

Electrostatic Contributions to Chain Stiffness and Excluded-Volume Effects in Sodium Hyaluronate Solutions

Kanako HAYASHI, Kikuko TSUTSUMI, Takashi NORISUYE, and Akio TERAMOTO

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

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ABSTRACT: Intrinsic viscosities $[\eta]$ for sodium hyaluronate in aqueous sodium chloride at 25°C were determined for 12 samples ranging in weight-average molecular weight M_w from 3.8×10^3 to 3.5×10^5 at NaCl concentrations C_s between 0.005 and 2.5 M. They were analyzed on the basis of the Yamakawa–Fujii–Yoshizaki theory for $[\eta]$ of an unperturbed wormlike chain combined with the Yamakawa–Stockmayer–Shimada theory for excluded-volume effects to estimate the total persistence length q and the excluded-volume strength B as functions of C_s . At any C_s studied, excluded-volume effects on $[\eta]$ became appreciable when M_w exceeded 1×10^4 – 2×10^4 . The C_s dependence of q yielded 4.0 nm for q_0 (the intrinsic persistence length) of the polysaccharide chain at infinite ionic strength. It was found that the values of $q - q_0$ (*i.e.*, the electrostatic contribution to q) at $C_s < 0.02$ M were roughly 70% larger than predicted by the Le Bret theory and the Odijk–Skolnick–Fixman theory. On the other hand, the estimated B values agreed fairly well with Fixman and Skolnick's theory for the excluded-volume interaction between a pair of charged rodlike segments unless C_s was lower than 0.05 M.

KEY WORDS Polyelectrolyte / Hyaluronic Acid / Wormlike Chain / Chain Stiffness / Electrostatic Persistence Length / Excluded-Volume Effect /

The current polyelectrolyte theory^{1–4} predicts that the persistence length q of a charged linear polymer in aqueous salt increases with lowering salt concentration C_s , provided the polyelectrolyte is modeled by the Kratky–Porod wormlike chain.⁵ This prediction for the electrostatic stiffening effect seems to be substantiated experimentally for intrinsically stiff polymers undergoing no appreciable intramolecular excluded-volume effect. Notably for double-stranded DNA,⁶ almost quantitative agreement was obtained between theoretical and experimental values for the electrostatic persistence length q_{el} (the electrostatic contribution to q).

On the other hand, our understanding of q_{el} for polyelectrolytes with intrinsically weak stiffness is far from satisfactory. The primary difficulty in the experimental determination of q_{el} or q for those polymers is that the effects of chain stiffness and volume exclusion on measured properties can hardly be separated without resort to a relevant excluded-volume theory. Some previous workers^{7–9} deliberately or undeliberately ignored the latter effect in their estimation of q and discussed apparent values so estimated for q or q_{el} . Others^{10–12} took account of volume effects, but they invoked the Fixman–Skolnick theory¹³ for the electrostatic binary cluster integral and early theories^{14,15} for the radius expansion factor α_s based on the random flight model. Here, α_s is defined as the ratio of the perturbed to unperturbed radius of gyration. It is probably fair to say that no established way of estimating the chain stiffness is as yet known for intrinsically flexible or weakly stiff polyelectrolytes unless the ionic strength is high enough. We note that although scattering functions in the form of the Kratky plot are often considered to allow direct estimation of q , the method cannot always be justified even without excluded volume.^{16,17}

The present study is concerned with our attempt at estimating q and the excluded-volume strength (or the binary cluster integral) for hyaluronic acid (sodium

salt) in aqueous NaCl at 25°C from measurements of the intrinsic viscosity $[\eta]$ covering a C_s range from 0.005 to 2.5 M. This polyacid consists of *N*-acetyl- β -D-glucosamine and β -D-glucuronic acid alternately linked at the 1,3 and 1,4 positions, respectively. Previous characterization work^{18,19} shows that its Na salt in 0.2 or 0.5 M aqueous NaCl behaves like a weakly stiff wormlike chain with $q \sim 4$ nm and undergoes significant intramolecular excluded-volume effects for molecular weight above 10^4 – 5×10^4 . We here analyze the molecular weight dependence of $[\eta]$ for sodium hyaluronate at fixed C_s by the Yamakawa–Fujii–Yoshizaki theory^{20,21} for the intrinsic viscosity $[\eta]_0$ of an unperturbed wormlike chain combined with the Yamakawa–Stockmayer–Shimada (YSS) quasi-two-parameter theory^{22–24} for excluded-volume effects, assuming that without volume effect, the macroion at any C_s studied mimics the unperturbed wormlike chain regardless of its chain length. The results for q and the excluded-volume strength thus obtained are compared with predictions from available polyelectrolyte theories.^{1–4,13} In regard to the adoption of the YSS theory, the following remarks are in order.

