Temperature Dependence of the Volumetric Parameters of Drug Binding to Poly[d(A-T)]·Poly[d(A-T)] and Poly(dA)·Poly(dT)

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ABSTRACT We report the temperature and salt dependence of the volume change (ΔV_b) associated with the binding of ethidium bromide and netropsin with poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)]. The ΔV_b of binding of ethidium with poly(dA)·poly(dT) was much more negative at temperatures ~70°C than at 25°C, whereas the difference is much smaller in the case of binding with poly[d(A-T)]·poly[d(A-T)]. We also determined the volume change of DNA-drug interaction by comparing the volume change of melting of DNA duplex and DNA-drug complex. The DNA-drug complexes display helix-coil transition temperatures (T_m) several degrees above those of the unbound polymers, e.g., the T_m of the netropsin complex with poly(dA)poly(dT) is 106°C. The results for the binding of ethidium with poly[d(A-T)]·poly[d(A-T)] were accurately described by scaled particle theory. However, this analysis did not yield results consistent with our data for ethidium binding with poly(dA)·poly(dT). We hypothesize that heat-induced changes in conformation and hydration of this polymer are responsible for this behavior. The volumetric properties of poly(dA)·poly(dT) become similar to those of poly[d(A-T)]·poly[d(A-T)] at higher temperatures.

INTRODUCTION

Over the past four decades, noncovalent interactions between DNA and drugs have been studied intensively; much of the impetus for these studies has arisen from the goal of rational drug design (1,2). Despite the research dedicated to understanding these interactions, the role of hydration in determining their affinity and specificity has been largely ignored. The interactions of the DNA binding site and the drug with water change upon formation of a complex; disregarding these changes limits the effectiveness and credibility of rational drug design. Although hydration changes are thermodynamically important, the quantitative assessment of the role of hydration in the energetics of the complexes presents a significant experimental challenge: hydration is difficult to detect structurally, it is generally too complex to be adequately simulated by computation, and it is not straightforward to separate the effect of hydration from other factors that influence the thermodynamics of a complex.

To a good approximation, the most important contribution to the volume change accompanying the formation of a noncovalent complex involving biological molecules arises from the accompanying changes in hydration. The difference between the partial molar volume of water in the hydration shell of the complex and of bulk water is the major source of the volume change. Thus, the thermodynamic parameters that provide the most information about hydration are the molar volume, expansivity, and compressibility changes. To date, research aimed at measuring the volume change associated with DNA-drug interactions has met with limited success (3–6). The two most successful methods employed to date are densitometry (4,5,7) and the spectrophotometric (3,6) measurement of the dependence of the equilibrium constant on pressure.

In this work, we measured the volume change arising from the formation of DNA-drug complexes using two methods. In the first method, the equilibrium between the drug and DNA at a given temperature and pressure was altered by changing the hydrostatic pressure and then determining the new equilibrium constant at the same temperature. In the second method, we measured the change in the DNA helix-coil transition temperature with and without bound drug at different pressures. The principal advantage of the second method is that it does not require the drug to have any difference in its spectroscopic properties between the free and bound states. The temperature range of these two methods is complementary; by combining them we can determine the effect of temperature on the volume change over the full temperature range in which the complex is stable. We have used these approaches to study the effect of pressure on the interaction of poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] with the intercalator ethidium bromide and netropsin a drug that noncovalently binds in the minor groove of DNA.

In an attempt to assess the factors that underlie the changes in volume parameters we used scaled particle theory to analyze temperature dependence of the volume parameters we obtained for ethidium binding with poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)]. Previous applications of scaled particle theory to this problem have met with limited success because the absolute values of the parameters rely

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upon detailed structural information that is often not available (8,9). However, the temperature dependence of the volume parameters is expected to be relatively independent of the structural details.

MATERIALS AND METHODS

Materials

Ethidium bromide (EB) and netropsin (nt) were obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification. Other small-molecular-weight chemicals were all reagent grade or better. Poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] were purchased from Amersham Biosciences Corporation. The DNA polymers were dissolved in and then dialyzed against aqueous solutions containing 20 mM Tris-HCl, pH 7.2, 0.1 mM EDTA, and the desired amount of NaCl. The concentration of DNA, in moles of basepairs, was determined spectrophotometrically using molar extinction coefficients: $\varepsilon_{259} = 12{,}000\ M^{-1}\ cm^{-1}$ for poly(dA)·poly(dT) and $\varepsilon_{262} = 13{,}200\ M^{-1}\ cm^{-1}$ for poly[d(A-T)]·poly[d(A-T)] (10,11). The concentrations of the ligand solutions were determined using $\varepsilon_{480} = 5{,}850\ M^{-1}\ cm^{-1}$ and $\varepsilon_{296} = 21{,}500\ M^{-1}\ cm^{-1}$ for EB and netropsin (12,13), respectively.

Fluorometric titrations

To measure the parameters describing the equilibrium binding of EB with DNA at room temperature we carried out fluorescence titrations. The data were acquired on a Spex FluoroMax 3 spectrofluorometer (Jobin Yvon, Edison, NJ). The excitation and emission wavelengths were 512 and 600 nm, respectively. DNA solutions were titrated with concentrated EB solutions. If r is the fraction of binding sites occupied, i.e., r = [bound EB]/[total DNA basepairs], and [L] is the concentration of unbound EB, then according to the site-exclusion model,

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

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

where K_a is the equilibrium binding constant, n is the size of the binding site in basepairs, and ω is a cooperativity parameter (14). The value of the parameters, K_a, n, and ω were determined by nonlinear fitting using MATLAB with n constrained to be an integer value. In our analysis, we assumed that the fluorescence properties of bound EB are independent of the fraction of sites bound, r; this is similar to other studies on measuring binding parameters of EB binding.

The value of the equilibrium constant for the binding of netropsin with DNA reported in the literature is at least three orders of magnitude larger than that of EB, and the excluded site parameter, n, is equal to 5 (15). Due to the larger value of K_a, netropsin binding was considered to be complete under our experimental conditions.

