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Large-Amplitude Picosecond Anisotropy Decay of the Intrinsic Fluorescence of Double-Stranded DNA

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ABSTRACT The conformational flexibility of the DNA double helix is of great interest because of its potential role in protein recognition, packaging into chromosomes, formation of photodefects, and interaction with drugs. Theory finds that DNA is very flexible; however, there is a scarcity of experimental results that examine intrinsic properties of the DNA bases for the inherent flexibility in solution. We have studied the dynamics of poly(dA)·poly(dT) and $(dA)_{20}$·$(dT)_{20}$ in a 50 mM cacodylate, 0.1 M NaCl, pH 7 buffer by using the time-correlated picosecond fluorescence anisotropy of thymine selectively excited at 293 nm. For both nucleic acids, a large-amplitude biphasic decrease in the anisotropy is observed that has a very fast, large-amplitude component on the picosecond time scale and a slower, smaller-amplitude component on the nanosecond time scale. These modes are sensitive to sucrose concentration, and are greatly attenuated at 77% sucrose by volume. This observation suggests that motions of the bases make a significant contribution to the observed fluorescence depolarization (in the absence of sucrose). Measurements on the single-stranded systems poly(dT) and $(dT)_{20}$ reveal a much smaller amplitude of the very fast depolarization mode. These observations are consistent with a mechanism that involves concerted motions in the interior of the double-stranded systems.

INTRODUCTION

There is considerable interest in delineating the conformational flexibility of the DNA double helix, a property that is thought to play a major role in its multifunctional task. DNA flexibility is an important parameter in a number of different processes or interactions, which include the following: i) protein-DNA interactions during replication, transcription, site-specific recombination, and DNA repair, all of which require DNA to possess a good degree of deformability (Harrington and Winicov, 1994); ii) The packaging of DNA into chromosomes, which necessitates considerable DNA bendability; iii) gene regulation at a distance, in which effects exerted at one DNA site alter the properties of another site that is far removed from it (Burd et al., 1975; Hogan et al., 1979a; Sullivan et al., 1988) and for which a number of mechanisms have been proposed that require DNA to be flexible (see reviews by Ptashne, 1986, and Schleif, 1988); iv) photodimer formation, which necessitates a conformational rearrangement that allows the interacting bases to attain the optimum geometry (Becker and Wang, 1989); v) the intercalation of a number of drugs between the DNA base pairs (the mode through which they carry out their action), which requires the generation of an opening in the DNA helix.

Several theoretical studies have presented evidence for the considerable flexibility of nucleic acids (Kollman et al., 1981; Srinivasan and Olson, 1987; Prabhakaran and Harvey, 1988; Sarai et al., 1989; Zakrweska, 1992). Very recent molecular dynamics simulations have provided us with fascinating insights into the plasticity of the structure of hydrated oligonucleotides in the presence of counterions. At the end of a 140-ps simulation, the so-called Dickerson/Drew dodecamer has been reported to exhibit a large inclination of its bases relative to the perpendicular to the helix axis (Swaminathan et al., 1991; Miaskiewicz et al., 1993) as well as a propeller twist (Swaminathan et al., 1991); it was also found to have a distorted structure with two kinks and a significantly unwound central AATT region (Miaskiewicz et al., 1993). A 50-ps simulation for the decamer $(dA)_{10}$·$(dT)_{10}$ of nonalternating sequence reported a large inclination of the bases, with the bifurcated hydrogen bonds (which are characteristic of this sequence) breaking, the spine of hydration in the minor groove becoming much less defined, and the conformation exhibiting a large deviation from the initial structure (Fritsch et al., 1993). Molecular dynamics simulations also suggest the occurrence of conformational transitions between substates of DNA on picosecond to nanosecond time scales (McConnell et al., 1994); the existence of substates was also previously suggested by an energy optimization method (Poncin et al., 1992). A recent review on molecular dynamics simulations for nucleic acids has appeared (Beveridge and Ravishanker, 1994).

The hydrogen bonds formed by the bases in DNA are known to be transiently disrupted on the millisecond time scale as a result of thermal fluctuations (Leroy et al., 1988); this suggests that the DNA structure is labile. Even in the crystalline state librational motions of considerable amplitude (about ±20°) for the sugar and the bases have been reported from crystallographic measurements (Holbrook and Kim, 1984). Evidence for the dynamic character of

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DNA was also obtained by an electron microscopy study (Thèveny et al., 1988) that found variations in the persistence length with base sequence.

The three major techniques that have been utilized to monitor DNA internal dynamics are NMR (^{1}H, ^{13}C, ^{31}P), EPR (using extrinsically labeled DNA bases), and fluorescence anisotropy (mainly using intercalated dyes, especially ethidium bromide). Early NMR studies revealed the presence of large-amplitude angular motions of the bases in DNA (Hogan and Jardetzky, 1980; Bolton and James, 1980; Bendel et al., 1982). In the original analysis of the data a variety of assumptions were required, some of which may not be justified (see Alam and Drobny, 1991). Subsequent theoretical extensions to the analysis of these data (Allison et al., 1982) suggested that large base excursions were not required to interpret the data, and much smaller angular amplitudes were obtained. Work with an extrinsically labeled DNA base using EPR spectroscopy (Hustedt et al., 1993) has also found only small-amplitude motions of the DNA bases, as have ^{2}H NMR relaxation in combination with ethidium bromide fluorometry (Nuutero et al., 1994) and ^{13}C NMR relaxation (Borer et al., 1994). (In the latter study, however, an alternative solution was found in the analysis of the data for both oligonucleotides examined, which involved large-amplitude excursions of the DNA bases and had virtually the same statistical significance as the one for small-amplitude excursions.) Of necessity, however, interpretation of the NMR data requires a number of assumptions concerning local structure as well as the relaxation mechanism in the liquid state.

Time-resolved fluorescence anisotropy is an attractive method for such studies because it allows a direct measurement of the reorientation of the transition dipole moment of the emitting base with picosecond/nanosecond resolution. Because of the very low values of the fluorescence quantum yield of nucleic acids ($<\sim 10^{-4}$; see Callis, 1983, and Cadet and Vigny, 1990), several workers used dyes (mostly ethidium bromide) as extrinsic fluorescent probes. The first such study with intercalated ethidium bromide was made by Wahl et al. (1970). Subsequently, Thomas et al. (1980) carried out an investigation with picosecond time resolution and analyzed the data in terms of the rod-spring model of Allison and Schurr (1979). Further studies by Millar et al. (1980, 1982) followed, and the results were discussed in the context of the elastic model of Barkley and Zimm (1979). A comprehensive review has recently appeared (Schurr et al., 1991). However, although ethidium bromide can be used to reveal the overall motional characteristics of DNA, it is not clear that it faithfully reports the very fast base dynamics. Intercalative dye binding elongates DNA by about 2.7 Å per dye molecule (Hogan et al., 1979b), partially unwinds the helix, displaces the helix axis, renders the DNA stiffer (Sobell et al., 1977), and straightens bent DNA (Barcelo et al., 1991); also, the dye wobbles in its binding site (Magde et al., 1983), and this rapid depolarization complicates the interpretation of the observed anisotropy. Two studies have been reported in which a modified adenine was used as a fluorescent probe (Nordlund et al., 1989; Guest et al., 1991). By relocating the amino group from position 6 to position 2, the base 2-aminopurine is obtained, which is highly fluorescent (Nordlund et al., 1989). By using this approach, these studies found large-amplitude motions with subnanosecond correlation times at room temperature for two double-stranded decamers (Nordlund et al., 1989) and for several heptamers with mismatched base pairs (Guest et al., 1991).

