Recognition and Unfolding of c-MYC and Telomeric G-Quadruplex DNAs by the RecQ C-Terminal Domain of Human Bloom Syndrome Helicase

Sungjin Lee, Jinwoo Kim, Suyeong Han, and Chin-Ju Park*

Cite This: ACS Omega 2020, 5, 14513–14522

ABSTRACT: G-quadruplex (G4) is a noncanonical DNA secondary structure formed by Hoogsteen base pairing. It is recognized by various DNA helicases involved in DNA metabolism processes such as replication and transcription. Human Bloom syndrome protein (BLM), one of five human RecQ helicases, is a G4 helicase. While several studies revealed the mechanism of G4 binding and unfolding by the conserved RecQ C-terminal (RQC) domain of BLM, how RQC recognizes different G4 topologies is still unclear. Here, we investigated the interaction of Myc-22(14/23T) G4 from the c-Myc promoter and hTelo G4 from the telomeric sequence with RQC. Myc-22(14/23T) and hTelo form parallel and (3+1) hybrid topologies, respectively. Our circular dichroism (CD) spectroscopy data indicate that RQC can partially unfold the parallel G4, even with a short 3′ overhang, while it can only partially unfold the (3+1) hybrid G4 with a 3′ overhang of 6 nucleotides or longer. We found that the intrinsic thermal stability of G4 does not determine RQC-induced G4 unfolding by comparing Tm of G4s. We also showed that both parallel and (3+1) hybrid G4s bind to the β-wing region of RQC. Thermodynamic analysis using isothermal titration calorimetry (ITC) showed that all interactions were endothermic and entropically driven. We suggest that RQC partially unfolds the parallel G4 more efficiently than the (3+1) hybrid G4 and binds to various G4 structures using its β-wing region. By this information, our research provides new insights into the influence of G4 structure on DNA metabolic processes involving BLM.

INTRODUCTION

Nucleic acids can form noncanonical structures other than conventional duplexes, including quadruplexes, triplexes, i-motifs, and pseudoknots. Wobble and Hoogsteen base pairing, in addition to Watson–Crick base pairing, are used to form noncanonical DNA structures. Among these, G-quadruplexes (G4s) are frequently formed within guanine-rich sequences.1–3 G4s are two or more stacked G-tetrads composed of four guanines using Hoogsteen base pairing in one plane with monovalent cations such as sodium and potassium localized in the center of each plane.4 The structure and conformation of G4s are quite heterogeneous based on the polarity of the strands and differences in the loops between G-tetrads.5,6 Tetramolecular and bimolecular G4s can be formed by four short strands and two strands, respectively, and intramolecular G4s can be generated by one long DNA sequence.7,8 If all contributing DNA strands have their backbones in the same orientation (5′ to 3′), the G4 is defined as parallel; conversely, if the two strands are in the same direction while two are in the opposite direction, the structure is antiparallel. The structures with three in the same direction and one in the opposite are called “(3+1) hybrid”. The type and concentration of monovalent cations at the center of the plane also affect the stability of the G4.9,10

In the human genome, there are many (approximately 376 000) potential G4-forming sequences, mostly in the promoter and telomere regions.11 The G4 structures formed by these sequences are thought to be diverse topologies in vivo. Because of G4s’ high stability and location in the genome, they must be resolved for replication or transcription to take place. In this way, G4s are involved in the critical processes of DNA metabolism.12,13 In particular, G4s in oncogene promoters and telomere regions have attracted interest from researchers because unregulated G4 unfolding could trigger cancer progression.12 For the proper handling of the heterogeneous G4 structures, cells can use appropriate helicases for each G4 topology in DNA metabolism. It was shown that an Saccharomyces cerevisiae DNA helicase, Pif1, preferentially unfolds the antiparallel G4 rather than a parallel G4 having similar intrinsic stability.14

Bloom syndrome protein (BLM) is a G4 helicase and one of the five human RecQ helicases along with RECQ1, WRN,
RECQ4, and RECQ5. Mutations in the BLM protein cause Bloom syndrome, a recessive genetic disorder with symptoms of premature aging and a predisposition to various types of cancer. BLM unwinds DNA in the 3′ to 5′ direction using the energy of ATP hydrolysis and maintains gene integrity by regulating DNA replication, recombination, and repair. In addition to canonical duplex DNA, BLM binds to several kinds of noncanonical DNA structures such as Holliday junctions, D-loops, and G4s, and it preferentially binds to G4s compared to other noncanonical DNA structures. BLM is composed of several domains: the helicase domain, RecQ C-terminal (RQC) domain, and the helicase and RNase D C-terminal (HRDC) domain. Among these, the RQC domain has been established as the main DNA binding module.

