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Pressure Dependence of the Melting Temperature of dA·dT Polymers[†]

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ABSTRACT: The pressure dependence of the helix-to-coil transition temperature (T_m) of poly[d(A-T)], poly(dA)·poly(dT), and poly(dA)·poly(dT)$_2$ was studied as a function of sodium chloride concentration. The molar volume change of the transition (ΔV) was calculated using the Clapeyron equation and calorimetrically determined enthalpies. The ΔV of the transition varied linearly with the logarithm of the salt concentration for both double-stranded polymers. The ΔV of poly[d(A-T)] changed from +0.36 to +3.86 cm^3 mol^{-1} as the sodium chloride concentration changed from 0.020 to 1.0 M. For poly(dA)·poly(dT), the ΔV varied from +2.60 to +4.59 cm^3 mol^{-1} over the range of 0.020–0.20 M NaCl. No pressure dependence of the van't Hoff enthalpy was observed for the double-helical form of either polymer. The ΔV for denaturation of the triple-helical species poly(dA)·poly(dT)$_2$ was found to be +7.81 and +10.4 cm^3 mol^{-1} at 1.0 and 3.0 M NaCl, respectively. The observed ΔV values are interpreted in terms of changes in the hydration of the polymers arising from release of counterions and changes in the stacking of the bases upon denaturation. Counterion release is assumed to make a net negative contribution to the overall ΔV, implying that disruption of the stacking interactions must make a positive volume change to the overall ΔV. The difference in the ΔV values for the two polymers remains constant as the salt concentration is changed, suggesting a difference in the partial molar volume of their single-stranded forms. The more positive ΔV for poly(dA)·poly(dT) is interpreted as arising from the greater base stacking of this polymer. The magnitude and salt-induced change in the ΔV for denaturation of triple-stranded poly(dA)·poly(dT)$_2$ with respect to the ΔV of the double-stranded form imply that the effect of adding another strand to the helix results in a linear change in the observed ΔV once the effect of sodium chloride concentration on this parameter has been considered.

The thermal stability of the double-stranded DNA depends upon the primary sequence of the DNA and the properties of the solvent such as ionic strength. The effect of hydrostatic pressure on the thermal stability of DNA remains largely unexplored; however, it offers an alternative method for the investigation of the role of solvation on DNA stability. Perturbation of the helix–coil transition with pressure yields the molar volume change of the equilibrium. Because the pressures employed are insufficient to induce covalent modifications or change the molecular volumes, the observed volume changes are related to changes in the interactions between the solvent and the solutes. Prior to the availability of synthetic DNA, studies on the effect of hydrostatic pressure on the stability of the secondary structure of nucleic acids focused on natural DNA and RNA sequences usually within a narrow range of conditions. These investigations found that elevated pressures induced small shifts in the helix-to-coil transition temperature (Heden et al., 1964; Hughes & Steiner, 1966; Weida & Gill, 1966; Chapman & Sturtevant, 1969; Gunter & Gunter, 1972; Hawley & MacLeod, 1974, 1977). For the majority of nucleic acids, increasing pressure was observed to lead to higher melting temperatures; thus, it was concluded that the molar volume change (ΔV) of denaturation was positive. However, the choice of natural DNA for these investigations precluded extracting any information on the influence of pressure changes on the stability of particular sequences.

Recent results demonstrate differences in the partial molar volumes of various DNA molecules consisting of simple repetitive sequences. A direct measure of these differences comes from the sound velocity data of Buckin et al. (1989a,b). They report the sign and magnitude of the partial molar volume of the water of hydration of several simple DNA sequences. In particular, the simplest dA·dT polymers, poly(dA)·poly(dT) and poly[d(A-T)], exhibit significant differences in this parameter despite being formally isomers. According to their data, poly(dA)·poly(dT) causes a larger decrease in the partial molar volume of the water molecules interacting with it than any other sequence of DNA; this is attributed to the formation of energetically favorable interactions between poly(dA)·poly(dT) and water.

Investigation of the molar volume change of ligand interactions with DNA yields a less direct measure of the partial molar volumes of specific sequences than the density measurements cited above; nevertheless, the results of such studies suggest the existence of large differences in the volumes of specific DNA sequences. Marky and Kupke (1990) found that the molar volume change for the formation of the complex between the minor groove binding drug netropsin and poly[d(A-T)] is approximately 100 cm^3 mol^{-1} greater than the molar volume change of formation of the netropsin–poly(dA)·poly(dT) complex. Similar, although less dramatic, differences were found for the molar volume change accompanying the intercalation of ethidium and propidium into these two DNA polymers (Marky & Macgregor, 1990).