An alternative method for monitoring internal DNA base dynamics is using the intrinsic fluorescence of DNA. A number of studies have been reported on the room-temperature steady-state fluorescence of bases, dinucleotides, single- and double-stranded polynucleotides, and DNA (see, e.g., Daniels and Hauswirth, 1971; Vigny and Duquesne, 1976; Vigny and Ballini, 1977; Morgan and Daniels, 1978; Callis, 1979; Aoki and Callis, 1982; Ballini et al., 1983; Williams et al., 1987; Daniels et al., 1988; Georghiou et al., 1990; Ge and Georghiou, 1991b; Georghiou et al., 1992, 1994; and reviews in Hauswirth and Daniels, 1976; Callis, 1983; Cadet and Vigny, 1990). The very low values of their fluorescence quantum yield ($<\sim 10^{-4}$) suggest that the decay times are short. Indeed, it has been reported that the major decay components are in the picosecond range, although small-amplitude nanosecond components are also present in the decay profiles (Georghiou et al., 1985; Rigler et al., 1985; Daniels et al., 1990).

So far there has been a complete lack of polarized intrinsic fluorescence time-resolved studies of nucleic acids. In the present work we report the results of such a study for the double-stranded nonalternating polynucleotide poly(dA)·poly(dT) and the double-stranded 20-mer $(dA)_{20}\cdot(dT)_{20}$ of this same sequence, as well as for the corresponding single-stranded systems poly(dT) and $(dT)_{20}$; excitation was at 293 nm, where thymine is selectively excited (Voet et al., 1963; Georghiou et al., 1992). For all of the nucleic acid systems investigated in the present study, a large-amplitude decay of the fluorescence anisotropy is observed on the picosecond/nanosecond time scales. We find this decay to be sensitive to sucrose concentration and to be greatly reduced at 77% sucrose by volume; this observation provides support for the notion that the motions of the bases contribute substantially to the observed depolarization of fluorescence. The results also provide evidence for a mechanism that involves the occurrence of concerted motions in the interior of double-stranded nucleic acid systems.

MATERIALS AND METHODS

The double-stranded nonalternating polynucleotide poly(dA)·poly(dT) and the double-stranded 20-mer $(dA)_{20}\cdot(dT)_{20}$ of this same sequence, as well as the corresponding single-stranded system $(dT)_{20}$ were obtained from Midland Certified Reagent Company (Midland, TX). The 20-mers were purified by high-performance liquid chromatography (HPLC). Poly(dT) was obtained from Calbiochem (La Jolla, CA). The buffer system used in all measurements was 0.05 M sodium cacodylate (Fluka, Ronkonkoma, NY), 0.1 M NaCl (Fisher, Pittsburgh, PA), pH 7, in triply distilled water.

Ultrapure sucrose from Boehringer Mannheim (Indianapolis, IN) was further purified by using activated charcoal (Sigma, St. Louis, MO).

Time-resolved measurements were performed on 150-μl samples in cuvettes with 3-mm path lengths and two adjacent sides made of black quartz. At the excitation wavelength of 293 nm, which was used for all measurements, the absorbance of the samples was about 0.05; at this wavelength, *T* is selectively excited (Voet et al., 1963; Georghiou et al., 1992). This corresponds to a concentration of about 0.3 mM in terms of DNA phosphate (Georghiou et al., 1992). This concentration is much smaller than that above which aggregation was reported to set in (Härd and Kearns, 1986; Eimer et al., 1990). A 1-ps, 293-nm excitation beam was generated from a Coherent (Palo Alto, CA) 702 dye laser, synchronously pumped by a Nd:YAG laser (Coherent Antares). The emission signal was passed through a Glan-Thompson Polarizer, 340 nm cut-off filter, and through a SPEX 0.22-m monochromator set at 360 nm (5 nm bandpass) to be detected by a 6 μ microchannel plate detector (Hamamatsu R2809U-01) operating in single photon-counting, time-correlated mode. Some experiments were repeated at 345 nm with essentially identical results. The detection electronics comprised a Phillips Scientific 2.5 GHz amplifier (Mahwah, NJ), a Tennelec (Oak Ridge, TN) constant fraction discriminator (TC455) and a time-to-amplitude converter (TC862), and a Nucleus (Oak Ridge, TN) pulse-height analysis analog-to-digital converter (MIS3–8K). The stop pulse was obtained from an Antel (Burlington, ON, Canada) fast photodiode and constant fraction discrimination (TC455) channel. Typical impulse response functions taken on triply distilled water with the emission monochomator set at 305 nm had a full width at half-maximum of about 60 ps. During acquisitions the emission polarizer was rotated from vertical to horizontal every 30 s (ISS Koalo unit, Urbana, IL). For vertically polarized exciting light, the fluorescence anisotropy $r(t)$ is defined as

$$r(t) = \frac{I_V(t) - gI_H(t)}{I_V(t) + 2gI_H(t)}, \tag{1}$$

where $I_V(t)$ and $I_H(t)$ are the vertically and horizontally polarized fluorescence components, respectively, and g is a correction factor that is close to unity. Data sets consisted of signal averaged data and simultaneously collected buffer backgrounds from 30 to 120 30-second acquisitions of vertically or horizontally polarized emissions. Typical data acquisition times were approximately 45 min per sample. The contribution of the buffer to the observed signal was approximately 2% for the solutions in buffer and as large as 10% for the 77% sucrose by volume. In these measurements a minimum of three to seven independently prepared samples were examined in each case. Instrumental g-factor (polarization bias) was determined by rotating the excitation beam to the horizontal position and acquiring signal averaged data for the same time period as that utilized for sample data acquisition. g-Factors were obtained after every sample measurement and were found to be very consistent and close to unity (a single average g-factor was utilized for data sets collected within a single experimental session). Fluorescence lifetimes and correlation times were obtained by simultaneous fitting of the vertical and horizontal emission decay curves from each sample using the Globals Unlimited (Urbana, IL) software package (Beechem et al., 1991) and a nonassociative anisotropy model.

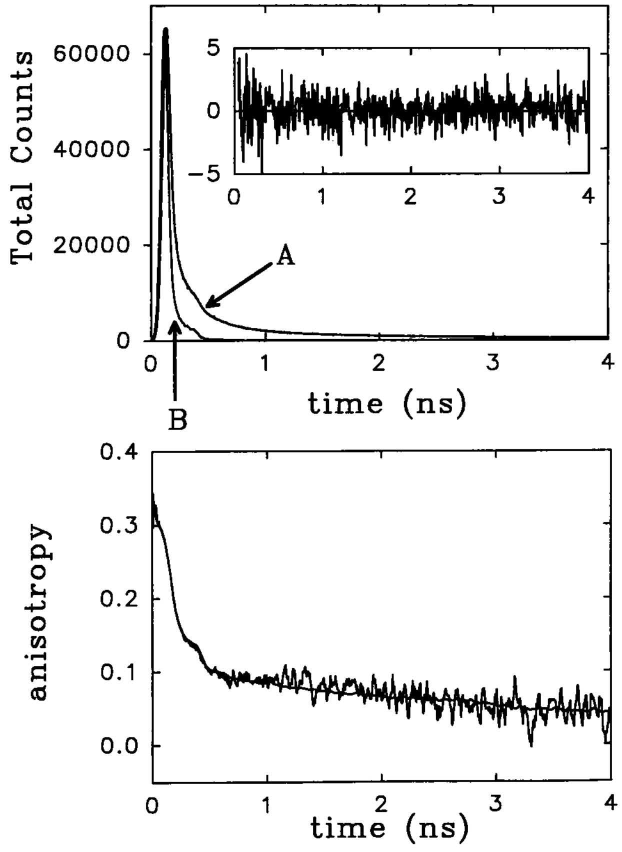

FIGURE 1 Time-resolved fluorescence measurements of the total decay and anisotropy for the nonalternating polynucleotide poly(dA)·poly(dT) at 20°C. Excitation was at 293 nm, where *T* is selectively excited. (*Top*) *B*, exciting laser pulse; *A*, total fluorescence intensity $I_V(t) + 2I_H(t)$, where $I_V(t)$ and $I_H(t)$ are the vertical and horizontal fluorescence components, respectively, for vertically polarized exciting light. (*Inset*) Residuals for the nonlinear least-squares fit. (*Bottom*) Fluorescence anisotropy. For all measurements carried out in the present study, the buffer consisted of 50 mM sodium cacodylate, 100 mM NaCl, pH 7, in triply distilled water.

