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The effect of hydrostatic pressure on the thermal stability of DNA hairpins

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DNA hairpins consist of two distinct structural domains: a double stranded stem and a single-stranded loop that connect the two strands of the stem. Previous studies of short DNA hairpins have revealed that loop and stem sequences can significantly affect the thermodynamic stability of short DNA hairpins. In this work we present the effect of hydrostatic pressure on the helix-coil transition temperature (T_M) for 11 16-base, hairpin-forming DNA oligonucleotides. All of the samples form a hairpin with a 6-base pair stem and a four-base loop. In addition, the four base pairs at the end of the stem distal from the loop are the same for every molecule. We have varied loop sequence and identity of the two duplex base pairs adjacent to the loop. Using the change in UV absorption to monitor the conformational state of the oligonucleotide the hairpin-coil transition temperature of these molecules was studied as a function of sodium ion concentration and pressure. From these data we calculated the volume change accompanying the transition. Model-dependent (van't Hoff) transition parameters such as ΔH_{vH} and transition volume (ΔV) were estimated from the analysis of conformational transitions. Experiments revealed that the ΔV for denaturation of these molecules range from -2.35 to $+6.74$ cm^3 mol^{-1}. The expansibility ($\Delta\Delta V/\Delta T$) and the pressure dependence of cation release are also presented. The difference in the volume change for this transition is related to the differences in the hydration of these molecules.

1. Introduction

The presence of inverted repeats in DNA or RNA sequences can lead to the formation of a snap-back region or hairpin [1]. Hairpins have two distinct structural domains: a base paired double stranded region and a single stranded loop that connects the base paired strands. They are common secondary structural elements in RNA and DNA. RNA hairpins have functions in initiating folding and forming tertiary structures and protein binding sites [2,3]; DNA hairpins are involved in regulating replication and transcription [4,5]. Hairpin loops are attractive candidates for the design of antisense therapeutics [6–8]. Understanding the factors underlying the stability of this conformation has received a great deal of attention [9–11]. As with other nucleic acid structures the thermal stability of the hairpin conformation depends upon the primary sequence of the oligodeoxyribonucleotide (ODN), hydrogen bonding, base pair stacking energies, and solvent properties such as the ionic strength. However, the peculiarities of the hairpin conformation are also important in their stability; thus, the base composition and base sequence of the loop and the base pairs adjacent to it also play a determining role in the stability of hairpins [12–15].

Water plays an important role in the stability of the higher-order structures formed by nucleic acids. The most direct methods to determine the role of hydration in the thermodynamic stability of a system involve measurements of volumetric parameters such as volume, compressibility, and expansibility. By studying the pressure dependence of the thermal stability we can determine volumetric parameters such as the molar volume change, molar expansibility change, and in some cases the compressibility change accompanying the transition. The molar volume change of a reaction or transition (ΔV) equals the difference between the molar volume of the products and the reactants; the value of ΔV can be positive or negative. In water, the sign of the volume change is related to the formation of more or less extensive hydration by the reactants or products. For example, a negative volume change would be attributed to the product, in the present case the coil form, forming stronger interactions with the solvent than the reactant (hairpin).

A general description of the thermodynamic behavior of the secondary structure of nucleic acids would take into account the length and the possible conformation states of oligomers and polymers. At this writing, it seems unlikely that a unified phase diagram will be developed in the short term; however, a more quantitative understanding of nucleic acid structures other than duplex polymers should be possible. Oligomeric nucleic acid duplexes and polymers exhibit many differences in thermodynamic properties [16–18]. The terminal bases play a much greater role in the properties of oligomers than they do in the polymeric forms of nucleic acids.

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Structural, thermodynamic, and kinetics studies have demonstrated that the terminal base pairs have properties significantly different from base pairs distant from the ends [20–24]. For polymers, because of the small fractional contribution to the overall number of base pairs, the properties of the terminal base pairs are ignored and are not included in the theory underlying the phase diagram presented by Dubins [19].

We report the effect of hydrostatic pressure on the thermal stability of 11 16-base hairpin forming ODNs; to our knowledge this is the first report of the volumetric properties of any hairpin structure. Each of the molecules we studied can form six base pairs and has a four-base loop; their thermal behavior at atmospheric pressure has been well characterized [11]. We have compared the results with those observed for the effect of pressure on polymeric double-stranded nucleic acids. Although by no means complete, the results presented here are a first step toward broadening our understanding of the role of sequence and non-standard secondary structure in the volumetric properties of nucleic acids.

2. Materials and methods

Cartridge-purified oligodeoxyribonucleotides (ODNs) were purchased from ACGT, Inc. (Toronto, Canada). The sequences of the 11self-complementary ODNs and the abbreviation we use in this work are shown in Table 1. Oligonucleotide concentrations were determined spectrophotometrically using calculated extinction coefficients [14,25]. All DNA samples were dissolved in 20 mM sodium cacodylate and 0.01 mM Na_2EDTA (pH6.42), at sodium ion concentrations ranging from 100 to 10 mM.[1] The Na^+ concentration was adjusted by the addition of NaCl. Previous work suggests that for these ODNs, with the exception of molecules with A_4 loops nucleated by AA/TT or AT base pair doublets, melting temperatures are independent of strand concentration from 0.5 to 63.5 μM [11]. In our experiments, the concentration of DNA samples was approximately 10 μM (strand). Prior to melting, samples were heated to 100 °C for 20 min and then placed on ice for 30 min.

