Long-distance radical cation transport in DNA: horizontal charge hopping in a dimeric quadruplex

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A series of DNA hairpins were synthesized and shown to associate to form quadruplexes formed by stacking five G-quartets in an antiparallel orientation. One of the hairpins in the quadruplex was linked covalently at the 5′-end to an anthraquinone (AQ) group and a ^{32}P label was incorporated either at the 3′-terminus of the AQ-containing hairpin or on its partner hairpin in the quadruplex. Irradiation of the AQ group with UV light leads to the one-electron oxidation of the DNA and concomitant introduction of a radical cation into the DNA. Analysis by PAGE and autoradiography shows that the radical cation reacts at guanines both on the AQ-containing strand and with its partner hairpin in the quadruplex. This observation demonstrates that charge migration in DNA occurs vertically along a DNA chain and horizontally within a G-quartet.

Introduction

Because of its significance in understanding oxidative damage and subsequent mutation, intense interest has been focused on the study of long-distance radical cation (electron "hole") transport in duplex DNA.[1–6] These studies have shown that radical cations are able to migrate long distances in DNA by a hopping mechanism.[7] Chemical trapping of the radical cation by reaction with H_2O or O_2 causes damage to DNA nucleobases, primarily guanines. This reactivity is attributed to the fact that guanines have the lowest oxidation potential (E_{ox}) of the four natural bases,[8,9] and a similar explanation has been offered to account for the observation that sequences containing consecutive guanines[10,11] show increasing reactivity in the order GGGG > GGG > GG > AG > TG ≈ CG.[12,13] However, it has recently become apparent that factors in addition to relative E_{ox} contribute to the reactivity of radical cations in DNA.[14]

G-quartets (Fig. 1) are planar structures formed by the hydrogen bonding of four guanine bases through Watson–Crick and Hoogsteen pairing that are stabilized by metal cations that serve to screen electrostatic repulsion between the negatively charged phosphate groups of the backbone.[15] When contained within appropriate nucleobase sequences the G-quartets can stack to form linear quadruplex DNA.[16] Quadruplexes are of special interest because of their suspected biological significance and because of their potential application in molecular electronics devices.[17,18] Quadruplexes may be formed from DNA with appropriate base sequences by the combination of one, two or four strands.

Numerous studies have shown that quadruplex DNA is richly polymorphic, with structural diversity introduced by the orientation of strands, bonds and loops.[16,19] Of particular relevance to this work is the observation that dimeric quadruplexes may be formed from hairpins in parallel or antiparallel orientations. In the former, the hairpin loops are on the same ends of the quadruplex, in the latter they are on opposite ends. In addition,

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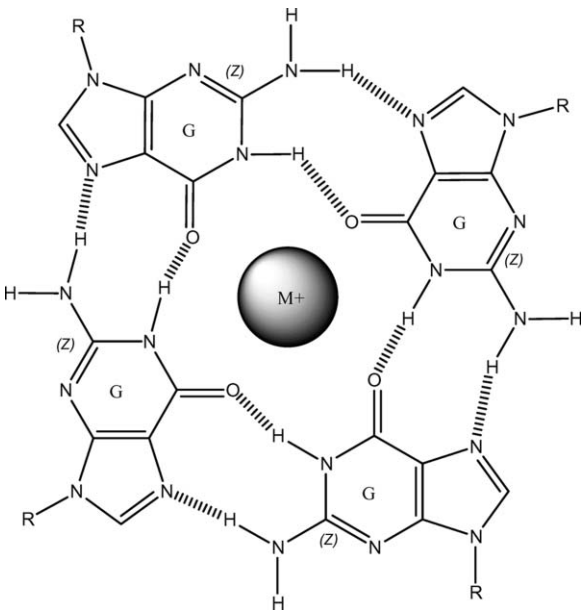


Fig. 1 Model structure of a G-quartet showing the central metal ion that stabilizes the assembly.

antiparallel quadruplexes formed from two hairpins may have loops oriented "edgewise" or "diagonally". Relevant structures are shown schematically in Fig. 2.

