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Pressure-induced helix–coil transition of DNA copolymers is linked to water activity

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ABSTRACT

We have investigated the effect of reduced water activity on the pressure-stability of double-stranded DNA polymers, poly[d(A-T)] and poly[d(I-C)]. Water activity was modulated by the addition of ethylene glycol and glycerol. The ionic strength of the medium was such that pressure had a destabilising effect on the polymers in the absence of cosolvents. The molar volume change of the heat-induced helix to coil transition (ΔV_T) becomes more positive as the activity of water was reduced, suggesting that the pressure-induced denaturation of DNA polymers would not occur at very low water activity. This would imply that water plays a crucial role in the pressure denaturation of DNA, much like that in pressure denaturation of proteins where the driving force of the process is the penetration of water molecules into the protein core [Hummer *et al.*, *Proc Natl Acad Sci USA* 1998, 95, 1552–1555].

1. Introduction

The first reported study of the effect of hydrostatic pressure on the stability of a biopolymer was published by Bridgman in 1914, who demonstrated that an increase in hydrostatic pressure resulted in an irreversible aggregation of ovalbumin [1]. Another 50 years passed before the first study exploring the effects of high pressure on the conformational stability of the DNA helical structure was published [2]. This work, conducted by Hedén, Lindahl, and Toplin, showed that pressure as high as 10,000 atm (0.9678 atm = 1 bar = 0.1 MPa) did not lead to the denaturation of *Bacillus subtilis* DNA under isothermal conditions. In addition, these authors showed that elevated hydrostatic pressure resulted in the stabilization of this polymer against temperature-induced denaturation, that is, the helix–coil transition temperature (T_M) of DNA increases as a function of pressure and the molar volume change of the transition (ΔV) is positive [2]. Subsequent studies reported similar results showing that pressure stabilizes double-stranded DNA helices. Those results are summarized in reviews by Macgregor [3] (Tables I and III particularly), Barciszewski *et al.* [4], Macgregor [5], and Chalikian and Macgregor [6]. Two notable exceptions to the general result that $\Delta T_M / \Delta P > 0$ were reported in studies published by Hughes and Steiner in 1966 [7], and Gunter and Gunter in 1972 [8]. Working with a synthetic double-stranded RNA polymer, poly[r(A)]·poly[r(U)], these groups observed that under certain conditions pressure destabilized the double helix, that is the ΔV of the helix–coil transition is negative. A negative ΔV implies that the helix–coil transition could be induced by pressure under isothermal conditions. The first pressure-induced helix–coil transition of a double-stranded nucleic acid polymer, the hybrid poly(dA)·poly(U), was reported by Dubins *et al.* [9]. The measurements were motivated by the authors' pressure–temperature phase diagram for the helix–coil transition of double-stranded nucleic acid polymers. According to this phase diagram, pressure can stabilize, destabilize, or have no effect on double-stranded nucleic acids; the sign of the volume change is dependent on the T_M of the polymer. Rayan and Macgregor presented the first pressure-induced denaturation of double-stranded DNA polymers, poly[d(A-T)] and poly[d(I-C)] [10]. The authors calculated the cooperative length of the pressure-induced transition (N_P) and found it to be strongly dependent on temperature. At temperatures nearing the value of T_M at atmospheric pressure, the magnitude of N_P is roughly twice that observed for the heat-induced transition (N_T). The difference between the two values was ascribed to differences in the mechanism between the pressure- and heat-induced helix–coil transitions [10].

In light of these results, we undertook this study to further elucidate the mechanism of the pressure-induced helix–coil transition of DNA, and especially to examine the role of water in this process. We have found that the pressure-induced transition of double-stranded DNA polymers becomes increasingly unfavourable as the activity of water decreases, a behaviour that is similar to that observed for proteins. This suggests that pressure denaturation involves the incorporation of water molecules between the DNA base pairs.

