Energetic and Hydration Contributions of the Removal of Methyl Groups From Thymine to Form Uracil in G-quadruplexes

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Supporting Information

EXPERIMENTAL

Materials. The deoxyoligonucleotides with 5' to 3' sequences:, $G_2U_2G_2UGUG_2U_2G_2$ (*G2-U*) and $G_2T_2G_2TGTG_2T_2G_2$ (*G2*) were synthesized by the Core Synthetic Facility of the Eppley Research Institute at UNMC, HPLC purified, and desalted by column chromatography using G-10 Sephadex exclusion chromatography. The concentration of the oligonucleotide solutions was determined spectrophotometrically at 260 nm and 80 °C using a Perkin-Elmer Lambda-10 spectrophotometer and the following molar absorptivities (in single strands): 156 $mM^{-1}cm^{-1}$ (*G2-U*) and 146 $mM^{-1}cm^{-1}$ (*G2*). These values were obtained using previously reported procedures.[1-3] Inorganic salts from Sigma were reagent grade, and used without further purification. All

measurements were made in buffer solutions containing 10 mM Cs-HEPES at pH = 7.5, adjusted to the desired salt concentration with KCl. Oligonucleotide solutions were prepared by dissolving the dry and desalted ODN in buffer, the Cs^+/K^+ ion exchange was done initially by heating the oligomer solution to 100 °C for 5 minutes and cooled to room temperature over 25 minutes.

Temperature-Dependent UV Spectroscopy. Absorbance versus temperature profiles (UV melting curves) were measured at 297 nm with a thermoelectrically controlled Aviv Spectrophotometer Model 14DS UV-VIS (Lakewood, NJ). The temperature was scanned from 10 to 100 °C at a heating rate of ~0.6 °C/min. Using standard procedures,[3-4] shape analysis of the melting curves yielded transition temperatures, T_M, which are the temperatures at the midpoint of the helix-coil transition. The transition molecularity for the unfolding of a particular complex was obtained by monitoring the T_M as a function of the strand concentration. Intramolecular complexes show a T_M-independence on strand concentration.

Circular Dichroism Spectroscopy (CD). The conformation of each G-quadruplex in the presence of K^+-ions was obtained from analysis of the CD spectra. CD spectra were measured with an Aviv Circular Dichroism Model 202SF spectrometer (Lakewood, NJ) equipped with a peltier temperature control system. Spectra were obtained from 340 nm to 220 nm in 1 nm increments using free strained quartz cuvettes with a pathlength of 0.1 or 1.0 cm. The reported spectra correspond to the average of at least two scans. The conformation of each complex was obtained by simple inspection of the CD spectra at low temperatures, by comparison with known spectra of G-quadruplexes with guanines in the antiparallel orientation.[5] CD melting curves were obtained by following the changes in ellipticity at 291 nm, as a function of temperature using a heating rate of ~0.9 °C/min. Analysis of the CD melting curves yielded T_Ms.[3]

Differential Scanning Calorimetry (DSC). The total heat required for the unfolding of each G-quadruplex was measured with a VP-DSC differential scanning calorimeter from Microcal (Northampton, MA). Standard thermodynamic profiles and T_Ms are obtained from a DSC experiment using the following relationships:[3] $\Delta H_{cal} = \int \Delta C_p \, dT$; $\Delta S_{cal} = \int \Delta C_p/T \, dT$, where ΔC_p is the anomalous heat capacity of the oligonucleotide solution during the unfolding process, ΔH_{cal} is the unfolding enthalpy, and ΔS_{cal} is the entropy of unfolding. The free energy term, $\Delta G°(T)$, is calculated at 20 °C using the Gibbs equation, $\Delta G°(T) = \Delta H_{cal} - T\Delta S_{cal}$. The ΔC_p terms are also obtained by measuring T_M and ΔH_{cal} under different salt conditions, then the variation of ΔH_{cal} with respect to temperature, $d\Delta H_{cal}/dT_M$, will give us the ΔC_p.

