Rotational Diffusion of Sterically Interacting Rodlike Macromolecules

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The rotational diffusion of rodlike M-13 viruses in solution has been measured at concentrations intermediate between the dilute and the concentrated regimes. The strong concentration dependence observed is in agreement with the Doi-Edwards model based upon simple steric hindrance arguments between rotating rodlike macromolecules. The dependence of the rotational diffusion coefficient on the virus length has also been investigated.

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The search for the concentration dependence of mass transport properties has been a long-time goal in macromolecular solutions. Indeed rich information can be obtained from such dependencies since they are related to interparticle interactions: hydrodynamic, electrostatic (in the case of charged particles), and steric, etc. The onset of macromolecular ordering in solutions of charged macromolecules at low salt concentrations[1] and the observation of the diffusion by reptation in flexible polymer solutions[2] provide recent examples of such studies applied to translational diffusion motion. The experiments are much less numerous for the rotational Brownian motion.[3-5] However, interesting theoretical predictions have been made recently for the dynamical behavior of thin rodlike macromolecules in moderately concentrated solutions.[6]

In this Letter, we describe measurements of the rotational diffusion coefficient for two rodlike viruses by the transient-electric-birefringence method (TEB). Our results show for the first time the existence of an intermediate-concentration regime in which the rotation of each rod is severely limited by the others even though the solution is still sufficiently dilute to be considered thermodynamically ideal. This is in agreement with a recent argument by Doi and Edwards[6] showing that the physical impossibility for rods to pass through each other is a strong topological constraint which slows down their rotational motion drastically.

The rod concentration considered here is such that $c^* \ll c \ll c^{**}$ where $c^* = M/NL^3$ and $c^* = M/NdL^2$. L is the rod length and is taken as much greater than the rod diameter d. N is the Avogadro number and M the molecular mass of the rod. The first inequality ensures that the rods are interacting since the hydrodynamic volume swept out by each particle is greater than the average density of rods. The second inequality means that the system is thermodynamically ideal since the volume fraction cd^2L occupied by the rods is negligibly small. The transition towards anisotropic nematic liquid phase due to excluded-volume effects[7] occurs only above c^{**} and is therefore not considered here.

The Brownian motion of the rods in this semidilute, entangled regime is rather complex. Each rod is confined to a certain tubelike region around its long molecular axis and can only change its orientation in a stepwise manner each time the relative translational diffusion of the other rods relaxes part of the topological constraints.[6] Doi and Edwards[6] have constructed the complete kinetic equation for infinitely thin rodlike particles, both at equilibrium and out of equilibrium when the orientational distribution function is homogeneous but anisotropic. This occurs, for instance, when the isotropic distribution is perturbed by an external electric field E, which tends to align the rodlike macromolecules because of their anisotropic polarizability (Kerr effect). In that case, the rotational diffusion coefficient D_R depends on the instantaneous value of the orientational distribution function. This makes the kinetic operator nonlinear and the equations which describe the collective effects of a many rod system become highly complicated. Fortunately, the final results are simple enough. Except for a fast initial decay which is very difficult to observe experimentally, $S(t)$ obeys a classical exponential relaxation

$$S(t) = \exp(-D_R t). \quad (1)$$

This is analogous to the case of infinitely dilute solutions where the rotational diffusion coefficient at zero concentration, D_{R0}, has been replaced by a coefficient D_R, which is both concentration and length dependent.

$$D_R \propto D_{R0} c^{-2} L^{-6}, \quad (2)$$

where $D_{R0} = (3kT/\pi\eta L^3)[\ln(2p) - \zeta]$, $\zeta(p)$ being a complicated function of the variable $p = L/d$.[8] Therefore, $D_R \propto c^{-2} L^{-9}$.

Experimentally two long rodlike virus particles M-13-WT (L = 8920 Å; d = 85 Å; molecular weight $M = 1.6 \times 10^7$) and M-13-T_n3-15 (L = 15 750 Å; d = 85 Å; $M = 2.28 \times 10^7$) were selected in preference to synthetic polymers. First, they are monodisperse in length[3,4] and their stiffness allows to neglect intramolecular motions. Moreover, samples of varying lengths but otherwise similar morphology can be prepared by modern recombinant DNA techniques. Last, their physical dimensions insure that the entangled regime of interest covers a broad concentration range $[(1-100)c^*]$ while it is still sufficiently dilute to minimize aggregation problems.

