Activation Volume of DNA Duplex Formation†

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

Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario M5S 2S2, Canada

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ABSTRACT: The denaturation−renaturation thermal hysteresis was used to investigate the kinetics of the helix−coil equilibrium of four 22-base pair homopurine−homopyrimidine duplex oligonucleotides with fractional G·C base pair content ($f_{G\cdot C}$) between 0.14 and 0.5. In 20 mM NaCl and 20 mM Tris-HCl at pH 7.0 and at hydrostatic pressures up to 200 MPa, a two-state bimolecular reaction mechanism adequately described the observed kinetics. At 1 MPa and 47 °C, the rate constant for helix formation, k_1, increased by a factor of 210, and the reverse rate constant, k_{-1}, decreased by a factor of 420 upon increasing $f_{G\cdot C}$ from 0.14 to 0.5. The activation energies for formation of the duplexes were negative and relatively insensitive to $f_{G\cdot C}$. The pressure-induced change in the rate constants is related to the activation volume of the reaction step. Pressure causes k_1 to become larger, and the magnitude of the change in k_1 with pressure increases the lower the $f_{G\cdot C}$ value. Thus, when $f_{G\cdot C} = 0.14$, the activation volume for forward reaction, $\Delta V^{\ddagger}_1$, equals −20 mL/mol, while when $f_{G\cdot C} = 0.5$, $\Delta V^{\ddagger}_1 = -6.7$ mL/mol. The rate constant for strand separation, k_{-1}, decreases at high pressure. The activation volume for this step, $\Delta V^{\ddagger}_1$, varies from 17 to 1.6 mL/mol when $f_{G\cdot C} = 0.14$ and 0.5, respectively. The ΔV for helix formation calculated from the activation parameters changed from −23 mL/mol when $f_{G\cdot C} = 0.14$ to −5.8 mL/mol when $f_{G\cdot C} = 0.5$. From extrapolation, it is estimated that the molar volume change for formation of G·C base pairs in homopurine−homopyrimidine sequences is approximately 0 mL/mol. Parameters calculated from kinetics of other two duplex molecules, when $f_{G\cdot C} = 0.23$ and 0.32, lie between these extremes.

The formation of double-stranded nucleic acids from two complementary strands is a spontaneous process involving formation of many structurally specific interactions between the single strands and a large number of reaction steps. Among the fundamental reaction steps are conformational changes that bring the structures of the two single strands into the proper orientation for base pairing and the redistribution of a large number of water molecules. Water molecules that form hydrogen bonds with the bases of single-stranded DNA must be removed prior to formation of the interstrand hydrogen bonds. Presumably, the inherent structural and physical differences between double- and single-stranded DNA will cause other water molecules to redistribute as well. If these water molecules undergo a density change, hydrostatic pressure will influence the position of equilibrium between the single- and double-stranded states due to the dependence of DNA duplex stability on the extent of hydration.

The majority of the research directed at studying the role of hydration in the stability of DNA has been directed at the equilibrium properties of single-stranded and duplex forms (Buckin *et al*., 1989; Chalikian *et al*., 1994; Hawley & MacLeod, 1973, 1977; Nordmeier, 1992; Rentzeperis *et al*., 1992a, 1993; Wu & Macgregor, 1993a,b, 1995). However, understanding how hydration influences the kinetics of the reaction steps leading to the duplex or single-stranded forms will provide more detailed knowledge concerning the relative importance of water molecules in the stabilization of the two conformations.

Despite the presumed existence of several reaction steps, transitions leading to helical structures can be treated to a very good approximation as a bimolecular reaction between the complementary strands. In a previous publication, we have reported the effect of hydrostatic pressure on the kinetics of the interaction between a duplex and a single-stranded oligonucleotide in forming a triple-stranded species (Lin & Macgregor, 1996). The kinetics were monitored via the thermal hysteresis between the denaturation and renaturation curves, and the transition was modeled as a single-step biomolecular mechanism. Pressure causes the rate of helix formation (the forward reaction) to increase; *i.e*. the activation volume is negative. Increased base stacking in the single-strand at elevated pressures may be responsible for this effect. The reverse rate constant (helix denaturation) decreased with pressure, indicating that the activation volume for this step is positive. This result was interpreted as arising from the lack of hydration of the transition state due to the inaccessibility of the major groove or the bases of the third strand until the complex is completely dissociated.