Previous studies have characterized the G4 structure and its conformational changes using circular dichroism (CD) spectroscopy. CD signals reflect the structural elements of G4s such as stacking, strand orientation, and loop arrangements. By monitoring the maximum molar ellipticity change, G4 unfolding by the titration of chemical compounds (TAP, TmPyP4, etc.) and proteins (UP1, Prion, etc.) has been reported. Single-molecule Förster resonance energy transfer (FRET) has been used to study the unfolding dynamics of G4 in the presence of BLM. The helicase core of BLM (residues 642–1290) can effectively unfold a G4 by different mechanisms, depending on the structural environment. Moreover, even without the helicase domain, the Zn-RQC–HRDC construct (858–1298) can unfold the G4 structure. Also, a significant FRET population change from high FRET to low FRET was observed by the RQC–HRDC domain (1066–1298) addition to G4, which indicates that RQC–HRDC can induce partial G4 unfolding. In these studies, G4s have adopted either parallel or (3+1) hybrid structures. Our previous nuclear magnetic resonance (NMR) study showed that RQC without the HRDC domain can destabilize the parallel G4 structure. Previous research also revealed that G4 unfolding by BLM depends on the length of the 3′ overhang of G4 DNA. The BLM core (residues 642–1290) cannot unfold (3+1) hybrid G4s with 3′ overhang lengths shorter than 6 nucleotides (nt). For 6 nt overhangs, 10% of G4 structures were unfolded, whereas for 15 nt overhangs, 50% unfolding was observed by FRET.

Even though these details of BLM–G4 interaction and G4 unfolding by BLM were studied, it is still not known how BLM, and specifically the RQC domain, recognizes and unfolds different G4 topologies. In this study, we investigated the interaction of RQC with (3+1) hybrid G4 structures with different 3′ overhang lengths and compared it to the parallel G4–RQC interaction. We investigated four different 3′ overhangs, even though the parallel G4 is a derivative of hTelo, which is based on the human telomeric sequence. We added one thymine to the 3′ overhang of the original hTelo described in a previous study to ensure a 3′ overhang equal to that of Myc-22(14/23T). The structure of hTelo and the chemical shifts of its imino protons were determined by previous research. Myc-22(14/23T) is a derivative of Myc-2345 from the c-Myc promoter with a highly stable parallel G4 conformation. Previous structural studies revealed the properties of the G4s formed in the c-Myc promoter, including Myc-2345 and its derivative Myc-22(14/23T). The imino proton chemical shifts were determined in a previous study, and the RQC–Myc-22(14/23T) interaction was previously studied by NMR spectroscopy.

Here, we monitored the changes in the CD spectra of each G4 during RQC titration and studied corresponding interactions with isothermal titration calorimetry (ITC) to investigate thermodynamic characteristics. Our CD data indicate that RQC more effectively unfolds the parallel G4 (Myc-22(14/23T)) than the (3+1) hybrid G4 (hTelo-3nt) with the same 3-nt 3′ overhang, even though the parallel G4 is more thermally stable. RQC only partially unfolds the hTelo G4 with 3′ overhangs of 6 nt or longer. By analyzing changes in the 1H–15N heteronuclear single quantum coherence (HSQC) spectra of RQC with increasing concentrations of G4, we determined that both parallel and (3+1) hybrid G4s bind to the β-wing region of RQC. In summary, we propose that, although the G4 binding mode of BLM RQC is the same for both the (3+1) hybrid and parallel structures, the parallel G4 is more susceptible to unfolding upon interaction with RQC. Our study provides insights into the influence of G4 structures on DNA metabolic processes involving the BLM helicase.