RESULTS

Results for poly(dA)·poly(dT)

We have chosen the nonalternating polynucleotide poly(dA)·poly(dT) for these studies because we can selectively excite its T bases at 293 nm, where A does not absorb (Voet et al., 1963; Georghiou et al., 1992). Fig. 1 (*top*) shows a plot of the total fluorescence intensity as a function of time, for excitation with vertically polarized light, in buffer at 20°C. Fig. 1 (*bottom*) shows the corresponding fluorescence anisotropy profile (which is convolved with the exciting light pulse). It is seen that the anisotropy exhibits a large drop with time over the time scale of 4 ns. As a first approximation, a multiexponential analysis of the data was performed. To obtain a random distribution of the residuals (differences between the experimental data and the computer fit), four decay times and two correlation times were required. All four lifetime terms were clearly distinct when examined using rigorous error analysis methodologies (Beechem et al., 1991). The overall recovered values of χ^2 were typically 1.0–1.25. For all analyses, 95–99% of the amplitude of the time-resolved fluorescence emission was associated with a very short decay time of 5–20 ps. The remaining 1–5% of the time-resolved amplitudes had decay times of approximately 200 ps, 1 ns, and 4 ns. Two rotational correlation times were always required, with approximately 70% being associated with a very fast depolarization (correlation time < 25–50 ps) and 30% being associated with a larger correlation time (2–4 ns). Analysis of the data shown in Fig. 1 recovered the following parameters for the fluorescence decay amplitudes α, the decay times τ, the anisotropy amplitudes β, and the correlation times ϕ: $\alpha_1 = 95.6\%$, $\tau_1 \approx 15$ ps,

$\alpha_2 = 3.6\%$, $\tau_2 = 200$ ps, $\alpha_3 = 0.6\%$, $\tau_3 = 0.9$ ns, $\alpha_4 = 0.2\%$, $\tau_4 = 4.3$ ns, $\beta_1 = 0.255$, $\phi_1 \approx 25$ ps, $\beta_2 = 0.10$, $\phi_2 = 3.7$ ns.

Although from the observed large drop in the anisotropy (Fig. 1, *bottom*) it is unequivocal that the emission oscillator of the thymine in the double-stranded DNA has rotated significantly during the time period of 4 ns, the observed rapid depolarization could have multiple origins: a) rapid internal base motions, b) Förster's electronic energy transfer and exciton interactions between adjacent bases, c) excited-state complex (excimer) formation, or d) fluorescent impurities. Possibilities b) and c) are considered below in the Discussion, where evidence is presented which suggests that these mechanisms are not the cause of the observed depolarization. Possibility d) was examined in two ways. Previous work from one of our laboratories has shown that the steady-state excitation spectrum for the polynucleotide poly(dA)·poly(dT) was successfully analyzed in terms of the absorption spectra of the A and T bases (Georghiou et al., 1992). In addition, as is shown in the present study (see below), the time-resolved fluorescence characteristics (both total intensity and anisotropy) for an HPLC-purified synthetic $(dA)_{20}\cdot(dT)_{20}$ have yielded results that exhibit trends very similar to those for the polynucleotide. The polynucleotide is produced enzymatically, whereas the synthetic 20-mer is produced using solid-phase synthesis, and therefore one would not expect the two preparations to have identical impurities.

To investigate rapid internal base motions as the origin of the observed fluorescence depolarization, experiments were performed as a function of viscosity and temperature. Fig. 2 shows plots of the anisotropy of poly(dA)·poly(dT) as a function of time at 20°C for the following concentrations of sucrose (in terms of grams in the total solution volume): 0%, 60%, and 77%. The corresponding viscosities are approximately 1, 14, and 58 cP (Barber, 1966). The decay of the anisotropy becomes progressively slower as more sucrose is added. The effect is very pronounced for 77% sucrose, where the decay of the fluorescence anisotropy is greatly attenuated; this attenuation is seen to be dramatic for 77% sucrose at 5°C, for which the viscosity is equal to about 149 cP (Barber, 1966).

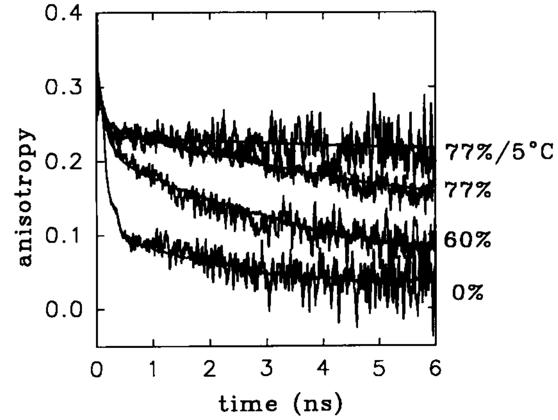

FIGURE 2 Fluorescence anisotropy for poly(dA)·poly(dT) in the absence and in the presence of sucrose expressed as grams of sucrose per total solution volume. The lower three curves are for measurements made at 20°C.

Results for the 20-mer $(dA)_{20}\cdot(dT)_{20}$

The experiments described so far were carried out on samples of the polynucleotide poly(dA)·poly(dT), for which the average number of base pairs is about 2500. We have supplemented them with experiments on the double-stranded 20-mer $(dA)_{20}\cdot(dT)_{20}$, which has the same base sequence as the polynucleotide. (The measured melting temperature, determined spectrophotometrically, is 45°C for this 20-mer, as compared to 70°C for the polynucleotide.)

The results for the 20-mer at 20°C in the absence of sucrose are shown in Fig. 3. It is seen that the anisotropy decay for the 20-mer, although similar in overall shape to that for the polynucleotide, has a larger percentage of the extremely rapid depolarization (90% versus 70%). We should note that global rotational diffusion of the 20-mer is much slower than the observed fluorescence depolarization. (Such depolarization is negligible for the polynucleotide.) A dynamic light scattering study (Eimer and Pecora, 1991) has reported a value of about 16 ns for the rotational correlation time of a double-stranded 20-mer. The spin correlation time for this 20-mer is calculated from the theory of Tirado and Garcia de la Torre (1980) to be about 4 ns. These values are much larger than the correlation times obtained from a global nonlinear least-squares fit to the data for the 20-mer $(dA)_{20}\cdot(dT)_{20}$ (Fig. 3): for the major component, about 90%, $\phi_1 \approx 130$ ps, and for the minor component, $\phi_2 \approx 0.8$ ns. These considerations suggest that the observed fluorescence depolarization (Fig. 3) does not stem from rotational or spinning motions.

