HYDRODYNAMIC STUDY OF SHORT RANGE INTERACTIONS IN SINGLE-STRANDED POLYNUCLEOTIDES

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SUMMARY

Equations are presented for the analysis of sedimentation-molecular weight and viscosity-molecular weight data on flexible polymers, which enable determination of the effective monomer length b in nonideal solvents. The treatment is applied to published data on single-stranded polynucleotides. b decreases from 14.4 ± 0.8 Å in 0.013 $\underline{M}$ Na^+ to 12.9 ± 0.8 Å in 0.2 $\underline{M}$ Na^+, but appears to remain constant at higher ionic strengths. These results are consistent with the damping out of repulsive Coulombic interactions between the phosphates, and may also reflect an increase in the amount of single-stranded helix, with increasing salt.

INTRODUCTION

Base-base interactions in nucleic acids are a subject of intense current interest[1]. Such interactions have been studied by a variety of thermodynamic and spectroscopic techniques. Hydrodynamic techniques, such as sedimentation and viscosity, are also capable of providing useful information on the configuration of polynucleotides in solution, and thus on base-base and other short range interactions. In this communication, equations are presented for the interpretation of viscosity and sedimentation measurements in terms of relevant macromolecular structural parameters--excluded volume and effective segment length. These equations are then applied to data in the literature on the molecular weight dependence of the sedimentation coefficient S^0 and intrinsic viscosity $[\eta]$ of single-stranded polynucleotides in various solvents.

THEORY

The structure of a flexible polymer may be characterized by three parameters: the number of monomeric units, N; the effective length of a monomer, b; and an expansion parameter, ϵ. The mean-square distance $\langle L^2 \rangle$

between the ends of such a polymer is given by

$$\langle L^2 \rangle = b^2 N^{1+\epsilon} \tag{1}$$

For an ideal, randomly-walking polymer, $\epsilon = 0$. ϵ may rise above this value due to repulsive, excluded volume and/or polyelectrolyte effects; or it may drop below zero in the presence of attractive forces between monomeric units.

The hydrodynamic properties of polymers depend on their dimensions, and thus on N, b, and ϵ. For high molecular weight linear chains, it has been shown[2] that the dependence of intrinsic viscosity on these parameters is accurately given by

$$[\eta] = 2.843 \times 10^{21} (1 - 2.68\,\epsilon + 2.64\,\epsilon^2) b^3 M_0^{-(3+3\epsilon)/2} M^{(1+3\epsilon)/2} \tag{2}$$

where M and M_0 are the polymer and monomer molecular weights, respectively, and $N = M/M_0$. $[\eta]$ in this equation is in $d\ell/g$. Eq. (2) may be compared with the Mark-Houwink-Sakurada equation

$$[\eta] = K_\eta M^{a_\eta} \tag{3}$$

which is the form in which most viscosity-molecular weight data are reported. Clearly, $a_\eta = (1 + 3\epsilon)/2$; and knowledge of K_η, ϵ, and M_0 then allows evaluation of b, the effective segment length.

A similar procedure may be used with sedimentation-molecular weight data. One may write the diffusion coefficient D^0 of a flexible polymer chain as[3]

$$D^0 = C_D(\epsilon)\, kT / b \eta_0 N^{(1+\epsilon)/2} \tag{4}$$

where η_0 is the solvent viscosity. $C_D(\epsilon)$ tabulated numerically in Ref. 3, may be fit to within 0.4 per cent for linear chains by the quadratic

$$C_D(\epsilon) = 0.1955(1 + 1.20\,\epsilon + 2.5\,\epsilon^2) \tag{5}$$

Using the Svedberg equation

$$S^0 = M(1 - \bar{v}\rho) D^0 / RT \tag{6}$$

one then obtains, if S^0 is in Svedbergs,

$$S^0 = \frac{0.1955 \times 10^{13}(1 + 1.20\epsilon + 2.5\epsilon^2)(1 - \bar{v}\rho) M_0^{(1+\epsilon)/2}}{N_A \eta_0 b} M^{(1-\epsilon)/2} \tag{7}$$

where N_A is Avogadro's number. Eq. (7) is of the form

$$S^0 = K_S M^{a_S}, \qquad (8)$$

where $a_S = (1-\varepsilon)/2$; so b can again be determined from a knowledge of K_S, a_S and the other parameters of the system.

