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Rotational and translational diffusion of short rodlike molecules in solution: Oligonucleotides

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Rotational and translational diffusion coefficients in aqueous solution of a series of three B duplex oligonucleotides 8, 12, and 20 base pairs in length are measured by depolarized and polarized dynamic light scattering, respectively. Theoretical relations for the diffusion coefficients of short rods by Tirado and Garcia de la Torre are used in combination with the experimental data to obtain the dimensions of the molecules. The theoretical relations are self-consistent through the series and give a hydrodynamic diameter for DNA of 20 ± 1.5 Å. The results, furthermore, show that the combination of depolarized and polarized dynamic light scattering provides a powerful method for obtaining hydrodynamic dimensions of short rodlike particles and may therefore be used in applications which follow molecular sizes and size changes.

INTRODUCTION

A wide variety of experimental and theoretical work suggests that, in many cases, the rotational and translational diffusion coefficients of both macromolecules and small molecules in dilute solution may be calculated by treating the solute as a hydrodynamic particle immersed in a continuum solvent.¹⁻⁵ In such cases, the diffusion coefficients of rigid solute molecules are determined by the temperature, the viscosity of the solvent, the hydrodynamic boundary conditions, and the shape and dimensions of the solute molecules. The relations between the shape and dimensions of a molecule for a particular set of hydrodynamic boundary conditions and its translational and rotational diffusion coefficients in dilute solution are of particular interest. Such relations, in addition to allowing ready estimation of the rates of molecular motions for molecules of known shape and size, provide the basis for the determination of molecular shape and dimensions from hydrodynamic measurements. It should be pointed out that knowledge of the overall molecular translational and rotational dynamics is also of importance in the interpretation of a wide variety of experiments which seek to measure local molecular dynamics [e.g., those utilizing nuclear magnetic resonance (NMR), fluorescence polarization anisotropy decay, electric birefringence decay, and dynamic light scattering].

Many molecules of materials and/or biological importance may, as a first approximation, be modeled as rigid rods. Such systems include small catalytic particles, duplex oligonucleotides, α -helical polypeptides, rigid rodlike monomers, oligomers and polymers (aromatic polyamides, etc.), and rodlike proteins. The difficulty in all hydrodynamic theories which attempt to model the rotation of rodlike molecules arises largely from the effects of the sharp ends of the rod ("end effect corrections"). The standard theory using stick boundary conditions is that of Broersma.⁶

There are as yet no theories which model such molecules using slip boundary conditions, although theories are available for ellipsoids.^{7,8} The Broersma theory even in its corrected form is valid only for cylinders with length (L) to diameter ratios (d) greater than about four. Yoshizaki and Yamakawa have performed stick boundary condition calculations for rods capped with hemispheres.⁹ This theory is not applicable to rods with small length to diameter ratios, $p \equiv L/d$, since the influence of the end caps on the end effect corrections becomes dominant at small p . Tirado and Garcia de la Torre¹⁰⁻¹² have circumvented some of the difficulties inherent in the calculation of the end effect corrections for small p rods in the case of stick boundary conditions by using models of rods composed of spherical hydrodynamic subunits. The spheres were first arranged into rings and the rings then stacked to form rods. Each sphere was characterized by a frictional coefficient and each interacts with other spheres via hydrodynamic interactions transmitted through the solvent. The frictional and the diffusion coefficients of the rodlike assembly of spheres were then calculated. In addition, Tirado and Garcia de la Torre give a polynomial approximation to their numerical calculations which provides an analytical form that fits their calculations down to $p = 2$. (Calculations were not performed for $p < 2$.) These relations have been applied to the analysis of hydrodynamic experiments on several rodlike systems.¹²⁻¹⁵

In this article, we present measurements of the rotational and translational diffusion coefficients of a homologous series of rodlike molecules in dilute aqueous solution: three B-duplex oligonucleotides 8, 12, and 20 base pairs in length (one base pair is approximately 3.4 Å along the helix axis). The approximate range of p for this series is from about 1.4 to 3.5 (see below).¹⁶⁻¹⁸ Thus, this series gives a further test of the Tirado-Garcia de la Torre relations in this low axial ratio region. Furthermore, given that these relations can be confirmed to be good approximations (at least in a self-consistent manner through the homologous series), they may then be used to establish "hydrodynamic dimen-

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sions for DNA." In particular the hydrodynamic diameter of DNA may be found.