Fig. 3 also shows a plot of the viscosity dependence of the observed fluorescence depolarization for the double-stranded 20-mer at the following concentrations of sucrose

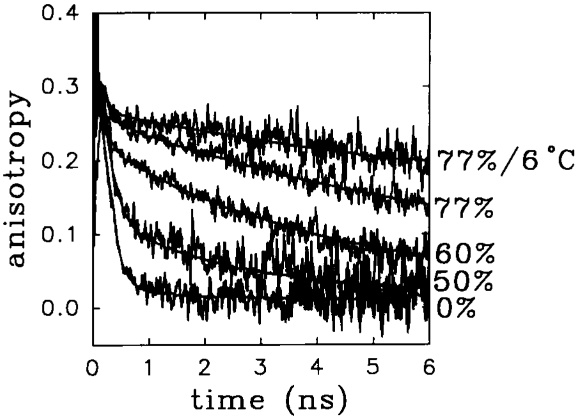

FIGURE 3 Fluorescence anisotropy for $(dA)_{20}\cdot(dT)_{20}$ in the absence and in the presence of sucrose expressed as grams of sucrose per total solution volume. The lower four curves are for measurements made at 20°C.

at 20°C: 0%, 50%, 60%, and 77%. The corresponding viscosities are approximately 1, 7.4, 14, and 58 cP; also shown is a plot for 77% sucrose at 6°C for which the viscosity is 139 cP. In this case also, the fluorescence anisotropy is seen from Fig. 3 to respond to sucrose concentration in a manner very similar to that for the polynucleotide (Fig. 2). These data suggest that the observed dynamics for both DNAs can be modulated by the solution viscosity. It should be emphasized that in both samples there is a fast component to the depolarization that cannot be resolved by our current instrumentation ($\phi < 50$ ps). The amplitude associated with the short (unresolved) correlation time decreases with increasing sucrose concentration, whereas the amplitude for the longer correlation time increases (see Fig. 4). Most of the amplitude change is complete by approximately 14 cP.

Next we proceeded to vary the temperature of poly(dA)·poly(dT) samples in sucrose solution. Our aim was to compare the anisotropy profiles for a combination of temperatures and amounts of sucrose added for which the viscosities η are nearly identical. Fig. 5 (*inset*) shows such a comparison between a 60% sucrose solution at 20°C and a 77% sucrose solution at 51°C. The corresponding values of η are 14.0 and 14.3 cP (Barber, 1966). The two profiles are seen to be very similar. These results provide additional support with regard to the importance of the role that solution viscosity plays in determining the rate of decay of the fluorescence anisotropy.

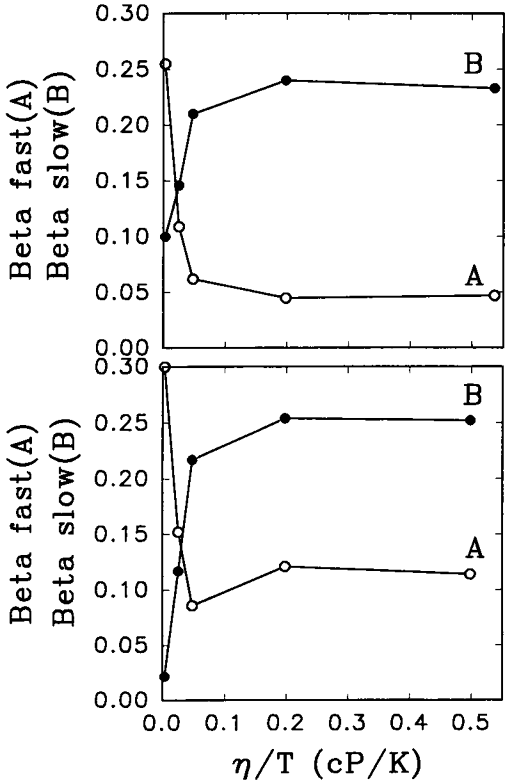

FIGURE 4 Amplitudes for the fluorescence anisotropy expressed in the form of a sum of two exponentials as a function of η/T, where η is the viscosity of the solution and T is the absolute temperature. A and B denote the amplitudes for the fast and slow components, respectively. (*Top*) Poly(dA)·poly(dT). (*Bottom*) $(dA)_{20}\cdot(dT)_{20}$. (All of the data were obtained at room temperature, except those obtained at 5°C and 6°C for the top and bottom graphs, respectively.)

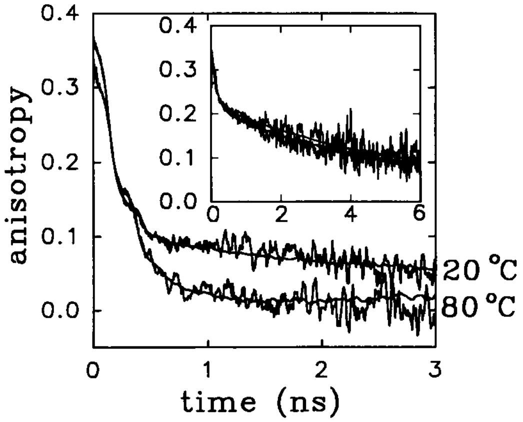

FIGURE 5 Comparison of the fluorescence anisotropy for poly(dA)·poly(dT) at 20°C with that at 80°C, which is 10°C above the melting temperature of the polynucleotide. (*Inset*) Comparison of the fluorescence anisotropy for poly(dA)·poly(dT) in 77% sucrose at 51°C (*upper trace*) and in 60% sucrose at 20°C (*lower trace*). For the former, the solution viscosity was 14.3 cP; for the latter, the solution viscosity was 14.0 cP. The time scale extends to 6 ns.

Time-resolved measurements were also performed above the melting temperature of poly(dA)·poly(dT). Fig. 5 shows a large increase in the rate of the decay of the fluorescence anisotropy for this polynucleotide upon going from 20°C to 80°C; this behavior is expected because this temperature is 10°C higher than the melting temperature of 70°C that we have measured spectrophotometrically for this polynucleotide. Upon melting the 20-mer $(dA)_{20}\cdot(dT)_{20}$ (at 55°C, which is 10°C above its melting temperature) we have found the change in the anisotropy to be much smaller than that for the polynucleotide (data not shown); this is consistent with the fact that the 20-mer at 20°C has a large amplitude of fast depolarization (Fig. 4, *bottom*).

Comparison of the results for double-stranded nucleic acid systems with those for single-stranded systems

We have also made a comparison of the fluorescence anisotropy for the single-stranded polynucleotide poly(dT) and the single-stranded 20-mer $(dT)_{20}$ with those for the double-stranded systems poly(dA)·poly(dT) and $(dA)_{20}\cdot(dT)_{20}$. The results in the absence of sucrose are shown in Fig. 6. The anisotropy profile for poly(dT) is seen to decay more slowly than that for poly(dA)·poly(dT). Also, the anisotropy profile for $(dT)_{20}$ is seen to decay more slowly than that for $(dA)_{20}\cdot(dT)_{20}$ (see *inset*). For poly(dT) in the absence of sucrose, we find a component with a correlation time $\phi_1 \approx 80$ ps, which makes a contribution of about 40%, and another component with a correlation time

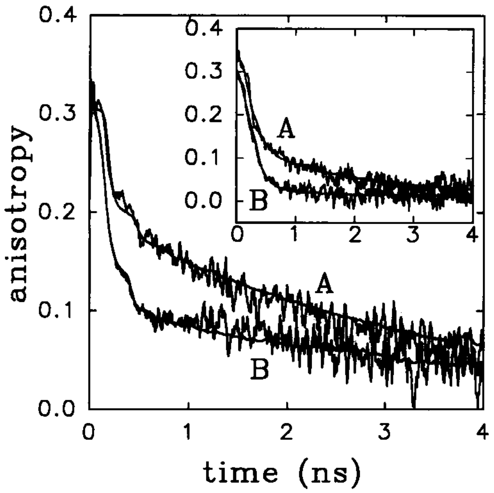

FIGURE 6 Comparison of the fluorescence anisotropy at 20°C for (*A*) poly(dT) with that for (*B*) poly(dA)·poly(dT). (*Inset*) Comparison of the fluorescence anisotropy at 20°C for (*A*) $(dT)_{20}$ with that for (*B*) $(dA)_{20}\cdot(dT)_{20}$.

