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Unraveling the thermodynamics of folding and interconversion of human telomere G-quadruplexes

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Abstract

Why human telomere DNA fragments fold into different G-quadruplex structures with parallel, hybrid and antiparallel strand orientations depending on the temperature and concentration of cosolutes remains poorly understood. Similarly, the formation of intermediate structures along their folding or interconversion pathways is not well understood. Here we address these questions by introducing a conceptual framework, based on the global thermodynamic analysis of DSC and CD spectroscopy data, which enables us to provide a detailed description of the topological phase space (phase diagram) of the stability of the human telomere fragment 5'-AGGG(TTAGGG)₃-3' (Tel22). This framework clarifies the driving forces of quadruplex folding and interconversion processes over a wide range of temperatures and ion (K⁺, Na⁺) and polyethylene glycol (PEG) concentrations and demonstrates their linkage to the human telomere DNA structural features.

Keywords

human telomere repeat; G-quadruplex; topology; thermodynamics; DSC

Guanine-rich human telomere (ht) repeat DNA sequences fold into varied G-quadruplex structures by complex mechanisms involving multiple intermediates.^[1] Key questions about quadruplex formation are: "How do the ht-quadruplexes fold and interconvert?" and "Why do they fold in a specific way?" Several studies dealing with mechanisms and kinetics of folding and interconversion attempt to answer the "how" question.^[2] On the other hand, the

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“why” question, related to the thermodynamic driving forces, remains mostly unanswered due to the complexity of thermodynamic analysis that has to take into account multiple contributions to the stability of the intermediates and folded structures. Here we attempt to address the “why” question by introducing a conceptual thermodynamic framework which enables description of the topological phase space of Tel22 from which estimates of the driving forces of its folding and interconversions can be made over a wide range of temperatures (T) and ion (K^+ , Na^+) and PEG concentrations.

In different solutions Tel22 forms an ensemble of quadruplex structures with a variety of strand-segment orientations and loop topologies, including antiparallel “basket” (A)^[3] and “hybrid” (H)^[4] forms or a parallel “propeller” (P)^[5] form. These forms are in equilibrium with one another and with stable folding intermediate species resulting in a complex phase space. Our previous results described the structural features and stability of Tel22 conformations in K^+ and Na^+ solutions^[1a,6] and here we present new thermodynamic data collected in the presence of the cosolute PEG (Fig. 1). The availability of such an extensive thermodynamic dataset raised two questions crucial for our understanding of the ht-DNA fragment (un)folding and interconversion processes: (i) What is the simplest model mechanism that can describe all the available experimental data simultaneously? (ii) How can thermodynamic parameters be used to dissect and correlate the molecular contributions to folding and interconversions to changes in structural features? Answering these questions will enable us to achieve our fundamental goal of predicting population of Tel22 conformations (phases) as a function of T , $[K^+]$, $[Na^+]$ and $[PEG]$ and estimating the dominant driving forces of Tel22 folding and interconversion processes.

To address question (i) we monitored thermal unfolding of Tel22 in solutions with $[K^+] = 25$ mM and various $[PEG]$ using DSC^[7] (Fig. 1a). In addition, we measured CD spectra^[8] of Tel22 in solutions at $[K^+] = 25$ mM and different $[PEG]$ (Fig. 1b) or at $[PEG] = 60$ mM with different Na^+ concentration (Fig. 1c). Global model analysis (see Supplementary Information (SI) for details) suggests that the simplest mechanism able to describe all experimental data involves seven macroscopic states (Scheme 1). Transition between any of these states was described by the corresponding apparent standard Gibbs free energy change $G_{(T,K,Na,PEG)}$ (eq. S4) defined by seven parameters including standard enthalpy (H), entropy (S), and heat capacity (C_p) changes. They define the corresponding model functions (eqs. S2,S3), that fit very well to the experimental data (Fig. 1), and the thermodynamic profiles (Tables S1,S2).