Differences in the physical characteristics of poly[d(A-T)] and poly(dA)·poly(dT) have become the rule as a large body of previous work has shown that these two polymers differ

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significantly for characteristics such as width of the grooves, propeller twist, base stacking (Coll et al., 1987; Nelson et al., 1987), helical repeat, axial rise (Rhodes, 1979; Rhodes & Klug, 1981), conformational plasticity (Arnott et al., 1974; Pilet et al., 1975), induction of bending (Wu & Crothers, 1984), and ligand interactions (Bresloff & Crothers, 1981; Sturm, 1982; Chairs, 1983; Marky et al., 1985; Wilson et al., 1985; Jones et al., 1986; Breslauer et al., 1987; Herrera & Chaires, 1989; Marky & Macgregor, 1990; Marky & Kupke, 1990).

We have examined the effect of pressure and ionic strength on the helix–coil transition temperature (T_m) for these two polymers. The T_m increases with pressure for both polymers, indicative of a positive volume change accompanying the transition. The transition temperature for poly(dA)·poly(dT) exhibits greater pressure sensitivity than the transition temperature of poly[d(A-T)]. We have calculated the molar volume change for the transition at several NaCl concentrations and have found that this parameter changes linearly with the logarithm of the salt concentration. The triple-stranded poly(dA)·poly(dT)$_2$ exhibits molar volume changes approximately 50% larger than the ΔV value of double-stranded poly(dA)·poly(dT) extrapolated to high salt concentrations.

EXPERIMENTAL PROCEDURES

The DNA melting curves were obtained using the temperature-regulated iso-hyperbaric spectrometer (TRIHBS) which we have described (Wu & Macgregor, 1993). With this instrument, we can measure the absorption of ~300 μL of a solution at pressures between atmospheric pressure and 250 MPa[1] (0.1 MPa = 1 bar = 0.987 atm). The high-pressure cell is positioned in the optical path of a spectrophotometer, and the temperature and pressure are continuously monitored and controlled by a microcomputer. The heating rate was maintained at less than 0.3 °C/min. The helix–coil transition was monitored at 260 nm; between measurements, the wavelength was set to 338 nm to minimize possible photodamage (Wu & Macgregor, 1993). The concentration of the polymers was approximately 80 μM (base pairs). No correction was made for the change in the concentration of solution components with pressure due to compression.

Using the equation $\theta(T) = [\mathrm{OD}(T) - \mathrm{H}(T)]/[\mathrm{H}(T) - \mathrm{L}(T)]$, we converted the optical density into the fraction of polymers in the single-stranded or denatured state at each temperature $\theta(T)$; L(T) and H(T) are the lines describing the base lines of the optical density vs temperature data in the low- and high-temperature regimes. At $\theta = 0$, all of the polymer is assumed to be double (or triple) stranded, and at $\theta = 1$, it is assumed to be entirely single stranded. The melting temperature is the point where $\theta = 0.5$. The standard deviation of the T_m values is ±0.3 °C.

The van't Hoff enthalpy of the helix–coil transition (ΔH_{vH}) was calculated in order to assess the pressure dependence of the enthalpy of the transition. For the transitions invovling double helices, we used $\Delta H_{vH} = 6RT_m^2(\partial\theta/\partial T)_{T=T_m}$; for the triple-strand transitions, $\Delta H_{vH} = 8RT_m^2(\partial\theta/\partial T)_{T=T_m}$. The slope was calculated in the region $0.4 \geq \theta \geq 0.6$. On the basis of the error in the slope, the standard error in the ΔH_{vH} values is on the order of 5%. Calorimetric enthalpies of the transitions, not the van't Hoff enthalpies, were employed in the calculation of the molar volume changes.

No significant difference was observed for the measured T_m values for samples subjected to more than one denaturation. We performed electrophoresis of the polymers before and after high-pressure melting and observed no apparent difference in the size distribution of the polymers. The polymers exhibited a fairly broad range of molecular weights (~100 base pairs) centered around 600 and 1200 base pairs as measured against standard molecular weight markers.

REAGENTS

All reagents were the highest quality available from commercial sources and were used without further purification. The DNA polymers, poly[d(A-T)] and poly(dA)·poly(dT), were from Pharmacia, Inc. The lyophilized powders were dissolved initially in 20 mM TrisHCl, pH 8.8, and 1 mM EDTA, heated to 65 °C, and then allowed to cool slowly to room temperature. These solutions were then extensively dialyzed against this buffer at 4 °C. The concentrations of the stock solutions were determined spectrophotometrically using $\epsilon_{259} = 12\,000$ M^{-1} cm^{-1} for poly(dA)·poly(dT) (Bresloff & Crothers, 1981) and $\epsilon_{262} = 13\,200$ M^{-1} cm^{-1} for poly[d(A-T)] (Schmechel & Crothers, 1971). The concentrations of all parameters are reported in terms of moles of base pair or base triplex.

