On the Stability of Double Stranded Nucleic Acids

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Abstract: We present the first pressure-versus-temperature phase diagram for the helix-to-coil transition of double stranded nucleic acids. The thermodynamic stability of a nucleic acid duplex is a complex function of temperature and pressure and strongly depends on the denaturation temperature, T_M, of the duplex at atmospheric pressure. Depending upon T_M, pressure, and temperature, the phase diagram shows that pressure may stabilize, destabilize, or have no effect on the conformational state of DNA. To verify the phase diagram, we have conducted high-pressure UV melting experiments on poly(dIdC)poly(dIdC), a DNA duplex, poly(rA)poly(rU), an RNA duplex, and poly(dA)poly(rU), a DNA/RNA hybrid duplex. The T_M values of these duplexes have been modulated by altering the solution ionic strength. Significantly, at low salt, these three duplexes have helix-to-coil transition temperatures of 50 °C or less. In agreement with the derived phase diagram, we found that the polymeric duplexes were destabilized by pressure if the T_M is $< \sim 50$ °C. However, these duplexes were stabilized by pressure if the T_M is $> \sim 50$ °C. The DNA/RNA hybrid duplex, poly(dA)poly(rU), with a T_M of 31 °C in 20 mM NaCl undergoes a pressure-induced helix-to-coil transition at room temperature. This is the first report of pressure-induced denaturation of a nucleic acid duplex and provides new insights into the molecular forces stabilizing these structures.

Introduction

The thermodynamic stability of nucleic acid duplexes refers to the energetics of the pseudo-first-order transition between the double helical form of the duplex and the separated complementary strands of the coil form.[1,2] In general, these transitions may be induced by either temperature or pressure. Consequently, although the majority of the biological processes involving DNA occur under conditions of relatively constant temperature and pressure, temperature- and pressure-dependent studies represent the only experimental way to identify and thermodynamically characterize the molecular interactions contributing to the stability of a given sequence-specific DNA conformation.[1−8]

In general, a thermodynamic system (e.g., a double stranded nucleic acid) is fully described by its canonical partition function, Q, which, in turn, is a function of temperature, T, and pressure, P. The canonical partition function of a system that exists in n isomeric states is given by the relationship[9−11]

$$Q(P,T) = 1 + \sum_{i=1}^{n} \omega_i \exp[-\Delta G_i(P,T)/RT] \tag{1}$$

where ω_i is the degeneracy of the ith accessible energy state and $\Delta G_i(P,T)$ is the Gibbs energy difference between the ith state and the reference state.

Experimentally, the canonical partition function can be calculated as a function of T and P if the average excess enthalpy, $\langle \Delta H \rangle$, is known as a function of temperature, T, and the average excess volume, $\langle \Delta V \rangle$, is known as a function of pressure, P. From eq 1, one can derive

$$Q(T) = \exp[\textstyle\int_T (\langle \Delta H \rangle / RT^2)\, dT] \tag{2}$$

$$Q(P) = \exp[\textstyle\int_P (\langle \Delta V \rangle / RT)\, dP] \tag{3}$$

where

$$\langle \Delta H \rangle = \sum_{i=0}^{n} \alpha_i \Delta H_i; \quad \langle \Delta V \rangle = \sum_{i=0}^{n} \alpha_i \Delta V_i;$$

α_i is the fraction of ith state; ΔH_i is the enthalpy difference between the ith state and the reference state, and ΔV_i is the volume difference between the ith state and the reference state.

Hence, parallel temperature- and pressure-dependent studies are required to characterize a nucleic acid structure as a thermodynamic system. In this respect, the pressure is as fundamental an intensive variable as is the temperature.[7,8,12,13] However, the overwhelming majority of helix-to-coil transitions of double stranded nucleic acids have been studied as a function of temperature at constant (atmospheric) pressure.[1−6,14−16] Con-

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sequently, our current thermodynamic knowledge of DNA stability is essentially limited to the temperature domain while the pressure-related properties of nucleic acids remain relatively unexplored. This limitation is serious and precludes a better understanding and more complete thermodynamic characterization of the molecular interactions determining the sequence-dependent stability and conformational preferences of nucleic acid domains.

In this work, we derive the first pressure-versus-temperature phase diagram for the helix-to-coil transition of double stranded nucleic acids. The phase diagram shows that the thermodynamic stability of a nucleic acid duplex depends in a complex manner upon the temperature and pressure as well as the duplex denaturation temperature, T_{M}. To verify the phase diagram, we have conducted high-pressure UV melting experiments on poly(dIdC)poly(dIdC), a DNA duplex, poly(rA)poly(rU), an RNA duplex, and poly(dA)poly(rU), a DNA/RNA hybrid duplex. The T_{M} values of the DNA and RNA duplexes have been modulated by altering the solution ionic strength. In agreement with the derived phase diagram, we found that the polymeric duplexes were destabilized by pressure if the T_{M} is $< \sim 50$ °C. However, these duplexes were stabilized by pressure if the T_{M} is $> \sim 50$ °C. The DNA/RNA hybrid duplex, poly(dA)poly(rU), with a T_{M} of 31 °C in 20 mM NaCl undergoes a pressure-induced helix-to-coil transition at room temperature. This is the first report of pressure-induced denaturation of a nucleic acid duplex and provides new insights into the molecular forces stabilizing these structures.

