

Comparison of the Heat- and Pressure-Induced Helix–Coil Transition of Two DNA Copolymers

Gamal Rayan and Robert B. Macgregor, Jr.*

Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto,
19 Russell Street, Toronto, Ontario M5S 2S2, Canada

Received: February 21, 2005; In Final Form: June 16, 2005

The helix–coil transition of poly[d(I-C)] and poly[d(A-T)] was studied as a function of hydrostatic pressure, temperature, and sodium ion concentration. These studies were undertaken in light of a recently published phase diagram for double stranded nucleic acids [Dubins et al. *J. Am. Chem. Soc.* **2001**, 123, 9254–9259]. The sign and magnitude of the volume change for the heat-induced helix–coil transition, ΔV_T , of poly[d(I-C)] and poly[d(A-T)] were dependent on the helix–coil transition temperature, T_M , at atmospheric pressure. The sign of ΔV_T changed from negative to positive as T_M was increased by increasing the sodium ion concentration. For poly[d(I-C)], $\Delta V_T = 0 \text{ cm}^3 \text{ mol}^{-1}$, when the sodium ion concentration is such that the spectroscopically monitored $T_M = 55 \text{ }^\circ\text{C}$ at atmospheric pressure. For poly[d(A-T)], the value of $\Delta V_T = 0$ under conditions such that $T_M = 47 \text{ }^\circ\text{C}$ at atmospheric pressure. Negative values of ΔV_T imply that the helical form is destabilized at high pressure. Under experimental conditions where the ΔV_T for the transition is negative, the transition could be caused by increasing the pressure under isothermal conditions. At temperatures below T_M measured at atmospheric pressure the midpoint of the pressure-induced helix–coil transition, P_M , decreases with increasing temperature. The volume change of the pressure-induced transitions helix–coil transition, ΔV_P , was calculated assuming a two-state model. The magnitude of ΔV_P (per cooperative length) was much larger than the volume change (per base pair) measured for the heat-induced transition, ΔV_T , calculated using the Clapeyron equation. The ratio of these two volume changes was used to calculate the cooperative length for the pressure-induced transition. This parameter depends strongly on temperature, becoming greater closer to T_M measured at atmospheric pressure. At temperatures approaching T_M the magnitude of the cooperative length of the pressure-induced transition is approximately twice that observed for the heat-induced transition (N_T). On the basis of the temperature dependence of the ΔV_T for the two polymers the coefficient of thermal expansion of the two polymers was found to be 0.17 and 0.16 $\text{cm}^3 \text{ K}^{-1} \text{ mol}^{-1}$ for poly[d(I-C)] and poly[d(A-T)], respectively.

Introduction

The importance of hydration in the conformational stability of DNA was recognized in the early X-ray fiber diffraction studies.^{1,2} Other studies employing techniques such as X-ray crystallography, NMR, IR spectroscopic, gravimetric, and thermodynamic methods have also pointed to the importance of hydration in the DNA structure.^{3–12} However, the number of water molecules interacting with DNA, the properties of those water molecules, and the mechanistic aspects of how they influence the conformation of DNA have proven technically difficult to study. The technical difficulties have several origins. The first major impediment is that the interactions between DNA and water takes place in a background of a very high relative concentration of chemically identical water molecules that are not interacting with DNA. Second, the different methods used to study these interactions are sensitive to different types of water molecules. Thus, the standard high-resolution structure techniques record only those water molecules that form the most stable interactions; other weaker interactions are not seen although they may play important roles in the stabilization of the structure. Finally, the physical properties of the water molecules do not, in general, differ dramatically from those of

the bulk water making it difficult to detect them and assess their thermodynamic importance.

We are interested in how water influences the thermodynamics and kinetics of conformational transitions of biological molecules and the role played by hydration in ligand binding to these molecules. The most direct methods to determine the role of water involve measurements of the thermodynamic parameters most sensitive to changes in hydration: volume and the pressure and temperature derivatives of volume, compressibility, and expansivity. There are two methods to assess these parameters; they may be measured directly or by studying the temperature and pressure dependence of the stability of a system. Until recently, it was technically unfeasible to carry out direct measurements of the volumetric parameters of most biological samples because of the large amount of material involved. Many of these obstacles have been overcome by technological advances and some of the most significant steps toward a deeper understanding of the contribution of water to the conformational stability of biological molecules have come from the direct measurement of volumetric parameters.^{13–15}

The consensus of the studies carried out to date is that DNA is hydrated by two distinct hydration shells.¹⁶ The primary hydration shell is next to DNA and consists of two classes of water molecules that interact with DNA; this water is thermo-

* Address correspondence to this author. Phone: 416-978-7332. Fax: 416-978-8511. E-mail: rob.macgregor@utoronto.ca.

dynamically distinct from bulk water. The secondary shell of hydration surrounds the primary hydration shell and the water molecules exhibit properties that are more similar to those of bulk water.¹⁷

In our studies, we have focused on investigating the effect of hydrostatic pressure on the conformational stability of biomolecules. Investigating the effect of pressure yields information pertinent to hydration because of the very low compressibility of liquids, especially water. At pressures below ~ 1000 MPa a shift in an equilibrium elicited by pressure occurs predominately through changes in the interactions between the solute and the solvent. In particular, we have carried out several investigations into the effect of pressure on the thermodynamics and kinetics of the equilibrium between double stranded DNA and its constituent single strands.^{18–22} In our studies, we generally measure the effect of pressure on the thermal stability of DNA as reflected in the pressure dependence of the helix–coil transition temperature, T_M . The value of dT_M/dP together with knowledge of the enthalpy of the transition are used to calculate the volume change of the transition using the Clapeyron equation. We have found that under most experimental conditions, high pressure favors the double stranded form of DNA. That is, the helix–coil transition temperature, T_M , increases with pressure. A detailed account on the effect of hydrostatic pressure on nucleic acids can be found in the recent reviews.^{23,24} It is worth mentioning that the volumetric parameters for poly[d(A-T)] measured by densimetry¹⁴ agree with those obtained using high-pressure methods¹⁸ (see below). A recent review summarizes the results of volumetric assessments of the hydration of nucleic acids.²⁵

Under most experimental conditions, most double stranded nucleic acid polymers are stabilized by pressure. A recently published pressure–temperature phase diagram for polymeric nucleic acids predicted that under conditions where T_M of the helix–coil transition is less than approximately 50 °C, pressure would be expected to destabilize the helix form.²⁶ This prediction was verified by the behavior of poly(dA)poly(U). The helix–coil transition temperature of the polymer was adjusted by changing the salt concentration of the solution. We found that if T_M was less than 50 °C, the polymer was destabilized by pressure, and it was stabilized if T_M was greater than 50 °C. The fact that pressure favors the coil form allowed us to carry out the first pressure-denaturation curve for a nucleic acid.

