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Detection of Quadruplex DNA Structures in Human Telomeres by a Fluorescent Carbazole Derivative

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Single-stranded telomeric DNA tends to form a four-base-paired planar structure termed G-quadruplex. This structure was easily formed in vitro in the presence of monovalent cations. However, the existence of this structure in native human telomeres is unclear. Here we address this important question through the distinctive properties of 3,6-bis(1-methyl-4-vinylpyridinium)carbazole diiodide (BMVC) upon binding to various DNA structures. Although the fluorescence of BMVC increases significantly in the presence of DNA, BMVC has high sensitivity and binding preference to quadruplex d(T_2AG_3)$_4$ over duplex DNA. In addition, the fluorescent emissions were characterized around 575 nm for quadruplex d(T_2AG_3)$_4$ and 545 nm for most of duplex DNA. The 575-nm fluorescence emissions were detected in the mixtures of 2 nM BMVC with the chromosomal DNA that were extracted from human cells, suggesting the presence of quadruplex structure in human nucleus. Further analyzing the BMVC fluorescence at the ends of metaphase chromosomes and other regions of chromosomes, we detected the quadruplex-binding BMVC fluorescence at telomere-proximal regions. Together these results provide the first evidence for the presence of quadruplex structures in human telomeres.

Telomeres, the ends of chromosomes, are essential for the stability and replication of eukaryotic chromosomes.[1−3] In human chromosomes, the length of telomeres is ∼15 kbp. It is known that telomeres progressively shorten with each cell cycle in somatic cells and eventually result in cellular senescence.[4−7] In contrast, the telomere length in tumor cells is maintained by telomerase and leads to cellular immortalization.[8−10] Note that telomerase appears in more than 85% of tumor cells and is not found in most somatic cells.[11] During the past few years, telomere−telomerase research has received great attention not only for its own importance but also for its potential use in therapeutic application.[12−15] Although it is believed that the formation of quadruplex structure in telomeric 3′-overhang repeats could effectively inhibit telomerase activity,[16−18] a challenging question regarding the existence of quadruplex structure in native human telomeres in vivo has not been verified yet.[19]

Telomeres generally consist of many tandem repeats of guanine-rich (G-rich) motifs, for example, the hexameric repeats of TTAGGG/CCCTAA in vertebrate telomeres.[20] Of particular interest is that a short 3′-overhang of G-rich single-stranded sequence 100−200 bases long could adopt intramolecular G-quadruplex structures in vitro. The quadruplex structure is stabilized by the $\pi-\pi$ interaction of the cyclic G-quartets stacked on top of each other, and the G-quartet is formed by Hoogsteen hydrogen bonding among four guanine bases.[21,22] Moreover, different types of quadruplex structures have been documented in the telomeric sequence of d[$AG_3(T_2AG_3)_3$] (Hum22). Chart