### RESULTS

**Structure of hTelo Derivative G4s.** We first confirmed whether the hTelo derivatives (3, 6, and 15 nt overhangs) used...
in this study have the same conformation as the originally characterized hTelo G4 using CD and NMR spectroscopies. Figure 2A,B shows the CD and 1D 1H NMR spectra of hTelo-3nt. CD data on hTelo-3nt showed a positive band at 290 nm and a negative band at 240 nm, which is typical of (3+1) hybrid G4s. Also, the imino proton NMR spectrum was identical to the previously reported one (hTelo in Figure 1B). Because the imino protons chemical shifts are very sensitive to G4 topology, it means that hTelo-3nt, which has one dT more at the 3′, maintains the same (3+1) hybrid geometry. The hTelo-6nt and hTelo-15nt G4s also showed similar CD and 1H 1D NMR spectral features (Figure 2C−F) to hTelo-3nt. These data suggest that all three hTelo G4s mainly have (3+1) hybrid topology.

**CD Analysis of Conformational Changes in G4s Induced by RQC Binding.** A previous report showed that RQC induces partial unfolding of the Myc-22(14/23T) G4 by analyzing hydrogen−deuterium exchange data of the imino protons in the middle plane of the G4. While this is an effective way to investigate the stability of the hydrogen bonds, the changes induced in the G4 stacking by the unfolding can also be directly observed with CD spectroscopy. The CD spectra of Myc-22(14/23T) showed that the main conformation is a parallel G4, which is typified by a positive band centered at 260 nm and a negative band centered at 240 nm (Figure S1). Figure 3A,B shows the change in the CD spectrum of Myc-22(14/23T) upon the addition of RQC. The molar ellipticity at 260 nm was found to decrease in a protein-concentration-dependent manner. At [DNA]/[RQC] = 1:2, the molar ellipticity at 260 nm decreased to 64% of the original value. The decrease continued until [RQC] = 60 μM ([DNA]/[RQC] = 1:3), beyond which no further reduction was observed. The molar ellipticity at 260 nm was 55% of the original value at this concentration. Because the CD signal of RQC itself was zero from 250 to 300 nm (Figure S2), the increasing [RQC] in the sample did not contribute to the signal changes in this region. Therefore, it can be considered that expanded spectra (Figure 3B,D,F,H) are the same as the net change of G4 signal, and the contribution of RQC is eliminated. These results were consistent with the conclusion that RQC binding induces partial unfolding of the G4 structure.

We also monitored CD spectral changes of the hTelo-3nt G4 upon the addition of RQC. Figure 3C,D shows the change of the CD spectrum of hTelo-3nt upon RQC addition. Surprisingly, unlike Myc-22(14/23T), the positive band at 290 nm showed no change even after 2 molar equivalents of RQC were added. It implies that the hTelo-3nt maintains its stacking interaction in the presence of RQC. Previous research shows that the BLM core can unfold telomeric G4s with 3′ overhangs of 6 nt or longer. Therefore, we performed a CD analysis of hTelo-6nt and hTelo-15nt. Figure 3E,F shows that the molar ellipticity at 290 nm of hTelo-6nt G4 decreased in a concentration-dependent manner. At [DNA]/[RQC] = 1:2, the maximum molar ellipticity value decreased to 74% of the ellipticity value in DNA-only spectrum. For hTelo-15nt, molar ellipticity at 290 nm was also decreased upon RQC addition. At [DNA]/[RQC] = 1:2, the maximum molar ellipticity value decreased to 49% of the ellipticity value in DNA-only spectrum (Figure 3G,H). To confirm the effect of longer 3′ overhang on the unfolding of RQC, we performed the CD experiment using Myc-15nt G4. An additional 12 dTs were added to Myc-22(14/23T) to make Myc-15nt. At [DNA]/[RQC] = 1:2, the molar ellipticity of Myc-15nt at 260 nm decreased to 57% of
the original value, while Myc-22(14/23T) decreased to 64% of
the original value (Figure S3). This is consistent with CD
results using hTelos where a longer 3′ overhang induces more
progressive unfolding of RQC. Figure S4 shows the residual
molar ellipticity of each G4 structure at [DNA]/[RQC] = 1:2.
These data suggest that RQC interacts with and partially
unfolds parallel G4s, but not (3+1) hybrid G4s, but that
addition of a long 3′ overhang facilitates an interaction
between the (3+1) hybrid G4 and RQC that results in partial
unfolding.