There has been some previous interest in determining the effects of radical cation reaction and transport in guanine-rich DNA structures. An early report on this topic describes electrochemical experiments on a quadruplex formed from a single DNA strand, which showed that this structure is not oxidized especially rapidly when compared with $(G)_n$ structures.[20] However, it was observed that the irreversible reaction of the radical cation with H_2O or O_2 is more likely to occur at the 5′- and 3′-guanines of the quadruplex than at the central guanine, which is distinct from the behavior of GGG segments in duplex DNA.[21] A further investigation of monomeric quadruplexes utilized an extended single strand

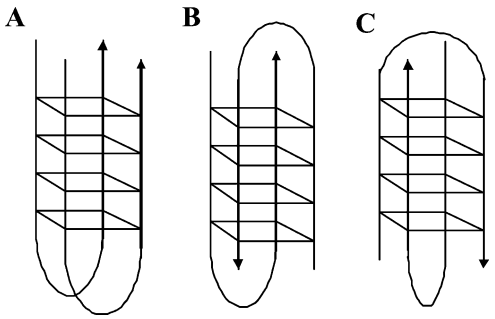


Fig. 2 Quadruplexes formed by the dimerization of hairpin duplex DNA. The squares represent the G-quartets and the arrows represent the direction of the DNA chain. **A**. Parallel quadruplex with edgewise loop regions. **B**. Antiparallel quadruplex with edgewise loop regions. **C** Antiparallel quadruplex with diagonal loop regions.

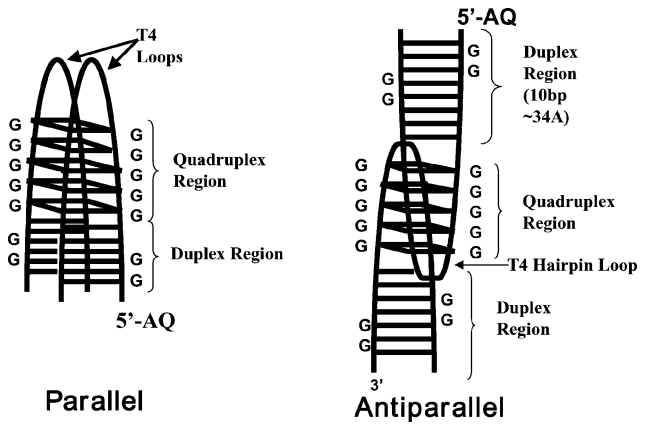


Fig. 3 Quadruplexes formed in parallel or antiparallel structures from hairpins G5 and G5–AQ.

structure appended with a discontinuous duplex overhang.[22] Site selective and non-site selective one-electron oxidation appears to show that these quadruplex structures are more effective radical cation traps than are GG steps in duplex DNA; that is, more damage is observed at guanines within the quadruplex region than those within the duplex. A reactivity variation between exterior and central guanines similar to that observed in the electrochemical experiments was attributed to the quadruplex core being less accessible to molecular oxygen. In contrast, a recent study[23] employing a non-selectively bound, inefficient, singlet-state photosensitizer[24] indicates that guanines in a quadruplex structure are less reactive than those in a corresponding duplex. From these experiments it is not possible to distinguish "vertical" charge transfer, which is the well-known process that occurs between adjacent guanines stacked in duplex DNA, from "horizontal" charge transfer, which might occur among hydrogen bonded guanines in a single G-quartet.

We report here the study of charge transfer and the reactivity of radical cations in a structure containing a $[d(G_5T_4G_5)]_2$ quadruplex core composed of five stacked G-quartets formed from two DNA hairpins. This structure has two contiguous overhanging ten base pair duplex regions (see Fig. 3). An anthraquinone (AQ) group is covalently attached to one end of a quadruplex-forming hairpin in order to enable site-specific photosensitized one-electron oxidation of the complex, and a radiolabel is attached either to the AQ-containing hairpin or to its partner hairpin. This dimer structure and radiolabel placement was designed to allow investigation of horizontal charge transfer in a G-quartet.

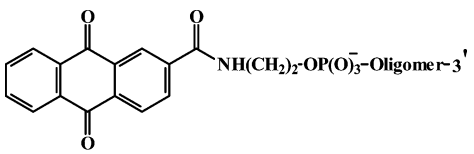
Experimental

General

Radioactive isotope, $^{\gamma-32}$P-ATP, was purchased from Amersham Biosciences. The enzymes, *T4 polynucleotide kinase* (T4-PNK), *terminal deoxynucleotidyl transferase* (TDT) and *DNase 1*, were purchased from New England Biolabs and stored at −20 ° C. 3,4,9,10-Perylenetetracarboxylic diimide and *N*-(3-aminopropyl)-morpholine were purchased from TCI America. *N*-Methylmesoporphyrin (NMM) was purchased from Frontier Scientific, Inc. Indodicarbocyanine-3-1-*O*-(2-cyanoethyl)-(*N,N*-diisopropyl)-phosporamidite (Cy3) and 1-dimethoxytrityloxy-3-[*O*-(*N*-carboxy-(di-*O*-pivaloyl-fluorescein)-3-aminopropyl)]-propyl-2-*O*-succinoyl-long chain alkylamino-CPG (3′-Fl) were purchased from Glen Research and stored at −20 °C. The oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer, and purified by reverse phase HPLC on a Dynamax C18 column.

