Macromolecules **1992,** *25,* 5613–5617

Influence of the Ionic Strength on the Dimensions of Sodium Hyaluronate

E. Fouissac, M. Milas, M. Rinaudo,* and R. Borsali

Centre de Recherches sur les Macromolécules Végétales Affiliated with the Joseph Fourier University of Grenoble, B.P. 53 X, 38041 Grenoble Cedex, France

Received September 30, 1991; Revised Manuscript Received June 25, 1992

ABSTRACT: In this paper, we present some results on the dimensions—radius of gyration $\langle\rho^2\rangle^{1/2}$ and persistence length L_p—and the intrinsic viscosity $[\eta]$ of different molecular weights of hyaluronate samples using light scattering and viscosity experiments. Particular attention has been given to the salt concentration effects. Extension of Odijk's model and application of the Yamakawa–Fujii theory lead to a good description of electrostatic expansion of the molecule assumed as a wormlike chain. A good agreement has been found between calculated and experimental values and allows the determination of the intrinsic persistence length $L_p \cong 80$ Å. In addition, the Mark–Houwink exponent a_η relating intrinsic viscosity with molecular weight and its dependence with ionic strength is predicted. A good agreement between calculated and experimental data is found, and the usually high value of a_η measured on this polymer is confirmed and justified.

Introduction

The hyaluronic acid is a carboxylic polysaccharide, recognized for its biological properties and more recently for cosmetic applications.[1] The presence of a uronic acid in the repeating unit leads to the consideration of this polysaccharide as a weakly charged polyelectrolyte. From light scattering measurements, assuming a wormlike chain behavior of sodium hyluronate, a value of 87 Å was obtained for the persistence length L_p in the high ionic strength limit.[2] Using small-angle X-rays and light scattering combined with intrinsic viscosity experiments, Cleland[3,4] determined a persistence length in the range of 40–53 Å. He also mentioned that the value of L_p obtained from light scattering data is larger than that obtained from intrinsic viscosity measurements. In this paper, we present the effect of ionic content on the dimensions of the chain within the framework of Odijk's model.[5-8] Mainly, we compare the experimental radii of gyration to the calculated ones and discuss the variation of the intrinsic viscosity as a function of the ionic concentration. This comparison was done on a series of sodium hyaluronate samples at different molecular weights and for which the intrinsic persistence lengths have been obtained.

Theoretical Approach

(A) Persistence Length. In Odijk's model the total persistence length L_t of a wormlike polyelectrolyte chain depends on the ionic strength and can be written as

$$L_t = L_p + L_e \tag{1}$$

where L_p is the intrinsic persistence length in the absence of an intramolecular electrostatic interaction and L_e the contribution of electrostatic repulsions to the total persistence length. Indeed, the local stiffness of the chain is increased via the Debye–Huckel potential between ionic sites on the charged polymer. This model was previously developed for highly charged polyelectrolyte chains (e.g., DNA). However, for the studied hyaluronate samples, where the charge parameter $\lambda = 0.71$ at the temperature $t = 25$ °C, the electrostatic persistence length takes into account λ and is given by

$$L_e\ (\text{Å}) = \lambda^2/4QK^2 = 0.675\lambda^2(C_p + 2C_s)^{-1} \tag{2}$$

where Q is the Bjerrum length ($\simeq 7$ Å), K^{-1} is the Debye radius, and C_p and C_s are the polymer and salt concentrations (equiv/L). One notes that this relation is valid for $L_t \gg K^{-1}$ and $AK \ll 1$ (A is the distance between two consecutive ionic sites on a polymeric chain). As a consequence of relation 2, the total persistence length L_t depends on C_p and C_s via the electrostatic persistence length L_e.

(B) Radius of Gyration. The radius of gyration can be expressed as a function of the molecule contour L as

$$\langle\rho^2\rangle = {}^1/_3 L L_t \alpha_{s,el}{}^2 \tag{3}$$

where it is assumed that $L \gg L_t$; $\alpha_{s,el}$ is the expansion coefficient with respect to ideal conditions. In fact this expansion is due to electrostatic repulsions acting as the principal contribution to the excluded volume. The expansion factor α_s is a function of the excluded volume parameter Z and is given by Yamakawa–Tanaka:[9]

$$\alpha_s{}^2 = 0.541 + 0.459(1 + 6.04Z)^{0.46} \tag{4}$$

which is one of the most appropriate approximations where

$$Z = (3^{3/2}/32\pi^{3/2})2\pi L_t{}^{-3/2} d L^{1/2} \tag{5}$$

and

$$d = K^{-1}(-\ln QK + \ln 4\pi + Y - {}^1/_2 + \ln \lambda^2) \tag{6}$$

Y being the Euler constant.