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(1) Blackburn, E. H.; Greider, C. W. *Telomeres*; Cold Spring Harbor Laboratory Press: New York, 1996.
(2) Blackburn, E. H. *Annu. Rev. Biochem.* **1984**, *53*, 163−194.
(3) Williamson, J. R. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 703−730.
(4) Harley, C. B.; Futcher, A. B.; Greider, C. W. *Nature* **1990**, *345*, 458−460.
(5) Lundblad, V.; Szostak, J. W. *Cell* **1989**, *57*, 633−643.
(6) Sandell, L. L.; Zakian, V. A. *Cell* **1993**, *75*, 729−739.
(7) Harley, C. B.; Villeponteau, M. P. *Curr. Opin. Genet. Dev.* **1995**, *5*, 249−255.
(8) Greider, C. W.; Blackburn, E. H. *Cell* **1987**, *51*, 887−898.
(9) Feng, J.; Funk, W. D.; Wang, S.-S.; Weinrich, S. L.; Avilion, A. A.; Chiu, C.-P.; Adams, R. R.; Chang, E.; Allsopp, R. C.; Yu, J.; Le, S.; West, M. D.; Harley, C. B.; Andrews, W. H.; Greider, C. W.; Villeponteau, B. *Science* **1995**, *269*, 1236−1241.
(10) Hahn, W. C.; Counter, C. M.; Lundberg, A. S.; Beijersbergen, R. L.; Brooks, M. W.; Weinberg, R. A. *Nature* **1999**, *400*, 464−468.
(11) Kim, N. W.; Piatyszek, M. A.; Prowas, K. R.; Harley, C. B.; West, M. D.; Ho, P. L.; Coviello, G. M.; Wright, W. E.; Weinrich, S. L.; Shay, J. W. *Science* **1994**, *266*, 2011−2015.
(12) Blackburn, E. H. *Nature* **1991**, *350*, 569−573.
(13) Breslow, R. A.; Shay, J. W.; Gazdar, A. F.; Srivastava, S. *J. Natl. Cancer Inst.* **1997**, *89*, 618−623.
(14) Mergny, J. L.; Hélène, C. *Nat. Med.* **1998**, *4*, 1366−1367.
(15) Han, H.; Hurley, L. H. *Trends Pharmacol. Sci.* **2001**, *21*, 136−142.
(16) Zahler, A. M.; Williamson, J, R.; Cech, T. R.; Prescott, D. M. *Nature* **1991**, *350*, 718−720.
(17) Neidle, S.; Parkinson, G. N. *Nat. Rev. Drug Discovery* **2003**, *1*, 383−393.
(18) Kerwin, S. M. *Curr. Pharm. Des.* **2000**, *6*, 441−478.
(19) Neidle, S.; Parkinson, G. N. *Curr. Opin. Struct. Biol.* **2003**, *13*, 275−283.
(20) Morin, G. B. *Cell* **1989**, *59*, 521−529.
(21) Gellert, M.; Lipsett, M. N.; Davies, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **1962**, *48*, 2013−2018.
(22) Arthanari, H.; Basu, S.; Kawano, T. L.; Bolton, P. H. *Nucleic Acids Res.* **1998**, *26*, 3724−3728.

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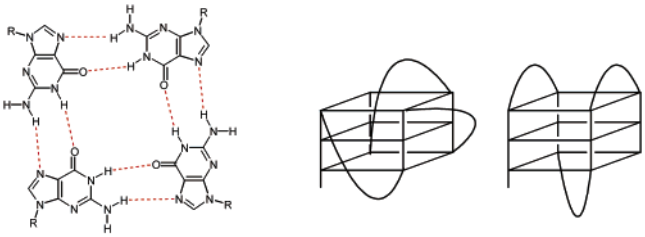
Chart 1



1A–C show the structure of the G-quartet, the X-ray crystal structure,[23] and the NMR solution structure[24] of Hum22 in vitro, respectively. However, direct evidence of quadruplex structure in native human telomeres has not been documented. Nevertheless, numerous indirect evidence support the idea that formation of G-quadruplex in vivo is likely to occur.[25–27] Recently, Schaffitzel et al.[28] used in vitro-generated antibodies to show that the cilitate *Stylonychia* forms quadruplex structures in vivo. In addition to telomeres, the quadruplex structure was also found in the c-myc promotor sequence.[29] The aim of this work is to provide direct evidence to elucidate the existence of the quadruplex structure in native human telomeres.

Recently, we have synthesized a novel fluorescence biomarker, 3,6-bis(1-methyl-4-vinylpyridinium)carbazole diiodide (BMVC).[30] We found that the fluorescence intensity of BMVC increases from 1 to almost 2 orders of magnitude upon interacting with DNA.[31] The fluorescence bands of BMVC locate at the red side of 570 nm upon interaction with eight different quadruplex structures, while the fluorescence bands of BMVC locate around 545 nm in the presence of $[d(AT)_6]_2$, GC-rich linear duplex and calf thymus DNA. Moreover, among these 11 DNA structures, 0.1 μM BMVC could not reveal duplex DNA, but did reveal some specific quadruplex structures in the prestained gels, such as a parallel quadruplex $[d(T_4G_4)]_4$ and an antiparallel quadruplex $d(T_2AG_3)_4$. Here we take advantage of BMVC's high sensitivity and binding preference to quadruplex $d(T_2AG_3)_4$ over duplex DNA and different fluorescence bands to investigate the fluorescence spectra of BMVC in human cancer cells. By using submicromolar concentration of BMVC, the distinct fluorescence spectra of BMVC upon interacting with nuclei and metaphase chromosomes allow us for the first time to demonstrate the presence of quadruplex structure in native human telomeres.

