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Direct observation of the torsional dynamics of DNA and RNA by picosecond spectroscopy

(internal motions of nucleic acids/fluorescence depolarization/ethidium intercalation)

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ABSTRACT **Picosecond time-dependent fluorescence depolarization techniques have been used to monitor the reorientation of ethidium bromide intercalated in DNA and RNA. The fluorescence polarization anisotropy reveals a *nonexponential*, $\exp(-at^{1/2})$, torsional relaxation of the DNA double helix and provides an accurate value for its torsional rigidity, $C = 1.3 \pm 0.2 \times 10^{-19}$ erg cm. Furthermore, from accurate measurements of the limiting anisotropy at zero time, we conclude that there is an additional fast (<10 psec) internal motion that depends on the viscosity of the medium. Denatured DNA is considerably more flexible than the intact double helix, thus demonstrating the influence of secondary structure on internal motions.**

In solution, double-stranded DNA is semiflexible. It behaves like an elastic worm-like chain and can undergo a variety of conformational fluctuations, with relaxation times spanning the subnanosecond and millisecond time domains. Although the long-range segmental motions of end-over-end persistence-length rotation and coil deformation have been well characterized by dynamic light scattering and electric dichroism techniques, very little is known about the more rapid torsional motions of the DNA helix. Our objective here is to investigate the torsional dynamics of DNA and RNA by the techniques of picosecond fluorescence depolarization spectroscopy and to compare the results with the predictions of the elastic model.

Rapid internal motions in DNA are suggested by two types of experiments. In nanosecond fluorescence depolarization experiments Wahl *et al.* (1) detected a rapid (≈28 nsec) decay of the fluorescence polarization anisotropy of ethidium bromide intercalated in DNA. Presumably the dye is rigidly bound to the double-stranded DNA and thus reports local torsional motions of the helix. Recent NMR experiments (2, 3) suggest that fast conformational fluctuations of the sugar-phosphate backbone occur in both DNA and RNA and have time constants of the order of 0.3–1 nsec. Taken together, these studies indicate that considerable flexibility of the DNA helix may be expected on nanosecond and subnanosecond time scales. Unfortunately, the NMR experiments (2, 3) provide only an indirect probe of the internal motion, and the limited time resolution (≈5 nsec) of the nanosecond fluorescence depolarization experiment (1) makes it unlikely that any subnanosecond motions could have been detected.

On the theoretical side, the torsional and bending dynamics of DNA have also aroused considerable interest. In a recent paper Barkley and Zimm (4) presented an elastic continuum model of DNA. They predict that the decay of the fluorescence polarization anisotropy of an intercalated dye should be complex, containing exponentials in $t^{1/2}$ due to twisting (torsion) and in $t^{1/4}$ due to bending motions. This model is formally equivalent to the more familiar bead-spring model (except at very early times), which has been analyzed in detail by Allison and Schurr (5) and by Le Bret (6). Allison and Schurr also obtain an exponential-$t^{1/2}$ torsional relaxation at intermediate times, but predict an initial exponential decay characteristic of uncoupled motions of the individual beads at sufficiently short times (5). They also consider the possibility that the DNA helix is not uniformly elastic but contains isolated torsion joints (i.e., separated by more than one base pair). In fact, Allison and Schurr (5) find that two sets of parameters—one corresponding to a bead length of 86 base pairs with the observed relaxation falling in the initial exponential decay zone, and another corresponding to a bead length of 1 base pair with relaxation in the intermediate exponential-$t^{1/2}$ zone—can fit the *existing* fluorescence depolarization data equally well. Le Bret (6) has also discussed the torsional dynamics of DNA within the context of a bead-spring model.

Clearly, fluorescence depolarization data with subnanosecond time resolution and extremely high signal-to-noise ratio are needed to test and distinguish between the theoretical models outlined above. Our approach has been to use picosecond pulses from a mode-locked argon ion laser and a subnanosecond single photon counting apparatus to monitor the fluorescence depolarization of ethidium bromide intercalated in DNA and RNA. The apparatus combines excellent time resolution (250 psec), good signal-to-noise ratio (>100:1), high sensitivity, and freedom from systematic distortions of the data. This has enabled the direct observation of the nonexponential torsional relaxation of both DNA and RNA in solution and has allowed us to obtain accurate values for the torsional rigidity of intact and denatured DNA. Our results also indicate the presence of an initial very rapid (<10 psec), but limited, internal motion.

