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Thermodynamic Characterization of Human Telomere Quadruplex Unfolding

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Abstract

The 3'-terminal extension of eukaryotic chromosomes are a unique example of functional single-stranded DNA. Human telomeres are constructed of the repeated DNA sequence 5'-d(TTAGGG). Four-repeats of human telomeric DNA have been characterized by high-resolution techniques to be capable of forming at least five distinct monomeric conformations. The predominant solution topology is influenced by solution conditions and the presence of 3'- or 5'-flanking residues. This study extends recently described sequential unfolding mechanisms for human telomeric quadruplexes to eight telomeric DNA sequences that form three unique topologies. Thermal unfolding monitored by circular dichroism is subjected to singular value decomposition to enumerate the number of significant spectral species required to model the unfolding process. Thermal denaturations are found to be best modeled by a four-state sequential mechanism with $G_{ave} = -8.8 \text{ kcal mol}^{-1}$. The thermal unfolding was also investigated in 50% (v/v) acetonitrile. Under dehydrated conditions, quadruplex thermal denaturations are best modeled by a three-state sequential unfolding mechanism. Dehydrated quadruplexes demonstrate increased thermal stability with $G_{ave} = -12.7 \text{ kcal mol}^{-1}$. A common spectral intermediate is shared amongst human telomere quadruplex thermal unfolding regardless of starting topology and hydration state. This unfolding intermediate was recently characterized as an ensemble of triple-helical structures for a 22-nt human telomeric quadruplex.

Introduction

The biological relevance of G-quadruplexes, which have potential functions at each step of the central dogma, has become an important research topic.^{1,2} For example, enzymes involved in DNA replication interact with quadruplex structures whose unwinding is critical for efficient replication through guanine rich regions of the genome.^{3,4} Cells deficient in the quadruplex unwinding enzyme REV1 demonstrate a loss of repressive markers and epigenetic instability due to uncoupled histone recycling during DNA synthesis.⁵ In addition, proper maintenance of the telomeric overhang requires the processive unwinding of quadruplex structures by the POT1-TPP1 complex.⁶ Quadruplexes also have important regulatory functions in gene transcription and translation. Formation of quadruplex structures in oncogenic promoters modulate gene expression,⁷ while quadruplex forming sequences in untranslated regions of mRNA regulate protein production.⁸ Quadruplexes offer unique structures for targeting by small molecules that can potentially modulate biological functions, gene expression, and protein production.

Studies often demand high-resolution structures for development of ligands with high selectivity for distinct quadruplex conformations.^{9,10} Quadruplex structures nominally contain two or more stacked G-tetrads stabilized by the coordination of monovalent cations within the central core. Quadruplex topologies can differ in molecularity, glycosidic bond angles, participating strand orientation, and connecting loop regions.¹¹ These variations in structure inherently complicate quadruplex systems through development of heterogeneous solution conformations.¹² Conformational heterogeneity is exemplified by human telomeric quadruplex (ht-quadruplex) systems. Human telomeres consists of repetitive DNA sequences in which 100–200 bases are found as a single-stranded 3'-overhang.¹³ Sequences taken from the human telomeric repeat, 5'-d(TTAGGG)_n, are capable of forming quadruplex structures *in vitro*.¹⁴ Through the use of high-resolution techniques, five distinct monomeric ht-quadruplex topologies have been resolved. Although the monomeric ht-quadruplexes contain the same core 21-nt sequence (5'-d(GGG(TTAGGG)₃)), the addition of flanking residues significantly perturb the quadruplex systems into distinct folding topologies.

The first high-resolution study of ht-quadruplexes evaluated a 22-nt sequence in the presence of Na⁺. Under these conditions, this sequence adopted a monomeric anti-parallel "basket" topology with a three-stacked G-tetrad core and loops ordered 5' to 3' of edgewise, diagonal, edgewise.¹⁵ This was followed by an X-ray crystallographic study that demonstrated the same 22-nt sequence formed an all-parallel "propeller" conformation in potassium solution.¹⁶ The propeller conformation has three stacked G-tetrads with all double-chain reversal loops, but has been shown to have unfavorable stability and is unlikely a predominant conformation in pure aqueous solutions.¹⁷

After elucidation of the basket and propeller conformations, three distinct "hybrid" conformations have been resolved using the core 21-nt sequence with different 5' and 3' flanking residues. Hybrid-1 and hybrid-2 conformations exhibit a unique (3+1) strand-orientation, in which three strands are oriented parallel with the fourth strand running anti-parallel, but differ in the orientation of the connecting loop regions. Hybrid-1 has loop regions ordered 5' to 3' of double-chain reversal, edgewise, edgewise,^{18–20} while the hybrid-2 conformation has the reversed 5' to 3' order of connecting loop regions.^{21,22} The hybrid-3 conformation does not have the (3+1) strand orientation, but instead displays an anti-parallel topology resembling the original 22-nt conformation established in Na⁺ with loops ordered 5' to 3' of edgewise, diagonal, edgewise.^{23,24} Hybrid-3 conformations have the unique property of a two-stacked G-tetrad core instead of the three G-tetrads encountered with all previous ht-quadruplex conformations.

Telomeric quadruplex systems are also known to be influenced by the addition of dehydrating co-solvents²⁵ and high concentrations of polyethylene glycols.²⁶ The transition was established to be a change from a hybrid to a propeller conformation in high concentrations of PEG.²⁷ The mechanism of the conformational transformation has been proposed to be driven by molecular crowding, although the propeller conformation occupies a larger molecular volume than the hybrid conformation, in opposition to the precept that molecular crowding favors more compact structures. An analytical method for partitioning excluded volume and preferential interactions²⁸ was recently employed to investigate the mechanism of co-solvent conversion. Findings suggest that the conversion in acetonitrile is

driven primarily by hydration effects,²⁵ whereas preferential interactions significantly contribute to the conformational change driven by large MW PEGs. Therefore, the mechanism of PEG induced conformational changes has been proposed to be conformational selection of the propeller form through a linked equilibrium with PEG binding (unpublished work).