EXPERIMENTAL SECTION

BMVC Binding Assay. To perform the competition binding assay, 2 or 0.2 μM BMVC was incubated with 20 μM quadruplex $d(T_2AG_3)_4$ (Hum) or linear duplex $[d(ATGCGCA_2T_2GCGCAT)]_2$ (LD) at room temperature for 10 min in buffer containing 10 mM Tris-HCl and 150 mM NaCl (pH 7.5). The reaction products were analyzed using 20% polyacrylamide gels. The gels were run at 100 V/cm for 15 h at 4 °C. To confirm the presence of quadruplex and linear duplex DNA, the same gels were then poststained in a solution containing 10 μM BMVC for 10 s.

BMVC Labeling of Living Cells. Stock cell cultures were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) in a 5% CO_2 incubator at 37 °C for 24–36 h prior to BMVC treatment. The cells were treated with trypsin after incubation and then the protocal of TRI reagent (standard procedures of Sigma product information) was used to extract the genomic DNA. The CL1-1 cells from human lung epithelial adenocarcinoma were kindly provided by Dr. P. C. Yang at National Taiwan University Hospital.

BMVC Labeling of Metaphase Chromosomes. To obtain cells arrested at metaphase of the cell cycle, cells were first cultivated in DMEM containing 10% FCS at 37 °C in a 5% CO_2 incubator. Subconfluent monolayer cells were subjected to 1 μg/mL Demecolcine (Colcemid, Life Technologies, Gaithersburg, MD) treatment for 1 h. The arrested cells were resuspended in 0.075 M KCl hypotonic buffer for 20 min and then fixed with methanol/acetic acid (3:1). An aliquot of 15–20 μL of cell suspension was dropped onto a slightly humidified slide and then the slide was placed on a hot metal plate to evaporate the fixatives. The sample was washed with PBS and then incubated with 0.1 μM BMVC for 10 min at room temperature. After incubation, the sample was washed again before imaging study.

Confocal Microscopy. A confocal microscopy (Leica TCS SP2) was used to study BMVC-stained metaphase chromosomes. The excitation source was Ar ion laser emitted at 488 nm, and the detector system was a sensitive cooled charged-coupled device camera. Fluorescence emissions of BMVC at 500–620 nm were recorded by scanning 3-nm wavelength windows for each image. The spectra collected at the ends of chromosomes (telomeric) and other places of chromosomes (chromosomal) were obtained from an average of 60–120 individual chromosomes. The selection of the telomere-proximal regions was based on the documented Q-FISH microscopy.[32]

RESULTS

Binding Preference. To determine the binding preference of BMVC to LD or Hum, we have conducted competition analysis under high (2 μM) and low (0.2 μM) concentrations of BMVC (Figure 1, prestain, lanes 1–6). Visualization of BMVC-bound DNA molecules was achieved under a UV light. It is clear that BMVC was capable of binding to both quadruplex Hum and duplex LD under both BMVC concentrations (Figure 1, lanes 1, 2, 4, 5). However, when a limiting amount of BMVC was incubated with an equal amount of Hum and LD in a mixture, most of the BMVC was bound to quadruplex Hum (Figure 1, lanes 3, 6). As a control, the same gels were then poststained with 10 μM BMVC to visualize the position and level of DNA loaded (Figure 1, lanes 7–12). Under our electrophoresis condition, BMVC-bound quadruplex Hum complex migrates faster than that of free quadruplex

