The Activation Volume of a DNA Helix−Coil Transition†

Meng-Chih Lin and Robert B. Macgregor, Jr.*

Faculty of Pharmacy, University of Toronto, 19 Russell Street, Toronto, Ontario M5S 2S2, Canada

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ABSTRACT: The role of hydration in the kinetics of a DNA helix−coil equilibrium is investigated by studying the effect of hydrostatic pressure on the rate constants describing the reaction. The kinetics were measured using the thermal hysteresis between the denaturation and renaturation curves of the triplex-forming oligonucleotides: $^{5\prime}$d[AAA-GGAGGAGAAGAAGAAAAAA] (sequence of purine strand) and $^{5\prime}$d[TTTCCTCCTCTTCTTCTTTTTT] (third strand). The kinetics at atmosphere pressure for this system have been recently reported [Rougée *et al*. (1992) *Biochemistry 31,* 9269−9278]. At all pressures the data are consistent with a single-step bimolecular reaction under the conditions of our experiments (100 mM NaCl, 10 mM cacodylate, pH 6.5). The rate of formation of the triplex from the duplex + single strand is accelerated by pressure. At the midpoint of the helix−coil transition (32.5 °C), the activation volume for helix formation, V^*_1, equals −11.8 (±2.4) cm^3 mol^{-1} at atmospheric pressure. At the same temperature, the activation volume for helix dissociation, V^*_{-1}, equals +39.9 (±5.0) cm^3 mol^{-1}; that is, the rate of strand separation is slowed by pressure. These findings emphasize the importance of solvent interactions in the stabilization and formation of DNA helices. It is proposed that the activation volume of the forward reaction may arise from the volume change due to charging the cytosine residues and the formation of base-stacking interactions in the third strand. The positive activation volume of strand separation may be a consequence of poor solvent packing of the DNA duplex major groove during dissociation of the third strand.

The formation of helical structures by nucleic acids is one of the central interactions in biology. Much attention has been devoted to the theoretical and experimental aspects of the thermodynamics of the transition between the helix and coil forms of nucleic acids. This effort has resulted in a detailed understanding of the factors that contribute to the stability of double-stranded helices in solution. For example, it is possible to predict with good accuracy the helix−coil transition temperature of a double-stranded oligonucleotide of defined sequence throughout a wide range of salt concentrations.

The kinetics of the helix−coil equilibrium have received much less attention although a detailed description of the molecular processes leading to helix formation would be valuable for several areas of biochemistry. Among the subjects directly related to the kinetics of the helix−coil equilibrium are the mechanism of enzymes acting on DNA and investigations into the origins of the stability of the helical structures.

The theory of helix−coil transitions developed by Applequist and Damle (1963) proposed that the helix-formation reaction involve two principal reaction steps: formation of nucleation complex followed by chain propagation. The nucleation step is bimolecular and rate-limiting because the stability of the initial base pairs is low. The bimolecular complex between the two strands becomes stable only after the formation of several consecutive base pairs. After formation of the nucleation complex, the monomolecular propagation of the helix in both directions from the nucleation complex is rapid.

This model provided the theoretical framework for interpretation of the temperature-jump studies reported simultaneously by Craig *et al*. (1971) and Pörschke and Eigen (1971). These two investigations considered the kinetics of double-helix formation from single-stranded oligonucleotides as a function of salt concentration and chain length. The results revealed several details characteristic of the kinetics of formation of double-stranded nucleic acids. Their data showed that the kinetics could be described adequately by a single-step bimolecular model under most conditions. This is consistent with the proposal that the helix propagation step is much faster than the initial nucleation step. They showed the size of the nucleation complex to be approximately 3−4 base pairs. From the temperature dependence of the kinetics, they found that the activation energy of the helix formation reaction is negative; *i.e*., it becomes slower with increasing temperature. This is perhaps the most unusual aspect of the helix formation reaction. It is a consequence of the fact that above the helix−coil transition temperature the double helix is unstable in solution and only single strands exist. Thus, the forward rate constant becomes negligible at temperatures above the helix−coil transition temperature. The reverse rate constant, separation of the strands, displays the more usual positive activation energy.