The relations between the diameter and the rotational and translational diffusion coefficients are much more sensitive to small differences in diameter for short rods than for long ones. (The diffusion coefficients— $\ln p$.) In addition, longer rods might exhibit significant deviations from rigid rod behavior. Consequently, using rodlike molecules with small p gives an excellent test of hydrodynamic relations, and results in hydrodynamic diameters that may then be used for the larger molecules in a homologous series. For instance, in the case of oligonucleotides the diameters obtained for the short ones may be used to calculate "overall" molecular relaxation times for the typically longer ones that are used in various experiments to analyze local motions. These diameters may also be used as one of the parameters in wormlike coil theories for much larger fragments. In earlier works some data on short rodlike molecules has been presented^{13–15} including some on the rotational diffusion coefficients of the 8-mer and the 12-mer.¹⁵ This oligonucleotide data was used in conjunction with the Tirado–García de la Torre theory to obtain a hydrodynamic diameter for DNA. In the current work we provide measurements of the rotational diffusion coefficients of the 20-mer and translational diffusion coefficients of all three oligonucleotides. Measurement of both translational and rotational diffusion coefficients provides a more stringent test of the consistency of the hydrodynamic relations, and allows extraction of d from the theoretical hydrodynamic relations without having to make assumptions about the length.

THEORY

The translational and rotational diffusion coefficients of rigid rods may, respectively, be written in the forms

$$D = (k_B T / 3\pi\eta_0 L) (\ln p + \nu) \quad (1)$$

and

$$\Theta = (3k_B T / \pi\eta_0 L^3) (\ln p + \delta), \quad (2)$$

where k_B is Boltzmann's constant, T the absolute temperature, and η_0 the pure solvent viscosity.

The differences between various theories for the diffusion coefficients are in the end effect corrections ν and δ . Expressions for these corrections have been given by Tirado, Lopez Martinez, and Garcia de la Torre.^{10–12} A polynomial approximation to their numerical results has been obtained for the range $2 \lesssim p \lesssim 30$:

$$\nu = 0.312 + 0.565p^{-1} - 0.100p^{-2} \quad (3)$$

and

$$\delta = -0.662 + 0.917p^{-1} - 0.050p^{-2}. \quad (4)$$

A technique for obtaining hydrodynamic dimensions of rodlike molecules using Eqs. (1)–(4) has been given by García de la Torre, Lopez Martinez, and Tirado.¹² They first compute a function $f(p)$ defined as

$$\begin{aligned} f(p) &= (9\pi\eta_0/k_B T)^{2/3} D / (\Theta^{1/3}) \\ &= \frac{\ln p + \nu}{(\ln p + \delta)^{1/3}}. \end{aligned} \quad (5)$$

The procedure is to then make a theoretical plot of $f(p)$ vs p based on Eqs. (3) and (4); obtain a value of $f(p)$ from measurement of D and Θ ; use the theoretical plot to obtain the experimental p ; and then use this p and the experimental diffusion coefficients to obtain L (and d) from either (or both) Eqs. (1) and (2). We use this procedure in the following.

Stokes–Einstein (D vs T/η_0) and Stokes–Einstein–Debye plots ($\tau \equiv 1/6\Theta$ vs η_0/T) may be used to check these relations. Deviations from straight line plots in either case for each of the oligomers is usually an indication of intermolecular interactions or aggregation. In addition, the slopes of any straight line plots obtained are related to the apparent hydrodynamic radius $R_h \equiv L/[2(\ln p + \nu)]$ and the hydrodynamic volume $V_h \equiv V_m(2/9)p^2/(\ln p + \delta)$ by

$$D = \frac{k_B}{6\pi R_h} (T/\eta_0) \quad (6)$$

and

$$\tau \equiv 1/6\Theta = (V_h/k_B)(\eta_0/T), \quad (7)$$

where $V_m = \pi d^2 L/4$ is the volume of the cylinder.

EXPERIMENTAL

Oligonucleotides

The 8-mer and 12-mer were purchased from Operon Technologies (San Pablo, California) and the 20-mer from the Midland Certified Reagent Company (Midland, Texas). All oligonucleotides are B-form duplexes with base sequence $d(\text{CG})_n$, where $n = 4, 6$ for the octamer and dodecamer and $d[\text{CGTACTAGTTAACTAGTACG}]$ for the 20-mer. All oligomers were verified to be at least 98% pure by HPLC. The experiments were performed in a phosphate buffer (50 mM phosphate, 100 mM NaCl, 2 mM EDTA, and 0.1% NaN_3) at $\text{pH} = 7$.

