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Human telomeric G-quadruplex: thermodynamic and kinetic studies of telomeric quadruplex stability

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Summary

Thermodynamic and kinetic studies complement high-resolution structures of G-quadruplexes. Such studies are essential for a thorough understanding of the mechanisms that govern quadruplex folding and conformational changes in quadruplexes. This perspective article reviews representative thermodynamic and kinetic studies of the folding of human telomeric quadruplex structures. Published thermodynamic data vary widely and are inconsistent. Possible reasons for these inconsistencies are discussed. The key issue of whether or not such folding reactions are a simple two-state process is examined. A tentative energy balance for the folding of telomeric quadruplexes in Na⁺ and K⁺ solution, and for conformational transition between these forms will be presented.

Keywords

quadruplex DNA; thermodynamics; kinetics; free energy; enthalpy

Introduction

Human telomeric DNA consists of several kilobases of tandem repeats of the sequence 5'-TTAGGG, including a terminal single-stranded overhang of approximately 200 nucleotides. This overhang can fold into a variety of quadruplex structures, the exact nature of which are under active and intensive investigation. An authoritative review of human telomere molecular biology and pharmacology appeared recently [1]. Telomeric quadruplexes are an attractive target for cancer chemotherapy [2–8]. Companion articles in this issue by Neidle and by Arora, Kumar, Agarwal and Maiti discuss drug and ligand binding to telomeric quadruplexes in detail. Full development of telomeric quadruplexes as a drug target requires a thorough understanding of not only their structures, but also of the energetics of their folding reactions and conformational transitions among different forms.

The structure of human telomeric quadruplexes under differing solution conditions has attracted considerable attention, and has been reviewed numerous times over the last few years [9–14]. The companion minireview in this issue by Phan offers the most recent discussion of the variety of G-quadruplex structures formed by human telomeric DNA and RNA. Na⁺ and K⁺ cations appear to dictate the unimolecular folding of the telomeric DNA sequence into different forms, along with the exact sequences of the strand termini. In Na⁺ solutions, a "basket" form is preferred, featuring an antiparallel quadruplex core with two lateral loops and one diagonal loop [15]. In K⁺ solutions, different "hybrid" forms are favored by variants of the human telomere sequence. These hybrids feature an antiparallel

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quadruplex core with two lateral loops and one side ("chain reversal") loop [14,16,17]. The position of the side loop varies in these hybrid forms. In crystals, a unique "propeller" form is favored that features a parallel-stranded quadruplex core and three side loops [18]. Biophysical studies [19] showed that the propeller form was not the major form in solution, a finding subsequently validated by high-resolution NMR studies [14,16,17,20]. The energy of folding these various forms and the energetic cost of converting one form to another is of fundamental importance for understanding telomere biology and the interactions of small molecules and proteins with telomeric DNA. The folding and unfolding of quadruplexes may be of profound physiological importance since quadruplex structures may form transiently in telomeres at specific times in the cell cycle as part of a regulatory mechanism [1,8].

The energetic and kinetic aspects of G-quadruplexes have received comparatively less attention. Kumar and Maiti provided a thermodynamic overview of naturally occurring intramolecular quadruplexes [21], focusing primarily on quadruplexes that might form within gene promoter sequences. Lane and colleagues provided a critical survey of the stability and kinetics of quadruplex structures, and posed some key unsolved problems that need to be addressed [22]. An algorithm for predicting quadruplex stability based on the rather sparse set of existing thermodynamic data was recently described [23]. This predictive algorithm promises to be an important new tool that recognizes that the biological significance of quadruplex structures is tightly linked to their thermodynamic stability.

Methods for studying quadruplex folding and unfolding

The folding and unfolding of G-quadruplexes can be conveniently monitored by spectroscopic methods. Changes in absorbance or circular dichroism as a function of salt concentration or changes in temperature provide a signal for the determination of the fraction of folded or unfolded DNA strand, allowing for the calculation of the equilibrium distribution of conformational forms. Standard methods using UV absorbance at a single wavelength to monitor the thermal denaturation of nucleic acid structures have been fully described, including the mathematical formalism needed for the quantitative analysis of melting curves [24–27]. Mergny and co-workers noted the desirability and utility of monitoring quadruplex formation by recording reversible absorbance changes at 295 nm [28], a wavelength that is selectively sensitive to disruption of the quadruplex stack. A particularly valuable protocol has recently been published that describes the practical details of UV melting studies of G-quadruplexes, with an thorough discussion of common problems and pitfalls [29]. The use of fluorescence to monitor the thermal denaturation of quadruplex forming oligonucleotides labeled with a FRET pair has been described [30,31]. A decided advantage of fluorescent methods is their high sensitivity, providing the ability to use small volumes and low concentrations of quadruplexes. One possible disadvantage of FRET methods is that the added labels may alter the stability of the structure. Circular dichroism (CD) is particularly sensitive to quadruplex structures, and can be used to conveniently monitor quadruplex formation [31], although expensive instrumentation is needed. General formalism for the quantitative analysis of thermal denaturation reactions using CD data has recently appeared [32]. All of these spectroscopic methods yield thermodynamic data only indirectly, and transitions curves must be fit to specific models to obtain enthalpy, entropy and free energy values. Almost inevitably, folding or unfolding reactions are assumed, for convenience, to be two-state processes, with negligible concentrations of any intermediate species. Calorimetry (DSC, differential scanning calorimetry; ITC, isothermal titration calorimetry) can be used to monitor quadruplex folding and unfolding reactions, with the significant advantage that enthalpy changes can be monitored directly and data obtained in a model-free fashion [33,34]. Disadvantages of calorimetry are the high concentrations and amounts of material necessary, and the need for expensive instrumentation.

