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LETTERS TO THE EDITOR

# The Dimensions of DNA in Solution

Combined measurement of the rotational and translational frictional coefficients of rod-like DNA molecules in dilute aqueous solution yields 22 to 26 Å for the hydrodynamic diameter and 3·34(±0·1) Å for the length per base-pair.

The dimensions of the *B* form DNA helix in a fiber are well known (Langridge *et al.*, 1969). The pitch is 33·6 Å, and there are 10·0 base-pairs per turn, with a rise of 3·36 Å per base-pair. However, there is increasing evidence for morphological diversity of DNA, both in the solid state (Wang *et al.*, 1979; Arnott *et al.*, 1980; Wing *et al.*, 1980) and in solution (Pohl, 1976). It is now clear that DNA structure in solution deviates slightly but appreciably from the *B* DNA model as based on fiber diffraction studies. Wang (1979) and Rhodes & Klug (1980) found that there are about 10·5 base-pairs per helical turn, and Hogan *et al.* (1978) reported evidence for propeller twisting of the base-pairs, using a technique that has subsequently reaffirmed structures with flat base-pairs in solution for both *A* and *Z* forms of DNA (Wu *et al.*, 1981).

In this context it seemed to us appropriate to examine as carefully as possible the dimensional properties of DNA in solution to determine the extent of possible further deviations from the *B* form. We used hydrodynamic techniques to accomplish this end, utilizing a two-stage strategy. First, we combined measurements of the translational and rotational frictional coefficients, allowing us to determine the hydrodynamic diameter of the DNA helix. Next, with the diameter fixed, we estimated the rise per base-pair, by extrapolation of the apparent rise per base-pair of slightly flexible molecules to zero bendability of the helix. The apparent rise per base-pair was determined from the rotational frictional coefficient, interpreted using an empirical formula generated by a study of the frictional properties of macroscopic cylinders (M. Mandelkern & D. M. Crothers, unpublished results).

The assumptions in our analysis are that rotational and translational motion are described by the same hydrodynamic dimensions, and that hydration has a negligible effect on the measured length because of the small size of the hydration layer compared to the molecular length. The molecular diameter, which is influenced by hydration and by deviations from cylindrical shape, is eliminated as an unknown parameter by measurement of both rotational and translational motion.

The DNA used in these experiments was derived from the sequenced (Sutcliffe, 1978) plasmid pBR322 by *Hae*III digestion. This enzyme, which cuts leaving flush ends, produced 22 restriction fragments (Sutcliffe, 1978) 11 of which were isolated and used in these experiments; the 184 and 192 base-pair fragments were pooled and used together, as were the 123 and 124 base-pair fragments. As illustrated in Figure 1, after preparative gel electrophoresis the fragments were quite pure.