Pressure dependence of the helix-coil transition

The helix-coil transition temperatures, T_m, of DNA and DNA-ligand complexes were determined by monitoring the change in absorbance at 260 nm as the temperature was increased at 0.6°C/min at pressures from 1 to 200 MPa (0.1 MPa = 1 bar = 0.987 atm). The high-pressure equipment has been described previously (16). By measuring T_m at different pressures, the volume change of these helix-coil transitions at ambient pressure was calculated using the Clapeyron equation: $dT_m/dP = T_{m1atm}\Delta V_b/\Delta H$. Calorimetrically determined values of ΔH (measured at atmospheric pressure) were taken from the literature. The helix-coil transition of the DNA-ligand complex involves unbinding ligand from DNA and DNA melting. The volume of DNA-drug binding was determined from the difference between volume change of the helix-coil transition of DNA ($\Delta V_{DNA\ HC}$) and the DNA-ligand complex ($\Delta V_{complex\ HC}$): $\Delta V_b = \Delta V_{DNA\ HC} - \Delta V_{complex\ HC}$. There are two assumptions implicit in this approach:

1. The extent of binding of EB with single-stranded DNA is negligible. The equilibrium constant for binding to single-stranded DNA is at least 10 times less than that for duplexes (17,18).
2. The enthalpy of helix-coil transition of DNA-ligand complex is equal to the sum of the enthalpy of the helix-coil transition of naked DNA and enthalpy of unbinding of ligand.

Fluorescence measurements at high pressure

We also measured the molar volume change of DNA-ligand binding on the basis of the standard thermodynamic relationship: $(\partial \ln K_a/\partial P)_T = -\Delta V_b/RT$, where R is the gas constant. To determine ΔV_b, we measured the change of $\ln K_a$ with pressure at constant temperature. The molar volume change of the intercalation of EB with the two DNA polymers was determined at four or five temperatures. The experimental settings of the spectrofluorometer were the same as those used in the fluorescence titration experiments. The change of $\ln K_a$ could be derived from the change of fluorescence signal intensity based on the results from the titration experiments. To determine ΔV_b, the data, $\ln K_a$ versus pressure, fitted with a second-order polynomial (OriginLab, Northampton, MA), were extrapolated to atmospheric pressure.

RESULTS

Binding data

The equilibrium binding parameters for ethidium bromide binding with poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] at different salt conditions are summarized in Table 1.

The binding constant, K_a, and cooperativity, ω, are obtained by fitting the raw data to the McGhee-von Hippel site-exclusion model using integer values of the binding-site size n (14). Binding with the homopolymer, poly(dA)·poly(dT), shows positive cooperativity, whereas no cooperativity is observed for interaction with the alternating polymer, poly[d(A-T)]·poly[d(A-T)]. The equilibrium constant for EB binding to poly[d(A-T)]·poly[d(A-T)] is >24 times larger than that for binding with homopolymer. The binding constants decrease with increasing salt concentration for both polymers.

TABLE 1 Equilibrium binding parameters for ethidium bromide binding with DNA

	[NaCl] (mM)	K_a (μM^{-1})	n (bp)	ω
Poly(dA)·poly(dT)	25	0.066*	5	6.2
	70	0.0254	5	4.6
Poly[d(A-T)]·poly[d(A-T)]	25	1.7	2	1
	70	0.60	2	1

All measurements were in 20 mM Tris-HCl, at pH 7.2, 25°C.
*The error in K_a and ω is ±10%.

Volume change of the DNA helix-coil transition

The pressure dependence of the helix-coil transition temperature, T_m, of the two polymers at two different salt concentrations is summarized in Table 2. We calculated the enthalpies of DNA denaturation at each of the transition temperatures given in Table 2 using calorimetrically measured, temperature-dependent enthalpies. Thus, $\Delta H_{ref} = 39.2$ kJ mol^{-1} at 58.2°C with $\Delta C_p = 228$ J mol^{-1} K^{-1} for poly(dA)·poly(dT) and $\Delta H_{ref} = 33.7$ kJ mol^{-1} at 50.9°C with $\Delta C_p = 178$ J mol^{-1} K^{-1} for poly[d(A-T)]·poly[d(A-T)] (19). Increasing the salt concentration stabilizes both polymers; the change in T_m upon changing the salt concentration from 25 to 70 mM equals 7.6 and 7.8°C for poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)], respectively.

Combining the data in Table 1 with other data describing the transition volume of these polymers (20,21), one obtains ΔV of the helix-coil transition at different temperatures (Fig. 1). For both polymers, the volume change varies linearly with the helix-coil transition temperature at atmospheric pressure. Within the temperature range studied, both polymers exhibit a positive volume change for the helix-coil transition and the magnitude of ΔV increases with increasing temperature. The magnitude of ΔV for poly[d(A-T)]·poly[d(A-T)] is less than that for poly(dA)poly(dT) at low temperatures; however, the value of ΔV for the two polymers becomes similar at higher temperatures. Extrapolating the temperature dependencies shown in Fig. 1, one finds that at 84.6°C the ΔV for the helix-coil transition equals $+5.6$ cm^3 (mol bp)$^{-1}$ for both polymers.

Volume change of denaturing the DNA-ethidium complex

In these experiments, we measured the helix-coil transition temperature of the DNA-ethidium complex at different pressures; the T_m of the complex is greater than that of the polymer alone. The pressure dependence of the T_m for the two polymers and their EB complexes is depicted in Fig. 2 and the data are summarized in Table 3. As expected, the binding of EB stabilizes the helix form of both polymers. As the extent of binding increases, the helix-coil transition temperature increases; thus, the T_m of the 2:1 complexes is greater than that of the 5:1 complexes. The effect of the salt concentration on the T_m is largest for the unbound polymers; the binding of EB decreases the influence of the salt concentration on the T_m. For example, increasing the salt concentration from 25 to 70 mM caused the T_m of poly(dA)·poly(dT) to increase by 7.6°C, whereas the T_m increased by 3.1°C and 0.3°C with low or high levels of bound ethidium, respectively.