2.1. Optical melting experiments under hydrostatic pressure

The temperature regulated iso-hyperbaric spectrophotometer was employed to obtain the heat-induced melting curves; this instrument has been described previously [26]. Briefly, the sample solution (~300 μL) was contained in a cylindrical quartz cuvette (path length 0.5 cm) positioned in the optical path of a pressure cell equipped with quartz windows. The high-pressure cell was filled with silicon oil as the pressure-transmitting medium. Pressure up to 200 MPa (0.1 MPa = 1 bar = 0.987 atm) was generated using an automated high-pressure pump (Porous Materials Incorporated, Ithaca, NY). The temperature was regulated using a Haake model DC5-k20 circulating bath (Thermo Scientific, Waltham, MA). A thermocouple connected to an Omega DP80 digital thermometer (Stamford, CT) is inserted into the pressure-cell in order to measure the temperature. The temperature, pressure and absorption of the sample were all recorded by the software controlling the experiment. Sample temperature was increased linearly at a heating rate of 0.9 °C/min and the hairpin-coil transition was monitored by measuring the change in absorption at 268 nm. The pressures employed in our experiments are insufficient to induce change in the molecular volumes or induce covalent modifications; consequently, the observed volume changes are related to differences in the interactions between the solvent and solutes.

Table 1
Sequences of the 11 DNA hairpins. Each has a six-base pair duplex-forming stem linked by a four-base loop. The first four base pairs in the stem are the same for every ODN.

Name	Stem	Nucleation stack	Loop	Nucleation stack	Stem
AT(T_4)	5′-GGAT	AT	TTTT	AT	ATCC
AT(C_4)	5′-GGAT	AT	CCCC	AT	ATCC
AT(G_4)	5′-GGAT	AT	GGGG	AT	ATCC
AT(TA_2T)	5′-GGAT	AT	TAAT	AT	ATCC
AT(TC_2T)	5′-GGAT	AT	TCCT	AT	ATCC
AT(TG_2T)	5′-GGAT	AT	TGGT	AT	ATCC
AA(C_4)	5′-GGAT	AA	CCCC	TT	ATCC
AA(G_4)	5′-GGAT	AA	GGGG	TT	ATCC
AA(TA_2T)	5′-GGAT	AA	TAAT	TT	ATCC
AA(TC_2T)	5′-GGAT	AA	TCCT	TT	ATCC
AA(TG_2T)	5′-GGAT	AA	TGGT	TT	ATCC

2.2. Analysis of the melting curves

The fraction of DNA in the coil form at a temperature T, α, was calculated using the following equation:

$$\alpha = \frac{[OD(\mathrm{T}) - L(\mathrm{T})]}{[H(\mathrm{T}) - L(\mathrm{T})]}. \tag{1}$$

Where $OD(\mathrm{T})$ is the optical density at temperature T, and $L(\mathrm{T})$ and $H(\mathrm{T})$ are equations for the lines describing the low- and high-temperature baselines respectively as a function of temperature. The samples are assumed to be in the hairpin form if $\alpha = 0$, while at $\alpha = 1$ the samples are assumed to be entirely in the coil form. The hairpin-coil transition temperature (T_M) is the temperature at which $\alpha = 0.5$. For this monomolecular, single-step transition, the T_M is the temperature at which half of the DNA samples are in the hairpin state and the other half are in the coil state. The transition temperature was also determined by differentiating the observed absorption with respect to temperature. We did this because for some transitions we could only observe a limited portion of the low-temperature base line, which made it difficult to fit a low-temperature base line equation, $L(\mathrm{T})$ in Eq. 1. However, we did not observe any difference between the transition temperatures obtained using Eq. 1 and those obtained by differentiating the data. For a more detailed explanation of melting curve analysis please refer to the review article by Mergney [27].

The molar volume change of the heat-induced helix-coil transition (ΔV) was calculated from the change in the transition temperature with pressure using the Clapeyron equation:

$$\frac{\Delta \mathrm{T_M}}{\Delta P} = \mathrm{T_M}\frac{\Delta V}{\Delta H_{cal}} \tag{2}$$

where ΔH_{cal} is the calorimetric enthalpy change of the hairpin-coil transition for these ODNs at atmospheric pressure reported in Vallone et al.[14]. At elevated pressures we have used the pressure dependence of the van't Hoff enthalpy change as a proxy for the calorimetric values at high pressure; the assumption being that pressure dependent changes in the calorimetric values will mirror any changes observed in the model-dependent van't Hoff values. The van't Hoff enthalpy (ΔH_{vH}) for each transition was calculated from $(\partial\alpha/\partial \mathrm{T})_{\mathrm{Max}}$ and the transition temperature using an equation for a two-state transition described in Marky and Breslauer [28].

$$\Delta H_{\mathrm{vH}} = (2 + 2\mathrm{n})\mathrm{R}\mathrm{T}^2_{\mathrm{T_M}}\left(\frac{\partial\alpha}{\partial \mathrm{T}}\right)_{\mathrm{Max}}. \tag{3}$$

Where n, the molecularity of the process, is presumed to equal 1 for this transition [11], R is the gas constant, and $(\partial\alpha/\partial \mathrm{T})_{\mathrm{Max}}$ is the maximum slope of the α versus temperature curve at $\mathrm{T_M}$.