According to recent experimental studies,^{25,26} data of α_s^2 and α_η^3 (the cube of the viscosity expansion factor defined by $[\eta]/[\eta]_0$) for uncharged polymers, either flexible or stiff, are explained almost quantitatively in the framework of the YSS theory, *i.e.*, by the YSS perturbation theory for the wormlike or helical wormlike chain²⁷ combined with the Domb–Barrett function²⁸ for α_s^2 or the Barrett function²⁹ for α_η^3 . This quasi-two-parameter theory is also a good approximation to Na hyaluronate in 0.2 and 0.5 M aqueous NaCl.^{19,26} Thus, it seems to be the most relevant to our purpose at present, though its applicability to polyelectrolytes at low ionic strength remains to be seen.

EXPERIMENTAL

Samples

The previously investigated 12 fractions¹⁹ of Na hyaluronate, designated HA1, HA2, ..., and HA12 in order of decreasing molecular weight, were used for the present study. Their weight-average molecular weights M_w (determined by light scattering or sedimentation equilibrium in 0.5 M aqueous NaCl) ranged from 3.8×10^3 to 3.5×10^5 (see Table I). The ratios of the z -average to weight-average molecular weight for six samples HA6 through HA11 were about 1.1.¹⁹

Viscometry

Aqueous NaCl solutions of Na hyaluronate were prepared as described previously,¹⁹ and their zero-shear rate viscosities at 25°C were determined at $C_s = 0.005, 0.0067, 0.01, 0.02, 0.05, 0.1, 1, \text{ and } 2.5 \text{ M}$; for the lowest molecular weight sample HA12, the measurement was made only at $C_s = 0.005$ and 0.01 M because of the limited sample quantity. A four-bulb low-shear capillary viscometer of the Ubbelohde type was used for sample HA1 at every C_s and for sample HA2 at $C_s \leq 0.01 \text{ M}$, and conventional capillary viscometers for the rest; we note that shear-rate effects on $[\eta]$ were small (less than 1.5%) for $[\eta] < 11 \times 10^2 \text{ cm}^3 \text{ g}^{-1}$ when $[\eta]$ values from the two types of viscometer were compared. As in our previous work, the relative viscosity was evaluated by taking account of the difference between the solution and solvent densities for $[\eta] < 60 \text{ cm}^3 \text{ g}^{-1}$. The Huggins plot, the Fuoss-Mead plot, and the Billmeyer plot were combined to determine $[\eta]$ and Huggins' constant k' . For the three lowest molecular weight samples at some low salt concentrations, however, precise values of k' could not be determined because the measurements were confined to low relative viscosities.

The $[\eta]$ data obtained were considered free from electroviscous effects. The reason for this was as follows.

According to Sho *et al.*,³⁰ the double helix of Na xanthan (linear charge density = 3 nm^{-1}) in aqueous NaCl at 25°C has an intrinsically high rigidity as characterized by a q of about 110 nm at infinite ionic strength. This implies that the polymer is hardly stiffened by lowering C_s . In other words, without electroviscous effect, $[\eta]$ for double-helical xanthan with a relatively low molar mass should be independent of C_s . The $[\eta]$ data of Sho *et al.* indeed show this to be the case for Na xanthan samples with M_w of 2×10^5 – 1×10^6 over a C_s range from 0.005 to 1 M.

RESULTS

Numerical data of zero-shear-rate $[\eta]$ for Na hyaluronate in aqueous NaCl are summarized in Table I, in which those at $C_s = 0.2$ and 0.5 M are the reproductions from our previous paper.¹⁹ All these $[\eta]$ values are plotted double-logarithmically against C_s in Figure 1. The curve fitting the plotted points for each sample rises with decreasing C_s , but it flattens as the molecular weight decreases.

