Petersheim, M. (1982) Ph.D. Thesis, University of Rochester.
Petersheim, M., & Turner, D. H. (1983) *Biochemistry 22*, 256–263.
Rawitscher, M. A., Ross, P. D., & Sturtevant, J. M. (1963) *J. Am. Chem. Soc. 85*, 1915–1918.
Rich, A., & Rajbhanday, T. (1976) *Annu. Rev. Biochem. 45*, 805–860.
Richards, E. G. (1975) in *Handbook of Biochemistry and Molecular Biology*: *Nucleic Acids* (Fasman, G. D., Ed.) 3rd ed., Vol. I, p 197, CRC Press, Cleveland, OH.
Ross, P. D., & Scruggs, R. L. (1965) *Biopolymers 3*, 491–496.
Salser, W. (1977) *Cold Spring Harbor Symp Quant. Biol. 42*, 985–1002.
Studnicka, G. M., Eiserling, F. A., & Lake, J. A. (1981) *Nucleic Acids Res. 9*, 1885–1904.
Suurkuusk, J., Alvarez, J., Freire, E., & Biltonen, R. (1977) *Biopolymers 16*, 2641–2652.
Tibanyenda, N., de Bruin, S. H., Haasnoot, C. A. G., van der Marel, G. A., van Boom, J. H., & Hilbers, C. W. (1984) *Eur. J. Biochem. 139*, 19–27.
Tinoco, I., Jr., Uhlenbeck, O. C., & Levine, M. D. (1971) *Nature (London) 230*, 362–367.
Traub, W., & Sussmann, J. L. (1982) *Nucleic Acids Res. 10*, 2701–2708.
Uhlenbeck, O. C., & Cameron, V. (1977) *Nucleic Acids Res. 4*, 85–98.
Zuker, M., & Stiegler, P. (1981) *Nucleic Acids Res. 9*, 133–148.

Poly(dA)·Poly(dT) Exists in an Unusual Conformation under Physiological Conditions: Propidium Binding to Poly(dA)·Poly(dT) and Poly[d(A-T)]·Poly[d(A-T)]†

W. David Wilson,* Yueh-Hwa Wang, C. R. Krishnamoorthy, and J. C. Smith
Department of Chemistry and Laboratory for Microbial and Biochemical Sciences, Georgia State University, Atlanta, Georgia 30303-3053

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ABSTRACT: The binding of propidium to poly(dA)·poly(dT) [poly(dA·dT)] and to poly[d(A-T)]·poly[d(A-T)] [poly[d(A-T)$_2$]] has been compared under a variety of solution conditions by viscometric titrations, binding studies, and kinetic experiments. The binding of propidium to poly[d(A-T)$_2$] is quite similar to its binding to calf thymus deoxyribonucleic acid (DNA). The interaction with poly(dA·dT), however, is quite unusual. The viscosity of a poly(dA·dT) solution first decreases and then increases in a titration with propidium at 18 °C. The viscosity of poly[d(A-T)$_2$] shows no decrease in a similar titration. Scatchard plots for the interaction of propidium with poly(dA·dT) show the classical upward curvature for positive cooperativity. The curvature decreases as the temperature is increased in binding experiments. A van't Hoff plot of the observed binding constants yields an apparent positive enthalpy of approximately +6 kcal/mol for the propidium–poly(dA·dT) interaction. Propidium binding to poly[d(A-T)$_2$] shows no evidence for positive cooperativity, and the enthalpy change for the reaction is approximately −9 kcal/mol. Both the magnitude of the dissociation constants and the effects of ionic strength are quite similar for the dissociation of propidium from poly(dA·dT) and from poly[d(A-T)$_2$], suggesting that the intercalated states are similar for the two complexes. The observed association reactions, under pseudo-first-order conditions, are quite different. Plots of the observed pseudo-first-order association rate constant vs. polymer concentration have much larger slopes for propidium binding to poly[d(A-T)$_2$] than to poly(dA·dT). These results are interpreted in terms of a fairly standard B conformation for poly[d(A-T)$_2$] which can bind propidium in a manner similar to DNA. Poly(dA·dT), however, must have some unusual structural features under normal conditions but can be converted to an intercalated B conformation with the unusual binding results described above. The polymer conformational transition is characterized by a large positive enthalpy and entropy values which result in a relatively small free-energy change for the transition.

The success of the Watson & Crick (1953) B-form model for deoxyribonucleic acid (DNA)[1] conformation in predicting biological and chemical properties of DNA has led to an ongoing analysis of DNA structure and possible structural changes. Early studies produced the related A, C, and D variations of the double helix (Arnott et al., 1982). More recently, the dramatically different left-handed Z-form helix (Wang et al., 1980) with its potential importance in gene expression (Nordheim et al., 1981) has attracted attention. Dickerson and co-workers (Dickerson et al., 1982), on the other hand, have shown that even random-sequence DNA can have structural variations which could serve as recognition signals in the biological functions of the nucleic acid.

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* Address correspondence to this author at the Department of Chemistry, Georgia State University.

[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-T)$_2$], poly[d(A-T)]·poly[d(A-T)]; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; 2D, two dimensional; NOE, nuclear Overhauser effect.

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Conformational variations which exist under physiological conditions and have unusual properties which could be important in such phenomena as nucleosome phasing, DNA packaging, and gene expression are of particular interest. Of the synthetic polymers containing the usual DNA base pairs, poly(dA·dT) has shown the greatest variety of unusual properties under standard solution conditions. The CD spectrum of this polymer cannot be predicted by using the summation rules which work well for other closely related double-helical polymers (Arnott, 1975). The Raman spectrum of this polymer has a band which suggests that, at least at low temperature, it contains a significant amount of the C3′ endo sugar conformation in contrast to the usual 2′ endo conformation of B-form DNA (Thomas & Peticolas, 1983). These results and X-ray fiber diffraction experiments have led Arnott and co-workers (Arnott et al., 1983) to propose that the secondary structure of poly(dA·dT) is heteronomous with standard Watson–Crick base pairing but with the C3′ endo sugar conformation on the poly(dA) chain and the C2′ endo conformation on the poly(dT) chain. It is known that under standard ionic and temperature conditions, DNA and polymers such as poly[d(A-T)$_2$] have an average of 10.6 base pairs per helical turn while poly(dA·dT) has 10.0 base pairs per turn (Klug et al., 1980). It has also been shown that nucleosomes will form on poly[d(A-T)$_2$] but not on poly(dA·dT), and, thus, segments of poly(dA·dT) may be of great importance in nucleosome phasing and packaging of DNA in chromatin (Kunkel & Martinson, 1981).

Unusual effects have also been found in the interaction of intercalators with poly(dA·dT). Most intercalators, such as ethidium, bind more weakly to poly(dA·dT) than to random-sequence DNA (Bresloff & Crothers, 1981). Strum (1982) has conducted a detailed study of the interaction of the intercalator tilorone with DNA and double-helical deoxypolynucleotides and has concluded that in contrast to the other polymers, tilorone does not intercalate with poly(dA·dT). He does conclude that there is a conformational change in poly(dA·dT) when tilorone binds (Strum, 1982). Chaires (1983) studied the binding of daunomycin to alternating- and nonalternating-sequence nucleotides and has also found that poly(dA·dT) is different from the other polymers whose binding is explained by a standard site-exclusion model. The binding of daunomycin to poly(dA·dT) exhibits positive cooperativity which Chaires (1983) interpreted in terms of a daunomycin-induced allosteric transition of the polymer between two conformations which bind the drug with significantly different equilibrium constants.