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[1] Note: There is an additional 0.02 mM sodium in all of the solutions that comes from the addition of 0.01 mM Na_2EDTA. Thus, 10 mM is actually 10.02 mM, 20 mM is 20.02 mM, etc.

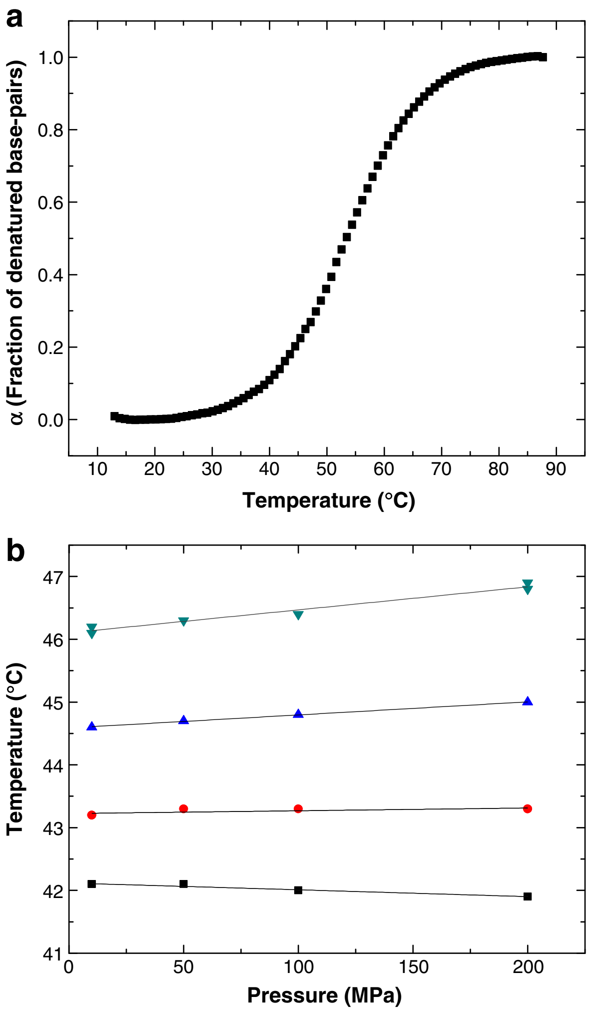

Fig. 1. a. A normalized heat-induced helix-coil transition of AT(T_4) hairpin sample in an aqueous solution containing 100 mM Na^+ at 200 MPa. The T_M of this sample under these conditions is 53.1 °C. b helix-coil transition temperature (T_M) of AT(TA_2T) as a function of pressure at four Na^+ concentrations: 100 mM (▼), 50 mM (▲), 20 mM (●), and 10 mM (■). The solid lines are least-squares fits to the data.

3. Results

3.1. Heterogeneous-loop hairpins

Fig. 1a shows a representative helix-coil transition curve for the AT(T_4) hairpin in an aqueous solution containing 100 mM Na^+ at 200 MPa. The T_M of this sample under these conditions is 53.1 °C. For all eleven hairpins the transitions are cooperative and the general shape of the curves does not change as a function of pressure. Fig. 1b illustrates the pressure dependence of the T_M for the AT(TA_2T) hairpins at four salt concentrations. These data are representative of those obtained for all of the ODNs studied in this work. The sign and magnitude of the change in the transition temperature with pressure, i.e. the slopes of the lines, are indicative of the effect of pressure on the stability of short DNA hairpins. Positive slopes indicate that increasing pressure stabilizes the hairpin form, while pressure destabilizes those systems for which the slope is negative. The slopes, $\Delta T_M/\Delta P$, are summarized in Table S1. Similar to the behavior of other DNA molecules studied at high pressure, we found the model-dependent enthalpy to be a weak function of the applied pressure; values are given in Table S2. The values of T_M and the molar volume change at each sodium ion concentration calculated from the values of $\Delta T_M/\Delta P$ for the six heterogeneous-loop hairpins are summarized in Table 2.

Table 2
Atmospheric pressure transition temperatures from optical melting experiments and the molar volume change of the transition for the six heterogeneous-loop hairpins (the error in temperature is approximately 0.1 °C).

		Loop sequence					
		TA_2T		TG_2T		TC_2T	
Nucleation stack	Na^+ (mM)	T_M (°C)	ΔV ($cm^3\ mol^{-1}$)	T_M (°C)	ΔV ($cm^3\ mol^{-1}$)	T_M (°C)	ΔV ($cm^3\ mol^{-1}$)
AT/AT	10	42.1	−0.44 ± 0.04	42.8	−1.41 ± 0.14	44.9	1.81 ± 0.29
	20	43.2	0.18 ± 0.08	44.0	−0.25 ± 0.05	46.1	2.27 ± 0.10
	50	44.6	0.83 ± 0.04	45.5	1.55 ± 0.21	48.7	3.05 ± 0.21
	100	46.1	1.46 ± 0.32	46.8	2.89 ± 0.20	51.3	3.76 ± 0.18
AA/TT	10	40.2	−1.96 ± 0.08	37.9	−2.35 ± 0.13	44.0	0.78 ± 0.08
	20	41.5	−1.15 ± 0.07	41.1	−0.86 ± 0.04	45.4	1.18 ± 0.09
	50	43.3	−0.19 ± 0.04	43.5	0.85 ± 0.04	47.9	1.75 ± 0.19
	100	44.7	0.74 ± 0.10	45.1	2.14 ± 0.09	49.9	2.35 ± 0.17

In Fig. 2, it can be seen that the volume change of the transition varies linearly with temperature; data are shown for two representative ODNs. From these data we calculated the expansibility of the transition, ΔE, $(=\Delta\Delta V/\Delta T)_p$. The expansibility values (ΔE) for the six heterogeneous-loop hairpins are summarized in Table 4.