The potential application of triple-stranded species as agents in antigene therapies has led to interest in determining the molecular factors important in the stability of triple-stranded structures. The studies of Craig *et al*. and Pörschke and Eigen report observation of a relaxation approximately 100 times longer than the relaxation due to double-helix

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* Corresponding author. Phone: 416/978-7332. Fax: 416/978-8511. Internet: macgreg@phm.utoronto.ca.

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R1: $^{5'}$d[AAAGGAGGAGAAGAAGAAAAAA]$^{3'}$
Y1: $^{3'}$d[TTTCCTCCTCTTCTTCTTTTTT]$^{5'}$
Y2: $^{5'}$d[TTTCCTCCTCTTCTTCTTTTTT]$^{3'}$

FIGURE 1: Sequence of the triplex-forming oligodeoxynucleotides used in this work.

formation. They ascribed this to formation of triple helices. Rougée *et al.* (1992) have completed the most thorough study of triple-helix formation kinetics to date. They have reported the kinetics of formation of a 22-base triplex from a duplex and single-stranded oligonucleotides. The triplex contained T−A·T and C^+−G·C base triples. They investigated the effect of salt concentration and single base−triple mismatches on the reaction rate. Their findings show that, mechanistically, triple-helix formation is similar to double-helix formation. For example, a single-step bimolecular mechanism describes the kinetics, and triplex formation is accompanied by a negative activation energy.

We have investigated the effect of hydrostatic pressure on the stability of DNA polymers and oligomers under several experimental conditions (Wu & Macgregor, 1993a,b, 1995; Najaf-Zadeh *et al.*, 1995; Macgregor, 1996). Because of the incompressibility of molecules at the pressures employed in our experiments, changes in the stability of DNA with pressure are interpreted in terms of alteration in the interaction of DNA forms with water. In the present work, we present the first report of the effect of hydrostatic pressure on the kinetics of DNA helix formation. The kinetics of the equilibrium between a triplex and the duplex + single strand were obtained from the hysteresis between the thermal denaturation and renaturation profiles (Rougée *et al.*, 1992). We have adapted the method described by Rougée *et al.* to investigate the pressure dependence, and thus the hydration dependence of triplex-formation kinetics. The results include the first report of activation volumes for a nucleic acid helix−coil equilibrium.

MATERIALS AND EXPERIMENTAL PROCEDURES

Synthetic triple-helix-forming oligonucleotides shown in Figure 1 were purified using thin-layer chromatography (Chou *et al.*, 1989). The concentrations were determined employing the extinction coefficients given in Rougée *et al.* (1992). Other reagents were obtained commercially and used without further purification. Equimolar amounts of the two duplex-forming strands, R1 and Y1, were mixed with a 10% excess of the third strand, Y2, in 100 mM NaCl, 10 mM sodium cacodylate, pH 6.5. The concentration of the duplex was 4.5 μM (or 100 μM base pair).

The helix−coil transition was followed by observing the increase in UV absorbance upon strand separation. We have previously described the details of the instrument used in these studies (Wu & Macgregor, 1993a,b). Within the high-pressure cell, the sample is contained in a 0.5-cm path length cuvette in the optical path of a spectrophotometer. The rate of temperature change was $\pm$0.12 °C min^{-1} for the heating and cooling curves, respectively. Besides the kinetics analysis described below, the helix−coil transition was characterized by the helix−coil transition temperature, T_m, which is the temperature at which half of the triple-stranded form is converted into duplex and single-stranded forms. We have described our method for calculating this parameter in previous publications (Wu & Macgregor, 1993a,b, 1995; Najaf-Zadeh *et al.*, 1995). The difference between the T_m of the triplex to duplex transition and the T_m of the duplex to single-strand transition is approximately 20 °C at atmospheric pressure. For mechanical reasons, the lowest pressure for which we report data is 1 MPa and not atmospheric pressure (0.1 MPa). This small over-pressure does not affect the equilibrium in any detectable manner.

