Verification of Intramolecular Hybrid/Parallel G-Quadruplex Structure under Physiological Conditions Using Novel Cyanine Dye H-Aggregates: Both in Solution and on Au Film

ARTICLE *in* ANALYTICAL CHEMISTRY · OCTOBER 2010

Impact Factor: 5.64 · DOI: 10.1021/ac1017716 · Source: PubMed

Anal. Chem. **2010,** *82,* 9135–9137

Letters to *Analytical Chemistry*

Verification of Intramolecular Hybrid/Parallel G-Quadruplex Structure under Physiological Conditions Using Novel Cyanine Dye H-Aggregates: Both in Solution and on Au Film

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The H-aggregates of a novel cyanine dye, 3,3′-di(3-sulfopropyl)-4,5,4′,5′-dibenzo-9-methyl-thiacarbocyanine triethylammonium salt (MTC), have been fabricated to verify hybrid/parallel intramolecular G-quadruplexes from linear duplex and single-strand DNAs under physiological conditions. The recognition is found to be successful both in solution and on Au film. These results have shown MTC H-aggregates, as a supramolecular system, may be used as a potential excellent probe for DNA structure, both in vitro and in vivo.

It is known that oligonucleotides containing runs of three or four adjacent guanines (G), as found in telomeric DNA, spontaneously formed four-stranded DNA structures called G-quadruplexes.[1,2] Recently, quadruplex-folded telomeric DNA has been found to perturb telomere function and inhibit the activity of telomerase, an enzyme overexpressed in above 85% of human cancers, hence opening up a novel avenue for cancer therapy in G-quadruplex stabilizing agents.[3−5] In addition, bioinformatics sequence analysis indicates that G-rich tracts capable of G-quadruplex formation are prevalent in the human genome.[6,7] For example, promoter regions spanning 1 kb upstream of transcription start sites of genes are significantly enriched in putative G-quadruplex-forming motifs, and these putative promoter G-quadruplex-forming regions strongly associate with nuclease hypersensitivity sites.[8] Such promoter-based G-quadruplexes may be directly involved in gene regulation at the level of transcription,[9] which have attracted extensive investigations of the structure and the role of promoter-mediated G-quadruplex in the promoters of many oncogenes, such as *c-myc*,[10] *c-kit*,[11] and *bcl-2*.[12]

G-quadruplex structure has been characterized in vitro,[13] which is stabilized by Hoogsteen hydrogen bonding among four guanine bases arranged in a square planar configuration. It has been revealed that G-quadruplex DNA structures are highly polymorphic.[14] The DNA-strand orientation may be either parallel or antiparallel, even both conformations (termed hybrid) in some cases, for example, the (3 + 1) G-quadruplex motif in human telomeres.[15] Depending on DNA sequence and extrinsic cation, an oligonucleotide even can exist as a mixture of several different quadruplex forms.[16] Furthermore, the DNA strands of G-quadruplexes can assemble into either an intramolecular (a single strand folds upon itself) or intermolecular (formed by two or more strands) configuration. Therefore, owing to the prevalent distribution and polymorphism of G-quadruplex, mapping their locations in the human genome and identifying their particular structures in specific sites in vivo are still complicated tasks, which are important in the study of cell proliferation, cancer research, and drug development.

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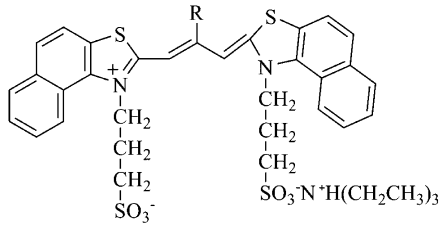
10.1021/ac1017716
Published on Web 10/22/2010



ETC R= —CH_2CH_3

MTC R= —CH_3

Figure 1. Molecular formula of ETC and MTC.