THEORETICAL CONSIDERATIONS

In this section we will briefly outline the relationship between time-dependent fluorescence depolarization experiments and the torsional dynamics of DNA.

The fluorescence polarization anisotropy $r(t)$ is defined by (7, 8)

$$r(t) = [i_{\parallel}(t) - i_{\perp}(t)]/[i_{\parallel}(t) + 2i_{\perp}(t)], \qquad [1]$$

in which $i_{\parallel}(t)$ and $i_{\perp}(t)$ are the fluorescence intensities polarized parallel and perpendicular, respectively, to the polarization of the exciting light. If the depolarization of the fluorescence

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of the intercalated ethidium is due solely to torsional motions of the DNA chain to which it is attached, then $r(t)$ is given by (4, 5)

$$r(t) = r_0\{\tfrac{1}{4}(3\cos^2\epsilon - 1) + \tfrac{3}{4}\sin^4\epsilon\, e^{-\Gamma} + 3\sin^2\epsilon\cos^2\epsilon\, e^{-\Gamma/4}\}, \quad [2]$$

in which r_0 is the anisotropy at time $t = 0$ (the limiting anisotropy), ϵ is the angle that the ethidium transition dipole moment makes with the helix axis, and Γ is the torsion decay function. When $\epsilon = 90°$, Eq. 2 reduces to

$$r(t) = r_0\{\tfrac{1}{4} + \tfrac{3}{4}e^{-\Gamma}\}. \quad [3]$$

The torsion decay function $\Gamma(t)$ has been derived by Barkley and Zimm (4) by using both an elastic continuum model and a bead-spring model, in which they consider a persistence length segment of the DNA helix with an ethidium at its center. This approach is justifiable only for long DNA chains. In a more recent paper, Allison and Schurr (5) have presented an exact solution of the bead-spring model for chains of arbitrary length. Nevertheless, we have chosen to adopt Barkley and Zimm's bead-spring model because we *are* interested in the torsional dynamics of long DNAs and it leads to a somewhat simpler expression for $\Gamma(t)$:

$$\Gamma(t) = 4D_1 t + 8D_b(N+1)^{-1}\sum_{k=1}^{(N+1)/2}(1 - e^{-t/\tau_{2k}})\tau_{2k}. \quad [4]$$

Here, D_1 is the diffusion coefficient for rotation of the entire chain, D_b is the diffusion coefficient for rotation of a single base pair (bead) about the helix axis, $N + 1$ is the total number of base pairs in the chain of length $2L$, and τ_j is the relaxation time of the jth normal torsional mode. The relaxation times τ_j are given (approximately) by

$$\tau_j = \lambda_j^2(4\eta b^2/\pi C), \quad [5]$$

in which $\lambda_j = 2L/j$ is the wavelength of the jth mode, b is the hydrodynamic radius of the helix of torsional rigidity C, and η is the viscosity of the solvent.

For various time intervals in the decay process, $\Gamma(t)$ may be approximated by simple functions (4, 5). At sufficiently short times ($t \ll \tau_{N+1}$, the highest frequency internal mode), the motions of the individual base pairs are uncorrelated and the anisotropy decays exponentially with a time constant $4D_b$. At intermediate times ($\tau_{N+1} < t < \tau_1$), the superposition of torsional relaxations leads to an exponential-$t^{1/2}$ decay of the anisotropy. At very long times ($t \gg \tau_1$, the lowest frequency internal mode) the anisotropy decay is also exponential, but with a time constant $4D_1$ associated with rotation of the entire chain. That is,

$$\Gamma(t) \simeq \begin{cases} 4D_b t, & t \ll \tau_{N+1} \quad [6a] \\ (2k_BT/\pi)\sqrt{t/b^2\eta C}, & \tau_{N+1} < t < \tau_1 \quad [6b] \\ 4D_1 t, & t \gg \tau_1 \quad [6c] \end{cases}$$

in which k_B is Boltzmann's constant and T is the absolute temperature. Identical results are obtained with the elastic continuum model (4), except that the initial exponential decay zone is absent.