$\phi_2 \approx 3.5$ ns. These values compare with those of $\phi_1 \approx 25$ ps (which makes a contribution of about 70%) and $\phi_2 \approx 3.7$ ns for poly(dA)·poly(dT). For $(dT)_{20}$, the results are $\phi_1 \approx 150$ ps (contribution about 60%) and $\phi_2 \approx 2.0$ ns. These compare with $\phi_1 \approx 130$ ps (contribution about 90%) and $\phi_2 \approx 0.8$ ns for $(dA)_{20}\cdot(dT)_{20}$. These results of the analysis reveal that the primary origin of the faster decrease in the double-stranded depolarization is due to a larger amplitude (1.5 to 1.8 times that of the single-stranded systems) associated with the fast motion. It should be noted that any contribution made by rotational or spinning motions to the fluorescence depolarization for $(dT)_{20}$ would have the effect of further accentuating the observed difference between the rate at which its anisotropy decays and that for $(dA)_{20}\cdot(dT)_{20}$; this is so because, as was noted above, such motions do not contribute significantly to the fluorescence depolarization of the double-stranded 20-mer. In this regard, rotational motion may be particularly important for $(dT)_{20}$, for in general the molecular volume of single-stranded nucleic acid systems is smaller than that of the corresponding double-stranded systems (Watson et al., 1987). We should note that poly(dT) was previously reported (Vigny and Ballini, 1977) to form photoproducts for excitation at 248 nm, which are manifested by a fluorescence band that grows with the time of irradiation and has a maximum at ~400 nm. We have confirmed this for both poly(dT) and $(dT)_{20}$ for excitation at 265 nm (Georghiou et al., 1994). However, we have looked for such a band in the fluorescence spectra of these single-stranded nucleic acids for the excitation wavelength (293 nm) that we have employed in the present time-resolved study, with negative results (Georghiou et al., 1994).

We have also sought to make further comparison between the rates of the decay of the anisotropy for double- and single-stranded oligomers by using a modified adenine as a fluorescent probe. To this end, we synthesized a) a double-stranded 20-mer that had the same base sequence as $(dA)_{20}\cdot(dT)_{20}$ except at position 10, where the adenine (which is 6-aminopurine) was replaced by 2-aminopurine (2-AP), and b) a single-stranded 20-mer that had the same base sequence as $(dA)_{20}$ except at position 10, where the adenine was replaced by 2-AP. 2-AP in the double-stranded 20-mer still forms two hydrogen bonds in the helix, but one of them is in the minor groove, absorbs at longer wavelengths than A or T, and is highly fluorescent (Nordlund et al., 1989). In the present experiments we selectively excited 2-AP at 310 nm. We observed qualitatively the same trend as that shown in Fig. 6, in which the intrinsic fluorescence of T was monitored, i.e., the anisotropy of the single-stranded 20-mer decays more slowly than that of the double-stranded 20-mer (data not shown). This probe also reveals large-amplitude depolarizing motions similar to what is observed with the intrinsic thymine fluorescence depolarization. We should add that two reports used 2-AP to study the dynamics of two double-stranded decamers and some heptamers with mismatched base pairs (Nordlund et al., 1989; Guest et al., 1991). These studies also reported large-amplitude motions with subnanosecond correlation times at room temperature.

We have found the decay of the anisotropy for $(dT)_{20}$ to become slower as the concentration of sucrose added to the sample is increased (Fig. 7). The anisotropy for poly(dT) has been found to exhibit a similar dependence on the amount of sucrose added (data not shown). This behavior has been found to be qualitatively similar to that exhibited by the double-stranded systems $(dA)_{20}\cdot(dT)_{20}$ (Fig. 3) and poly(dA)·poly(dT) (Fig. 2).

Fig. 8 shows the recovered long correlation times for $(dA)_{20}\cdot(dT)_{20}$ and $(dT)_{20}$ as a function of η/T, where η is the solvent viscosity and T the absolute temperature. Fig. 9 shows a similar plot for poly(dA)·poly(dT) and poly(dT). It is seen that in both cases the slopes for the single-stranded systems, which are found to be similar, are much larger than

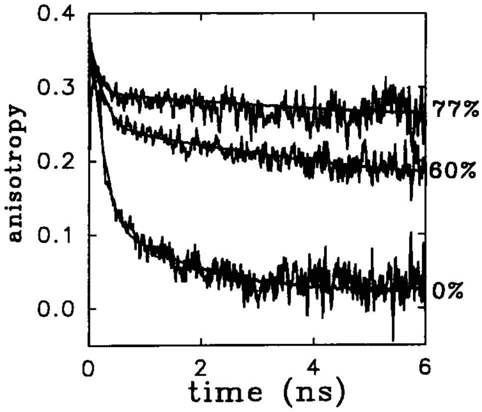

FIGURE 7 Fluorescence anisotropy for $(dT)_{20}$ at 20°C in the absence and in the presence of sucrose expressed as grams of sucrose per total solution volume.

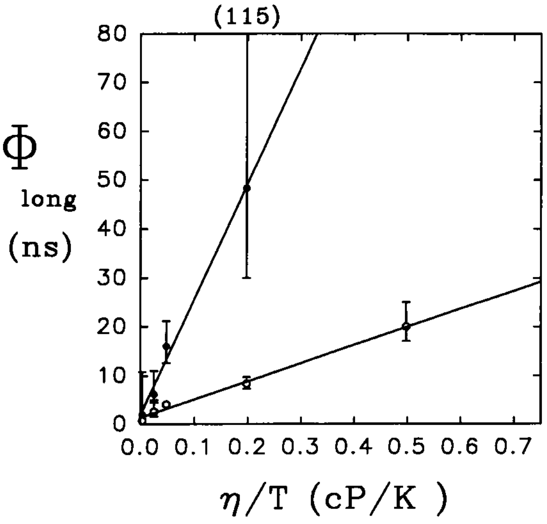

FIGURE 8 Plot of the long rotational correlation time as a function of η/T, where η is the solution viscosity and T is the absolute temperature. ●, $(dT)_{20}$; ○, $(dA)_{20}\cdot(dT)_{20}$. The range of the error bars for each rotational correlation time is that which was determined using the rigorous nonlinear error estimation described by Beechem et al. (1991). (All of the data were obtained at room temperature, except those obtained at 6°C for $(dA)_{20}\cdot(dT)_{20}$.)

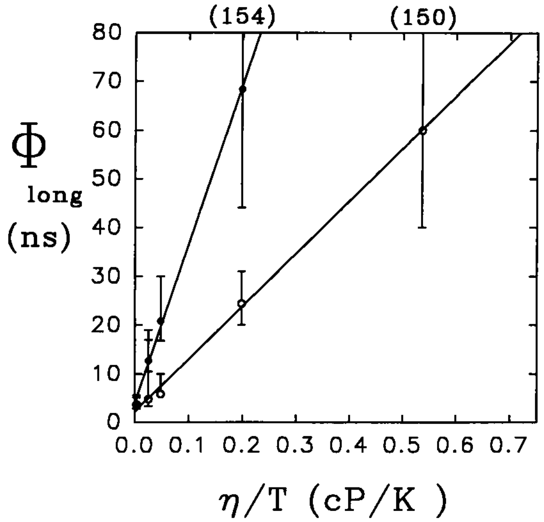

FIGURE 9 Plot of the long rotational correlation time as a function of η/T, where η is the solution viscosity and T is the absolute temperature. ●, Poly(dT); ○, poly(dA)·poly(dT). The range of the error bars for each rotational correlation time is that which was determined using the rigorous nonlinear error estimation described by Beechem et al. (1991). (All of the data were obtained at room temperature, except those obtained at 5°C for poly(dA)·poly(dT).)

those for the double-stranded systems. In terms of the motional origin of the observed fluorescence depolarization, this suggests that, as would be expected, the motions of the single-stranded systems are more strongly coupled to the solvent than are those of the double-stranded systems.