In Table 3, the enthalpy of the transition was calculated as the sum of the enthalpy of the helix-coil transition of naked DNA and unbinding of ethidium per basepair at the transition temperature: $\Delta H_{tot} = \Delta H_{hc} + r\Delta H_{uneb}$. The enthalpy of helix-coil transition of naked DNA, ΔH_{hc}, was calculated as described before. The enthalpy change resulting from the unbinding of EB, ΔH_{uneb}, was calculated using $\Delta H = 5.4$ kJ mol$^{-1}_{complex}$ at 20°C, and $\Delta Cp \approx 0$ J K^{-1} mol^{-1} for poly(dA)·poly(dT) (22), and $\Delta H = 38$ kJ mol$^{-1}_{complex}$ and $\Delta Cp \approx -285$ J K^{-1} mol^{-1} at 20°C for poly[d(A-T)]·poly[d(A-T)] (3). At each temperature, r is calculated using the van 't Hoff relationship, the values of the binding parameters at 298 K (Table 1), and literature values of the binding enthalpy (3). The cooperativity and binding-site size were assumed not to vary with temperature. This assumption appears reasonable because the magnitude of ΔH_{hc} is several times greater that that of $r\Delta H_{uneb}$. Thus, any temperature dependence of these two parameters would not significantly change ΔH_{tot}. The last column of Table 3 shows the volume change of melting of DNA-ethidium complexes per basepair of DNA. We do not report data in the case of intermediate amounts of bound ethidium (basepair/ligand ratio of ~5) with poly[d(A-T)]·poly[d(A-T)] at low salt concentration (25 mM), because the transitions are biphasic. All other transitions were monophasic.

Table 4 summarizes the volume change of DNA-EB binding (ΔV_b) obtained at the helix-coil transition temperature, which we calculated according to $\Delta V_b = (\Delta V_{DNA\ HC} - \Delta V_{complex\ HC})/r$. In all cases, we observed a negative volume change; thus, higher pressure stabilizes the complex. For the intercalation of EB with poly(dA)·poly(dT), ΔV_b ranges from -5.0 to -16.8 cm^{3}mol^{-1} and is more negative at higher salt concentration and at lower degrees of binding. Note that if the cooperativity of binding of EB with poly(dA)·poly(dT) is completely lost at helix-coil transition temperature, the calculated value of r will be different and the resulting ΔV_b will be slightly more negative with an average decrease of ~3 cm^3 mol^{-1}. The volume change arising from the interaction of EB with poly[d(A-T)] equals ~-13 cm^3 mol^{-1};

TABLE 2 Volume change of DNA melting at different salts concentrations

	[NaCl] (mM)	T_m (°C)	$100 \times (\Delta T_m/\Delta P)$ (°C/MPa)	ΔH^* (kJ mol^{-1})	ΔV (cm^3 mol^{-1})
Poly(dA)·poly(dT)	25	56.9 ± 0.1	2.57 ± 0.13	38.9	3.03 ± 0.15
	70	64.5 ± 0.1	3.02 ± 0.11	40.6	3.63 ± 0.13
Poly[d(A-T)]·poly[d(A-T)]	25	50.1 ± 0.0	0.432 ± 0.029	33.6	0.449 ± 0.030
	70	57.9 ± 0.2	2.01 ± 0.12	35.0	2.12 ± 0.13

All measurements were in 20 mM Tris-HCl, at pH 7.2.
*ΔH and ΔV are per mole of basepairs.

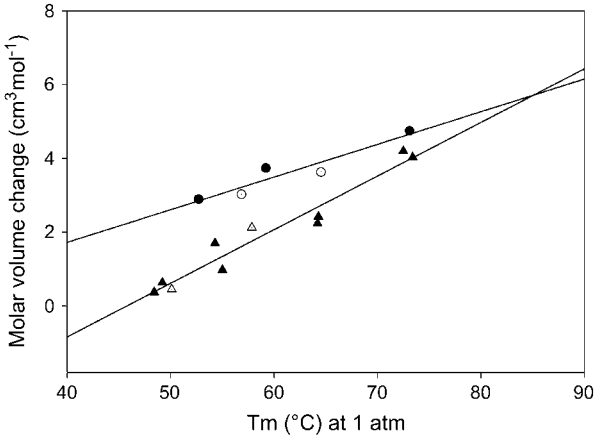

FIGURE 1 Temperature dependence of the molar volume change of DNA denaturation. The volume change is per mole of basepairs. The open circles are data for poly(dA)·poly(dT) obtained in this work and the solid circles are from the literature (20). The open triangles are data for poly[d(A-T)]·poly[d(A-T)] obtained in this work and the solid triangles are from the literature (21). The lines fit to the data are given by ΔV (cm^3 mol bp^{-1}) = -1.82 ($\pm$ 1.04) + (0.088 $\pm$ 0.017) $\times$ T_m (°C) for poly(dA)·poly(dT) and ΔV (cm^3 mol bp^{-1}) = -6.64 ($\pm$ 0.72) + (0.145 $\pm$ 0.012) $\times$ T_m (°C) for poly[d(A-T)]·poly[d(A-T)].

this value was not influenced by salt concentration or degree of binding.

Volume change of denaturing the DNA-netropsin complex

The pressure dependence of the T_m of the two polymers and their netropsin complexes is shown in Fig. 3; the data are summarized in Table 5. The T_m increases significantly, ~40°C, upon the binding of nt; this is much greater than the change caused by the binding of EB, because nt binds more strongly to DNA. The binding of nt with poly(dA)·poly(dT) is energetically more favorable than with poly[d(A-T)]·poly[d(A-T)]; ethidium displays the opposite preference for these polymers. There is no significant difference between the data obtained at 25 and 75 mM salt; this is expected for positively charged ligands at high degrees of binding. Under experimental conditions where the ratio of DNA basepairs to nt was >2:1, we observed a biphasic transition.