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2. Materials and methods

Poly[d(A-T)], poly[d(I-C)], and poly[d(G-C)] were purchased from Amersham/GE Healthcare (Piscataway, NJ). Sodium cacodylate trihydrate, ethylene glycol, and glycerol were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). Na_2EDTA was obtained from Bio-Rad laboratories (Hercules, CA). The polymers, poly[d(A-T)] and poly[d(I-C)] were dialyzed against three changes of 5 mM sodium cacodylate and 0.1 mM Na_2EDTA, pH 6.7, while poly[d(G-C)] was dialyzed in 1 mM sodium cacodylate and 0.1 mM Na_2EDTA, pH 6.7. Dialysis was carried out at 4 °C. The dialysis tubing (molecular weight cut-off of 1000 Da) was acquired from Genotech (St. Louis, MO). DNA concentrations were determined spectrophotometrically using the following extinction coefficients (per base pair) $\varepsilon_{262} = 13{,}300\ M^{-1}\ cm^{-1}$ for poly[d(A-T)] [11], $\varepsilon_{251} = 13{,}800\ M^{-1}\ cm^{-1}$ for poly[d(I-C)] [12], and $\varepsilon_{254} = 16{,}800\ M^{-1}\ cm^{-1}$ for poly[d(G-C)] [13]. The experimental polymer concentrations were approximately 70 µM (base pair). The cosolvents were dissolved in the same buffer as the DNA polymers. Pressure-dependent thermal-denaturation experiments were performed in triplicate employing the temperature-regulated isohyperbaric spectrophotometer, which has been described previously [10,14]. In all experiments the heating rate was 0.1 °C/min; the UV absorption was monitored at 260 nm for poly[d(A-T)], 251 nm for poly[d(I-C)], and 254 nm for poly[d(G-C)]. Pressure denaturation experiments were performed in the same instrument employing a pressurization rate of 1.0 MPa/min.

The fraction of DNA polymers in the coil form at a given temperature, $\theta(T)$, was calculated using the following equation;

$$\theta(T) = \frac{OD(T) - L(T)}{H(T) - L(T)} \tag{1}$$

where OD(T) is the optical density at temperature T, $H(T)$ is the equation for the line describing the temperature dependence of the optical density at temperatures above the helix–coil transition, and $L(T)$ is the equation for the line describing the temperature dependence of the optical density at temperatures below the helix–coil transition. The polymer is assumed to be in double-stranded (helix) form if $\theta = 0$, while at $\theta = 1$ the polymer is assumed to be entirely in the single stranded (coil) form. The helix–coil transition temperature (T_M) is defined as the temperature at which $\theta = 0.5$. The T_M values at various cosolvent concentrations are listed in Table 1.

An analogous procedure was carried out to determine the helix–coil transition pressure, P_M. The original data are transformed into the extent of transition at a given pressure $\theta(P)$, thus,

$$\theta(P) = \frac{OD(P) - L(P)}{H(P) - L(P)} \tag{2}$$

where OD(P) is the optical density at pressure P, $H(P)$ is the baseline observed at pressures greater than the helix–coil transition, and $L(P)$ is the baseline observed at pressures less than the helix–coil transition. The polymer is assumed to be in the helix form when $\theta = 0$, while at $\theta = 1$ the polymer is assumed to be in the coil form. The helix–coil transition pressure, P_M, is defined as the pressure at which $\theta = 0.5$.

The model-independent enthalpy change of the helix to coil transition was calculated using the following equation:

$$\Delta H_{Tm} = \Delta H_R + \Delta C_P(T_m - T_R) \tag{3}$$

where T_R is the reference temperature of 25 °C, ΔH_R is the calorimetrically determined enthalpy change at the reference temperature (25 °C) in the absence of cosolvents, and ΔC_P is the calorimetrically determined, temperature-independent heat capacity change. The ΔH_R values were taken from Chalikian *et al.* [15]. In the calculation of ΔH_{Tm} the following average ΔC_P values were used [16]: $213 \pm 66\ J\ mol^{-1}\ K^{-1}$ for poly[d(A-T)], $293 \pm 58\ J\ mol^{-1}\ K^{-1}$ for poly[d(I-C)], and $268 \pm 33\ J\ mol^{-1}\ K^{-1}$ for poly[d(G-C)]. We would like to reiterate that the enthalpy change values used in the calculation of the volume change are estimates and were not measured calorimetrically. The data in Table 1 show that the enthalpy change of the transition decreases with increasing cosolvent concentration and are qualitatively in good agreement with a number of calorimetric studies on the helix–coil transition of DNA polymers in the presence of cosolvents. Del Vecchio *et al.* have shown that the enthalpy change of the calf-thymus DNA melting in the presence of glycerol (and other cosolvents) decreases with an increase in cosolvent concentration [17]; Spink *et al.* have illustrated a decrease in the enthalpy change of the helix–coil transition of poly[d(A-T)] and poly[d(G-C)] in the presence of glycerol, ethylene glycol, and other cosolvents [18].

Table 1

Volume change of the helix–coil transition (ΔV) of poly[d(A-T)] and poly[d(I-C)] as a function of cosolvent concentration.

DNA	Cosolvent	T_M (°C)	$100 \times \Delta T_M/\Delta P$ (°C/MPa)	ΔH_{Tm} (kJ/mol)* (b.p.)	ΔV_T (cm^3/mol) (b.p.)
poly [d(A-T)]	None	35.1 ± 0.1	-1.48 ± 0.06	29.5	-1.42 ± 0.06
	5% EG**	32.6 ± 0.0	-1.51 ± 0.04	28.8	-1.42 ± 0.04
	10% EG	32.0 ± 0.1	-1.23 ± 0.07	28.7	-1.15 ± 0.06
	20% EG	28.9 ± 0.1	-0.60 ± 0.02	27.8	-0.55 ± 0.00
	40% EG	20.6 ± 0.1	0.91 ± 0.02	25.6	0.79 ± 0.02
	5% glycerol	33.2 ± 0.1	-1.44 ± 0.14	29.0	-1.37 ± 0.06
	10% glycerol	28.7 ± 0.1	-1.44 ± 0.06	28.4	-1.34 ± 0.06
	20% glycerol	27.0 ± 0.2	-1.12 ± 0.05	27.3	-1.02 ± 0.04
	40% glycerol	20.3 ± 0.0	0.34 ± 0.03	25.5	0.29 ± 0.03
poly [d(I-C)]	None	27.7 ± 0.1	-4.30 ± 0.08	31.9	-4.57 ± 0.09
	5% EG	26.7 ± 0.1	-3.91 ± 0.07	31.7	-4.14 ± 0.07
	10% EG	25.6 ± 0.0	-3.76 ± 0.01	31.4	-3.96 ± 0.01
	20% EG	23.2 ± 0.1	-3.19 ± 0.04	30.9	-3.32 ± 0.04
	40% EG	15.8 ± 0.1	-1.76 ± 0.08	29.1	-1.77 ± 0.08
	50% EG	11.0 ± 0.1	-1.07 ± 0.02	28.0	-1.05 ± 0.02
	5% glycerol	25.8 ± 0.1	-4.35 ± 0.02	31.5	-4.58 ± 0.02
	10% glycerol	23.9 ± 0.1	-4.41 ± 0.04	31.0	-4.60 ± 0.04
	20% glycerol	19.3 ± 0.0	-4.45 ± 0.04	29.9	-4.56 ± 0.05
	40% glycerol	13.8 ± 0.1	-0.02 ± 0.30	28.6	0.08 ± 0.09

*The errors in ΔH_{Tm} do not exceed 0.1 kJ/mol (b.p.).

**Ethylene glycol.

The molar volume change of the heat-induced helix–coil transition (ΔV_T) was calculated from the change in T_M as a function of pressure (Figs. 1 and 2) using the Clapeyron equation:

$$\frac{\Delta T_M}{\Delta P} = T_M \frac{\Delta V_T}{\Delta H_{Tm}} \tag{4}$$

where ΔH_{Tm} is the model-independent estimate enthalpy change of the helix–coil transition. The values of the volume change at various cosolvent concentrations are shown in Table 1. Use of the Claperyon equation assumes that the DNA helix–coil transition is a two-state process (Eqs. (1) and (2)) that behaves like a first-order phase transition. Consistent with this treatment is the observation that the helix–coil transition temperatures measured using model-dependent techniques agrees well with those determined using calorimetry (see references [3–6,8]).