Our interpretation of the results for the triplex ⇌ duplex + single strand equilibrium suggested that the duplex to single strand equilibrium should exhibit the same general trend; *i.e*. the rate of helix formation should increase with pressure, and the rate of helix denaturation should decrease with pressure. However, the magnitude of the pressure effect should be less because of the greater solvent accessibility of the grooves in the duplex molecule. We report here the pressure dependence of the kinetics of the equilibrium between the duplex and single-stranded forms of four 22-bp[1] duplexes. All four duplexes are homopurine−homopyrimidine oligonucleotides with base compositions ranging

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* Address correspondence to this author at Department of Pharmaceutical Sciences, University of Toronto, 19 Russell St., Toronto, Ontario M5S 2S2, Canada. Phone: (416) 978-7332. Fax: (416) 978-8511. E-mail: macgreg@phm.utoronto.ca.

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Table 1: Summary of the Kinetic Parameters for the Helix−Coil Equilibrium of Four 22-Base Pair DNA Duplexes with Different Fractions of G·C Base Pairs

duplex	fraction G·C	T_m[a] (°C)	ln (k_1)[b] (M^{-1} s^{-1})	ln (k_{-1})[b] (s^{-1})	ΔH_{vH} (kcal/mol)	ΔV[c] (mL/mol)	$\Delta V^{\ddagger}_1$[b] (mL/mol)	$\Delta V^{\ddagger}_{-1}$[b] (mL/mol)	$E^{\ddagger}_1$ (kcal/mol)	$E^{\ddagger}_{-1}$ (kcal/mol)
R1·Y1	0.5	56.8	10.8	−9.9	−200	−5.8	−6.7	1.6	−94	82
R2·Y2	0.32	49.5	7.42	−6.9	−170	−5.9	−8.0	0.40	−96	72
R3·Y3	0.23	46.1	6.90	−5.2	−180	−19	−13	15	−72	92
R4·Y4	0.14	43.1	5.45	−4.1	−170	−23	−20	17	−80	82

[a] The values given here are estimated to have the following errors: T_m, ±0.3 °C; ln(k_1) and ln(k_{-1}), ±0.2; ΔH_{vH}, ±30 kcal/mol; ΔV, ±10%; $\Delta V^{\ddagger}_1$ and $\Delta V^{\ddagger}_{-1}$, ±15%; and $E^{\ddagger}_1$ and $E^{\ddagger}_{-1}$, ±10%. [b] At atmospheric pressure and 320 K (47 °C). The data are reported at this temperature to facilitate comparison of the different sequences. The temperature 47 °C was chosen because it is near the T_m of three of the oligonucleotides and thus requires the least extrapolation. [c] Volume change for helix formation, calculated using the Claperyon equation, $dT_m/dP = T_m\Delta V/\Delta H$.

R1:Y1 5'd[AGAGGAGGAGAAGAAGAGGAGA]3'
f_{GC} = 0.5 3'd[TCTCCTCCTCTTCTTCTCCTCT]5'

R2:Y2 5'd[AAAGGAGGAGAAGAAGAAAAAA]3'
f_{GC} = 0.32 3'd[TTTCCTCCTCTTCTTCTTTTTT]5'

R3:Y3 5'd[AAAGGAGGAAAAAAAGAAAAAA]3'
f_{GC} = 0.23 3'd[TTTCCTCCTTTTTTTCTTTTTT]5'

R4:Y4 5'd[AAAGGAAAAAAAAAAGAAAAAA]3'
f_{GC} = 0.14 3'd[TTTCCTTTTTTTTTTCTTTTTT]5'

FIGURE 1: Names, fractional G·C base pair contents, and sequences of the four duplex 22-mers used in these experiments.

from 14 to 50% G·C base pairs. The kinetics of the helix−coil equilibrium were determined by measuring the thermal hysteresis in the UV denaturation−renaturation curves. As was observed for the triplex−duplex equilibrium, the kinetics were adequately described by a single-step bimolecular mechanism. The trend predicted from the effect of pressure on the triplex−duplex equilibrium was borne out; namely, pressure affects the kinetics of duplex equilibrium to a smaller extent than the triplex equilibrium. By varying the base composition, we also report that the magnitude of the influence of pressure decreases with increasing G·C content.