So far, a lot of direct evidence to show the presence of G-quadruplex structure both in vitro and in vivo has been reported. However, these methods mainly focus on biochemical technologies[17,18] or organic quadruplex-specific ligands.[19−21] Besides these strategies, previously, we had provided a novel strategy to recognize specific G-quadruplex structure in vitro by supramolecular assembly[22] where J-aggregates of cyanine dye 3,3′-di(3-sulfopropyl)-4,5,4′,5′-dibenzo-9-ethyl-thiacarbocyanine triethylammonium salt (ETC) (Figure 1) have been fabricated to recognize specific G-quadruplexes from other DNA motifs[23] in solution via the transformation between ETC J-aggregates and monomer. However, such recognition succeeded only in the case of the buffer solution with low concentration of metal ion (17.2 mM K^+ and no Na^+), which is far away from physiological conditions. To further push this strategy in vivo, recognition should be carried out under real physiological conditions with ionic strength and ion composition: 140 mM K^+, 10 mM Na^+, and 2 mM Mg^{2+}. Unfortunately, owing to the relatively high ionic strength, the intermolecular forces within J-aggregates of ETC molecules are so strong that the transformation from J-aggregates to monomer did not occur in the presence of hybrid/parallel G-quadruplexes under the similar condition as that with low [K^+]. Consequently, no recognition signatures were observed. In order to regulate the intermolecular force among cyanine dye in the supramolecular assembly under physiological conditions, the 9-position substituent (ethyl group) of ETC was replaced by a methyl group to decrease the assembly ability and a new cyanine dye 3,3′-di(3-sulfopropyl)-4,5,4′,5′-dibenzo-9-methyl-thiacarbocyanine triethylammonium salt (MTC) (Figure 1) was synthesized to recognize the intramolecular hybrid/parallel G-quadruplex.

Due to the extended planar π-electron conjugated system, MTC tends to be self-assembled under a physiological solution (termed as PS, 10 mM KH_2PO_4/K_2HPO_4 with 122.8 mM KCl, 10 mM NaCl, and 2 mM $MgCl_2$, pH 7.4) and exhibits only a broad absorption band around 445−480 nm assigned to H-aggregates[24] (Supporting Information). Addition of oligonucleotides with intramolecular specific G-quadruplex (Table 1), including ***M24*** (a mixture of several G-quadruplex structures), derived from a human telomere sequence (***c-myc 2345*** (parallel G-quadruplex), ***c-kit1*** (parallel G-quadruplex), and ***bcl-2 2345*** (hybrid G-quadruplex)), which are derived from oncogene promoter-based sequences, caused great changes in the absorption spectra of MTC solution; the absorbance of H-aggregates decreased while the appearance of a new absorption band at 580 nm was assigned to a monomer. Consequently, the apparent yellow color of the solution has been changed to pink (Figure 2A). These results indicated that these oligonucleotides with hybrid/parallel G-quadruplex had transformed the assembled state of MTC into monomer. The detailed absorption titration experiments (Supporting Information) also showed that these oligonucleotides with hybrid/parallel G-quadruplex could disassemble MTC H-aggregates to monomer completely. On the other hand, linear duplex ***CT*** (calf thymus DNA), ***SS*** (salmon sperm DNA), and ***D22*** (derived from human telomere sequence) and single-strand ***S17*** (relative random sequence) could hardly disassemble MTC H-aggregates or change the solution color, indicating much weaker interaction between MTC and these DNAs. Figure 2B shows the changes of MTC monomer absorbance against the ratio of [oligonucleotides]/[MTC]. Obviously, ***M24***, ***c-myc 2345***, ***c-kit1***, and ***bcl-2 2345*** could interact with MTC strongly and induce unique, dramatic, and visible recognition signature arisen from MTC monomer, while ***CT***, ***SS***, ***D22***, and ***S17*** could not.