Determination of the Differential Binding of Counterions and Water. Additional UV melting curves, as a function of salt and osmolyte concentrations, were performed to determine the differential binding of counterions, Δn_{K^+}, and water molecules, Δn_W, between the quadruplex and coil states. These linking numbers, Δn_{K^+} and Δn_W represent the uptake (or release) of counterions and water, respectively, for the helix-coil transition of each complex, and are measured experimentally using the following relationships:

$$\Delta n_{K+} = 1.11 \, [\Delta H_{cal}/RT_M^2] \, (\partial T_M/\partial \ln [K^+]) \qquad (1)$$

$$\Delta n_W = [\Delta H_{cal}/RT_M^2] \, (\partial T_M/\partial \ln a_W) \qquad (2)$$

The term in brackets in Eqs. 1 and 2 are determined directly from DSC experiments while the terms in parentheses are obtained from UV melting curves. These later terms correspond to the slopes of the lines of the T_M dependences on the concentration of potassium and water activity, respectively.[6] The value 1.11 in Eq. 1 is a constant, which is used to convert concentration into ionic activity terms. The activity of water is varied by using different concentrations of a co-solute, ethylene glycol, which does not interact with DNA.[7] The osmolality of these solutions

were obtained using a UIC vapor pressure osmometer Model 830, calibrated with standardized NaCl solutions.

Pressure Perturbation Calorimetry (PPC). The heat change (ΔQ) resulting from a pressure change (ΔP) above a solution is measured using the VP-DSC differential scanning calorimeter from Microcal (Northampton, MA) equipped with a PPC accessory. A complete description of this novel technique can be found elsewhere.[8] Prior to the PPC scan, a DSC melt is carried out to determine the temperature range and temperature step to be used in the PPC experiment. A sample solution (sample cell, 0.7 or 1.1 mM in strands) is allowed to equilibrate against the same buffer solution (reference cell) at constant temperature and external pressure. The external pressure is then increased by ~50 psi, causing heat to be absorbed differentially by the sample and reference cells. These heats, ΔQ, are obtained from integration of the compression and decompression peaks resulting from switching the external pressure on and off at particular temperatures determined by the DSC curve. The resulting values of ΔQ are then used to measure the apparent coefficient of thermal expansion, $\alpha(T)$, from integration of the relationship: $(\partial Q/\partial P)_T = -T(\partial V/\partial T)_P = -T\alpha(T)V$; yielding: $\Delta Q = -TV\alpha(T)\Delta P$; where V is the apparent molar volume of the solute.

For a two component system: $\alpha(T) = \alpha_o - \Delta Q/(TV\Delta P)$, where α_o is the thermal coefficient of the solvent. Integration of $\alpha(T)$ over the temperature range of the unfolding reaction, $\int \alpha(T)dT$, yields the relative volume changes of the solute, $\Delta V/V$, where ΔV is the unfolding volume of the macromolecule. The value of V is obtained from the equation[9]: $V = M/\rho_o - (\rho_o - \rho)/\rho_o C$, where M is the molecular mass of a protein, ρ_o and ρ are the densities of the solvent and solution, respectively. The density of the solutions used in the PPC experiment is measured with an Anton Paar (Graz, Austria) DMA densitometer in the differential mode, using two 602-M micro

cells, each with a volume of ~150 μL. The reference cell is filled with water while the measuring cell is filled with solution or buffer. The density, ρ, is calculated from the oscillation period T of the cell using the following relationship: $\rho = AT^2 + B$, where A and B are constants determined from calibrating densities (and periods) of water and air.

