The Flow Birefringence of Persistence Length Deoxyribonucleic Acid. Hydrodynamic Properties, Optical Anisotropy, and Hydration Shell Anisotropy

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Abstract: Flow birefringence studies have been conducted upon bihelical fragments of T2 bacteriophage DNA of viscosity molecular weight 9.9×10^4 corresponding to a contour length of 520 Å based upon the Watson–Crick B model. The rotational diffusion constant is estimated as approximately 10^5 corresponding to an ellipsoidal axial ratio of about 12. The birefringence appears to be interpretable in terms of effective molecular polarizabilities using classical dielectric continuum theory, and the results imply that the contributions to the optical anisotropy of bound solvent and electron delocalization along the helical axis are relatively small and of roughly comparable magnitudes. Discrepancies between the present results and flow birefringence studies on other macromolecular systems are considered and discussed.

The optical anisotropy over a defined region of a macromolecule is a quantity of fundamental significance since it is closely related to the optical, electrical, and spectroscopic behavior of the system and to details of molecular structure as well. In principle, this quantity is obtainable from a variety of measurements including dielectric constants, light scattering, and the determination of birefringence. However, electrical measurements and their interpretation are difficult for polyelectrolytes in solution, and light scattering determinations, for various practical reasons, are normally restricted to polymers of not more than a few million molecular weight.

Flow birefringence is a method of considerable potential utility in obtaining not only the optical properties of macromolecular systems but their hydrodynamic properties as well. This is especially true for those systems which are difficult to study using electrical or light-scattering techniques. Most native DNA's fall into this category, and it has been shown that the flow birefringence of high molecular weight DNA can provide the essential hydrodynamic properties of this molecule in neutral aqueous solution, and in addition, a relation between the persistence length and the optical anisotropy per base pair.[1,2] Thus, the effective optical anisotropy has an operational significance in terms of the persistence length or the Kuhn segmental length in DNA and in other stiff-chain polymers, and this conclusion is probably generally true also for relatively flexible macromolecules.[2,3] However, the analysis requires the use of a continuum ellipsoid of revolution as a model for the Kuhn statistical segment, and the optical theory for both the intrinsic and the shape birefringence is in its essentials at the same level of approximation as that originally proposed for rigid ellipsoidal particles in suspension by Peterlin and Stuart.[4] It is clearly necessary, therefore, that an independent determination of the optical anisotropy be made on persistence length fragments of DNA, and furthermore, in terms of the optical properties of this molecule, it is of considerable interest to examine the molecular level significance of this quantity and the applicability of the Peterlin and Stuart model to this system.

In an earlier communication,[5] we presented the results of flow birefringence measurements upon fragments obtained from the hydrodynamic degradation of T2 bacteriophage DNA. These fragments were obtained at a limiting molecular weight of 3.8×10^5 daltons, corresponding to a Watson–Crick B helix of contour length 1990 Å, and were studied in neutral aqueous solution. Although our data were complicated by evidence of polydispersity at lower velocity gradients (which we attributed to shear-dependent aggregation, induced by trace protein contamination from the chromatographic fractionations of the samples), no real indication of chain flexibility could be detected at velocity gradients on the order of 2000 sec^{-1}. Nevertheless, the persistence length of linear bihelical DNA has been variously estimated to be near 500 Å,[1,2,6,7] and intrinsic viscosity data[8,9] are available which appear to imply that DNA may not be perfectly rigid at the molecular weight studied in the previous work. In view of this, we made no attempt in the earlier discussion to treat the optical anisotropy in terms of the Peterlin and Stuart theory. We calculated the polarizability anisotropy per base pair rather as an operational quantity, which, as was subsequently shown,[2] is perfectly consistent also with data on high molecular weight native DNA. However, the magnitude of the anisotropy as obtained in this way was found to be in remarkably close quantitative agreement with several bond polarizability summations based upon an isolated Watson–Crick helix.

In the present work, refinements in preparative methods and in instrumental techniques have permitted us to extend these studies into the persistence length region of molecular size for helical DNA while still maintaining experimental conditions of relatively low

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concentration and velocity gradient. Since the Peterlin and Stuart hydrodynamic theory should apply quite rigorously to the present system, the associated optical treatment is evaluated for DNA in a solvent of substantially different refractive index by comparing the experimental polarizability anisotropy to various available theoretical values. The results appear generally to substantiate the Peterlin and Stuart model in this case, and to imply that the flow birefringence monomer anisotropy, at least for DNA, is a fundamental property of the molecule and is either largely unaffected by the structure of associated solvent and by electron delocalization along the helical axis, or else these two effects are very nearly compensatory.

Experimental Section

Materials. DNA was obtained from T2 bacteriophage as described elsewhere.[2,5] The solvent used was neutral aqueous phosphate–EDTA buffer 0.1 *M* in NaCl. A single sample of DNA was used for all preparations; this sample showed normal melting characteristics and had an intrinsic viscosity $[\eta]$ = 314 dl/g prior to degradation. Concentrations were determined from the absorbance at 260 mμ in a Zeiss PMQ-II spectrophotometer using an extinction coefficient of 1.81×10^{-2} cm²/μg[10] for both the undegraded and degraded material.

