A more unified picture for the thermodynamics of nucleic acid duplex melting: A characterization by calorimetric and volumetric techniques

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ABSTRACT We use a combination of calorimetric and volumetric techniques to detect and to characterize the thermodynamic changes that accompany helix-to-coil transitions for five polymeric nucleic acid duplexes. Our calorimetric measurements reveal that melting of the duplexes is accompanied by positive changes in heat capacity (ΔCp) of similar magnitude, with an average ΔCp value of 64.6 ± 21.4 cal deg⁻¹ mol⁻¹. When this heat capacity value is used to compare significantly different transition enthalpies (ΔHP) at a common reference temperature, we find ΔTref for duplex melting to be far less dependent on duplex type, base composition, or base sequence than previously believed on the basis of the conventional assumption of a near-zero value for ΔCp. Similarly, our densimetric and acoustic measurements reveal that, at a given temperature, all the AT- and AU-containing duplexes studied here melt with nearly the same volume and compressibility changes. In the aggregate, our results, in conjunction with literature data, suggest that a more unified picture for the thermodynamics of nucleic acid duplex melting. Specifically, when compared at a common temperature, the apparent large differences present in the literature for the transition enthalpies of different duplexes become much more compressed, and the melting of all-AT- and all-AU-containing duplexes exhibits similar volume and compressibility changes despite differences in sequence and conformation. Thus, insofar as thermodynamic properties are concerned, when comparing duplexes, the temperature under consideration is as important as, if not more important than, the duplex type, the base composition, or the base sequence. This general behavior has significant implications for our basic understanding of the forces that stabilize nucleic acid duplexes. This behavior also is of practical significance in connection with the use of thermodynamic databases for designing probes and for assessing the affinity and specificity associated with hybridization-based protocols used in a wide range of sequencing, diagnostic, and therapeutic applications.

Thermodynamic studies of nucleic acids have produced data of both fundamental and practical importance. On the fundamental side, such studies have provided insight into the nature and strength of the forces that stabilize nucleic acids in the myriad of structural states they can assume (1–7). On the practical side, such studies have produced databases that are used to predict the stability and selectivity of hybridization events required in a broad range of nucleic acid-based diagnostic and therapeutic protocols (3, 8–11, 19, 42–44). Three aspects of the current nucleic acid thermodynamic library that are conspicuously deficient are values for the heat capacity change(s), ΔCp, the volume changes, ΔV, and the compressibility changes, ΔKs, which accompany nucleic acid conformational transitions. These deficiencies are of particular concern because ΔCp, ΔV, and ΔKs provide unique insights into the role of solvent in duplex stabilization (12–15) and because quantitative knowledge of ΔCp is required to compare thermodynamic properties of nucleic acids at a common reference temperature (16–18). This latter capacity is of specific practical value because thermodynamic databases reported at a given temperature often are used to predict hybridization properties at a different temperature as part of various diagnostic and therapeutic protocols (19). Without a significant body of directly measured heat capacity values, this parameter generally has been assumed to be zero for the melting of duplex structures to their component single strands (1–3). In this paper, we use a new generation of highly sensitive differential scanning calorimeters to demonstrate that this assumption is false. In fact, we measure ΔCp to be positive, with an average value of 64.6 ± 21.4 cal deg⁻¹ mol⁻¹, a value that we find to depend somewhat on base composition and sequence. When we use this ΔCp value to compare the transition enthalpies, ΔH, of different duplexes at a common reference temperature, Tref. This comparison yields duplex transition enthalpies at Tref, ΔHref, which depend significantly less on duplex type, base composition, or base sequence than previously believed on the basis of the conventional assumption of a near-zero value for ΔCp. We also present volumetric data (ΔV and ΔKs) on nucleic acid duplexes that we derive from acoustic and densitometric measurements (14, 15, 20). These volumetric data reveal a similar decrease in the dependence of duplex thermodynamic properties on the conformation, composition, and/or sequence of the duplex. Besides the fundamental importance of and insights derived from these ΔCp, ΔV, and ΔKs data, they also allow us to resolve duplex interactions into two general categories: those that depend primarily on duplex conformation, composition, and/or sequence (so-called “duplex-specific” interactions) and those that are relatively independent of conformation, composition, and sequence; that is to say, those that are common to all duplexes (so-called “duplex-nonspecific” interactions).