Furthermore, 12 μM ***M24*** was added into MTC in the presence of 800 $\mu g \cdot mL^{-1}$ ***CT*** (or ***SS***) and vice versa; 800 $\mu g \cdot mL^{-1}$ ***CT*** (or ***SS***) was added into MTC in the presence of 12 μM ***M24***. In both cases, the absorption spectra were just the same as that of MTC with 12 μM ***M24*** only (data not shown). The results indicated that ***CT*** and ***SS***, even in a large amount, could not influence the interaction between ***M24*** and MTC assembly.

Since MTC could verify hybrid/parallel G-quadruplexes in physiological solution, one may wonder if it was possible to be applied at the interface as well, which is very important for the application of such a system to study the prevalent distribution of G-quadruplex in the human genome and map their locations. Therefore, the recognition ability of hybrid/parallel G-quadruplexes (which were 5′-biotin modified and self-assembled on specific areas of Au chip separately) by MTC H-aggregates has been investigated.

The different 5′-biotin modified DNA samples (including linear duplex ***D22***; single-strand **S17**, and hybrid/parallel G-quadruplexes **M24**, ***c-myc 2345***, **c-kit1**, and **bcl-2 2345**) were self-assembled, respectively, on the streptavidin-coated sensor chips via the surface plasmon resonance (SPR) instrument. Then, the MTC staining was

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Table 1. DNA Samples with Different Sequences and Their Abbreviations

abbr.	sequences	motifs in PS
D22	[5′-AGGG(TTAGGG)$_3$-3′]/[5′-(CCCTAA)$_3$CCCT-3′]	
CT	calf thymus DNA	linear duplex
SS	salmon sperm DNA	
S17	[5′-CCAGTTCGTAGTAACCC-3′]	single-strand
M24	[5′-(TTAGGG)$_4$-3′]	mixed G4[27−30]
c-myc 2345	[5′-TGAGGGTGGGGAGGGTGGGGAA-3′]	parallel G4
c-kit1	[5′-AGGGAGGGCGCTGGGAGGAGGG-3′]	parallel G4
bcl-2 2345	[5′-GGGCGCGGGAGGAATTGGGCGGG-3′]	hybrid G4

carried out outside the instrument due to the low molecular weight of MTC and the trace amount of methanol in the system.

Before being stained by MTC H-aggregates, no visible fluorescence was observed in the assembled areas of all Au chips when excited by white light or a mercury lamp (530−550 nm; Supporting Information). After being stained by MTC H-aggregates, the areas of Au chips assembled with ***M24***, ***c-myc 2345***, ***c-kit1***, and ***bcl-2 2345*** emitted distinct fluorescence signatures when excited by a mercury lamp (530−550 nm; Figure 3b−e) while the chips without any DNA sample (as a control; Figure 3a) or assembled with ***D22*** and ***S17*** emitted no obvious signature (Figure 3f,g). This phenomenon is consistent with detailed fluorescence titration results (Supporting Information), which showed that ***M24***, ***c-myc 2345***, ***c-kit1***, and ***bcl-2 2345*** could strongly enhance MTC monomer fluorescence intensity (more than 1000 times) while ***D22*** and ***S17*** could hardly. On the basis of the results, further verification and mapping of specific G-quadruplex structures in vivo could be achieved by MTC H-aggregates, which may also offer MTC supramolecular assembly a vaster application foreground in molecular recognition and disease monitoring.