Materials and Methods

Materials. The synthetic single stranded polymers poly(rA), poly(dA), and poly(rU) and the double stranded polymer poly(dIdC)poly(dIdC) were purchased from Amersham Pharmacia Biotech, Inc. (Baie d'Urfé, Québec, Canada). These polymers were used without further purification. All experiments were performed in a pH 6.8 buffer consisting of 10 mM cacodylic acid−sodium cacodylate and 1 mM $\mathrm{Na_2EDTA}$. The ionic strength of the buffer was adjusted to the desired level by adding known amounts of NaCl. All solutions were exhaustively dialyzed at 4 °C against the same buffer using dialysis tubing with a molecular weight cutoff of 1000 (VWR Canlab, Mississauga, Ontario, Canada).

Preparation of Poly(rA)poly(rU) and Poly(dA)poly(rU) Duplexes. Equimolar amounts of complementary single strands were mixed in buffer to obtain the poly(rA)poly(rU) and poly(dA)poly(rU) duplexes. The concentrations of the single and double stranded polynucleotides were determined spectrophotometrically using the following molar extinction coefficients: poly(rA), $\epsilon_{258} = 9800$ $\mathrm{M^{-1}\ cm^{-1}}$, poly(rU), $\epsilon_{260} = 9350$ $\mathrm{M^{-1}\ cm^{-1}}$; poly(dA), $\epsilon_{257} = 8600$ $\mathrm{M^{-1}\ cm^{-1}}$; poly(dIdC)poly(dIdC), $\epsilon_{291} = 6900$ $\mathrm{M^{-1}\ cm^{-1}}$ (per nucleotide); poly(rA)poly(rU), $\epsilon_{257} = 7000$ $\mathrm{M^{-1}\ cm^{-1}}$ (per nucleotide); and poly(dA)poly(rU), $\epsilon_{257} = 6500$ $\mathrm{M^{-1}\ cm^{-1}}$ (per nucleotide). These values were either provided by the manufacturer or taken from the literature.[17]

To confirm that poly(dIdC)poly(dIdC) and poly(rA)poly(rU) adopt the double stranded conformation under the experimental conditions employed, we measured the circular dichroism (CD) spectra of both duplexes (not shown) and compared them with the literature data.[18,19]

Poly(dA)poly(rU) is Double Stranded under the Conditions of Our Experiments. Before conducting experiments at high pressure, we employed UV titration and CD spectroscopic measurements to confirm that an equimolar mixture of poly(dA) and poly(rU) forms a double stranded structure. This confirmation was required since it had been reported that poly(dA)poly(rU) exists exclusively in the triple stranded or single stranded conformations.[17]

We have titrated poly(dA) with poly(rU) in a solution containing 10 mM cacodylate, pH 6.7, and 20 mM NaCl (see Figure 1a). The polynucleotide concentrations were between 0.1 and 0.2 mM in nucleotide. The interaction between the two strands was monitored by measuring the change in absorbance at 260 nm. The titration was conducted at 5 °C because the kinetics of formation of the poly(dA)poly(rU) duplex is slow at room temperature. The Job plot shown in Figure 1a exhibits a pronounced break at the point corresponding to the 1:1 stoichiometric ratio of the two polymers. This result indicates that, at neutral pH and 20 mM NaCl, the single stranded poly(dA) and poly(rU) polymeric chains associate to form the poly(dA)poly(rU) duplex.

In addition, we have measured the CD spectrum of poly(dA)poly(rU) as a function of salt concentration. The CD spectra of nucleic acids provide a convenient, sensitive, and nondestructive method to assess the conformational state of nucleic acids in solution.[20−22] Figure 1b presents the CD spectra of poly(dA)poly(rU) at 20 °C and NaCl concentrations between 10 and 300 mM. Inspection of Figure 1b reveals that poly(dA)poly(rU) undergoes a salt-induced conformational transition with the midpoint of around 30 mM NaCl. The isodichroic point observed at 245 nm is consistent with a two-state transition. The spectrum of the "high-salt" conformation (above 50 mM NaCl) equals the sum of the CD spectrum of the poly(dA) plus the spectrum of triple stranded poly(rU)poly(dA)poly(rU) measured at 1:2 molar ratio of poly(dA) and poly(rU)[19]. On the basis of these observations, we propose that, at high salt, poly(dA)poly(rU) disproportionates to form triple stranded poly(rU)poly(dA)poly(rU) and single stranded poly(dA). Figure 1c shows the profile of this salt-induced disproportionation transition as monitored by the ellipticity at 255 nm. Inspection of Figure 1c reveals that the salt-induced disproportionation of poly(dA)poly(rU) is highly cooperative with a midpoint of 28 mM NaCl. Thus, under the conditions of our experiments, namely, 20 mM NaCl, an equimolar mixture of poly(dA) and poly(rU) predominantly forms a double stranded structure. A small fraction of triple stranded molecules ($\sim$10%) may be present in the solution under these salt conditions. Significantly, the triplex form predominates only at higher NaCl concentrations (≥ 50 mM NaCl), a conclusion in agreement with the accepted point of view on the stability of poly(dA)poly(rU).[17,19,23] It is noteworthy that all previous thermodynamic characterizations of poly(dA)poly(rU) have been carried out at sodium ion concentrations of 100 mM and higher.