In the present work, we have studied the heat-induced and pressure-induced denaturation of poly[d(I-C)] and poly[d(A-T)] by monitoring UV absorption as a function of temperature and pressure, respectively. We have observed the pressure destabilization of these polymers and report pressure denaturation from which we calculated the volume change of the pressure-induced transition. We have measured for the first time the cooperative length of a pressure-induced helix–coil transition. We report that it is strongly dependent on temperature, increasing at temperatures closer to T_M . These data provide additional validation of the phase diagram for the helix–coil transition of double stranded nucleic acids.

Materials and Methods

Poly[d(I-C)] and poly[d(A-T)] were obtained from Amersham (Piscataway, NJ). Prior to use they were dialyzed three times at 4 °C in 5 mM sodium cacodylate and 0.1 mM Na₂EDTA, pH 6.7, at sodium ion concentrations ranging from 5.2 to 30.2 mM for poly[d(A-T)] and 5.2 to 205.2 mM for poly[d(I-C)]. The sodium ion concentration was adjusted by altering the concentration of NaCl. We used dialysis tubing with a molecular

TABLE 1: Volume Change of the Helix–Coil Transition of Poly[d(A-T)] and Poly[d(I-C)] as a Function of Sodium Ion Concentration

Na ⁺ (mM)	T_M (°C)	$100 \times dT_M/dP$ (°C/MPa)	ΔV (cm ³ mol ⁻¹)
poly[d(A-T)]			
5.2	35.8 \pm 0.2	-1.48 \pm 0.19	-1.39 \pm 0.22
10.2	43.5 \pm 0.4	-0.68 \pm 0.12	-0.66 \pm 0.13
15.2	45.7 \pm 0.2	-0.17 \pm 0.06	-0.17 \pm 0.06
30.2	52.6 \pm 0.3	0.54 \pm 0.08	0.54 \pm 0.09
poly[d(I-C)]			
5.2	29.0 \pm 0.2	-4.06 \pm 0.13	-4.36 \pm 0.31
10.2	35.3 \pm 0.2	-2.94 \pm 0.15	-3.26 \pm 0.26
15.2	38.0 \pm 0.1	-2.57 \pm 0.09	-2.90 \pm 0.21
20.2	41.4 \pm 0.1	-2.10 \pm 0.03	-2.41 \pm 0.15
30.2	43.8 \pm 0.1	-1.67 \pm 0.07	-1.94 \pm 0.15
40.2	47.8 \pm 0.1	-1.12 \pm 0.07	-1.33 \pm 0.12
65.2	49.0 \pm 0.1	-0.94 \pm 0.04	-1.12 \pm 0.09
105.2	54.1 \pm 0.1	0.00 \pm 0.04	0.00 \pm 0.05
205.2	56.4 \pm 0.1	0.39 \pm 0.04	0.48 \pm 0.06

weight cutoff of 1000 Da (Spectrum, Inc., Rancho Dominguez, CA). The concentration of the stock solution was determined spectrophotometrically using $\epsilon_{262} = 13\,200\text{ M}^{-1}\text{cm}^{-1}$ for poly[d(A-T)]²⁷ and $\epsilon_{251} = 13\,800\text{ M}^{-1}\text{cm}^{-1}$ for poly[d(I-C)].²⁸ In our experiments, the concentration of the polymer was approximately 70 μM (base pairs).

The temperature-regulated isohyperbaric spectrophotometer was employed to obtain the (thermal- and pressure-induced) melting curves; this instrument has been described previously.²⁹ Briefly, the sample solution is placed into a 300- μL quartz cuvette that is placed in the optical high-pressure cell. The pressure cell is positioned in the optical path of a Uvikon 860 spectrophotometer. The temperature, pressure, and absorption of the sample are measured in real-time and the pressure and temperature can be adjusted to desired values by the software controlling the experiment. Pressures generated by this equipment are in the range of 0.1 and 250 MPa (0.1 MPa = 1 bar = 0.987 atm). For the heat-induced transitions, the heating rate was 0.1 °C/min, while the pressurization rate of 1.0 MPa/min was used in the pressure denaturation. The helix–coil transition was monitored by measuring the change in absorption at 260 nm.

The fraction of polymer in the coil form at a temperature T , $\alpha(T)$, was calculated using the following equation; $\alpha(T) = [\text{OD}(T) - L(T)]/[H(T) - L(T)]$, where $\text{OD}(T)$ is the optical density at temperature T , $H(T)$ is the equation for the line describing the high-temperature baseline as a function of temperature, and $L(T)$ is the equation for the line describing the low-temperature baseline as a function of temperature. If $\alpha = 0$, the polymer is assumed to be in double stranded (helix) form, while at $\alpha = 1$ the polymer is assumed to be entirely in the single stranded (coil) form. The helix–coil transition temperature (T_M) is the temperature at which $\alpha = 0.5$. The errors in T_M values are shown in Table 1.

We calculated the helix–coil transition pressure, P_M , in an analogous manner. The original data are transformed into the extent of transition at a given pressure, thus, $\alpha(P) = [\text{OD}(P) - L(P)]/[H(P) - L(P)]$, where $\text{OD}(P)$ is the optical density at pressure P , $H(P)$ is the high-pressure baseline, and $L(P)$ is the low-pressure baseline. The helix–coil transition pressure, P_M , is the pressure at which $\alpha = 0.5$.