Degradation was accomplished by sonication using a slight modification of the method described by Li and Crothers.[11] A Branson Model W185D sonicator with normal (approximately ½ in. diameter) tip was employed (Heat Systems, Ultrasonics, Inc., Plainfield, N. Y.). DNA at a concentration of 50 μg/ml was placed in a rosette cell mounted in a cooling bath. The solution was saturated with nitrogen using a sintered-glass gas-dispersion tube. One minute of sonication at full power was alternated with 10 min of cooling and gas saturation. Approximately a 10° temperature rise occurred during the sonication cycle, and the solution was cooled to slightly below 0° during the saturation phase. Most of the degradation occurred during the first few minutes of sonication, but a further slow increase was observed even after 45 min of sonication. The samples studied were sonicated for a total of 60 min.

Since considerable shedding of the titanium probe tip occurred, the samples, after degradation, were clarified by high-speed centrifugation and by filtration through a 0.45-μ Millipore filter under gravity flow. The samples were concentrated by ultrafiltration using an ordinary high-pressure filtering cell (Millipore Filter Corp., Bedford, Mass.) equipped with dialysis membrane as a filtering element and maintained at approximately 30 lb/in.² nitrogen pressure and refrigerator temperature; roughly 50 ml of solvent could be removed in a 24-hr period using a 47-mm diameter filter with negligible loss of DNA as evidenced by the optical absorbance of the filtrate. After concentration, the samples were equilibrium dialyzed against pure buffer using dialysis bags which had been presoaked for several days with occasional changes of bath. Dialysis was continued until both the absorbance at 260 mμ and the refractive index at 546 mμ of the bath were identical with those of the stock solvent.

Prior to flow birefringence determinations, the degraded samples were characterized by thermal denaturation and by intrinsic viscosity. For the sample studied, $[\eta]$ = 0.412 dl/g corresponding to $M = 9.90 \times 10^4$ daltons using the low molecular weight form of the Eigner–Doty equation[8] corrected as suggested by Cohen and Eisenberg.[12] Melting curves appeared normal for a preparation of this molecular weight. No attempt was made to fractionate the samples prior to study.

Methods. Intrinsic viscosities were obtained using the cartesian diver concentric cylinder viscometer employed in previous work.[1] Relative viscosity data were obtained for the degraded samples at shear rates between 0.4 and 3.1 sec^{-1}; no shear dependence could be observed within experimental error. Concentration extrapolations were made using the Huggins equation[13] with $k' = 0.47$ for the low molecular weight material.

The refractive index increment dn/dc and the absolute refractive index of the solvent were obtained at 546 mμ using a Phoenix differential refractometer (Phoenix Precision Instrument Co., Philadelphia, Pa.) calibrated against standard KCl solutions. The data for DNA were obtained on the same samples as used for flow birefringence; $(n - n_0)$ was linear within reading error over the concentration range investigated and dn/dc was obtained as 0.172 ± 0.001. This value is considerably lower than that obtained by us previously for chromatographically fractionated low molecular weight DNA[6] but is in satisfactory agreement with other determinations on unfractionated material of higher molecular weight.[1,2,12] The solvent refractive index was measured as 1.33385 by comparison with standard KCl solutions.

Flow birefringence data were obtained using a somewhat more sensitive version of the instrument employed in our earlier investigations.[1,6] The apparatus, its operation, and data reduction techniques are discussed elsewhere.[5,14] Principal modifications for the present study included the use of a relatively heavy-walled Teflon rotor sleeve and stator in the flow cell; the annular gap width was 0.49 mm. Only the flow-cell bearings were cooled; the samples were allowed to attain a thermal steady state at each shear rate since a substantial reduction in optical noise at higher velocity gradients was obtained in this way. The flow-cell temperature was monitored roughly by means of a thermistor probe located at the bottom of the stator. At 4000-sec^{-1} shear with the aqueous DNA solutions in the cell, the final cell temperature stabilized at approximately 5° above the 25° ambient. The instrument signal was time averaged to four significant figures for each determination.

All data were corrected for solvent effects as described previously[6] by determining A and B curves *vs.* shear rate based upon repetitive measurements upon pure solvent. At the higher velocity gradients, these effects became quite large, on the order of two to three times the corresponding values attributable to the solute; without correction, the extinction angles would have been somewhat reduced in magnitude, and the solute birefringence numerically increased by roughly the amount of the solvent signals. Although the solvent effect appeared to be a quasi-flow birefringence phenomenon, and the data were remarkably reproducible when taken as described above, we attribute it largely to optical noise in the flow cell; it is theoretically possible for flow birefringence to be associated with the solvent alone,[15,16] but our results implied otherwise since the apparent extinction angles were invariably less than 45° and the data and their reproducibility were to some extent dependent upon the flow-cell temperature relative to its final steady-state value.

Basic Equations and Theory

The flow birefringence theory of Peterlin and Stuart[4] formally treats the opticodynamical properties of macroscopic ellipsoids of revolution suspended in continuous viscous fluids. In a uniform velocity gradient, the distribution of particle orientations under stationary-state conditions is obtained from the competition between hydrodynamic orientation and rotational diffusion. Since this distribution function can be analytically[4] or numerically[17] evaluated over a wide range of the shear rate to rotary diffusion constant ratio, the extinction angle is directly obtainable under the assumption that the principal optical and geometrical axes of the particle are coincident. The treatment of the birefringence, however, requires additional assumptions. Both the particle and the solvent are represented as dielectric continua, with the latter assumed also to be isotropic. Furthermore, the electric field of the light is treated as quasi-static over the longest dimension of the particle. This formally introduces the restriction that the particle dimension be small

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with respect to the wavelength of the incident light, *i.e.*, $a \ll \lambda/4\pi n$ where a is the major semiaxis and n is the solvent refractive index. Although this latter restriction is largely satisfied in the present work, Taylor and Cramer[18] have shown that the Peterlin and Stuart optical theory can be rederived in terms of light scattering theory and this condition removed for rods of diameter less than approximately 400 Å oriented perpendicular to the incident beam.