Optical Spectroscopy. Optical absorbance and circular dichroism spectra were recorded using, respectively, an AVIV model 14 DS spectrophotometer and model 62 DS spectropolarimeter (Aviv Associates, Lakewood, NJ). A 10 mm path length cell was used for all UV absorbance measurements. A cell with a path length of 1 mm was used for the CD measurements. For all CD spectroscopic experiments, the polynucleotide concentrations were between 1 and 1.5 mM in nucleotide.

High-Pressure UV Melting. The UV melting profiles for the poly(dA)poly(rU) duplex, as monitored by the temperature and pressure dependencies of the extinction coefficient at 260 nm, were recorded at pressures between 1 and 2000 bar in a high-pressure optical cell as previously described.[24] The optical cell is filled with silicon oil as the pressure-transmitting medium. Within the optical cell, the samples are contained in a 5 mm path length quartz cuvette designed to allow for pressure equilibration between the exterior and interior of the cuvette.

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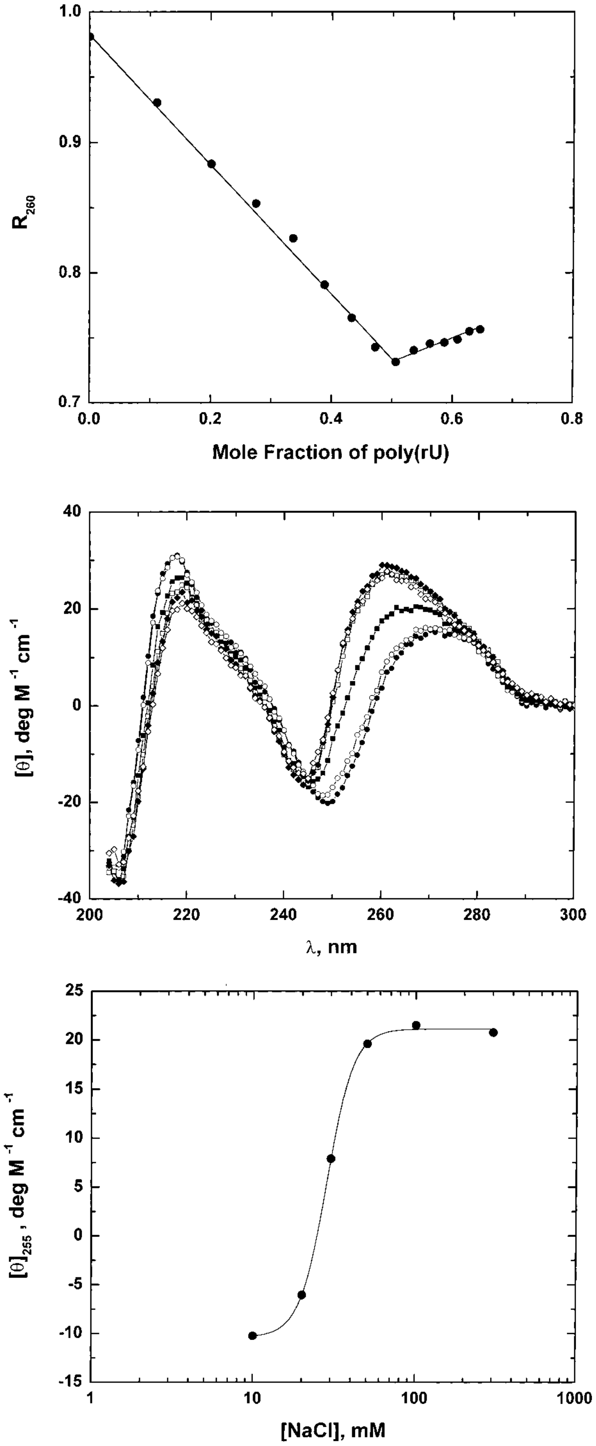

Figure 1. (a) Mixing curve for poly(dA) and poly(rU) (Job plot) as measured by UV absorbance at 260 nm recorded at 5 °C in an aqueous solution containing 10 mM pH 6.7 cacodylic buffer and 20 mM NaCl. The relative absorbance R_{260} is equal to $\epsilon/(\epsilon_A X_A + \epsilon_U X_U)$, where $\epsilon = A/(C_A + C_U)$, A is the UV absorbance at 260 nm, C_A and C_U are the total concentrations of poly(dA) and poly(rU), respectively, ϵ_A and ϵ_U are the extinction coefficients of poly(dA) and poly(rU), respectively, and X_A and X_U are the mole fractions of poly(dA) and poly(rU), respectively; (b) CD spectra of an equimolar mixture of poly(dA) and poly(rU) at 20 °C in 10 mM pH 6.7 cacodylic buffer and 10 (●), 20 (○), 30 (■), 50 (□), 100 (◆), and 300 (◇) mM NaCl; (c) salt dependence of the molar ellipticity at 255 nm of equimolar amounts of poly(rU) and poly(dA) at 20 °C in a 10 mM pH 6.7 cacodylic buffer.

