A Premelting Conformational Transition in Poly(dA)–Poly(dT) Coupled to Daunomycin Binding†

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ABSTRACT: Circular dichroism and UV absorbance spectroscopy were used to monitor and characterize a premelting conformational transition of poly(dA)–poly(dT) from one helical form to another. The transition was found to be broad, with a midpoint of t_m = 29.9 °C and ΔH_{VH} = +19.9 kcal mol^{-1}. The transition renders poly(dA)–poly(dT) more susceptible to digestion by DNase I and facilitates binding of the intercalator daunomycin. Dimethyl sulfoxide was found to perturb poly(dA)–poly(dT) structure in a manner similar to temperature. These combined results suggest that disruption of bound water might be linked to the observed transition. A thermodynamic analysis of daunomycin binding to poly(dA)–poly(dT) shows that antibiotic binding is coupled to the polynucleotide conformational transition. Daunomycin binding renders poly(dA)–poly(dT) more susceptible to DNase I digestion at low bindng ratios, in contrast to the normal behavior of intercalators, indicating that antibiotic binding alters the conformation of the polynucleotide. The unusual thermodynamic profiles previously observed for the binding of many antibiotics to poly(dA)–poly(dT) can be explained by our results as arising from the coupling of ligand binding to the polynucleotide conformational transition. Our data further suggest a physical basis for the temperature dependence of DNA bending.

The structure and solution properties of poly(dA)–poly(dT) are of intense current interest, in part because of the involvement of oligo(dA)–oligo(dT) tracts in DNA bending (Wu & Crothers, 1984; Hagerman, 1984; Olson et al., 1988). Oligo(dA)–oligo(dT) tracts have been shown to occur in vivo with a nonrandom frequency and may be an important feature of regulatory DNA sequences (Struhl, 1985; Plaskon & Wartell, 1987; Behe, 1987). DNA bending may be important in the specific binding of regulatory proteins to DNA [see reviews by Widom (1985) and Olson et al. (1988)].

Poly(dA)–poly(dT) has an unusual solution structure compared to standard B-form DNA. Its helical periodicity in solution (10 bp/pitch) is markedly different from the standard value of 10.6 bp/pitch observed for other DNAs (Rhodes & Klug, 1981; Peck & Wang, 1981). Poly(dA)–poly(dT) is thermodynamically more stable than poly(dA-dT)–poly(dA-dT) (Wartell, 1972; Marky & Breslauer, 1982; Nakanishi et al., 1984). The spectral properties of poly(dA)–poly(dT) are unique (Arnott, 1975; Edmondson & Johnson, 1985; Edmondson, 1987; Thomas & Peticolas, 1983; Jolles et al., 1985; Katahira et al., 1986; Wartell & Harrell, 1986; Sarma et al., 1985; Behling & Kearns, 1986; Roy et al., 1987), and its persistence length is different from standard B-form DNA (Hogan et al., 1983; Theveney et al., 1988). The unique solution structure of poly(dA)–poly(dT) renders it immunogenic, and antibodies raised against poly(dA)–poly(dT) will not recognize standard B-form DNA (Diekmann & Zarling, 1987).

Fiber diffraction studies of poly(dA)–poly(dT) at low (77%) relative humidity led to the "heteronomous" model for its conformation, in which the poly(dA) strand of the polynucleotide was in the A conformation, while the poly(dT) strand was in the B form (Arnott et al., 1983). However, NMR and Raman spectroscopic studies in solution are inconsistent with the heteronomous model (Wartell & Harrell, 1986; Sarma et al., 1985; Behling & Kearns, 1986; Roy et al., 1987). More recent fiber diffraction results obtained from samples at higher relative humidity (>80%) show that both strands of poly(dA)–poly(dT) are B-like but that the conformation of the polynucleotide is still different from standard B DNA, primarily in the orientation of the bases and in the width of the minor groove (Park et al., 1987; Alexeev et al., 1987). Crystal structures of oligo(dA)–oligo(dT) tracts within dodecanucleotide duplexes have recently been reported (Coll et al., 1987; Nelson et al., 1987) and provide the most detailed insight into the structure of poly(dA)–poly(dT). The special features of poly(dA)–poly(dT) observed in these structures include (i) a high propeller twist in the base pairs, resulting in maximal base stacking interactions, (ii) a novel system of bifurcated hydrogen bonds, and (iii) a narrow minor groove.

Molecular mechanics calculations suggest that the structures of poly(dA)–poly(dT) in the condensed form and in solution are similar, based on a correlation of NMR and fiber diffraction studes (Behling et al., 1987; Lipanov & Chuprina, 1987). A notable result from these calculations is the identification of a spine of hydration as a unique feature of poly(dA)–poly(dT) structure (Chuprina, 1987). The spine of hydration stabilizes the alternative B-form conformation of poly(dA)–poly(dT) and results in a narrowed minor groove (Chuprina, 1987).

The unique structure of poly(dA)–poly(dT) correlates with its limited conformational flexibility. Poly(dA)–poly(dT) cannot be reconstituted into nucleosomes (Rhodes, 1979) nor will nucleosomes form on oligo(dA)–oligo(dT) tracts within recombinant DNA fragments (Kunkel & Martinson, 1981). Poly(dA)–poly(dT) will not undergo the B to A transition (Pilet et al., 1975; Arnott et al., 1974). The condensation properties of poly(dA)–poly(dT) are unusual, and the polynucleotide will condense only to the ψ^+ form regardless of the solvent conditions used to effect condensation (Chaires, 1988).