The optical properties of the system are treated using ordinary macroscopic dielectric theory. The principal components of polarization are calculated as the sum of the solvent polarization and the excess polarization due to the particles oriented with respect to the incident light; in terms of the external field, therefore, the excess polarization is calculated from the corresponding polarizability components of the ellipsoidal shaped particles where the internal field is determined by an ellipsoidal shape factor L.

Since the macromolecule or particle in the Peterlin and Stuart theory is, in effect, treated as a single dipole with respect to its components of polarization, the molecular level significance of the polarizability anisotropy is not immediately apparent. It certainly depends upon the true mean anisotropy of the (isolated) particle, the particle shape, and upon the electrical nature and orientation of adjacent solvent molecules. In general, this question can only be resolved from a knowledge of the true particle anisotropy, and in this sense, it is useful to compare the experimental value obtained from birefringence to reasonable theoretical molecular level calculations. However, such a comparison requires that the true intrinsic birefringence be obtainable from the experimental quantity. As is noted below, a formal separation of intrinsic and form anisotropy contributions is easily obtained in terms of the Peterlin and Stuart model; in the present work, the results of such a separation are somewhat at variance with a direct calculation of the form birefringence, however.

In terms of the assumptions discussed above, the Peterlin and Stuart theory gives for the extinction angle χ in radians ($G\cdot/D_r \leqslant 1.5$)

$$\chi = \frac{\pi}{4} - \frac{G\cdot}{12D_r}\left[1 - \frac{G\cdot^2}{180D_r^2}\left(1 + \frac{24d^2}{35}\right) + \dots\right] \quad (1)$$

and for the birefringence

$$\Delta n = \frac{2\pi\bar{\nu}c}{n}(g_1 - g_2)F \quad (2)$$

in which $G\cdot$ is shear rate, D_r is the rotational diffusion coefficient, $\bar{\nu}$ is the partial specific volume of the macromolecule, c is the weight concentration, d is a function of axial ratio p, *i.e.*, $d = (p^2 - 1)/(p^2 + 1)$, and the g_i are polarizability components per unit volume. In eq 2, F is an orientation function derived from the orientation distribution and is given by Peterlin and Stuart as ($G\cdot/D_r \leqslant 1.5$)

$$F = \frac{G\cdot d}{15D_r}\left[1 - \frac{G\cdot^2}{72D_r^2}\left(1 + \frac{6d^2}{35}\right) + \dots\right] \quad (3)$$

In order to avoid ambiguities due to finite concentration and shear, it is convenient to define the reduced birefringence in terms of eq 2 and 3

$$[n] = \left(\frac{\Delta n}{cG\cdot\eta_0}\right)_{c,G\cdot\to 0} = \frac{2\pi\bar{\nu}d}{15n\eta_0 D_r}(g_1 - g_2) \quad (4)$$

in which η_0 is the absolute viscosity of the solvent. Although D_r is obtainable from extinction angle data and eq 1, it is desirable in the present work to obtain this quantity as a function of intrinsic viscosity from the relation[19] in which M is molecular weight and δ

$$D_r = \frac{\delta RT}{6\eta_0[\eta]M}$$

is a relatively insensitive function of axial ratio only. Thus, eq 4 can be rewritten

$$\frac{[n]}{[\eta]} = \frac{4\pi}{5nkT}\frac{\bar{\nu}M}{\delta N_A}(g_1 - g_2) \quad (5)$$

The ith polarizability component of an ellipsoid of revolution of volume ν_P suspended in a fluid of refractive index n is[4]

$$\gamma_i = \frac{\nu_P}{4\pi}\frac{(n_i^2 - n^2)}{1 + [(n_i^2 - n^2)/n^2)]L_i} \quad (6)$$

in which n_i is the particle refractive index and L_i is an ellipsoidal shape factor[20]

$$L_1 = \frac{(1 - e^2)}{e^2}\left[\frac{1}{2e}\ln\frac{1 + e}{1 - e} - 1\right]$$

$$e^2 = \frac{(a^2 - b^2)^{1/2}}{a}$$

$$L_1 + 2L_2 = 1$$

where a and b are the major and minor semiaxes, respectively. Hence, one obtains from eq 6

$$(g_1 - g_2) = \frac{1}{4\pi} \times \frac{(n_1^2 - n_2^2) + \dfrac{(n_1^2 - n^2)(n_2^2 - n^2)(L_2 - L_1)}{n^2}}{\left(1 + \dfrac{n_1^2 - n^2}{n^2}L_1\right)\left(1 + \dfrac{n_2^2 - n^2}{n^2}L_2\right)} \quad (7)$$

The optical factor $(g_1 - g_2)$ contains both the intrinsic and the form anisotropy of the particle. The latter must vanish when $(g_1 - g_2)$ becomes independent of n. For large axial ratios, $L_1 = 0$ and $L_2 = 0.5$; it is easily shown that under these conditions, eq 7 is minimized when $n = n_2$, and hence the intrinsic anisotropy can be written in terms of the principal refractive indices of the particle. If the axial ratio is

$$(g_1 - g_2)_{int} = \frac{1}{4\pi}(n_1^2 - n_2^2) \quad (8)$$

less than approximately 20, the minimum in eq 7 is most easily obtained numerically using calculated values of the L_i. In either case, the form anisotropy is obtained directly as the difference between the observed anisotropy and the intrinsic contribution determined as above. The polarizability anisotropy of the particle can be obtained from $(g_1 - g_2)_{int}$ and the particle