The choice of a buffer for these experiments is problematic. We used TrisHCl because the ΔV of ionization for this buffer is ~+1 cm^3/mol (Isaacs, 1981) and because we are interested primarily in emphasizing the effect of pressure on the melting temperature of DNA. However, the pK_a of this buffer is a strong function of temperature, $\Delta pK_a/\Delta T \approx -0.025/°$C; thus, 20 mM TrisHCl made to have a pH of 8.8 at room temperature (21 °C) will have a pH between ~8.1 and 7.3 for the temperature range used in the experiments. Other buffers exhibit much larger ΔV values. Phosphate, which has a small ΔH, has a ΔV of −28 cm^3/mol. Although the overall pH change between the T_m values determined at low salt and those at 1 M salt is fairly large, at a given salt concentration the pH of the solution changes by not more than 0.3 unit. Given the weak dependence of the T_m of DNA on pH (Zimmer & Venner, 1962; Privalov et al., 1969), the influence of the pH change on the T_m is of secondary importance.

RESULTS

Representative melting curves at 0.1 and 200 MPa at each sodium chloride concentration are shown in Figure 1. The transitions are highly cooperative, and their width and overall shape are unaffected by pressure. At high pressures, poly(dA)·poly(dT) continues to display the characteristic apparent discontinuity observed in spectroscopic and calorimetric studies of this polymer (Riley et al., 1966; Marky & Breslauer, 1982). The data are summarized in Figure 2, where the T_m is plotted as a function of pressure at each salt concentration. From this plot, it is evident that the thermal stabilities of poly[d(A-T)] and poly(dA)·poly(dT) are functions of sodium ion concentration and pressure.

The rate of change of T_m as a function of pressure, i.e., the slopes of the lines shown in Figure 2, was used to calculate the molar volume change (ΔV) of the transformation from the double helix to single strands via the Clapeyron equation: $\Delta T_m/\Delta P = T_m\Delta V/\Delta H$, where ΔV and ΔH are the molar volume and enthalpy changes of the transition, respectively [see, for example, Sheehan (1961)]. The enthalpy of denaturation of poly[d(A-T)] is reported to equal 32 (±4) kJ mol^{-1} independent of the salt concentration (Filimonov, 1986). We have used this value to calculate ΔV as a function of the

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[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; TrisHCl, tris(hydroxymethyl)aminomethane hydrochloride; MPa, megapascal, 0.1 MPa = 1 bar = 0.987 atm.

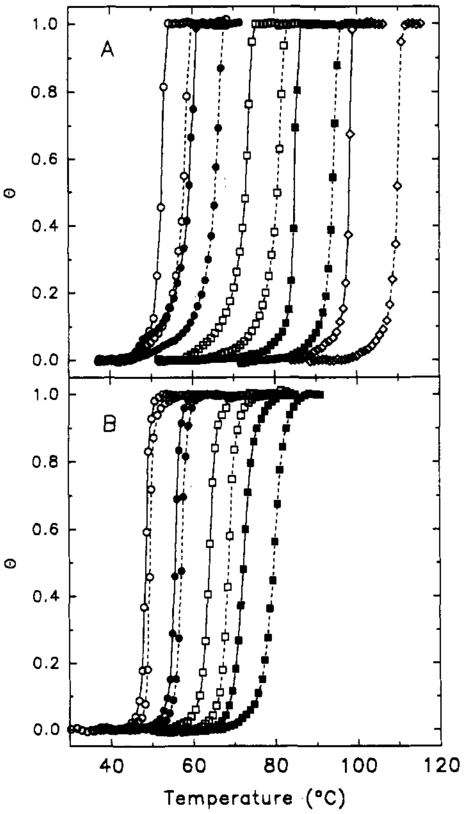

FIGURE 1: Representative melting curves at different NaCl concentrations; solid lines are data at 0.1 MPa, and dashed lines are at 200 MPa. (A) Poly(dA)·poly(dT): (○) 20 mM; (●) 50 mM; (□) 200 mM. Poly(d(A)·poly(dT)$_2$: (■) 1 M; (◇) 3 M. (B) Poly[d-(A-T)]: (○) 20 mM; (●) 50 mM; (□) 200 mM; (■) 1 M.