Another manifestation of the unusual solution structure of poly(dA)–poly(dT) is anomalous antibiotic binding to the polymer. Ethidium (Bresloff, 1974; Bresloff & Crothers,

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1981), tilorone (Sturm, 1982), daunomycin (Chaires, 1983), netropsin (Marky et al., 1985), and propidium (Wilson et al., 1985, 1986; Jones et al., 1986) all have shown unusual binding to poly(dA)–poly(dT). Unique features of these antibiotic/poly(dA)–poly(dT) interactions include binding isotherms with pronounced positive cooperativity (Chaires, 1983; Wilson et al., 1985) and thermodynamic binding profiles indicative of an entropically driven association reaction (Wilson et al., 1985; Marky et al., 1985; Breslauer et al., 1987). Two views are offered to explain these effects. The cooperative binding of daunomycin to poly(dA)–poly(dT) was explained (Chaires, 1983) by invoking the theory of allosteric interactions developed by Crothers and co-workers (Dattagupta et al., 1980), in which antibiotic binding is coupled to a conformational transition in the DNA, with preferential binding to one of the two possible conformations. Similarly, Wilson et al. (1985) proposed a "preequilibrium" model to account for the unusual binding of propidium to poly(dA)–poly(dT). A second view has emphasized the unusual hydration of poly(dA)–poly(dT) and has hypothesized that water release is coupled to antibiotic binding, giving rise to entropically driven antibiotic association reactions (Wilson et al., 1985; Marky et al., 1985; Breslauer, 1987). These two points of view are not necessarily mutually exclusive, given the close connection between hydration and conformation delineated in the molecular calculations (Chuprina, 1987). The molecular basis of cooperative antibiotic binding to poly(dA)–poly(dT) and the origins of entropically driven antibiotic binding to the polymer remain, however, incompletely described (Wilson, 1987). The major goal of the work described here is to explain these phenomena.

We report here the thermodynamic characterization of a premelting conformational transition in poly(dA)–poly(dT). The endothermic transition we observe is not highly cooperative, results in pronounced changes in the UV absorbance and circular dichroism of poly(dA)–poly(dT), and renders the polynucleotide more susceptible to digestion by deoxyribonuclease I (DNase I).[1] The conformational change facilitates the binding of daunomycin to the polynucleotide. A thermodynamic analysis of the interaction of daunomycin with poly(dA)–poly(dT) shows that antibiotic binding is coupled to the premelting conformational transition in the polynucleotide. Our results illustrate the conformational polymorphism of poly(dA)–poly(dT) and support the previously proposed allosteric model to explain the cooperative binding of antibiotics to the polynucleotide (Chaires, 1983). The conformational transition in poly(dA)–poly(dT) also provides a physical basis for the temperature dependence of DNA bending, previously observed (Koo et al., 1986; Diekmann, 1987) but unexplained.

Materials and Methods

Polydeoxynucleotides. Poly(dA)–poly(dT) and poly(dA-dT)–poly(dA-dT) were purchased from Sigma Chemical Co. (St. Louis, MO) or Pharmacia, Inc. (Piscataway, NJ), and were used without further purification. Samples were dissolved in BPES buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 0.185 M NaCl, and 1 mM Na_2EDTA, pH 7.0) and dialyzed for 24 h against the same buffer before use. Polydeoxynucleotide concentrations (in terms of base pairs) were determined by absorbance, using ϵ_{260nm} = 12 000 M^{-1} cm^{-1} for poly(dA)–poly(dT) and ϵ_{262nm} = 13 200 M^{-1} cm^{-1} for poly(dA-dT)–poly(dA-dT).

UV/Vis Absorbance Measurements. All absorbance measurements were made by using a Cary 219 UV/Vis recording spectrophotometer, equipped with a temperature readout accessory. The temperature was regulated by using a Neslab circulating water bath, equipped with a Neslab ETP 4RC temperature programmer and a Neslab DCR1 digital controller/readout.

Circular Dichroism Measurements. CD spectra were recorded on a Jasco J500A spectropolarimeter, interfaced to and controlled by an IBM PC computer. Sample temperatures were regulated by using jacketed cuvettes attached to a Polyscience Polytemp circulating water bath. The temperature at the cuvette was monitored by a digital thermometer. The molar ellipticity was calculated from the equation $[\theta] = 100\theta/Cl$, where θ is the measured ellipticity in degrees, C is the polydeoxynucleotide concentration in base pairs, and l is the path length in centimeters.

Digestion by Deoxyribonuclease I. The susceptibility of poly(dA)–poly(dT) and poly(dA-dT)–poly(dA-dT) to digestion by DNase I was determined by the procedures of Kunitz (1950). The initial rate of digestion was monitored by the absorbance at 260 nm using a Cary 219 spectrophotometer. Typical assay mixtures contained 24 μM (bp) polydeoxyribonucleotide in BPES buffer + 5 mM $MgCl_2$ in a volume of 0.5 mL. The reaction was initiated by the addition of a small aliquot of stock DNase I solution (Sigma Chemical Co.) to give a final enzyme concentration of 30–120 units mL^{-1}. Initial rates were determined from the slope of the linear portions of plots of absorbance vs time. V_{max} and K_m values were estimated by linear least-squares fits of initial rate data cast into the form of a Lineweaver–Burke plot.

Thermodynamics of Daunomycin Binding. The enthalpy of daunomycin binding to poly(dA)–poly(dT) and poly(dA-dT)–poly(dA-dT) was determined by difference spectroscopy using a thermal dissociation method previously described (Chaires, 1985). Briefly, a fixed amount of daunomycin (Sigma Chemical Co.) was added to a polynucleotide solution (100 μM bp) in BPES buffer and equilibrated at 20 °C. The sample was scanned over the range 400–750 nm, providing a spectrum from which the concentration of free and bound daunomycin could be determined. The sample was then heated at a rate of 0.25 °C min^{-1}, while continuously monitoring the absorbance at 480 nm, a wavelength sensitive to drug binding. The extinction of daunomycin as a function of temperature was then calculated, from which the binding ratio and free drug concentration are readily obtained by using known values of the extinction coefficients (Chaires, 1983, 1985; Chaires et al., 1982). These data were used to estimate the equilibrium constant as a function of temperature by assuming a value for the exclusion parameter (Chaires, 1985), which has been well characterized for daunomycin (Chaires et al., 1982; Chaires, 1983, 1985).

Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) experiments were done using a Microcal MC2 scanning calorimeter (Amherst, MA) interfaced to, and controlled by, an IBM PC computer. Polynucleotides in BPES buffer at a concentration of 0.5–1.12 mM bp were used, with dialyzate used as a reference solution. A scan rate of 1 °C min^{-1} was used for most experiments. A buffer–buffer base line was subtracted from all thermograms, and Microcal, Inc., software was used for subsequent analysis. A model assuming a single transition with $\Delta C_p = 0$ and $\Delta H_{cal} \neq \Delta H_{VH}$ was used to analyze melting curves for the polynucleotides.

Results and Analysis

Premelting Transition in Poly(dA)–Poly(dT). CD spectra

[1] Abbreviations: CD, circular dichroism; DMSO, dimethyl sulfoxide; DNase I, deoxyribonuclease I; DSC, differential scanning calorimetry.

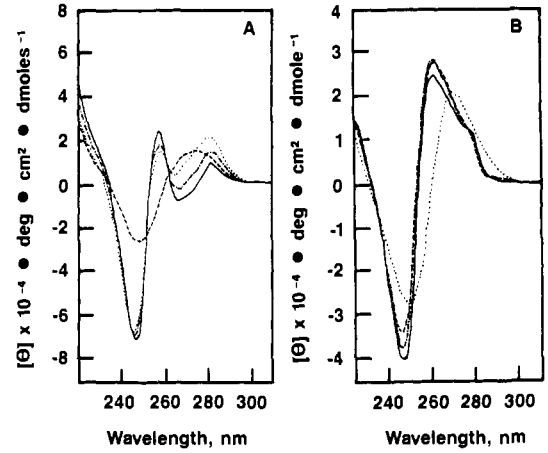

FIGURE 1: CD spectra as a function of temperature. (A) Poly(dA)–poly(dT) in BPES buffer at 20 (—), 40 (–·–), 60 (···), and 80 °C (– – –). Under these solution conditions, the melting temperature is 74.0 °C. (B) Poly(dA-dT)–poly(dA-dT) in BPES buffer at 20 (—), 40 (–·–), 60 (– – –), and 80 °C (···). Under these conditions, the melting temperature is 65.9 °C.

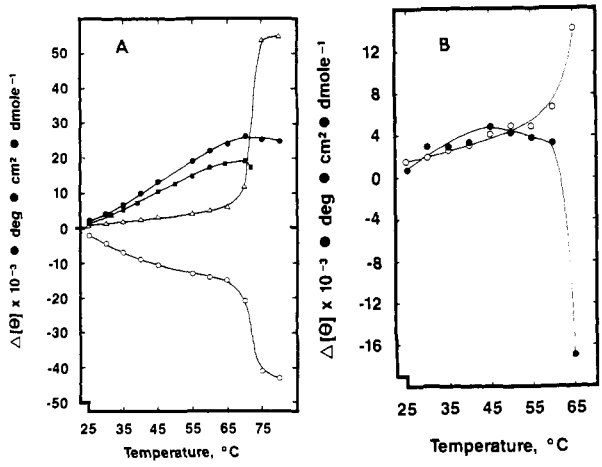

FIGURE 2: Molar ellipticity at selected wavelengths as a function of temperature. (A) Poly(dA)–poly(dT) at (●) 287 nm, (■) 271 nm, (○) 255 nm, and (△) 245 nm. (B) Poly(dA-dT)–poly(dA-dT) at (○) 260 nm and (●) 246 nm. The difference in molar ellipticity relative to that observed at 20 °C for each polynucleotide is shown.

as a function of temperature are shown in Figure 1 for poly(dA)–poly(dT) and poly(dA-dT)–poly(dA-dT). Under these solution conditions, the melting temperatures for poly(dA)–poly(dT) and poly(dA-dT)–poly(dA-dT) are 74.0 and 65.9 °C, respectively. Increasing temperature below the melting temperature has a dramatic effect on the CD spectrum of poly(dA)–poly(dT). Ellipticity increases at 287 nm, while decreasing at 255 nm. Apparent isoelliptic points are seen near 260 and 250 nm, suggesting that a conformational transition between two helical states is occurring. Changes in the CD spectra of poly(dA-dT)–poly(dA-dT) are less dramatic over the same temperature range. Figure 2 shows changes in the molar ellipticity at selected wavelengths as a function of temperature for both polynucleotides. These data indicate that the transition that occurs in poly(dA)–poly(dT) is broad and not highly cooperative.

Figure 3 shows the temperature dependence of changes in UV extinction for poly(dA)–poly(dT) and poly(dA-dT)–poly(dA-dT), as measured by UV difference spectroscopy. Maximal changes were observed at 286 nm for poly(dA)–poly(dT) and at 291 nm for poly(dA-dT)–poly(dA-dT). Again, a broad transition is seen for poly(dA)–poly(dT), with changes in extinction of larger magnitude than observed for poly(dA-dT)–poly(dA-dT). Transformation of the data of

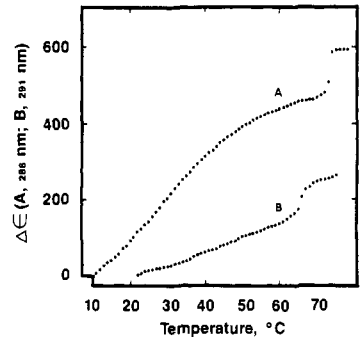

FIGURE 3: Temperature dependence of UV absorbance. (A) Poly(dA)–poly(dT), monitored at 286 nm. (B) Poly(dA-dT)–poly(dA-dT), monitored at 291 nm. The change in molar extinction, $\Delta\epsilon$, as a function of temperature was determined by UV difference spectroscopy as described under Materials and Methods. The wavelengths selected to monitor the premelting transition are those at which the maximal change occurs.