Quadruplexes from the human telomere repeat constitute a unique set of diverse topologies that can be used for a systematic study of thermodynamic differences based on conformation and sequence variation. Such a unique set has been used for hydrodynamic bead modeling to evaluate hydrodynamic differences in size, shape, radius of gyration and sedimentation coefficients between the distinct ht-quadruplex conformations.²⁹ Hydrodynamic characterization through computation methods is capable of accurate estimation of solution properties, bridging the gap between high and low-resolution methodologies. However, many unanswered questions still remain as to how quadruplex structures unfold. Computational studies have shown that triple-helix intermediates are energetically stable compared with quadruplex structures.³⁰ Recent spectroscopic investigations demonstrated that a 22-nt ht-quadruplex unfolds by a complex mechanism requiring multiple intermediate states, and proposed unfolding contains a triple-helix intermediate.^{31,32} Understanding the thermodynamics involved in quadruplex unfolding can aid in the rational design of quadruplex-interactive compounds and improve predictive algorithms used to evaluate quadruplex formation.^{33,34}

Investigated here are the unfolding thermodynamics of eight sequences derived from the human telomeric repeat known to form monomeric quadruplexes. Quadruplexes are evaluated in both aqueous and 50% (v/v) acetonitrile solutions. Singular value decomposition is employed for determination of the number of spectral components necessary to describe the unfolding mechanism. This work includes a detailed thermodynamic characterization of ht-quadruplex unfolding, putting to use recently established unfolding mechanisms.³¹ The results of the thermodynamic characterization are used to rationalize potential intermediate states and elucidate similarities between distinct quadruplex topologies. Finally, differential scanning calorimetry is used to evaluate enthalpy changes for comparison with spectral studies.

Methods

Oligodeoxynucleotides

Nucleic acid sequences, length, extinction coefficients and corresponding PDB names for the ht-quadruplexes are given in Table 1. The eight sequences correspond to high-resolution NMR studies demonstrating a predominant quadruplex topology in potassium solutions. Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA) on a 1- μ mol scale with standard desalting. Lyophilized DNA was dissolved at a concentration of 1 mM in 10 mM tetrabutylammonium phosphate, 1 mM EDTA, pH 7.0 (folding buffer) and stored at 4 °C. All concentrations were calculated from 260 nm absorbance data using nearest-neighbor extinction coefficients.³⁵ Because the extinction coefficients reflect the single-stranded state, normalized CD values may contain error estimated to be $\pm 15\%$ due to DNA concentration.³⁶ When stated, annealing was completed by placement of DNA into

pre-boiling water for 20 minutes, followed by the removal of heat and cooling to room temperature over night. All reagents were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO) at the highest available purity. All plots were prepared in Origin 7.0 software.

Circular Dichroism and Thermal Denaturation Protocol

Thermal denaturation of quadruplexes was monitored using a Jasco J-810 spectropolarimeter (Jasco Inc., Easton, MD) equipped with a programmable Peltier thermostatted cell holder and electronic stirrer. Sample preparation and CD parameters were described previously.³¹ Background CD and absorbance spectra were recorded using 25 mM KCl in folding buffer prepared by addition of a 1M KCl-folding buffer solution. The relatively low potassium concentration was chosen to ensure quadruplex structures are fully folded in solution,³⁷ while allowing the full thermal denaturation profile of the quadruplex system to be investigated.

Thermal denaturation samples were prepared at 3–4 μ M in 25 mM KCl-folding buffer using a 1 cm path length quartz cuvette. CD spectra were collected using instrumental parameters: $280 < \lambda < 350$ nm, 1.0 nm step size, 200 nm/min scan rate, 1.0 nm bandwidth, 2 s integration time, with 4 total scans averaged. For melting experiments, samples were equilibrated in the cuvette holder at 4 °C prior to starting the melt. Melts were collected in duplicate on successive days with data presented from the second melt. Melting experiments were collected with the additional thermal parameters: 4 °C/min ramp, 0.05 °C equilibration with a 60 s delay prior to acquisition. CD data were normalized to ϵ based on DNA concentration using Equation 1, where θ is the CD ellipticity in millidegrees, c is DNA concentration in mol/L, and l is the path length in cm.

$$\Delta\epsilon = \theta / (3298.2 * c * l) \quad (\text{Equation 1})$$

Isothermal CD samples were prepared at 3–4 μ M in 25 mM KCl-folding buffer followed by annealing. For experiments that contained acetonitrile, samples were prepared by dilution into 50% (v/v) acetonitrile in 25 mM KCl-folding buffer. All acetonitrile containing samples were annealed prior to the first melting experiment. Reference scans were collected using 50% (v/v) acetonitrile 25 mM KCl-folding buffer in the absence of DNA.

NMR sample preparation and spectroscopy

NMR samples were prepared by dilution of lyophilized DNA to 0.4–0.5 mM using folding buffer. Once fully dissolved samples were brought to 25 mM KCl and annealed. D₂O and DSS were added to final concentrations of 10% and ≈ 30 μ M, respectively. Samples were loaded into 5 mm Shigemi NMR tubes. NMR spectra were recorded at 20 °C using a 5 mm inverse triple resonance (HCN) probe on a Varian Inova spectrometer at 14.1 T using a cold probe. Diluted NMR samples gave CD spectra matching low concentration samples, indicating the high concentration used did not influence conformation.³⁸

Singular Value Decomposition

Thermal denaturation results were used to construct three-dimensional data matrices as described previously.³⁹ The three-dimensional data matrix contains elements D_{ij} , where each row i corresponds to a melting profile at λ_i and each column j represents a CD spectra collected at temperature T_j . The matrix $\mathbf{D}$ was analyzed by SVD, generating three additional matrices such that $\mathbf{D} = \mathbf{USV}^T$.⁴⁰ Matrix $\mathbf{S}$ is a diagonal matrix whose elements represent the weight of each spectral component. $\mathbf{U}$ is a matrix of basis spectra for each spectral component. $\mathbf{V}$ is a matrix of amplitude values corresponding to a spectral component's change with temperature. SVD was calculated using built-in algorithms in *Mathematica 8.0* (Wolfram Research, Champaign, IL).

SVD represents a model-free interpretation of the spectroscopic results capable of extracting information on the presence of significant spectral species prior to the application of analytical models. Autocorrelation values were calculated for all $\mathbf{V}$ matrices using Equation 2, where $X_{j,i}$ and $X_{j,i+1}$ are the i th and $i+1$ row elements from column j .

$$C(X_j) = \text{Sum}(X_{j,i}X_{j,i+1}) \quad (\text{Equation 2})$$

V-vector autocorrelations (V-autocorrelations) are a measure of the apparent noise of each V-vector. A decrease in V-autocorrelation magnitude indicates a loss in confidence that the V-vector is significant to the spectral changes observed.