The model-dependent enthalpy and volume changes were calculated assuming a single-step bimolecular reaction between the single strands and the double stranded species. The model-dependent van't Hoff enthalpy of the transition (ΔH_{vH}) was calculated as described in Marky and Breslauer³⁰ and in Wu

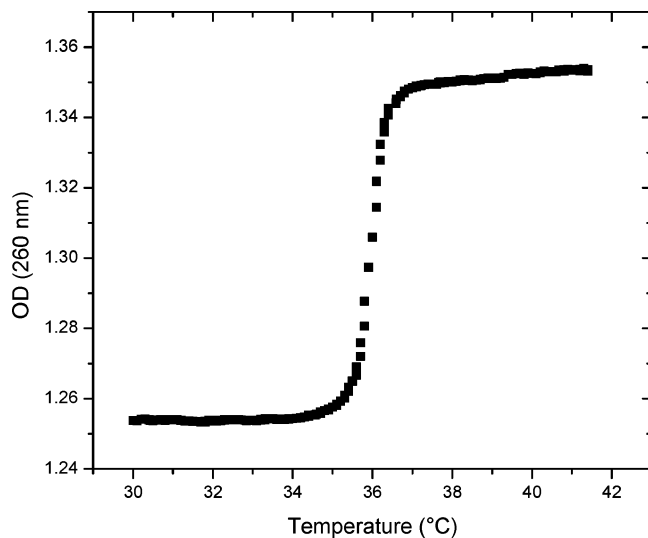


Figure 1. A representative heat-induced helix–coil transition curve of poly[d(A-T)] in an aqueous solution containing 5.2 mM Na⁺ at 5.0 MPa. The heating rate was 0.1 °C/min. The melting temperature, T_M , was 36.0 °C. The observed absorbance includes the absorbance of the pressure cell. Thermally induced helix–coil transition curves of poly[d(I-C)] universally exhibit the same sharp transition.

and Macgregor.¹⁸ The model-dependent volumes were calculated in an analogous way from the pressure dependence of the helix–coil transition. The model-dependent (two state) enthalpy and volume changes were used to calculate the cooperative length for the temperature- and pressure-induced transitions. The thermal cooperative length, N_T , is equal to the ratio of the model-dependent enthalpy to the calorimetrically measured enthalpy, $\Delta H_{vH}/\Delta H_{cal}$. Similarly, the pressure cooperative length, N_P , equals the ratio of the model-dependent volume to the volume calculated based on calorimetric data, $\Delta V_P/\Delta V_T$.

Haq et al.³¹ have demonstrated that the helix–coil transition of poly[d(A-T)] (and some other synthetic polynucleotides) is not a two-state process. However, in the absence of another model we have analyzed our data with the assumptions of the two-state transition model to obtain numerical values for thermodynamic parameters.

The enthalpy of the helix–coil transition was determined by the following equation, $\Delta H(T_M) = \Delta H(T_R) + \Delta C_P(T_M - T_R)$, where T_R is the reference temperature of 25 °C, T_M is the melting temperature, $\Delta H(T_R)$ is the calorimetrically determined enthalpy at 25 °C, and ΔC_P is the calorimetrically determined temperature-independent heat capacity change. In our calculation of $\Delta H(T_M)$ we have used the average values of ΔC_P for poly[d(A-T)] and poly[d(I-C)] of 213 ± 66 and 293 ± 58 J mol⁻¹ K⁻¹, respectively.³³

Results

A representative heat-induced helix–coil transition of poly[d(A-T)] at 5 MPa is shown in Figure 1. The transition is highly cooperative as seen from the shape of the curve. Although the helix–coil transition temperature, T_M , increases with hydrostatic pressure the general shape of the curve is unaltered.

In Figure 2 we present the pressure dependence of T_M for poly[d(I-C)] and poly[d(A-T)]; the sign and magnitude of $\Delta T_M/\Delta P$ are indicative of the effect of pressure on the conformational stability of the polymer. Positive values of $\Delta T_M/\Delta P$ indicate that pressure stabilizes the helix while negative values of $\Delta T_M/\Delta P$ reflect destabilization of the helical form of the polymer with pressure. The numerical values of $\Delta T_M/\Delta P$ are summarized in Table 1. In all cases, the pressure dependence of T_M is linear

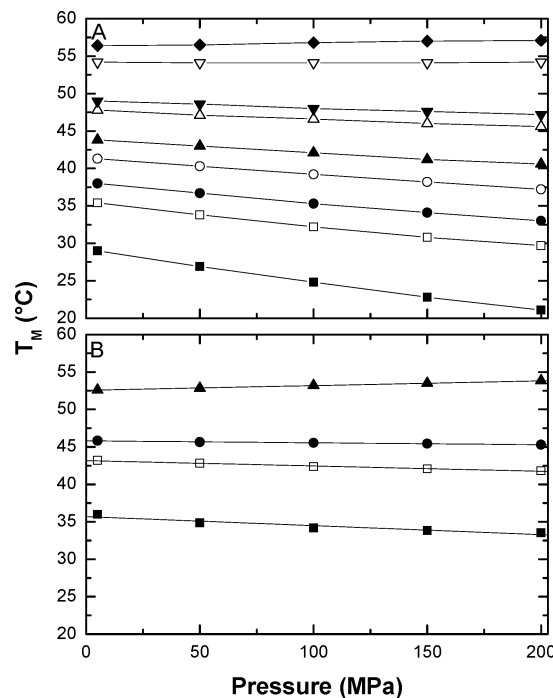


Figure 2. Helix–coil transition temperature (T_M) as a function of pressure at various Na⁺ concentrations: (A, poly[d(I-C)]) 5.2 (■), 10.2 (□), 15.2 (●), 20.2 (○), 30.2 (▲), 40.2 (△), 65.2 (▼), 105 (▽), and 205 mM (◆); (B, poly[d(A-T)]) 5.2 (■), 10.2 (□), 15.2 (●), and 30.2 mM (▲). The solid lines are least-squares fits to the data.

within experimental error over the pressure range we investigated, indicative of a negligible compressibility for this transition under these conditions.