Depolarized dynamic light scattering

All rotational diffusion coefficients were measured using an apparatus similar to that described previously.^{15,19} The light source was an argon ion laser (Spectra Physics Model 2000 Series) operating single mode at $\lambda = 488.0$ nm. The scattered light at 90° in the VH geometry was frequency analyzed by passing it through a piezoelectrically driven Fabry–Perot interferometer (RC-110, Burleigh Instruments). The interferometer was equipped with a set of 750 MHz free spectral range confocal mirrors to allow the analysis of slow relaxation processes on the order of nanoseconds. The typical finesse was about 80–90. The detection system consisted of an EMI photomultiplier tube (Model 9865), an SSR (Model 1105) amplifier discriminator, and Burleigh data acquisition and stabilization unit (DAS-1). A reference beam of unscattered light was used to monitor the instrumental linewidth at the beginning of each interferometer scan. Data analysis was performed on a Charles River Data Systems Microcomputer (Universe 68). All data were deconvoluted by an iterative method²⁰ using the spectrum of the reference beam as the instrumental function. In some cases, due to the influence of dust, the spectrum of the scattered light contained a narrow line with the instrumental profile.

Polarized dynamic light scattering

Translational diffusion coefficients were measured using a photon correlation spectroscopy apparatus similar to one described in detail elsewhere.²¹ The light source was an argon ion laser (Spectra Physics Model 165) operating at $\lambda = 488.0$ nm at powers varying between 0.2 and 1.5 W. The detection system consisted of a photomultiplier (ITT FW 130), and an Ortec amplifier-discriminator-photon counting system. The time correlation function of the photon counts was computed by a Brookhaven Bi 2030AT digital correlator. The computed correlation function was then electronically passed to a VAX Station 3200 and the relaxation time distributions in the scattered correlation functions (and the resulting translational diffusion coefficients) were obtained with use of the standard data analysis programs CONTIN and DISCRETE.²²⁻²⁴ Results obtained from these programs were the same within the scatter of the data. Most experiments were done at a scattering angle of 27 deg. Experiments on the 8-mer were done at a series of scattering angles from 27 to 90 deg, and the resulting light scattering relaxation frequencies followed the expected linear dependence on the square of the scattering vector length.

RESULTS

The rotational diffusion coefficients of the three oligonucleotides at various concentrations corrected to 20 °C are shown in Fig. 1. The figure shows that except for the 8-mer and 20-mer at the highest concentrations studied the rotational diffusion coefficients are independent of concentration, and can therefore be presumed to be single molecule rotational diffusion coefficients.⁴ The decrease in the rotational diffusion coefficients at high concentrations is due to aggregation of the oligonucleotides.¹⁵

Figure 2 shows a plot of the translational diffusion coef-

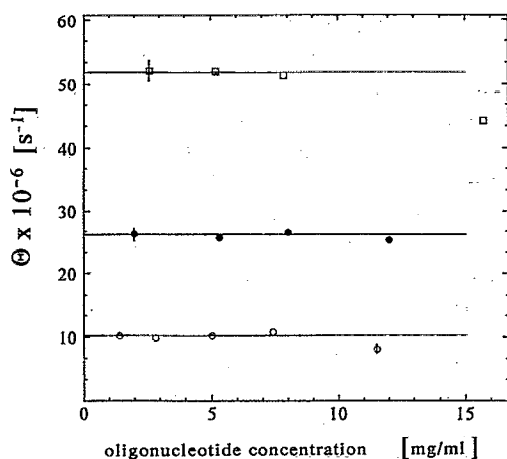


FIG. 1. Rotational diffusion coefficients vs concentration corrected to 20 °C for the three oligonucleotides. Plot symbols: \circ = 20-mer, \bullet = 12-mer, and \square = 8-mer. The highest concentration points for the 8-mer and 20-mer are indicative of aggregation. For the 20-mer all measurements were done at 20 °C except for the 11.5 mg ml⁻¹ sample. For this concentration sample measurements were performed in the range 10–40 °C, which when corrected to 20 °C gave the same result within the experimental error.