Melting Temperatures of Each G4. Our CD data on
Myc-22(14/23T) and hTelo G4s with RQC raised the
question of how the intrinsic stability of each G4 DNA
contributes to the unfolding by RQC. We hypothesized that a
G4 that is inherently more unstable is more likely to be
unfolded by RQC. To investigate this possibility, the thermal
stability of each G4 was analyzed by measuring its melting
temperature using CD spectroscopy. The maximum molar
ellipticity of each G4 over a temperature range of 25–90 °C
was fitted to a Boltzmann sigmoidal curve to obtain the
melting temperature (Figure 4). For the hTelo G4s, as the 3′
overhang became longer, the melting temperature slightly decreased (Table 1), indicating that the extra nucleotides at the 3’ end affected the G4 stability. The partial unfolding of hTelo G4(-6nt and -15nt) by RQC monitored by CD spectroscopy could be related to their intrinsic thermal stabilities. For Myc-22(14/23T), a much higher melting temperature of 78.6 °C was estimated compared to those of the hTelo G4s (melting temperature range from 53.1 to 57.3 °C). This implies that Myc-22(14/23T) is much more intrinscally stable than hTelo G4s. These results suggest that the preferred unfolding function of the RQC to parallel G4 over the (3+1) hybrid ones is not related to the intrinsic stability of the G4 DNA.

Identification of Binding Surface on RQC. We monitored the changes in the backbone amide signals of RQC upon addition of the (3+1) hybrid G4 by $^1$H–$^{15}$N HSQC spectra to investigate the binding surface of RQC toward (3+1) hybrid G4. We used hTelo-3nt to investigate the binding surface on RQC because it is the least unfolded one. hTelo-6nt and -15nt are unfolded by RQC and have higher molecular weights than hTelo-3nt. They could contribute to either disappearance of peaks or perturbation of other regions besides the initial binding area. The binding surface of RQC for Myc-22(14/23T) was revealed by a previous study.30 Peaks were assigned based on previously published data.22 Figure 5A shows $^1$H–$^{15}$N HSQC spectra of RQC during titration of hTelo-3nt at 25 °C. Several peaks, most prominently N1162, were significantly perturbed, and several peaks disappeared upon addition of 2 equiv of G4. Figure 5B shows the histogram of the average chemical shift perturbations ($\Delta\delta_{\text{avg}}$) vs residue

![Figure 4.](image)

**Figure 4.** Boltzmann sigmoidal plot of the normalized maximum molar ellipticity of each G4 vs temperature.

**Table 1. Melting Temperature of Each G4**

| G4             | $T_m$ (°C) |
|----------------|-----------|
| hTelo-3nt      | 57.2 ± 0.3|
| hTelo-6nt      | 54.5 ± 0.4|
| hTelo-15nt     | 53.1 ± 0.3|
| Myc-22(14/23T) | 78.6 ± 4.4|

![Figure 5.](image)

**Figure 5.** (A) Overlaid $^1$H–$^{15}$N HSQC spectra of $^{15}$N-labeled RQC at increasing molar ratios of hTelo-3nt. (B) Chemical shift perturbations ($\Delta\delta_{\text{avg}}$) of $^{15}$N-labeled RQC induced by 1.0 equiv of hTelo-3nt. Residues with broadened cross-peaks upon hTelo-3nt addition are shaded in purple. The dotted lines indicate one and two standard deviations higher than the average. (C) Mapping of residues affected by G4 binding on the solution structure of RQC (PDB ID 2MH9). Residues perturbed by two standard deviations above the average are marked in red and those perturbed by one standard deviation above the average are marked in blue.

https://dx.doi.org/10.1021/acsomega.0c01176

ACS Omega 2020, 5, 14513–14522
Terminals are binding rather than direct interaction with G4 because both terminal regions are likely by the allosteric effect. Standard deviation above the average. Perturbations in the terminal regions are likely by the allosteric effects of G4 binding rather than direct interaction with G4 because both terminals are flexible unstructured regions. Significantly perturbed residues including V1103, N1162, and N1164 were mapped on the solution structure of RQC (Figure S5). N1162 and N1164 are located in the β-wing region, and V1103 is located in the α1–α2 loop. It is noteworthy that both β wing and α1–α2 loop regions were identified as the essential regions for duplex DNA and parallel G4 binding by previous research. In addition, V1103, N1162, and N1164 were the residues most perturbed by the addition of Myc-22(14/23T).  