The DNA sequences that were analyzed are shown in Table 1. Electron spray ionization (ESI) mass spectrometry was used

Table 1 Structures of the DNA oligomers used in this work



AQ- DNA

	DNA sequences
G5	5′-AATGGCCTAT**GGGGG**TTTT**GGGGG**ATAGGCCATT-3′
G5C	3′-TTACCGGATACCCCCAAAACCCCCTATCCGGTAA-5′
G5T	5′-TTTTTAATGGCCTAT**GGGGG**TTTT**GGGGG**ATAGGCCATT-3′
G5–AQ	5′-*AQ*-AATGGCCTAT**GGGGG**TTTT**GGGGG**ATAGGCCATT-3′
G5T–AQ	5′-*AQ*-AATGGCCTAT**GGGGG**TTTT**GGGGG**ATAGGCCATTTTTTT-3′
G5(2)–AQ	5′-*AQ*-AATGGCCTATCCGGTAT**GGGGG**TTTT**GGGGG**ATACCGGATAGGCCATT-3′
G5–5Cy	5′-**Cy3**-AATGGCCTAT**GGGGG**TTTT**GGGGG**ATAGGCCATT-3′
G5–3Fl	5′-AATAATGGCCTAT**GGGGG**TTTT**GGGGG**ATAGGCCATT-**Fl**-3′

to confirm composition. The concentrations of the oligomers were determined by UV spectroscopy and monitored at 260 nm. Fluorescence experiments were performed on a SPEX Fluorolog-2 spectrofluorimeter.

Spectroscopic experiments

Quadruplex DNA samples (typically 50 μM DNA) were prepared in sodium cacodylate buffer solution (10 mM, pH 7), and in some cases 25 mM of potassium phosphate was added before hybridization. Quadruplex samples were labeled with either 5′-Cy3 or 3′-Fl in the fluorescence resonance energy transfer (FRET) experiments. The fluorescence spectra were measured with excitation at 450 nm. All spectra were recorded at 25 °C.

DNase 1 assay

The duplex DNA samples were prepared by adding 6 μL of $^{\gamma-32}$P radiolabeled DNA (G5T*) to 25 μM DNA (G5), 25 μM of its complementary sequence (G5C), and 10 mM sodium cacodylate buffer at pH 7. Quadruplex DNA samples were prepared by adding 6 μL of $^{\gamma-32}$P radiolabeled DNA (G5T*) to 50 μM DNA (G5), 25 mM potassium phosphate salt, and 10 mM sodium cacodylate buffer. In addition, a control sample was prepared with 6 μL of $^{\gamma-32}$P radiolabeled DNA (G5T*), 50 μM DNA (G5A) and 10 mM sodium cacodylate buffer. The DNase 1 solution was freshly prepared with 20 units/μL of DNase 1, 100 mM of Tris buffer, 25 mM $MgCl_2$, and 5 mM $CaCl_2$ salt solution. A formamide–EDTA dye solution was prepared by 10 mM EDTA solution, bromophenol blue dye and a 4 : 1 ratio of formamide to water. Upon addition of 2 μL DNase 1 solution, the samples were stirred and incubated for 5 min at room temperature. The DNase 1 reaction was quenched by the addition of 10 μL of formamide–EDTA dye solution to each sample. The samples were then heated for 3 min at 90 °C. Each sample was loaded onto a 20% polyacrylamide gel and strand cleavage was revealed by autoradiography.

DMS methylation

The duplex and quadruplex samples were prepared as stated earlier for the DNase 1 experiment. Each sample was incubated with 2.5 μL of the 10% (v/v) dimethylsulfate (DMS) for 10 min at room temperature. The reaction was quenched by the addition of 1 M 2-mercaptoethanol, and 1.5 M sodium acetate, (pH 7). The samples were precipitated by adding 750 μL of ethanol and 1 μL of glycogen followed by cooling to −80 °C for 4 h before decanting the liquid and drying the samples. The samples were treated with piperidine and analyzed by PAGE and autoradiography.