References

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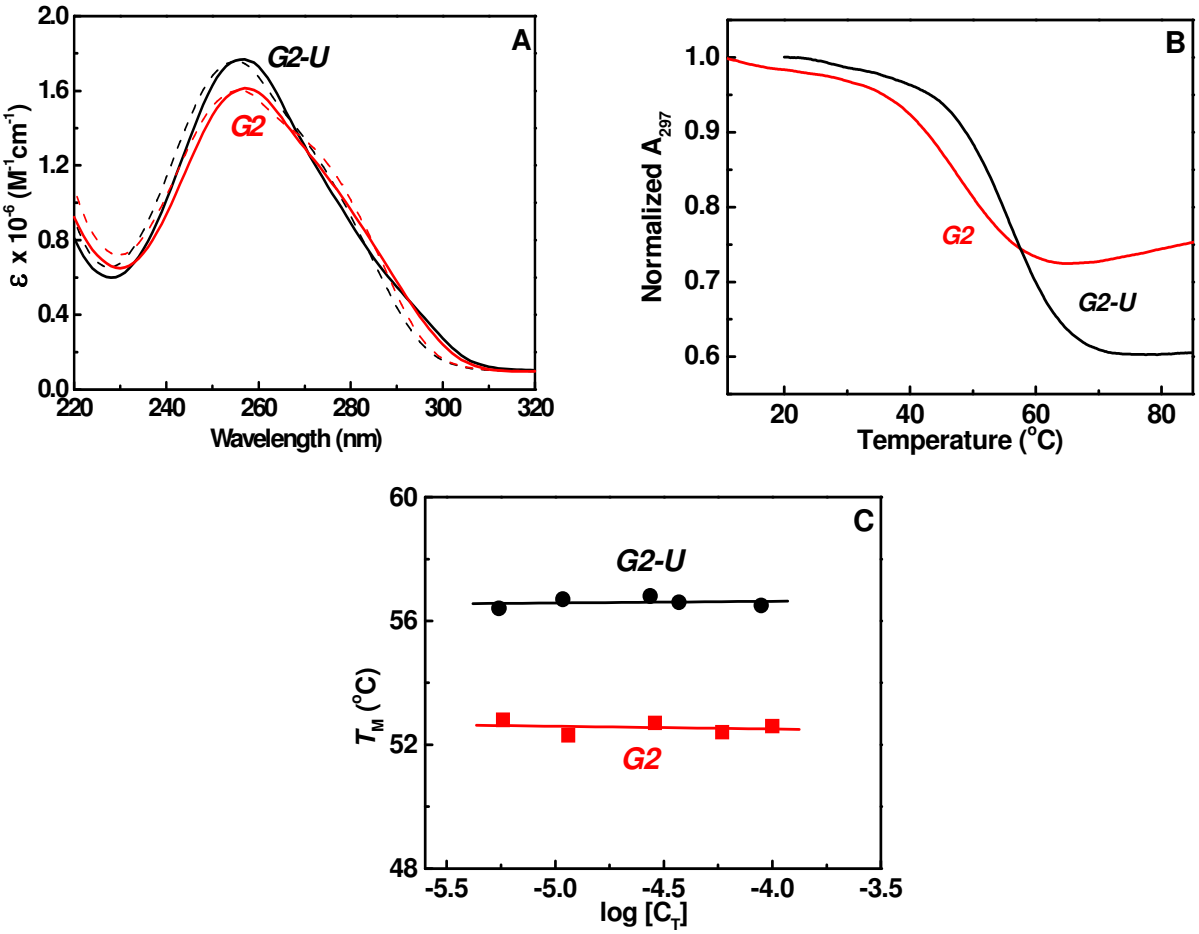

Figure S1. UV Spectroscopy of G-quadruplexes in 10 mM Cs-HEPES buffer, 100 mM KCl, at pH 7.5. A) UV spectra of *G2-U* and *G2* at 20 ºC (solid line) and 80 ºC (dashed line). B) Typical UV melts at 297 nm. C) T_M-dependence on strand concentration.