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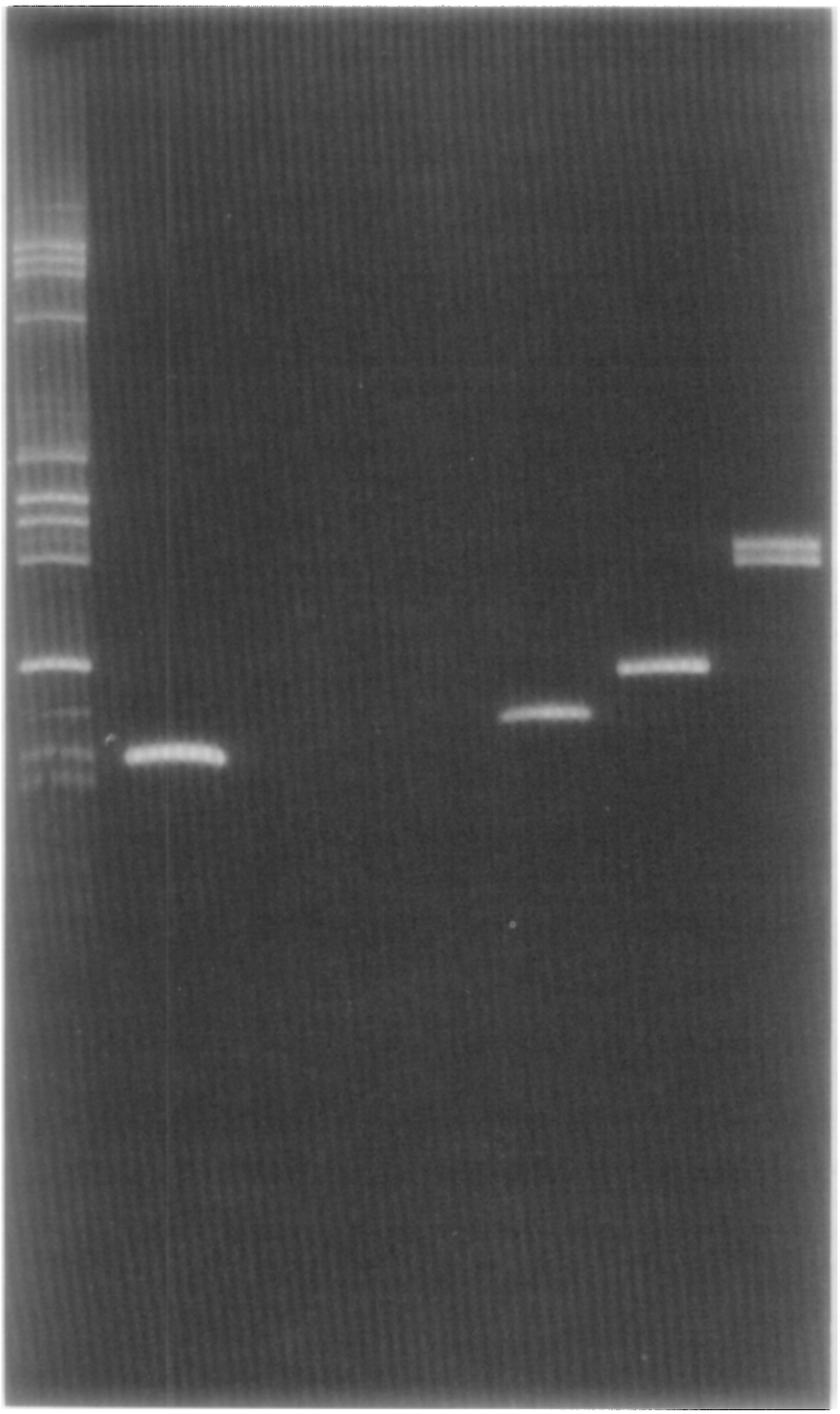

FIG. 1.

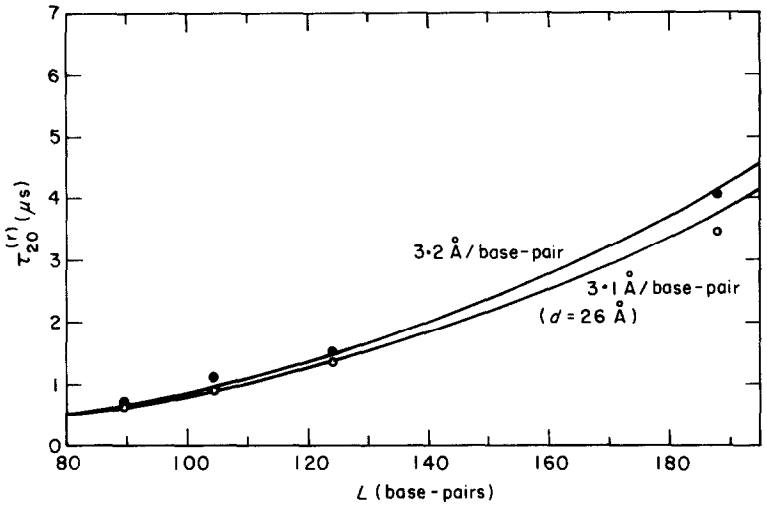


FIG. 2. Rotational relaxation time *versus* number of base-pairs. $\tau_{20}^{(r)}$ is $\tau^{rot}$ corrected to 20°C, accounting for the influence of solvent viscosity and absolute temperature on rotational diffusion. (●) Rise times, measured using electric dichroism or electric birefringence. (○) Fall times, measured using electric birefringence. The measurements were made in 1 mM-$Na^+$, at 4°C, and corrected to 20°C. Lines were calculated as described in the text.