MATERIALS AND METHODS

The eight synthetic 22-base oligonucleotides shown in Figure 1 were purified using thin-layer chromatography (Chou *et al*., 1989). All other reagents were used without further purification. The concentrations were calculated according to the method described in Fasman (1975). Equimolar amounts of the two complementary duplex-forming strands were mixed in 20 mM NaCl and 20 mM Tris-HCl at pH 7.0. The concentration of the duplex was 4.5 μM (or 100 μM base pair). None of the concentrations are corrected for compression at high pressure, which is on the order of 7% at 200 MPa.

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 ±0.35 °C min^{-1} for the heating and cooling curves, respectively. Throughout the range of heating and cooling rates for which we obtain useful hysteresis data, the rate constants are independant of the heating and cooling rates. 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 the midpoint of the transition. We have described our method for calculating the T_m in previous publications (Wu & Macgregor, 1993a,b, 1995; Najaf-Zadeh *et al*., 1995). For mechanical reasons, the lowest pressure for which we report data is 1 MPa and not atmospheric pressure (0.1 MPa). This small overpressure does not affect the equilibrium in a detectable manner.

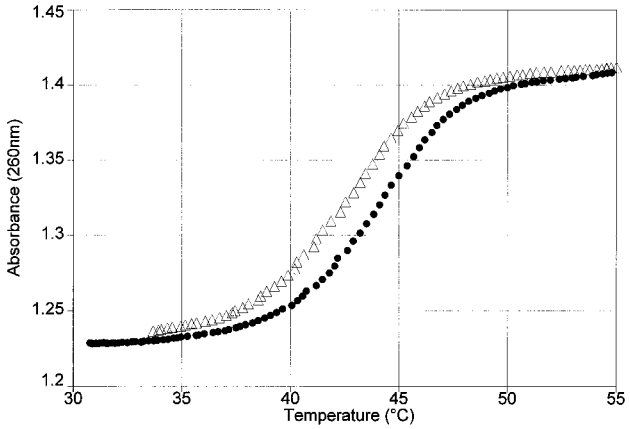

FIGURE 2: Representative denaturation−renaturation hysteresis data. This figure shows the raw denaturation (●) and renaturation (△) curves for the R4·Y4 duplex in 20 mM NaCl and 20 mM Tris-HCl at pH 7.0 and 100 MPa and a heating−cooling rate of ±0.35 °C/min.

RESULTS

In solutions containing 20 mM NaCl and 20 mM Tris-HCl at pH 7.0, the four duplex molecules shown in Figure 1 displayed helix−coil transitions with midpoints (T_m) ranging from 43 °C for R4·Y4 (14% G·C) to 56.8 °C for R1·Y1 (50% G·C) (see Table 1). The transitions were reversible, and the T_m values increased with pressure as has been observed for DNA molecules with other base compositions and sequences. However, as discussed below, the effect of pressure decreases with increasing G·C content.

The kinetics of the helix−coil equilibrium were determined by measuring the thermal hysteresis of the UV melting curves. A representative denaturation−renaturation curve is shown in Figure 2. The method for analyzing the kinetics from these curves is described in Rougée *et al*. (1992) and Lin and Macgregor (1996). A single-step bimolecular model adequately fit the observed kinetics, *i.e*.

$$ss_1 + ss_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{duplex} \quad (1)$$

[1] Abbreviations: Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; bp, base pair(s).

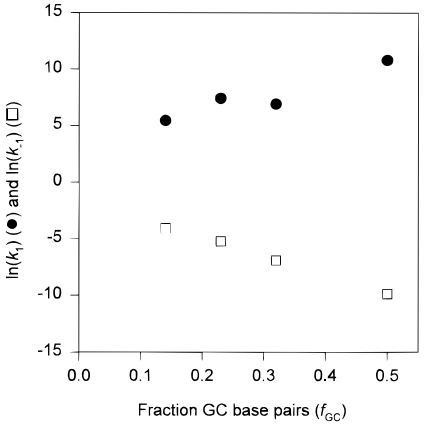

FIGURE 3: ln(k_1) (●) and ln(k_{-1}) (□) at 47 °C vs the fraction of G·C base pairs. The units for k_1 are M^{-1} s^{-1}, and the units for k_{-1} are s^{-1}.

