Sequence-Dependent Cooperative Interactions in A/T-Containing Oligo- and Polydeoxyribonucleotides†

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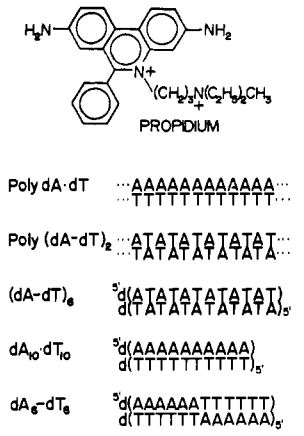
ABSTRACT: To evaluate the length and sequence dependence of the unusual interaction properties observed for nonalternating A/T sequences in deoxyribonucleic acid (DNA) [Wilson, W. D., Wang, Y. H., Krishnamoorthy, C. R., & Smith, J. C. (1985) *Biochemistry 24*, 3991–3999], we have synthesized the oligomers $d(A\text{-}T)_6$, $dA_{10}\cdot dT_{10}$, and $d(A_6\text{-}T_6)$ and evaluated their interaction with the intercalator propidium. Propidium visible spectral shifts on adding all three oligomers are quite similar. Low-temperature spectrophotometric binding measurements indicate that $d(A\text{-}T)_6$ has a significantly larger binding constant for propidium than $dA_{10}\cdot dT_{10}$, as with the analogous alternating and nonalternating DNA polymers. The oligomer $dA_{10}\cdot dT_{10}$ displays positive cooperativity in its propidium binding isotherm, and its binding constant increases with increasing temperature while $d(A\text{-}T)_6$ does not display positive cooperativity, and its binding constant decreases with temperature, again as with the analogous polymers. van't Hoff plots indicate that the propidium binding enthalpies are approximately −9 and +6 kcal/mol for the alternating and nonalternating DNA samples, respectively. The mixed-sequence self-complementary oligomer $d(A_6\text{-}T_6)$ has an unusual low-temperature binding isotherm which suggests a single strong binding site and a larger number of weaker binding sites which bind propidium cooperatively. A van't Hoff plot indicates that the cooperative sites $d(A\text{-}T)_6$ have binding constants and binding enthalpies similar to $dA_{10}\cdot dT_{10}$. Similar rate constants are observed in the sodium dodecyl sulfate driven dissociation reaction of propidium from $d(A\text{-}T)_6$ and $d(A_6\text{-}T_6)$, but the association reaction of propidium is significantly slower with $d(A_6\text{-}T_6)$ than with $d(A\text{-}T)_6$. These results illustrate that, under physiological conditions, short sections of nonalternating A/T base pairs can exist in the unusual state and exhibit the unusual interaction properties observed for poly(dA·dT). The apparent B-form structure and positive binding enthalpy and entropy of nonalternating A/T sequences suggest that the unusual state may be due to a highly specific hydration of this DNA sequence.

The ability of the four bases of deoxyribonucleic acid (DNA),[1] when organized into a specific sequence, to code for the amino acid sequence of a protein was recognized soon after the discovery of the double helix (Crick et al., 1961). The molecular basis for selectively packaging DNA, thus turning off gene expression of specific sequences and, in response to specific stimuli, turning them on for protein synthesis, remains an active area of research (Lewin, 1980). Since these specific effects require sequence-selective interactions of DNA with other molecules, we have used an analysis of variations in molecular interactions as one method to probe for unusual properties of DNA sequences (Wilson et al., 1985b, 1986a). This method, using selected probe molecules, is a useful complement to spectroscopic and crystallographic methods for structural analysis for several reasons: (1) it focuses directly on the binding properties of the sequence; (2) it directly establishes the thermodynamic parameters characterizing the interaction properties of the sequence; and (3) it can identify sequences with unusual solvent interactions which may have a fairly standard B-form double helix and not be identified as unusual by other techniques.

We have used the interaction probe method, with the intercalator propidium (Scheme I) and the polymers poly[$d(A\text{-}T)_2$] and poly(dA·dT), to show that sequences of nonalternating A/T base pairs have usual properties (Wilson et al., 1985b, 1986a). The following are the observed characteristic unusual features of nonalternating A/T sequences in their interaction with propidium: (1) a viscosity decrease followed by an increase in a viscometric titration of DNA with propidium; (2) unusual association with fairly normal dissociation kinetics for the propidium–DNA binding reaction; (3) lower equilibrium constants and positive ligand cooperativity in the binding reaction; and (4) a large positive enthalpy and entropy for the thermodynamics of the interaction. These altered interaction properties of nonalternating A/T sequences exist

Scheme I



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[1] Abbreviations: CD, circular dichroism; DNA, deoxyribonucleic acid; NMR, nuclear magnetic resonance; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); poly(dA·dT), poly(dA)·poly(dT); poly[$d(A\text{-}T)_2$], poly[d(A-T)]·poly[d(A-T)]; SDS, sodium dodecyl sulfate; SSR, sum of the squared residuals; EDTA, ethylenediaminetetraacetic acid.

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under physiological conditions even though the polymer apparently has a fairly normal B conformation under these conditions (Sarma et al., 1985; Behling & Kearns, 1986). These results suggest that the unusual properties of nonalternating A/T sequences may be due to some particularly favorable interactions of water molecules with the thymine C-2 carboxyl oxygen and/or adenine N-3 groups in the minor groove. Since Dickerson and co-workers (Dickerson et al., 1982) have shown that A/T regions generally have more structured water in the minor groove than G/C regions, the hydration of nonalternating A/T sequences would have to be particularly strong and selective. It is interesting to note that alternating G/C but not alternating A/T sequences can assume the Z conformation (Wang et al., 1980) while nonalternating A/T but not nonalternating G/C sequences can exhibit the nonstandard interactions described above (Wilson et al., 1985b, 1986a).

In our effort to identify the molecular basis and possible biological significance for the altered interactions seen with nonalternating A/T sequences, the following questions must be answered: (1) Since the unusual properties have only been observed in the polymer poly(dA·dT), how many base pairs in a nonalternating sequence are required to give the unusual effects? (2) How are the unusual properties varied by breaks or alterations in the nonalternating sequence such as an alternating base pair in a nonalternating sequence and/or an interchange of the A and T chains in a sequence? (3) How are the unusual properties modified when the nonalternating A/T sequence is embedded in a different DNA sequence, and how strongly does the modification depend on the surrounding sequence?

To begin to answer the first two of these questions, we have synthesized the oligomers dA_{10}·dT_{10}, d(A-T)$_6$, and d(A_6-T_6) (Scheme I) and have characterized their binding with our standard probe molecule, propidium, in the low-temperature region, where the oligomers are in the double-helical conformation. We have also extended the binding analysis of propidium with poly[d(A-T)$_2$] and poly(dA·dT) into the region between 0 and 18 °C to allow direct comparison with the oligonucleotide results. Our major finding is that short sequences of nonalternating A/T base pairs can also exhibit the unusual interaction properties described above.