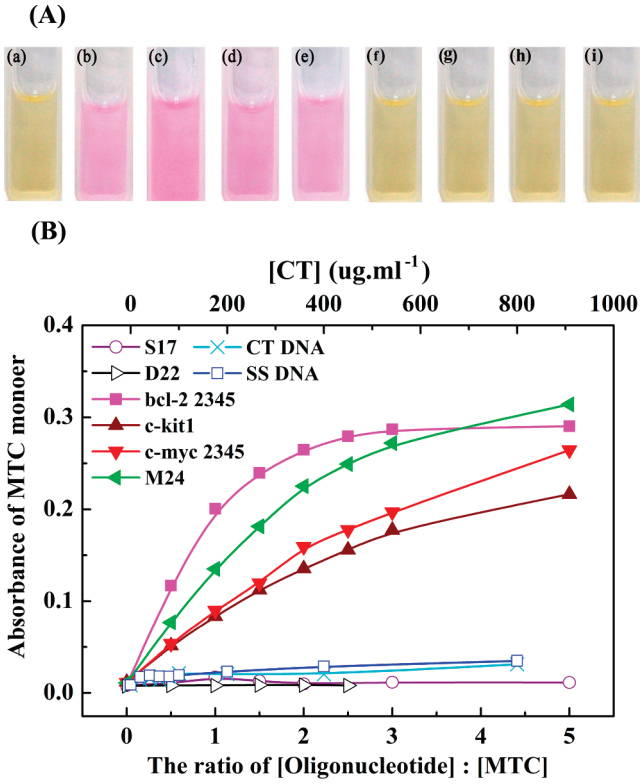

Figure 2. (A) Apparent color of (a) 4 μM MTC H-aggregates and 4 μM MTC with 20 μM (b) ***M24***; (c) ***c-myc 2345***; (d) ***c-kit1***; (e) ***bcl-2 2345***; (f) ***D22***; (g) ***S17***; (h) ***CT***; and (i) ***SS***. (B) The changes of 4 μM MTC monomer absorbance against the ratio of [DNAs]/[MTC].

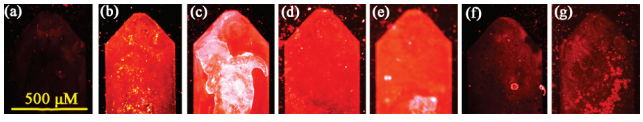

Figure 3. Fluorescence microscopy images of MTC-stained oligonucleotides on Au film: (a) no DNA sample; (b) ***M24***; (c) ***c-myc 2345***; (d) ***c-kit1***; (e) ***bcl-2 2345***; (f) ***D22***; and (g) ***S17***.

On the basis of the results, it is believed that the recognition of G-quadruplex by MTC H-aggregates is quite different from that by organic probes. In this strategy, since the recognition signatures come from the distinct spectral properties of supramolecular arrangements, spectral changes due to recognition would be more visible and much clearer than those from different states of the same molecule.

As shown in the absorption spectra, the absorption peak of MTC in solution red shifts more than 100 nm in the presence of specific G-quadruplex. A carbocyanine dye 3,3′-diethyloxadicarbocyanine (DODC), reported as G-quadruplex ligand, gives rise to a new absorption peak when specifically binding to dimeric hairpin G-quadruplexes.[25] However, the small shoulder peak of DODC, which is blue shifted by about 30 nm from the primary peak, is relatively hard to be resolved and could not be a clear signature in recognizing certain G-quadruplex structures. In the case of MTC, however, the well-resolved independent peak and the large peak shift make the recognition of signatures observed just by judging the apparent color of the solution.

Furthermore, the fluorescence intensity of MTC monomer could be enhanced more than 1000 times by a specific G-quadruplex, which is about 200 times of that enhanced by other DNA motifs. At the interface, owing to the dramatic fluorescence signature, common fluorescence microscopy could easily map the locations of specific G-quadruplex on Au film. Compared with the known molecule used for the G-quadruplex fluorescence probes, such as 3,6-bis(1-methyl-4-vinylpyridinium)carbazole diiodide (BMVC), which can recognize and verify antiparallel G-quadruplex structure in cancer cell chromosomes through distinct fluorescence lifetimes (about 0.5 ns)[26] by two-photon excitation fluorescence lifetime imaging microscopy, clearly, imaging by the use of MTC H-aggregates may be more feasible as a DNA structure probe.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review July 5, 2010. Accepted October 20, 2010.

AC1017716