Figure 2 shows the C_s dependence of k' for samples HA1 through HA9. The values of k' for the three highest molecular weight samples are essentially constant

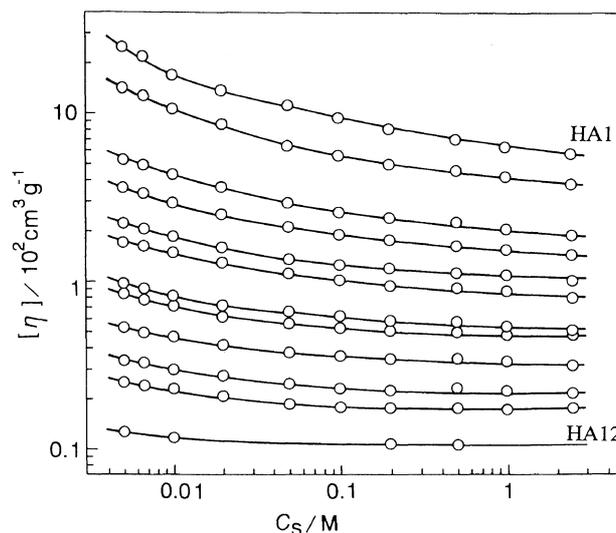


Figure 1. Dependence of $[\eta]$ on C_s for Na hyaluronate samples (HA1, HA2, ..., HA12 from top to bottom) in aqueous NaCl at 25°C. The data at $C_s = 0.2$ and 0.5 M are the reproductions from ref 19.

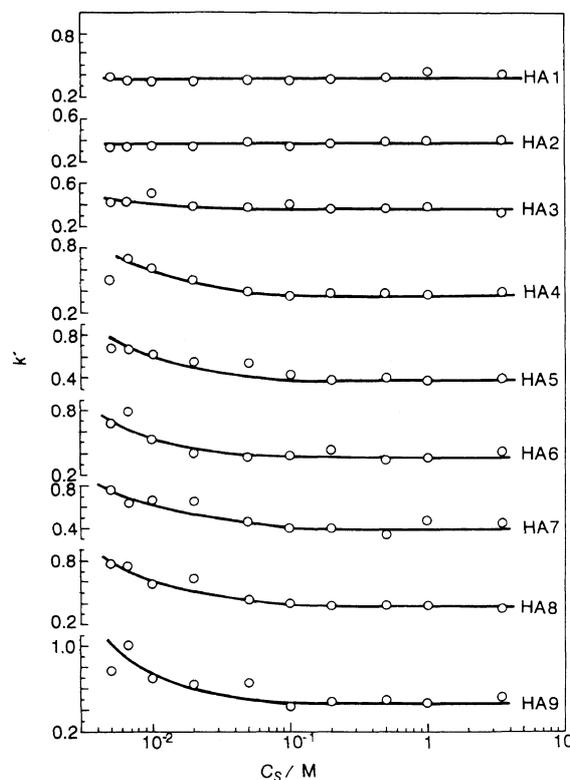


Figure 2. Huggins' constant plotted against $\log C_s$ for indicated Na hyaluronate samples in aqueous NaCl at 25°C. The data at $C_s = 0.2$ and 0.5 M were obtained in our previous work.¹⁹

throughout the entire range of C_s studied. Those for the other samples stay almost constant down to $C_s \sim 0.1 \text{ M}$ and then sharply increase with decreasing C_s . Similar M_w -dependent increases in k' were observed by Sho *et al.*³⁰ for Na salt xanthan in aqueous NaCl.

Figure 3 depicts the molecular weight dependence of $[\eta]$ at the indicated salt concentrations. The plotted points for each C_s follow a curve which is slightly convex upward for $M_w < 10^4$ and roughly linear for $M_w > 10^4$. The slope in this high M_w region is 0.84 at the highest C_s of 2.5 M and 1.14 at the lowest C_s of 0.005 M. Even

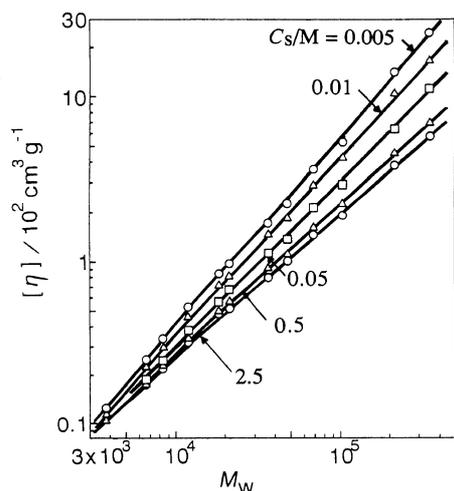


Figure 3. Molecular weight dependence of $[\eta]$ for Na hyaluronate in aqueous NaCl with indicated C_s at 25°C.

the former viscosity exponent is slightly larger than the upper bound value of 0.80 predictable for uncharged flexible chains.