Following Odijk and Houwaart[7] and assuming that all the long-range interactions are of electrostatic type, $\alpha_s{}^2$ and Z can be, with a good approximation, replaced by $\alpha_{s,el}{}^2$ and Z_{el}.[7] In eq 5, d is the effective diameter of a Kuhn segment calculated by Fixman and Skolnick[10] and adapted from their original work to the case of $\lambda < 1$. From relations 1–6, one sees that $\langle\rho^2\rangle$ depends on L_p, K, λ, and L. Therefore, it should be possible to extract the persistence length L_p from each set of data when K, λ, and L are known. One notes that this model is not valid for the values of $C_s{}^{-1/2}$ less than 1.5. Indeed the value of d (see relation 6), which is independent of L_p, must be larger than the hydrodynamic diameter d_h and has been deliberately set equal to 10 Å. In Figure 1, we have plotted the variations of the radius of gyration $\langle\rho^2\rangle^{1/2}$ as a function of $C_s{}^{-1/2}$ for different values of L_p = 45, 70, 80, and 100 Å. One observes that, for a given salt concentration, $\langle\rho^2\rangle^{1/2}$ increases with L_p and, for a given L_p, $\langle\rho^2\rangle^{1/2}$ decreases when the salt concentration increases. This behavior is of course expected and confirms the screening effect by adding the salt. Moreover, it is interesting to note that

0024-9297/92/2225-5613$03.00/0 © 1992 American Chemical Society

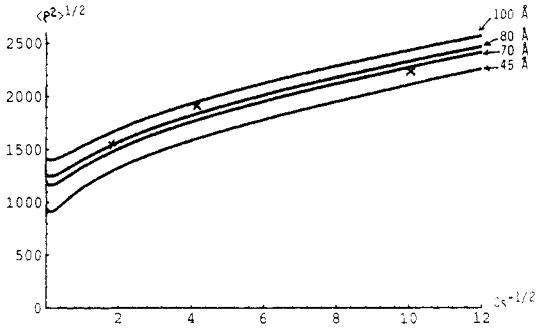

Figure 1. Theoretical variation of the radius of gyration $\langle\rho^2\rangle^{1/2}$ as a function of $C_s^{-1/2}$ for different values of L_p (45, 70, 80, and 100 Å): ×, experimental values for $M_w = 1.86 \times 10^6$.

the extrapolation of $\langle\rho^2\rangle^{1/2}$ for infinite salt excess $\langle\rho^2\rangle_\theta$, corresponds to $\alpha_{s,el} = 1$, i.e., unperturbed conditions (or θ conditions), and is given by[16]

$$\langle\rho^2\rangle_\theta = {}^1/_3LL_p - L_p^{\,2} + 2L_p^{\,3}L^{-2}[1 - \exp(-L/L_p)] \quad (7)$$

and allows us to write for the total expansion factor $\alpha_{s,t}$ (when $L \gg L_p$)

$$\alpha_{s,t}^{\,2} = \langle\rho^2\rangle/\langle\rho^2\rangle_\theta = 3\langle\rho^2\rangle/LL_p = (L_t/L_p)\alpha_{s,el}^{\,2} \quad (8)$$

(C) Intrinsic Viscosity. In order to show the effect of the ionic strength (μ) on the intrinsic viscosity $[\eta]_\mu$, let us consider their relationship within the framework of Odijk,[8] e.g.