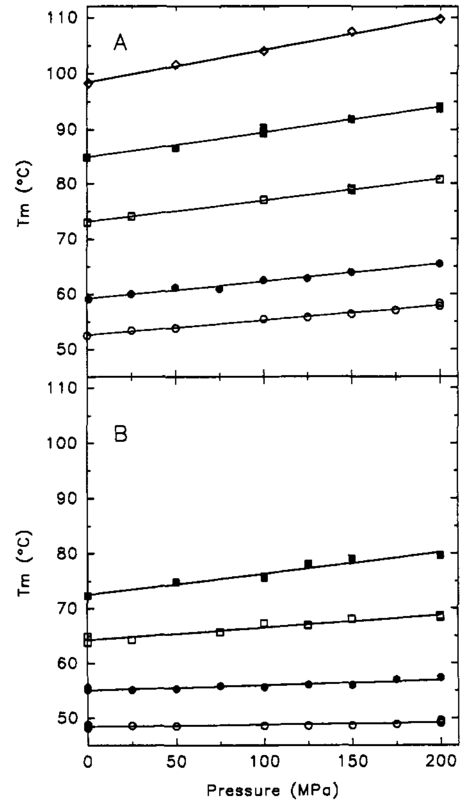

FIGURE 2: Melting temperature, T_m, as a function of pressure at a series of salt concentrations; the solid lines are least-squares fits to the data. (A) Poly(dA)·poly(dT): (○) 20 mM; (●) 50 mM; (□) 200 mM. Poly(dA)·poly(dT)$_2$: (■) 1 M; (◇) 3 M. (B) Poly[d(A-T)]: (○) 20 mM; (●) 50 mM; (□) 200 mM; (■) 1 M.

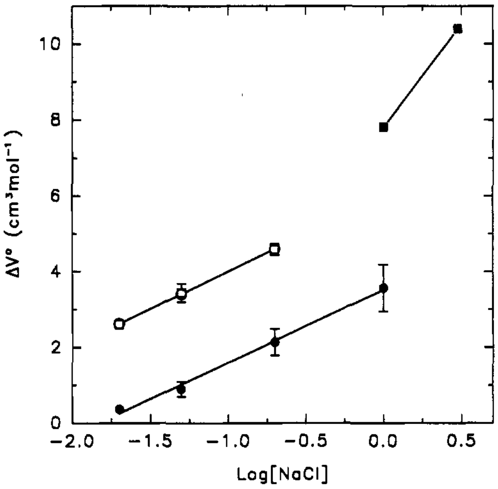

FIGURE 3: Molar volume change of the helix–coil transition, ΔV, as a function of NaCl concentration: (□) poly(dA)·poly(dT); (■) poly(dA)·poly(dT)$_2$; (●) poly[d(A-T)].

concentration of sodium chloride; the molar volume change for the transition varied from +0.3 (±0.09) cm^3 mol^{-1} in 20 mM NaCl to 3.47 (±0.56) cm^3 mol^{-1} in 1 M NaCl. These results are summarized in Figure 3 and Table I.

The ΔV of the helix–coil transition for poly(dA)·poly(dT) was calculated in a similar manner although the system is more complicated due to the existence of double- and triple-stranded forms. At the three lowest NaCl concentrations used in these experiments, the double-stranded polymer is the most stable form. At the two highest salt concentrations, the highest temperature transition arises from denaturation of triple-stranded poly(dA)·poly(dT)$_2$ into single strands (Riley et al., 1966; Klump, 1988a). Empirical relations for the ΔH values as a function of the melting temperature of the ribopolymer analogues, poly(A)·poly(U) and poly(A)·poly(U)$_2$, were used because they have been measured over wide range of salt concentrations (Krakauer & Sturtevant, 1968; Filimonov, 1986). We are unaware of similar data for the deoxyribose polymers. For the transition of poly(A)·poly(U) to single strands, $\Delta H = 0.35T_m - 80.0$ kJ mol^{-1} (±1.0 kJ mol^{-1}), and for the triple helix to single-strand transition, $\Delta H = 0.33T_m - 56.0$ kJ mol^{-1} (±3.0 kJ mol^{-1}), where T_m is in kelvin (Filiminov, 1986). The value at low salt concentration agrees with the ΔH for the deoxyribose polymer measured calorimetrically (Marky & Breslauer, 1982). The parameters are summarized in Figure 3 and Table I. At the high salt concentrations, we occasionally observed a small transition at lower temperatures accounting for ~5% of the main high-temperature transition. In these cases, the lower temperature transition was ignored. We plan to investigate the pressure dependence of this transition in the future.

For both polymers, the assumed independence of the molar volume change and molar enthalpy change on the pressure is supported by the linearity of the data in Figure 2. A measurable dependence of one of these parameters on the hydrostatic pressure would lead to changes in the transition widths or a nonlinear dependence of T_m on pressure (Figure 2).