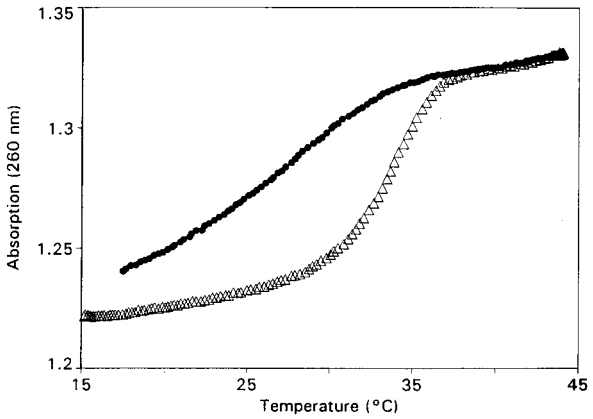

FIGURE 2: Example of a thermal denaturation renaturation hysteresis profile. In this example, the data were obtained at 50 MPa, and the heating and cooling rates were $\pm$0.12 °C min^{-1}, respectively. The denaturation (strand separation) curve is the lower of the two curves and was obtained first. The upper curve was acquired after the temperature had reached the maximum value.

The pressure dependence of the pH of the solution was measured using the method described by Neuman *et al.* (1973). This technique is based on the pH-induced spectral changes in the dye 2,5-dinitrophenol at the temperature and pressure of our experiments.

RESULTS

An example of a denaturation/renaturation hysteresis obtained at a constant heating rate of +0.12 °C min^{-1} followed by cooling at −0.12 °C min^{-1} is shown in Figure 2. Denaturation of the triplex to form the single-stranded plus duplex species occurs at temperatures approximately 7 °C higher than renaturation. This temperature shift and the difference in the shape of the two curves depend on the kinetics of the helix formation reaction. The transition from the triplex to the duplex plus single strands shifts to higher temperatures with increasing pressure. This shift implies that the helical forms have smaller molar volumes than the unbound strands. This finding is in qualitative agreement with other studies by ourselves and others on the effect of pressure on the stability of helical forms of DNA. It is also consistent with densitometric measurements of helix formation (Zieba *et al.*, 1990; Rentzeperis *et al.*, 1993; Chalikian *et al.*, 1994). In these experiments, the renaturation transition, *i.e.*, triplex formation, also shifted to higher temperatures with pressure. The fact that the denaturation and renaturation curves change in the same direction with increasing pressure is a characteristic of the kinetics of the reaction and not a general feature of the method.

In our experiments, the helix−coil transition temperature was higher than that reported by Rougée *et al.* (1992). We found the T_m at atmospheric pressure to be 32.5 °C, which is about 10 °C higher than that previously reported. The pH of our experiments, 6.5, was somewhat lower than the pH 6.8 they employed. Lower pH stabilizes C^+−G·C triplets

and probably accounts for most of the difference in the T_m values. The present experiments were also carried out at higher DNA concentrations than those of Rougée *et al*. This would also result in a higher T_m.

The equilibrium between single-, double-, and triple-stranded forms of DNA can be written as a single-step bimolecular reaction:

$$DS + SS \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} TS \quad (1)$$

where DS, SS, and TS are the double-, single-, and triple-stranded forms and k_1 and k_{-1} are the rate constants. Following the development presented by Rougée *et al*. (1992), setting α equal to the fraction of DNA present in the triplex form, the rate of triplex formation is given by

$$\frac{d\alpha}{dt} = k_1 M_0 (1 - \alpha)\left(1 - \frac{\alpha}{\rho}\right) - k_{-1}\alpha \quad (2)$$