Non-linear Regression Analysis

Five unique quadruplex unfolding mechanisms were recently investigated.³¹ These mechanisms included both sequential unfolding and parallel-species unfolding with an increasing number of intermediate species. The mechanism proposed to best described the unfolding of ht-quadruplexes was a four-species sequential mechanism, in which a transition from folded to unfolded occurs through two intermediate states (Mechanism 1). The present study additionally investigates the thermodynamics involved in the melting of ht-quadruplexes in the presence of 50% (v/v) acetonitrile. A kinetic and thermodynamic study of the unfolding of ht-quadruplexes under co-solvents conditions proposed three significant species are required for accurate modeling.⁴¹ A mechanistic investigation was conducted using a three-state sequential mechanism (Mechanism 2) and parallel unfolding mechanism (Mechanism 3).

Quadruplex $\leftrightarrow$ Intermediate 1 $\leftrightarrow$ Intermediate 2 $\leftrightarrow$ Unfolded (Mechanism 1)

Quadruplex $\leftrightarrow$ Intermediate 1 $\leftrightarrow$ Unfolded (Mechanism 2)

Quadruplex 1 $\leftrightarrow$ Unfolded $\leftrightarrow$ Quadruplex 2 (Mechanism 3)

Each mechanism was used to describe the unfolding process by analytical models (Supplemental Equations 1–3). The analytical models were fit using non-linear regression that included the simultaneous minimization of four S-value weighted V-vectors. Analysis resulted in a set of signal amplitude values for each V-vector with globally determined melting temperatures (T_m 1–3) and enthalpy changes (ΔH 1–3). Signal amplitude values are used to construct generated CD spectra for each species involved in the thermal denaturation

process. Generated spectra are mechanism-dependent such that $\mathbf{CF}^T = \mathbf{D} = \mathbf{USV}^T$, where $\mathbf{C}$ is a matrix whose columns contain the isolated spectra of each species, and $\mathbf{F}$ is a matrix of titration profiles from a pure species recovered from non-linear regression analysis.⁴⁰ Best-fit parameters and χ^2 values were incorporated into Monte Carlo simulations as described by Saroff for investigation of parameter uncertainty.⁴²

Differential Scanning Calorimetry

DSC was conducted using a MicroCal VP-DSC (MicroCal Inc., Piscataway, NJ). The calorimeter was thermally equilibrated through sequential scanning of buffer blanks prior to sample scans. Samples were prepared at 0.25–0.3 mM strand concentration in 25 mM KCl-folding buffer with annealing. DSC sample CD spectra were found to match low concentration samples. Samples were scanned successively from 4 – 98 °C using instrumental parameters of 30 °C/hr, 10 min pre-scan equilibration, with medium gain. Thermograms were exported to Origin 7.0 software for baseline corrections and further analysis.

Results

Reproducible Basis Set of Human Telomeric G-quadruplexes

All ht-quadruplex sequences used in this work have been shown to form intramolecular (single-stranded) structures. The sequences can be grouped based on the connecting loop regions into three distinct topologies: hybrid-1 (2GKU, 2HY9, 2JSM), hybrid-2 (2JPZ, 2JSL), and hybrid-3 (2KF8, 2KKA). Figure 1 shows concentration-normalized CD spectra for each ht-quadruplex recorded at 20 °C. The CD spectra have been reproducibly observed using multiple syntheses of the deoxyoligonucleotides, but are sensitive to changes in K^+ concentration and temperature. Ht-quadruplex CD spectra are consistent with hybrid conformations,⁴³ but demonstrate a variety of subtle differences. All ht-quadruplexes give CD spectra with two regions of positive ellipticity near 265 and 290 nm, with the higher intensity at 290 nm. Although three distinct topologies may be present in the set of quadruplexes studied, CD spectra do not contain distinguishable differences to evaluate quadruplex conformation.

The reproducible CD spectra along with their ^1H NMR are shown in Supplemental Figure 1. The ^1H NMR spectra can be used as an informative measurement of quadruplex conformation. The ht-quadruplex imino-region ^1H NMR agrees with published high-resolution topologies for each sequence. Broad peak widths and the presence of smaller intensity peaks indicate the presence of minor amounts of additional conformations in solution, accounting for approximately 15–20% of the total signal. Interestingly, this heterogeneity may reflect the sensitivity of quadruplex conformations to temperature, as will be shown through CD thermal denaturation experiments.

Unfolding of ht-quadruplexes requires a non-two-state mechanism

Unfolding of ht-quadruplexes was monitored using CD spectra collected over the temperature range 4 – 94 °C in 25 mM KCl-folding buffer. A representative set of primary CD results for the thermal denaturation of the hybrid-2 sequence 2JSL is shown in Figure

2A. Temperature-dependent CD spectra demonstrate a decrease in 290 nm intensity concurrent with increased intensity at 265 nm prior to denaturation to a CD spectrum resembling G-rich single-stranded DNA. This is visualized by monitoring the two major peaks, 265 and 290 nm, against temperature (Figure 2B). Multi-wavelength CD intensity demonstrates a non-linear correlation (Supplemental Figure 2), indicating unfolding is not a two-state process.⁴³ Derivative plots of the change in ϵ with respect to temperature for 260 and 295 nm show three unique maximum/minimum values related to distinct melting transitions (Figure 2C). The presence of multiple inflection points recovered from the melting profile indicates the presence of distinct states during the thermal denaturation process. The derivative-derived melting temperatures for the ht-quadruplex are summarized in Supplemental Table 1, and were used as initial estimates for non-linear regression analysis.

SVD Spectral Species and V-Autocorrelations

Thermal denaturation of ht-quadruplexes was used to construct individual three-dimensional matrices of circular dichroism signal intensity based on changes in temperature and wavelength. The matrices were subjected to SVD to investigate the number of significant spectral species required to accurately model the unfolding process. V-autocorrelations were calculated and compared for each ht-quadruplex (Figure 3). V-autocorrelations are greater than 0.9 for the first three spectral species of all ht-quadruplexes. A slight drop in autocorrelation is seen for species 4; however, the loss in magnitude is less than 0.15 for six of the eight quadruplexes (Tel22, 2GKU, 2HY9, 2JSL, 2KF8, 2KKA) with all but one quadruplex (2JSM) having a V-autocorrelation magnitude greater than 0.75. The fifth spectral species shows a drop in V-autocorrelation of 0.35 or greater for six of the eight quadruplexes study, indicating species beyond the first four do not contribute significantly to the spectral signal. V-autocorrelations from quadruplex thermal denaturations indicate the unfolding mechanism requires four unique spectral species.