Materials and Methods

Materials. Propidium iodide (Calbiochem) was prepared as previously described (Davidson et al., 1977). All experiments were conducted in PIPES 00 buffer (10 mM PIPES and 1 mM EDTA adjusted to pH 7.0 with NaOH) or PIPES 20 buffer (PIPES 00 with 0.2 M NaCl). Poly[d(A-T)$_2$] (lot 782-38) and poly(dA·dT) (lot 675-79) from P-L Biochemicals were prepared and analyzed as previously described (Wilson et al., 1985b, 1986a). We have found that these polymer preparations vary considerably, particularly with poly(dA·dT), and each lot must be carefully characterized by UV and NMR spectroscopic methods and by analysis of the T_m curve. Stock solutions of the polymers were made by dissolving the DNA polymers in PIPES buffer to make approximately 3 mM solutions. The oligonucleotides were synthesized and purified by using previously described methods [see Stec et al. (1985) and references cited therein]. Oligonucleotide stock solutions (100 units/mL) were prepared and dialyzed against PIPES 20 buffer using Spectra/Por 6 dialysis bags (molecular weight cutoff 1000) at 4 °C for 2 days. The oligomer dA_{10}·dT_{10} was prepared by mixing equimolar amounts of dA_{10} and dT_{10}. To determine the proper mixing ratios, dA_{10} was titrated with dT_{10}, and dT_{10} was titrated with dA_{10} (PIPES 20, 5 °C), with both titrations resulting in a maximum hypochromicity at equimolar amounts of each oligomer. Therefore, under our conditions, the oligomer dA_{10}·dT_{10} forms a double and not a triple helix. Oligonucleotide stock solution concentrations were determined by UV spectroscopy, using extinction coefficients of the analogous DNA polymers [in the case of dA_6-dT_6, the extinction coefficient used was that of poly(dA·dT)]. As a check, concentrations were also determined by using inductively coupled plasma emission spectroscopy of phosphorus content, and for these oligomers, both methods agreed within experimental error. Prior to each series of binding experiments, a fresh propidium solution was prepared, and both the propidium (PIPES 20, 25 °C) and the oligonucleotide (PIPES 20, 0 °C) concentrations were determined by absorption spectroscopy. Under these conditions of salt and temperature, the oligonucleotide is in a duplexed state as determined by T_m measurements (Wilson et al., 1986c).

Spectrophotometric Measurements. Both Cary 17D and Cary 219 spectrophotometers were used for UV–visible spectral measurements. Cell holders were thermostated by using Haake A81 circulating water baths. Temperature measurements were made by using either YSI 42 SC or Electromedics IT 610 temperature monitors. Before each experiment, a thermister probe was inserted into the buffer, the temperature was monitored until the buffer had equilibrated at the desired temperature, and the probe was removed. Due to the low temperature of these experiments, nitrogen gas was used to flush the cell compartment to eliminate any possible condensation on the optics and sample cell during the optical measurements. Care was taken to allow the solutions to return to thermal equilibrium after each aliquot of propidium was added (generally 10–15 min at the lower temperatures). Wavelength scans and absorbance measurements were made in quartz cells from 1- to 5-cm path lengths. Extinction coefficients of both free and intercalated propidium were determined at 480 nm, a point of optimum spectral change, as previously described (Wilson et al., 1985b).

For determination of binding isotherms, absorbance values of propidium in the presence of DNA samples were entered into a real-time interactive computer program that converted these values into ν (moles of propidium bound per mole of DNA base pairs) and free ligand concentrations using the free and bound extinction coefficients for the compound (Wilson & Lopp, 1979; Wilson et al., 1985b). Only binding data in the fraction bound range between 0.2 and 0.8 were used. Binding data outside of this range are subject to large errors as a result of experimental error in determining the free and bound extinction coefficients. A complete binding isotherm can be obtained by varying the initial DNA concentration. Upon termination of the binding experiment, the computer calculated a first-order approximation of the K, n, and ω values (eq 1 under Results) and then plotted the data along with the theoretical curve from the site-exclusion model of McGhee and von Hippel (1974). The computer then calculated nonlinear least-squares best-fit values for K, n, and ω and presented a graphical display of the results in the form of a Scatchard plot.

Stopped-Flow Kinetics. Kinetic measurements were made by using an Aminco-Morrow stopped-flow apparatus adapted to a Johnson Foundation MB2 air turbine spectrophotometer as previously described (Wilson et al., 1985a,b). The output of the instrument was fed to an Olis 3820 data acquisition system, stored on magnetic disk, and analyzed subsequently. Two hundred data points collected in a preselected time range could be fitted by using from one to three exponential curves. Fitting was done as described earlier (Krishnamoorthy et al.,

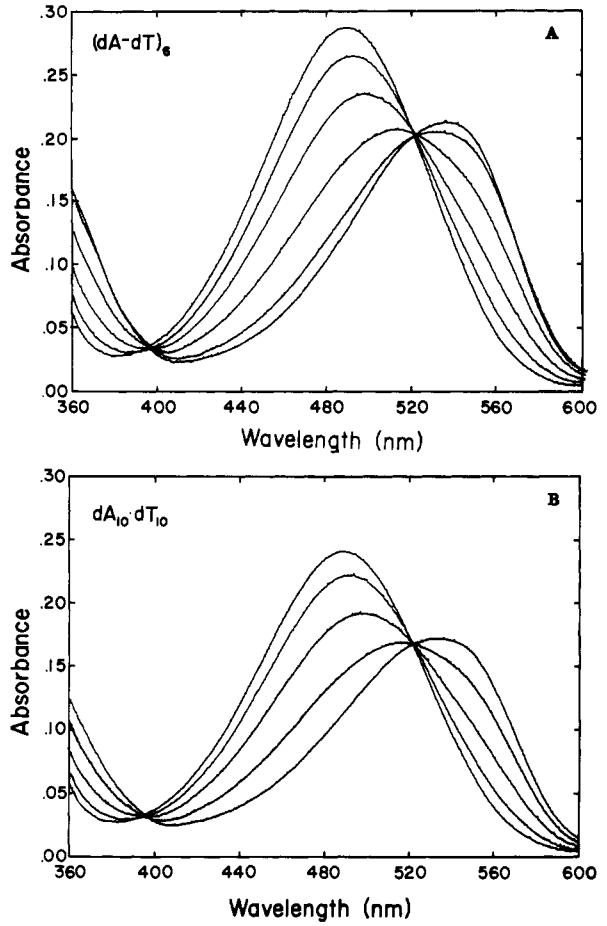

FIGURE 1: (A) Spectrophotometric titration of propidium with $d(A\text{-}T)_6$ at 6 °C in PIPES 00 buffer. The titrations were conducted in a 5-cm cell as described under Materials and Methods, and concentrations are as follows: 1.0×10^{-5} M propidium and $d(A\text{-}T)_6$–base concentrations of zero, 9.27×10^{-6}, 2.47×10^{-5}, 4.63×10^{-5}, 7.4×10^{-5}, and 1.2×10^{-4} M, respectively, from the top to the bottom curves at 480 nm. (B) Spectrophotometric titration of propidium with $dA_{10}\cdot dT_{10}$ at 6 °C in PIPES 00 buffer. Because of the weaker binding of propidium to this oligomer, the titrations were conducted in a 1-cm cell, and concentrations are as follows: 4.6×10^{-5} M propidium and $dA_{10}\cdot dT_{10}$–base concentrations of zero, 2.6×10^{-6}, 7.7×10^{-5}, 1.5×10^{-4}, and 2.8×10^{-4} M, respectively, from the top to the bottom curves at 480 nm.

