

Optimization of surface plasmon resonance based assay for investigating T-antigen helicase activity

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Due to the increasing number of helicases shown to unwind quadruplex DNA structures in addition to duplex DNA, the biological significance of this activity is currently under investigation. One limitation of traditional gel analysis of helicase activity is the inability to effectively monitor unwinding of intramolecular G-quadruplex DNA substrates. Optimization of our novel SPR-based assay for monitoring the helicase activity of simian virus 40 (SV40) large T-antigen (T-ag) was undertaken to explore limitations and improvements in the ability to investigate G-quadruplex helicase activity. Although T-ag helicase was used, the assay is general in nature. An improved method for assessing unwinding of intramolecular G-quadruplex DNA substrates was developed.

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Abbreviations: Simian virus 40, SV40; origin of replication, OR; large T-antigen, T-ag; origin binding domain, OBD; surface plasmon resonance, SPR; dimethylsulfide, DMS; polyacrylamide gel electrophoresis, PAGE; adenosine triphosphate, ATP.

Introduction

Investigation of the role helicases play in maintaining genomic stability has been stimulated by the growing number of such proteins shown to be involved in DNA repair [1], replication and recombination [2], and telomere function [3]. In addition to duplex DNA substrates, tetra- and bi-molecular G-quadruplex DNA structures are unwound by a number of helicases, including *E. coli* RecQ [4], yeast Sgs1p [5], human BLM [6], WRN [7] and FANCD1 [8], as well as simian virus 40 (SV40) large T-antigen (T-ag) [9]. The biological role of G-quadruplex DNA has not been conclusively demonstrated; however, the potential for G-quadruplex formation in the human genome correlates with maintenance of genetic stability and function [8, 10-12].

Cellular DNA replication studies often employ SV40 replication as a model system since the SV40 genome is extensively characterized and possesses similarities to eukaryotic chromosomes [13, 14]. Potential G-quadruplex forming regions identified in the SV40 genome occur primarily near the origin of replication and polyadenylation signals for early and late transcripts [15]. A particularly likely site of G-quadruplex formation encompasses six GGGCGG repeats near the origin of replication. An unusual tetramolecular quadruplex structure formed from an oligonucleotide encompassing a portion of this repeat sequence has been observed by NMR [16]. In addition, DMS protection observed for this region of the SV40 genome indicates likely formation of an intramolecular quadruplex structure [15]. The quadruplex unwinding activity of T-ag, an

essential protein for viral replication and transformation [17], may thus be necessary for the efficient replication of the SV40 genome.

Helicase activity is primarily visualized by gel shift analysis of labeled DNA substrates. The ability of T-ag to unwind duplex, bimolecular and tetramolecular quadruplex DNA substrates has been demonstrated using PAGE analysis of radiolabeled DNA [15]; however, unwinding of intramolecular quadruplex substrates is not easily visualized in this manner. We recently developed a novel SPR-based assay for observing T-ag helicase activity in real-time [18]. Advantages of the SPR-based assay include the requirement of only small sub-stoichiometric amounts of helicase and the ability to monitor unwinding of an intramolecular G-quadruplex DNA substrate which is difficult to achieve using traditional PAGE techniques. Since G-quadruplexes of *in vivo* relevance are likely to be intramolecular in composition [19-20] as well as bimolecular, the ability to monitor intramolecular quadruplex helicase activity and inhibition is important. Here we report improvements to our original SPR-based assay of helicase activity that provide a means to investigate G-quadruplex DNA helicase activity in the absence of any additional reporter molecules and optimize reproducibility. This ability to monitor G-quadruplex helicase activity using a DNA substrate containing an intramolecular G-quadruplex-forming region followed by duplex region is important since this more closely approximates *in vivo* conditions in which there is most likely competition between duplex/ quadruplex DNA structures.

Materials and Methods

Chemicals, Reagents, and DNA substrates

Chemicals were obtained from Sigma-Aldrich or GE Healthcare. Stock solutions and running buffers included HBS-EP buffer (0.01 M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, and 0.005% v/v P20 surfactant), HBS-EP-Mg²⁺ Buffer (0.01 M HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 10 mM MgCl₂, and 0.005% v/v P20 surfactant), or HBS-EP-K⁺ Buffer (0.01 M HEPES, pH 7.4, 0.15 M KCl, 3 mM EDTA, and 0.005% v/v P20 surfactant). Potassium chloride and magnesium chloride were obtained from EM Science. All buffers were degassed and passed through 0.2 µm filters (Nalgene) prior to use. DNA oligonucleotides were obtained from Integrated DNA Technologies. Biotinylated sequences were HPLC purified; additional sequences were either PAGE purified or desalted and used without further purification.