EXPERIMENTAL

Samples. Ethidium bromide (2,7-diamino-10-ethyl-9-phenyl phenanthridinium bromide), calf thymus DNA, and total soluble yeast RNA were obtained from Sigma and used without further purification. Calf thymus DNA is approximately 10,000 base pairs long and has the familiar Watson–Crick double-helical (duplex) structure. Total yeast RNA is heterogeneous: the dominant component is ribosomal RNA (≈80%) which, itself, consists of three fragments with molecular weights of about 1×10^6, 5×10^5, and 4×10^4. Although RNA does not have a duplex structure, the single chain can fold back on itself to form short, irregularly distributed base-paired regions constituting ≈50% of the total. Samples, prepared according to Wahl *et al.* (1), contained 230 μg of DNA (or RNA) per ml and 3 μg of ethidium bromide per ml dissolved in 0.1 M Tris·HCl, pH 7.7/0.15 M NaCl. Under these conditions, essentially all of the ethidium is bound specifically to double-stranded regions of the nucleic acids; the average distance between ethidium molecules is >200 Å so that dye–dye excitation energy transfer is neglibible (1, 9). DNA–ethidium samples were denatured by heating to 100°C for 5 min and then cooling rapidly to room temperature. All fluorescence measurements were performed at room temperature (22°C).

Time-Resolved Fluorescence Measurements. Time-resolved fluorescence depolarization measurements were performed with a mode-locked argon ion laser (Spectra Physics model 171/342) and single photon counting apparatus similar to that described by Robbins *et al.* (10). A detailed description of our own apparatus will be given elsewhere. Ethidium bromide–DNA (or RNA) samples were excited with vertically polarized laser pulses (Δt = 100 psec; λ = 5145 Å) at a repetition rate of 1 MHz, and the emission was monitored by a Philips XP2020Q photomultiplier. Cutoff filters were placed in front of the photomultiplier to isolate emission at wavelengths longer than 610 nm and remove scattered laser light. The fluorescence components polarized parallel $I_\parallel(t)$ and perpendicular $I_\perp(t)$ to the polarization of the exciting light were selected by a rotatable polarizer and acquired alternately in separate halves of the memory of a multichannel analyzer for equal total analyzer live times (≈5000 sec each). The fluorescence decay law $S(t)$ could also be obtained, independent of the depolarization effects, by setting the emission polarizer at 54.7° to the direction of parallel polarization (8). The instrument response function $g(t)$, obtained by scattering the laser pulses from a dilute suspension of coffee creamer, had a half-width of 250 psec.

Data Analysis. The fluorescence polarization anisotropy $r(t)$ is defined in terms of the fluorescence intensities $i_\parallel(t)$ and $i_\perp(t)$ that would be obtained with an infinitely short duration excitation pulse (Eq. 1). Due to the finite response time of the photon counting system, the measured intensities $I_\parallel(t)$ and $I_\perp(t)$ are given by the convolution of the ideal decay functions $i_\parallel(t)$ and $i_\perp(t)$ with the instrument response function $g(t)$,

$$I_{\parallel,\perp}(t) = \int_{-\infty}^{t} g(t')i_{\parallel,\perp}(t - t')dt'. \quad [7]$$