1986). Dissociation experiments were conducted by mixing equal volumes (100 μL) of propidium–oligomer complex and 1.0% SDS solution. Dual-wavelength difference spectra were obtained as a function of time using the wavelength pair 469 and 612 nm. Data collection was begun within 5 ms after initiation of mixing, and the instrument response time was set at 2 ms. Association kinetic measurements were conducted under pseudo-first-order conditions with a 10-fold excess of oligomers to propidium. Equal amounts (100 μL) of an oligomer solution and a solution of propidium were mixed under the desired conditions and data collected as above. Typically, five to eight runs of this kind were averaged by the computer to improve the signal to noise ratio.

RESULTS

Propidium Spectral Changes. Titration of propidium with the alternating and nonalternating sequence polymers poly-[$d(A\text{-}T)_2$] and poly(dA·dT) has been previously shown to result in essentially identical spectral shifts for the intercalator (Wilson et al., 1985b). Titrations of propidium with the analogous alternating and nonalternating oligomers $d(A\text{-}T)_6$ and $dA_{10}\cdot dT_{10}$ are shown in panels A and B, respectively, of

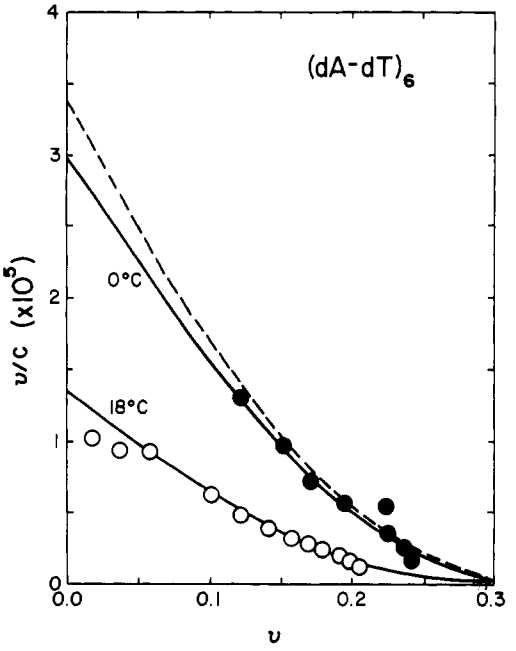

FIGURE 2: Scatchard plot for propidium binding to $d(A\text{-}T)_6$ at 0 °C (●) and 18 °C (○). Experiments were conducted in 2-cm cells in PIPES 20 buffer as described under Materials and Methods. Points in the figure are experimental, and the solid curves are the nonlinear least-squares best-fit values using eq 1. The dashed line in the figure represents a correction to the 0 °C results according to eq 2 as discussed under Results.

Figure 1 in the 360–600-nm spectral region. The spectrum for free propidium in solution gives an absorbance maximum at 490 nm, and as oligomer is added to the solution, there is a reduction in the propidium extinction coefficient and a shift in the spectrum to longer wavelengths. When the propidium is fully complexed with an oligomer, its absorbance maximum is shifted to 537 nm. Isosbestic points are observed at 397 and 525 nm. The absorbance maximum, isosbestic points, total hypochromicity, and spectral shape are, thus, very similar for propidium complexed with both alternating and nonalternating oligomers. As can be seen in Figure 1A,B, the maximum change in extinction coefficient occurs near 480 nm, and this wavelength was used for binding studies. It should be emphasized that the following binding analyses assume a unique bound extinction coefficient for propidium at all A/T sites, and for a probe molecule to be useful in structural investigations, this is a requirement.

Propidium Binding to Alternating A/T Samples: $d(A\text{-}T)_6$ and Poly[$d(A\text{-}T)_2$]. Spectrophotometric titrations of the type shown in Figure 1 can be used to determine binding parameters for propidium–DNA interactions. For accuracy and coverage of the broadest range in a binding isotherm, the titrations are conducted at a constant wavelength with addition of a ligand solution into the appropriate DNA sample as described under Materials and Methods. Example binding isotherms in the form of Scatchard plots are shown in Figure 2 for the propidium interaction with $d(A\text{-}T)_6$ at 0 and 18 °C. The solid lines in Figure 2 are calculated by a nonlinear least-squares computer program using eq 1 where ν is the moles of propi-

$$\frac{\nu}{c} = K(1 - n\nu) \times \left[\frac{(2\omega - 1)(1 - n\nu) + (\nu - R)}{2(\omega - 1)(1 - n\nu)}\right]^{n-1}\left[\frac{1 - (n + 1)\nu + R}{2(1 - n\nu)}\right]^{2} \quad (1)$$

$$R = \{[1 - (n + 1)\nu] + 4\omega\nu(1 - n\nu)\}^{1/2}$$

Table I: Effect of Temperature on the Interaction of Propidium with Alternating and Nonalternating A/T Base Pair Containing Oligonucleotide and Polymer DNA Samples[a]

DNA	T (°C)	K ($\times 10^{-3}$)	n	ω
	Alternating			
$d(A\text{-}T)_6$	0	298	2.8	0.7
	6	221	2.5	0.6
	12	216	2.8	0.5
	15	165	2.3	0.8
	18	134	3.1	0.8
$poly[d(A\text{-}T)_2]$	0	745	2.0	0.3
	10	734	2.0	0.1
	18	704	1.9	0.1
	29	337	2.3	0.2
	38	267	2.6	0.1
	48	158	2.5	0.1
	Nonalternating			
$dA_{10}\cdot dT_{10}$	0	10	3.8	4.7
	6	15	3.3	3.2
	12	12	3.1	2.6
	15	17	3.7	2.5
	18	15	3.6	3.2
poly(dA·dT)	0	4	3.3	14.0
	10	13	2.9	5.0
	18	18	2.5	3.7
	25	25	2.3	2.4
	30	36	2.4	1.6
	36	35	2.5	1.3
	41	39	2.6	1.2
	48	53	2.5	1.1

[a] Results calculated by using eq 1.

dium per polymer base pair, c is the free propidium concentration, K is the intrinsic equilibrium association binding constant, n is the number of base pair units per binding site, and ω is a cooperativity parameter which represents the equilibrium constant for transfer of a bound propidium molecule from an isolated to a singly contiguous binding site (McGhee & von Hippel, 1974).