To address the question (ii) we describe the ht-DNA folding and interconversion between the species (Scheme 1) at $T = 25^\circ C$, $[K^+] = [Na^+] = 25$ mM and $[PEG] = 0$ M in terms of more fundamental driving forces. First we dissected the corresponding apparent $G_{(T,K,Na,PEG)}$ of folding into its enthalpy and entropy components (Table S1). These data show that under these conditions Tel22 folding into P is unfavorable and becomes favorable only at high $[PEG]$ (Fig. S1). Each stage of $U \rightarrow I_P \rightarrow P$ folding is, just like the $U \rightarrow I_H \rightarrow H$ and $U \rightarrow I_A \rightarrow A$ transitions, characterized by extensive enthalpy-entropy compensation. It should be emphasized that our calorimetric data and global analysis uniquely provide a reasonable estimation of C_p , a fingerprint of changes in hydration. C_p correlates with changes in solvent accessible surface area and may be translated into G contribution (G_{hyd}) that at T

$\approx 25^\circ\text{C}$ reflects mainly the entropy of (de)hydration of hydrophobic groups. Fig 2a shows that most of G_{hyd} change upon folding occurs in the formation of stable intermediates suggesting that hydrophobic dehydration is the primary driving force of these processes. By contrast, specific ion binding, base stacking and H-bonding appear to be the main driving forces for $\text{I}_\text{P} \rightarrow \text{P}$, $\text{I}_\text{H} \rightarrow \text{H}$ and $\text{I}_\text{A} \rightarrow \text{A}$ folding steps. Taken together, the hydrophobic dehydration effects are significant in the initial steps of ht-quadruplex folding, a process which may be thought of as analogous to molten globule formation in folding of globular proteins.

Next we used thermodynamic cycles to estimate thermodynamic parameters for the interconversion of folded structural forms (Fig. 2, Table S2). They show that the $\text{P} \rightarrow \text{H}$ conversion is strongly favored, driven by a large favorable entropic term. The $\text{A} \rightarrow \text{H}$ conversion is energetically favorable, but with only about 1 kcal separating the two forms. The free energy changes in this case results from equal enthalpy and entropy contributions. In addition, we attempted to estimate the four main contributions to the observed free energies of interconversions under the specific solution conditions: $G = G_{\text{hyd}} + G_{\text{ion}} + G_{\text{int}} + G_{\text{conf}}$. (Changes in Tel22 and ion translational and rotational freedom may be neglected since the starting and end states are similarly ligated). G_{hyd} has already been mentioned above. G_{ion} is the contribution of (K^+ , Na^+) ion (de)hydration. G_{conf} represents the entropic contribution due to changes of conformational freedom. G_{int} is the contribution due to specific interactions arising mainly from differences in potential energy of van der Waals (base stacking), hydrogen bonding and cation coordination interactions (see SI for details on the estimation of these contributions). We are well aware that due to approximations involved in this dissection the estimated values are tenuous and the interpretation of G contributions should be taken with great care. Nevertheless, we believe that for the interconversions the G contributions are reliable enough to characterize their driving forces. The contributions describing $\text{A} \rightarrow \text{H}$ transition (Fig. 2b) show that H conformation is more populated than A mainly due to favorable ion (de)hydration effect (Na^+ hydration, K^+ dehydration; $G_{\text{ion}} \ll 0$) which is in agreement with the generally accepted interpretation of Na^+ and K^+ influence on the quadruplex stability^[9]. H conformation is slightly favored over A also by other hydration contributions ($G_{\text{hyd}} < 0$) and higher conformational freedom ($G_{\text{conf}} < 0$). All these contributions together overcompensate highly unfavorable G_{int} (mainly due to Na^+ de-coordination from the quadruplex core). Relatively small G_{hyd} and G_{conf} values are in accordance with similar exposure of surfaces to water in A and H structures (Table S3) and with their similar compactness.^[5d]

Driving forces for $\text{P} \rightarrow \text{H}$ conversion differ significantly from those for $\text{A} \rightarrow \text{H}$ conversion. P is not significantly populated because G_{hyd} that strongly favors the H form overcompensates positive G_{conf} . This interpretation is in accordance with P and H structural features which indicate that H structure is more compact than P and that the P form has more water exposed (hydrophobic) surface area than H.^[5d] This is supported by C_p estimated from the corresponding change in solvent accessible surface area (Table S3) which is in accordance with our experimentally determined C_p . Lowering the exposure of hydrophobic groups in Tel22 may thus increase the P type population. One way to achieve this is to add PEG.^[10] The suggestion that in the presence of PEG $\text{H} \rightarrow \text{P}$ transition becomes

favorable (Fig. S1) due to selective PEG binding to P conformation^[5e] and not due to crowding is in full agreement with the analysis of $P \leftrightarrow H$ conversion presented here (see SI). Hydrophobicity may be reduced also by replacing thymines in Tel22 sequence with less hydrophobic uracils.^[11] Indeed the shape of CD spectrum of the modified sequence (Fig. S5) suggests the coexistence of P and H type conformations. Thus lowering of hydrophobicity may represent an important factor governing the formation of the parallel structure from RNA Tel22.^[12]

