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Hydration of dA·dT Polymers: Role of Water in the Thermodynamics of Ethidium and Propidium Intercalation†

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ABSTRACT: We report differences in the interaction of two structurally similar phenanthroline intercalators, ethidium and propidium, with poly(dA)·poly(dT) and poly[d(A-T)] as a function of ionic strength based on titration microcalorimetry, fluorescence titration, and hydrostatic pressure measurements. Both ethidium and propidium bind more strongly to poly[d(A-T)]·poly[d(A-T)] than to poly(dA)·poly(dT). Ethidium intercalation into the latter polymer displays titrations with positive cooperativity; this is not found with propidium. The enthalpy of intercalation ($\Delta H°$) is exothermic for both dyes with poly[d(A-T)]·poly[d(A-T)]; however, the value of this parameter is nearly zero in the case of poly(dA)·poly(dT). The molar volume change ($\Delta V°$) accompanying dye intercalation is negative under all conditions for poly[d(A-T)]·poly[d(A-T)] whereas it is positive for poly(dA)·poly(dT). The changes observed in $\Delta V°$ correlate well with the entropy changes derived from the titration and calorimetric data for this reaction. The results, interpreted in terms of the relative hydration of these two polymers, are consistent with a higher extent of hydration of poly(dA)·poly(dT) relative to poly[d(A-T)]·poly[d(A-T)].

The hydration of helical nucleic acids remains a subject of intensive experimental and theoretical investigation. The particular conformation of double-stranded DNA depends on its primary sequence and its degree of hydration; sequence-dependent hydration has been inferred from X-ray analysis of the crystal structures of oligonucleotides and other physical techniques (Drew & Dickerson, 1981; Kopka et al., 1983; Kennard et al., 1986; Wang et al., 1979; Buckin et al., 1989). Presumably, specific water–DNA interactions arise primarily from hydrogen bonding to the groups on the edges of the bases facing the grooves of the double helix and to a lesser extent from hydrogen bonding to the sugars and phosphate groups. The conformational plasticity of DNA and sequence-dependent ligand binding can be rationalized in terms of the hydration at these positions (Saenger et al., 1986). Experimental techniques that change the water activity shift the equilibrium between the various conformations of DNA and alter the characteristics of the interaction of DNA with ligands (Pohl & Jovin, 1972; Ivanov et al., 1973; Pohl, 1976; Rich et al., 1984).

The partial molar volume of water involved in the hydration of a solute is smaller than that of bulk water; volume changes during association reactions reflect changes in electrostriction of water molecules around charges or changes in the solvent cage surrounding hydrophobic molecules (Drude & Nernst, 1894; Frank & Evans, 1945; Kauzmann, 1959). As a consequence of their size and complexity, both of these effects often contribute significantly to the volume change of reactions and conformational changes involving biological molecules. The volume change accompanying formation of a DNA duplex from single strands is nearly zero because of compensation between electrostriction and hydrophobic effects (Heden et al., 1964; Weida & Gill, 1966; Chapman & Sturtevant, 1966; Noguchi et al., 1971; Gunter & Gunter, 1972; Hawley & MacLeod, 1974, 1977). Determination of the molar reaction

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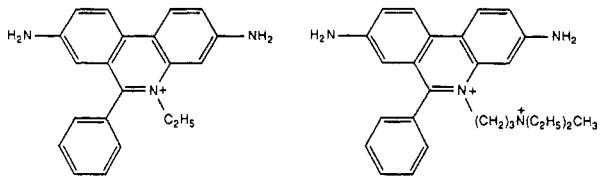

FIGURE 1: Structure of ethidium and propidium cations.

volume (ΔV°) from the pressure dependence of the binding constant of a series of structurally similar ligands can potentially offer an informative method for studying the relative hydration of specific nucleic acid sequences. The sign and magnitude of the pressure-induced change in an association between DNA and ligands can be compared with the response of simpler reactions toward pressure in order to evaluate the relative contributions of the different components of the reaction, e.g., ionic, or hydrophobic. Perhaps the clearest example of such a comparison is the effect of increasing the total number of ionic interactions formed in the DNA–ligand complex; this would be expected to lead to a more positive ΔV° if these interactions make a significant contribution to the free energy of the reaction (Isaacs, 1981). The ΔV° together with other thermodynamic data can be interpreted on the basis of the structural information obtained from NMR and X-ray crystallography. The interaction of the intercalators ethidium bromide (EB)[1] and propidium iodide (PI) with the two simplest dA·dT polymers, poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)], is well suited for a comparative investigation because of the large body of data on the structure and thermodynamics of these polymers and their complexes with these ligands.