In the aggregate, our heat capacity and volumetric results, in conjunction with literature data, suggest a more unified picture of duplex melting thermodynamics in which, at a common temperature, the thermodynamic similarities between duplexes are greater than the thermodynamic differences induced by disparities in duplex conformation, base composition, and/or base sequence. In other words, insofar as thermodynamic properties are concerned, when comparing duplexes, the temperature under consideration is as important as, if not more important than, differences in the conformations, base compositions, or base sequences of the duplexes being compared. This general behavior has significant implications for our basic understanding of the forces that stabilize nucleic acid duplexes. This behavior also is of practical significance, as noted above, because thermodynamic

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databases are used to design probes and to assess the affinity and specificity associated with hybridization-based protocols used in a wide range of sequencing, diagnostic, and therapeutic applications.

**MATERIALS AND METHODS**

**Materials.** The synthetic single-stranded polymers poly[d(A)], poly[d(T)], poly[r(A)], poly[r(U)], and double-stranded polymers poly[d(AT)]poly[d(AT)], poly[d(A)]poly[d(T)], poly[d(AC)]poly[d(CT)], poly[d(GC)]poly[d(GT)], and poly[d(IC)]poly[d(IC)] are of the highest grade commercially available and were purchased from Amersham Pharmacia-LKB. All ultrasonic velocimetric and densimetric measurements were performed in a pH 6.8 buffer consisting of 10 mM cacodylic acid-sodium cacodylate, 20 mM NaCl, and 0.1 mM Na2EDTA. The differential scanning calorimetric experiments were performed in the same basic buffer containing variable amounts of NaCl ranging from 20 to 200 mM. All solutions were exhaustively dialyzed at 4°C against the same buffer by using tubing with a molecular weight cutoff of 1,000 (Spectrum Laboratories, Houston).

**Preparation of Double-Helix Solutions and Concentration Determinations.** Equal amounts of complementary single strands were mixed in buffer to obtain the poly[d(A)]poly[d(T)], poly[r(A)]poly[r(U)], poly[d(A)]poly[r(U)], and poly[r(A)]poly[d(T)] duplexes.

The concentrations of all single- and double-stranded polynucleotides were determined spectrophotometrically by using the following molar extinction coefficients: poly[r(A)], ε260 = 9,800 M⁻¹ cm⁻¹ (per base); poly[r(U)], ε260 = 9,350 M⁻¹ cm⁻¹ (per base); poly[d(A)], ε260 = 8,600 M⁻¹ cm⁻¹ (per base); poly[d(T)], ε260 = 8,520 M⁻¹ cm⁻¹ (per base); poly[r(A)]poly[r(U)], ε260 = 14,000 M⁻¹ cm⁻¹ (per base pair); poly[d(A)]poly[d(T)], ε260 = 12,000 M⁻¹ cm⁻¹ (per base pair); poly[d(AT)]poly[d(AT)], ε260 = 13,300 M⁻¹ cm⁻¹ (per base pair); poly[r(A)]poly[r(U)], ε260 = 13,000 M⁻¹ cm⁻¹ (per base pair); poly[r(A)]poly[d(T)], ε260 = 13,800 M⁻¹ cm⁻¹ (per base pair); poly[d(AC)]poly[d(GT)], ε260 = 13,000 M⁻¹ cm⁻¹ (per base pair); poly[d(GC)]poly[d(GC)], ε260 = 16,800 M⁻¹ cm⁻¹ (per base pair); poly[d(IC)]poly[d(IC)], ε260 = 13,800 M⁻¹ cm⁻¹ (per base pair). These values were either provided by the manufacturer or taken from the literature (21). For all calorimetric, densimetric, and ultrasonic velocimetric experiments, polynucleotide concentrations were between 1 and 2 mM in base pair.