SV40 T-ag was obtained from CHIMERx, where it was isolated from cultured insect cells and stored in 10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1.0 mM EDTA, 1.0 mM dithiothreitol, and 50% (v/v) glycerol. T-ag was used without further purification. Aliquots of the T-ag enzyme were stored at -80°C and diluted with HBS-EP-Mg²⁺ buffer to the desired concentrations just prior to use. Concentrations of T-ag were expressed in terms of T-ag hexamer concentration.

All experiments were performed on a BiacoreX instrument. Streptavidin-derivatized sensor chips were immobilized with single-stranded 5'-biotinylated oligonucleotides and hybridization to the desired complement was accomplished as previously described [18]. Levels of immobilization were ~500-1000 RUs. Hybridization levels were ~90-100%. Regeneration of chip surfaces was accomplished with 0.005% SDS. Immobilized sequences and DNA substrates are shown in Table 1.

Optimization of Duplex Unwinding

ATP at 1, 2, 4, 8, 16, and 32 mM, was mixed with 2.93 nM of T-ag in HBS-EP-Mg²⁺ buffer just prior to injections. The T-ag solutions were injected at a flow rate of 20 µL/min over sensor chips, immobilized with biotinylated single-stranded DNA (5'-BioTEG-TTT TTT TTG AGC AGC AAT ACA CGA-3').

T-ag (1.0, 2.93, 4.2, 6.43, 8.74, 12.93, and 14.2 nM, respectively) was injected over sensor chips immobilized with the same biotinylated single-stranded DNA as above that was hybridized to a partially complementary sequence containing a free 3'-tail to form dsDNA1 (Table 1). Aliquots of T-ag (100 µL) in HBS-EP-Mg²⁺ buffer containing 40 mM ATP were injected at a flow rate of 20 µL/min, with a delayed wash of 300 sec. The percent DNA substrate unwound was calculated as previously reported [18].

At a flow rate of 20 µL/min, T-ag in HBS-EP-Mg²⁺ buffer containing 40 mM ATP was injected for 25 sec, 50 sec, 125 sec, 200 sec, and 250 sec (which corresponded to 8 µL, 17 µL, 42 µL, 67 µL, and 75 µL of 2.9 nM T-ag, respectively) over immobilized dsDNA1. The percent DNA substrate unwound in each case was determined as previously described.

HBS-EP-Mg²⁺ buffer containing NaCl at 1.5, 15, 50, 150, and 300 mM was used for diluting T-ag (2.9 nM). Injections of T-ag containing 40 mM ATP were performed as described above. Running buffers were adjusted to contain equivalent amounts of NaCl.

Influence of DNA substrate sequence on T-ag duplex helicase activity

The biotinylated sequence containing the SV40 site II palindromic origin of replication was

immobilized as described above. Due to the high propensity of this sequence to form secondary structures, most likely small hairpin loops, the temperature of the Biacore was raised to 37°C prior to hybridization to form dsDNA4 and the complimentary DNA was heated to 85°C prior to injection. An injection of T-ag (9.7 nM, HBS-EP-Mg²⁺ buffer) at a flow rate of 20 µL/min was performed immediately after dsDNA4 was formed. T-ag was removed from the DNA substrate and sensor surface by regeneration with a solution of 2 M guanidine-hydrochloride, 10% (v/v) formamide and 0.3% (v/v) p20 surfactant.

A shorter 5'-biotinylated SV40 origin sequence-containing oligonucleotide was immobilized on a sensor chip and hybridized to a full complement to form dsDNA5. T-ag was injected as before (9.7 nM, HBS-EP-Mg²⁺ buffer) at a flow rate of 20 µL/min. After the injection ceased, T-ag remained bound to the DNA and was removed by regeneration as stated above. Hybridizing a partial complement to produce a forked substrate (dsDNA6) was achieved in the same manner.