The cosolvents ethylene glycol and glycerol were used solely for the purpose of modulating water activity and we have interpreted the results as such. These cosolvents were chosen because they have low molecular weights and would not introduce an excluded volume effect *per se*. However, we cannot unequivocally rule out preferential interactions between the cosolvent and the DNA; several calorimetric studies have demonstrated the enthalpy of the helix–coil transition of calf-thymus DNA [17], poly[d(A-T)], and poly[d(G-C)] [18] to be dependent on the nature of the cosolvent, a finding that would be consistent with preferential interactions between the biopolymer and the cosolvents. Additionally we cannot exclude preferential interactions between the cosolvents and the DNA as a function of increasing

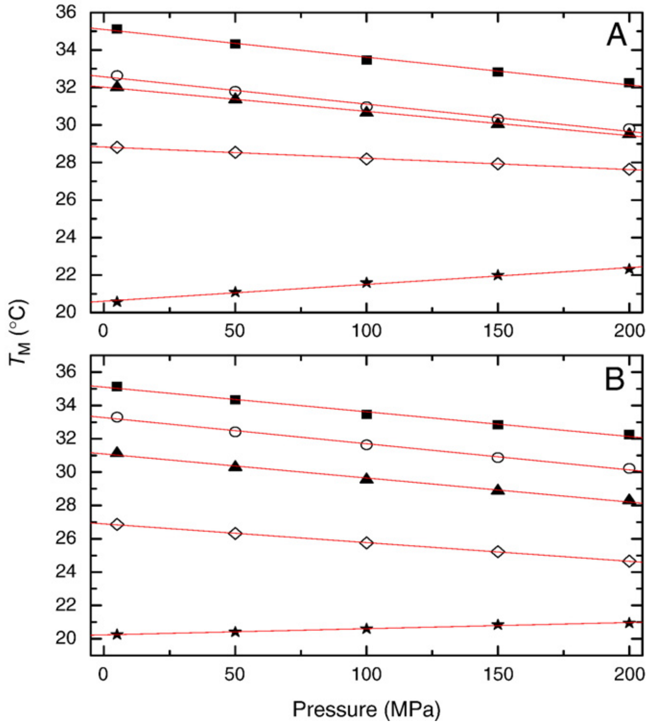

Fig. 1. Helix–coil transition temperature (T_M) of poly[d(A-T)] as a function of pressure at various cosolvent concentrations: (A) ethylene glycol 0% (■), 5% (○), 10% (▲), 20% (◊), and 40% (★); (B) glycerol 0% (■), 5% (○), 10% (▲), 20% (◊), and 40% (★). The solid lines are least-squares fits to the data.

hydrostatic pressure. Recently a high pressure differential scanning calorimeter has become available [19], and it would yield useful information on the interactions of cosolvents and biopolymers as a function of elevated hydrostatic pressure.

3. Results

Figs. 1 and 2 display representative plots of the helix–coil transition temperature (T_M) of the polymers poly[d(A-T)] and poly[d(I-C)] as a function of pressure and cosolvent concentration. The values of $\Delta T_M / \Delta P$ are given in Table 1.

In Figs. 3 and 4 we present the effect of increasing cosolvent concentration on the volume change of helix–coil transition of poly[d(A-T)] and poly[d(I-C)], respectively. While we cannot rule out potential interactions between the cosolvents and the DNA polymers, it is evident that the molar volume change of the transition becomes more positive with an increase in cosolvent concentration. Table 1 lists the ΔV_T values.

Fig. 5 is a plot of the pressure-induced helix–coil transition of poly[d(A-T)] at 31.9 °C: in solutions containing 0 and 5% glycerol. The lower baseline of the pressure-induced melting curve at 5% glycerol is absent, as the transition has already commenced at the starting experimental pressure.

