Supporting Information for

Salt-Dependent Heat Capacity Changes for RNA Duplex Formation

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Supporting Data

Differential Scanning Calorimetry Experiments.

DSC experiments were run in support of the idea that single-stranded stacking interactions might be in part responsible for the salt-dependent duplex ΔC_Ps observed by ITC. Figure S1 shows the DSC traces for duplex 1 at 0.1 and 1.5 M NaCl. The duplex data show exactly what one expects to see for duplex melting, with an increase in the T_M (11 °C in this case) at higher salt. These data can be fit nicley to a two-state model with a heat capacity change. However, the ΔC_P values that derive from this analysis are highly dependent upon the temperature range used during fitting and are not reproducible across multiple runs. This exemplifies the need to focus on ITC as the method of choice for obtaining ΔC_P values for duplex formation.

Figure S1 also shows the DSC scans of the purine rich oligo 1A at the same salt concentrations as used for the duplex. In order to obtain sufficient signal to observe, the DSC experiments were run at concentrations 10 – 30-fold higher than those used for the same oligo in the ITC experiments. The feature of importance with respect to the results in this paper is the low temperature signal near 10 °C observed under high salt conditions. This transition cannot be fit due to the low temperature of the T_M and the inability to obtain a lower baseline. The differences are extremely salt dependent,

however, as displayed by the contrast between the 0.1 M and 1.5 M NaCl conditions. Experiments with oligo 1B do not show a significant signal for the low temperature transition most likely due to the reduced stacking propensity of the pyrimidine bases that dominate this sequence. This leads to the possibility that part of the sequence dependence of ΔC_P values may in fact derive from component equilibria in the single-stranded state. In other words, the ability of the single stranded precursors to form intermediate, stacked states may impact strongly on the overall ΔC_P observed for duplex folding transitions.

Materials and Methods.

Preparation of RNAs. Oligonucleotides were chemically synthesized (Dharmacon, Inc.), deprotected according to the manufacturer's protocol and resuspended in sterile ddH_2O. Purity was assessed by PAGE and was generally >95% after deprotection. The sequences of the RNAs were as follows: 1A: 5'-GGGAACG-3'; 1B: 5'-CGUUCCC-3'; 2A: 5'-CGGAAC-3'; 2B: 5'-GUUCCG-3' where strands 1A and 1B form duplex 1 and 2A and 2B form duplex 2. Concentrations of the RNA stock solutions were determined by A_{260} using calculated extinction coefficients for each oligonucleotide (1A: 73,600 M^{-1} cm^{-1}, 1B: 58,500 M^{-1} cm^{-1}, 2A: 59,200 M^{-1} cm^{-1}, 2B: 55,800 M^{-1} cm^{-1}). (Biopolymer calculator: http://paris.chem.yale.edu/extinct.html)

ITC Studies. A VP-ITC titration calorimeter (MicroCal) was used for all measurements. RNA samples were prepared by diluting a small volume of the RNA stock into 50 mM HEPES, pH 7.5 (Sigma-Aldrich) plus a specific amount of NaCl (EMD Chemicals) which varied from 0.1 M to 1.5 M, depending on the experiment. Samples were then

annealed 2 min at 95°C and cooled at room temperature prior to use. All buffers were prepared from stock solutions on the day of use and extensively degassed under vacuum. Each experiment consisted of approximately forty 7 μl injections of oligonucleotide at 75 μM into 1.4 mL of the complementary strand at 5 μM. Both the syringe and cell RNA samples were prepared in identical buffers to minimize background from the heat of dilution due to mismatched buffers. Sample stirring was set at 310 rpm for all measurements. For duplex 1, strand 1B was titrated into strand 1A and for duplex 2, strand 2B was titrated into strand 2A. ITC experiments were repeated over a temperature range of 278 K to 293 K. The short length of the duplexes precluded measurements at higher temperatures due to the need to stay well below their T_Ms to obtain high quality data.

ITC data were analyzed with ORIGIN software (MicroCal Inc., version 7.0). The raw data were integrated and normalized resulting in a plot of ΔH per moles of injectant versus molar ratio. In each experiment, a long upper baseline was collected after the binding transition of the duplexes had been saturated (see Figure 1). The slope of this upper baseline reflected background heat present throughout the experiment due to factors such as the heat of mixing and heat of dilution. The terminal ten to fifteen points of the upper baseline in each experiment were fit to a straight line, which was subsequently subtracted from the entire dataset. A total ΔH for each reaction was obtained by fitting the plot of ΔH per mole of injectant versus molar ratio using ORIGIN software. Data were fit to a single-site, two-state binding model to obtain ΔH, K_a and N for duplex formation under each condition. Across all of these experiments, the stoichiometry (N) was found to be 1.0 ± 0.1, as expected for duplex formation.