The molar volume change of the heat-induced helix–coil transitions (ΔV_T) was calculated from the slopes of the data shown in Figure 2 by using the Clapeyron equation: $\Delta T_M/\Delta P = T_M \Delta V_T/\Delta H$, where ΔH is the enthalpy change of the transition. The values of the volume change at various salt concentrations are given in Table 1. Several studies have shown that ΔH of the transition is temperature dependent and that ΔC_P is positive.^{14,33,34} The enthalpy of the helix–coil transition at the temperature of interest was calculated by using the standard equation: $\Delta H_T = \Delta H_R + \Delta C_P(T - T_R)$, where ΔH_T is the enthalpy at a temperature T and ΔH_R is the enthalpy at a reference temperature, T_R . The value of ΔV_T for poly[d(A-T)] varied linearly with the log[Na⁺] from $-1.39(\pm 0.22)$ cm³ mol⁻¹ in 5.2 mM Na⁺ to $0.54(\pm 0.09)$ cm³ mol⁻¹ in 30.2 mM Na⁺. For poly[d(I-C)] it ranged from $-4.36(\pm 0.31)$ cm³ mol⁻¹ in 5.2 mM Na⁺ to $0.48(\pm 0.06)$ cm³ mol⁻¹ in 205 mM Na⁺ (Figure 3). The slope of the dependence of ΔV on log[Na⁺] for poly[d(I-C)] is greater than that for poly[d(A-T)] (Figure 3). It should be mentioned the ΔV values we report represent differential hydration between the denatured and the native state; they do not directly imply anything about the absolute extent of hydration of either the helix or coil states.

In Figure 4 we present the effect of T_M on the volume change (ΔV_T). The plots show data measured in these experiments and previously reported data for poly[d(A-T)].¹⁸ From these data we calculate that $\Delta V_T = 0$ cm³ mol⁻¹ at 47 °C for poly[d(A-T)]. For poly[d(I-C)], $\Delta V_T = 0$ cm³ mol⁻¹ at 55 °C. An increase in ΔV_T for the helix–coil transition of DNA with increasing ionic strength was reported previously.^{18,19,32} An earlier investigation of the effect of pressure on the conformational stability of poly[d(A-T)] at higher sodium ion concentrations found $\Delta T_M/\Delta P$ and ΔV_T to be positive under conditions where the T_M value ranged from 48.4 to 72.5 °C ([Na⁺] from 20 to 100 mM).¹⁸

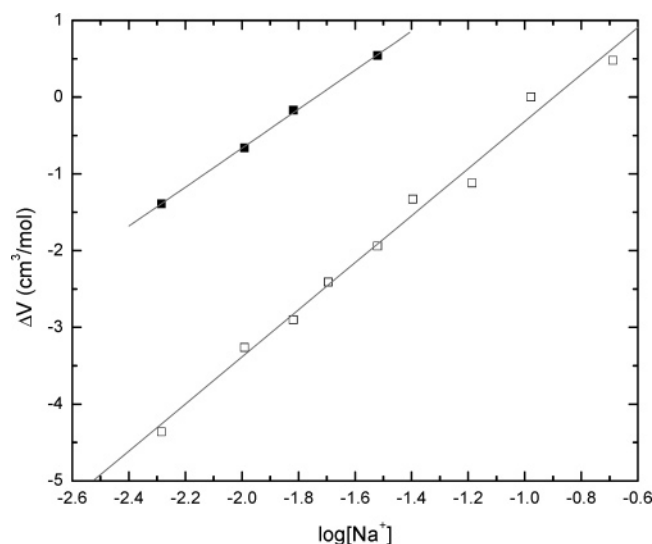


Figure 3. Molar volume change of the heat-induced denaturation (ΔV_T) as a function of salt concentration: (\square) poly[d(I-C)] and (\blacksquare) poly[d(A-T)]. The lines are least-squares fit of the data; the slope of the line for poly[d(I-C)] is $3.07 \pm 0.12 \text{ cm}^3 \text{ mol}^{-1}$ and that for poly[d(A-T)] is $2.54 \pm 0.05 \text{ cm}^3 \text{ mol}^{-1}$.

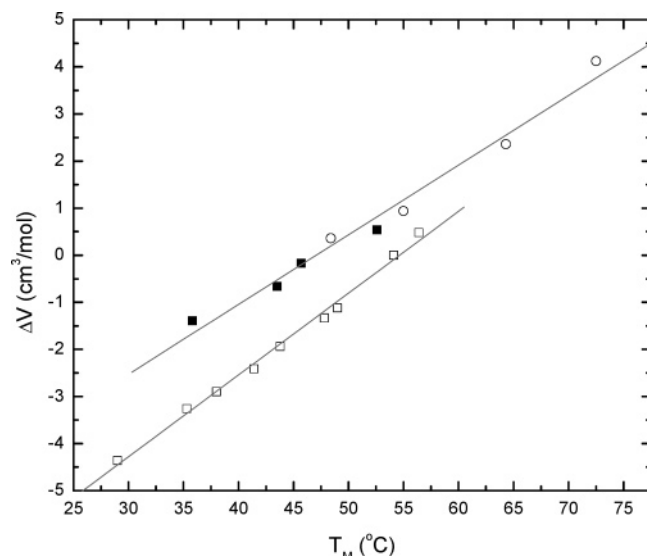


Figure 4. Molar volume change of the heat-induced transition (ΔV_T) as a function of T_M , (\square) poly[d(I-C)] and (\blacksquare) poly[d(A-T)], for the combined results of the authors (black squares) and those of Wu and Macgregor (open circles).¹⁸ The solid lines are a least-squares fit of the data. The slope of the line, the expansivity of the transition, is equal to $0.17 \text{ cm}^3 \text{ K}^{-1} \text{ mol}^{-1}$ for poly[d(I-C)] and $0.16 \text{ cm}^3 \text{ K}^{-1} \text{ mol}^{-1}$ for poly[d(A-T)].