Human telomere quadruplex unfolding thermodynamics

A four-state sequential mechanism, in which transition from a folded to unfolded state occurs through two distinct intermediate states, was recently proposed for the unfolding of ht-quadruplexes (Mechanism 1).³¹ The analytical model (Supplemental Information Equation 1) was fit by simultaneous analysis of the first four V-vectors for accurate determination of thermodynamic parameters. Weights were implemented based on the significance of the V-vectors using their corresponding singular values (Supplemental Figure 3). The best-fit residuals of global non-linear regression for the hybrid-2 forming G-quadruplex 2JSM are shown in Figure 4. Fit residuals demonstrate the first four V-vectors are well determined by the analytical fitting algorithm. Small spikes in the fit residuals are seen for V-vectors 3 and 4, corresponding to a high temperature melting species. However, because of the rigorous requirements discussed for species and model determination, there is no basis for introduction of a fifth species in an attempt to improve fit residuals.

Resulting best-fit parameters and Monte Carlo obtained error estimates for each ht-quadruplex are shown in Table 2. The eight ht-quadruplexes were accurately modeled by the global analysis of the first four weighted V-vectors, with only one quadruplex (2JPZ)

displaying a large parameter uncertainty. Unfolding of the ht-quadruplexes occurs with three transitions, corresponding to melting temperature ranges of 33.8 – 44.2, 43.7 – 58.5, and 50.7 – 61.7 °C for the first, second, and third transitions, respectively. Although the sequences are treated with the same mechanism, global non-linear regression results define unique thermodynamic differences between the ht-quadruplexes. The Gibbs free energy changes for the three observed transitions are summarized in Table 3. The initial transition demonstrates a relatively low Gibbs free energy change (-0.4 to -1.7 kcal mol⁻¹) for all ht-quadruplexes. The second and third transitions occur with Gibbs free energy changes ranging from -2.26 to -4.30 kcal mol⁻¹ and -2.86 to -6.80 kcal mol⁻¹, respectively. The average Gibbs free energy change for ht-quadruplex unfolding is -8.8 kcal mol⁻¹, with most ht-quadruplexes being separated by less than 2.0 kcal mol⁻¹.

Ht-quadruplexes have a unified unfolding mechanism with a common intermediate

SVD analysis and Monte Carlo results using best-fit parameters were used to reconstruct the melting process based on the four-state sequential unfolding mechanism. Analytical models resulted in signal values used to generate CD spectra for each species of the unfolding process (Supplemental Information Figure 4). Reconstructed CD spectra was used for generalized inspection of quadruplex conformational changes, but cannot be used to rationalize absolute quadruplex topologies. Generated CD spectra show the starting and first intermediate species resemble hybrid-like quadruplex conformations, displaying the highest ellipticity at 290 nm.⁴⁴ The second intermediate species resembles the spectrum recently obtain for a G-rich triple-helical structure.⁴⁵ The generated spectra for the second intermediate species of each ht-quadruplex are shown in Figure 5. The recovery of similar CD spectra from all ht-quadruplexes indicates a common intermediate state exists during the unfolding of ht-quadruplexes, regardless of starting conformation. Although high-resolution structures have not been established for the intermediate spectra, recent biophysical investigations suggest the CD spectra results from an ensemble of triple-helix intermediate states.³¹

The relative concentration of each species and their dependence on temperature is shown in Supporting Information Figure 5. Modulation of relative concentrations for each species with increasing temperature is similar for all ht-quadruplexes. The relative concentration of each species was used to define an unfolding diagram based on the system of ht-quadruplexes investigated (Figure 6). The relative concentration diagram illustrates the unfolding of ht-quadruplexes, starting from an ensemble of quadruplex species that unfold through the presence of two distinct intermediate states. The average relative concentration diagram demonstrates a potential cause of quadruplex solution heterogeneity at 20 °C observed by NMR. The diagram contains nearly 15% of the first intermediate species at 20 °C, with almost equal concentrations of starting and first intermediate species near 37 °C. This heterogeneity results from two species with similar CD spectra (Supplemental Information Figure 4). In summary, unfolding of ht-quadruplexes in the presence of 25 mM potassium requires two distinct intermediate species, with the second intermediate having a common CD spectrum for all ht-quadruplexes.

Thermal denaturation of quadruplexes in 50% (v/v) acetonitrile

Ht-quadruplexes undergo a conformational change upon the addition of 50% (v/v) acetonitrile resulting in CD spectra with maximum and minimum intensities at 265 and 245 nm, respectively (Figure 7). The resulting CD spectra match recent investigations of ht-quadruplexes under dehydrating conditions,²⁵ and was recently determined by NMR to be a propeller topology in the presence of high concentrations of polyethylene glycol.²⁷ The results of thermal denaturation experiments for each ht-quadruplex in 50% acetonitrile were analyzed by SVD and V-autocorrelations were calculated (Figure 8). V-autocorrelations for 50% (v/v) acetonitrile melts suggest that the unfolding of the dehydrated quadruplex is simplified, having only three species with magnitudes greater than 0.75. Six of the eight quadruplexes demonstrate a 0.25 or greater drop in V-autocorrelation magnitude for species four and higher.

Two mechanisms exist for the unfolding of the converted ht-quadruplexes in 50% acetonitrile: Mechanisms 2 and 3 describe the three-species sequential and parallel models, respectively. The parallel model differs from the sequential model by asserting multiple starting conformations are capable of formation, which melt independently to a common unfolded species. Table 4 evaluates the Gibbs free energy change calculated at 20 °C for each model. Both models give accurate fits to the data, but differ in average free energy changes by $-10 \text{ kcal mol}^{-1}$. Because each model resulted in identical χ^2 values and have equivalent degrees of freedom, it is not possible to statistically differentiate the models based on F-statistic or Akaike Information Criterion.⁴⁶

We propose that the three-species sequential model (Mechanism 2) best represents dehydrated ht-quadruplex unfolding based on two criteria. First, NMR studies indicate the presence of homogenous samples in the presence of co-solvents,^{18,25,47} with sharp imino-region peaks with minimal background intensity. Second, the Gibbs free energy changes from the sequential model demonstrate a -4 kcal mol^{-1} increase in stability compared to aqueous results. Use of the parallel model would require an additional $-14 \text{ kcal mol}^{-1}$ energy be accounted for upon conversion of the ht-quadruplexes, a value that cannot be rationalized based on changes in conformation. Therefore, it is proposed that the unfolding of dehydrated ht-quadruplexes occurs through a three-state sequential model.