Titrations at a range of temperatures were conducted as in Figure 2 for both the alternating base pair oligomer and polymer, and the results, fitted by using eq 1, are collected in Table I. The binding constants for propidium bound to both the oligomer and the polymer decrease with increasing temperature, and both show strong binding of propidium, n values between 2 and 3, and ω values less than 1. The n and ω values determined from the best-fit results with the oligomer are both larger than for the polymer. As we have previously noted (Wilson et al., 1985a,b), n and ω are highly correlated when fitting data with eq 1 whereas K is much more highly determined. Fixing ω for the oligomer at 0.1, the average value for the polymer above 0 °C, results in an n value of 2.1, in good agreement with the neighbor-exclusion model and the polymer results; the equilibrium binding constants for the fixed ω value varied less than 1% from the values in Table I.

The equilibrium constants for propidium binding to the oligomer are lower by approximately an average factor of 3 relative to the polymer, and this could be due to end effects in the oligomer. In the original derivation of eq 1 by McGhee and von Hippel (1974), for example, it was assumed that the number of residues in the binding lattice, N, was much greater than the number of lattice units, n, in the binding site. In their original treatment, McGhee and von Hippel (1974) pointed out that at low binding densities, ν_N for small lattice sites would be related to ν for large lattices by

$$\nu_N/\nu = (N - n + 1)/N \qquad (2)$$

For propidium binding to $d(A\text{-}T)_6$, this requires multiplication by 12/11 to obtain ν from the observed results (ν_N). The dashed line in Figure 2 is a theoretical curve corrected by this factor. Note that to obtain 10 potential sites, propidium must bind at both ends of the lattice even though the last propidium at one end of the oligomer would not have a neighboring site to "exclude". This is required because the propidium binding sites are actually spaces between base pairs and not base pairs themselves. With the assumption that propidium can bind at sites on both ends of the lattice, a maximum of six propidium molecules can bind at saturation with neighbor exclusion, an n of 2. The number of propidium molecules bound at saturation in the original McGhee and von Hippel (1974) treatment would be the integral part of N/n or six if counting base pairs and five (Wilson & Lopp, 1979; McGhee & von Hippel, 1974) if counting sites between base pairs. This raises interesting questions about the requirements for neighboring base pairs in intercalator–DNA interactions. NMR studies of base pair imino proton shifts on adding propidium to oligomers indicate that both terminal sites between base pairs can bind propidium (Wilson et al., 1986b). In addition to the statistical reduction in ν as shown in eq 2, propidium binding to the oligomers may be reduced relative to the polymers by the lower stability of the terminal base pairs of the oligomers. Both of these effects would reduce the apparent binding constant of propidium to $d(A\text{-}T)_6$ relative to $poly[d(A\text{-}T)_2]$. The basic conclusion from all of these results is, however, that the interactions of propidium with both the alternating polymer and oligomer are very similar and show considerable similarity to binding results with random-sequence natural DNA samples (Wilson et al., 1985a,b).

Propidium Binding to Nonalternating A/T Samples: $dA_{10}\cdot dT_{10}$ and Poly(dA·dT). Spectrophotometric binding titrations were also conducted with $dA_{10}\cdot dT_{10}$, and example results at the same two temperatures as in Figure 2, 0 and 18 °C, are shown for comparison in Figure 3. Titrations for the analogous nonalternating polymer, poly(dA·dT), are shown at the same two temperatures in Figure 4, and equilibrium results for both the oligomer and polymer at a range of temperatures are collected in Table I. Unlike the alternating polymer and oligomer, poly(dA·dT) and $dA_{10}\cdot dT_{10}$ both interact with propidium with positive cooperativity and increasing binding strength with increasing temperature. They also bind propidium more weakly at lower temperatures than for the alternating sequence A/T DNA samples. Comparison of Figures 3 and 4 shows that although the positive cooperativity is more pronounced at low temperature with the polymer than with the oligomer, the oligomer does display significant positive cooperativity in its interaction with propidium. The binding constants for the nonalternating oligomer and polymer are more similar than for the nonalternating samples. It is possible that end effects in the oligomer, which, as discussed above, would normally lower the binding equilibrium constant, convert the nonalternating oligomer ends to a more standard DNA state and give an offsetting increase in binding. The nonalternating A/T samples are, thus, quite similar to each other in their interaction with propidium and are quite unlike the alternating A/T DNA duplexes.

Propidium Binding to a Mixed Alternating and Nonalternating Oligomer: $d(A_6\text{-}T_6)$. Binding isotherms were generated from spectrophotometric titrations of propidium with $d(A_6\text{-}T_6)$ as described above. Results for titrations at 0, 6, and 18 °C are shown in panels A, B, and C, respectively, of Figure 5. The plots are presented in three separate panels since they overlap significantly. The Scatchard plots for the 0 °C titration are the most unusual we have seen for an intercalator binding to any DNA sequence. It has a very steep slope at low ν values followed by a region with curvature

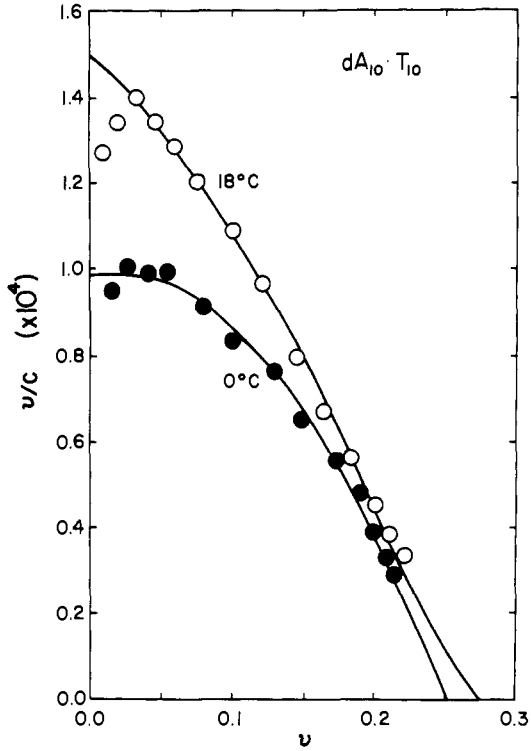

FIGURE 3: Scatchard plot for propidium binding to dA_{10}·dT_{10} at 0 °C (●) and 18 °C (○). Experiments were conducted in 1-cm cells in PIPES 20 buffer as described under Materials and Methods. Points in the figure are experimental, and the solid curves are the nonlinear least-squares best-fit values using eq 1. The two points at the lowest ν values in the 18 °C experiment could not be included on the best-fit curve using eq 1. The differences are, however, close to the experimental error at limits of the binding isotherm.