Although they are formally isomers, the properties of poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] are quite different. As a model for the structure of poly(dA)·poly(dT), X-ray crystal structures of dA·dT tracts in oligomers reveal an optimum base-stacking interaction arising from a large propeller twist in the base pairs, an unusual system of bifurcated hydrogen bonds, and a narrow minor groove (Coll et al., 1987; Nelson et al., 1987). The helical repeat and axial rise of poly(dA)·poly(dT) in solution are 10 base pairs (bp) per turn and 3.2 Å, respectively; these values can be compared with 10.5 bp per turn and 3.4 Å for poly[d(A-T)]·poly[d(A-T)] and other sequences (Rhodes, 1979; Rhodes & Klug, 1981; Peck & Wang, 1981). Lowering the water activity by the addition of ethanol does not cause poly(dA)·poly(dT) to undergo a conformational transition to the A form as do the alternating and other DNA sequences (Arnott et al., 1974; Pilet et al., 1975). Short, phased repeats of dA·dT within DNA are implicated in the bending of these molecules (Wu & Crothers, 1984; Hagerman, 1984; Olson et al., 1988). Unusual properties of poly(dA)·poly(dT) have been observed also in its interactions with ligands (Bresloff & Crothers, 1981; Sturm, 1982; Chaires, 1983; Marky et al., 1985; Wilson et al., 1985; Jones et al., 1986; Breslauer et al., 1987; Herrera & Chaires, 1989).

In this work, we present data on the influence of hydrostatic pressure on the interaction of EB and PI (Figure 1) with poly[d(A-T)]·poly[d(A-T)] and poly(dA)·poly(dT) in an attempt to understand the role of water and other molecular forces important for the binding of these intercalators (Lerman, 1961; LePecq & Paoletti, 1967) to dA·dT binding sites and thereby indirectly gain an understanding of sequence-dependent hydration. Complementary studies by titration microcalorimetry have been performed in order to correlate entropy changes with the volume-change data.

Materials and Methods

Materials. Ethidium bromide (EB) and propidium iodide (PI) were obtained from Sigma Biochemicals and used without further purification. Poly[d(A-T)]·poly[d(A-T)] was purchased from Pharmacia-LKB Biochemicals; poly(dA)·poly(dT) was purchased from Boehringer Mannheim and Pharmacia-LKB Biochemicals; polymers were obtained in lyophillized form and were used without further purification. All other chemicals were reagent grade or better. The buffer solutions consisted of 20 mM Tris-HCl, pH 7.2, and 0.1 mM EDTA, adjusted to the desired ionic strength with NaCl. Proper annealing of the polymer samples was ensured by dissolving them in buffer, heating to 65 °C for 0.5 h, and slowly cooling to room temperature. This solution was then dialyzed against the same buffer. The concentrations of stock solutions were determined by measuring the optical absorption with ϵ_{262} = 13 200 M^{-1} cm^{-1} for poly[d(A-T)]·poly[d(A-T)] (Schmechel & Crothers, 1971), ϵ_{259} = 12 000 M^{-1} cm^{-1} for poly(dA)·poly(dT) (Bresloff & Crothers, 1981), ϵ_{480} = 5850 M^{-1} cm^{-1} for EB (Bresloff & Crothers, 1975), and ϵ_{493} = 5900 M^{-1} cm^{-1} for propidium iodide (Patel & Canuel, 1977). All DNA concentrations are given in moles of base pairs.

Fluorometric Titrations. The equilibrium binding parameters at 21 °C were obtained by monitoring the fluorescence intensity as the polymer was titrated with concentrated solutions of dye in buffer at the appropriate NaCl concentration. Fluorescence, measured with an SLM Model 8000 spectrofluorometer (Urbana, IL), was excited at the isosbestic wavelength of the free and bound forms of the dye (512 nm for EB, 526 nm for PI), and the emission was monitored through a Schott OG-570 cutoff filter. Titration data were analyzed according to the excluded-site formalism presented by McGhee and von Hippel (1974):

$$\frac{r}{[L]} = K_a(1 - nr) \times \left[\frac{(2\omega + 1)(1 - nr) + r - R}{2(\omega - 1)(1 - nr)}\right]^{n-1}\left[\frac{1 - (n + 1)r + R}{2(1 - nr)}\right]^2 \quad (1)$$

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

r = [complex]/[sites]$_0$ equals the fraction of bound binding sites, K_a is the equilibrium constant describing the binding of a dye to a naked lattice, n is the number of bp removed from further binding events by a single dye, and ω is a cooperativity parameter. The raw data, in the form of a Scatchard plot (Scatchard, 1949), were fit to (1) with a nonlinear least-squares routine.