The rotational frictional coefficient ($f^{rot}$) was measured by observing the field-free decay of birefringence after orientation in an electric field, and the field-induced rise time in birefringence or electric dichroism. The rise times were systematically about 5% longer than the fall times, independent of field at low fields. Generally, the fall time is taken as proportional to the diffusion constant, $D$ (Fredericq & Houssier, 1973):

$$\tau_{fall}^{rot} = 1/6D^{rot} \tag{1}$$

with:

$$D^{rot} = kT/f^{rot}, \tag{2}$$

where $k$ is Boltzmann constant, and $T$ is absolute tempperature. However, coupled ion-diffusion effects may be responsible for the difference between $\tau_{rise}^{rot}$ and $\tau_{fall}^{rot}$, and we prefer to consider that the true rotational diffusion constant lies between the limits set by the rise and decay times. The measurements were performed in 0·2 to 10 mM-$Na^+$. It was found that $\tau^{rot}$ decreased slightly (2 to 3%) in higher salt for the larger fragments ($N > 124$ base-pairs). The results are summarized in Figure 2.

FIG. 1. Gel electrophoresis of pBR322/*Hae*III digest, and purified fragments. From left to right: the whole digest, 89, 104, 123+124, 184+192 base-pairs. The plasmid was grown in *Escherichia coli*, and isolated as the closed circular DNA band on a CsCl/ethidium equilibrium gradient. The DNA was digested with *Hae*III restriction endonuclease (N.E. Biolabs) for 18 h at 37°C (180 units/mg DNA). The digest was run on a large, cylindrical 5% polyacrylamide gel, and the bands were cut and eluted.

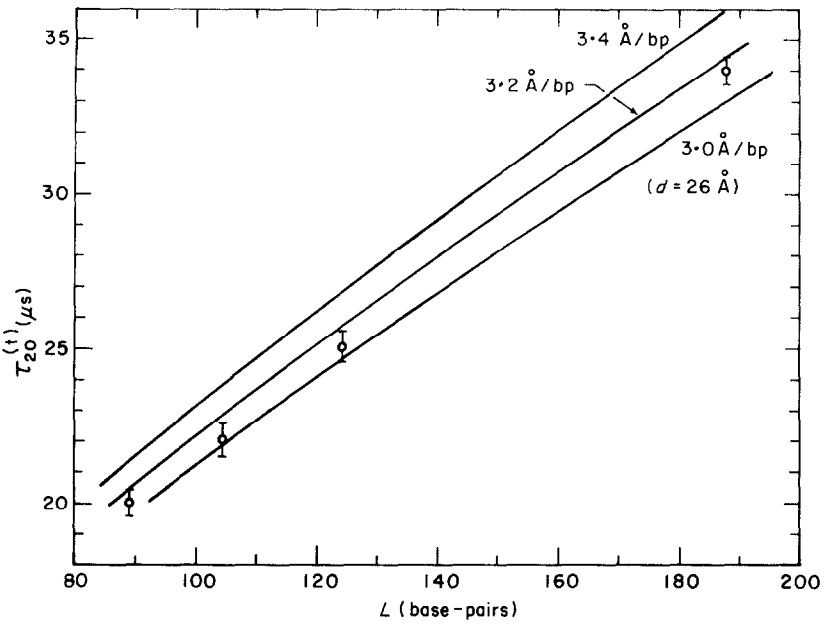


FIG. 3. $\tau_{20}^{(t)}$ ($\tau^{trans}$ at 20°C and $\theta = 90°$) *versus* number of base-pairs. Correlation times were measured at 20°C, using laser light, $\lambda = 4880$ Å. The autocorrelation function was calculated by a Malvern real-time multibit correlator. The DNA concentration was 0·5 to 1·0 mg/ml; the ionic strength was 50 mM. The samples were cleaned before measurement by the emulsification technique of Froelich *et al.* (1963). Correlation times were measured at $\theta = 90°$, 60°, 45° and 30°; the time reported here reflects the average of these measurements, expressed as the value at $\theta = 90°$. The correlation rate was a linear function of $\sin^2 \theta/2$, with intercept zero. No systematic dependence of $\tau_{20}^{(t)}$ on DNA concentration could be detected. bp, base-pairs.

The translational frictional coefficient was measured by homodyne dynamic light-scattering (Berne & Pecora, 1976). The decay time of the autocorrelation function of the scattered intensity at angle $\theta$ is proportional to the diffusion constant, $D^{trans}$:

$$\frac{1}{\tau^{trans}} = 2q^2 D^{trans}, \tag{3}$$

where:

$$q = \frac{4\pi n}{\lambda} \sin(\theta/2), \tag{4}$$

$n$ is the refractive index and $\lambda$ the wavelength. The correlation time was measured at several angles for each sample, generally at 50 mM ionic strength. Addition of NaCl to 100 mM had no effect on $\tau^{trans}$. Figure 3 summarizes the results, expressed as the correlation time at $\theta = 90°$; the observed angular dependence was as predicted by equation (3).