Our task is to extract the fluorescence polarization anisotropy $r(t)$ from the experimental functions $I_\parallel(t)$, $I_\perp(t)$, and $g(t)$. For this purpose, it is convenient to introduce the linear combinations $D(t)$ and $S(t)$

$$D(t) = I_\parallel(t) - I_\perp(t),$$
$$S(t) = I_\parallel(t) + 2I_\perp(t); \quad [8]$$

which are related to corresponding ideal functions $d(t)$ and $s(t)$ by the convolution integral (Eq. 7), such that

$$d(t) = r(t).s(t). \quad [9]$$

These functions were taken to be of the form

$$d(t) = r_0[\tfrac{1}{4} + \tfrac{3}{4}\exp(-at^{1/2})].s(t)$$
$$s(t) = A\exp(-t/\tau) \quad [10]$$

in which A was a pre-exponential factor, τ was the fluorescence

lifetime of the bound dye, and a was a parameter related to the torsional rigidity (see Eq. **6b**),

$$a = 2k_BT/\pi\sqrt{b^2\eta C}\,. \qquad [11]$$

The values of the four parameters A, τ, r_0, and a were adjusted so as to *simultaneously* obtain the best fit between the experimental functions $D(t)$ and $S(t)$ and the convolution of the ideal functions $d(t)$ and $s(t)$ with the instrument response function, by using a nonlinear least-squares curve-fitting program based on Marquardt's algorithm (11). The quality of the fit was judged by the reduced chi-squared (χ_r^2) criterion.

RESULTS

Free Compared to Bound Ethidium. The fluorescence decays $S(t)$ of ethidium bromide in aqueous solution (3 μg/ml in 0.1 M Tris·HCl, pH 7.7/0.15 M NaCl) and bound to DNA and RNA were measured by setting the emission polarizer at 54.7°, as described above. All decays were well described by a single exponential decay. The lifetime of the free dye was 1.7 ± 0.2 nsec, but increased to 22.6 ± 0.2 nsec when intercalated in DNA, denatured DNA, or RNA. The dramatic increase in the fluorescence lifetime of the bound dye is accompanied by an ≈20-fold increase in fluorescence quantum yield (9) and provides a sensitive test for intercalation. Our lifetime measurement of 22.6 ± 0.2 nsec for the ethidium–DNA complex is the same within experimental error as that reported by Genest and Wahl (12).

Ethidium–DNA Complex in Saturated Sucrose. In a rigid medium, where Brownian rotational motion is suppressed, the fluorescence polarization anisotropy should reach its theoretical maximal value of 0.4. For the ethidium–DNA complex in saturated sucrose ($\eta \simeq 480$ cP) we did in fact obtain $r_0 = 0.39 \pm 0.01$, but the anisotropy decayed within a few nanoseconds to a constant (plateau) value of 0.36. Similar behavior was observed for ethidium bromide bound to RNA. A limiting anisotropy $r_0 = 0.39 \pm 0.01$ was also obtained for free ethidium in both saturated sucrose and glycerol ($\eta \simeq 1000$ cP).

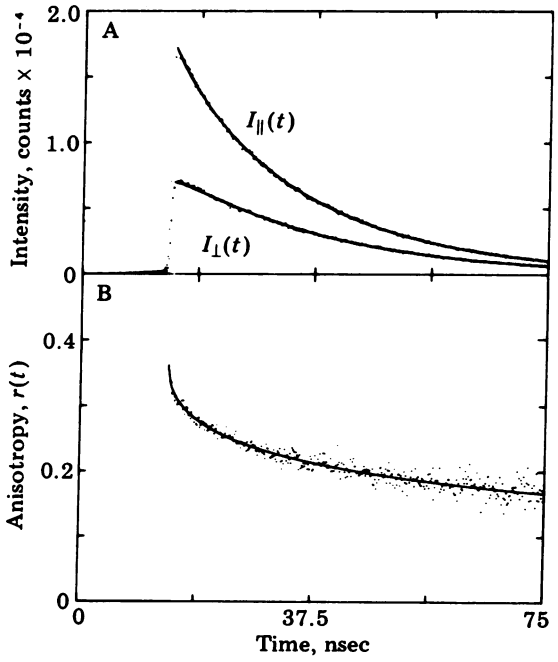

FIG. 1. (A) Experimental $I_\parallel(t)$ and $I_\perp(t)$ curves for the ethidium–DNA complex in 0.1 M Tris·HCl, pH 7.7/0.15 M NaCl. The solid lines are the best fit ($\chi_r^2 = 1.16$) of the data to Eq. **10**, with τ = 22.6 nsec, $b^2\eta C = 2.6 \times 10^{-35}$ erg^2 sec, and $r_0 = 0.36$. (*B*) Fluorescence polarization anisotropy $r(t)$ constructed from the experimental data in *A*; the solid line is calculated from the best-fit parameters given above.