**Ultrasonic Velocimetry.** For the poly[d(A)]poly[d(T)], poly[r(A)]poly[r(U)], poly[d(A)]poly[r(U)], and poly[d(AT)]poly[d(AT)] duplexes, the solution sound velocities between 20 and 70°C were determined at a frequency of 7.5 MHz with a precision of ±0.4% by using previously described ultrasonic velocimetric instruments, resonator cells and a differential mode of measurement (20, 22).

Ultrasonic velocimetric titrations of 3 mM poly[r(A)] with 12 mM poly[r(U)], at 20, 30, and 40°C, were performed as previously described (23–26).

**Densimetry.** The solution densities of the poly[d(A)]poly[d(T)], poly[r(A)]poly[r(U)], poly[d(A)]poly[r(U)], poly[r(A)]poly[d(T)], and poly[d(AT)]poly[d(AT)] duplexes were measured between 20 and 70°C with a precision of ±1.5 × 10⁻⁶ g/cm³ by using a vibrating tube densimeter (DMA-60, Anton Paar, Graz, Austria). The partial molar volumes, V°, of each polynucleotide duplex were calculated by using the well known relationship (13):

\[ V^\circ = M/\rho_0 - (\rho - \rho_0)/(\rho_0 C) \]

where \( M \) is molecular weight of the DNA and/or RNA duplex (average per nucleotide) and \( \rho_0 \) and \( \rho \) are the densities of the solution and the solvent (buffer), respectively.

**Densimetric titrations of 3 mM poly[r(A)] with 12 mM poly[r(U)], at 20, 30, and 40°C were performed as previously described (23, 24).**

**Determination of the Partial Molar Adiabatic Compressibility.** The relative molar sound velocity increments, \( \Delta V \), determined as described above, were used in conjunction with the partial molar volume, \( V^\circ \), data to calculate the partial molar adiabatic compressibility, \( K_{SV} \), of polynucleotides by using the relationship (27, 28):

\[ K_{SV} = \beta_0(2V^\circ - 2[U] - M/\rho_0) \]

where \( \beta_0 \) is the coefficient of adiabatic compressibility of the solvent.

Differentiating Eq. 2 yields the expression

\[ \Delta K_S = 2\beta_0(\Delta V - \Delta [U]) \]

where \( \Delta K_S, \Delta V, \) and \( \Delta [U] \) are, respectively, the changes in adiabatic compressibility, volume, and relative molar sound velocity increment accompanying the helix-to-coil transitions of each polynucleotide.

**Differential Scanning Calorimetry.** Calorimetric melting profiles of the heteropolymeric poly[d(IC)]poly[d(IC)], poly[d(AT)]poly[d(AT)], poly[d(AC)]poly[d(CT)], and poly[d(GC)]poly[d(GC)] duplexes and the homopolymers poly[d(A)]poly[d(T)] duplexes were determined by using either a model 5100 NanoDSC (CSC, Provo, UT) differential scanning calorimeter with nominal cell volume 0.9 ml, or a model 6100 NanoDSCII (CSC) differential scanning calorimeter with nominal cell volume 0.3 ml. Results for both instruments were indistinguishable within the error of the experiment (±3%).

For each polynucleotide/salt condition, several denaturation/renaturation scans were performed, and the denaturation scans were averaged. This approach was justified as the repeat scans were indistinguishable, provided the sample was not heated to high temperatures. Appropriate buffer vs. buffer baselines were determined before and immediately after the polymer vs. buffer scans were averaged. After subtraction of the (averaged) buffer scan, the (averaged) polymer scan was normalized for concentration and analyzed as follows. The heat capacity difference, \( \Delta C_P \), was determined from the difference in the pre- and posttransition baseline at the midpoint of the transition: the calorimetric enthalpy, \( \Delta H_{cal} \), was determined by integration of the area enclosed by the transition curve and the pre/posttransition baseline; and the melting temperature, \( T_m \), was determined as the midpoint of the melting transition.