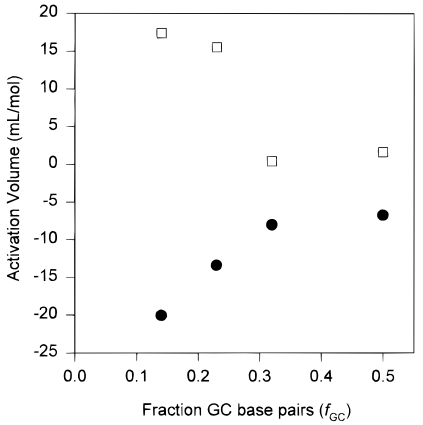

FIGURE 4: Activation volume of helix formation $\Delta V^{\ddagger}_1$ (●) and the activation volume of helix denaturation $\Delta V^{\ddagger}_{-1}$ (□) vs the fraction of G·C base pairs.

where ss_1 and ss_2 refer to the two complementary single-strands, k_1 is the forward rate constant, and k_{-1} is the reverse rate constant. Measurement of a thermal hysteresis at constant pressure yields the temperature dependence of the two rate constants, and from these data, the activation energies for the forward and reverse reactions can be calculated. By repeating the process of measuring the thermal hysteresis throughout a range of constant pressures, we can obtain the activation volumes of the equilibrium at constant temperatures.

The composition dependence of the forward rate constant, k_1, at atmospheric pressure is shown in Figure 3 and in Table 1. The data have been normalized to 47 °C (320 K) using the activation energies; we have done this with all of the parameters to facilitate comparison of the values. The rate constants increase approximately 200-fold for these 22-base oligonucleotides as the fractional G·C content ($f_{G\cdot C}$) increases from 0.14 to 0.5. Parallel to this, the activation energy changes a small amount from −80 to −94 kcal/mol. Negative activation energies are often observed in complexes arising from base pairing interactions of nucleic acids. It is a consequence of the instability of the nucleation complex formed initially between the two strands. This behavior is also observed in the kinetics of triple-helix formation.

Figure 3 also shows the dependence of the reverse rate constant, k_{-1}, upon the fraction of G·C base pairs in these 22-mers at atmospheric pressure and normalized to 47 °C. k_{-1} decreases by a factor of 420 as $f_{G\cdot C}$ increases from 0.14 to 0.50. Thus, the increased thermal stability of the duplexes with higher G·C content is reflected in the forward and reverse rate constants. The activation energy for the reverse step equals approximately 80 kcal/mol; it is independent of the base composition to a first approximation (Table 1).

From the two activation energies, we calculated the enthalpy change of the helix formation reaction, $\Delta H_{vH} = E^{\ddagger}_1 - E^{\ddagger}_{-1}$. As shown in Table 1, a lower $f_{G\cdot C}$ decreases the magnitude of ΔH_{vH}. Thus, at atmospheric pressure, when $f_{G\cdot C}$ is 0.5, ΔH_{vH} equals −200 kcal/mol, and when $f_{G\cdot C}$ is 0.14, ΔH_{vH} is −170 kcal/mol. These values are similar to those reported by Rentzeperis *et al*. (1992b) from the analysis of the UV transitions of a 25-bp duplex.

We monitored the thermal hysteresis at several pressures, usually up to 200 MPa (0.1 MPa = 1 bar = 0.987 atm). From the rate constants at a given temperature measured as a function of hydrostatic pressure, we have calculated the activation volume of helix formation and denaturation. The value of this parameter is related to the difference in the molar volume of the transition state and either the duplex or single-stranded form. Negative activation volumes imply that the apparent molar volume of the transition state is smaller than the initial state; the opposite is true when the activation volume is positive.

For all four duplexes, formation of the helix is accompanied by a negative activation volume; these results are summarized in Table 1 and Figure 4. The value of $\Delta V^{\ddagger}_1$ at 47 °C ranges from −6.7 mL/mol when $f_{G\cdot C} = 0.5$ to −20 mL/mol when $f_{G\cdot C} = 0.14$. These numbers imply that the transition state leading to helix formation is smaller than that of the two strands, *i.e.* $V_{ss_1} + V_{ss_2} > \Delta V^{\ddagger}_1$, where V_{ss_1} and V_{ss_2} are the apparent molar volumes of the two strands. Experimentally, this finding means that helix formation is accelerated by elevated pressure.