Ethidium–DNA Complex in Aqueous Solution, pH 7.7. Fig. 1*A* shows the experimental $I_\parallel(t)$ and $I_\perp(t)$ curves obtained for ethidium bromide intercalated in DNA. At this time resolution (155 psec/channel) the instrument response function has a half-width of <2 channels and, therefore, the fluorescence polarization anisotropy $r(t)$ can be constructed directly according to Eq. **1**. The $r(t)$ curve (Fig. 1*B*) is of the general form expected on the basis of the bead-spring model for the intermediate exponential-$t^{1/2}$ decay zone (Eqs. **3** and **6b**), showing an initial rapid decay and tending to level off at longer times. We can, however, verify the exponential-$t^{1/2}$ torsional relaxation more directly by plotting the data of Fig. 1 in the form $\log[r(t) - 0.1]$ against $t^{1/2}$; a straight line should result. The plot (Fig. 2) is indeed linear within the experimental uncertainty. Quantitative values of the torsional rigidity C are more easily obtained via the curve-fitting procedure based on Eq. **10**. Analysis of the data shown in Fig. 1 gave τ = 22.6 nsec, $b^2\eta C = 2.6 \times 10^{-35}$ erg^2 sec, and $r_0 = 0.36$; the calculated curve is an excellent fit to the data ($\chi_r^2 = 1.16$) and provides further support for the exponential-$t^{1/2}$ decay law. The average values of the parameters computed from the results of 15 separate experiments on widely different time scales (20–300 psec/channel) were: $\tau = 22.6 \pm 0.2$ nsec, $b^2\eta C = 2.4 \pm 0.5 \times 10^{-35}$ erg^2 sec, and $r_0 = 0.36 \pm 0.01$.

Denatured DNA and RNA. Fluorescence depolarization data were also recorded for ethidium bromide bound to thermally denatured DNA and (intact) RNA. The fluorescence polarization anisotropy $r(t)$ obtained for the denatured ethidium–DNA complex was of the same form as that obtained for intact DNA (Fig. 1*B*), although the anisotropy decay was significantly more rapid ($b^2\eta C = 1.2 \pm 0.2 \times 10^{-35}$ erg^2 sec). However, with RNA the situation was quite different. Here the fluorescence polarization anisotropy $r(t)$ decays completely within ≈50 nsec, rather than tending to a constant value at long times as observed for the ethidium–DNA complex.

DISCUSSION AND CONCLUSIONS

For ethidium bromide, either free in solution or bound to DNA or RNA, we find that the limiting anisotropy $r_0 = 0.39 \pm 0.01$ is equal within the experimental uncertainty to the theoretical value of 0.4. This observation is consistent with the results of recent picosecond time-dependent fluorescence depolarization experiments (8, 13) in which values of 0.40 ± 0.02 have been obtained for a number of dye molecules in solution. Therefore, the observation of lower *apparent* initial anisotropy, which is frequently the case in steady-state and nanosecond time-resolved experiments (14), must indicate the presence of a very

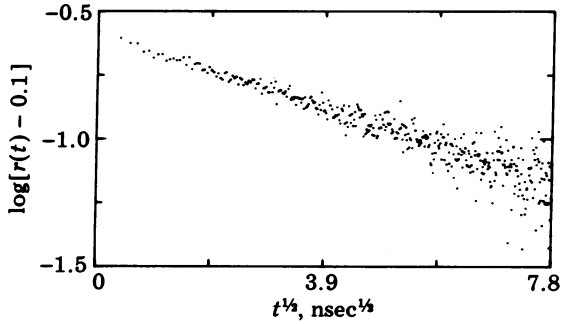

FIG. 2. Plot of the experimental data of Fig. 1*B* in the form $\log[r(t) - 0.1)$ against $t^{1/2}$. Note the linear relationship, which confirms the exponential-$t^{1/2}$ torsional relaxation of the DNA double helix.

rapid, but limited, reorientation of the transition dipole. In fact, for the ethidium–DNA complex in saturated sucrose ($\eta \simeq 480$ cP), the anisotropy decays from its initial value of 0.39 ± 0.01 to a plateau value of ≈ 0.36 within just a few nanoseconds. A rapid decay of this sort would escape detection in experiments with poorer time resolution.

