Chem.* **1953**, *57*, 958.
(35) Domb, C.; Barrett, A. J. *Polymer* **1976**, *17*, 179.
(36) Kitagawa, T.; Sadanobu, J.; Norisuye, T. *Macromolecules* **1990**, *23*, 602.
(37) Abe, F.; Einaga, Y.; Yoshizaki, T.; Yamakawa, H. *Macromolecules* **1993**, *26*, 1884.

MA946168K