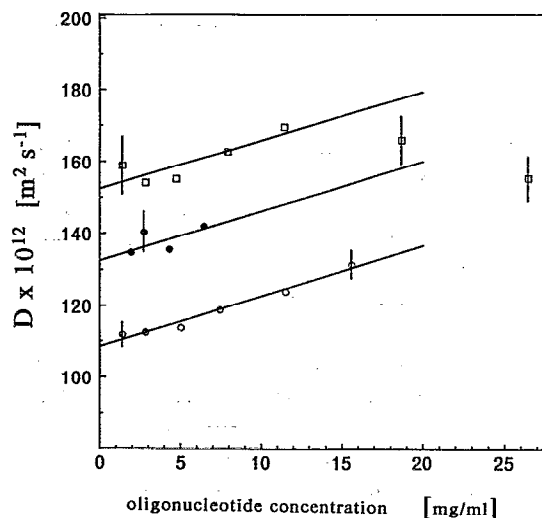


FIG. 2. Translational diffusion coefficients for the oligonucleotides vs concentration corrected to 20 °C. The temperature range of the measurements was 10–50 °C. Plot symbols are the same as in Fig. 1.

ficients vs concentration at 20 °C for all three oligomers obtained by applying CONTIN and DISCRETE analysis to the polarized photon correlation spectroscopy time correlation functions. Except for the two highest concentrations of the 8-mer, all diffusion coefficients fit well to a straight line

$$D = D_0 (1 + kc). \quad (8)$$

The zero concentration intercepts D_0 and the diffusion second virial coefficients k are given in Table I. The zero concentration intercept is the translational self-diffusion coefficient of the oligomer and is given with an error of at most $\pm 4\%$. Note that the diffusion coefficients increase with increasing concentration (below 15 mg ml⁻¹), an indication that aggregation is not important at these concentrations. The second virial coefficients are relatively small and given the considerable experimental error (about $\pm 20\%$) cannot be said to differ significantly.

To further test the hydrodynamic theories, Stokes–Einstein–Debye (SED) and Stokes–Einstein (SE) plots of the rotational relaxation times ($\tau \equiv 1/6\Theta$) and translational self-diffusion coefficients were made and are shown, respectively, in Figs. 3 and 4. The data in both cases fit well to straight lines except for data at high concentration and low temperature, which is most likely due to aggregation under these conditions. The hydrodynamic volumes and hydrodynamic radii extracted from the rotational and translational plots according to Eqs. (7) and (6) are shown in Table II along with a comparison of values calculated from assumed dimensions. The radii and volumes from the slopes of the SE and SED plots are slightly larger than those obtained from the values of the extrapolated zero concentration values of the translational and rotational diffusion coefficients at 20 °C.

Figures 5 and 6 give, respectively, universal plots of the rotational relaxation times and the translational self-diffusion coefficients. Values of the abscissa were calculated using $L = n \cdot 3.4$ Å and $d = 20$ Å. The graphs show that the Tir-

TABLE I. Diffusion coefficients at 20 °C and derived dimensions.

	D_0 ($10^{-12} \text{ m}^2 \text{ s}^{-1}$)	k ($10^{-3} \text{ ml mg}^{-1}$)	Θ (10^6 s^{-1})	$f(p)$	p	L (Å) $-L/n$ (Å)	d (Å)
8-mer	152.6	8.5	51.8	1.50	1.43	28.6 3.57	20.0
12-mer	134.1	8.0	26.1	1.63	2.10	42.1 3.50	20.1
20-mer	108.6	12.9	10.3	1.83	3.59	68.8 3.44	19.2

ado-Garcia de la Torre theory is applicable in a self-consistent manner. There is no significant deviation from the straight lines for the different length cylinders. In addition, numerical values of the slopes may be compared with those predicted from Eqs. (6) and (7). The experimental slope of the straight line in the rotational correlation time plot in Fig. 5 is $1.47 \times 10^{15} \text{ K s}^2/\text{g}^2 \text{ cm}^2$ while the theoretical slope ($= \pi/18k_B$) is $1.26 \times 10^{15} \text{ K s}^2/\text{g}^2 \text{ cm}^2$. The experimental slope of the translational diffusion coefficient plot in Fig. 6 is $1.54 \times 10^{-17} \text{ P m}^2 \text{ Å/s K}$ while the theoretical slope ($= k_B/3\pi$) in the same units is 1.47×10^{-17} . Thus, the experimental slopes are slightly larger than the theoretical ones predicted from Eqs. (6) and (7), possibly due to intermolecular interactions or aggregation. The experimental slopes would be in good agreement with the theoretical ones if the data were plotted using diameters of 22 Å, but not with larger diameters.