DISCUSSION

Data Analysis

Within the framework of the YSS theory^{22–24} for nonionic wormlike or helical wormlike bead chains, α_η^3 is a universal function of the scaled excluded-volume parameter \tilde{z} defined by

$$\tilde{z} = (3/4)K(\lambda L)z \quad (1)$$

with

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

and

$$K(\lambda L) = \begin{cases} (4/3) - 2.711(\lambda L)^{-1/2} \\ \quad + (7/6)(\lambda L)^{-1} & \text{for } \lambda L > 6 \\ (\lambda L)^{-1/2} \exp[-6.611(\lambda L)^{-1} \\ \quad + 0.9198 + 0.03516\lambda L] & \text{for } \lambda L \leq 6 \end{cases} \quad (3)$$

Here, λ^{-1} is the stiffness parameter, L is the contour length of the chain related to the molecular weight M by $M_L = M/L$, with M_L being the molar mass per unit contour length, z is the conventional excluded-volume parameter, and B is the excluded-volume strength for the interaction between a pair of beads. The $K(\lambda L)$ function is essentially zero for λL below unity due to the stiffness effect and approaches the coil-limiting value of 4/3 with increasing λL . For the wormlike chain (a special limit of the helical wormlike chain²⁷) we have

$$\lambda^{-1} = 2q \quad (4)$$

$$B = \beta/a^2 \quad (5)$$

where β and a are the binary cluster integral and the bead spacing, respectively. The Barrett function²⁹ in the YSS scheme reads³¹

$$\alpha_\eta^3 = (1 + 3.8\tilde{z} + 1.9\tilde{z}^2)^{0.3} \quad (6)$$

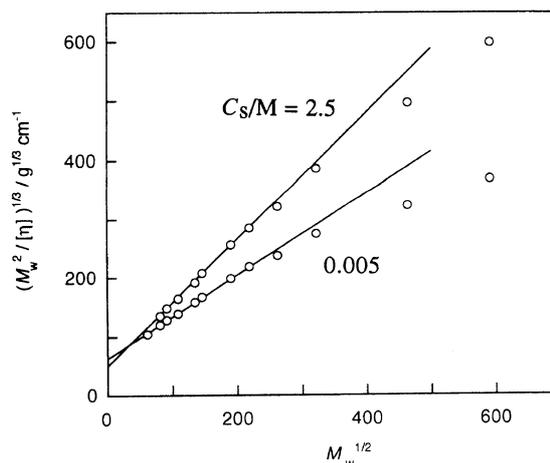


Figure 4. Bushin–Bohdanecký plots for Na hyaluronate in 0.005 and 2.5 M aqueous NaCl at 25°C.

which reduces to the original Barrett equation in the coil limit where $\tilde{z} = z$.

The Yamakawa–Fujii–Yoshizaki theory^{20,21} for $[\eta]_0$ of an unperturbed wormlike chain contains M_L , q , and d (the hydrodynamic diameter in the cylinder model) as the parameters, so that $[\eta]$ in a perturbed state is determined by M_L , q , d , and B ; note that this theory is essentially equivalent to that³² based on the touched-bead model if the difference in hydrodynamic diameter is taken into account. In the data analysis presented below, we first determine M_L of Na hyaluronate at each C_s from $[\eta]$ data at relatively low M_w without explicit consideration of excluded-volume effects and then q and B from all $[\eta]$ data with their consideration, assuming that the d value of 1.0 nm estimated previously¹⁹ at $C_s = 0.2$ M is applicable to any other C_s .

Figure 4 illustrates the Bushin–Bohdanecký plots^{33,34} of $(M_w^2/[\eta])^{1/3}$ against $M_w^{1/2}$ at $C_s = 0.005$ and 2.5 M, constructed according to the equation (an approximation to the Yamakawa–Fujii–Yoshizaki theory)

$$(M^2/[\eta]_0)^{1/3} = I + SM^{1/2} \quad (7)$$

where I and S are given by

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

$$S = 1.516 \times 10^{-8} S_0 (M_L/2q)^{1/2} \quad (\text{g}^{1/3} \text{cm}^{-1}) \quad (9)$$

with I_0 and S_0 being known functions of $d/2q$. The plotted points for either C_s are seen to follow a straight line below $M_w^{1/2} \sim 220$, but above that they deviate downward from the line progressively with increasing $M_w^{1/2}$, showing pronounced intramolecular excluded-volume effects. Plots for other C_s exhibited similar trends. The intercept I and slope S for each C_s together with $d = 1.0$ nm yield a set of M_L and q values, but, as mentioned above, we re-evaluate q in such a way that for $d = 1.0$ nm and the estimated M_L value, a set of q and B allows the Yamakawa–Fujii–Yoshizaki theory with eq 6 to give the closest fit to the $[\eta]$ data in the entire M_w range studied; note that, as remarked previously,¹⁹ stiffness and excluded-volume effects may lead to similar chain length dependences of $[\eta]$ in a certain, narrow molecular weight range.