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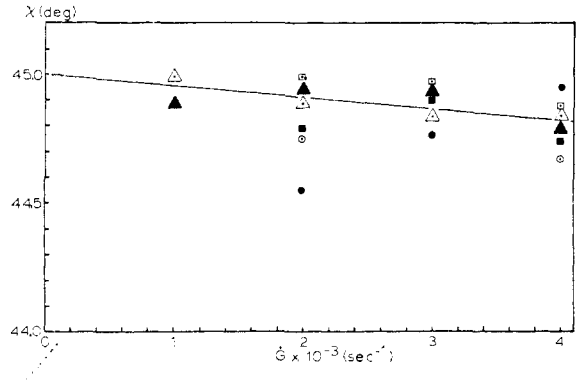

Figure 1. Extinction angle χ against shear rate G: solid curve, calculated from eq 1 assuming rotary diffusion constant $D_r = 10^6$ and axial ratio $p = 12$. Experimental points: ⊙, 48.7 μg/ml; ●, 81.2 μg/ml; ⊡, 122 μg/ml; ■, 185 μg/ml; △, 251 μg/ml; ▲, 297 μg/ml.

volume

$$(\gamma_1 - \gamma_2) = \frac{M\bar{\nu}}{N_A}(g_1 - g_2)_{int} \quad (9)$$

and if it is assumed, as appears reasonable with rigid helical DNA, that the effective monomer polarizability anisotropies α are linearly additive

$$(\alpha_{||} - \alpha_{\perp}) = \frac{M_m\bar{\nu}}{N_A}(g_1 - g_2)_{int} \quad (10)$$

where M_m is the monomer molecular weight, and the subscripts on the α are relative to the helical axis.

Since the calculation of the intrinsic anisotropy from the Peterlin and Stuart theory requires a knowledge of n_1 and n_2, an additional experimental quantity relating g_1 and g_2 is necessary. The needed relationship can be obtained from the solution refractive index increment.[18,21] Equations 5 and 11 can now be solved

$$\frac{dn}{dc} = \frac{2\pi\bar{\nu}}{3n}(g_1 + 2g_2) \quad (11)$$

simultaneously for the g_i, and the particle refractive indices can be obtained directly from eq 6 and a knowledge of the particle axial ratio.

As an alternative to the above procedure, the form anisotropy can be calculated directly and the intrinsic anisotropy obtained by difference. For this purpose, we consider an electrically isotropic ellipsoid of refractive index n_P. The ith polarizability becomes for this model, analogously to eq 6[20,22]

$$\gamma_i = \frac{\nu_P}{4\pi}\left(\frac{n_P^2 - n^2}{1 + \frac{n_P^2 - n^2}{n^2}L_i}\right) \quad (12)$$

If n_P is not too different from n, *i.e.*, in this case $[(n_P^2 - n^2)/n^2]L_i \ll 1$, then the mean polarizability becomes to this approximation

$$\langle\gamma\rangle_{av} = \frac{\gamma_1 + 2\gamma_2}{3} = \frac{\nu_P n(n_P - n)}{2\pi} + \frac{\nu_P(n_P - n)^2(L_1 + 2L_2)}{3\pi} \cong \frac{\nu_P n(n_P - n)}{2\pi} \quad (13)$$

and the anisotropy

$$(\gamma_1 - \gamma_2)_f = \frac{\nu_P}{4\pi}\left[\frac{(n_P^2 - n^2)}{n}\right]^2(L_2 - L_1) \cong \frac{\nu_P}{\pi}(n_P - n)^2(L_2 - L_1) \quad (14)$$

which reduces for high axial ratios to

$$(\gamma_1 - \gamma_2)_f \cong \frac{\nu_P}{2\pi}(n_P - n)^2 \quad (15)$$

The equivalent of eq 14 has also been given by Peterlin and Stuart.[4]

Equation 11 and the relation $\gamma = \nu_P g$ can be combined with eq 13 to give the relation

$$\frac{dn}{dc} = \bar{\nu}(n_P - n) = \frac{\bar{n} - n}{c} = \frac{\bar{\nu}(\bar{n} - n)}{\varphi}$$

where φ is the volume fraction of particles and $\bar{n}$ is the mean refractive index of the solution. Hence, the level of approximation used is that $\varphi(n_P - n) = (\bar{n} - n)$, and with this relationship, eq 15 can be restated in terms of experimental parameters.

$$(\gamma_1 - \gamma_2)_f = \frac{(\bar{n} - n)^2}{2\pi\bar{\nu}^2c^2} = \frac{M(dn/dc)^2}{2\pi N_A\bar{\nu}} \quad (16)$$

From eq 9, 10, and 16, eq 5 can now be explicitly written in terms of the intrinsic and form anisotropies of the macromolecules. Apart from the presence of

$$\frac{[n]}{[\eta]} = \frac{4\pi}{5nkT\delta}[(\gamma_1 - \gamma_2)_{int} + (\gamma_1 - \gamma_2)_f] = \frac{4\pi}{5nkT\delta}\frac{M}{M_m}\left[(\alpha_{||} - \alpha_{\perp}) + \frac{M_m(dn/dc)^2}{2\pi N_A\bar{\nu}}\right] \quad (17)$$

the parameter δ and the absence of a Lorentz factor, eq 17 is formally identical with an expression applied by Tsvetkov[23] to DNA and also used by us previously.[1,2,15] However, Tsvetkov's equation is based upon the theory of stiff but nonetheless Gaussian chains;[24] the form anisotropy strictly applies only to the local anisotropy due to the Kuhn segments, and the overall form anisotropy is neglected in this case. The above arguments, therefore, establish the quantitative relationship between the Tsvetkov approach and the theory of rigid particles which is necessary to correlate the results of the present study with investigations upon high molecular weight random coiled DNA.[1,2]