The reverse reaction, *i.e.* denaturation, exhibits the opposite behavior, the activation volume is positive for all four sequences investigated (Table 1 and Figure 4). The trend in the change of $\Delta V^{\ddagger}_{-1}$ with sequence is opposite of that observed for $\Delta V^{\ddagger}_1$. Thus, at 320 K, $\Delta V^{\ddagger}_{-1} = 1.6$ mL/mol when $f_{G\cdot C}$ is 0.5 and 17 mL/mol when $f_{G\cdot C}$ is 0.14. The positive magnitude of $\Delta V^{\ddagger}_{-1}$ means that this reaction step is slowed by elevating the pressure; the apparent molar volume of the transition state is larger than the molar volume of the duplex, *i.e.* $V_{duplex} < \Delta V^{\ddagger}_{-1}$.

Viewed another way, the changes in $\Delta V^{\ddagger}_{-1}$ and $\Delta V^{\ddagger}_1$ with sequence are similar; as the $f_{G\cdot C}$ increases, these two parameters approach zero and the kinetics of helix formation and denaturation becomes less sensitive to pressure. This trend is also reflected in the equilibrium values for the transition. As shown in Figure 5 (see also Table 1), the molar volume change approaches zero with increasing $f_{G\cdot C}$. This is apparently a characteristic of G·G and A·G sequences because it runs counter to what we observed for the effect of pressure on the equilibrium properties of poly[d(G-C)] (Najaf-Zadeh & Macgregor, 1995). The ΔV for this alternating polymer equals −4.8 mL/mol (for helix formation). This value was determined at 106 °C, the helix−coil transition temperature of the polymer in 52 mM NaCl; however, even extrapolating back to 47 °C, the ΔV of poly[d(G-C)] is approximately −3.9 mL/mol. This is much larger than we would have predicted on the basis of the data presented here. Thus, we hypothesize that the effect is due

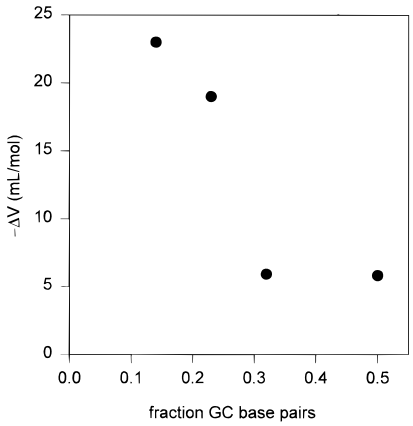

FIGURE 5: Molar volume change for helix formation, ΔV, vs the fraction of G·C base pairs.

to differences in solvent interactions with homopurine−homopyrimidine molecules relative to the interactions with alternating sequences.

We calculated the molar volume change two ways, using the activation volumes (*i.e.* $\Delta V = \Delta V^{\ddagger}_{1} - \Delta V^{\ddagger}_{-1}$) and using the Claperyon equation ($dT_m/dP = T_m\Delta V/\Delta H$, where T_m is the temperature at the midpoint of the denaturation transition). The ΔV values calculated in these two ways agree reasonably well with each other. It is also encouraging that the values follow the same trend for the different duplexes. The ΔV values calculated from the activation volumes are systematically larger than those found using the Claperyon equation. In the absence of other data, it is difficult to ascribe this difference to any particular factor. This discrepancy could arise from factors such as the enthalpies employed in the Claperyon equation or the limitations of the kinetic model we used. The helix−coil equilibrium is definitely not a single-step bimolecular reaction; however, the kinetics of this reaction can usually be accurately modeled with this mechanism. Perhaps the difference between the two ΔV values is a reflection of this, similar to the apparent negative activation energies of the forward reaction. The ΔV values of helix formation for the four duplexes appear to be reasonable in light of the other molar volume changes for other DNA sequences. The magnitude of ΔV decreases with increasing G·C content, implying that the molar volume change for the formation of the duplex $d(G_{22})\cdot d(C_{22})$ must be zero or positive. We have attempted to estimate this ΔV by assuming that the net volume change is a function only of the fraction of G·C base pairs and that there is no contribution arising from the different base stacking environments in the duplexes. Given these assumptions, we estimate the ΔV of formation for $d(G_{22})\cdot d(C_{22})$ to be 0 mL/mol on the basis of either the kinetics or equilibrium data. Due to the propensity of molecules containing several consecutive guanines to self-associate into tetraplex structures, it will be difficult to verify this result by a direct measurement of the ΔV. If this approximation can be extrapolated to the polymer, these data allow us to compare the volume changes for the four simplest polymers. Thus, the difference between the ΔV of poly(dA)·poly(dT) and poly[d(A-T)] ($\Delta\Delta V = 2.1$ mL/mol) has a different sign and a magnitude of half of that of the difference in ΔV for poly(dG)·poly(dC) and poly[d(G-C)] ($\Delta\Delta V = -3.9$ mL/mol). The disparity in the $\Delta\Delta V$ values underscores the dependence of the volume parameters on the composition and sequence of DNA.