M_0 is the total concentration of triplex-forming strand ([TS] + [SS]) and ρ is the ratio of the total concentration of duplex and triplex forms. The rate of change of α with temperature at a known rate of heating or cooling is related to eq 2 by

$$\frac{d\alpha}{dT} = \left(\frac{d\alpha}{dt}\right)\left(\frac{dt}{dT}\right) \quad (3)$$

where t is the time and T is temperature. The terms $d\alpha/dT$ and dt/dT are determined experimentally. Substituting eq 2 into eq 3, it can be seen that by measuring $d\alpha/dT$ and dt/dT for the heating and cooling curves one can determine the two unknown parameters in eq 2, k_1 and k_{-1}, by solving the system of equations:

$$\left(\frac{d\alpha}{dT}\right)_c = \left(\frac{dt}{dT}\right)_c \left[k_1 M_0 (1 - \alpha_c)\left(1 - \frac{\alpha_c}{\rho}\right) - k_{-1}\alpha_c\right] \quad (4)$$

$$\left(\frac{d\alpha}{dT}\right)_h = \left(\frac{dt}{dT}\right)_h \left[k_1 M_0 (1 - \alpha_h)\left(1 - \frac{\alpha_h}{\rho}\right) - k_{-1}\alpha_h\right] \quad (5)$$

The subscripts c and h refer to the cooling and heating curves.

Using eqs 4 and 5 to analyze the kinetics yields the temperature dependence of the two rate constants at all of the temperatures investigated. The temperature dependence of k_1 and k_{-1} is related to the activation energy of the reaction

$$k(T) = \exp\left(\frac{-E^*}{RT}\right) \quad (6)$$

where E^* is the activation energy. Each pair of heating/cooling curves yields the activation energies of the reaction. Figure 3 displays typical Arrhenius plots for the forward and reverse reaction steps. Due to experimental error, the rate constants found in this manner are accurate only in the range $0.1 \leq \alpha \leq 0.9$. By measuring the denaturation/renaturation cycle at several pressures, the pressure dependence of E^*_1 and E^*_{-1} can be calculated.

The aim of these experiments was to investigate the effect of hydrostatic pressure on the rate of the helix−coil transition. At a given temperature, the effect of pressure on the forward rate constant is given by an expression similar to eq 6:

$$k(P) = \exp\left(\frac{-V^*}{RT}P\right) \quad (7)$$

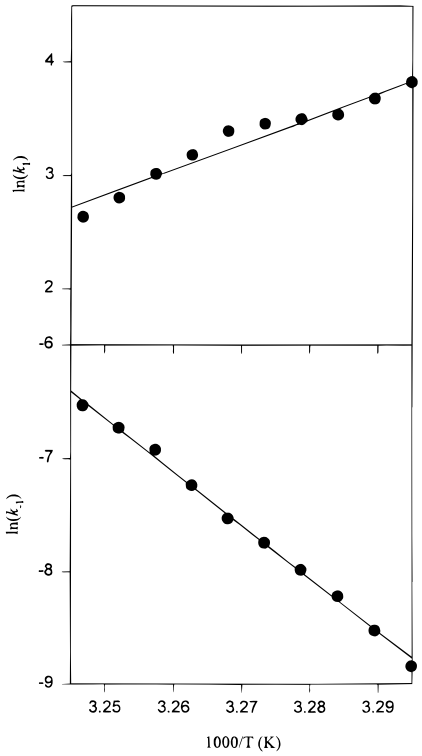

FIGURE 3: Arrhenius plots of the denaturation−renaturation hysteresis curves given in the example in Figure 2. The slopes of the fitted lines were used to calculate the activation energies, E^*_1 and E^*_{-1}, of the reaction. Note that the forward step displays the unusual negative activation energy typical of the helix−coil transitions of nucleic acids.

where V^* is the activation volume. To calculate the activation volumes, we compared the rate constants at a given temperature obtained from the hysteresis data at several pressures. This gave us the experimental slope, $(dk/dP)_T$, necessary to calculate the V^* values. The temperature dependence of the activation volumes, V^*_1 and V^*_{-1} is determined by comparison of the rate constants at a given temperature at the temperatures within the transition region.

Figure 4 shows the pressure dependence of the forward and reverse rate constants at a temperature at the midpoint of the transition. The magnitude of the forward rate constant, k_1, increases with increasing pressure, indicating a negative activation volume. This implies that the volume of the transition state in the forward direction is smaller than that of the sum of the volumes of the duplex plus single strand. The reverse rate constant (strand separation), k_{-1}, exhibits a positive activation volume. Table 1 summarizes these data for the activation volumes.