The values of M_L , q , and B thus obtained are summarized in Table II, along with the previous results at $C_s=0.2$ and 0.5 M, and in Figure 5, the best fit theoretical curves (the solid lines) are compared with the experimental data at the indicated salt concentrations. These solid curves appear somewhat above the data points for M_w between 3×10^4 and 1.5×10^5 and somewhat below those for $M_w > 2 \times 10^5$, but except for $C_s=0.005$ M, the general agreement is fairly good. The dashed lines in the figure represent the theoretical $[\eta]_0$ values for $C_s=0.005$ and 2.5 M. Excluded-volume effects on $[\eta]$ at either C_s are seen to become appreciable at a molecular weight of 1×10^4 – 2×10^4 . The same was true for all the other salt concentrations. With regard to the poor agreement between the theoretical and experimental $[\eta]$ values at $C_s=0.005$ M, a few remarks may be made.

1. As already mentioned, the experimental $\log [\eta]$ vs. $\log M_w$ relation at $C_s=0.005$ M is almost linear for $M_w > 1 \times 10^4$ with a viscosity exponent of 1.14. The contribution from $[\eta]_0$ to this exponent is about 0.79 in

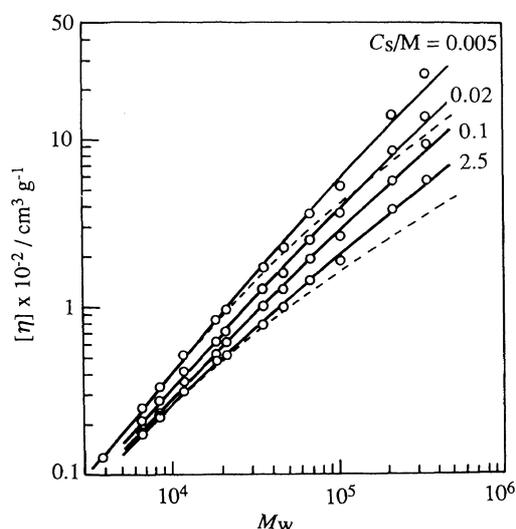


Figure 5. Comparison between the measured $[\eta]$ for Na hyaluronate at indicated C_s (the circles) and the theoretical values (the solid lines) calculated from the YSS theory (the Yamakawa–Fujii–Yoshizaki theory for $[\eta]_0 + \text{eq 6}$) for perturbed wormlike chains with $d=1.0$ nm and the parameters in Table II. The two dashed lines refer to the unperturbed wormlike chains at $C_s=0.005$ (upper) and 2.5 M (lower).

the molecular weight range between 6×10^4 and 4×10^5 when estimated from the dashed line (for $C_s=0.005$ M) in Figure 5. The difference of 0.35 in exponent, which comes from excluded-volume effects, exceeds the asymptotic value 0.30 predicted by eq 6, though the theoretical exponent for α_η^3 can be somewhat larger than 0.3 at finite L because of the L dependence of $K(\lambda L)$ in \bar{z} (see eq 3). A similar argument was found to apply to $C_s=0.0067$ M, at which the agreement between the theoretical and experimental $[\eta]$ for $M_w > 4 \times 10^4$ was not good either. Thus the high experimental exponents for α_η^3 seem to be responsible for the poor agreements between the YSS theory and the present data at the two lowest salt concentrations, suggesting need of some specific theoretical consideration for electrostatic excluded-volume interactions at such low ionic strength.

2. Because of these poor agreements, the values of B at $C_s=0.005$ and 0.0067 M in Table II are inaccurate. They can be larger by 30% or more, provided that even at these low NaCl concentrations eq 6 is valid for very high molecular weights.

3. On the other hand, the q values at the two lowest salt concentrations do not seem to rely strongly on the YSS theory since, as may be seen in Figure 5, both solid and dashed lines for $C_s=0.005$ M fit the data points up to $M_w \sim 2 \times 10^4$ and their difference remains rather small up to $M_w \sim 5 \times 10^4$. In actuality, the estimated q value of 9.7 nm at this C_s (see Table II) differs only by 8% from that (10.5 nm) pre-determined by use of the Bushin–Bohdanecký plot in Figure 4. Furthermore, if a smaller q of 9.0 nm is chosen instead of 9.7 nm, the fit of the YSS curve in the region of M_w below 2×10^4 becomes definitely worse regardless of the value of B . Thus the errors of our q values at $C_s=0.0067$ and 0.005 M should be less than 10%.