Wild-type M-13-WT was prepared by the method reported by Berkowitz and Day[9] and details of the preparation and characterization of the macrophage M-13-T_n3-15 will be published elsewhere.[10] Figure 1 shows electron micrographs for both viruses. With use of the literature value L = 8920 Å[4] for the M-13-WT, the length of the macrophage is found to be 15 750 Å. The c^* and c^{**} values are then calculated to be 38 μg/cm^3 and 4.0 mg/cm^3 for M-13-WT and 9.7 μg/cm^3 and 1.8 mg/cm^3 for M-13-T_n3-15, respectively. It should be borne in mind, however, that the definition of c^* is rather loose and the actual values may well differ by an unknown numerical constant. Solutions of viruses were prepared by dialysis against buffers of variable ionic strength and concentrations were determined by uv absorption measurements using an extinction coefficient of 3.84 mg^{-1} cm^2 at 269 nm[4] at high concentrations and by volumetric dilution at low concentrations.

The relaxation of the field-induced rod orientation towards the equilibrium, isotropic distribution is followed by monitoring the transient optical birefringence of the solution. The light intensity $I(t)$ transmitted by the sample between crossed polarizers is directly proportional to the orientational-order parameter $S(t)$. The analysis of the decay curve of $I(t)$ yields a straightforward measurement of D_R since $I(t) \propto \exp(-6D_R t)$. The

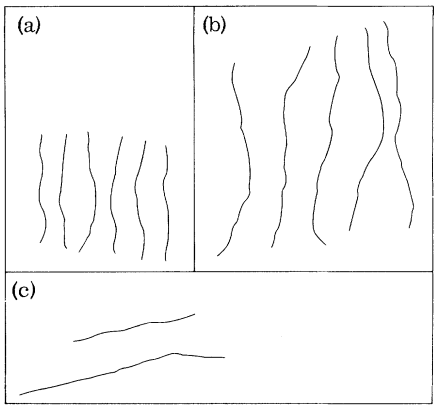

FIG. 1. Electron micrograph pictures of (a) M-13-WT, (b) M-13-T_n3-15, and (c) a mixture of (a) and (b).

experimental TEB setup was standard. Several improvements originally suggested by Newman and Swinney[3] were, however, added, including the use of a 10-mW He-Ne laser for the light source, signal averaging with a Biomation Model-610B transient recorder, and online data processing by a PDP-8 computer. The cell was constructed from Teflon and the two strain-free glass windows (Schott grade 00) were glued by soft silastic adhesive (Dow Corning RTV 732). Platinum-plated electrodes of area 1 cm^2 were used with a 0.5-cm gap. Square-wave pulses (0.50 V) of duration 0.1 ms were applied to the cell with alternating polarity and time lags of 5 s to minimize electrolysis and ion concentration buildup at either electrode.

Since the theory has been established in the absence of Coulombic interaction, we first investigated the behavior of D_R as a function of added electrolyte. Even for the highest virus concentration, the results became independent of salt concentration in the range 5–10 mM KCl. For these ionic strengths, the Debye screening length is very small, of the order of the rod diameter, and the surface charges of the macromolecules are effectively screened out.[11] All further studies were therefore carried out at 10 mM KCl. The field-free birefringence intensity decay curves were always observed to be exponential with a single relaxation time. Semilogarithmic plots versus time yield straight lines with a slope inversely proportional to the diffusion coefficient D_R. The measured D_R values for the two viruses have been plotted in Fig. 2 in reduced units D_{R0}/D_R versus reduced concentration squared $(c/c^*)^2$. The data for M-13-WT virus indicate a c^{-2} de-