Table I: Thermodynamics of the Helix to Coil Transition for Poly(dA)–Poly(dT) and Poly(dA-dT)–Poly(dA-dT)[a]

polynucleotide	t_m (°C)	ΔH_{cal} (kcal mol^{-1})	N_0 (bp)
poly(dA-dT)–poly(dA-dT)	65.9 ± 0.1	7.8 ± 0.4	30 ± 3
poly(dA)–poly(dT)	74.0 ± 0.1	13.6 ± 0.2	83 ± 3

[a] Data were obtained in BPES buffer. t_m is the temperature at the transition midpoint, ΔH_{cal} is the calorimetric enthalpy, and N_0 is the length of the cooperative unit, calculated from the ratio $\Delta H_{VH}/\Delta H_{cal}$.

Figure 3 into a derivative plot of $d\Delta\epsilon/dT^{-1}$ vs T^{-1} (not shown), where T is the absolute temperature, allows us to estimate a transition midpoint of t_m = 29.9 °C (Cantor & Schimmel, 1980). The data of Figure 3 may be transformed into a van't Hoff plot by assuming a two-state transition and that the fractional concentration of the two helical forms is a linear function of the apparent extinction. The van't Hoff plot (not shown at the request of the editor) is linear over the temperature range 15–50 °C, with a slope of −9990 and a correlation coefficient of 0.9998. ΔH_{VH} is obtained from the slope and is +19.9 kcal mol^{-1}. We calculate, at the transition midpoint, $\Delta S = \Delta H_{VH}/(t_m + 273.15)$ = 65.7 cal deg^{-1} mol^{-1}.

DSC Studies. Attempts to monitor the premelting transition in poly(dA)–poly(dT) by scanning calorimetry were unsuccessful (data not shown). Computer simulations based on our estimates for ΔH_{VH} and t_m show, however, that the premelting transition would be extremely difficult to distinguish from the experimental base line. The low cooperativity of the transition results in a transition width at the half-height of over 30 °C. The failure to observe the transition by DSC methods confirms that it is broad and not highly cooperative, and is accompanied by only a modest enthalpy change. Table I summarizes estimates for the enthalpy of the helix to coil transition in poly(dA)–poly(dT) and poly(dA-dT)–poly(dA-dT) obtained by DSC methods. A notable difference between the two polynucleotides is in the length of the cooperative unit, N_0, which differs by over a factor of 2 (Table I).

DNase I Digestion Studies. An alternate probe of DNA conformation is the susceptibility of polynucleotides to digestion by nucleases. We have used DNase I to probe the conformation of poly(dA-dT)–poly(dA-dT) and poly(dA)–poly(dT). The initial rate of digestion by DNase I was found to be nearly 1 order of magnitude slower for poly(dA)–poly(dT) than for poly(dA-dT)–poly(dA-dT) under identical solution conditions. Poly(dA)–poly(dT) is thus a poorer substrate for DNase I compared to poly(dA-dT)–poly(dA-dT). We determined V_{max} = 0.2 A_{260} s^{-1} and K_m = 22 μM for

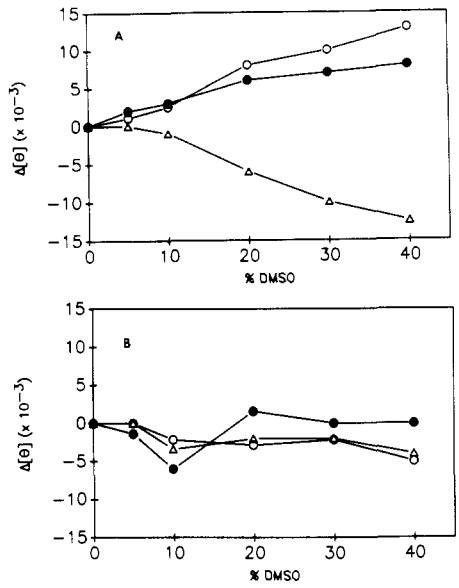

FIGURE 4: Perturbation of poly(dA)–poly(dT) structure by DMSO. The molar ellipticity at selected wavelengths as a function of DMSO concentration is shown. (A) Poly(dA)–poly(dT) at (○) 281 nm, (●) 265 nm, and (△) 257 nm. (B) Poly(dA-dT)–poly(dA-dT) at (○) 260 nm, (●) 245 nm, and (△) 277 nm.

poly(dA-dT)–poly(dA-dT) and V_{max} = 0.5 A_{260} s^{-1} and K_m = 1600 μM for poly(dA)–poly(dT). The slower initial rate observed for poly(dA)–poly(dT) arises primarily from a difference in K_m, possibly reflecting a lower affinity of DNase I toward this polynucleotide. An Arrhenius plot (not shown at the request of the editor) for the initial rate of digestion of poly(dA-dT)–poly(dA-dT) by DNase I is linear over the temperature range 15–50 °C and provies an estimate for the activation energy of DNase I digestion of 14.2 kcal mol^{-1} deg^{-1}. In contrast, the Arrhenius plot for poly(dA)–poly(dT) is biphasic, with a break point near 40 °C. The activation energy over the range 15–40 °C is 38.0 kcal mol^{-1} deg^{-1} but decreases to a value of 14.2 kcal mol^{-1} deg^{-1} above 40 °C. This latter value is equal to that observed for poly(dA-dT)–poly(dA-dT). A plausible interpretation of these data is that poly(dA)–poly(dT) undergoes a thermally driven transition to a form more susceptible to DNase I digestion. Such an interpretation is fully consistent with the optical data presented above.

Solvent Perturbation. Addition of dimethyl sulfoxide (DMSO) to poly(dA)–poly(dT) solutions alters their CD spectra in the same ways as does temperature. Figure 4 shows the molar ellipticity at selected wavelengths as a function of DMSO concentration. The magnitude of the perturbations of the CD spectrum of poly(dA)–poly(dT) upon addition of DMSO is comparable to that observed for temperature increases, as seen by comparison of Figures 2 and 4. DMSO perturbs the spectrum of poly(dA-dT)–poly(dA-dT) to a negligible extent (Figure 4B). We conclude that both temperature and DMSO are effective in transforming poly(dA)–poly(dT) from one conformation to another.

