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Salt-Dependent Heat Capacity Changes for RNA Duplex Formation

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RNAs were recently shown to undergo a low-temperature unfolding reaction, a phenomenon called cold denaturation.[1] Cold denaturation occurs when a macromolecule folds in such a way that it is accompanied by a large change in the heat capacity of the polymer (ΔC_P).[2–5] Typically, this ΔC_P term derives from solvent effects and the burial of hydrophobic surfaces upon folding. In the case of nucleic acids, added complexity may arise from residual stacking in the single-stranded state that reduces the amount of hydrophobic surface exposed in the unfolded state.[6] While ΔC_P of protein folding has been studied extensively,[7] the number of studies addressing the ΔC_P of nucleic acid folding is much smaller.[6,8–12] Previous predictions for nucleic acid duplexes had estimated that this unfolding would not occur above temperatures around −120 °C.[13] When cold denaturation of RNA was discovered, the finding indicated that the ΔC_P for RNA folding was significantly larger than previously expected.[14] Thus, the discovery of RNA cold denaturation prompted us to begin a detailed investigation of the role that ΔC_P might play in RNA stability and the solution conditions that affect it. Herein, we report our discovery that the ΔC_P for two simple RNA duplexes (Scheme 1) exhibit a marked ionic strength dependence.

The nature of ΔC_P effects in protein folding has been well studied.[7,15,16] The ΔC_P upon folding can be measured directly through calorimetry or estimated from chaotropic unfolding studies conducted at several temperatures. Empirically, ΔC_P scales with the size of the protein and the extent of hydrophobic burial during folding.[7] One explanation for this phenomenon involves the release of water molecules that form clathrate structures around the hydrophobic side chains in the unfolded state. While the magnitude of the ΔC_P for protein folding is modulated by organic cosolvents,[17] this parameter is relatively insensitive to the solution ionic strength, unless specific ion binding occurs upon folding.[18] Much less is known about the solution parameters that affect ΔC_P for nucleic acid folding. One might predict that ΔC_Ps would be modulated by the ionic strength of the solution through site-specific binding or ion condensation effects.

RNA duplex formation is the most fundamental element in RNA folding. Although residual base stacking can be present in unfolded RNAs, the base pairing process generally is coupled to base stacking and thus involves occlusion of the planar hydrophobic surface. Since water is excluded as part of this process, analogy to protein folding suggests that a significant ΔC_P should accompany duplex formation. In many studies of duplex thermodynamics, ΔC_P effects are ignored. When they are considered, it is often as a small per base pair contribution of −20 to −200 cal mol^{-1} bp^{-1} K^{-1}.[6,8,12] The quantitative disparity might imply significant solution effects or nearest neighbor contributions which affect the magnitude of the ΔC_P.

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Experimental procedures and DSC experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

A major difference between proteins and nucleic acids derives from the polyanionic charge of the phosphodiester backbone. Nucleic acids condense counterions to their surfaces to screen this charge. These ions play an important role in the fast collapse and folding processes of the RNA[19] and strongly influence folding parameters such as ΔG, ΔH, and ΔS.[20,21] Despite an earlier report probing the ΔC_P of DNA duplexes under low salt conditions,[6] we asked in this study whether the ions might affect the ΔC_P parameter since the condensed ion layer is such an integral component of the solvation shell around the RNA structure.

The heat capacity of duplex formation was studied by analyzing the temperature dependence of enthalpies measured by isothermal titration calorimetry (ITC) for two short RNA duplexes. Figure 1 shows the raw power curve measured during the titration as well as the integrated data fit to a single-site two-state binding model for duplex formation. A linear extrapolation of the long base line was used to correct for background contributions due to mixing and dilution. The titration was repeated over a series of temperatures under otherwise equivalent conditions. Since the heat capacity change can be defined as the temperature dependence of the enthalpy, the slopes of the lines in Figure 2 allow accurate determination of ΔC_P. Over the range of 0.1–1.5 M added NaCl, ΔC_P changes rather dramatically for both duplexes and follows a log-linear relationship (Figure 3). The absolute magnitude of the ΔC_P appears sequence-dependent. The small positive values observed for the lowest NaCl conditions of duplex 2 may relate to end effects since solvation and ion binding are expected to differ significantly in these regions relative to the center of an A-form duplex.[22]

A previous study by Holbrook et al. surveyed salt effects in DNA duplex formation and found no significant trend.[6] That study only assessed from 50 to 120 mM added salt, a range much narrower than the one probed here. The difference between DNA and RNA duplexes is unlikely to explain the contrasting results. More likely, the disagreement derives from leveling effects due to buffer contributions to ionic strength and the relatively narrow range of added salt probed previously.

It should be noted that the low-temperature ΔC_Ps measured in our study differ from those that would be measured by thermal melting. At high temperature, the single-stranded state is prone to be less structured than at low temperature, conditions where more base stacking may be present. The current study has focused on short duplexes to avoid some of the complexities due to selfstructure in the single-stranded state, but additional work on longer duplexes is currently underway.