Preparation and verification of quadruplex DNA substrates

Two quadruplex-containing DNA substrates, dsDNA2G and dsDNA3G, were formed *in situ* on sensor chips as described above. The DNA substrates were equilibrated in HBS-EP-K⁺ buffer (150 mM KCl) and the running buffer was changed to HBS-EP-Mg²⁺ just prior to injections of T-ag (2.9 nM, 40 mM ATP, HBS-EP-Mg²⁺, 20 µL/min). The percent DNA substrate unwound was calculated as previously described.

In order to ascertain the extent of folded quadruplex, the ability of the oligonucleotide 5'-[CCC TAA]₄-3' to bind to dsDNA3G at different

concentrations of KCl or LiCl was determined. The quadruplex-forming substrate dsDNA3G was equilibrated in HBS-EP buffer containing 15 mM, 50 mM, or 150 mM KCl, respectively, or 150 mM LiCl. Injections of the oligonucleotide 5'-[CCC TAA]₄-3' in the same equilibration buffer were performed at 20 μ L/min. The amount of unfolded dsDNA3G was calculated from:

$$[RU_A / [RU_L \times (MW_A/MW_L)]] \times 100$$

where RU_A is the response after hybridization of 5'-[CCC TAA]₄-3' to dsDNA3G, RU_L is the RU increase obtained after hybridization of 3'-(T)₆(GGG ATT)₄(T)₆TCG TCG TTA TGT GCT-5' to the immobilized sequence 5'-BioTEG-TTT TTT TTG AGC AGC AAT ACA CGA-3' in order to form dsDNA3G, MW_A is the molecular weight of 5'-[CCC TAA]₄-3', and MW_L is the molecular weight of the quadruplex-forming oligonucleotide 3'-(T)₆(GGG ATT)₄(T)₆TCG TCG TTA TGT GCT-5'. The interaction between the two strands is a 1:1 binding interaction; therefore multiplication by a binding stoichiometry factor (S) is not necessary.

Results and Discussion

The optimization of our novel SPR-based assay for monitoring unwinding of DNA substrates was undertaken to explore limitations and improvements in the ability to investigate helicase activity, including unwinding of intramolecular G-quadruplex DNA. SV40 T-ag was employed as the model helicase for these studies. Different DNA substrates, varying between random sequence duplex oligonucleotides, SV40 origin of replication repeat sequence-containing duplex substrates, and substrates capable of adopting an

intramolecular G-quadruplex conformation were utilized to assess the affect of sequence composition on the observed helicase activity. Optimization of ATP concentration, T-ag helicase concentration, DNA/T-ag contact time and ionic strength, was performed to probe the effect on observed duplex and G-quadruplex helicase activity. Finally, an improved method for assessing unwinding of intramolecular G-quadruplex DNA substrates was developed (Figure 1).

DNA Substrates

The duplex dsDNA1 is a random sequence forked substrate that is easily unwound by T-ag, resulting in loss of the complementary strand from the sensor chip (Figure 1). However, we were also interested in determining the effect of T-ag/DNA sequence specificity on the observed real-time unwinding of the DNA substrate. Therefore, the influence of incorporating the SV40 OR repeat sequence into DNA substrates was investigated using dsDNA4, dsDNA5, and dsDNA6 (Table 1).

The intramolecular G-quadruplex-forming G4DNA (Table 1) employed in our initial assay of T-ag helicase activity was directly immobilized to the sensor surface, requiring a secondary means of determining the extent of unwinding since no change in mass results at the sensor surface as a result of T-ag helicase activity for this substrate [18]. This requirement complicates the assay, leading us to develop an improved G-quadruplex helicase assay involving a substrate that incorporates an intramolecular G-quadruplex-forming region on the complementary strand of a duplex (dsDNA2G and dsDNA3G, Table 1). Unwinding of the G-quadruplex-forming region on the free 3'-end of