The thermodynamic parameters describing the unfolding process are shown in Table 5. Melting occurs by denaturation of the starting quadruplex structure to form a stable intermediate species. The intermediate CD spectra are similar regardless of sequence and resemble the second intermediate found from experiments done in purely aqueous environments (Figure 9). The first transition has a melting temperature range of 44.3 – 52.4 °C, similar to that of the first intermediate found from aqueous investigations, while the second transition demonstrates elevated melting temperatures ranging from 83.4 – 98.4 °C. This indicates the increase in ht-quadruplex stability may be through stabilization of the intermediate state rather than the fully-folded quadruplex.

Assessing human telomere quadruplex heat capacity changes

The thermal stability of human telomere quadruplexes was studied through the use of DSC to assess the heat capacity changes associated with ht-quadruplexes. Each DSC thermogram was normalized for strand concentration and baseline corrected using a cubic regression technique (Supporting Information Figure 6). Baseline correction routines are often arbitrary, with techniques varying based on how endpoints are determined and the order of the polynomial chosen for extrapolation. Baseline-corrected DSC thermograms were integrated to evaluate total calorimetric enthalpy changes. Calorimetric enthalpies are found to be much lower in magnitude compared to CD-derived van't Hoff enthalpy changes (Table 6). If the chosen mechanism accurately reflects quadruplex unfolding, calorimetric and van't Hoff enthalpy changes should agree. The significant disagreement between the two techniques indicates a correction is required in the handling of DSC thermograms and that heat capacity changes may significantly influence the thermodynamic parameters.

To investigate this possibility, thermodynamic parameters determined from CD melting were used to simulate a DSC thermogram for ht-quadruplex unfolding, and the difference between the generated thermogram and experimentally collected thermogram was calculated (Figure 10). The difference curve reflects the baseline required to achieve agreement between CD and DSC results and contains a complex multi-phase behavior with three distinct changes in magnitude. We propose that this difference curve reflects unique heat capacity changes that correspond to the three transitions required to accurately model ht-quadruplex unfolding. Distinct heat capacity changes would require baseline corrections for each species, rather than treating DSC thermograms for a single baseline correction. It is possible that heat capacity changes could be obtained for each species involved in the unfolding mechanism, which could lead to agreement between the thermodynamic parameters recovered from spectroscopic and calorimetric techniques.

Discussion

The thermodynamic characterization of ht-quadruplex thermal unfolding investigated here compliments the mechanistic study recently conducted by our laboratory.³¹ Circular dichroism has been established as a key method capable of capturing the intermediate states involved in ht-quadruplex unfolding. SVD analysis of spectrally relevant components necessitates the use of a multi-state mechanism for accurate modeling of the unfolding mechanism. A four-state sequential mechanism is found to describe the unfolding of each of the ht-quadruplexes. This complexity is reduced in the presence of acetonitrile. This study represents the first detailed thermodynamic analysis of a set of diverse quadruplex topologies by complex unfolding mechanisms, expanding upon the recent hTel22 characterization.³¹ The study highlights unique similarities upon the unfolding of distinct ht-quadruplex topologies, while parsing the free energy contributions to illustrate differences in the overall stability of each sequence. The thermodynamic parameters describing the unfolding of ht-quadruplexes can aid in the design of more specific quadruplex-interactive compounds and improve predictive algorithms for quadruplex stability.

Ht-quadruplexes represent a system of diverse topologies

The human telomeric system of quadruplexes contains a rich history of high-resolution investigations that includes distinct monomeric topologies in potassium solutions.^{18–24} Investigated here was a set of eight quadruplex forming sequences adopted from the human telomeric repeat. The set of ht-quadruplexes represents a model system for characterizing similarities and differences between distinct quadruplex topologies. Spectroscopic techniques were employed to evaluate differences between the ht-quadruplex forming sequences under standardized conditions. CD signatures were reproducibly obtained for the ht-quadruplexes (Figure 1), but cannot be used to establish solution structure. Resulting CD signatures from equivalent hybrid topologies display small variations in CD at two predominant wavelengths, 265 and 290 nm, with intensity that depends on sequence. The differences in peak intensities are reflections of both quadruplex conformation (differential stacking of loop bases) and the presence of additional flanking residues to the quadruplex forming sequence. CD variations may also reflect influences of unique base-pair or potential A-triplet capping structures.¹⁹ The reproducible CD signatures were found to correlate with ¹H NMR spectra that match published high-resolution topology studies (Supporting Information Figure 1). Collected NMR spectra demonstrate solution heterogeneity (≈15%) for each of the sequences studied at 20 °C. No influence of solution structure was seen for strand concentrations up to 0.4–0.5 μM, although high strand concentration has been proposed to influence solution structure.³⁸

Ht-quadruplexes are best modeled by a four-state sequential unfolding mechanism

Thermal denaturation experiments were analyzed by multiple methods to establish a multi-state pathway that was required to accurately describe the unfolding mechanism.^{39,43,48,49} Simultaneously collected wavelength data show non-linear correlations (Supporting Information Figure 2), while derivative curves ($\delta \epsilon / \delta T$) demonstrated the presence of multiple unique minimum and maximum values (Figure 2). These two analysis techniques indicate that ht-quadruplex unfolding cannot be described by a two-state mechanism. The application of SVD, a model-independent deconvolution technique, resulted in V-autocorrelations indicating four significant spectral components are necessary to describe ht-quadruplex unfolding (Figure 3).

A four-species sequential mechanism was recently proposed for ht-quadruplex unfolding. Mechanisms containing two, three or four species unfolding sequentially or with formation of multiple starting species (parallel) were rigorously investigated for the Tel22 and 2GKU sequences.³¹ The four-species mechanism accurately described the thermal denaturation process while resulting in minimized error surfaces based on Akaike Information Criterion (AIC) to account for increasing numbers of adjustable fitting parameters. Although AIC could not be used to determine statistical differences between the sequential and parallel mechanisms, evaluation of the Gibbs free energy changes for the parallel mechanism resulted in differences between multiple starting species that deviates from the proposed 1:1 equilibrium between hybrid conformations for the Tel22 sequence.^{18,50} Thus, monitoring of ht-quadruplex unfolding through collection of CD intensity is likely not sensitive to the formation of multiple distinct quadruplex conformations, but instead senses changes in the

topology of an ensemble of quadruplexes that occurs due to thermal disruption of the system.