High-Pressure Measurements. The molar volume change (ΔV°) of intercalation was determined by measuring the change in K_a with pressure, employing the standard thermodynamic relation $(\partial \ln K_{eq}/\partial P)_T = -\Delta V^\circ/RT$ (Planck, 1887), where R is the gas constant (8.1 cm^3 MPa K^{-1} mol^{-1}) and T is the absolute temperature. For these measurements the solutions were contained in an optical high-pressure cell (Nova Swiss, Effretikon, Switzerland) positioned in the sample chamber of the spectrofluorometer and maintained at 21 °C with a circulating temperature bath. Within the pressure cell, the sample was contained in an 800-μL quartz cuvette closed with a polyethylene cap to allow pressure equilibration between

[1] Abbreviations: bp, base pair(s); EB, ethidium bromide; EDTA, ethylenediaminetetraacetic acid; NMR, nuclear magnetic resonance; PI, propidium iodide; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

Table I: Equilibrium Binding Parameters for Ethidium and Propidium Intercalation into dA·dT Polymers[a]

	[NaCl] (mM)	K_a (±10%) (μM^{-1})	n (±0.2) (bp)	ω (±0.2)
poly(dA)·poly(dT)				
EB	0	0.021	4.4	4.6
	50	0.0058	4.5	5.9
PI	0	4.6	3.7	1
	10	1.7	3.9	1
	50	0.29	3.7	1
poly[d(A-T)]·poly[d(A-T)]				
EB	0	2.2	2.1	1
	50	1.1	2	1
PI	0	13	3	1
	50	4.4	3.1	1
	100	1.4	4.7	1

[a] All measurements in 20 mM Tris-HCl, pH 7.2.

the pressurizing fluid and the sample (Paladini, 1987). The pressure within the pressure cell was measured with a Bourdon-style gauge (Heise, Norwalk, CT); pressures are reported in megapascals; 0.1 MPa is equal to 1 bar or 0.9678 atm. The wavelengths of excitation and emission were the same for these measurements as for the titrations. The fluorescence intensities and total concentrations were corrected for compression effects with data for the density of water at 20 °C as a function of pressure (Landolt-Bornstein, 1971).

Calorimetric Measurements. All calorimetric experiments were carried out on an OMEGA titration calorimeter from Microcal Inc. (Northampton, MA). A detailed description of this instrument has been presented elsewhere (Wiseman et al., 1989). Each polymer was titrated with concentrated solutions of EB and PI in a 100-μL stirring syringe rotating at 400 rpm. The concentration of the dye in the syringe was 22–30 times larger than that of the polymer solution (in base pairs) in the reaction cell. Typically, a single titration consisted of 16–18 injections of 5 μL each. The reference cell of the calorimeter acts as a thermal reference; it was filled with a 0.01% (w/v) aqueous solution of NaN_3. The instrument was calibrated with a standard electrical pulse. Its sensitivity is such that it can deliver heats with a precision of less than 1 μcal. Molar binding enthalpies were determined by averaging the heats of injections 2–6 of a given titration and then normalizing for the amount of bound drug or, alternatively, by using a nonlinear fit program provided with the instrument. Both methods yielded similar results. Molar enthalpies are reported in kilocalories; 1 kcal is equal to 4.18 kJ.

Results

Binding Curves. Results of titrations of the two dyes with each polymer at different NaCl concentrations are summarized in Table I. The upward curvature of the titrations shown in Figure 2a,b,d is a consequence of the ligand requiring more than a single free base pair for binding; random occupation of the available binding sites by the ligand leads to isolation of stretches of unbound DNA that are shorter than the minimum length (number of base pairs) necessary for additional ligands to bind. This causes the apparent concentration of remaining binding sites to decrease faster than expected on the basis of mass-action considerations (McGhee & von Hippel, 1974). The presence of the extra charge in the bulkier side group of propidium compared to ethidium is responsible for the stronger binding (larger K_a) and larger number of bp per bound dye (n). Noninteger values of n are the result of the fitting routine; the difference between the results obtained with integer values of n and those reported in Table I for the poly[d(A-T)]·poly[d(A-T)] titrations is within experimental