We have also calculated the van't Hoff enthalpy (ΔH_{vH}) of the transitions (Figure 4). With the exception of the data for poly(dA)·poly(dT)$_2$ in 1 M NaCl, there is no apparent pressure dependence of the ΔH_{vH} values. The lines drawn through the points are least-squares fits to the data, and in each case, the slopes were zero within experimental error. In 1 M sodium chloride, poly(dA)·poly(dT)$_2$ shows a linearly decreasing ΔH_{vH} as a function of pressure. The origin of this

Table I: Volume Change of the Helix–Coil Transition of Poly(dA)·Poly(dT) and Poly[d(A-T)] as a Function of NaCl Concentration

[NaCl] (M)	$100(\Delta T_m/\Delta P)$ (°C/MPa)	T_m (°C)	ΔH (kJ mol^{-1})	ΔV (cm^3 mol^{-1})
		Poly(dA)·Poly(dT)[a]		
0.02	2.49 (±0.1)	52.7	34.0	2.60 (±0.13)
0.05	3.15 (±0.20)	59.2	36.3	3.44 (±0.24)
0.20	3.86 (±0.08)	73.1	41.2	4.59 (±0.15)
1.0	4.50 (±0.24)	85.1	62.2	7.81 (±0.5)
3.0	5.80 (±0.22)	98.4	66.6	10.4 (±0.6)
		Poly[d(A-T)]		
0.02	0.36 (±0.08)	48.4	32	0.36 (±0.09)
0.05	0.93 (±0.17)	55.0	32	0.90 (±0.20)
0.20	2.26 (±0.24)	64.3	32	2.14 (±0.35)
1.0	3.86 (±0.46)	72.5	32	3.57 (±0.62)

[a] 1 and 3 M NaCl are for the triple-stranded transition: poly(dA)·poly(dT)$_2$ → poly(dA) + 2 poly(dT).

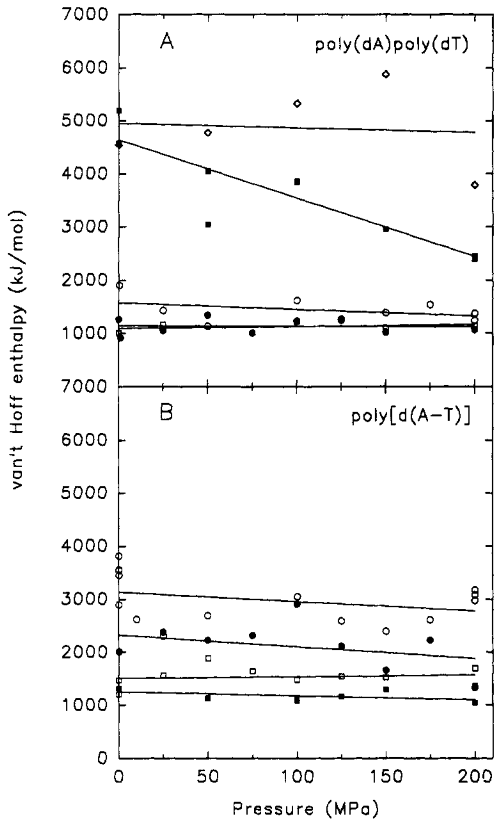

FIGURE 4: van't Hoff enthalpy of the helix–coil transition as a function of pressure and salt concentration. (A) poly(dA)·poly(dT): (○) 20 mM; (●) 50 mM; (■) 200 mM. Poly(dA)·poly(dT)$_2$: (■) 1 M; (◇) 3 M. (B) Poly[d(A-T)]: (○) 20 mM; (●) 50 mM; (□) 200 mM; (■) 1 M.

effect is unknown. Perhaps the equilibrium of the disproportionation reaction is shifted toward the double-stranded form by pressure, or perhaps the length of a cooperative unit is sensitive to pressure. Because the equilibrium between the single, double, and triple helices is near the triple point at this salt concentration (Klump, 1988a), unexpected behavior is not surprising. However, because we were not directly looking at both of the transitions in these experiments, the validity of these hypotheses cannot be assessed with the present data. In the absence of other data, we assume that the pressure dependence of the van't Hoff enthalpies reflects the same trend in the calorimetrically measured enthalpies.

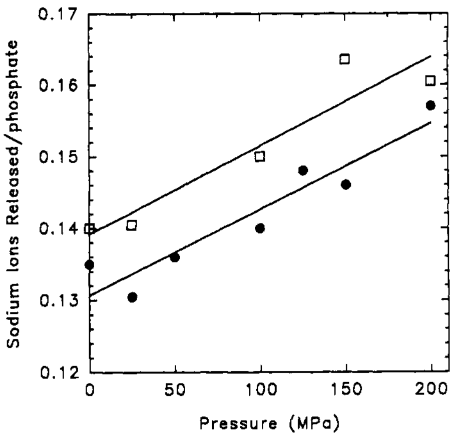

FIGURE 5: Number of sodium ions released upon transition as a function of pressure: (□) poly(dA)·poly(dT); (●) poly[d(A-T)].