Photocleavage

Quadruplex samples with the label on the non-AQ-containing strand were radiolabeled by incubating a mixture of 2 μL of T4 PNK (*ca.* 400 × 10^3 U mL^{-1}), 2 μL of T4 PNK buffer, 1 μL $^{\gamma-32}$P–ATP, and 25 μM of G5T DNA in a total volume of 100 μL at 37 °C for 45 min. Quadruplex samples with the label on the AQ-containing strand were radiolabeled by incubating a mixture of 2 μL TdT enzyme PNK (*ca.* 400 × 10^3 U mL^{-1}), 2 μL of Phor all buffer, pH 7, 1 μL$^{\alpha-32}$P–ATP, and 25 μM of G5T–AQ DNA in a total volume of 100 μL at 37 °C for 45 min. The labeled samples were suspended in 10 μL of loading dye, and then loaded onto a purification gel, which was run for 1.5 h at 400 V. Purified DNA was removed from the gel by placing it in 750 μL elution buffer (1 mM EDTA, 0.5 M sodium acetate, 10 mM magnesium acetate, 0.1% SDS), and incubating the solution at 37 °C overnight. The DNA was precipitated by the addition of 750 μL of 100% ethanol and 1 μL glycogen stored at −80 °C for 4 h. The product was isolated by centrifugation at 13000 RCF for 30 min. After decanting the supernatant, the samples were washed twice with 100 μL of 80% ethanol. Quadruplex samples with the $^{\gamma-32}$P radiolabel on the non-AQ-containing strand of DNA (G5T*) were prepared for hybridization by adding 6 μL of labeled G5T to 50 μM unlabeled G5–AQ, 25 mM of potassium phosphate salt, and 10 mM sodium cacodylate buffer. The quadruplex samples with the $^{\alpha-32}$P radiolabel on the anthraquinone-containing strand of DNA (G5T*–AQ) were prepared by adding 6 μL of labeled G5T–AQ to 50 μM unlabeled G5, 25 mM potassium phosphate salt, and 10 mM sodium cacodylate buffer. The experimental samples for analysis of intermolecular electron transfer were comprised of 6 μL labeled G5T, 25 mM potassium phosphate salt, 10 mM sodium cacodylate buffer and 50 μM unlabeled G5(2)–AQ quadruplex DNA for analysis of the non-AQ-containing strand. Irradiation of hybridized samples was performed for 10 min using a Rayonet photoreactor with eight 360 nm lamps. The precipitated samples were treated with 1 M piperidine for 30 min at 90 °C. After evaporating the piperidine, the samples were suspended in loading buffer. Samples with 4000 cpm were analyzed on a 20% polyacrylamide gel. The gels were dried, and the cleavage was revealed by an autoradiograph and quantified by a Fuji phosphorimager.

Results

I. Characterization of quadruplex-containing DNA structures

A. Melting behavior. Dimerization of hairpin-forming oligomer G5 could result in the formation of a quadruplex-containing five stacked G-quartets connected by $(T)_4$ loops with two linked 10 base pair duplexes in either a parallel or antiparallel arrangement, see Fig. 3. The melting behavior of the structure formed from oligomer G5 was investigated and compared with the duplex that results from the hybridization of oligomer G5 with G5C, which is its complement. A 50 μM sample of oligomer G5 in sodium cacodylate buffer solution (10 mM) containing 25 mM of potassium phosphate shows a single, reversible melting transition (T_m) at 72 °C when monitored by UV spectroscopy at 295 nm. For comparison under these conditions, the G5–G5C duplex exhibits a T_m of 57 °C. These results suggest that G5 forms a stable structure that exhibits a single melting transition. This structure is shown to be a dimeric parallel quadruplex through the combination of chemical and spectroscopic experiments.

B. Dye binding analysis. The optical absorption spectrum of *N*-methylmesoporphyrin (NMM) changes characteristically when it binds to quadruplex DNA, presumably by intercalation.[25] In buffer solution, NMM exhibits a symmetrical absorption peak at *ca.* 380 nm, which broadens slightly when it is in the presence of duplex DNA. This band shifts to *ca.* 400 nm when NMM is in the presence of a DNA quadruplex. Fig. 4 shows three absorption