**RESULTS AND DISCUSSION**

**Nucleic Acid Duplex Melting Is Accompanied by a Positive \( \Delta C_P \) with an Average Value of 64.6 ± 21.4 cal.deg⁻¹.mol⁻¹ per base pair².** Fig. 1A shows the family of heat capacity curves we have measured as a function of salt [Na⁺] concentration for the melting of the poly[d(AT)]poly[d(AT)] duplex. Fig. 1B shows three of these curves with an expanded y-axis to facilitate visualization of the positive change in heat capacity. We observe similar heat capacity changes for the other four duplexes (not shown).

In addition to the poly[d(AT)]poly[d(AT)] curves shown in Fig. 1A, we have also measured the heat capacity profiles for the poly[d(A)]poly[d(T)] and poly[d(IC)]poly[d(IC)] duplexes as a function of salt concentration, between 32 mM and 200 mM [Na⁺]. Analysis of each of these 20 curves yields \( T_m \), \( \Delta H_{cal} \), and \( \Delta C_P \) data. In Table 1, we list in the second and third columns the \( T_m \) and \( \Delta H_{cal} \) data at 32 mM Na⁺, whereas in the fourth column we list the \( \Delta C_P \) data averaged over the entire salt range studied. Note that the average \( \Delta C_P \) values are not zero, as often has been...
measuring ΔCₚ, we focus here on the average ΔCₚ value of 64.6 ± 21.4 cal·deg⁻¹·mol⁻¹ that we measure across duplexes and across Na⁺ concentration.

Comparison with Literature Data. Armed with our calorimetrically measured average ΔCₚ value of 64.6 ± 21.4 cal·deg⁻¹·mol⁻¹ for nucleic acid duplex melting, we surveyed the literature for published calorimetric ΔH° data on duplex melting over a range of temperatures (2, 3, 9). This survey revealed that the most extensive compilation of calorimetric Tₘ and ΔH° data on polynucleotides is that of Klump (3, 9). Because the published calorimetric studies were conducted on a variety of polymeric duplexes over a range of solution conditions, they yield different Tₘ values over which ΔH° is reported. Fig. 2 shows a plot of these ΔH° values vs. the duplex melting temperature, primarily on the basis of the data of Klump (3, 9). Within the experimental uncertainty, these data yield a straight line with a slope of 47 cal·deg⁻¹·(mol per base pair)⁻¹, a value within the range of our directly measured average ΔCₚ value of 64.6 ± 21.4 cal·deg⁻¹·(mol per base pair)⁻¹.

The Average Heat Capacity Change of 64.6 ± 21.4 cal·deg⁻¹·mol⁻¹ That We Derive from the Difference in the Pre- and Posttransition Baselines Agrees with the Value We Calculate from the Salt Dependence of the Calorimetrically Measured Transition Enthalpies. As noted above, we have measured heat capacity curves as a function of salt concentration for three of the duplexes studied here. Because increasing the salt concentration also increases the melting temperature (29, 30), we obtain the transition enthalpy as a function of temperature as well. The slopes of these plots (not shown), which correspond to ΔCₚ, are listed in column 5 of Table 1. The average ΔCₚ value of 64.7 cal·deg⁻¹·mol⁻¹ derived from these data is in excellent agreement with the average of 64.6 ± 21.4 cal·deg⁻¹·mol⁻¹ derived from the directly measured values determined from the difference in the pre- and posttransition baselines of the experimental heat capacity curves. Although the near identity of these two average values is undoubtedly fortuitous, the good agreement lends credence to both the quality of our measurements and our data analysis. These two average ΔCₚ values also are in good agreement with the few early estimates in the literature (31–36).

In the sections that follow, we use our average heat capacity value of 64.6 ± 21.4 cal·deg⁻¹·mol⁻¹ to make comparisons between duplex properties at a common reference temperature. As previously stated, we judge the use of an average heat capacity value to be justified given the experimental uncertainties in this parameter. However, it is important to note that the general nature of our observations and conclusions remains unaltered when we use the individual heat capacity values listed in Table 1 in our analysis.