Thermodynamic Parameters of RQC Binding to G4. To further investigate the RQC–hTelos interaction’s thermodynamic properties, ITC experiments were employed. Figure 6A shows the binding isotherm of RQC with hTelos-3nt. Figure 6B,C shows the results of RQC binding to hTelos-6nt and hTelos-15nt, respectively. We measured the heat of dilutions separately, and the heat of dilution was subtracted from the integrated heat change. The resulting data were fitted to the nonlinear regression fit (Figure S5). The thermodynamic parameters, including dissociation constants ($K_d$), are listed in Table 2. Our results showed that all $K_d$s of RQC with hTelos were in the micromolar range. The apparent $K_d$ of hTelos-3nt with RQC is the lowest among the G4s that we tested (0.59 ± 0.06 μM). $K_d$ values increased as the 3’ overhangs became longer: the $K_d$ of hTelos-6nt–RQC was 1.3-fold larger and that of hTelos-15nt–RQC was 2-fold larger than the hTelos-3nt–RQC complex. Also, the enthalpy and entropy values increased as the 3’ overhangs got longer. While the interaction was endothermic throughout the experiment for hTelos-6nt and hTelos-15nt, the hTelos-3nt–RQC interaction was endothermic at earlier titration points and changed to exothermic at later points. ΔG of each interaction was calculated using ΔH and ΔS values obtained from the ITC experiment (Table 2). The results of the ITC experiment using RQC domain and Myc-22(14/23T) have been published previously. The reaction was also endothermic and assumed to be an entropically driven process.  

### DISCUSSION

Previous reports showed that the BLM RQC domain is the main binding module for duplex and G4 DNA and that the BLM core without helicase domain (858–1298) can unfold the G4 in an ATP-independent manner. Moreover, RQC can partially unfold the parallel G4 structure in the absence of other domains. However, whether this binding and unfolding by the RQC domain has any difference for the type of G4 structure has not yet been revealed. In this study, using CD spectroscopy, we investigated partial disruption of (3+1) hybrid G4s by RQC. It is expressed quantitatively using the remaining molar ellipticity value in CD spectra. First, we confirmed that RQC could not unfold the (3+1) hybrid G4 with a short 3’ overhang (3 nt) and only partially unfolded the (3+1) hybrid G4s with long 3’ overhangs (≥6 nt). This is in line with previous research showing that the BLM core can unfold the telomeric G4 with 3’ overhangs of 6 nucleotides or longer. Also, as the 3’ overhang got longer, the melting temperature of the (3+1) hybrid G4 was decreased, which indicates that it became more unstable. Previously, it has been shown that adding extra nucleotides to the G4 terminal makes the structure less stable. Because RQC does not make additional contact with the ssDNA overhang of the DNA duplex in the crystal structure, it is reasonable that the
intrinsic G4 stability affects the extent of the unfolding by RQC in the (3+1) hybrid G4 case.

As shown through CD spectroscopy, RQC–G4 bindings that we examined in this study are associated with the conformational change of the G4. It implies that at least two events such as binding and unfolding occur during the titration of G4 into RQC. However, our ITC data only show a combined binding isotherm of all events at each titration, which was well-fitted to one event binding model. It is likely to contain the sum of all events occurring during each titration.41 The previous study showed that reverse titration separated heat isotherms of each event.12 Unfortunately, we could not obtain the separated isotherms of the binding and unfolding event by the titration of RQC into hTelo G4s (data not shown).

Figure 7. (A) Schematic drawing of Gibbs free energy state of each reaction coordinate. “Q” means folded G4 (quadruplex), “P” means RQC (protein), and “I” means the partially unfolded G4 (intermediate). \( \Delta G_b \) is the \( \Delta G \) of RQC–G4 binding and \( \Delta G_u \) is the \( \Delta G \) of G4 partial unfolding. (B) Comparison of \( \Delta G \) values of each G4 interaction with RQC.

In summary, our study provided the data on the interaction between BLM RQC and (3+1) hybrid G4s. CD spectroscopy showed that RQC could unfold a parallel G4 partially and (3+1) hybrid G4 with the same binding surfaces as revealed by our NMR data.30 In the case of hTelo-6nt and -15nt with RQC, positive heat changes throughout the titrations were observed. The unfolding process after the initial binding could contribute to the positive enthalpy and entropy change by the cation release when the hydrogen bond breaks in the guanine plane.

Contrary to hTelo-3nt, Myc-22(14/23T) could be partially disrupted by RQC. The decreased molar ellipticity in the CD spectrum (Figure 3A,B) is in line with the accelerated deuterium exchange of the guanines in the middle plane with RQC.47 These imply that RQC induces the disruption of stacking interactions and hydrogen bonds. It is remarkable because Myc-22(14/23T) G4 is intrinsically more stable than hTelo-3nt, as revealed by CD melting experiments.