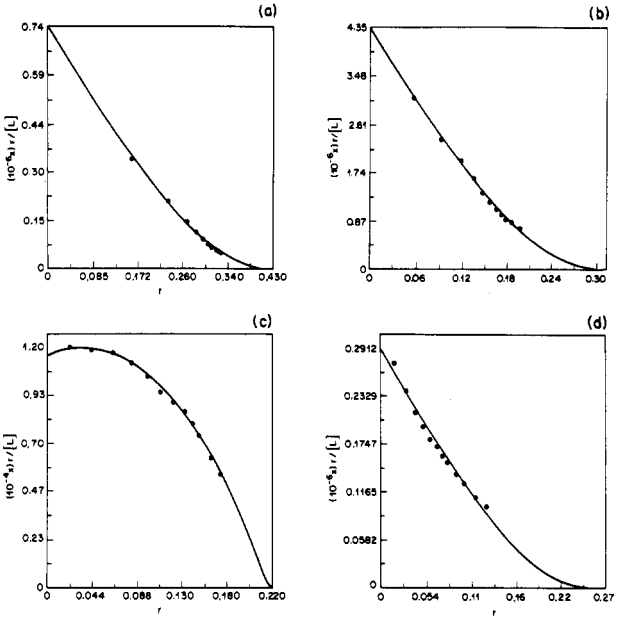

FIGURE 2: Fluorescence titrations: (a) poly[d(A-T)]·poly[d(A-T)] with EB; (b) poly[d(A-T)]·poly[d(A-T)] with PI; (c) poly(dA)·poly(dT) with EB; (d) poly(dA)·poly(dT) with PI. These titrations were carried out in 50 mM NaCl–20 mM Tris-HCl, pH 7.2, 21 °C. The solid lines are the result of fitting the data to eq 1; the difference in the shape of the curves is due to cooperativity; see text for details.

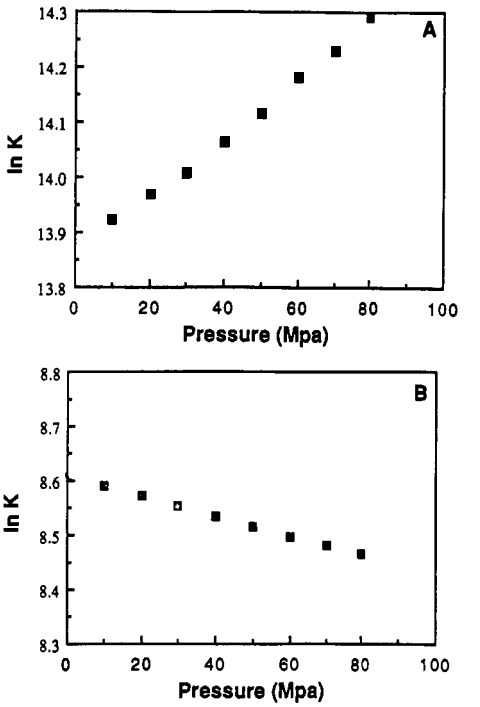

FIGURE 3: Pressure dependence of the equilibrium constant for EB binding to dA·dT polymers in 20 mM Tris-HCl–50 mM NaCl, pH 7.2, 21 °C. The fluorescence was excited at 512 nm and the emission observed through a 570-nm cutoff filter. (Panel A) Poly[d(A-T)]·poly[d(A-T)], 2.0 μM bp; $[EB]_0$ = 0.2 μM. (Panel B) Poly(dA)·poly(dT), 34.7 μM bp; $[EB]_0$ = 5.1 μM.

error. However, holding this parameter constant at integer values above or below that of the best fit for the titrations with poly(dA)·poly(dT) gave unacceptable results. Cooperativity was found only for the titrations of poly(dA)·poly(dT) with EB at 0 and 50 mM NaCl. For all other titrations the data were consistent with a value of ω = 1 (i.e., no cooperativity) from the model of McGhee and von Hippel (1974).

Volume Changes. Representative pressure data are shown in Figure 3, and the resulting $\Delta V°$'s of intercalation are given

Table II: Molar Volume Change of Intercalation into dA·dT Polymers[a]

	[NaCl] (mM)	$\Delta V°$ (±0.5) (cm^3 mol^{-1}) ethidium	propidium
poly[d(A-T)]·poly[d(A-T)]	0	−7.2	
	50	−13.0	−8.3
	100	−15.0[b]	−8.9
poly(dA)·poly(dT)	0	+4.0	+5.6
	50	+4.5	+6.8

[a] 20 mM Tris-HCl, pH 7.2, 21 °C. [b] From Macgregor et al. (1985).

Table III: Intercalation Enthalpies into dA·dT Polymers[a]

	[NaCl] (mM)	$\Delta H°$ (kcal/mol) ethidium	propidium
poly[d(A-T)]·poly[d(A-T)]	0	−10.5 (±0.6)	−7.5 (±0.5)
	50	−9.7 (±0.4)	−7.1 (±0.2)
	100	−9.1 (±0.3)	−6.3 (±0.4)
poly(dA)·poly(dT)	0	−2.9 (±0.5)	−1.0 (±1.9)
	50	−1.3 (±0.5)	−1.1 (±1.0)
	100	−0.9 (±0.5)	−0.4 (±1.4)

[a] 20 mM Tris-HCl, pH 7.2, 29 °C.

in Table II. A negative $\Delta V°$ implies that the partial molar volume of the dye–DNA complex is smaller than that of the sum of the individual reactants. The sign of the $\Delta V°$ also indicates the direction the reaction will proceed when perturbed with pressure; thus, a negative value means that the complex is energetically more favorable as the pressure is increased. Reaction volumes reported here are model dependent insofar as it is assumed that the intercalation reaction is the only process contributing significantly to the pressure data.