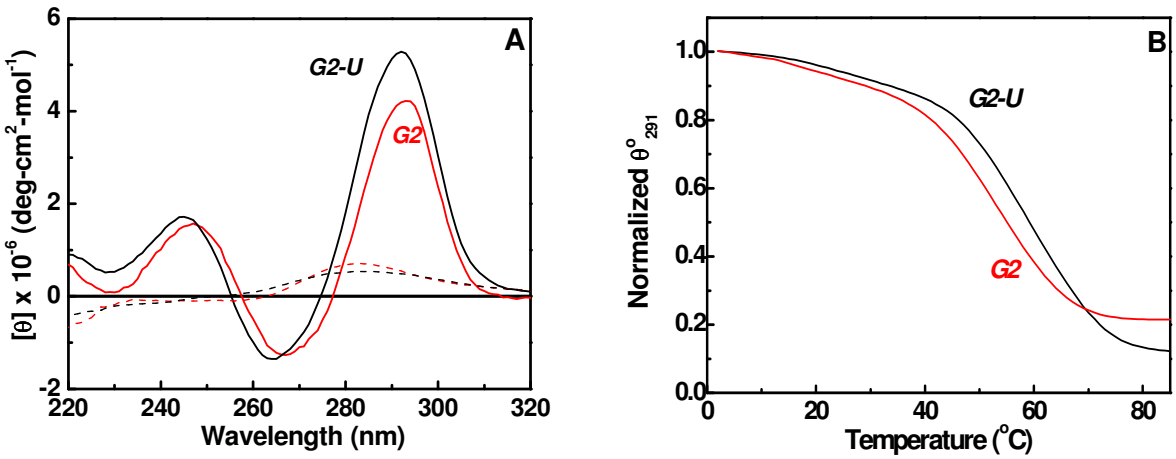

Figure S2. Circular Dichroism of G-quadruplexes in 10 mM Cs-HEPES buffer, 100 mM KCl at pH 7.5 and total strand concentration of ~6 μM.. A) CD spectra of *G2-U* and *G2* at 20 ºC (solid line) and 80 ºC (dashed line). B) Typical CD melting curves at 291 nm.

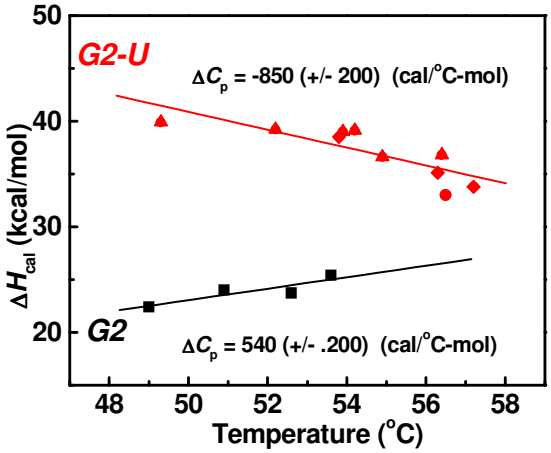

Figure S3. Heat capacity effects of G-quadruplexes in 10 mM Cs-HEPES buffer at pH 7.5 and at varied concentrations of KCl.

Table 1S. Pressure Perturbation Calorimetric Results for the Unfolding of *G2-U* and *G2*.

Pre Transition		Post Transition				
α x10^3 (deg^{-1})	$d\alpha/dT$ x 10^5 (deg^{-2})	α x10^3 (deg^{-1})	$d\alpha/dT$ x 10^5 (deg^{-2})	$\Delta V/\Phi_V$ x 10^3	Φ_V x 10^{-3} (cm^3-mol^{-1})	ΔV_{Unf} (cm^3- mol^{-1})
			G2-U			
1.37	-3.29	0.60	-0.33	-10.4	2.6	-27
			G2			
1.04	-0.91	0.51	-0.84	-6.8	2.7	-18

All experiments were performed in 10 mM Cs-HEPES buffer, 50 mM KCl, at pH 7.5. Experimental errors as follows: α (±3%), $d\alpha/dT$ (±11%), $\Delta V/\Phi_V$ (±13%), Φ_V (±5%), ΔV (±12).