Thermodynamics of Daunomycin Binding. The thermodynamics of daunomycin binding to poly(dA)–poly(dT) and to poly(dA-dT)–poly(dA-dT) was explored by difference spectroscopy using the thermal dissociation method previously described (Chaires, 1985). Figure 5 shows the change in extinction of daunomycin as a function of temperature for both poly(dA)–poly(dT) and poly(dA-dT)–poly(dA-dT) at several values of the initial binding ratio. For poly(dA-dT)–poly(dA-dT) (Figure 5b), the apparent extinction coefficient increases with increasing temperature, regardless of the initial binding ratio. The positive slope in this case corresponds to

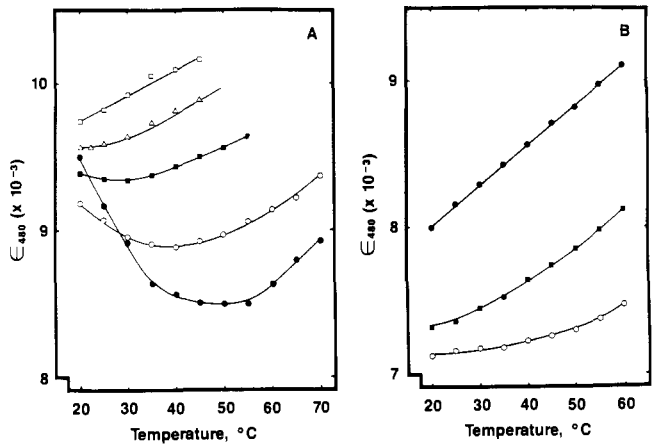

FIGURE 5: Extinction of daunomycin–polynucleotide solutions as a function of temperature. Visible absorbance at 480 nm was used to monitor daunomycin binding as a function of temperature at a variety of initial binding ratios. (A) Poly(dA)–poly(dT) at initial binding ratios of (●) 0.017, (○) 0.122, (■) 0.208, (△), 0.287, and (□) 0.314. (B) Poly(dA-dT)–poly(dA-dT) at initial binding ratios of (○) 0.142, (■) 0.266, and (●) 0.334.

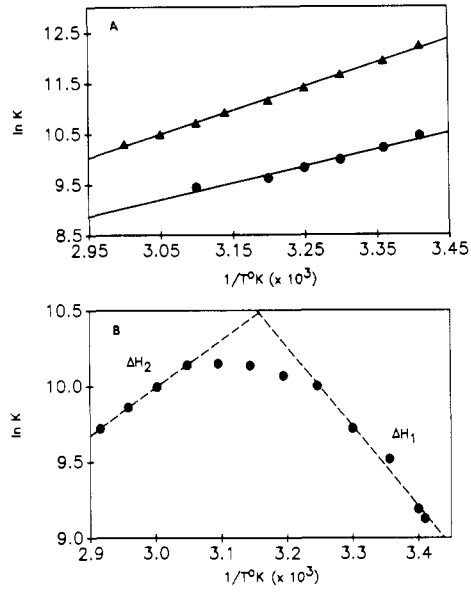

FIGURE 6: van't Hoff plots for the interaction of daunomycin with poly(dA-dT)–poly(dA-dT) and poly(dA)–poly(dT). (A) Poly(dA-dT)–poly(dA-dT) (▲) and poly(dA)–poly(dT) (●) at initial binding ratios of 0.334 and 0.314, respectively. (B) Poly(dA)–poly(dT) at an initial binding ratio of 0.017. The slope at limiting high (ΔH_2) and low (ΔH_1) temperatures was evaluated by linear least-squares analysis, as indicated by the dashed lines. Enthalpy estimates obtained from these data are summarized in Table II.

a net dissociation of bound antibiotic with increasing temperature, qualitatively indicative of a negative enthalpy for daunomycin binding. The behavior of poly(dA)–poly(dT) is considerably more complex (Figure 5A). At low binding ratios, the apparent extinction *decreases* with increasing temperature, passess through a minimum, and then increases. Such behavior indicates that increasing temperature at first favors binding (positive enthalpy) but then favors antibiotic dissociation (negative enthalpy). With increasing amounts of initially bound antibiotic, the curvature is lessened, and the behavior simplified, yielding, at $r_b \simeq 0.3$, a straight line with positive slope. These data indicate that the sign of the apparent enthalpy of antibiotic binding is dependent on the binding ratio. The data of Figure 5 are readily transformed into a van't Hoff plot to obtain values for ΔH_{VH} of daunomycin binding (Chaires, 1985), shown in Figure 6. The ΔH_{VH} for dauno-

Table II: Thermodynamic Profiles for the Interaction of Daunomycin with AT-Containing Deoxypolynucleotides[a]

polynucleotide	K ($M^{-1}/10^4$)	n	$\Delta G°$ (kcal mol^{-1})	ΔH_{VH} (kcal mol^{-1})	$\Delta S°$ (eu)
$[poly(dA\text{-}dT)]_2$	76.0 ± 6	2.4	−7.9	−9.1	−4.1
poly(dA)–poly(dT)	2.0 ± 0.1	2.0	−5.8		
$r_b \rightarrow 0$				+9.2	+51.2
$r_b \rightarrow$ saturation				−6.6	−2.8

[a] These values refer to solution conditions of BPES buffer at 20 °C.