Results and Discussion

Flow birefringence data were obtained over a shear rate range of from 100 to 4000 sec^{-1} and at several concentrations from 48.7 to 297 μg/ml. Extinction angles as functions of shear rate are given in Figure 1 for the various concentrations studied. Under the present experimental conditions, these are all greater than 44°, but the scatter is sufficiently large that no sensible concentration dependence is evident. The curve of eq 1 with $D_r = 10^5$ is shown also; this fits the data reasonably well, and on this basis, we estimate $\delta = 1$ corresponding to an effective "ellipsoidal" axial ratio $p \cong 12$.[19]

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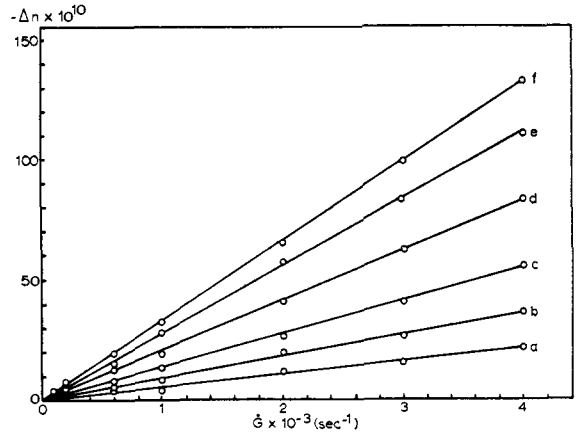

Figure 2. Experimental flow birefringence Δn against shear rate $G\cdot$ for T2 phage DNA of length ~550 Å: (a) 48.7 μg/ml; (b) 81.2 μg/ml; (c) 122 μg/ml; (d) 185 μg/ml; (e) 251 μg/ml; (f) 297 μg/ml.

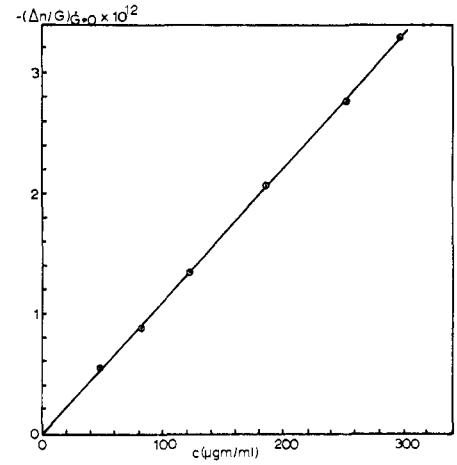

Figure 3. Shear extrapolated ratio $-(\Delta n/G\cdot)_{G\cdot\to 0}$ against concentration c in μg/ml for T2 phage DNA of length ~550 Å. The zero concentration slope $-(\Delta n/cG\cdot)_{c,G\cdot\to 0}$ is obtained as $1.1072 \pm 0.0005 \times 10^{-8}$ (cgs units) from a quadratic least-squares analysis of the data shown.

Birefringence data are shown against shear rate in Figure 2. At all concentrations, these data are for practical purposes linear and intercept properly at the origin; the low shear data and the intercept were significantly improved by evaluating the instrument correction[5,14] for each run such that the extinction angle data fall on the best monotonic curve, a procedure applicable in the present work but not employed previously.[5] The birefringence curves were fitted by least squares to a quadratic function of the form $-\Delta n = a + bG\cdot + cG\cdot^2$; the zero shear slopes b are shown against concentration in Figure 3, from which the ratio $-(\Delta n/cG\cdot)_{c,G\cdot\to 0} = 1.107 \pm 0.0005 \times 10^{-8}$ is obtained also by quadratic least squares, ignoring the individual statistical errors in the ordinate which are all quite small. Using the experimental intrinsic viscosity $[\eta] = 41.2$ cm³/g and the solvent viscosity 0.900×10^{-2} P,[5] the ratio $[n]/[\eta] = -2.986 \pm 0.0008 \times 10^{-8}$.

Frisman[25] has reported $[n]/[\eta] = -14 \times 10^{-8}$ for sonicated calf thymus DNA of intrinsic viscosity 1 dl/g. Extrapolation of the curve given for this quantity to the intrinsic viscosity of the present DNA shows roughly a factor of 4 disagreement with our results above. However, a comparison of our present and earlier[5] data to Frisman's results shows very nearly the same percentage disagreement in both cases. Tsvetkov[23] has also studied the flow birefringence of sonicated calf thymus DNA down to an estimated molecular weight of 2×10^5; an extrapolation of this curve to our experimental conditions indicates a discrepancy with our result of less than a factor of 2. We are able only to speculate on these differences; they do not appear to be due to solvent corrections since we find that these are of positive sign. On the other hand, we also find in our work instrumental corrections deriving, we believe, from residual birefringence in the optical system. In our instrument, these are usually of negative sign. Corrections for such effects are difficult to make using optical compensation in flow birefringence measurement,[16] and the above authors make no mention of attempting to do so. Based upon our experience, both the magnitudes and directions of the discrepancies can generally be accounted for in this way.