3.2. Homogenous-loop hairpins

We also studied the effect of hydrostatic pressure on the helix-coil transition temperature of hairpins with homogeneous four-base loops. As with the heterogeneous-loop hairpins, hydrostatic pressure did not change the general shape of the transitions and the dependence of T_M on pressure, $\Delta T_M/\Delta P$, was linear. The T_M and transition volume for this series of hairpin-forming ODNs are summarized in Table 3. The experimental values, $\Delta T_M/\Delta P$, and the model-dependent enthalpy change of the transition for the homogeneous-loop hairpins are presented in Tables S3 and S4, respectively. The transition enthalpy decreased slightly with pressure for these hairpins, similar to the behavior of the heterogeneous-loop hairpins.

The transition volume for these hairpins also varied linearly with temperature (Fig. S1). From the change of ΔV with temperature we calculated the ΔE; the values for this set of ODNs are summarized in Table 4.

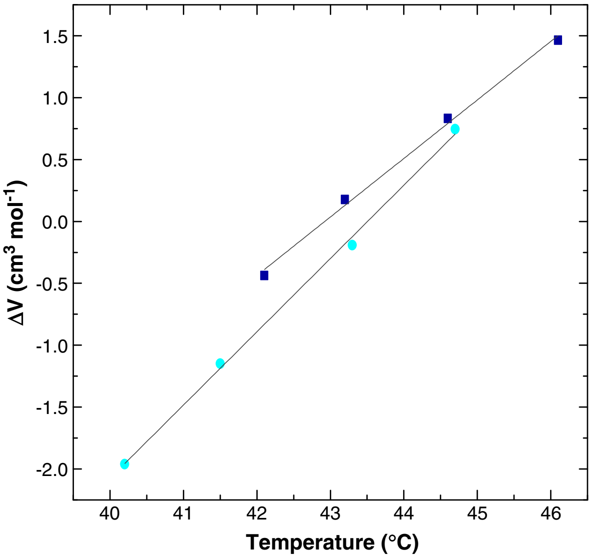

Fig. 2. Molar volume change of the heat-induced denaturation (ΔV) as a function of temperature, (■) AT(TA_2T) and (●) AA(TA_2T). The solid lines are least-squares fits to the data. The slope of the line, ΔE of the transition, is equal to 0.48 $cm^3\ K^{-1}\ mol^{-1}$ and 0.58 $cm^3\ K^{-1}\ mol^{-1}$ for AT(TA_2T) and AA(TA_2T) hairpin samples, respectively.

Table 3
Atmospheric pressure transition temperatures from optical melting experiments and the molar volume change of the transition for the six homogeneous-loop hairpins (the error in temperature is approximately 0.1).

		Loop sequence					
		C_4		G_4		T_4	
Nucleation stack	Na^+ (mM)	T_M (° C)	ΔV ($cm^3\ mol^{-1}$)	T_M (°C)	ΔV ($cm^3\ mol^{-1}$)	T_M (°C)	ΔV ($cm^3\ mol^{-1}$)
AT/AT	10	42.4	3.07 ± 0.13	44.0	−1.72 ± 0.09	43.9	1.38 ± 0.07
	20	43.7	4.10 ± 0.20	45.1	0.29 ± 0.06	45.8	2.30 ± 0.13
	50	46.1	5.58 ± 0.25	46.5	2.79 ± 0.19	48.2	3.92 ± 0.20
	100	49.3	6.74 ± 0.38	47.7	4.52 ± 0.22	51.0	5.40 ± 0.22
AA/TT	10	42.9	1.38 ± 0.07	38.4	−0.83 ± 0.04		
	20	44.1	1.67 ± 0.08	40.0	0.22 ± 0.06		
	50	45.6	2.18 ± 0.10	42.0	1.44 ± 0.09		
	100	47.2	2.45 ± 0.10	43.7	2.68 ± 0.11		

3.3. Salt dependence of transition temperature

As expected, increasing the Na^+ concentration shifted the transition temperature of all hairpins to higher temperatures. The dependence of T_M on the sodium ion concentration is shown in Figure S2. The number of sodium cations released, Δn, can be calculated from the salt dependence of the transition temperature ($\partial T_M / \partial \log [Na^+]$) according to the following relationship [29–31]

$$\partial T_M / \partial \log \left[Na^+\right] = \left(2.303 R T_M^2 / \Delta H_{cal}\right) \Delta n \tag{4}$$

where R is the gas constant (8.314 J K^{-1} mol^{-1}). Consistent with Eq. 4, we observed a linear dependence of T_M on the log of the sodium ion concentration. The values of Δn as a function of pressure are shown in Fig. 3. The values of $\partial T_M / \partial \log [Na^+]$ are given in Table S5. The number of sodium cations released, Δn, during the hairpin-coil transition increases modestly with pressure for all eleven of our samples. A similar pressure dependence of Δn was reported previously for DNA polymers [32,33].