APPLICATIONS

In Table I we see the application of these equations to a variety of single-stranded polynucleotides, both ribo- and deoxyribo-polymers, in solvents of varying ionic strengths. In evaluating K_S and K_η and applying Eqs. (2) and (7), we have used M_0 = 331 for DNA and M_0 = 351 for RNA and polyadenylic acids (Na salts). The buoyancy factor $(1-\bar{v}\rho)$ was taken as 0.45 in all cases[6,7]. Sedimentation coefficients were adjusted to $S^0_{20,w}$ and measurements were made at 20-25°C unless otherwise noted. The values of K_S and K_η for the first five entries in Table I are somewhat different than those reported by Eigner and Doty[4]. These authors determined molecular weights from S^0 and $[\eta]$ using a Scheraga-Mandelkern[8] β of 2.3×10^6. This is lower than the best theoretical[3] and experimental[7] estimates, and leads to overestimation of the molecular weight and thus to underestimation of K_S and K_η. We have used β as a function of ε as tabulated in Ref. 3.

The data in Table I are internally consistent, insofar as ε_S and ε_η differ from each other by less than 0.02 save in one case. For the first five entries in Table I, such consistency is to be expected because of the use of the Scheraga-Mandelkern equation to determine M. Agreement between b as determined from sedimentation and from viscosity is also reasonably good.

DISCUSSION

The average values of the step length b at ionic strengths of 0.013 M, 0.2 M, and 1.0 M, are 14.4 ± 0.8 Å, 12.9 ± 0.8 Å, and 12.7 ± 1.2 Å. Averages were calculated weighting all data equally. There thus appears to be a probably significant decrease in b in going from low to medium salt, but no further decrease in going from medium to high salt. DNA and RNA (or poly-

Table I. Sedimentation-molecular weight and viscosity-molecular weight characterizations of single-stranded polynucleotides in various solvents, and effective segment lengths derived from them.

Polymer	Solvent	Ref.	a_S	K_S	a_η	$K_\eta \times 10^4$	ϵ_S	ϵ_η	b_S,Å	b_η,Å
DNA, 39% GC	0.013$\underline{M}$Na$^+$, pH6.8	4	.34	.073	.97	.30	.32	.31	15.1	14.7
DNA, 42% GC	0.013$\underline{M}$Na$^+$, pH6.8	4	.35	.068	.94	.37	.30	.29	14.7	14.4
DNA, 50% GC	0.013$\underline{M}$Na$^+$, pH6.8	4	.36	.064	.91	.45	.28	.27	14.4	14.0
DNA, 66% GC	0.013$\underline{M}$Na$^+$, pH6.8	4	.41	.039	.88	.39	.18	.25	15.0	12.5
DNA, all compositions	0.195$\underline{M}$Na$^+$, pH7	4	.48	.025	.55	5.71	.04	.03	12.8	12.1
RNA	1.1$\underline{M}$HCHO, 0.2$\underline{M}$Na$^+$	5	.4	.05	--	--	.2	--	13.8	--
DNA	0.9$\underline{M}$NaCl, 0.1$\underline{M}$NaOH	6	.4	.0528	--	--	.200	--	12.0	--
polyadenylic acid	1$\underline{M}$NaCl, 0°C	7	--	--	.713	2.11	--	.142	--	14.5
polyadenylic acid	1$\underline{M}$NaCl, 7.8°C	7	.437	.0410	.680	2.38	.126	.120	11.5	13.7
polyadenylic acid	1$\underline{M}$NaCl, 20°C	7	.467	.0309	.569	5.41	.066	.046	11.7	13.2

adenylic acid) behave roughly the same in changing salt, and there is no definite trend in b with base composition.

The decrease in effective step length with increasing salt concentration is consistent with a decreasing Coulombic repulsion between neighboring phosphate groups. It may also be consistent with the formation of a greater fraction of helical structure at higher ionic strengths (corresponding to lower temperatures)[9], and with the smaller extension of a short helical region relative to a short random coil region[7,10]. The actual values of b observed are about twice the 6.53Å calculated using the known bond lengths and bond angles in the ribose-phosphate backbone, and assuming free rotation[11,12].

Eisenberg and Felsenfeld[7] have determined unperturbed radii of gyration of polyadenylic acid chains in θ-solvents by light scattering. From these measurements, b values may be obtained which are concordant with those obtained here. The procedure described here for determining b has the advantage that it is not restricted to ideal solvents. This means that the influence of solvent on short-range, as well as long-range, interactions between the polynucleotide monomers can be investigated over a wide range of conditions.

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