Fig. 6 shows the change in ΔV_T as a function of the T_M of poly[d(G-C)]. The figure includes data (the open circles) reported by Wu and Macgregor (1995) [20]. The inset of Fig. 6 illustrates the pressure dependence of the T_M of poly[d(G-C)] in aqueous solution containing 1.2 mM Na^+.

4. Discussion

Previous studies have shown that the pressure-induced denaturation of proteins is strongly dependent on water activity [21,22]. The molar volume change of the pressure-induced denaturation becomes more positive with decreasing water activity in experiments where the change in water activity is brought about by increasing cosolvent concentrations. Consequently, pressure denaturation of proteins becomes less attainable as water activity decreases, suggesting that water is essential for this process. It has been suggested that the pressure-induced denaturation of proteins occurs as a consequence of

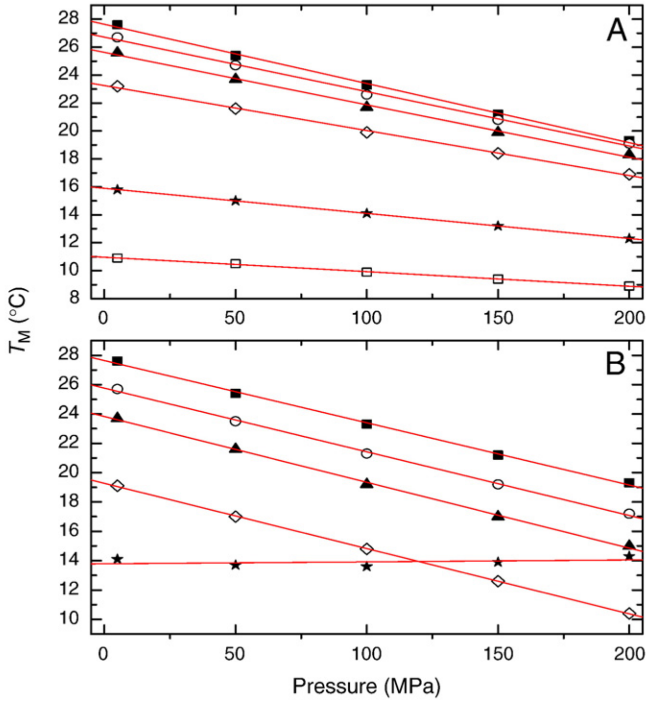

Fig. 2. Helix–coil transition temperature (T_M) of poly[d(I-C)] as a function of pressure at various cosolvent concentrations: (A) ethylene glycol 0% (■), 5% (○), 10% (▲), 20% (◊), 40% (★), and 50% (□); (B) glycerol 0% (■), 5% (○), 10% (▲), 20% (◊), and 40% (★). The solid lines are least-squares fits to the data.