In Table II, the M_L values are all in the range between 400 and 410 nm^{-1} . They are consistent with the chemical structure of Na hyaluronate,^{18,19} indicating that the wormlike chain is a good model for Na hyaluronate in the C_s range studied. It can also be seen in the table that q and B monotonically increase with lowering C_s . This shows that both stiffness and volume effects are responsible for the increases in $[\eta]$ with decreasing C_s , observed for high molecular weight samples in Figure 1.

Table I. Intrinsic viscosities for sodium hyaluronate samples in aqueous NaCl with different salt concentrations at 25°C

Sample	$10^{-4} M_w^a$	$[\eta] \times 10^{-2} / \text{cm}^3 \text{g}^{-1}$									
		C_s / M									
		0.005	0.0067	0.01	0.02	0.05	0.1	0.2 ^a	0.5 ^a	1.0	2.5
HA1	35.0	24.4	21.4	16.7	13.6	11.1	9.39	8.07	6.98	6.27	5.72
HA2	21.5	13.8	12.6	10.4	8.49	6.36	5.60	4.94	4.54	4.19	3.78
HA3	10.4	5.23	4.88	4.28	3.60	2.92	2.60	2.39	2.24	2.05	1.88
HA4	6.89	3.58	3.31	2.92	2.50	2.10	1.90	1.76	1.62	1.54	1.43
HA5	4.81	2.22	2.04	1.84	1.59	1.35	1.26	1.19	1.12	1.08	1.00
HA6	3.64	1.70	1.61	1.48	1.28	1.11	1.01	0.935	0.911	0.870	0.790
HA7	2.13	0.964	0.899	0.817	0.717	0.661	0.621	0.579	0.570	0.540	0.506
HA8	1.83	0.838	0.766	0.710	0.617	0.557	0.524	0.508	0.497	0.478	0.476
HA9	1.19	0.524	0.487	0.463	0.417	0.374	0.361	0.345	0.344	0.333	0.316
HA10	0.838	0.335	0.324	0.296	0.274	0.244	0.229	0.223	0.232	0.221	0.215
HA11	0.657	0.249	0.237	0.229	0.206	0.184	0.177	0.175	0.175	0.175	0.174
HA12	0.377	0.124		0.115				0.107	0.105		

^a Taken from ref 19.

For $C_s > 0.2$ M, however, q hardly changes with C_s while B increases with decreasing C_s . Thus, in this high C_s region, the observed viscosity increases may be ascribed primarily to excluded-volume effects.

Electrostatic Contribution to the Persistence Length

Odijk¹ and Skolnick and Fixman² independently showed that q of a charged wormlike chain is the sum of q_0 (the intrinsic persistence length) and q_{el} , *i.e.*, $q = q_0 + q_{el}$. These authors derived an analytical expression for q_{el} of a thin wormlike chain with line charge distribution on the basis of the Debye–Hückel approximation to the Poisson–Boltzmann equation. More elaborate analyses were made by Le Bret³ and Fixman,⁴ who computed q_{el} for a wormlike cylinder with radius r , dielectric constant ϵ , and smeared charge distribution (on the surface), using the complete Poisson–Boltzmann equation. The resulting q_{el} values are tabulated in Le Bret's paper as a function of σ (the linear charge density) and κr for $\epsilon/\epsilon_0 = 0$ and ∞ . Here, ϵ_0 is the dielectric constant of water and κ is the reciprocal of the Debye screening length defined by $\kappa^2 = 8\pi Q N_A C_s / 1000$ for aqueous 1–1 electrolyte, with Q and N_A being the Bjerrum length (0.714 nm for water at 25°C) and the Avogadro constant, respectively; C_s is expressed in units of M. All these theories predict q_{el} to be a decreasing function of C_s and vanish at infinite ionic strength, so that q at this limit equals q_0 . From the data of q in Table II we estimate q_0 of Na hyaluronate (at infinite ionic strength) to be 4.0 nm.

In Figure 6, our q data are compared with the Le Bret theory³ for $q_0 = 4.0$ nm, $\sigma = 1.0$ nm⁻¹, $\epsilon/\epsilon_0 = 0$, and $r = 0.5$ nm; q_0 has been taken to be independent of C_s in first approximation and the σ value has been evaluated from the average M_L of 405 nm⁻¹ (see Table II) and the molar mass (401 g mol⁻¹) of the repeating unit of Na hyaluronate. The theoretical q_{el} values are about 70% smaller than the experimental ones for $C_s^{-1/2} > 7$ M^{-1/2} (*i.e.*, for $C_s < 0.02$ M), the discrepancy being much larger than possible errors in our estimation of q . Interestingly, however, both theoretical and experimental q_{el} 's, when plotted double-logarithmically against C_s (not shown), are essentially parallel with a slope of -0.6 for $C_s < 0.1$ M. This slope happens to be close to those (about -0.5) estimated for several intrinsically flexible or weak-

ly stiff polyelectrolytes^{7,11} without consideration of excluded-volume effects.

