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Thermodynamic Contributions of Deoxyuridine Loops to the Folding of DNA Straight Hairpin Loops with d(GCGC/GCGC) Stems

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ABSTRACT

The global stability of DNA molecules depends on base stacking, base-pairing, ionic and hydration contributions. To understand the mechanisms that govern the many biological roles of nucleic acids, it is essential to have a complete physical description of the folding of nucleic acids, including ion and water binding. We used a combination of temperature-dependent UV spectroscopy and differential scanning calorimetry to investigate the effect of the loop length on the unfolding thermodynamics of a set of DNA stem-loop motifs with the following sequence: d($^{5'}$-GCGCUnGCGC), where n = 3, 5, 7, and 9.

The folding of each hairpin loop resulted in a monophasic transition that was monomolecular. The favorable folding of each hairpin (negative ΔG^o) in this set resulted in a thermodynamic compensation of a favorable enthalpy with an unfavorable entropy contributions, enthalpy contributions correspond to formation of base-pair stackings while the unfavorable entropy contributions correspond mainly to the immobilization of counterions, as the length of the uracil hairpin loop increases, the stability of the hairpin decreases (the free energy term becomes more positive), due to a less favorable entropic contribution because more counterions are binding. Relative to a similar set of thymine hairpin loops, we measured less favorable free energy terms because of the lower thermal stability of the uracil hairpins (lower T_M's) and their lesser impact of ion binding. Supported by Grant MCB-1912587 from the NSF.

MATERIALS & METHODS

Materials. All DNA molecules were synthesized by IDT, reverse-phase HPLC purified, desalted on a G-10 Sephadex column, and lyophilized to dryness prior to experiments. The sequences of the four stem-loop DNA oligonucleotides, Scheme 1, and their designations are reported in Table 1. The concentration of each oligomer solution was determined from absorbance measurements at 260 nm at 90 ºC using molar absorptivity's in the range of 96.8 to 157.4 mM^{-1} cm^{-1} of strands. These values were calculated by extrapolation of the tabulated values of the dimers and monomer bases from 25 ºC to high temperatures, using procedures reported earlier. Buffer solutions consisted of 10 mM sodium phosphate buffer and pH 7.0, adjusted with different salt concentrations up to 0.2 M NaCl. All chemicals used in this study were reagent grade.

***Temperature-Dependent UV Spectroscopy* (UV Melting Curves).** Absorbance versus temperature profiles (UV melting curves) were measured at 260 nm with a thermoelectrically controlled Aviv 14 DS UV/vis spectrophotometer (Lakewood, NJ). The absorbance was scanned with a temperature ramp of approximately 0.6 ºC-mim-1. The analysis of the shape of the melting curves yielded transition temperatures, T_M, which corresponds to the inflection point of the helix− coil transitions. To determine the molecularity of the transition(s) of each DNA stem-loop motif, we investigated the dependence of T_M over at least a 10-fold range of total strand concentration. If the TM remains constant in this range of strand concentration, it indicates a monomolecular or intramolecular transition. Additional UV melting curves were obtained as a function of salt concentration to determine the differential binding of ions.

***Differential Scanning Calorimetry* (DSC).** The total heat required for the unfolding of each hairpin loop was measured with a VP-DSC differential scanning calorimeter from Microcal (Northampton, MA). These thermograms were obtained with a temperature ramp of ~ 0.75 ºC min-1 with hairpin ranging in concentration from 0.13 to 0.050 mM. Standard thermodynamic profiles and T_M's are determined from the DSC experiments using the following relationships:

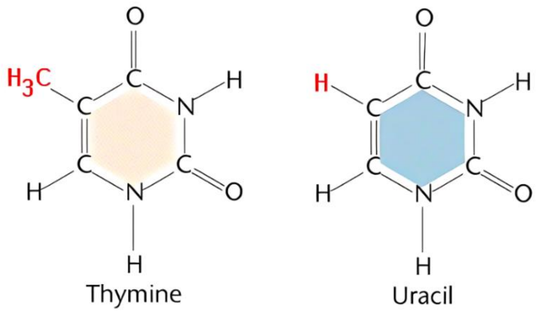
$$\Delta H_{cal} = \int \Delta C_p dT,\ \Delta S_{cal} = \int (\Delta Cp/T) dT \text{ and } \Delta G^\circ_{(25)} = \Delta H_{cal} - T\Delta S_{cal}$$

where ΔCp is the anomalous heat capacity of the oligonucleotide solution during the unfolding process, ΔH_{cal} and ΔS_{cal} are the unfolding enthalpy and entropy, respectively, both assumed to be temperature-independent, and $\Delta G^o_{(25)}$ is the free energy at 25ºC.