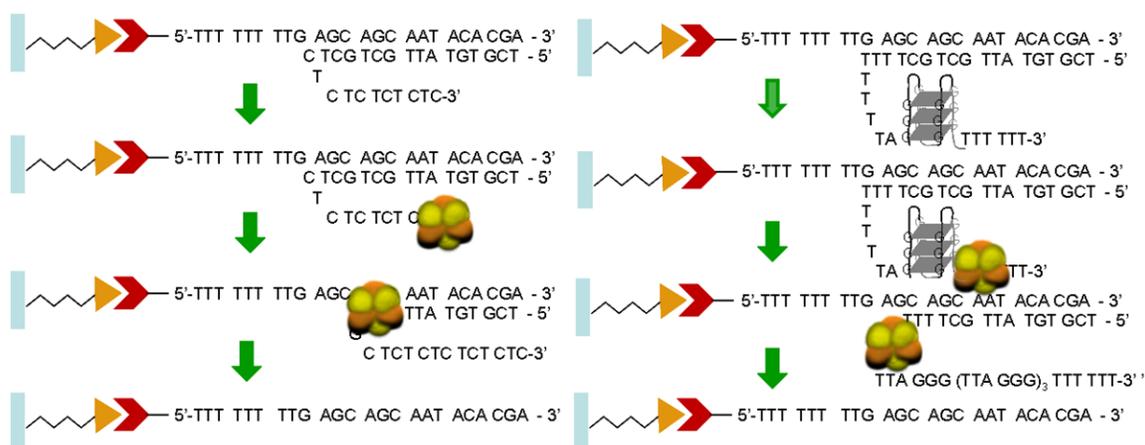


Figure 1. SPR-based analysis of T-ag helicase activity. The scheme depicts the strategy for assessing unwinding of duplex (left) and intramolecular quadruplex (right) DNA substrates. Unwinding of each substrate by T-ag (hexamer) liberates the complement from the chip while the 5'-biotinylated-oligonucleotide remains immobilized. The corresponding decrease in SPR response reflects the amount of DNA substrate unwound.

Table 1. Immobilized DNA substrates. Single-stranded DNA was immobilized through capture of 5'-biotinylated (BioTEG)-oligonucleotides. Duplex substrates were formed on the chip through hybridization of the desired complement. (Sequences shown in green are complimentary to the preceding sequences shown in red).

DNA Substrate	Sequence
G4DNA	5'-BioTEG-(TTAGGG) ₄ TT-3'
dsDNA1	5'-BioTEG-TTTTTTTT GAGCAGCAATACACGA -3' 3'-CTCTCTCTCT CTCGTCGTTATGTGCT -5'
dsDNA2G	5'-BioTEG-TTTTTTTT GAGCAGCAATACACGA -3' 3'-TTTT(GGGATT) ₄ CCCGTTATGTGCT -5'
dsDNA3G	5'-BioTEG-TTTTTTTT GAGCAGCAATACACGA -3' 3'-(T) ₆ (GGGATT) ₄ (T) ₆ TCGTCGTTATGTGCT -5'
dsDNA4	5'-BioTEG-TGAGCTATTCCAGAAGTAGTGTAATTTTTTT TATTTATGCA GAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTG -3' 3'-TCCGGTTCAG- ATAAATACGT CTCCGGCTCCGGCGGAGCCGGAGACTCGATAAGGTCTTCATCAC -5'
dsDNA5	5'-BioTEG- GCTCAGAGGCCGAGGCCGCTCGGCC -3' 3'- CGAGTCTCCGGCTCCGCCGAGCCGG -5'
dsDNA6	5'-BioTEG-GCTCA GAGGCCGAGGCCGCTCGGCC -3' 3'-TTTTA CTCCGGCTCCGCCGAGCCGG -5'

the complementary strand is necessary before liberation of the complementary strand from the sensor surface can occur (Figure 1). This intramolecular quadruplex-forming substrate, dsDNA3G, probably more closely approximates *in vivo* conditions encountered by T-ag during helicase activity, in which G-quadruplex-forming DNA regions would be surrounded by duplex DNA.

Optimization of Duplex Unwinding Assay

In order to optimize conditions for monitoring T-ag duplex helicase activity the influence of ATP concentration, T-ag concentration, DNA/T-ag contact time, ionic strength and DNA sequence on the observed unwinding efficiency was investigated.

ATP is required for assembly of the active hexamer T-ag helicase [21] and for 3'-5' translocation along the DNA substrate [22, 23]. Binding of T-ag (2.9 nM) to a 5'-immobilized random sequence DNA strand resulted in an increased SPR response with increasing amounts of ATP; the response leveled off in the presence of 16-32 mM ATP, at 10 mM Mg²⁺ (Figure 2). T-ag did not bind to the DNA in any appreciable amount in the absence of ATP.