As noted above, the polydispersity of the present DNA sample was not examined and no preparative fractionation was attempted in order to avoid the apparent molecular aggregation effects observed in our previous study.[5] However, considerable evidence now exists that the size distribution in sonicated DNA is not large,[9,26] and since the degradation mechanism appears to be hydrodynamic,[27] theoretical considerations[28] predict a relatively narrow limiting molecular length range with a negligible high molecular weight tail; this type of situation has actually been observed in other polymer systems.[29]

The most sensitive test for polydispersity effects upon flow birefringence is the behavior of the extinction angle, *i.e.*, the rotational diffusion constant must be independent of shear for rigid particles. It is clear from Figure 1 that there are no serious anomalies in this respect comparable to those observed previously,[5] although the relative scatter in the present extinction angle data is quite large. A less sensitive test is the shear dependence of the optical factor $(g_1 - g_2)$. In the present work, this factor as calculated from eq 2 and 3 is sensibly independent of shear at all concentrations studied. The higher molecular weight data[5] indicate a slight shear dependence of $(g_1 - g_2)$, but it is difficult to separate the effects of polydispersity and slight molecular flexibility in this case. Although no evidence for appreciable chain flexibility was obtained in our earlier study, Cohen and Eisenberg's data[9] on DNA of about the same size as that used in our earlier work showed an effective viscosity length about 87% of the contour length from electron microscopy, and hence suggest a slight flexibility in the molecule. In any event, the above considerations lead us to believe that polydispersity effects are negligible in the present study. Of particular importance to this conclusion

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Table I[a]

	A	B	C	D
$(g_1 - g_2)_{obsd}$	−0.007892	−0.007892	−0.002570	−0.002841
n_1	1.6222	1.6203	1.6308	1.6590
n_2	1.7490	1.7511	1.7340	1.7853
Δn_{int}	−0.1268	−0.1308	−0.1032	−0.1263
$\langle n \rangle_{av}$	1.7068	1.7075	1.6996	1.7432
$(g_1 - g_2)_{int}$	−0.03408[b]	−0.03510	−0.02763	−0.03462
$(g_1 - g_2)_{form}$	0.02619	0.02721	0.02506	0.03178
$(\alpha_\parallel - \alpha_\perp)_{int}$	−17.93 Å^3	−19.46 Å^3	−16.07 Å^3	−18.21 Å^3

[a] Calculated optical parameters for DNA from the theory of Peterlin and Stuart[4] based upon the observed optical factor $(g_1 - g_2)_{obsd}$ (see text): A, present work assuming axial ratio $p = 12$; B, present work assuming axial ratio $p = \infty$; C, data of Sarquis and Harrington[5] on DNA of mol wt 3.8×10^5 assuming partial specific volume $\bar{\nu} = 0.556$ as taken in that work; D, data of Sarquis and Harrington assuming $\bar{\nu} = 0.503$ as revised by Cohen and Eisenberg[12] and used in the present study. [b] Obtained at $n = 1.7528$.

is the absence of an appreciable fraction of higher molecular weight species in exhaustively degraded sample preparations, since Sadron[30] has shown that such molecules contribute disproportionately both to the extinction angle and to the measured birefringence.

The optical factor $(g_1 - g_2)$ is calculated from eq 5 using the viscosity molecular weight and $\bar{\nu} = 0.503$ for DNA.[12] From this, the nucleotide molecular weight $M_m = 630$, and the experimental value $dn/dc = 0.172$, the particle refractive indices, absolute intrinsic birefringence, and polarizability anisotropies are obtained from eq 6–11. Results are shown in Table I for assumed axial ratios $p = 12$ and $p = \infty$; it is clear that the optical anisotropy values are relatively insensitive to axial ratio over the range considered here. However, for the case $p = 12$, eq 7 leads to a minimum in $(g_1 - g_2)$ at a solvent refractive index of 1.753, which is somewhat higher than either n_2 or $\langle n \rangle_{av}$.

The nucleotide polarizability anisotropy from eq 17 is -12.4 ± 0.1 Å^3 where the error is computed only on the basis of the statistical scatter in the birefringence and refractive index increment. This is in excellent agreement with but considerably more precise than the corresponding quantity obtained earlier.[5] However, it is only two-thirds as large numerically as the values obtained from the Peterlin and Stuart treatment above. A discrepancy of nearly the same magnitude exists if the earlier data are subjected to a similar analysis; for comparative purposes, these calculations are included also in Table I with $dn/dc = 0.188$ as determined in that work, for both $\bar{\nu} = 0.503$ and the value $\bar{\nu} = 0.556$ used previously; the corresponding nucleotide anisotropy from eq 17 is -12.8 Å^3 with $\bar{\nu} = 0.503$.

It is not clear to what extent the discrepancy in calculated nucleotide anisotropies reflects the requirement in eq 17 that the form anisotropy be small as against an inherent inability of eq 7 to partition properly the observed anisotropy into form and intrinsic components. Recent experimental data on TMV[31] and on a number of helical polypeptides[31,32] in aqueous solvents of various refractive indices suggest that the Peterlin and Stuart optical theory overestimates the form contribution, and the discrepancies between theory and experiment cannot be explained by quasi-solvent or residual instrument birefringence since the functional form of the calculated $(g_1 - g_2)$ *vs.* n curve is in all cases different from that observed experimentally. These studies were unable to discriminate unambiguously between a fundamental deficiency in the Peterlin and Stuart optical treatment and the possibility of solvent effects upon the anisotropy, however, and although Taylor and coworkers have reported improved agreement between data on poly-γ-benzyl-L-glutamate and a dipole necklace model[33] it is clear that a bound layer of highly ordered water can make a substantial contribution to the negative intrinsic birefringence.[18,31] Hence, it is not inconceivable that such a layer might undergo considerable change, both in structure and in size, over the range of glycerol concentrations spanned by these investigations.[34,35]