DISCUSSION

The effect of hydrostatic pressure on a reaction manifests itself in terms of the position of the equilibrium and the rate of the reaction. The molar volume change, ΔV, is a measure of the pressure effect on the former, and the activation volumes, $\Delta V^{\ddagger}_{1}$ and $\Delta V^{\ddagger}_{-1}$, provide a measure of the influence of pressure on the latter. At the pressures employed in our experiments, molecules and ions retain their ambient pressure volume due to their very low compressibility. This implies that pressure effects arise from changes in the intermolecular interactions and to a lesser extent by intramolecular conformational changes. Whatever the molecular process, however, upon the application of hydrostatic pressure, the net result must be a net increase in the density of the entire system. For biological molecules, this includes water and salt in addition to the protein or DNA. In aqueous solution, water is responsible for the greatest number of interactions with DNA. Each base has many water molecules associated in inner and outer hydration layers, and relatively minor changes in the density of these hydrating water molecules can have a significant impact on the total density of the system.

Molar volume changes and activation volumes can be either positive or negative. Structural changes that lead to energetically more favorable interactions between the DNA and water will cause a decrease in the average distance between the water molecules and DNA and hence an increase in the density of the system. The clearest example of this effect is the electrostriction of water molecules around ions; the interaction between the permanent dipole of water and the electrostatic charge of the ion results in the waters of hydration being significantly more dense than bulk water. Similar but smaller effects are observed in the hydration layers surrounding partial charges. Molecular processes that lead to volume decreases such as conformational changes and ionization become more favorable with increasing pressure, and these processes will have negative molar volume change. Similarly, if the transition state of a reaction step has a smaller molar volume than the initial state, the activation volume is negative and the reaction step will be accelerated by pressure. Conversely, conformational or other changes that lead to weaker interactions between the solute and water generally result in increases in the volume of the system. Such reactions are less favorable or are slowed by high pressure.

We have measured the thermal hysteresis between the denaturation and renaturation of four different 22-bp duplex oligonucleotides with different base compositions at pressures between 1 and 200 MPa (0.1 MPa = 1 bar = 0.987 atm). The data presented here show that pressure influences the position of the helix−coil equilibrium and the rate of denaturation and renaturation. For all four duplexes, the overall trends observed were the same. The molar volume change for helix formation, ΔV, and the activation volume for helix formation, $\Delta V^{\ddagger}_{1}$, are negative, and the activation volume for helix denaturation, $\Delta V^{\ddagger}_{-1}$, is positive. The activation energies, $E^{\ddagger}_{1}$ and $E^{\ddagger}_{-1}$, were negative for helix formation and positive for denaturation.

The rate and extent of the helix−coil equilibrium are a function of several factors, including chain length, base composition, sequence complexity, and solvent conditions such as the salt concentration. The rate constants we have found appear to be consistent with rate constants from other

studies; however, it is difficult to assess the level of quantitative agreement because of the variety of experimental conditions, oligonucleotide lengths, and sequences used in previous investigations of helix−coil kinetics. At 320 K, we found that, as the $f_{G\cdot C}$ increases, the forward rate constant, k_1, increases and the reverse rate constant, k_{-1}, becomes smaller (Table 1). This is consistent with the observed increase in stability of the four oligonucleotides. These two trends become much smaller upon comparison of the rate constants at the T_m of each duplex. At their respective T_m's, $\ln(k_1)$ varies from 7.05 to 6.31 and $\ln(k_{-1})$ changes from −5.4 to −5.9 as $f_{G\cdot C}$ changes from 0.14 to 0.5. Thus, at similar points in the helix−coil transition equilibrium, the forward and reverse rate constants are weakly dependent on base composition.