The polynucleotide concentrations were between 0.2 and 0.3 mM (nucleotide) in these measurements.

Results and Discussion

Nucleic Acid Duplexes with T_M < 50 °C Should Be Destabilized by Pressure. The slope of the change in the helix-to-coil transition temperature, T_M, induced by pressure, $\Delta T_M/\Delta P$, can be used as a measure of the effect of pressure upon the stability of a nucleic acid structure. The value of $\Delta T_M/\Delta P$ is a linear function of the volume change, ΔV, accompanying DNA denaturation, as can be seen by rearranging the Clapeyron equation (24):

$$\Delta V = (\Delta T_M/\Delta P)\, \Delta S(T_M) \tag{4}$$

where $\Delta S(T_M)$ is the transition entropy determined at T_M.

For all heat-induced DNA helix-to-coil transitions, $\Delta S(T_M)$ is positive.[1,16] Thus, according to eq 4, positive values of $\Delta T_M/\Delta P$ indicate that the volume changes, ΔV, associated with helix-to-coil transitions of nucleic acids should be also positive and vice versa. There have been relatively few studies of nucleic acids at elevated pressures.[7,8,24−26] In these studies, the melting temperature, T_M, of DNA duplexes has been reported to increase with pressure. Consequently, it has been assumed that high-pressure stabilizes the double stranded conformation of nucleic acids relative to constituent single strands with the transition volume, ΔV, being positive.[7,8]

In contrast to these findings, densimetric measurements carried out at atmospheric pressure on five polymeric AT- and AU-containing nucleic acid duplexes revealed that the values of ΔV for helix-to-coil transitions are negative at temperatures lower than ~50 °C and positive at higher temperatures.[6,27] Furthermore, when compared at a common temperature, all AT- and AU-containing duplexes denature with nearly the same volume change, independent of the specific duplex.[6] Between 20 and 80 °C, ΔV increases linearly with temperature from negative to positive values with the slope equal to the expansibility change, ΔE, of the helix-to-coil transition: $\Delta V(T) = \Delta V(T_R) + \Delta E(T - T_R)$, where T_R is the reference temperature.[6] The value of ΔE expressed *per mole of nucleotide* is equal to 0.15 ± 0.03 cm^3 mol^{-1} K^{-1}.[6,27] If the reference temperature, T_R, is chosen to be 25 °C, then $\Delta V(T_R)$ is equal to -3.5 cm^3 mol^{-1} (expressed *per mole of nucleotide*). Hence, according to eq 4, duplexes with $T_M > \sim$50 °C will be stabilized at elevated pressures, while duplexes with $T_M < \sim$50 °C will be destabilized at elevated pressures.

Pressure-versus-Temperature Phase Diagram of Nucleic Acids. The stability of a nucleic acid duplex as a function of temperature and pressure is given by the Gibbs free energy difference, $\Delta G(P,T)$, between the helix and coil states. Assuming, as a first approximation, that ΔE is independent of temperature, the following equation for $\Delta G(P,T)$ can be derived from the relationship $d\Delta G = -\Delta S\, dT + \Delta V\, dP$:

$$\Delta G(P,T) = \Delta H(T_M)(1 - T/T_M) + \Delta C_P[(T - T_M - T\ln(T/T_M)] + [\Delta V(T_R) + \Delta E(T - T_R)](P - P_R) - 0.5\Delta K_T(P^2 - P_R{}^2) \tag{5}$$

where T_M is the denaturation temperature of the duplex at the reference pressure, P_R; $\Delta H(T_M)$ is the enthalpy of the helix-to-coil transition determined at T_M and P_R; ΔC_P is the heat capacity change associated with the transition (in eq 5, ΔC_P is assumed independent of temperature); $\Delta K_T = -(\partial \Delta V/\partial P)_T$ is the change in *isothermal* compressibility associated with duplex melting (in eq 5, ΔK_T is assumed independent of pressure). Experimen-

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tally, it is convenient to choose $P_R = 1$ bar as the reference pressure.

Recently, we have shown that at atmospheric pressure the enthalpy of duplex denaturation, $\Delta H(T_M)$, when compared at a common temperature is only slightly dependent on duplex type, base composition, or base sequence.[6] In general, we found that $\Delta H(T_M)$ of the duplex transition can be approximated by a linear function of temperature, $\Delta H(T_M) = \Delta H(T_R) + \Delta C_P(T_M - T_R)$. At the reference temperature, T_R, of 25 °C, $\Delta H(T_R) \approx 3.4$ kcal mol^{-1} (expressed *per mole of nucleotide*).[6] The average heat capacity change, ΔC_P, associated with the helix-to-coil transition of polymeric duplexes was determined from our calorimetric measurements to be 30 ± 10 cal K^{-1} mol^{-1} (expressed *per mole of nucleotide*).[6] It is worth noting that similar values of ΔC_P have been subsequently derived by the Privalov group[28] from experimental studies on oligomeric duplexes and by the Bloomfield group[29,30] on the basis of theoretical calculations.