The nucleotide anisotropies obtained here for DNA are in remarkably close accord with available theoretical values for this molecule. Bond polarizability calculations for the DNA helix have been reported as $(\alpha_\parallel - \alpha_\perp) = -19$ Å^3 [Tsvetkov[36]], -15 Å^3 [Frisman[37]], and -14.5 Å^3 [Harrington[5]], the latter using Denbigh's bond polarizability data[38] and the helical coordinates of Langridge,[39] and weighted according to the base-pair ratio corresponding to T2 phage DNA. Such calculations are highly approximate for the heterocyclic DNA base system, however, since they necessarily neglect π-electron polarizability. Takashima[40] has reported evidence based upon SCF calculations for electron delocalization across the hydrogen bonds in the adenine–uracil base pair; the mean polarizability anisotropy is given as -24.2 Å^3 compared with -11.3 Å^3 obtained as the simple sum of adenine and uracil polarizability components, and it appears from this comparison that electron delocalization in the base planes increases the effective nucleotide anisotropy. Nevertheless our experimental nucleotide anisotropy is in semiquantitative agreement with the available theoretical estimates and this poses something of a dilemma at the present time. If the optical theory used here is in error, its deficiency is evidently closely compensated by other factors; of these the most obvious would be effects due to anisotropically bound water or to axial electron delocalization. As we have noted, other investigations seem to indicate that the optical treat-

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ment overestimates the form anisotropy, and if such is the case here, our experimental intrinsic anisotropy is numerically too large.

In aqueous solution, the DNA molecule is known to be associated with a hydrodynamically bound layer of water.[6] From the viscosity molecular weight, the contour lengths of the DNA molecules used here are 520 Å, and assuming $p = 12$ as discussed above, the effective hydrodynamic diameter is on the order of 40 Å; according to Burgers,[41] the latter dimension might be reduced by approximately 25% for an equivalent cylinder, but this is based upon the theory of highly elongated particles. This estimate of the hydrodynamic diameter is exceedingly crude, but is nevertheless comparable to that calculated by Hearst[42-44] and by us previously,[5] and corresponds roughly to a monolayer of hydrodynamically bound water. The principal attraction between water and the helix is probably charge–dipole interaction. From an exceedingly naive viewpoint, therefore, we can postulate that water molecules are aligned with their permanent dipoles normal to the helical axis. In terms of this model, a simple calculation of the hydration shell anisotropy similar to that described by Taylor and Cramer,[18] with the water molecules approximated as oblate ellipsoids, indicates that a monolayer hydration shell should be negatively birefringent and might, to an upper limit, account for all or most of the observed flow birefringence. This conclusion is, of course, exceedingly naive since among other things, the effect of the ionic atmosphere upon the hydration layer, the heterogeneous charge distribution upon the macromolecule, and the perturbation of the electrostatic field of the phosphates upon the sign and magnitude of the water anisotropy are not known. Nevertheless, if it is even qualitatively correct, the effect of an oriented hydration shell should be additive rather than compensatory to the direction of presumed theoretical imperfection. On the other hand, axial π-electron delocalization would be expected to decrease the negative anisotropy of the helix. This phenomenon has been predicted and discussed theoretically,[45-48] and experimental conductivity measurements appear to corroborate its existence.[49,50] The axial delocalization energy has only been considered comparatively recently as a possibly significant component of the base stacking energy in the stabilization free energy of the DNA helix,[47,48,51] and its effect in this respect appears to be relatively small; it seems unlikely, therefore, that axial delocalization has a large influence upon the optical anisotropy of the DNA molecule, comparable in magnitude, for example, to the discrepancies between theory and experiment reported by Taylor and coworkers,[31,33] and this conjecture is only exaggerated if bound solvent anisotropy plays a role.

On the basis of the foregoing, we believe that the present work provides some justification, at least in a semiquantitative sense, for the optical theory and associated approximations used here as applied to DNA. Furthermore, as is clear from eq 17 and the numerical values given in Table I, the effective nucleotide anisotropy depends in a relatively sensitive fashion upon both the experimental birefringence and its separation into intrinsic and form components. If the optical theory is correct in its application to the DNA molecule, therefore, we must conclude either that the solvent layer is nearly optically isotropic and axial delocalization contributes only in a minor way to the polarizability, or that these effects are of roughly comparable magnitude with respect to the optical anisotropy.

It is difficult to distinguish between these two situations on the basis of what is presently known about the hydration of DNA; an excellent review of the subject has recently been given by Tunis and Hearst.[52] The principal techniques which have been used to study DNA hydration, *i.e.*, spectroscopic, hydrodynamic, thermodynamic, X-ray structure or scattering, and diffusion experiments, are in agreement that some water is tightly bound to the macromolecule, although quantitative estimates vary rather widely according to the method. Of these techniques, nmr measurements appear to be the least ambiguous in terms of average properties of tightly bound water on the DNA molecule in solution, and these results should be of primary interest in the present case. Lubas and Wilczok,[53] using pulsed nmr, have estimated the nonrotationally bound hydration layer as 0.1 g of H_2O/g of DNA corresponding to only about 2 molecules of water per base; more recently Sprinz, *et al.*,[54] using similar methods and D_2O solutions have concluded that the rotational mobility of bound water may be much higher than previously believed, and may be reduced by as little as roughly 10^{-2} over that in the bulk solvent. These findings are entirely consistent with the present work if we assume a relatively small effect of axial electron delocalization upon the effective optical anisotropy of DNA. Although hydrodynamic, equilibrium, and scattering experiments generally associate a greater amount of water with the hydration shell,[52] there is no reason to believe that this is less labile than the layer most intimately associated with the polymer, which appears to be bound largely by the phosphate groups.[55]