Values of the forward rate constant have been reported for several different oligoribonucleotide and oligodeoxyribonucleotide duplexes under similar experimental conditions. The value of $\ln(k_1)$ has been reported to be 13.8 for $A_7{\cdot}U_7$ (Pörschke & Eigen, 1971), 13.3 for d(GGAATTCC) (Braunlin & Bloomfield, 1991), and 14.3 for d(GCATGC) (Williams *et al*., 1989), at temperatures near or at the respective helix−coil transition temperatures. The fact that the values of $\ln(k_1)$ for the four duplexes we studied are smaller than this is consistent with their increased length and complexity relative to the duplexes cited above. Our data were acquired at salt concentrations lower than those used in the other studies; a lower salt concentration is expected to lead to lower values of k_1 and larger values of k_{-1} [see for example Braunlin and Bloomfield (1991)].

There is a much larger variation in the rate of helix denaturation k_{-1} for different duplexes reported in the literature. Thus, at temperatures near or at the T_m, $\ln(k_{-1})$ equals 1 for $A_7{\cdot}U_7$ (Eigen, 1971), 8.43 for d(GGAATTCC) (Bloomfield, 1991), and 5.35 for d(GCATGC) (Turner, 1989). We have found that $\ln(k_{-1})$ is between −5.5 and −6.0 for the four duplexes used in this study. The values we measured for the rate of denaturation of the helix are approximately 3 orders of magnitude smaller than the rate constant reported for the 14-base pair self-complementary oligoribonucleotide, $A_7{\cdot}U_7$. The difference between the rates of denaturation of the other duplexes cited above and those we investigated is larger still. We attribute this difference to the length of the oligonucleotides we investigated. Due to the cooperative nature of the helix−coil transition, one would anticipate a large length effect on the rate of denaturation.

At their respective transition temperatures, the values of $\ln(k_{-1})$ for the four duplexes show a trend with $f_{G\cdot C}$ that agrees with what one might expect, namely that duplexes with larger $f_{G\cdot C}$ values have lower denaturation rate constants. However, given the narrow range of the values, it is doubtful that a great deal of significance can be attached to this. This trend is much clearer at the normalization temperature, 320 K, but this finding is expected because of the greater stability of the duplexes with higher $f_{G\cdot C}$ values.

We can also compare the rate constants for duplex formation with those recently reported for the equilibrium between the duplex R2·Y2 and a triplex-forming single-strand (Rougée *et al.*, 1992; Lin & Macgregor, 1996). With respect to the equilibrium triplex ⇄ duplex + single strand, the rate constant for formation of the duplex, R2·Y2, is approximately 10^5-fold larger, and the reverse rate constant for denaturation of the duplex is more than 100-fold smaller. These values are consistent with the greater stability of the duplex molecules and with the estimates of Pörschke and Eigen (1971) of the relative magnitude of the rate constants of the duplex and triplex transitions.

The activation volumes for the forward reaction ($\Delta V^{\ddagger}_1$) of the duplexes we studied fall between −6.7 and −20 mL/mol, becoming more negative as $f_{G\cdot C}$ decreases. That they are negative means that the reaction is accelerated by pressure; *i.e*. the volume of the transition state leading from the single-strands to the duplex molecule is less than the volume of the isolated strands. As with the activation energy for helix formation, the value of $\Delta V^{\ddagger}_1$ includes the assumptions of the single-step bimolecular mechanism, the most significant being that the formation of a helix nucleation complex and the helix propagation steps can be adequately described by a single parameter. While this is phenomenologically valid insofar as the kinetics are accurately described by this mechanism, we cannot make any statements concerning the contribution of the individual steps of this process.

For $\Delta V^{\ddagger}_{-1}$, the values are positive for all duplexes, implying that the volume of the transition state for denaturation is larger than the volume of the duplex. The magnitude of $\Delta V^{\ddagger}_{-1}$ increases with decreasing $f_{G\cdot C}$ values. We hypothesize that the volume of the transition state for denaturation is larger than the volume of the helix due to the necessity of forming an open structure in which the base pairs are no longer hydrogen bonded but are not sufficiently separated to allow interactions with water. This putative intermediate would have a larger volume than the helix and would cause the rate of denaturation to be suppressed by hydrostatic pressure.