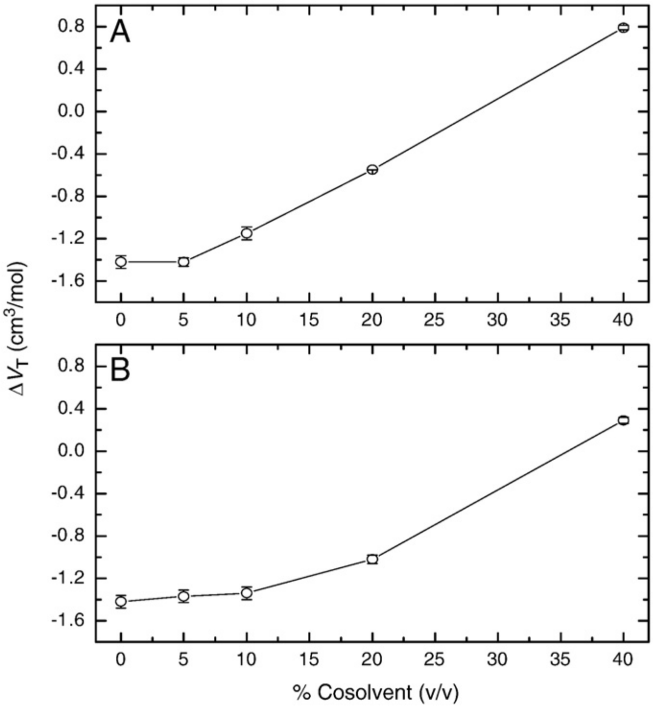

Fig. 3. Molar volume change of the heat-induced helix–coil transition (ΔV_T) of poly[d(A-T)] as a function of increasing concentration of (A) ethylene glycol, and (B) glycerol. The lines are only to guide the eye; the error bars indicate ±1 standard deviation.

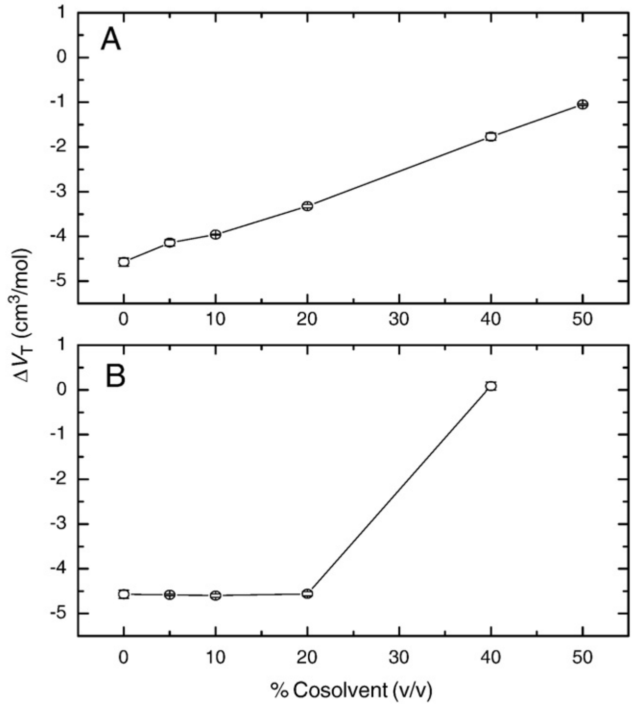

Fig. 4. Molar volume change of the heat-induced helix–coil transition (ΔV_T) of poly[d(I-C)] as a function of increasing concentration of (A) ethylene glycol, and (B) glycerol. The lines are only to guide the eye; the error bars indicate ±1 standard deviation.

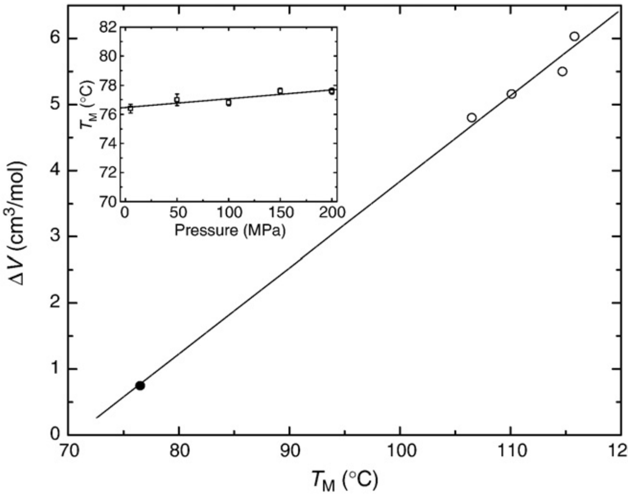

Fig. 6. Molar volume change of the heat-induced helix–coil transition (ΔV_T) of poly[d(G-C)] as a function of T_M for the combined results of the authors (black circle) and those of Wu and Macgregor (open circles) [16]. The line is a least-square fit of the data. The slope of the line is the expansivity change of the transition (ΔE) and it equals 0.13 ± 0.01 cm^3 K^{-1} mol^{-1}. Inset: helix–coil transition temperature (T_M) as a function of pressure of poly[d(G-C)] in an aqueous solution containing 1.2 mM Na$^+$. The line is a least-square fit to the data.