3.4. The role of the loops and nucleation stack in the transition volume

We have evaluated our data in two ways; either by considering the influence of different loops by comparing the ΔV values of the hairpins with the same nucleation stack or by examining the effect of the nucleation stack by comparing the ΔV values of hairpins with the same loop sequence. By holding one of these factors constant, either the loop or the nucleation sequence, we gain insight into the contribution of the changes in the other structural component to the observed volumetric behavior. It must be pointed out that it is unlikely that these two structural elements behave independently of each other; however, in the absence of any other data on the influence of pressure on the stability of short DNA hairpins we chose this as an initial basis of comparison.

Table 4
Expansibility values, ΔE, of the hairpins; the units are $cm^3\ mol^{-1}\ K^{-1}$.

	Loop sequence		
Nucleation stack	TA_2T	TG_2T	TC_2T
AT/AT	0.48 ± 0.02	1.10 ± 0.04	0.30 ± 0.01
AA/TT	0.58 ± 0.02	0.62 ± 0.05	0.26 ± 0.01

	Loop sequence		
Nucleation stack	C_4	G_4	T_4
AT/AT	0.53 ± 0.06	1.72 ± 0.06	0.58 ± 0.02
AA/TT	0.26 ± 0.02	0.65 ± 0.01	

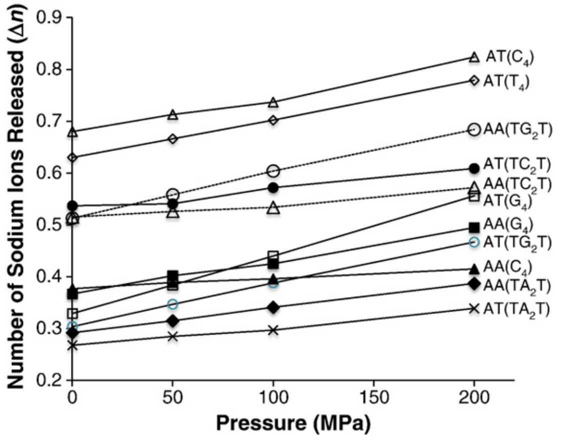

Fig. 3. Pressure dependence of the release of sodium ions. Open triangle, solid line, AT(C_4); open diamonds, AT(T_4); open circle, dotted line, AA(TG_2T); solid circle, AT(TC_2T); open triangle, dotted line, AA(TC_2T); open square, AT(G_4); solid square, AA(G_4); open circle, solid line, AT(TG_2T); solid triangle, solid line, AA(C_4); solid diamond, AA(TA_2T);×, AT(TA_2T). The lines are drawn to guide the eye.

In Fig. 4, we compare the effect of different nucleation stacks for the same loop sequences. The largest differences in the transition volumes, $\Delta\Delta V$, are observed for the hairpins with C_4 and G_4 loops. The differences become larger with higher salt concentration.

Fig. 5a and b illustrates the difference in transition volumes between the different homogenous and heterogeneous loop hairpins, respectively. In this case, assuming we consider the same nucleating base pairs, we can isolate changes resulting from loop sequences and, hence, see the effect of loop sequences on volumetric properties. Since the stem and the nucleation stacks are identical for the samples summarized in Fig. 5a and b, we assume that the changes measured arise due to the differences in the behavior of the loops. The data presented in Figs. 4, 5a, and b are also collected in Tables S6–S8.

4. Discussion

In this project, we have studied the effect of hydrostatic pressure on the hairpin-coil transition temperature (T_M) for eleven 16-base DNA hairpins at different salt concentrations by performing UV-monitored melting. The hairpins we studied have a six-base pair duplex-forming stem linked by a four-base loop. The molecules investigated in this study were chosen because of the extensive thermodynamic studies carried out on them by Vallone et al. [11]. They studied the ODNs listed in Table 1 (and several other ODNs)

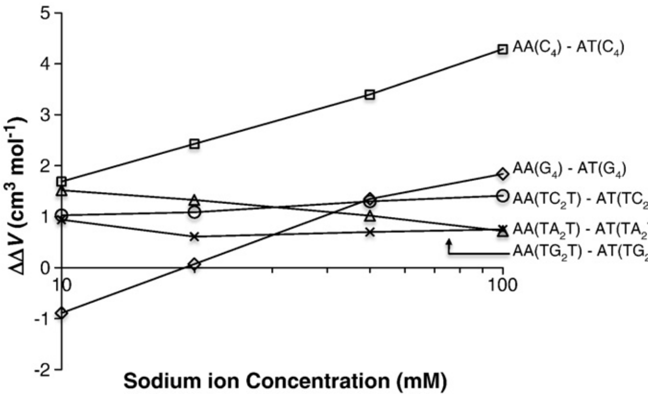

Fig. 4. The change in the molar volume change as a function of sodium ion concentration for the same loop sequences, varying the sequence of the nucleation bases. Squares, ΔV[AA(C_4)]–ΔV[AT(C_4)]; diamonds, ΔV[AA(G_4)]–ΔV[AT(G_4)]; circles ΔV[AA(TC_2T)]–ΔV[ATTC_2T)]; triangles, ΔV[AA(TA_2T)]–ΔV[AT(TA_2T)]; ×, ΔV[AA(TG_2T)]–ΔV[AT(TG_2T)]. The lines are drawn to guide the eye.