Energetics of unfolding human telomeric quadruplexes

Representative thermodynamic values for the unfolding of human telomere quadruplexes taken from the literature are shown in Table 1. Data were gathered for similar sequences and for studies done using similar solution conditions. The results are both disturbing and discouraging. There is an unacceptably wide range in values reported from different laboratories. Reported T_m values range from 56–63.7 °C in 100 mM Na⁺ and from 63–81.8 °C in 100 mM K⁺. Enthalpy values range from 38 to 72.7 kcal mol⁻¹ in Na⁺ and from 49 to 77.5 kcal mol⁻¹ in K⁺. The origin of these discrepancies is not at all clear. While slight sequence variations might be offered as one source of the differing values, inspection of Table 1 shows that even identical sequences in solutions with the same cation concentration yield different results in different laboratories. It is impossible to say why this is. Different annealing procedures could contribute, or the addition of fluorescent labels that alter quadruplex stability could be another culprit.

There are several potential pitfalls in reliably obtaining thermodynamic parameters from spectroscopic transition curves. The first is the difficulty in establishing reliable pre- and post-transition baselines. Any transformation of the primary data or any attempt to directly analyze the primary data by curve fitting must include choices concerning these baselines. Slopes in baseline regions may arise from intrinsic physical phenomenon, such as the intrinsic temperature dependence of fluorescence or from absorbance changes resulting from solvent expansion. More insidiously, though, such slopes could arise from additional reactions that complicate the study of the denaturation transition. These may involve thermally driven processes like helix-helix transition or single-strand base unstacking that precede the actual helix melting transition. Such transitions may have small enthalpy values, leading to broad, featureless melting transitions. Attempts to “correct” sloping baselines that arise from such complications would lead to an oversimplification of the true reaction mechanism, and to a loss of information. Even without such complications, establishing proper baselines presents practical problems. There are worrisome reports that document that the lengths of the pre- and post-transition baselines selected and used in data analysis directly affect the values of the thermodynamic parameters extracted from the data [35,36]. Investigators of G-quadruplex denaturation should be fully aware of these difficulties, and should describe in detail their procedures for establishing baselines for analysis.

Another pitfall is the common assumption that denaturation reactions are simple two-state processes, and simply pass from a folded “native” state to an unfolded denatured state without any intermediates. The two-state assumption must be justified by some experimental test. A classical test, first utilized for protein denaturation studies, is to obtain denaturation curves by two (or more) different physical methods [37]. If transition curves obtained by the multiple methods are exactly superimposable, that is consistent with a two-state mechanism. More recent tests utilizing multiple wavelength data have appeared. A dual- wavelength parametric test for a two-state denaturation transition monitored by spectroscopy was described [38]. In this test, data obtained at two different wavelengths are plotted against one another. For a two-state transition, such a plot should be strictly linear. Deviations from strict linear behavior signal that the denaturation process is not two-state, and likely has intermediate states that are significantly populated. Singular value decomposition provides an additional test of the two-state assumption [39–41]. With modern diode array spectrophotometers, it is easy to collect entire spectra as a function of temperature, instead of single wavelength data. A set of spectra as a function of temperature defines a three dimensional surface that is easily converted to a matrix. Singular value decomposition (SVD) of the matrix rigorously enumerates the number of significant spectral species required to account for the spectral changes without reference to any specific model. For a two-state transition, there should be only two significant spectral species, corresponding to

the folded and unfolded forms. Any number of species greater than two indicates a violation of the two-state assumption, and signals the presence of intermediates. SVD (or a similar multivariate analysis method) has been used to characterize the denaturation of G-quadruplex or other four-stranded structures [42–44]. SVD analysis is not easily explained in limited space, so the literature cited should be consulted for details of using the procedure.