Thermodynamic parameters used for the estimation of driving forces enable also the calculation of species population at any T , $[K^+]$, $[Na^+]$ and $[PEG]$ (Fig. S4) so that Tel22 phase diagrams can be constructed (Fig. 3). These diagrams indicate the possible macroscopic pathways of Tel22 (un)folding and interconversions. For example, Fig. 3a shows that Tel22 thermal unfolding at low $[PEG]$ follows mainly a $H \rightarrow I_H \rightarrow U$ pathway, but at high $[PEG]$ a $P \rightarrow I_P \rightarrow U$ pathway predominates. The Tel22 conformational change at 25°C induced by increasing $[PEG]$ follows a $H \rightarrow P$ pathway (Fig. 3a), but follows a $P \rightarrow A$ pathway by increasing $[Na^+]$ at $[PEG] = 60$ mM (Fig. 3b). $[Na^+]$ vs. $[K^+]$ diagrams (Fig. 3c,d) show that at 25°C phase A is accessible to Tel22 only at $[Na^+] \gg [K^+]$. These phase diagrams emphasize that ht-DNA adopts an ensemble of quadruplex structures in equilibrium, an equilibrium that can be easily perturbed by changes in solution conditions, cosolutes and quadruplex binding ligands.

The phase diagrams also show that populated intermediates in the absence of PEG appear mainly at $[Na^+]$ or/and $[K^+]$ between 10^{-3} M and 10^{-2} M while at high $[PEG]$ the I_P phase appears at $[K^+] < 10^{-3}$ M. This analysis suggests that phase U collapses into phase I at conditions at which the Tel22 chain is able to coordinate specifically one K^+ or Na^+ ion and simultaneously remove a significant amount of hydrophobic surface from water. Tel22 forms in phase I appear to be structured since their measured CD spectra are similar to the folded quadruplexes (Fig. S3). These intermediates may thus be considered analogous to the molten globule structures in proteins.

In summary, using global thermodynamic analysis of structural transitions of a human telomere fragment we describe in detail its topological phase space and suggest why it populates certain intermediate and folded quadruplex states at given temperatures and cosolute concentrations. We show that hydrophobic desolvation represents crucial driving force for the formation of stable compact folding intermediates that could be, in analogy with protein folding, characterized as human telomere quadruplex molten globules. Significantly, this thermodynamic analysis appears to give reliable predictions of driving forces for human telomere quadruplex interconversions that are consistent with the observed structural features. Therefore, we believe that this work represents an important step in elucidating thermodynamic principles by which human telomere G-quadruplexes fold and interconvert to different structures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Tel22 unfolding and interconversions at $[K^+] = 25$ mM monitored by DSC at different $[PEG]$ (a), and by CD spectroscopy at $T = 25$ °C at varying $[PEG]$ (b) or $[Na^+]$ at $[PEG] = 60$ mM (c). The globally fitted model functions (eqs. S3, S4; lines) derived on the basis of the model presented in Scheme 1 show good agreement with the experimental data (symbols).

Figure 2.

Thermodynamic profiles of Tel22 ($T = 25^\circ\text{C}$, $[\text{K}^+] = [\text{Na}^+] = 25 \text{ mM}$, $[\text{PEG}] = 0 \text{ mM}$). Free energy of Tel22 folding to intermediate, folded and ligand (L=Phen-DC3)-bound^[6b] folded forms as a function of the “dehydration” contribution estimated as $G_{\text{hyd}} = C_p \cdot 80\text{K}^{[13]}$ (a); Energetic contributions to the P → H (black) and A → H (red) interconversions: Free energy, enthalpy and entropy values (b); Best estimates for G_{ion} , G_{hyd} and G_{conf} contributions (c; see Table S2).

Figure 3.

Phase diagrams present the most populated Tel22 species (phases) at given T , $[K^+]$, $[Na^+]$ and $[PEG]$.

Scheme 1.

The simplest model mechanism (left) that successfully describes (un)folding and structural interconversions of Tel22 at various T , $[K^+]$, $[Na^+]$ and $[PEG]$ includes hybrid (H), antiparallel (A) and parallel (P) G-quadruplexes (Q), intermediates (I_H, I_A, I_P) and unfolded structure (U). A free energy diagram for the folding of quadruplex forms under specific solution conditions ($T = 25^\circ\text{C}$, $[K^+] = [Na^+] = 25\text{ mM}$ and $[PEG] = 0\text{ M}$) is shown on the right.