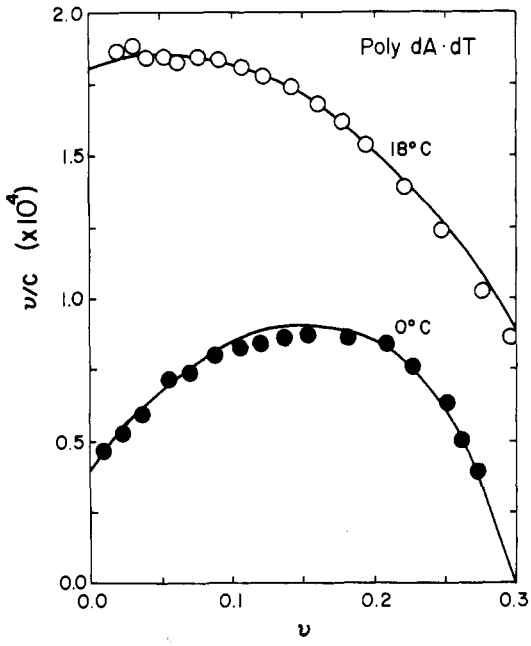

FIGURE 4: Scatchard plot for propidium poly(dA·dT) at 0 °C (●) and 18 °C (○). Experiments were conducted in 1-cm cells in PIPES 20 buffer as described under Materials and Methods. All points in the figure are experimental, and the solid curves are the nonlinear least-squares best-fit values using eq 1.

characteristic of positive cooperativity at higher ν values. This oligonucleotide obviously does not follow behavior that can be directly fitted by eq 1. For this reason, we have divided the isotherm into the two regions described above for analysis, and the rationale behind this division is presented in more detail under Discussion. The initial steeply sloped region was

Table II: Effect of Temperature on the Binding of Propidium to $d(A_6\text{-}T_6)$

T (°C)	K ($\times 10^{-3}$)	n	ω
0[a]	4.2	3.1	4.0
6	4.3	3.5	3.5
12	5.3	3.9	4.0
15	5.2	4.3	4.2
18	6.3	4.2	3.3
0[b]	5.4	2.6	3.0
6	5.0	3.2	3.7
12	6.2	3.5	4.0
15	6.1	3.8	4.1
18	7.5	3.5	2.9
0[c]	150	1[d]	

[a] Results calculated for the cooperative sections of the binding isotherm by using eq 1. [b] Results calculated for the cooperative sections of the binding isotherm by using eq 1 assuming two nonalternating regions only for the oligomer concentration. [c] Approximate results calculated by using eq 3 for a single site per duplexed oligomer using the classical Scatchard model. Results for the cooperative region of the curve were subtracted prior to fitting with this model. [d] Approximate n_s from eq 3.

fitted by using the classical Scatchard equation for independent binding sites without cooperativity (Scatchard, 1949):

$$\nu_s/C = K_s(n_s - \nu_s) \quad (3)$$

where C is the free ligand concentration, ν_s is the moles of propidium bound per oligomer duplex, n_s is the number of propidium binding sites per oligomer duplex, and K is the propidium binding constant. Note that, with the exception of C, these definitions differ significantly from those in eq 1. For the 0 °C binding experiment, this analysis predicts approximately one strong binding site per oligomer with an equilibrium constant much larger than for the nonalternating DNAs and more similar to those observed for the alternating A/T samples. This result is listed in Table II for reference. Because of the overlap of the two isotherms and the small number of data points in the steeply sloped region, these results have a larger error than fits to other, similar binding plots.

The second region was independently fit by using the same methods as for dA_{10}·dT_{10} and $d(A\text{-}T)_6$. This cooperative region of the $d(A_6\text{-}T_6)$–propidium binding isotherm can be fitted reasonably well with eq 1 over the 0–18 °C range, and results over this temperature range are also collected in Table II. This sample, in the cooperative binding region, has the lowest propidium binding constant of any of the A/T DNA samples. Analysis of all of the results in Table II suggests that the cooperative binding region for the oligomer is more similar to the nonalternating A/T samples than to the alternating DNA samples with their higher binding constants and negative binding enthalpy. The strong binding region for this oligomer cannot be observed over a broad enough temperature range for an enthalpy determination. The isotherm at 6 °C shows similar behavior to that seen at 0 °C, but the points at low ν value are more difficult to observe and fit. The points at higher ν values were fitted by using eq 1. At 18 °C, no region with steep slope at low ν values can be resolved. The entire curve at 18 °C was fitted with eq 1. Although this approach is an oversimplification of the binding of propidium to $d(A_6\text{-}T_6)$, it does permit a qualitative comparison of the relative interactions of propidium with the oligomer in the two distinct regions of the binding isotherm.

Enthalpy Determinations. The logarithms of the equilibrium constants as a function of temperature from Tables I and II are plotted in Figure 6 according to the van't Hoff equation:

$$\ln K = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (4)$$

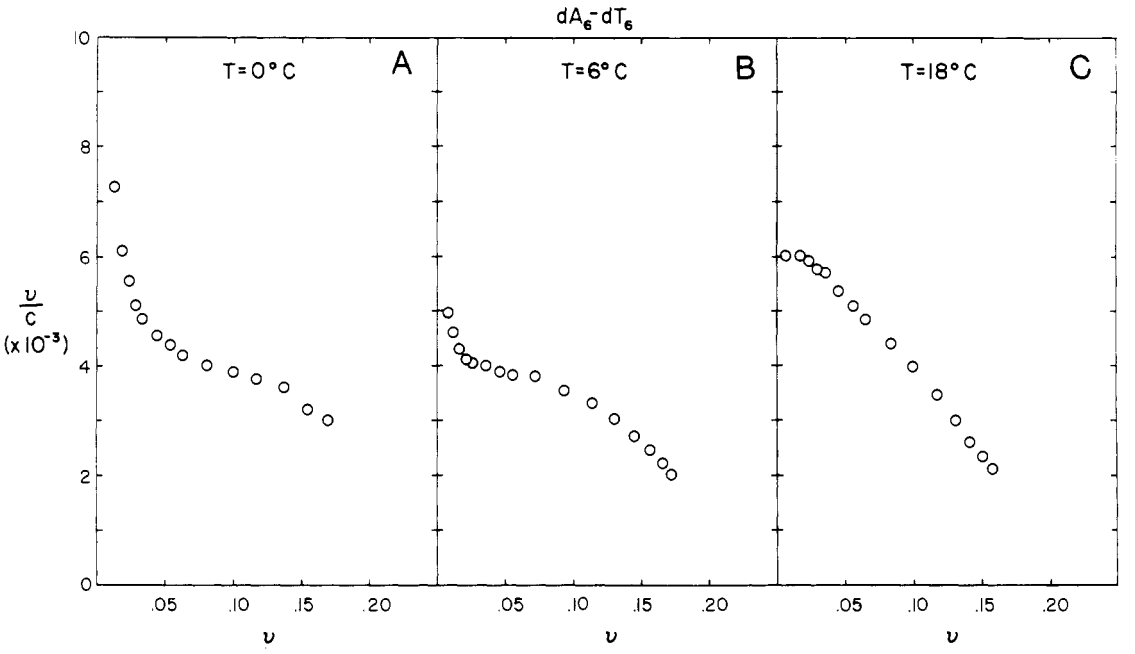

FIGURE 5: Scatchard plots for propidium binding to $d(A_6\text{-}T_6)$ at 0, 6, and 18 °C in 1-cm cells in PIPES 20 buffer. All experiments were conducted as described under Materials and Methods.