A final pitfall is the neglect of heat capacity changes (ΔC_p) that may accompany quadruplex denaturation. Heat capacity changes are correlated with exposure of hydrophobic surface areas [45,46] as well as increasing fluctuations among microstates associated with the less compact forms [47], so it would be surprising indeed if the unfolding of quadruplex structures, with the concomitant exposures of the bases, was not accompanied by a nonzero ΔC_p value. Unfortunately, it is enormously difficult to reliably fit transition curves to obtain derivative values of the primary thermodynamic parameters [48]. Small heat capacity changes could manifest themselves as contributors to sloping baselines, and might easily be “corrected out” at the expense of systematic errors in enthalpy values. Even when a van’t Hoff plot of $\ln K$ versus T^{-1} is constructed by transformation of the primary transition curve problems remain. Nonzero ΔC_p values should lead to curvature in the van’t Hoff plot. However, Monte Carlo simulations of van’t plots showed that for “small” (less than $|200| \text{ cal mol}^{-1} \text{ K}^{-1}$) ΔC_p values, which is typical of values observed for nucleic acid unfolding, no curvature could in fact be observed within the typical error of experimental data, but that slopes were systematically biased away from true enthalpy values [49].

All of these pitfalls may contribute to the discrepant results shown in Table 1. Additional thermodynamic studies are needed to resolve the discrepancies and to obtain a coherent picture of the thermodynamic profile for quadruplex formation. Thermodynamic studies of quadruplex folding are perhaps best done using calorimetric methods where enthalpy values may be determined as directly as possible. Spectrophotometric methods can certainly be used as well, provided proper attention is paid to baseline determinations and to fitting the primary data to appropriate unfolding models that include intermediate states, if necessary.

Multiple states in quadruplex denaturation

Circular dichroism and differential scanning calorimetry were used in a recent study to measure the thermodynamics of human telomere quadruplex unfolding in K^+ solution [42]. SVD analysis of CD spectra collected a function of temperature revealed the presence of an intermediate species along the melting pathway. Quadruplex denaturation is thus not a simple two-state process. The shapes of thermograms obtained by DSC also indicated multiple species. Multiple intermediate species were also found for denaturation of the human telomere quadruplex in Na^+ solution (Li & Chaires, unpublished data).

A significant advantage of DSC is that model-free thermodynamic information can be obtained for complex reactions from the experimental thermograms without fitting to any particular model. The thermogram yields the overall enthalpy directly as the integral of the thermogram:

$$\Delta H = \int C_p dT \quad [1]$$

The overall entropy may be calculated as:

$$\Delta S = \int \frac{C_p}{T} dT \quad [2]$$

The free energy change may then be calculated from the Gibbs equation:

$$\Delta G = \Delta H - T\Delta S \quad [3]$$

Figure 1 shows representative thermograms for the denaturation of human telomere quadruplexes in Na⁺ and K⁺ solutions, along with the overall free energy for the denaturation process.

Multiple states in single molecule experiments

A number of studies that use single-molecule fluorescence resonance energy transfer (smFRET) methods to examine the stability and dynamics of human telomere quadruplexes have appeared [50–54]. Collectively, these studies reveal that in both Na⁺ and K⁺ solution, multiple conformational forms coexist. Temperature and changes in the cation concentration both perturb the equilibria between these species. A dynamic switching model between unfolded and folded quadruplex conformational forms was proposed that included six distinct states [51]. Single base mutations within the G-tetrad stack were subsequently found to dramatically alter the distribution and dynamics of quadruplex forms [50].

Telomeric G-quadruplex structures with strategic BrG substitutions were studied by smFRET [52]. The observed FRET distributions included at least five components, the relative population of which depended strongly on the exact position of the BrG substitution. The results were integrated into a coherent model that incorporated the most recent high-resolution structural information. The model includes a proposed triple-stranded core conformation along the folding pathway to hybrid quadruplex structures, and equilibrium between hybrid and "chair" quadruplex forms.

Simple two-state models for quadruplex denaturation discussed above (Table 1) are inconsistent with the complexity revealed by smFRET methods. The presence of intermediates along the denaturation pathway inferred from SVD analysis [42] is at least qualitatively consistent with the multiple species observed in smFRET studies. One caveat in comparing smFRET results with ensemble solution studies needs to be considered. All smFRET studies to date have used constructs in which single-stranded telomeric DNA sequences are tethered to a segment of duplex DNA. The biophysical properties of a similar constructed were described earlier [43]. The attachment of the duplex can alter the stability of the folded quadruplex segment relative to folding of an unadorned single-stranded sequence. The concepts of "telestability" [55,56] and allostery in DNA [57] can account for differences in the stability of tethered and free sequences.