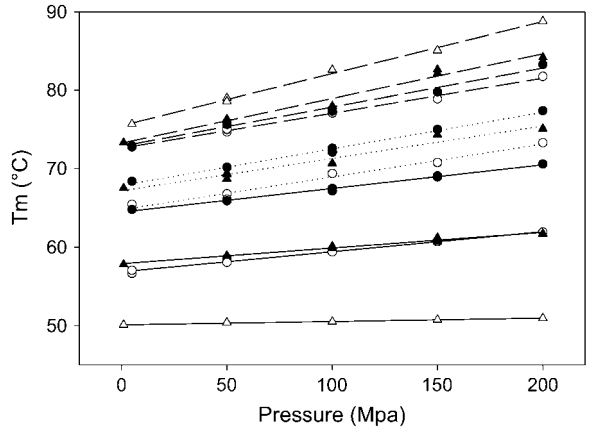

FIGURE 2 Pressure dependence of the helix-coil transition temperature of DNA with or without ethidium bromide at two sodium chloride concentrations. Poly(dA)·poly(dT), *circles*; poly[d(A-T)]·poly[d(A-T)], *triangles*; 25 mM NaCl, *open symbols*; 75 mM NaCl, *solid symbols*; DNA only, *solid line*; basepair/drug ratio of ~5:1 (*dotted line*); and basepair/drug ratio of 2:1 (*dashed line*). Please refer to the text for details.

The enthalpy of the helix-coil transition of the DNA-nt complex was calculated as described above for EB; the results are shown in Table 5. For the binding of nt with poly(dA)·poly(dT), poly[d(A-T)]·poly[d(A-T)], $\Delta H = -0.4$ kJ mol$^{-1}_{complex}$ and -51.1 kJ mol$^{-1}_{complex}$, respectively (14), assuming $\Delta Cp \approx 0$ for complex formation with either polymer (23). Each bound nt molecule is considered to occupy five consecutive basepairs with a binding constant ~10^9 M^{-1} (15). Saturation was reached in the four cases we studied with ~0.2 bound nt molecules per basepair.

Table 4 summarizes the values of ΔV_b for the DNA-netropsin complex at the helix-coil transition temperature. Under the conditions studied, the binding of nt exhibits small negative ΔV_b values ranging from -4.0 to -7.7 cm^3 mol^{-1}. The ΔV_b values are slightly more negative for binding with poly(dA)·poly(dT) than with poly[d(A-T)]·poly[d(A-T)] and slightly more negative at higher salt concentrations, but the difference is not significant.

Temperature dependence of ΔV_b for EB binding with DNA

Due to the nature of the method employed, the values reported above are obtained at temperatures much higher than those at which most literature data have been reported, i.e., 20–25°C. We also measured ΔV_b of EB binding with the two polymers at different temperatures by monitoring the effect of pressure on the fluorescence of the EB-DNA complex; this enabled us to make direct comparisons between the data obtained at the helix-coil transition temperature and literature results obtained at ~25°C. The binding parameters of EB intercalation summarized in Table 1 are similar to those reported by Marky and Macgregor (3).

The volume change associated with EB binding with the two polymers at different temperatures is shown in Fig. 4. The binding constant, K_a, at different temperatures was calculated from the fluorescence intensity; the temperature dependence of K_a is shown in Fig. 5. We calculated the enthalpies of binding from the slopes in Fig. 5 using the van 't Hoff equation. The data are summarized in Table 6.

The data in Fig. 4 show that the ΔV_b of poly[d(A-T)]·poly[d(A-T)] complexation with EB remains negative at all temperatures. For this system, ΔV_b becomes more negative with increasing temperature; the coefficient of expansivity, $\Delta E = -0.15$ cm^3 mol^{-1} K^{-1}. In the case of the formation of the complex between poly(dA)·poly(dT) and EB, ΔV_b

TABLE 3 Volume change of melting of DNA-ethidium complexes

Polymer/EB ratio	[NaCl] (mM)	T_m (°C)	$100 \times (\Delta T_m/\Delta P)$ (°C/MPa)	ΔH^* (kJ mol^{-1})	ΔV_b (cm^3 mol^{-1})
Poly(dA)·poly(dT)					
5:1	25	64.8 ± 0.4	4.19 ± 0.32	41.4	5.13 ± 0.39
2:1	70	67.9 ± 0.2	4.66 ± 0.19	41.8	5.72 ± 0.23
	25	72.6 ± 0.2	4.46 ± 0.15	43.4	5.60 ± 0.19
Poly[d(A-T)]·poly[d(A-T)]					
6.03:1	70	72.9 ± 0.4	4.98 ± 0.34	43.3	6.23 ± 0.43
2:1	70	67.2 ± 0.4	4.13 ± 0.36	39.6	4.81 ± 0.42
2.53:1	25	75.5 ± 0.2	6.63 ± 0.21	45.1	8.57 ± 0.27
	70	73.3 ± 0.4	5.69 ± 0.37	43.0	7.06 ± 0.46

All measurements were in 20 mM Tris-HCl, at pH 7.2.
*ΔH and ΔV_b are per mole of basepairs.

changes sign from negative to positive at ~41°C and $\Delta E = -0.49$ cm^3 mol^{-1} K^{-1}. The value of ΔE is 3–4 times larger for EB binding to poly(dA)·poly(dT) than to poly [d(A-T)]·poly[d(A-T)]. The trends observed in all of the data for the two experimental methods agree well. As seen in Fig. 4, it is evident that at higher temperatures the ΔV_b becomes similar for these two polymers.

DISCUSSION

We report the temperature and salt dependence of the equilibrium volume parameters for the complexes formed by ethidium bromide and netropsin and poly[d(A-T)]·poly[d(A-T)] and poly(dA)·poly(dT). Our data greatly extend the temperature range of these values for these two DNA-binding ligands. For many noncovalent interactions, a large fraction of the net free energy change results from the differential hydration of the free and bound states. The volume parameters of the binding interaction are the values most directly related to changes in hydration. By extending the temperature range of the volumetric parameters, we can better understand the thermodynamic origins of the stability of the complexes.

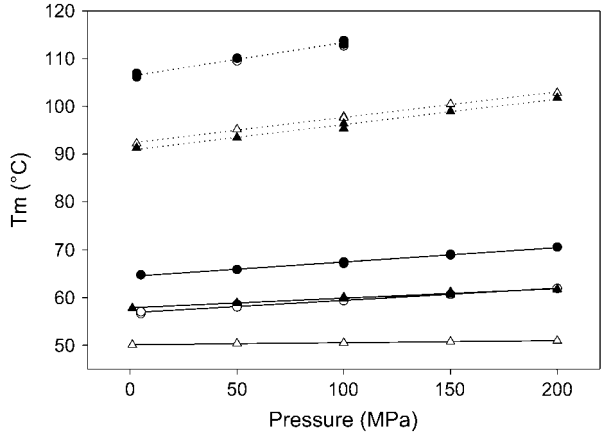

FIGURE 3 Pressure dependence of the helix-coil transition temperature of DNA with or without netropsin at various salt concentrations. Poly(dA)·poly(dT), *circles*; poly[d(A-T)]·poly[d(A-T)], *triangles*; 25 mM salt, *open symbols*; 75 mM salt, *solid symbols*; DNA only, *solid line*; basepair/drug ratio of 2:1, *dotted line*).