DISCUSSION

Before we discuss the nature of the motions in the double-stranded nucleic acids poly(dA)·poly(dT) and $(dA)_{20}\cdot(dT)_{20}$, which are suggested by the fluorescence anisotropy measurements, it is instructive to examine i) the nature of the emitting species and ii) other potential interpretations of the observed large-amplitude decay of the anisotropy (in the absence of sucrose; Figs. 1 and 3).

Nature of the emitting species

The fluorescence decay profiles contain a number of components; we have extracted four, ranging from tens of picoseconds to several nanoseconds. Most probably there is a distribution of decay times. In this regard, ongoing research (Gerke and Georghiou, to be published) finds that the fluorescence of free thymidine is modulated by both the fluidity and the polarity of its environment. These results, taken together, suggest that the distribution of decay times of T in the polymers is at least in part a consequence of motions of the bases, which give rise to a population of stacked geometries of varying rigidity and degree of exposure to water. The observed nanosecond components have very small amplitudes but should make a large contribution to steady-state fluorescence. That these components stem from T emission is suggested by the successful analysis of the steady-state fluorescence excitation spectrum of poly(dA)·poly(dT) (for which T is the sole emitter) in terms of the absorption spectra of the individual bases A and T (Georghiou et al., 1992): these absorption spectra were found to be very similar in shape and in peak wavelength to those of the free A and T bases (with the spectrum of T considerably shifted to longer wavelengths relative to that of A). This finding is in agreement with the general observation that the absorption spectra of double-stranded nucleic acids are very similar to those of the corresponding equimolar mixtures of bases (Gueron et al., 1974; Cantor and Schimmel, 1980).

Significantly, it was observed (Georghiou et al., 1992) that the steady-state fluorescence anisotropy for the T bases of poly(dA)·poly(dT), as well as their fluorescence spectrum and their intrinsic fluorescence quantum yield, are independent of the excitation wavelength. Consequently, selective excitation of T at 293 nm (as has been done in the present study) does not probe a subpopulation of thymines that have unusual photophysical properties.

The fluorescence anisotropy profiles in buffer have been found in the present study to be biphasic and to consist of a) a very fast large-amplitude component on the picosecond time scale (about 70% and 90% for poly(dA)·poly(dT) and $(dA)_{20}\cdot(dT)_{20}$, respectively) and b) a slower component with corresponding correlation times of 3.7 and 0.8 ns. Both the slow and the fast components are sensitive to the viscosity η of the aqueous environment, which was controlled through the addition of sucrose. For 77% sucrose by volume, both modes of fluorescence depolarization are greatly attenuated (Figs. 2 and 3); in fact, the fluorescence anisotropy decay of poly(dA)·poly(dT) (Fig. 2) (and, to a lesser extent, that of $(dA)_{20}\cdot(dT)_{20}$; Fig. 3) exhibits a dramatic reduction upon going from room temperature ($\eta \approx 1$ cP) to 5°C ($\eta \approx 149$ cP). Furthermore, as is seen from Fig. 5 (*inset*), two solutions of poly(dA)·poly(dT), for which the temperatures and sucrose concentrations differed but which had essentially the same solvent viscosities, showed virtually identical anisotropy profiles. These observations suggest that motions of the bases make a significant contribution to the observed fluorescence depolarization in buffer at 20°C, in the absence of sucrose, for these double-stranded nucleic acids (Figs. 1 and 3).

The angle θ by which the transition dipole moment of the excited base has rotated, because of the DNA motion during the lifetime of the excited state, is related to the anisotropy through the following relationship (Lakowicz, 1983):

$$r = r_o \frac{3\langle \cos^2\theta \rangle - 1}{2}. \tag{2}$$

Here, r_o and r are the values of the fluorescence anisotropy at time $t = 0$ (i.e., at the time the base is excited with a picosecond light pulse) and at a particular time interval after excitation, respectively, and the brackets denote the ensemble average. This equation yields $\langle \cos^2\theta \rangle \approx 0.4$ over a time span of 4 ns for poly(dA)·poly(dT) at 20°C in the absence of sucrose (Fig. 1). Thus, the angular amplitudes of the motions that are suggested by these observations appear to be considerable.

It might be argued, however, that the fast-decaying components in these fluorescence anisotropy profiles could be accounted for by either electronic resonance interaction or excited-state molecular complex (excimer) formation between adjacent T bases. We consider these alternatives below.

Coulombic interaction as a possible origin of the observed large-amplitude fluorescence depolarization

A mechanism that involves coulombic coupling between an excited molecule and a nonexcited one is known as exciton interaction and encompasses three cases: a) strong coupling; b) weak coupling; and c) very weak coupling.

Strong coupling

The criterion for the applicability of this mechanism, which is known to operate in dimers (as well as in crystals), is $V \gg \Delta w/2$, where V is the magnitude of the interaction energy and Δw is the full width at half-maximum of the absorption band (Simpson and Peterson, 1957; Förster, 1965). This process would result in delocalization, with the energy being spread over many bases. Typically, for nucleic acids $\Delta w \approx 5000$ cm^{-1} (Gueron et al., 1974). Thus, for the strong coupling case to be applicable to nucleic acids, $V \gg 2500$ cm^{-1}. The interaction energy V between two adjacent T bases may be calculated from $V = \mu^2 k/n^2R^3$ (Förster, 1965), where μ is the transition dipole moment of T, R is the center-to-center T-to-T distance, n is the refractive index of the medium between the bases ($n \approx 1$), and k is an orientational factor. For μ we use the value of 3.3 Debye reported for uridine (Johnson and Tinoco, 1969). The values of R and k are 4.1 Å and 0.17, respectively (Georghiou et al., 1990). V is then found from the above equation to be about 130 cm^{-1}. Thus, V is about one order of magnitude smaller than $\Delta w/2$. Therefore, the inequality $V \gg \Delta w/2$, which is the necessary condition for the applicability of strong interaction, is clearly not satisfied for the present systems. Strong coupling causes a large splitting of the absorption band, which for a homopolymer is equal to $4V$ (Förster, 1965); according to the inequality $V \gg \Delta w/2$, for the present case this splitting should be greater than 10^4 cm^{-1}. However, no significant splitting is observed experimentally in the absorption spectra of nucleic acids (Gueron et al., 1974; Cantor and Schimmel, 1980; Georghiou et al., 1992). This is in accord with the present calculation for the interaction between two adjacent T bases: the splitting is equal to $4V = 4 \times 130 = 520$ cm^{-1}, which is a small value. (In the ultraviolet region, where nucleic acids absorb, this energy corresponds to about 4 nm.) CD calculations (Cantor and Schimmel, 1980) also show that in nucleic acids exciton splitting is very small. These considerations suggest that no efficient strong excitonic interactions operate in the nucleic acid systems under study in the present work. Thus, the excitation energy does not appear to be delocalized over many bases, but instead can be considered to be localized on the base that absorbs the exciting photon. The successful analysis of the absorption spectrum of poly(dA)·poly(dT) into the constituent absorption spectra of the A and T residues through the use of the fluorescence excitation spectrum of the polynucleotide (Georghiou et al., 1992) provides further support for this conclusion; this is so because in that analysis the explicit assumption was made that the observed fluorescence emanates from the initially excited base. (Finally, we should note that the existence of hypochromism does not imply exciton coupling, as is sometimes stated in the literature, for Tinoco (1960, 1961), Rhodes (1961), and Rhodes and Chase (1967) have shown that this phenomenon stems from dispersion interactions between adjacent bases.)