We have found the activity of T-ag to be variable depending on the batch of T-ag used, conditions for solution preparation, and handling. Therefore, our intent was to establish a baseline of activity in the SPR assay with which to compare subsequent measurements of T-ag activity. T-ag at various hexamer concentrations was injected over SA chips immobilized with dsDNA1. The minimum amount of T-ag hexamer required to remove slightly less than 100% of the hybridized complement from the substrate dsDNA1 was determined to be 2.9 nM. It should be noted

that this concentration of T-ag hexamer was sufficient to unwind slightly less than 100% of the DNA substrate at immobilization levels of ~500 – 1000 RUs. Higher levels of immobilized DNA substrate may require a greater amount of T-ag for complete unwinding to occur.

The contact time between injected T-ag and the immobilized DNA substrates is controlled by the flow rate and the volume of injected T-ag. In order to determine the minimum length of time needed to observe complete duplex unwinding for random duplex substrates, T-ag (2.9 nM) was injected for 25, 50, 125, 200, and 250 seconds at a flow rate of 20 µl/ min over immobilized dsDNA1. Complete duplex unwinding was observed within the 25 second interval. The rate of T-ag duplex unwinding has been reported as a “few hundred” base pairs per minute [24]. Our DNA substrates are considerably shorter and the observed time frame for complete unwinding is thus reasonable. In order to measure smaller time increments, prohibitively small injection volumes for the Biacore X would be required.

Table 2. Influence of contact time and ionic strength on DNA substrate unwinding. The duplex substrate dsDNA1 was formed on the sensor chip as described and subjected to T-ag helicase activity under variable contact time and ionic strength, as noted. Unwinding of the DNA occurred immediately upon injection of T-ag and the extent of unwinding decreased with increasing ionic strength.

Contact Time (s)	NaCl (mM)	% unwound*
25	1.5	100
50	1.5	98
125	1.5	95
200	1.5	96
225	1.5	92
225	15	93
225	50	89
225	300	86

*values are averaged from 2-3 determinations and are within +/- 5%.

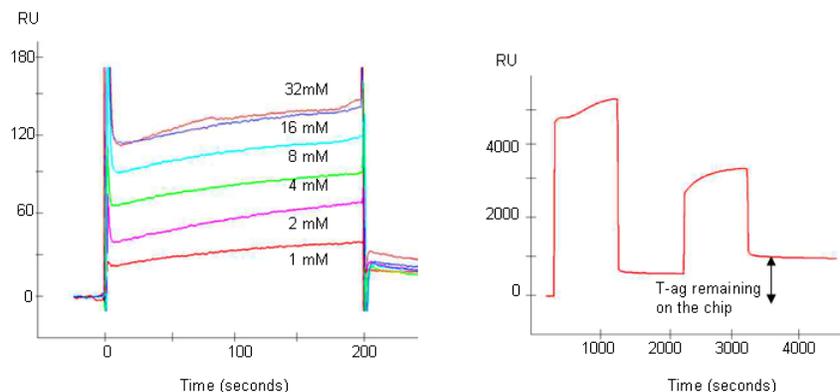


Figure 2. Binding of T-ag to 5'-immobilized single-stranded DNA at increasing ATP concentration (left), and SV40 OR-containing duplex substrate (right). The binding of T-ag to immobilized single-stranded DNA increased at increasing concentration of ATP (1 mM – 32 mM, indicated on sensorgram) until leveling off at 16-32 mM ATP. At the end of the injection, T-ag dissociated quickly from the DNA in the absence of additional ATP in the flow solution. Conversely, a substantial amount of T-ag remained bound to a duplex substrate containing a portion of the SV40 OR, even after the end of the injection (right). If the duplex substrate had been unwound, a corresponding decrease in RUs relative to the initial value at zero would have been observed. The sensorgram on the right is not reference-subtracted to reflect the absolute binding of T-ag to the substrate.

The amount of dsDNA1 unwound by T-ag clearly decreased at *increasing* ionic strength (>96% unwound at 1.5 mM NaCl, decreasing to 85% unwound at 300 mM NaCl), consistent with the reported detrimental effect of increased ionic strength on the binding of the T-ag origin binding domain (OBD) to DNA substrates [25]. However, even at somewhat elevated ionic strength (150 mM NaCl), T-ag bound with high affinity to DNA substrates containing either the full SV40 origin pentanucleotide sequence (dsDNA4) or a partial origin-containing sequence (dsDNA5, Figure 2).