On the basis of relaxation amplitudes observed in pressure-jump experiments, the $\Delta V°$ of intercalation of EB into poly[d(A-T)]·poly[d(A-T)] and poly[d(G-C)]·poly[d(G-C)] in 100 mM NaCl was found to be −17 and −15 cm^3 mol^{-1}, respectively (Macgregor et al., 1985). Delben et al. (1982) reported a $\Delta V°$ of −17 cm^3 mol^{-1} for EB intercalation into calf thymus DNA using dilatometry.

Calorimetry. To facilitate the interpretation of the molar reaction volumes, we carried out titration microcalorimetric experiments at three NaCl concentrations (Table III) and 29 °C. Calorimetric titration curves for the addition of EB to the poly[d(A-T)]·poly[d(A-T)] duplex and to buffer are shown in Figure 4. The heats obtained for each injection are dependent on the total concentration of added ligand. After correction for the heat of dilution of the dye, molar binding enthalpies ($\Delta H°$) are calculated as a function of r, the fraction of binding sites occupied, Figure 5. Exothermic enthalpies are measured for intercalation of either dye into poly[d(A-T)]·poly[d(A-T)]; near-zero $\Delta H°$ values are found with poly(dA)·poly(dT). For both polymers $\Delta H°$ depends on the fraction of bound ligand (r), more so in the case of poly[d(A-T)]·poly[d(A-T)]. The enthalpy values at high levels of bound ligand agree with the corresponding values obtained with batch calorimetry and flow calorimetry at higher DNA concentrations (Delben et al., 1982; Chou et al., 1985). Raising the salt concentration leads to a small decrease in the overall exothermicity of the binding enthalpies. In order to correlate the enthalpies with the molar volume changes at 21 °C, heat capacities were obtained by measuring binding enthalpies of the two dyes into poly[d(A-T)]·poly[d(A-T)] at 19 and 29 °C; we obtained heat capacities equal to 68 (±5) and 48 (±9) cal K^{-1} mol^{-1} for EB and PI, respectively.

Complete Thermodynamic Profiles. In order to compare directly our thermodynamic results, the observed variables,

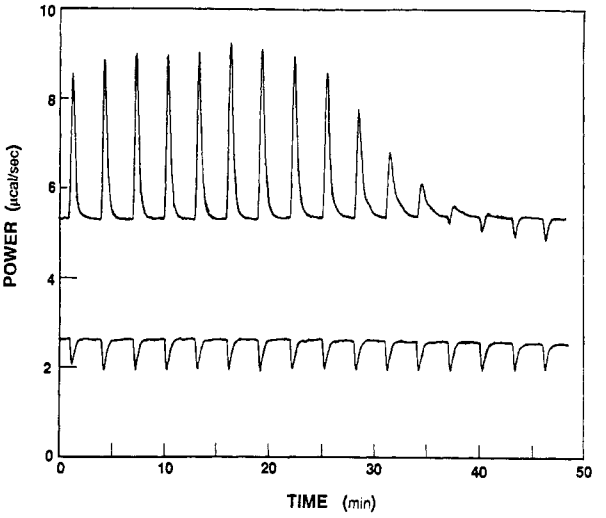

FIGURE 4: Calorimetric titrations. The top curve corresponds to a sequence of 16 injections (5 μL each) of a 2.52 mM solution of EB into 1.4 mL of poly[d(A-T)]·poly[d(A-T)] (0.11 mM bp) in 20 mM Tris-HCl–50 mM NaCl, pH 7.2, 29 °C; the bottom curve is the dilution of EB into the same buffer under the same conditions.

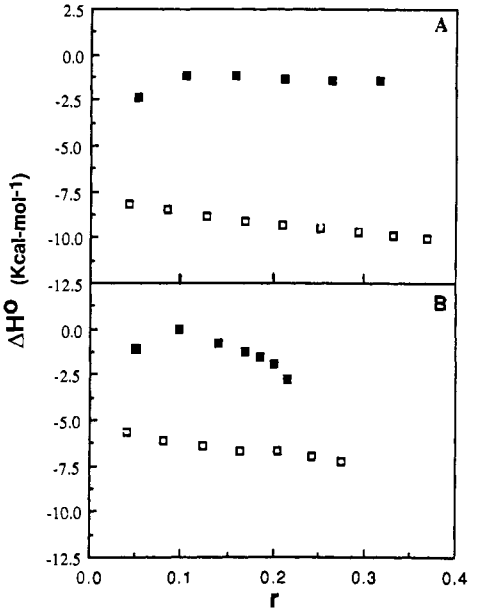

FIGURE 5: Dependence of the binding enthalpies, $\Delta H°$, on the fraction of bound sites, r, in 20 mM Tris-HCl–50 mM NaCl, pH 7.2, 29 °C. (Top panel) EB with poly[d(A-T)]·poly[d(A-T)] (closed squares) and with poly(dA)·poly(dT) (open squares). (Bottom panel) PI with poly[d(A-T)]·poly[d(A-T)] (closed squares) and with poly(dA)·poly(dT) (open squares).