Measurement of the thermal hysteresis at several pressures yielded the pressure dependence of the activation energies (dE^*/dP) (see Table 2). Neither E^*_1 nor E^*_{-1} exhibits a marked pressure dependence. E^*_1 becomes slightly more negative as the pressure increases, changing from −53.4 kcal mol^{-1} at 1 MPa to −57.8 kcal mol^{-1} at 200 MPa. This change is within the error of the measurements. The value of E^*_{-1} decreases from +101 kcal mol^{-1} at 1 MPa to +88.2 kcal mol^{-1} at 200 MPa and thus becomes less temperature-dependent with increasing pressure. Again, the change observed is of the same magnitude as the error. The small pressure-induced change in the activation energies is consistent with our earlier observations that the enthalpy of the helix−coil transition is unchanged by pressure.

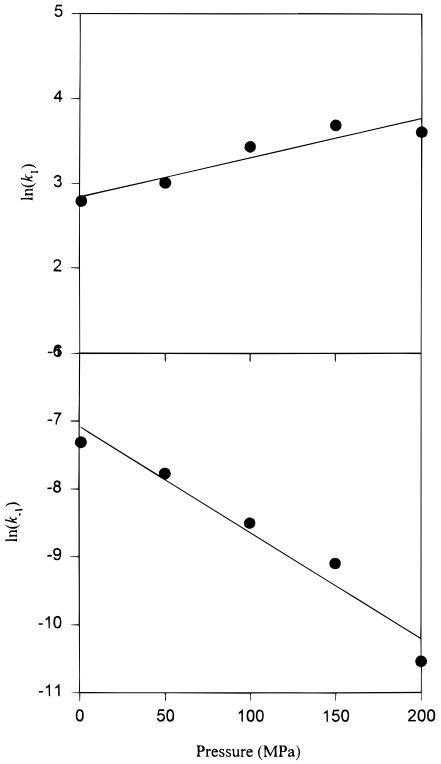

FIGURE 4: Pressure dependence of the forward and reverse rate constants at 32.5 °C (the temperature of the atmospheric helix−coil transition temperature). The slopes of the fitted lines are proportional to the activation volumes, V^*_1 and V^*_{-1}, of the reaction.

Table 1: Activation Volumes for the Triplex−Duplex Equilibrium at Temperatures within the Transition Region

temp (°C)	V^*_1 (cm^3 mol^{-1})	V^*_{-1} (cm^3 mol^{-1})
30	-13.4 ± 2.7	37.0 ± 4.6
31	-12.7 ± 2.5	38.2 ± 4.8
32	-12.1 ± 2.4	39.3 ± 4.9
32.5[a]	$\mathbf{-11.8 \pm 2.4}$	$\mathbf{39.9 \pm 5.0}$
33	-11.5 ± 2.3	40.4 ± 5.1
34	-10.9 ± 2.2	41.3 ± 5.2
35	-10.2 ± 2.1	42.5 ± 5.3
36	-9.6 ± 1.9	43.7 ± 5.5
37	-9.0 ± 1.8	44.6 ± 5.6
38	-8.4 ± 1.7	45.7 ± 5.7
39	-7.8 ± 1.6	46.7 ± 5.8
40	-7.1 ± 1.4	47.9 ± 6.0

[a] T_m at atmospheric pressure (0.1 MPa).

The change in the two activation volumes with temperature within the transition region is similar; both V^*_1 and V^*_{-1} become more positive with temperature (Table 1). In contrast to the behavior of the activation energies, the temperature-induced changes in the activation volumes are larger than the error. The rate of the helix formation (forward) reaction increases with pressure at the low temperatures of the transition but becomes much less pressure dependent at the high-temperature region of the transition. V^*_1 increases from -13.4 cm^3 mol^{-1} at 30 °C to -7.1 cm^3 mol^{-1} at 40 °C. The rate of the reverse reaction or denaturation is slowed by pressure at the temperatures throughout the transition, but it is slowed to a greater extent at higher temperatures. The activation volume for the reverse reaction (denaturation) increased from $+37.0$ cm^3 mol^{-1} to $+47.9$ cm^3 mol^{-1} in this temperature range.