The parameter ΔK_T equals the change in ΔV with pressure at constant temperature. Experimentally, however, it is easier and more accurate to measure the change in *adiabatic* compressibility, ΔK_S, associated with duplex melting using a combination of densimetric and ultrasonic velocimetric techniques.[27,31−33] The relationship between the values of ΔK_T and ΔK_S is given by the relationship[32]

$$\Delta K_T = \Delta K_S + (T\,\alpha_0^{\,2}/\rho_0 c_{P0})(2\Delta E/\alpha_0 - \Delta C_P/\rho_0 c_{P0}) \quad (6)$$

where ρ_0 is the density of the solvent, α_0 is the coefficient of thermal expansion of the solvent, and c_{P0} is the specific heat capacity of the solvent.

Analogous to enthalpy and volume, the value of ΔK_S associated with the denaturation of AT- and AU-containing duplexes has a weak dependence on the specific duplex type and linearly depends on temperature:[6] $\Delta K_S(T) = \Delta K_S(T_R) + (\partial \Delta K_S/\partial T)_P(T - T_R)$. The value of $(\partial \Delta K_S/\partial T)_P$ expressed *per mole of nucleotide* equals $(0.60 \pm 0.05) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} K^{-1}.[6,27] At the reference temperature, T_R, of 25 °C and atmospheric pressure, $\Delta K_S(T_R)$ equals -19×10^{-4} cm^3 mol^{-1} bar^{-1} (expressed *per mole of nucleotide*).[6,27] Using eq 6, and our previously reported experimental values of ΔK_S, ΔE, and ΔC_P, the temperature dependence of ΔK_T is given by $\Delta K_T(T) = \Delta K_T(T_R) + (\partial \Delta K_T/\partial T)_P(T - T_R)$. The value of the temperature slope, $(\partial \Delta K_T/\partial T)_P$, expressed *per mole of nucleotide* equals $(0.75 \pm 0.05) \times 10^{-4}$ cm^3 mol^{-1} bar^{-1} K^{-1}. At 25 °C, $\Delta K_T(T_R)$ is -17×10^{-4} cm^3 mol^{-1} bar^{-1} (expressed *per mole of nucleotide*).[6,27]

Assuming, as a first approximation, that $\Delta K_T(T_R)$ is independent of pressure and that $(\partial \Delta K_T/\partial T)_P$ is independent of pressure and temperature, eq 5 can be used to calculate the phase diagram of an "average" duplex by setting the value of $\Delta G(P,T)$ equal to zero:

$$\Delta H(T_M)(1 - T/T_M) + \Delta C_P[(T - T_M - T\ln(T/T_M)] + [\Delta V(T_R) + \Delta E(T - T_R)](P_M - P_R) - 0.5[\Delta K_T(T_R) + (\partial \Delta K_T/\partial T)_P(T - T_R)](P_M^{\,2} - P_R^{\,2}) = 0 \quad (7)$$

where P_M is the melting pressure for the duplex at temperature T.

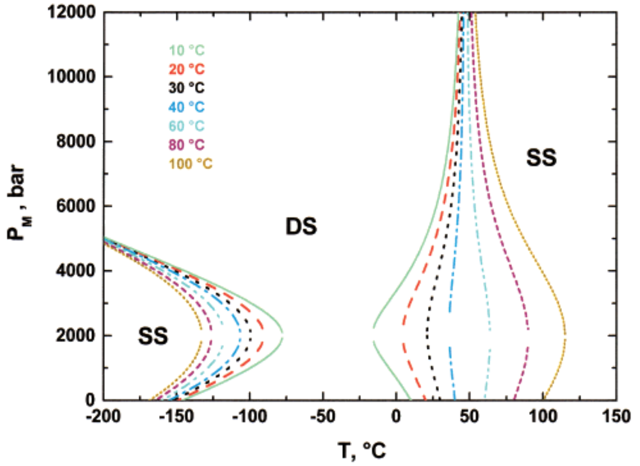

Figure 2. Phase diagram for the helix−to-coil transition of double stranded nucleic acid polymers. The denaturation pressure, P_M, is plotted as a function of temperature, T, for several values of the helix-to-coil transition temperature, T_M, at atmospheric pressure: 10 °C (solid line); 20 °C (dashed line); 30 °C (dotted line); 40 °C (dash−dot); 60 °C (dash−dot−dot); 80 °C (short dash); 100 °C (short dot). The phase diagrams were calculated using eq 7. In the figure, SS denotes the single stranded conformation and DS denotes the double stranded conformation.

This quadratic equation can be solved with respect to the denaturation pressure, P_M, as a function of temperature, T, for different values of the melting temperature, T_M. Figure 2 shows the temperature dependence of P_M calculated for different values of T_M. This phase diagram can be used to determine the conformational state of nucleic acid polymers at any temperature and pressure provided that the denaturation temperature, T_M, at atmospheric pressure is known.

Properties of the Phase Diagram of Nucleic Acid Duplexes. Inspection of Figure 2 reveals a number of important observations. First, the thermodynamic stability of double stranded nucleic acids is a complex function of temperature and pressure (as reflected in the shape and relative position of the boundaries between the single-stranded and double-stranded conformations) and strongly depends on the atmospheric pressure value of T_M.