Because poly(dA·dT) displays its unusual properties under normal physiological conditions, this sequence is of obvious biological importance, and it is essential to understand its thermodynamic and kinetic properties in detail. In a continuing study of dicationic intercalators (Wilson & Lopp, 1979; Yen et al., 1982; Wilson et al., 1985), we have found propidium (structure in Figure 1) to be a particularly well-behaved model system for thermodynamic, kinetic (Wilson et al., 1985), and NMR (Chandrasekaran et al., 1985) studies of DNA interactions. We report here a kinetic, thermodynamic, and viscometric comparison of propidium binding to poly[d(A-T)$_2$] and poly(dA·dT) under a variety of temperature and ionic strength conditions. The inescapable conclusion from all of our experimental results is that poly(dA·dT) exists in an unusual conformation under physiological conditions. The conformation appears to be structurally similar to the standard DNA solution conformation but quite different from DNA in its binding properties. Poly[d(A-T)$_2$] interacts with propidium in a manner quite similar to random-sequence DNA.

Materials and Methods

Materials. Propidium iodide (Calbiochem) was prepared as previously described (Davidson et al., 1977). PIPES buffers used throughout these experiments contained 10 mM PIPES, 1 mM EDTA, and NaCl as follows: PIPES 00, no added NaCl; PIPES 03, 0.03 M NaCl; PIPES 10, 0.1 M NaCl; PIPES 20, 0.2 M NaCl; PIPES 30, 0.3 M NaCl; PIPES 50, 0.5 M NaCl. All were adjusted to pH 7.0. Poly[d(A-T)$_2$] and poly(dA·dT) were purchased from P-L Biochemicals and were analyzed by spectrophotometric, T_m (Wells et al., 1970), and ^{31}P NMR (Chen & Cohen, 1983) methods. The results agreed with the above literature values. The polymers were dissolved at approximately 1 mM stock solutions in the buffer of choice for a particular experiment.

Viscometric Titrations. Experiments were conducted in Cannon–Ubbelohde semimicrodilution viscometers (series 75) as previously described (Jones et al., 1980). Briefly, 1 mL of a polymer solution, approximately 2×10^{-4} M DNA phosphate groups, was placed in a viscometer, and the titration was conducted in a constant-temperature water bath (Cannon Instrument Co.) by adding aliquots of a propidium stock solution. The propidium additions were made directly into the polymer solution by using a Hamilton syringe modified to fit into the viscometer mixing chamber (Jones et al., 1980).

Spectrophotometric Measurements. Absorbance measurements in the UV–visible region were made on a Cary 219 spectrophotometer interfaced to an Apple IIe microcomputer through a bidirectional digital communications port. Cell holders were thermostated by using Neslab or Haake circulating water baths. Wavelength scans and extinction coefficient measurements were made in cells from 1- to 10-cm path length at the wavelength or wavelength range appropriate for the compound being investigated. Extinction coefficients of compounds bound to DNA were determined at the same wavelength as the extinction coefficient measurements of the free compound, but a large molar excess of DNA was present ([DNA-P]/[compound] > 100). The Cary 219 spectrophotometer was also used in spectrophotometric binding studies. To remove some of the random error, for each absorbance measurement in the absence or presence of DNA, the microcomputer calculated the average of 100 acquired absorbance readings at the preselected wavelength for the compound under study. These averaged absorbance values were converted by the microcomputer to ν (moles of compound bound per mole of DNA base pairs) and free ligand concentrations using the free and bound extinction coefficients for the compound (Wilson & Lopp, 1979). At the end of a titration, the computer plotted the digitalized data which were in the fraction-bound range 0.2–0.8. Any binding results outside of this range are subject to large systematic errors as a result of experimental errors in extinction coefficients. The computer then calculated nonlinear least-squares best fit K, n, and ω values from the site-exclusion method of McGhee & von Hippel (1974) as defined in eq 1 under Results.

Kinetic measurements were made by using an Aminco–Morrow stopped-flow apparatus adapted to a Johnson Foundation MB2 air turbine spectrophotometer. The output from the instrument was fed to an OLIS 3820 data acquisition system for storage on magnetic disk and subsequent analysis. Typically, several runs were stored and averaged before final analysis. Two hundred data points in a preselected time range could be fitted by using from one to three exponential curves. Fitting was done by using software supplied with the OLIS system and using a program based on the Marquardt–Le-

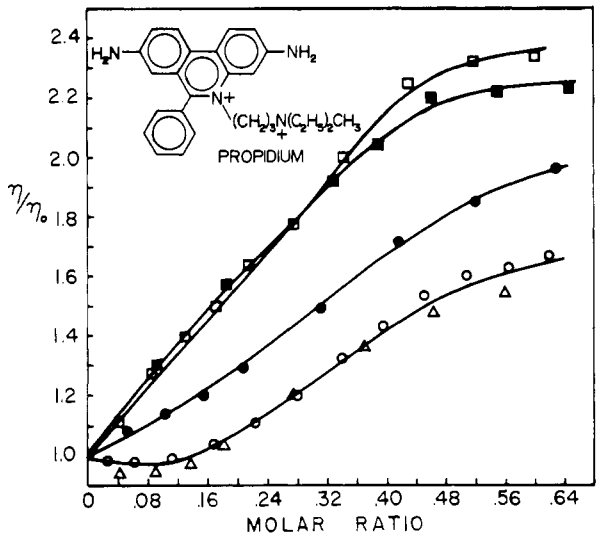

FIGURE 1: Viscometric titrations of poly[d(A-T)$_2$] with propidium at 26 (□) and 41 °C (■) and of poly(dA·dT) at 26 (○, △) and 46 °C (●). The titrations were conducted in PIPES 00 buffer, and the reduced specific viscosity ratio (η/η_0, specific viscosity of a polymer–propidium complex divided by the polymer specific viscosity) is plotted as a function of the molar ratio of propidium to polymer base pair concentration.

venberg algorithm (written and generously given to us by Professor R. H. Shafer, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA). Both methods gave the same results within the experimental error of the measurements. Dissociation experiments were conducted by mixing equal volumes (100 μL) of a propidium–polymer complex and a 1.0% sodium dodecyl sulfate solution. Dual wavelength difference spectra were obtained as a function of time by using the wavelength pair 469 and 612 nm. Typically, data collection was begun 3 ms after triggering the A/D converter due to the stopped-flow mixing event. The instrument time constant was set from 2 to 6 ms depending on the reaction rate. At the gain settings used in this experiment, the voltage to absorbance conversion factor on the attached traces is 0.01 absorbance unit to 50 mV. The zero voltage balance setting was typically set near the center of the difference between readings for the bound and dissociated propidium. Association kinetic measurements were conducted under pseudo-first-order conditions in a similar manner. Equal volumes (100 μL) of a polymer solution and a propidium solution were mixed under the desired conditions and data collected as above. Typically, five to eight runs of this type were averaged in the computer to improve the signal to noise ratio.