Figure 5 shows the pressure and salt dependence of the two-state or van't Hoff enthalpy of the transitions (ΔH_{vH}). The ΔH_{vH} values are weakly dependent on the pressure and become less pressure dependent at higher salt concentrations. However, the value of ΔH_{vH} decreases with increasing salt concentration (Figure 5). The same effect was observed previously for poly[d(A-T)],¹⁸ poly(dA)poly(dT),¹⁸ and poly[d(G-C)].¹⁹

At each pressure, the salt dependence of the transition temperature $\Delta T_M / \Delta \log[\text{Na}^+]$ was used to calculate the number of cations released when the polymer undergoes the helix–coil transition. The number of sodium cations released upon the transition was calculated from the following equation: $\Delta T_M / \Delta \log[\text{Na}^+] = (2.303RT_M^2 / \Delta H) \Delta n$,^{18,38,39} where Δn is the number of sodium ions released, and R is the gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$). The slopes of the plots of T_M vs $\log[\text{Na}^+]$ for poly-

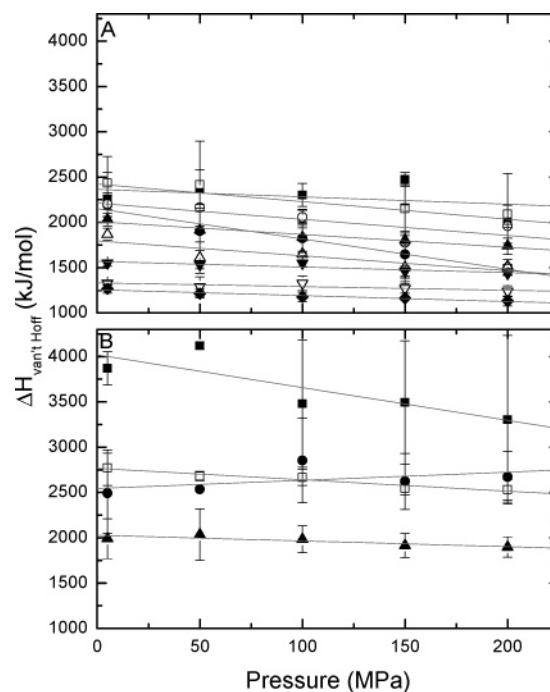


Figure 5. van't Hoff enthalpy of the heat-induced helix–coil transition as a function of pressure and salt concentration: (A, poly[d(I-C)]) 5.2 (\blacksquare), 10.2 (\square), 15.2 (\bullet), 20.2 (\circ), 30.2 (\blacktriangle), 40.2 (\triangle), 65.2 (\blacktriangledown), 105.2 (\triangledown), and 205.2 mM (\blacklozenge); (B, poly[d(A-T)]) 5.2 (\blacksquare), 10.2 (\square), 15.2 (\bullet), and 30.2 mM (\blacktriangle). The solid lines are least-squares fits to the data.

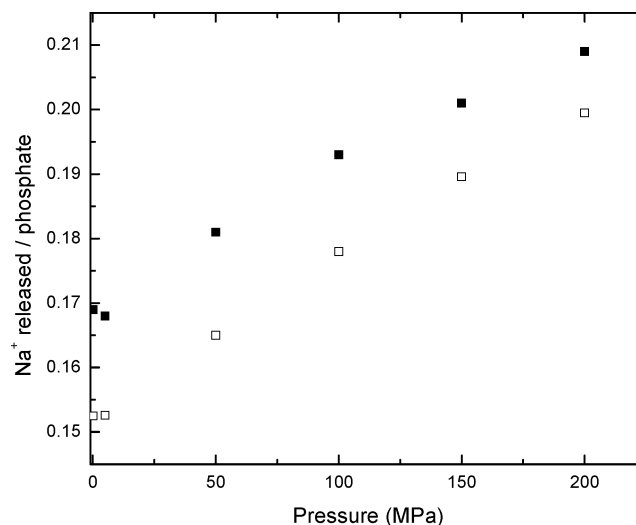


Figure 6. Number of sodium ions released upon helix–coil transition of as a function of pressure: (\square) poly[d(I-C)] and (\blacksquare) poly[d(A-T)].

[d(A-T)] varied from 21.1°C at 0.1 MPa to 26.0°C at 200 MPa , while for poly[d(I-C)] $\Delta T_M / \Delta \log[\text{Na}^+]$ ranged from 17.6°C at 0.1 MPa to 23.0°C at 200 MPa . Figure 6 illustrates that the number of sodium ions released during the helix–coil transition increases with pressure for both polymers. Similar pressure dependence was reported by Wu and Macgregor;¹⁸ however, the magnitudes of Δn values they report are somewhat lower than what we report here. There is a wide variation in the values of $\Delta T_M / \Delta \log[\text{Na}^+]$ reported in the literature.^{18,35–38} Our values for poly[d(A-T)] are larger than those reported for poly[d(A-T)] by Wu¹⁸ and Klump,³⁵ while they are lower than those obtained by Gruenwedel³⁶ and are close to those acquired by Elson.³⁷ For poly[d(I-C)], our $\Delta T_M / \Delta \log[\text{Na}^+]$ value of $17.6(\pm 0.9)^\circ \text{C}$ is in excellent agreement with Klump's value of 18.0°C for the same polymer.³⁵

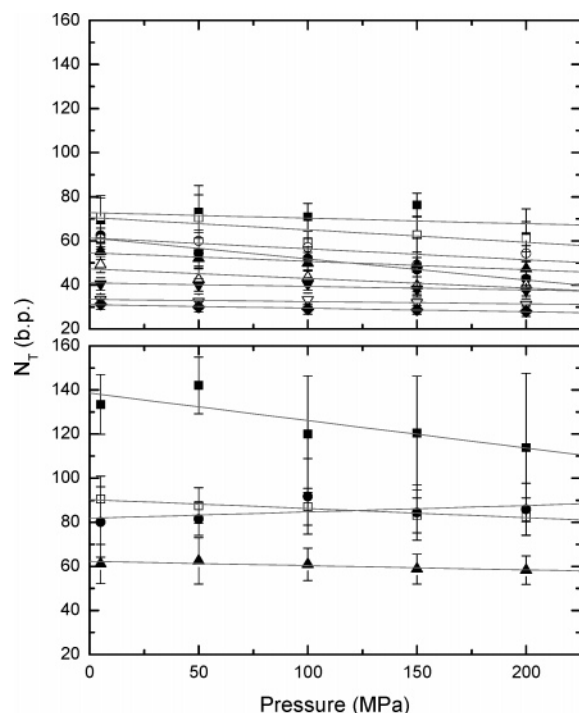


Figure 7. Cooperative length of heat-induced helix-coil denaturation (N_T) as a function of pressure and salt concentration: (top figure, poly[d(I-C)]) 5.2 (■), 10.2 (□), 15.2 (●), 20.2 (○), 30.2 (▲), 40.2 (△), 65.2 (▼), 105 (▽), and 205 mM (◆); (bottom figure, poly[d(A-T)]) 5.2 (■), 10.2 (□), 15.2 (●), and 30.2 mM (▲). The solid lines are least-squares fits to the data.