Second, within the experimentally accessible temperature range (roughly, −20 to 120 °C) and at pressures below 2000 bar, the thermal stability of duplexes with the atmospheric pressure T_M values below 50 °C *decreases* as the pressure increases as reflected in negative values of $\Delta T_M/\Delta P$. By contrast, the thermal stability of duplexes with T_M greater than 50 °C *increases* as the pressure increases: the values of $\Delta T_M/\Delta P$ are positive. This observation provides the rationalization for the increase in T_M with pressure reported in the literature for a number of natural and synthetic polymeric nucleic acid duplexes.[7,8] Examination of the literature data reveals that all polymeric duplexes, for which the pressure dependence of stability has been investigated, exhibit T_M values greater than ~50 °C at reported experimental conditions.

Third, at pressures above 2000 bar, the situation is reversed. Duplexes with T_M lower than 50 °C are *stabilized* as the pressure increases, $\Delta T_M/\Delta P > 0$, whereas duplexes with T_M larger than 50 °C are thermally *destabilized* as the pressure increases: $\Delta T_M/\Delta P < 0$.

Finally, for any duplex, there is a pressure domain within which the helix-to-coil transition can be caused by decreasing temperature. For proteins, this process is known as "cold denaturation" and results from a positive value of the heat capacity change, ΔC_P, associated with an order−disorder

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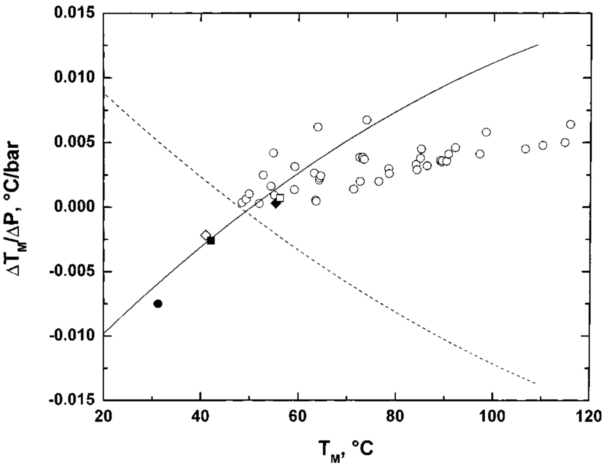

Figure 3. Temperature dependencies of $\Delta T_M/\Delta P$: theoretical curve for 1 bar (—); theoretical curve for 4000 bar (- - -); experimental points for 1 bar from ref 7 (○); poly(dA)poly(rU) in 20 mM NaCl (●); poly(dIdC)poly(dIdC) in 10 (◇) and 100 (◆) mM NaCl; poly(rA)poly(rU) in 10 (■) and 100 (□) mM NaCl.

transition.[34] As is seen in Figure 2, the cold denaturation of nucleic acids occurs at very low temperatures due to a relatively small magnitude of ΔC_P. At atmospheric pressure, the temperatures of cold denaturation of nucleic acids are typically below −120 °C although they may somewhat increase at elevated pressure.

In Figure 3, we present the pressure slopes, $\Delta T_M/\Delta P$, as a function of T_M calculated at 1 bar (solid line) and 4000 bar (dashed line). The individual points in Figure 3 are experimental values of $\Delta T_M/\Delta P$ at atmospheric pressure determined in this (●, □, ■, ◇, ◆) and previous (○) works for a number of natural and synthetic duplexes plotted against T_M.[7] In qualitative agreement with the theoretical curve (solid line, Figure 3), the experimental values of $\Delta T_M/\Delta P$ *increase* with T_M even though at high temperatures the absolute values of $\Delta T_M/\Delta P$ are somewhat smaller than the predicted values. These quantitative differences between the predicted and experimentally determined T_M dependencies of $\Delta T_M/\Delta P$ are not unexpected, especially at high temperatures, in light of the assumptions used in the analysis and the structural and compositional diversity of the experimentally studied duplexes. The most important is the assumption that the transition volume, ΔV, in eq 7 changes linearly with temperature within the entire temperature range, even though our experimentally studied temperatures for volume measurements have been limited to the 20 to 80 °C range.[6,27] Apparently, the ΔV-versus-T function starts to deviate from linearity above 80 °C, which would account, at least in part, for the observed difference between the predicted and experimentally determined T_M dependencies of $\Delta T_M/\Delta P$ (see Figure 3). In addition, we have measured ΔV as a function of temperature only for all AT- and AU-containing synthetic duplexes[6,27] while the experimental points in Figure 3 have been obtained using natural and synthetic duplexes that also contain GC pairs. One cannot exclude the possibility that the temperature dependencies of ΔV for AT- and GC-rich duplexes may be somewhat different, in particular at elevated temperatures. This also may cause a disparity between the predicted and experimentally determined values of $\Delta T_M/\Delta P$. Nevertheless, the qualitative agreement between the experiment and calculation is gratifying and lends credence to our analysis.