$$[\eta]_\mu = [\eta]_\infty(L_t/L_p)^{3/2}\alpha_\eta^{\,3}\Phi(\mu)/\Phi(\infty) \quad (9)$$

where $[\eta]_\infty$ is the extrapolated value of the intrinsic viscosity to infinite ionic strength and α_η is the expansion factor which can be deduced from $\alpha_{s,el}$ as described by Weill and des Cloizeaux.[12] The ratio $\Phi(\mu)/\Phi(\infty)$ takes into account the effect of draining modification. It goes to unity for large persistence length values and large ionic content. For a given set of parameters, the values of Φ have been deduced from the Yamakawa–Fujii model developed for wormlike chains in the absence of excluded volume effect.[13] One notes that Φ is a function of the reduced length $L_r = L/2L_t$ and of the reduced diameter $d_r = d_h/2L_t$ (d_h is the hydrodynamic diameter). The ratio $[\eta]_\mu/[\eta]_\infty$ is a function of K, L_p, L, d_h, and λ and can be calculated via α_η obtained from $\alpha_{s,el}$ values. The best fit between experimental and calculated values of the ratio $[\eta]_\mu/[\eta]_\infty$ allows the determination of L_p for the hyaluronate samples. From Yamakawa–Fujii, the value of $[\eta]_\infty$ is assumed to be identical to that in θ conditions $[\eta]_\theta$ and is given by

$$[\eta]_\theta = \Phi_\infty(L_r,d_r)(M_L/2L_p)^{-3/2}M^{1/2} \quad (10)$$

where M_L is the mass per unit length. One recalls that relation 10 is valid only for lower L_r values, i.e., $L_r < 100$, where no excluded volume effect exists in absence of electrostatic repulsions.[14] In addition, the exponent of the Mark–Houwink relation is also deduced from this model and allows the determination of $(a_\eta)_\theta = \mathrm{d}(\log[\eta]_\theta)/\mathrm{d}(\log M)$; $(a_\eta)_\theta$ has been calculated in the range of molecular weight 10^5–10^6 and is equal to 0.63 for $L_p = 80$ Å.[15] From the dependence of $[\alpha_\eta^{\,3}\Phi(\mu)/\Phi(\infty)]$ with the molecular weight, it is also possible to calculate the value of the a_η exponent in the range of molecular weight covered for each ionic content; since $\alpha_\eta^{\,3}\Phi(\mu)/\Phi(\infty)$ varies as M^ϵ, it follows that $a_\eta = (a_\eta)_\theta + \epsilon$. This method of data treatment allows the calculation of $[\eta]_\theta$ and the behaviors of $[\eta]$ and a_η as a function of the ionic strength for a given L_p.

In the remainder of this paper, the experimental results obtained on a series of hyaluronates are discussed and compared with theoretical calculations regarding the determination of the intrinsic persistence length of the hyaluronic acid.

Experimental Section

The native sodium hyaluronates of different molecular weights are produced by A.R.D. Co. (Paris, France). Some of these samples are partially depolymerized by ultrasonic radiations in order to increase the available range of molecular weights. Partial depolymerization was achieved with a B12 Branson sonifier using the standard microtip (3-mm diameter). The temperature was maintained near 0 °C during sonication periods (from 1 min to 2 h) of 2 g/L sodium form hyaluronate solutions in pure water. The different hyaluronate samples were purified using the usual way: after dissolution in pure water (2 g/L) or after sonication, the solutions were filtrated through Sartorius membranes (3–0.2 μm as pore diameters). Then sodium chloride was added (15 g/L) in the solutions, and hyaluronate was precipitated with ethanol (50% v/v, ethanol–water). The precipitates were successively washed with ethanol–water mixtures from 70% to 100% v/v. Finally, the hyaluronate samples were dried under vacuum for 48 h at 30 °C. Before the experiments, the moisture content was determined by thermogravimetry with a thermobalance Setaram Model G70.

The characterization of the polymer, e.g., the molecular weight distribution, was done for hyaluronate solutions (0.5 g/L) in NH_4NO_3 (0.1 N) by steric exclusion chromatography, using multidetection equipment previously described;[16] the polydispersities of different samples are $M_w/M_n \approx 1.3$ and $M_z/M_w \approx 1.3$. The refractive index increment measured at 25 °C with a Brice Phoenix differential refractometer was found equal to 0.155 mL/g in NH_4NO_3 (0.1 N) and 0.144 mL/g in NaCl and is independent of NaCl concentrations in the range 10^{-2}–0.3 N.

Light scattering measurements on hyaluronate solutions were done using the Fica 5000 automatic scattering photometer in the angular range from 30° to 150° at the wavelength $\lambda = 546$ nm. The static information was extracted from the angular and concentration dependence of scattered intensities by the use of a Zimm plot type, i.e., KC/R_θ versus $\sin^2(\theta/2) + k'C$. K is the optical constant, C the polymer mass concentration, R_θ the reduced scattering intensity at scattering angle θ, and k' an arbitrary constant. Optical clarification of aqueous NaCl solutions of hyaluronate was performed by filtration through a 0.2-μm Sartorius membrane filter.