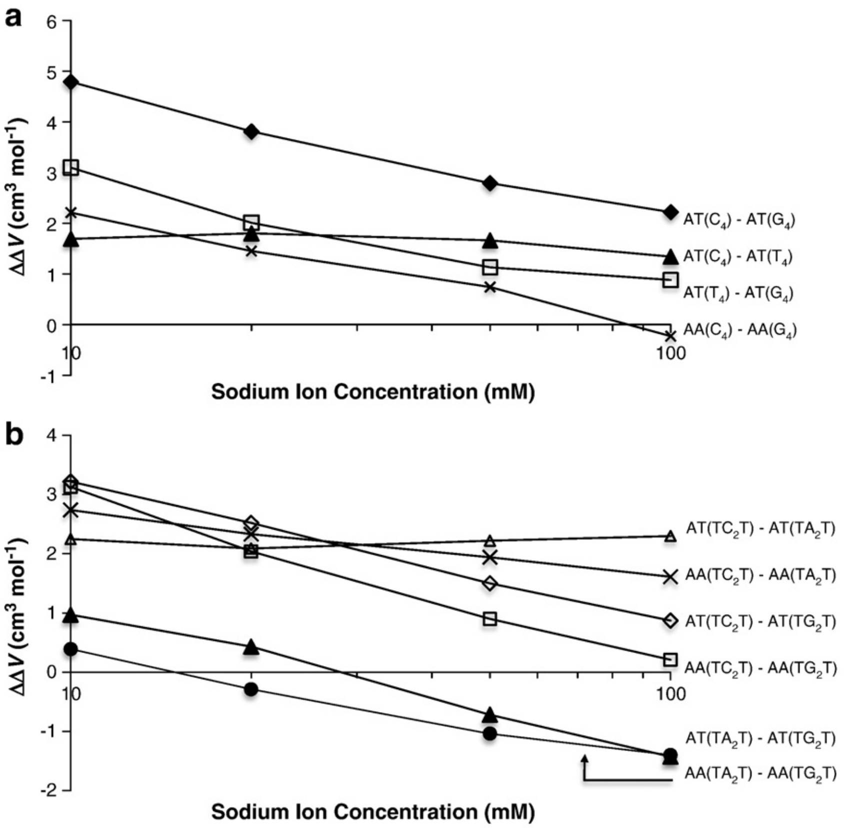

Fig. 5. a. The difference in transition volumes (ΔV) between the homogenous-loop hairpins in which the sequence of the nucleation stack is constant but the sequence of the loop is varied. Diamonds, ΔV[AT(C_4)]–ΔV[AT(G_4)]; triangles ΔV[AT(C_4)]–ΔV[AT(T_4)]; open squares, ΔV[AT(T_4)]–ΔV[AT(G_4)]; ×, ΔV[AA(C_4)]–ΔV[AA(G_4)]. The lines are drawn to guide the eye. b. The difference in transition volumes (ΔV) between the heterogynous-loop hairpins in which the sequence of the nucleation stack is constant but the sequence of the loop is varied. Open triangle, ΔV[AT(TC_2T)]–ΔV[AT(TA_2T)]; ×, ΔV[AA(TC_2T)]–ΔV[AA(TA_2T)]; diamond, ΔV[AT(TC_2T)]–ΔV[AT(TG_2T)]; square, ΔV[AA(TC_2T)]–ΔV[AA(TG_2T)]; solid triangle, ΔV[AT(TA_2T)]–ΔV[AT(TG_2T)]; circle, ΔV[AA(TA_2T)]–ΔV[AA(TG_2T)]. The lines are drawn to guide the eye.

using calorimetry and UV melting experiments and found that for 10 of the ODNs analysis of the calorimetry and UV melting data yielded the same thermodynamic values. This finding is consistent with the hairpin-coil transition of these ODNs being a single-step process. On the basis of their results we felt confident in our assumption of a single-step transition in the analysis of our results. However, Vallone et al. found that one of the ODNs, AA(A_4), exhibited experimentally significantly different values of the derived thermodynamic and thus its transition may not a single-step process. The lack of agreement between the parameters measured by the two methods may be due to the much higher concentration required for calorimetry, which could potentially lead to aggregation of the ODN, which in turn could contribute to the measured parameters. Nevertheless, we included this hairpin to assess what the effect would be on the values we measure. In general, we found no obvious effect; this ODN behaves like the ten others we investigated. The putative existence of other states in the mechanism of the transition from a hairpin to the coil form may change the values of the volume and expansibility change we report; however, we cannot assess the magnitude or direction of these changes at this time.

Thus, assuming a single-step transition, we have used the pressure dependence of the hairpin-coil transition temperature to determine the transition volume and expansivity of this process for the 11 ODNs listed in Table 1. The change in T_M as a function of pressure was linear under our conditions so that we were unable to assess the compressibility of the transition. These measurements extend the thermodynamic characterization of these short DNA hairpins [11].