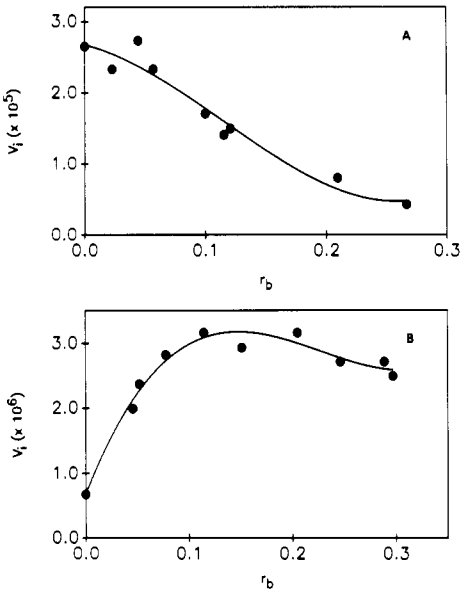

FIGURE 7: Effect of bound daunomycin on the initial rate of DNase I digestion of poly(dA-dT)–poly(dA-dT) (A) and poly(dA)–poly(dT) (B).

mycin binding to poly(dA-dT)–poly(dA-dT) is estimated by this procedure to be −9.1 kcal mol^{-1}, a value in excellent agreement with the published value of −8.9 kcal mol^{-1} determined calorimetrically (Marky et al., 1983). For poly(dA)–poly(dT), we estimate, at $r = 0.314$, $\Delta H_{VH} = -6.6$ kcal mol^{-1}. At low r values, the van't Hoff plot is complex (Figure 6B). We estimate from the respective slopes at low and high temperatures ΔH_{VH} values of +9.2 and −6.5 kcal mol^{-1}, respectively. Table II summarizes the thermodynamic profiles for daunomycin binding to poly(dA)–poly(dT) and poly(dA-dT)–poly(dA-dT), incorporating previously published estimates for the binding constants (Chaires, 1983).

Perturbation of Poly(dA)–Poly(dT) Structure by Daunomycin. Susceptibility toward digestion by DNase I and circular dichroism were used to study the effect of daunomycin on the structure of poly(dA)–poly(dT) and poly(dA-dT)–poly(dA-dT). Figure 7 shows the effect of daunomycin binding on the initial rate of DNase I digestion of both polynucleotides. Intercalation generally results in inhibition of DNase I digestion, as is observed in the case of poly(dA-dT)–poly(dA-dT) (Figure 7A). In contrast, binding of daunomycin to poly(dA)–poly(dT) results in an enhanced rate of DNase I cleavage at low binding ratios (Figure 7B). The simplest explanation for this observation is that the bound antibiotic perturbs the polynucleotide structure in a way that renders it more susceptible to nuclease attack. At higher binding ratios, antibiotic binding begins to inhibit DNase I action. Figure 8 shows the CD spectra of poly(dA)–poly(dT) and poly(dA-dT)–poly(dA-dT) alone and saturated with daunomycin. The CD spectra of the polynucleotides alone differ

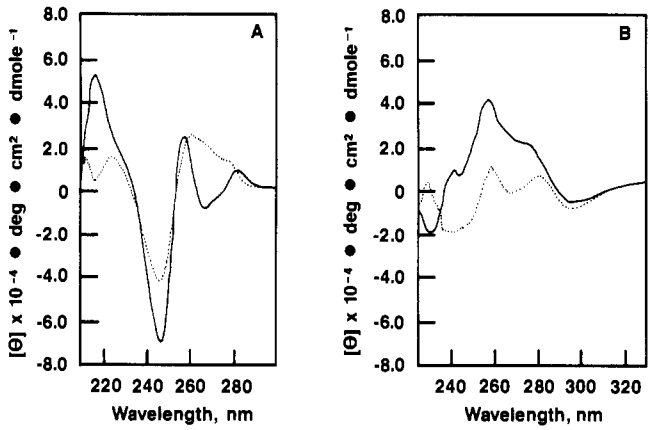

FIGURE 8: CD of poly(dA-dT)–poly(dA-dT) (···) and poly(dA)–poly(dT) (—) alone (A) or saturated with daunomycin (B). The contribution of free daunomycin to the CD spectra has been subtracted from each curve in panel B; these spectra thus arise from the daunomycin–polynucleotide complex.

in both shape and magnitude, reflecting their structural differences. The CD spectra of the polynucleotide–daunomycin complexes also differ in both shape and magnitude, which indicates that the structures of the two intercalation complexes must differ in significant ways. Daunomycin alters the structure of poly(dA)–poly(dT), but the resultant structure remains distinct from that of an intercalation complex formed from standard B-form DNA.

Discussion

Our results show that poly(dA)–poly(dT) undergoes a premelting conformational transition. The endothermic transition is broad, with a transition midpoint at 29.9 °C. Substantial changes in both the molar ellipticity and molar extinction of the polynucleotide accompany the transition. The transition renders poly(dA)–poly(dT) more susceptible to digestion by DNase I and facilitates daunomycin binding. The existence of a two-state conformational transition in poly(dA)–poly(dT) provides a physical basis for the previously observed cooperative antibiotic binding to the polynucleotide. The thermodynamic values determined for the transition may be used to quantitatively account for the unusual thermodynamics of daunomycin binding to poly(dA)–poly(dT).

Thermodynamics of the Transition. The existence of an isoelliptic point in the CD spectra shown in Figure 1 suggests that a two-state transition is an appropriate description of the conformational change in poly(dA)–poly(dT). The enthalpy of the transition was estimated by van't Hoff analysis assuming a two-state transition and found to be +19.9 kcal mol^{-1}. At the transition midpoint of 29.9 °C, we calculate that ΔS = +65.7 cal mol^{-1} deg^{-1}. These estimates for ΔH_{VH} and ΔS allow the free energy of the transition to be calculated at any temperature from the standard relation $\Delta G = \Delta H - T\Delta S$, assuming that there is no change in the heat capacity.

The width of the premelting transition in poly(dA)–poly(dT) observed in Figure 3 suggests that the conformational change is not highly cooperative. However, the magnitude of ΔH_{VH} (which refers to moles of cooperative unit) is too large to be reasonably assigned to a transition involving a single base pair. We conclude that the cooperative unit must contain several base pairs but is certainly not as large as the number of base pairs comprising the cooperative unit involved in the melting transition (Table I).