Kinetics of human telomere quadruplex folding

Formation of tetramolecular quadruplex structures is fourth order with respect to strand association and is exceedingly slow [58]. In contrast, unimolecular folding of the human telomeric sequence is expected to be first order, and could be rapid. Stopped-flow kinetic studies showed that folding of the human telomere quadruplex is indeed fast [59]. In that study, cation-induced folding into quadruplex structures for three model human telomeric oligonucleotides, d[AGGG(TTAGGG)₃], d[TTGGG(TTAGGG)₃A] and d[TTGGG(TTAGGG)₃], was characterized by equilibrium titrations with KCl and NaCl and by multi-wavelength stopped-flow kinetics. Cation binding was cooperative with Hill coefficients of 1.5–2.2 in K⁺ and 2.4–2.9 in Na⁺ with half-saturation concentrations of 0.5–1 mM for K⁺ and 4–13 mM for Na⁺ depending on the oligonucleotide sequence. Oligonucleotide folding in 50 mM KCl at 25 °C consisted of single exponential processes with relaxation times (τ) of 20–60 ms depending on the sequence. In contrast, folding in 100

mM NaCl consisted of three exponentials with τ values of 40–85 ms, 250–950 ms and 1.5–10.5 s. The folding rate constants approached limiting values with increasing cation concentration; in addition, the rates of folding decreased with increasing temperature over the range 15–45 °C. Taken together, these results suggest that folding of G-rich oligonucleotides into quadruplex structures proceeds via kinetically significant intermediates. These intermediates may consist of antiparallel double hairpins in rapid equilibrium with less ordered structures. The hairpins may subsequently form nascent G-quartets stabilized by hydrogen bonding and cation binding followed by relatively slow strand rearrangements to form the final completely folded topologies. Fewer kinetic intermediates were evident with K^+ than Na^+ , suggesting a simpler folding pathway in K^+ solutions. These studies show directly and unambiguously that more than two states must be considered in the folding and unfolding of telomeric quadruplex structures.

Quadruplex conformational transitions

Cations and a variety of small molecule ligands have been shown to facilitate quadruplex folding or to facilitate the transition from one quadruplex conformational form to another [60–69]. In order to fully understand such allosteric effects, the energetics of the underlying conformational transitions in quadruplex structures must be determined. The thermodynamics and kinetics of the transition of the human telomere quadruplex between the Na^+ basket form and the K^+ hybrid form was recently described [70]. Circular dichroism and differential scanning calorimetry were used to determine the energetics of the conformational switch of the human telomere quadruplex formed by the sequence d[AGGG(TTAGGG)₃] between the Na^+ basket form and the K^+ hybrid form. The energy barrier separating the two conformations was found to be modest, only 1.4–2.4 kcal mol⁻¹. The kinetics of exchange of bound K^+ for Na^+ cations and the concomitant conformational switch was assessed by measuring time-dependent changes in the circular dichroism spectrum accompanying the cation exchange reaction. The time course of these changes was found to consist of three distinct kinetic processes: a rapid phase that was complete in less than 5 ms followed by two slower phases with relaxation times of 40–50 s and 600–800 s at 25 °C and pH 7.0. These kinetics were interpreted in terms of a model in which the bound Na^+ cations are rapidly replaced by K^+ followed by relatively slow structural rearrangements to generate the final K^+ -bound product(s). Circular dichroism studies showed that addition of the porphyrin TmPyP4 promoted conversion of the basket to the hybrid form. The kinetics of the TmPyP4-induced conformational change were the same as those observed for the cation exchange reaction.

Summary and conclusions

All recent evidence points to the fact that the folding and unfolding of human telomeric quadruplex structures are not simple two-state processes, but rather proceed along a pathway with multiple intermediate states. Once formed, quadruplex structures can interconvert between forms. Surprisingly, small energy barriers separate the basket and hybrid conformations of the human telomeric quadruplex. Similarly, unfolding of quadruplex forms requires only modest expenditures of energy. That fact may be of biological significance, since quadruplex unfolding may be tightly coupled to protein binding that stabilizes the single-stranded overhang at specific times during the cell cycle to facilitate replication.

Figure 2 portrays a tentative free energy cycle for human telomere quadruplex folding and for the transition from the basket and hybrid forms. Best available estimates for the *overall* free energies and for the relaxation times for each transition are shown (possible intermediate steps are not shown). The magnitudes of the free energies show that an intricate

and subtle balance of forces drive the transition between folded forms and the unfolded state.