where $\Delta H°$ is the binding enthalpy and $\Delta S°$ is the entropy of binding. The plots dramatically illustrate the difference between alternating, $d(A\text{-}T)_6$ and poly[$d(A\text{-}T)_2$], and nonalternating, $dA_{10}\cdot dT_{10}$ and poly(dA·dT), DNA samples. The alternating samples have significantly stronger binding, especially at lower temperatures, and have a negative binding enthalpy [−8.9 kcal/mol for the linear least-squares best-fit line to the poly[$d(A\text{-}T)_2$] point in Figure 6]. Because of their lower T_m values, oligomers can only be studied over a small temperature range, and their binding enthalpies cannot be determined as accurately as with the DNA polymers. General comparisons with the polymer results can, however, be made. The $d(A\text{-}T)_6$ results, for example, definitely indicate a negative binding enthalpy and appear to have a slope very similar to the alternating polymer.

The nonalternating polymer has lower binding constants than the alternating samples, and it has a positive binding enthalpy [+6.1 kcal/mol for the linear least-squares best-fit line for poly(dA·dT) in Figure 6]. The line for the poly(dA·dT)–propidium equilibrium binding constants plotted in Figure 6 was calculated by omitting the equilibrium constant for the binding at 0 °C. It should be noted that the equilibrium binding constants for the binding to poly(dA·dT) at temperatures near 0 °C deviate downward from the best-fit line calculated for the temperatures above 5 °C. This temperature dependence of the enthalpy could be due to an enhanced cooperative solvent interaction with poly(dA·dT) at temperatures near the freezing point of water. The results for the nonalternating oligomer $dA_{10}\cdot dT_{10}$ indicate a positive binding enthalpy and, in fact, are quite close to the best-fit line for the poly(dA·dT) results in Figure 6.

Binding of propidium to the oligomer $d(A_6\text{-}T_6)$ also has a positive binding enthalpy as shown in Figure 6. The equilibrium constants plotted in Figure 6 for $d(A_6\text{-}T_6)$ were calculated by eq 1 using only the cooperative region of the binding isotherm (Table II). An additional set of calculations using eq 1 was performed on the cooperative regions of these binding isotherms in which the oligomer was viewed as having two independent nonalternating regions containing five base pairs with each region being able to bind propidium as in $dA_{10}\cdot dT_{10}$.

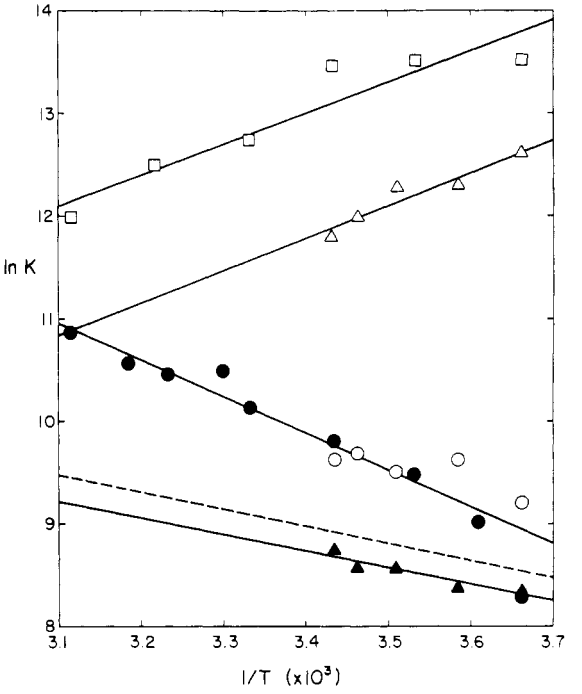

FIGURE 6: van't Hoff plot using the equilibrium constants as a function of temperature for poly[$d(A\text{-}T)_2$] (□), $d(A\text{-}T)_6$ (△), poly(dA·dT) (●), $dA_{10}\cdot dT_{10}$ (○), and $d(A_6\text{-}T_6)$ (▲), from Tables I and II. The line for $d(A_6\text{-}T_6)$ was calculated by using only results from the cooperative binding region. The dashed line is a calculation with the $d(A_6\text{-}T_6)$ oligomer concentration corrected to include only the nonalternating base pairs as discussed under Results.

The results of these calculations, using an oligomer concentration 10/12 the value for the above calculations, are shown as a broken line in Figure 6 and are tabulated in Table II for reference. It can be seen from the broken line in Figure 6 that if only the nonalternating regions of this oligomer are considered in calculating concentration, it has equilibrium binding constants similar to those for the propidium–$dA_{10}\cdot dT_{10}$ complex.

Kinetic Studies: *$d(A\text{-}T)_6$ and $d(A_6\text{-}T_6)$*. Kinetic studies were conducted with the self-complementary oligomers d(A-

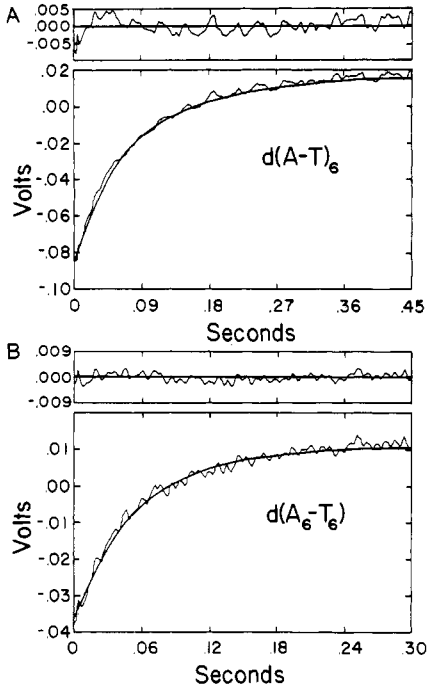

FIGURE 7: SDS-driven stopped-flow dissociation reactions at 10 °C in PIPES 20 buffer for propidium complexes with $d(A\text{-}T)_6$ (A) and $d(A_6\text{-}T_6)$ (B). The oligomer concentrations, kept at 2×10^{-4} M, were 10 times that of propidium. The smooth lines in panels A and B are the single-exponential nonlinear least-squares best-fit values to the experimental data and yield dissociation rate constants of 12.4 s^{-1} for $d(A\text{-}T)_6$ and of 17.3 s^{-1} for $d(A_6\text{-}T_6)$. A total of 200 data points were collected and plotted in the preselected time ranges, and the points are consecutively connected in panels A and B for ease of visualization. Residual plots for both data sets are shown above the experimental plots.