As we have mentioned above, the stability and hence the helix−coil transition temperature of C$^+$−G•C triples is sensitive to the pH. The pH of an aqueous solution will change with pressure if the ΔV of ionization of the buffer is not equal to zero. To assure that the changes we observed were not due to pressure−induced pH changes, we have measured the pH of the buffer solution (10 mM sodium cacodylate, 100 mM NaCl) throughout the range of pressures used in these experiments. At the temperature of the helix−coil transition temperature, 32.5 °C, we observed a negligible pH change (<0.05 pH unit) over the range of 0.1−200 MPa (Figure 5). Thus, the changes observed in the rate constants are not due to the effect of pressure on the pH of the solution. The data in Figure 5 imply that at 32.5 °C the ΔV of ionization is approximately zero. Neuman *et al*. (1973) report the ΔV of ionization of cacodylate to be -13.2 cm^3 mol^{-1} at 20 °C. The difference between their value ΔV of ionization and our value stems from the higher temperature used in our studies and the presence of 100 mM NaCl in the solution. Both factors would cause the magnitude of this parameter to decrease.

The equilibrium parameters for the transition, ΔH and ΔV, can be calculated from the activation energies and volumes. Thus, $\Delta H = E^*_1 - E^*_{-1} = -154$ kcal mol^{-1} (strands); dividing by the number of bases (22) yields -7.0 kcal mol^{-1} (base triple) for helix formation at atmospheric pressure. The volume change is given by $\Delta V_t = V^*_1 - V^*_{-1} = -51.7$ cm^3 mol^{-1} (strand) or -2.35 cm^3 mol^{-1} (base triple). The volume change is for helix formation at 32.5 °C, the temperature corresponding to the T_m at atmospheric pressure.

Both the ΔH and the ΔV_t values calculated from the activation parameters compare well with literature values for similar transitions. Enthalpy values for triplex−duplex transitions in the literature range between -2.0 kcal mol^{-1} base triple to -8.1 kcal mol^{-1} base triple (Singleton & Dervan, 1994; Plum *et al*., 1990; Manzini *et al*., 1990). Rougée *et al*. (1992) obtained -5.1 kcal mol^{-1} base triple for the same oligonucleotide sequence at a somewhat higher pH (*see above*). The ΔV_t is lower than that measured for the transition between triple helices and single strands in the poly(dA)poly(dT)$_2$ system (Wu & Macgregor, 1993a,b). However, it is comparable to the values found for double-strand to single-strand transitions. The equilibrium we are considering here is more analogous to the duplex transition since it involves only two strand-forming entities. Our data for the denaturation of poly(dA)poly(dT)$_2$ described the transition from a triplex to three single strands.

We have also examined the influence of pressure on the T_m of the TS → DS + SS transition (Figure 6). The value of dT_m/dP is $+0.0290$ °C/MPa. Thus, the triplex is stabilized by elevated pressure, and as previously observed, dT_m/dP is linear over the range of pressures employed. As we have done in our previous work, we used the Clapeyron equation ($dT_m/dP = T_m\Delta V/\Delta H$) to calculate the volume change of the dT_m/dP data. Employing the enthalpy derived from the activation energies yields a volume change of $+2.8$ cm^3 mol^{-1}, which agrees with the volume change found using the activation volumes (see above). As is the case for the enthalpy and volume changes of the transition, the magnitude of the dT_m/dP value is smaller than what we found for complete melting of triple-stranded DNA at higher salt concentrations. Because of the difference in the experimental conditions, we cannot assess whether the smaller value of dT_m/dP is due to the number of strands involved in the

Table 2: Pressure Dependence of the Activation Energy for the Triplex−Duplex Transition

	pressure (MPa)				
	1	50	100	150	200
E^*_1 (kcal mol^{-1})[a]	−53.4 ± 7.2	−53.5 ± 7.6	−56.6 ± 16.0	−68.7 ± 2.5	−57.8 ± 2.5
E^*_{-1} (kcal mol^{-1})[a]	101 ± 3	93.6 ± 6.2	89.8 ± 5.3	84.8 ± 2.0	88.2 ± 11.8
ΔH (kcal mol^{-1})[b]	−154 ± 8	−147 ± 10	−146 ± 17	−153 ± 3	−146 ± 12

[a] Per mole strand. [b] $\Delta H = E^*_1 - E^*_{-1}$.