Zero-shear rate viscosities were measured using a low-shear (LS 30) viscometer from Contraves. Light scattering and viscosity measurements were performed at 25 °C. In all cases the polymer concentrations used were below the overlap concentration which is about $[\eta]^{-1}$. The dilutions were performed at constant salt concentration assuming the contribution of the ionic polysaccharide as negligible.

Results and Discussion

(A) Radius of Gyration. Light scattering measurements were performed for different molecular weight samples and three external salt contents. The values of $\langle\rho^2\rangle_z^{1/2}$ were determined by extrapolation to zero angle and polymer concentration. The experimental data are given in Table I. The calculated values of $\langle\rho^2\rangle^{1/2}$ and $\alpha_{s,el}$ obtained from relations 2–6 are also reported in Table I for $L_p = 45$ and 80 Å. The experimental and calculated values are plotted in Figure 1 for the sample with the highest molecular weight. In Figure 2, the variation of the radius of gyration as a function of M_w in the log–log plot is given for two L_p; the experimental values are compared with the calculated values. The data are given for the $C_s = 0.3$ N case for which the electrostatic contribution is practically screened out, allowing a better estimation of L_p.

From the comparison between experimental and calculated values of the radius of gyration for all studied samples (Table I, Figures 1 and 2), it is concluded that the

Table I
Comparison between Experimental and Calculated $\langle\rho^2\rangle^{1/2}$ Values of the Electrostatic Expansion Coefficient as a Function of the Molecular Weight and Salt Content for L_p = 45 Å and L_p = 80 Å

				L_p = 45 Å		L_p = 80 Å	
M_w	L (Å)	C_s (N)	$\langle\rho^2\rangle_z^{1/2}$(exptl) (Å)	$\langle\rho^2\rangle^{1/2}$ (Å)	$\alpha_{s,el}$	$\langle\rho^2\rangle^{1/2}$ (Å)	$\alpha_{s,el}$
1.86 × 10⁶	46 500	0.3	1554	1297	1.353	1543	1.212
		0.06	1907	1592	1.621	1827	1.414
		0.01	2207	2105	1.881	2324	1.661
810 000	20 250	0.3	980	808	1.276	974	1.157
		0.06	1039	979	1.510	1134	1.330
		0.01	1067	1285	1.740	1426	1.546
360 000	9 000	0.3	578	512	1.216	627	1.118
		0.06	592	611	1.414	716	1.257
		0.01	712	796	1.618	890	1.445
260 000	6 500	0.3	509	428	1.191	527	1.104
		0.06		507	1.378	597	1.237
		0.01	608	658	1.571	737	1.410
160 000	4 000	0.3	458	328	1.162	406	1.086
		0.06		384	1.330	455	1.200
		0.01	522	496	1.510	558	1.356
130 000	3 250	0.3	424	292	1.153	364	1.077
		0.06	444	341	1.315	406	1.183
		0.01	557	439	1.483	495	1.341

Table II
Calculated $\langle\rho^2\rangle^{1/2}$ (Å) Values for M_w = 1.86 × 10⁶ (M_z/M_w = 1.3) and Different L_p (Relations 2–6)

L_p (Å)	C_s (N) = 0.3	C_s (N) = 0.06	C_s (N) = 0.01
45	1297	1592	2105
70	1479	1767	2267
80	1543	1827	2324
100	1664	1940	2430
$\langle\rho^2\rangle_z^{1/2}$(exptl) (Å)	1554	1907	2207

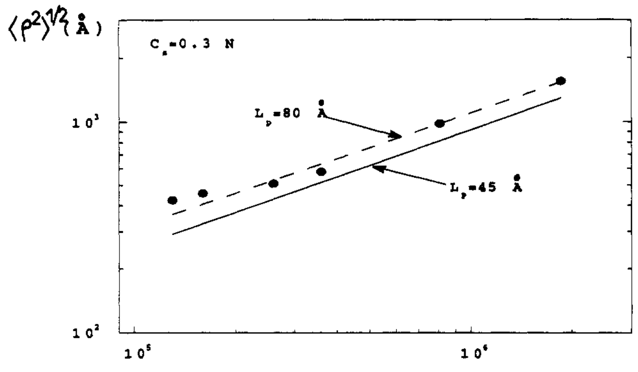

Figure 2. log–log representation of $\langle\rho^2\rangle^{1/2}$ as a function of M_w at C_s = 0.3 N: calculated values for L_p = 45 Å (—) and L_p = 80 Å (- - -); experimental values (●).