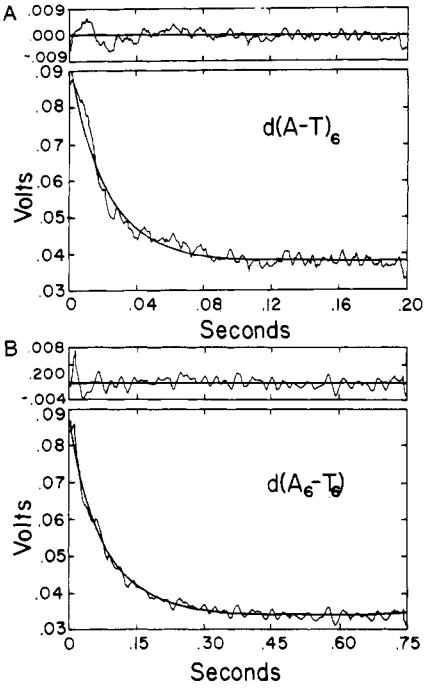

FIGURE 8: Stopped-flow kinetic traces for the association reactions of propidium with $d(A\text{-}T)_6$ (A) and $d(A_6\text{-}T_6)$ (B). The concentrations of both oligomers (in base pairs) after mixing were 1×10^{-4} and 1×10^{-5} M for propidium in PIPES 20 at 5 °C. The smooth lines in panels A and B represent the single-exponential nonlinear least-squares best-fit values to the experimental data and yield pseudo-first-order rate constants of 47.0 s^{-1} for $d(A\text{-}T)_6$ and 13.1 s^{-1} for $d(A_6\text{-}T_6)$. A total of 200 data points were collected and plotted in the preselected time ranges in panels A and B, and the points are consecutively connected for ease of visualization. Corresponding residual plots are shown above each experimental plot.

$T)_6$ and $d(A_6\text{-}T_6)$ which, as shown above, give characteristically different binding behavior for alternating and nonalternating sequences. Results for the SDS-driven dissociation of propidium from both oligomers are shown in Figure 7A,B. The smooth line through the experimental results is for fitting with a single exponential function, and the residuals for the fit are shown at the top of each plot. No significant improvement in the SSR or in the distribution of the residuals was obtained for a two-exponential fit. The basic conclusion from these results is that dissociation of propidium from the oligomers, as with the alternating and nonalternating A/T polymers, is quite similar.

Example kinetic plots for association of propidium with the same two oligomers are shown in Figure 8A,B. As can be seen, the association reactions of these oligomers, as with the alternating and nonalternating A/T polymers (Wilson et al., 1985b), are significantly different. The association reactions were conducted at two other DNA oligomer concentrations, as in Figure 8, and the second-order rate constants calculated from plotting the pseudo-first-order rate constants vs. DNA concentration are 2.1×10^5 M^{-1} s^{-1} for $d(A\text{-}T)_6$ and 4.3×10^4 M^{-1} s^{-1} for $d(A_6\text{-}T_6)$. The rates for $d(A\text{-}T)_6$, even at low concentration, are near our instrumental limit and may be underestimated. This would only make the observed rates more different and emphasizes the difference in association behavior of these oligomers.

Discussion

Viscometric, kinetic, and spectral results suggest that although the final propidium intercalation complexes with both poly[$d(A\text{-}T)_2$] and poly(dA·dT) are quite similar, the initial interactions are significantly different (Wilson et al., 1985b). The spectrophotometric titration results in Figure 1 also suggest that the propidium complexes with alternating and nonalternating A/T sequences in DNA oligomers are very similar. The binding results of Figures 2 and 3 and the kinetic results of Figure 8, however, demonstrate a pronounced difference in interaction properties of the alternating and nonalternating A/T sequences. These results convincingly demonstrate that the unusual interaction properties observed for poly(dA·dT) with intercalators can also exist in shorter DNA segments. The van't Hoff plots for propidium binding to alternating and nonalternating oligomers, shown in Figure 6, again demonstrate that the oligomers have the dramatic enthalpy differences observed for the corresponding alternating and nonalternating polymers.

We are in the process of analyzing the interactions of a range of other intercalators with nonalternating DNA sequences and have found all display much weaker binding to this sequence than to alternating A/T base pairs. Ethidium (Bresloff & Crothers, 1981; Wilson et al., 1985), tilorone (Strum, 1982), and daunomycin (Chaires, 1983a) have also been shown to bind significantly more weakly to nonalternating than to alternating A/T sequences. In an ongoing, detailed investigation of the interaction of daunomycin with different DNA sequences, Chaires (1983a,b; J. B. Chaires, personal communication) has found that daunomycin binds to poly(dA·dT) with positive enthalpy and entropy. Breslauer and co-workers (Marky et al., 1985) have conducted a detailed thermodynamic investigation of the interaction of netropsin with both alternating and nonalternating sequence DNA polymers. They find that netropsin binds strongly to both sequences but that the binding is enthalpy driven for the alternating and entropy driven for the nonalternating polymer.

This groove-binding antibiotic can, thus, interact strongly with the nonalternating A/T sequence, unlike intercalators, but retains the positive enthalpy of binding.

The cooperativity in binding of propidium and other intercalators to nonalternating A/T sites suggests an allosteric conversion, of the type discussed by Crothers and co-workers (Dattagupta et al., 1980), from some nonstandard DNA state, D*, to a more usual B-form double helix, D, which then binds intercalators to form a fairly standard intercalation complex, I (Wilson et al., 1986b):

$$D^* \rightarrow D \quad (5)$$

$$D + L \rightarrow I \quad (6)$$

The allosteric conversion is not required for other DNA sequences, and they bind propidium by the standard site-exclusion mechanism. The cooperativity in this model results from the positive free energy of the D* to D conversion which results from the energy required to create a boundary between D* and D sections of double helix (Dattagupta et al., 1980). Initial binding of propidium results in creation of the boundary, and other propidium molecules can bind in this region with a more favorable free energy. This results in the observed positive cooperativity for propidium binding to nonalternating DNA sequences and accounts for the large ω values seen at low temperature for this interaction (Table I). The quantitative model of Dattagupta et al. (1980) is the ideal method for analysis of allosteric transitions of this type. It does not at present allow for correction of potential end effects on the shape of oligomer binding isotherms, however, and the large number of variable parameters in the model requires extremely precise binding data for a unique fit to an isotherm to be obtained. Such very precise data are difficult to obtain for the weak binding nonalternating oligomers under low-temperature conditions. The apparent equilibrium constants obtained by fitting binding isotherms using eq 1 are, however, analogous to K_1 from the model of Dattagupta et al. (1980) and represent the most interesting binding constant, that for propidium binding to the unusual state of poly(dA·dT). For fitting purposes, all cooperative effects have been incorporated into the ω parameter of eq 1 to allow nonlinear least-squares analysis of the experimental results.