Abbreviations

SVD	singular value decomposition
FRET	fluorescence resonance energy transfer
smFRET	single-molecule fluorescence resonance energy transfer
DSC	differential scanning calorimetry
ITC	isothermal titration calorimetry
CD	circular dichroism

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References

1. De Cian A, Lacroix L, Douarre C, Temime-Smaali N, Trentesaux C, Riou JF, Mergny JL. Targeting telomeres and telomerase. *Biochimie*. 2008; 90:131–155. [PubMed: 17822826]
2. Huppert JL. Four-stranded DNA: cancer, gene regulation and drug development. *Philos Transact A Math Phys Eng Sci*. 2007; 365:2969–2984. [PubMed: 17855220]
3. Huppert JL. Four-stranded nucleic acids: structure, function and targeting of G-quadruplexes. *Chem Soc Rev*. 2008; 37:1375–1384. [PubMed: 18568163]
4. Wong HM, Payet L, Huppert JL. Function and targeting of G-quadruplexes. *Curr Opin Mol Ther*. 2009; 11:146–155. [PubMed: 19330720]
5. Hurley LH. DNA and its associated processes as targets for cancer therapy. *Nat Rev Cancer*. 2002; 2:188–200. [PubMed: 11990855]
6. Mergny JL, Helene C. G-quadruplex DNA: a target for drug design. *Nat Med*. 1998; 4:1366–1367. [PubMed: 9846570]
7. Neidle S, Parkinson G. Telomere maintenance as a target for anticancer drug discovery. *Nat Rev Drug Discov*. 2002; 1:383–393. [PubMed: 12120414]
8. Oganessian L, Bryan TM. Physiological relevance of telomeric G-quadruplex formation: a potential drug target. *Bioessays*. 2007; 29:155–165. [PubMed: 17226803]
9. Neidle S, Parkinson GN. The structure of telomeric DNA. *Curr Opin Struct Biol*. 2003; 13:275–283. [PubMed: 12831878]
10. Neidle S. The structures of quadruplex nucleic acids and their drug complexes. *Curr Opin Struct Biol*. 2009; 19:239–250. [PubMed: 19487118]
11. Burge S, Parkinson GN, Hazel P, Todd AK, Neidle S. Quadruplex DNA: sequence, topology and structure. *Nucleic Acids Res*. 2006; 34:5402–5415. [PubMed: 17012276]
12. Patel DJ, Phan AT, Kuryavyi V. Human telomere, oncogenic promoter and 5'-UTR G-quadruplexes: diverse higher order DNA and RNA targets for cancer therapeutics. *Nucleic Acids Res*. 2007; 35:7429–7455. [PubMed: 17913750]
13. Dai J, Carver M, Yang D. Polymorphism of human telomeric quadruplex structures. *Biochimie*. 2008; 90:1172–1183. [PubMed: 18373984]
14. Xu Y, Noguchi Y, Sugiyama H. The new models of the human telomere d[AGGG(TTAGGG)₃] in K⁺ solution. *Bioorg Med Chem*. 2006; 14:5584–5591. [PubMed: 16682210]
15. Wang Y, Patel DJ. Solution structure of the human telomeric repeat d[AG3(T2AG3)₃] G-tetraplex. *Structure*. 1993; 1:263–282. [PubMed: 8081740]