Weak (vibronic resonance) coupling

This involves resonance between individual vibronic bands (Förster, 1965), which are absent in nucleic acids. Even if one considers such bands, however, to underlie the absorption envelope, this mechanism would not operate in poly(dA)·poly(dT) for 293 nm excitation because the interaction energy is proportional to the extinction coefficient (Simpson and Peterson, 1957; Förster, 1965), which is very small at this wavelength (~600 M^{-1} cm^{-1}; Georghiou et al., 1992).

Very weak coupling

This is the well-known Förster's mechanism of energy transfer (Förster, 1965). We first note that A-to-T interstrand energy transfer cannot operate under the conditions of our experiments because we have excited at 293 nm, where A does not absorb (Voet et al., 1963; Georghiou et al., 1992). Evidence against T-to-T intrastrand energy transfer is provided by Weber's effect (Weber and Shinitzky, 1970), according to which energy transfer between identical molecules (at room temperature as well as at low temperatures) fails when exciting at the long-wavelength region of the absorption spectrum, as we did in the present experiments. Failure of homotransfer for long-wavelength excitation was also invoked to explain the excitation wavelength dependence of the fluorescence polarization of the dinucleotide dpTpT (Wilson and Callis, 1976). Förster's theory has previously been found (Georghiou et al., 1990) to describe energy transfer in double-stranded random-sequence DNA. Below we calculate the efficiency f of T-to-T transfer and find it to be very low. The efficiency is given by $f = (R_o^6)/(R_o^6 + R^6)$, where R is the center-to-center donor-acceptor distance, and R_o is the critical transfer distance, which is calculated from spectral data. For T-to-T transfer, $R = 4.1$ Å, the square of the orientational factor k is equal to 0.03, the overlap between the fluorescence spectrum of T and its absorption spectrum is 1.6×10^{-17} cm^3 M^{-1} (Georghiou et al., 1990), and the fluorescence quantum yield of T in the polymer is equal to 4×10^{-4} (Georghiou et al., 1992). For these values, R_o is found to be equal to 2.4 Å (further details are found in Georghiou et al., 1990), for which the above equation yields $f \approx 4\%$ for the efficiency of T-to-T transfer. Thus, such transfer is very inefficient. We should add that this transfer mechanism (as well as the strong and weak coupling mechanisms) are not sensitive to viscosity changes (Förster, 1965). Consequently, for the sucrose solutions the notion of viscosity effects on the T-to-T transfer as being responsible for the observed large attenuation of the anisotropy decay (Figs. 2 and 3) does not appear to be tenable. For completeness, we note that electron exchange, another potential transfer mechanism (Dexter, 1953), was previously shown to be much less efficient than Förster's mechanism for double-stranded DNA (Georghiou et al., 1990).

Finally, work in progress (Georghiou and Beechem, to be published) finds that the time-resolved anisotropy profile for the alternating polynucleotide poly(dA-dT)·poly(dA-dT) is qualitatively similar to that shown in Fig. 1 for poly(dA)·poly(dT). This finding adds additional weight to the argument against ascribing the observed fluorescence depolarization to energy transfer: in the alternating polynucleotide the nearest neighbors of T are A's that are not excited at 293 nm and consequently cannot transfer energy to T. (Furthermore, T-to-T transfer is very inefficient because the distance R is much larger than R_o.)

Intermolecular excimer formation as a possible origin of the observed fluorescence depolarization

Another possible origin of the observed fluorescence depolarization is excimer formation. We previously presented evidence from one of our laboratories (Ge and Georghiou, 1991b) against the occurrence of this process in poly(dA)·poly(dT) in the absence of sucrose: the shape of its fluorescence spectrum lacks a long-wavelength structureless emission band, which is known to accompany the formation of such a molecular complex (Birks, 1970). We have found this to be the case for all the nucleic acids that we have studied in the present work, both in the absence and in the presence of sucrose (Gerke and Georghiou, unpublished observations). It should be noted that in general excimer fluorescence tends to have a low anisotropy (see, e.g., Morgan and Daniels, 1980a,b). This depolarization is the result of i) rotation of the bases to achieve the most favorable orientation and ii) strong perturbations that stem from charge-transfer and/or exciton-resonance interactions (Birks, 1970) that hold the two molecules together. Indeed, we previously reported from one of our laboratories (Ge and Georghiou, 1991a) a value very close to zero for the steady-state fluorescence anisotropy of the excimeric band of the alternating polynucleotide poly(dA-dT)·poly(dA-dT). As a precaution, in the present study the measurements were made at the short-wavelength region (at 360 nm) to minimize the contribution to the fluorescence signal of any excimer that might be present; in this regard, some experiments were repeated at 345 nm, where any excimer contribution would be negligible (Ge and Georghiou, 1991a), with essentially identical results.

Nature of the detected motions

We now consider the origin of the motions in the double-stranded systems poly(dA)·poly(dT) and $(dA)_{20}\cdot(dT)_{20}$. Barkley and Zimm (1979) and Schurr and Fujimoto (1988) have developed theoretical treatments of the fluorescence anisotropy of the extrinsic fluorescent probe ethidium bromide, intercalated between the DNA bases, by considering the fluorescence depolarization as stemming from collective twisting and bending modes. These theories in their present forms predict decay rates for the anisotropy that are on the order of (tens of nanoseconds)$^{-1}$ and therefore cannot account for the motions that are suggested by the present study (in the absence of sucrose), the rates of which are on the order of (tens of picoseconds to a few nanoseconds)$^{-1}$.

Below, we consider alternative mechanisms for the motions of the bases. Sobell and co-workers (Lozansky et al., 1979) have proposed that collisions with DNA by energetic solvent molecules with their momenta oriented along the dyad axes may give rise to the generation of elastic waves that travel along DNA with the speed of sound. When, as a result of constructive interference between waves that travel in opposite directions, their amplitudes exceed the elastic limit of DNA, DNA undergoes a transition to a β-kinked form. The kinks are considered to be hyperflexible joints. These are regions that facilitate transient unstacking of the bases and breaking of the hydrogen bonds. In this regard, conformational energy calculations suggest that, upon disruption of a single base pair, DNA becomes very flexible (Ramstein and Lavery, 1988). A somewhat similar mechanism was proposed by Chou and Mao (1988). As in the theory of Sobell and co-workers (Lozansky et al., 1979), collisions with solvent molecules give rise to elastic waves. When the stress generated along the hydrogen bonds is very large, a "free jerk" is generated that resembles a sudden "quake." Thus, collective motions result in the transient opening of several base pairs. The solvent molecules are considered to be the source of both the "push" and the "pull," so that the oscillatory motions are not overdamped. A lattice dynamics approach (Prohofsky, 1986) also finds that compressional acoustic waves can propagate over many base pairs in DNA. The speed of acoustic waves in DNA is about 1800 m/s (Lee et al., 1987); therefore, it would only take about 3 ps to cover a distance of 50 Å. These considerations suggest that structural modifications that are initiated at one site can be communicated with such a mechanism to a distant site over a time period of picoseconds. These theoretical treatments have not developed expressions for the decay of the fluorescence anisotropy, however, and consequently at this stage no comparison can be made with the experimental results reported in the present study.

The finding of the present study that the motions of the single-stranded systems poly(dT) and $(dT)_{20}$ are slower than those of the corresponding double-stranded systems poly(dA)·poly(dT) and $(dA)_{20}\cdot(dT)_{20}$ (Fig. 6) is consistent with wave propagation in the double-stranded structures. Cooperative interactions, which are necessary for the occurrence of concerted motions, are to a great extent absent from single-stranded structures. Consequently, poly(dT) and $(dT)_{20}$ cannot support the propagation of traveling waves and are expected to have slower motions, in agreement with our observations. It should be noted in this regard that, as was discussed under Results, differences in the rates of rotational or spinning motions between $(dA)_{20}\cdot(dT)_{20}$ and $(dT)_{20}$ cannot account for the observed slower rate of decay of the fluorescence anisotropy of $(dT)_{20}$.