(23) Wang, Y.; Patel, D. J. *Structure* **1993**, *1*, 262–283.
(24) Parkinson, G. N.; Lee, M. P. H.; Neidle, S. *Nature* **2002**, *417*, 876–880.
(25) Fang, G.; Cech, T. R. *Cell* **1993**, *74*, 875–885.
(26) Giraldo, R.; Rhodes, D. *EMBO J.* **1994**, *13*, 2411–2420.
(27) Lin, Y.-C.; Shih, J.-W.; Hsu, C.-L.; Lin, J.-J. *J. Biol. Chem.* **2001**, *276*, 47671–47674.
(28) Schaffitzel, C.; Berger, I.; Postberg, J.; Hanes, J.; Lipps, H. J.; Plückthun, A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8572–8577.
(29) Siddiqui-Jain, A.; Grand, C. L.; Bearss, D. J.; Hurley, L. H. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11593–11598.
(30) Chang, C.-C.; Wu, J.-Y.; Chang, T.-C. *J. Chin. Chem. Soc.* **2003**, *50*, 185–188.
(31) Chang, C. C.; Wu, J. Y.; Chien, C. W.; Wu, W. S.; Liu, H.; Kang, C. C.; Yu, L. J.; Chang, T.-C. *Anal. Chem.* **2003**, *75*, 6177–6183.
(32) Shay, J. W.; Zou, Y.; Hiyama, E.; Wright, W. E. *Hum. Mol. Genet.* **2001**, *10*, 677–685.

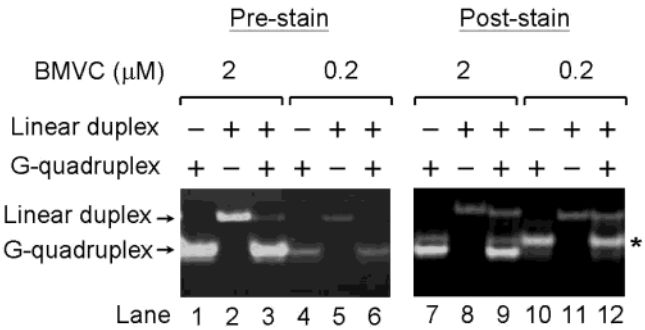


Figure 1. Binding preference of BMVC to quadruplex DNA. 20 μM each of quadruplex or linear duplex DNA were incubated with 2 or 0.2 μM BMVC and analyzed on a 20% polyacrylamide gel (prestain, lanes 1−6). The same gels were then poststained with 10 μM BMVC to visualize the position and level of DNA loaded (poststain, lanes 7−12). An asterisk indicates position of the free quadruplex DNA.

Hum. Thus, both BMVC-bound and free quadruplex Hum are revealed in the poststained gel. We found that a small amount of Hum was prestained by using 0.2 μM BMVC, and most of the Hum was poststained by BMVC (Figure 1, lanes 10, 12). Under the condition of 2 μM BMVC, the prestained Hum became dominant, as shown in lanes 7 and 9. More significantly, the BMVC fluorescence was only detected in quadruplex DNA when a limiting amount of BMVC was used. Thus, it is clear that BMVC has a binding preference toward quadruplex Hum over duplex LD. Note that a similar binding preference was also found in the presence of K^+ ions (data not shown).

Presence of Quadruplex DNA Structure in Human Nucleus. With the binding preference and high sensitivity of BMVC toward quadruplex Hum, we anticipate that BMVC, especially at low concentration, may be useful to verify the hypothesis of quadruplex structure formed in the G-rich 3′-overhang of human telomeres. Moreover, different fluorescence bands of BMVC upon binding to quadruplex and duplex DNA provide an additional key for elucidating the possible quadruplex structure formed in the human nucleus. For clarity, Figure 2a shows the fluorescence bands of BMVC around 575 and 545 nm upon binding to quadruplex Hum and duplex LD, respectively. Figure 2b shows the fluorescence spectra of BMVC and its mixture with DNA that were extracted from human lung epithelial adenocarcinoma CL1-1 cells at different incubation times. The fluorescence emissions were not well discernible before adding DNA. The spectrum taken right after the addition of DNA to BMVC differs from the spectrum recorded after 10-min incubation time. The 575-nm fluorescence observed at very short incubation time supported the existence of a quadruplex structure. At long incubation time, the 545-nm fluorescence band dominates. Note that similar spectral features were also observed in human oral cancer Ca9-22 cells. Since the duplex structures of DNA are overwhelmed in chromosomes, the observation of the 575-nm fluorescence supported that BMVC was capable of binding to the quadruplex structure even in the presence of excess amount of duplex DNA.