Coupling of Antibiotic Binding to the Poly(dA)–Poly(dT) Conformational Change. Daunomycin binding to poly-

(dA)–poly(dT) was previously shown to be cooperative, and the cooperativity was proposed to arise from the coupling of antibiotic binding to a conformational transition in the polynucleotide (Chaires, 1983). The direct observation of a conformational change in poly(dA)–poly(dT) reported here strongly supports that argument. Knowledge of the thermodynamics of the polynucleotide conformational transition, coupled with previously determined binding data (Chaires, 1983), provides considerable insight into the system. At 20 °C, the system is described by the following equilibria:

$$B^* \rightleftharpoons B \qquad \Delta G_1 = +0.3$$

$$D + B^* \rightleftharpoons DB^* \qquad \Delta G_2 = -5.1$$

$$D + B \rightleftharpoons DB \qquad \Delta G_3 = -5.8$$

where B* and B denote conformations of poly(dA)–poly(dT), D denotes daunomycin, and DB and DB* denote antibiotic complexes with the two conformational forms of poly(dA)–poly(dT). The overall antibiotic binding reaction free energy may be calculated by using these values:

$$D + B^* \rightleftharpoons DB \qquad \Delta G_T = -5.5$$

The coupling free energy (Weber, 1975) is defined for this system as $\Delta G_T - \Delta G_2 = -0.4$ kcal mol^{-1}. The magnitude of the coupling free energy is modest, but the negative sign indicates that the conformational transition in poly(dA)–poly(dT) facilitates daunomycin binding. For comparison, daunomycin allosterically converts Z-form DNA to an intercalated B form, with a coupling free energy of −2.3 kcal mol^{-1} (Chaires, 1986). The larger magnitude of the coupling free energy in that case indicates that the relative preference of daunomycin for B DNA over Z DNA is stronger than its relative preference for one of the two conformations of poly(dA)–poly(dT). Nonetheless, in both of these cases, daunomycin discriminates against non-B-form conformations, and antibiotic binding is facilitated by a conformational transition in the polynucleotide.

The enthalpy we estimate for the premelting transition can quantitatively account for the unusual thermodynamic profile observed for the interaction of daunomycin with poly(dA)–poly(dT) (Table II), and for the dependence of the apparent enthalpy on the initial binding ratio seen in Figure 5. For the assumed reactions

$$B^* \rightleftharpoons B \qquad \Delta H^* = +19.9 \text{ kcal mol}^{-1}$$

$$B + D \rightleftharpoons BD \qquad \Delta H^\circ = -6.6 \text{ kcal mol}^{-1}$$

the apparent enthalpy is given by

$$\Delta H_{app} = \Delta H^\circ + f\Delta H^* \qquad (1)$$

where f is the fraction of the polynucleotide in the B* conformation. ΔH° is taken as the observed value near saturation of daunomycin binding sites. From inspection of the transition curve shown in Figure 3, a value of $f \approx 0.8$ may be estimated at 20 °C. Substitution into the above equations predicts ΔH_{app} = +9.3 kcal mol^{-1}, a value in excellent agreement with the estimate of +9.2 kcal mol^{-1} obtained at a binding ratio of 0.017 (Figure 6B; Table II). This calculation indicates that our data are internally self-consistent. The dependence of the apparent enthalpy on the initial binding ratio may now be understood from eq 1. Binding of increasing amounts of daunomycin will decrease f, the fraction of polynucleotide in the B* conformation, and will lessen the contribution of ΔH^* to the overall apparent ethalpy. Breslauer et al. (1987) have suggested that the enthalpy change for a helix to helix transition in poly(dA)–poly(dT) was not large enough to account for the reduced enthalpy observed for the binding of many antibiotics to the polynucleotide. Our data show that this is not the case. The value of ΔH^* = +19.9 kcal mol^{-1} we determined can quantitatively account for the observed thermodynamics of daunomycin binding. The unusual thermodynamic profiles observed for distamycin, netropsin, ethidium, and propidium binding to poly(dA)–poly(dT) (Breslauer et al., 1987; Wilson et al., 1985) are most readily explained by the coupling of the antibiotic binding to the endothermic premelting transition we observe. Furthermore, the enthalpy–entropy compensation observed for the interaction of antibiotics with polynucleotides (Breslauer et al., 1987) can arise as a direct consequence of the coupling of ligand binding to a macromolecular conformational transition such as we observe here (Eftink et al., 1983).

Comparison with Earlier Results. Premelting conformational transitions in DNA have been long recognized (Palecek, 1976). What distinguishes the transition we observe for poly(dA)–poly(dT) from these previously observed transitions is the magnitude of the optical changes in the polynucleotide and the comparatively large enthalpy for the conformational change. A premelting transition in poly(dA-dT)–poly(dA-dT) has been well characterized (Gennis & Cantor, 1972; Brahms et al., 1976) and found to be noncooperative, with $\Delta H \leq 5$ kcal mol^{-1}. The temperature dependence of the CD spectra of poly(dA)–poly(dT) has been previousely observed (Sarocchi & Guschlbauer, 1973; Greve et al., 1976), but the thermodynamics of the transition were not characterized. Greve et al. (1976) concluded from their results that poly(dA)–poly(dT) undergoes a two-state transition between two different B-type geometries, in agreement with our findings. Several indirect indications of a conformational transition in poly(dA)–poly(dT) have appeared. Temperature-jump relaxation experiments on the binding of ethidium (Bresloff, 1974) and daunomycin (Chaires, 1983) to poly(dA)–poly(dT) showed amplitudes of opposite sign compared to other DNA samples. The interpretation in both cases, was that the apparent enthalpy of intercalator binding was positive and perhaps arose from a conformational change in poly(dA)–poly(dT). It is notable that Bresloff (1974) found that the sign of the apparent enthalpy for the interaction of ethidium with poly(dA)–poly(dT) was dependent upon the initial binding ratio and became negative near saturation, behavior identical with that reported here for daunomycin (Figure 5). Sturm (1982) conducted an insightful temperature-jump study of the interaction of tilorone with poly(dA)–poly(dT). He concluded that tilorone binding was coupled to a conformational transition in the polynucleotide and estimated from his kinetic data an enthalpy of 17.3 kcal mol^{-1} for the transition, a value in excellent agreement with our estimate derived from direct observation of the transition (Figure 3). Wilson et al. (1985) found that the rate of propidium binding to poly(dA)–poly(dT) was independent of concentration and proposed that intercalator binding was linked to a preequilibrium conformational change in the polymer. They estimated from their data an enthalpy of ΔH^* = +15 kcal mol^{-1} for the preequilibrium transition in poly(dA)–poly(dT). Our data provide a direct observation and thermodynamic characterization of the poly(dA)–poly(dT) conformational change that is fully consistent with these previous indirect characterizations.