The four-state sequential mechanism is proposed to be sensitive to the "breathing" of the quadruplex structure. Unlike previously proposed mechanisms where multiple quadruplex conformations interconvert rapidly in solution, the sequential mechanism asserts that quadruplex conversion can occur only upon reaching an intermediate or unfolded state. Therefore, the equilibrated solutions obtained by annealing refer to a preformed ensemble of quadruplexes, where the CD signatures reflect a weighted average of the quadruplex conformations. The first unfolding transition, $F \leftrightarrow I_1$, corresponds to a shift in state rather than multiple solution topologies. The first transition is likely a relaxing of the quadruplex conformation, with the generated CD spectra from SVD analysis reflecting changes in the stacking of the G-tetrad core. This change from a rigid to a relaxed state affects the chirality of the stacked G-tetrad core and potential weak interactions such as would be found in loop regions or capping structures. Disrupting the chirality results in small shifts in the CD signatures of the system,⁴⁴ such as that seen from generated spectra resembling hybrid quadruplex CD signatures for the initial folded and first intermediate states (Supporting Information Figure 4).

The second transition, $I_1 \leftrightarrow I_2$, was recently proposed to be a change from a quadruplex to a triple-helix conformation.³¹ Spectroscopic investigation using CD and fluorescent techniques demonstrated the second intermediate state (I_2) is best characterized by an ensemble triple-helical conformations, with a recovered CD spectra that resembles a G-rich triple-helix. The second intermediate state for all ht-quadruplexes investigated is found to give CD signatures that resemble the triple-helical state (Figure 5), indicating the unfolding of ht-quadruplexes may contain similar triple-helix intermediates independent of starting conformation. However, the rigorous biophysical investigation conducted using the Tel22 to characterize the populated intermediate state has not been done for any of the additional ht-quadruplexes used in this study.

Thermodynamic characterization of ht-quadruplexes

A standard solution condition of 25 mM KCl-folding buffer, pH 7.0 was used to evaluate energetic differences between the eight ht-quadruplexes. Although the ht-quadruplexes have been characterized to adopt similar conformations, thermodynamic analysis resulted in a wide range of stabilities using the four-state sequential mechanism (Table 3). Overall, the ht-quadruplexes were found to have similar energetic contributions from the three transitions that occur upon unfolding, with each transition having an increase in the Gibbs free energy change magnitude such that $G_1 \ll G_2 < G_3$. The low energetic contribution of the first transition ($G_{1,ave} = -1.0 \text{ kcal mol}^{-1}$) indicates the ht-quadruplexes are unlikely to adopt fully-stacked rigid conformations, with small changes in temperature inducing thermal disruption to a more relaxed state. The second transition was found to have an increased free energy change ($G_{2,ave} = -2.8 \text{ kcal mol}^{-1}$), where removal of one of the terminal strands causes formation of the triple-helical state. The free energy change from the initial folded state to the second intermediate triple-helix state ($F \leftrightarrow I_2$) contains a low total free energy change ($G_{1+2,ave} = -3.8 \text{ kcal mol}^{-1}$), agreeing with computational investigations that the

triple-helix state may have similar stability to the quadruplex.³⁰ Unfolding of the second intermediate requires the largest energetic change ($\Delta G_{3,ave} = -4.9 \text{ kcal mol}^{-1}$), having a larger magnitude than the first two transitions combined. This large energetic change corresponds with the total disruption of structured DNA, where the folded triple-helical state unfolds to a random-coil single stranded state.

Although the ht-quadruplexes contain similarities in their energetic contributions, the overall stability of the quadruplexes show significant variations. The ht-quadruplex Gibbs free energy changes ordered from lowest to highest are $2\text{HY9} < 2\text{JPZ} < 2\text{JSL} < 2\text{JSM} < \text{Tel22} < 2\text{KKA} < 2\text{KF8} < 2\text{GKU}$. The ht-quadruplexes have an average Gibbs free energy change of $-8.8 \text{ kcal mol}^{-1}$. Differences in the total Gibbs free energy changes are proposed to arise from unique flanking residues and differences in stabilizing interactions such as capping structures. The total Gibbs free energy change correlates well with sequence length, with longer sequences having decreased total Gibbs free energy changes. The longest sequence (2HY9) is highly destabilized in comparison with the other ht-quadruplexes, and contains the addition of five adenine residues to the core 21-nt quadruplex forming sequence. This result agrees with a study that showed addition of adenine residues to the 3'-end of the Tel22 decrease stability.⁵¹

Co-solvent-converted ht-quadruplexes display reduced unfolding complexity

The ht-quadruplexes were also studied in the presence of 50% (v/v) to evaluate changes to the unfolding thermodynamics caused by dehydration. All ht-quadruplexes are found to convert to a CD spectrum having a maximum intensity near 260 nm (Figure 7). This conversion has been shown to be a change in conformation to a propeller quadruplex topology in the presence of PEG.²⁷ SVD analysis of thermal denaturation profiles resulted in V-autocorrelations demonstrating reduced unfolding complexity, with only three significant components required to describe the unfolding process (Figure 8). The reduction in unfolding complexity is associated with a loss of the first heterogeneous step discussed for the four-state sequential mechanism, with the converted ht-quadruplexes not displaying low-temperature variations in conformation.

A three-state sequential unfolding mechanism was chosen to describe the unfolding of ht-quadruplexes in the presence of acetonitrile based on Gibbs free energy changes (Table 4). The sequential mechanism demonstrates an average Gibbs free energy change of $-12.7 \text{ kcal mol}^{-1}$, a difference of -4 kcal mol^{-1} from results determined in the absence of acetonitrile. The initial transition occurs with an average Gibbs free energy change of $-4.1 \text{ kcal mol}^{-1}$, similar to the $-3.8 \text{ kcal mol}^{-1}$ recovered from combination of the first two transitions in an all aqueous environment. The stabilization of the ht-quadruplexes is through a large increase in Gibbs free energy change for the intermediate state. The intermediate state is found to have an average Gibbs free energy change of $-8.6 \text{ kcal mol}^{-1}$, compared to the $-4.9 \text{ kcal mol}^{-1}$ recovered from aqueous results. Although rigorous biophysical investigations have not been conducted, the intermediate CD spectra recovered from SVD analysis is similar to the recovered CD spectra from aqueous investigations that is proposed to be an ensemble of triple-helical conformations. Overall, ht-quadruplexes are found to have increased stability

upon dehydration by acetonitrile with a significant contribution to the increased stability coming from the intermediate state.