At low ionic strengths, the change in T_m with salt concentration is proportional to the number of cations released upon melting (Record et al., 1978). The number of sodium cations can be calculated using the equation:

$$\Delta T_m/\Delta \log([\mathrm{Na}^+]) = (2.303RT_m^2/\Delta H)\Delta n$$

where Δn is the number of ions released and $R = 8.31$ J K^{-1} mol^{-1}. The results of analyzing the data according to this equation are shown in Figure 5. The number of cations released during the transition increases with pressure in a parallel manner for poly[d(A-T)] and poly(dA)·poly(dT). The slope of the dependence of T_m of poly[d(A-T)] on log([NaCl]) varies from 16.8 °C at 0.1 MPa to 19.7 °C at 200 MPa. For poly(dA)·poly(dT), the slope of T_m vs log([NaCl]) varies from 16.5 °C at 0.1 MPa to 19.7 °C at 200 MPa. Klump (1988b) has reported slopes of 16.0 °C for poly[d(A-T)] and 16.5 °C for poly(dA)·poly(dT), both at atmospheric pressure.

The observed shifts in T_m with pressure cannot be rationalized in terms of changes in the molar enthalpy because this would require a 10-fold difference in the enthalpy of the transition throughout the experimental range tested here. This, in turn, would lead to large changes in the width of the melting curves, which was not observed (Figure 1). Although the magnitude of the change in Δn is such that it could be explained by changes in the molar enthalpy, we nevertheless interpret the slopes, $\Delta T_m/\Delta \log([\mathrm{NaCl}])$, in terms of differences in ionic interactions with the polymers because this is consistent with the decreasing magnitude of electrostriction component in the overall molar volume change with increasing salt concentrations (see Discussion).

DISCUSSION

The response of a system to an increase in pressure is to reduce the volume. At the modest pressures employed in these experiments, the atoms and molecules constituting the system behave like rigid spheres; i.e., the sizes, bond lengths, bond angles, etc. remain constant. The system responds by reducing the free volume (e.g., between solvent molecules) and by favoring interactions between the solvent and the solute that minimize the volume. There are two mechanisms which are thought to be responsible for the majority of the volume change observed in the formation of noncovalent complexes between biological molecules (Weber & Drickamer, 1983). In the first, the volume is reduced by favoring conformational changes that allow the solvent to gain access to volumes where the macromolecule is imperfectly packed. This mechanism is thought to be applicable to the dissociation of proteins under pressure.

In the second mechanism, pressure induces conformational or structural changes that lead to an increase in the number of full or partial charges exposed to the solvent. Ions and partial charges dissolved in polar solvents have spheres of solvent molecules surrounding them in order to accommodate the charge. These electrostricted solvent molecules have a higher density than bulk solvent molecules, and the increment in density arising from solvation of charges in a polar solvent leads to the observed increase in dissociation with pressure. For noncovalent complexes of biological interest, formation of charge complexes often provides a large fraction of the net interaction energy, and these complexes exhibit increasing dissociation with pressure.

It is evident that the interactions between DNA and water will likely change upon disruption of the double helix, but it is unclear how the change will depend upon the sequence. We have examined the difference in the dA·dT polymers by studying the effect of hydrostatic pressure on the transition temperature. Our results show that three of the polymers poly[d(A-T)], poly(dA)·poly(dT), and poly(dA)·poly(dT)$_2$ are stabilized by high pressure at the salt concentrations reported here. Dissimilarities in the behavior of these polymers become evident upon comparison of the magnitude of the salt dependence of the melting temperature at different hydrostatic pressures. In previous studies on the effect of pressure on the thermal stabiilty of DNA and RNA, the lack of a change in the charge state as a function of their conformation was offered as an explanation for the modest effect of pressure on the stability of these polymers. However, differences in the behavior of different nucleic acid sequences that may have been present were masked by the choice of natural-sequence DNA for the studies. In the studies where repeating sequences were employed, e.g., poly(A)·poly(U), and poly[d(A-T)], interpretation was limited by the use of different buffer conditions, pH, etc. (Heden et al., 1964; Hughes & Steiner, 1966; Weida & Gill, 1966; Chapman & Sturtevant, 1969; Gunter & Gunter, 1972; Hawley & MacLeod, 1974, 1977).