Table IV: Thermodynamic Parameters for Intercalation into dA·dT Polymers in 50 mM NaCl[a]

	$\Delta G°$ (kcal mol^{-1})	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (kcal mol^{-1})	$\Delta V°$ (cm^3 mol^{-1})
ethidium				
poly[d(A-T)]·poly[d(A-T)]	−8.1	−9.0	−0.9	−13
poly(dA)·poly(dT)	−5.0	−1.3	+3.7	+4.5
propidium				
poly[d(A-T)]·poly[d(A-T)]	−8.9	−6.6	+2.3	−8.3
poly(dA)·poly(dT)	−7.4	−1.1	+6.3	+6.8

[a] 20 mM Tris-HCl, pH 7.2, 20 °C.

$\Delta V°$ and $\Delta H°$, along with the calculated variables, $\Delta G°$ and $\Delta S°$, are presented in Table IV. The equilibrium constants from the fluorescence titrations are used to calculate the standard free energy change ($\Delta G°$) for intercalation employing

Table V: Differential Thermodynamic Parameters for Intercalation into dA·dT Polymers in 50 mM NaCl

	poly(dA)·poly(dT) – poly[d(A-T)]·poly[d(A-T)]			
	$\Delta\Delta G°$ (kcal mol^{-1})	$\Delta\Delta H°$ (kcal mol^{-1})	$\Delta(T\Delta S°)$ (kcal mol^{-1})	$\Delta\Delta V°$ (cm^3 mol^{-1})
ethidium	3.1	7.7	4.6	17.5
propidium	1.5	5.5	4.0	15.1

the relation $\Delta G° = -RT \ln K_a$, where R is the gas constant (1.987 cal K^{-1} mol^{-1}) and T is the absolute temperature. Together with the $\Delta H°$ determined by microcalorimetry, the $\Delta S°$ for each reaction can be found by rearranging the equation $\Delta G° = \Delta H° - T\Delta S°$. Despite differences in the free energy values for a given dye to each polymer, the changes in $\Delta V°$ parallel those for $\Delta S°$ of this reaction, as is most clearly seen in Table V. This result reflects the generally observed relation between these two parameters for ionic interactions in polar solvents (Hepler, 1964; Spiro et al., 1968).

Discussion

We will discuss the observed thermodynamic parameters in terms of the following overall mechanism for the formation of the intercalation complex:

$$\text{site}[a\text{Na}^+(\alpha\text{H}_2\text{O}),b\text{H}_2\text{O}] + \text{dye}[c\text{H}_2\text{O}] \rightleftharpoons \text{complex}[x\text{Na}^+(\beta\text{H}_2\text{O}),y\text{H}_2\text{O}] + m\text{Na}^+(z\text{H}_2\text{O}) + n\text{H}_2\text{O} \quad (2)$$

where *site* refers to a sufficient number of consecutive unoccupied base pairs to permit intercalation and *complex* is the intercalative association formed between the site and the dye (EB or PI). The molecularity of the reactants and products is denoted by a letter prefix (Roman or Greek); for example, 1 mol of site has associated with it a mol of Na^+ and b mol of H_2O; the sodium ion in turn has α mol of water in its hydration shell. The hydration of each species is indicated in order to emphasize their role in the reaction, and in general, the extent of hydration of the species on the left side of eq 2 will not equal that on the right side. The formal concentrations of water and Na^+ were not included in the calculation of the binding constants or other thermodynamic parameters.

In this discussion the binding properties of two structurally similar dyes to two isomeric DNA molecules are compared; for each parameter a comparison is made between the two dyes binding to the same polymer. A second comparison is also made for the binding properties of each dye to the two different polymers.

Binding Affinities. The parameters characterizing the binding of EB and PI to the two polymers are given in Tables I and IV. The purpose of the titrations was to establish the constants describing equilibrium binding (K_a, n, ω); these values were then used in the calculations of the thermodynamic parameters. Similar data for other DNA polymers have been presented by other authors, and where values existed, our results agree the earlier measurements (Jovin & Striker, 1977; Bresloff & Crothers, 1981; Macgregor et al., 1985; Wilson et al., 1985).