Experiments were repeated at several temperatures and ΔH was plotted versus temperature using Kaleidagraph (Synergy Software). Data were fit to a linear model to obtain ΔC_P under each condition. Standard errors for the ΔC_P values were obtained from the linear regressions of the ΔH versus T data sets using Kaleidagraph.

Differential Scanning Calorimetry.

A VP-DSC differential scanning calorimeter (MicroCal) was used for all of the DSC experiments. The DSC experiments required 800 μL samples at RNA concentrations ranging from 50 – 150 μM for single-stranded experiments or at 50 μM for duplex experiments. Single-stranded RNA oligos were diluted into appropriate buffers (50 mM HEPES and 0.1 – 1.5 M NaCl) and annealed for 90 seconds at 90 °C. The samples were then allowed to equilibrate to 20 °C in a Thermovac apparatus and degassed by applying partial vacuum. The DSC was allowed to establish thermal history by running it through the experimental cycle on buffer alone before beginning the experiment. Thermal scans were measured from 4 °C to 90 °C scanning at a rate of 90 °C/hr with a 15 min pre-equilibration prior to each thermal cycle. Samples were loaded in cycle between 30 and 15 °C with a sample pre-equilibrated at 20 °C. The manufacturer's protocols were used to purge the cell of bubbles and level it at exactly 0.513 mL using the specially modified needle provided by the manufacturer. Data were analyzed in Origin following the manufacturer's procedures. A background correction was applied by subtracting a reference scan of buffer against buffer. The thermal scans were normalized based on the RNA concentration in a given experiment and then baseline corrected to allow scans to

be overlaid with one another. Data were collected in duplicate or triplicate to ensure reproducibility.

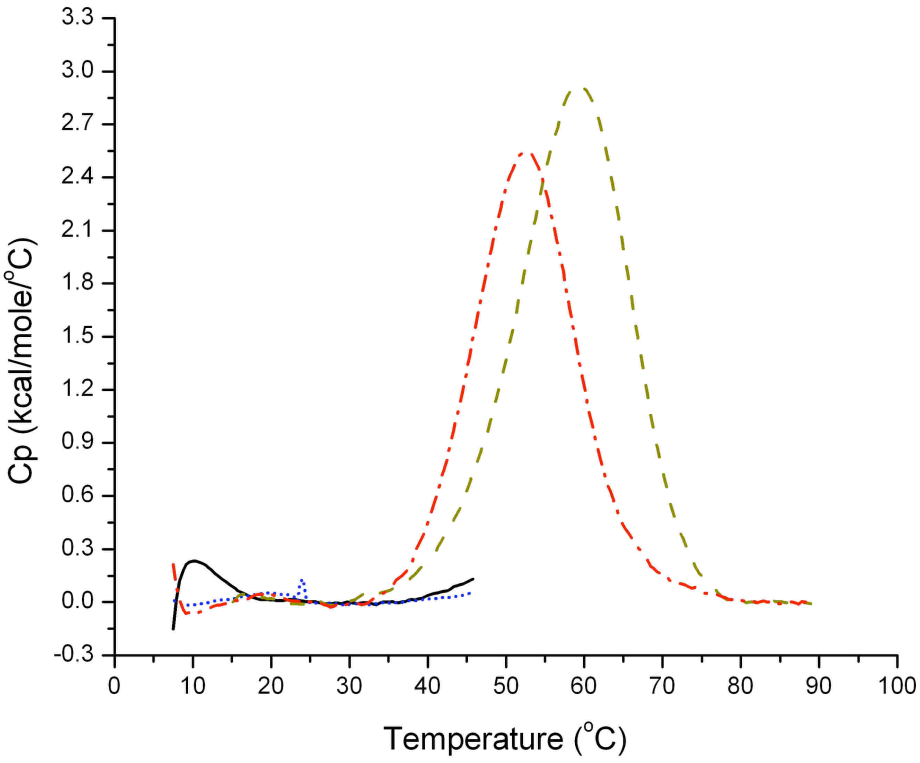

Figure S1. Overlaid DSC thermal profiles for 150 μM oligo 1A in 1.5 M NaCl (solid black line), 150 μM oligo 1A in 0.1 M NaCl (dotted blue line), 50 μM Duplex 1 in 1.5 M NaCl (dashed green line) and 50 μM Duplex 1 in 0.1 M NaCl (dashed/dotted red line). All samples contained 50 mM HEPES buffer, pH 7.5. The duplexes show the expected change in T_M as a function of increased NaCl. The purine rich single-strand shows an extra transition at about 10 °C in the presence of high salt that is not observed under low salt conditions. This single stranded unstacking transition cannot be fit due to the inability to collect a sufficient low-temperature baseline.