In addition, different concentrations of BMVC incubated with the nuclei that were extracted from Ca9-22 cells were subjected to fluorescence spectral analysis directly. Figure 2c shows the 575-nm fluorescent emissions detected at low concentration of BMVC, while the 545-nm fluorescence band was obtained at high

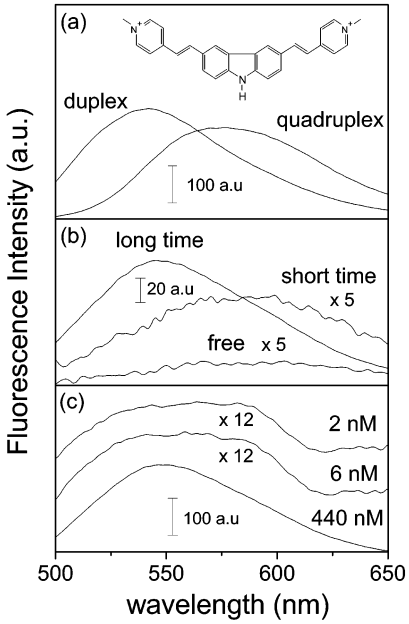


Figure 2. (A) Chemical structure of BMVC and the fluorescence spectra of BMVC upon binding to duplex LD and quadruplex Hum, (B) Fluorescent spectra of 50 nM BMVC and its mixture with DNA that were extracted from human lung cancer CL1-1 cells at very short incubation time (right after adding the DNA to BMVC) and long incubation time (10 min). The first two fluorescence spectra were scaled up by 5 times. (C) Fluorescent spectra of various concentrations (2, 6, and 440 nM) of BMVC bound to Ca9-22 nuclei. The fluorescence signals of low concentration of BMVC were scaled up by 12 times to visualize the emission at 500−650 nm.

concentration of BMVC upon interacting with nuclei. Since duplex DNA is the major component of chromosomal DNA, it is not surprising that the 545-nm fluorescence predominated at a high concentration of BMVC. The important issue is that the detection of the fluorescence around 575 nm at short incubation time or low concentration of BMVC upon interacting with chromosomal DNA provides evidence for the existence of quadruplex structure in human nucleus.

Presence of Quadruplex DNA Structure in Human Telomeres. Since telomeres are the ends of chromosomes, we next compare the fluorescence emissions of BMVC collected at the ends of chromosomes to other places on chromosomes to verify the hypothesis of the existence of quadruplex structure in human telomeres. Figure 3A shows fluorescence microscopy of BMVC in metaphase chromosomes of CL1-1 cells. Metaphase spreads prepared from CL1-1 cells on microscope slides were incubated with 50 nM BMVC for 10 min at room temperature. To compare the fluorescence spectra at 500−620 nm from the staining BMVC at the ends of chromosomes and other places on chromosomes, the fluorescence spectra of telomere-proximal regions and other places on chromosomes were obtained from an average of 120 individual chromosomes, as shown in Figure 3B. Telomere-proximal regions showed fluorescence emissions around 565 nm whereas the other chromosomal regions showed fluorescent emissions around 545 nm. Since we were not able to completely eliminate the possible contribution from the interaction with duplex DNA in the telomere-proximal regions, the red shift of the fluorescence band to ∼565 nm was likely due to the interference from the interaction of BMVC with duplex DNA. It

