A look at the effect of sequence complexity on pressure destabilisation of DNA polymers

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HIGHLIGHTS

- Under certain conditions synthetic nucleic polymers can be denatured with pressure.
- We show that the change of T_M of *Clostridium perfringes* DNA with pressure is negative.
- We were unable to induce a helix–coil transition *C. perfringes* DNA with pressure.
- The sequence complexity of this genomic DNA underlies its insensitivity to pressure.

GRAPHICAL ABSTRACT
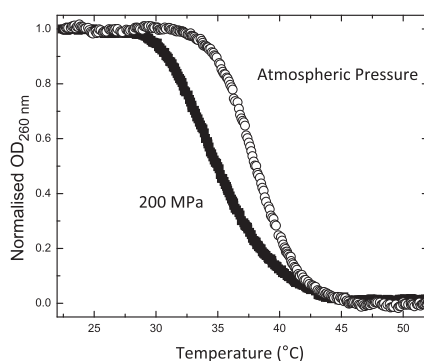
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ABSTRACT

Our previous studies on the helix–coil transition of double-stranded DNA polymers have demonstrated that molar volume change (ΔV) accompanying the thermally-induced transition can be positive or negative depending on the experimental conditions, that the pressure-induced transition is more cooperative than the heat-induced transition [Rayan and Macgregor, *J Phys Chem B* **2005**, 109, 15558–15565], and that the pressure-induced transition does not occur in the absence of water [Rayan and Macgregor, *Biophys Chem*, **2009**, 144, 62–66]. Additionally, we have shown that ΔV values obtained by pressure-dependent techniques differ from those obtained by ambient pressure techniques such as PPC [Rayan et al. *J Phys Chem B* **2009**, 113, 1738–1742] thus shedding light on the effects of pressure on DNA polymers. Herein, we examine the effect of sequence complexity, and hence cooperativity on pressure destabilisation of DNA polymers. Working with *Clostridium perfringes* DNA under conditions such that the estimated ΔV of the helix–coil transition corresponds to −1.78 mL/mol (base pair) at atmospheric pressure, we do not observe the pressure-induced helix–coil transition of this DNA polymer, whereas synthetic copolymers poly[d(A-T)] and poly[d(I-C)] undergo cooperative pressure-induced transitions at similar ΔV values. We hypothesise that the reason for the lack of pressure-induced helix–coil transition of *C. perfringens* DNA under these experimental conditions lies in its sequence complexity.

1. Introduction

While the main aim of utilising elevated hydrostatic pressure is to gain additional information on the stability of the system being probed at atmospheric conditions, the fact that certain organisms can withstand

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pressures as high as 100 MPa (1 MPa = 10 bar = 9.678 atm) in the Mariana Trench (the deepest depression in the Pacific Ocean) makes this method particularly appealing to understanding the adaptations of those organisms to elevated pressures at the molecular level. A pioneering study conducted by Hedén, Lindahl, and Toplin [1] 50 years ago ushered in the era of investigation of high pressure effects on the stability of nucleic acids. This study, as well as most of the studies that followed [2–9] reported that elevated hydrostatic pressure led to the stabilisation of the helical from of DNA against the heat-induced transition, as witnessed by an increase in the melting temperature (T$_M$) of nucleic acids. With the exception of two studies, which showed that the molar volume change (ΔV) of the helix–coil transition of poly[r(A)]·poly[r(U)] (a synthetic double-stranded RNA homopolymer) can also be negative [10,11], the consensus was that pressure stabilises the helical state of DNA (hence a positive ΔV of the helix–coil transition). The temperature–pressure phase diagram for the helix–coil transition of double-stranded nucleic acids indicates that polymers with T$_M$ values <~50 °C are destabilised by pressure (negative ΔV), while those polymers with T$_M$ values >~50 °C are stabilised by pressure (positive ΔV) [12]. Consequently, the authors were able to perform the first pressure-induced helix–coil transition of a double-stranded nucleic acid polymer [12]. Following reviews summarise the studies on the effects of high pressure on the stability of DNA and other biopolymers [13–17].

We have shown that ΔV of the helix–coil transition of DNA polymers can be positive or negative, depending on the conditions, and that the pressure-induced and heat-induced transitions of DNA polymers appear to occur via different mechanisms, as the latter is more cooperative than the former [18]. As a follow-up to this study, we demonstrated that the pressure-induced helix–coil transition of DNA copolymers would not occur in the absence of water [19], much like the pressure-induced denaturation of proteins [20]. Furthermore, we have illustrated that ΔV values for the helix–coil transition of DNA as obtained from pressure-dependent studies are different than those obtained by methods that operate at nearly atmospheric conditions (such as pressure-perturbation calorimetry) thus providing additional insights into the effects of pressure on DNA polymers [21].