Even though more investigation is necessary to answer why Myc-22(14/23T) shows preferential unfolding by RQC, the preferred unfolding of the parallel G4 by RQC could be physiologically meaningful. It has been revealed that many of the G4s observed in promoter regions, including those of c-myc, KRAS, VEGF, HIF-1α, and PDGF-A, have parallel topology.8,33,47–52 The G4 structures at transcription start sites and the first introns were shown to be the genomic target for BLM and BLM-dependent transcription regulation.53 Based on this, we would predict that RQC’s preferential unfolding of parallel G4s would lead to the preferred activity of BLM during transcription.
MATERIALS AND METHODS

Sample Preparation. The unlabeled BLM RQC (residues 1067–1210) and 15N-labeled BLM RQC were expressed and purified as previously described. All of the DNA sequences were purchased from IDT Inc. and dissolved in 20 mM Tris, 100 mM KCl, and pH 7.0 buffer to prepare the G4 solution at 1 mM. The dissolved G4s were heated to 95 °C for 10 min and cooled to room temperature for 1 h. Samples were stored at 4 °C after cooling.

CD Spectroscopy. Unless stated otherwise, all of the spectra were collected from 200 to 320 nm at a scanning speed of 100 nm/min and with a spectral bandwidth of 2 nm for each sample, and the spectra were observed after overnight incubation of samples. The average of three scans was recorded. For the Myc-22(14/23T) sample, spectra were collected from 200 to 300 nm.

For investigating the melting temperature of G4, a JASCO J-1500 CD spectrometer (KBSI, Ochang) was used. The concentration of four G4s was adjusted to have a maximum molar ellipticity value of 20 mdeg. The sample was heated from 25 to 90 °C at a rate of 1 °C/min. The maximum molar ellipticity values at 260 nm for Myc-22(14/23T) and at 290 nm for hTelo were normalized and fitted to the Boltzmann sigmoidal curve (eq 1) using Origin 2019 software where dx implies the slope of the curve and describes the steepness of the curve. Y is the molar ellipticity value, x is the temperature, and \( V_{eo} \) is the melting temperature. Error bar shows the standard error of the 95% confidence interval

\[
y = y_{\text{min}} + \frac{(y_{\text{max}} - y_{\text{min}})}{1 + e^{(V_e - x)/dx}}
\]  

(1)

To examine G4 unfolding by RQC, the prefolded Myc-22(14/23T) concentration was fixed at 20 μM, and BLM RQC was added to yield molar ratios of 0.05, 0.1, 0.2, 0.4, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 in the 20 mM Tris, 100 mM KCl, and pH 7.0 buffer. CD experiments were performed using a JASCO J-815 CD spectrometer (KBSI, Ochang) at 20 °C. For hTelo (3, 6, and 15 nt overhang), the prefolded G4 concentration was fixed at 60 μM, and BLM RQC was added to yield molar ratios of 0, 0.5, 1, 1.5, and 2 in the same buffer.

NMR Spectroscopy. NMR experiments were performed using a Bruker Avance II 900 MHz spectrometer equipped with a cryogenic probe (Korea Basic Science Institute, Ochang). 1D proton spectra of 0.3 mM hTelo-3nt G4 were obtained at 25 °C. 1H–15N HSQC spectra of 0.3 mM 15N-labeled RQC in the absence or presence of G4s were also obtained at 25 °C. All NMR data were processed with Topspin (Bruker) and analyzed with SPARKY software. The following equation was used to calculate the average chemical shift perturbation values (\( \Delta \delta_{\text{avg}} \)). \( \Delta \delta_{\text{avg}} \) values higher than one standard deviation above the average are selected as the significantly perturbed residues

\[
\Delta \delta_{\text{avg}} = \sqrt{(\Delta \delta_{\text{obs}})^2 + (\Delta \delta_{\text{pert}}/5.88)^2}
\]  

(2)

Isothermal Titration Calorimetry. A Nano-ITC SV instrument (GIST, Gwangju) was used for the ITC experiments. Aliquots of a highly concentrated G4 solution (400 μM) were titrated into a diluted 40 μM solution of RQC. Protein and DNA samples were dialyzed against 4 L of 20 mM Tris, 100 mM KCl, and pH 7.0 buffer before the experiment. One microliter of DNA stock was added to the protein sample for the first injection. Subsequent titration points were done with 5 μL injections into the cell for a total of 21 titration points. We applied other experimental conditions, which are as follows: interval, 300 s; stirring speed, 300 rpm; and cell temperature, 25 °C. The heat of dilutions was measured by titrating G4s into the buffer with the same experimental conditions. The measured dilution heat was subtracted from the data with the average area mode of area correction function in NanoAnalyze software (TA Instrument). The subtracted heat isotherm was obtained by point-to-point subtraction of the heat of dilution from the integrated heat change.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c01176.