Results

Viscometric Titrations. Results for viscometric titration of both poly[d(A-T)$_2$] and poly(dA·dT) with propidium at two different temperatures are shown in Figure 1. Increases in viscosity are obtained in all four titrations as expected for intercalation (Wilson & Jones, 1981). The results for poly[d(A-T)$_2$] are not significantly different for the two temperatures and are also not significantly different from a titration of DNA at 26 °C (not shown). A greater effect of temperature is seen with poly(dA·dT). At 26 °C, the titration of poly(dA·dT) with propidium first results in a slight decrease in viscosity and then at a molar ratio of 0.15 to 0.2 begins to increase the polymer solution viscosity. At the higher temperature titration, the viscosity increases from the beginning and is consistently higher than the results for the lower temperature titration. The titrations level off in the molar ratio range of 0.5–0.6. The leveling off is more apparent for the poly[d(A-T)$_2$] polymer and for poly(dA·dT) at the higher temperature as expected for their stronger binding to be shown in the next section. The titration of poly(dA·dT) at low temperature was repeated with two different lots of the polymer, and the results did not show any significant difference (Figure 1).

Table I: Effect of Temperature on the Interaction of Propidium with Poly[d(A-T)$_2$] and Poly(dA·dT)[a]

temp (°C)	$K \times 10^{-3}$	n	ω
	Poly[d(A-T)$_2$]		
18	704	1.9	0.12
29	337	2.3	0.18
38	267	2.6	0.07
48	158	2.5	0.09
	Poly(dA·dT)		
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] Values are for nonlinear least-squares fits using eq 1 to the binding isotherms in Figure 2A,B. Experiments were conducted in PIPES 20 buffer, all constants are defined in DNA base pair units in eq 1, and concentrations are in molarity.

Spectrophotometric Titrations. Both polymers produced similar shifts in the propidium absorption, and the results were similar to those obtained on titration with DNA (Wilson et al., 1985). Free propidium has a wavelength maximum at 493 nm, and this shifts to 535 nm for propidium bound to DNA and the polymers. The extinction coefficient is 5920 M^{-1} cm^{-1} at 480 nm for free propidium and drops to 2020 M^{-1} cm^{-1} at the same wavelength for bound propidium. Using these extinction coefficients at 480 nm where the optimum changes were obtained, we converted spectrophotometric titration results at several temperatures to Scatchard plots (Wilson & Lopp, 1979; Wilson et al., 1985) which are shown for poly[d(A-T)$_2$] in Figure 2A and for poly(dA·dT) in Figure 2B. The solid lines through the data points in the figures are calculated by a nonlinear least-squares computer program using eq 1. where ν is the moles of propidium per polymer base

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

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

pair, c is the free propidium concentration, K is the intrinsic equilibrium 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 & vaon Hippel, 1974). Values from the fits in Figure 2 for K, n, and ω are collected in Table I for propidium binding to the two A·T polymers. Values for binding results at two additional temperatures for poly(dA·dT), which were not included in Figure 2 for the sake of clarity, are also included in Table I.

The observed equilibrium constants as a function of temperature are plotted according to the van't Hoff equation, eq 2, in Figure 3:

$$\ln K = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (2)$$

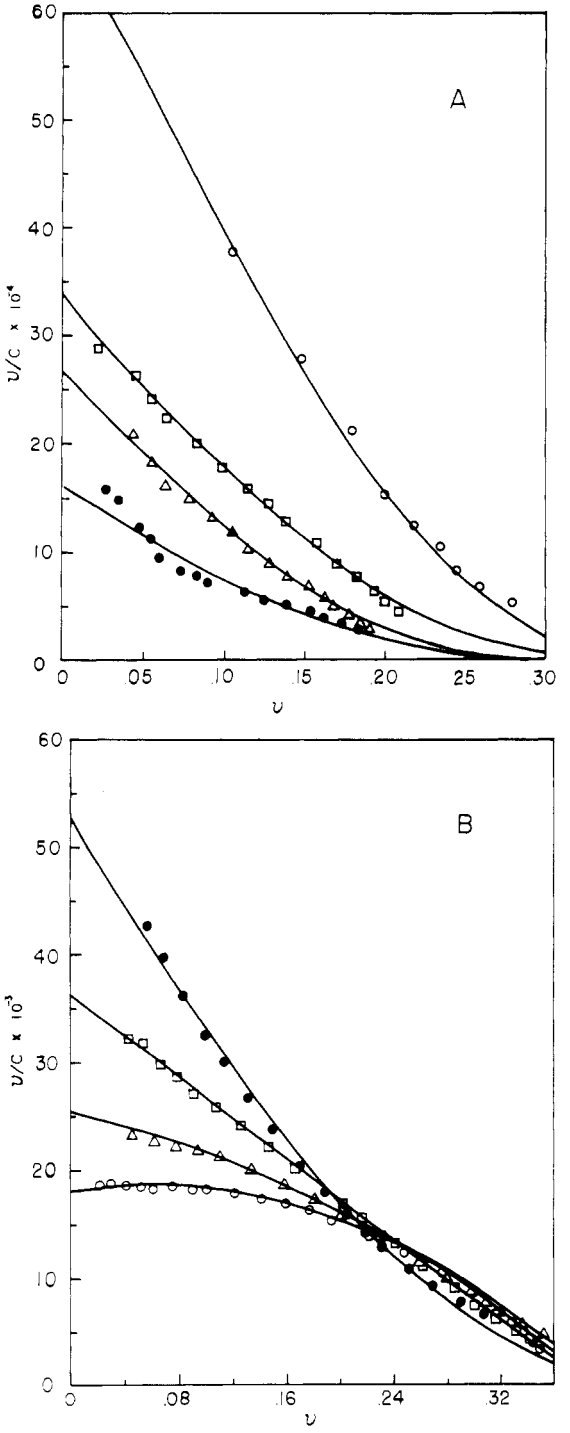

FIGURE 2: Scatchard plots for propidium binding to (A) poly[d(A-T)$_2$] at 18 (○), 29 (□), 38 (△), and 48 °C (●) and (B) poly(dA·dT) at 18 (○), 25 (△), 36 (□), and 48 °C (●). Experiments were conducted in PIPES 20 buffer as described under Materials and Methods. Points in the figure are experimental, and the smooth curves are nonlinear least-squares best fits using eq 1.

where $\Delta H°$ and $\Delta S°$ are the observed enthalpy and entropy changes, respectively, for the propidium–polymer binding reaction. The apparent enthalpy values calculated from the slopes of these lines are −8.9 kcal/mol for poly[d(A-T)$_2$] and +6.1 kcal/mol for poly(dA·dT).

The effect of salt concentration on the binding of propidium to poly(dA·dT) is shown in Figure 4A,B. Scatchard plots at four different ionic strengths and 18 °C are shown in Figure 4A. The points in the figure are experimental, and the solid lines are from best-fit values to the points using a nonlinear least-squares program with eq 1 as in Figure 2. The fitting

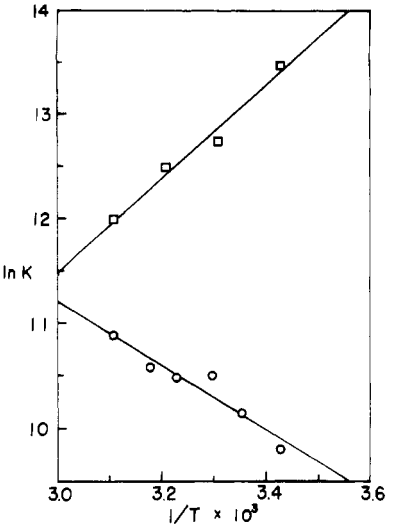

FIGURE 3: van't Hoff plot using the equilibrium constants and temperatures for poly[d(A-T)$_2$] (□) and poly(dA·dT) (○) from Figure 2 and Table I.