Changes in pressure lead to changes in volume. At the pressures employed in these experiments, the molecules and ions constituting the system are essentially incompressible and behave as rigid spheres, i.e. bond lengths and bond angles remain constant. In accordance with Le Chatelier's principle, increasing pressure will shift the equilibrium toward the state with the smallest molar volume. In the absence of measurable compressibility,[2] pressure-induced changes in the equilibrium constantmay be attributed to differences in the extent of interaction between the solvent and solutes. In other words, the system responds by reducing the free volume and by favoring interactions between the solvent and the solute that minimize the volume. The molar volume change (ΔV) for the hairpin-coil transition equals the difference between the partial molar volume of the denatured or coil form and the partial molar volume of the hairpin form. The data in Tables 2 and 3 show that the volume changes for the hairpin-coil transition can be either positive or negative depending on the sodium ion concentration. Since the four base pairs distal from the loop are the same for all of these hairpins (Table 1), we assume that the difference in the behavior of these hairpins is due to the properties of the nucleating sequence or the bases in the loop. It is likely that these two structural elements interact with each other; however, the number of hairpins investigated is insufficient for assessing the importance of these interactions and our analysis treats them as independent domains.

The volume change that results from formation of a complex, ΔV, can be decomposed into a sum of three components [34–39]:

$$\Delta V = \Delta V_T + \Delta V_1 + \Delta V_M. \tag{5}$$

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[2] Compressibility changes are below the level of detection of our system; our data (dT_M/dP) are linear within accuracy and range of our experiments.

Where ΔV_T is the thermal volume change, ΔV_I is the hydration (interaction) volume change and, ΔV_M is the intrinsic volume change of the solute. The thermal volume, V_T, is the volume of the void space surrounding the solvent accessible surface of the solute molecules; it arises from the thermal motion of the solute and solvent molecules and is proportional to the solvent accessible surface area (S_A) of the solute [38]. The hydration volume change, ΔV_I, is the volume change generated from exchange between relatively high-density water in the hydration shell of solutes and lower density bulk water. The intrinsic volume, V_M, is the geometric volume of the solute molecules; the change in ΔV_M is negligible for DNA due to the absence of internal voids [37,39]. Thus, Eq. 4 simplifies to:

$$\Delta V = \Delta V_T + \Delta V_1. \tag{6}$$

Each term in Eq. 6 can be rewritten as the difference between the volume of the hairpin and coil state yielding:

$$\Delta V = (\Delta V_C - \Delta V_H) = (V_{TC} - V_{TH}) + (V_{IC} - V_{IH}) \tag{7}$$

where the subscripts C and H refer to the coil and hairpin states, respectively.

The thermal volume change, ΔV_T, is positive for the helix-coil transition since the coil form has a larger solvent accessible surface area than the hairpin form; hence $V_{TC} - V_{TH} > 0$. Thus, ΔV_I is the parameter that changes sign. At low sodium ion concentrations ΔV could be positive or negative, depending on the sequence of the hairpin. For the ODNs that exhibit a salt-dependent change in the sign of the ΔV, the value of ΔV_I is negative at low salt concentrations, that is, $V_{IC} - V_{IH} < 0$. This suggests that at low sodium ion concentrations the interactions with water are stronger for the coil form than for the helix form. The ODNs for which this is true are: AT(G_4), AA(G_4), AT(TA_2T), AA(TA_2T), AT(TG_2T), and AA(TG_2T). For each of these ODNs however, the transition volume changes sign and becomes positive at higher salt concentration. If our line of reasoning is correct then the salt-dependent change of sign in the transition volume means that they undergo a salt-dependent change in their interaction with water. The consequence of this change in water interaction is that as ΔV_I becomes more positive either V_{IH} decreases or V_{IC} increases, or both. Unfortunately, the present data do not allow us to distinguish between these possibilities.

A second group of ODNs has a positive molar volume change at all salt concentrations we employed. In this case the sign of ΔV_I could be positive or negative; however, its magnitude would be limited if it was negative ($\Delta V = \Delta V_I + \Delta V_T > 0$). Following the same reasoning as above, we assume that as the sodium ion concentration increases ΔV_I becomes increasingly positive. However, in the absence of knowledge of the magnitude of the thermal volume change, we cannot assess whether ΔV_I changes sign or remains positive throughout this range of salt concentration. As the value of ΔV_I becomes increasingly positive, one can infer that the interactions of water with the hairpin conformation become increasingly favorable, i.e. V_{IH} becomes smaller relative to V_{IC}. The hairpins formed by ODNs for which the transition volume is positive at all temperatures are: AT(T_4), AT(C_4), AA(C_4), AT(TC_2T), and AA(TC_2T). Again, because the four base pairs distal from the loop are the same for all of the ODNs, we propose that this sign and magnitude of ΔV_I depend on the properties of the loop or the nucleation bases or both.

There does not appear to be any single feature of the ODNs that accounts for the behavior we have observed for either group. However, in the set of ODNs we have examined, only hairpins with purines in the loop showed a transition from negative to positive volume changes with increasing salt concentrations. The generality of this observation and its rationalization must await additional experimentation although, as discussed below, it could be due to specific interactions between the loop and cations.