When ΔCₚ Is Used to Compare Duplex Transition Enthalpies at the Same Temperature, the Apparent Composition/Sequence-Dependent Differences Becomes Much Less Pronounced. We used our average calorimetrically measured ΔCₚ value to extrapolate and compare at a common reference temperature, Tₑₐ, the disparate transition enthalpies at Tₘ that we and others have measured (3, 9). These data are listed in column 6 of Table 1 for

Table 1. Thermodynamic parameters for polynucleotides in 32 mM Na⁺ buffer

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Tₑₐ, °C</th>
<th>ΔHₑₐ, kcal/molbp</th>
<th>ΔCₚ*, cal/molbp K</th>
<th>ΔCₚ†, cal/molbp K</th>
<th>ΔHₑₐ*, kcal/molbp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly[d(IC)]/poly[d(IC)]</td>
<td>44.7</td>
<td>8.76 ± 0.26</td>
<td>31.2 ± 16.4</td>
<td>96.7</td>
<td>7.48 ± 0.49</td>
</tr>
<tr>
<td>Poly[d(AT)]/poly[d(AT)]</td>
<td>50.9</td>
<td>8.06 ± 0.24</td>
<td>61.3 ± 18.3</td>
<td>42.6</td>
<td>6.38 ± 0.60</td>
</tr>
<tr>
<td>Poly[d(A)/poly[d(T)]</td>
<td>58.2</td>
<td>9.36 ± 0.28</td>
<td>101.7 ± 24.5</td>
<td>54.5</td>
<td>7.21 ± 0.76</td>
</tr>
<tr>
<td>Poly[d(AC)]/poly[d(GT)]</td>
<td>79.9</td>
<td>9.62 ± 0.28</td>
<td>40.08</td>
<td>—</td>
<td>6.06 ± 1.21</td>
</tr>
<tr>
<td>Poly[d(GC)]/poly[d(GC)]</td>
<td>103.8</td>
<td>12.10 ± 0.36</td>
<td>78.28</td>
<td>—</td>
<td>7.00 ± 1.72</td>
</tr>
</tbody>
</table>

*Measured ΔCₚ derived from the difference in pre- and posttransition baseline averaged over all salt concentrations (32 mM, 50 mM, 75 mM, 105 mM, 125 mM, and 200 mM).
†ΔCₚ derived from a plot of ΔHₑₐ vs. Tₑₐ.
‡ΔHₑₐ (at 25°C) calculated by using the average ΔCₚ = 64.6 cal·deg⁻¹·mol⁻¹ (see text).
§ΔCₚ value derived from a measurement at a single salt concentration (32 mM Na⁺).
When we use our average values are compared at a common reference temperature. In fact, more appropriate comparison reveals the transition enthalpies at differences in base composition and sequence. By comparison, D by a cleotide thermodynamic data compiled by Klump (3, 9). The solid line in Fig. 2 equals 25°C. Note the substantial compression of the generally accepted strong dependence of transition enthalpy on duplex composition and sequence. The transition enthalpies of the duplex specific contributions to duplex energetics than previously assumed. An alternative complementary framing of this same point states that insofar as the enthalpy is concerned, duplex-specific interactions contribute less to duplex energetics than previously assumed.

Minor Differences in the Small Duplex-Specific Contributions to the Transition Enthalpy Can Yield Substantial Shifts in Tm. In the paragraphs above, we have shown that all nucleic acid duplexes calorimetrically studied to date exhibit nonzero positive heat capacity changes of similar magnitudes. Further, when these ΔCp values are used to extrapolate duplex transition enthalpies to a common temperature, we find ΔHmel to depend less on composition, sequence, and conformation than previously believed.

We attribute these observations to the strong contribution of duplex-nonspecific interactions to ΔHP and its temperature dependence. Nevertheless, it is an experimental fact that, under identical solution conditions, duplexes with different base compositions and base sequences exhibit significantly different Tm or Tm values (37). We propose that this difference in thermal stability derives from differences in the duplex-specific contribution to ΔHP, which, because of its logarithmic relationship to the equilibrium constant, translates into a significant shift in Tm, despite being a less significant fraction of the overall ΔΔTm than previously assumed (1–3).

If the observations of conformation/composition/sequence-independent and dependent contributions to duplex melting energetics described above are general, then they also should be reflected in other thermodynamic observations, including volumetric changes associated with duplex melting. To make this assessment, we have used a combination of densimetric and acoustic techniques to determine the change in volume, ΔV; and compressibility, Ks, accompanying the melting and/or formation of several nucleic acid duplexes. The resulting data, which are listed in Tables 2 and 3, are presented and discussed below.