Hybridization to form dsDNA4 substrate was problematic, presumably due to the high propensity of the immobilized single-stranded sequence to form hairpin or other higher order structures. Partial hybridization (~50%) was achieved after elevating the temperature briefly before slowly cooling to room temperature; however, no unwinding of dsDNA4 was observed after contact with T-ag, even though dsDNA4 contains a free 3'-end. In fact, T-ag could only be removed from the DNA with harsh regeneration conditions. The shorter

origin-containing DNA substrates dsDNA5 and dsDNA6 contain three of the four pentanucleotide repeats from the SV40 origin and lack a flanking AT-region. These substrates hybridized completely but neither dsDNA5 nor dsDNA6, which also possesses a free 3'-end, was unwound after contact with T-ag. Again, T-ag was removed only under harsh regeneration conditions, indicating the strong binding that occurs between T-ag and the SV40 origin.

During initiation of SV40 replication, T-ag assembles at the OR sequence as a double hexamer prior to origin unwinding and subsequent helicase activity [26-27]. It is still not known exactly when in the sequence of initiation events the four pentanucleotide repeats of the origin are unwound. It has been suggested that T-ag remains as a double hexamer complex, with single-stranded DNA threaded through it in a 3'-5' direction [26]. Alternatively, each hexamer would translocate in the opposite direction from the origin. However, the affinity of the T-ag origin binding domain for the pentanucleotide repeat sequence is 31 nM [28] compared to 25 μ M for

single-stranded DNA [27] and this difference may be the reason we don't observe any unwinding of dsDNA4 or dsDNA6 in the absence of any additional factors, such as the single-strand binding protein RPA.

Our primary goal was a label-free, real-time means to observe T-ag intramolecular G-quadruplex helicase activity and inhibition. Since the helicase activity of T-ag involves primarily non-specific contacts with DNA substrates, we focused our attention on DNA substrates lacking the SV40 OR sequence.

Quadruplex Unwinding Assay

The first verification of G-quadruplex DNA on SPR sensor chips employed *E. coli* single-strand binding protein (SSB) as a reporter for DNA conformation [29]. This approach requires a secondary reporter; in addition, substrates such as dsDNA2G and dsDNA3G contain single-stranded and duplex regions as well as the intramolecular G-quadruplex-forming region. Therefore, binding of SSB would not necessarily indicate whether or not the quadruplex was folded. In the absence of verifiable intramolecular G-quadruplex formation, T-ag helicase activity to unwind dsDNA2G and dsDNA3G could just as easily be attributed to unwinding of a small duplex with a long, extended single-stranded tail.

The equilibrium between intramolecular folding (quadruplex) and unfolding (single-stranded DNA) for a number of immobilized intramolecular quadruplex DNA sequences has been distinguished using SPR in a coupled reaction of bimolecular ligand binding to the DNA substrate [30]. The half-life of the folded molecules during ligand binding ranged from 124 – 1260 seconds, depending on the quadruplex sequence and ligand. During

competition with hybridization to form a duplex, the half-life of the folded molecules decreased to 59 – 98 seconds, depending on sequence. These time frames are commensurate with those used in our SPR helicase assay. In all cases, equilibrium association constants for hybridization to form the duplex were favorable [30]. Therefore, the extent of hybridization of an oligonucleotide *complementary* to the G-quadruplex-forming region of dsDNA2G and dsDNA3G (forming a duplex region instead) should give a maximum limit for the proportion of unfolded substrate under conditions of the SPR experiments.

We challenged our G-quadruplex-forming DNA substrate, dsDNA3G, with an oligonucleotide that is complementary to the intramolecular G-quadruplex-forming region. Subsequent hybridization of this additional complement to dsDNA3G should only occur with DNA substrates that are not folded into the G-quadruplex secondary structure. The DNA substrate dsDNA3G was folded *in situ* on the sensor chip with HBS-EP buffer containing varied salt composition and the additional complement was then flowed over the sensor chip (Figure 3). The maximum percent unfolded G-quadruplex substrate was calculated as specified in the methods. In 150 mM KCl-containing HBS-EP buffer, the maximum amount of unfolded G-quadruplex substrate was 26%. The maximum amount of unfolding increased to 47% at 15 and 50 mM KCl. By comparison, the amount of unfolded substrate in 150 mM LiCl-containing HBS-EP buffer was 86%. Folding of quadruplex structures in solution and on sensor chips under different buffer conditions has been investigated [31] and our results indicate that the sensor-bound substrate dsDNA3G utilized in this study is folded as expected.