We now describe our choice of the best value for the diameter (which was already used in the theoretical comparisons above) based on values for the diffusion coefficients extrapolated to zero concentration and 20 °C and the Garcia

de la Torre-Lopez Martinez-Tirado procedure described in the Theory section. We may, using this procedure, determine values of the hydrodynamic dimensions without making any assumptions about the molecular length. Experimental values of p were obtained from $f(p)$.¹² The function $f(p)$ was calculated as a function of $\ln p$ and the function for $1 < p < 5$ was interpolated with a fourth order polynomial. Then p and then L and d are obtained. Table I lists the values of $f(p)$, p , L , and d . Note that the derived length corresponds to a rise per base pair of 3.44 Å for the 20-mer and slightly higher values for the shorter two oligomers. The average obtained is about 3.5 Å. If we accept the experimentally derived p as correct, then using a rise per base pair of 3.4 Å in the above procedure would give a slightly smaller hydrodynamic diameter (closer to 19 Å). The diameter obtained here is within the experimental error the same as that obtained previously from rotational diffusion coefficient values of the 8-mer and 12-mer assuming a rise per base pair of 3.4 Å.¹⁵

The sensitivity of the derived diffusion coefficients to small changes in the dimensions of short rods is illustrated in Fig. 7. Figure 7 shows a theoretical plot of the rotational diffusion coefficients vs the translational ones all at 20 °C.

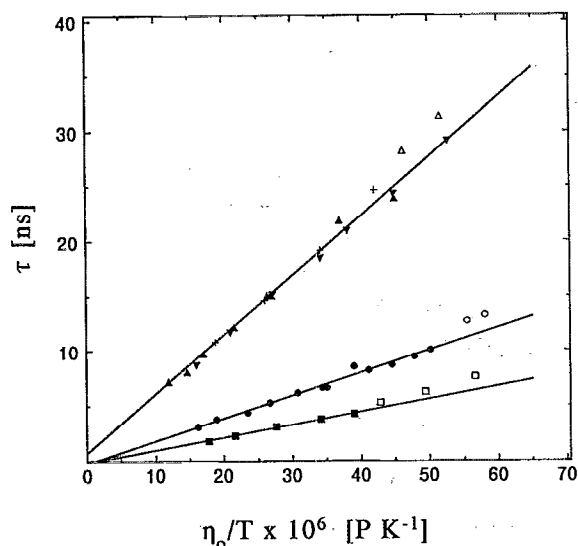


FIG. 3. Stokes-Einstein-Debye plot. Rotational relaxation times ($= 1/6\Theta$) vs solvent viscosity divided by the absolute temperature. ■ 8-mer, 15 mg ml⁻¹, high temperature; □ 8-mer, 15 mg ml⁻¹, low temperature (aggregation); ● 12-mer, 12 mg ml⁻¹, high temperature; ○ 12-mer, 12 mg ml⁻¹, low temperature (aggregation); ▲ 20-mer, 11.5 mg ml⁻¹, high temperature; △ 20-mer, 11.5 mg ml⁻¹, low temperature (aggregation); ▼ 20-mer, 2.9 mg ml⁻¹; + 20-mer, 1.7 mg ml⁻¹.

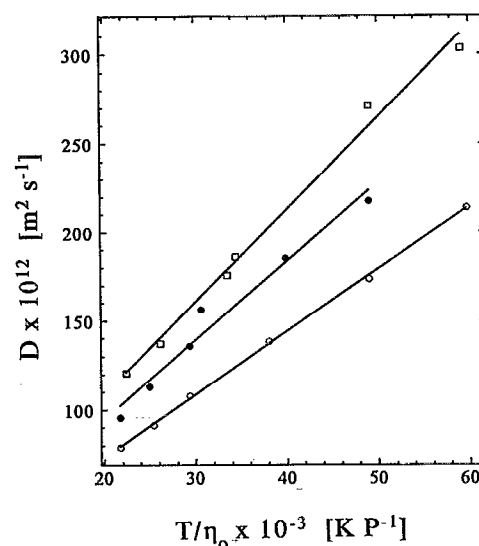


FIG. 4. Stokes-Einstein plot. Translational diffusion coefficients extrapolated to zero concentration vs T/η_0 . Plot symbols are the same as in Fig. 1. The following indicate the concentrations at which measurements were performed at different temperatures: 20-mer—11.5 mg ml⁻¹, 12-mer—2.7 mg ml⁻¹, 8-mer—7 mg ml⁻¹.