CD spectrum of Myc-22(14/23T) G4 DNA; CD spectra of RQC; CD spectra of Myc-15nt in the absence and presence of RQC; comparison of remaining molar ellipticity of each G4; raw heat data on ITC of each G4s with the heat of dilution (PDF)

AUTHOR INFORMATION

Corresponding Author

Chin-Ju Park — Department of Chemistry, Gwangju Institute of Science and Technology, Gwangju 61005, Korea; orcid.org/0000-0002-7750-1554; Phone: +82 62 715 3630; Email: cjpark@gist.ac.kr; Fax: +82 62 715 2866

Authors

Sungjin Lee — Department of Chemistry, Gwangju Institute of Science and Technology, Gwangju 61005, Korea

Jinwoo Kim — Department of Chemistry, Gwangju Institute of Science and Technology, Gwangju 61005, Korea

Suyeong Han — Department of Chemistry, Gwangju Institute of Science and Technology, Gwangju 61005, Korea

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c01176

Author Contributions

S.L., J.K., and C.-J.P. designed the study. S.L., J.K., and S.H. prepared samples. S.L., J.K., S.H., and C.-J.P. planned and performed experiments. S.L. and C.-J.P. analyzed the data. S.L., J.K., and C.-J.P. planned and helped with CD and NMR experiments. The authors thank Dr. Eunha Hwang, Dr. Hae-Kap Cheong at KBSI, Ochang, for helping with CD and NMR experiments. The authors thank

Funding

This work was supported by the National Research Foundation (NRF) of Korea (Grant 2018R1A2B6004388 to C.-J.P.), which is funded by the Korean Government (MSIT); by a GIST Research Institute grant, funded by GIST in 2020; and by the Korea Basic Science Institute under the R&D program (Project No. D39700) supervised by the Ministry of Science and ICT, Korea.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank the high-field NMR facility at the Korean Basic Science Institute (KBSI, Ochang) for performing NMR experiments. The authors also thank Dr. Eunha Hwang, Dr. Eun-Hee Kim, and Dr. Hae-Kap Cheong at KBSI, Ochang, for help with CD and NMR experiments. The authors thank...
Dr. Melissa Stauffer of Scientific Editing Solutions for editing the manuscript.

**ABBREVIATIONS**

G4, G-quadruplex; BLM, Bloom syndrome protein; RQC, RecQ C-terminal domain; NMR, nuclear magnetic resonance; ITC, isothermal titration calorimetry; CD, circular dichroism; FRET, Förster resonance energy transfer; HRDC, helicase and RNase D C-terminal domain

**REFERENCES**

(1) Heddi, B.; Phan, A. T. Structure of Human Telomeric DNA in Crowded Solution. J. Am. Chem. Soc. 2011, 133, 9824−9833.

(2) Artese, A.; Costa, G.; Distinto, S.; Moraca, F.; Ortuoso, F.; Parrotta, L.; Alcaro, S. Toward the Design of New DNA G-Quadruplex Ligands through Rational Analysis of Polymorphism and Binding Data. Eur. J. Med. Chem. 2013, 68, 139−149.

(3) Karolisitiotis, A. I.; da Silva, M. W. Structural Probes in Quadruplex Nucleic Acid Structure Determination by NMR. Molecules 2012, 17, No. 13073.

(4) Haider, S.; Modr, Y. S.; Patel, D. J. Propeller-Type Parallel-Stranded G-Quadruplexes in the Human c-Myc Promoter. J. Am. Chem. Soc. 2004, 126, 8710−8716.

(5) Davis, J. T. G-Quartets 40 Years Later: From S-GMP to Molecular Biology and Supramolecular Chemistry. Angew. Chem., Int. Ed. 2004, 43, 668−698.

(6) Wang, Y.; Patel, D. J. Solution Structure of a Parallel-Stranded G-Quadruplex DNA. J. Mol. Biol. 1993, 234, 1171−1183.