theoretical approach proposed gives a better agreement for L_p = 80 Å. As an example, the influence of L_p on $\langle\rho^2\rangle^{1/2}$ at a given molecular weight (M_w = 1.86 × 10⁶) is reported in Table II; L_p = 80 Å is confirmed as being the most convenient value. In Table I are also given the values of $\alpha_{s,el}$; these values are used to determine α_η, which is the viscosity expansion coefficient.

Using the values of $\langle\rho^2\rangle_z^{1/2}$ in the domain of high salt content where the long-range electrostatic interactions are nearly completely screened (C_s = 0.3 N), the values of L_t (with $L_t \simeq L_p$) were determined from relation 7 assuming $\alpha_{s,el} \simeq 1$. The values obtained are given in Table III. These values are obviously larger than those of the θ state, and therefore the L_t values are slightly larger than 80 Å.

From the series of values $\langle\rho^2\rangle^{1/2}$ (experimental and calculated), the exponents ν of relation $\langle\rho^2\rangle^{1/2} = K''M^\nu$ were determined and are given in Table IV; the agreement is good between experimental and calculated values. As one can see, the value of L_p has no large influence on ν.

Table III
Estimation of the Persistence Length from the Radius of Gyration in Salt Excess (Relation 7)

$\langle\rho^2\rangle_z^{1/2}$ (Å) (NaCl (0.3 N))	L_t(calcd) (Å)	$\langle\rho^2\rangle_z^{1/2}$ (Å) (NaCl (0.3 N))	L_t(calcd) (Å)
1554	106	509	92
980	106	458	120
578	85	424	124

Table IV
Estimation of the Radius of Gyration–Molecular Weight Exponents (ν) for Different Ionic Contents and Different L_p (Comparison with Experimental Results)

C_s	ν (L_p = 45 Å)	ν (L_p = 80 Å)	ν (L_p = 100 Å)	ν(exptl)
∞	0.5	0.5	0.5	
0.3	0.56	0.542	0.536	0.5
0.06	0.58	0.565	0.560	0.55
0.01	0.590	0.581	0.577	0.585

For hyaluronic acid, the validity of this treatment may be questionable since the charge parameter λ is lower than 1 and the condition $A/K^{-1} < 1$ has to be respected (i.e., $C_s < 9.4 \times 10^{-2} \simeq 10^{-1}$ N or $C_s^{-1/2} > 3.3$). In spite of the fact that one of the salt concentrations (C_s = 0.3) is out of this domain, there is still a good agreement between experimental and theoretical predictions regarding the light scattering measurements.

(B) Intrinsic Viscosity. From relation 9, the ratio $[\eta]_\mu/[\eta]_\infty$ can be calculated using the Weill–des Cloizeaux relation for the determination of α_η. The values are given in Table V. From experimental values of $[\eta]_\mu$ obtained for different ionic strengths extrapolated to infinite salt content (see Figure 3), the values of $[\eta]_\infty$ were determined and compared in Table VI with those of $[\eta]_\theta$ calculated using the Yamakawa–Fujii theory (wormlike chain model in the absence of the excluded volume effect and taking into account the draining effect). The agreement is found satisfactory rather for L_p = 45 Å. On the other hand, when recalculating the values of $[\eta]_\mu$ from the experimental value of $[\eta]_\infty$ times the ratio $[\eta]_\mu/[\eta]_\infty$ (Table V), the agreement is, in this case, better for L_p = 80 Å. The values of $[\eta]_{\mu(\text{exptl})}$ and $[\eta]_{\mu(\text{calcd})}$ are given in Table V. This result means that the expansion predicted from $\alpha_{s,el}$ and relation 9 is satisfactory.