The association constant for the intercalation of PI into poly[d(A-T)]·poly[d(A-T)] is slightly larger than that of EB; in addition, PI excludes 3 bp for the 2 bp of EB. In light of the higher charge and larger nonintercalating part of PI (see Figure 1), these two results are reasonable.

The binding of EB and PI to poly(dA)·poly(dT), however, is quite dissimilar; the equilibrium constants differ by more than 2 orders of magnitude, and the number of excluded base pairs and the cooperativity parameter are also different. Presumably, the cooperativity arises from a ligand-induced conformational transition in the DNA. According to this hypothesis, the binding of the first EB to a naked DNA lattice ($r \rightarrow 0$) stabilizes a conformational change extending several bp in both directions from the intercalation site; subsequent intercalation events are energetically more favorable within this intercalator–induced conformation. This implies that the intercalation reaction can be broken down into a two-step mechanism: the first step involves a conformational change of the DNA, and the second step is the actual intercalation of the dye. Kinetic and thermodynamic evidence for this mechanism has been presented by Capelle et al. (1979), Wilson et al. (1985), and Macgregor et al. (1987), and it is the mechanism implicit in isotope-exchange studies of DNA (Englander & Kallenbach, 1984). Our results suggest that the fraction of poly(dA)·poly(dT) in the conformation favoring intercalation is smaller than for other DNA polymers; dye binding stabilizes the conformation exhibiting stronger intercalation and thus facilitates subsequent intercalation in vicinal base pairs.

Titrating poly(dA)·poly(dT) with PI yielded values of K_a considerably larger than observed with EB. The exclusion parameter for PI intercalation into this polymer is also larger (3.7 vs 3.1), but there was no evidence of positive cooperativity. It is postulated that no cooperativity is observed because the extra charge of PI renders the ionic component of its interaction with this polymer sufficiently strong that low-energy conformational changes do not noticeably influence the binding. Under experimental conditions that lower the K_a by weakening the ionic component of the interaction such as the high salt concentration, PI does display cooperativity in binding to this polymer (Wilson et al., 1985). Therefore, we would conclude that as the salt concentration is increased the observed decrease the K_a is accompanied by an onset of measurable cooperativity due to the larger role of the energetics of the conformational change in the DNA to the overall binding energy. Decreasing the charge on the intercalator should cause the cooperativity to become measurable at lower ionic concentrations, as is observed for the binding of EB with this polymer.

Binding Enthalpies. The observed binding enthalpies for the association of EB and PI to each polymer are strikingly different (Table III). We consider the total enthalpy change of binding, $\Delta H°$, as arising from the sum $\Delta H°_{\text{intrinsic}} + \Delta H°_{\text{ionic}} + \Delta H°_{\text{conformation}} + \Delta H°_{\text{hydration}}$. The term $\Delta H°_{\text{intrinsic}}$ is the result of exothermic contributions from stacking interactions; this is aproximately equal for the four complexes considered here because the phenanthroline ring stacks with the same bases in both polymers. Likewise, $\Delta H°_{\text{ionic}}$ for all of the complexes is similar according to the salt dependence we report in Table III. Transitions between the various DNA conformations proceed with small enthalpy changes (Marky et al., 1981; Chaires & Sturtevant, 1986); thus, contributions to the overall $\Delta H°$ arising from conformational changes in the DNA and dye ($\Delta H°_{\text{conformation}}$) are also small. With these assumptions, the difference in $\Delta H°$ of intercalation of a given dye into these two polymers is interpreted is being primarily due to enthalpic differences arising from hydration changes at the polymer level since changes in hydration of the dye arising from intercalation of the phenanthroline ring will be the same in all cases under consideration. For a given polymer, the differences in $\Delta H°$ for the binding of these two dyes depend primarily upon hydration changes due to the extra charge of PI. These hydration differences may be present in the complexes as well as in the free dyes.

Binding Entropies. In a similar fashion, the derived entropy change, $\Delta S°$, is considered to be equal to the sum of the following contributions: $\Delta S°_{molecularity}$, the loss of entropy due to a bimolecular association reaction; $\Delta S°_{conformation}$, changes in the polymer configuration during binding; $\Delta S°_{counterion}$, the release of Na^+ and Tris-H^+ accompanying binding of a cationic dye; $\Delta S°_{hydration}$, the release or uptake of water molecules. The first two terms are considered to be identical for the reactions considered here. On the basis of the change in K_a with NaCl concentration (Record et al., 1978), two cations are released when PI intercalates whereas only one is released upon intercalation of EB; thus, the third term ($\Delta S°_{counterion}$) depends upon the ligand under consideration. However, for a given ligand cation release is independent of the polymer.