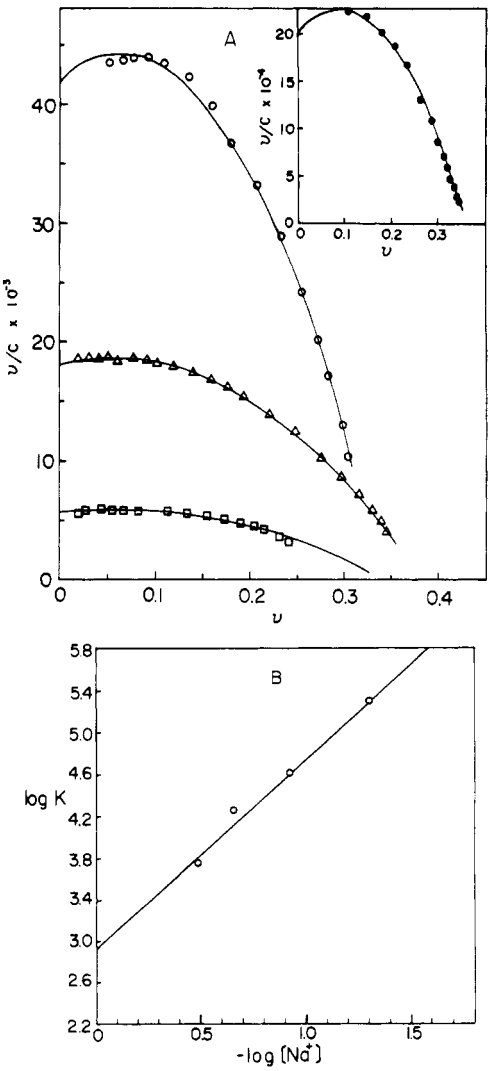

FIGURE 4: (A) Scatchard plots for the binding of propidium to poly(dA·dT) in PIPES 03 (●), PIPES 10 (○), PIPES 20 (△), and PIPES 30 (□) at 18 °C. The methods and best-fit values are as in Figure 2. (B) Plot of log K using the equilibrium constants from (A) and Table II as a function of −log [Na$^+$].

results are collected in Table II. The value of n is essentially constant, $n = 2.7 \pm 0.2$, as a function of salt. The ω value

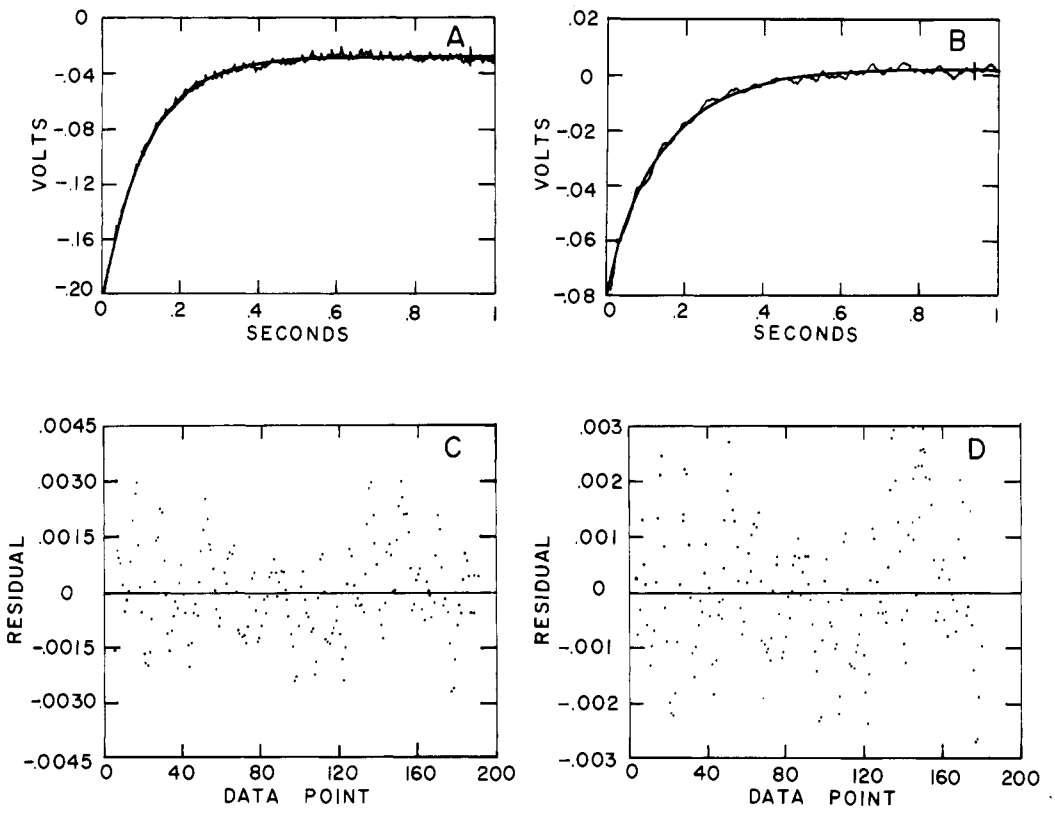

FIGURE 5: SDS-driven stopped-flow dissociation reactions at 15 °C in PIPES 20 buffer for propidium complexes with a 1 to 10 ratio of propidium to polymer base pairs with (A) poly[d(A-T)$_2$] and (B) poly(dA·dT). The smooth lines in panels A and B are the single-exponential nonlinear least-squares best-fit values to the experimental data. Panels C and D show residuals for single- and double-exponential best fits to the data of panel B, respectively. A total of 200 data points were collected in a 1-s time span as described under Materials and Methods, and the data points are consecutively connected in panels A and B for ease of visualization.

Table II: Effect of Ionic Strength on the Interaction of Propidium with Poly(dA·dT)[a]

buffer	$K \times 10^{-3}$	n	ω
PIPES 03	202	2.7	4.7
PIPES 10	42	2.9	4.4
PIPES 20	18	2.5	3.7
PIPES 30	5.6	2.8	4.2

[a] Values are for nonlinear least-squares fits to the curves in Figure 4A using eq 1. Experiments were conducted at 18 °C, all constants are defined in DNA base pair units in eq 1, and concentrations are in molarity.

shows a general small downward trend as ionic strength is increased, but this is close to the experimental error, and ω can be fit in all cases by $\omega = 4.2 \pm 0.5$. The observed equilibrium constant decreases markedly with increasing ionic strength as has been seen with propidium (Wilson et al., 1985) and other dications (Wilson & Lopp, 1979) binding to DNA. For highly charged polymers like DNA, the dependence of the observed equilibrium constant (K_{obsd}) on counterion concentration is given by eq 3 where m' depends on the number of

$$\frac{\delta \log K_{obsd}}{\delta \log [Na^+]} = -m'\psi - C \qquad (3)$$

ionic interactions found in the ligand–DNA complexes, ψ is a characteristic of the polymer which depends on the number of counterions thermodynamically associated with each charge unit on the polymer, and C depends on the change in associated counterions which occurs as a result of a polymer conformational change which is coupled to the binding of the ligand (Record et al., 1978; Wilson & Lopp, 1979). In most cases, the slope will be quite close to the number of ion pairs formed in the complex as has been found for the propidium–DNA interaction (Wilson et al., 1985). A plot of the observed equilibrium constants of Table II according to eq 3 is shown in Figure 4B. The slope of the plot is 1.8 compared to a slope of 2.0 for a similar set of results for calf thymus DNA (Wilson et al., 1985).