If the time constant for the rapid depolarization detected in saturated sucrose is linear with viscosity, then it must be extremely rapid in aqueous solution ($\eta = 1$ cP), of the order of 10 psec or less. Such a rapid initial decay of the anisotropy, which would not be resolved by our single photon counting apparatus, may explain the apparent $r_0 = 0.36 \pm 0.01$ observed for the ethidium–DNA complex in aqueous solution at pH 7.7. The cause of the initial depolarization is uncertain, although its apparent dependence on solvent viscosity seems to rule out a purely electronic origin. Wobbling (i.e., rapid libration) of the ethidium within its intercalation site is an obvious possibility. In any case, the amplitude of this motion corresponds to a reorientation angle of about 15°.

An important finding in the present work is that the decay of the anisotropy $r(t)$ is definitely not exponential. It has, instead, an exponential-$t^{1/2}$ form characteristic of torsional relaxation in the intermediate zone (Eq. **6b**). This conclusion comes from both the linearity of the plot of $\log[r(t) - 0.1]$ against $t^{1/2}$ (Fig. 2) and the excellent agreement between the experimental and calculated decay functions (Fig. 1). On this basis we can rule out the hypothesis advanced by Allison and Schurr (5) that calf thymus DNA contains isolated torsion joints or other inhomogeneities in the torsional rigidity, since it is predicated on a fit of the nanosecond fluorescence depolarization data of Wahl *et al.* (1) to a bead-spring model for a bead length of 86 base pair with relaxation in the initial *exponential* decay zone.

Calculation of the torsional rigidity C from our values of $b^2\eta C$ requires a knowledge of the hydrodynamic radius b of the DNA helix. Estimates of b obtained from the crystallographic structure range from 12.5 to 15 Å, depending on whether or not an attached layer of water is assumed (4), and the *translational* hydrodynamic radius is reported to be 13.5 Å (6). Taking the latter value ($b = 13.5$ Å), we calculate $C = 1.3 \pm 0.2 \times 10^{-19}$ erg cm for the intact DNA double helix; the corresponding value for denatured DNA is $0.7 \pm 0.1 \times 10^{-19}$ erg cm. Another quantity related to the torsional rigidity is the equilibrium root-mean square fluctuation in the torsion angle between adjacent base pairs (4), $\langle \Delta\gamma \rangle_{rms} = (hk_BT/C)^{1/2}$, in which $h = 3.4$ Å is the distance between base pairs. For $C = 1.3 \times 10^{-19}$ erg cm, we calculate $\langle \Delta\gamma \rangle_{rms} = 5.9°$. The properties of the ethidium–DNA complex derived from the present work are summarized in Table 1.

The use of Eq. **10** to fit the experimental data is based on the implicit assumption that the transition dipole moment of the intercalated ethidium is perpendicular to the helix axis, $\epsilon = 90°$. This requires some comment in light of recent experiments (15, 16) which suggest that, in solution, this angle is actually about 70–75°. Barkley and Zimm (4) have shown by numerical calculation that the anisotropies calculated from Eq. **2** with $\epsilon = 75°$ and 90° differ by less than 1.5% over the first 100 nsec for reasonable values of $b^2\eta C$. Therefore, the use of $\epsilon = 90°$ in the curve-fitting procedure seems justified considering the simpler function that results and the negligible error introduced.