The Odijk–Skolnick–Fixman (OSF) theory predicts the C_s^{-1} dependence (or more generally the κ^{-2} dependence) of q_{el} differing from the Le Bret theory, but it (without ion condensation^{3,5}) gives q values close to the Le Bret line, as indicated by a dashed curve in Figure 6; note that the charge parameter σQ for the hyaluronate chain is less than unity (about 0.7). Skolnick and Fixman's calculation² for a discrete charge model gives a curve (not shown here) that is indistinguishable from the dashed line. A point to note is that the discrete charge distribution significantly lowers the OSF theoretical q_{el} for C_s above 0.2 M where q of the hyaluronate chain is dominated by q_0 . In short, all the above-mentioned theories fail to describe the present q_{el} data and thus appear to be only applicable to (long) intrinsically stiff chains.

Schmidt and coworkers^{9,36} computed q_{el} for flexible polyelectrolytes using a mean-field approach and Koyama's distribution function³⁷ for the end-to-end vector of a wormlike chain, and showed that q_{el} can be much larger than OSF's values unless the ionic strength is extremely low. Though this appears to be in line with our data, their theoretical q_{el} contains contributions from electrostatic excluded-volume effects.

Excluded-Volume Strength

The circles in Figure 7 show that B for Na hyaluronate in aqueous NaCl increases almost linearly with increasing $C_s^{-1/2}$. If extended to infinite ionic strength, this linear relation yields a value of about 1 nm for the nonionic excluded-volume strength, which is one order of magnitude smaller than the B values at $C_s^{-1/2} > 7$ M^{-1/2} (*i.e.*, at $C_s < 0.02$ M). Thus, β ($= a^2 B$) in this low C_s region is dominated by its electrostatic contribution β_{el} . As is well known, the conventional bead-bead interaction model in the Debye–Hückel approximation gives β_{el} values that are a few orders of magnitude larger than experimental estimates at such low ionic strength.^{13,38,39} This is also the case for Na hyaluronate (the charge spacing is 1.0 nm), for which we found that the dis-

Table II. Wormlike chain parameters and excluded-volume strength for sodium hyaluronate in aqueous NaCl at 25°C

C_s	M_L	q	B
M	nm ⁻¹	nm	nm
0.005	400	9.7	16
0.0067	400	8.5	14
0.01	400	7.4	11
0.02	405	6.0	9.0
0.05	410	5.0	6.0
0.1	410	4.8	4.8
0.2 ^a	405	4.2	3.4
0.5 ^a	400	4.1	2.8
1	400	4.0	2.3
2.5	405	4.0	1.6

^a Taken from ref 19.

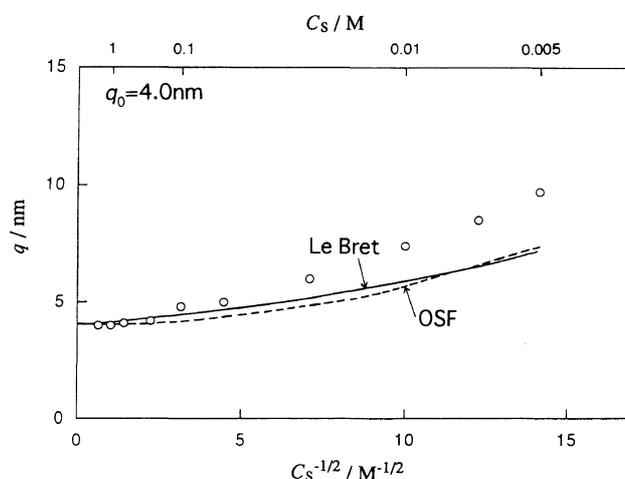


Figure 6. Experimental values (the circles) for the total persistence length of Na hyaluronate in aqueous NaCl compared with the Le Bret theory³ (the solid line) and the Odijk–Skolnick–Fixman theory^{1,2} (the dashed line). The intrinsic persistence length is taken to be 4.0 nm irrespective of C_s .