Kinetics. The spectral changes observed for propidium on binding to A·T polymers can also be used to follow the kinetics of binding (Wilson et al., 1985). Stopped-flow kinetic results are shown, for example, in Figure 5 for the SDS-driven dissociation of propidium from poly[d(A-T)$_2$] (Figure 5A) and from poly(dA·dT) (Figure 5B). The smooth lines in Figure 5A,B are nonlinear least-squares best-fit values for single-exponential fits to the kinetic results. Residual plots for single- and double-exponential fits to the results of Figure 5B are shown in panels C and D, respectively, of Figure 5. In no case where the fits to the results (correlation coefficient or distribution of residuals) significantly improved in going from a single- to a double-exponential fit. Similar results have been found for the propidium–DNA dissociation (Wilson et al., 1985). Experiments such as those illustrated in Figure 5A,B were repeated at several salt concentrations, and the log values of the single-exponential best-fit dissociation constants are plotted as a function of –log [Na$^+$] in Figure 6. The linear least-squares best-fit slopes for the lines in Figure 6 are 0.80 for poly(dA·dT) and 0.83 for poly[d(A-T)$_2$] as compared to a slope of 0.85 for a similar plot with calf thymus DNA (Wilson et al., 1985) and are, thus, identical within experimental error. The experiments above were conducted at a ratio of 1 propidium to 10 polymer base pairs. Changing the ratio from 1 to 40 had no effect within experimental error on the observed dissociation rate constants in PIPES 20 buffer. We have found with DNA that using half the SDS concentration, while increasing the salt concentration to maintain constant

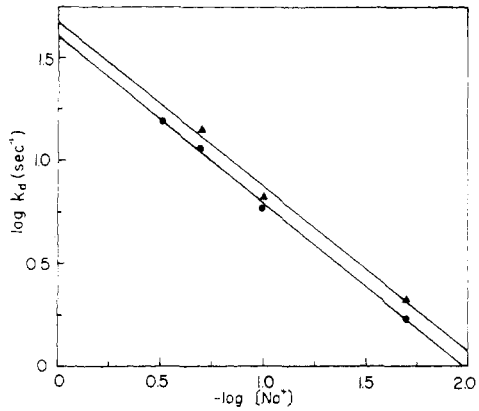

FIGURE 6: Plot of the observed dissociation rate constants vs. −log [Na^+] for poly[d(A-T)$_2$] (●) and poly(dA·dT) (▲) complexes with propidium. Experiments were performed at various ionic strengths in the same way as described in Figure 5. All results are at 20 °C in PIPES buffers. Rate constants for both poly[d(A-T)$_2$] and poly(dA·dT) were determined from single-exponential fits to dissociation decay curves as shown in panels A and B of Figure 5.

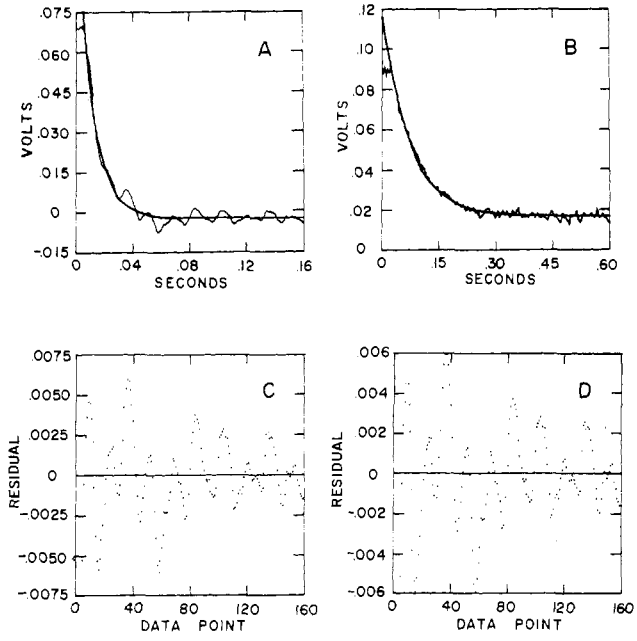

FIGURE 7: Stopped-flow kinetic traces of the association reactions of propidium with (A) poly[d(A-T)$_2$] and (B) poly(dA·dT). The concentrations after mixing were 5×10^{-5} M for both polymers (base pairs) and 5×10^{-6} M for propidium in PIPES 20 at 15 °C. The smooth lines in panels A and B represent the single-exponential nonlinear least-squares best-fit rate constants to the experimental data. Panel C shows the residuals for the single-exponential fit of panel A, and panel D shows the residuals for a double-exponential fit of the data in panel A. A total of 160 data points were collected and plotted in 0.16 and 0.6 s in panels A and B, respectively, as described in Figure 5.

sodium ion concentration, had no significant effect on the observed propidium dissociation constants (Wilson et al., 1985).

The association of propidium with DNA can also be followed directly by spectral changes as shown by the stopped-flow traces for poly[d(A-T)$_2$] and poly(dA·dT) in panels A and B, respectively, of Figure 7. The smooth lines in Figure 7A,B represent single-exponential best-fit values to the results as in Figure 5. Residual plots for single- and double-exponential best-fit values to the results in Figure 7A are shown in panels C and D, respectively, of Figure 7. The residual plots in both Figure 5 and Figure 7 are for the worst cases, and residual plots for other experiments have lower amplitude

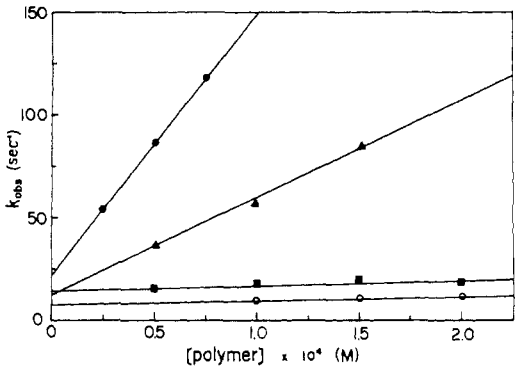

FIGURE 8: Observed pseudo-first-order association rate constants determined at 15 °C as in Figure 7 and plotted as a function of polymer concentrations (base pairs) for poly[d(A-T)$_2$] in PIPES 20 (●), poly[d(A-T)$_2$] in PIPES 50 (▲), poly(dA·dT) in PIPES 20 (■), and poly(dA·dT) in PIPES 50 (○) buffers. The straight lines correspond to linear least-squares fits to the points. The ratio of propidium to DNA was maintained constant in all of these experiments.