The molar volume change (ΔV) for the helix–coil transition equals the difference in the partial molar volumes of the single strands and the partial molar volume of the double (or triple) helix. For the sake of the present discussion, we write the total partial molar volume of each state in terms of a partial molar volume arising from electrostatic interactions, V_e, and a volume due to stacking interactions, V_s. Thus, for the helical form, the total partial molar volume $V^h = V^h_e + V^h_s$, similarly for the denatured or coil state where $V^c = V^c_e + V^c_s$ (the superscripts h and c refer to the double-stranded helix and single-stranded coil forms, respectively). The ΔV of the helix–coil transition is equal to the difference $\Delta V = V^c - V^h = (V^c_e - V^h_e) + (V^c_s - V^h_s)$.

The difference in the ionic interactions of the double- and single-stranded forms presumably predominates the electrostatic term. Similarly, the stacking between adjacent bases is taken to make the largest contribution to the partial molar volume of stacking. In the absence of other data, the separation of the observed values into these two components is rather artificial; nonetheless, it provides a useful framework for interpreting the results and conceiving new experiments.

The electrostatic term, $V^c_e - V^h_s$, is negative; cations are released upon formation of the single strands (Figure 5). Each of the released ions coordinates water, and the partial molar volume of this water will be less than the partial molar volume of bulk water because of electrostriction. This is in analogy to a very large number of other ionic interactions in water which display negative volumes of ionization (Hepler, 1965).

Interpretation of the stacking term, $V^c_s - V^h_s$, is not as straightforward. Visser et al. (1977) studied the effect of hydrostatic pressure on the formation of stacking complexes of flavinyltryptophan peptides and found that increasing pressure favored the formation of stacked tryptophan–flavin complexes. They report a ΔV of approximately -5 cm^3 mol^{-1} for this process. Thus, unstacking will proceed with a ΔV of $+5$ cm^3 mol^{-1}. Høiland et al. (1984) have reported a positive ΔV for stacking of nucleic acid bases in aqueous solution; however, the exact geometry of the stack is evidently important as the ΔV of stacking for nucleosides is negative. Clearly, the presence of the deoxyribose moiety and its orientation in a complex of noncovalently stacked molecules are important factors in determining the net volume change. Because the sign of the volume change arising from stacking interactions is positive, our data imply that the data pertaining to base stacking are more relevant in determining the volume change due to stacking in a polymer.

The magnitude of the ΔV of denaturation of double-stranded polymers (i.e., $\Delta V \sim 0$ cm^3 mol^{-1}) suggest that the electrostatic and stacking effects contribute approximately equally to the net volume changes. With increasing sodium chloride concentration, the molar volume changes become more positive. This change is attributed to a decrease in the number of cations released upon melting as reflected in the decreasing magnitude of $\Delta T_m/\Delta \log([\mathrm{NaCl}])$ (Record et al., 1978) with increasing salt concentration. Each ion coordinates several water molecules, and even water molecules involved in outer-sphere hydration of ions have higher densities than bulk water (Spiro et al., 1968). Consequently, the release of fewer cations upon denaturation causes the electrostatic contribution to the volume change, and thus the overall ΔV becomes more positive. For poly[d(A-T)] at 1 atm, the slope decreases from 15.7 °C at 20 mM NaCl to 11.4 °C at 1 M NaCl. The stacking component is expected to display only minimal dependence on the salt concentration (Williams & Epand, 1986). Therefore, we suggest that to a first approximation the effect of sodium chloride concentration on the molar volume change of denaturation arises from the decrease in ion release with increasing ionic strength.

Crystallographic structure determinations of (dA)$_n$·(dT)$_n$ regions in oligonucleotides indicate that the propeller twist of the bases of this sequence leads to greater base stacking relative to that found in poly[d(A-T)] (Coll et al., 1987; Nelson et al., 1987). Thus, the initial low-salt ΔV value is more positive for poly(dA)·poly(dT) on account of greater stacking; however, as the salt concentration is increased, this positive contribution to the net ΔV of poly(dA)·poly(dT) does not change, and the volume changes increase in a parallel manner for both polymers due to electrostatic interactions. The interpretation of the molar volume changes is consistent with the available data; we are cognizant of the existence of other possible interpretations. For example, the volume changes may arise because the partial molar volume due to stacking in the helical form (V^h_s) is smaller than that of the denatured form and thus the volume change atttributable to stacking would be positive. However, this requires the net electrostatic contribution to be negative or zero (i.e., $V^c_e - V^h_e \leq 0$); taking into consideration the cation release upon melting and the negative contribution of electrostriction to volume changes, this seems less reasonable than the interpretation offered above.