We have also used the modified fluorescent DNA base 2-aminopurine to further compare the dynamics of these single- and double-stranded oligonucleotides. We have found that this same general trend is observed for 2-aminopurine located at position 10 of the 20-mers as that found using intrinsic thymine fluorescence (data not shown), i.e., the anisotropy profile decays more slowly for the single-stranded 20-mer. Because the 2-aminopurine fluorescence was obtained by exciting at 310 nm, there are no potential complications in these studies due to energy transfer between adjacent bases.

Regarding the entity that undergoes the observed motions, an approximate and phenomenological calculation of its volume V may be made from Figs. 8 and 9 in terms of classical rotation. From the equation $\phi = \eta V/kT$, which is valid for a sphere, the resulting values are then found to be ~1500 $Å^3$ and 500 $Å^3$ for poly(dA)·poly(dT) and $(dA)_{20}\cdot(dT)_{20}$, respectively, and ~5000 $Å^3$ for poly(dT) and 3000 $Å^3$ for $(dT)_{20}$. The volume of free T (thymine) is calculated to be ~100 $Å^3$ (Edward, 1970), with approximately an additional 100 $Å^3$ contributed by the sugar. These values are much smaller than the recovered volumes associated with the rotational modes found in these nucleic acids. This is not surprising, as the bases cannot be considered to undergo unrestricted diffusional motion. The much larger slopes for the single-stranded systems, as compared to those for the double-stranded systems (Figs. 8 and 9), are in accord with the expected stronger coupling to the solvent by the former systems. We should add that work in progress (Kubala and Georghiou, to be published) regarding excimer formation by the alternating polynucleotide poly(dA-dT)·poly(dA-dT) has been found to provide further evidence for the modulation of the motions of the bases by the solvent viscosity.

Other evidence for fast motions in double-stranded nucleic acids

A recent molecular dynamics simulation for a fully hydrated decamer $(dA)_{10}\cdot(dT)_{10}$ with counterions present (Fritsch et al., 1993) reported that the features that are thought to impart enhanced stability to such a base sequence (i.e., bifurcated hydrogen bonds and a spine of hydration) change during the simulation time of about 50 ps: i) the bifurcated bonds are broken and ii) the spine becomes much less regular. Furthermore, very large oscillations in the RMS deviations from the initial structure were observed, with the bases significantly inclined relative to the perpendicular to the helix axis. That study also reported that the simulation did not converge, i.e., the conformational changes were continuing at the end of the simulation. With regard to the inclination of the bases, a transient electric dichroism study (Hogan et al., 1978) reported that, for random-sequence DNA, the bases are inclined by 17° with respect to the perpendicular to the helix axis. A flow dichroism study (Chou and Johnson, 1993) reported similar results: 16°, 25°, 18°, and 25° for the A, T, G, and C bases, respectively. The latter study also reported that the bases of poly(dA)·poly(dT) exhibit large inclinations: 23° and 42° for the A and T bases, respectively. The results of the molecular dynamics simulation (Fritsch et al., 1993) suggest that this structural distortion is of dynamic origin. Another molecular dynamics simulation (Miaskiewicz et al., 1993) reported that the AATT central region of the so-called Dickerson/Drew dodecamer (hydrated and with counterions present) becomes significantly unwound over a period of 150 ps and the helix axis becomes very distorted. These theoretical studies suggest that double-stranded DNA undergoes large conformational changes on the picosecond time scale. The large fluorescence depolarization, which the present study reports for the polynucleotide (Fig. 1) and the 20-mer with this sequence (Fig. 3), is compatible with this inference. We should note that large angular amplitudes of motion for the oligonucleotides d(CG)$_4$ and d(CG)$_6$ have also been reported by ^{2}H NMR relaxation and depolarized dynamic light scattering (Eimer et al., 1990). This result was also reached (Garcia de la Torre et al., 1994) through the use of a double-helical bead model or the model of Schurr and Fujimoto (1988) in combination with an analysis of the NMR relaxation data of Eimer et al. (1990) and Birchall and Lane (1990). Large-amplitude fast motions were also reported for several oligonucleotides by time-resolved fluorometry through the use of the modified base 2-aminopurine as a fluorescent probe (Nordlund et al., 1989; Guest et al., 1991); molecular dynamics simulations in the presence of counterions (Nordlund et al., 1989) found that the interbase hydrogen bonds remain intact on a time scale of 120 ps. In the absence of an adequate model, it is not clear from our results whether the hydrogen bonds remain intact in the systems we have studied.

As was mentioned in the Introduction, several studies (reviewed in Schurr et al., 1991) have used the fluorescence anisotropy of intercalated dyes (which distort DNA and render it stiffer; Hogan et al., 1979b; Sobell et al., 1977) to study the dynamics of nucleic acids. From such measurements, in conjunction with ^{2}H NMR relaxation measurements for the Dickerson/Drew dodecamer, Nuutero et al. (1994) have concluded that the RMS angular amplitude of motions does not exceed 11°; this value is very similar to that reported at low levels of hydration in the solid state (Alam and Drobny, 1991). Another study that also reported small-amplitude motions (in contrast to the findings of the present study and those of the studies discussed above) is that by Hustedt et al. (1993) for some other oligonucleotides through the use of a covalently attached EPR probe.

CONCLUDING REMARKS

The polynucleotide poly(dA)·poly(dT) has attracted considerable interest, mainly because it was initially believed that the dA·dT tracts (so-called A-tracts) are involved in DNA bending. The current view is that these tracts are straight, with DNA bending occurring at the G·C/A·T junction (Dickerson et al., 1994). This polynucleotide is considered to have enhanced stability, which is thought to stem from a) a large propeller twist of the bases (Coll et al., 1987; Nelson et al., 1987) that allows the formation of bifurcated hydrogen bonds and enhances base stacking and b) a spine of hydration in the minor groove (Drew and Dickerson, 1981; Chuprina, 1987). In fact, it is a general practice to refer to this polynucleotide in the literature as rigid. This belief stems, in part, from initial reports that this polynucleotide cannot be reconstituted into nucleosomes; it was subsequently shown, however, to be reconstituted (Puhl et al., 1991). Nevertheless, a study (Satchwell et al., 1986) that determined the base sequences of 177 different examples of nucleosomal core DNA found that runs of adenines are distributed near the ends of nucleosomal DNA, where DNA is not very curved. This finding suggests that the A-tracts are less flexible than most other base sequences. Thus, in view of the fast large-amplitude motions, which are suggested by the results of the present study and predicted by molecular dynamics simulations (Fritsch et al., 1993) for the $(dA)_n{\cdot}(dT)_n$ sequences, we expect a number of other base steps to be flexible as well.

It should be emphasized that the present study is preliminary in nature. This is of necessity, however, because time-correlated anisotropy measurements of intrinsic DNA fluorescence have not previously been reported. Clearly, a complicating factor in the interpretation of these data involves the presence of multiple fluorescence decay times; additional experiments will need to be performed that will directly address the origin of these decay components. As was discussed above, ongoing research (Gerke and Georghiou, to be published) suggests that this decay time heterogeneity is at least in part a consequence of the motions that the bases are undergoing. All of the data analysis presented in this study was in terms of a phenomenological, exponential-type model with no explicit coupling between the fluorescence decay times and rotational correlation times; there does not appear to be a significant fluorescence decay time ↔ rotational correlation time association in these systems, as patterns that are characteristic of associative-type anisotropy (Ludescher et al., 1987) were not observed in the present study. Finally, the possibility that an as yet unknown electronic process makes some contribution to the observed large-amplitude decay of the anisotropy cannot be completely excluded. The observed strong response of the anisotropy decay to the addition of sucrose, however, suggests a significant motional component in its origin.

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