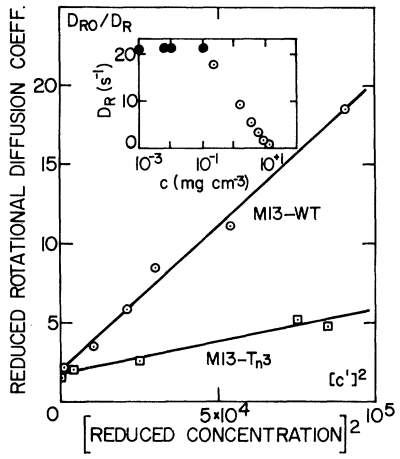

FIG. 2. Reduced rotational diffusion coefficient D_{R0}/D_R vs reduced rod concentration $c'=c/c^*$ squared for M-13-WT (dotted circles) and M-13-T_n3-15 (dotted squares). $c^*=M/NL^3$. The inset: D_R variation for M-13-WT over an extended concentration range c in semilogarithmic scales including some data points from Ref. 4 (solid circles).

pendence for D_R between 0.2 and 11 mg/cm^3. The data for the M-13-T_n3-15 virus also confirm this result between 30 μg/cm^3 and 3.2 mg/cm^3. The ratio of the two slopes in Fig. 2 for D_R yields an experimental length exponent of 5.7. The extrapolated values to infinite dilution are in good agreement with Newman, Swinney, and Day[4] data for M-13-WT giving $D_{R0}=21$ s^{-1} (see inset of Fig. 2) and with the calculated values of 21 s^{-1} and 4 s^{-1} for M-13-WT and M-13-T_n3-15, respectively.

The central result of these experiments is the confirmation of the c^{-2} dependence for D_R in the entangled regime, as predicted by Doi and Edwards.[6] It may seem surprising that such a simple result has not been achieved before since there have been indeed several similar studies on tobacco mosaic virus.[3] However, its geometrical dimensions $L=3300$ Å and $d=180$ Å are not as favorable as for M-13. The reduction in length between the two viruses increases c^* to 1.8 mg/cm^3 for tobacco mosaic virus, while the reduction in L/d ratio squeezes the range of the entangled regime by a factor of 5. Another unsuccessful attempt has been made recently by Wang and Pecora[12] on light meromyosin which is a rod of length 785 Å and diameter 20 Å. At least two experiments have been previously performed on M-13 viruses, one with the TEB technique,[4] the other with quasielastic light scattering.[5] However, the concentrations were always kept very low since the authors were mainly interested in the determination of the hydrodynamic dimensions of the isolated particle.

Our results also provide some information on the length dependence of the rotational diffusion coefficient. At low concentrations, our data for the two viruses are in good agreement with the calculated values. Therefore, $D_{R0}\propto L^{-3}$ as expected for fully extended rods. At large concentrations D_R is observed to vary as $L^{-5.7}$, a rather conflicting result since the Doi-Edwards model predicts a L^{-9} dependence in the entangled regime. A possible explanation for this discrepancy is that the macromolecules lose part of their rigidity. The overall rotation of the entire macromolecule is severely hindered by the presence of the others and it may be that small portions of the rod are then allowed to rotate individually between weaker links. The longer virus could be more affected by this process and, therefore, the apparent length dependence would be reduced. A more trivial explanation could also be that calculations of Ref. 6 are only valid to within an unknown numerical factor. Were these numerical factors different for the two viruses studied because of overlooked details in the intermolecular interactions, the length exponent for D_R would appear erroneous. More experiments are needed to clear up these points.

In conclusion, we have shown that the physical impossibility for rigid rodlike macromolecules to interpenetrate each other leads to a sharp decrease of the rod rotational diffusion coefficient, even at low volume fractions. The measured concentration dependence is in agreement with the Doi-Edwards predictions. An extremely large dependence on particle length has also been qualitatively observed.

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[11]Rigorously, the apparent dimensions of the rod should be increased by one Debye screening length (see Onsanger, Ref. 7). This effect is negligible here.
[12]C. C. Wang and R. Pecora, to be published.

ERRATA

COMPARISON OF ^{24,25,26}Mg(p,n) ^{24,25,26}Al CROSS SECTIONS WITH GIANT $M1$ STRENGTH. U. E. P. Berg, Sam M. Austin, R. DeVito, A. I. Galonsky, Y. Iwasaki, W. A. Sterrenburg, and L. E. Young [Phys. Rev. Lett. 45, 11 (1980)].

On page 11, two additional names should be inserted into the author list and the byline should read

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