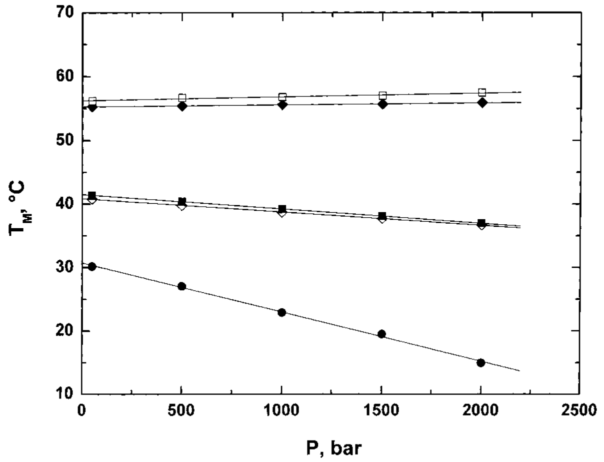

Figure 4. Pressure dependence of T_M for the heat-induced helix-to-coil transition of the poly(dIdC)poly(dIdC) [in 10 (◇) and 100 (□) mM NaCl], poly(rA)poly(rU) [in 10 (□) and 100 (■) mM NaCl], and poly(dA)poly(rU) [in 20 mM NaCl (●)] duplexes as monitored by the temperature dependencies of the extinction coefficient at 260 nm.

As a further test of the ability of the phase diagram to predict the behavior of nucleic acid polymers, we conducted temperature- and pressure-dependent UV melting experiments on polymers which, at low salt, exhibit values of T_M below 50 °C. Specifically, we have studied the pressure dependence of the T_M of poly(dIdC)poly(dIdC), a DNA duplex, poly(rA)poly(rU), an RNA duplex, and poly(dA)poly(rU), a DNA/RNA hybrid duplex. The phase diagram shown in Figure 2 predicts that, at low salt, these duplexes should be destabilized by pressure. For example, in 20 mM NaCl and at neutral pH, the T_M of poly(dA)poly(rU) is approximately 31 °C.[6] According to Figure 3 the T_M of poly(dA)poly(rU) is expected to decrease with increasing pressure ($\Delta T_M/\Delta P \approx -0.006$ °C/bar). Furthermore, as is seen in Figure 2, at temperatures slightly below T_M, the duplex should undergo a pressure-induced helix-to-coil transition.

Poly(dA)poly(rU) Is Destabilized by Pressure. We have measured the UV absorbance spectra of the poly(dA)poly(rU) DNA/RNA hybrid duplex as a function of temperature and pressure in 20 mM NaCl. As discussed above, at this salt, the poly(dA) + poly(rU) system may contain a small fraction (~10%) of triple stranded molecules. However, the use of lower salt would compromise the duplex stability by decreasing the T_M.

Figure 4 presents the pressure dependence of the T_M of poly(dA)poly(rU) in 20 mM NaCl (●). Inspection of Figure 4 reveals that the T_M of poly(dA)poly(rU) decreases with pressure, changing from 31.2 °C at 1 bar to 15.0 °C at 2000 bar. Thus, $\Delta T_M/\Delta P$ equals -0.0075 °C bar^{-1}, in good agreement with the predicted value of -0.006 °C bar^{-1} (see Figure 3). Figure 5 shows the UV absorption at 260 nm-versus-pressure profiles for poly(dA)poly(rU) at 20 and 25 °C. Inspection of Figure 5 reveals that, in agreement with the phase diagram (see Figure 2), the poly(dA)poly(rU) DNA/RNA hybrid duplex undergoes a pressure-induced helix-to-coil conformational transition with the denaturation pressures, P_M, of 1200 and 600 bar at 20 and 25 °C, respectively. This is the first report of pressure-induced denaturation of a nucleic acid duplex and provides strong support to our analysis. This observation clearly demonstrates that pressure does not always stabilize nucleic acid duplexes as had been previously assumed. On the contrary, under the appropriate conditions, pressure destabilizes duplexes and pressure-induced helix-to-coil transitions are possible.

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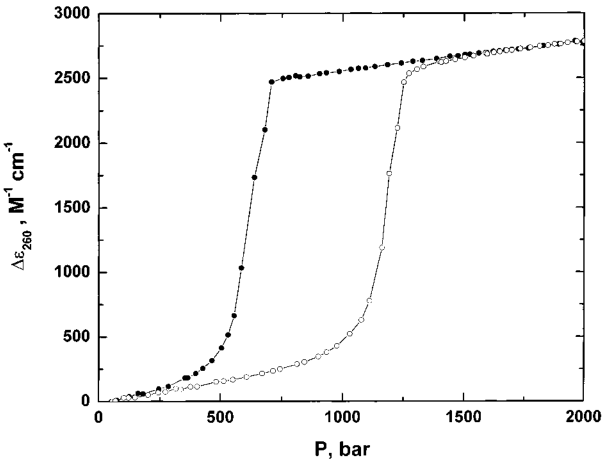

Figure 5. Pressure dependencies of the extinction coefficient, ϵ_{260}, of the poly(dA)poly(rU) duplex at 20 (○) and 25 °C (●).