At salt concentrations where only double-stranded forms need to be considered, examination of the data in Figure 3 reveals that the ΔV of poly(dA)·poly(dT) is larger than that of poly[d(A-T)] by 2.3 cm^3 mol^{-1} and that this difference ($\Delta\Delta V$) is independent of the concentration of sodium chloride. For poly[d(A-T)], the partial molar volume of the water of hydration (V_{h}) is equal to -14 cm^3 mol^{-1}, and for poly(dA)·poly(dT), V_{h} equals -21 cm^3 mol^{-1} (Buckin et al., 1989). The difference ($V_{\mathrm{h,poly(dA)poly(dT)}} - V_{\mathrm{h,poly[d(A-T)]}}$) is then -7 cm^3 mol^{-1}. If the hydration difference were the same in the single-stranded forms as in the double-stranded forms of these two polymers (that is, $V_{\mathrm{c,poly(dA)poly(dT)}} - V_{\mathrm{c,poly[d(A-T)]}} = -7$ cm^3 mol^{-1}), then one would anticipate that the $\Delta\Delta V$ values would be approximatley 0 cm^3 mol^{-1}. On the other hand, if the transition to the denatured forms gave rise to structures with more or less identical solvent interactions (i.e., $V_{\mathrm{c,poly(dA)poly(dT)}} - V_{\mathrm{c,poly[d(A-T)]}} = 0$), we would expect the $\Delta\Delta V$ values to be $+7$ cm^3 mol^{-1}. We have measured $\Delta\Delta V$ values of $+2.3$ cm^3 mol^{-1}; this implies that the difference, $V_{\mathrm{c,poly(dA)poly(dT)}} - V_{\mathrm{c,poly[d(A-T)]}}$, is on the order of -5 cm^3 mol^{-1}. Thus, the data indicate that even in the single-stranded form, these two polymers do not interact identically with the solvent.

At high salt concentrations, the homopolymer poly(dA)·poly(dT) undergoes a disproportionation reaction to yield a triple-stranded form, poly(dA)·poly(dT)$_2$, and a single-stranded form, poly(dA) (Riley et al., 1966). The melting of the triple-stranded species to form the composite single strands is the predominant transition at the two highest salt concentrations we used, namely, 1 and 3M NaCl (Figure 3). The molar volume changes are positive and have a larger magnitude than those for the double-stranded polymer. The increment in ΔV upon addition of the third strand is what would be expected on the basis of mass action if the line describing the sodium chloride dependence of the ΔV of the double-stranded from is extrapolated to these higher salt concentrations and then multiplied by a factor of 1.5. Thus, it appears that whatever the source of the pressure dependence of the melting temperature, the components of the overall change contribute linearly with the addition of another strand to the helix. The denaturation of triple-helical DNA proceeds with the release of counterions (Durand et al., 1992); in analogy with the arguments given above for the double-stranded conformations, we assume that any electrostatic component will contribute a negative molar volume change to the overall ΔV. The additional stacking component arising from adding another strand to the double helix is then responsible for the larger overall ΔV.

The data presented here agree with previously measured molar volume changes for DNA. Hawley and MacLeod (1974) report that the rate of change of the T_{m} of *Clostridium perfringens* DNA with respect to pressure ($\Delta T_{\mathrm{m}}/\Delta P$) ranges from 5.5×10^{-3} to 4.7×10^{-2} °C/MPa as the sodium chloride concentration is changed from 10 mM to 3.6 M. These values lie within the range we observed for this slope. This is expected because *Cl. perfringens* DNA is only 72% AT base paris and clearly does not contain the sequence homogeneity of the polymers we studied. In a later report, Hawley and MacLeod (1977) found $\Delta T_{\mathrm{m}}/\Delta P$ equal to 2.43×10^{-2} °C/MPa for poly[d(A-T)] in 150 mM NaCl. Interpolation of our data for poly[d(A-T)] yields a slope of 2.0×10^{-2} °C/MPa.

Rentzeperis et al. (1993) have recently measured the volume for the formation of duplex poly(dA)·poly(dT) using densitometry. They found ΔV values equal to -0.5 cm^3 mol^{-1} at 16 mM Na$^+$ and -17.4 cm^3 mol^{-1} at 116 mM Na$^+$. These volume changes are negative because they are for the *formation* of a double helix whereas our data are for the *denaturation* of the dobule helix. Despite quantitative differences between our data and those of Rentzeperis et al., the qualitative agreement is good. The double-stranded form has a smaller partial molar volume than the single strands, and the volume increases with salt concentration. The quantitative discrepancy may be related to the temperature difference of the two studies, the difference in DNA concentration used, a systematic difference of the two techniques, or a combination of these or other effects.

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