An additional process that occurs as a consequence of the transition is the release of cations that interact with the hairpin due to polyelectrolyte effects. The results of Tikhomirova and Chalikian have shown that it is unlikely that the change in the location of these cations from the molecular environment of the hairpin to bulk water will be accompanied by a significant change in volume [40]. We should point out, however, that their results were for polymers and did not take into account the possibility of specific interactions between DNA and an ion.

The molar volume change of the transition becomes more positive with temperature for all samples; therefore the expansibilities, ΔE, are positive (Table 4). In general, the positive change in the expansibility accompanying strand separation is due to electrostatic and hydrophobic contributions. One would anticipate that higher temperature would cause the types of interactions grouped together in the ΔV_I term, such as hydrogen bonding and electrostriction, to become less favorable and cause this term to become more positive. With increasing temperature the bases will become more disordered, less stacked, in the coil state leading to an increase in the solvent accessible surfaces area; this will result ΔV_T ($= V_{TC} - V_{TH}$) becoming more positive. While both the hydration and thermal components exhibit similar temperature dependencies, we cannot predict which one predominates, nor can we say assess the influence the sequence of the ODN on these values given the small data set.

The transition volumes also increase linearly with the log [Na^+] (see for example Figs. S3 and S4), which is expected as the transition temperature is dependent on the log [Na^+]. The average number of Na^+ counter-ions released, Δn, for the hairpins we have studied is 0.437 ± 0.143 mol Na^+/mol at atmospheric pressure. The magnitudes of the values of Δn for these hairpins samples are greater by a factor of ~3 compared to DNA polymers [32,33]. This significant difference may be due to the high charge density of the hairpin structure relative to polymers since, presumably, the charge density of the unfolded states would be very similar regardless of the initial folded structure.

From our data it appears that the sequence of the nucleation bases has a role in determining the volumetric properties (Fig. 4). The salt dependence of the slope of the differential volumes for ΔV[AA(C_4)]–ΔV[AT(C_4)], and ΔV[AA(G_4)]–ΔV[AT(G_4)] is twice that of the other samples. Despite the fact that we separated the loop and the nucleation base effects in our analysis, these values of $\Delta\Delta V$ suggest the existence of an interaction between the effects attributable to the loops and the nucleation bases. The transition volumes of the polymers poly[d(A-T)] and poly(dA)·poly(dT) differ by approximately 2 $cm^3\ mol^{-1}$ at these salt concentrations (both are positive and the homopolymer has the larger transition volume) [32]; a value somewhat smaller than what we observed for the hairpins. A nearest-neighbor analysis of the transition volumes of double-stranded ODNs [41] found the difference between the transition volumes of AA TT and AT TA dinucleotide steps in 25 mM sodium chloride to be negligible. Thus, neither the behavior of polymers nor the results of the nearest-neighbor analysis are consistent with the transition volumes of the hairpin-forming ODNs we have examined.

Vallone and Benight have proposed that hydrophobic interactions of bases within the loop, and the exclusion of water from tight loops, may be a significant factor in the stability of hairpins with small loops, i.e. four of five bases [42]. They proposed that the loop immobilizes a water molecule due to a larger exposure of bases to solvent [43]. Bevilacqua and colleagues further suggest that a network of hydrogen bonds in the loop region, in addition to interactions between the loop and nucleating base pairs contribute significantly to the stability of hairpins [44–46]. It is worth mentioning that loop formation is unfavorable due to backbone charge–charge repulsions arising from the phosphate groups. The contribution of these Coulombic repulsions can be modulated by the ions in solution. Introduction of salt (Na^+ in this case) can increase the loop stability by neutralizing the phosphate charges, consequently causing the loop formation to be less unfavorable (i.e. the increase in salt concentration leads to the decrease of the free energy cost for loop formation). The results of Tan [47] and Kuznetsov [48] corroborate this idea; these authors showed that the ion-dependence of loop free energy plays an important role in the overall salt-dependence of hairpin stability. Furthermore, in their work, Kuznetsov et al. suggest that sodium cations interact specifically with loops, and stabilize them. Specific interactions with cations would favor loop formation; a higher sodium ion concentration would reduce repulsive forces discussed above due to stronger charge neutralization and thus improves loop stability. We suspect this is the main reason for the larger expansibility values of these hairpin samples compared to those of double stranded nucleic acid polymers [32,33,49].

5. Conclusions

We report the effect of pressure on the thermal stability of 11 different 16-base DNA hairpins and have observed both negative and positive transition volumes. In our study we have varied the composition and sequence of the four-base loops and the sequence of the two base pairs adjacent to the loop (the nucleation sequence). For this first study of the volumetric properties of short DNA hairpins, the ODNs were chosen specifically because of the extensive thermodynamic characterization that had been carried out by previous authors. The transition volumes of all 11 samples change linearly with temperature (salt concentration) and from these data we calculated the expansibility of the transition, which was positive in all cases. The molar volume change of the hairpin-coil transition is negative and positive, depending on the sample; however, at 100 mM NaCl the values are all positive. The expansibilities for the hairpins we studied are much greater than those of the double-stranded nucleic acids. Both the loop and the nucleation sequence appear to play important roles in the volumetric parameters and additional data. Our data show that the effects depend on the sequence of the hairpin; studies of a broader representative sample of hairpins would allow for a more quantitative analysis of the role of the different structural and environmental factors in the volumetric properties of this nucleic acid conformation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.bpc.2011.02.001.

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