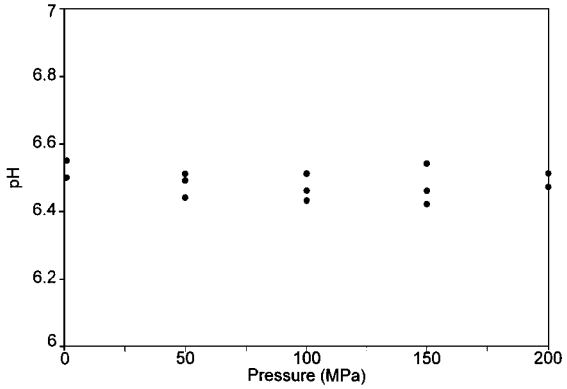

FIGURE 5: Pressure dependence of the pH of the buffer used in these experiments at the temperature of the helix−coil transition at atmospheric pressure (32.5 °C). The pH at different pressures was measured by monitoring the absorption at 440 nm of 2,5-dinitrophenol dissolved in the buffer.

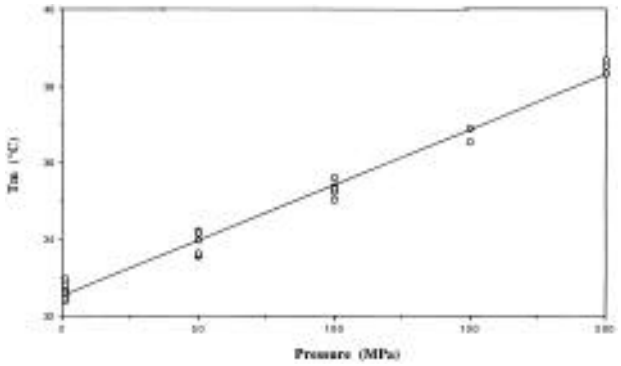

FIGURE 6: Change in the helix−coil transition temperature (T_m) as a function of hydrostatic pressure. The line is a least-squares fit of the data, the slope is equal to 0.0290 °C/MPa, and the intercept is 32.5 °C.

transition, the salt concentration, the sequence of the DNA, or a combination of these factors.

DISCUSSION

The helix−coil transformations of nucleic acids are among the most fundamental and most extensive structural changes in biology. A thorough understanding of the mechanism of the helix−coil transition is important for a full biochemical characterization of DNA and RNA *in vivo*. This knowledge may also become useful in attempting to design therapeutic agents based on antisense or antigene complexes. The use of a variety of experimental techniques and conditions aids in the discovery of how various factors influence the reaction. The recent interest in the potential of antisense-based therapies has led to several reports addressing the kinetics of triple-helix formation kinetics (Maher *et al*., 1990; Rougée *et al*., 1992; Shindo *et al*., 1993; Fox, 1995; Yang *et al*., 1994).

In the present work, we have varied the temperature and the pressure in an investigation of the kinetics of a triple-helix-forming 22-base oligonucleotide (Figure 1). The temperature dependence of the kinetics of triple-helix formation from the duplex and single-stranded oligonucleotides was recently reported by Rougée *et al*. (1992). Qualitatively and quantitatively, our results at low pressure agree well with their findings. The activation energy for the forward (helix-forming) reaction is negative while the activation energy for the reverse reaction step is positive. The value of the activation energy for the forward reaction that we obtained is approximately twice as large as that reported by Rougée *et al*. For the reverse reaction, the value of E^*_{-1} is equal to theirs within experimental error.

Effect of Hydrostatic Pressure on the Rate of Helix Formation. We have studied the effect of pressure on the reaction between double-stranded and single-stranded DNA leading to triplex formation as given in eq 1. The formation of triplex is accelerated by pressure; that is, this process occurs with a negative activation volume. A positive activation volume is found for separation of the triplex into double plus single strands.