A most interesting question concerning the above mechanism is the following: What molecular properties account for the unusual features of D*? The cooperative transitions illustrated in the Scatchard plots of Figures 2 and 3 are quite similar to those observed for intercalators interacting with poly[d(G-C)$_2$] and similar polymers under conditions which result in the Z DNA conformation (Walker et al., 1985). The binding results with the Z form have been interpreted in terms of an intercalator-induced allosteric transition from the Z to the B conformation (Chaires, 1983b; Walker et al., 1985) and are explained by the model in eq 5 and 6. Arnott and co-workers (Arnott et al., 1982), based on fiber diffraction results, have suggested that poly(dA·dT) is in an unusual heteronomous double helix with the poly(dA) chain in an A-type helix and the poly(dT) chain in a B-type helix conformation. Recent NMR studies (Sarma et al., 1985; Behling & Kearns, 1986) have, however, failed to detect this unusual helical form and, in fact, have suggested that poly(dA·dT) is in a fairly standard B conformation. In high-resolution NMR studies of nonalternating A/T oligomer sequences, we have only been able to detect C-1′ sugar residues with coupling constants characteristic of the C-2′ endo sugar conformation (R. L. Jones and W. D. Wilson, unpublished results). Preliminary NOE measurements with these oligomers are also consistent with the C-2′ endo sugar and B helix conformation (R. L. Jones and W. D. Wilson, unpublished results). The intercalator-induced conversion of Z- to B-form DNA is quite slow (Mirau & Kearns, 1983) while propidium binding to convert D* to D and I has association kinetics which are much faster (Figure 8). These results suggest that while the nonalternating A/T sequence may have small structural variations relative to other B-type sequences, they are minor when compared to major conformational variations such as the Z-form double helix.

The thermodynamic results for the interaction of the intercalators propidium (Wilson et al., 1985b), daunorubicin (Chaires, 1983a,b; J. B. Chaires, personal communication), and the groove-binding compound netropsin (Marky et al., 1985) to poly(dA·dT) suggest another possible explanation for the D* to D conversion. All of these compounds bind to nonalternating A/T sequences with positive enthalpy (Figure 6) and positive entropy but bind to natural DNA, other polymers (Wilson et al., 1986a), and oligomers (Figure 6) in an enthalpy-driven process. These unusual thermodynamic results with nonalternating A/T sequences could be due to sections of highly structured solvent associated with D* which are released to a large extent in the conversion to D. This would account for the unusual thermodynamics and cooperativity in the Scatchard plots, the essentially B-form structure observed in NMR experiments (Sarma et al., 1985; Behling & Kearns, 1986; R. L. Jones and W. D. Wilson, unpublished results), and the relatively fast kinetics of the D* to D conversion (assuming that the small solvent molecules could undergo the order–disorder transition rapidly). Either the highly structured solvent or the associated slight distortions of the double helix could account for the unusual fiber diffraction patterns (Arnott et al., 1982) and unusual CD curves (Arnott, 1975) for poly(dA·dT). The bending of DNA observed in kinetoplasts contain nonalternating sequences (Wu & Crothers, 1984; Hagerman, 1984; Marini et al., 1984; Diekman & Wang, 1985) and suggested in propidium viscometric titrations of poly(dA·dT) (Wilson et al., 1985b) could be due to helix distortions where the nonalternating sequences join random sequence DNA with its normal hydration layer. Although release of excess bound counterions from poly(dA·dT) (relative to other sequences) could possibly account for the observed positive binding entropies, we feel that this is not the case because the effects of ionic strength on propidium binding to poly(dA·dT) and poly[d(A-T)$_2$] are very similar (Wilson et al., 1985b).

In natural DNA, sections of nonalternating DNA would generally be shorter than the dA$_{10}$·dT$_{10}$ oligomer. To accurately monitor the weak and cooperative binding properties of nonalternating A/T sequences, large amounts of noncooperative, strong binding alternating A/T sequences cannot be present. We, therefore, investigated propidium binding to d(A$_6$-T$_6$) which has two discontinuous sections of nonalternating A/T jointed by a single alternating base pair. The binding results with this oligomer are extremely unusual (Figure 5), and at 0 °C the binding isotherm can be resolved into two regions: (1) at low ν values, there is a sharply sloped region of apparently strong binding; and (2) at higher ν values, there are weaker sites which apparently bind propidium with positive cooperativity. One fact from these results is clear, the d(A$_6$-T$_6$) oligomer behaves most like the nonalternating A/T oligomer and polymer DNA samples in the magnitude of the binding constant, the positive binding enthalpy, and in association kinetics. One interpretation of the unusual d(A$_6$-T$_6$) propidium binding isotherm is that the single central alternating base pair is a stronger binding site for propidium

and causes the initial slope in the Scatchard plots seen at low temperature. The nonalternating end sections of the oligomer could then cooperatively bind propidium as in dA_{10}·dT_{10}. As the temperature is raised, the binding to the alternating site would become weaker and binding to the nonalternating site stronger, so that above 10 °C, no resolution of the two types could unequivocally be made. Although there are other possible explanations for the d(A_6-T_6) binding isotherm, there can be no question that this oligomer displays the unusual binding properties characteristic of larger sections of nonalternating A/T base pairs, and, therefore, very short sections of nonalternating A/T sequences in natural DNA could exert significant biological effects.

The observation that the unusual interaction properties can also occur in short DNA sections under physiological conditions suggests that these modified interactions could have a role in gene function. It has been observed, for example, that (1) nucleosomes will not form on sections of nonalternating A/T DNA (Simpson & Kunzler, 1979), (2) short repeating sections of nonalternating A/T base pairs in kinetoplast DNA result in apparent periodic bends in the double helix (Wu & Crothers, 1984; Marini et al., 1984), and (3) short sections of nonalternating A/T base pairs near potential nuclease cleavage sites can affect the rate of DNA scission at the enzyme site (Baralle et al., 1980; Drew et al., 1985). We are continuing high-resolution NMR studies and gel electrophoresis experiments with the A/T oligomers to evaluate these ideas in more detail.

ACKNOWLEDGMENTS

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Registry No. d(A-T)$_6$, 98921-76-7; poly[d(A-T)$_2$], 26966-61-0; dA_{10}·dT_{10}, 86029-40-5; poly(dA·dT), 24939-09-1; d(A_6-T_6), 104575-84-0; propidium iodide, 25535-16-4.

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