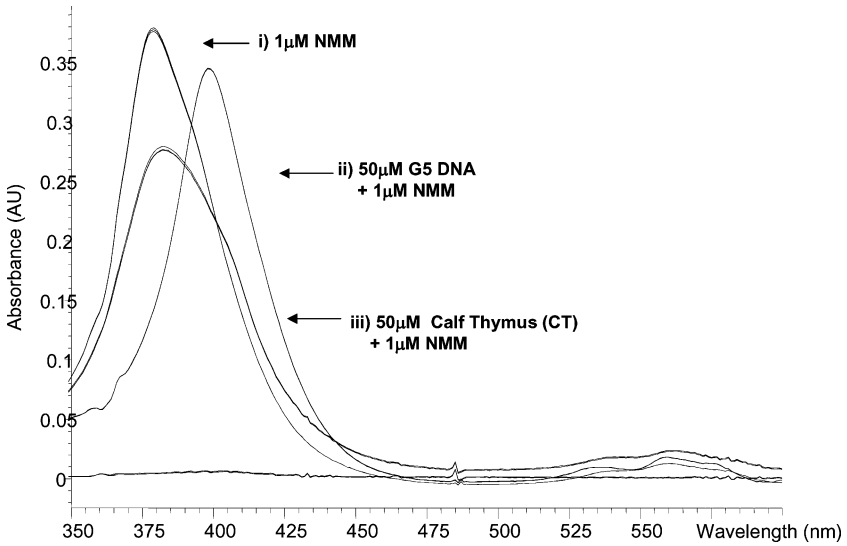


Fig. 4 Absorption spectra of NMM in buffer solution, in the presence of G5 DNA and in the presence of calf thymus (duplex) DNA.

spectra: (i) NMM (1 μM) in buffer solution containing 25 mM of potassium phosphate; (ii) NMM in buffer solution containing 50 μM of oligomer G5; (iii) a solution of NMM-containing calf thymus DNA. The absorption of the dye shifts to 400 nm only when in the solution of the G5 hairpin oligomer, which is characteristic of quadruplex formation. A similar experiment was carried out using a morpholino-substituted perylenetetracarboxylic acid amide (Tel01)[26] as the indicator dye. Its properties are similar to NMM in that its spectrum shifts characteristically only in the presence of quadruplex DNA structures. Tel01 gives results similar to NMM; the spectral shift characteristics of quadruplex formation occur only for solutions containing hairpin G5. These experiments reveal that G5 in the presence of NMM or Tel01 forms a dimeric quadruplex, but they do not show conclusively that G5 forms a quadruplex in the absence of these dyes. We carried out chemical reactivity experiments to confirm that G5 forms dimeric quadruplexes in the absence of an intercalating dye.

C. DNase 1 and *N*-methylation assays of quadruplex formation. The DNase 1 enzyme cleaves both single-stranded and double-stranded DNA into mono-, di- or tri-nucleotides, but it does not efficiently cause strand cleavage at quadruplex regions.[27] We used this property of the enzyme to assay quadruplex formation and to confirm the preservation of the two contiguous overhanging ten base pair duplex regions that result from dimerization of the hairpin oligomers. To facilitate the radiolabeling that is required for PAGE analysis, a new structure, G5T (see Table 1), was prepared that contains a $(T)_5$ single strand segment at the 5′-terminus of the hairpin. The $(T)_5$ segment overhangs the duplex and this structure was readily labeled with ^{32}P at its 5′-terminus. Fig. 5 shows the results of the reaction of DNase 1 with solutions of G5T in the presence and absence of 25 mM potassium phosphate and the reaction of the enzyme with the G5–G5C duplex. As expected, DNase 1 efficiently cleaves the DNA duplex. Similarly, characteristic cleavage occurs when G5T in the absence of K^+ is treated with the enzyme, but the two $(G)_5$ segments of the hairpin are not cleaved when potassium phosphate is present, which indicates quadruplex formation. In a related

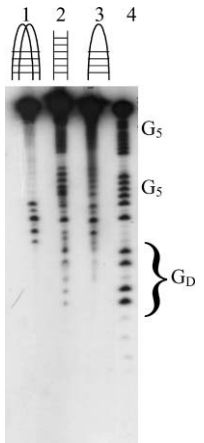


Fig. 5 Autoradiogram of PAGE gel from reaction of DNA with DNase 1. Lane 1: quadruplex formed from 50 μM G5T in 10 mM sodium cacodylate buffer solution containing 25 mM potassium phosphate. Lane 2: duplex DNA formed from 25 μM G5T and 25 μM G5C in 10 mM sodium cacodylate buffer solution. Lane 3: hairpin formed form from 50 μM G5T in 10 mM sodium cacodylate buffer solution. Lane 4: A + G sequencing. The labels G_5 and G_D indicate the guanines participating in the quadruplex and duplex region, respectively.

experiment, we showed that the $(G)_5$ segments in hairpin G5T in the presence of K^+ are not methylated at the N7 positions by dimethylsulfate, which is a characteristic of guanines participating in the Hoogsteen hydrogen bond patterns of G-quartets.[28] These experiments confirm that in the presence of K^+ the G5 hairpins dimerize spontaneously to form quadruplexes and this process does not require an intercalating dye.