Figures 2 and 3 contain illustrative calculated values of $\tau^{rot}$ and $\tau^{trans}$ for different assumed values of the rise per base-pair (from 3·0 Å to 3·4 Å) and an assumed

diameter of 26 Å. These curves were calculated using the standard forms for the frictional coefficients $f^{trans}$ (Tirado & de la Torre, 1979) and $f^{rot}$ (Broersma, 1960):

$$f^{trans} = \frac{3\pi\eta L}{\ln p + \gamma_t}, \tag{5}$$

$$f^{rot} = \frac{\pi\eta L^3}{3(\ln 2p - \gamma_r)}, \tag{6}$$

where $L$ is the length of the rod, $p$ its axial ratio (length divided by diameter $d$) and $\eta$ is the solvent viscosity. The correction factors, $\gamma_t$ and $\gamma_r$, were taken from Tirado & de la Torre (1979) and Broersma (1960), respectively.

The next step in the analysis was determination of the helix diameter by combination of the rotational and translational correlation times. Several methods for accomplishing this end have been described (Chen *et al.*, 1980; Newman *et al.*, 1977; Lee *et al.*, 1981); one is illustrated in Figure 4. For different choices of the diameter, $\tau^{rot}$ and $\tau^{trans}$ are calculated theoretically as functions of length, producing the dependence of $\tau^{rot}$ on $\tau^{trans}$ shown in the Figure, calculated for $d = 22$ Å and $d = 26$ Å. The experimental values of $\tau^{rot}$ and $\tau^{trans}$ corresponding to a given fragment are plotted on the same graph. In general, the data lie between the predictions for $d = 22$ Å and $d = 26$ Å, with the latter being the best value when the field-free decay times are used. Since the fiber diffraction data give a diameter of

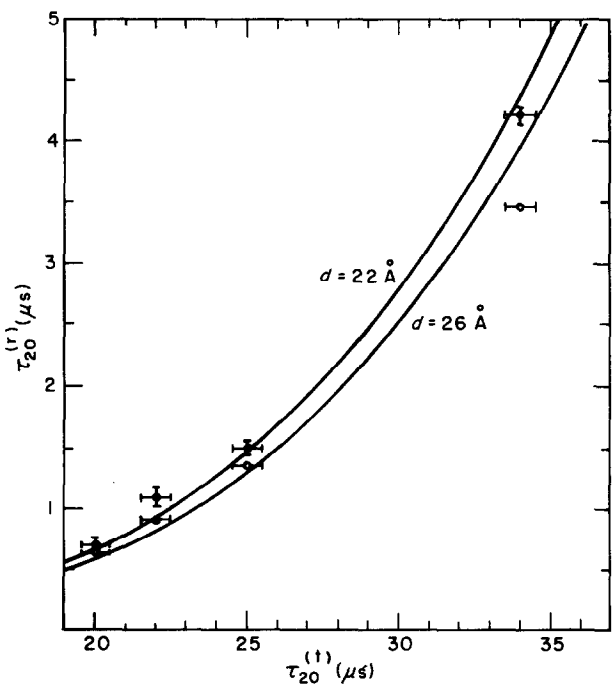


FIG. 4. $\tau_{20}^{(r)}$ *versus* $\tau_{20}^{(t)}$. The solid lines represent theoretical curves for diameters of 22 Å and 26 Å, respectively. (●) Rise times; (○) fall times.

20·4 Å (Arnott & Hukins, 1972), a range from 22 to 26 Å is reasonable for the hydrodynamic diameter, which is expected to include a hydration shell.

In summary of the results in Figures 2 to 4, we conclude that the helix hydrodynamic diameter lies between 22 Å and 26 Å, with a rise per base-pair of about 3·1 to 3·2 Å. The difference between this result and the 3·4 Å that we estimated earlier (Hogan *et al.*, 1978) is due primarily to the use of only sequenced fragments in our present work, allowing precise specification of the number of base-pairs, and also to the use of rise times in our earlier work. Because of the weak (logarithmic) dependence of $f$ on diameter, our measurements are not capable of specifying $d$ more precisely. We can, however, pursue calculation of the rise per base-pair more carefully.