The activation volumes for the duplex transitions can also be compared with those we measured for a related triplex (Lin & Macgregor, 1996). The sign and magnitude of $\Delta V^{\ddagger}_1$ are similar for the duplexes and the triplex. For the triplex, $\Delta V^{\ddagger}_1$ is −11.8 mL/mol at 32.5 °C; the $\Delta V^{\ddagger}_1$ values of the four duplexes are both higher and lower than this at 47 °C, suggesting that the molecular events giving rise to the activation volume are similar in the two cases. However, there is a larger difference between the activation volumes for denaturation of the duplexes and the triplex. For the triplex, $\Delta V^{\ddagger}_{-1}$ equals approximately 40 mL/mol, whereas the duplexes have $\Delta V^{\ddagger}_{-1}$ values ranging from approximately 0 to 14 mL/mol (Table 1). The larger magnitude of $\Delta V^{\ddagger}_{-1}$ for the triplex with respect to the duplex transition may arise from the greater facility with which the transition state of the duplex can be hydrated. Hydration of the transition state of the triplex during denaturation is impeded by the fact that the third strand is bound in the major groove of the duplex. The presence of the two duplex strands sterically blocks the interactions of water with the third strand and the major groove of the duplex until the two are completely unbound. The separation of the two strands of the duplex on the other hand can be hydrated from both grooves during denaturation. The greater access of the transition state during denaturation of the duplex leads to the smaller values of $\Delta V^{\ddagger}_{-1}$. It is apparent that the propensity of this sort of solvent-inaccessible open state to form will decrease with increasing pressure. The role of this effect in stabilizing DNA helices at high pressure has been previously proposed by ourselves and others (Hawley & MacLeod, 1974, 1977; Wu & Macgregor, 1993a,b, 1995; Najaf-Zadeh *et al*., 1995).

Chalikian and Breslauer (1996) have proposed that the small negative volumes of formation observed for the formation of DNA helices may be due to the thermal volume, υ_T, which arises principally from the larger solvent-exposed surface area of the single-strands. The suggestion is supported by calculations of the change in surface area of DNA as a consequence of helix formation (Alden & Kim, 1979). In our previous publications, we have hypothesized that the molar volume change of helix formation is due to electrostatic effects and the increase in the extent of base stacking. Clearly, base stacking is related to the extent of solvent exposure of the bases. Our argument is qualitatively consistent with the proposal of Chalikian and Breslauer; although we have not assessed the relative importance of stacking (solvent exposure) and electrostatics.

Not all of the parameters describing the helix−coil equilibrium change smoothly as functions of the fraction of G·C base pairs. This is particularly apparent for $\Delta V^{\ddagger}_{-1}$ and ΔV which change abruptly when $f_{G\cdot C}$ is between 0.32 and 0.23 (Figures 4 and 5). The origin of this behavior is perhaps related to the disruption of consecutive A_n·T_n runs by G·C base pairs. The structural and physical properties of A_n·T_n runs are different from those of other base pairs in a number of ways (Nelson *et al*., 1987; Wu & Crothers, 1984; Arnott *et al*., 1974; Marky & Macgregor, 1990); the interruption of these A·T runs by G·C base pairs will cause the physical and structural properties of the duplex to more closely resemble those of a canonical B-form DNA in some ways. However, it is unlikely that the properties of A_n·T_n runs offer the entire explanation for the discontinuity in some parameters. G_n·C_n runs also have structural peculiarities, one of which is manifested in the molar volume change approaching zero as $f_{G\cdot C}$ increases. An additional difference between the four duplexes we investigated is their terminal sequences. Three of the duplexes, R2·Y2, R3·Y3, and R4·Y4, have similar ends, while R1·Y1 has an additional G·C base pair near both ends. If we considered only the three duplexes with similar ends, the apparent correlations of the parameters with $f_{G\cdot C}$ would improve. However, given the limited data set, the validity of further restricting the data set to emphasize this correlation is questionable. In a future study, we plan to investigate the kinetics of oligonucleotides with purines and pyrimidines in both strands and to control better for the influence of ends on the paramters.

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