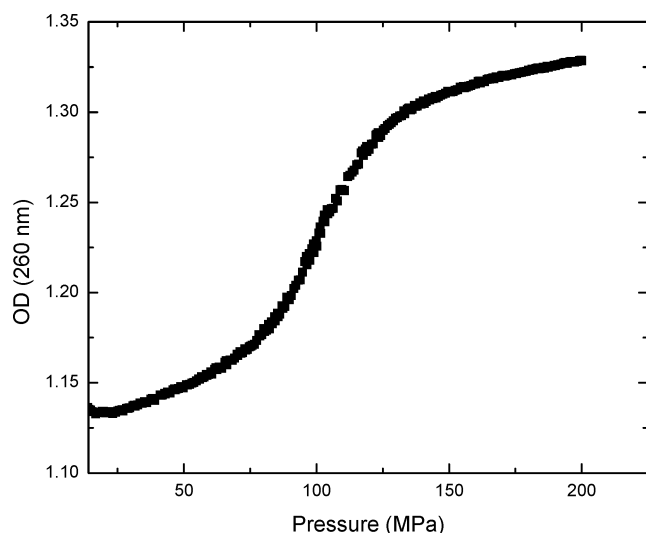


Figure 8. A pressure-induced helix-coil transition of poly[d(A-T)] in an aqueous solution containing 5.2 mM Na^+ at 33.8 °C. The rate of pressurization was 1.0 MPa/min. The helix-coil transition pressure, P_M , was 100.9 MPa. The observed absorbance includes the absorbance of the pressure cell. Typical pressure-induced helix-coil transition curves of poly[d(I-C)] exhibit the same shape.

We calculated the number of base pairs in the cooperative unit of a heat-induced transition (or the cooperative length) N_T by taking the ratio of $\Delta H_{VH}/\Delta H_{cal}$.^{19,31,40} The data in Figure 7 show that N_T is independent of pressure to a good approximation and that it decreases with salt concentration.

Figure 8 shows an example of a pressure-induced helix-coil transition of poly[d(A-T)] in 5.2 mM Na^+ at 33.8 °C; the transitions for poly[d(I-C)] are similar. This is the first experimental demonstration of pressure denaturation of DNA polymers.²⁶ In a manner analogous to our measurements of the

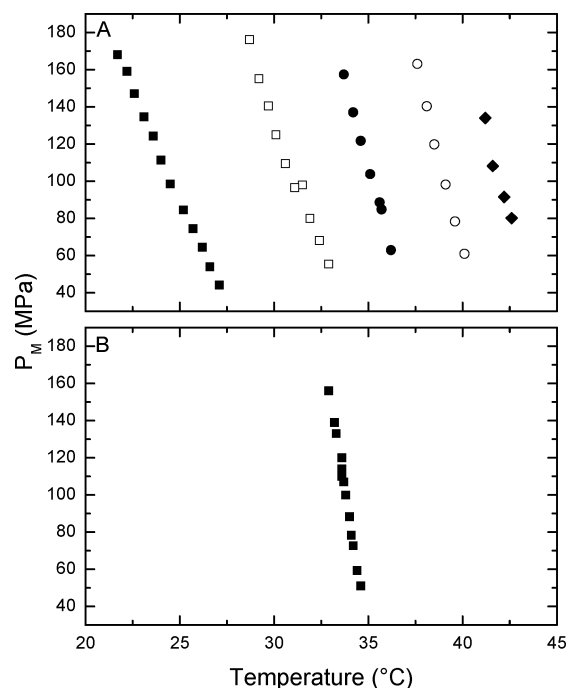


Figure 9. Temperature dependence of P_M for (A) poly[d(I-C)] (5.2 (■), 10.2 (□), 15.2 (●), 20.2 (○), and 30.2 mM (◆)) and (B) poly[d(A-T)] in an aqueous solution containing 5.2 mM sodium ions.

effect of pressure on T_M we measured the influence of temperature dependence of on P_M . These data are illustrated in Figure 9; it is clear from the data that P_M depends strongly on the temperature. With our pressure apparatus we are able to induce the helix-coil transition with pressure only at temperatures that are in the vicinity of T_M at atmospheric pressure.

Assuming the helix-coil transition to be a two-state equilibrium we calculated the volume change of the reaction in a manner analogous to that used to obtain the van't Hoff enthalpy for heat-induced transitions. Thus, the volume change of the pressure-induced transition (per cooperative length), ΔV_P , was obtained from the following equation: $[\Delta \ln K / \Delta P]_T = -\Delta V_P / RT$, where R is the gas constant ($8.314 \text{ m}^3 \text{ Pa K}^{-1} \text{ mol}^{-1}$), T is the absolute temperature, and K is the equilibrium constant for the monomolecular helix denaturation reaction. By dividing the molar volume change of pressure-induced transition by the molar volume change of heat-induced transition, we calculate the cooperative length of pressure-induced transition: $N_P = \Delta V_P / \Delta V_T$. The temperature dependence of N_P is shown in Figure 10. Over the narrow temperature range we could investigate, the value of N_P increases rapidly and approximately linearly with temperature. At temperatures approaching T_M , the cooperative length of the pressure-induced transition, N_P , is approximately 2-fold larger than that of a heat-induced transition, N_T .

Discussion

Evidence of the importance of hydration in the conformational stability of biological polymers has come from several lines of investigation.^{41–46} In our studies, we have been particularly interested in the role played by water in the stability of the helical forms of DNA. Our experimental focus has been on the influence of hydrostatic pressure on the thermodynamics and kinetics of the helix-coil transition.