Heat capacity change of ht-quadruplexes

Estimates of the heat capacity change for ht-quadruplex unfolding are required for a full thermodynamic characterization. DSC was employed to evaluate the heat capacity changes of each of the ht-quadruplexes, as calorimetric techniques are most sensitive to heat capacity effects.⁵² Evaluation of DSC thermograms using a common baseline correction technique resulted in a large discrepancy in the total enthalpy change compared to the values recovered by global SVD analysis (Table 6). This is a consequence of the uncertainty in heat capacity changes for ht-quadruplex systems.

Recent combined calorimetric and spectroscopic studies employed global CD and DSC fits to estimate the total heat capacity change for the folding of Tel22 to be $-310 \text{ cal mol}^{-1} \text{ K}^{-1}$.³² Problems with this methodology are that baseline corrections are done prior to global analysis, where baselines are chosen based on end point values and regression done typically through the use of polynomial splines (Supporting Information Figure 6). If heat capacity changes are present for each transition in the unfolding mechanism, it may be that the use of polynomial regressions for baseline correction influences the estimated total heat capacity change. It may also be true that each transition has a unique heat capacity change, requiring the baseline correction to contain multiple distinct transitions as is observed by evaluating the difference curves between CD and DSC recovered in this study (Figure 10). At this time, the heat capacity change for ht-quadruplex unfolding is still uncertain, with estimates ranging from 310 to $1600 \text{ cal mol}^{-1} \text{ K}^{-1}$.^{32,53} While agreement is being reached that ht-quadruplex unfolding must be analyzed for multi-state transitions, it will be necessary for detailed calorimetric studies to be employed in an attempt to accurately assess heat capacity changes.

Conclusions

Human telomeric quadruplex thermal unfolding cannot be described by a two-state mechanism. Under aqueous and dehydrated conditions, sequential mechanisms with one or two intermediate species accurately describe the unfolding of three unique hydrated and one dehydrated topology. This indicates sequential unfolding mechanisms may be common for biologically-relevant unimolecular quadruplexes, although deconvolution of melting profiles for other distinct quadruplexes systems is required. Additionally, thermal deconvolutions indicate a common intermediate spectral species during human telomeric quadruplex unfolding. Similar CD spectra recovered from eight distinct human telomeric sequences, under both hydrated and dehydrated conditions, indicate these intermediates are relevant in quadruplex unfolding. Recent biophysical characterization of Tel22 suggests this intermediate is an ensemble of triple-helical conformations. The thermodynamic description of these intermediate states improves the interpretation of the dynamic quadruplex equilibrium exhibited by telomeric sequences. Whether such intermediates could be of biological relevance or used as small-molecule targets remains to be seen.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Concentration-normalized CD spectra for the eight ht-quadruplex sequences. CD spectra were collected in the presence of 25 mM KCl in folding buffer held constant at 20 °C. CD spectra are grouped by conformation: hybrid-1 (squares), hybrid-2 (circles), and hybrid-3 (stars).

Figure 2.

Three-dimensional thermal denaturation primary results of the hybrid-2 quadruplex forming sequence 2JSL. (A) Primary CD spectra. (B) Single wavelength analysis of 290 nm (filled squares) and 265 nm (open squares) demonstrate a non-two state behavior. (C) Derivative plots allow for the estimation of melting temperatures prior to SVD analysis and represent accurate starting values for non-linear regression.

Figure 3.

V-autocorrelations used to evaluate the presence of spectral species. A cutoff value of 0.75 (solid black line) was established for which a singular value species is above the noise threshold of the system. Comparison of V-autocorrelations for all eight ht-quadruplex forming sequences demonstrates four species are required for the accurate modeling of the unfolding process.

Figure 4. Best-fit residuals from the global analysis of four S-value weighted V-vectors. Best-fit lines are shown for the first (black), second (red), third (green), and fourth (blue) V-vectors.

Figure 5.

Generated spectra of the second unfolding intermediate recovered from mean values of Monte Carlo analysis. Generated spectra retain similar features independent of quadruplex conformation, having peak maximum and minimum of 265 and 245 nm, respectively. The largest uncertainty in the generated spectra are at the 290 nm shoulder (data not shown).

Figure 6.

Relative concentration of each species present during the unfolding of human telomere quadruplexes. Concentrations are based on the average of eight human telomere quadruplex forming sequences used in the study. Diagram shows the relative concentration of each species with respect to temperature, illustrating the unfolding mechanism of human telomere quadruplexes. Lines represent the starting (black), first intermediate (red), second intermediate (green), and unfolded (blue) species.

Figure 7.
Comparison of quadruplex CD spectra in 50% (v/v) acetonitrile 25 mM KCl-folding buffer. Conversion of the quadruplex conformation occurs upon addition of 50% (v/v) acetonitrile to a CD spectra with a maximum peak at 265 nm and minimum at 245 nm.

Figure 8.

V-autocorrelations from SVD analysis of quadruplex thermal denaturations in the presence of 50% (v/v) acetonitrile. The solid black line represents the cutoff of 0.75. Autocorrelation analysis contains a dramatic drop in autocorrelation values for 4 or more species for all but one sequence (2HY9). This result demonstrates only three significant spectral species are required to accurately model the thermal unfolding of quadruplexes in the presence of acetonitrile.

Figure 9.

Comparison of generated CD spectra for the melting intermediates determined from thermal denaturation of the ht-quadruplexes in 50% (v/v) acetonitrile.

Figure 10.

Comparison of buffer-blanked DSC thermogram for 2JSL and thermogram generated from global analysis of CD thermal denaturations. The difference curve reflects the baseline necessary to achieve agreement between the DSC and CD results, and may indicate the presence of three-distinct heat capacity changes occurring during the unfolding of ht-quadaruplexes.

Human telomeric quadruplex forming deoxyoligonucleotides. The ht-quadruplexes studied in this work are given with their PDB designation and corresponding sequence. The hybrid conformations are grouped based on loop arrangement into hybrid-1, hybrid-2, and hybrid-3.