D. Quadruplex structural analysis by fluorescence resonance energy transfer (FRET). FRET experiments are popular for the structural analysis of biomolecules.[29] They can provide information about the average distance between fluorescent dyes that are attached to structural elements within the moiety being studied. We used FRET to distinguish between formation of parallel

and antiparallel conformations of the quadruplex resulting from dimerization of G5.

Two modified hairpins were prepared (see Table 1). The first contains fluorescein (Fl), a fluorescent donor, covalently attached to the 3′-terminus of the G5 hairpin (G5–3Fl). The second contains the cyanine dye Cy3 covalently attached to the 5′-terminus of the G5 DNA (G5–5Cy). The Cy3 functions as the fluorescence acceptor (quencher) in the FRET measurements, see Fig. 6.

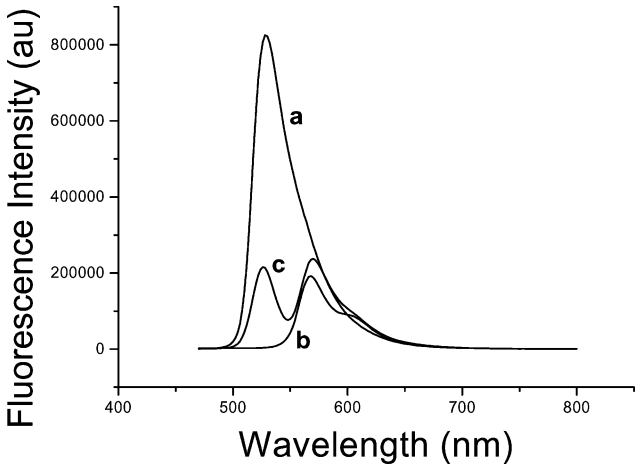


Fig. 6 Results from the FRET experiment. Spectrum (a) corresponds to a solution of 25 μM G5–Fl and 25 μM G5 in potassium phosphate-containing buffer solution, (b) corresponds to a solution of 25 μM G5–Cy and 25 μM G5 in potassium phosphate-containing buffer solution and (c) corresponds to a solution of 25 μM G5–Cy and 25 μM G5–Fl in buffer solution containing potassium phosphate.

Three experiments were carried out on these structures. In the first, the fluorescence intensity was measured for a mixture containing equal amounts (25 μM) of G5 and G5–3Fl in potassium phosphate-containing buffer solution. This mixture should contain a statistical distribution of quadruplex structures formed by the two hairpins and serves to provide a reference value for the unquenched fluorescence intensity. In the second experiment, the fluorescence intensity was measured for a mixture containing equal amounts (25 μM) of G5 and G5–5Cy in potassium phosphate-containing buffer solution. This measurement serves to provide a reference for fluorescence resulting from excitation of Cy3. The third experiment was carried out on a 1 : 1 mixture of G5–3Fl and G5–5Cy where, statistically, 50% of the quadruplexes contain a G5–3Fl hairpin and a G5–5Cy hairpin, 25% of the quadruplexes contain two G5–3Fl hairpins, and the remaining 25% are composed of two G5–5Cy hairpins. The energy transfer efficiency was calculated from eqn. 1:

$$E = 1 - F_{DA}/F_{D} \quad (1)$$

where E is the energy transfer efficiency, F_{DA} is the fluorescence intensity for both the donor and the acceptor, and F_{D} is the fluorescence intensity of the donor at the donor maxima (approximately 520 nm). The FRET quenching efficiency is 75% for the G5 quadruplex complex, which corresponds to a distance (R) between the donor and acceptor of 46 Å determined from the Forster equation with $R_{o} = 56$ Å.[30] Thus, the FRET experiment indicates that the G5 quadruplex is primarily in the form of a parallel structure.