According to a recently proposed theoretical phase diagram for double stranded nucleic acid polymers,²⁶ hydrostatic pressure can destabilize, stabilize, or have no effect on DNA. The effect

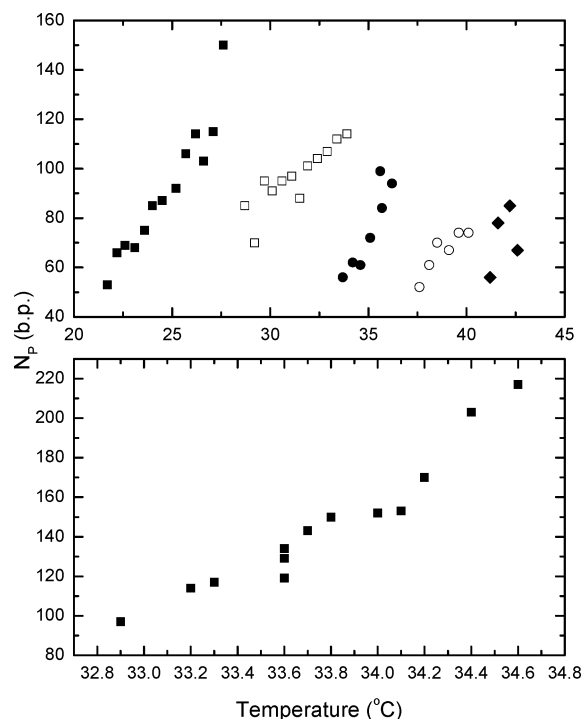


Figure 10. The temperature dependence of the cooperative length of the pressure-induced helix–coil transition of (top figure) poly[d(I-C)] (5.2 (■), 10.2 (□), 15.2 (●), 20.2 (○), and 30.2 mM (◆) and (bottom figure) poly[d(A-T)] in an aqueous solution containing 5.2 mM Na⁺. Note that the temperature axes are not the same for the two plots.

is contingent on the helix–coil transition temperature, T_M , i.e., the temperature at which half of the molecules have lost their helical structure. One of the predictions of the phase diagram is that polymers with T_M values $< \sim 50$ °C are destabilized by pressure, while polymers with T_M values $> \sim 50$ °C are stabilized by pressure. Previous studies of the effect of pressure on the conformational stability of DNA have focused on experimental conditions that would not lead to the observation of pressure destabilization of DNA.

Figure 4 shows that by altering the ionic strength of the solution, and hence the T_M , the volume change for the helix–coil transition of poly[d(I-C)] and poly[d(A-T)] can be positive or negative, passing through zero at about 55 and 47 °C, respectively. There are several factors that can contribute to an observed volume change (ΔV). According to scaled particle theory^{47–50} the molar volume can be written as $V = V_M + V_T + V_I + \beta_{TO}RT$.²⁵ The first term, V_M , is the intrinsic volume of the solute, V_T is the thermal volume corresponding to the volume of the void space around the solute as a result of thermal vibration of the solute and solvent molecules and is presumably proportional to the solvent accessible surface area of the solute (DNA),¹⁵ V_I is the interaction volume, which represents the contraction of the solvent (water) around the solute (DNA) due to hydrogen bonding or electrostriction, β_{TO} is the coefficient of isothermal compressibility of the solvent (water), R is the gas constant, and T is the temperature. The final term is negligible for macromolecules and we can omit it in this analysis.⁴⁶

We can express the measured volume change as the difference between its components; thus, $\Delta V = \Delta V_M + \Delta V_T + \Delta V_I$, where each of the terms refers to the difference in that term between the helix and coil forms. We assume that the change in the intrinsic volume ΔV_M is zero; the intrinsic volume of the helix, V_{Mh} , is equal to that of the coil, V_{Mc} . This further simplifies the expression to $\Delta V = \Delta V_T + \Delta V_I$. Each of these terms is in turn

dependent on the difference between the volume of the helix and coil states. Thus, we can write $\Delta V = \Delta V_c - \Delta V_h = (V_{Tc} - V_{Th}) + (V_{Ic} - V_{Ih})$, where the subscripts c and h refer to the coil and helix forms, respectively. For the transition from the helix to coil conformations, it seems reasonable to anticipate that ΔV_T is positive because the coil form should have a greater solvent accessible surface area than the helix form, i.e., $V_{Tc} > V_{Th}$. If $V_{Tc} - V_{Th}$ is positive then ΔV_I must be negative at low temperatures because the net ΔV is negative. This implies that $V_{Ic} < V_{Ih}$; the interactions with water are stronger for the coil form than for the helix form. This may be a consequence of the larger number of water–DNA interactions formed with the larger solvent accessible surface area of the coil.

We have shown that the molar volume change of the transition increases with temperature, hence the expansivity coefficient, ΔE , is positive. Combining our data and those of Wu and Macgregor,¹⁸ we calculate that $\Delta E = 0.16(\pm 0.01)$ cm³ K⁻¹ mol⁻¹ for poly[d(A-T)]. For poly[d(I-C)], $\Delta E = 0.17(\pm 0.01)$ cm³ K⁻¹ mol⁻¹. What is the origin of positive change in expansivity? From the previous discussion, $\Delta V = \Delta V_T + \Delta V_I$ at the temperature where $\Delta V = 0$, $\Delta V_T = -\Delta V_I$. It seems reasonable to anticipate that the change in the thermal volume for the transition will be positive, i.e., $\Delta V_T = V_{Tc} - V_{Th} > 0$. It also seems reasonable to expect that with increasing temperature the coil form will adopt an increasingly random structure resulting in a larger solvent accessible surface area; consequently, the magnitude of ΔV_T will increase with temperature, i.e., $d\Delta V_T/dT > 0$. A similar temperature dependence is expected for ΔV_I ; the value of $d\Delta V_I/dT$ should be positive because interactions such as electrostriction will become less favorable with increasing temperature. Experimentally, the measured volume change of the helix–coil transition increases with temperature. Both components also increase; however, we cannot assess whether the thermal volume or interaction volume term predominates.