There are two main potential sources of error in the analysis up to this point: the DNA molecules may retain significant flexibility, causing them to deviate from rigid rod behavior, and the hydrodynamic equations for the rod may not be sufficiently accurate. We have investigated the latter point by analyzing the frictional properties of macroscopic cylinders, with the result that (Mandelkern, 1980; M. Mandelkern & D. M. Crothers, unpublished data):

$$\gamma_r = 2{\cdot}837 - 6{\cdot}389\sigma^{-1} + 6{\cdot}514\sigma^{-2}, \quad (5 \leq p \leq 20), \tag{7}$$

where $\sigma = \ln 2p$. These values for $\gamma_r$ may be substituted into the equation:

$$\left(\frac{L}{N}\right)_{ap} = \left[\frac{\tau^{rot}}{N^3}\left(\frac{18kT[\sigma - \gamma_r]}{\pi\eta}\right)\right]^{1/3} \tag{8}$$

obtained by combining equations (1), (2) and (6) to obtain the apparent rise per base-pair $(L/N)_{ap}$ for each fragment of length $L$ containing $N$ base-pairs. We determined $(L/N)_{ap}$ for a series of fragments that were as small in size as possible, so as to approach as closely as possible the rigid rod limit. The results are presented in Table 1. A diameter of 26 Å was used since the data refer to field-free decay times (see Fig. 2). Axial ratios were estimated with a rise per base-pair of 3·3 Å (see below). For $p > 20$, we used a value of $\gamma_r = 1{\cdot}61$, close to the value given by the Broersma equation.

TABLE 1

*Apparent rise per base-pair of small DNA fragments*

| $N$ (base-pairs) | $\tau^{rot}_{fall}$ ($\mu$s) | $(L/N)_{ap}$ (Å) |
|---|---|---|
| 64 | 0·50 ± 0·02 | 3·32 |
| 80 | 0·80 ± 0·03 | 3·23 |
| 89 | 1·17 ± 0·02 | 3·35 |
| 104 | 1·59 ± 0·06 | 3·26 |
| 124 | 2·22 ± 0·03 | 3·13 |
| 188 | 5·76 ± 0·01 | 3·00 |
| 213 | 8·38 ± 0·1 | 3·05 |
| 234 | 9·22 ± 0·2 | 2·91 |
| 267 | 11·94 ± 0·2 | 2·83 |

$T$ = 276·9 K, $\eta$ = 0·0158 poise, 1 mM salt.

The results in Table 1 show a tendency for the apparent rise per base-pair to decrease when the DNA molecule contains more than about 100 base-pairs; a phenomenon doubtless due to increasing flexibility as molecular length increases. The results for fragments 104 base-pairs and smaller show no experimentally detectable systematic variation of $(L/N)_{ap}$ with length, and therefore should be close to the actual value. Their range is 3·25 to 3·35 Å, slightly higher than the 3·1 to 3·2 Å estimated in Figures 2 and 3. Part of the calculated length dependence of $(L/N)_{ap}$ is due to the use of our empirical relationship for $\gamma_r$. The $\tau^{rot}_{fall}$ value for the $N = 64$ fragment yields $(L/N)_{ap} = 3{\cdot}21$ Å using Broersma's value of $\gamma_r$, rather than 3·32 Å as given in the Table, whereas the two calculations give very similar results for the larger fragments. Hence the Broersma values of $\gamma_r$ overestimate the constancy of $(L/N)_{ap}$, and tend to mask the small but detectable influence of molecular flexibility in the size range below 150 base-pairs.

It would be desirable to extrapolate the data in Table 1 in some manner in order to obtain the true rise per base-pair $(L/N)_0$ of a perfectly stiff helix. We have used the Hearst (1963) theory for a weakly bending rod to estimate the "bendability" as a function of chain length. From that theory we use the ratio of $\tau^{rot}$ for a rigid rod to the value for a weakly bending rod, obtaining the result that is valid near the rigid rod limit:

$$(L/N)_{ap} = (L/N)_0\left[1 - \frac{\lambda n(4{\cdot}5 \ln n - 6{\cdot}2)}{9 \ln n - 2{\cdot}76}\right]. \tag{9}$$

The chain is modeled as a string of $2n+1$ touching spherical beads of diameter $a$ and spacing $b$ ($b = a$). The chain length is $L = 2nb$, and the persistence length is $b/2\lambda$.