the penetration of water molecules into the protein core [23]; a hypothesis that was collaborated by a theoretical study illustrating that water penetration into the protein is responsible for the pressure-induced denaturation of ubiquitin [24]. Molecular dynamics (MD) calculations have added support to this idea by showing that changing the pressure from 3 to 300 MPa leads to the internalization of 1–5 additional water molecules [25]. It should be noted that ubiquitin is in the folded state at both pressures. A study of T4 lysozyme mutant L99A employing high pressure crystallography and MD showed that at elevated pressures under which the protein was not denatured resulted in the entry of about four water molecules into the hydrophobic cavity of the protein [26]. Another MD study illustrated that additional water molecules penetrated into the heme pocket of cytochrome c at elevated hydrostatic pressure [27].

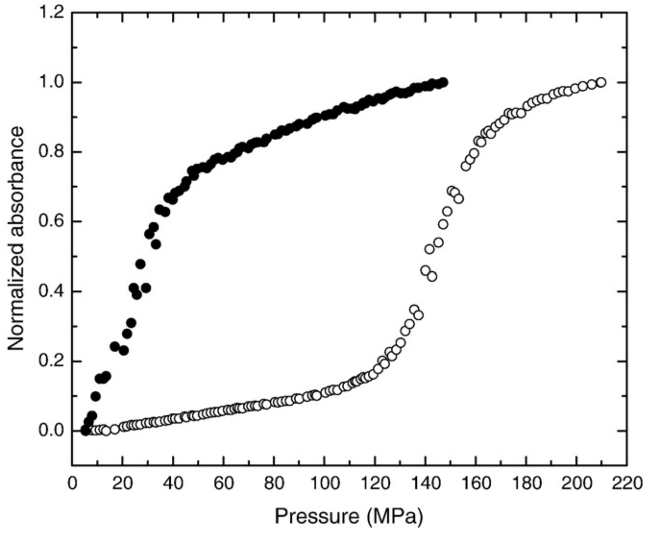

Fig. 5. Pressure-induced helix–coil transition curves of poly[d(A-T)] at 31.9 °C: 0% glycerol (○) and 5% glycerol (●). The helix–coil transition pressure (P_M) equals 142 MPa at 0% glycerol, and 20.7 MPa at 5% glycerol. The respective melting temperatures are 35.1 °C and 33.2 °C.

Thus, several groups have argued that pressure denaturation of proteins occurs due to (or is accompanied by) the penetration of water into the protein [23,24,28–30]. This mechanism of pressure-induced denaturation of proteins was proposed by Wu in 1931; “when a protein solution is subjected to high pressure, molecules of water are crushed into the protein molecule and cause denaturation. [28,31]” This work presented the first comprehensive review on protein denaturation. Employing statistical thermodynamics, Harano and Kinoshita have argued that pressure denaturation of proteins is primarily driven by the translational entropy of water, and that it cannot occur in solvents other than water due to greater size of other solvent molecules [32].

Figs. 1 and 2 illustrate the pressure dependence of T_M for poly[d(A-T)] and poly[d(I-C)] as a function of cosolvent concentration; the numerical values of $\Delta T_M/\Delta P$ are listed in Table 1. At higher cosolvent concentrations, *i.e.* under conditions of reduced water activity, the value of $\Delta T_M/\Delta P$ becomes less negative, hence showing that as the water activity is reduced the destabilization of these polymers by pressure is also attenuated.

The effect of reduced water activity/increased cosolvent concentrations on the volume change (ΔV_T) of the helix–coil transition is shown in Figs. 3 and 4. Evidently the ΔV_T of the helix–coil transition is less negative at lower water activity implying that the pressure-induced denaturation of DNA is less attainable as the activity of water is reduced. The data from this study agree with those obtained by Oliviera *et al.*, who showed that the pressure-induced denaturation of the Arc repressor (a DNA binding protein) will not occur in the absence of water [21]. Consequently, the role of water in the pressure-induced denaturation of DNA appears to be similar to that in the pressure denaturation of proteins.