Hydrostatic pressure influences the rate of a reaction when there is a difference between the molar volume of the reactants, or products, and the transition state. Because molecules are incompressible at the relatively low pressures employed in these experiments, differences in the molar volume of the species along the path of the reaction arise from changes in the interactions of these species with the solvent. For example, a transition state that is more highly charged than the reactants would likely be favored by high pressure because of electrostriction of water molecules around the transition state. Such a transition state would exhibit a negative activation volume.

The formation of a helix leads to an increase in the number of cations associated with the DNA structure. We have proposed that the association of these cations will be accompanied by a positive volume change based on the loss of electrostricted water molecules. The ionization of the cytosine residue required for formation of stable Hoogsteen interactions in a C^+−G·C triple probably also contributes to the activation volume. However, the net effect of this ionization on the observed activation volume is difficult to assess. Ionization reactions in polar solvents generally proceed with negative activation volumes (Asano & LeNoble, 1978). However, the negative volume change arising from ionization of the cytosine on the third strand will be partially or completely offset by the fact that this residue has limited exposure to the solvent. The importance of the charge on the cytosine residues in the triplex formation kinetics is currently being studied by using triplex-forming oligonucleotides with different numbers of cytosines and by varing the pH. Based on our current understanding of the effect of hydrostatic pressure on helix formation, we expect V^*_1 to be proportional to the pH and the number of cytosine residues. At lower pH values, a larger fraction of the

cytosines will be charged in solution as single strands. This will decrease the magnitude of the volume change due to charging these residues prior to triplex formation. In the same way, at a given pH, changing the number of cytosine residues will directly affect the total contribution of cytosine ionization to V^*_1.

The transition between the TS and DS + SS forms given in eq 1 clearly involves more than just electrostatic interactions. Stacking interactions between adjacent bases are also important to the stability of helical species. The molar volume for the formation of stacked intermolecular complexes between aromatic molecules in water is negative (Visser *et al*., 1977). The effect of pressure on the stacking interactions could be to favor the stacked conformation and increase the rate of both the nucleation step and the propagation step due to the increased stacking at high pressure.

Dissociation of the triplex to form the double-stranded and single-stranded species exhibits a positive activation volume. The size of the effect is larger than the activation volume for triplex formation, and in contrast to V^*_1, the magnitude of V^*_{-1} increases with temperature. To dissociate the third strand, the Hoogsteen interactions it forms with the duplex and the base stacking of the third strand must be disrupted. The volume occupied by the third strand must then be filled with water. The large positive value of V^*_{-1} may be a consequence of the poor packing of the major groove of the duplex during dissociation of the third strand.

The temperature dependencies of the activation volumes suggest either that pressure has a larger effect on the helix−coil transition as $\alpha \rightarrow 0$ or that there is a significant difference in the expansivities ($V^{-1}[\delta V/\delta T]_P$) of the species along the reaction pathway. Given that we have assumed a single-step mechanism for this reaction, and that we have no evidence for other reaction steps, we conclude that the temperature dependence of the activation volumes arises from expansivities. However, it is difficult to ascribe this effect to any mechanistic detail of the reaction given the current state of understanding of the relative volumes of the initial, final, and transition states.

The extent to which our results can be extrapolated to the pressure dependence of DNA duplex formation kinetics is unknown. However, even though triplex formation is much slower than duplex formation, at atmospheric pressure the kinetics of duplex and triplex equilibria appear to share several common features, including a negative activation energy for helix formation and similar dependencies on salt concentration. The molar volume change, ΔV_t, measured here for the dissociation of one strand of a triplex, is similar to the values characterizing SS $\rightleftharpoons$ DS transitions. However, this is the only parameter that we can compare directly with the observed effect of pressure on duplex transitions; thus, it is difficult to assess the generality of this observation in terms of the other parameters that describe the transition. We are currently carrying out further experiments to study the pressure dependence of triplex formation and to compare this with the behavior of duplex DNA.

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