This treatment allows the prediction of the viscosity exponent a_η at different ionic contents from the variation of $(\alpha_\eta^3\Phi(\mu)/\Phi(\infty))$ with the molecular weight. The values of $(a_\eta)_\theta$ for infinite salt content were recalculated as the

Table V
Calculated Values of the Ratio $[\eta]_\mu/[\eta]_\infty$ (Relation 9) and Prediction of the Intrinsic Viscosities at Different Ionic Concentrations

	$[\eta]_\mu/[\eta]_\infty$ (0.3 N)		$[\eta]_\mu/[\eta]_\infty$ (0.06 N)		$[\eta]_\mu/[\eta]_\infty$ (0.01 N)			$[\eta]_\mu$(calcd)			$[\eta]_\mu$(calcd)			$[\eta]_\mu$(calcd)	
M_w	L_p = 45 Å	L_p = 80 Å	L_p = 45 Å	L_p = 80 Å	L_p = 45 Å	L_p = 80 Å	$[\eta]_\mu$-(exptl)	L_p = 45 Å	L_p = 80 Å	$[\eta]_\mu$-(exptl)	L_p = 45 Å	L_p = 80 Å	$[\eta]_\mu$-(exptl)	L_p = 45 Å	L_p = 80 Å
1 8600 000	2.19	1.59	3.92	2.54	8.69	4.91	2200	2407	1755	3500	4310	2800	6875	9563	5402
810 000	1.88	1.44	3.19	2.17	6.74	3.96	1010	1243	951	1500	2110	1434	3000	4465	2622
360 000	1.65	1.31	2.63	1.85	5.28	3.23	600	678	536	900	1078	760	1500	2165	1327
260 000	1.56	1.27	2.50	1.76	4.89	3.01	450	525	426		839	590	1100	1642	1013
160 000	1.47	1.22	2.27	1.64	4.36	2.69	310	363	303		560	405	710	1077	665
130 000	1.43	1.20	2.17	1.60	4.08	2.49	260	309	260	365	469	345	590	880	538

Table VI
Intrinsic Viscosity in θ Conditions Calculated from the Yamakawa–Fujii Theory: Comparison with Experimental Values Extrapolated to Infinite Salt Excess Ionic Strength (Relation 10)

M_w	$[\eta]_\theta$ (L_p = 45 Å) (mL/g)	$[\eta]_\theta$ (L_p = 80 Å) (mL/g)	$[\eta]_\infty$(exptl) (mL/g)
1 860 000	1120	2408	1100
810 000	694	1440	662
360 000	427	848	410
260 000	344	661	336
160 000	250	466	247
130 000	216.6	404	216

Table VII
Viscosity Exponent a_η as a Function of Ionic Content: Comparison between Experimental and Calculated Values

C_s	a_η(exptl)	a_η(calcd) (L_p = 45 Å)	a_η(calcd) (L_p = 80 Å)
∞	0.67	0.593	0.646
0.3	0.78	0.753	0.746
0.06	0.83	0.813	0.826
0.01	0.92	0.873	0.896

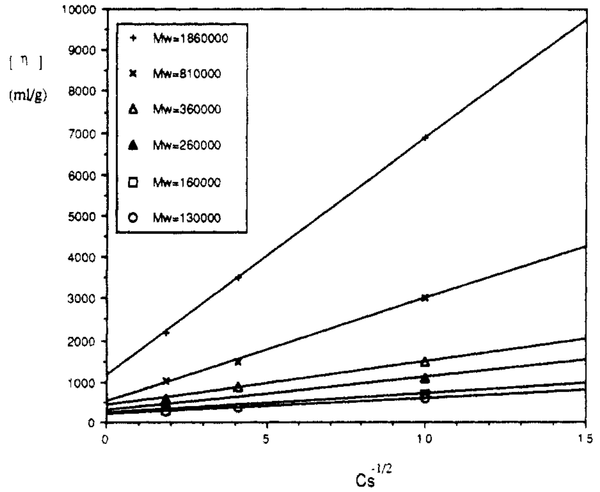

Figure 3. Dependence of $[\eta]_\mu$ with $C_s^{-1/2}$ for different samples of sodium hyaluronate.