The form of equation (9) suggests a plot of $(L/N)_{ap}$ against the bendability index $f(n) = n(4{\cdot}5 \ln n - 6{\cdot}2)/(9 \ln n - 2{\cdot}76)$. The intercept at $f(n) = 0$ gives $(L/N)_0$, and the slope $\lambda(L/N)_0$ allows estimation of the persistence length. We used $b = 26$ Å to estimate $n$ and thus calculate $f(n)$. Figure 5 shows the results. The extrapolated value $(L/N)_0$ is 3·34(±0·03) Å. Given the additional uncertainties due to lack of precision in the diameter and the choice of orientational rise or decay times, we estimate the total probable range of error to be about ±0·1 Å. The resulting rise per base-pair of 3·34(±0·1) Å is clearly within the range of values found for natural DNA in the *B* form. Hence we find no detectable variation in the length of DNA in solution from the structural parameters deduced from the study of fibers.

Another technique by which the rise per base-pair in solution can be determined is light or low-angle X-ray scattering. Eisenberg & Cohen's (1968) analysis of light-scattering data gives a value of 3·25 Å per base-pair, with X-ray scattering results varying from 2·7 to 3·25 Å per base-pair. Precision in the measurement of absolute scattering intensities, concentrations and density increments may account for the variability. Furthermore, the theory used for the analysis assumes that DNA is a rod; the problem of possible corrections due to bendability has not been studied quantitatively.

The DNA persistence length calculated from the slope in Figure 5 is 450(±50) Å, on the low side of the range of values reported for that parameter (see Kovacic &

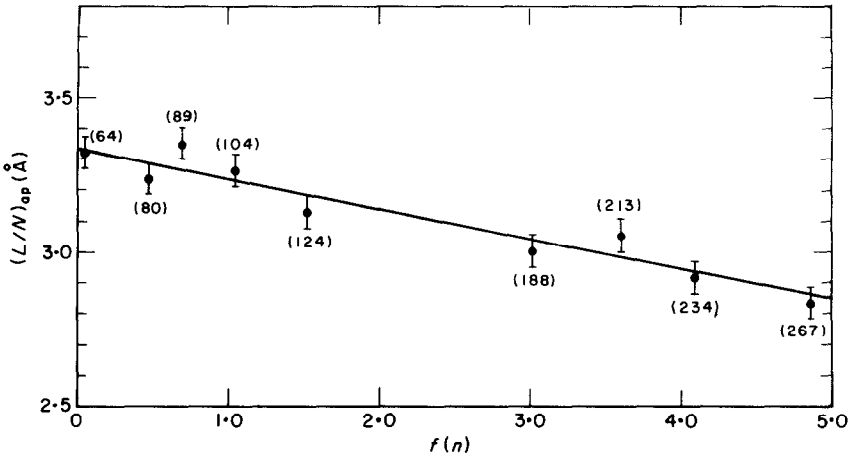


FIG. 5. Extrapolation of the apparent rise per base-pair $(L/N)_{ap}$ to zero bendability of the helix, as measured by the bendability index $f(n)$, defined in the text. Numbers indicate the size in base-pairs of each fragment. The extrapolated rise per base-pair at $f(n) = 0$ is $(L/N)_0 = 3{\cdot}34$ Å, and the persistence length is 450 Å.

van Holde, 1977; Hearst & Reese, 1980; Elias & Eden, 1981; Hagerman, 1981). (It should be realized that the exact value found for the persistence length is model- and theory-dependent, and may differ if evaluated exclusively for very short rods, as in our experiment.) Also, we note that the Hearst (1963) theory for calculation of the bendability index, $f(n)$, is approximate and breaks down when $n$ is small. Hence the points, especially for small fragments, are not positioned precisely on the $f(n)$ axis in Figure 5. However, given the scatter of the data and the other sources of error in determination of $(L/N)_0$, the weakness of the theory is relatively unimportant for the extrapolation process, except that the resulting persistence length is only approximate.

The error bars in Figure 5 correspond to an error of approximately 5% in the measurement of the orientational decay time, substantially greater than the apparent reproducibility of that measurement. The significant scatter of the values of $(L/N)_{ap}$ about the average line raises the possibility that the rise per base-pair (or possibly the persistence length) is not strictly constant, but sequence-dependent. This might be of importance for protein–DNA recognition when the protein contacts the DNA at two separate sites, since the apparently non-interacting base-pairs between the two sites would nonetheless be essential for establishing the correct distance relationship between the sites.

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