16. Ambrus A, Chen D, Dai J, Bialis T, Jones RA, Yang D. Human telomeric sequence forms a hybrid-type intramolecular G-quadruplex structure with mixed parallel/antiparallel strands in potassium solution. *Nucleic Acids Res.* 2006; 34:2723–2735. [PubMed: 16714449]
17. Phan AT, Kuryavyi V, Luu KN, Patel DJ. Structure of two intramolecular G-quadruplexes formed by natural human telomere sequences in K⁺ solution. *Nucleic Acids Res.* 2007; 35:6517–6525. [PubMed: 17895279]
18. Parkinson GN, Lee MP, Neidle S. Crystal structure of parallel quadruplexes from human telomeric DNA. *Nature.* 2002; 417:876–880. [PubMed: 12050675]
19. Li J, Correia JJ, Wang L, Trent JO, Chaires JB. Not so crystal clear: the structure of the human telomere G-quadruplex in solution differs from that present in a crystal. *Nucleic Acids Res.* 2005; 33:4649–4659. [PubMed: 16106044]
20. Matsugami A, Xu Y, Noguchi Y, Sugiyama H, Katahira M. Structure of a human telomeric DNA sequence stabilized by 8-bromoguanosine substitutions, as determined by NMR in a K⁺ solution. *FEBS J.* 2007; 274:3545–3556. [PubMed: 17561958]
21. Kumar N, Maiti S. A thermodynamic overview of naturally occurring intramolecular DNA quadruplexes. *Nucleic Acids Res.* 2008; 36:5610–5622. [PubMed: 18757890]
22. Lane AN, Chaires JB, Gray RD, Trent JO. Stability and kinetics of G-quadruplex structures. *Nucleic Acids Res.* 2008; 36:5482–5515. [PubMed: 18718931]
23. Stegle O, Payet L, Mergny JL, MacKay DJ, Leon JH. Predicting and understanding the stability of G-quadruplexes. *Bioinformatics.* 2009; 25:i374–i382. [PubMed: 19478012]
24. Marky LA, Breslauer KJ. Calculating thermodynamic data for transitions of any molecularity from equilibrium melting curves. *Biopolymers.* 1987; 26:1601–1620. [PubMed: 3663875]
25. Plum, GE. Optical Methods. In: Bergstrom, DE., editor. *Current Protocols in Nucleic Acid Chemistry*. New York: John Wiley & Sons; 2000. p. 7.3.1-7.3.17.
26. Puglisi JD, Tinoco I Jr. Absorbance melting curves of RNA. *Methods Enzymol.* 1989; 180:304–325. [PubMed: 2482421]
27. Mergny JL, Lacroix L. Analysis of thermal melting curves. *Oligonucleotides.* 2003; 13:515–537. [PubMed: 15025917]
28. Mergny JL, Phan AT, Lacroix L. Following G-quartet formation by UV-spectroscopy. *FEBS Lett.* 1998; 435:74–78. [PubMed: 9755862]
29. Mergny, JL.; Lacroix, L. *Current Protocols in Nucleic Acid Chemistry*. New York: John Wiley & Sons; 2009. UV Melting of G-Quadruplexes; p. 1711
30. De Cian A, Guittat L, Kaiser M, Sacca B, Amrane S, Bourdoncle A, Alberti P, Teulade-Fichou MP, Lacroix L, Mergny JL. Fluorescence-based melting assays for studying quadruplex ligands. *Methods.* 2007; 42:183–195. [PubMed: 17472900]
31. Rachwal PA, Fox KR. Quadruplex melting. *Methods.* 2007; 43:291–301. [PubMed: 17967699]
32. Greenfield NJ. Using circular dichroism collected as a function of temperature to determine the thermodynamics of protein unfolding and binding interactions. *Nat Protocols.* 2007; 1:2527–2535.
33. Haq I, Chowdhry BZ, Jenkins TC. Calorimetric techniques in the study of high-order DNA-drug interactions. *Methods Enzymol.* 2001; 340:109–149. [PubMed: 11494846]
34. Majhi PR, Qi J, Tang CF, Shafer RH. Heat capacity changes associated with guanine quadruplex formation: an isothermal titration calorimetry study. *Biopolymers.* 2008; 89:302–309. [PubMed: 18183583]
35. Allen DL, Pielak GJ. Baseline length and automated fitting of denaturation data. *Protein Sci.* 1998; 7:1262–1263. [PubMed: 9605334]
36. Cooper A. Thermodynamic analysis of biomolecular interactions. *Curr Opin Chem Biol.* 1999; 3:557–563. [PubMed: 10508661]
37. Lumry R, Biltonen R. Validity of the "two-state" hypothesis for conformational transitions of proteins. *Biopolymers.* 1966; 4:917–944. [PubMed: 5975643]
38. Wallimann P, Kennedy RJ, Miller JS, Shalongo W, Kemp DS. Dual wavelength parametric test of two-state models for circular dichroism spectra of helical polypeptides: anomalous dichroic properties of alanine-rich peptides. *J Am Chem Soc.* 2003; 125:1203–1220. [PubMed: 12553823]