II. Radical cation hopping and reaction in quadruplex DNA

Previous studies of DNA have shown that irradiation (350 nm where only the AQ absorbs) of a covalently-linked anthraquinone derivative results in efficient one-electron oxidation that injects a radical cation into the duplex.[1] The radical cation migrates through the DNA by hopping[6,7] and is quenched by reaction with H_2O or O_2 at a guanine or G_n step. The reaction at guanine is revealed by subsequent treatment of the irradiated sample with piperidine, which results in strand cleavage at the modified nucleotide. At low conversion (single-hit conditions) the amount of strand cleavage, measured by autoradiography and phosphorimagery on ^{32}P-labeled samples, is proportional to the reactivity of the nucleobase. This protocol was applied to the analysis of radical cation hopping and reaction in the parallel quadruplex structure formed from G5 hairpins. In particular, we analyzed cross-over of the radical cation from one hairpin to the other in the quadruplex by monitoring reactions in the duplex regions.

Two classes of dimeric quadruplex structures were constructed to analyze the path of radical cation migration through the five stacked G-quartets. The first was formed from combination of G5 with G5T–AQ (see Table 1 and Fig. 7); the latter hairpin has an AQ group at its 5′-end and was radiolabeled at its 3′-end (identified as G5T*–AQ). The second quadruplex is composed of G5–AQ and G5T, with the latter hairpin radiolabeled at its 5′-end (G5T*). Both quadruplex solutions contain mixtures of three structures. For example, combination of equal amounts of hairpins G5T* and G5–AQ will give quadruplexes G5T*/G5T*, G5–AQ/G5T* and G5–AQ/G5–AQ presumably in the statistical ratio of 1 : 2 : 1. The G5T*/G5T* quadruplex does not contain an AQ photosensitizer and a control experiment (see below) shows that its irradiation does not lead to detectable strand cleavage. Similarly, the G5–AQ/G5–AQ quadruplex does not contain a radiolabel and thus it will not produce an image in the autoradiography or phosphorimagery measurements. Only quadruplex G5–AQ/G5T* can give detectable strand cleavage when irradiated because it contains both a photosensitizer and a radiolabel. In this case, the sensitizer and the label are on separate hairpins and strand cleavage observed in G5T* must result from

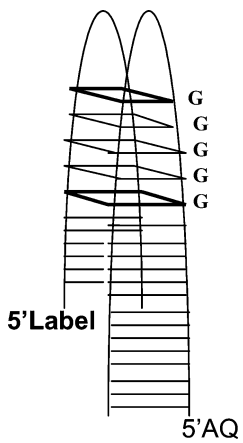


Fig. 7 Quadruplex structure formed from combination of G5T* and G5(2)–AQ. The AQ group is not able to intercalate in the duplex region of the hairpin formed from G5T*.

a crossover of the radical cation injected into G5–AQ hairpin to the G5T* hairpin. Similarly, there are three quadruplex structures formed from mixing the two hairpins G5 and G5T*–AQ: G5/G5, G5T*–AQ/G5 and G5T*–AQ/G5T*–AQ. Only the latter two can give detectable strand cleavage after irradiation. In these cases, the photosensitizer and the label are in the same hairpin structure and crossover of the radical cation is not required for detectable strand cleavage.

The results of irradiation and analysis of the quadruplexes are shown as autoradiograms of high resolution PAGE gels in Fig. 8. Similar results are observed for G5–AQ/G5T* and G5T*–AQ/G5 quadruplexes. Strand cleavage is observed at guanines participating in the G-quartets and at guanines in the duplex regions of both hairpins. These findings suggest that crossover, that is, horizontal radical cation transfer from one guanine to another, occurs from one hairpin to its partner in the quadruplex. Control experiments were carried out to confirm this conclusion.

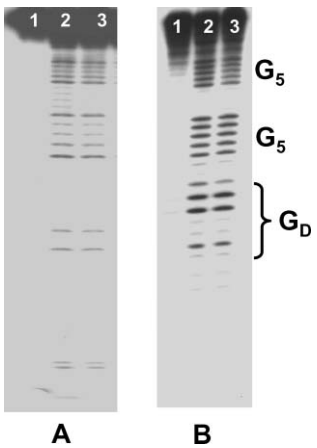


Fig. 8 Autoradiograms of PAGE gels from the irradiation of the AQ-linked quadruplexes at 350 nm in 10 mM sodium cacodylate buffer solution containing 25 mM potassium phosphate. The labels G_5 and G_D indicate the guanines participating in the quadruplex and duplex region, respectively. In 8A, lane 1 corresponds to the "dark control," which is an unirradiated solution of G5T*/G5–AQ in potassium phosphate-containing buffer solution. The unirradiated control sample was analyzed by treatment with piperidine precisely as were the experimental samples. Lane 2 in 8A is the same as lane 1 but it was irradiated for 10 min with UV light before analysis. Lane 3 is the same as lane 2 but with the addition of excess duplex DNA. The lane designations have the same meaning in 8B as in 8A except the sample is composed of G5T*–AQ/G5. The absolute intensity of strand cleavage in the gels shown in A and B cannot be compared.