Volume Changes Accompanying Helix-to-Coil Transitions of Polynucleotide Duplexes. In general, the partial molar volume, Vm, of a solute can be represented by a sum of the intrinsic volume, Vm, occupied by the solute molecule itself and the hydration-induced changes in the solvent volume, Vh:

\[ V^\circ = V_m + V_h \]

As previously discussed (33), the overall volume change, ΔV, which accompanies helix-to-coil transitions of biopolymers, can be expressed as the sum of the intrinsic and hydration contributions.

\[ \Delta V = \Delta V_m + \Delta V_h. \]  

| Table 2. Changes in the relative molar volume, ΔV, sound velocity increment, ΔU, and adiabatic compressibility, ΔKs, accompanying the thermally induced helix-to-coil transitions of polynucleotide duplexes |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Polymer          | Tm, °C          | ΔV, cm³·mol⁻¹·bar⁻¹ | ΔU, cm³·mol⁻¹·bar⁻¹ | ΔKs × 10⁶, cm³·mol⁻¹·bar⁻¹ |
| Poly[d(A)]poly[r(U)] | 30.5 ± 0.5      | -10.6 ± 2.0      | 20.0 ± 2.0        | -27.0 ± 4.0      |
| Poly[r(A)]poly[d(U)] | 48.0 ± 0.5      | 0 ± 2.0          | 14.0 ± 2.0        | -13.0 ± 4.0      |
| Poly[d(AT)]poly[d(AT)] | 51.0 ± 0.5      | 8.0 ± 2.0        | 10.0 ± 2.0        | -2.0 ± 4.0       |
| Poly[r(A)]poly[d(T)] | 55.0 ± 0.5      | 0 ± 2.0          | 12.0 ± 2.0        | -11.0 ± 4.0      |
| Poly[d(A)]poly[d(T)] | 56.5 ± 0.5      | 2.0 ± 2.0        | 10.0 ± 2.0        | -7.0 ± 4.0       |

| Table 3. Changes in the relative molar volume, ΔV, sound velocity increment, ΔU, and adiabatic compressibility, ΔKs, accompanying the helix-to-coil transitions of poly[r(A)]poly[r(U)] duplex resulting from the addition of single-strand poly[r(U)] to single-strand poly[r(A)] at 20, 30, and 40°C |
|-----------------|-----------------|-----------------|-----------------|
| Temperature     | ΔV, cm³·mol⁻¹·bar⁻¹ | ΔU, cm³·mol⁻¹·bar⁻¹ | ΔKs × 10⁶, cm³·mol⁻¹·bar⁻¹ |
| 20°C            | -9.6 ± 2.6     | -8.0 ± 2.6      | 42.2 ± 1.6      |
| 30°C            | 20.0 ± 2.0     | 31.2 ± 1.6      | 20.0 ± 2.0      |
| 40°C            | -4.0 ± 3.0     | -34.6 ± 4.0     | 20.3 ± 4.0      |

Superficially, the above conclusion may appear to conflict with an observation consistent with an apparent dependence of transition enthalpy on Tm, which, despite being a less significant fraction of the overall ΔΔTm than previously assumed (1–3). However, when minor differences in the small duplex-specific contributions to duplex energetics than previously assumed.

![Fig. 2. Comparison of the published calorimetric enthalpy values for all RNA/RNA, RNA/DNA, and DNA/DNA polynucleotide duplexes plotted against their melting temperatures. The experimental data in Fig. 2 (●) are taken from the extensive collection of polynucleotide thermodynamic data compiled by Klump (3, 9). The solid line corresponds to the best fit straight line to these values. This line has a slope, ΔH/Tm (= ΔCp), equal to 47 cal·K⁻¹·mol⁻¹ (of base pair)⁻¹.](image-url)
Transition-induced structural changes in the polynucleotide strands are reflected in the $\Delta V_M$ term in Eq. 4, whereas the hydration changes are reflected in the $\Delta V_H$ term. It should be noted that the $\Delta V_K$ term also reflects changes in the amount and nature of counterion and free ion hydration (29, 30).