residuals. As with the dissociation results, no significant improvement in any of the association experiments is obtained on going from single- to double-exponential fits. Unlike the dissociation experiments, the association kinetic results indicate significant differences in observed rate constants for propidium binding to poly[d(A-T)$_2$] and poly(dA·dT). The observed pseudo-first-order association rate constant for poly[d(A-T)$_2$] in Figure 7A is 89.7 s^{-1}, for example, while the comparable value for poly(dA·dT) in Figure 7B is 13.9 s^{-1}. Experiments such as those illustrated in Figure 7A,B were repeated at several polymer concentrations and at two ionic strengths, and the results are plotted in Figure 8. The results for poly[d(A-T)$_2$] in 0.2 M salt are near the upper limit for our instrument and, therefore, have more error than the other experiments. The slopes in Figure 8 for poly[d(A-T)$_2$] are 12.5 × 10^5 and 4.7 × 10^5 M^{-1} s^{-1} at 0.2 and 0.5 M NaCl, respectively. These are only slightly greater than the values of 10.6 × 10^5 and 3.1 × 10^5 M^{-1} s^{-1} obtained for calf thymus DNA in a similar set of experiments (Wilson et al., 1985). Basically, the absolute results and the effect of salt on the association kinetics of propidium with poly[d(A-T)$_2$] and calf thymus DNA are quite similar. As can be seen in Figure 8, however, the association results with poly(dA·dT) are quite different. Although the trend in the results for poly(dA·dT) at both ionic strengths in Figure 8 is increasing with increasing DNA concentration (linear least-squares fits), the slopes could be zero within experimental error. A zero slope has been obtained in a similar plot for the tilorone association with poly(dA·dT), for example (Strum, 1982).

To investigate this point with more accuracy, we have followed the propidium association with poly(dA·dT) over a wider concentration range and at a higher temperature (25 °C) where the rate constants are larger and any trend, or lack thereof, in the slope should be more easily detected. The results of association experiments at 15 and 25 °C are compared in Figure 9. The plot at the higher temperature clearly illustrates a positive slope (1.8 × 10^5 M^{-1} s^{-1}) outside of experimental error. The best-fit linear least-squares slope for the results at 15 °C is 4.8 × 10^4 M^{-1} s^{-1}. Association of propidium with poly[d(A-T)$_2$] and with DNA is too fast to be followed over a significant polymer concentration range at 25 °C.

Discussion

The hydrodynamic, thermodynamic, and kinetic results illustrated in Figures 1–9 clearly show that there is a dramatic difference in the interaction of propidium with poly[d(A-T)$_2$]

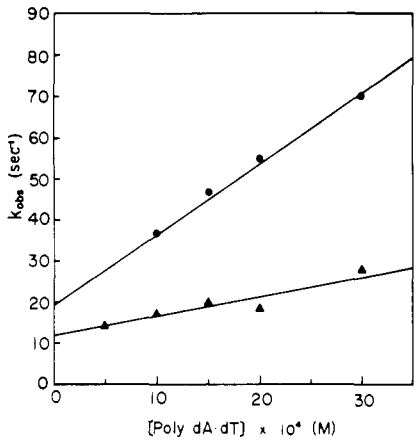

FIGURE 9: Observed pseudo-first-order rate constants for the association reaction of propidium with poly(dA·dT) determined in PIPES 20 and plotted as a function of poly(dA·dT) concentration (base pairs) at 15 (▲) and at 25 °C (●). The straight lines represent linear least-squares fits to the points.

and poly(dA·dT). Strum (1982) with tilorone, Chaires (1983) with daunomycin, and Bresloff & Crothers (1981) with ethidium have also shown that other intercalators bind quite differently with these two polymers. We have found similar differences with naphthalenediimide and anthraquinone derivatives (S.-F. Yen and W. D. Wilson, unpublished results). These results have suggested that the binding of intercalators to poly(dA·dT) is strongly cooperative and much weaker than with natural DNA or DNA polymers such as poly[d(A-T)$_2$] and poly[d(G-C)$_2$]. Strum (1982) interpreted the results with tilorone to indicate that tilorone did not intercalate with poly(dA·dT). Chaires (1983), on the other hand, suggested that daunomycin did intercalate with poly(dA·dT) but shifted the polymer from one conformation to another to which it could bind more favorably. The allosteric conformational model of Dattagupta et al. (1980) could then account for the observed cooperativity in binding (Chaires, 1983).

The similar spectral changes and the viscosity increases at high ratios obtained for propidium binding to poly[d(A-T)$_2$] and poly(dA·dT) strongly suggest that propidium binds to both polymers by intercalation. The differences in binding of propidium to these two polymers can be explained most simply by the following mechanism:

$$D^* \rightleftarrows D \tag{4}$$

$$D + L \underset{k_d}{\overset{k_a}{\rightleftharpoons}} I \tag{5}$$

where D represents a binding site in the normal average solution conformation of DNA, D* represents a binding site in a different DNA conformation to which propidium binds much more weakly, and L is a ligand such as propidium which can form a complex, I, with a binding site on natural DNA or a synthetic polymer. Binding of propidium to calf thymus DNA, poly[d(A-T)$_2$], and probably most other DNA samples under standard solution conditions can be explained by eq 5. Binding of propidium to poly(dA·dT), however, must include the additional step in eq 4 because of the unusual state of poly(dA·dT) under normal conditions. The ω value from eq 1 is high for propidium binding to poly(dA·dT) then because propidium binding in regions where the D* to D transition has occurred would be favored. At low ν values, this would lead to regions on the polymer rich in propidium and other regions free of propidium.

There is considerable evidence to support this mechanism. First, as stated above, CD studies (Arnott, 1975), measurements of the number of base pairs per turn of the double helix in solution (Klug et al., 1980), X-ray fiber diffration (Arnott et al., 1983), Raman spectroscopy (Thomas & Peticolas, 1983), and nucleosome reconstitution experiments (Kunkel & Martinson, 1981), in addition to the intercalator binding results discussed above, have all suggested that poly(dA·dT) is in an unusual conformation under normal solution conditions. Poly[d(A-T)$_2$], on the other hand, behaves much more like random-sequence DNA in these experiments, and recent 2D NOE experiments with this polymer have suggested that it is in a fairly standard B conformation in solution under normal conditions (Assa-Munt & Kearns, 1984). The viscosity results, shown in Figure 1, with poly[d(A-T)$_2$] are very similar to those obtained with a wide variety of intercalators with natural DNA (Wilson & Jones, 1981). The unusual viscosity results obtained with poly(dA·dT) suggest that propidium does intercalate but that the binding may drive an additional conformational change in the polymer as illustrated in eq 4. The temperature effects on the viscometric titrations suggest that the cooperativity of the transition may decrease as the temperature increases, and this is supported by the Scatchard plots as a function of temperature in Figure 2B. The initial decrease in viscosity with poly(dA·dT) at low temperature could be the result of DNA bends or kinks at conformational junctions betweed D* and D at low levels of propidium bound. At higher levels, the conformation would be mostly or all D (standard B-form DNA), and addition of propidium would cause an increase in viscosity. This argument is supported by the recent finding by Wu & Crothers (1984) that trypanosome kinetoplast DNA has a fixed region where the DNA has a static bend which can be detected by methods such as gel electrophoresis. The sequence in this bent region contains repeated units of nonalternating dA·dT base pairs 5–6 base pairs long which are separated by random (dG + dC)-rich regions (Wu & Crothers, 1984). All of these results suggest that when poly(dA·dT) in its unusual conformation joins a standard B-form helix [created by an intercalator in poly(dA·dT) or a random-sequence section of DNA] a bend is created.