We can decompose the volume change that results from formation of a complex, ΔV_b, into a sum of three components: $\Delta V_b = \Delta V_I + \Delta V_T + \Delta V_H$, where ΔV_I is the intrinsic volume change, ΔV_T is the thermal volume change, and ΔV_H is the hydration volume change. The intrinsic volume, V_I, is the geometric volume of the solute molecules (9); ΔV_I will be negligible because the DNA and the DNA-ligand complex are tightly packed and have no significant internal voids. The thermal volume, V_T, is the volume of the layer of void space surrounding the solvent accessible surface of the solute molecules. This volume arises from the thermal motion of solute and solvent molecules. Because the thickness of this void layer depends primarily on the solvent, the thermal volume is proportional to the accessible surface area of the solutes to a first approximation.

Intercalation and binding to the minor groove result in a loss of solvent-accessible surface area, hence a loss of V_T and a negative ΔV_T. Hydration volume, V_H, is the change in solvent volume arising from the interactions between the solute and the solvent; in water, these interactions lead to the formation of a hydration shell composed of molecules with a higher density than bulk water. The hydration volume change, ΔV_H, is the volume change generated from exchange between relatively high-density water in the hydration shell of solutes and lower-density bulk water. Both intercalation and minor-groove binding require that the ligands lose some solvent accessibility and some fraction of their hydration shell. Binding may also disrupt specific hydration structures, for example, the specific hydration in the minor-groove DNA. The release of counterions upon binding may have a small negative contribution to ΔV_H, although this effect may be offset by the uptake of a similarly charged ligand.

The formation of a DNA-ligand complex may lead to the creation of an extensive multilayer hydration structure surrounding unbound DNA; however, the total number of water molecules involved in hydration will decrease due to the loss of solvent-accessible surface. Thus, the overall result of binding is a net release of high-density hydration water to the bulk phase and a positive ΔV_H. In sum, ΔV_b has a major

TABLE 4 Volume change of DNA-ligand binding

	[NaCl] (mM)	T_m (°C)	$\Delta V_{DNA\ HC}$ ($cm^3\ mol^{-1}$)	$\Delta V_{complex\ HC}$ ($cm^3\ mol^{-1}$)	ΔV_b ($cm^3\ mol^{-1}$)
Poly(dA)·poly(dT)/					
EB = 5:1 (7.75:1)	25	64.8 ± 0.4	3.95 ± 0.82	5.13 ± 0.39	−9.2 ± 2.0
(11.4:1)	70	67.9 ± 0.2	4.25 ± 0.88	5.72 ± 0.23	−16.8 ± 3.5
EB = 2:1 (5.72:1)	25	72.6 ± 0.2	4.73 ± 0.98	5.60 ± 0.19	−5.0 ± 1.0
(7.11:1)	70	72.9 ± 0.4	4.75 ± 0.98	6.23 ± 0.43	−10.5 ± 2.3
Poly[d(A-T)]·poly[d(A-T)]/					
EB 6.03:1 (8.14:1)	70	67.2 ± 0.4	3.11 ± 0.2	4.81 ± 0.42	−13.8 ± 2.2
2:1 (3.15:1)	25	75.5 ± 0.2	4.32 ± 0.59	8.57 ± 0.27	−13.4 ± 1.9
2.53:1 (4.28:1)	70	73.3 ± 0.4	4.00 ± 0.54	7.06 ± 0.46	−13.1 ± 2.0
Poly(dA)·poly(dT)					
Netropsin = 2:1 (5:1)	25	106.4 ± 0.4	8.09 ± 1.67	9.17 ± 0.73	−5.41 ± 1.20
	70	106.3 ± 0.3	8.08 ± 1.67	9.62 ± 0.51	−7.74 ± 1.65
Poly[d(A-T)]·poly[d(A-T)]					
Netropsin = 2:1 (5:1)	25	92.3 ± 0.2	6.76 ± 0.92	7.55 ± 0.18	−3.97 ± 0.58
	70	90.8 ± 0.3	6.54 ± 0.89	7.52 ± 0.41	−4.88 ± 0.72

All measurements were in 20 mM Tris-HCl, at pH 7.2

In the first column the ratios before brackets are the ratio of basepairs to total ligands and the ratio in the brackets are ratio of basepairs to bound ligands. $\Delta V_{DNA\ HC}$ and $\Delta V_{complex\ HC}$ are per mole of basepair; ΔV_b is per mole of binding event.

negative contribution from ΔV_T and a major positive contribution from ΔV_H. The sign of ΔV_b depends on the relative magnitude of ΔV_T and ΔV_H. Both negative and positive ΔV_b have been reported (3,24).

Ideally, one would like to link the thermodynamic values with molecular changes; however, such an interpretation is not straightforward. Thermodynamic values for biological systems are rarely sufficiently detailed to permit a molecular interpretation. Structural methods generally only reveal those water molecules that are strongly associated with the solute while the majority water molecules interacting more weakly are not observed. We considered that, in the present case, theory might provide insight where these two experimental approaches fall short.

The scaled particle theory (SPT) is a statistical mechanical theory of liquids developed to interpret the thermodynamic parameters of aqueous and nonaqueous solutions (8,25,26). With SPT one can generate an approximate expression for the reversible work required to generate a cavity in a fluid of spherical particles to accommodate a new spherical particle (the solute); the volume of the cavity is equal to $V_I + V_T$ (8). According to the assumptions of SPT, the thermal volume, V_T, of a spherical solute is given as (8,9)

$$V_T = 82.054\beta_{T_0} T f(T), \quad (1)$$

where $f(T) = 6By/(1-y)^2 + 36Cy^2/(1-y)^3 + y/(1-y)$ and $y = \pi d_1^3 N_A/(6V_0^o)$.