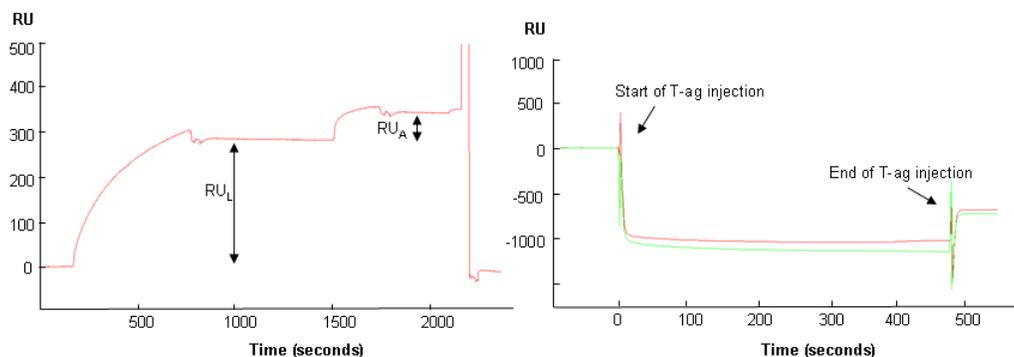


Figure 3. Formation of intramolecular G-quadruplex forming DNA substrate (left) and quadruplex unwinding by T-ag (right). The increase in RU observed after addition of the intramolecular G-quadruplex forming complement to immobilized single-stranded DNA in order to form dsDNA3G is reflected by RU_L . Addition of a duplex forming complement that hybridized to unfolded dsDNA3G is reflected by RU_A . The amount of unfolded substrate was calculated as specified in the methods. The sensorgram on the left was obtained using 50 mM KCl-containing buffer. At 150 mM KCl, dsDNA3G is greater than 86% folded. Under these conditions, the addition of T-ag to the sensor chip leads to nearly complete removal of the intramolecular G-quadruplex forming complement (right), reflected by the steep and persistent decrease in RU observed in the sensorgram. Both sensorgrams are reference-subtracted.

The intramolecular G-quadruplex containing DNA substrate dsDNA2G contains an 11-base pair duplex region proximal to the intramolecular G-quadruplex forming region of the oligonucleotide (Table 1). This substrate did not efficiently form on the SPR sensor chip, presumably since the folded quadruplex region is so close to the duplex region that anchors the substrate to the chip. However, the addition of a poly-dT spacer between an intramolecular G-quadruplex forming region and a longer 15-base pair duplex region allowed complete hybridization to form the dsDNA3G substrate (Table 1). As described above, this substrate was determined to be at least 84% folded (maximum of 26% unfolded) in 150 mM KCl-containing buffer.

Unwinding of dsDNA3G occurred efficiently under conditions similar to those utilized for assessing duplex unwinding (Figure 3). Helicase activity was not observed in the absence of ATP. In addition, the loss in response observed after T-ag helicase activity could be exactly replaced

by rehybridizing the G-quadruplex-forming complement to reform the dsDNA3G substrate (not shown).

Quadruplex structure and stability is affected by sequence composition, including the length of connecting loops [20]. The human telomeric repeat sequence TTA GGG, used to form the intramolecular G-quadruplex region in the dsDNA3G substrate is repeated thousands of times in human telomeres [32]. However, quadruplex-forming regions in other parts of the genome, including promoter regions of oncogenes, consist of variable sequences [20]. It is not clear what affect, if any, quadruplex stability has on G-quadruplex helicase activity. Work is currently underway to investigate the relevance of G-quadruplex stability in regards to helicase activity.

Conclusion

The optimization of our SPR-based assay of helicase activity reported here for T-ag is

important for minimizing variability in the assay due to different experimental parameters. Most importantly, an improved method for determining the unwinding of biologically relevant intramolecular G-quadruplex DNA substrates was developed. It should be noted that although the model T-ag helicase was employed for these studies, the assay itself is general and should be useful for assessing the ability of other helicases to unwind various DNA substrates of interest in a real-time, label-free manner.

Acknowledgements

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