Nature of the Premelting Transition. The unusual structure of poly(dA)–poly(dT) is stabilized by purine–purine base stacking interactions and by additional hydrogen bonds, both of which arise from the high propeller twist of the base pairs (Nelson et al., 1987). A spine of hydration in the minor groove is thought to provide an energetically favorable contribution

that stabilizes the nonstandard B form of the polynucleotide (Chuprina, 1987), although the details of solvation within the minor groove are not visible at the current resolution of the structural studies (Nelson et al., 1987; Coll et al., 1987). The minor groove is considerably narrower in poly(dA)–poly(dT) than in standard B-form DNA (Chuprina, 1987; Alexeev et al., 1987). We find that both temperature and DMSO perturb poly(dA)–poly(dT) structure, both giving rise to pronounced changes in the CD and absorbance spectra of the polynucleotide. Since both temperature and organic solvents are known to dehydrate DNA (Bloomfield et al., 1974), we interpret these results as arising from a disruption of the spine of hydration of poly(dA)–poly(dT), with a concomitant alteration of the conformation of the base pairs. The optical changes we observe presumably arise from the latter event. Hydration and conformation are strongly linked in poly(dA)–poly(dT) (Chuprina, 1987), so the proposed effects are strongly coupled and are probably inseparable. The positive sign and the magnitudes of the measured enthalpy and entropy of the premelting transition are consistent with the melting and release of structured water from poly(dA)–poly(dT). The perturbed structure of poly(dA)–poly(dT) binds daunomycin more readily and is more susceptible to DNase I digestion. Since both daunomycin (Quigley et al., 1980) and DNase I (Drew, 1984; Suck & Oefner, 1986) bind to DNA through interactions at the minor groove, the perturbed poly(dA)–poly(dT) structure may have an altered minor groove geometry, perhaps widened as a result of disruption of the spine of hydration.

Comparison with Oligo(dA)–Oligo(dT) Tracts in Situ. What is the relevance of our observations on poly(dA)–poly(dT) to the structure of oligo(dA)–oligo(dT) tracts within native DNA sequences? Do these smaller tracts behave like the synthetic homopolynucleotide? Existing evidence suggests that they do. Within the 160 bp tyr T promoter fragment, oligo(dA)–oligo(dT) tracts are cut by DNase I with considerably less frequency than other regions of the sequence (Drew & Travers, 1984). However, increased temperature or the addition of DMSO enhances the susceptibility of nonalternating AT runs to DNase digestion to a proportionally larger extent than other sequences (Drew & Travers, 1984). These effects are in accord with our observation on poly(dA)–poly(dT). Footprinting studies indicate that daunomycin does not bind to oligo(dA)–oligo(dT) tracts within the tyr T fragment (Chaires et al., 1987), consistent with its low affinity for poly(dA)–poly(dT) (Chaires, 1983). However, binding of daunomycin to other sites within the fragment results in pronounced enhancement of the cutting of nonalternating AT runs, beyond what might arise solely from simple mass action effects (Chaires et al., 1987). Such behavior is similar to the enhanced susceptibility of poly(dA)–poly(dT) to DNase action upon daunomycin binding (Figure 7). It is plausible that daunomycin binding to sites within the tyr T fragment results in structural alterations that are propagated into the oligo(dA)–oligo(dT) tracts, altering their susceptibility to nuclease attack. Poly(dA)–poly(dT) and oligo(dA)–oligo(dT) tracts within natural DNA sequences thus behave similarly in their response to temperature, solvent perturbation, and intercalator binding. Their structural dynamics are likely to be the same.

Implications for DNA Bending. DNA bending is thought to arise from the properties of the junction that forms where oligo(dA)–oligo(dT) tracts, in a nonstandard B conformation, join with standard B-form DNA [reviewed in Olson et al. (1988)]. DNA bending is strongly dependent upon temperature (Koo et al., 1986; Diekmann, 1987). The premelting transition we observe offers a plausible physical basis for the temperature dependence of bending. Increased temperature would disrupt the nonstandard conformation of oligo(dA)–oligo(dT) tracts and drive these regions into a more standard B conformation which will fuse with surrounding sequences in a normal way. Discrete junctions would then be absent, reducing the tendency to bend. The data of Diekmann (1987) show that the temperature-dependent bending transition is broad, and not highly cooperative, similar to the premelting transition we observe in poly(dA)–poly(dT). If we take the liberty of using the data of Figure 3a in Diekmann (1987) to estimate an enthalpy for DNA bending, we calculate $\Delta H \approx 16$ kcal mol^{-1}, assuming a two-state process with a limiting k value of 1.8. This estimate is of the same sign and magnitude as we observe for the premelting transition in poly(dA)–poly(dT) and suggests that the two phenomena may be driven by the same molecular forces.

Added in Proof

We have found that the magnitude of the enthalpy for the helix to coil transition in poly(dA)–poly(dT) appears to depend on the source of the polynucleotide, with samples from Sigma Chemical Co. (St. Louis, MO), Boehringer-Mannheim (Indianapolis, IN), and Pharmacia LKB Biotechnology, Inc. (Piscataway, NJ), showing ΔH values that differ by nearly a factor of 2 (data not shown). The premelting transition we describe does not, in contrast, appear to depend on the source of the material. The enthalpy value for the melting of poly(dA)–poly(dT) reported in Table I is higher than previously published estimates. The reason for this higher value is not clear, but is under investigation. Dr. Harry Hopkins, Georgia State University, has provided helpful discussion concerning this point.

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Registry No. Poly(dA-dT)–poly(dA–dT), 26966-61-0; poly(dA)–poly(dT), 24939-09-1; daunomycin, 20830-81-3.

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