Table 1

PDB ID	Sequence (5' to 3')	Type	ϵ_{260} (M ⁻¹ cm ⁻¹)	Length	Reference
2GKU	TTGGGTTAGGGTTAGGGTTAGGGA	hybrid-1	244300	24	20
2HY9	AAAGGGTTAGGGTTAGGGTTAGGGAA	hybrid-1	278200	26	19
2ISM	TAGGGTTAGGGTTAGGGTTAGGG	hybrid-1	236500	23	22
2JPZ	TTAGGGTTAGGGTTAGGGTTAGGGTT	hybrid-2	261200	26	21
2JSL	TAGGGTTAGGGTTAGGGTTAGGGTT	hybrid-2	253100	25	22
2KF8	GGGTTAGGGTTAGGGTTAGGGT	hybrid-3	223500	22	23
2KKA	AGGGTTAGGGTTAGGGTTAGGGT	hybrid-3	237000	23	24
Tel22*	AGGGTTAGGGTTAGGGTTAGGG	Mixture	228500	22	15,16

* Tel22 sequence corresponds to K⁺-crystal structure 1KF1 and Na⁺ structures 143D. Tel22 is believed to be a mixture of anti-parallel quadruplexes in aqueous K⁺ solutions.^{25,47}

Table 2

Thermodynamic values for the thermal denaturation of eight distinct ht-quadruplex forming sequences. All thermodynamic parameters were determined from thermal denaturations conducted in 25 mM KCl TBAP buffer using equilibrated samples. All H values are in kcal mol⁻¹ and T_m values are in °C.

	H_1	T_{m1}	H_2	T_{m2}	H_3	T_{m3}
2GKU	-21.1 ± 8.1	33.8 ± 6.6	-41.4 ± 6.2	53.8 ± 1.9	-55.8 ± 6.6	60.8 ± 1.2
2HY9	-12.6 ± 2.6	35.7 ± 8.6	-28.2 ± 4.0	45.7 ± 3.3	-29.8 ± 4.2	50.7 ± 3.7
2ISM	-19.1 ± 2.2	40.7 ± 7.3	-29.2 ± 2.1	48.8 ± 2.8	-41.6 ± 3.6	58.4 ± 1.3
2JPZ	-7.8 ± 1.8	37.5 ± 22.5	-36.4 ± 4.7	43.7 ± 2.9	-39.9 ± 4.8	54.3 ± 1.7
2JSL	-13.3 ± 2.6	39.2 ± 8.8	-30.7 ± 3.3	47.4 ± 3.2	-38.8 ± 4.4	52.9 ± 2.3
2KKA	-24.9 ± 4.2	42.0 ± 3.7	-22.9 ± 3.5	58.5 ± 5.2	-44.8 ± 4.8	61.7 ± 2.0
2KF8	-22.5 ± 3.8	44.2 ± 6.7	-31.6 ± 3.4	46.0 ± 2.7	-48.7 ± 4.3	60.8 ± 1.0
Tel22	-21.9 ± 4.7	35.2 ± 5.3	-33.4 ± 4.2	47.6 ± 1.6	-45.3 ± 4.2	61.4 ± 1.0

Table 3

Gibbs free energy changes for the thermal denaturation of ht-quadruplexes. All Gibbs free energy changes were calculated at 20 °C assuming no heat capacity change. Standard ΔG values were calculated at 20 °C in kcal mol⁻¹.

	G_1	G_2	G_3	G_{Tot}
2GKU	-0.9 ± 0.4	-4.3 ± 0.6	-6.8 ± 0.9	-12.0 ± 1.0
2HY9	-0.6 ± 0.2	-2.3 ± 0.5	-2.9 ± 0.7	-5.7 ± 0.5
2JSM	-1.2 ± 0.4	-2.6 ± 0.3	-4.8 ± 0.5	-8.7 ± 0.5
2JPZ	-0.4 ± 0.5	-2.7 ± 0.5	-4.2 ± 0.7	-7.3 ± 0.6
2JSL	-0.8 ± 0.3	-2.6 ± 0.3	-3.9 ± 0.7	-7.3 ± 0.6
2KKA	-1.7 ± 0.2	-2.6 ± 0.4	-5.6 ± 0.8	-9.9 ± 0.8
2KF8	-1.7 ± 0.4	-2.6 ± 0.4	-6.0 ± 0.6	-10.2 ± 0.7
Tel22	-1.1 ± 0.4	-2.9 ± 0.4	-5.6 ± 0.6	-9.5 ± 0.7

Table 4

Comparison of Gibbs free energy changes determined from a three-state sequential model and a three-state parallel model. All G values were calculated at 20 °C in kcal mol⁻¹.

	Sequential Model			Parallel Model		
	G ₁	G ₂	G _{Tot}	G ₁	G ₂	G _{Tot}
2GKU	-4.1	-9.7	-13.7	-13.7	-9.7	-23.4
2HY9	-3.5	-8.2	-11.7	-11.7	-8.2	-19.8
2ISM	-4.5	-8.6	-13.1	-13.1	-8.6	-21.7
2JPZ	-4.3	-8.2	-12.5	-12.5	-8.2	-20.8
2JSL	-4.5	-8.6	-13.1	-13.1	-8.6	-21.7
2KKA	-4.3	-9.5	-13.9	-13.9	-9.5	-23.4
2KF8	-4.4	-8.6	-13.0	-13.0	-8.6	-21.6
Te22	-3.0	-7.4	-10.5	4.2*	-7.7*	-3.5*

* Results could not be accurately fit for a three-species parallel model.

Table 5

Thermodynamic parameters describing the unfolding of human telomeric quadruplexes in the presence of 50% (v/v) acetonitrile. Parameters were obtained using a three-state sequential unfolding model. All ΔH values are in kcal mol⁻¹ and T_m values are in °C.

	ΔH_1	T_{m1}	ΔH_2	T_{m2}
2GKU	-42.9	50.7	-49.2	91.7
2HY9	-42.6	45.9	-45.3	84.8
2JSM	-54.2	46.2	-45.2	88.8
2JPZ	-55.9	44.3	-46.4	83.4
2JSL	-54.2	46.2	-45.2	88.8
2KKA	-43.6	52.4	-46.5	95.4
2KF8	-55.1	45.5	-42.1	95.5
Tel22	-39	44.6	-35.3	98.4

Table 6

Calorimetric and van't Hoff enthalpy changes for the unfolding of human telomeric quadruplexes.

	H_{cal} (kcal/mol)	H_{vH} (kcal/mol)
2GKU	−34.2	−100.6
2HY9	−23.8	−118.3
2JSM	−36.3	−82.9
2JPZ	−32.5	−70.7
2JSL	−37.4	−84.1
2KF8	−44.1	−89.9
2KKA	−36.4	−92.6
Tel22	−34.4	−102.8