In this work, we have measured the volume changes, $\Delta V$, which accompany the helix-to-coil transitions of several of the polynucleotide duplexes studied here, in two different ways: (i) volume changes at $T_m$ were measured for the thermally induced denaturations of the poly[d(A)]poly[r(U)], poly[r(A)]poly[r(U)], poly[d(AT)]poly[d(AT)], poly[r(A)]poly[d(T)], and poly[r(A)]poly[d(T)] duplexes, and (ii) volume changes at 20, 30, and 40°C were measured for duplex formation of poly[r(U)]poly[r(U)] by addition of the poly[r(U)] single strand to the poly[r(A)] single strand.

Table 2 presents the $T_m$ (second column) and $\Delta V$ (third column) data we have measured for the thermally induced helix-to-coil transitions of the five duplexes. Our $T_m$ values (obtained at pH 6.8 and a total Na$^+$ concentration of 28 mM) are in good agreement with previous reports (3, 21). Table 3 shows the $\Delta V$ data for the isothermal duplex formation of poly[r(A)]poly[r(U)] at 20, 30, and 40°C.

Fig. 3 shows a plot of temperature vs. volume changes, $\Delta V$, accompanying the helix-to-coil transitions of the five polynucleotide duplexes. Note that when two transition temperatures are close, the $\Delta V$ values reported by Wu and Macgregor (39) for poly[d(AT)]poly[d(AT)] and poly[d(A)]poly[d(T)] are in excellent agreement with our densimetric data. In the aggregate, the data in Fig. 3 reveal that, between 20 and 70°C, $\Delta V$ for all the A/U and A/T duplexes studied here increases linearly from negative values below 50°C to positive values at higher temperatures. The slope, $\Delta V/\Delta T$, is equal to 0.30 ± 0.05 cm$^3$mol$^{-1}$K$^{-1}$. This value represents the change in thermal expansivity, $\Delta \varepsilon$, that accompanies the helix-to-coil transitions of the A/U and A/T duplexes. To our knowledge, this determination of $\Delta \varepsilon_{\text{hel}}$ represents the first such measurement of nucleic acids.

As noted above, our data reveal that A/T and A/U duplexes exhibit very similar volumetric temperature dependencies, $\Delta V(T)$, which, within experimental uncertainty, do not depend on the method of measurement, the buffer, the salt concentration, or the duplex type. We offer below one possible interpretation of this observation. We present this interpretation only as a basis for further discussion because clearly other possibilities exist.

The volume change, $\Delta V$, accompanying the helix-to-coil transitions of nucleic acids can be expressed as the sum of specific, $\Delta V_{\text{spec}}$, and nonspecific, $\Delta V_{\text{nonspec}}$, contributions:

$$\Delta V(T) = \Delta V_{\text{spec}}(T) + \Delta V_{\text{nonspec}}(T).$$

The striking similarity of the observed temperature dependencies, $\Delta V(T)$, for all the A/T- and A/U-containing duplexes shown in Fig. 3 suggests that the duplex-nonspecific contribution, $\Delta V_{\text{nonspec}}(T)$, overwhelms the duplex-specific contribution, $\Delta V_{\text{spec}}(T)$. One interpretation of this observation is that, at least for the helix-to-coil transitions of A/T- and A/U-containing duplexes, the intrinsic and hydration volumetric changes are predominantly nonspecific; that is to say, they do not strongly depend on the specific type of duplex, the arrangement of the component bases, or the structure of the sugar ring.

The significant general observation concerning the $\Delta V$ data presented above is that for diverse A/T- and A/U-containing duplexes, the value of $\Delta V$, like that of $\Delta H$, depends primarily on temperature, although it depends less on duplex conformation, base composition, or base sequence than previously assumed (40, 41).