We would like to emphasise that the ΔV values are estimations obtained using the Clapeyron equation, which is applicable to reversible processes. Heat-induced denaturation of genomic DNA is not a reversible process, and consequently any thermodynamic parameter (such as ΔV or ΔH$_{vH}$) obtained using reversible thermodynamics is approximate. The fact that the dT$_M$/dP values are negative implies that the corresponding ΔVs are negative as well, and that elevated hydrostatic pressure should denature these polymers, as is the case with poly[d(A-T)] and poly[d(I-C)] under similar experimental conditions [18]. Here we report the first experimentally obtained negative ΔV for the helix-to-coil transition of genomic DNA (*Clostridium perfringes*). We chose this particular genomic DNA due to its low G-C content (28%), which renders it less stable than those biopolymers with a higher G-C content and consequently more amenable to studies exploring the pressure behaviour of low/negative ΔV (or dT$_M$/dP) values. Under the conditions used in these experiments, the ΔV equals − 1.78 mL/mol (base pair), and the T$_M$ is 38 °C at atmospheric pressure. Based on the sign and magnitude of the molar volume change, it is not unreasonable to expect this polymer to undergo a cooperative pressure-induced helix–coil transition. Indeed, we reported that synthetic DNA copolymers can undergo a pressure-induced helix–coil transition under the conditions where the magnitude of the negative volume change was less than the value of − 1.78 mL/mol (base pair) obtained in this study [18]. We ascribe the inability of this genomic DNA to undergo the pressure-induced transition under these conditions to the greater sequence complexity of genomic DNA relative to that of the synthetic polymers poly[d(A-T)] and poly[d(I-C)]. We presume that this phenomenon arises because there are regions of the polymer that have sequences for which the dT$_M$/dP values are not negative, underscoring the role of the sequence complexity in the physical chemistry of DNA.

2. Materials and methods

2.1. Clostridium perfringes

DNA and sodium cacodylate trihydrate were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada), while Na$_2$EDTA was obtained from Bio-Rad Laboratories (Hercules, CA). The DNA solution was dialysed 3× at 4 °C against an aqueous solution containing 1 mM sodium cacodylate and 0.1 mM Na$_2$EDTA, pH 6.7. The dialysis tubing with a molecular weight cut-off of 1000 Da was acquired from Genotech, Inc. (St. Louis, MO). The experimental DNA concentration was approximately 70 μM (base pair).

The melting experiments were performed using the temperature-regulated iso-hyperbaric spectrophotometer (TRIHBS), which has been described previously [22]. The sample is loaded into a 300-μL quartz cuvette that is placed in the optical high-pressure cell, which is positioned in the path of the spectrophotometer (Uvikon model 860). The spectrophotometer, the pressure pump and the thermometer are connected to a computer, enabling us to control absorption, pressure, and temperature in real time. The samples were heated at a rate of 0.1 °C/min and the helix–coil transition was monitored at 260 nm. Because the heat-induced denaturation of genomic DNA is irreversible a fresh DNA sample was used for each experiment. The measurements were performed in triplicate at constant pressures ranging from 5 to 200 MPa.

The observed helix–coil transition curves were analysed using the following equation:

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

where θ(T) corresponds to the fraction of polymer in the coil form at a temperature T, OD(T) is the optical density at temperature T, L(T) is the equation of the line for the low-temperature baseline, and H(T) is the equation of the line for the high-temperature baseline. When θ = 0, the polymer is assumed to be in the native, double stranded state, while θ = 1 signifies that the polymer is in the denatured, single-stranded state. The helix–coil transition temperature (T$_M$) is the temperature at which θ = 0.5.

The model-dependent, van't Hoff enthalpy change (ΔH$_{vH}$) of the transition was calculated using the following equation and under the assumption that the helix–coil transition is a single-step, reversible, bimolecular transition: [8,23]

$$\Delta H_{vH} = 6RT_M{}^2 (d\theta/dT)_{T=T_M}$$

where R is the gas constant and dθ/dT is the slope of a θ versus T curve at T$_M$ [23]. As already mentioned the ΔH$_{vH}$ values we report here are apparent values because the heat-induced helix–coil transition of genomic DNA is not a reversible process.

The calorimetric enthalpy change (ΔH$_{cal}$) of the transition was taken from the following reference [24]. The ratio of the van't Hoff enthalpy change to the calorimetric enthalpy change was used to calculate the cooperative melting unit (N):

$$N = \frac{\Delta H_{vH}}{\Delta H_{cal}}$$

where N is the number of base pairs that melt as one.

Isothermal pressure-induced denaturation experiments were attempted at the pressurisation rate of 1.0 MPa/min, at several temperatures ranging from 30.8 to 38.1 °C by monitoring the absorption at 260 nm (data not shown).