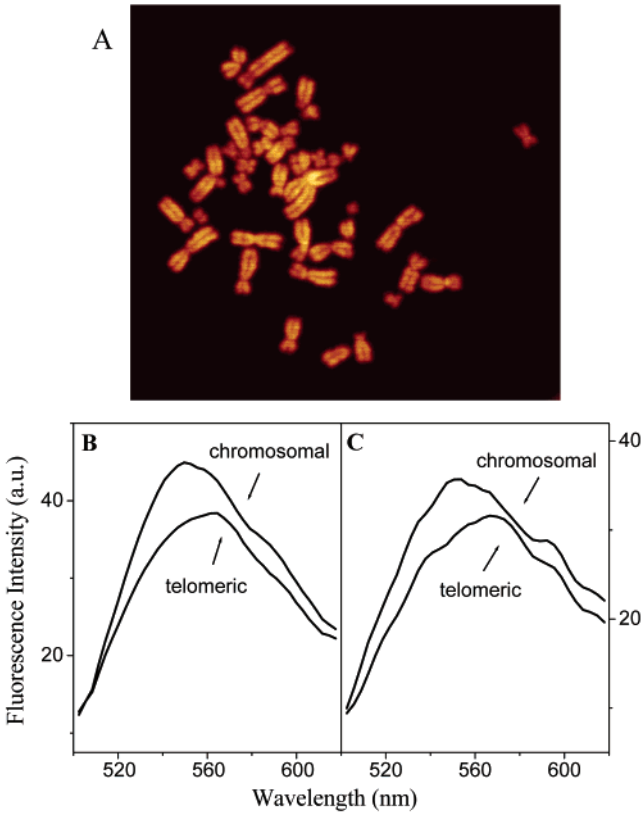


Figure 3. (A) Fluorescence microscopy of BMVC-stained metaphase chromosomes of CL1-1 cells. Fluorescence emissions of BMVC at 500−620 nm were collected at the ends of chromosomes (telomeric) and other places on chromosomes (chromosomal) of human CL1−1 (B) and Ca9−22 (C) cells by scanning 3-nm wavelength windows for each image. The spectra were obtained from the average of 120 individual CL1-1 chromosomes and 60 individual Ca9-22 chromosomes.

appears that the fluorescence emission around 565 nm at the ends of chromosomes and around 545 nm at other regions of chromosomes could reveal the presence of quadruplex structures in human telomeres. In addition, very similar spectral features were also observed in the average of 60 metaphase chromosomes of human Ca9-22 cells, as shown in Figure 3C. Here the fluorescence band of telomere-proximal regions was ∼570 nm. Thus, our finding demonstrates for the first time the existence of quadruplex structure in native human telomeres.

DISCUSSION

Single-stranded telomeric DNA tends to form quadruplex structures in vitro, but direct evidence of quadruplex structures in vivo is much less. Nevertheless, a number of findings have been presented to support the existence of the G-quadruplex structure in vivo. For instance, both the β-subunit of the *Oxytricha* telomere-binding protein[25] and the Rap1p of *Saccharomyces cerevisiae*[26] promote the formation of G-quadruplex structure at very low G-rich DNA concentration. Recently, Schaffitzel et al.[28] used in vitro-generated antibodies to show that the telomeres of cilitate *Stylonychia* form quadruplex structures in vivo. In addition to telomeres, the quadruplex structure was also found in the c-myc promotor sequence.[29] However, to our knowledge, direct evidence for the presence of quadruplex structures in human telomeres has not been documented.

The verification of the existence of quadruplex structures of human telomeres is very important in supporting the recent development of telomerase inhibitors for antitumor agents. This is because the folding of telomeric DNA into a quadruplex structure has been shown to inhibit telomerase activity in vitro.[16−18] Therefore, molecules that stabilize G-quadruplex have the potential to interfere with telomere replication and possibly to serve as antitumor agents.[14,15] Here we have used a novel fluorescence biomarker BMVC to verify the quadruplex structure in human telomeres.

The fluorescence of BMVC increases ∼2 orders of magnitude in the presence of Hum and LD.[31] In addition, distinct fluorescence emissions of Hum-binding and most of duplex-binding BMVC were characterized to be around 575 and 545 nm, respectively. Although the 580-nm fluorescence emissions was detected in the presence of $[d(GC)_6]_2$,[30] its fluorescence signal was ∼1 order of magnitude less than the fluorescence signals of Hum-binding and LD-binding BMVC. Furthermore, the competition assay showed that the binding preference of BMVC to quadruplex Hum is over duplex LD and much stronger than $[d(GC)_6]_2$. In addition, 0.1 μM BMVC could reveal 0.01 μM Hum, but not 10 μM duplex DNA in the prestained gels. The higher sensitivity of BMVC to quadruplex Hum than to duplex DNA allows us to reveal the quadruplex Hum by using a very low concentration of BMVC. It appears that detection of the 575-nm fluorescence emissions while adding a very low concentration of BMVC to the chromosomal DNA that was extracted from human cells supports the presence of quadruplex structures in the human nucleus.