One can interpret our data on the basis of residual stacking in the single-stranded state. Higher ionic strength conditions have been shown to promote stacking and single-stranded helix formation.[23] Thus at low ionic strength little single-stranded stacking may be present over all of the temperatures studied. Under the higher salt conditions, additional stacking is possible at the lowest temperatures, thus yielding a more negative observed ΔC_P for those transitions. This interpretation was confirmed by comparing DSC scans under high and low salt conditions (see Supporting Information).

In previous studies of heat capacity changes for nucleic acid duplexes, it has been commonplace to assume a constant per base pair contribution. Analysis of Figure 3 shows that such an approximation is dangerous and highly dependent on experimental conditions. It may be more valid for long duplexes where sequence dependencies average out, but at the limit of the short helices currently under investigation, this approximation breaks down. Under those conditions, one expects to observe either basecomposition or nearest neighbor-related phenomena. Based on the sequences used here, it is likely that nearest neighbor effects are involved, but it is unclear whether the effects derive from changes in the folded versus the unfolded state.

Ionic strength is one of the fundamental properties of an aqueous solution. Understanding how it modulates macromolecular stability helps to measure the accuracy of our conceptual models of biochemical phenomena. Our current study highlights the fact that large gaps still exist in our knowledge and understanding of RNA folding thermodynamics, even on systems as simple as A-form RNA duplexes.

Acknowledgment

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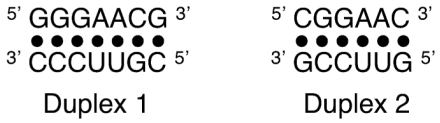

Scheme 1.

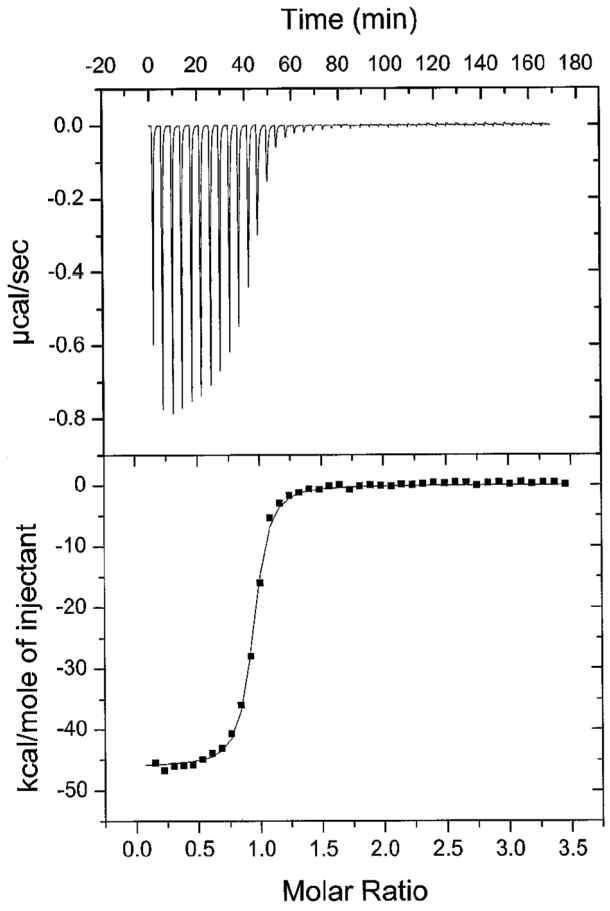

Figure 1.

Typical data from an ITC experiment. A 75 μM solution of strand 1B was titrated into 1.4 mL of 5 μM strand 1A equilibrated at 15 °C. Both RNAs were in 50 mM HEPES, pH 7.5 and 1 M NaCl. This run yielded fitting parameters: $\Delta H = -46.1 \pm 0.2$ kcal mol^{-1}; $\Delta S = -125$ cal mol^{-1} K^{-1}; $K_a = 4.3 \times 10^7$ M^{-1}; $N = 0.91 \pm 0.01$.

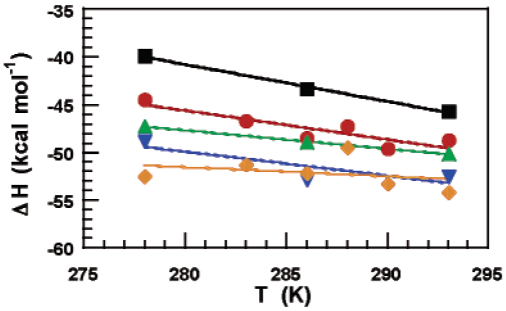

Figure 2.

Plot of Δ*H* versus temperature used to determine ΔC_P for duplex 1 formation in 50 mM HEPES, pH 7.5 as a function of added NaCl: 100 mM (orange ♦), 300 mM (green ▲), 500 mM (blue ▼), 1 M (red ●), 1.5 M (black ■). Data points are the mean of three independent trials.

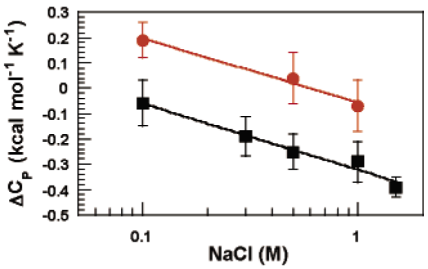

Figure 3.
Semilog plot showing the relationship between ΔC_P and concentration of added NaCl for duplex 1 (black ■) and duplex 2 (red ●).