The propidium binding isotherms with poly[d(A-T)$_2$] (Figure 2A) are quite similar to those obtained with calf thymus DNA. The observed binding constant for propidium with poly[d(A-T)$_2$] and natural DNA is for the reaction in eq 5. The observed binding constant for propidium with poly(dA·dT), on the other hand, is the product of the equilibrium constants for the reactions in eq 4 and 5. The enthalpy value obtained from Figure 3 for propidium binding to poly[d(A-T)$_2$] is quite similar to the values obtained for several common intercalators (Wilson & Jones, 1982). The observed apparent positive enthalpy for propidium binding to poly(dA·dT) is quite unusual but includes contributions from enthalpies for both reactions shown in eq 4 and 5. The apparent equilibrium constant and enthalpy for the reactions of eq 4 and 5 are given by

$$K_{app} = \frac{K}{1/K^* + 1} \tag{6}$$

and

$$\Delta H_{app} = \Delta H^\circ + f^* \Delta H^* \tag{7}$$

where K^*, ΔH^* and K, ΔH° represent equilibrium constants and enthalpy changes for eq 4 and 5, respectively, and f^* represents the fraction of the DNA sample initially present as D*. If it is assumed that the enthalpies for binding of propidium to poly[d(A-T)$_2$] and poly(dA·dT) are approxi-

mately the same and that f^* for poly[d(A-T)$_2$] is zero, then ΔH° is approximately −9 kcal/mol. In the same manner, if it is assumed that K for eq 5 is approximately the same for propidium binding to poly[d(A-T)$_2$] and poly(dA·dT), then K^* is small and f^* is approximately 1 for poly(dA·dT). Using these values for f^* and ΔH° and the value of ΔH_{app} for the propidium–poly(dA·dT) reaction from Figure 3 gives a values of ΔH^* of approximately +15 kcal/mol. This large positive enthalpy is partially offset by a large positive entropy for the conformational transition which leads to a relatively small (+1 to +2 kcal) free energy for the transition of eq 4 over the temperature range 20–40 °C. Even if the value of K for eq 5 for propidium binding to poly(dA·dT) is less than the K value for poly[d(A-T)$_2$], the thermodynamic values for the two polymers are so different that f^* will remain over 0.9 for a large range of variations in K and the above qualitative conclusions will remain correct.

These results indicate that poly(dA·dT) is in an unusual conformation under standard solution and temperature conditions. Propidium binds to this unusual conformation weakly or not at all. Addition of propidium to poly(dA·dT) thus results in a shift of the conformation equilibrium to a more standard B conformation to which propidium can bind in a fairly standard intercalation complex. With this model, it is possible to make some general predictions about the kinetics associated with the mechanism of eq 4 and 5. First, the association reaction, which depends on the conformational rearrangement of eq 4, will be greatly perturbed relative to propidium binding to poly[d(A-T)$_2$] and DNA. Second, in the SDS-driven dissociation of propidium from both polymers, the observed step in the experiment is the dissociation reaction in eq 5 with dissociation rate constant k_d. The mechanism predicts that dissociation occurs from similar intercalated complexes with both poly[d(A-T)$_2$] and poly(dA·dT). The conformational rearrangement of poly(dA·dT) cannot be observed in this dissociation because of the design of the experiment. The predicted similarity of dissociation of propidium from the two polymers is observed experimentally as shown in Figures 5 and 6. Both the magnitude of the observed dissociation rate constants and the effects of counterions are the same within experimental error. These results and the similar spectral changes obtained on binding suggest that the interactions of propidium with alternating and nonalternating base pairs in the double-helical polymers are quite similar.

The dramatic difference in the interaction of propidium with these two A·T polymers must then be due to differences in the association reactions. This is predicted by the mechanism in eq 4 and 5 and is observed experimentally with the association rate constants from Figures 7–9. The behavior of propidium with poly[d(A-T)$_2$] as a function of polymer concentration (Figure 8) is typical for a second-order association and is quite similar to the propidium association reaction with calf thymus DNA (Wilson et al., 1985). Both the magnitude of the association constants and salt effects are similar to those observed for association with DNA. All observed thermodynamic and kinetic constants for the interaction of propidium with poly[d(A-T)$_2$] and calf thymus DNA are, therefore, quite similar.

The two-step association predicted for propidium with poly(dA·dT) has two limiting cases depending on the rate of the conformational rearrangement in eq 4 relative to the intercalation reaction in eq 5. If the conformational change is the rate-limiting step, this can lead to an observed relaxation time which is independent of the polymer concentration (Eigen, 1968). Strum (1982) has suggested that this is the case for the binding of tilorone to poly(dA·dT). Alternatively, the conformational change may be faster than the binding step leading to a preequilibrium concentration of D in eq 4. The reaction would then appear to be a typical second-order association except that the slope in plots such as those shown in Figure 8, which is usually the second-order rate constant, would be multiplied by the equilibrium constant for eq 4, K^*.

The slopes for the plots for the reaction of propidium with poly(dA·dT) in Figure 8 are positive at both salt concentrations, but a zero slope falls within the experimental error of the method under both conditions, and it is, therefore, not possible from these results to confidently choose between the two limiting cases listed above. For this reason, the association of propidium with poly(dA·dT) was also conducted at a higher temperature and over a wide concentration range to determine the slope with more confidence (Figure 9). At the higher temperature, it can be seen that the slope is positive well outside of the error range. This suggests that the observed positive slope at lower temperature is also correct. These results also suggest that the preequilibrium mechanism is the correct case for the propidium–poly(dA·dT) interactions and that under our conditions the conformational equilibrium in eq 4 is quite fast. Preliminary ^{31}P NMR studies of the interaction of propidium with poly(dA·dT) have also indicated a fast exchange between the intercalated and nonintercalated phosphate conformation on titration of poly(dA·dT) with propidium (R. L. Jones and W. D. Wilson, unpublished results). The Z to B conformational change on binding intercalators is much slower (Mirau & Kearns, 1983), and this indicates that the D* conformation is much closer to the intercalated B form and involves a much less drastic rearrangement of the double-helix backbone than the B to Z transition.

If the preequilibrium model is correct and it is assumed that the association of propidium with both poly(dA·dT) and poly[d(A-T)$_2$] in the B conformation has the same association constant, K^* can be calculated at 15 °C from the ratios of observed association constants from the slopes of poly(dA·dT) in Figure 9 and poly[d(A-T)$_2$] in Figure 8 [the slope for the poly[d(A-T)$_2$] plot would be k_a, and the slope for the poly(dA·dT) plot would be k_aK^*]. The value of K^* at 15 °C calculated in this way is 0.038. K^* can also be calculated from the thermodynamic results in Table I if it is also assumed that the binding constant for propidium is the same for the B conformations of poly(dA·dT) and poly[d(A-T)$_2$]. Using the 18 °C results gives a value of 0.026 for K^*. Considering the experimental error in the different experiments, these two K^* values are in excellent agreement and lend further support to the preequilibrium model and to the idea that the propidium interactions with the two polymers in the intercalated conformations are quite similar.