Examination of Figs. 3 and 4, as well as Table 1 shows that low cosolvent concentrations generally do not affect the ΔV_T of the helix–coil transition and consequently do not influence the effect of pressure on the stability of DNA polymers. Fig. 5 illustrates the pressure-induced DNA helix–coil curves of poly[d(A-T)] at 31.9 °C and at 0% and 5% glycerol. The respective melting temperatures at atmospheric pressure correspond to 35.1 °C and 33.2 °C. The helix–coil transition pressure (P_M) is 142 MPa at 0% glycerol, and 21 MPa at 5% glycerol. Thus, at low cosolvent concentrations, the midpoint of the pressure-induced transition (P_M) depends on T_M at atmospheric pressure.

Chen and Prohofsky argued that that the penetration of water molecules occurs at elevated hydrostatic pressures and that pressure destabilization of DNA can occur under conditions below a critical salt concentration [33]. Several experimental results are in agreement with their suggestion. A study of the double-stranded DNA oligonucleotide d(GGTATACC) using high pressure crystallography demonstrated that although it is not pressure denatured, the oligonucleotide is more hydrated at elevated pressures [34]. Increased hydration of DNA at elevated pressures was also reported by a high pressure FTIR study of poly(dA)·poly(dT) and the composite monomers, poly(dA) and poly(dT) [35]. Rayan and Macgregor have shown that the ΔV_T of the heat-induced helix–coil transition of two DNA copolymers changes from negative to positive as the sodium ion concentration is increased [10], thus higher salt concentration inhibit pressure denaturation of DNA, which is what was advocated by Chen and Prohofsky [33]. This effect is clearly not due to lower water activity; the molar volume change equals zero at ~18 mM NaCl and ~105 mM NaCl, for poly[d(A-T)] and poly[d(I-C)], respectively [10]. These salt concentrations do not significantly affect water activity.

To further examine the effect of salt on the pressure-stability of DNA polymers, we have looked at the stability of poly[d(G-C)] at a low ionic strength of 1.2 mM Na^+. Under these conditions the polymer undergoes a cooperative helix–coil transition at 76.5±0.2 °C at atmospheric pressure. Fig. 6 shows the effect of T_M on the ΔV_T of the helix–coil transition of poly[d(G-C)]; the inset of Fig. 6 is a plot of the pressure dependence of T_M of poly[d(G-C)]. The data shown in Fig. 6 are the combined results of this study and those of Wu and Macgregor [20]. From the data of the Fig. 6 inset we calculated $100\times\Delta T_M/\Delta P = 0.61\pm 0.16$ °C/MPa, and $\Delta V_T = 0.75\pm0.2$ cm^3/mol at 1.2 mM Na^+. This polymer cannot undergo a pressure-induced denaturation under these experimental conditions. From the slope of the plot of ΔV_T vs. T_M (Fig. 6) one obtains an expansivity change of the transition (ΔE), equal to 0.13 ± 0.1 cm^3 $K^{-1}mol^{-1}$. This value is very similar to values we have measured for poly[d(I-C)] and poly[d(A-T)], which are 0.17 ± 0.1 and 0.16 ± 0.1 cm^3 K^{-1} mol^{-1}, respectively [10], and the value of 0.14 ± 0.0 cm^3 K^{-1} mol^{-1} for the helix–coil transition of calf-thymus DNA obtained by Nordmeier [36]. A positive value of ΔE is consistent with the coil state being more hydrated than the helix state. Volumetric studies carried out by Chalikian *et al*. have also reported positive values of ΔE [15]. A recently published pressure-perturbation calorimetric (PPC) study on the helix–coil transition of poly[d(A-T)] also reported a positive ΔE [37].

In conclusion, we have shown that the ΔV of the helix–coil transition of poly[d(A-T)] and poly[d(I-C)] became less negative as the activity of water was decreased, illustrating that water plays an important role in the pressure-induced denaturation of DNA, and that pressure denaturation of DNA polymers would not occur in the absence of water.

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