Inspection of the parallel quadruplex structure suggests that it may be possible for an AQ-group on one hairpin to reach over and bind to the duplex region of its partner hairpin. Such inter-strand sensitization has been observed previously in certain DNA three-way junctions.[31] If this inter-strand sensitization occurred, then the strand cleavage observed in the G5T* hairpin of the G5–AQ/G5T* quadruplex might not be due to horizontal radical cation transport. To assess this possibility, hairpin G5(2)–AQ (Table 1, Fig. 7) was prepared and investigated. In the quadruplex formed from G5(2)–AQ/G5T*, the AQ-linked duplex region is 17 base pairs long and the duplex region of the G5T* hairpin is only 10 base pairs long. Consequently, inter-strand sensitization is geometrically impossible in the G5(2)–AQ/G5T* quadruplex. The results of irradiation of G5(2)–AQ/G5T* give an observed pattern of strand cleavage that is essentially indistinguishable from that observed from irradiation of G5–AQ/G5T*, which shows that horizontal radical cation transfer in these quadruplexes is not due to inter-strand sensitization.

Finally, there is a possibility some sort of "higher-order" structure or aggregate formed in solution could enable an AQ group of one quadruplex to photosensitize strand cleavage in another. This was ruled out by investigating the irradiation of G5T*–AQ in the presence of a large excess of G5. If some higher order structure formed between G5T*–AQ and G5 that resulted in inter-strand sensitization, the amount of strand cleavage in G5T*–AQ would be reduced. No such reduction was observed, which shows that at the concentrations of DNA employed in these experiments, higher-order structures play no meaningful role.

Discussion

The combination of spectroscopic and chemical analyses reported here shows conclusively that the G5 hairpins dimerize in the presence of potassium phosphate to form primarily parallel quadruplex structures. These assemblies contain five stacked G-quartets and contiguous duplex regions. The irradiation of AQ-linked quadruplexes labeled appropriately with ^{32}P yields DNA strand cleavage at guanines in both the quadruplex and duplex regions of these assemblies.

Consider the results from irradiation of the quadruplex formed from hairpin dimers G5–AQ and G5T*. In these assemblies the AQ group is at the 5′-terminus of a 10 base pair duplex region. Injection of the radical cation into the DNA must occur at a base pair adjacent to the AQ, and numerous studies have shown that this radical cation will hop through the duplex and react occasionally with H_2O or O_2 at the GG steps located five and seven base pairs from the AQ group in the duplex region. It is important to recognize that the trapping efficiency is relatively low and that only a small fraction of the radical cations introduced by irradiation of the AQ will be consumed by reaction at the GG steps in the duplex region, the rest will continue to hop through the DNA eventually encountering the five stacked G-quartets.

The experimental results show that the radical cation also reacts at the guanines of the G-quartet. Quantitatively, the radical cation is approximately 50% more likely to be trapped at a guanine in the quadruplex region than one in the duplex region, which is consistent with previous investigations. These experiments show a radical cation can migrate from a duplex region that contains guanines, through a G-quartet region, and then to a second guanine-containing duplex region. In the G5–AQ/G5T* quadruplex, the guanines at the "top" and "bottom" of the stack of 5 G-quartets are somewhat more reactive than the three "interior" G-quartets, which is an effect that has also been observed previously. However this effect is not universal, all of the guanines in the G-quartets of the G5T*–AQ/G5 quadruplex appear to have similar reactivity.

These quadruplex complexes were developed to investigate the possibility that horizontal radical cation transfer can occur in G quartets. The experimental results obtained from investigation of the G5–AQ/G5T* assembly show for the first time that transfer of a radical cation from one hairpin (G5–AQ) to another (G5T*)

does occur in the quadruplex region. This is consistent with results from experiments and calculations indicating that radical cations in DNA are delocalized as polarons. The findings reported here suggest that in regions containing stacked G-quartets the polaronic delocalization[32] extends both vertically and horizontally.

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