For the association of ion pairs in aqueous solution, changes in $\Delta S°$ are proportional to changes in $\Delta V°$ (Helper, 1964; Spiro et al., 1968); as shown in Table IV, the measured $\Delta V°$ values depend on the polymer and dye under consideration. The changes in both of these thermodynamic parameters have the same molecular origin, namely, a difference in the electrostriction of the surrounding water. Formation of an ion pair in water results in the release of several molecules of water from the hydration spheres of the interacting ions; the net increase in the number of free water molecules results in an increase in the entropy. This process additionally contributes a positive volume change to the overall $\Delta V°$ of the reaction because the partial molar volume of the released (bulk) water is larger than that of water electrostricted around an ion. The pressures employed in these experiments are not high enough to change the volume of any of the molecules due to shortening of bond lengths or compression of the molecular van der Waals surfaces. Measurable values of $\Delta V°$ arise exclusively as a consequence of changes in solvent interactions. The data presented in Table IV demonstrate that the derived $\Delta S°$ and measured $\Delta V°$ for the intercalation of EB and PI vary in a parallel manner; this result is interpreted as directly reflecting differences in $\Delta S°_{hydration}$.

Comparison of the Differential Thermodynamic Parameters. When the thermodynamic parameters are considered in terms of a differential effect, that is, if the parameters of dye–poly[d(A-T)]·poly[d(A-T)] are subtracted from those of dye–poly(dA)·poly(dT), we obtain the values exhibited in Table V. Because reaction volumes are equal to the difference between the sums of the partial molar volumes of the products and reactants ($\sum V_{prod} - \sum V_{react}$), the positive $\Delta\Delta V°$ implies either that $\sum V_{prod}$ is larger for poly(dA)·poly(dT) than for poly[d(A-T)]·poly[d(A-T)] or that $\sum V_{react}$ is smaller, or both.

The presence of an intercalated dye will minimize the hydration and structural differences between the two polymers; base-stacking interactions and unusual or sequence-specific solvent interactions will be lost upon opening of the helix to allow intercalation of the phenanthroline ring. After intercalation the interaction of the water with the two dye–polymer complexes will be similar. These considerations imply that $\sum V_{prod}$ is similar for the two polymers; consequently, the fact that $\Delta\Delta V°$ is positive must be due to the smaller $\sum V_{react}$ for the reaction involving poly(dA)·poly(dT). It is evident that the partial molar volume of unbound EB of PI will not depend on the polymer. Therefore, the smaller $\sum V_{react}$ arises from the more negative partial molar volume, i.e., more extensive hydration of poly(dA)·poly(dT). This proposal has recently been corroborated by the densitometric measurements of the hydration volumes of DNA oligomers and polymers (Buckin et al., 1989; Marky & Kupke, 1989). The correlation between the entropy and volume changes is also evident in Table V.

It has been suggested that a spine of immobilized water molecules lies along the minor groove of poly(dA)·poly(dT) duplexes (Fratani et al., 1982; Chuprina, 1987). Via hydrogen bonding, such a spine of constrained water molecules may stabilize a warped B-type structure and narrow the minor groove. Intercalation would necessarily destroy this spine of water molecules at least between the bp where the dye is inserted. This proposed structural feature of poly(dA)·poly(dT) could be responsible for the different behavior of dye intercalation into these two polymers toward pressure.

In an analogous manner, the differential parameters for the binding of the two dyes to the same polymer can also be compared. In all cases the $\Delta V°$ of PI is more positive than that of EB, presumably due to the smaller charge of the latter. The contribution of the additional charge to the volume of intercalation can be estimated by comparing the $\Delta V°$'s of the two dyes with the same polymer; thus, the $\Delta\Delta V°$ (PI − EB) is 4.7 cm^3 mol^{-1} with poly[d(A-T)]·poly[d(A-T)] and 2.3 cm^3 mol^{-1} with poly(dA)·poly(dT). That is, the additional charge of PI increases the partial molar volume of the complex approximately 3.5 cm^3 mol^{-1} with respect to that of the complex formed with EB.

Conclusions

From the pressure dependence of the equilibrium constants for the intercalation of ethidium and propidium, we obtain positive molar volume changes ($\Delta V°$) for poly(dA)·poly(dT) and negative values for poly[d(A-T)]·poly[d(A-T)]. For both intercalators we measure exothermic enthalpies for intercalation into poly[d(A-T)]·[d(A-T)], indicating that enthalpy is primarily driving the reaction. However, in the case of poly(dA)·poly(dT) the enthalpies are only slightly exothermic, implying that intercalation into this polymer is driven by entropy. In all cases, trends in the measured $\Delta V°$'s parallel the changes in the reaction entropies. Assuming that the structures of the dye–DNA complexes are similar, our results imply that the free poly(dA)·poly(dT) helix is more hydrated than poly[d(A-T)]·poly[d(A-T)]. The extra hydration of poly(dA)·poly(dT) may offer an explanation for the unusual physical properties of this polymer.

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