TABLE II. Hydrodynamic radii and volumes.

	R_h (exp) Å ^a	R_h (theory) Å ^b	V_h (exp) Å ^{3c}	V_h (theory) Å ^{3b}
8-mer	14.1	13.9	16 450	12 000
12-mer	16.6	16.0	28 400	24 270
20-mer	20.8	20.1	73 500	66 340

^a From Eq. (6).^b Calculated for a cylinder using $L = n \cdot 3.4$ Å and $d = 20$ Å in Eqs. (1) and (2).^c From Eq. (7).

The diameter of the rod varies from 18 to 26 Å. The intercepting lines are calculated from the Tirado–Garcia de la Torre theory for different lengths of the three oligonucleotides based on rises per base pair between 3.1 (upper line), 3.2, 3.4, and 3.5 Å. The three filled squares represent the experimental data extrapolated to zero concentration for all three oligomers. For the 20-mer (point at the lower left of the figure), the intercepting solid lines are shown only for rises per base pair of 3.1 (top) and 3.4 Å. Note that the line which is calculated for $d = 20$ Å gives a good fit to the data. Figure 7 illustrates that for these short DNA fragments, the diffusion coefficients are very sensitive to both the length and the diameter. It is clear that neither diameters of 24 or 26 Å nor rises per base pair of 3.1 and 3.2 Å fit the experimental data within the experimental error.

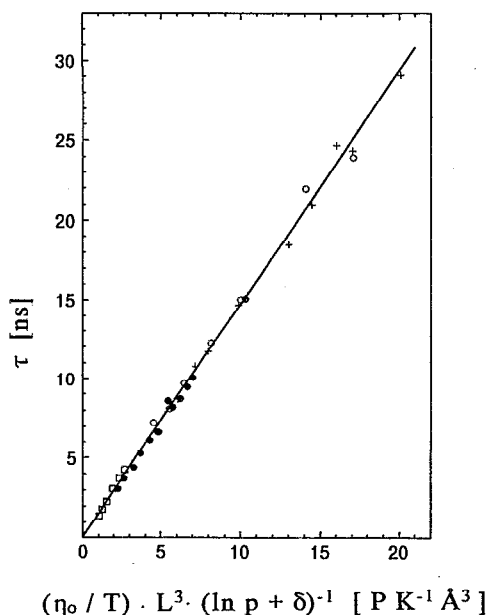


FIG. 5. Rotational relaxation times vs $L^3 T (\ln p + \delta)^{-1} / \eta_0$. Circles and squares the same as in Fig 1, + = 20-mer at 1.7 and 2.9 mg ml⁻¹. All oligomers fit well to the same straight line as predicted by theory of Tirado and Garcia de la Torre.

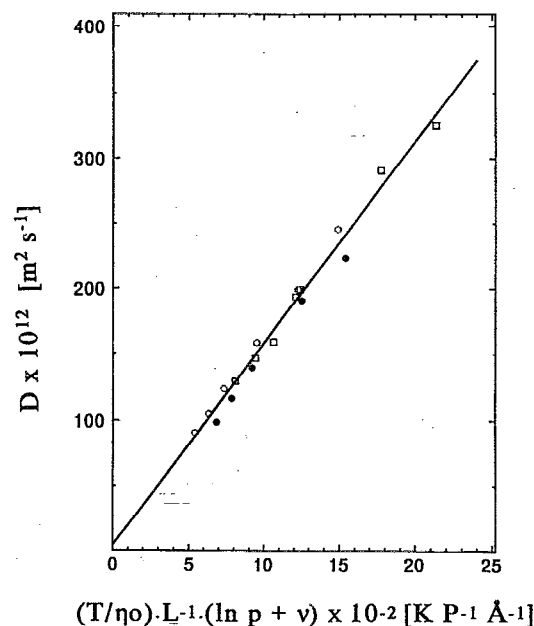


FIG. 6. Translational self-diffusion coefficients vs $(\eta_0 / TL) (\ln p + v)$. ○ 20-mer, 11.5 mg ml⁻¹; ● 12-mer, 2.9 and 1.7 mg ml⁻¹; □ 8-mer, 7.9 and 2.8 mg ml⁻¹. All oligomers fit well to the same straight line as predicted by the theory of Tirado and Garcia de la Torre.