Table 1. Properties of the ethidium–DNA complex

Property	$b = 13.5$ Å
Fluorescence lifetime, τ (nsec)	22.6 ± 0.2
Limiting anisotropy, r_0	0.36 ± 0.01
Torsional rigidity, $C(10^{-19}$ erg cm)	1.3 ± 0.2
$\langle \Delta\gamma \rangle_{rms}$	5.9°

In our experiments we do not observe an initial exponential decay of the anisotropy, which is expected due to uncoupled motions of the base pairs at short times (Eq. **6a**). The decay time $1/4D_b$ and upper time limit τ_{N+1} for the initial exponential decay zone are estimated to be about 470 psec and 2 psec, respectively, for the values of b and C given above. Apparently, the exponential decay persists for only a very short time compared with its decay time and therefore the relaxation *amplitude* is too small (<1%) to be detected in our experiments. It is also pertinent to note that the exponential decay time $1/4D_b \simeq 470$ psec for rotation of an isolated base pair about the helix axis defines the fastest torsional motion that can appear within the context of the bead-spring model. Therefore, experiments with higher time resolution than the present one (≈250 psec) are not expected to provide any additional information concerning the torsional dynamics.

Recent NMR studies of DNA (2, 3, 17, 18) have shown that the internal structure is not rigid, but experiences large conformational fluctuations (±20–35°) with time constants of the order of 0.3–1 nsec. These fluctuations are thought to involve *coupled* changes in the deoxyribose-phosphate backbone conformation, sugar pucker geometry, and base plane orientation. A corresponding large amplitude relaxation with a time constant of ≈1 nsec is not observed in the fluorescence depolarization experiments, which has been taken to imply that the internal motions are not strongly coupled to the torsional modes of the helix (2, 3). However, in a recent paper Hogan and Jardetzky (19) have shown that internal motions of the DNA chain are greatly hindered when ethidium bromide is intercalated in DNA and that the effect is localized to two base pairs, one either side of the binding site. Therefore, fluorescence depolarization experiments with intercalated dyes cannot sensibly be used to probe short-wavelength phenomena, but are restricted to monitoring longer wavelength torsional motions associated with changes in helix twist over segments longer than a few base pairs.

Estimates of the torsional rigidity of DNA can be obtained from several sources. First, values in the range $1.8–4.1 \times 10^{-19}$ erg cm have been deduced by Barkley and Zimm (4) and Allison and Schurr (5) from analyses of the nanosecond fluorescence depolarization data of Wahl *et al.* (1); the large range of possible values reflects the uncertainty involved in the original experimental data and the resulting difficulty in finding a unique interpretation (5). A second estimate of C comes from classical elasticity theory, wherein the torsional rigidity is related through Poisson's ratio σ to the flexural or bending rigidity, which is in turn related to the persistence length (4). Taking a persistence length of 650 Å and a Poisson ratio $\sigma = 0.5$ characteristic of bulk polymeric materials, Barkley and Zimm calculate $C = 1.75 \times 10^{-19}$ erg cm (4). The final estimate of C comes from the supercoiling data of Depew and Wang (20) and Pulleyblank *et al.* (21). If it is assumed that the free energy for introducing superhelical twists into a nicked circular DNA comes only from torsion, then a torsional rigidity in the range $0.64–1.1 \times 10^{-19}$ erg cm is obtained. Because bending energy is ignored in this calculation, the higher value is considered to represent the best estimate of C from supercoiling data (4).

Our experimental value for the torsional rigidity of DNA, $C = 1.3 \pm 0.2 \times 10^{-19}$ erg cm, is in excellent agreement with the values estimated from supercoiling data (1.1×10^{-19} erg cm) and persistence length data (1.75×10^{-19} erg cm), although all of these values are significantly smaller than those obtained from analysis of the fluorescence depolarization data of Wahl *et al.* ($1.8–4.1 \times 10^{-19}$ erg cm).

In conclusion, the results of this work show the power of picosecond fluorescence depolarization measurements for obtaining accurate information on the internal dynamics of biological macromolecules. By using such accurate and sensitive techniques, we hope to extend the investigation to other related systems in order to determine the effects of molecular structure (primary, secondary, and tertiary) on the internal dynamics.

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