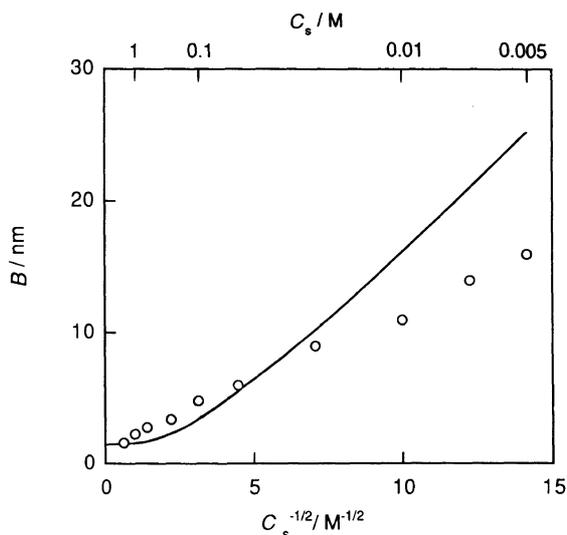


Figure 7. Values of the excluded-volume strength B for Na hyaluronate (the circles) compared with the Fixman-Skolnick theory¹³ (the curve) for $\sigma = 1.0 \text{ nm}^{-1}$ and $d_c = 1.0 \text{ nm}$.

crepancy amounts to one order of magnitude at $C_s = 0.005 \text{ M}$. This factor of 10 is too large to be attributed to the Debye-Hückel approximation, since the charge parameter for the polysaccharide is only about 0.7.

Fixman and Skolnick,¹³ evaluating the excluded volume β' for a pair of rodlike segments with line charge distribution in the Debye-Hückel approximation, explained the early observed large discrepancies^{38,39} in β_{el} as due to the direct application of the bead-bead interaction model to long-range charge interactions at low ionic strength. Since, as mentioned in the Introduction, their theory has often been used for evaluation of z in the study of q_{el} ,¹⁰⁻¹² its comparison with our B values (at $C_s \leq 0.01 \text{ M}$) is of practical interest.

If β' for a pair of segments of length l , each consisting of n_0 beads ($l = n_0 a$), is equated to $n_0^2 \beta$,¹³ it follows that $\beta/a^2 = \beta'/l^2$. The Fixman-Skolnick theory for β' is then expressed in terms of B as

$$B = (\pi/2)d_c + (2/\kappa)R(y) \quad (10)$$

where d_c is the hard core diameter of each rodlike segment and $R(y)$ is a known function of y defined by

$$y = 2\pi\sigma^2 Q \kappa^{-1} \exp(-\kappa d_c) \quad (11)$$

In eq 10, the first and second terms represent the hard core and electrostatic contributions, respectively, and the attractive contribution is assumed to be negligible; note that B requires no specification of l . If y is greater than 3, $R(y)$ is approximated by $R(y) = (\pi/4)(\ln y + 0.7704)$ and B does not depend on d_c .

The curve in Figure 7 represents eq 10 with $\sigma = 1.0 \text{ nm}^{-1}$ and $d_c = 1.0 \text{ nm}$. It comes close to the plotted points for $C_s^{-1/2}$ below $8 \text{ M}^{-1/2}$. Thus we find that the Fixman-Skolnick theory allows a fairly satisfactory description of α_η^3 for Na hyaluronate at $C_s \geq 0.05 \text{ M}$ if it is combined with the YSS theory. Though the theoretical B values are appreciably larger than our estimates at $C_s \leq 0.01 \text{ M}$, we are able to say that the Fixman-Skolnick theory is entirely responsible for this discrepancy. Our B data based on the YSS theory are

inaccurate at $C_s = 0.0067$ and 0.005 M .

CONCLUSIONS

The molecular weight dependence of $[\eta]$ for Na hyaluronate in aqueous NaCl with $C_s \geq 0.01 \text{ M}$ at 25°C is described fairly satisfactorily by the Yamakawa-Fujii-Yoshizaki viscosity theory^{20,21} for unperturbed wormlike chains combined with the Yamakawa-Stockmayer-Shimada excluded-volume theory,²²⁻²⁴ throughout the entire molecular weight range studied (3.8×10^3 – 3.5×10^5). At lower salt concentrations of 0.0067 and 0.005 M , however, the agreement is poor in the region of M_w above 4×10^4 where (electrostatic) excluded-volume effects are significant. The electrostatic contribution to the persistence length determined from the analysis of the $[\eta]$ data is roughly 70% larger (at $C_s < 0.02 \text{ M}$) than predicted by the known theories,¹⁻³ while the estimated values for the excluded-volume strength (or the binary cluster integral) are in moderate agreement with the Fixman-Skolnick theory¹³ for a pair of charged rodlike segments unless C_s is lower than 0.05 M .

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