DISCUSSION

The Tirado–Garcia de la Torre theory gives a consistent picture of the translational and rotational diffusion coefficients of short DNA oligonucleotides in dilute solution. The relations are consistent down to length to diameter ratios of about 1.4, the smallest ratio studied. Thus, this hydrodynamic model appears to be applicable to rodlike molecules with small p .

The Tirado–Garcia de la Torre relations applied to the

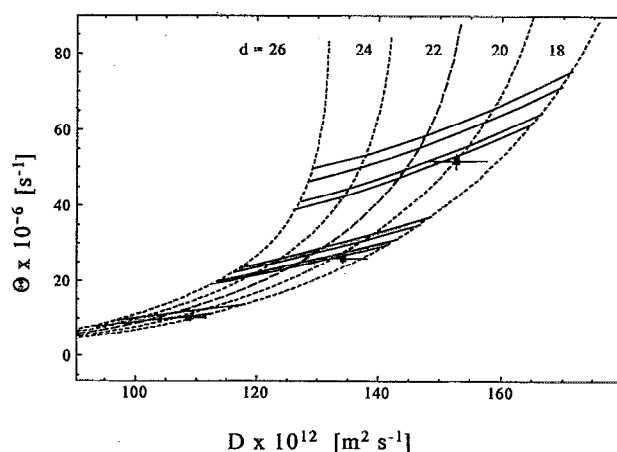


FIG. 7. Rotational diffusion coefficients vs the translational self-diffusion coefficients (zero concentration intercepts). All diffusion coefficients are corrected to 20 °C. ■ experimental data with error bars; --- theoretical values as a function of length for various diameters d from 18 to 26 Å;—theoretical values calculated for different rises per base pair for each of the three fragments. The top four solid lines correspond, reading from top to bottom, to rises per base pair of 3.1, 3.2, 3.4, and 3.5 Å for the 8-mer; the second four correspond to the same rises per base pair for the 12-mer, and the last two to rises per base pair of 3.1 and 3.4 Å calculated for the 20-mer.

three oligomers studied here give a hydrodynamic diameter of DNA of 20 ± 1.5 Å, which is just the distance given by fiber diffraction data^{16,18} for the outer diameter as determined by the extent of the phosphate groups. Our results indicate that self-consistent hydrodynamic diameters obtained by our procedures do not include the extra 4 to 6 Å due to water of hydration beyond the extent of the phosphate groups that have been usually assumed by workers in this field. Several possibilities might be invoked to explain this discrepancy. First, it is possible that deviations of the oligomers from a perfectly cylindrical form are responsible for this effect. It would be interesting to extend the subunit models of Tirado and Garcia de la Torre to include some modeling of the DNA grooves and determine what their effect should be. Second, it may be that the second faster rotation about the axis of the helix is contributing to the depolarized dynamic light scattering spectrum. If this were the case, however, one would expect that the contribution of this rotation would be different for the three fragments and that the resulting effective diameters would also be different for each of the fragments. Third, it is possible that the Tirado-Garcia de la Torre relations, although self-consistent in the homologous series, are giving a diameter that is too small to represent the dimensions of the true rotating unit.

The results also demonstrate that the combination of depolarized dynamic light scattering using Fabry-Perot interferometry and polarized dynamic light scattering using photon correlation spectroscopy constitute very sensitive techniques to monitor sizes and, by extension, size changes of relatively small rodlike molecules such as the oligonucleotides in solution. Possible applications include studies of the transitions between the A, B, and Z forms of DNA²⁵ and the size and shape changes that occur on binding of proteins,^{26,27} platinum anticancer drugs,²⁸ and antibiotics to DNA.²⁹ The Z form of DNA, for instance, forms a helix that is thought to be approximately 10% thinner and approximately 29% more extended than that of the B form. Changes of this extent should be relatively easy to follow by our methods. The use of NMR techniques¹⁵ along with the dynamic light scattering techniques would be an even more powerful combination, allowing the study of both overall and local dynamics.

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