The parameter d_1 is the effective hard-sphere diameter of the solvent; for water $d_1 = 0.274$ nm; $V_0^o$is the partial molar volume of the solvent; N_A is Avogadro's number, and y is the packing density of the solvent. The parameters B and C depend only on the relative size of the solute and the solvent molecules. It is important to note that the only temperature-dependent parameters are V_0^o and the coefficient of isothermal compressibility of the solvent β_{T_0}.

SPT has limited utility for predicting the absolute value of V_T because most molecules are not well approximated as spheres but the temperature dependence of V_T is not expected to depend on shape and should be explained by this theory. The relative temperature dependence of V_T can be expressed as a sum of three terms:

$$\frac{dV_T}{V_T} = \frac{dT}{T} + \frac{d\beta_{T_0}}{\beta_{T_0}} + \frac{df(T)}{f(T)}. \quad (2)$$

The first term on the right-hand side of Eq. 2 contributes to an increase in V_T with temperature and is almost constant

TABLE 5 Volume change of melting of DNA-netropsin complexes

	[NaCl] (mM)	T_m (°C)	$100 \times (\Delta T_m/\Delta P)$ (°C/MPa)	ΔH^* (kJ mol^{-1})	ΔV_b ($cm^3\ mol^{-1}$)
Poly(dA)·poly(dT)/netropsin = 2:1	25	106.4 ± 0.4	6.79 ± 0.54	51.2	9.17 ± 0.73
	70	106.3 ± 0.3	7.13 ± 0.38	51.2	9.62 ± 0.51
Poly[d(A-T)]·poly[d(A-T)]/netropsin = 2:1	25	92.3 ± 0.2	5.38 ± 0.13	51.3	7.55 ± 0.18
	70	90.8 ± 0.3	5.36 ± 0.29	51.0	7.52 ± 0.41

All measurements were in 20 mM Tris-HCl, at pH 7.2.

*ΔH and ΔV_b are per mole of basepairs.

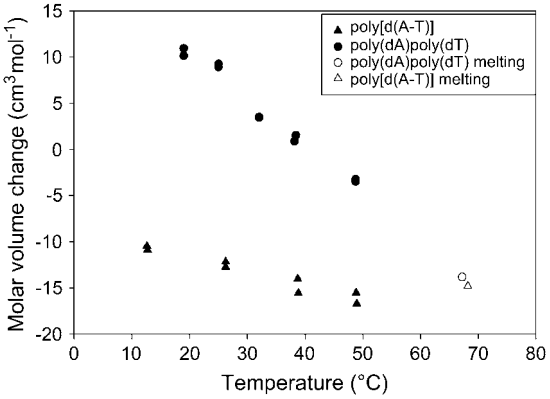

FIGURE 4 Temperature dependence of volume change of ethidium binding with poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] in 20 mM Tris-HCl, 50 mM NaCl, pH 7.2.

within our temperature range ($1/T$ ~0.0035 at 10°C and 0.0028 at 80°C). The second term depends on the compressibility of the solvent. For an aqueous NaCl solution, the coefficient of isothermal compressibility, β_{T_0}, decreases approximately linearly between 0 and 40°C, reaches a minimum, and then increases approximately linearly from 50 to 100°C (27). The solution becomes less compressible with increasing salt concentration and the temperature dependence of $(d\beta_{T_0})/\beta_{T_0}$ becomes slightly weaker with increasing salt concentration. For a 70-mM NaCl solution, the second term increases linearly from −0.47% (10°C) to +0.42% (80°C) (27).

The third term, $(df(T))/f(T)$, is the only one that includes the influence of the relative size of solute molecules to solvent molecules on V_T. The solvent and solute are considered to be hard spheres with diameters d_1 and d_2, respectively; a fivefold change in the ratio of the diameters, d_2/d_1, from 2 to 10 results in a relatively small (1%) change in $f(T)$ at constant temperature. Thus, the relative size of the solute has little influence on temperature dependence of the thermal volume V_T. If we take $d_2/d_1 = 3$ for ethidium in water, then the third term changes linearly from −0.04% at 10°C to −0.18% at 80°C. Thus, we expect V_T to decrease slowly with temperature from 10 to ~25°C and then increase with temperature.

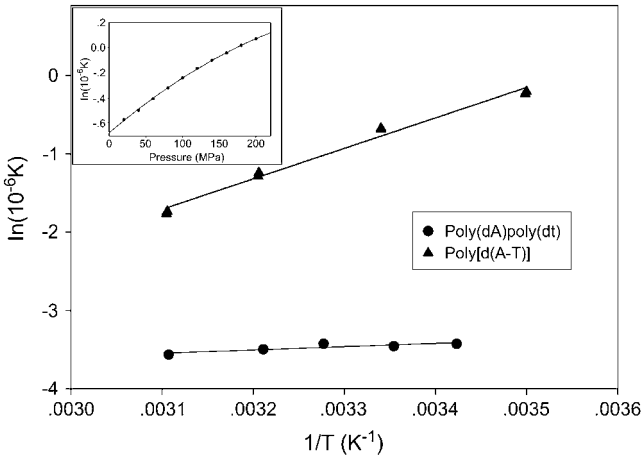

FIGURE 5 Temperature dependence of equilibrium constant of ethidium binding with poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] in 20 mM Tris-HCl, 50 mM NaCl, pH 7.2. A typical plot of $\ln K_a$ versus pressure (poly[d(A-T)]·poly[d(A-T)], 26.2°C) is shown in the inset.