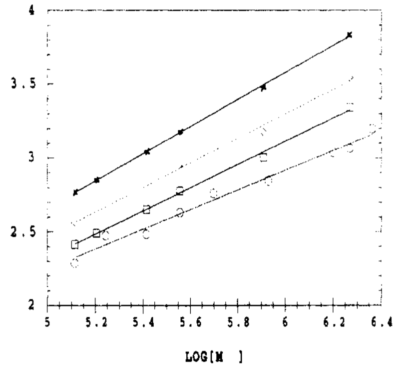

Figure 4. log–log representation of the experimental values of $[\eta]_\mu$ as a function of the molecular weight M_w for different salt contents: (×), 0.01 N; (◇) 0.06 N; (□) 0.3 N; (○) infinite salt concentration.

average of the values obtained for $M_w > 3 \times 10^5$ using the values of $d(\log [\eta]_\theta)/d(\log M)$.[17] These values are compared in Table VII with the experimental ones obtained from the slope of the curves log $[\eta]$ versus log M (Figure 4). The L_p value equal to 80 Å appears to be the best value to predict the exponent of the Mark–Houwink law at least at infinite salt concentration. Therefore, Yamakawa–Fujii relations predict quite well the $[\eta]_\theta$ with L_p = 45 Å, but the dependence of $[\eta]_\theta$ on the molecular weight is better evaluated when we take L_p = 80 Å.

Then, the main question concerns the discrepancy about the values of $[\eta]_\infty$ which is justified by L_p = 45 Å; this value agrees with the previous conclusion from Cleland but is lower than L_p = 80 Å extracted from the data of the radius of gyration and also of the role of the ionic strength on the expansion factor and of the molecular weight on $[\eta]_\theta$. At the moment no significant interpretation of this phenomenon is proposed.

Conclusion

The electrostatic treatment from Odijk seems to be convenient to predict the effect of ionic strength on the radius of gyration and intrinsic viscosity of sodium hyaluronate. In this paper, the excluded volume effect was limited to the effect of electrostatic repulsions. The reduced length of the molecule (i.e., the number of Kuhn segments) always remains low enough to consider hyaluronic acid as a wormlike chain polymer with a large influence of the ionic strength on the draining effect. The Odijk treatment was adapted to a polyelectrolyte with charge parameter λ lower than 1 in the entire range of salt excess considered. The best value of L_p allowing a good fit for $\langle\rho^2\rangle^{1/2}$ and the dependence of $[\eta]$ on the ionic content is found to be about 80 Å, taking into account the role of the polydispersity in molecular weights, i.e., $\langle\rho^2\rangle^{1/2}$, z-average, and $[\eta]$, w-average. But the comparison of $[\eta]_\infty$ with the values predicted from the Yamakawa–Fujii theory indicates a better agreement for L_p = 45 Å. No interpretation of this apparent disagreement is proposed at the moment.

Finally the dependence of the viscosities and the radii of gyration on molecular weight as well as their behavior with salt concentration is well explained by this model.

Acknowledgment. We thank the reviewer for helpful suggestions which contributed to the improvement of the paper.

References and Notes

(1) Bettelheim, F. A. In *Biological polyelectrolytes*; Veis, A., Ed.; Marcel Dekker: New York, 1970; Chapter 3, pp 131–209.
(2) Ghosh, S.; Li, X.; Reed, C. E.; Reed, W. F. *Biopolymers* **1990**, *30*, 1101.
(3) Cleland, R. L. *Biopolymers* **1984**, *23*, 647.

(4) Cleland, R. L. *Arch. Biochem. Biophys.* **1977**, *180*, 57.
(5) Odijk, T. *Polymer* **1978**, *19*, 989.
(6) Odijk, T. *J. Polym. Sci., Polym. Phys. Ed.* **1977**, *15*, 477.
(7) Odijk, T., Houwaart, A. C. *J. Polym. Sci., Polym. Phys. Ed.* **1978**, *16*, 627.
(8) Odijk, T. *Biopolymers* **1979**, *18*, 3111.
(9) Yamakawa, H. *Modern theory of polymer solutions*; Harper and Row: New York, 1971.
(10) Fixman, M.; Skolnick, J. *Macromolecules* **1978**, *11*, 863.
(11) Benoit, M.; Doty, P. *J. Phys. Chem.* **1983**, *57*, 958.
(12) Weill, G.; des Cloizeaux, J. *J. Phys. (Paris)* **1979**, *40*, 99.
(13) Yamakawa, H.; Fujii, M. *Macromolecules* **1974**, *7*, 128.
(14) Fujita, H. *Macromolecules* **1988**, *21*, 179.
(15) Rinaudo, M. *Polym. Bull.* **1992**, *27*, 585.
(16) Tinland, B.; Mazet, J.; Rinaudo, M. *Makromol. Chem., Rapid Commun.* **1988**, *9*, 69.
(17) Bohdanecky, M. Private communication.

Registry No. Sodium hyaluronate, 9067-32-7.