Tanious et al.[33] found that the binding mode of carbazole dications to the minor groove of AT sequences is quite different from the intercalation of other planar aromatic chromophores. Binding of carbazole dications to poly(dG-dC)$_2$ is ∼1 order of magnitude weaker than binding to poly(dA-dT)$_2$. They further suggested that the intercalation is more likely the binding mode of bisamidinocarbazoles to poly(dG-dC)$_2$. In our previous work,[31] we found that the ∼ 545-nm fluorescence emissions detected in the GC-rich duplexes differ from the 580-nm emissions in the pure GC duplexes, indicating that BMVC preferentially interacts with AT than with GC in the DNA duplexes. To our knowledge, the interaction of carbazole dications with quadruplex structures has not been documented, with the exception of BMVC.[30,31] However, Neidle et al.[34] have applied molecular modeling to study the binding mode of 3,6-disubstituted acridine derivatives with the Hum quadruplex. Their results showed that the ligand externally stacks between the diagonal loop and the end of the G-quartet. It is conceivable that the major binding mode of the G-quadruplex interaction with BMVC is similar to that of acridine derivatives. The exact binding modes of BMVC to various DNAs deserve further investigation.

Since several telomerase inhibitors could assist the formation of G-quadruplex structures,[29] it is possible that the formation of quadruplex structures of chromosomal DNA is induced by BMVC. However, the fluorescence band detected around 575 nm immediately after the addition of DNA to BMVC could eliminate

(33) Tanious, F. A.; Ding, D.; Patrick, D. A.; Bailly, C.; Tidwell, R. R.; Wilson, W. D. *Biochemistry* **2000**, *39*, 12091−12101.
(34) Read, M.; Harrison, R. J.; Romabnoli, B.; Tanious, F. A.; Gowan, S. H.; Reszka, A. P.; Wilson, W. D.; Kelland, L. R.; Neidle, S. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 4844−4849.

this possibility. This is because the circular dichroism spectra of the $d(T_2AG_3)_4$ in the absence of salt and then incubated with BMVC even for 1 h show no appreciable difference. Our finding provides more evidence for the existence of quadruplex structure in the human nucleus.

The aim of this work is to verify the existence of the quadruplex structure in the human telomere. Note that the duplex DNA structures are overwhelmed in chromosomes. To avoid the ensemble average effect, we have applied the idea from single-molecule spectroscopy[35] to measure the fluorescence spectra of telomere-proximal regions in metaphase chromosomes. This is because telomeres are the ends of chromosomes. The most striking feature is the detection of the fluorescence emissions around 570 and 545 nm at telomere-proximal regions in metaphase chromosomes and other chromosomal regions, respectively. Our results show the first evidence for the presence of quadruplex structure in human telomeres.

In addition, two possible quadruplex structures of Hum (Chart 1B and IC) were documented. Although the fluorescence bands of BMVC around 575 nm are similar upon interacting with parallel and antiparallel quadruplexes, their binding affinities could be very different. Note that almost no fluorescence was detected in the poststained gels of tetramolecular parallel quadruplex $[d(TG_4T)]_4$,[31] implying that the binding affinity of BMVC to $[d(TG_4T)]_4$ is much weaker than LD. Since there is no tails in the parallel quadruplex Hum (Chart 1B), we anticipated that the binding affinity of BMVC to the parallel quadruplex Hum is also weak. Considering the competition assay of BMVC to these two quadruplex Hum structures with duplex DNA in chromosomes, the observation of the fluorescence around 575 nm under a low concentration of BMVC or short incubation time with chromosomal DNA suggests that the unimolecular antiparallel quadruplex structure (Chart 1C) is more likely the structure in human telomeres.

A promising biomarker for recognizing quadruplex structure of human telomeres is applicable to elucidate the structure of human telomeres. We have taken the advantage of BMVC's distinct fluorescence properties and binding preference to quadruplex structure and applied the idea of single-molecule spectroscopy to demonstrate the existence of quadruplex structure in human telomeres. Verifying the existence of quadruplex structures in the human telomeres is important in the study of cell proliferation, cancer research, and drug development.

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(35) Moerner, W. E.; Orrit, M. *Science* **1999**, *213*, 1670−1676.