39. DeSa RJ, Matheson IB. A practical approach to interpretation of singular value decomposition results. *Methods Enzymol.* 2004; 384:1–8. [PubMed: 15081677]
40. Haq I, Chowdhry BZ, Chaires JB. Singular value decomposition of 3-D DNA melting curves reveals complexity in the melting process. *Eur Biophys J.* 1997; 26:419–426. [PubMed: 9404005]
41. Hendler RW, Shrager RI. Deconvolutions based on singular value decomposition and the pseudoinverse: a guide for beginners. *J Biochem Biophys Methods.* 1994; 28:1–33. [PubMed: 8151067]
42. Antonacci C, Chaires JB, Sheardy RD. Biophysical characterization of the human telomeric (TTAGGG)₄ repeat in a potassium solution. *Biochemistry.* 2007; 46:4654–4660. [PubMed: 17381076]
43. Ren J, Qu X, Trent JO, Chaires JB. Tiny telomere DNA. *Nucleic Acids Res.* 2002; 30:2307–2315. [PubMed: 12034817]
44. Jaumot J, Escaja N, Gargallo R, Gonzalez C, Pedroso E, Tauler R. Multivariate curve resolution: a powerful tool for the analysis of conformational transitions in nucleic acids. *Nucleic Acids Res.* 2002; 30:e92. [PubMed: 12202780]
45. Prabhu NV, Sharp KA. Heat capacity in proteins. *Annu Rev Phys Chem.* 2005; 56:521–548. [PubMed: 15796710]
46. Spolar RS, Record MT Jr. Coupling of local folding to site-specific binding of proteins to DNA. *Science.* 1994; 263:777–784. [PubMed: 8303294]
47. Lane AN, Jenkins TC. Thermodynamics of nucleic acids and their interactions with ligands. *Q Rev Biophys.* 2000; 33:255–306. [PubMed: 11191844]
48. Prehoda KE, Mooberry ES, Markley JL. Pressure denaturation of proteins: evaluation of compressibility effects. *Biochemistry.* 1998; 37:5785–5790. [PubMed: 9558311]
49. Chaires JB. Possible origin of differences between van't Hoff and calorimetric enthalpy estimates. *Biophys Chem.* 1997; 64:15–23. [PubMed: 9127935]
50. Lee JY, Kim DS. Dramatic effect of single-base mutation on the conformational dynamics of human telomeric G-quadruplex. *Nucleic Acids Res.* 2009; 37:3625–3634. [PubMed: 19359361]
51. Lee JY, Okumus B, Kim DS, Ha T. Extreme conformational diversity in human telomeric DNA. *Proc Natl Acad Sci U S A.* 2005; 102:18938–18943. [PubMed: 16365301]
52. Okamoto K, Sannohe Y, Mashimo T, Sugiyama H, Terazima M. G-quadruplex structures of human telomere DNA examined by single molecule FRET and BrG-substitution. *Bioorg Med Chem.* 2008; 16:6873–6879. [PubMed: 18555689]
53. Shirude PS, Balasubramanian S. Single molecule conformational analysis of DNA G-quadruplexes. *Biochimie.* 2008; 90:1197–1206. [PubMed: 18295608]
54. Ying L, Green JJ, Li H, Klenerman D, Balasubramanian S. Studies on the structure and dynamics of the human telomeric G quadruplex by single-molecule fluorescence resonance energy transfer. *Proc Natl Acad Sci U S A.* 2003; 100:14629–14634. [PubMed: 14645716]
55. Burd JF, Larson JE, Wells RD. Further studies on telestability in DNA. The synthesis and characterization of the duplex block polymers d(C20A10)-d(T10G20) and d(C20A15) - d(T15G20). *J Biol Chem.* 1975; 250:6002–6007. [PubMed: 50322]
56. Burd JF, Wartell RM, Dodgson JB, Wells RD. Transmission of stability (telestability) in deoxyribonucleic acid. Physical and enzymatic studies on the duplex block polymer d(C15A15) - d(T15G15). *J Biol Chem.* 1975; 250:5109–5113. [PubMed: 50320]
57. Schurr JM, Delrow JJ, Fujimoto BS, Benight AS. The question of long-range allosteric transitions in DNA. *Biopolymers.* 1997; 44:283–308. [PubMed: 9591480]
58. Mergny JL, De Cian A, Ghelab A, Sacca B, Lacroix L. Kinetics of tetramolecular quadruplexes. *Nucleic Acids Res.* 2005; 33:81–94. [PubMed: 15642696]
59. Gray RD, Chaires JB. Kinetics and mechanism of K⁺- and Na⁺-induced folding of models of human telomeric DNA into G-quadruplex structures. *Nucleic Acids Res.* 2008; 36:4191–4203. [PubMed: 18567908]
60. Arthanari H, Bolton PH. Porphyrins can catalyze the interconversion of DNA quadruplex structural types. *Anticancer Drug Des.* 1999; 14:317–326. [PubMed: 10625924]