The volume changes determined by measuring the effect of pressure on the helix–coil equilibrium of poly[d(A-T)] now extend over approximately 35 °C. It is clear from Figure 4 that the ΔV values we report here are consistent with those reported by Wu and Macgregor.¹⁸ However, the expansion coefficient we have measured for poly[d(A-T)] appears to differ from the value found by Chalikian et al.¹⁴ by approximately a factor of 2; we measured 0.16 cm³ K⁻¹ mol⁻¹ for poly[d(A-T)] and 0.17 cm³ K⁻¹ mol⁻¹ for poly[d(I-C)] while they reported 0.30 cm³ K⁻¹ mol⁻¹. The value they report is based on data for several nucleic acid sequences and represents an average for these polymers and included data from densometric and high-pressure studies. Examination of Figure 3 in their publication¹⁴ reveals that while the ΔV values assessed from pressure experiments are consistent with densometrically determined values, the expansivity of the pressure data is less than that of the other experiments. Given the consistency of our data, the temperature range over which we have measured the volume change, and that the values for poly[d(I-C)] and poly[d(A-T)] are similar, we propose that the difference in the values of ΔE measured by densitometry and high-pressure results from the effect of pressure on the single-stranded form of DNA. Specifically, pressure leads to a more structured single-stranded form. If this is true, the change in molar volume of the helix form with temperature measured in pressure experiments is less than that measured by ambient pressure volume techniques such as densitometry. This suggestion is corroborated by the results of a high-pressure FTIR study of poly(dA)poly(dT) and its composite monomers, poly(dA) and poly(dT).⁵¹ In that study

the hypochromicity of several IR bands of the single strands decreased with increasing pressure at room temperature implying that the single strands have a greater amount of stacking at high pressure than at atmospheric pressure.

Figure 7 illustrates that for both polymers the cooperative length for heat-induced transitions (N_T) is independent of pressure to a first approximation and that it decreases with increasing salt concentrations. Increasing the salt concentration stabilizes the double helix and consequently causes a decrease in the cooperative melting unit. Wu and Macgregor¹⁹ working with poly[d(G-C)] also observed a decrease in the length of the cooperative unit with increasing salt concentration. These results contrast with those of Nordmeier,³² who reported a gradual increase in the value of N_T for calf thymus DNA with potassium ion concentration over the range of 5–500 mM. Interestingly, Gruenwedel observed that poly[d(AT)] exhibits decreased cooperativity with increasing salt concentrations,³⁶ while the cooperativity of calf thymus DNA increased with increasing salt concentrations.³⁹ Perhaps sequence complexity gives rise to the different behavior of natural and synthetic DNA sequences.

For pressure-induced helix–coil transitions, we have defined a parameter P_M that is analogous to T_M , i.e., it is the pressure at which half of the DNA is in the coil form. We determined the value of P_M by measuring the UV absorption as a function of pressure at a constant temperature (see Figure 8). The temperature dependence of P_M is illustrated in Figure 9. It is evident that as the temperature approaches T_M (at atmospheric pressure) less pressure is needed to induce the helix–coil transition, as an increasing fraction of the population of polymers is present in the denatured form.

Using the values of P_M , we calculated the cooperative length of the pressure-induced transition, N_P . This parameter equals the ratio of the values of the volume change of the pressure-induced helix–coil transition, ΔV_P , measured assuming a bimolecular reaction to the value of the “model-independent” volume change, ΔV_T . This is analogous to the definition of the cooperative length for heat-induced transitions, N_T . The data in Figure 10 and Table 2 show that the magnitude of N_P increases rapidly and apparently linearly as the temperature approaches the T_M of the polymer. Experimentally, this increase arises because the volume change calculated from the pressure-denaturation data increases as the temperature approaches T_M . Mechanistically, we believe that the increase in ΔV_P (or N_P) occurs because of an increase in the molar volume of the helix form as the temperature approaches T_M .

The difference between the two measures of the cooperative length appears to point to a different mechanism for the helix–coil transition when induced by pressure or heat. Our calculation of the cooperative length is dependent on knowledge of the enthalpy of the transition. Unfortunately, there are no data for the calorimetrically determined enthalpy as a function of pressure. However, the van't Hoff enthalpies for the transitions show only modest pressure dependence. To account for the difference in N_T and N_P they would have to change by a factor of at least 2 over the pressure range that we studied, which they do not. In light of the weak pressure dependence of the van't Hoff enthalpy it seems unlikely that the calorimetrically determined (model independent) enthalpy would behave substantially different with pressure.

In summary, we have shown that the effect of pressure on the double stranded DNA polymer poly[d(A-T)] and poly[d(I-C)] helices is a function of T_M at atmospheric pressure. The volume change of the heat-induced transition (ΔV_T) is positive,

TABLE 2: The Volume Change Calculated from the Pressure-Induced Transition (ΔV) and the Cooperative Length of Pressure-Induced Transition (N_P) of Poly[d(A-T)] and Poly[d(I-C)] in an Aqueous Solution Containing 5.2 mM Na^+ ^a

polymer	T (°C) ^b	ΔV_P (cm ³ mol ⁻¹) ^c	N_P (bp) ^d
poly[d(A-T)] $T_M = 35.8$ °C	32.9	-135	97
	33.2	-159	114
	33.3	-163	117
	33.6	-166	119
	33.6	-179	129
	33.6	-186	134
	33.7	-199	143
	33.8	-209	150
	34.0	-211	152
	34.1	-212	153
	34.2	-236	170
	34.4	-282	203
	34.6	-302	217
poly[d(I-C)] $T_M = 29.0$ °C	21.7	-230	53
	21.7	-289	66
	22.6	-301	69
	23.1	-297	68
	23.6	-326	75
	24.0	-370	85
	24.5	-378	87
	25.2	-401	92
	25.7	-464	106
	26.2	-495	114
	26.6	-449	103
	27.1	-501	115
	27.6	-651	149

^a Figure 10 displays N_P as a function of temperature. ^b The error in temperature is approximately 0.1 °C. ^c The estimated errors in ΔV do not exceed 23 cm³ mol⁻¹. ^d The estimated errors in N_P are not greater than 30 bp.

that is the helix is stabilized by pressure if T_M is above 47 °C for poly[d(A-T)] and 55 °C for poly[d(I-C)], and it is negative if T_M is below these temperatures, respectively. The cooperative length (N_P) measured by pressure-induced transition is strongly dependent temperature, increasing closer to the T_M measured at atmospheric pressure.

Acknowledgment. We would like to thank Drs. Tigran V. Chalikian and Gregory M. K. Poon for useful discussions. This research was supported by a grant from the Canadian Institute for Health Research.

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