To compare our calculations with the experimentally measured temperature dependence of volume, we must consider the temperature dependence of V_H also. The value of V_H can be expressed as $V_H = \beta_{T_0}\left((A_{dipole})/T + G_{other}\right)$, in which $(A_{dipole})/T$ is the free energy of dipole-dipole interactions between solvent water and solute and G_{other} is the free energy of other van der Waals interactions between solvent water and solute; both terms are negative and, consequently, so is V_H (7). If A_{dipole} and G_{other} are approximated to be temperature-independent, the relative temperature dependence of V_T can be expressed as a sum of two terms:

$$\frac{dV_H}{V_H} = \frac{d\beta_{T_0}}{\beta_{T_0}} + \frac{dT}{-T}\left(\frac{V_{H,\,dipole}}{V_H}\right), \tag{3}$$

in which $V_{H,\,dipole} = \beta_{T_0}(A_{dipole})/T$. The first term of the right-hand side of Eq. 3 is the same as the second term of Eq. 2. The second term is similar to the first term of Eq. 2 except for a negative sign and a factor that varies between 0 and 1 depending on the solute molecules. Combining Eqs. 2 and 3 we obtain

$$dV = dV_T + dV_H = \frac{d\beta_{T_0}}{\beta_{T_0}}(V_T + V_H) + \frac{dT}{T}(V_T - V_{H,dipole}) + \frac{df(T)}{f(T)}V_T. \tag{4}$$

Since V_H and $V_{H,dipole}$ are negative and V_T is positive, the influence of $d\beta_{T_0}/\beta_{T_0}$ is diminished and the influence of $1/T$ is strengthened. The overall effect is that dV changes sign from negative to positive at low temperatures, reaches a maximum, and then decreases slowly with temperature. Thus, dV becomes less sensitive to temperature.

To assess our results, we are interested in evaluating the volume change, ΔV, instead of V in Eq. 4; thus, all of the terms change signs: ΔV_T is negative; ΔV_H is positive; and $d\Delta V$ is likely negative for our temperature range. For EB intercalation with poly[d(A-T)]·poly[d(A-T)], the loss of solvent-accessible surface of EB predominates ΔV_T.

In the absence of structural data for the DNA-ethidium complex we have estimated the fraction of solvent-accessible surface area lost in the following manner. First, the thermal volume of EB is found by multiplying the Connolly molecular area of ethidium, 296.2 Å^2, by 0.51 Å, as outlined in Lee and Chalikian (28), which results in $\Delta V_T = -91$ cm^3 mol^{-1} (note the change in units). If three of the four aromatic rings of EB are partially covered by basepairs above and below, then V_T will be reduced by ~50% upon intercalation, thus, $\Delta V_T = -45.5$ cm^3 mol^{-1}. For this system, $\Delta V = \Delta V_T + \Delta V_H$, to a

TABLE 6 Temperature dependence of ethidium-DNA binding

	T (°C)	K_a* (10^2 μM^{-1})	ΔV_b (cm^3 mol^{-1})	$\Delta H_{van\ 't\ Hoff}$ (kcal mol^{-1})
Poly(dA)·poly(dT)	19.0	3.26	10.6 ± 0.6	−0.83 ± 0.26
	25.0	3.17	9.1 ± 0.3	
	32.0	3.26	3.5 ± 0.1	
	38.3	3.04	1.2 ± 0.4	
	48.7	2.84	−3.4 ± 0.2	
	67.9†	80.6	−16.8 ± 3.5	
Poly[d(A-T)]·poly[d(A-T)]	12.7	50.9	−10.7 ± 0.2	−7.7 ± 0.4
	26.2	28.3	−12.4 ± 0.3	
	38.8	17.4	−14.8 ± 0.8	
	48.9		−16.1 ± 0.6	
	67.2†		−13.8 ± 2.2	

All measurements were in 20 mM Tris-HCl and 50 mM NaCl at pH 7.2.
*Equilibrium constant, K_a, at atmospheric pressure.
†Data are from the transition temperature shift experiments.

good approximation, and the value of ΔV ranges from −10.7 to −16.1 cm^3 mol^{-1} between 12.7 and 48.9°C. Using our estimate of ΔV_T we find that ΔV_H is ~32 cm^3 mol^{-1}.

Since dipole-dipole interactions are the major force in causing extraction of hydration water (29), $\Delta V_{H,dipole}$ makes a significant contribution to ΔV_H. Suppose $\Delta V_{H,dipole}$ represents ~60% of ΔV_H or ~19 cm^3 mol^{-1}, then $d\Delta V$ is ~−0.16 cm^3 mol^{-1} at 12.7°C and ~–0.15 cm^3 mol^{-1} at 48.9°C. This is in agreement with our experiment result of −0.15 cm^3 mol^{-1}. Changing the ratio of $\Delta V_{H,dipole}$ to ΔV_H to 40% or 80% only slightly broadens $d\Delta V$ to ~–0.13 (40%) or −0.18 (80%) cm^3/mol, respectively. Thus, the slight decrease in ΔV observed with increasing temperature for EB binding with poly[d(A-T)]·poly[d(A-T)] is mainly due to the predictable changes of solvent properties and solvent-solute interactions.

Despite this apparent quantitative success, SPT does not yield credible results for analysis of EB binding with poly(dA)·poly(dT). For this system, the experimentally measured value of $d\Delta V$ is −0.49 cm^3 mol^{-1} between 19° and 49°C; this is three times larger than the value of −0.20 ± 0.04 cm^3 mol^{-1} calculated using SPT as outlined above. The difference in $d\Delta V$ appears too large to be explained by error; it implies some changes of the system, other than in thermal motion, with temperature. Thermodynamic measurements do not provide insight into the molecular processes leading to binding and there are no data on the structure of these systems at high temperatures. Our observations may arise as a consequence of a temperature-dependent loss of specific structures involved in the hydration of poly(dA)·poly(dT) or a heat-induced conformational change. We hypothesize that the change occurs with the unbound polymer and not with the complex. The polymer is less hydrated at higher temperatures so that it loses fewer water molecules at higher temperatures, resulting in a more negative value of ΔV_b. The alternating polymer, poly[d(A-T)]·poly[d(A-T)], does not undergo the same temperature-dependent changes as poly(dA)·poly(dT) and does not have irregular temperature dependence of ΔV_b. Although there is good agreement between our results and the analysis based on SPT for EB binding to poly[d(A-T)]·poly[d(A-T)], it may be fortuitous. The assumptions we made for the parameters appear reasonable and the fact that the theory does not adequately describe the behavior of EB binding to poly(dA)·poly(dT) is not altogether surprising considering the generally anomalous properties of this polymer.