The results of the present study demonstrate presumptively, although by no means unambiguously, that the classical theory of macroscopic dielectrics can account for the intrinsic optical anisotropy of DNA. With this assumption and the results of additional investigations on the DNA hydration layer, both anisotropy of bound water and electron delocalization

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along the helical axis appear to be minor in their effects upon the polarizability of this macromolecule. Although these results appear to be at variance with flow birefringence investigations on proteins and viruses in mixed solvents, these differences may be explainable in terms of anisotropic hydration and differential solvation in the latter. Since differential solvation effects may be less important in DNA,[52] it is clear that additional flow birefringence studies are required on low molecular weight DNA involving mixed solvents, and including the effects of the pH, ionic strength, and ionic size and nature. Such studies are currently in progress.

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Communications to the Editor

Rearrangements of Molecular Ions of Dialkyl-*N*-nitrosamines[1]

Sir:

We wish to report evidence for rearrangement of molecular ions of a series of dialkyl-*N*-nitrosamines. The occurrence of this rearrangement, which is analogous to the first step of the McLafferty rearrangement,[2] was suggested by the observation that the molecular ion, $R_2NNO\cdot^+$, undergoes an ion–molecule reaction (eq 1) in which a proton is transferred only when the

$$R_2NNO\cdot^+ + R_2'NNO \longrightarrow R_2'NNOH^+ + \text{products} \quad (1)$$

neutral species $R_2'NNO$ is equally as basic as, or more basic than R_2NNO. The reaction is not observed when R_2NNO is less basic than $R_2'NNO$.

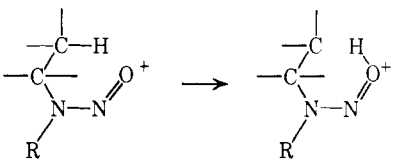
Ion cyclotron resonance (icr) techniques[3,4] were utilized to identify the occurrence of ion–molecule reactions. Observations on individual compounds and mixtures were made at a total pressure of 2×10^{-5} Torr, using a nominal ionization energy of 14 eV. In all mixtures, the single-resonance intensities of molecular ions were adjusted to an approximately 1:1 ratio.

Relative gas-phase basicities of substituted nitrosamines were established by observing proton transfer between one compound, $R_2'NNO$, and the conjugate acid of another, R_2NNOH^+ (eq 2).[5] The gas-phase

$$R_2NNOH^+ + R_2'NNO \longrightarrow R_2'NNOH^+ + R_2NNO \quad (2)$$

results, where $Me_2NNO < Et_2NNO < n\text{-}Pr_2NNO < i\text{-}Pr_2NNO \approx n\text{-}Bu_2NNO$ is the observed order of base strengths, parallel those observed in solution.[6] Proton transfer occurred only from the conjugate acid of the weaker base to the stronger for all possible pairs except $i\text{-}Pr_2NNO$ and $n\text{-}Bu_2NNO$, where the reaction was seen to proceed in either direction. $n\text{-}Bu_2NNO$ was found to be of equal base strength as $EtNH_2$. Extrapolation of the data of Haney and Franklin[7] sets an upper limit of 214 ± 3 kcal/mol for the proton affinity of $n\text{-}Bu_2NNO$.

The specificity of reaction 1 is not readily explicable on the basis that $R_2'NNO$ directly abstracts a proton bonded to a carbon atom in the alkyl chain. Such a reaction would not be expected to depend upon the relative basicities of the proton acceptor and conjugate neutral base but rather on the availability of primary, secondary, or tertiary protons. On the other hand, proton abstraction from an O–H moiety, resulting from a rearrangement of the molecular ion (eq 3) prior to proton transfer, would be a reflection of



(3)

relative basicities of the nitrosamines. Support for this argument is obtained from the observed loss of a fragment of 17 mass units in the mass spectra of ethyl-, *n*-propyl-, isopropyl, and *n*-butyl-*N*-nitrosamines; this fragment has been identified by high-resolution mass spectrometry as an OH radical. Identification of collision-induced fragmentation pathways[8] by icr double resonance indicates that *m/e* (M − 17) is coming only from the parent molecular ion. Dimethyl-*N*-nitrosamine molecular ion, which lacks the necessary hydrogen for a six-membered transition state for transfer of a hydrogen from carbon to oxygen, does not readily undergo loss of 17 mass units. However, transfer of a proton from carbon for the least basic member of the family is still feasible.

This rearrangement is analogous to a rearrangement postulated for nitropropane[9] on the basis of a M − 17 peak, but the icr evidence now suggests strongly that the loss of OH occurs from a rearranged molecular ion, not from a protonated form. The loss of OH from dialkylnitrosamines has been observed previously,[10] but its origin was previously unknown.

In the course of this study several other observations have been made. Single-resonance spectra of reaction pairs (obtained under the specified conditions) all show the ratio of $M\cdot^+$ to $(M + 1)^+$ to be greater than one for the less basic of the pair, while the protonated form

(1) This work was performed under NSF Grant No. GP 7551.
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