Poly(dIdC)poly(dIdC) and Poly(rA)poly(rU) Are Destabilized by Pressure in 10 mM NaCl but Stabilized by Pressure in 100 mM NaCl. Using UV absorption, we have measured the helix−to-coil transitions of the poly(dIdC)poly(dIdC) DNA duplex and the poly(rA)poly(rU) RNA duplex as a function of temperature and pressure in solutions containing 10 and 100 mM NaCl. At atmospheric pressure in 10 mM NaCl, we determined the T_M values for poly(dIdC)poly(dIdC) and poly(rA)poly(rU) to be 41.0 and 42.1 °C, respectively. These values are in good agreement with the literature data.[16] On the basis of the phase diagram (Figure 2), these duplexes should be destabilized by pressure at low salt. On the other hand, in 100 mM NaCl, the atmospheric pressure values of T_M for poly(dIdC)poly(dIdC) and poly(rA)poly(rU) are 55.3 and 56.2 °C, respectively. According to the phase diagram, these duplexes should be stabilized by pressure at this salt concentration.

In Figure 4, we have plotted the values of T_M as a function of pressure for poly(dIdC)poly(dIdC) (◇ and ◆) and poly(rA)poly(rU) (□ and ■) in 10 and 100 mM NaCl. Inspection of Figure 4 reveals that, in 10 mM NaCl, both duplexes are destabilized by pressure: the T_M decreases linearly with increasing pressure. This observation is in agreement with the caluculated phase diagram. As is seen in Figure 4, for poly(dIdC)poly(dIdC), the T_M decreases from 41.0 °C at 1 bar to 36.7 °C at 2000 bar, while, for poly(rA)poly(rU), the T_M decreases from 42.1 °C at 1 bar to 37.0 °C at 2000 bar. The pressure slopes, $\Delta T_M/\Delta P$, for poly(dIdC)poly(dIdC) and poly(rA)poly(rU) equal −0.0022 and −0.0026 °C bar^{-1}, respectively, in excellent agreement with the theoretical values of −0.0027 and −0.0024 °C bar^{-1} (see Figure 3).

Pressure has the opposite effect on these polymers in solutions containing 100 mM NaCl. Poly(dIdC)poly(dIdC) and poly(rA)poly(rU) are slightly stabilized by pressure as can be judged by linear *increases* in T_M with increasing pressure. Specifically, for the DNA duplex, T_M increases from 55.3 °C at 1 bar to 55.9 °C at 2000 bar, while, for the RNA duplex, T_M increases from 56.2 °C at 1 bar to 57.5 °C at 2000 bar. The pressure slopes, $\Delta T_M/\Delta P$, for poly(dIdC)poly(dIdC) and poly(rA)poly(rU) are positive and equal to +0.0003 and +0.0007 °C bar^{-1}, respectively, in good agreement with the theoretical values of +0.0014 and +0.0016 °C bar^{-1} (see Figure 3).

In the aggregate, these results show that the same duplex may be either stabilized or destabilized by pressure depending on the value of T_M at specific experimental conditions. This result is of fundamental importance and suggests that the pressure stability of any duplex should always be defined at given experimental conditions in the context of T_M and specific pressure−temperature domain. In general, these results reveal the limitations of thermodynamic analyses of nucleic acid stability that are based exclusively on base sequence and composition of nucleic acids.

Thermodynamic Rationale for the T_M Dependence of the Pressure Stability of a Nucleic Acid Duplex. The strong dependence of the DNA phase diagram upon T_M is not entirely unexpected and can be rationalized as follows. Helix-to-coil transitions of double stranded nucleic acids are governed by a large number of intra- and intermolecular interactions including hydration, base stacking, interstrand hydrogen bonding, electrostatic phosphate−counterion interactions, entropic factors, etc.[1,3,35−37] Pressure and temperature dependencies of these interactions are reflected in the values of changes in enthalpy, ΔH, entropy, ΔS, volume, ΔV, and other macroscopic observables associated with duplex melting and ultimately determine the thermodynamic stability of double stranded nucleic acids. In this respect, the helix-to-coil transition temperature, T_M, equal to the ratio $\Delta H(T_M)/\Delta S(T_M)$, is an especially sensitive measure of the overall balance of interactions that stabilize the helix conformation relative to the coil conformation. Consequently, the strong dependence of the phase diagram of a nucleic acid duplex on its T_M reflects fundamental interrelationships that exist between the numerous microscopic interactions stabilizing the duplex and its macroscopically measurable thermodynamic stability.

Concluding Remarks

We present the first phase-diagram of double stranded nucleic acids as a function of temperature and pressure. The effect of pressure on the stability of a nucleic acid duplex strongly depends on its T_M and must always be defined in the context of the solution ionic strength and a specific pressure−temperature domain. Nucleic acid duplexes with T_M values below ~50 °C are destabilized by pressure, while those with higher T_M values are stabilized by pressure. These predictions are in good quantitative agreement with our experimental results for poly(dIdC)poly(dIdC), poly(rA)poly(rU), and poly(dA)poly(rU), as well as with the literature data on synthetic and natural nucleic acid duplexes. These findings are of fundamental importance for understanding the molecular origins of conformational stability of nucleic acids. On a practical level, the results will enhance our ability to predict the local stability and conformational propensities of genomic sequences and, ultimately, may have implications for the development of improved therapeutic and diagnostic nucleic-acid-based protocols (e.g., development of new sequence-specific DNA-binding agents and DNA microarrays).

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