Determination of the Differential Binding of Counterions. Additional UV melting curves were obtained as a function of salt concentration to determine the differential binding of counterions, $\Delta n_{Na}{}^+$. This $\Delta n_{Na}{}^+$ linking number is measured experimentally using the following relationship:

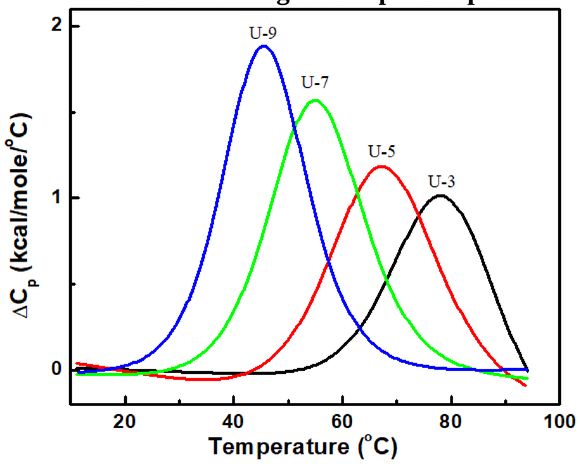
$$\Delta n_{Na+} = (\Delta H_{cal}/RT_M{}^2)[\partial T_M/\partial \ln[Na^+]]$$

The $(\Delta H_{cal}/RT_M{}^2)$ term is determined directly from DSC experiments, whereas the term in brackets is determined from the slopes of the plots of T_M as a function of the activity of salt, ranging from 16 to 216 mM. The activity term is converted to its concentration term by multiplying it by the 0.9 constant over the 10 - 210 mM range of salt concentration.

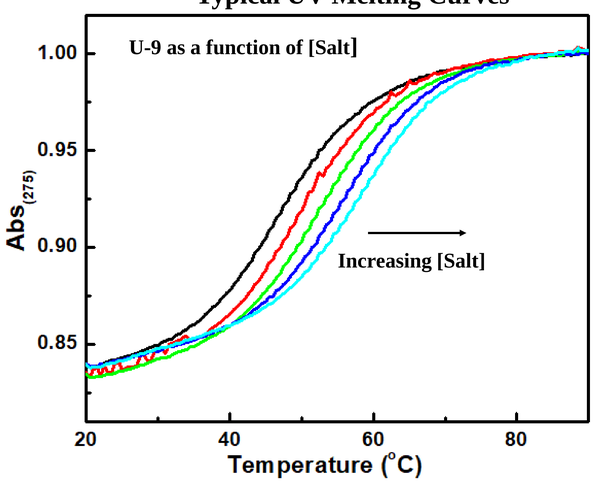


Scheme 1: Uracil Stem-Loop Motif, Hairpin

DSC Unfolding of Hairpin Loops



Typical UV Melting Curves



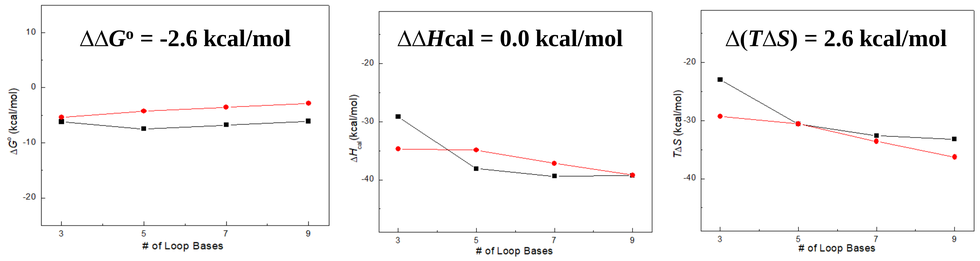
RESULTS

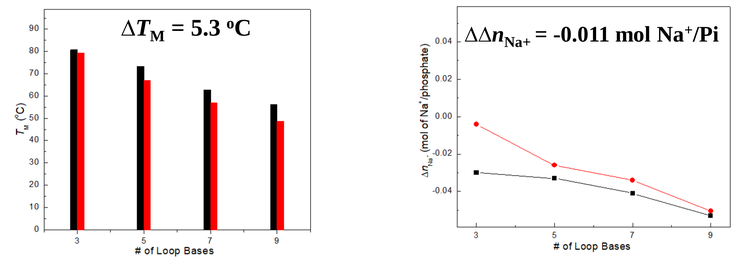
Table 1. Thermodynamic Profiles for the Folding of Hairpins with Uracil Loops

	Tm (°C)	ΔH_{Cal} (kcal/mol)	$\Delta G_{(25)}$ (kcal/mol)	$T\Delta S$ (kcal/mol)	Δn_{Na+} (mol/Phosphate)
U-3	**79.4**	**-34.7**	**-5.4**	**-29.3**	**-0.0042**
U-5	**66.9**	**-34.9**	**-4.3**	**-30.6**	**-0.0261**
U-7	**56.9**	**-37.2**	**-3.6**	**-33.6**	**-0.0342**
U-9	**48.6**	**-39.2**	**-2.9**	**-36.3**	**-0.0505**

Differential Thermodynamic Profiles of Hairpin Loops with Thymines or Uracils

The above thermodynamic profiles (containing uracil loops) are subtracted from the thermodynamic profiles of a similar set of hairpins with thymine loops (C.Reiling et.al., **2015**, *J Phys. Chem 119*, 1939-46), yielding the graphs below.





CONCLUSIONS

- **The folding of each hairpin for the set of uracil loops resulted in a monophasic transition that was monomolecular.**
- **The favorable folding of each hairpin (negative ΔG^o) in this set resulted in a thermodynamic compensation of a favorable enthalpy with an unfavorable entropy compensation. This is due mainly to an uptake of counterions.**
- **The increase in the length of the loop resulted in a negative correlation with the folding free energy of each hairpin, due to less favorable entropy contributions.**
- **Relative to a similar set of thymine hairpin loops, we measure less favorable free energy terms with these hairpins. This is due to a lower thermal stability of the uracil hairpins and the lower impact of ion binding.**