In our analyses, we have assumed that the intrinsic volume change is independent of temperature. Although this assumption appears reasonable, it is possible that the rise per basepair could increase significantly with temperature. Such an increase would contribute to the intrinsic volume and change the relative contribution of the volume components to the observed volume change. We are not aware of any measurements of the temperature dependence of intrinsic volume in the literature that would permit us to estimate the magnitude of this effect.

The experimental values of ΔV_b for EB binding with poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] converge at high temperatures. We observed the same trend for nt binding and the helix-coil transition of the naked DNA polymers; it appears as though these two polymers become increasingly similar at higher temperatures. The physical and structural properties of poly(dA)·poly(dT) differ from those of other DNA polymers, such as poly[d(A-T)]·poly[d(A-T)] (30–32). The behavior of poly(dA)·poly(dT) has drawn a great deal of interest and it is tempting to attribute the anomalous properties of this polymer to the structure observed in oligonucleotides with consecutive AT sequences, such as the middle section of the Dickerson dodecamer, d[CGCGAATTCGCG]$_2$ (33). The x-ray fiber diffraction study of Alexeev et al. (30) showed that poly(dA)·poly(dT) is a B-type double helix with a distinctively narrow minor groove; the narrow minor groove is also a characteristic of oligonucleotides with consecutive adenosine residues (34). The narrow minor groove has been linked to formation of a spine-like hydration pattern in the minor groove (35). Molecular simulation has shown that it is energetically favorable to form a spine of hydration in poly(dA)·poly(dT) but unfavorable for poly

TABLE 7 Comparison of volume change data for DNA-ligand binding

	[NaCl] (mM)	T (°C)	ΔV_b ($cm^3\ mol^{-1}$)	Methods	Reference
Poly(dA)·poly(dT) + EB	70	67.9 ± 0.2	−16.8 ± 3.5	Melting	
		26.2	9.54 ± 0.64	Fluorescence	
		21	4.5 ± 0.5	Fluorescence	Macgregor (3)
Poly[d(A-T)]·poly[d(A-T)] + EB	70	67.2 ± 0.4	−13.8 ± 2.2	Melting	
		26.2	−12.4 ± 0.3	Fluorescence	
		21	−13.0 ± 0.5	Fluorescence	Macgregor (3)
Poly(dA)·poly(dT) + Netropsin	25	106.4 ± 0.4	−5.41 ± 1.20	Melting	
	70	106.3 ± 0.3	−7.74 ± 1.65	Melting	
	50	25.0	50 ± 10	Densitometry	Chalikian (4)
	16	20	97	Densitometry	Marky (5)
	116	20	68	Densitometry	Marky (5)
Poly[d(A-T)]·poly[d(A-T)] + Netropsin	25	92.3 ± 0.2	−3.97 ± 0.58	Melting	
	70	90.8 ± 0.3	−4.88 ± 0.72	Melting	
	50	25.0	−5 ± 10	Densitometry	Chalikian (4)
	16	20	−16	Densitometry	Marky (5)
	116	20	−1	Densitometry	Marky (5)

ΔV_b is per mole of binding event.

[d(A-T)]·poly[d(A-T)] (36,37). This may imply that the conformation and hydration differences are partly responsible for the properties of poly(dA)·poly(dT). Unfortunately, unlike oligonucleotides, DNA polymers are not amenable to high-resolution structural studies. In the absence of high-resolution structure data, the exact difference between these two polymers remains unclear.

Premelting transitions have been observed for poly(dA)·poly(dT) using spectroscopic techniques (38,39). Herrera and Chaires (39) showed that ultraviolet absorbance and molar ellipticity of poly(dA)·poly(dT) exhibit a strong temperature dependence; this is not observed for poly[(dAT)]·poly[(dAT)]. It was proposed that the temperature-dependent conformation changes and concomitant disruption of the hydration spine were responsible for the spectroscopic responses. It seems reasonable to propose that similar temperature-dependent changes in structure and hydration are responsible for the volumetric changes we report.

For ethidium intercalation, our results near ambient temperature agree with values in the literature (Table 7). The van 't Hoff enthalpies for binding with poly[d(A-T)]·poly[(dA-T)] and poly(dA)·poly(dT) obtained from the fluorescence method, −7.7 and −0.83 kcal mol^{-1}, respectively, are similar to the calorimetric results, −9.0 and −1.3 kcal mol^{-1}, respectively (3), validating the use of the fluorescence method. From these data, the volume change at melting temperature can be predicted to be $\sim -19 \pm 1$ $cm^3\ mol^{-1}$ and -18 ± 2 $cm^3\ mol^{-1}$ for binding with the poly[d(A-T)]·poly[d(A-T)] and poly(dA)·poly(dT), respectively. The melting method gives a similar result for poly(dA)·poly(dT) at -16.8 ± 3.5 $cm^3\ mol^{-1}$ and slightly more positive values for binding with poly[d(A-T)]·poly[d(A-T)] at -13.8 ± 2.2 $cm^3\ mol^{-1}$. This implies that determining the volume change by observing the coupling between binding and the helix-coil transition yields an accurate value of the volume change at the transition temperature. This value cannot be obtained by other volumetric methods. This method also requires less material than densitometry and can be used for any ligand-DNA system without the need of spectroscopic signal from the ligand.

As shown in Table 7, the densitometry data from literature for netropsin binding with DNA show a much more positive ΔV_b for poly(dA)·poly(dT) than poly[d(A-T)]·poly[d(A-T)] at ambient temperature (5). The ΔV_b measured at melting temperature by melting experiments are similar for both polymers, apparently due to the polymers becoming increasingly similar at high temperature. Unlike simple intercalators, such as EB, nt binding in the minor groove results in the creation of a certain amount of void between netropsin and the minor groove. Thus, it is impossible to predict its volume-change temperature dependence without additional knowledge of the void volume or structure of the complex at high temperatures.

In future studies we intend to apply the scaled particle theory to the analysis of the temperature dependence of volumetric properties of a greater range of molecules and binding events. A theoretical interpretation of the pressure dependence of these parameters will also be investigated. With further improvements, we feel that this approach may be very useful in the dissection of the relative contributions of the thermal and hydration volumes.

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