(7) Wang, L.; Wang, Q. M.; Wang, Y. R.; Xi, X. G.; Hou, X. M. DNA-Unwinding Activity of Saccharomyces cerevisiae Pif1 Is Modulated by Thermal Stability, Folding Conformation, and Loop Lengths of G-Quadruplex DNA. J. Biol. Chem. 2018, 293, 18504−18513.

(8) Monnat, R. J. Human RECV Helicases: Roles in DNA Metabolism, Mutagenesis and Cancer Biology. Semin. Cancer Biol. 2010, 20, 329−339.

(9) Ellis, N. A.; Gorden, J.; Ye, T-Z.; Straughen, J.; Lennon, D. J.; Ciocci, S.; Poyrtcheva, M.; German, J. The Bloom’s Syndrome Gene Product Is Homologous to RecQ Helicases. Cell 1995, 83, 655−666.

(10) Bohr, V. A. Rising from the RecQ-Age: The Role of Human RecQ Helicases in Genome Maintenance. Trends Biochem. Sci. 2008, 33, 609−620.

(11) Karsisiotis, A. I.; Hessari, N. M.; Novellino, E.; Spada, G. P.; Randazzo, A.; da Silva, M. W. Topological Characterization of Nucleic Acid G-Quadruplexes by UV Absorption and Circular Dichroism. Angew. Chem., Int. Ed. 2011, 50, 10645−10648.

(12) Mou, T.-C.; Shen, M.; Abdalla, S.; Delamora, D.; Bochkareva, E.; Bochkarev, A.; Gray, D. M. Effects of ssDNA sequence on non-sequence-specific protein binding. Chirality 2006, 18, 370−382.

(13) Pradhan, D.; Hansen, L. H.; Vester, B.; Petersen, M. Selection of G-Quadruplex Folding Topology with LNA-Modified Human Telomeric Sequences in K+ Solution. ACS Omega 2020, 5, 14513−14522.
Human Telomere d[AG3(TTAG3)3] Quadruplex Folding. *Nucleic Acids Res.* 2014, 42, 14031–14041.

(38) Yoo, S.; Lee, S.; Park, C.-J. Backbone Dynamics and Model-Free Analysis of the RecQ C-Terminal Domain of Bloom Syndrome Protein. *Bull. Korean Chem. Soc.* 2018, 39, 1243–1247.

(39) Swan, M. K.; Legits, V.; Tanner, A.; Reaper, P. M.; Vial, S.; Benett, R.; Pollard, J. R.; Charlton, P. A.; Golec, J. M. C.; Bertrand, J. A. Structure of Human Bloom’s Syndrome Helicase in Complex with ADP and Duplex DNA. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* 2014, 70, 1465–1475.

(40) Hayden, K. L.; Graves, D. E. Addition of Bases to the 5′-End of Human Telomeric DNA: Influences on Thermal Stability and Energetics of Unfolding. *Molecules* 2014, 35, 3347–3356.

(41) Datta, K.; LiCata, V. J. Thermodynamics of the Binding of *Thermus aquaticus* DNA Polymerase to Primed-Template DNA. *Nucleic Acids Res.* 2003, 31, 5590–5597.

(42) Datta, K.; Wowor, A. J.; Richard, A. J.; LiCata, V. J. Temperature Dependence and Thermodynamics of Klenow Polymerase Binding to Primed-Template DNA. *Biophys. J.* 2006, 90, 1739–1751.

(43) Giri, M.; Maulik, A.; Singh, M. Signatures of Specific DNA Binding by the AT-Rich Interaction Domain of BAF250a. *Biochemistry* 2020, 59, 100–113.

(44) Kerckour, A.; Marqueville, J.; Ivashchenko, S.; Yatsunyk, L. A.; Mergny, J. L.; Salgado, G. F. High-Resolution Three-Dimensional NMR Structure of the KRAS Proto-Oncogene Promoter Reveals Key Features of a G-Quadruplex Involved in Transcriptional Regulation. *J. Biol. Chem.* 2017, 292, 8082–8091.

(45) Sun, D.; Guo, K.; Rusche, J. J.; Hurley, L. H. Facilitation of a Structural Transition in the Polypurine/Polypyrimidine Tract within the Proximal Promoter Region of the Human VEGF Gene by the Presence of Potassium and G-Quadruplex-Interactive Agents. *Nucleic Acids Res.* 2005, 33, 6070–6080.