61. Engelhart, AE.; Plavec, J.; Persil, O.; Hud, NV. Metal Ion Interactions with G-quadruplex Structures. In: Hud, NV., editor. *Nucleic Acid-Metal Ion Interactions*. Cambridge: Royal Society of Chemistry; 2008. p. 114-149.
62. Han H, Cliff CL, Hurley LH. Accelerated assembly of G-quadruplex structures by a small molecule. *Biochemistry*. 1999; 38:6981-6986. [PubMed: 10353809]
63. Hud, NV.; Plavec, J. The Role of Cations in Determining Quadruplex Structure and Stability. In: Neidle, S.; Balasubramanian, S., editors. *Quadruplex Nucleic Acids*. Cambridge: Royal Society of Chemistry; 2006. p. 100-130.
64. Nakatani K, Hagihara S, Sando S, Sakamoto S, Yamaguchi K, Maesawa C, Saito I. Induction of a remarkable conformational change in a human telomeric sequence by the binding of naphthyridine dimer: inhibition of the elongation of a telomeric repeat by telomerase. *J Am Chem Soc*. 2003; 125:662-666. [PubMed: 12526665]
65. Rezler EM, Seenisamy J, Bashyam S, Kim M-Y, White E, Wilson WD, Hurley LH. Telomestatin and Diseleno Sapphyrin Bind Selectively to Two Different Forms of the Human Telomeric G-Quadruplex Structure. *J Am Chem Soc*. 2005; 127:9439-9447. [PubMed: 15984871]
66. Rodriguez R, Pantos GD, Goncalves DP, Sanders JK, Balasubramanian S. Ligand-driven G-quadruplex conformational switching by using an unusual mode of interaction. *Angew Chem Int Ed Engl*. 2007; 46:5405-5407. [PubMed: 17562537]
67. Sen D, Gilbert W. A sodium-potassium switch in the formation of four-stranded G4-DNA. *Nature*. 1990; 344:410-414. [PubMed: 2320109]
68. Yu H, Wang X, Fu M, Ren J, Qu X. Chiral metallo-supramolecular complexes selectively recognize human telomeric G-quadruplex DNA. *Nucleic Acids Res*. 2008; 36:5695-5703. [PubMed: 18776218]
69. Zhang HJ, Wang XF, Wang P, Ai XC, Zhang JP. Spectroscopic study on the binding of a cationic porphyrin to DNA G-quadruplex under different K⁺ concentrations. *Photochem Photobiol Sci*. 2008; 7:948-955. [PubMed: 18688502]
70. Gray RD, Li J, Chaires JB. Energetics and Kinetics of a Conformational Switch in G-Quadruplex DNA. *J Phys Chem B*. 2009; 113:2676-2683. [PubMed: 19708205]
71. Balagurumoorthy P, Brahmachari SK. Structure and stability of human telomeric sequence. *J Biol Chem*. 1994; 269:21858-21869. [PubMed: 8063830]
72. Li W, Wu P, Ohmichi T, Sugimoto N. Characterization and thermodynamic properties of quadruplex/duplex competition. *FEBS Lett*. 2002; 526:77-81. [PubMed: 12208508]
73. Risitano A, Fox KR. Stability of intramolecular DNA quadruplexes: comparison with DNA duplexes. *Biochemistry*. 2003; 42:6507-6513. [PubMed: 12767234]
74. Olsen CM, Gmeiner WH, Marky LA. Unfolding of G-quadruplexes: energetic, and ion and water contributions of G-quartet stacking. *J Phys Chem B*. 2006; 110:6962-6969. [PubMed: 16571009]

Figure 1.

Thermodynamics of denaturation of human telomere quadruplex structures in buffered Na⁺ (black) or K⁺ (red) solutions. Total cation concentration was 200 mM, pH 7.0. (A) Results from DSC experiments. Integration of the thermograms shown yield direct estimates of the total enthalpy required for denaturation (eq. [1]). (B). Total free energy cost of denaturing quadruplex structures as a function of temperature. Total free energy was estimated from the thermograms in panel (A) using eq. [3].

Figure 2.

Free energy cycle for telomeric quadruplex folding and interconversion between the basket and hybrid forms. Total free energy estimates for each step in the cycle are shown in **bold**. Relaxation times observed for each path are shown in *italics*. No intermediate states are shown along each pathway, only the initial and final states. Guanine bases are colored green, adenines red, and thymines blue.

Table 1

Energetics of human telomere quadruplex unfolding. ΔH , ΔS and ΔG are for the *unfolding* direction.

Sequence $5' \rightarrow 3'$	Cation (mM)	T_m °C	ΔH kcal mol ⁻¹	ΔS cal mol ⁻¹ K ⁻¹	$\Delta G_{(310K)}$ kcal mol ⁻¹	Reference
1. (TTAGGG) ₄	Na ⁺ (70)	49	38.0	119	1.1	[71]
	K ⁺ (70)	63	49.0	147	3.4	
2. AGGG(TTAGGG) ₃	Na ⁺ (100)	56	54.0	163	3.5	[28]
	K ⁺ (100)	63	57.0	169	4.6	
3. GGG(TTAGGG) ₃	Na ⁺ (100)	58	51	155	3.0	[28]
	K ⁺ (100)	65	60.5	179	5.0	
4. GGG(TTAGGG) ₃	Na ⁺ (100)	63.7	72.7	192	13.2	[72]
	K ⁺ (100)	69.3	77.5	202	14.8	
5. TGGG(TTAGGG) ₃ [*]	Na ⁺ (100)	62.8	51.4	153	3.9	[73]
	K ⁺ (100)	81.8	66.2	186.5	8.4	
6. AGGG(TTAGGG) ₃ ^{**}	K ⁺ (100)	66.1	34.4	101.4	3.0	[74]

* Determined using FRET with labeled strand

** Determined by DSC