**Adiabatic Compressibility Changes Accompanying Helix-to-Coil Transitions of Polynucleotide Duplexes.** Analogous to the treatment of the volumetric data noted above (see Eq. 4), and as previously discussed (15), the changes in adiabatic compressibility, $\Delta K_{\text{ad}}$, that accompany biopolymer transitions can be expressed as the sum of intrinsic ($K_{{\text{intrinsic}}}$) and hydration ($K_{{\text{hydration}}}$) contributions:

$$\Delta K_{\text{ad}} = \Delta K_{{\text{intrinsic}}} + \Delta K_{{\text{hydration}}}$$

Using Eq. 3 from Materials and Methods, we have calculated changes in adiabatic compressibility, $\Delta K_{\text{ad}}$, by combining our data on the changes in volume, $\Delta V$, and the relative molar sound velocity increment, $\Delta [\text{U}]$, listed in Table 2. The fourth and fifth columns in Table 2 list the changes in $\Delta [\text{U}]$ and $\Delta K_{\text{ad}}$, respectively, for the thermally induced helix-to-coil transitions of the duplexes studied here. Table 3 lists the corresponding $\Delta [\text{U}]$ and $\Delta K_{\text{ad}}$ data.
for the isothermal duplex formation of poly[r(A)poly[r(U)]] at 20, 30, and 40°C.

Fig. 4 shows a plot of the adiabatic compressibility changes, $\Delta K_a$, vs. $T_m$, that we have measured for the duplexes studied here. Inspection of this plot reveals that, independent of the type of the duplex or the means of duplex disruption/formation, $\Delta K_a$ linearly increases with temperature. In other words, analogous to $\Delta V^\circ$ and $\Delta H^\circ$, we find the magnitude of the temperature dependence of $\Delta K_a$ to be relatively insensitive to the type of duplex.

This observation, as in the case of $\Delta H^\circ$ and $\Delta V^\circ$, can be rationalized by defining the observable as the sum of specific, $\Delta K_{spec}$, and nonspecific, $\Delta K_{non}$, contributions to $\Delta K_a$:

$$\Delta K_a(T) = \Delta K_{spec}(T) + \Delta K_{non}(T)$$

The similarity of the observed temperature dependences, $\Delta K_a$, for A/T and A/U duplexes shown in Fig. 4 suggests that the nonspecific contribution, $\Delta K_{non}(T)$, overwhelms the specific contribution, $\Delta K_{spec}(T)$. This observation also is consistent with our early suggestion that, for the A/T and A/U duplexes, intrinsic and hydration changes accompanying helix-to-coil transitions are predominantly nonspecific, i.e., they do not strongly depend on the specific type of duplex.

Given the volumetric results described above, it is instructive now to revisit the heat capacity data presented earlier.

Dissection of the Helix-to-Coil Transition Enthalpies for the Polynucleotide Duplexes. Recall that Fig. 2 shows a plot of calorimetrically determined $\Delta H^\circ$ values vs. $T_m$ for all combinations of A/U, A/T, G/C, and I/C-containing DNA, RNA, and DNA/RNA hybrid polymeric duplexes studied to date. Note that the A/U- and A/T-containing duplexes, as well as the G/C- and I/C-containing duplexes, exhibit similar linear temperature dependencies for $\Delta H^\circ(T)$, with a standard deviation of $\pm 0.3$ kcal mol$^{-1}$ base pair. Analogous to our analysis of the $\Delta V^\circ$ and $\Delta K_a$ data (see Eqs. 1 and 5), we can also represent $\Delta H^\circ(T)$ as a sum of conformation/composition/sequence specific and nonspecific contributions:

$$\Delta H^\circ(T) = \Delta H_{spec}^\circ(T) + \Delta H_{non}^\circ(T).$$

We propose that the similarity in the magnitude of the observed temperature dependence of $\Delta H^\circ$ [$\Delta H^\circ(T)$] for all polymeric duplexes studied to date (Fig. 2) suggests that, relative to specific enthalpic contributions, $\Delta H_{spec}^\circ(T)$, nonspecific enthalpic contributions, $\Delta H_{non}^\circ(T)$ contribute more than previously assumed. In other words, those interactions that are common to all duplexes are more significant contributors to $\Delta H^\circ(T)$ than those that are different from one duplex to another. Simply stated, the similarities between duplexes exceed the differences.

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