As can be seen from Figure 3, the free-energy difference for propidium binding to poly(dA·dT) and poly[d(A-T)$_2$] under physiological conditions is only 1–2 kcal, depending on the temperature. This relatively small free-energy change results from a large positive enthalpy and a large positive entropy change for the D* to D transition. Arnott et al. (1982) have suggested that poly(dA·dT) has the dA chain sugar in the C3′ endo sugar conformation with the dT chain in the usual C2′ endo conformation. It is difficult, however, to see how sugar conformational transitions could lead to the observed enthalpy and entropy changes for the D* to D transition. Another possibility, at least under our conditions, is that poly(dA·dT) has a significant amount of structured, hydrogen-bonded, water associated with it in the D* conformation. Dickerson and co-workers (Dickerson et al., 1982) have pro-

posed that A·T base pairs have a significant amount of structured water in the minor groove but that would not distinguish between poly(dA·dT) and poly[d(A-T)$_2$] unless the minor groove water were bound much more tightly in poly(dA·dT) than in poly[d(A-T)$_2$]. A more structured solvent layer in poly(dA·dT) relative to DNA and poly[d(A-T)$_2$] which is released on binding ligands such as propidium could explain the unusual thermodynamics of propidium binding. The solvent release could also occur quite rapidly and account for the predicted fast kinetics for the D* to D transition. It is more difficult to see how bound water could lead to the unusual CD and Raman spectra and base pair per turn discussed above for poly(dA·dT). It is quite possible that solvent interactions could be accompanied by minor conformational shifts in poly(dA·dT) to give a modified B conformation with highly ordered bound water molecules. More detailed studies of the conformation of poly(dA·dT) under a variety of conditions and in different flanking sequences are clearly needed to answer these questions.

It will also be of interest to determine what importance this unusual structure for poly(dA·dT) has in the biological function of nucleic acids. It is known that poly(dA·dT) sequences frequently occur in both 5′- and 3′-terminal regions of genes (Schmidt, 1984; Kunkel & Martinson, 1981) and may, thus, have significance for both initiation and termination of transcription. The fact that nucleosomes will not form on poly(dA·dT) has been documented, and dA·dT segments may be important in chromatin ordering and structure (Kunkel & Martinson, 1981). Sequences of dA·dT three to seven residues long are also resistant to cleavage by mung bean nuclease in a PZM DNA early denaturation region where extensive nuclease digestion occurs (Sheflin & Kowalski, 1984). It is possible that poly(dA·dT) repels binding of many molecules and that dA·dT segments in chromosomes occur in fairly free states and serve useful structural roles and as markers for genes. It is obvious that intercalators such as propidium are useful probes for unusual DNA conformations such as that of poly(dA·dT).

Registry No. Poly(dA)·poly(dT), 24939-09-1; poly[d(A-T)], 26966-61-0; propidium iodide, 25535-16-4.

References

Arnott, S. (1975) *Nucleic Acids Res. 2*, 1493–1502.

Arnott, S., Chandrasekaran, R., Hall, I. H., Puigjaner, L. C., Walker, J. K., & Wang, M. (1982) *Cold Spring Harbor Symp. Quant. Biol. 47*, 53–65.

Arnott, S., Chandrasekaran, R., Hall, I. H., & Puigjaner, L. C. (1983) *Nucleic Acids Res. 11*, 4141–4155.

Assa-Munt, N., & Kearns, D. R. (1984) *Biochemistry 23*, 791–796.

Bresloff, J. L., & Crothers, D. M. (1981) *Biochemistry 20*, 3537–3553.

Chaires, J. B. (1983) *Biochemistry 22*, 4204–4211.

Chandrasekaran, S., Jones, R. L., & Wilson, W. D. (1985) *Biopolymers* (in press).

Chen, C-W., & Cohen, J. S. (1983) *Biopolymers 22*, 879–893.

Dattagupta, N., Hogan, M., & Crothers, D. M. (1980) *Biochemistry 19*, 5998–6005.

Davidson, M. W., Griggs, B. G., Lopp, I. G., & Wilson, W. D. (1977) *Biochim. Biophys. Acta 479*, 378–390.

Dickerson, R. E., Drew, H. R., Conner, B. N., Wing, R. M., Fratini, A. V., & Kopka, M. L. (1982) *Science (Washington, D.C.) 216*, 475–485.

Eigen, M. (1968) *Q. Rev. Biophys. 1*, 3–33.

Jones, R. L., Lanier, A. C., Keel, R. A., & Wilson, W. D. (1980) *Nucleic Acids Res. 8*, 1613–1624.

Klug, A., Rhodes, D., Smith, J., Finch, J. T., & Thomas, J. O. (1980) *Nature (London) 287*, 509–516.

Kunkel, G. R., & Martinson, H. G. (1981) *Nucleic Acids Res. 9*, 6869–6888.

McGhee, J. D., & van Hippel, P. H. (1974) *J. Mol. Biol. 86*, 469–489.

Mirau, P. A., & Kearns, D. R. (1983) *Nucleic Acids Res. 11*, 1931–1941.

Nordheim, A., Pardue, M. L., Lafer, E. M., Möller, A., Stollar, D., & Rich, A (1981) *Nature (London) 294*, 417–422.

Record, M. T., Jr., Anderson, C. F., & Lohman, T. M. (1978) *Q. Rev. Biophys. 11*, 103–178.

Schmidt, E. R. (1984) *J. Mol. Biol. 178*, 1–15.

Sheflin, L. G., & Kowalski, D. (1984) *Nucleic Acids Res. 12*, 7087–7104.

Strum, J. (1982) *Biopolymers 21*, 1189–1206.

Thomas, G. A., & Peticolas, W. L. (1983) *J. Am. Chem. Soc. 105*, 993–996.

Wang, A., Quigley, G. J., Kolpak, F. J., van der Marel, G., van Boom, J. H., & Rich, A. (1980) *Science (Washington, D.C.) 211*, 171–176.

Watson, J. D., & Crick, F. (1953) *Nature (London) 171*, 737–738.

Wells, R. D., Larson, J. E., Grant, R. C., Shortle, B. E., & Cantor, C. R. (1970) *J. Mol. Biol. 54*, 465–497.

Wilson, W. D., & Lopp, I. G. (1979) *Biopolymers 18*, 3025–3041.

Wilson, W. D., & Jones, R. L. (1981) *Adv. Pharmacol. Chemother. 18*, 177–222.

Wilson, W. D., & Jones, R. L. (1982) in *Intercalation Chemistry* (Whittingham, M. S., & Jacobson, A. J., Eds.) pp 445–501, Academic Press, New York.

Wilson, W. D., Krishnamoorthy, C. R., Wang, Y.-H., & Smith, J. C. (1985) *Biopolymers* (in press).

Wu, H.-M., & Crothers, D. M. (1984) *Nature (London) 308*, 509–513.

Yen, S.-F., Gabbay, E. J., & Wilson, W. D. (1982) *Biochemistry 21*, 2070–2076.